UNIVERSITÉ PARIS 6 - PIERRE ET MARIE CURIE

THÈSE DE DOCTORAT en ÉCOLOGIE

INSTITUTO DE
ECOLOGIA, A.C.

Institut de recherche
pour le développement

# COMMENT LES VERS DE TERRE INFLUENCENT LA CROISSANCE DES PLANTES: ÉTUDES EN SERRE SUR LES INTERACTIONS AVEC LE SYSTÈME RACINAIRE

Présentée pour l'obtention du titre de
Docteur de l'Université Pierre et Marie Curie (Paris VI)

par

GEORGE GARDNER BROWN

Soutenue le 03 Décembre 1999 devant le jury composé de :

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# RÉSUME

Les vers de terre stimulent la production aérienne des plantes la plupart du temps, celle des graines aussi, mais dans une moindre mesure, alors que la production racinaire est parfois diminuée. Ces effets sont d'autant plus importants que la biomasse des vers dépasse 30 g $m^{-2}$, que le sol est pauvre en C (<1.5%), sableux (>65% sable, <10% argile) et modérément acide ($5.6<pH<7$). C'est dans la rhizosphère essentiellement que se développent les interactions entre vers et plantes. Les expériences réalisées en serre avec l' espèces de ver pantropicale *Polypheretima elongata* ont mis en évidence une augmentation de 88% de la biomasse racinaire et de 119% des parties épigées de haricots mais aucune effet sur la production de graines. On observe une augmentation de la densité et une distribution plus homogène des racines qui donnent une meilleure résistance des racines aux stress, et des effets positifs des vers sur la disponibilité en élements nutritifs. *Pontoscolex corethrurus*, autre espèce endogée pantropicale, n'a pas affecté significativement la production des haricots malgré un effet positif sur la densité et distribution des racines. Avec le maïs sans apport de litière en surface, *P. corethrurus* a peu modifié la production; l'addition de litière a un effet négatif, en raison, probablement , de la richesse intitiale du sol utilisé. Dans ce cas, les vers stimulent plus la production relative de racines (baisse du rapport tige/racine) et leur densité, sans augmentation de la production des tiges et des graines. Cependant, les racines du maïs ont contribué à la nutrition des vers par l'exsudation: 8% du C des vers provient des racines et le transfert dans leur biomasse de $^{15}N$ contenu dans des résidus déposés en surface est 25 fois supérieur en présence de plantes vivantes. Une expérience avec la graminée *Brachiaria decumbens* et les mycorrhizes montre une régulation par les vers de la colonization des racines par les MVA et les effets des engrais sur cette interaction. Les interactions MVA-vers expliquent probablement largement les effets positifs des vers sur la production des plantes. L'identification plus précise des mécanismes réalisée dans ce travail ouvre la voie à une modélisation qui permettra de mieux cibler les futures expériences et d'aboutir, in fine, à des méthodes de gestion appropriées des vers de terre dans les systèmes agricoles.

Mots-clés:
Vers de terre, Production des plantes, Rhizosphère, Fertilité du sol, Isotopes stables, Mycorhizes.

# SUMMARY

Earthworms stimulate shoot biomass in most cases, but grain production is less affected and roots frequently respond negatively. Their effects on production become more important when earthworm biomass is greater than 30 g $m^{-2}$, and when the soil is poor in C (<1.5%), sandy (>65% sand, <10% clay) and moderately acid (5.6<pH<7). Earthworm-plant interactions occur primarily in the rhizosphere. Greenhouse experiments with the pantropical earthworm species *Polypheretima elongata* showed an 88% and 119% increase in bean root and shoot biomass, respectively, but no effect on grain yields. Higher root density and a more even root distribution, as well as an increase in nutrient availability in worm-worked soils promotes plant resistance to stresss. *Pontoscolex corethrurus*, another endogeic pantropical species, did not significantly affect bean production, although a positive effect was seen on root density and distribution. With maize, *P. corethrurus* had no effect on production when no surface litter was added, and reduced biomass when residues were applied, probably due to the richness of the soil used. In this case, earthworms stimulated the relative production of roots (lower shoot/root ratio) and their density, without increasing yields. Nevertheless, maize roots contributed to earthworm nutrition by rhizodeposition: 8% of earthworm tissue C was derived from roots and tissue N derived from $^{15}N$-labelled surface residues increased 25 times in presence vs. absence of growing plants. An experiment with the pasture grass *Brachiaria decumbens* ± mycorrhiza showed the regulatory role of earthworms on root colonization by arbuscular mycorrhizal fungi (AMF) and the effect of fertilizers on this interaction. Interactions AMF-earthworms are probably responsible for most positive effects of earthworms on plant production. The more detailed identification of mechanisms undertaken in this thesis opens the way for modelling of earthworm effects on plant production, which will help direct further research and accomplish at last, a more appropriate management of earthworm benefits for agroecosystems.

Key Words:
Earthworms, Plant production, Rhizosphere, Soil fertility, Stable Isotopes, Arbuscular Mycorrhizal Fungi

# RESUMEN (EN ESPAÑOL)

Las lombrices de tierra estimulan la biomasa aérea de las plantas en la mayor parte de los casos, pero la producción de grano es generalmente menos estimulada y la de raíces frecuentemente afectada negativamente. Los efectos de las lombrices en la producción de las plantas se torna más importante cuando su biomasa sobrepasa los 30 g $m^{-2}$ y cuando el suelo es pobre en C (<1.5%), arenoso (>65% arena, <10% arcilla) y moderadamente ácido ($5.6<pH<7$). Las interacciones entre las lombrices y las plantas se desarrollan principalmente en la rizósfera. Experimentos en el invernadero con la especie pantropical *Polypheretima elongata* mostraron un aumento de 88% y 119% en la biomasa de raíces y aérea, respectivamente, de frijoles negros, pero ningún efecto significativo en el rendimiento de granos. En presencia de las lombrices se encontró además una mayor densidad de raíces del frijol con distribución más uniforme y un aumento en la disponibilidad de nutrientes en los turrículos, lo que confiere a la planta una mayor resistencia al estrés. *Pontoscolex corethrurus*, otra especie endógea pantropical, no afectó significativamente la producción de frijoles aunque se observó un efecto positivo en la densidad y distribución de las raíces. En el experimento con maíz, *P. corethrurus* tampoco afectó positivamente la producción cuando no se añadió resíduo superficial y cuando éstos resíduos fueron aplicados, el rendimiento del maíz fue reducido debido a las lombrices, probablmente debido a la alta fertilidad nativa del suelo utilizado para éste experimento. Las lombrices estimularon la producción de raíces en relación a las partes aéreas (mayor relación raíz/parte aérea) y su densidad fue también mayor, aunque no se observó efectos positivos en el rendimiento de granos. Sin embargo, las raíces del maíz contribuyeron a la nutrición de las lombrices a través de la rizodeposición: 8% del C del tejido de las lombrices fue derivado de las raíces del maíz y la cantidad de N derivado de resíduos superficiales de maíz marcados con $^{15}N$ fue 25 veces mayor en presencia que en ausencia de plantas de maíz. Otro experimento con el pasto *Brachiaria decumbens* ± hongos micorrízicos arbusculares (HMA) mostró el papel regulador de las lombrices en la colonización de las raíces por los HMA y el efecto de los fertilizantes en ésta interacción. Interacciones entre los HMA y las lombrices son probablemente responsables por la mayoría de los efectos positivos de las lombrices en la productividad de las plantas. La identificación más detallada de los mecanismos desarrollada en ésta tesis abre el camino para la modelización de los efectos de las lombrices de tierra en la producción vegetal, lo que ayudará a direccionar futuras investigaciones y finalmente lograr un manejo más apropiado de los benefícios de las lombrices de tierra en los agroecosistemas.

Palabras clave:
Lombrices de tierra, Producción Vegetal, Rizósfera, Fertilidad del Suelo, Isótopos Estables, Hongos Micorrízicos Arbusculares

# RESUMO (EM PORTUGUÊS)

As minhocas estimulam a biomassa aérea das plantas na la maior parte dos casos, mas a produção de grão é geralmente menos estimulada e as raízes são frequentemente afectadas negativamente. Os efeitos das minhocas na produção das plantas se torna mais importante quando a sua biomassa é >30 g $m^{-2}$ e quando o solo é pobre em C (<1.5%), arenoso (>65% areia, <10% argila) e moderadamente ácido ($5.6<pH<7$). As interações entre as minhocas e as plantas se desenvolvem principalmente na rizosfera. Experimentos em casa de vegetação com a espécie pantropical *Polypheretima elongata* mostraram um aumento de 88% e 119% na biomassa das raízes e aérea, respetivamente, do feijão preto, mas nenhum efeito significativo no rendimento de grãos. Na presença das minhocas se encontrou também uma maior densidade de raízes do feijão com uma distribuição mais uniforme, e um aumento na disponibilidade dos nutrientes nos turrículos (excretas), o que confere à planta uma maior resistência ao estrés. *Pontoscolex corethrurus*, outra espécie endogéia pantropical, não afetou significativamente a produção de feijão mesmo que se observou um efeito positivo na densidade e na distribuição das raízes. No experimento com o milho, *P. corethrurus* também não afetou positivamente a produção quando não se adicionaram resíduos superficiais e quando estes foram adicionados, o rendimento do milho foi reduzido devido às minhocas, provavelmente devido à alta fertilidade nativa do solo utilizado para este experimento. As minhocas estimularon a produção das raízes em relação às partes aéreas (maior taixa raíz/parte aérea) e a sua densidade também foi maior, mesmo que não se observou efeito positivo no rendimento dos grãos. A pesar desses resultados, as raízes do milho contribuíram à nutrição das minhocas através da rizodeposição: 8% do C do tecido das minhocas foi derivado das raízes do milho e a quantidade de N derivado dos resíduos superficiais do milho marcado com $^{15}N$ foi 25 vezes maior na presença que na ausença das plantas de milho. Outro experimento com a grama *Brachiaria decumbens* ± fungos micorrízicos arbusculares (FMA) mostrou o papel regulador das minhocas na colonização das raíces pelos FMA e o efeito dos fertilizantes nesta interação. Interações entre os FMA e as minhocas são provavelmente responsáveis pela maioría dos efeitos positivos das minhocas na produtividade das plantas. A identificação mais detalhada dos mecanismos desenvolvida nesta tese abre o caminho para a modelização dos efeitos das minhocas sobre a produção vegetal, o que ajudará a direcionar futuras pesquisas neste tema e finalmente realizar um manejo mais apropriado dos benefícios das minhocas nos agroecosistemas.

Palavras chave:
Minhocas, Produção Vegetal, Rizosfera, Fertilidade do Solo, Isótopos Estáveis, Fungos Micorrízicos Arbusculares

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**Nohemí Ibáñez Brown**,

*et à mes parents,*

**Keith et Kay† Brown**
**Efraín et Enriqueta Ibáñez**

# TABLE DE MATIÈRES

# LISTE DES ANNEXES

## ANNEXE 1.

### CHAPITRE 2.

Table 1. Summary of all data collected during the Macrofauna Project and other data from the literature on the effects of earthworms on earthworm and plant species studied, residues applied, size of plot or pot used, and the response of plants (grain, shoot and root biomass, shoot:root ratios) and earthworms (initial and final biomass) over the experimental period.

## ANNEXE 2.

### CHAPITRE 3.

Description of the profile of savanna soil at La Víbora, Veracruz.

Figure 1. Texture (according to USDA classification) of the horizons from soil profile dug in the pasture at La Víbora, Veracruz (texture obtained as in Gee and Bauder, 1986).

Table 1. Selected physico-chemical properties of the surface and subsurface soil horizons of the pasture paddock at La Víbora, Veracruz.

Table 2. Soil water contents (%H2O, w/w) at which different pF values were obtained (ORSTOM, Laboratoire de Physique du sol), and the container capacity (determined as in Cassel and Nielsen, 1986) of the topsoil (A horizon, 0-10 cm) from La Víbora, Veracruz.

Table 3. $\delta^{13}C$ and $\delta^{15}N$ signatures (mean ± standard deviations) of the soil profile, A horizon light OM fractions and earthworm tissue (Gen. nov.1) from the pasture at La Víbora, Veracruz. Numbers accompanied by different letters within the same column indicate significant differences at the $p<0.05$ level).

Table 4. Percentage light OM (floating and particulate) fractions (LF) and heavy fractions (HF) in the topsoil (0-10cm) horizon from La Víbora, Veracruz. Fractions were gravitationally separated in $H_2O$ after shaking for 17 hours. Values are means of 3 replicates for % LF, while C and N values are results of a composite sample analysis. nd = not determined.

Figure 2. Texture of the topsoil from La Víbora and castings of Gen. nov.1, *Pontoscolex corethrurus* and *Polypheretima elongata* produced in laboratory cultures using the topsoil (0-10cm) horizon.

## ANNEXE 3.

### CHAPITRE 3.

Results of the mineralogical analysis (ORSTOM-Bondy) of the soil profile horizons at La Víbora, Veracruz.

## ANNEXE 4.

### CHAPITRES 3-7.

Brief description of the earthworm *Pontoscolex corethrurus* (from Sims and Gerard, 1985).

Figure 1. Several views of the external and internal morphology of *P. corethrurus.* A. Lateral view of external and internal structures drawn by Ana Moreno, from Borges (1988). B. Ventral view anteriorly and posteriorly (note distribution of setae) as in Sims and Gerard (1985).

## ANNEXE 5.

### CHAPITRE 3.

Table 1. Detailed results of the laboratory cultures using *Pontoscolex corethrurus* earthworms in soil from La Víbora, Veracruz. A. Earthworm biomass; B. Cocoon production; C. Cast production. (Barois, Irisson and Brown, unp. data)

Figure 2. Growth and mortality of *P. corethrurus* in laboratory cultures using soil from La Víbora, Veracruz. (Data was modified from Table 1 to fit estimated ages)

Figure 3. Surface cast production per weight (g) of *P. corethrurus* earthworms per day in the laboratory cultures using soil from La Víbora, Veracruz (Table 1).

## ANNEXE 6.

### CHAPITRE 3, 5 et 7.

Brief description of the earthwom *Polypheretima elongata* (from Borges, 1988).

Figure 1. External ventral and dorsal views of *P. elongata* (drawings by Ana Moreno, in Borges, 1988).

Figure 2. Internal anatomy of *P. elongata*. A. Ventral view, showing circulatory system, crop, gizzards and intestine. B. Detail of two spermatheca. C. Detail of the genitalia in segments 14-23.

## ANNEXE 7.

### CHAPITRE 3.

Table 1. Detailed results of the laboratory cultures of *P. elongata* earthworms in soil from La Víbora, Veracruz. A. Earthworm biomass; B. Cocoon poduction; C. Cast production. (Barois, Irisson and Brown, unp. data)

Figure 1. Growth and mortality of *P. elongata* in laboratory cultures using soil from La Víbora, Veracruz. (Data modified from Table 1 to fit estimated ages)

Figure 2. Surface cast production per weight of *P. elongata* earthworms per day in the laboratory cultures using soil from La Víbora, Veracruz (Table 1). No relationship between age and cast production was observed.

## ANNEXE 8.

### CHAPITRE 4.

Description of the soil profiles at the Estación de Biología Tropical "Los Tuxtlas," Veracruz.

Figure 1. Texture of the topsoil (USDA classification) from the forest at Los Tuxtlas and castings of *Pontoscolex corethrurus* produced in laboratory cultures using the topsoil (texture obtained as in Gee and Bauder, 1986).

Table 1. Soil water contents (%H2O, w/w) at which different pF values were obtained (ORSTOM, Laboratoire de Physique du sol), and the container capacity (determined as in Cassel and Nielsen, 1986) of the topsoil (A horizon, 0-10 cm) from the forest at Los Tuxtlas, Veracruz.

Table 2. Soil and earthworm (*P. corethrurus*) tissue $\delta^{13}C$ and $\delta^{15}N$ (means ± standard deviations) from the topsoil (A Horizon; 0-10cm) of the tropical rainforest at Los Tuxtlas, Veracruz.

Table 3. Percentage light OM (floating and particulate) fractions (LF) and heavy fractions (HF) in the topsoil (0-10cm) horizon from the forest at Los Tuxtlas, Veracruz. Fractions were gravitationally separated in $H_2O$ after shaking for 17 hours. Values are means of 3 replicates for % LF, while C and N values were taken from a composite sample (n=1) of the three separates in each fraction. nd = not determined

## ANNEXE 9.

### CHAPITRE 4.

Results of the mineralogical analysis (ORSTOM-Bondy) of the soil profile horizons at Los Tuxtlas, Veracruz.

## ANNEXE 10.

### CHAPITRE 4.

Table 1. Detailed results of the laboratory cultures using *Pontoscolex corethrurus* earthworms and topsoil (0-10cm) from Los Tuxtlas, Veracruz (Hernández, Barois and Brown, unp. data). A. Earthworm biomass; B. Cocoon production; C. Cast production; D. Hatchling production (weights).

Figure 1. Growth of *P. corethrurus* in the laboratory cultures using soil from the forest at Los Tuxtlas, Veracruz (Data modified from Table 1 to fit estimated ages).

# LISTE DES FIGURES

**INTRODUCTION GÉNÉRALE.**

**CHAPITRE 1.**

**CHAPITRE 2.**

**CHAPITRE 3.**

**CHAPITRE 4.**

**CHAPITRE 5.**

**CHAPITRE 6.**

## CHAPITRE 7.

# LISTE DES TABLEUX

## CHAPITRE 1.

## CHAPITRE 2.

**CHAPITRE 3.**

**CHAPITRE 4.**

**CHAPITRE 5.**

## CHAPITRE 6.

## CHAPITRE 7.

# INTRODUCTION GÉNÉRALE

# INTRODUCTION GÉNÉRALE

Chaque jour, le population mondiale s'accroit de 230,000 personnes dont l'alimentation est problèmatique. Cette croissance dèmographique est estimèe comme intervenant à 95-98 % dans les pays les moins dèveloppès (Tiers Monde) (NGS, Octobre 1998). Bien qu'assez de nourriture soit produite annuellement pour fournir plus que le minimum des besoins alimentaires pour approximativement 6 milliards d'habitants, soit 2 200 calories par jour et par individu selon l'ONU, la production et la disponibilitè de la nourriture sont mal rèparties à l'èchelle mondiale pour des raisons politiques, èconomiques et sociales et provoquent une malnutrition chronique pour environ 20 % de la population des pays en voie de dèveloppement (Reid, 1998).

L'augmentation de la production agricole ces derniéres dècennies rèsulte essentiellement de l'augmentation des ètendues de terres placèes sous cultures arables et de la modernisation des techniques agricoles (produits issus de l'agrochimie, matèriel agricole, nouvelles semences). Malheureusement, les variètès nouvelles sont dèpendantes de forts intrants; cette dèpendance les a rendues inabordables financiérement pour la grande majoritè des petits propriètaires de fermes et a limitè l'adoption de ces mèthodes par des propriètaires terriens plus riches (Sanchez, 1994). Ainsi, la situation en Afrique sub-saharienne a peu èvoluè; elle est peut etre meme pire qu'il y a trente ans (cf. Buresh *et al.*, 1997). Il en rèsulte que des millions de fermiers à travers le monde cultivent toujours des variètès traditionnelles nècessitant peu d'intrants et travaillent la terre avec l'aide d'animaux.

La dure rèalitè de cette situation conduit au dèveloppement d'un nouveau modéle proposè par Sanchez (1994) liant dèveloppement agricole et production accrue. Dans ce modéle, la dèpendance, prècèdemment basèe sur l'utilisation de produits issus de l'agrochimie, repose maintenant sur les concepts biologiques tels que (1) l'utilisation de semences amèliorèes, mieux adaptèes à des sols peu fertiles, (2) l'optimisation du cycle des nutriments et (3) l'amèlioration de l'activitè biologique du sol dans le but de minimiser les apports externes tout en optimisant leur efficacitè. La solution pour un fonctionnement convenable de ce modéle rèside dans une utilisation efficace des apports externes et des ressources internes telles que la matiére organique et la faune du sol. Alors que Sanchez (1994; 1997) propose une mise en oeuvre de ce modéle basèe principalement sur les deux premiéres composantes, Swift (1999) met plus l'accent sur les implications de la biologie du sol (la troisiéme composante) sur la règulation de la fertilitè du sol et sur sa gestion.

L'introduction de ce second modéle requiert une gestion biologique intègrèe du sol montrant que la manipulation de la faune du sol peut augmenter à la fois la production agricole et la rentabilitè, et pouvant etre mise en pratique dans une entreprise agricole (Swift, 1999). Il existe plusieurs exemples d'interventions biologiques profitables, directes ou indirectes, qui rendent prometteuse une instauration à grande èchelle (règionale ou nationale). Parmi ces derniers, on peut trouver l'inoculation de bactèries fixatrices d'azote (*Rhizobium*) à des lègumineuses (Giller *et al.*, 1994; Giller et Cadish, 1995), l'utilisation combinèe de fertilisants organiques et inorganiques pour amèliorer l'efficacitè de l'apport et la protection du sol (Palm *et al.*, 1997), l'addition de matiére organique et de vers de terre dans des champs de thè en Inde (Senapati *et al.*, 1999), l'inoculation de mycorhizes (VAM) à des jeunes plants destinès à etre transplantès, et l'inoculation d'agents biologiques spècifiques pour amèliorer la croissance des plantes (Swift *et al.*, 1994).

Pour rèellement profiter des interventions biologiques proposèes dans le modéle, diffèrentes ètapes doivent etre respectèes: premiérement, connaïtre les caractèristiques de base du sol; deuxiémement, identifier les contraintes limitant la production et dresser la liste des solutions possibles pour chaque constrainte; et finalement, tester les solutions possibles afin d'identifier les plus performantes et d'èvaluer leur compatibilitè avec les systémes agricoles (Swift, 1997). Le cycle Accumulation des connaissances-Application-Transfert prèsentè en Figure 1 peut facilement etre employè pour chacun des organismes identifiès comme intervenant biologique direct, comme le sont les vers de terre.

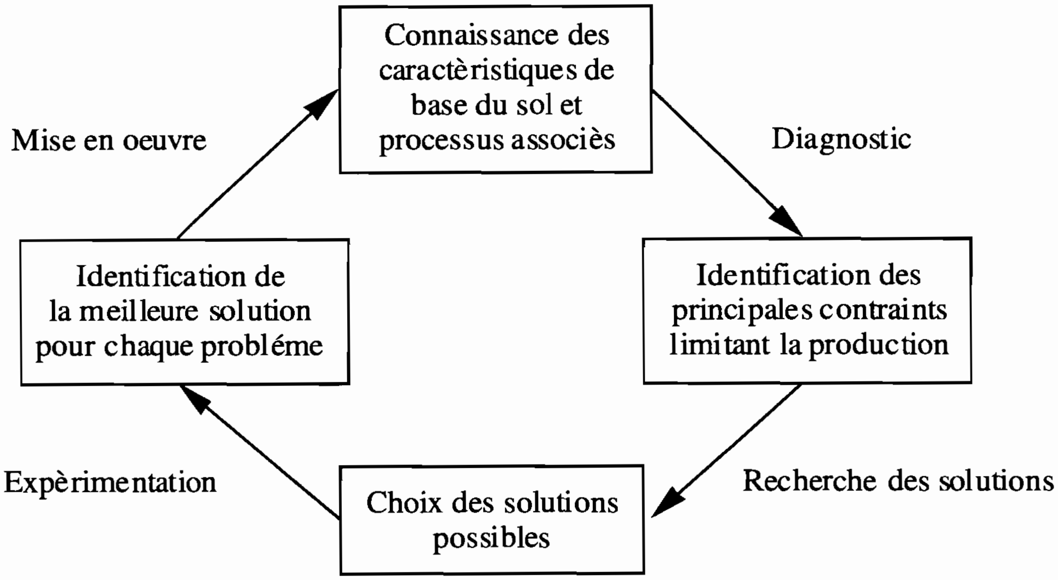

Figure 1. Etapes de mise en öeuvre du 'second paradigme' (Sanchez, 1994) utilisant des interventions et manipulations biologiques en vue d'obtenir une fertilitè du sol durable et une production agricole accrue (d'aprés Swift, 1997).

Ainsi, certaines contraintes èdaphiques limitant la production agricole pourraient etre levèes par la stimulation des effets biologiques et èdaphiques des vers de terre. Dans certains cas, une intervention directe (par exemple, par inoculation de populations approprièes) peut etre une solution adaptèe et rèalisable, alors que dans d'autres cas, une manipulation indirecte (gestion basèe sur des pratiques agricoles favorisant leurs populations et leurs activitès) est prèfèrable.

Le sol est à la base de la chaine alimentaire essentiel à la vie des plantes et des animaux, il constitue l'habitat des organismes du sol (y compris les racines). C' est un milieu dense, peu aèrè et gènèralement pauvre en matiére organique et en nutriments; ces caractèristiques engendrent d'importantes contraintes physiques et chimiques pour les organismes vivants qui y vivent (Lavelle et Spain, 1999). Dans les zones tropicales humides, les sols prèsentent des contraintes encore plus grandes lièes à une rèserve en humus faible et peu accessible, à une sensibilitè à l'èrosion couramment observèe et à de faible quantitès en nutriments assimilables (Lavelle, 1984). Cependant, les limitations abiotiques du fonctionnement du sol sont diminuèes par une tempèrature quasi constante et par l'humiditè gènèralement importante de ces environnements; le rôle de la règulation biologique dans l'ensemble fonctionnel du sol peut donc etre augmentè par le dèveloppement de symbioses, l'utilisation de mèthodes efficaces de conservation des nutriments et par la crèation et le maintien d'une structure du sol stable et aèrèe (Lavelle, 1984). La macrofaune, notamment les vers de terre, termites et fourmis prolifèrent, atteignant des populations et biomasses èlevèes (Lavelle *et al.*, 1994) et commencent alors à jouer un rôle plus important dans le fonctionnement du sol. Les structures crèes par leur activitè (galeries, turricules, chambres, etc.) modifient les caractèristiques physiques du sol et le cycle des nutriments (Figure 2), affectant ainsi la disponibilitè des ressources offertes aux autres organismes. Les activitès de ces Ingènieurs de l'Ecosystéme (Jones *et al.*, 1994; 1997) peuvent entrainer des changements considèrables dans la structure du sol (Blanchart *et al.*, 1999) et provoquer une cascade d'effets trophiques en modifiant les processus de dècomposition et de minèralisation de la matiére organique (Lavelle, 1996; Lavelle *et al.*, 1997). Ces effets se manifestent à diverses èchelles et ainsi, la matiére organique dont la dècomposition s'accèlére fortement lorsqu'elle est ingèrèe peut etre ensuite protègèe dans des structures biologiques compactes, turricules ou parois de galeries (Martin, 1991).

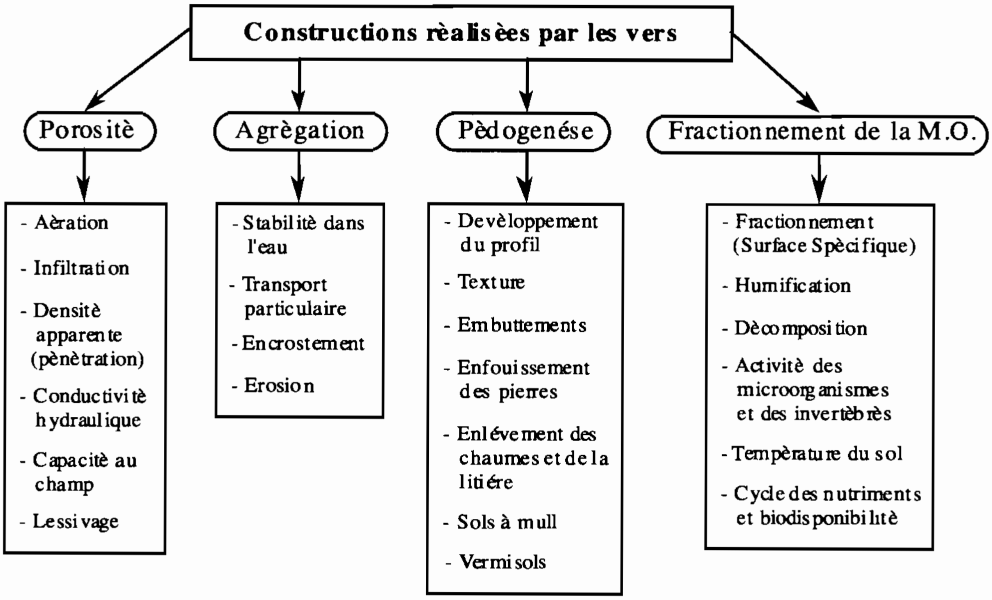

Figure 2. Les principales consèquences des constructions des vers sur les caractèristiques physiques de sol et la matière organique (modifiè d'aprés Brown, 1995).

Parmi les Ingènieurs de l'Ècosystéme, les vers de terre sont gènèralement les plus abondants. Ils forment la plus grande partie de la biomasse de la faune dans les paturages et les zones humides; leur contribution est moins èlevèe en forêt et dans les zones séches (Lavelle *et al.*, 1994). Dans quarante ècosystémes sèlectionnès en Afrique australe et orientale, les vers sont peu abondants et leur biomasse n'excéde que rarement 5 g $m^{-2}$ (Brown *et al.*, 1998b). Dans ces pays, la biomasse de la faune est souvent dominèe par les termites. En regroupant toutes les donnèes disponibles pour le continent africain (142 èchantillons), les vers se montrent plus abondants dans les pays d'Afrique centrale et occidentale. Les biomasses moyennes les plus èlevèes sont observèes sous jachére (spècialement au Sènègal), savanes, paturages et plantations d'arbres avec des valeurs s'èchelonnant de 16 (paturages) à 40 g $m^{-2}$ (jachéres) (Brown *et al.*, 1998b). Les biomasses les plus faibles sont observèes dans les systémes cultivès (en moyenne, environ 4 g $m^{-2}$). C'est dans les paturages que la contribution des vers de terre à la biomasse totale est la plus èlevèe (>80%); dans les cultures et la plupart des ècosystémes naturels (exceptè pour les savanes), les vers reprèsentent moins de 50% de la biomasse totale (gènèralement environ 40%).

Un grande campagne d'èchantillonnage rèalisèe au Mexique, regroupant 80 èchantillons sur prés de 30 sites (Brown *et al.*, 2000f), montre que les vers sont plus abondants dans les plantations de canne à sucre, d'agrumes et de cafèiers et dans les paturages et moins reprèsentès dans les cultures de maïs et en forët (Figure 3A; Brown *et al.*, 2000f). Les biomasses les plus èlevèes sont rencontrèes dans les plantations de canne à sucre (>50 g $m^{-2}$), d'arbres et sous paturages (31 g $m^{-2}$) avec une contribution à la biomasse totale supèrieure à 75% (Figure 3B; Brown *et al.*, 2000f). Les plus faible valeurs de biomasse et contribution sont observèes dan les culture de maïs (respectivement, <5 g $m^{-2}$ et <30%).

L'importance relative des vers de terre dans la macrofaune et leur biomasse èlevèe dans plusieurs sites ètudiès indiquent qu'ils peuvent jouer un rôle important dans ces ècosystémes. Sous climat tempèrè, l'impact des vers sur la fertilitè du sol et la production des plantes n'est cependant pas trés bien corrèlè avec leur biomasse et leur abondance. Dans une ètude regroupant plus de 10 sites mondialement rèpartis, les paramétres descriptifs des populations n'apparaissent pas comme de bons indicateurs de la productivitè des plantes (Doube et Schmidt, 1997). Dans les zones tropicales, la relation liant l'abondance et la biomasse des vers à la fertilitè et à la productivitè des ècosystémes est encore moins documentèe. En fait, la valeur des vers de terre en tant qu'indicateur de la qualitè d'un sol est discutèe nègativement ou positivement selon les auteurs (Blair *et al.*, 1996; Doube et Schmidt, 1997; Linden *et al.*, 1994), et ils semblent etre de meilleurs indicateurs de la qualitè lors de comparaisons entre les sites plutôt qu'à l'intèrieur des sites (Paoletti *et al.*, 1998; Stork et Eggleton, 1992). Manifestement, la capacitè à considèrer les vers de terre comme indicateurs dèpend du sens donnè au mot qualitè et de la façon dont les rèsultats sont interprètès (Hill et McKevan, 1987; Linden *et al.*, 1994).

Un meilleur indicateur serait peutetre basè sur la quantitè et le type de structures produites (Barros, 1999, Decaëns, 1999, Lavelle, 1996); cette hypothése nècessiterait cependant d'etre plus largement testèe. Ces structures vont du petit agrègat fragile à de larges accumulations de turricules et produisent des effets trés distincts sur les propriètès du sol. Les galeries peuvent etre creusèes depuis la surface (jusqu'à plusieurs métres de profondeur), elles ont le plus souvent une orientation verticale mais peuvent aussi s'ètendre horizontalement dés la surface. La drilosphére, correspondant au sol et à la microflore affectès par l'activitè des vers, est l'un des systémes biologiques de règulation du fonctionnement du sol proposès par Lavelle *et al.* (1989); ses caractèristiques dèpendent ènormèment des communautès et espéces de vers prèsentes et du type de sol. Les trois principales stratègies ècologiques proposèes par Bouchè (1977), èpigès, endogès et anèciques, se trouvent seules ou ensemble, selon diffèrentes combinaisons, formant ainsi diffèrents types de drilosphéres. Sous les tropiques, les espéces endogèes dominent souvent la biomasse totale des vers de terre (Lavelle, 1983; Fragoso et Lavelle, 1992), et au Mexique peu d'espéces èpigèes ou anèciques ont ètè observèes (Fragoso, 1993). Les endogès ont tendance à ingèrer de grandes quantitès de sol de diffèrentes qualitès, principalement prés de la surface, dans les galeries orientèes horizontalement. Les turricules donnent au sol une structure grumeleuse rèsistante (Lavelle *et al.*, 1989). Les quantitès produites dans un paturage mexicain peuvent atteindre 400 T $ha^{-1}$ (Lavelle *et al.*, 1987).

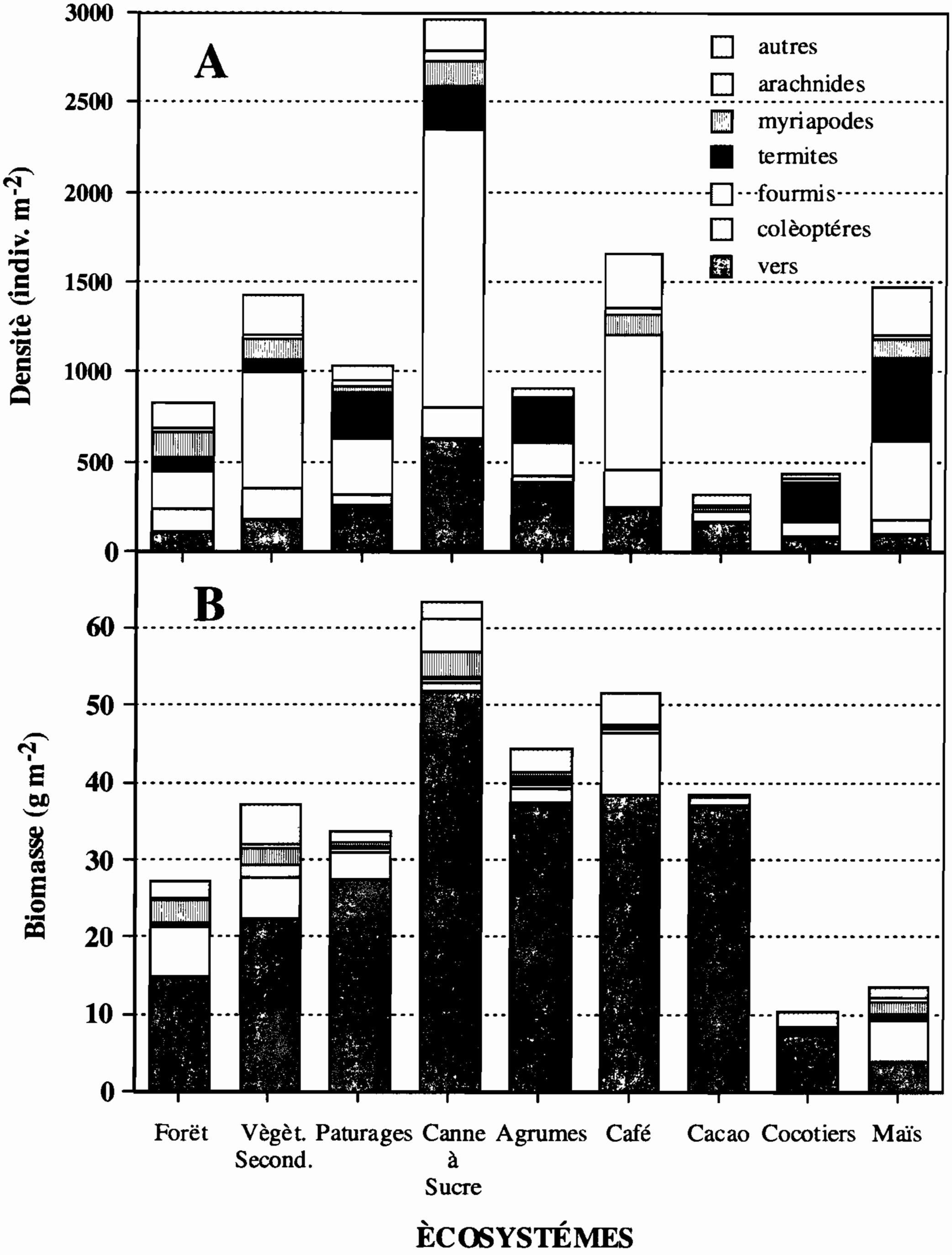

Figure 3. Abondance des principaux groupes de la macrofaune du sol d'ècosystémes mexicains des règions humides: populations (A; individus $m^{-2}$) et biomasses (B; g $m^{-2}$). Les moyennes sont calculèes à partir de plus de 80 èchantillons rèpartis sur 29 sites. Les donnèes proviennent de sources varièes (Brown *et al.*, 2000f).

Dans les agrosystémes (paturages, cultures), les populations de vers de terre sont dominès par quelques espéces, souvent introduites (non natives) (Fragoso *et al.*, 1997). Dans le premier cas, la biomasse atteint les valeurs les plus hautes (Figure 3B), dans le second cas, elle dèpasse rarement 10 g $m^{-2}$. Parmi les espéces de vers endogès communèment prèsentes au Mexique, l'espéce introduite *Pontoscolex corethrurus* est probablement la plus rèpandue (Fragoso, 1993). *Polypheretima elongata*, autre espéce introduite, prèsente une distribution gèographique dans le pays plus limitèe. *P. corethrurus* peut atteindre de trés fortes abondances et biomasses (plus de 60 g $m^{-2}$ dans certains cas) sous paturages, cultures de canne à sucre, jachéres et dans certains systémes arboricoles (Fragoso, 1993; Ordaz *et al.*, 1996a). La biomasse de *P. elongata* peut ègalement atteindre des valeurs èlevèes (90 g $m^{-2}$) mais dans une nombre de situations plus limitè (Elizondo, 1999). Ces espéces sont connues pour leur grande consommation de sol (Lavelle *et al.*, 1987; Krisnamoorthy, 1986) qui affecte la structure et les processus physiques de sol (Barois *et al.*, 1993; Barros *et al.*, 1998; Blanchart *et al.*, 1999; Duboisset, 1995; Ordaz *et al.*, 1996b; Robertson *et al.*, 1994; Rose et Wood, 1980; Zund *et al.*, 1997), la minèralisation et la disponibilitè en N et (Brossard *et al.*, 1995; Chapuis-Lardy et al, 1998; Dubash et Ganti, 1964; Lavelle *et al.*, 1992; López-Hernández *et al.*, 1993; Krishnamoorthy, 1986), le cycle d'autres nutriments essentiels à la plante (Barois *et al.*, 1999; Hernández, 1999; Duboisset, 1993; Kale et Krishnamoorthy, 1980) et la croissance des plantes (Pashanasi *et al.*, 1992; 1996; Spain *et al.*, 1992; Blakemore, 1994; Puttarudriah et Sastry, 1963).

Les ètudes concernant les effets des vers de terre sur la croissance des plantes en zone tempèrèe sont abondantes (plus de 100) et ont dèbutè à la fin du siécle dernier (Wollny, 1890). Cependant, ce n'est qu'au dèbut des annèes 50 qu'elles ont concernèes les zones tropicales (Nijhawan et Kanwar, 1952; Joshi et Kelkar, 1952). Durant les dix derniéres annèes, et depuis le dèbut du projet 'Macrofauna' (STD-2 et STD-3), les travaux sur le rôle des vers de terre sur la production des plantes en zone tropicale se sont largement dèveloppès; plus de 50 de ces ètudes devraient s'achever et etre publièes jus'quau l'an 2000. De ces expèriences dècoule une mine d'informations concernant les effets positifs, neutres ou nègatifs sur la production des plantes. Manifestement, il reste encore à apprendre des nouvelles combinaisons entre espéces de vers, espéces de plantes et types de sol, notamment au regard de la grande diversitè des espéces de vers prèsents sous les tropiques, et de l'èventail relativement ètroit des espéces de plantes et de vers ètudiès jusqu'à lors. Cependant, des ètudes dètaillèes sur les mècanismes responsables des effets observès s'avérent nècessaires, ce théme ètant peu abordè et documentè dans les travaux actuellement publiès.

Il existe vraisemblablement une multitude de propriètès du sol et de processus influencès par l'activitè des vers de terre, qui peuvent entraïner des changements pour la croissance des plantes. Rechercher le mècanisme spècifiquement impliquè dans une situation donnèe n'est pas tache facile. Cette thése a dèbutè par le projet d'aborder certains des mècanismes pouvant intervenir dans la rèponse des plantes à la prèsence des vers de terre. La mise en pratique a nècessitè diffèrentes ètapes: èchantillonnage sur le terrain, expèriences en serre, expèrimentations complèmentaires au laboratoire et revues bibliographiques. Les diverses expèriences et ètapes de ce travail ne vous sont pas prèsentèes dans l'ordre chronologique de leur exècution mais dans l'ordre qui suit. Tout d'abord, une revue bibliographique des mècanismes potentiellement impliquès vous est prèsentèe, et les principaux manques dans les connaissances actuelles sont identifiès. Ensuite, les donnèes issues d'une sèrie d'expèriences en serre ou au champ, rèalisèes dans le cadre du programme 'Macrofauna', sont combinèes à d'autres issues d'expèriences pertinentes sur le rôle des vers de terre sur la production des plantes; les principales tendances sont soulignèes et discutèe. Les limitations et perspectives d'utilisation du ver comme promoteur de la croissance des plantes sont prèsentèes, et suivies par une conclusion exposant plusieurs lignes de conduite et expèriences possibles qui pourraient/devraient etre menèes afin de mieux comprendre les interactions complexes plantes-sol-vers.

Comment les racines peuvent reguler la croissance de toute la plante (Aiken and Smucker, 1996), l'une des principales hypothéses à tester repose sur les possibles interactions vers de terre-système racinaire des plantes et l'activitè des vers de terre au niveau rhizosphèrique, et les interactions avec le système racinaire, du fait que beaucoup des mècanismes influant sur la croissance des plantes peuvent s'opèrer à ce niveau. Quatre expèriences ont ètè conduites sous serre dans des seaux en plastiques pour confirmer et estimer 1) l'activitè des vers au sein de la rhizosphére de diffèrentes plantes et les interaction avec le système racinaire, 2) leur association avec les microorganismes symbiotiques des racines (VAM et rhizobia), et 3) les consèquences sur la production des plantes. De nombreuses et diverses analyses de laboratoire, incluant des analyses de sol traditionnelles et des analyses utilisant des isotopes stables, ont ètè effectuèes pour confirmer l'hypothése que les interactions avec le système racinaire et les activitès rhizosphèriques peuvent etre une composante importante des effets des vers sur la production des plantes. La biomasse des racines et leur distribution spatiale ont ètè dètaillèes par analyses d'images, et corrèlèes à la production des plantes et à l'activitè des vers. Les principaux rèsultats de ces expèrimentations en pots sont discutès dans une courte conclusion à la lumiére des hypothéses prèsentèes.

Finalement, cette thése se termine par une courte conclusion gènèrale qui expose quelques-unes des questions restèes sans rèponse et les prioritès en terme de recherches futures.

# PREMIÈRE PARTIE:

# RAPPELS BIBLIOGRAPHIQUES

## CHAPITRE 1

# HOW EARTHWORMS AFFECT PLANT GROWTH: DIGGING INTO THE MECHANISMS*

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## SUMMARY

Earthworm effects on plant production are well documented. Beginning with the classic work from the end of the 19th century, up to the present over 100 publications support the notion that earthworms generally increase plant growth or have no measurable effect, rather than decrease yields. However, the mechanisms for the observed effects, be they negative, neutral or positive are mostly unknown and in dire need of further investigation. The difficulty in describing and pinpointing mechanisms are due to the multitude of physical, chemical and biological soil properties and processes modified by earthworm activity, and the common interactions between edaphic functions and earthworm activities. Simplistically, mechanisms can be divided into direct and indirect and may be physical, chemical or biological in nature. Direct positive or negative effects are probably rare, and usually less important than indirect ones. Positive indirect effects have to do mostly with changes in nutrient, biological or physical conditions limiting root (plant) growth, while negative indirect effects are often related to disfunctions in the soil created or induced by earthworm activity. The present paper discusses in detail, for the first time, the theoretical and empirical mechanisms proposed in the literature, and lists some basic principles that can be applied to help isolate potential mechanisms involved in a particular plant growth response experiment.

## INTRODUCTION

> "Dear Sir - ... Earthworms, though in appearance a small and despicable link in the chain of Nature, yet, if lost, would make a lamentable chasm. For to say nothing of half the birds, and some quadrupeds which are almost entirely supported by them, worms seem to be the great promoters of vegetation, which would proceed but lamely without them, by boring, perforating, and loosening the soil, and rendering it pervious to rains and the fibers of plants, by drawing straws and stalks of leaves and twigs into it; and most of all, by throwing up such infinite numbers of lumps of earth called worm-casts, which, being their excrement, is a fine manure for grain and grass ... Gardeners and farmers express their detestation of worms; the former because they render their walks unsightly, and make them much work; and the latter because, as they think, worms eat their green corn. But these men would find that the earth without worms would soon become cold, hard-bound, and void of fermentation, and consequently sterile; and, besides, in favour of worms, it should be hinted that green corn, plants, and flowers, are

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> not so much injured by them as by many species of *coleoptera* (scarabs), and *tipulae* (long-legs) in their larva, or grub-state; and by unnoticed myriads of small and shell-less snails, called slugs, which silently and imperceptibly make amazing havoc in the field and garden." (White, 1789; in "The Natural History of Sanborn")

This fragment of letter 35 of Rev. Gilbert White to the Hon. Daines Barrington, written on May 20th, 1777, is probably among the first anectodal reports of the potential importance of earthworms to plant production and in soil fertility, and the possible mechanisms by which this occurs. The first scientific reports on the importance of earthworms to soil processes, fertility and plant production were not to come until more than a century later, in Charles Darwin's book (1881) and with Hensen's (1877; 1882), Müller's (1878; 1884) and Wollny's (1890) papers. While Darwin's and Hensen's approach was based mostly on describing the behavior of earthworms and their physico-chemical effects on soils (burrow and cast production, leaf ingestion, burial of artifacts, nutrients in castings, formation of vegetable mould), Müller's work consisted mainly in classifying the two types of humus (mull and mor) in Danish woodlands, and the inherent differences between them, mostly due to the soil-leaf litter mixing and casting activities of earthworms. On the other hand, Wollny's research was based on quantifying (for the first time) the effect of earthworm presence on plant growth. Despite being initially skeptical about the reports of Darwin and Hensen (Wollny, 1882a,b), Wollny became convinced that earthworms were important for plant production after his experiment (Wollny, 1890) showed increased yields of 12 plants ranging from negligible amounts up to 733% (rape) in treatments with earthworms. However, he continued to warn about the generalization of these results to field situations.

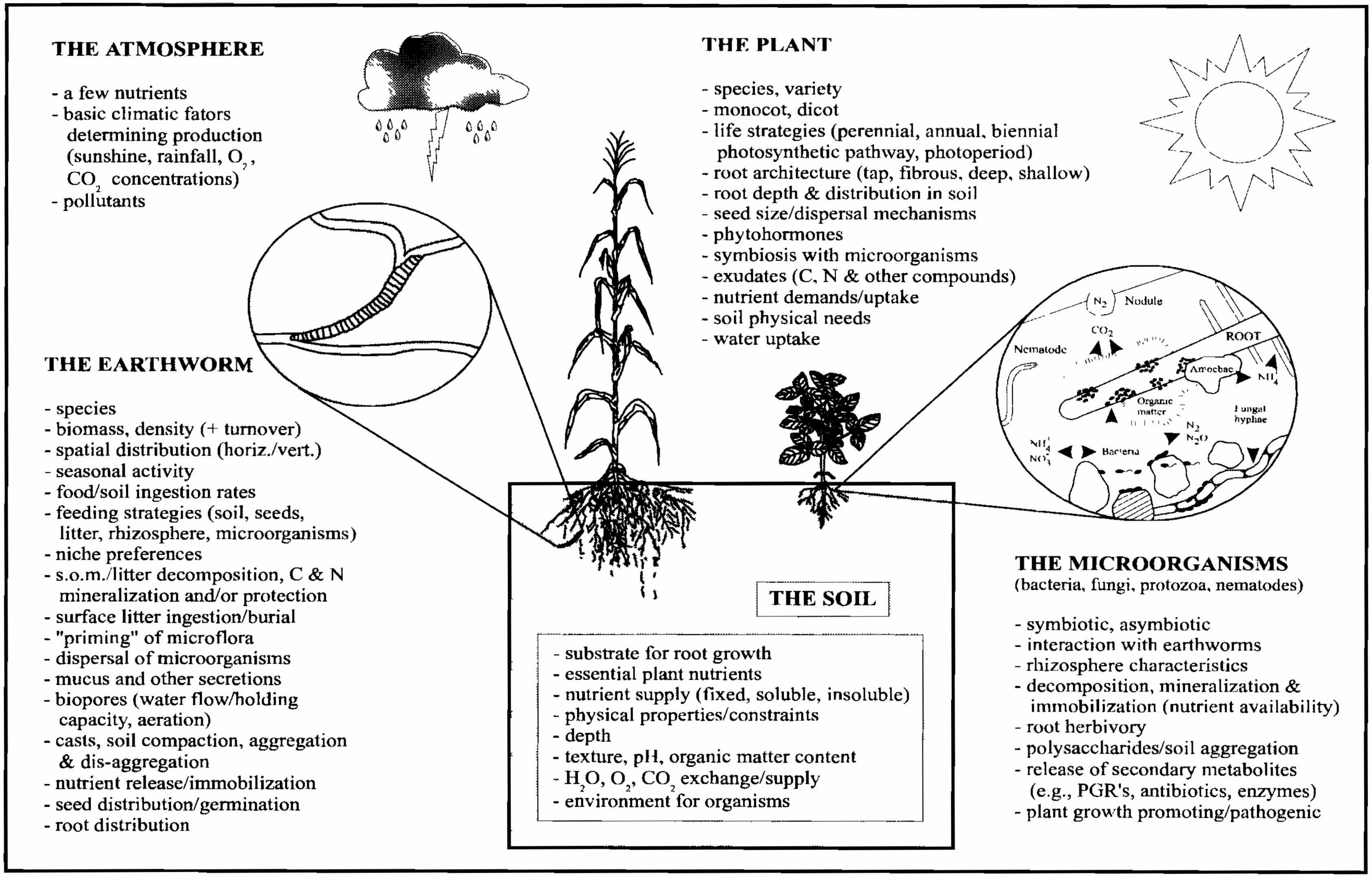
From the early 20th Century the time between experiments decreased and the number of papers published progressively increased, beginning mostly with work by European scientists: Djemil (1896), Stebler *et al.* (1904), Ribaudcourt and Combault (1907), Russell (1910), Kashnitz (1922), Archangelski (1929; quoted in Satchell, 1958) and Dreidax (1931). Finally, the experiments of Powers and Bollen (1935), Chadwick and Bradley (1948) and Hopp and Slatter (1948, 1949) in the USA, brought this applied research topic to the new world, while the papers of Waters (1951), Nielson (1951; 1953), Duff (1958) and Stockdill (1959), transported the importance of the European lumbricids to New Zealand pastures. The first reports of the effects of earthworms on plant production in the tropical region did not appear until Joshi and Kelkar (1952) and Nijhawan and Kanwar (1952) published papers on their work in India. Since then, over 100 papers on the topic have been published and the literature on earthworm effects on plant production continues to be increasing. Nevertheless, the aim of most of these studies has been to answer the questions:
a) do earthworms affect plant growth (positively or negatively), and if so, how much?
b) which plants are most affected (positively or negatively)?
c) which earthworm species are most efficient at promoting plant growth?

Rarely has the question of how these effects come about, that is, what are the mechanisms behind the observed effects, been properly addressed. In most papers, mechanisms are only briefly alluded to, and in several instances the possible reasons for the observed effects are not even mentioned. Furthermore, in many cases, the proposed mechanism could not be proven or validated. The reason for this is because, to answer this question (how?), one must know about plant and earthworm physiology, soil physics, biology (especially microbiology) and chemistry due to the multitude of soil, plant, human and environmental factors (conditions) which determine the growth of a particular plant in a given situation (Fig. 1). Furthermore, earthworms of different species and perhaps even different ages respond differently to these conditions, altering their potential effects on soil properties and plant growth. Thus, pinpointing the exact reasons for (mechanisms) a specific plant response in an experiment is not easy and more often than not, several rather than a single mechanism are probably operating simultaneously. The purpose of this paper therefore, is to search for and reveal the mechanisms by which earthworms promote or suppress plant growth by reviewing the literature on the topic and propose ways of reaching a better understanding of this complex issue.

## DIGGING INTO THE MECHANISMS WHICH ALTER PLANT GROWTH: CONCEPTUAL BACKGROUND

Lee (1985), Edwards and Bohlen (1996) and Blakemore (1994) have produced good reviews of the effects of earthworms on plant growth. Although these reviews mention various mechanisms, they are dispersed throughout the text and seemed to receive less attention than the observed growth and production responses (positive or negative) of the plants involved. To the author's knowledge, no publication up to the present has exposed a compilation and comprehensive explanation of the potential mechanisms involved in plant growth promotion or suppression. The mechanisms given in the above publications included the role of earthworms in pasture production by dung and lime incorporation, thatch removal and OM mineralization, effects on compaction, soil aeration and water infiltration rates, acceleration of litter decomposition and nutrient cycling, changes in the viability of plant seeds, relationship with plant pathogens and plant growth promoting microorganisms, the production of metabolites that stimulate plant growth, nutrient enrichment in earthworm structures (casts, burrows), nutrient release from earthworm mortality and facilitation of root growth in burrows.

If viewed simplistically, earthworm impacts on soils can take on three main forms: effects on physical, biological and chemical properties and processes. The combination of earthworm populations and the soil volume, microbial and invertebrate populations affected by their activities has been called the drilosphere (Lavelle, 1988), an edaphic functional domain (Beare et al., 1995) with significance in regulating major soil processes and functions such as structure, organic matter (OM) decomposition, nutrient cycling, microbial populations and plant growth. Earthworm activities can also regulate the soil as an environment for other organisms by controlling its physical structure, nutrient fluxes and energic status (resource availability). Thus, they are "ecosystem engineers" (sensu Lavelle *et al.*, 1997; Jones *et al.*, 1994). Furthermore, burrows and macro-aggregates produced (especially by endogeics) may outlive the earthworms themselves and lead to long term conservation of soil physical structure and SOM by physical protection from decomposition (Blanchart *et al.*, 1999; Villenave *et al.*, 1999).



**THE ATMOSPHERE**

- a few nutrients
- basic climatic fators determining production (sunshine, rainfall, $O_2$, $CO_2$ concentrations)
- pollutants

**THE PLANT**

- species, variety
- monocot, dicot
- life strategies (perennial, annual, biennial photosynthetic pathway, photoperiod)
- root architecture (tap, fibrous, deep, shallow)
- root depth & distribution in soil
- seed size/dispersal mechanisms
- phytohormones
- symbiosis with microorganisms
- exudates (C, N & other compounds)
- nutrient demands/uptake
- soil physical needs
- water uptake

**THE EARTHWORM**

- species
- biomass, density (+ turnover)
- spatial distribution (horiz./vert.)
- seasonal activity
- food/soil ingestion rates
- feeding strategies (soil, seeds, litter, rhizosphere, microorganisms)
- niche preferences
- s.o.m./litter decomposition, C & N mineralization and/or protection
- surface litter ingestion/burial
- "priming" of microflora
- dispersal of microorganisms
- mucus and other secretions
- biopores (water flow/holding capacity, aeration)
- casts, soil compaction, aggregation & dis-aggregation
- nutrient release/immobilization
- seed distribution/germination
- root distribution

**THE SOIL**

- substrate for root growth
- essential plant nutrients
- nutrient supply (fixed, soluble, insoluble)
- physical properties/constraints
- depth
- texture, pH, organic matter content
- $H_2O$, $O_2$, $CO_2$ exchange/supply
- environment for organisms

**THE MICROORGANISMS**
(bacteria, fungi, protozoa, nematodes)

- symbiotic, asymbiotic
- interaction with earthworms
- rhizosphere characteristics
- decomposition, mineralization & immobilization (nutrient availability)
- root herbivory
- polysaccharides/soil aggregation
- release of secondary metabolites (e.g., PGR's, antibiotics, enzymes)
- plant growth promoting/pathogenic

Figure 1. Diagramatic representation of various atmospheric, soil, plant, microbial and earthworm-related factors with potential to impact plant production.

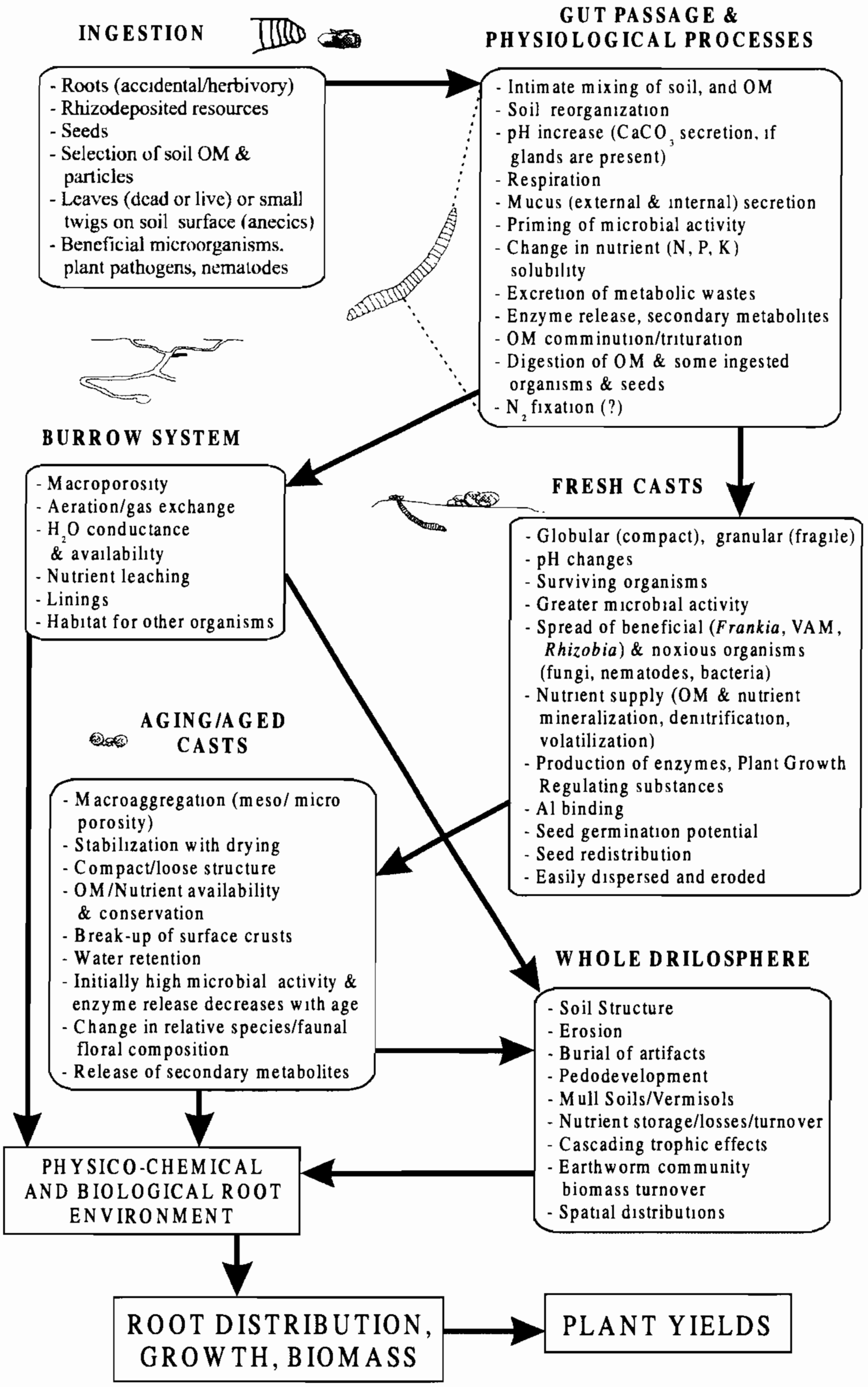


Figure 2. Flow diagram by which earthworms can affect root distribution, growth and biomass and consequently plant yields, using the spatio-temporal approach to earthworm function in soil.

The drilosphere is a dynamic sphere that is constantly changing as the earthworms ingest and re-ingest soil, burrow and cast at different rates and in different locations in the soil. Therefore, the drilosphere can affect soil function at different spatio-temporal scales from the gut to the soil profile (Lavelle, 1997; see Fig. 2, below). Furthermore, although often easily identifiable and/or separable from the edaphosphere (e.g. burrows, casts, earthworm individuals), the drilospshere is connected with the rest of the soil system and can interact profoundly with other soil functional domains (Brown *et al.*, 2000e), e.g., the rhizosphere, porosphere, aggregatusphere and litter system (detritusphere). Thus, physical structures created by earthworms don't only affect soil physical properties and process, they can also modify biological interactions (e.g., with microorganisms and plants) and soil chemical cycles. This interconnectedness is evident as one attempts to separate out the mechanism (s) responsible for plant responses in a given situation.

One of the proposed ways of assessing the functional significance of earthworm activities and their modes of action on the soil and plant yields, is to trace the various structures they create and the processes they influence at different spatio-temporal scales (Decaëns, 1999; Lavelle, 1996; Lavelle et al., 1997). This concept is further developed in Figure 2.

1. Ingestion (selection/feeding process) and gut passage + physiological processes. These processes will control to a large extent what happens in the physical structures produced by earthworms. In turn, these processes are controlled in themselves mainly by earthworm habits, species, ecological categories and the soil environment (type, resource availability, temperature and moisture regimes). For example, the proportion of soil/OM ingested, the organisms and materials digested, microbial activation by intestinal mucus and the assimilation rates of epigeic, endogeic and anecic earthworms may be very different (Trigo et al., 1999; Lavelle et al., 1995; Lavelle and Spain, 1999).

2. Fresh and aging casts and associated properties and processes. Their ultimate characteristics and function in soils are defined by the results of the first level (ingestion/gut passage). Their type (globular, granular) and the earthworm that produced them (ecological category, species), placement (surface, below ground, deep in soil, near roots, etc.), the quantities deposited, the interaction with microbes and other organisms, age (fresh or old), the physico-chemical soil environment (esp. fertility & structure) and land management also play an important role.

3. Burrows and their associated properties and processes. The characteristics and effects on soil function are especially dependent on the species and ecological category of earthworms that produce them, initial soil physical properties (e.g., compact or loose soil), land management, the age (fresh or old), length and diameter, direction (vertical, horizontal, other) whether they are open or closed, confined to belowground or open to the surface and still in use or abandoned.

4. Whole drilosphere. This is the combination of all spatial scales, over a long temporal scale. At this level, earthworm actions are responsible (to a certain extent, depending on the previous scales) for determining soil physical structure & conservation (erosion rates, soil hydrologic regimes), humus types, nutrient turnover rates and cascading trophic effects (regulation of

the populations & activity other soil organisms) (Lavelle *et al.*, 1997). The assessment of the spatial distribution of these drilospheric effects is essential.

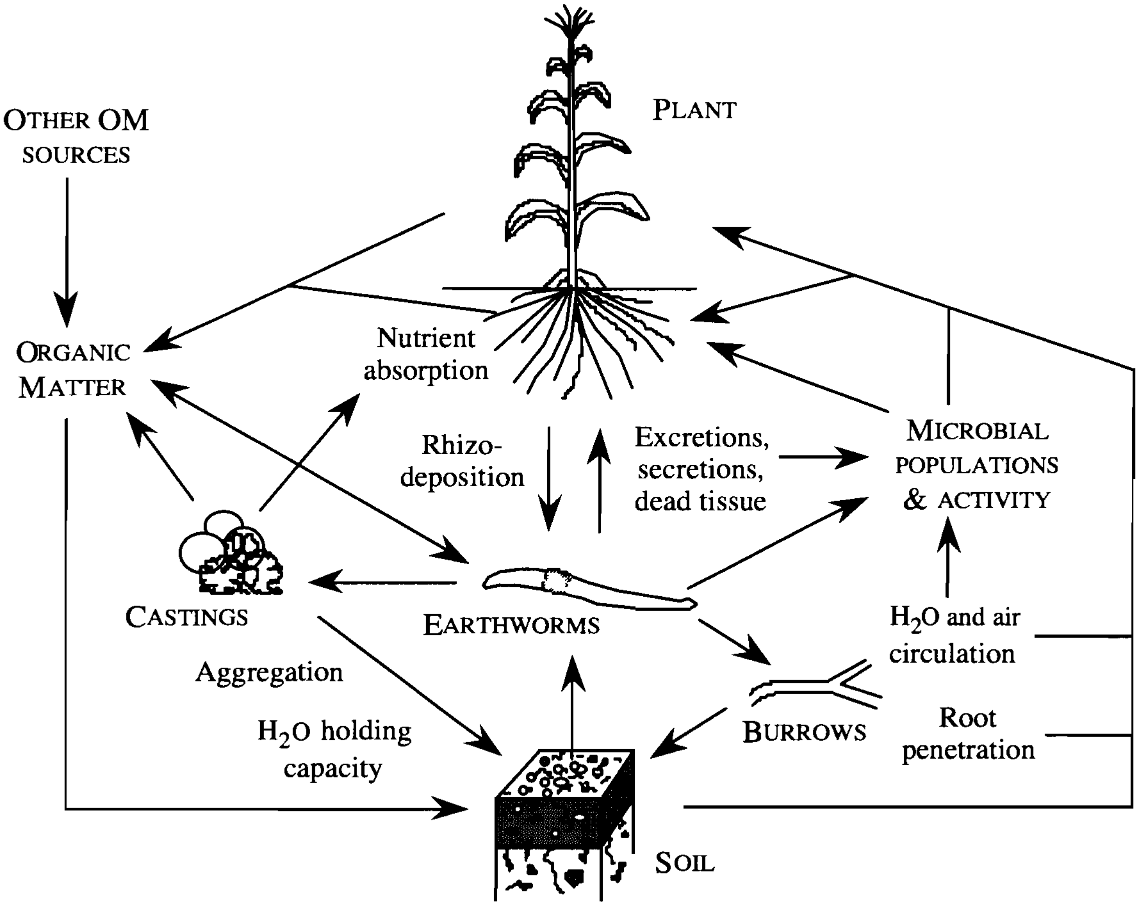


Figure 3. Simplified conceptual model connecting the physical, chemical and biological earthworm effects on soils with their potential effects on plant growth and nutrition (modified from Cuendet and Bieri, 1990 and Syers and Springett, 1983).

A simple conceptual model connecting the physical, chemical and biological earthworm effects on soils with their potential effects on plant growth and nutrition is shown in Figure 3 (modified from Cuendet and Bieri, 1990 and Syers and Springett, 1983). The interdependence of earthworm activities (production of casts and burrows; excretions, secretions and tissue death) with soil, plants, OM (soil and residue inputs) and microbial populations, is evident. Chemical effects on soil properties and processes are based on the mucus secretion and excretion products of their metabolism, selection of soil particles and OM and the different nutrient composition of their egesta compared to bulk soil. Biological effects are due primarily to interactions with microorganisms, depending especially on feeding and digestive habits of the earthworms (Fig. 2). Biological effects are intimately linked with the chemical due to the importance of resource availability in the drilosphere (especially in casts), and to the physical because of physical environmental controls (water, air, and temperature) on biological processes. Similarly, chemical effects are also dependent on the physical characteristics of the structures created by earthworm action and on the biological effects, since processes defined as chemical (e.g., decomposition and mineralization) are often biologically mediated and regulated. Physical effects are linked to the biological and chemical because these interact to control the preservation or destruction of the structures produced (see Figure 4 for details).

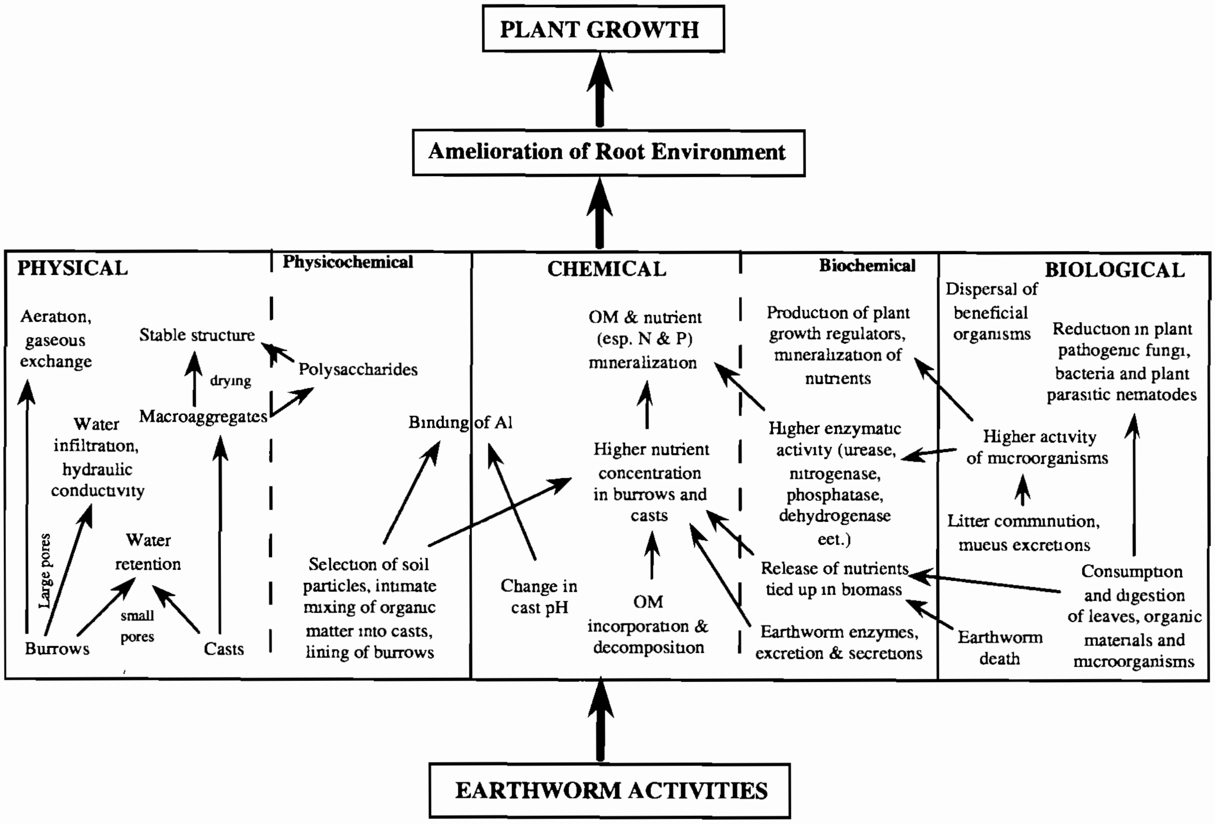


Figure 4. Diagramatic representation of ways by which earthworms can positively affect plant growth via a "bottom up" control of edaphic biological, chemical and physical properties and processes (and their interactions) and the root environment.

A more functional diagramatic model shown in Figure 4, provides more details on the specific mechanisms, concentrating on the positive effects of earthworms on chemical, physical and biological edaphic properties and their interactions (physico-chemical, biochemical) in altering the environment for roots. A specific diagram for the biological (and microbiological) mechanisms involving interactions with microflora and fauna (beneficials, pests, plant pathogens or parasites) and the potential effects on plant production, both positive and negative, was developed by Brown (1995) and is shown in Figure 5.

Roots, being sensors of the soil environment and the producers of many signals which ultimately control shoot growth (Aiken and Smucker, 1996), are the primary and immediate receivers of drilosphere contributions to soil function. By controlling nutrient and water supply to the shoots, it is the biomass, density, distribution and activity (growth rate and longevity) of roots within the soil profile that will largely determine plant production (Brown and Scott, 1984).

Thus, it is the response of roots to the drilosphere which will generally control the ultimate plant response. This bottom-up approach is gaining increased recognition as scientists (e.g., Jones *et al.*, 1994; 1996; Lavelle *et al.*, 1997) confirm the truth of Darwin's (1881) statements so long ago: that seemingly small, insignificant quantitative changes, on accumulation, provoke significant qualitative changes (Ghilarov, 1983).

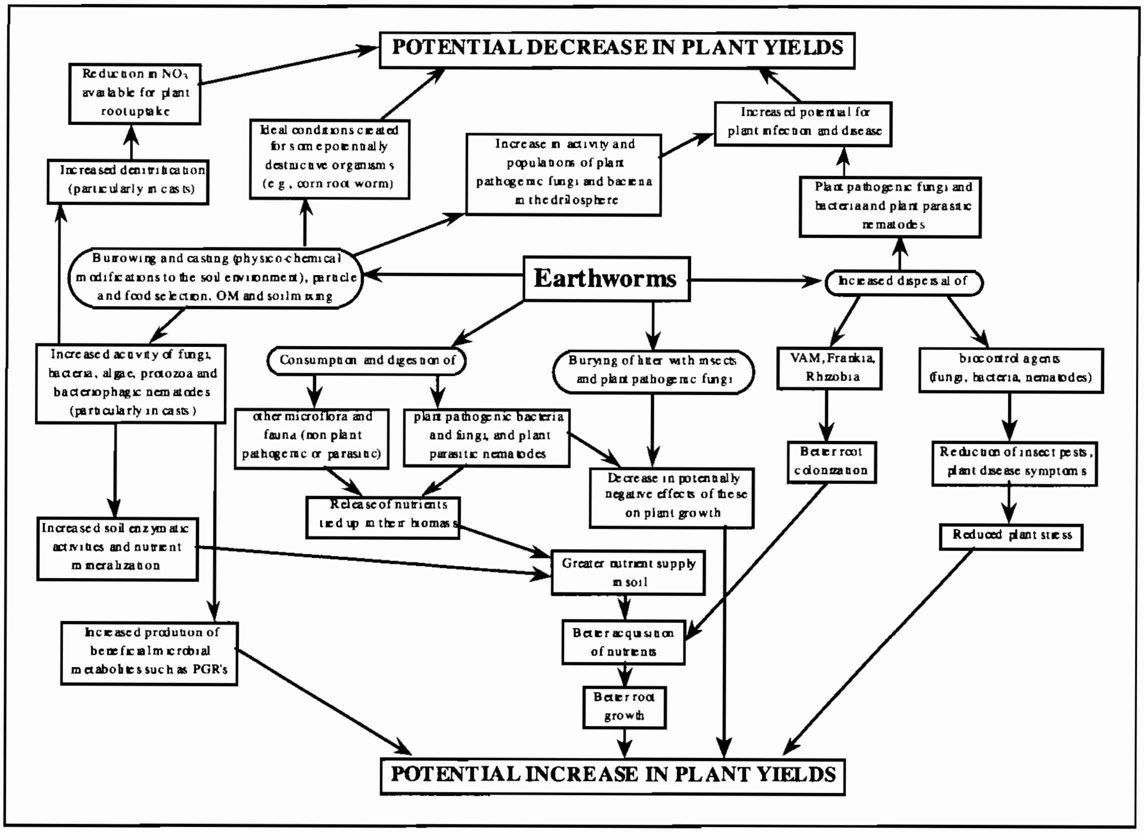


Figure 5. Biologically controlled mechanisms (with special focus on microbiological) by which earthworm activities can lead to both positive and negative potential effects on plant yields (modified from Brown, 1995).

The following sections explore the ways in which earthworms affect both directly and indirectly plant growth. To achieve this task, over 100 publications on the effects of earthworms on soil fertility and plant growth, ranging from the work of Darwin (1881) up to the most recent papers (1999) from scientific journals were reviewed. The mechanisms proposed were identified and classified into three main types: biological, physical and chemical (both empirical and theoretical).

## RESULTS

The results, combined for the first time, are presented in Table 1 (biological mechanisms), Table 2 (chemical mechanisms) and Table 3 (physical mechanisms). In each table, both positive (yield enhancement) and negative (yield reduction) mechanisms are given with the respective plants studied and the reference (s) from where they were obtained. Most key papers within each mechanism proposed are cited. Several potential (P) mechanisms, which have been proposed but not experimentally verified (V) with plants are also shown. Further research will be necessary to confirm that these mechanisms actually occur in the plant-soil-earthworm system.

Earthworm activities can influence the growth of living plants both directly and indirectly. Direct effects are mostly related to how much earthworms feed on roots (or shoot tissues) and are active in the rhizosphere, a matter of much present contention and speculation, which few experiments have addressed. The rhizosphere, a thin soil layer (generally <0.5mm) surrounding plant roots, is a region preferentially rich in microflora and fauna, so earthworm feeding in this zone can have important consequences to microbial and faunal activity and populations (including mycorrhizal infection) and thus, indirectly, on plant production.

Indirect effects are related to earthworm-induced changes in soil physical and chemical properties which influence (or limit) plant growth, and in soil biological conditions, such as pest or pathogen populations (e.g., nematodes, fungi) and beneficial microorganisms (e.g., plant growth promoting rhizobacteria, biocontrol agents), particularly in the root zone.

### A. Positive Mechanisms

*Direct vs. indirect effects*

Direct positive effects of earthworms on plant growth are probably few and have not yet been properly studied. Higher production of plant growth promoting (PGP) substances have been related to earthworm activity. Earthworms may liberate PGP substances themselves (e.g., vitamins, proteins), although most accounts seem to suggest that the substances are probably produced by microorganisms associated with the earthworm gut or its structures (casts, burrows) (El Harti, 1999a,b; Lee, 1985; Edwards and Bohlen, 1996). If these substances were produced by the earthworms and released in the rhizosphere, this would be a case of direct effects on plant production. However, the issue of the origin (earthworms or mediated via microorganisms) of biologically active substances such as phytohormones or plant growth regulators (PGR's, auxins, gibberellins, cytokinins and ethylene) is far from being well established or understood. Much more research in this area is needed and future experiments including a hormone isolation and quantification (e.g., with bioassays) component will help enlighten the possible linkages of PGP subtances with the plant growth promotion observed in the presence of earthworms. Since these substances are effective at very low concentrations, even small increases in their concentrations can be important to plant growth, especially if released in the rhizosphere (Arshad and Frankenberger, 1993). However, since the effects of each substance on plants is different (and some opposite), highly dependent on their concentration and different earthworms may affect their production differently under various plant-soil conditions (Krishanmoorthy and Vajranhabhaiah, 1986), identifying the potential plant response of these PGP substances will demand wholistic approaches and appropriate methodologies. For example, Springett and Syers (1979) observed how ryegrass roots grew upwards (negative geotropism) into *Lumbricus rubellus* castings deposited on the soil surface, but saw no such effects with casts of *Aporrectodea caliginosa*. Nevertheless, both species significantly increased ryegrass growth. Obviously, many other soil factors are involved in plant growth promotion as well, hence the importance of studying the indirect effects of earthworms on the rhizosphere and soil properties, that operate simultaneously (with the direct effects).

From the moment a seed germinates, it comes into contact with the soil physico-chemical environment and organisms, all of which may have a variable degree of influence on its growth and success as a plant, depending on inherent genetic codes and the subsequent plant-soil (+organism) interactions and within-soil (+organism) interactions. However, even before a seed germinates, some of these forces already may be influencing its fate. For example, some earthworm species (e.g., *Lumbricus terrestris*) appear to show a preference for ingesting particular plant species seeds, depending on their size, shape, texture and taste (Piearce *et al.*, 1994). Observations dating back over a century (Darwin, 1881; Hensen, 1877) have shown the potential importance of surface feeding anecics and even endogeic earthworms in ingesting, transporting and altering seed distribution in soils. Seed germination may be higher or lower, slower or more rapid (see later) in egested earthworm castings (Atlavinyte and Zimkuviene, 1985; McRill, 1974; Piearce *et al.*, 1994). Given this selective consumption and digestion, the preferential germination of different seed species in earthworm structures, the movement of seeds throughout the soil and the physical-chemical effects of earthworms on the soil environment it has been proposed that earthworms may influence the composition of plant communities (Piearce *et al.*, 1994). In fact, various authors have stated that earthworms seem to favor particularly the proportion (and often biomass) of clover in pastures (Hopp and Slater, 1948; Nielson, 1953; Nuutinen *et al.*, 1998; Stebler *et al.*, 1904; Thompson *et al.*, 1993), although grazing may be more the culprit than earthworms themselves (Bates, 1933; Satchell, 1955). Positive associations of worm casts with the frequency distribution of *Plantago* spp., *Trifolium* and *Ranunculus* were observed in a meadows in the UK (Bates, 1933; Piearce *et al.*, 1994). Conversely, when comparing earthworm-inoculated vs. uninoculated pastures on Dutch polders, van der Reest and Rogaar (1988) found no significant differences between the plant communities of the two sites.

### *Biological mechanisms (indirect)*

Both root distribution and density in soils may be significantly affected by earthworm activites. After inoculating newly reclaimed polders in the Netherlands, van Rhee (1977) observed significantly higher total and small (<0.5mm) root density of fruit trees. Nevertheless, this had little influence on fruit production. On the other hand, in a pot study, Brown *et al.* (2000a; chap. 3) observed significantly greater root density and higher root and shoot biomass of beans in the presence of *Polypheretima elongata*. There were, nevertheless, no significant effects on grain yields. Root distribution throughout these pots showed a more even (homogeneous distribution), and this was assumed to confer greater plant resistance to stress (Brown *et al.*, 2000c; chap. 5). Root depth in soil seems to be intimately associated with the presence of deep macropores in many instances (Ehlers *et al.*, 1983; Kirkham, 1981; see later), and it has been stated that root distribution is closely related with the zone (especially depth) of earthworm activity (Edwards and Lofty, 1978; 1980).

Table 1. Biological mechanisms by which earthworm activity in soils affects the growth of plants in a positive (enhancement) or negative (decrease) manner, either directly and/or indirectly. Potential (P) are possible mechanisms which have not yet been experimentally verified (V) to affect plant production. n.d. = not determined

| **Negative Mechanisms** | **Plants** | **Status** | **References** |
|---|---|---|---|
| Burial of live leaves | lettuce, *Triticum repens*, various | V | Darwin (1881); Edwards and Bohlen (1996); Zicsi (1954) |
| Rhizophagy | wheat, clover, pasture grasses | V | Baylis *et al.* (1986); Carpenter (1985); Cortez and Bouché (1992); Gunn and Cherrett (1993); Hameed *et al.* (1993); Sackville-Hamilton and Cherret (1991) |
| Seed ingestion and/or burial, reducing germination | grasses, composites, ferns, carrot | V | Decaëns and Lavelle (1998); Grant (1983); Piearce *et al.* (1994); Thompson *et al.* (1993); van Tooren and During (1988); Willems and Huijsmans (1994) |
| Direct damage to seeds/seedlings | various vegetables, tobacco, onions, others | V | Patel and Patel (1959); Olson (1928); Pradhan (1986); Trifonov (1957); Walton (1928) |
| Spread of phytoparasitic nematodes, increased numbers in casts and worm-worked soils | n.d. | P | Ellenby (1945); Lal (1987); Russom (1993) |
| Spread of phytopathogenic fungi | n.d. | P | Hampson and Coombes (1989); Hoffman and Purdy (1964); Hutchinson and Kamel (1956); Khambata and Bhat (1957); Melouk and Horner (1976); Moody *et al.* (1995); Rao (1979); Rathbun (1918); Reddy (1983); Thornton (1970); Toyota and Kimura (1994) |
| Increased numbers of northern corn rootworm (*Diabrotica* spp.) eggs | maize | P | Kirk (1981) |
| Spread of plant viruses and bacterial diseases | n.d. | P | Brown (1995) |
| Stimulating mole activity | grasslands | P | Hoogerkamp (1984) |
| Reduced VAM root colonization | *Brachiaria decumbens* | V | Patrón *et al.* (1999); Brown *et al.* (2000d; chap. 6) |
| Increased C drain by VAM | *B. decumbens* | P | Brown *et al.* (2000d; chap. 6) |
| Increased incidence of weeds | grasslands, pastures, maize | P | Edwards and Bohlen (1996); McRill (1974); Piearce *et al.* (1994); Stinner *et al.* (1997) |

Table 1. continuation

| Positive Mechanisms | Plants | Status | References |
|---|---|---|---|
| VAM root colonization increase (and dispersal) | *B. decumbens, Bactris gasipaes, Bixa orellana, Eugenia stipitata* | V | Brown *et al.* (2000d; chap. 6); Ydrogo (1994) |
| Increased spread of *Frankia* (actinomycetales) | *Casuarina equisetifolia* | P | Reddell and Spain (1991b) |
| Increased spread and root colonization by Ectomycorrhizae | n.d. | P | Reddell *et al.* (1999) |
| *Rhizobium* dispersal and increased root nodulation | subterranean clover, clover, alfalfa, soybean | V | Doube *et al.* (1994a); Madsen and Alexander (1982); Rouelle (1983); Stephens and Davoren (1994); Stephens *et al.* (1994f); Thompson *et al.* (1993) |
| Dispersal of entomo-pathogenic nematodes for biocontrol of pests | n.d. | P | Shapiro *et al.* (1993) |
| Reduced infestation with stalk borer | maize | V | Boyer (1998) |
| Dispersal of nematode-trapping fungi | n.d. | P | Edwards and Fletcher (1988) |
| Decrease in phytoparasitic nematode populations | rice, maize, pasture | V | Boyer (1998); Maraun *et al.* (1999); Yeates (1981; 1991) |
| Decreased virus infection | beans, tobacco | P | Amaravadi *et al.* (1991) |
| Dispersal and increased root colonization by biocontrol agents | wheat | V | Stephens *et al.* (1993a) |
| Decrease in plant pathogenic fungi populations in casts | n.d. | P | Moody *et al.* (1996); Tiwari *et al.* (1990) |
| Burial of leaves containing plant pathogens and insect pests | apple orchards | V | Hirst and Stedman (1962); Kennel (1990); Niklas and Kennel (1981); Laing *et al.* (1986); Mills (1976); Raw (1962) |
| Decreased plant fungal diseases | wheat, subterranean clover raddish | V | Nakamura (1996); Stephens and Davoren (1995; 1997); Stephens *et al.* (1993b; 1994b-e,g) |
| Increased seedling germination | grasses, cress, cereals, barley | V | Atlavinyte and Zimkuviene (1985); Edwards and Bater (1992); Kollmannsperger (1980); McRill (1974); McRill and Sagar (1973) |

Table 1. continuation

| **Positive Mechanisms** | **Plants** | **Status** | **References** |
|---|---|---|---|
| Increased depth of root penetration | wheat, pastures, grasses, soybean, maize, barley, ryegrass-white clover, oats | V | Edwards and Lofty (1978; 1980); Ehlers (1975); Ehlers *et al.* (1983); Hoogerkamp (1984); Kirkham (1981); Kladivko and Timmenga (1990); Springett (1985); van de Westeringh (1972); Wang *et al.* (1986) |
| Higher root density | maize, beans, fruit trees | V | Brown *et al.* (2000c; chap. 5); van Rhee (1977) |
| More homogeneous root distribution | maize, beans | V | Brown *et al.* (2000c; chap. 5) |
| Greater shoot/root ratios | beech, maize, *Panicum maximum*, wood barley | V | Klebsch *et al.* (1995); Marshall (1971); Spain *et al.* (1992); Tomati *et al.* (1996); Wolters and Stickan (1991) |
| Increased protein synthesis and nitrate reductase activity | radish, lettuce, mushrooms, maize, carrot | V | Dell'Agnola and Nardi (1987); Galli *et al.* (1990); Tomati *et al.* (1990; 1996); Tomati and Galli (1995) |
| Plant growth promoting (PGP) substances, humic matter, hormone production, PGR's (IAA, auxin, cytokinin, gibberellin, ethylene) | barley, rye, wheat, ryegrass mushrooms, pea, carrot, spinach, ornamentals | V | Atlavinyte and Daciulyte (1969); Ghilarov (1963); Graff and Makeschin (1980); Krishnamoorthy and Vajranabhaiah (1986); Muscolo *et al.* (1996; 1999); Nardi *et al.* (1995); Nielson (1965); Simek and Pizl (1989); Springett and Syers (1979); Tomati *et al.* (1987; 1988) |
| Dispersal and/or enhancement of Plant growth promoting rhizobacteria (PGPR) populations | n.d. | P | Bhat *et al.* (1960); Bhatnagar (1975); Hand and Hayes (1983); Kale *et al.* (1989); Kozlovskaya and Zaguralskaya (1966); Kozlovskaya and Zdhannikhova (1961); Loquet *et al.* (1977); Pederson and Hendriksen (1993); Savalgi and Savalgi (1991) |
| Earthworm- and plant-species specific effects | beans, clover, wheat, maize, peanuts, rice, cowpea, rye, *P. maximum*, oats, barley, grasses, sorghum, tree seedlings, spinach, mustard, others | V | Baker (1998); Baker *et al.* (1995; 1997); Blakemore (1994; 1997); Brown *et al.* (1999; chap. 2; 2000a; chap. 3); Derouard *et al.* (1997); Doube *et al.* (1997); Graff and Makeschin (1983); Hopp and Slater (1948; 1949); James and Seastedt (1986); Kashnitz (1922); Pashanasi *et al.* (1992; 1994; 1996); Russell (1910); Spain *et al.* (1992); van Rhee (1965); Wollny (1890) |

Table 1. continuation

| **Positive Mechanisms** | **Plants** | **Status** | **References** |
|---|---|---|---|
| Earthworm abundance (number) or biomass | wheat, sugarcane, barley, sweet potato, pastures, tea, maize, *P. maximum*, various grains | V | Atlavinyte (1971); Atlavinyte and Vanagas (1973; 1982); Atlavinyte *et al.* (1968); Brown *et al.* (1999; chap. 2); Buckerfield *et al.* (1997); Decaëns *et al.* (1999); Doube and Schmidt (1997); Giri (1995); Hoogerkamp (1983); Hoogerkamp *et al.* (1983); Pashanasi *et al.* (1992) Rose and Wood (1980); Satchell (1955); Sears amd Evans (1953); Senapati and Dash (1981); Shuixin *et al.* (1991); Spain *et al.* (1992); Temirov and Valiakhmedov (1988); Waters (1955); Ydrogo (1994) |

Rhizosphere feeding (see below) appears to be common in many earthworm species (especially polyhumic endogeics), as reports in the literature often mention earthworms as "active" or "concentrating" in the root zone (e.g., James and Seastedt, 1986; Robertson *et al.*, 1994; Rovira *et al.*, 1987). Experimentally, rhizosphere activity has been inferred from visual observations of *Aporrectodea trapezoides* feeding on wheat rhizosphere soil (Doube and Brown, 1998), from radio-isotope ($^{14}C$) analysis of *Lumbricus terrestris* tissues under wheat (Cortez and Bouché, 1992) and stable isotope ($^{15}N$, $^{13}C$) analyses of *Pontoscolex corethrurus* tissues under maize, *B. decumbens* and sugarcane (Brown, 2000a; chap. 7; Spain *et al.*, 1990; Spain and Le Feuvre, 1997).

As earthworms burrow and cast near or within the rhizosphere, the soil disturbance and abrasion may affect the mycorrhizal hyphal network, decreasing root colonization (Brown *et al.*, 2000d; chap. 6; Pattinson *et al.*, 1997; see later). However, they may also act as facilitators of VAM propagule (hyphae, infected root fragments, spores) dispersal, and in the presence of various tropical tree seedlings or the pasture grass *B. decumbens* (with no fertilizer and PK fertilizers) the pantropical geophagous endogeic earthworm *P. corethrurus* increased root colonization by VAM (Brown *et al.*, 2000d; chap. 6; Ydrogo *et al.*, 1994), also increasing plant harvest biomass in various treatments.

The actinomycete *Frankia* and ectomycorrhizae were also shown to be dispersed by this earthworms species (Reddell and Spain, 1991b; Reddell *et al.*, 1999), though no experiments have addressed this issue under field conditions. Plant growth promoting rhizobacteria (PGPR; see above) such as *Enterobacter cloacae*, *Azotobacter*, *Azospirillum*, *Acinetobacter*, *Bacillus* and *Pseudomonas* spp. may be dispersed and/or their populations enhanced in the casts and burrows (Bhat *et al.*, 1960; Bhatnagar, 1975; Hand and Hayes, 1983; Kozlovskaya and Zaguralskaya 1966; Kozlovskaya and Zdhannikhova, 1961; Loquet *et al.*, 1977; Pederson and Hendriksen, 1993; Savalgi and Savalgi, 1991). The metabolites released by these microorganisms may be of particular importance in the potential plant response. Dispersal of these and other microorganisms such as biocontrol agents that colonize roots preventing root diseases (e.g., *Pseudomonas corrugata*), or *Rhizobium* spp. that nodulate legume roots (e.g., *R. trifolii* in clover) are mechanisms which need further investigation, particularly in the field (Stephens and Davoren, 1994; Stephens *et al.*, 1993a). Inoculation strategies with these microorganisms all suffer with their inability to actively and rapidly spread through the soil and colonize extensively plant roots, so earthworms may act as important vectors for this purpose (Doube *et al.*, 1994b; Rouelle, 1983; Stephens *et al.*, 1993a).

Earthworms can affect root health by directly or indirectly affecting populations of plant pathogens (viruses, bacteria, fungi), parasitic nematodes, or insect pests (Brown, 1995). For example both *Aporrectodea rosea* and *A. trapezoides* were shown to increase yields of wheat, ryegrass and subterranean clover under greenhouse and field conditions by reducing incidence of *Rhizoctonia solani* (bare patch disease). Furthermore, wheat yields were also increased by the same earthworms through a reduction in *Gauemannomyces graminis* var. tritici (take-all disease) incidence (Stephens and Davoren, 1997; Stephens *et al.*, 1994a-d,f); *A. trapezoides* appeared to be more effective in the disease suppressiveness, probably due to more surface feeding and casting activities. In addition to the possibility of direct disease suppression, burial of infected litter, increased porosity, greater availability of plant nutrients in worm-worked soils and possible changes to the microfloral community in the drilosphere and rhizosphere in presence of active rhizosphere feeding earthworms are other factors which may help earthworms to indirectly control plant disease development. In apple orchards, this has been recognized for decades, where the burial of various insect pests and fungal pathogens overwintering in the surface leaf litter by *L. terrestris* (Raw, 1962), reduces their survival and ability to disperse and colonize/infect the trees in spring (Hirst and Stedman, 1962; Kennel, 1990; Laing *et al.*, 1986; Niklas and Kennel, 1981).

The reduction of plant parasitic nematode populations by earthworm activity has been documented for various tropical and temperate earthworm and nematode species combinations (Boyer, 1998; Dash *et al.*, 1980; Roessner, 1981; 1986; Senapati, 1992). Several of these studies also reported a preferential increase in bacteriophagic nematodes over other nematode trophic groups. However, most of the laboratory experimental designs were not very realistic (very high earthworm/soil weights) and probably increased nematode-earthworm contact to levels much higher than typically occur in the field. Boyer (1998) on the other hand, observed significantly lower *Pratylenchus vulnus* populations in maize rhizosphere when earthworms (*Amynthas corticis*) were introduced into the field and Yeates (1980; 1981) observed lower populations of total nematodes and some plant parasitic species in pasture fields inoculated with lumbricid earthworms in New Zealand. In both cases, significantly higher plant production (of maize and pasture, respectively) was observed. Earthworm-induced reduction in nematode populations may be due to direct ingestion and digestion (Boyer, 1998; Dash *et al.*, 1980) or the release of fluids (enzymes, etc.) which affect the fertility, viability and germination of cysts present in worm worked soils and castings (Boyer, 1998; Ellenby, 1945; Roessner, 1981), or indirect, through modifications in soil structure, water regimes, and nutrient cycling processes (Yeates, 1981). Edwards and Fletcher (1988) have also suggested that earthworms may spread nematode-trapping fungi that are of major importance in controlling nematode populations (Mankau, 1980). Nematodes that pass unharmed through the gut or able to take advantage of or adapt to earthworm changes in soil properties and processes may be dispersed by earthworms. In the case of plant parasites, this could lead to potential problems (see below), but for entomo-pathogenic nematodes (for insect pest biocontrol) this may be beneficial (Shapiro *et al.*, 1993).

### *Chemical mechanisms (indirect)*

Plants grown in the presence of earthworms are often found to have higher nutrient contents (e.g., Atlavinyte and Vanagas 1982; Baker *et al.*, 1997; Blakemore, 1994; Graff and Makeschin, 1983; McColl, 1982; Spain *et al.*, 1992; Stephens *et al.*, 1995), particularly of N and P. A few authors have proposed that earthworm N excretions and mucus secretions may be rapidly utilized (taken up) by plants, although this contribution is probably not very large unless the earthworm biomass is very high and activity concentrated in the root zone. Further research on this topic, particularly using homogeneously labelled ($^{15}$N, $^{32}$P) earthworms is necessary to properly ascertain this contribution. Most of the increases in nutrient content and uptake (esp. N and P) by plants in presence of earthworms is probably due to increased P and N mineralization rates and availability in castings, burrow linings and worm-worked soils (e.g., Aldag and Graff, 1975; Barois *et al.*, 1999; Brossard *et al.*, 1995; Chapuis-Lardy *et al.*, 1998; Graff, 1967; 1970; Lavelle *et al.*, 1992; Lee, 1985; López-Hernández *et al.*, 1993). This is especially evident in newly invated sites. For example, lumbricid or pheretimoid earthworm invasion in forests of North

America can result in dramatic changes to the chemical status of soil and humus may be transformed from the mor- to the mull-type (Nielson and Hole, 1964; Langmaid, 1964). Corresponding C and N losses and increased turnover rates resulting from this type of invasion may be on the order of several dozen to hundreds of T $ha^{-1}$ (Alban and Berry, 1994; Burtelow *et al.*, 1998, O'Brien and Stout, 1978; Scheu and Parkinson, 1994a).

The availability of many other elements has also been shown to increase in the structures produced by various earthworm species (e.g., Barois *et al.*, 1999; Mulongoy and Bedoret, 1989). This higher nutrient availability may come from selective feeding on OM- and nutrient-rich regions and greater incorporation, decomposition and mineralization of dung, thatch, litter and OM mediated by the earthworms and stimulated by the soil microflora whose populations, enzymatic activities and/or activity quotients have often been found to be higher in earthworm-worked soils (Hendrix *et al.*, 1998; Scheu and Parkinson, 1994a; Wolters and Joergensen, 1992). Greater availability and uptake of nutrients by plants in the presence of earthworms has often led to a greater transfer of C and N to shoots (the location of reproductive structures), and higher shoot biomass relative to roots (> shoot:root ratios). It has also been hypothesized that the synchrony of nutrient availability (especially N) with their uptake by plants, may be enhanced by earthworm activities (Fragoso et al., 1997).

Earthworm castings and burrows both serve as places of root concentration (Darwin, 1881; Ehlers, 1975; Lavelle *et al.*, 1998; Spiers *et al.*, 1986) and their importance to plant nutrition increases proportional to the difference in nutrient status between earthworm structures and bulk soil, the quantity of structures produced and their synlocalization with root growth pathways. Thus, in deeper soil zones, earthworm castings and burrows may serve as hot-spots of nutrient availability to plant roots (Mouat and Keogh, 1987). When living in nutrient rich soils, the relative stimulation (compared with the original soil) of nutrient bio-availability by earthworms is lower than in poor soils (due to the higher background fertility), and expected plant growth enhancements may lower (e.g., Atlavinyte and Vanagas, 1973; Brown *et al.*, 1999; chap. 2; Buse, 1990; Doube *et al.*, 1997), since the plant can obtain most of its nutrients by itself. Chemical effects of earthworms are probably among the most important mechanisms for enhanced plant growth, especially in cases where nutrients are limiting optimum yields. This is because earthworms are not only increasing plant nutrient availabilities in casts but also because soil egestion (cast production) rates can reach dramatic proportions (ranging from a few tons $ha^{-1}$ in temperate arable land up to more than 1000 T $ha^{-1}$ in tropical savannas with predominance of geophagous endogeic earthworms; Lavelle, 1988).

Nutrient release from dead earthworm tissues has often been stated as having an important role in the plant growth promotion observed in many experiments (Barley, 1961; Callaham and Hendrix, 1998; Russell *et al.*, 1910; Satchell, 1958). However, despite the fact that empirical visual observations show earthworm bodies decompose very rapidly in soil, only a few studies so far have been published on the amounts of nutrients made available from dead worm biomass (e.g., Christensen, 1988; Martin, 1990; Satchell, 1967; Whalen *et al.*, 1999). Furthermore, earthworm biomass is likely to only be an important and significant supplier of plant nutrients (enough to alter plant production) in field and pot experiments when inoculation rates or earthworm biomass to soil biomass ratio is excessively high and when mortality and turnover rates are high. When small soil volumes in pots are insufficient to maintain earthworm biomass well above the carrying capacity, earthworms die liberating nutrients which, although may be only a small amount compared to typical soil nutrient supplies, are enough to cause plant growth increases because of the low soil weights. This has occurred in many experiments, even those that based earthworm addition rates on field numbers but not field biomass, thus adding much higher biomass than would normally be found in the field (e.g., Baker *et al.*, 1996; 1997; Callaham and Hendrix, 1998; Doube *et al.*, 1994c; Satchell, 1958). In cases where larger soil volumes are used or more realistic earthworm biomass is added, nutrients from dead earthworms play a small role in plant nutrition. This is probably the case even in field situations with large earthworm biomass turnovers of up to 600 kg $ha^{-1}$ $yr^{-1}$ (fresh mass), which will supply only a few kg $ha^{-1}$ $yr^{-1}$ mineral N (Brown *et al.*, unp. data).

The incorporation of OM by the anecic earthworm *Martiodrilus carimaguensis* in savannas of Colombia has been associated with reduced Al saturation and its limitation to grass growth (Decaëns *et al.*, 1999). In pastures in New Zealand, earthworms accelerated the incorporation of lime, fertilizers and DDT (for grass grub control, Melollonthidae), increasing their concentrations with depth (MacKay *et al.*, 1982; Springett, 1985; Stockdill, 1966; 1982). Finally, in reclaimed polders in New Zealand, Hoogerkamp (1984) observed fewer urine scorched areas in pastures with inoculated earthworms.

Earthworm casts have been used in many experiments to show the importance of nutrients or PGP substances in casts in the plant response (e.g., Dash and Das, 1989; Kang and Ojo, 1996; Kang et al., 1994; Nijhawan and Kanwar, 1952; Reddy *et al.*, 1994; Tomati *et al.*, 1987). This response was generally proportional and positively related to the quantity of casts applied or the ratio of castings to soil or other substrates used. Experiments of this nature, however, have the disadvantage of unrealistic conditions compared to the field (lack of live earthworms and their effects) and the vastly different natures (chemical, physical and biological) of the casts, depending on their source and age.

### *Physical mechanisms (indirect)*

Physical engineering effects of earthworms are the basis for a series of chemical and biological effects which depend on the structure (e.g., type of cast, burrow etc.) created. Earthworms can deeply influence soil physical properties, affecting soil structure through effects on aggregation (mostly by casting) and porosity (mostly by burrowing), with associated effects on aeration, gaseous exchange, water infiltration and holding capacity. Thus, the importance of earthworms in altering soil physical conditions and ameliorating existing limitations is of utmost importance and probably sits at the base of the pyramid of mechanisms. For example, compacted soils will benefit from the activity of de-compacting earthworm species (Blanchart *et al.*, 1997; 1999), the incorporation of OM (aggregating agent) by anecics, and the burrowing strength and stable aggregate formation of endogeics (Zund *et al.*, 1996). Loose soils, on the other hand may benefit from the action of compacting earthworms. The physical incorporation of pasture thatch, lime, fertilizers and DDT reduced physical, chemical and biological limitations to pasture productivity in New Zealand (see above). Burial of leaves with plant pathogens and insects or dispersal of beneficial and malefic organisms throughout the drilosphere (above) are other biological effects partly regulated by the physical actions of earthworms.

Table 2. Chemical mechanisms by which earthworm activity in soils affects the growth of plants in a positive (enhancement) or negative (decrease) manner, either directly and/or indirectly. Potential (P) are possible mechanisms which have not yet been experimentally verified (V) to affect plant production. n.d. = not determined

| **Negative Mechanisms** | **Plants** | **Status** | **References** |
|---|---|---|---|
| Competition for P and N by earthworms or microflora | oats, pastures, ryegrass | P | Blakemore (1994; 1997); Hameed *et al.* (1993) |
| Inhibition due to high $NH_4$ | ryegrass | P | Hameed *et al.* (1993) |
| Increased nutrient leaching | grasslands | V | Anderson *et al.* (1983); Edwards *et al.* (1990); Haimi and Boucelham (1991); Hoogerkamp (1984); Knight *et al.* (1989) |
| Increased denitrification and/or volatilization rates | grasslands | V | Elliott *et al.* (1990); Lensi *et al.* (1992) |

Table 2. continuation

| **Positive Mechanisms** | **Plants** | **Status** | **References** |
|---|---|---|---|
| Quantity of casts used or deposited (more casts = greater production) | rice, mushrooms, sorghum, spinach, wheat, soybean, maize, *Vinca rosea* | V | Dash and Das (1989); Grappelli *et al.* (1987); Hauser *et al.* (1997); Khan (1966); Krishnamoorthy and Vajranabhaiah (1986); Nijhawan and Kanwar (1952); Reddy (1983); Reddy *et al.* (1994); Shuixin *et al.* (1991); Tomati *et al.* (1987) |
| Higher pH and concentrations of DDT with depth (for grass grub control) | pastures | V | Springett (1984; 1985); Stockdill (1966) |
| Decomposition of pasture thatch | pastures | V | Hoogerkamp (1984); Noble *et al.* (1970); Potter *et al.* (1990); Stockdill (1959; 1966; 1982); Temple-Smith *et al.* (1995); van de Westeringh (1972) |
| Incorporation of dung, green manure and OM sources into soil and enhancement of decomposition and mineralization | pastures, rice, wheat, maize, ryegrass, barley, grasses, oats | V | Atlavinyte and Vanagas (1973); Boyle *et al.* (1997); Curry (1988); Hoogerkamp (1984); Hu and Wu (1994); Graff and Kühn (1977); Graff and Makeschin (1983); Senapati *et al.* (1985); Sharma and Madan (1988); Stockdill (1959; 1966; 1982); Syers and Springett (1983); Temirov and Valiakhmedov (1988); van Rhee (1977); Waters (1951) |
| Reduced scorching of grass sward by cattle urine | grasslands | V | Hoogerkamp (1984) |
| Lower Al saturation | pastures | V | Decaëns *et al.* (2000) |
| Excreted or secreted N | grasses | V | Bouché and Ferrière (1986); Bouché *et al.* (1987); Hameed *et al.* (1994) |
| N recovery from organic residues or inorganic fertilizers | *B. decumbens*, ryegrass, maize, *P. maximum* | V | Brown *et al.* (1998; 2000b; chap. 4; 2000d; chap. 6); Gilot-Villenave *et al.* (1996); Hameed *et al.* (1993; 1994); Hu and Wu (1994); Spain *et al.* (1992) |
| C and N transfer to shoots (over roots) | beech, maize, *P. maximum*, wood barley | V | Klebsch *et al.* (1995); Marshall (1971); Spain *et al.* (1992); Tomati *et al.* (1996); Wolters and Stickan (1991) |
| Greater enzymatic activity in casts | various | V | Busenelli *et al.* (1984); Mulongoy and Bedoret (1989); Satchell and Martin (1984); Simek and Pizl (1989); Syers *et al.* (1979); Syers and Springett (1983); Tomati *et al.* (1987) |

Table 2. continuation

| **Positive Mechanisms** | **Plants** | **Status** | **References** |
|---|---|---|---|
| Enhanced nutrient (esp. N & P) availability | oats, ryegrass, grasses, wood barley, wheat, maize, rape, various | V | Alphei *et al.* (1996); Blakemore (1994); Brossard *et al.* (1995); Chapuis-Lardy *et al.* (1998); Curry and Boyle (1987); Edwards (1981); Gilot (1997); Graff (1971a); Hameed *et al.* (1994); Joshi and Kelkar (1952); Lavelle *et al.* (1992); López-Hernández *et al.* (1993); MacKay *et al.* (1982; 1983); Mansell *et al.* (1981); Marshall (1971); Scheu and Parkinson (1994b); Stephens *et al.* (1994a); Tomati *et al.* (1996); Wolters and Stickan (1991) |
| Increased nutrient uptake | clover, grasses, ryegrass, oats, wheat, maize, *Panicum maximum*, birch, various | V | Atlavinyte and Vanagas (1982); Abdul Rida (1996); Baker *et al.* (1997); Blakemore (1994); Brown *et al.* (1999; chap. 2); Buse (1990); Callaham and Hendrix (1998); Dreidax (1931); Doube *et al.* (1997); Graff (1971a); Graff and Kühn (1977); Graff and Makeschin (1983); Haimi and Einbork (1992); Haimi *et al.* (1992); McColl (1982); McColl *et al.* (1982); Spain *et al.* (1992), Stephens *et al.* (1994a); Tomati *et al.* (1996) |
| Nutrients in dead earthworms | hay/clover, millet, soybean, lima beans, wheat, spinach, rye, mustard, barley | V | Dreidax (1931); Hopp and Slatter (1949); Martin (1990); Russell (1910); Satchell (1958); Whalen *et al.* (1999) |
| Enhanced synchrony of nutrient availability with plant demand | n.d. | P | Fragoso *et al.* (1997) |
| Soil type: greater richness, lower response | pastures, grasses, maize, barley, various | V | Atlavinyte and Vanagas (1973); Brown *et al.* (1999; chap. 2); Buse (1990); Doube *et al.* (1997); Mackay and Kladivko (1985); Tomati *et al.* (1996); Zaller and Arnone (1999) |

Macropores are important for hydraulic conductivity and infiltration (when connected with the soil surface), and in increasing aeration (Kretzschmar, 1998), while castings, when dried, produce a stable soil structure with increase water holding capacity (Syers and Springett, 1983). The changes in soil pore size distribution and aggregation are most evident in studies using sieved soil, or where the original soil was compact. The effect of earthworms on porosity and infiltration has been associated with increased yields in New Zealand pastures (Stockdill, 1959; 1982) and reclaimed dutch polders (e.g., Hoogerkamp, 1984; van de Westeringh, 1972) and with greater hay and bean yields in large bucket experiments (Hopp and Slatter, 1948; 1949), although the interaction with incorporated or surface OM (another aggregating agent) is also likely to be implicated (Cogle *et al.*, 1994) in the observed responses in several cases. Increased water use efficiency has also been observed by various authors and crops both in pots and the field (Doube *et al.*, 1997; Stockdill, 1966; van Rhee, 1969).

Burrows can serve as preferential pathways for root elongation (e.g., Edwards and Lofty, 1980; Ehlers, 1975; Ehlers *et al.*, 1983; Jiménez, 1999; Kirkham, 1981; Kladivko and Timmenga, 1990; Wang *et al.*, 1986), especially in compacted zones (e.g., deeper soil horizons). The proportion of roots found in deep burrows (e.g., in the B horizons) compared with those in the bulk soil matrix can be very high (Kirkham, 1981; Logsdon and Linden, 1992), and these roots may be important in plant water dynamics. However the estimates of the proportion of roots in burrows may be exagerated because roots in burrows are easily observed while the rest of the root system is concealed in the soil matrix (Kretzschmar, 1998; Logsdon and Linden, 1992). A three-dimensional estimation of the interaction between roots and burrows is still not available (Kretzschmar, 1998), and considerable efforts must still be made to reach an understanding of this interaction and the mechanisms which control it (Tisdall and McKenzie, 1995).

Earthworms casts, once they have undergone a stabilization process (still not well understood) become water-stable (resistant) aggregates, although this stability is highly dependent on the soil type, earthworm species and feeding habits (Blanchart *et al.*, 1999). Often an important part (5% or more) of the surface (A) horizon of soils passes annually through earthworm intestines, particularly in tropical regions dominated by endogeics (Lavelle, 1988). Over time and under particular circumstances, most of the topsoil may be composed of earthworm castings, sometimes even long after the earthworms have disappeared (Buntley and Papendick, 1960; Graff, 1971b; Lavelle, 1988; Pop and Postolache, 1987). Casting on the soil surface opens new pores in the soil and can break surface crusts, helping germinating seedlings reach the soil surface (Kladivko *et al.*, 1986).

### *Species-specific effects and biomass relationships*

Earthworm species differ in their ability to modify soil properties and processes depending on their life-strategies and cycles. Plants also vary tremendously in their nutrient and water requirements and rooting strategies (among other factors). Earthworm effects and plant performance may further differ depending on the soil type. Thus, species-specific effects on plants depending on the earthworm and plant species and soil type used have been often observed (e.g., Brown *et al.*, 1999; chap. 2; Blakemore 1994; 1997; Derouard *et al.*, 1997; Doube and Schmidt, 1997; James and Seastedt, 1986; Spain *et al.*, 1992). Furthermore, the abundance (individuals $m^{-2}$) or biomass (g $m^{-2}$) of earthworms has also been found as being a significant factor in plant response. For example, Spain *et al.* (1992) observed an increase in *P. maximum* (green panic) grass production in plastic pots with increasing *Millsonia anomala* biomass up to 100g $m^{-2}$. Several other studies have shown positive correlations between earthworm abundance and/or biomass with the production of various plants (e.g., pasture, wheat, sweet potato, sugarcane, grains) in field situations (Atlavinyte 1974; Brown *et al.*, 1999; chap. 2; Buckerfield *et al.*, 1997; Rose and Wood, 1980; Satchell, 1955; Senapati and Dash, 1981).

In their review, Brown *et al.* (1999; chap. 2), using root and grain yields from a number of trials in the tropics, showed how the biomass of earthworms may have both upper and lower thresholds for plant-yield effects. Below the lower threshold, few significant effects are observed on grain (or root) yields, even though there may be significant effects on soil properties and processes. The value of earthworm biomass (g $m^{-2}$) representing this lower threshold is most likely variable depending on the soil type and cropping systems in question. In nutrient-poor or physically limited soils, earthworm effects on plant production may be obtained at biomass values lower than those necessary in rich soils or those with few physical limitations. Above the upper threshold, yield responses may turn from positive to negative, as the earthworms, particularly monospecific populations or communities dominated by one species (e.g., the compacting *P. corethrurus*), create a drilosphere with characteristics (e.g., soil structure) that complicate root growth and nutrient uptake (see section B., below).

*Problems of scale*

In seeking to reduce variability, experimental difficulty and guarantee good results, many studies of earthworms with plants have probably overestimated earthworm effects by applying only earthworm castings (not live worms) or high earthworm biomass/soil weights in pots. These artificial situations which rarely occur in the field are tempting and often work wonderfully, giving beautiful results, but have very little application to real-world situations. For example, if two adult earthworms weighing 1 g each are placed in a soil mass of 1 kg, the equivalent weight of earthworms in one hectare considering a bulk density of 1.2 Mg $m^{-3}$ and a 30cm plow layer, would be 7.2 Mg. Highest typical earthworm biomass in cultivated agroecosystems rarely surpasses 1 Mg $ha^{-1}$ (unless OM matter management and absence of tillage are implemented) and more typical values are usually <0.3 Mg $ha^{-1}$, especially in conventionally cultivated systems. In pastures, biomass may exceptionally reach up to 4 Mg $ha^{-1}$ (Barois *et al.*, 1988) although more typical values are probably around one third of that (1.2 Mg $ha^{-1}$). To adequately characterize the potential mechanisms operating in the field, realistic population and species combinations which mimic actual situations are imperative.

## B. Negative Mechanisms

Up to the early 1900's most accounts from books on agriculture and natural history, placed earthworms among plant pests, organisms that needed to be removed from the soil (Edwards and Bohlen, 1996; Walton, 1928). Graff (1983) showed how many authors attempted to disqualify Darwin's (1881) early accounts of the potential benefits of earthworms to plant productivity (in the conclusion section of his book), by highlighting negative effects of earthworms on plants, especially in flower pots. To this very day, there are still many places in the world where farmers do not attribute any potential usefulness to earthworms in agricultural production, sometimes even considering them as pests (Ortiz *et al.*, 1999). Truly there are instances in which earthworms can cause plant damage directly or indirectly, although most of the results published so far report positive effects (Brown *et al.*, 1999; chap. 2). In fact, in recent decades, this turn of attention to beneficial aspects may have led some authors not to report negative effects thinking that they may not be publishable (Blakemore, 1994; C.A. Edwards, pers. comm.), artificially biasing available results to the plant growth promotion side.

Both Lee (1985) and Edwards and Bohlen (1996) state that earthworms can occasionally damage healthy plants although, in most cases, it is more likely that they will attack moribund plants (already damaged previously by some other factor) and their tissues and that "there is no reason to regard them as pests of plants in any significant way" (pg. 277 and 219, respectively). Nevertheless, the results in Tables 1-3 provide ample evidence that earthworms, under particular situations, can cause important damage to plants and that care should be taken to prevent these situations whenever possible.

### *Direct effects*

Some authors have proposed that lumbricid earthworms (temperate spp.) feed on live roots (Baylis *et al.*, 1986; Carpenter, 1985; Cortez and Bouché, 1992; Gunn and Cherret, 1993; Hameed *et al.*, 1993; Sackville-Hamilton and Cherret, 1985; Stephenson, 1930), although only in a few cases this was associated with decreased plant growth/production. Furthermore, this phenomenon does not seem to be widespread since studies on earthworm crop, gizzard or gut contents of over 30 spp. revealed that roots form a very minor component of the ingested materials in most species (see Brown *et al.*, 1999; chap. 2), although rhizosphere activities may be more common than previously thought. These activities however, may be responsible for physical damage to plants due to root abrasion. Several authors have reported damage to rice crops in SE Asia (Barrion and Litsinger, 1996; Chen and Liu, 1963; Inoue and Kondo, 1962, cited in Lee, 1985; Otanes and Sison, 1947; Pradhan, 1986; Stephenson, 1930), which may likely be due root abrasion, although casting up the tillers and complicatios with drainage are also probably responsible. Other direct negative effects, probably due mostly to anecic earthworm species, involve the burial of living plant leaves (Darwin, 1881; Zicsi, 1954, cited in Edwards and Bohlen, 1996), or damage to germinating seedlings and plant seeds (Grant, 1983; McRill and Sagar, 1973; Olson, 1928; Patel and Patel, 1959; Shumway and Koide, 1994; Trifonov, 1957, cited in Lee, 1985; Walton, 1928). For example, Grant (1983) and Decaëns and Lavelle (1998) observed lower germination rates and slower germination of several plant species seeds in earthworm castings. Furthermore, many seeds to not appear to survive gut passage (Grant, 1983).

Table 3. Physical mechanisms by which earthworm activity in soils affects the growth of plants in a positive (enhancement) or negative (decrease) manner, either directly and/or indirectly. Potential (P) are possible mechanisms which have not yet been experimentally verified (V) to affect plant production.

| **Negative Mechanisms** | **Plants** | **Status** | **References** |
|---|---|---|---|
| Soil compaction and problems with water drainage/infiltration | *P. maximum*, oats, pastures, radish, carrots, beans, tree seedlings | V | Agarwal *et al.* (1958); Barros *et al.* (1996; 1998); Blakemore (1994); Chauvel *et al.* (1999); Doube *et al.* (1997); Duboisset (1995); Pashanasi *et al.* (1992); Puttarudriah and Sastry (1961); Rose and Wood (1980) |
| Abrasion of roots, direct damage of plant | rice, potted plants | V | Barrion and Litsinger (1996); Chen and Liu (1963); Inoue and Kondo (1962); Otanes and Sison (1947); Pradhan (1986); Stephenson (1930); Walton (1928) |
| Casting on surface interfering with harvesting | hay, cereals | V | Stephenson (1957); Edwards and Bohlen (1996) |
| Increased risk of treading in wet periods | grazed pastures | P | Evans and Guild (1947); Hoogerkamp (1984); Lee (1959) |
| Competition with plants for water | maize, pasture | P | Auerswald *et al.* (1996); Brown *et al.* (2000b; chap. 4) |
| Increased soil erosion | grasslands, cultivated soils, forests | P | Binet and Le Bayon (1999); Darwin (1881); Hazelhoff *et al.* (1981); Sharpley and Syers (1976); Sharpley *et al.* (1979); van Hoof (1983) |

Table 3. continuation

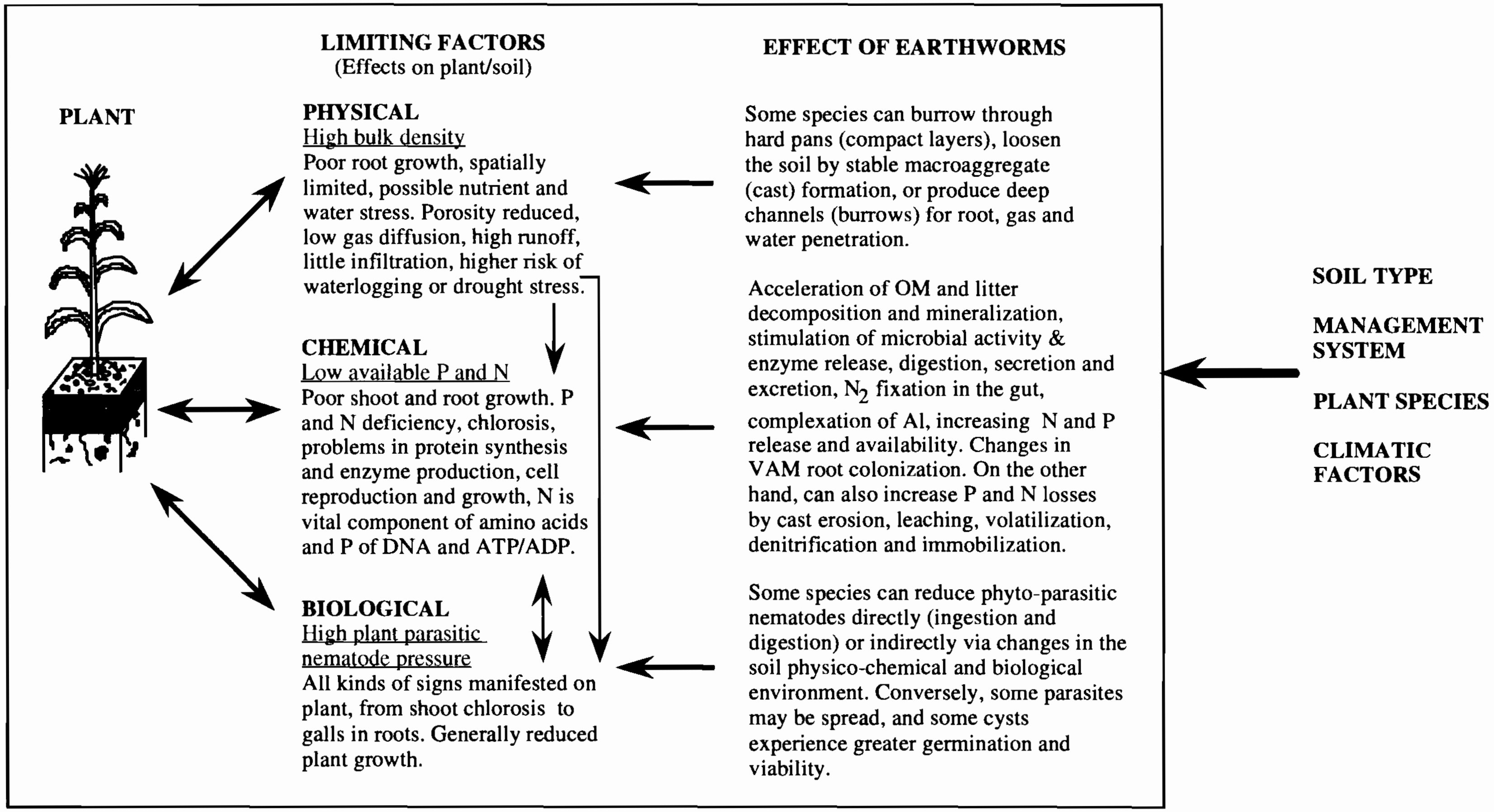
| **Positive Mechanisms** | **Plants** | **Status** | **References** |
|---|---|---|---|
| Increased incorporation of lime and DDT (vertical distribution) | grasslands | V | Springett (1984; 1985); Stockdill (1959; 1966; 1982) |
| Reduced soil erosion | cropland, yam mounds | P | Gilot (1997); Hopp (1946; 1954) |
| Regulation of soil bulk density (increase or decrease) | pastures, ryegrass, barley, maala system, maize, rice, cowpea, grasses | V | Alegre *et al.* (1996); Atlavinyte and Zimkuviene (1985); Blakemore (1994); Duboisset (1995); Hoogerkamp (1984); Mboukou (1998); McColl (1982); McColl *et al.* (1982); Stewart *et al.* (1988); Stockdill (1959; 1966; 1982); van de Westeringh (1972); Zund *et al.* (1997) |
| Deep channels (burrows) for roots | soybeans, maize, barley, oats, ryegrass-white clover | V | Edwards and Lofty (1978; 1980); Ehlers (1975); Ehlers *et al.* (1983); Kirkham (1981); Kladivko and Timmenga (1990); Springett (1985); Wang *et al.* (1986) |
| Increased available water and/or water use efficiency | pastures, wheat, barley, yam | V | Doube *et al.* (1997); Gilot (1997); Stockdill (1959; 1966; 1982); Syers and Springett (1983); van Rhee (1969) |
| Changes in soil porosity (greater macro and meso, less micro), aeration and/or infiltration | hay, pastures, barley, fruit trees, soybeans, wheat, rye, mustard, spinach, wheat, maize, rice, cowpea, *Mimosa scabrella* | V | Alegre *et al.* (1996); Atlavinyte and Zimkuviene (1985); Chaudhry *et al.* (1987); Duboisset (1995); Hoogerkamp (1984); Hopp and Slatter (1948; 1949); Jakobsen and Dexter (1988); Kobiyama (1994); McColl (1982); McColl *et al.* (1982); Russell (1910); Springett (1984; 1985); Stockdill (1959; 1966; 1982); Syers and Springett (1983); van de Westeringh (1972); Van Rhee (1977); Zund *et al.* (1997) |
| Reduced soil crusting |  | V | Kladivko *et al.* (1986); Roth and Joschko (1991) |

*Indirect biological, physical and chemical mechanisms*

Confirmed indirect negative effects of earthworms on plant production may occur in situations where a single earthworm species or functional type (e.g., compacting) dominates the community and the soils are prone to compaction. Under these conditions, Blakemore (1994), Barros *et al.* (1996; 1998) and Puttarudriah and Sastry (1961) observed increased compaction and associated lower soil porosity and water infiltration due to the activities of particularly *P. corethrurus*, with reduced radish, carrot, bean, pasture and sorghum production. Excessive casting on the soil surface and up plant stalks by lumbricid earthworms in England caused complications in cereal and hay harvesting (Stephenson, 1957; Edwards and Bohlen, 1996). Large amounts of castings on the soil surface of grazed pastures may also lead to "poaching" from cattle trampling, decreasing grass growth (Hoogerkamp, 1984 in the Netherlands; Lee, 1959 in New Zealand). Increased soil erosion of surface casts rich in nutrients (esp. N and P), higher denitrification and volatilization rates or greater leaching of soluble nutrients (Anderson *et al.*, 1983; Darwin, 1881; Edwards and Shipitalo, 1998; Elliott *et al.*, 1990; Hoogerkamp, 1984; Knight *et al.*, 1989; Sharpley and Syers, 1976; Sharpley *et al.*, 1979; van Hoof, 1983) may reduce their availability for plant root uptake.

Potential effects, still not confirmed are related to the ability of earthworms to compete with plants for water and nutrients (esp. N and P), and their ability to transport and infect plants or the rhizosphere with plant pathogenic fungi, viruses and bacteria, or disperse parasitic nematodes. A large number of plant pathogenic fungi and bacteria have been found in earthworm casts, and phytoparasitic nematodes have also been shown to survive gut passage (see Table 1), but it is not known whether or not, by spreading these organisms, earthworm activity increases plant disease incidence or nematode damage. The possible increased C drain by VAM in *B. decumbens* pasture plants in the presence of *P. corethrurus* needs further confirmation (Brown *et al.*, 2000d; chap. 6). Kirk (1981) reported large numbers of the northern maize rootworm (Diabrotica: Coleoptera) eggs in earthworm burrows and hypothesized that this may contribute to the spottiness of rootworm distribution and damage often observed in maize fields. Finally, the preferential ingestion and/or survival, germination and growth of weed seedlings in earthworm casts or worm-worked soils (Piearce *et al.*, 1994) may lead to increased infestation of crop fields or grasslands with weeds, potentially increasing competition with the crops or desired plants (Edwards and Bohlen, 1996; Stinner *et al.*, 1997).

Clearly the negative mechanisms, both potential and confirmed are wide-ranging and deserve further attention. The presence and extent of root feeding, the identification of the species involved, the conditions promoting this phenomenon, and its possible damage to plant production needs to be properly studied. The prediction of plant growth suppression by indirect effects (most common mechanisms) will require detailed comparison of the compatability of existing earthworm communities with established plants, cropping systems and crops to be used. There may be situations where it is better to plant gramineous species rather than legumes or vegetables (Puttarudriah and Sastry, 1961), or to manage the soil in a way to reduce potentially negative effects of earthworm activity on plant production.



**PHYSICAL**
High bulk density
Poor root growth, spatially limited, possible nutrient and water stress. Porosity reduced, low gas diffusion, high runoff, little infiltration, higher risk of waterlogging or drought stress.

Some species can burrow through hard pans (compact layers), loosen the soil by stable macroaggregate (cast) formation, or produce deep channels (burrows) for root, gas and water penetration.

**CHEMICAL**
Low available P and N
Poor shoot and root growth. P and N deficiency, chlorosis, problems in protein synthesis and enzyme production, cell reproduction and growth, N is vital component of amino acids and P of DNA and ATP/ADP.

Acceleration of OM and litter decomposition and mineralization, stimulation of microbial activity & enzyme release, digestion, secretion and excretion, $N_2$ fixation in the gut, complexation of Al, increasing N and P release and availability. Changes in VAM root colonization. On the other hand, can also increase P and N losses by cast erosion, leaching, volatilization, denitrification and immobilization.

**BIOLOGICAL**
High plant parasitic nematode pressure
All kinds of signs manifested on plant, from shoot chlorosis to galls in roots. Generally reduced plant growth.

Some species can reduce phyto-parasitic nematodes directly (ingestion and digestion) or indirectly via changes in the soil physico-chemical and biological environment. Conversely, some parasites may be spread, and some cysts experience greater germination and viability.

Figure 6. Example of three factors limiting plant production (biological, chemical and physical), and the effects of earthworms on these limitations (within box). Factors which control the ultimate limiting factors and the possibility of earthworms amelioration are shown outside the box. The arrows indicate where thresholds, both upper and lower, can control action of particular organisms/properties and processes on these limitations.

## CONCLUSION: IDENTIFYING EARTHWORM PHYTO-PROMOTION POTENTIAL

One can easily observe from Tables 1-3 the wide gamut of mechanisms of plant growth alterations by earthworm activities. The presence and determination of which (mechanism) will be most important in a given situation demands, first of all, a comprehensive knowledge of the existing limitations to plant growth in a given situation. Next, since earthworm effects on soils vary tremendously depending on species and ecological category, and whether alone or in combination with other species (Abbott and Parker, 1981; Lavelle and Spain, 1999), only a proper taxonomical and functional identification of the earthworm species and/or communities involved in each experiment will permit a mechanistic study to be applicable to field situations. Finally, the effects of earthworms on soil physical, biological and chemical limiting factors to plant production must be assessed and the possible mechanisms of earthworm amelioration identified and isolated. Likely, the most important earthworm effects on yields will be those that are most modified by earthworms and which also happen to be most limiting plant growth.

The possible effects of earthworms on plants are hence regulated by the factors limiting plant growth and their thresholds, both upper and lower. There are generally not only one, but several factors limiting plant growth in a particular situation. Each of these factors is exerting a particular level of limitation on the plant, and one factor may be dominant (the most important limiting factor). The possible amelioration (reduction) of each factor is thus controlled by a thresholding action, whereby above or below this particular threshold, ameliorating action begins and the plant responds positively. The combination of drilosphere effects (both positive and negative) and the threshold level at which each of these factors is operating its limitation on the plant (i.e. which factors are most limiting, and at what level) will ultimately determine the effect of earthworm action on the plant. This notion is explored further in Figure 6, using three examples of limiting factors (biological, chemical and physical) and the possible effects of earthworms on these limitations. For example, if a parasitic nematode abundance or infestation by a particular fungal or bacterial pathogen has reached plague proportions and are among the most limiting factors for ideal plant production, and earthworms are able to reduce their populations, it is likely that this will be the dominant mechanism, although other factors (primarily indirect) such as the influence on soil structure and fertility and biological interactions with the edaphic microflora and fauna will also be important and operate simultaneously. Similarly, if nutrients such as N or P are the most limiting factors, earthworm-induced increases in their availability, or changes in the mycorrhizal colonization of the roots may be important controlling mechanisms. Finally, if soils are compact or prone to compaction and associated hydrological limitations are complicating plant growth, biological tillage (bioturbation) achieved by earthworm-induced changes in the soil structure may be the most important mechanisms enhancing plant production.

The next step to take in the process of assessing earthworm effects on plant production would be to develop a model where the soil constraints are matched with the earthworm and plant species (or community) present at a site, to predict the potential direct and indirect influences of earthworm activities on soil physical, chemical and biological limitations and plant production. Unfortunately, there are still many gaps in our knowledge of these processes which need to be filled in order to adequately develop this model and accurately predict whether a particular earthworm species or community will enhance or suppress plant production. Since many of the above mechanisms are still in the "potential" stage, it is obvious that much more research is needed to be able to properly assess the mechanisms that operate under particular conditions, and those that are more general, i.e., operating most of the time.

Table 4. Soil, water and plant parameters and processes determining plant production and the key indicators which can be used to determine the quality and sustainability of land use (after Doran and Jones, 1996; Doran and Safley, 1997; Elliott, 1994; Lal, 1994; Nannipieri, 1994).

| **Parameters/ Processes** | **Indicators** |
|---|---|
| **Soil** | |
| Acidification | pH, total acidity, base saturation, Exchangeable Al and Mn |
| Salinization | Electrical conductivity, SAR, total soluble salts |
| Fertility | Total and available macro and micronutrients, soil OM content, OM fractions |
| Structure | Aggregation, mean weight diameter, bulk density and strength, compaction/penetration resistance, porosity and pore size distribution, erodibility, aggregate water stability, crust strength, cracking patterns and intensity |
| Water | Available water capacity, infiltration rate, saturated and unsaturated hydraulic conductivity, anerobiosis, surface ponding, soil color, mottling, bypass and macropore flow |
| Erosion | Rates of soil loss, erodibility, dispersability, stoniness, depth of topsoil, texture, $CaCO_3$ content, exposed tree roots, soil color |
| Biology | Earthworm biomass, density and community species composition, cast and burrow production rates and turnover, litterfall, respiration (& SIR), decomposition, humification and mineralization rates (esp. N), enzyme production, biologically active substances, $N_2$ fixation, mycorrhizal spore number and % AM and EM root colonization, microbial biomass C, N & P, respiratory quotients, nematode community trophic structure, microbial and faunal populations and community structure, PFLA, status of pests, parasites and pathogens, soil suppressiveness |
| **Water** | |
| Balance | Runoff rate and amount, interflow, soil water storage capacity, water deficit |
| Quality | Dissolved and suspended sediment loads, type and concentration of agro-chemicals, eutrophication, concentration of soluble salts in surface and ground waters |
| **Plant** | Shoot, root, grain biomasss, total yields, potential vs. actual production, root growth, depth, photosynthesis rates, nitrate reduction, plant nutrient content and balance (DRIS), toxicity/deficiency symptoms, disease symptoms and status (root, shoot, grain), rhizobia nodulation, nutrient- and water use-efficiency, water balance, drought stress, galls, parasitic nematode and pest damage |

However, there are some basic principles that can be applied to help isolate potential mechanisms involved. A proper identification of the soil chemical limitations to plant growth can be assessed by soil and plant analyses and limiting nutrient pot studies. If experiments are to be performed in pots in the greenhouse, environmental controls and possible treatment combinations are much greater. For example, soil sterilization and inoculation with a variety of different microorganism treatments is possible, to assess the role of their interaction with earthworms on plant production. Furthermore, fertilization with all but one nutrient and inclusion/exclusion of earthworms allows an assessment of their role in chemical soil fertility. In these experiments (especially short term) the physical effects of earthworms on soils are probably less important than the chemical and biological, although as the experimental periods increase, physical effects (especially limitations when only one earthworm species is included) may become more important. To separate chemical, physical and biological mechanisms simultaneous experiments using different methodologies and designs, such as pots with earthworms and pots with soil previously processed by earthworms and various treatment combinations (± residues, ± fertilizers, ± micro- or other organisms), can be developed. One must also keep in mind that there are probably thresholds for each mechanism, beyond or below which effects on plant yields are observed. Furthermore, these may be regulated by the inherent soil properties and the biomass of earthworms applied, which also display thresholding behavior.

In the field, a great many more variables (many of them uncontrolable) are at play, although the results obtained are much more realistic and useful for practicing farmers, foresters or other environmental managers. In such instances a proper assessment of the physical limitations is essential since, under these conditions, earthworm physical effects are of utmost importance (Barros, 1999; Alegre *et al.*, 1996) and may outweigh the other effects, which are often diluted by the multitude of interactions in soils controlling their action on plant production (e.g., climate, soil minerology, native microbial communities, other soil fauna). Obviously, characterization of earthworm community changes by periodic sampling, the quantification of casting and burrowing activities (e.g., by physical description of cores and X-ray commuted tomography) and their chemical and biological analyses are important to assess the extent of earthworm changes to soil structure, biology and fertility. In these studies, clean controls (without introduced or native earthworms) must be present and maintained and, if earthworms are to be introduced, realistic biomass, numbers and species assemblages should be used.

In conclusion, drilosphere effects, although wide-ranging, can be conveniently separated into physical, chemical and biological in nature which exert a series of ecosystem engineering effects that can ultimately regulate not only soil structure, but also nutrient resource availability for other soil organisms and plants. Following the structures created and the processes modified by earthworm activities (Fig. 2), especially those which can serve as indicators of soil quality and sustainable productivity (Table 4), and matching them with the immediate and long-term limiting factors to plant production (Fig. 6) provides a framework in which the identification of potential mechanisms and their operation in space and time can be studied for future modelling and predicting of earthworm effects on plant yields.

# CHAPITRE 2

# EFFECTS OF EARTHWORMS ON PLANT PRODUCTION IN THE TROPICS[†]

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## SUMMARY

As part of the European Union-funded Macrofauna Project, 16 experiments were conducted over a seven year period on the effects of earthworm inoculation on plant production, both at the greenhouse and field scale. These experiments were undertaken in six countries, involved 14 plant species, six great groups of soils, and at least 13 species of earthworms. Additional data were taken from the literature, totalling over 240 data points on the percent change (+ or -) in above-ground production in the presence of over 34 earthworm species. The overall average increase in shoot and grain biomass due to earthworms was +56.3%±9.3% (s.e.) and 35.8±8.9%, significant at $P<0.07$ and $P<0.08$, respectively. Highest increases were observed in soils with sandy textures, poor in organic matter, and with a moderately acid pH.

Earthworm biomass of around 30 g $m^{-2}$ or more was shown to be necessary to promote agriculturally important (> 40%) grain yield increases. Earthworm species which appeared most promising in enhancing plant growth at both the field and pot experiment scales were *Pontoscolex corethrurus* and *Drawida willsi*, both introduced with substantial results in India. Several other species showed significant advantages under particular situations, and are likely to be useful under wider conditions of crop and soil management. Plants most affected were tropical trees (in Peru), tea in India, and *Panicum maximum* grass, planted both in Australia and the Ivory Coast. Benefits of earthworm introduction are therefore particularly important in perennial cropping systems. High and significant increases in grain biomass were observed in several situations, especially for sorghum, rice and maize. Leguminous crops appeared to be less enhanced by earthworm activities.

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Numerous mechanisms are involved in plant growth stimulation (observed in 72% of all cases), ranging from large scale effects on soil physical properties (aggregation and water infiltration), to the microsite level where earthworms enhance microbial activity, nutrient availability and rhizosphere processes. When earthworms are to be introduced, a suite of adapted species, at sustainable numbers and biomass must be added to ensure a stable population which will induce favorable soil properties and enhanced plant production. Once earthworms are established, cropping systems involving crop rotations with long cycle crops or perennials with sufficient organic matter additions will help secure long-lasting benefits from earthworm activities.

## INTRODUCTION

The importance of earthworms for plant growth has been recognized for over 100 years, since the publication of Charles Darwin's book "The formation of vegetable mould through the action of worms" in 1881. Over the last century, many researchers, primarily in the temperate zone, have described the effects of earthworms on plant production, at the field and small-scale (pot) levels. Their experiments (summarized in Blakemore and Temple-Smith, 1995) dealt almost exclusively with four to six widespread lumbricid earthworm species in pasture or cereal crop situations. The results show that these earthworms exert primarily beneficial effects on plant growth, although in a few cases, negative or null effects could be induced under particular situations. In addition, shoot biomass tended to benefit more than roots to earthwom activities.

Nevertheless, various shortfalls have become obvious from these studies. Pot trials are generally run for relatively short periods of time (only one crop cycle), often receive unrealistically high earthworm densities and biomass or are performed using earthworm casts or composts rather than live earthworms, and the earthworm species used are often not adequately identified. In field experiments there is little control over many variables, it is difficult to completely exclude earthworms from control plots, and earthworm structures produced before the trials are long-lasting, possibly masking current earthworm exclusion treatment effects. Also, there are currently over 3000 earthworm species classified, and probably an equal or larger number still to be described, many of which appear to have some potential for management in tropical agroecosystems (Fragoso *et al.*, 1999a,b). It is thus essential that more species be tested for potential effects on plant production, particularly in the tropics where a limited number of studies has been conducted using common tropical earthworm species and plants. Given that much of the world's population lives in, and their food production originates from this zone, it is imperative that more attention be given to understanding the role of tropical earthworms (both native and exotic, widespread and locally common species; Fragoso *et al.*, 1999b) in enhancing production of tropical food, fodder and tree crops.

## OBJECTIVES

Following the above demands for information, and the need for further research in this area, during the six years of research of the "Macrofauna" programme, various experiments both at the glasshouse and field level were performed on the influence of earthworms on soil fertility and plant growth. This chapter synthesizes the data obtained and, together with other experiments performed both before and after the programme began, attempts to address the following questions:

1) are earthworms in the tropics important for plant growth, and if so, to what degree?
2) what plant species (trees, crops, grasses, etc.) are affected the most, and by which species of earthworms?
3) by what means (the mechanisms) are plants affected by earthworm activity, positively and negatively?
4) how many earthworms, or what biomass is necessary to have a measurable (and agriculturally important) positive effect?

## MATERIALS AND METHODS

### Experimental Designs

To address these questions, soil biological, physical and chemical parameters, earthworm survival and production of different plant parts (above- and below-ground) were evaluated to reveal mechanisms of plant growth enhancement (if observed) in 16 experiments completed during the Macrofauna Programme. A review of the literature for the tropics revealed a further 12 trials which had suitable data on earthworms and plant biomass for the statistical analyses (Blakemore, 1994; dos Santos, 1995; Kobiyama, 1994; Patrón, 1998; Senapati *et al.*, 1985 and unp. data; Spain *et al.*, 1992). Thus, in 28 experiments chosen, at least 34 earthworm and 19 plant species were tested in approximately 23 different soils belonging to eight great groups. For each of these experiments selection criteria were applied to determine which earthworm, soil type and crop species would be used. Earthworm species used were common at or near the site and known to be generally adaptable to cropping systems and to affect soil properties. In general, the soils used were C-poor, often having some kind of deficiency or limitation for crop growth, although in a few cases, C-rich soils (e.g., pasture or forest soils) were used. The characteristics of the soils used in the experiments are given in Table 1. Finally, the crops tested were ones that were widely sown by farmers in nearby regions. Since much of the food consumed in the tropics is grown at household or small field-levels for self-subsistence or local markets, and few external inputs are added to the cultivated plants, low-input practices were usually mimicked in the experiments detailed below. A brief summary of the main materials and methods used is shown in Table 2.

Generally speaking, the trials were performed at three levels, spatially and temporally.

**1.** At the smallest scale, short-term experiments lasting from 15 days to eight months, using various containers (nursery bags, buckets, PVC pipes) and involving either one or two cropping cycles were performed in the greenhouse and open air. More than 12 plant and at least 27 earthworm species were tested in small- to medium volumes of soil (oven dry weight from 0.9 up to 17.5 kg) of approximately 12 different types. The purpose of these experiments was to reduce soil and climatic variability, illustrate the mechanisms of earthworm effects on soil and plants in greater detail, and find the most promising earthworm and plant species associations to use in field situations. The plants tested had different rooting strategies (fibrous or taproot) and life cycles (short or long season, perennial), and the earthworms were of various ecological strategies (mostly endogeic, some epigeic and anecic).

**2.** At the intermediate scale, 13 species of earthworms were inoculated into field plots with and without enclosures which isolated a set volume of soil. Plot size varied from circular plots of 60 cm diameter ($0.28 m^2$) at Yurimaguas (Peru) to 50 $m^2$ at St. Anne (Martinique). Earthworm biomass added varied greatly, but for most cases, equivalent values found nearby were taken as a basis. More than eight plant species were tested for periods lasting from four months to seven years. Several trials were performed with similar plant and earthworm species used in the smaller scale experiments to confirm that previously observed effects would also be present at field scales (e.g., in Ivory Coast, Australia and India).

**3.** At the broadest scale, earthworms were reared in special culture beds and introduced *en masse* into the field, to assess their colonization potential and effects on plant production in a situation more comparable to farmer's fields. In addition, costs and benefits of such large-scale undertakings were studied to reveal the economic viability of such ventures (Senapati *et al.*, 1999). Results from these studies would be immediately applicable to situations common around the research sites. Two trials were performed at this level, one in Lower Sheikalmudi, in the state of Tamil Nadu, India, and the other, at Yurimaguas, Peru (Senapati *et al.*, 1999). At the first site, 1200 pits of 0.54 $m^2$ each were dug in one hectare including 5500 tea trees approximately 80 years old. A large quantity of residues and four species of earthworms (primarily *P. corethrurus*) were applied at the rate of about 150 kg $ha^{-1}$ (350 g $pit^{-1}$) in one-half of the pits and tea production studied intensively over a 10 month harvest cycle (Giri, 1995). At Yurimaguas, a forest area of about 0.5 ha was cleared, and two areas, one receiving earthworms and one not, were separated by a pesticide-poisoned soil strip. Two types of agricultural practices, traditional (shifting cultivation) and "improved" (use of fertilizers), were applied to the area, and *P. corethrurus* was inoculated at the rate of 1-10 g $m^{-2}$ on several planting dates (e.g., together with maize seed), on top of the resident earthworm fauna. During the three year trial, maize, rice, cowpea, cassava and forest trees were planted, depending on the system. Unfortunately, the soil textural difference between inoculated and uninoculated plot (Table 1) led to a low survival of inoculated earthworms in addition to greater crop harvests in the control treatments, so the experiment had to be abandoned.

One of the most pernicious problems in performing both pot and field experiments was preventing contamination of control plots with resident or introduced earthworms. For instance, in La Mancha, plots inoculated with *P. corethrurus* were increasingly contaminated with *Polypheretima elongata*. Several methods were imposed to prevent contamination and to kill or remove resident or potential invading earthworms, with variable effectiveness. The most efficient methods utilized were to sterilize the soil by heating (for pots), to choose sites with low native earthworm populations (e.g., Narayen, Australia, for pots and field; Blakemore, 1994), or to chemically extirpate them with carbamate pesticides (e.g., Lamto, St. Anne and Yurimaguas). The least effective method was soil tillage and/or hand removal (e.g., La Mancha).

Table 1. Types and characteristics of soils used in field and greenhouse investigations on the role of earthworms in soil fertility and plant production. a=Earthworm inoculated treatment; b=Non-inoculated treatment; ND=not determined.

| Soil type | Location | Vegetation | Rainfall (mm) | %Sand | %Silt | %Clay | %C | %N | C/N | pH | CEC (meq 100g-1) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alfisol | Lamto, Ivory Coast | Savanna | 1228 | 75.4 | 14.0 | 7.5 | 1.09 | 0.08 | 14.3 | ND | 3.2 | Spain *et al.* (1992) |
| | Lamto, Ivory Coast | Secondary Forest | 1228 | 87.6 | 8.5 | 4.7 | 1.26 | 0.13 | 9.9 | 7.5 | 5.1 | Gilot (1994) |
| | Lamto, Ivory Coast | Savanna | 1228 | 78.1 | 17.0 | 6.0 | 0.91 | 0.05 | 17.3 | 6.7 | 4.4 | Gilot (1997) |
| | Lamto, Ivory Coast | Secondary Forest | 1228 | 85.0 | 10.5 | 4.5 | 1.18 | 0.12 | 9.8 | 7.15 | 5.3 | Gilot-Villenave *et al.* (1996) |
| | Lamto, Ivory Coast | Savanna | 1228 | 72.6 | 12.1 | 11.7 | 0.91 | 0.11 | 14.1 | 7.5 | ND | Derouard *et al.* (1997) |
| Psamment | La Mancha, Mexico | Weed fallow | 1345 | 75.4 | 8.6 | 16.0 | 1.65 | 0.11 | 15.7 | 7.9 | 30.9 | Patrón *et al.* (unp. data; 1994) |
| Andosol | Los Tuxtlas, Mexico | Tropical Rainforest | 4700 | 18.5 | 37.4 | 41.9 | 5.27 | 0.46 | 11.4 | 5.9 | 13.5 | Brown et. al. (2000b) |
| Haplustalf | La Víbora, Mexico | Pasture | 1400 | 81.5 | 7.4 | 10.6 | 1.07 | 0.10 | 10.7 | 5.1 | 12.1 | Brown et. al. (2000a) |
| Ultisol | Mbalmayo, Cameroon | Secondary Forest | 1600 | 61.8 | 16.0 | 22.2 | 4 | ND | ND | 6.34 | ND | Kidza (unp. data) |
| Paleudult | Yurimaguas, Peru | Secondary Forest | 2100 | 55 | 22 | 23 | 1.68 | 0.13 | 12.9 | 4.0 | 5.5 | Pashanasi *et al.* (1994) |
| | Yurimaguas, Peru | Secondary Forest | 2100 | 67.7 | 23 | 9.1 | 2.07 | 0.13 | 15.9 | 4.17 | 5.14 | Chapuis (1994) |
| | Yurimaguas, Peru | Secondary Forest | 2100 | 59.1a<br>54.3b | 22.1<br>23.6 | 19<br>22 | 1.55 | 0.11 | 14.1 | 4.3 | ND | Pashanasi *et al.* (unp. data) |
| Vertisol | St. Anne, Martinique | New pasture | 1580 | 25 | 15 | 60 | 1.4 | 0.14 | 10 | 6.25 | 37 | Hartmann *et al.* (1998) |
| Vertisol | Sambalpur, India | Rice paddy | 1500-2000 | 92.6 | 1.52 | 5.88 | 1.44 | 0.23 | 6.48 | 7.21 | 3.3 | Senapati *et al.* (unp. data) |
| | Sambalpur, India | Rice paddy | 1500-2000 | 92.8 | 1.4 | 5.8 | 1.43 | 0.22 | 6.5 | 7.1 | 3.3 | Senapati *et al.* (1985) |
| Oxisol | Tamil Nadu, India | Deciduous Forest | 2000-3000 | 60-70 | ND | 30-40 | 1.77 | 0.19 | 9.3 | 6.4 | 4.9 | Giri (1995) |
| | Tamil Nadu, India | 80 year old tea | 2000-3000 | 65.6 | 19.3 | 9.93 | 1.34 | 0.24 | 5.2(?) | 6.58 | 4.9 | Giri (1995) |
| Oxisol | Curitiba, Brasil | Fallow | 1400 | 46 | 14 | 40 | 6.1 | ND | ND | ND | 23.3 | Kobiyama (1994) |
| Oxisol | Guarapuava, Brasil | Wheat | 1880 | 17.8 | 44.2 | 38 | 4.2 | ND | ND | ND | 19.4 | dos Santos (1995) |
| Vertisol | Narayen, Australia | *Panicum maximum* | 710 | 13 | 23 | 43 | 4.8 | 0.35 | 13.7 | 7.0 | 38 | Blakemore (1994) |
| | Biloela, Australia | No-till sorghum | 600 | 20-45 | ND | >30 | 2.4 | 0.145 | 16.6 | 7.9 | ND | Blakemore (1994) |
| | Kingaroy, Australia | *P. maximum* | ND | ND | ND | 55-60 | ND | ND | ND | 5.5 | ND | Blakemore (1994) |
| Ultisol | Samford, Australia | 20 year old grass pasture | 1105 | 82 | 4 | 10 | 1.3 | 0.08 | 16.3 | 5.7 | 8.6 | Blakemore (1994) |
| Mollisol | Samford, Australia | Mixed-sward pasture | 1105 | 34 | 17 | 42 | 6 | 0.33 | 18.2 | 6 | 30 | Blakemore (1994) |

Table 2. Simplified summary of materials and methods used for experiments performed to assess the role of earthworms in plant production.

| Scale | Site | Duration | Plants tested | Earthworm species used | Mass added (g m$^{-2}$) | Author |
|---|---|---|---|---|---|---|
| Nursery bags | Yurimaguas, Peru | 15d-8 mo. | Fruit trees (3 species) | *P. corethrurus* | 3.5-22 | Pashanasi *et al.* (1992), Ydrogo (1994) |
| | Tamil Nadu, India | 120, 150d | Tea | *P. corethrurus* | 127 | Giri (1995) |
| Buckets | Lamto, Ivory Coast | 79, 84d | Maize, *Panicum maximum*<br>Peanuts, Rice, Maize | *P. corethrurus, H. africanus, M. anomala, C. zielae, S. porifera* | 12.5-128 | Spain *et al.* (1992) |
| | Lamto, Ivory Coast | 69-74d | | | 56.5 | Derouard *et al.* (1997) |
| | Xalapa, Mexico | 30d-6 mo. | Beans, Maize | *P. elongata, P. corethrurus* | 48-63 | Brown *et al.* (2000a,b) |
| | Salazar, Mexico | 90d | *Brachiaria decumbens* | *P. corethrurus* | 114 | Patrón (1998) |
| | Mbalmayo, Cameroon | 65d | Maize | At least 2 species | 164 | Kidza (unp. data) |
| | Sambalpur, India | ~90d | Rice | *D. willsi* | 42.4 | Senapati *et al.* (1985) |
| PVC tubes | Brisbane, Australia | 26d-30 mo. | Oats, Sorghum, 3 grass species | at least 27 species | 13.5-326 | Blakemore (1994) |
| Single crop cycle field studies | Lamto, Ivory Coast | 35 - 90d | Maize | *M. anomala* | 52 | Gilot (1994; 1997) |
| | Sambalpur, India | 90d | Rice | *D. willsi* | 13 | Senapati *et al.* (unp. data) |
| | Narayen, Australia | 14.5 mo. | *P. maximum*, various grasses | 9 species | 8-166 | Blakemore (1994) |
| | Samford, Australia | 13.2 mo. | *P. maximum*, various grasses | 10 species | 7-166 | Blakemore (1994) |
| | Curitiba, Brasil | 9 mo. | *Mimosa scabrella* | *Amynthas* spp. | 30-90 | Kobiyama (1994) |

Table 2. continuation

| Scale | Site | Duration | Plants tested | Earthworm species used | Mass added (g m$^{-2}$) | Author |
|---|---|---|---|---|---|---|
| Multi-crop field enclosures | Lamto, Ivory Coast | 3 yr. | Yam, Maize | *M. anomala* | 16-31.4 | Gilot (1994) |
| | Yurimaguas, Peru | 3-7 yr. | Rice, Cowpea, Maize | *P. corethrurus* | 36 | Pashanasi *et al.* (1994; 1996), Charpentier (1996) |
| | La Mancha, Mexico | 3 yr. | Maize | *P. corethrurus* (*P. elongata*) | 35.5 | Patrón *et al.* (unp. data) |
| | Guarapuava,ı Brasil | 1 yr. | Beans, Wheat | *Amynthas* spp. | 30 - 90 | dos Santos (1995) |
| Long-term field inoculation | Yurimaguas, Peru | 3 yr. | Maize, Cassava, Cowpea, Trees | *P. corethrurus* | 1-36 | Pashanasi *et al.* (unp. data) |
| | Tamil Nadu, India | >3 yr. | Tea | *P. corethrurus* + 4 species | 648 | Giri (1995) |
| | St. Anne, Martinique | >4 yr. | Pangola (*Digitaria decumbens*) | *P. elongata* | ~90 | Blanchart (1997) |

## Data Analyses

For the statistical analyses, data on earthworm biomass initially applied and at (each) harvest, the plant biomass obtained in each treatment (in units of Mg ha$^{-1}$), the plant and earthworm species tested, plot size, amount of residues applied, and the characteristics of the soils (% sand, silt and clay, %C and pH) used in the 28 experiments were entered into a spreadsheet. Analysis of variance (ANOVA) and Principal Component Analyses (PCA) were conducted using the previous factors and the percent increase in plant biomass in the earthworm-inoculated versus non-inoculated treatments (controls) for each of the plant parts studied (e.g., grain, stubble, root).

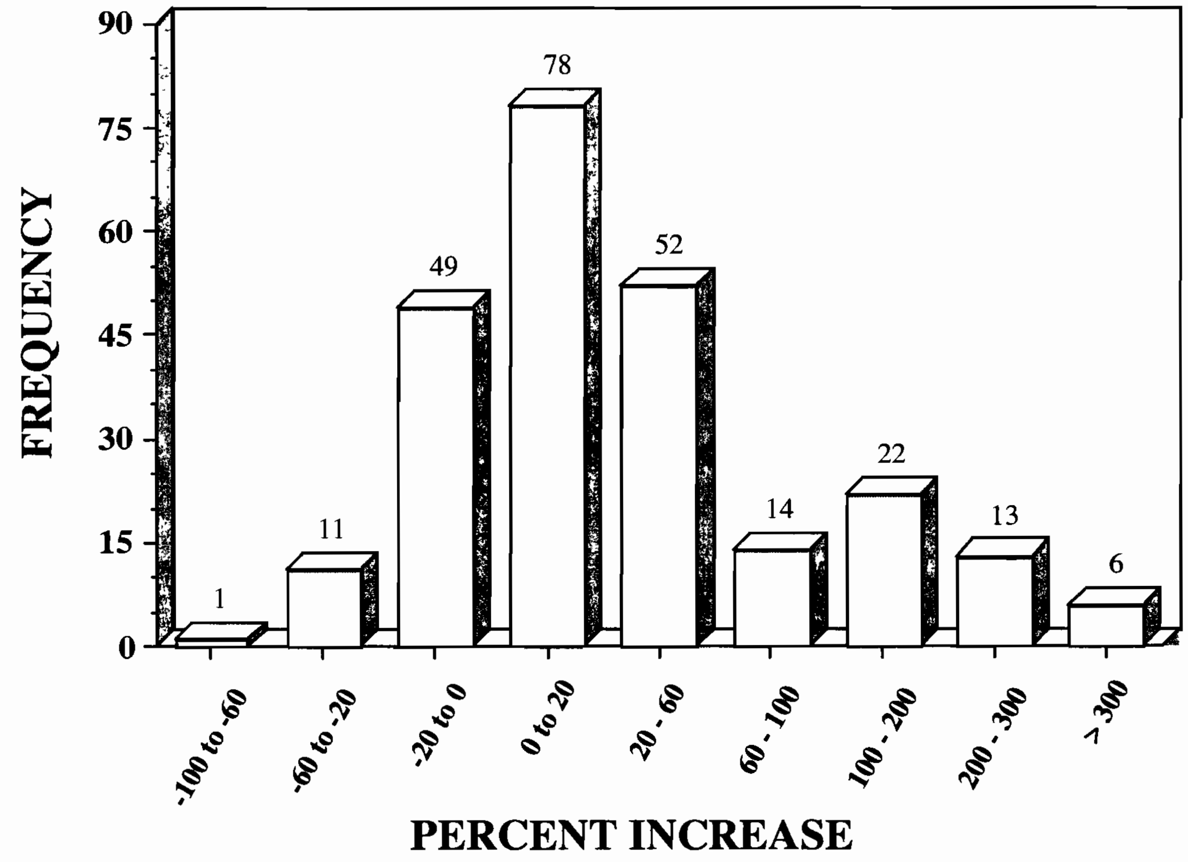

Figure 1. Histogram of frequency of increases (the number above the bars indicates the number of cases) in above-ground (shoot) plant biomass due to earthworms. Data from the Macrofauna and other available experiments performed in the tropics (246 data points).

## RESULTS AND DISCUSSION

### Identification of major factors

A total of 246 data points, means of specific treatments resulting from 28 different experiments were obtained for total above-ground (shoot) plant parts. In contrast, fewer data were available on grain production as well as root or total plant biomass (Table 3). The overall % increase due to earthworms was higher for total shoot biomass (56.7%) than for grain alone (35.8%). However, due to the high variability of the results (voir Annexe 1), both effects were significant only at P<0.08. Similarly, the high increases observed in root and total plant biomass production were not significantly different from the no-worm controls (Table 3).

The percentages of instances in which shoot and grain production increased in response to earthworm inoculation were 75.2 and 71.6%, respectively (Table 3). In the frequency histogram of the results of shoot biomass (Figure 1), about one half of the results fell within -20% to +20%, where earthworm effects are not so important (and rarely significant). The other half of results fell within a range where earthworm effects became increasingly important, i.e., over +20% or under -20%. Of these, most were positive effects, contributing 43% of the total, only 5% being negative. These results show that the effect of earthworms on above-ground production is generally positive, and in many cases may be highly so, but also that it may be near to neutral (no effect, or unimportant, both positive and negative) in a large number of cases as well. An important finding was that above-ground plant biomass is rarely greatly reduced by earthworms, such phenomena occurring only under specific circumstances (explained later in the text). Root production, on the other hand, was skewed partially to the negative, neutral and unimportant increase values. Over 40% of the results were negative (reduction in root biomass), and 60% had increases of 20% or less.

Table 3. Summary of overall percentage increases in biomass of different plant parts with standard error of the mean (s.e.) and p-value of the increase due to earthworm presence. In addition, the frequency of biomass increase or decrease is shown using all available data (number of experimental results used shown under "n"). Values with different letters within a same column indicate significant differences at p<0.05. s.e.=standard error of the mean

| Plant part | n | Overall % increase | s.e. | P-value | Increases (%) | Decreases (%) |
|---|---|---|---|---|---|---|
| Shoot | 246 | 56.7b | 9.31 | 0.07 | 75.2 | 24.8 |
| Grain | 88 | 35.8b | 8.88 | 0.08 | 71.6 | 28.4 |
| Root | 115 | 66.1a | 21.8 | 0.83 | 59.1 | 40.9 |
| Total | 116 | 62.8b | 18.8 | 0.42 | 74.6 | 25.4 |

Factors that control these responses, and the variability of earthworm effects were explored using principal component analysis of the shoot results including 221 data points. The analysis showed that the % increase due to earthworms was positively correlated with residue applications and sand content, and inversely related to clay and C contents of the soil (Table 4). However, correlation coeficients of residues and sand with % increase were low (0.42 and 0.11, respectively). Earthworm biomass applied had no particular relationship to shoot biomass increase. The first principal component (FI) of the analysis corresponded mostly to soil factors (texture and C content) and accounted for 43.9% of the explained variance, while the second component (FII) was related to OM (organic matter) applications and % increase accounting for 18% of the variance. A similar analysis was performed with 89 data points on grain production, and yielded different results: few variables were closely correlated to % increase, the most related being earthworm biomass applied (correlation coeficient=0.17) and biomass recovered (cc=0.20) at the end of the experiment. These analyses appear to point to the important role of earthworm biomass, residue applications and the soil's %C and texture in governing the role of earthworms in plant production. These were further explored using ANOVA's (below).

Table 4. Correlation coefficients between the different factors and the shoot production increase and earthworm biomass applied, resulting from the Principal Component Analysis (PCA) using a total of 221 data points on shoot biomass % increase, earthworm biomass and quantity of residues applied, and the soil's texture, %C and pH.

| Factors | Shoot % increase | Earthworm mass applied |
|---|---|---|
| Residues | 0.42 | 0.10 |
| % Increase | - | 0.20 |
| Mass Applied | 0.02 | - |
| Sand | 0.11 | -0.18 |
| Silt | 0.04 | 0.05 |
| Clay | -0.24 | 0.24 |
| % C | -0.21 | 0.14 |
| pH | 0.01 | 0.14 |

To further understand the differences in the results obtained, the soils of all the experiments were separated into three distinct classes according to texture, OM content and pH and the % increase due to earthworm activities calculated for the different plant parts in each of the soil classes. Sandy soils had >65% sand and <10% clay, clayey soils had >30% clay, and intermediate soils grouped all the other textures represented. C-poor soils had <1.5%C, C-intermediate soils 1.5<%C<3 and C-rich soils >3%C. Strongly acid soils had pH<5.6, moderately acid soils 5.6<pH<7.0 and alkaline soils pH>7.0. The results, presented in Figure 2 and Table 5 show significant differences in earthworm effects depending on the plant part as well as soil status. The increase of the different plant parts was higher in C-poor and intermediate than C-rich soils, and in sandy than in loamy or clayey soils. Regarding pH, % increase was higher in moderately acid and strongly acid than alkaline soils. Earthworm effects therefore seem to be particularly enhanced in sandy soils, with less than 10% clay, in strongly to moderately acid soils with pH <5.6 up to 7, and in poor-C status soils, with <1.5%C.

Several separate analyses confirm the above observations. For example in Yurimaguas, when no residues were applied, the average increase in grain production due to *P. corethrurus* was +46%, but when crop residues (additional C inputs) were applied it was reduced to +21%; when both residues and green manure were added, it was even lower, at +15% (Pashanasi *et al.*, 1996). When all available data for pasture grass species were analysed separately, average shoot and root biomass increase due to earthworms was calculated to be 72% in C-poor sandy soils, while in C-rich (clay) soils it was 24%, although the production gain due to earthworms was similar (1-1.2 Mg ha$^{-1}$). Root biomass change in the same soils was +50.5% (C-poor) and -11.2% (C-rich), respectively, indicating that in C-rich soils, earthworms tended to have a slight negative effect on roots. When all rice grain biomass data were combined, the increase was found to be higher in sandy (86.8%) than in loamy (30.7%) soils, even though (as for the pastures) average production increase in both soils was similar, approximately 0.2-0.3 Mg ha$^{-1}$ higher in earthworm treatments. Although both the pastures and the rice had different earthworm species and biomasses applied, and the different responses may be due to factors other than the soils involved, these results highlight the importance of soil factors on the effect of earthworms on plant biomass. Several reasons may account for these phenomena. First, soil nutrient reserves in no residue treatments and in C-poor and sandy soils are lower than in the other treatments, where the earthworm effects may be diluted by nutrients in residue inputs. Secondly, earthworms such as *P. corethrurus* are able to exploit highly stable organic reserves in poor soils with the help of microorganisms (Lavelle and Gilot, 1994; Barois and Lavelle, 1986), thus liberating and cycling nutrients that would otherwise be tied up and unavailable to plants.

Table 5. Percent increase in biomass of different plant parts due to earthworms depending on the % Carbon, texture and pH of the soil utilized. Values with different letters within a same column indicate significant differences at $p < 0.05$.

| | Plant part | | |
|---|---|---|---|
| | Shoot | Grain | Root |
| Soil Staus | | Increase (%) | |
| C-Poor | 60.5a | 29.9a | 22.6b |
| C-Intermediate | 25.5b | 47.2a | 48.9a |
| C-Rich | 19.9b | 7.7a | -14.1c |
| Sandy | 70.0a | 53.2a | 33.4b |
| Loamy | 23.3b | 24.4a | 24.1b |
| Clay | 16.2b | 29.0a | 11.7bc |
| Strongly Acid | 24.9b | 38.3a | 35.9b |
| Moderately Acid | 67.5a | (22.4)a[1] | 28.6b |
| Basic | 30.9b | 33.8a | 15.3bc |

1. n=1

## Species-specific responses

### *Plant species*

The combined effect of all earthworm species together on the shoot biomass of each plant species in both field and pot trials is shown in Figure 3. Despite several large increases in biomass, only a few plants showed significant earthworm treatment effects, due to the high variability between different experiments. The lack of significance at this level of analysis, therefore does not imply that earthworm effect on biomass were not significant at the individual experiment level (in fact this was very often the case, particularly in pot experiments). Rather, it shows that combining all the mean plant biomass yields (in Mg ha$^{-1}$) from each trial with the same species resulted in no significant differences between biomass of treatments with and without earthworms.

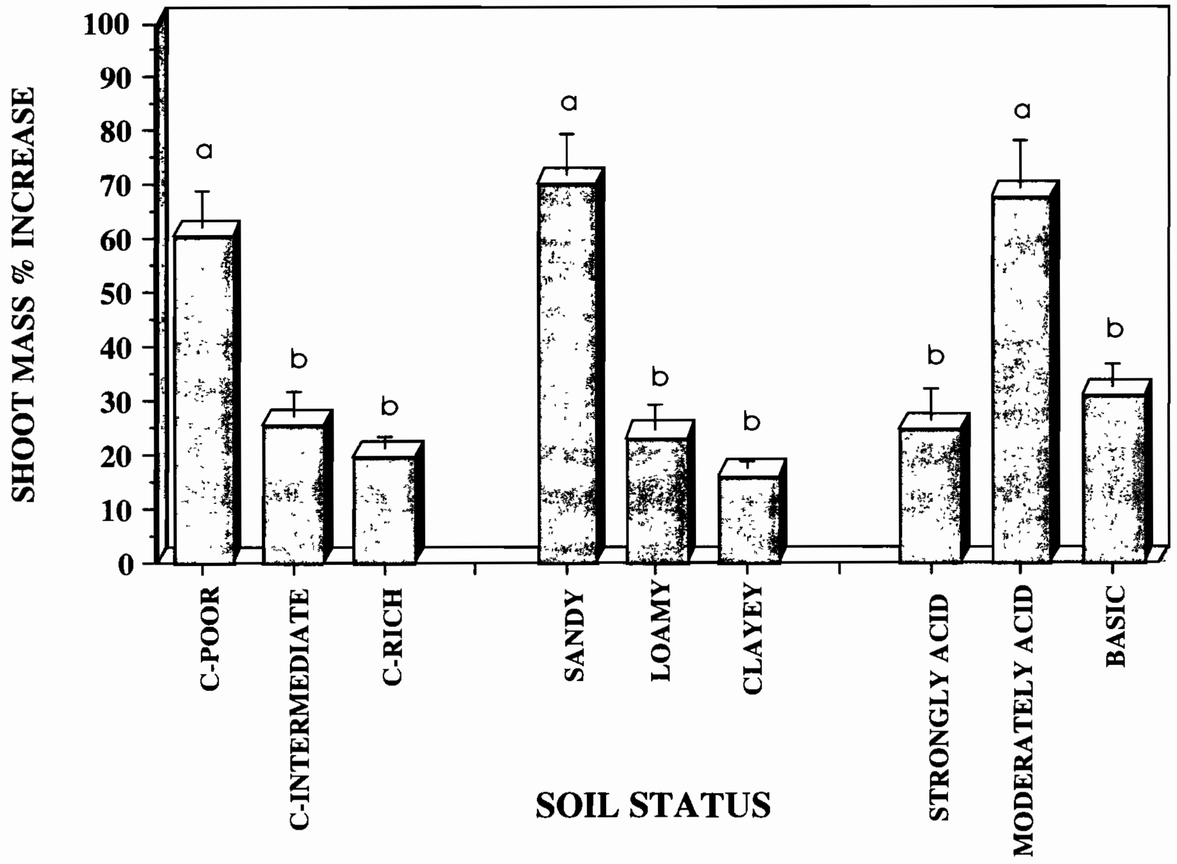

Figure 2. Average % increase (+ s.e. bars) in above ground (shoot) production due to earthworm activities in relation to soil texture, richness (% C), and pH, taken from a total of 221 data points. Poor soils had <1.5%C, rich soils, >3%C, and intermediate soils, 1.5<%C<3; sandy soils had >65% sand and <10% clay, clayey soils had >30% clay, and intermediate soils, all other textures represented; stongly acid soils had pH<5.6, basic soils, pH>7.0, moderately acid soils, 5.6<pH<7.0. Bars with different letters indicate significant differences at P< 0.05.

The plants most positively affected by earthworm activity were the trees *Bixa orellana* (760.7%), *Eugenia stipitata* (117%), tea (162%) and *Mimosa scabrella* (53.7%), and the pasture grass *Panicum maximum* (103%), the production increase being equivalent to 1.7 Mg ha$^{-1}$ (in a single cut) for the latter plant. Interestingly, these are all perennial plants. Little work other than the studies mentioned here has dealt with the effect of earthworms on perennials in the tropics, and more work is warranted. Shoot biomasses of annual crops were less affected, the highest increases being those found for common beans and rice (47.9 and 35%, respectively, though the effects were not statistically significant). In Australia and Brazil, significant increases (15.6 and 11.5%, respectively) were observed at the field scale for four pasture grasses and wheat, showing production gains of approximately 0.8 and 0.4 Mg ha$^{-1}$, for each trial, respectively, due to earthworms.

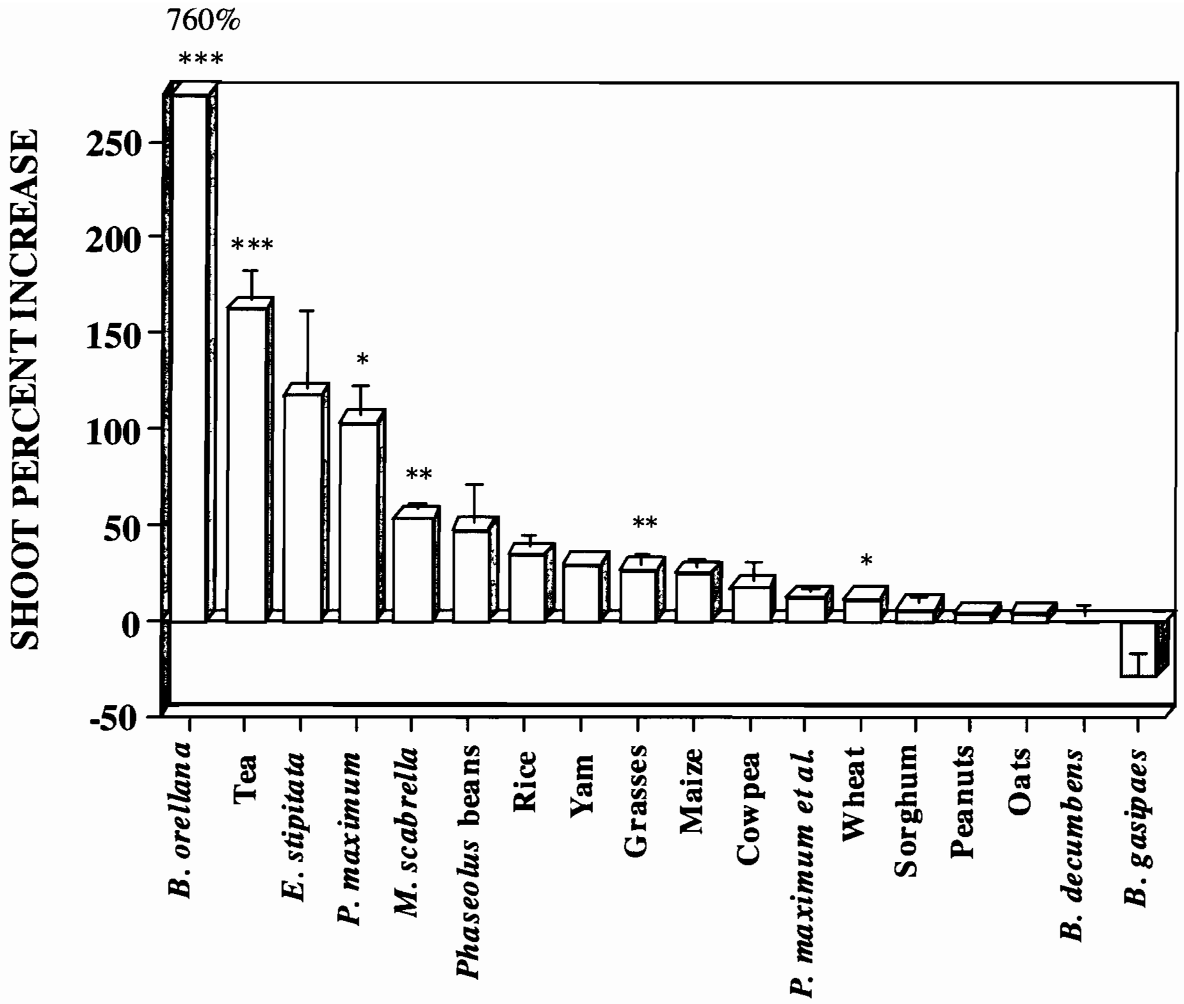

Figure 3. Percentage increase (mean + s.e. bars) due to all earthworm species combined, of above-ground biomass of 17 plant species (from a total of 246 data points). Statistical significance of the F test comparing the means of earthworm and non inoculated treatments are shown as follows: *** $P<0.001$, ** $P<0.01$, * $P<0.05$. (Note: Grass species tested were *Digitaria didactyla* and *Cynodon*, *Paspalum* and *Setaria* spp. The species harvested with *P. maximum* were *Chloris gayana* and *Cenchrus ciliaris*; *Brachiaria* species used was *B. decumbens*).

Only the palm tree *B. gasipaes* responded negatively to earthworm activity in the nursery bags, due to its coarse root system being perhaps unable to take advantage of worm structures which increased soil compaction and reduced water infiltration. Similar growth reductions were encountered for crops such as oats, maize and rice in other idividual experiments (Blakemore, 1994; Gilot, 1994; Pashanasi *et al.*, unp. data), although the reasons for these decreases were not well explained. The occurrence of and mechanisms by which earthworm activity leads to decreased plant production are poorly understood and need futher research.

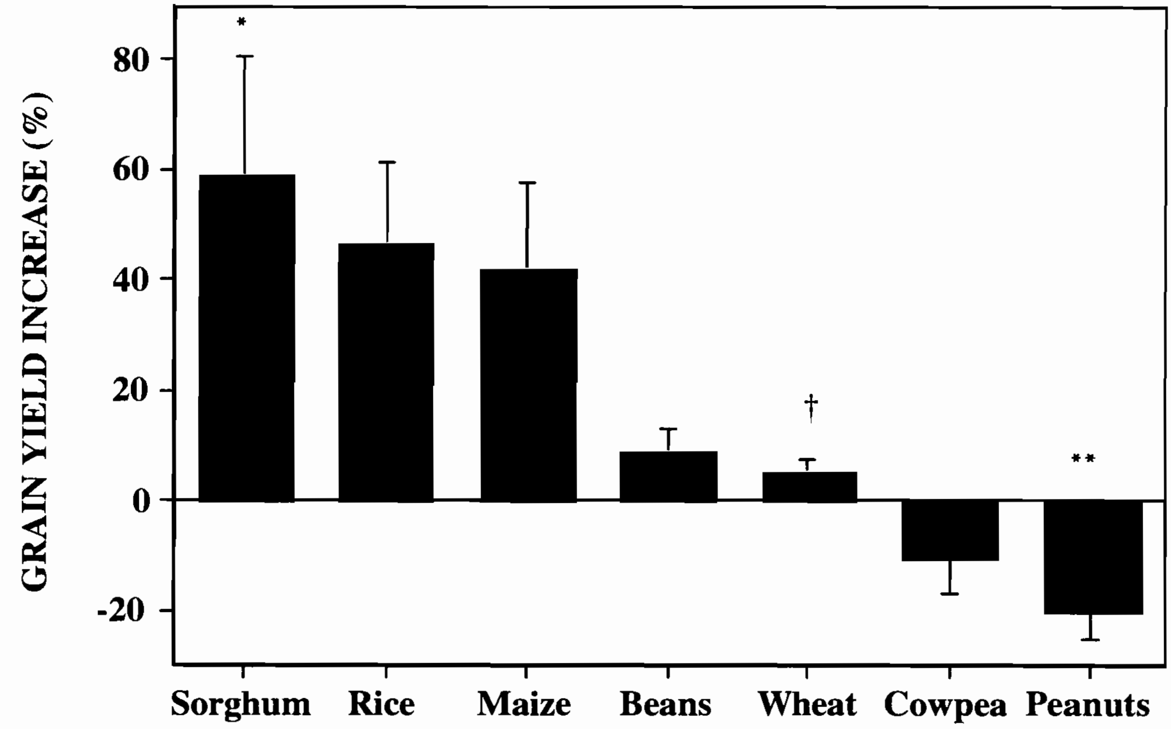

Figure 4. Average percentage increase (+ s.e. bars) in grain biomass of seven annual crops due to earthworms (from a total of 89 data points). Statistical significance, when applicable, shown above the column († $P< 0.1$); significance values as in Fig. 3.

Grain production was increased by earthworms in five of the seven annual crops tested (Figure 4), although a significant increase was only observed for sorghum (59%, equivalent to a 1.44 Mg ha$^{-1}$ production gain). Grain biomass increases for rice and maize were over 42%, but the combined differences over all the studies (about 0.2 Mg ha$^{-1}$ more grains with earthworms in both crops) were not significant. Yields of leguminous plants were little affected (beans), or negatively affected by earthworm activities (peanuts and cowpea), while graminaeous grain crops were always positively affected. Reasons for this may be different (generally higher) nutrient demands and root architecture, and the lack of symbiotic $N_2$-fixing microorganisms in the grass crops, i.e., greater N independence in the legumes. Further mechanisms may involve symbiotic or other organisms (e.g., mycorrhizae, protozoa, nematodes, parasitic fungi) affected directly or indirectly by earthworm activities (see later discussion).

### *Effect of earthworm species*

Increases in shoot biomass due to the presence of different earthworm species varied substantially (Table 6). Intraspecific variation in the results was also high, depending on the crop, soil type and experimental conditions; only in one case (*P. corethrurus* +*Notoscolex* sp., *Metaphire* sp., *Megascolex* sp. and *Amynthas* sp. additions to tea in India) were significant earthworm effects detected. This does not mean however, that a given species of earthworm did not increase shoot production in individual experiments. In fact this was often the case, so the potential of each species for introduction and/or management in tropical and subtropical soils was assessed based on: 1) earthworm survival rates and 2) ability to increase plant growth above a certain percentage in a given number of cases. High potential was ascribed to a species when the population biomass was maintained at >98% of the biomass applied, and when the species promoted shoot yield increases (>20%) in over 80% of the cases. Moderate potential was applied when the species population was maintained over 64%, yet promoted plant growth on average less than 20% or >20% but in less than 60% of the cases. Low potential was given when small or negative effects on biomass were obtained, or when survival of the introduced species was poor.

Earthworm addition treatments that most increased biomass, and that also had a high potential for use in tropical soils were those including the species *P. corethrurus*. Under tea cropping in India, this species together with four other species resulted in an increase of 217% in green leaf production (Table 6). When applied alone with a range of different plants in five countries, the average increase was 82%. The other six species which also showed high potential for management were *Chuniodrilus zielae* and *Stuhlmannia porifera* (69% increase) and *Millsonia anomala* (56%) at Lamto, *Dichogaster affinis* and *Dichogaster saliens* (24%) in Australia, and *Drawida willsii* (24%) in India. Treatments with *Heteroporodrilus bongeen* and the combination of *Drawida barwelli* and *Amynthas minimus* also in Australia, led to important biomass increases, but the earthworm populations added were not sustainable; these species thus showed low potential for management. Many species that had medium potentials, including five native and locally distributed species (*M. anomala* +*C. zielae* and *S. porifera*, *Diplotrema sp. nov. 1* and *Diplotrema sp. nov.* 2) and eight widespread exotic peregrine species, could easily be of high value if managed properly, for example with appropriate plant species and soil types.

Interestingly, in several cases, the addition of more than one species of earthworms increased plant production more than the addition of each species separately (e.g., *A. trapezoides*+*E. rosea*, *D. barwelli*+*A. minimus*, *P. corethrurus* +others). Thus, species diversity within the soil should be taken into account, and promoted if possible, to achieve effective plant production enhancement. It is likely that, by producing a variety of structures and using different ecological niches within the soil, combinations of species are more efficient at stimulating both nutrient cycling and the conservation of a good soil sturcture (Blanchart *et al.*, 1999; Villenave *et al.*, 1999).

Average survival rates of earthworms inoculated into both pot and field experiments varied widely, depending on their ability to adapt to particular soil conditions. In the field, most of the species displayed poor survival rates, the only species surviving well and reproducing being *D. willsii* (7-fold increase), *Amynthas* spp. (109% of inital mass added), and *P. corethrurus* (107% of initial). *M. anomala* biomass decreased to 58% of that applied. Poor earthworm survival was due to harsh climatic conditions (drought at Narayen and Samford), competition with other species (La Mancha), or the inability of the soils to support the biomasses introduced (Martinique, Ivory Coast and India). In the pot experiments, under more controlled conditions, survival rates were much higher and 15 species maintained their biomass above or close to 100% of the initial mass added, often reproducing successfully (Annex 1). In particular, *P. corethrurus*, *Dichogaster affinis*, and *D. saliens* displayed large increases in biomass, from four to 6-fold on average. Finding and maintaining the proper soil conditions (e.g., texture, C content, residues, pH, temperature, moisture) for each earthworm species is therefore essential if they are to be introduced, especially in field conditions. Earthworm biomass additions (properly chosen and tested previously for adaptibility) should not exceed that which is sustainable for the particular soil or plant conditions in question. Probably the most important practice is to ensure adequate food (C-sources) availability for the earthworms (Lavelle, 1997; Villenave *et al.*, 1999). Residues have been added with some success in Peru, Mexico and India, which in addition to helping maintain earthworm biomass, also can increase crop yields (Giri, 1995; Pashanasi *et al.*, 1994; 1996; Patrón *et al.*, unp. data).

**Effect on different parts of the plant**

Using all data available for each plant part, no significant earthworm effects between the parts were found (Table 3). However, if the values for % increase of the tree *B. orellana* were removed from the data set (on the basis of being outliers from the rest of the data), the overall increase in shoot, root and total plant production became 42.1%, 28.2% and 29.4%, respectively, while grain production increase remained unchanged (35.8%). The difference between % increase of shoot and root biomass now becomes significant at $P< 0.09$. Therefore, considering all the other remaining crops, the average increase was higher for shoot than root biomass, as observed in several of the individual studies (Spain *et al.*, 1992; Derouard *et al.*, 1997, Pashanasi *et al.*, 1996). For example, when *P. corethrurus* was introduced into an Ultisol in Yurimaguas, grain and stover production over 6 cropping cycles averaged 46 and 34% higher, respectively, than where worms were not introduced, the equivalent of a production gain in harvested biomass of 2.1 and 2.9 Mg $ha^{-1}$. On the other hand, root biomass harvested at the end of each cropping cycle averaged only 23% higher in the presence of earthworms (equivalent to +0.3 Mg $ha^{-1}$). Although the harvesting procedure did not include intermediate harvests to estimate root growth over the cropping cycle, and no estimates of root turnover were made, this phenomenon may still pose potential hazards to OM sustainability within the soil, particularly if the grain and stover are removed from the system and root biomass is the main OM input remaining. Over time, this could lead to a decrease in OM inputs into the soil due to earthworm activities, resulting in an overall loss of organic-C as well as other nutrients found in plant matter, such as N and P, from the soil (Charpentier, 1996; Gilot 1994; Villenave *et al.*, 1999). However, if a reasonable portion of the stover is maintained, this potential loss could be arrested.

Table 7 summarizes the results on the % increase of different plant parts due to earthworm activity (irrespective of earthworm species), as well as the proportion of positive results (increases) obtained for 12 plants. The data clearly demonstrate that for plants such as maize, beans, *P. maximum* and two other grasses, cowpea and peanuts, the above ground parts received a greater stimulation than roots due to earthworm activities. Since the harvesting of the first four plants involves the removal of above ground parts, and the latter plant is below ground harvested (peanuts), special attention must be taken to manage the soil organic matter (SOM) pool, to prevent potential soil C-losses induced by earthworm activities. In contrast, root biomass of rice and all four tree plants (*B. gasipaes*, *B. orellana*, tea and *E. stipitata*) was slightly stimulated by earthworm activities. The reason for the stimulation of rice root biomass is not known and should be further investigated. The other four plants are perennial dicotyledenous species, with different life cycles, root growth and nutrient requirements than the previously mentioned crops, factors which may have affected the ability of the earthworms (*P. corethrurus*, primarily) to stimulate root growth. As mentioned earlier, *P. corethrurus* does not favor overall growth of *B. gasipaes*. Both shoot and root biomass of this plant were reduced by presence of the earthworm.

The enhancement of shoot/root ratios by earthworm activity in several of the crops mentioned above supports the hypothesis that plants invest more energy in above-ground (especially fruit or grain) growth because plants are healthier and able to absorb more essential elements and water from soils colonized by earthworms. Spain *et al.* (1992) found higher N and P uptake by *P. maximum* shoots and roots in the presence of several earthworm species, and Gilot-Villenave *et al.* (1996) found that *M. anomala* activities enhanced $^{15}N$ uptake from decomposing plant residues incorporated into the soil. On the other hand, at Yurimaguas no differences in nutrient uptake by the different crops were found over six cropping cycles (Pashanasi *et al.*, 1996). Nevertheless, plant tissue analyses should always be performed to reveal the stocks of nutrients taken up by the plants and to assess the potential need for fertilization or OM addition to maintain soil fertility. Such additions should be related to the increased uptake and export of nutrients from the soil system due to earthworm activies, especially N and P (Blakemore, 1994; Charpentier, 1996) harvested in the above-ground biomass (grain and/or shoot).

### Mechanisms involved

Earthworm activities modify many soil properties which affect plant growth rates and ultimately crop yields. These range from large-scale effects such as acceleration of soil profile formation (e.g., mollic and vermic A horizons) to enhancement of soil microbial activities (e.g., respiration, production of plant-growth regulators, antibiotics) at the microscopic level (Brown, 1995). A major problem, however, has been determining which soil, plant or earthworm characteristics are the most important mechanism for the observed effects in a given situtation. The drilosphere, i.e., the soil fraction modified by earthworm activities (Lavelle, 1988), including casts, burrow systems and gut processes, is generally very different from soil unmodified by the worms (Brown, 1995), and its extent and characteristics (e.g., fertility, physical properties) depend on earthworm species and ecological category together with soil and climatic conditions (Barois *et al.*, 1999; Blanchart *et al.*, 1999).

The factors and processes of the drilosphere and the ways in which they influence plant growth (especially roots) are summarized in Figure 5. The changes important to soil fertility and plant production begin when the earthworm ingests the soil, selectively choosing particular particle sizes or regions rich in OM or with high microbial activity, and these are subjected to various transformations as they pass through the earthworm gut. These processes (ingestion and gut passage) determine the richness of the egested castings, which are characterized by higher available nutrient contents and microorganisms populations (Barois *et al.*, 1999). Beneficial or antagonistic organisms as well as plant seeds may also be dispersed throughout the soil by earthworm adctivities. The combination of aggregates produced (castings) within the soiil and the burrows dug through the profile detemine the physical structure of the soil, influencing its capacity to hold air and $H_2O$, and to permit adequate root growth. The sum of these phenomena thus determine the overall effect of a worm community on potential plant response, depending on the worm species (and ecological category) composition and the particular requirements of the plant community.

Table 6. Average plant shoot biomass increase due to earthworm species or species combinations, mean earthworm survival rates, percentage of positive results (increases) obtained from the total number of observations (n), crops most positively affected and the potential of each species for management or introduction into tropical or subtropical cropping systems. Rows are arranged according to shoot % increase, in decreasing order.

| Earthworm species (ecological category) | Location | Crops most affected[1] | n$^0$ | Shoot increase (%) | P-value | Change in mass (g m$^{-2}$) | Mean survival (%) | Positive results (%) | Potential (see text) |
|---|---|---|---|---|---|---|---|---|---|
| *Pontoscolex corethrurus* + others[2] | India | Tea | 20 | 217.4 | 0.0001 | -585.3 | 9.7 | 100 | High[3] |
| *Pontoscolex corethrurus* (mesohumic endogeic) | Mexico, Ivory Coast, India, Australia, Peru, | Tea, trees, maize, rice | 69 | 81.8 | 0.45 | +22.6 | 323.4 | 65 | High |
| *Chuniodrilus zielae* + *Stuhlmania porifera* (polyhumic endogeics) | Ivory Coast | Maize, *P. maximum* | 6 | 69.1 | 0.37 | -5.6 | 123.3 | 100 | High |
| *Drawida barwelli* + *Amynthas minimus* | Australia | grasses | 2 | 63.6 | 0.298 | -23.9 | 0.32 | 100 | Low |
| *Millsonia anomala* (mesohumic endogeic) | Ivory Coast | maize, yam, *P. maximum* | 29 | 58.2 | 0.38 | -1.0 | 98.9 | 63 | High |
| Undetermined endogeics (at least 3 spp.) | Cameroon | maize | 2 | 45.2 | 0.63 | ? | n.d. | 100 | ? |
| *Heteroporodrilus bongeen* | Australia | oats | 1 | 39.6 | - | -253.3 | 0 | 100 | Low |
| *Polypheretima elongata* (mesohumic endogeic) | Mexico, Australia | beans, sorghum | 9 | 35.4 | 0.84 | +19.1 | 126.7 | 50 | Medium |
| *Aporrectodea trapezoides* + *Eisenia rosea* | Australia | grasses | 2 | 29.7 | 0.59 | -164.0 | 1.2 | 100 | Low |
| *Diplotrema sp. nov.* 1 | Australia | grasses, oats | 9 | 25.1 | 0.49 | -2.7 | 68.4 | 80 | Medium |
| *Dichogaster* spp. (polyhumic endogeics) | Australia | grasses | 10 | 24.4 | 0.25 | +46.5 | 321.4 | 70 | High |

Table 6. continuation

| Earthworm species (ecological category) | Location | Crops most affected[1] | $n^0$ | Shoot increase (%) | P-value | Change in mass (g $m^{-2}$) | Mean survival (%) | Positive results (%) | Potential (see text) |
|---|---|---|---|---|---|---|---|---|---|
| *Drawida willsi* (epianecic) | India | rice | 6 | 23.8 | 0.71 | +55.9 | 483.8 | 100 | High |
| *Eisenia rosea* mesohumic (endogeic) | Australia | oats | 4 | 22.5 | 0.57 | -134.8 | 1.2 | 75 | Low |
| *Amynthas* spp. (polyhumic endogeics) | Australia, Brazil | grasses, *M. scabrela* | 13 | 19.2 | 0.26 | -18.4 | 68.4 | 84 | Medium |
| *Millsonia anomala* + Eudrilidae[4] | Ivory Coast | maize | 3 | 13.5 | 0.70 | -6.0 | 89.4 | 100 | Medium |
| *Eudrilus eugeniae* (polyhumic endogeic) | Australia | grasses | 9 | 12.9 | 0.62 | -77.3 | 35.5 | 66 | Low |
| *Drawida barwelli* | Australia | grasses | 4 | 12.8 | 0.72 | +4.8 | 113.7 | 75 | Medium |
| *Polypheretima taprobanae* (mesohumic endogeic) | Australia | grasses | 5 | 11.2 | 0.64 | -26.9 | 80.3 | 80 | Medium |
| *Aporrectodea trapezoides* (mesohumic endogeic) | Australia | sorghum, grasses | 7 | 9.6 | 0.81 | -48.4 | 93.4 | 100 | Medium |
| *Hyperiodrilus africanus* (polyhumic endogeic) | Ivory Coast | | 4 | 6.9 | 0.97 | -46.7 | 14.5 | 50 | Low |
| *Pontoscolex corethrurus* + *Polypheretima elongata* | Mexico | maize | 12 | 5.9 | 0.89 | ? | n.d. | 80 | Low |
| *Fletcherodrilus unicus* | Australia | | 4 | 4.2 | 0.91 | -140.6 | 23.2 | 75 | Low |
| *Diplotrema sp. nov.* 2 | Australia | | 2 | 3.6 | 0.94 | +20.1 | 183.2 | 100 | Medium |
| *Metaphire californica* (epigeic?) | Australia | | 4 | 3.2 | 0.98 | -25.6 | 80 | 75 | Medium |
| *Perionyx excavatus* (epigeic) | Australia | | 1 | -1.2 | - | -12.0 | 61.5 | 0 | Low |
| *Eukerria saltensis* (polyhumic endogeic) | Australia | | 4 | -2.4 | 0.89 | +0.2 | 101.7 | 25 | Low |

Table 6. continuation

| Earthworm species (ecological category) | Location | Crops most affected[1] | n$^{0}$ | Shoot increase (%) | P-value | Change in mass (g m$^{-2}$) | Mean survival (%) | Positive results (%) | Potential (see text) |
|---|---|---|---|---|---|---|---|---|---|
| *Octochaetus beatrix* | Australia | oats | 4 | -3.5 | 0.84 | -38.1 | 40.0 | 50 | Low |
| *Ocnerodrilus occidentalis* + others[5] (polyhumic endogeics) | Australia | | 1 | -11.6 | - | -9.4 | 60.0 | 0 | Low |
| *Digaster brunneus* | Australia | | 2 | -12.2 | 0.81 | -111.6 | 0 | 0 | Low |
| *Spenceriella minor* | Australia | | 2 | -22.5 | 0.60 | -35.0 | 22.7 | 0 | Low |

n = number of observations; 1. Crops are mentioned only when increase is above 10%; 2. Other species added in low quantities were *Notoscolex* sp., *Metaphire houlleti*, *Megascolex konkanensis* and *Amynthas corticis*; 3. This is a special case, see text for explanation; 4. Other species added were *C. zielae* and *S. porifera*; 5. Other species added were *Gordiodrilus elegans* and *Dichogaster bolaui*.

Table 7. Average percentage of positive results (increases) and percent increase in shoot, root and grain biomass of 13 plant species (for which all three parts were available). Statistical significance for earthworm effects as in Figure 3.

| PLANT | n | Increase shoot (%) | % Positive | Increase root (%) | % Positive | Increase grain (%) | % Positive |
|---|---|---|---|---|---|---|---|
| Maize | 17 | 12.6b | 80 | 12.6b | 48 | 42.0a | 84 |
| Rice | 18 | 34.9* | 78 | 59.7* | 77 | 55.2 | 78 |
| Sorghum | 5 | 14.5 | 83 | - | - | 58.8* | 100 |
| *P. maximum et al.*[1] | 24 | 10.5 | 79 | -0.9 | 50 | - | - |
| *P. maximum* | 7[2] | 129.2* | 86 | 107.6* | 100 | - | - |
| Peanuts | 4 | 3.6 | 75 | -5.3 | 25 | -20.3* | 0 |
| Beans | 2 | 103.4a* | 100 | 61.4a* | 100 | 13.8b | 100 |
| Cowpea | 3 | 16.9 | 66 | -14.3 | 0 | -4.9 | 33 |
| Tea | 8[2] | 25.0* | 100 | 53.0* | 75 | - | - |
| *B. orellana* | 5 | 760.7*** | 100 | 900.2*** | 100 | - | - |
| *E. stipitata* | 5 | 117.4 | 100 | 164.3 | 80 | - | - |
| *B. gasipaes* | 5 | -28.1 | 40 | -22.0 | 20 | - | - |

1. The other two species harvested were *Chloris gayana* and *Cenchrus ciliaris*; 2. Includes only data from the potted plants.

The Macrofauna programme has contributed greatly to the understanding of many mechanisms of plant growth changes (both positive and negative) due to earthworm activities. These can be divided into three general categories, i.e., chemical, physical and biological.

**1.** Biological factors affecting earthworm-induced changes in plant biomass include:

- differential responses of specific plant parts, especially above ground portions;
- markedly different effects depending on plant and earthworm species used in combination;
- earthworm biomass (see later discussion);
- competition between earthworms and plants for water;
- extent of rhizosphere and bulk soil feeding activities;
- preference of different earthworm species for particular plant rhizospheres;
- changes in (increased or reduced) microbial biomass and priming of microbial activity in the gut and casts;
- release of enzymes by microorganisms and earthworms in the gut, leading to changes in C and nutrient status of ingested food and casts;
- increased dispersal and promotion of root infection by vesicular-arbuscular mycorrhizal (VAM) fungi (Figure 6) and ectomycorrhizal fungi, in appropriate plants;
- reduced damage from plant parasitic nematodes (Figure 7);
- increased nutrient uptake by plants;

(Boyer, 1998; Brown *et al.*, unp. data; Brussaard *et al.*, unp. data; Charpentier *et al.*, unp data; Derouard *et al.*, 1997; Gilot, 1994; Giri, 1995; Kidza, unp. data; Lattaud *et al.*, 1998; Lavelle and Gilot, 1994; Pashanasi *et al.*, 1992; 1994; 1996; Patrón *et al.*, unp. data; Ydrogo, 1994).

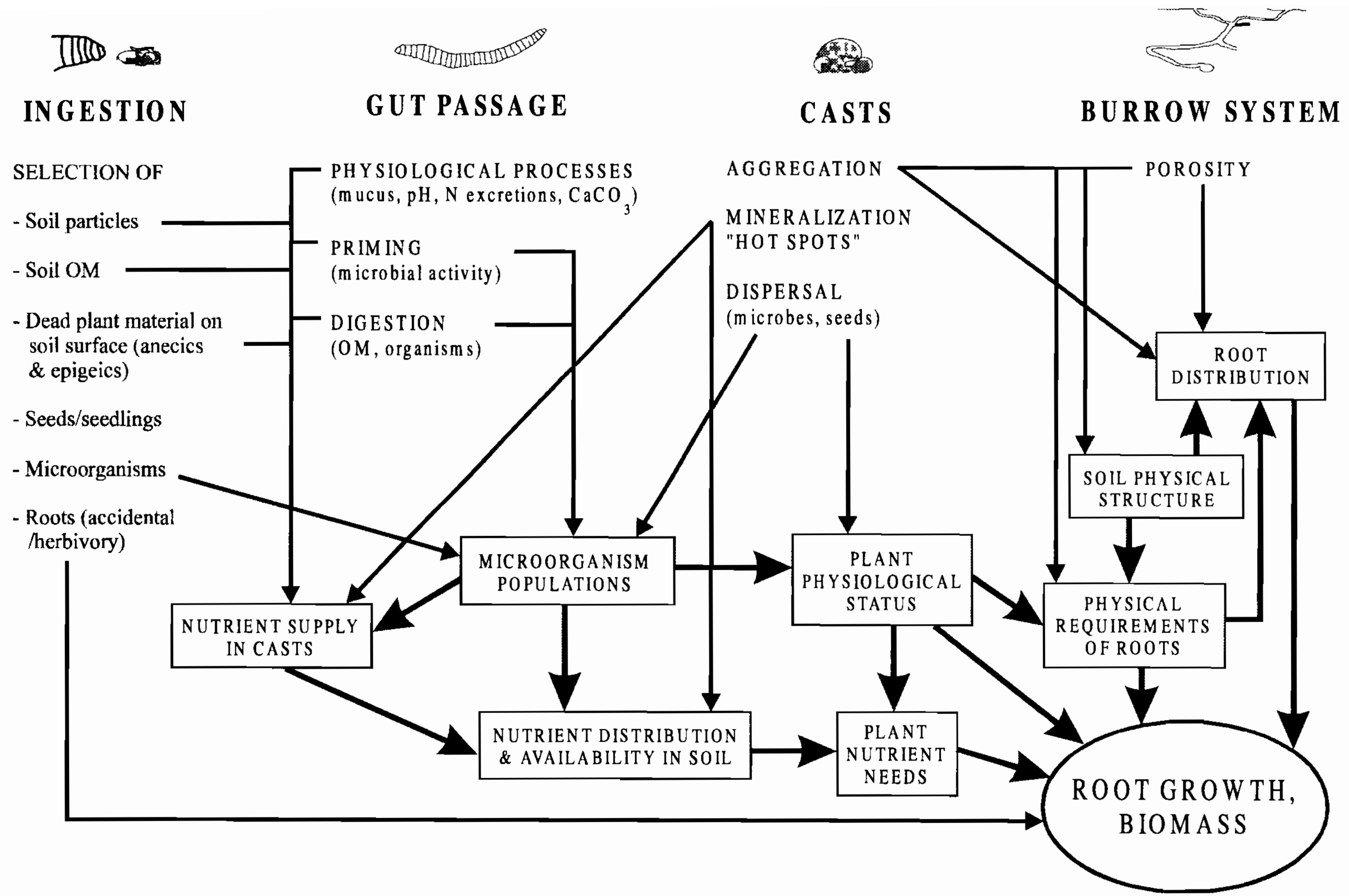

Figure 5. Mechanisms by which the drilosphere properties (earthworm casts, burrows, gut) and its associated processes influence plant growth (especially roots).

**2.** Among the chemical factors observed were increased nutrient (especially N, P, K; a few micronutrients) availabilities in casts and burrows due to microbial activation or earthworm induced changes in nutrient solubility; selection of richer soil portions by the earthworms; addition of nutrients from dead worm tissues, mucus and other excretions; and accelerated nutrient release from decomposing plant residues (Barois *et al.*, 1999; Brossard *et al.*, 1996; Brussaard *et al.*, unp. data; Gilot, 1994; Chapuis 1994; Chapuis and Brossard, 1995; Kidza, unp. data; Lavelle *et al.*, 1992; López-Hernández *et al.*, 1993; Pashanasi *et al.*, 1994; 1996).

**3.** Physical factors included amelioration of soil physical properties (limiting plant growth under certain conditions) leading to an increased proportion of water-stable macroaggregates, changes in porosity, aeration and water infiltration, an increase or decrease in bulk density and crusting, and the creation of burrows which act as preferential pathways for plant root growth (Blanchart *et al.*, 1999; Brussaard *et al.*, unp. data; Derouard *et al.*, 1997; Gilot, 1994; Giri, 1995; Kidza, unp. data; Pashanasi *et al.*, 1994; 1996; Patrón *et al.*, unp. data).

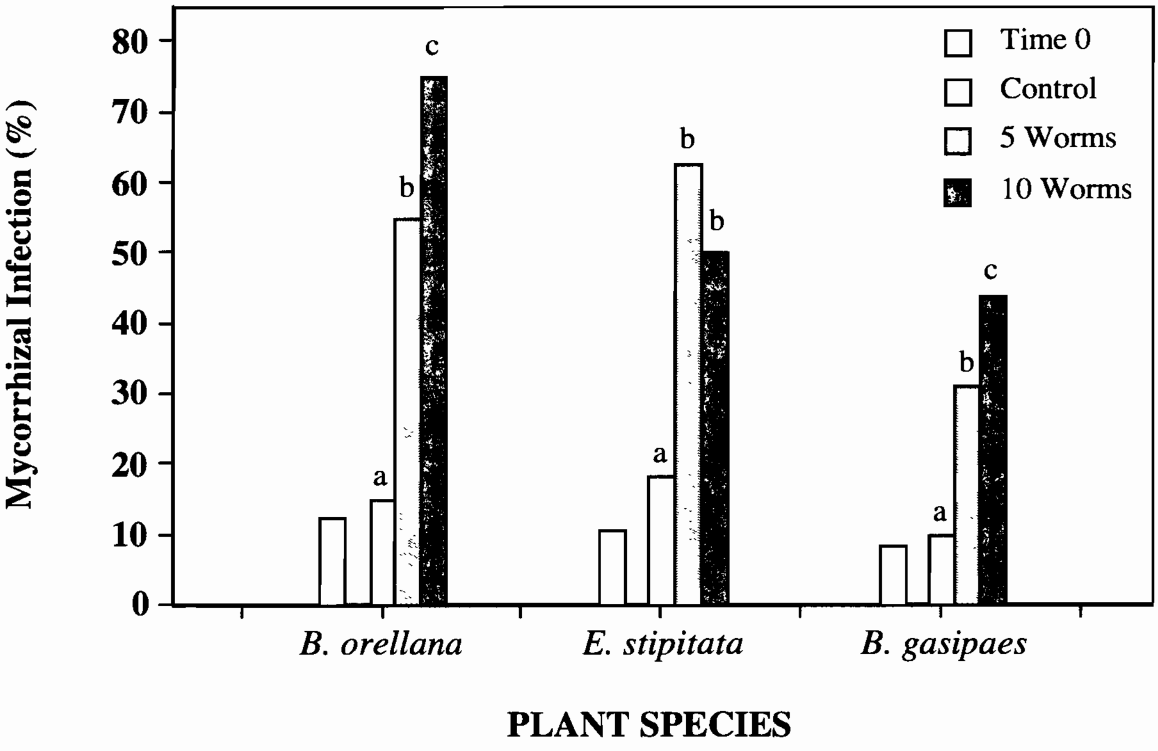

Figure 6. Mycorrhizal infection in roots after various durations of greenhouse culture of tree seedling species *Bixa orellana* (120d), *Eugenia stipitata* (240d) and *Bactris gasipaes* (210d) in the absence of earthworms (control), or in presence of five (0.375g) and ten (0.75g) *P. corethrurus* in Yurimaguas, Peru (Ydrogo, 1994). Bars with different letters indicate significant differences at P< 0.05.

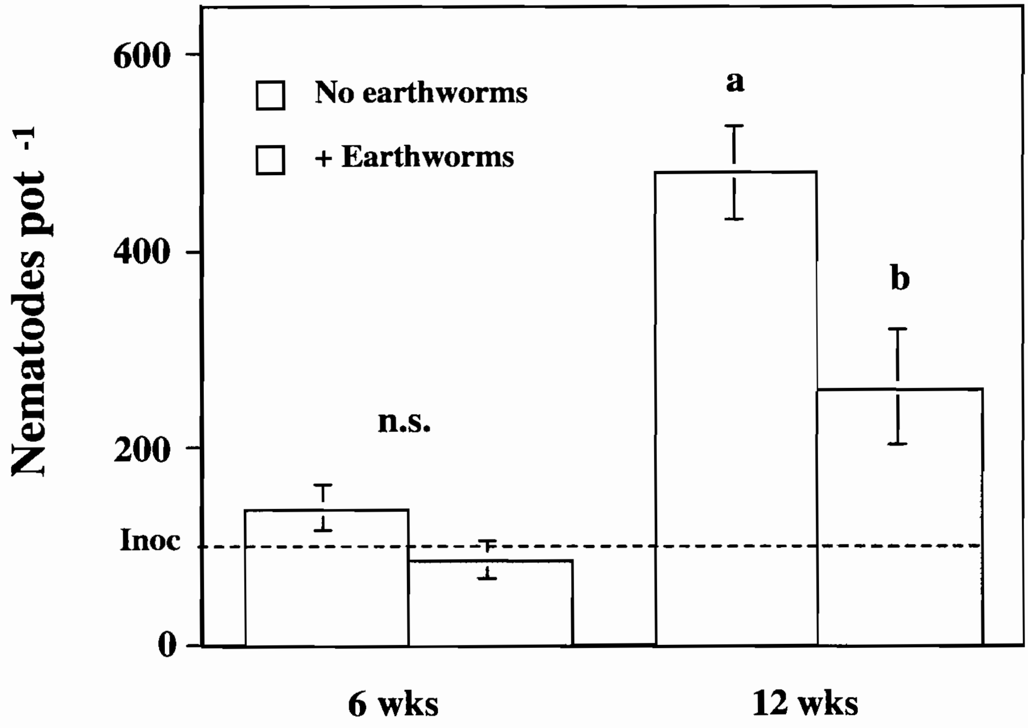

Figure 7. Number of plant parasitic nematodes (*Pratylenchus zeae*) recovered pot$^{-1}$ (means ± st. dev.) after one and two generation times (six and 12 weeks) in small pots planted with rice with and without *P. corethrurus* (Boyer, 1998). n.s.= differences not significant; different letters indicate significant differences at $P < 0.01$. Initial inoculum density = 100 nematodes pot$^{-1}$.

Other mechanisms have also been associated with plant growth enhancement due to earthworm activity (see Figure 5). These have been shown mostly for lumbricid earthworm species and are primarily biological or biochemical in nature and include:

**1.** Dispersal and enhancement of plant growth promoting rhizobacteria (PGPR) such as *Enterobacter cloacae*, *Acinetobacter*, *Azotobacter*, *Azospirillum*, *Pseudomonas* and *Bacillus* spp. in the casts and drilosphere (Bhat *et al.*, 1960; Bhatnagar, 1975; Hand and Hayes, 1983; Kozlovskaya and Zaguralskaya 1966; Kozlovskaya and Zdhannikhova, 1961; Loquet *et al.*, 1977; Pederson and Hendriksen, 1993), and the promotion of plant growth regulator (auxins, cytokinins, giberellins and ethylene) production by microorganisms in the casts (Krishnamoorthy and Vajranabhaiah, 1986; Nardi *et al.*, 1994; Simek and Pizl, 1989; Tomati *et al.*, 1988; Tomati and Galli, 1995), which may dramatically alter plant growth and architecture;

**2.** Stimulation of enzyme production (e.g., phosphatases, nitrogenase, urease) by cast- and burrow-inhabiting microorganisms (Loquet *et al.*, 1977; Mulongoy and Bedoret, 1989; Satchell and Martin, 1984; Simek and Pizl, 1989; Syers and Springett, 1984; Zou, 1992);

**3.** Spread and enhancement of *Rhizobia* and $N_2$ fixation in leguminous plants (Doube *et al.*, 1994a; Rouelle, 1983; Stephens *et al.*, 1994f; Thompson *et al.*, 1993) and spread of actinomycetes such as *Frankia spp.* in earthworm casts resulting in increased infection (nodule formation) in susceptible plants (such as *Casuarina equisetifolia*; Reddell and Spain, 1991b), as well as the addition of N to the drilosphere through associative (non-symbiotic) $N_2$ fixation by microorganisms such as *Chlostridia* spp. in the earthworm gut (Barois *et al.*, 1987; Striganova *et al.*, 1989);

**4.** Dispersal of biocontrol agents (e.g., *Pseudomonas corrugata*) which reduce plant disease (Doube *et al.*, 1994b; Stephens *et al.*, 1993a), or direct reduction of plant root diseases such as the fungi *Rhizoctonia solani* (the causative agent of "*Rhizoctonia* bare patch" disease) and *Gaeumannomyces graminis* var. tritici (the causal agent of take-all disease) by *Aporrectodea* spp. (Stephens and Davoren, 1995; Stephens *et al.*, 1994b-e, g), and the reduction in infectivity of cowpea and tobacco mosaic viruses by earthworm (*Eisenia fetida*) enzyme extracts (Amaravadi *et al.*, 1990);

**5.** Ingestion and/or burial of leaves, causing reduction in populations of surface litter-inhabiting pathogenic fungi (Niklas and Kennel, 1981; Kennel, 1990), including *Venturia inaequalis* (causal agent of apple scab) by litter-feeding earthworm species such as *Lumbricus terrestris*;

**6.** Seed consumption and/or burial, leading to the preferential germination of some plant species' seeds (Grant, 1983; Piearce *et al.*, 1994, Shumway and Koide, 1994; Thompson *et al.*, 1993; 1994; van der Reest and Rogaar, 1988);

**7.** Dead or live root consumption (Carpenter, 1985) and feeding on germinating plant seedlings (Shumway and Koide, 1994) by lumbricid earthworms;

**8.** An increase in nitrate reductase activity and protein synthesis leading to a more efficient photosynthesis by plants (Galli *et al.*, 1990; Tomati *et al.*, 1990; 1996; Tomati and Galli, 1995).

Not all the fore-mentioned mechanisms act on the soil and the plant at one time. These mechanisms are complex and dependent on the crop/soil/worm combinations. Thus it is unlikely that the same suite of mechanisms will be applicable in two different locations, even for the same crop and earthworm species. Earthworms modify soil properties at large and small spatiotemporal scales. Over the short term, a cropping cycle for example, modification of soil in or near the rhizosphere is likely to lead to significant earthworm effects on plant growth. If nutrients or physical conditions are limiting plant growth to some extent and earthworms help reduce these limiting factors, plants will respond positively. Thus, at the rhizosphere level quantification of earthworm activity at both the physical (spatial) and biochemical scales is essential if we are to assess what impact earthworms have on crop root growth and hence on above-ground yields.

Several approaches have been made to the question of spatial synchrony of earthworm activities with plant rhizospheres, and some progress has been made in this area. $^{13}C$ analysis of *P. corethrurus* (a polyhumic endogeic which lives primarily in the top 10 cm of the soil - essentially the zone of highest root density) tissue in sugar-cane plantations (Spain *et al.*, 1990) and under maize (Brown, 2000a; chap. 7), suggests that this earthworm feeds at least partly on C derived from the rhizosphere of these crops. On the other hand, under beans this same species and *P. elongata* do not seem to concentrate in the rhizosphere of benefit from their exudates (Brown, 2000a; chap. 7). Furthermore, under maize, *P. elongata* also did not show preferential consumption and assimilation (using $^{15}N$ as a tracer) of root-derived materials (Brown, 2000a; chap. 7). Carpenter (1985) observed lumbricid earthworms feeding on living roots in a rhizotron, in the only known case of direct visual observation of this phenomenon. Doube and Brown (1998) show photographic evidence of wheat rhizosphere feeding by *Aporrectodea trapezoides*. In a field study over 1 yr using $^{32}P$ as a tracer, Baylis *et al.* (1986) found that three species of lumbricid earthworms actively fed on clover roots, while two other species did not. Another species, *L. terrestris* was shown to feed on both rhizosphere microorganisms and ryegrass roots, using $^{14}C$ as a tracer (Cortez and Bouché, 1992), and Shumway and Koide (1994) discovered partially-consumed plant seedlings in the bottom of *L. terrestris* burrows.

The possibility of root herbivory has been associated with the analysis of gut contents for ingested root fragments. This type of analysis does not prove active herbivory by the earthworms since they may be ingesting root fragments randomly or accidentally, but it is useful as an indirect tool to complement other research approaches, and does suggest activity in plant rhizospheres. Over 20 earthworm species have been subjected to gut analyses and the results indicate absence of root fragments in one-half of the species (Table 8). In those species in which root fragments were detected, they generally tended to be a minor component of the gut contents. In most cases the fragments were of dead roots. Both absence and presence were detected for three species (*A. caliginosa*, *A. rosea* and *A. longa*), indicating that in different environments they may be feeding on different resources, excluding or including roots, depending on the quality and quantity of available food. For example, in the savanna region of Lamto, Lavelle *et al.* (1989) showed that roots of the predominant grass species (*Loudetia simplex*) were a poor food resource for *M. anomala* and other organic sources (leaves, SOM and dead OM) were generally preferred and ingested in greater quantities (Ka Kayondo, 1984), as well as being more effectively assimilated and earthworm growth-promoting.

Finally, not only must earthworm activities be effective at the rhizosphere level, they should also coincide both spatially and temporally with the demands for root expansion and nutrient uptake. So far, few studies have been performed addressing the temporal synchrony of earthworm activites with plant nutrient needs. These have revealed an improved uptake of $^{15}N$ by maize from labelled maize residues incorporated into the soil (Gilot-Villenave *et al.*, 1996), and by *P. maximum* shoots from labelled soil (Spain *et al.*, 1992) in the presence of *M. anomala* over a short time (<90 days). Brown *et al.* (2000a; chap. 3; 2000b; chap. 4) observed an important effect of *P. corethrurus* and *P. elongata* on maize and bean root distribution and density, leading to greater bean biomass, but no significant difference in maize production. Further experimentation in this field is required to clarify the extent of synchrony between earthworm effects on soil properties and the physical and chemical needs of plants.

The above results lead us to conclude that several earthworm species may be active in the rhizosphere of at least some plant species, and that they may be grazing on dead or live roots (though the latter is less likely), or on rhizosphere exudates, asimilable organic matter or microorganisms (protozoa, fungi, bacteria, nematodes) (Brown, 1995; 2000a; chap. 3). In addition, earthworms may be important in mycorrhizal (both ecto- and endo-) fungi dispersal and the infection potential in host plants (Brown, 1995; Reddell and Spain, 1991a; Ydrogo, 1994). Given the importance of these fungi in enhancing plant nutrient uptake in poor soils and the fact that as much as 90% of all plants are mycorrhizal symbionts, there is potential for exploring the roles of earthworms in these processes, especially in tropical forestry (in relation to Casuarinales, *Eucalyptus*, and *Pinus* spp.) and in cultivated soils, where the inoculum potential is generally low.

Table 8. Presence and absence of root fragments in the intestinal contents of several earthworm species from tropical and temperate regions.

| Root fragments | Earthworm Species | Reference |
|---|---|---|
| Present | *Aporrectodea rosea, A. chlorotica, Lumbricus terrestris* | Ferrière (1980) |
| | *Nicodrilus caliginosus, Eisenia nordenskioldi* | Striganova (1982, 1984) |
| | *L. rubellus, A. caliginosa, A. chlorotica* | (Piearce, 1978) |
| | *Aporrectodea longa* | |
| | *P. corethrurus* | Reddell and Spain (1991a) |
| | *Millsonia lamtoiana, M. ghanensis, Dichogaster terrae-nigrae, Agastrodrilus opisthogynus, A. multivesiculatus* | Ka Kayondo (1984), Lavelle (1973)<br>Blakemore (1994) |
| | *Digaster sp., Heteroporodrilus* spp., *Anteoides* sp. | Németh (1981) |
| | *Diplocardia longiseta, D. smithii, D. rugosa, D. prosenteris, D. verrucosa, A. turgida, Octalasion cyaneum* | James and Cunningham (1988) |
| Absent | *Dendrobaena mammalis, Lumbricus castaneus* | Piearce (1978) |
| | *L. castaneus, Nicodrilus longus ripicola, N. longus longus, N. caliginosus, A. icterica, N. nocturnus, D. mammalis* | Ferrière (1980) |
| | *Aporrectodea rosea* | Judas (1992), Bouché and Kretzschmar (1974) |
| | *M. anomala, Stuhlmania porifera, Chuniodrilus palustris, C. zielae, Dichogaster agilis* | Lavelle (1971; 1973) |
| | Several tropical species | Lavelle (1978; pers. obs.) |
| | *Andiorrhinus amazonius, Andiorrhinus* sp. 1, sp. 2 | Németh (1981) |

**Earthworm abundance and biomass vs. plant response (dose/effect relationships)**

The fact that earthworms may be important in plant production is by now clearly evident. However, the question of how many, and what biomass are necessary for earthworms to become important remains. The first reports by Hopp (1954), suggested that a minimum of approximately 100 earthworms $m^{-2}$ were necessary to be important in the physical conditioning of soil (and thus in affecting crop growth). In New Zealand, Waters (1951) found a significant correlation ($r = 0.87$) between pasture dry matter production and earthworm biomass; however, it appears that the chief agents in raising the yield in pastures with earthworms were the presence of clover and nutrient additions (dung and urine), which also raised the earthworm biomass.

Only recently have such biomass/yield relationships been established for tropical earthworm species. In Papua New Guinea, Rose and Wood (1980) found a relationship between sweet potato topgrowth and earthworm (>99% *P. corethrurus*) biomass in potato mounds. When the biomass was <43 g $m^{-2}$, the relationship with shoot weight was positive ($r = 0.48$, $P < 0.01$); above 43 g $m^{-2}$, this relationship was lost. The correlation also varied depending on soil type and plant part; in an alluvial soil (sandier), a positive correlation ($r = 0.6$) with topgrowth was found, but in a clayey peat soil, worm biomass was negatively correlated ($r = -0.61$) with tuber production.

At Lamto, Spain *et al.* (1992), found a significant correlation ($r = 0.81$; $P < 0.01$) between total dry matter produced by maize and the biomass of *M. anomala* and Eudrilidae earthworms found at the end of the experiment. They also found that increasing application of *M. anomala* biomass increased *P. maximum* yields up to a point, whereafter the effect was reduced, suggesting a curvilinear (polynomial) relationship ($r = 0.96$). In this case, biomass applied above 100 g $m^{-2}$ caused a reduction in growth stimulation, attributed to compaction from the excess soil working by these earthworms (Blanchart *et al.*, 1989, 1990). Nevertheless, if final biomass of *M. anomala* obtained at harvest was associated with the same *P. maximum* shoot biomass used above, the relationship became exponential ($r = 0.97$).

In a tropical pasture in Sambalpur, India, with a predominance (>80% of biomass) of the grass species *Eragrostis amabilis*, *Cynodon* sp. and *C. dactylon*, Senapati and Dash (1981) established a significant positive relationship ($r = 0.78$) between mean monthly earthworm biomass (five species, dominance of *Octochaetona surensis*) and above-ground plant biomass for both grazed and ungrazed plots. Root biomass was positively correlated with earthworm biomass only in the ungrazed plot ($r = 0.38$). Both earthworm and shoot biomass followed a similar monthly cycle throughout the year, both being correlated with and depending on primarily soil moisture (positively) and temperature (negatively).

In a native pasture (*Sporobolus jacquemonti*, *Paspalum notatum* and *Setaria* sp. predominant) pasture at La Vibora, Mexico, monthly sampling of approximately six earthworm species (dominated by an undescribed Glossoscolecidae sp.) and green and dry grass during 10 months of a year revealed significant ($P < 0.001$) positive correlations ($r = 0.52$) of annual (yearly total) earthworm biomass and numbers with green grass yields (Brown *et al.*, unp. data). Nevertheless, both earthworm and plant factors were significantly correlated with soil moisture (a main factor limiting both plant production and earthworms for at least six months of the year), confounding the relationship between the two. Nevertheless, when peak earthworm biomass and numbers (September) were present, and the average pasture production was high, the relationship between green production and earthworm populations was significant ($r = 0.4$, $P < 0.05$), while production was not related to soil mositure. This showed that earthworms had the potential to concentrate in the regions of higher plant production, in a synergistic association, in which the plants can benefit from worm activity in the rhizosphere and fom the higher nutrient contents in the drilosphere, and the earthworm benefit from higher OM inputs both in shoot litter, roots and rhizosphere deposition.

Using data from field trials at Yurimaguas, Lamto and La Mancha, Lavelle (1997) developed a relationship between earthworm biomass and percentage increase of grain yield ($r = 0.53$, $P<0.05$). The important increases in yields were mostly obtained when earthworm biomass was above about 30 g $m^{-2}$. Using all the data obtained from pot and field experiments performed during the Macrofauna programme and from the literature for tropical regions, several regression analyses were performed, using root, shoot and grain biomass increase and earthworm biomass applied and recovered at the end of each trial. No significant relationship between earthworm biomass and shoot and root biomass was found. However, when only the grain % increase data (for cowpea, beans, rice, maize, sorghum, wheat) were correlated with the difference in earthworm biomass between inoculated and uninoculated treatments, a small but significant linear relationship was found ($r = 0.31$, $P < 0.015$) (Figure 8). Moderate (20-40%) and agriculturally important (>40%) grain production increases were found with just over 13 g $m^{-2}$ and 47 g $m^{-2}$ earthworm biomass, respectively. Using the same data, the curvilinear relationship (2nd order polynomial; Figure 8) had slightly higher correlation ($r = 0.41$), where moderate (20-40%) and important (>40%) grain production increases were found with a biomass value above 17 g $m^{-2}$ and 32 g $m^{-2}$, respectively, with maximum grain increases (approx 70%) at around 80 g $m^{-2}$. Root biomass increase of these grain crops was also positively correlated with earthworm biomass difference (linear $r = 0.39$, $P < 0.006$; curvilinear $r = 0.42$). Similarly, maximum values (55%) were found with a biomass of about 75 g $m^{-2}$.

In a first instance, these results appear to indicate that earthworms may positively influence grain production at biomass values that occur in some agricultural fields, or at least at a biomass achievable through soil management techniques that stimulate earthworm populations. Secondly, we arrive at the following question: Can there be too much of a good thing? Spain *et al.* (1992), proposed that there may be a biomass beyond which the soil working activities of earthworms (particularly monospecific communities) become detrimental to plant production. The limit is most likely variable depending on the plant and earthworm species or assemblage, soil type, and the length of time earthworms have been active (the extent of the drilosphere effects on soil properties). At present, four case studies have shown negative effects on plant production of high earthworm biomass in the field:

**1.** The first refers to a *Dichogaster* sp. (*D. curgensis*) as a potential pest in rice fields, but also referring to various annelids playing the same role. These earthworms are adapted to living in fooded conditions, but under particular situations can infest rice fields reaching densities of up to >10,000 $m^{-2}$ (assuming average weights of about 0.3g worm, this equals 3,000 g $m^{-2}$), at which point the mere fact of their movement within the soil damages the rice roots resulting in total crop failure at densities above 7,000 $m^{-2}$ (Barrion and Litsinger, 1996).

**2.** The second case was in a 15-year old abandoned pasture (*Brachiaria* sp.) in the Brazilian Amazonia, north of Manaus, where the the lack of decompacting species, and the activity of *P. corethrurus* (the only species present) with a mean biomass of 45 g m$^{-2}$, led to the degradation of the topsoil structure (compaction, reduced infiltration) and reduction of pasture grass growth (Barros *et al.*, 1996).

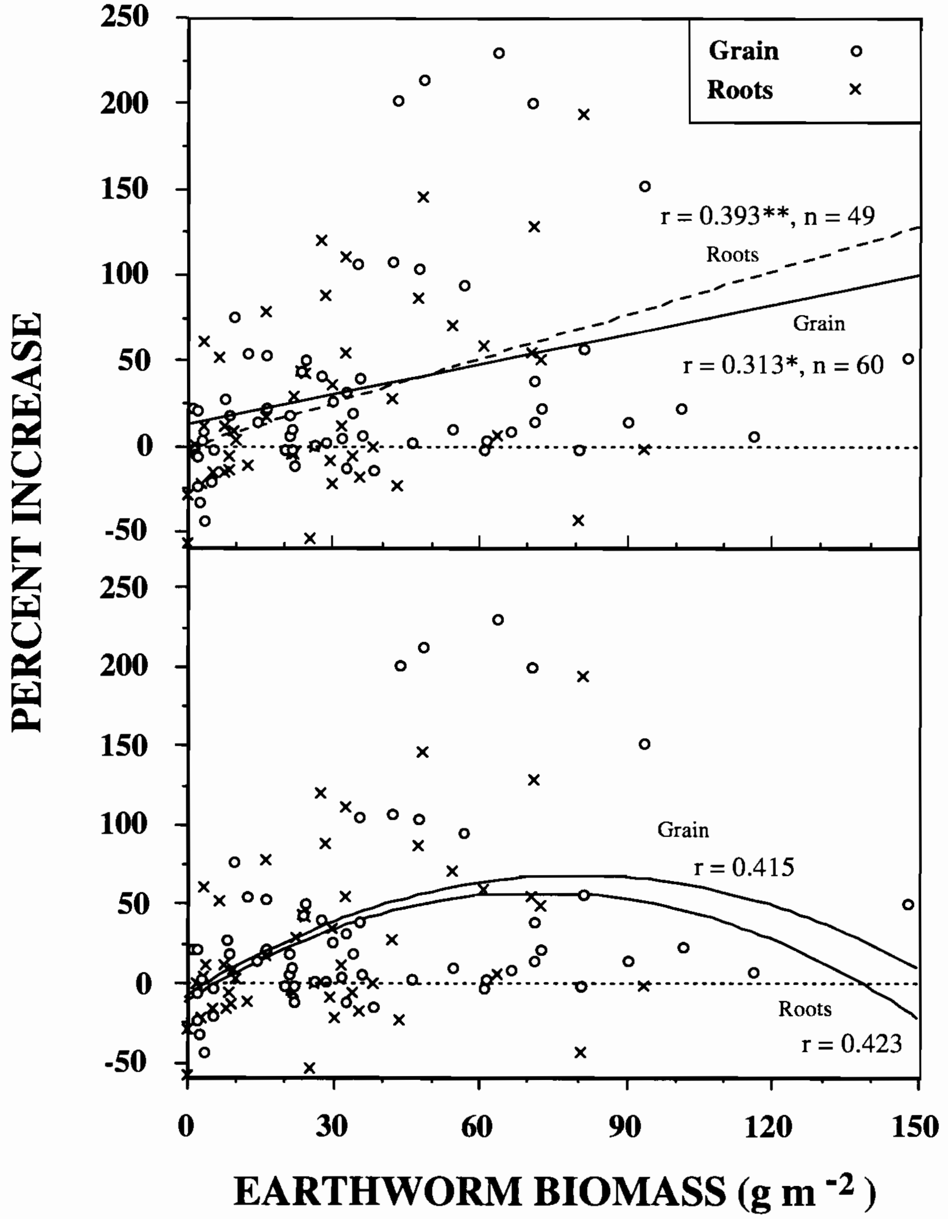

Figure 8. Relationship between the increase in grain and root biomass due to the presence of earthworms and the difference in the biomass of earthworms obtained between worm addition and no-worm treatments for 60 (grain) and 49 (root) data points taken from 12 trials with six crops (maize, rice, sorghum, wheat, beans and cowpea). Significance values as in Figure 3.

**3.** The third case was found in Papua New Guinea, where sweet potato tuber yields decreased in a clayey soil where *P. corethrurus* biomass was higher than about 40 g $m^{-2}$ (Rose and Wood, 1980).

**4.** The final case was in a vegetable garden of about 1.8 ha in India, where a *P. corethrurus* population of 1308 $m^{-2}$ in association with 247 $m^{-2}$ *P. elongata* (equivalent to biomasses of approximately 520 g $m^{-2}$ and 240 g $m^{-2}$) caused severe soil compaction reducing the yields of carrots, raddish, beans and knol-khol (*Brassica oleracea*) (Puttarudriah and Shivashankara-Sastry, 1961). Interestingly, in this garden, yield reductions were observed only in dicotyledenous plants; monocot plants such as maize and ragi (*Eleusine coracana*) with a fibrous root system grew well, without an adverse effect of the high worm biomass. These cases not only confirm the probability of a biomass vs. yield relationship upper limit, but also highlight the importance of promoting a diverse assemblage of earthworm species, with both soil compacting and decompacting strategies, to arrest any possible detrimental effects of a high biomass and activity of a single species (or several species with the same strategy), e.g., the soil compacting *P. corethrurus*.

## Effects of spatio-temporal scales of investigation

Two spatial scales were investigated: field trials and pot experiments. The field trials consisted of mesocosms or small plots, and the massive inoculation trials (hectare scale). Approximately half of the data on shoot, root and grain % increase comes from pot experiments and the other half from field experiments. When taken separately, results suggest different trends for the effects of earthworms on biomass increase of the different plant parts, depending on the spatial scale of investigation (Table 9). In almost every case, higher (but not always significantly different) results were obtained, at the pot scale for a given plant and earthworms combination. Nevertheless, F-tests revealed that grain and shoot production in the field trials were significantly higher in earthworm treatments than controls at lower p-values than in the pot trials (less variable results). Grain production was significantly higher at $P< 0.1$ and shoot biomass at $P< 0.11$. Reasons for the higher results at the pot scale are likely related to overall higher biomass of earthworms applied, reduced soil and environmental variability, close contact enforced between the rhizosphere and drilosphere systems, and the easier general care of the trials. Nevertheless, the greater number of species of both plants and worms used inevitably lead to a greater variability of the results.

Two large scale earthworm introduction trials were made as part of the second phase of the Macrofauna project. The first experiment, at Yurimaguas, was abandoned. The other experiment , still in place, in a tea plantation in India inoculated at high rates (150 kg $ha^{-1}$ fresh weight) with *P. corethrurus* and four other species showed dramatic production increases over all the 10 months in which tea was harvested, when earthworms were introduced (Giri, 1995; Senapati *et al.*, unp. data; Figure 9). After three years, the positive effect on tea production is still present, although the earthworm population is not sustained and must be reintroduced (Senapati *et al.*, 1999). No differences were found between treatments with and without application of OM (prunings), so earthworms appear to be the main agents influencing tea production in this system.

Table 9. Mean ± standard error (s.e.) of percent increases of different plant parts (shoot, root, grain), due to the presence of earthworms in field trials and pot experiments.

| Plant | Field Trials | | | | Pot Experiments | | | |
|---|---|---|---|---|---|---|---|---|
| part | n | % Increase[1] | se | p-value[2] | n | % Increase | s.e. | p-value[2] |
| Shoot | 104 | 59.6a | 8.5 | 0.11 | 142 | 54.6a | 14.9 | 0.33 |
| Grain | 66 | 29.7b | 10.5 | 0.10 | 23 | 53.3a | 16.3 | 0.40 |
| Root | 35 | 29.8ab | 9.3 | 0.96 | 80 | 81.9a | 31.0 | 0.79 |

1. Values with the same letters are not significantly different at $P< 0.05$;
2. Results of the F-test comparing means of earthworm inoculated and uninoculated treatments for each plant part.

Two temporal scales were used for the trials described in Table 2. The first examined effects of earthworms over one cropping cycle, but with intermediate harvests before the final harvest at plant maturity. The second compared effects of earthworms over short term (single cycle) and long term (multiple cycles) experiments. The latter studies provide data on survival of earthworms over time and duration of effects on plant production (positive and/or negative), resulting in an estimate of the sustainability of earthworm introductions.

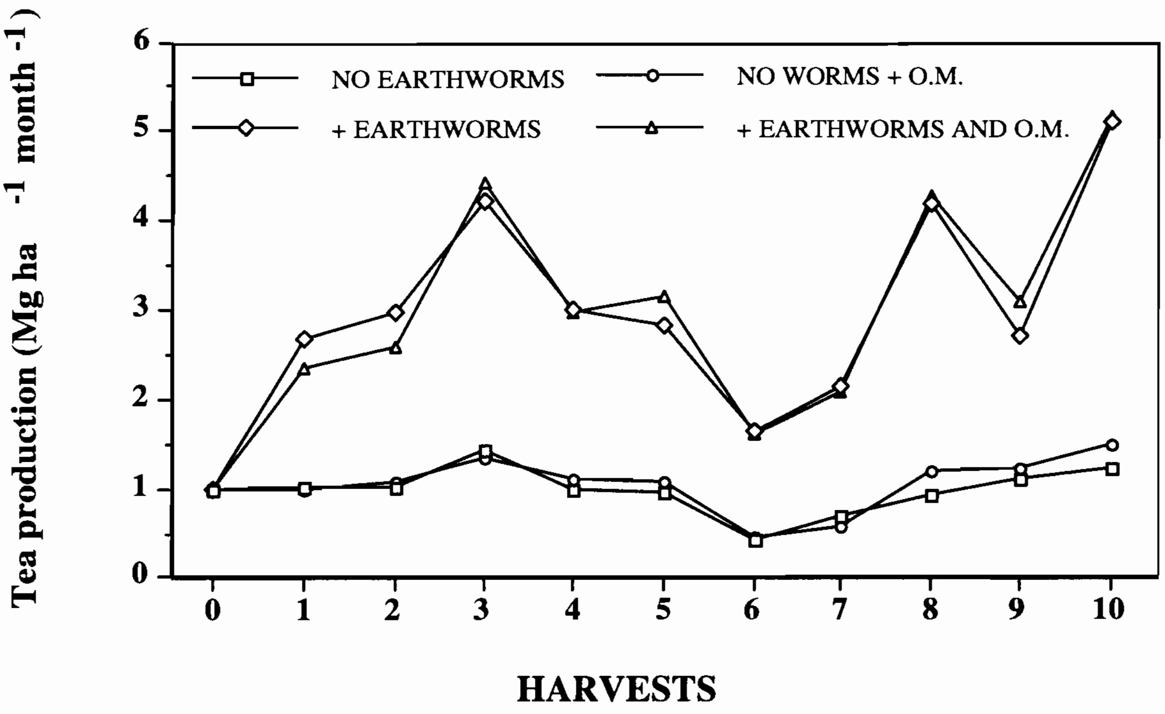

Figure 9. Monthly green leaf tea production (in Mg ha$^{-1}$) as affected by the introduction of earthworms (primarily *P. corethrurus*) and organic matter into trenches of 0.54 m$^2$ at Lower Sheikamuldi Tea Estate, Parry Agro Industries Ltd., Tamil Nadu, India (Giri, 1995; Senapati *et al.*, unp. data).

At the first scale, increases in plant biomass due to earthworm activity were initially neutral or low, but increased with time such so that beneficial effects were usually highest at harvest time. Furthermore, plant maturity was often more rapid in treatments that included earthworms (e.g., Pashanasi *et al.*, 1996). This is likely due to reorganization of the soil (in trials using sieved soil), and enhanced microbial activity and nutrient release which create cumulative effects on the plant. This phenomenon was observed for three tree seedlings by Ydrogo (1994) and Pashanasi *et al.* (1992), although for *B. gasipaes*, the latter authors found a cumulative decrease in biomass after 60 days. Brown *et al.* (2000a; chap. 3), also found increasing positive differences in shoot biomass of common beans over three harvests in the presence of *P. corethrurus* and *P. elongata*. Blakemore (1994) similarly found greater biomass increases of two grass species (*P. maximum* and *Chloris gayana*) in treatments with *D. affinis* and *D. saliens* up to five months, after which the growth stabilized until final harvest (8.5 months). However, when he tested the effect of 12 earthworm species in three different soil types on the growth of oats over 14 weeks (three harvests, at 42, 70 and 98d), not only were few significant effects on biomass observed, but earthworm effects were cumulatively negative in one soil type (Narayen) for all except one worm species (*Eudrilus eugeniae*). In the other soils (Samford, Kingaroy), cumulative effects on biomass increase were mostly positive. Finally, when these same pots were seeded with two grasses (*P. maximum* and *Cenchrus ciliaris*), and harvested at 42 and 70 days, the increase in biomass was higher at the latter harvest for all earthworm species in both Narayen and Kingaroy soils. Therefore, although effects of earthworms on plant biomass increase are generally cumulative, there are situations in which they may be the reverse, depending on the soil type, earthworm and plant species.

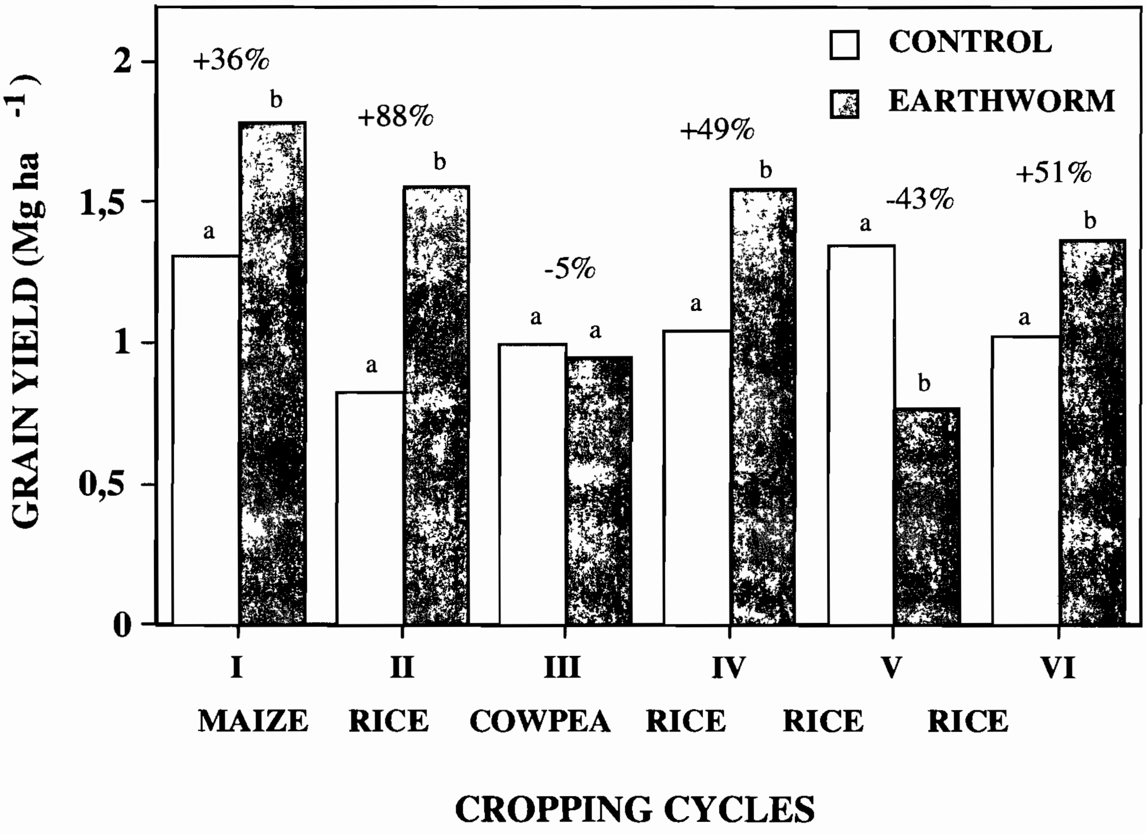

Figure 10. Effect of earthworm (*P. corethrurus*) activities on grain production (in Mg ha$^{-1}$) in field plots of 0.28 m$^2$ during six successive harvests over a three year period, irrespective of organic treatments, at Yurimaguas, Peru (Pashanasi *et al.*, 1994; 1996). Bars with different letters indicate significant differences at $P < 0.05$.

Effects of earthworm inoculation on plant yields over several cycles were investigated at five sites (Guarapuava, Lamto, La Mancha, St. Anne and Yurimaguas). At Guarapuava, both wheat and bean yields were only slightly (not significant) higher with the introduction of *Amynthas* sp. Survival of the introduced earthworms after 12 months of cropping, however, was good, averaging >100%, indicating population increase. At Lamto, yam tuber production was significantly ($P < 0.1$) higher in two of the three cycles (Gilot, 1997), while at both Lamto and La Mancha, few significant effects of earthworms on maize yields were observed over six continuous cropping cycles (three years), and survival of introduced earthworms was poor (Gilot, 1994; Patrón *et al.*, unp. data). Nevertheless, average percent increases in grain yields were generally higher at the final three harvests at both sites, indicating that earthworms helped sustain higher production levels for a longer time-period in these low-input systems. Reasons for this may be the cumulative effects of earthworm activity on nutrient and SOM dynamics, and soil biological and physical properties. At St. Anne, *Digitaria decumbens* (pangola grass) root biomass, the only plant parameter measured, was not significantly influenced by the inoculation of 90 individuals m$^{-2}$ (about 90g m$^{-2}$) of *P. elongata* throughout the experiment, and earthworm biomass was reduced due to the very low quality initial soil, although there is evidence of recovery in the last samples, probably due to soil aggradation (C increase; Blanchart, 1997). At Yurimaguas earthworm biomass was maintained throughout six cycles, and significant positive effects of earthworm addition on crop production obtained in four of the six cycles (Figure 10; Pashanasi *et al.*, 1996). In the fifth cycle, when rice was sown out of season, *P. corethrurus* caused complications in water dynamics in the soil, reducing yields (-43%). When sown in the previous and following seasons, however, rice outyielded the controls (+49 and +51%, respectively) in earthworm treatments. Despite continued cropping for three years and six crop cycles on the same soil, production was maintained at satisfactory levels, with slightly higher yields than crops of the same type harvested locally.

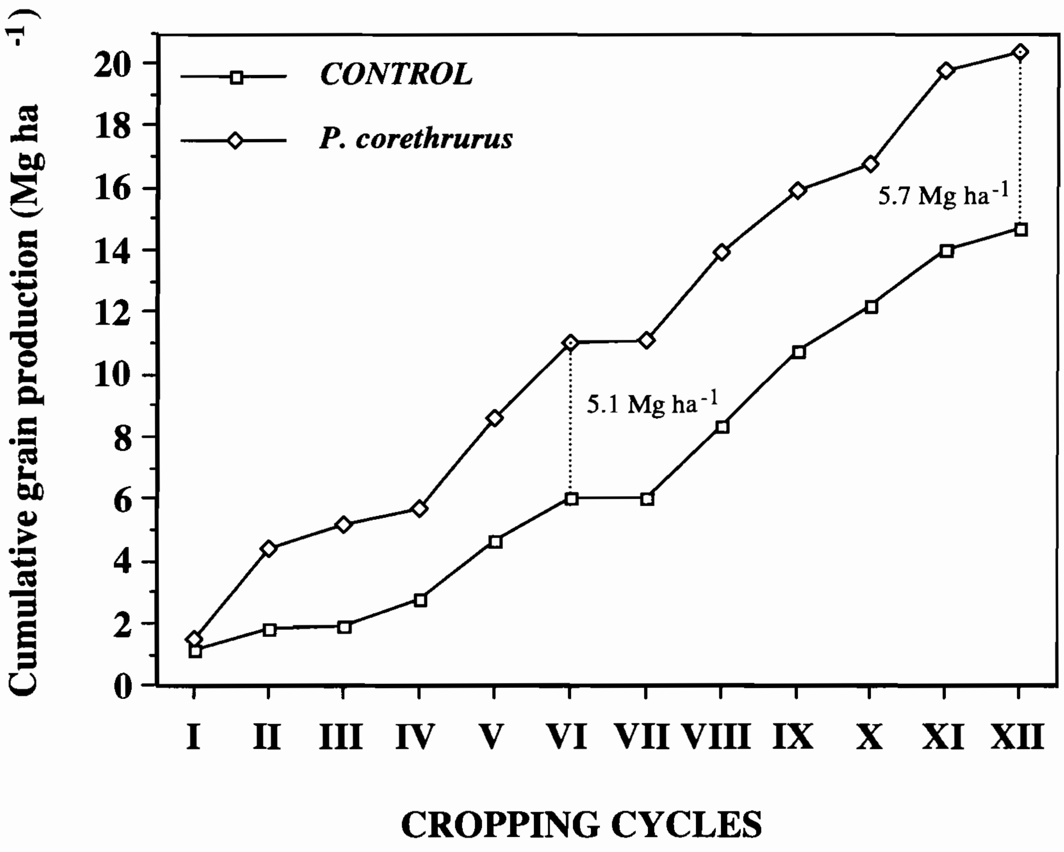

Figure 11. Cumulative grain production of maize (in Mg ha$^{-1}$) over seven years, including 12 cycles, in treatments with and without addition of 36 g m$^{-2}$ of *P. corethrurus* at Yurimaguas, Peru (Charpentier, 1996; Pashanasi *et al.*, unp. data).

On the other hand, when maize was grown continuously over seven years (12 cycles) in the same type of enclosures (60 cm diameter) nearby, earthworm populations were reduced (as measured by surface casting activity), and had to be reintroduced at the 10th cycle (Pashanasi *et al.*, unp. data). Introduction of *P. corethrurus* did not also arrest the loss of soil fertility due to cropping. By the third harvest, grain production was practically nil in both treatments with and without earthworm addition. Fertilizers then had to be added for all the following eight cycles. Despite fertilization, earthworms continued to positively affect yields, although the cumulative effect was lower after the sixth harvest than over the first six harvests. By the end of the sixth cycle, the cumulative difference in grain production was as much as 5.1 Mg ha$^{-1}$, the equivalent to approximately two or three single harvests (Figure 11). The following six harvests accumulated only 0.6 Mg ha$^{-1}$ more, for a total of 5.7 Mg ha$^{-1}$ above the uninoculated treatments. Thus, the effect of earthworms on production was positive in nine of the 12 cycles, and importantly so (>1 Mg ha$^{-1}$ increase) in four of the 12 cycles. However, despite this large production increase, there was evidence of greater losses of SOM due to earthworm activity at the end of the experiment (Charpentier, 1996; Villenave *et al.*, 1999), despite the fact that at the long-term (decades), these losses may be balanced out by the conservation of C in earthworm castings versus uningested soil (Villenave *et al.*, 1999). Therefore, attempts must be made to manage not only earthworms, but also OM (with use of residues) and cropping systems (rotations) in a holistic manner.

## Limitations and Future Prospects

The large number of earthworm and crop species tested in tropical and temperate regions, confirm the dependence of plant response on earthworm species and biomass, soil type and plant species and part. Additional factors such as microclimate, or slight genetic differences may also be important. Field population associations of earthworms at a given site are generally adequate since they have generally adapted to the local conditions, although this may not always be the case. Given that effects can range from positive to negative when the factors are varied, we are still far from being able to propose a general combination of factors which could be applicable at many different sites.

Nevertheless, a few studies have yielded promising results that may have large-scale applicability, for example the use of *P. corethrururs* along with four other species to enhance soil fertility and tea production in degraded tea plantations in India. Despite the large investment of human labour required, the cost-benefit ratios are promising (Senapati *et al.*, 1999). *P. corethrurus* also shows promise for use in certain tree seedling nurseries. However, the applicability of this tropical species at the global level is still uncertain, and more field experiments in different cropping systems and regions, particularly at the long term (decades) scale, are needed to confirm the observed SOM losses at Yurimaguas (Charpentier, 1996).

Based on results at the greenhouse (pot) and field levels, *Drawida barwelli* in Australia (Blakemore, 1994) and *Drawida spp.* in India (Kale *et al.*, 1989; Senapati et al, 1985; unp. data) showed promise for introduction or management at larger scales. The latter species may be particularly useful in paddy rice-based cropping systems, since they are adapted to living under water-logged conditions for some period of time (Kale *et al.*, 1989; Pani, 1986). Trials with these species in other regions and with other plants may confirm their positive role on biomass production at a larger scale.

Several other species, such as the eudrilids *E. eugeniae* in Australia, *C. zielae* and *S. porifera*, and the megascolecid *M. anomala* in Ivory Coast, have not been tested beyond a small region; despite their high potential (Table 6), ways must be found to increase survival and maintain their populations in field cropping systems. Furthermore, testing of these species with other plants such as *P. maximum* or other pasture grasses at the field level, may result in sustainable biomass, as well as considerable yield gains.

Finally, the small polyhumic *Dichogaster* spp. have not been tested beyond a few trials in Australia, where they showed a high potential to increase yields, yet a poor survival rate when introduced into pastures. These species are widespread throughout the tropics, in both perennial and annual cropping systems (Fragoso *et al.*, 1999a,b), yet their role in soil fertility and plant production is practically unknown. Under rice, some species of this genus may reach a pest status, but little is known of their effects on other crops, and of other species from this genus. Effects of the widespread *Amynthas* spp. and other *Metaphire* spp. on crops and soil processes is also virtually unknown. Further research may reveal that these species have a much wider applicability and potential for management and for increasing yields. Of the latter group, *P. elongata*, a widely distributed and deep burrowing species (unlike most other candidate species), deserves further attention.

A large number of other species which inhabit tropical soils have never been tested for effects on plant growth. Given the probably 6000+ species of earthworms in the world (Fragoso *et al.*, 1999b), only 10 of which have been tested in depth, further investigations such as those by Blakemore (1994; 1997) may reveal other species useful both in tropical and temperate regions. In fact, it may be preferable in some cases to use or test locally adapted or endemic species which have, by their presence demonstrated their ability to survive under local conditions of climate and soil. Great care must be taken if earthworms are transported between different countries, or even between different regions in the same country, to prevent dispersal and transmission of crop and animal diseases or pests.

Pot experiments, although limited in scope (see Blakemore and Temple-Smith, 1995), have proven to be a useful tool for screening earthworm species and crops for their potential association, and to test survival of earthworms in situations where this would be impossible at the larger scale of investigation. Nevertheless, the comparison of data between experiments is often difficult, due to differences in earthworm and crop species used, lack of detailed information in specific studies, absence of a standard methodology for addressing the question of earthworm effects on plant growth, and diverse approaches and objectives of the trials. Very often, few clear links were made between observed results and underlying mechanisms. We therefore suggest for future trials a more standardized approach and a minimum data set, which will permit comparisons of trials from different regions and provide a broader understanding of earthworm influences on plant growth and biomass. For pot experiments this should cosist of:

- pasturization or irradiation of test soils to remove residual earthworms and their cocoons;
- statistically valid replication;
- realistic crop, earthworm and soil combinations;
- proper identification of the earthworm species;
- clear specification of the quantity of earthworms applied (based on realistic fresh field biomass, not numbers) (Dalby *et al.*, 1996), and reasons for the chosen biomass;
- full physical, chemical and biological description of soils used;
- longer time periods of investigation, preferably until plant maturity, but not longer than the time by which all soil in the pot will have been consumed by the earthworms;
- analyses of key soil properties which will be affected by earthworm activity (such as bulk density, infiltration, inorganic P and N) to reveal mechanisms of the observed effects; for chemical properties, the use of stable and radio isotopes is particularly useful;
- measurement of all plant parts and plant growth throughout the cycle, with intermediate harvests;
- proper assessment of earthworm biomass at the end of the experiment.

From the Macrofauna and other experiments in the tropics described previously, several drawbacks arose regarding inoculation of earthworms into the field at both large and small-scales. First was the difficulty and cost (money and time) of obtaining sufficient earthworm biomass to apply to the plots. A possible solution to this is mass rearing of earthworms (Senapati *et al.*, 1999). Next, few suitable sites for field inoculations, with low or nil background earthworm populations were found, and it was almost impossible to eliminate completely the native earthworm fauna, making it difficult to obtain and maintain control (no worm) treatments. Very often, control plots or even worm+ plots became contaminated with introduced or resident worms. Thus, comparisons of the effects on plants between worm and no-worm treaments must take into account the biomass 'difference' between the two. In addition, earthworm exclusion treatments often conserve for a certain period of time the structures and soil properties (porosity, water infiltration, abundance and composition of macroaggregates) created by the previous earthworm community, possibly masking differences between treatments until the structures and properties were broken down. Finally, low survival of introduced species implied that specific management practices such as application of OM and the use of crop rotations were necessary to promote population stabilization and/or increase.

Field trials should be performed over several cropping cycles, on large plots, preferably $>1\mathrm{m}^{-2}$, and special care should be taken to obtain controls without earthworms; if this is not possible, or if earthworms are applied over a resident fauna, results should be compared with biomass difference between earthworm and control plots. Earthworm abundance and biomass (and species interaction, if the case) must be assessed throughout the duration of the trial, and earthworms should not be re-introduced, or the feasability of the trial for large-scale application will be sacrificed. Biomass measurements of all appropriate plant parts must be made, and the soil well characterized at the beginning of the trial (including assessment of spatial variability) and at each harvest. These data are used to ascertain effects of earthworms on soil physical properties and fertility, including C status in long-duration trials (>3 years), and to correlate these with observed plant responses.

## CONCLUSIONS

When introduced into new systems, earthworms generally improve plant productivity, especially of above-ground parts. A survey of literature in the tropics revealed for >34 species of earthworms and 19 plants, positive effects on above ground biomass in 72% of the cases. In 28% of the cases earthworms reduced plant growth, but the mechanisms are unclear. Therefore, studies on the mechanisms by which earthworms affect plant growth (both positively and negatively) are an urgent research imperative.

Effects of earthworms (even of the same species) on different crop species depend on both environmental requirements of plants and the ability of earthworms to modify the soil environment for root growth. Earthworm effects appear particularly promising in perennial crops such as tree seedlings or pasture grasses. Monocrops are not generally beneficial to earthworm populations, and thus earthworm effects on these crops are generally less. If crop rotations are implemented, the potential for beneficial earthworm effects becomes more important.

Influences of earthworms on plant growth also depend on soil characteristics. Their effects are more important in C-poor than in C-rich soils, in sandy than loamy and clayey soils, and in moderately acid than in alkaline or highly acid soils. Mechanisms by which plant growth is affected by earthworm activity are numerous, a variety of factors often being relevant in a given situation. Mechanisms range from modification of soil function at the molecular and microscopic level (e.g., greater nutrient availability in the drilosphere, increased microbial activity in casts, enhancement of VAM fungal-root colonization, and reduction in plant parasitic nematodes), to visible soil structural changes (e.g., increased macroporosity, stable aggregates), the enhancement of specific plant parts (e.g., grain), or reduction in root diseases (particularly fungal pathogens). To obtain optimal earthworm benefits on plant production they must be synchronized both spatially and temporally with root growth and nutrient uptake.

Increased plant shoot biomass is often associated with increased earthworm biomass, especially in pastures. Moderately positive effects on plant production can begin at biomass values >15 g $m^{-2}$, while important (> 40% increase) effects appears around 30 g $m^{-2}$. However, a maximum earthworm biomass for particular soil, crop, earthworm and climate combinations also appears to be present beyond which negative effects on plant biomass may result, or earthworm populations decrease to carrying capacity of the site.

Pot experiments, should be used to screen a range of earthworm species for potential effects on plants in different soils, considering that they may have a limited applicability to field situations. A standardized methodology involving realistic earthworm, crop and soil combinations, earthworm numbers and biomass equivalent to common field values, detailed descriptions of soil modification by earthworms, and harvesting of plants preferably at maturity (unless the objective is to differentiate effects on vegetative growth, in which case harvest should take place just prior to flowering) should help increase the comparability of these trials to the field.

Several earthworm species (particularly *Pontoscolex corethrurus*) show high potential for introduction into specific plant systems (e.g., tree seedlings, pastures, tea), but further experimentation in additional cropping and plant systems is necessary to assess their role in increasing plant production on a wider geographic scale. Furthermore, given the large number of earthworm species in the tropics which have not been tested for plant growth response, it is likely that more species with useful effects will be discovered with more field work.

Finally, given the obvious benefits of earthworms to plant growth and yields, agriculturists and other ecosystem managers interested in harvesting these benefits must implement practices that favor the development of a diverse assemblage of earthworm species (and other macroinvertebrates important in regulating soil properties and processes) in their target areas. This can be achieved by applying management practices such as mulching, OM conservation, crop rotation, minimum tillage, restricted use of pesticides, incorporation of legume into pastures, as well as other practices that favor a stable and adequate earthworm biomass. If earthworms are to be introduced, care must be made to introduce several adapted species (of various ecological strategies) in sufficient but not excessive numbers (and biomass) for them to persist in new soil environments, so that favorable soil properties and positive effects on plant production can be sustained.

# CONCLUSIONS DE LA PREMIÈRE PARTIE

Beaucoup des expériences cherchant à caractériser les effets des vers de terre sur la production des plantes ont été développées en serre, très peu l'ont été au champ. Les expériences réalisées dans des serres sont utiles surtout pour la description des mécanismes précis responsables des effets observés mais trouvent peu d'application directe au champ. De plus, ces expériences utilisent souvent les déjections des vers plutôt que les vers eux-mêmes, et lorsque ces derniers sont utilisés, le rapport entre la biomasse de vers et le poids de sol est souvent élevé et peu réaliste. Dans ce cas, les résultats obtenus sont issus de situations rarement (ou jamais) observées sur le terrain. Enfait, peu d'auteurs ont décrit en détails toutes les caractéristiques des systèmes, ces observations peuvent pourtant être importantes pour identifier le ou les mécanisme(s) impliqué(s) dans la réponse des plantes.

D'autre part, les expériences au champ permettent d'avoir confirmation des tendances observées en conditions contrôlées dans des conditions d'environnement et de sol pouvant être très changeantes. Dans certains cas, le mécanisme responsable des effets des vers est clairement identifié, mais dans la plupart des cas, il reste masqué par les activités propres au sol. La synthèse présentée dans le chapitre 2 montre que les espèces herbacées pérennes et les plantations d'arbres sont particulièrement stimulées par l'activité des vers. Au Mexique, ce sous pâturages et plantations d'arbres où les vers sont très actifs et développent une biomasse élevée. Les effets des vers sur la production de ces plantes ont jusqu'alors été peu étudiés dans le contexte mexicain; un essai au champ a débuté en juin 1996 dans un pâturage à La Vibora pour évaluer le rôle des vers sur la production herbacée (Brown et al., données non publiées). L'activité et la croissance des plantes et des vers suivent un rythme saisonnier, le facteur temps doit donc être considéré comme essentiel dans la détermination des effets. La variabilité spatiale, autre facteur important, doit aussi être prise en compte.

Une multitude de mécanismes peuvent opérer, souvent simultanément (chap. 1). Puisque les effets des vers sur la production des plantes sont essentiellement indirects (peu d'effets directs ont été observés), via les changements qu'ils induisent dans les caractéristiques et les processus physiques, chimiques et biologiques du sol, les interactions des vers avec les racines des plantes et les facteurs limitant leur croissance et leur développement requièrent une grande attention si l'on veut déterminer les mécanismes impliqués. Bien qu'il y ait plusieurs façons, directes ou indirectes, de les estimer, l'identification des interactions entre les vers et les racines des plantes n'est pas une tâche facile. L'observation des profils des sols montre que les racines suivent souvent les galeries construites par les vers et prolifèrent dans les turricules; "l'attraction" des racines pour les galeries des vers reste cependant l'objet d'un vaste débat. D'un autre côté, l'incessante recherche de ressources par les vers peut conduire certaines espèces à concentrer leurs activités à proximité des racines; certaines espèces sont en effet actives essentiellement dans la zone où les racines se développent (Edwards et Lofty, 1980). La vérification expérimentale de cette relation de cause à effet a cependant soulevé de grandes difficultés.

Les méthodes indirectes d'estimation des interactions vers-racines emploient: 1) les isotopes stables ou radioactifs; 2) l'analyse d'images d'échantillons de sol non remaniés, et 3) l'étude des changements des propriétés du sol sous l'effet des vers pouvant affecter la croissance et le développement du système racinaire.

1) Les isotopes sont utilisés pour matérialiser l'accumulation d'éléments nutritifs provenant des racines dans les tissus du ver et cibler les changements induits par le ver dans la nutrition de la plante et le cycle des nutriments dans le sol.
2) L'analyse d'images est particulièrement utile pour mettre en valeur les relations entre les structures construites par les vers et les racines.
3) L'évaluation des changements induits par les vers dans les caractéristiques physiques, chimiques et biologiques du sol est une mesure de leur habilité à réduire les limitations freinant la croissance des plantes. Evidemment, pour une estimation correcte de l'amélioration produite par l'action des vers, une connaissance préalable des contraintes limitant la production des plantes (Figure 1, pg. 2) est indispensable.

L'un des problèmes dans l'estimation des effets des vers sur la croissance des plantes, via les interactions racines-vers, est basé sur le fait que ce qui est bon pour la croissance des racines en l'est pas forcément pour celle des parties aériennes (Logsdon et Linden, 1992). Lorsque les conditions sont idéales, les plantes investissent généralement moins dans la croissance de leurs racines parce que les parties aériennes contiennent les structures servant à leur propagation (Brown and Scott, 1984). Le rapport tige/racine est donc habituellement supérieur à 1/1 et atteint environ 11/1 à maturité (Aiken et Smucker, 1996; Brown and Scott, 1984). L'effet des vers, mesuré via le rapport tige/racine, montre que les racines sont en général moins favorisées que les parties aériennes. Ceci semble indiquer que les vers opèrent des changements dans le sol qui favorisent plus la production aérienne. Par exemple de meilleures conditions d'humidité favorisent la croissance racinaire mais aussi la disponibilité des éléments nutritifs pour la plante et le développement de microorganismes symbiotiques (mycorhize, *Rhizobia, Frankia*); ces conditions avantagent la croissance de la plante et favorisent ainsi les populations bactériennes (telles que *Azotobacter, Azospirillum*, et quelques *Pseudomonas*) et la production d'hormones améliorant le développement des parties aériennes et limitant celui des parasites et organismes pathogènes.

Le but de ce travail était d'identifier les mécanismes intervenant dans les expériences en conditions contrôlées. Pour cela, on a évalué les changements dans des propriétés et des processus physiques, chimiques et biologiques (p.ex. biomasse racinaire et sa distribution spatiale) sélectionnés, matérialisé les interactions avec les bactéries symbiotiques (rhizobia) et les champignons (mycorhize) et estimé l'amélioration de la disponibilité des nutriments due aux fertilisants et aux résidus. Les isotopes stables $^{13}C$ et $^{15}N$ ont été utilisés dans la plupart des expériences, seuls ou en association, pour fournir des données supplémentaires sur les flux de nutriments du sol vers les plantes et de la plante ver les vers de terre.

# DEUXIÈME PARTIE:

# ÉTUDES EN SERRE

# CHAPITRE 3

# EFFECT OF TWO TROPICAL EARTHWORMS, *PONTOSCOLEX CORETHRURUS* (GLOSSOSCOLECIDAE) AND *POLYPHERETIMA ELONGATA* (MEGASCOLECIDAE) ON COMMON BEAN (*PHASEOLUS VULGARIS*) PRODUCTION[‡]

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## SUMMARY

The effects of two earthworm species *Pontoscolex corethrurus* and *Polypheretima elongata* on common bean (*Phaseolus vulgaris*) production, with and without *Rhizobium phaseoli* inoculation was evaluated in the greenhouse. Earthworms were introduced into small and large plastic pots filled with a sandy loam nutrient-poor soil derived from a savanna-pasture ($C_4$ plants dominant), and plant growth evaluated at four harvest dates (30, 42, 62 and 97 d). Soil bulk density, $NO_3$ and $NH_4$-N, pH ($H_2O$, KCl), moisture, and plant shoot, root, pod and grain biomass as well as root density were measured. Earthworm tissue $\delta^{13}C$ signature changes were used to determine food sources and rhizosphere activities. Despite less-than ideal conditions for both plant growth and earthworm activity due to the high ambient and soil temperatures and nutrient limitations, positive effects of both earthworm species on plant growth were found: bean plants tended to be significantly taller in the presence of both *P. elongata* and *P. corethrurus,* leading to higher plant shoot and root biomass at the final harvest, although neither bean pod nor bean seed production were significantly greater than controls (no earthworms). Earthworms also increased N-uptake by the plants as well as root density, although no active nodules were found, even when inoculant was added, indicating unsuitable conditions for *R. phaseoli* in this soil, probably due to extremely low available P and low pH. Earthworm castings collected from soil cultures, showed a much higher availability of nutrients (particularly N and P), which probably improved plant nutrition. Earthworm weight losses averaged around 40-60% with both species, due to the extreme conditions of the greenhouse, competition for $H_2O$ with the plants, the high biomasses applied, and the poor C resources of the soil used. No significant differences were obtained between $\delta^{13}C$ ratios of either earthworm species body tissues at any of the sample dates, indicating that they were not assimilating an important portion of bean plant C ($C_3$), low in $^{13}C$. They still may have been feeding in the rhizosphere, but this was not measurable using this technique.

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## INTRODUCTION

It is well known that earthworm activity in soils can affect plant growth, nutrient uptake and yields, through a variety of mechanisms. These mechanisms can range from large-scale and long-term effects on soil processes including soil profile formation (e.g., mollic A horizons or mull soils) and incorporation of, protection from and/or stimulation of organic matter decomposition, down to the enhancement of soil microbial activities (e.g., respiration, production of plant-growth regulators, antibiotics) and nutrient mobilization or immobilization. A major problem, however, in determining effects of earthworms on plant growth has been narrowing down which soil, plant or earthworm characteristics are the most important causal agent for the observed effects in a given situation.

A recent review of the literature (Brown *et al.*, 1999; chap. 2) revealed that *Pontoscolex corethrurus* was among the tropical earthworms most affecting plant yields. Other species that showed promissing effects on plant yields were of the *Dichogaster* and *Pheretima* (including *Polypheretima elongata*) genera. Yield enhancements tended to occur primarily with perennial plants, and in C-poor, sandy, and moderately acid soils. Leguminous plants were generally little affected by earthworm activities.

The state of Veracruz, is the most important in Mexico in terms of common bean (*Phaseolus vulgaris*) production. The coastal region, where most of the beans are planted, is also extensively inhabited by the peregrine tropical earthworms *P. corethrurus* and *P. elongata*. The former species is widely distributed throughout the state, while the latter has a more confined distribution, although both are most often encountered in disturbed or agricultural ecosystems (Fragoso, 1993).

This study was undertaken as part of the EU project "Conservation of soil fertility in low-input agroecosystems of the humid tropics through management of earthworm populations", which lasted six years (1990-1996; see Lavelle *et al.*, 1998). One of the major objectives of this project was to establish mechanisms by which earthworms affect soil fertility and the growth and development of agriculturally important plants, using traditional and novel techniques such as organic matter management and stable isotopes in field, laboratory and greenhouse earthworm manipulation and introduction experiments. The present experiment was performed to determine the relationships between two pantropical earthworms and common bean production in a sandy loam soil from South-Central Veracruz, Mexico.

## MATERIALS AND METHODS

The background concepts used to develop this experiment are derived from the work of Martin *et al.* (1992a,b) and Spain *et al.* (1992). To utilize natural abundance $\delta^{13}C$ labelling, soils and earthworms under $C_4$ plants were combined with a $C_3$ plant. This permits the tracing of changes in $\delta^{13}C$ of earthworm body tissues, indicating the source of their diets. For example a shift from low to high $\delta^{13}C$ signatures indicates the earthworm is feeding on high $^{13}C$-C materials. In a brief pilot study in the greenhouse both *P. corethrurus* and *P. elongata* survived well in the target soil and increased average plant heights, total plant biomass and bean pod weights over the no earthworm controls. Thus, a larger experiment was established, using the following methodology.

### Soil sampling and processing

Soil with a moderately high $^{13}C$ content ($\delta^{13}C$ = -19.1‰) was taken from a human-managed pasture derived from a savanna, with a mixture of both native and exotic $C_4$ pasture grasses (*Sporobolus jacquemonti* and *Setaria parviflora*, dominant), interspersed with isolated palm and hardwood trees and shrubs, located near La Víbora, Tlalixcoyan County, Veracruz (18°50' N 96°07' W). This pasture constitutes one of the few places in the country where native savanna-type vegetation has been conserved, and more than six native earthworm species have been found there (Brown *et al.*, unp. data). The soil, a kanhaplic Haplustalf (USDA) or a haplic Lixisol (FAO) with high smectite content and vertic characters in the B horizon, has a loamy sand surface horizon of variable depth (7-18 cm), and a very low C, N and plant-available nutrient content, especially P (Table 1; Annexe 2). Earthworm casts cover the soil surface for much of the year and the clay mineralogy (Annexe 3), especially of the surface horizon, has been intensely modified by biological activities (D. Dubroeucq, pers. comm.). Tlalixcoyan county is one of the important bean-growing regions in Central Veracruz (Salinas *et al.*, 1994), although the soil of the site was considered as having medium to low potential for bean production (Uresti and López, 1995). Topsoil (A horizon) was collected by digging and removing grass root clumps in October 1994, at the end of the rainy season, brought to the laboratory (at Xalapa), sieved at 5 and 2 mm and stored in burlap bags in the greenhouse.

### Earthworm sampling

Since *P. corethrurus* is not abundant and *P. elongata* is absent at the savanna-pasture, both were collected at other locations in the state. *P. corethrurus* was taken from a well studied $C_4$ grass pasture at 800 m above sea-level at Plan de las Hayas (19°43° N and 96°36'W) (Lavelle *et al.*, 1981), and *P. elongata* from an abandoned maize field used previously for another experiment, part of the same EU project (Patrón *et al.*, 1994), at the Centro de Investigaciones Costeras "La Mancha" (19°35' N and 96°23' W). All earthworms were brought to the laboratory and placed for several days in the savanna soil in large plastic containers, to permit re-adaptation before use.

### Experiment 1 (Photo 1)

The sieved soil was mixed vigorously and the equivalent to 12.5 kg (oven dry weight) was packed to a bulk density of approximately 1.2 g $cm^{-3}$, in 20 l white plastic buckets and watered to field capacity (pF 2.0), about 20% $H_2O$. Certified seeds of *Phaseolus vulgaris* (black bean: variety "Jamapa") were purchased locally and planted at the rate of 3 seeds $bucket^{-1}$. Earthworms were dried with paper towells and approximately 9-10 *P. corethrurus* (150 indiv. $m^{-2}$) and 3 *P. elongata* (50 indiv. $m^{-2}$), with total equivalent wet weights of 3.6-4.0 g (equivalent to around 60 g $m^{-2}$; gut contents included), were placed on the soil surface in the evening. Any earthworms not found to enter the soil overnight were replaced the following morning. Buckets were established with and without earthworms to assess the effect of each species on bean plant production. To measure the amount of C derived from bean plant presence, independent treatments were set up with each earthworm species but without bean plants. Thus, a total of 51 buckets were used for the following treatments:

1) Savanna soil with *P. corethrurus* (PONTO) from Plan de las Hayas (14 replicates) or *P. elongata* (POLY) from La Mancha (15 replicates) or without (CONT) earthworms (16 replicates), planted with beans;
2) Savanna soil with *P. corethrurus* only (3 replicates), and no bean plants (PONTO-NP);
3) Savanna soil with *P. elongata* only (3 replicates), and no bean plants (POLY-NP);

Table 1. Selected chemical properties of the A horizon of the kanhaplic Haplustalf from a savanna pasture near La Vibora, Veracruz, Mexico, its fertility rating according to Landon (1991), and the changes after passage through the intestines (casts) of *P. corethrurus* and *P. elongata* (data from the present experiment and from Barois *et al.*, 1999; Hernández, 1999; Patrón, 1998; Patrón *et al.*, 1999). n.d. = not determined; VL = Very Low; Def = Deficient; Adeq = Adequate. Values accompanied by different letters within the same row are significantly different at $P<0.05$. † = significantly different at $P<0.07$.

| | Control soil (A horizon) | Fertility rating | *P. corethrurus* casts | *P. elongata* casts |
|---|---|---|---|---|
| C (%) | 0.92c | VL | 1.10b | 1.83a |
| N (%) | 0.13a | Low | 0.15a† | 0.12a |
| NH$_4$ (ppm) | 33.1b | Adeq | 93.1a | 69.3a |
| NO$_3$ (ppm) | 7.6b | Low | 6.0b | 14.3a |
| C:N | 6.4b | Adeq | 7.6b | 15.2a |
| pH (H$_2$O) | 5.1b | Low | 5.41a | n.d. |
| Exch. Al (meq 100g$^{-1}$) | 0.13a | Low | 0.12a | n.d. |
| CEC (meq 100g$^{-1}$) | 11.7a | Low | 13.0a† | 13.7a† |
| K (meq 100g$^{-1}$) | 0.08a | Def | 0.06a | 0.07a |
| Ca (meq 100g$^{-1}$) | 7.8a | Adeq | 8.7a | 4.1b |
| Mg (meq 100g$^{-1}$) | 3.6b | High | 4.0b | 9.2a |
| Na (meq 100g$^{-1}$) | 0.15a | Adeq | 0.34a | 0.31a |
| P-resin (ppm) | 20.5a | Adeq | 30.2a | n.d. |
| P-bray (ppm) | 6.7a | Low | 10.7a | n.d. |
| P-total (ppm) | 61.4b | Low | 91.2a | n.d. |
| P-org (ppm) | 16.9b | Low | 49.8a | n.d. |
| P-inorg (ppm) | 43.7a | Low | 39.4a | n.d. |
| Mn (mg kg$^{-1}$) | 14.4b | Adeq | 22.8$^{a}$ | n.d. |
| Zn (mg kg$^{-1}$) | trace | Def | 0.02 | n.d. |
| Fe (mg kg$^{-1}$) | 118.7a | High | 117.3$^{a}$ | n.d. |
| Cu (mg kg$^{-1}$) | trace | Def | 0.09 | n.d. |
| Sand (%) | 81.8 | n.d. | 78.5 | 81.8 |
| Silt (%) | 7.5 | n.d. | 8.6 | 6.1 |
| Clay (%) | 10.7 | n.d. | 12.9 | 12.1 |

All the buckets were placed in a random order in a clear plastic-roofed greenhouse at UNCADER (Unión Nacional de Capacitación y Desarollo Rural), in the nearby city of Coatepec, Veracruz. A thermometer was placed 8 cm up from the bottom of one CONT bucket treatment and the soil and ambient air temperatures were recorded every few days. Plant heights and health were monitored every few days. After 14 d all extra bean plants remaining were removed, leaving only one plant for further measurements. After 30 and 62 d, 3 replicates of the PONTO, POLY and CONT bean plants were harvested, and one replicate each of PONTO-NP and POLY-NP buckets were taken down. After 97 d, when the bean plants were mature, the remaining replicates of all treatments were harvested. At each harvest date, the pots were cut in half and soil bulk density, moisture, $NO_3$ and $NH_4$-N (62 and 97 d only) and pH (KCl and $H_2O$), as well as plant height, root density, nodule presence, root and shoot biomass, pod and bean biomass, number of pods and beans (97 d only), earthworm wet and dry weights and numbers, and $\delta^{13}C$ of body tissues were measured. Roots were separated by manually sieving through the soil. Root density was measured following the methodology described in Brown *et al.* (2000c; chap. 5).

To prepare earthworm, plant and soil samples for $^{13}C$ analyses, each material was dried for 48 h at 60°C, ground manually with a mortar, passed through a 0.02 mm sieve, and stored in plastic vials. Isotopic analyses ($\delta^{13}C$) was performed on a high-precision (± 0.05‰) MICROMASS SiRA10 mass spectrometer coupled to a Carlo Erba NA 1500 elemental CHN analyser at the Laboratoire de Biogéochimie Isotopique at the University of Paris VI (Mariotti, 1991) and on a MICROMASS EA-CF Isochrom dual isotope ratio ($^{13}C$, $^{15}N$) mass spectrometer (precision ± 0.15‰ for $^{13}C$), coupled to a Carlo Erba 1108 CHNS-O elemental analyser at the Universidad Autónoma de Madrid. Bulk density was obtained by inserting a round metal cylinder of approximately 5.2 cm diam. by 5 cm deep into the soil surface. Soil mineral-N ($NO_3$-N & $NH_4$-N) was obtained by colorimetric methods detailed in the TSBF (Tropical Soil Biology and Fertility) handbook of methods (Anderson and Ingram, 1993). For $NO_3$, 10 g of moist soil were extracted with 0.5M $K_2SO_4$, shaken for 30 min, filtered using Whatman #42 paper, and the solution placed into plastic vials and stored in the refrigerator until analysis (within a few days). For $NH_4$, the 10 g of soil were extracted with 2M KCl. For pH measurements, 50 ml of $H_2O$ were added to 10 g of moist soil placed into a beaker, shaken intermitently for 30 min, and pH measured 1 h after the last shaking.

### Experiment 2

Small pots with approximately 1 kg (equivalent oven dry weight) of soil, moistened to 20% $H_2O$, were placed in the greenhouse at the Jardín Botánico of the Instituto de Ecología, A.C. Earthworms were added at the rate of 2 *P. corethrurus* (220 indiv. $m^{-2}$) and a single individual of *P. elongata* (110 indiv. $m^{-2}$) with equivalent weight of roughly 49 g $m^{-2}$. Four replicates of each earthworm treatment and the control (no earthworms) were established. Three bean seeds were planted and, after 7 d, *Rhizobium phaseoli* (Nitragin Co.) was inoculated with 100 ml of $H_2O$ poured onto the surface of each container. The purpose of this was to assess the role of earthworms in the dissemination and nodulation of the beans by the rhizobia. Throughout the 42 d of the experiment, plant heights, ambient temperatures, number of leaves, and presence of earthworm casts were noted. At harvest, the plant above ground portion was cut at the soil surface, the pot overturned and the soil removed to extract living earthworms and roots. Plant shoots and roots were dried (60°C) for 48 h and weighed. Earthworms were counted and weighed (fresh). Presence of nodules was recorded.

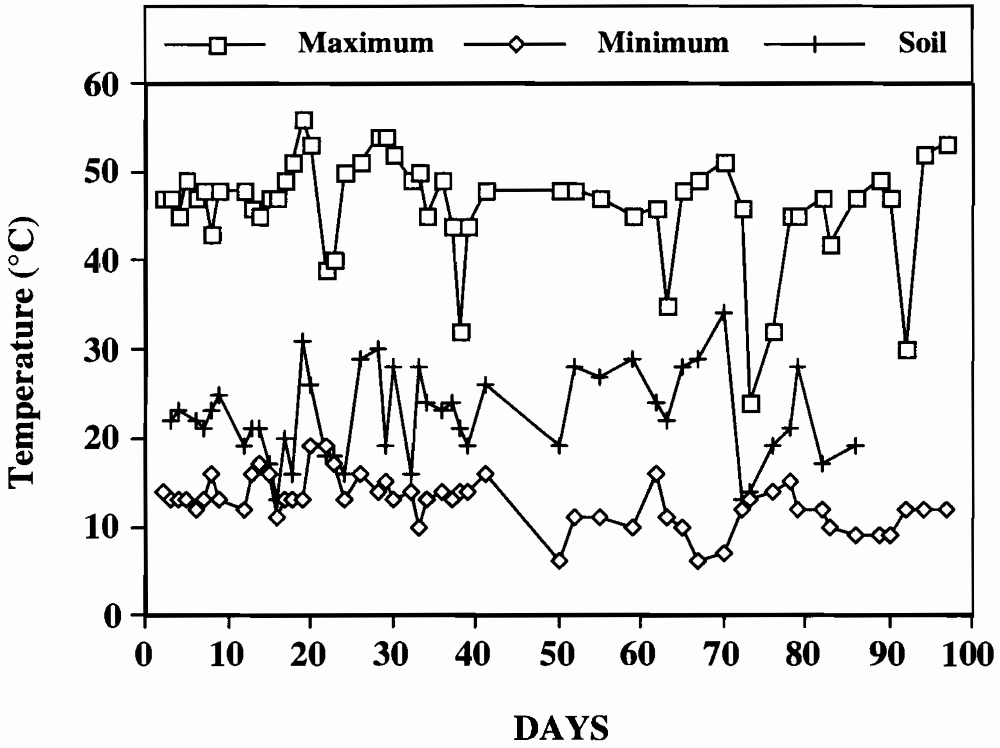

Figure 1. Ambient maximum and minimum temperatures in the greenhouse at UNCADER (Coatepec, Veracruz) and in the soil throughout the 97d of *Phaseolus* bean culture.

## RESULTS

### Experiment 1

Over the 97 d experimental period, temperature ranges in the greenhouse were extreme (Fig. 1), warming up during the day-time to consistently over 40°C, and at night cooling to around 15°C (the minimum measured was 7°C). Many clear days caused the greenhouse (which was closed with a clear plastic on the sides and on top) to heat excessively and, because no extracting fans were present, temperatures reached up to 56°C. Soil temperatures measured at various times during the day, however, did not exceed 34°C. Nevertheless, plant growth and earthworm activity under these conditions is not ideal, so the results given below indicate the reaction of these parameters to stressful conditions.

*Plant parameters*

Several pests affected the bean plants throughout the experimental period. Leaf miners were found on most plants, and lasted for several weeks. These were killed manually and, although damaged, the plants recovered well. A few plants were affected by bean mosaic virus, but one of them was able to reach maturity and produce beans. Of all the plants, only two were severely stunted (probably due to *Rhizoctonia solani*), and two died before the end of the experiment, for unknown reasons. No artificial *Rhizobium* inoculant was added to the seeds or soil, and only two roots were found to have nodules, but these were inactive, indicating that native *Rhizobia* spp. were inefficient at nodulating and fixing $N_2$ in the bean roots. Soil samples sent to Dr. Ken Giller at the Dept. of Biological Sciences, Wye College, Ashford (U.K.), confirmed the absence of *Rhizobia phaseoli* (K. Giller, pers. comm.). In a few pots, soil-dwelling coleoptera larvae of *Diabrotica* and *Elateridae* family were found (J. Villalobos, pers. comm.). These larvae are normally root feeders, and may have been damaging roots of some plants, although this was not investigated.

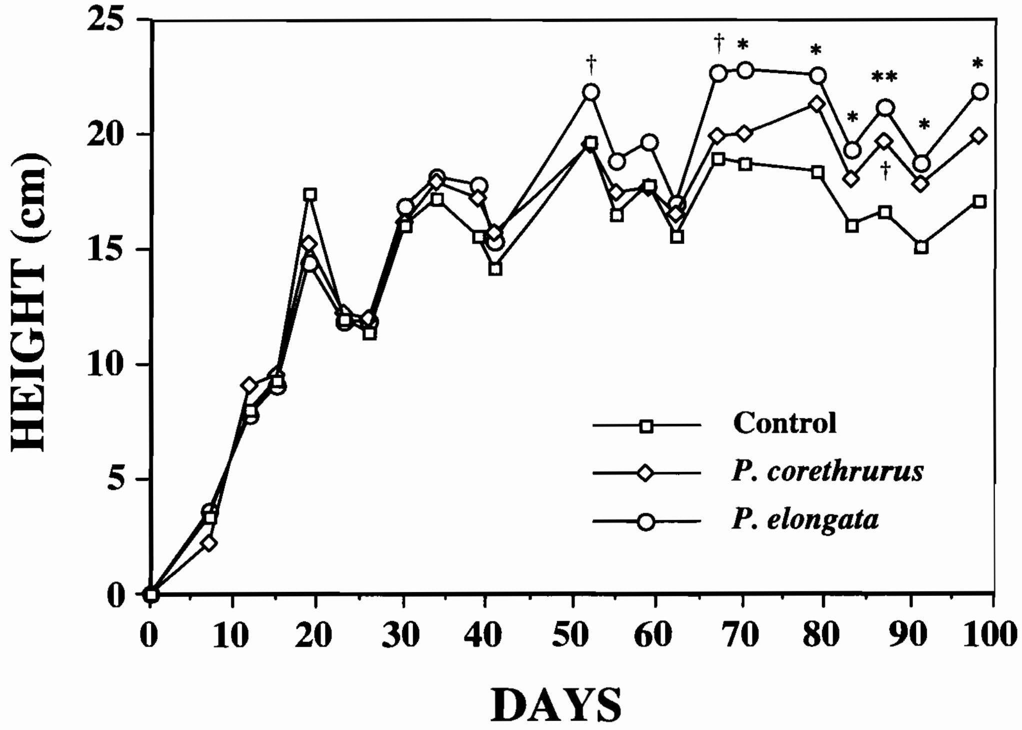

Figure 2. Average bean plant heights (cm) in the absence (Control) or presence of *P. corethrurus* or *P. elongata* (significant differences between earthworm and control treatments; * $p< 0.05$; ** $p< 0.01$; † = $p< 0.1$).

Heights of the bean plants over the experimental period (97 d) are shown in Figure 2. Treatments with POLY had plants significantly (at $p< 0.05$) taller than the CONT beans throughout the last 25 d of the experiment. Plants with PONTO were also taller than CONT for the same period, although these differences were not significant at $p< 0.05$. Compared with the plants grown in the pre-trial experiment, which grew to more than 80 cm in only 64 d, these plants clearly show the effects of extreme climatic variations on their growth (Photo 1). It is interesting that, despite these conditions, earthworm effects on plant production parameters were still evident. These effects were cummulative in nature, with highest biomass increases observed at the final harvest. Shoot biomass was on average 85% and 119% higher (significant; $p< 0.05$)

in PONTO and POLY at 97 d, respectively, while at 62 d, although slightly higher, the differences were not significant (at p< 0.05) (Fig. 3). ). Root biomass was 35% and 88% higher at 97 d for PONTO and POLY treatments, although only significant with POLY (p<0.001) (Fig. 3). Shoot/root ratios ranged from1.0-2.9, depending on the harvest date and treatment, and tended towards higher values with earthworms (62 and 97 d), although no significant differences between treatments were observed at any date.

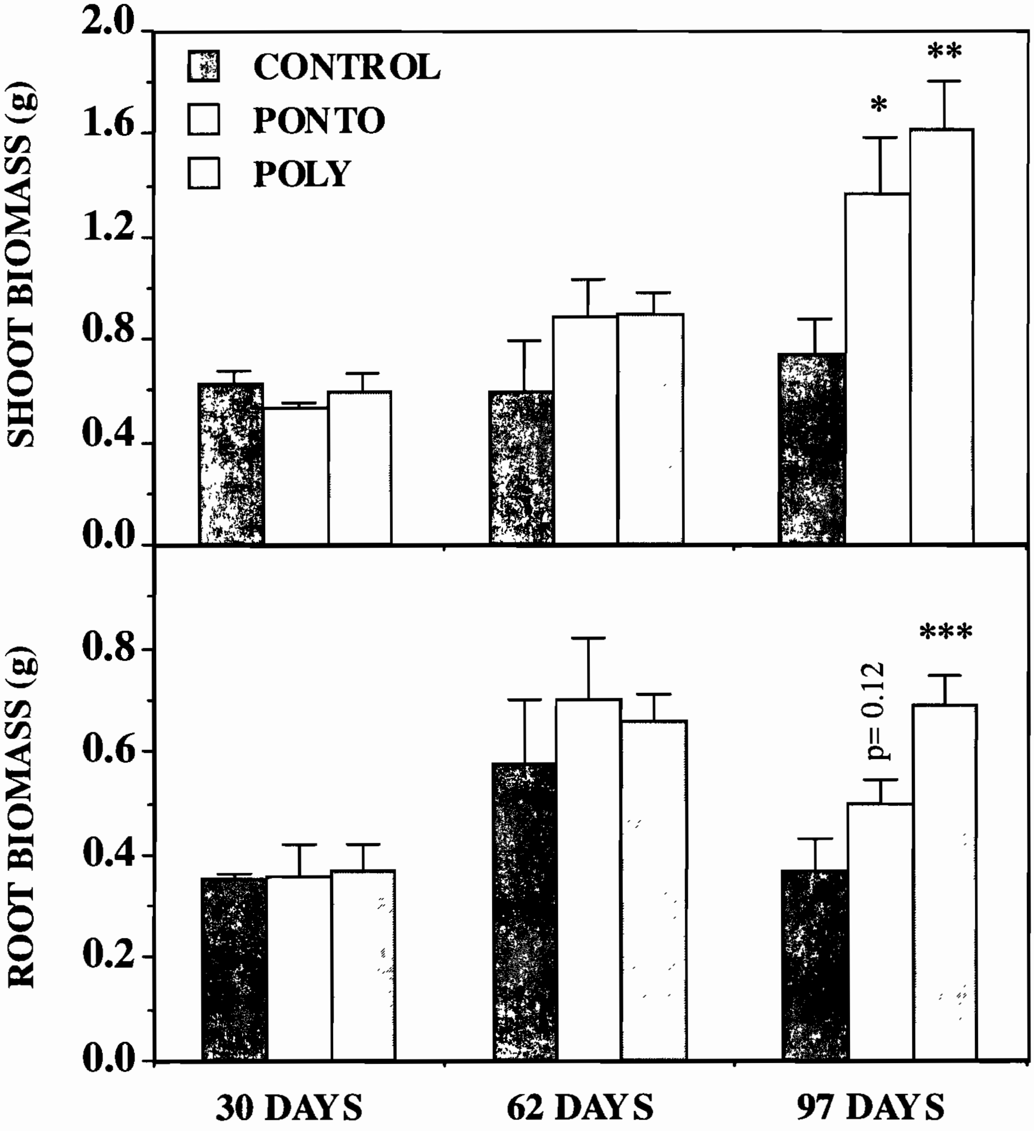

Figure 3. Shoot and root biomass (g pot$^{-1}$) of beans grown in the absence (Control) or presence of *P. corethrurus* (PONTO) or *P. elongata* (POLY) after 30, 62 and 97d (* = P< 0.05, *** = P< 0.001 for comparisons between earthworm and control treatments). Values shown are means + SE bars.

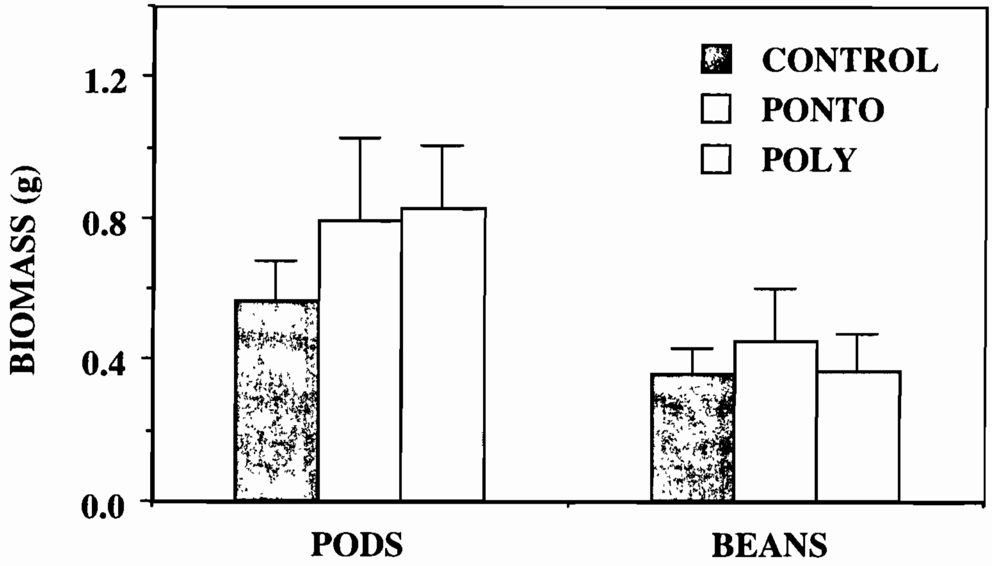

Figure 4. Biomass of pods and total weight of beans (g $pot^{-1}$) grown in the absence (Control) or presence of *P. corethrurus* (PONTO) or *P. elongata* (POLY) at the final harvest date (97d). Values shown are means + SE bars.

Root density (from Brown *et al.*, 2000c; chap. 5) was significantly higher than CONT in POLY pots in both the vertical and horizontal planes, but for PONTO this occurred only in the horizontal plane. Neither flowering date, nor pod set dates were significantly different due to earthworm presence. Thus, biomass of pods at the final harvest were not significantly greater due to earthworms, although there was a trend for higher yields in both PONTO and POLY (Fig. 4). The number of pots that produced pods and beans were 6 (of 8) in PONTO, 8 (of 9) in POLY and 7 (of 10) in CONT, respectively. Bean grain dry weights were not significantly different between treatments. Bean grain and pod P and N content were not significantly different, although the total N uptake was significantly higher in both PONTO and POLY than in CONT. Too few replicates were available to permit valid statistical comparisons of P uptake by the plants.

When the final biomass of *P. corethrurus* was related to bean biomass (in pots where earthworms were still alive and beans were found at harvest), a significant positive linear relationship was observed ($n = 4$; $r = 0.96$; $P< 0.05$). No relationships were found with *P. elongata* biomass. On the other hand, when all pots were included, a negative relationship was observed between *P. elongata* biomass and bean numbers ($r = 0.7$; $P< 0.05$), weights ($r = 0.75$; $P< 0.04$) and shoot/root ratios ($r = 0.7$; $P< 0.04$), and a positive relationship with root biomass ($r = 0.72$; $P< 0.03$). No relationship was found between any plant parameter and *P. corethrurus* biomass.

### *Soil parameters*

Results of various soil properties at each harvest date are shown in Table 2. No differences between treatments were found in bulk density at any date. However, % $H_2O$ in both top (0-10 cm) and bottom (10-25 cm) horizons were significantly lower in POLY than in CONT or PONTO treatments at the final harvest (97 d). As for pH, only once were higher values found due to earthworms (POLY, 97 d). pH was higher at 62 d and lower at 97 d. Although no differences in either $NO_3$ or $NH_4$ were found between the treatments at both dates measured (62 and 97 d), mineral-N concentrations in the upper horizon were generally higher in both CONT and PONTO than in POLY treatments, and in the lower horizon, higher in POLY and PONTO than in CONT treatments. Earthworm castings, obtained from laboratory cultures in a separate experiment (Hernández, 1999), had higher nutrient contents than the control soil (Table 1). The most dramatic differences were observed for $NH_4$-N and organic P in *P. corethrurus* casts and Total C, C:N, $NH_4$-N and $NO_3$-N in *P. elongata* casts.

### *Earthworm survival and $\delta^{13}C$*

Despite the high soil and ambient temperature variations, both species were able to survive and *P. corethrurus* reproduced on some occasions, evidenced by presence of cocoons (particularly in pots with no plants). No *P. elongata* cocoons were found in any of the pots at any time, even though adult worms were collected on various occasions, indicating sub-optimal breeding conditions. When plants were present there appears to have been a competition between the earthworms and plants for soil $H_2O$ near the final harvest. With bean plants, the activities of both species were mostly limited to the bottom 10 cm of the buckets. When plants were not present, not only were they found to be active over a greater portion of the bucket soil volume but also to maintain higher biomass at most sampling dates (Fig. 5). Trends in biomass loss by PONTO and POLY as well as those of POLY-NP were similar (linear regressions, $r = 0.99$, 0.90 and 0.91, respectively). An estimated 49, 84 and 60% of the initial biomass were lost in these treatments by the final harvest. Even if 100% of this biomass was mineralized over the experimental period, this would equal an addition of about only 6.4 mg $kg^{-1}$ N, a small value compared with that of available N in the soil.

Body tissue $\delta^{13}C$ signatures were not significantly different between dates for either PONTO or POLY (Fig. 6). At each harvest date, however, *P. corethrurus* $\delta^{13}C$ signatures were significantly lower than those of the original worms ($\delta^{13}C$ = -15.1‰), indicating that they had assimilated lower $^{13}C$-carbon. Nevertheless, both in presence and absence of bean plants, $\delta^{13}C$ signatures of PONTO and POLY were not significantly different, indicating the earthworms were not feeding on the lower $^{13}C$ bean-plant derived C. Thus, the lower $\delta^{13}C$ signatures observed in PONTO were likely due to assimilation of the soil-C ($\delta^{13}C$ = -19.1 ± 0.3‰). Despite this assimilation, $\delta^{13}C$ signatures were still higher than in the bulk soil, indicating a slow assimilation rate of new C (soil-C) or that the soil presents higher $^{13}C$ food sources in some fractions the earthworms were feeding on (Brown, 2000a; chap. 7; Annexe 2).

Table 2. Soil moisture, pH, and mineral N ($NO_3$, $NH_4$) in the upper (A = 0-10 cm) and lower (B = 10-15 cm) horizons of buckets with or without (Control) addition of *P. corethrurus* or *P. elongata* after 30, 62 and 97 d of bean culture in a sandy loam Inceptisol from La Vibora, Mexico. Different letters within each column indicate significant differences within sample dates and depth (A, B). n.d. = not determined.

| TREATMENT | | $H_2O$ (%) | | | pH ($H_2O$) | | | $NH_4$ (ppm) | | | $NO_3$ (ppm) | | | Mineral-N | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 30 d | 62 d | 97 d | 30 d | 62 d | 97 d | 30 d | 62 d | 97 d | 30 d | 62 d | 97 d | 30 d | 62 d | 97 d |
| Control | A | 6.3a | 5.2a | 3.9a | 4.8a | 5.4a | 4.4a | | 20.8a | 17.0a | | 17.0a | 23.5a | | 37.8 | 39.5 |
| | B | 7.2a | 6.5a | 5.8a | 4.8a | 5.4a | 4.4a | n.d. | 15.4a | 16.7a | n.d. | 18.7a | 28.9a | n.d. | 34.1 | 39.2 |
| *P. corethrurus* | A | 6.3a | 4.6a | 2.9b | 5.0a | 5.3a | 4.4a | | 23.9a | 18.0a | | 14.1a | 21.9a | | 38.0 | 39.9 |
| | B | 8.2a | 6.7a | 4.4b | 4.9a | 5.3a | 4.1a | n.d. | 21.4a | 25.7a | n.d. | 22.8b | 29.5a | n.d. | 44.2 | 54.4 |
| *P. elongata* | A | 6.7a | 4.6a | 2.6b | 5.0a | 5.4a | 4.4a | | 14.8a | 9.1a | | 14.3a | 24.3a | | 29.0 | 33.4 |
| | B | 7.5a | 5.9a | 3.9b | 4.9a | 4.9a | 4.9b | n.d. | 22.2a | 17.9a | n.d. | 17.3a | 25.2a | n.d. | 39.6 | 44.2 |

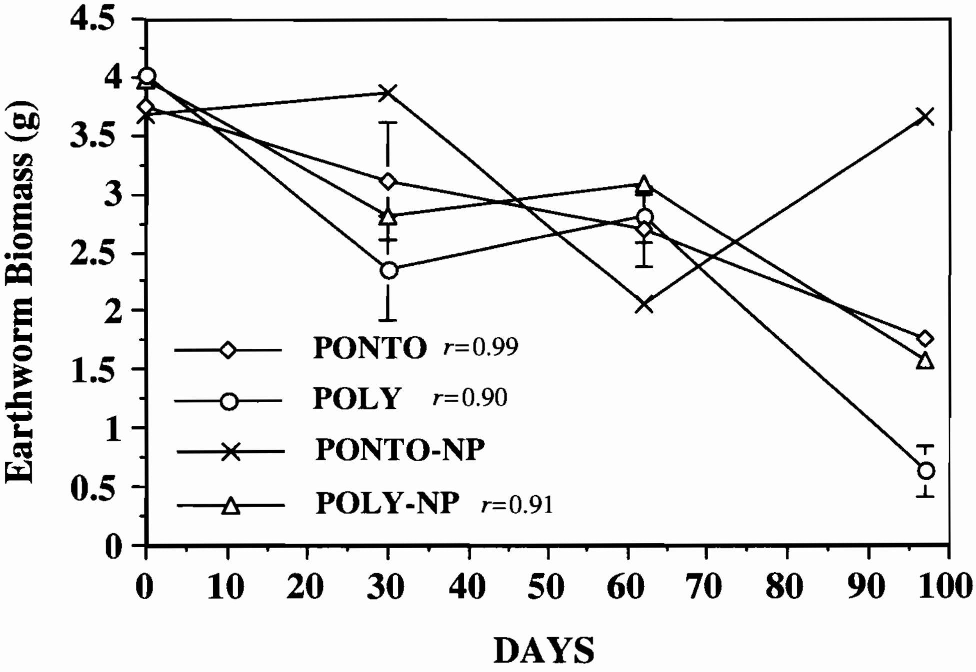

Figure 5. Biomass (means ± SE) of *P. corethrurus* (PONTO) and *P. elongata* (POLY) in the presence and absence (-NP) of bean plants at each harvest date (30, 62 and 97d). Linear regression (*r*) values of mass losses are displayed.

**Experiment 2**

All but two earthworms and one plant survived to the final harvest. Both *P. elongata* and *P. corethrurus* biomass decreased an average of 60%. Temperatures in the greenhouse were more ammene than those at UNCADER; mean maximum and minimum temperatures were 24° and 15°C, respectively, with absolute maxima of 38°C and minima of 9°C. Nevertheless, growth of the beans was only slightly better initially, and plant heights of both CONT and POLY at the final harvest (42 d) were lower than beans grown under the extreme conditions at UNCADER (Table 3). Plants in the PONTO treatments, on the other hand, grew taller than those in the same treatments at UNCADER, and were consistently taller than the other treatments throughout the experimental period (data not shown). Biomass of both roots and shoots at harvest, however, were not significantly different between treatments, although a clear trend for both higher shoot biomass and shoot:root ratio was seen in the PONTO treatment (Table 3). No nodules were found on any plants, indicating unsuitable conditions for *Rhizobium* activity in the soil and nodule formation on roots.

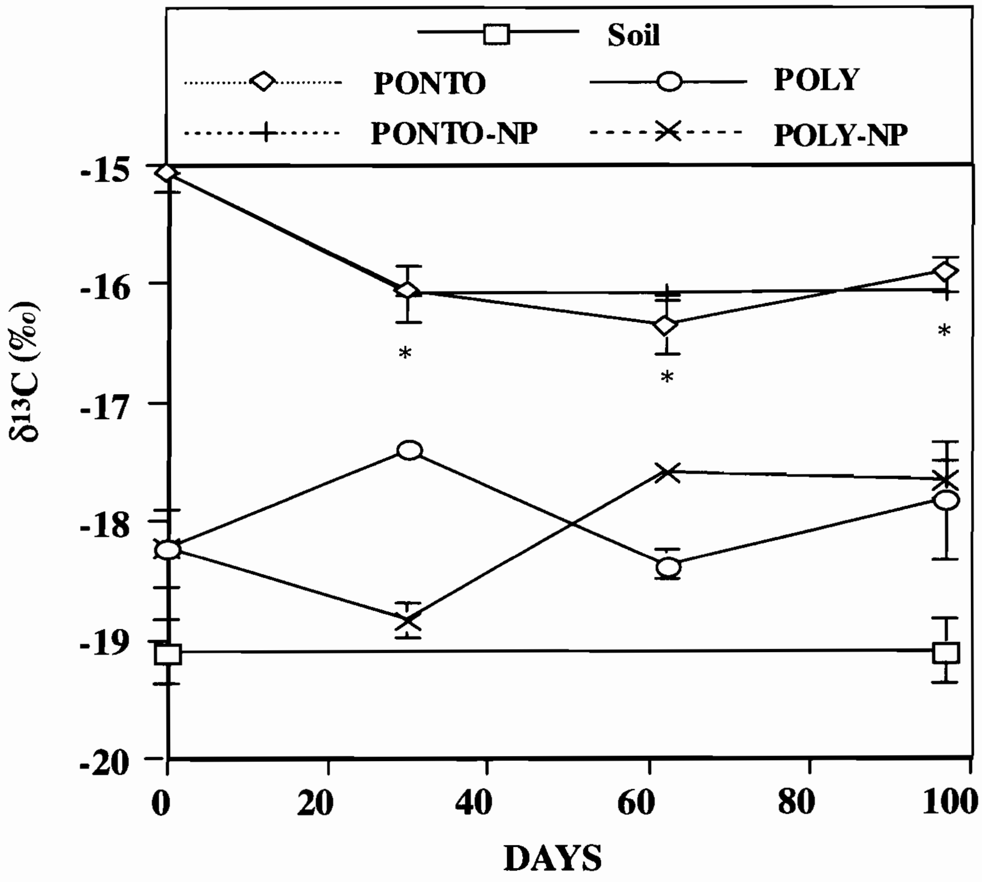

Figure 6. $^{13}C$ values (means ± SE, in δ‰) of the savanna-pasture soil and of *P. corethrurus* (PONTO) and *P. elongata* (POLY) in the presence and absence (-NP) of bean plants at each harvest date (30, 62 and 97d). Asterisks (*) denote significant differences ($p < 0.05$) between PONTO $\delta^{13}C$ values at the beginning and each harvest date.

Table 3. Plant heights, shoot and root biomass and shoot:root ratios of *Rhizobium phaseoli*-inoculated *Phaseolus* beans after 42 d growth in small pots in the greenhouse, in the absence (CONT) or presence of *P. corethrurus* (PONTO) or *P. elongata* (POLY). Different letters within a same column mean significant differences between treatments at $P < 0.05$.

| TREATMENT | Plant height (cm) | Shoot Biomass (g) | Root Biomass (g) | Shoot:Root |
|---|---|---|---|---|
| CONT | 10.1b | 0.116a | 0.054a | 2.13a |
| PONTO | 18.7a | 0.166a | 0.056a | 3.07a |
| POLY | 12.9ab | 0.107a | 0.041a | 2.66a |

## DISCUSSION

Of the more than 100 publications addressing various aspects of the relationship between earthworms and plant growth (Brown *et al.*, 1999; chap. 2; Brown, 2000b; chap. 2; Blakemore and Temple-Smith, 1995), most are devoted to cereal crops or pasture grasses; only a few deal with leguminous plants. Of these, even fewer involve legume grain crops; only eight experiments from temperate and tropical regions (4 in each, 2 unpublished) were found in an exhaustive literature review.

Using various lumbricid earthworms, authors in the temperate regions observed a 90% increase in garbanzo bean production (Kashnitz, 1922), a small and not significant increase in lima bean production and a 248% increase in soybean shoot biomass (Hopp and Slater, 1949), a 39% decrease in shoot biomass in artificial cultures of peas (van Rhee, 1965), and no effects on faba bean production in three soil types (Doube *et al.*, 1997). In Yurimaguas, Peru, Pashanasi *et al.* (1996) observed only slight and not significant decreases in grain (-5%) and root (-14%), and a 17% increase in shoot biomass in a single crop cycle of cowpeas grown in 0.28 $m^2$ field enclosures inoculated with *P. corethrurus* with or without added crop residues. When the widespread exotic endogeic *Amynthas* sp. earthworms were inoculated into 1 $m^2$ field enclosures at Guarapuava, Brasil, no significant effects were found on *Phaseolus* bean grain or shoot biomass (dos Santos, 1995). Nearby, two adjacent fields, with and without a naturally present *Pheretima* (or *Amynthas*) sp. earthworm population, soybean yields were 51, 47, and 22% higher over a consecutive 2.5 y period in the field with earthworms (Peixoto and Mariochi, 1994). Finally, when several species native to Lamto, Ivory Coast, were inoculated into buckets placed in the open-air, a significant decrease in peanut grain (-20%) and root biomass was observed with several of the species, but with no significant effects on shoot biomass (Derouard *et al.*, 1997).

Compared with the experiments involving grass grain crops, which generally led to important increases in production, results with legumes show mostly null or negative effects of inoculated earthworms (with a few exceptions). Several reasons may account for this phenomenon. Perhaps the wide-spread mutualistic association of these plants with *Rhizobia* increases their independence for N supply, reducing the possibility of earthworm-induced increased N-supply in soil as a mechanism for plant growth promotion. In addition, the inoculation of earthworms into fertile soils, generally tends to reduce their potential effects on plant production (Brown *et al.*, 1999; chap. 2), due to reduced nutrient limitations to plant growth. The present experiment, undertaken with a highly nutrient-poor soil, confirms this trend. Given the absence of *Rhizobium phaseoli* nodulation in roots in both experiments, the higher shoot and root biomass are likely due to both higher N and probably P availabilities induced by earthworm activities within the pots. Although not measured throughout the experiment these could be deduced by the higher nutrient contents in casts of both species (Table 1; also Brossard *et al.*, 1996; Lavelle *et al.*, 1992; López-Hernández, 1993; Patrón, 1998), and in soil mineral-N concentrations in the lower part (>10cm) of the pots with both earthworms. On many instances, roots were found to grow within earthworm burrows. Root density, higher in soils with both earthworm species (Brown *et al.*, 2000c; chap. 5) could also be responsible for better plant nutrition and yield promotion. Finally, both earthworm species could also be increasing root colonization by mycorrhizae (*Phaseolus* beans are mycorrhizal), and these could be a major factor influencing both the uptake of nutrients and the final plant biomass (Guzmán-Plazola *et al.*, 1993) in this experiment, although this was not measured. *P. corethrurus* has been shown to affect mycorrhizal colonization and biomass of a pasture grass (*Brachiaria decumbens*) grown in the same soil used for this experiment (Brown *et al.*, 2000d; chap. 6). Nothing is known of the capacity of *P. elongata* to affect mycorrhization of plant roots.

Nonetheless, despite better root and shoot production, bean grain biomass was not significantly affected by either earthworm species, and a negative relationship was observed between bean grain and final *P. elongata* biomass at the final harvest. Reasons for these observations are uknown, but could be related to changes in the transfer of photosynthates and nutrients into the reproductive portions of the plants, induced by the earthworms. Further research on the mechanisms behind these effects is necessary.

Compared with the expected growth and yield of this variety under field conditions in Veracruz (Salinas *et al.*, 1994), flowering dates were at least two weeks later and bean production was low, approx. 10-20x less. This is likely due to the late planting date (early November vs. early September to mid-October), stressful soil (poor nutrient contents) and greenhouse conditions (temperature extremes) to which the plants and earthworms were subjected in the present experiment. Soil P-status, very low in the savanna-pasture soil, is especially important in determining bean yields (Fageria, 1989; Fageria *et al.*, 1997). Under more amene climatic conditions, or by taking soil with a higher potential for bean production (Uresti and López, 1995) higher yields and more pronounced differences would probably have been observed between earthworm and control treatments.

No effect of earthworms on bean nodulation by *Rhizobia* was observed, and the few nodules found in the first experiment (without inoculation) were inactive. Common bean associations with rhizobia are generally difficult to establish (Graham, 1981), and N fertilization is often recommended (Salinas *et al.*, 1994). In the present case, the low P status of the soil, as well as its low pH, probably had an important effect on *Rhizobium* inoculation potential, although varietal and/or genetic incompatabilities cannot be discounted.

The important amounts of N in legume roots, and the high additions of N into the soil by these plants, is often attributed to increased earthworm populations in the field (Hopp, 1954; Kladivko and Timmenga, 1991). Thus, earthworm biomass recovered at the end of each experiment would have been expected to increase. Nevertheless, the opposite was found due to less than ideal conditions for reproductive activities and growth. In the present experiment and in long-term laboratory cultures (Barois *et al.*, unp. data), *P. elongata* did not reproduce in this soil (Annexe 7), although *P. corethrurus* did well (Annexe 5) and in a few cases (particularly when no plants were present), cocoons were collected.

No measurable rhizosphere feeding was detected for either species using stable isotopes in the present study. These results are in contrast to those of Brown *et al.* (2000b; chap. 4; 2000d; chap. 6) Spain *et al.* (1990) and Spain and Le Feuvre (1997) who showed preference of *P. corethrurus* for the rhizosphere of three $C_4$ grasses: sugarcane, maize and *Brachiaria decumbens* pasture. As with previous studies, $\delta^{13}C$ ratios of earthworms were always a few $\delta$ units higher than the soil they were feeding on (Martin et. al, 1992a;b). This may be because the earthworms were not feeding on bulk soil, but selecting different particle size fractions of the soil which contain different $\delta^{13}C$ ratios than those of the bulk soil (Balesdent, 1991), or due to preferential discrimination for $^{13}C$ in earthworm C assimilation. The *P. corethrurus* in this experiment had a higher $\delta^{13}C$ signatures than the soil used, and thus changes in tissue $\delta^{13}C$ signatures were observed. Nevertheless, these were more likely due to soil $^{13}C$ and not to plant $^{13}C$, since no differences were observed between $\delta^{13}C$ of earthworms in the presence or absence of bean plants. *P. elongata* do not seem to be preferential rhizosphere grazers. Another experiment (Brown *et al.*, 2000b; chap. 4), using maize, also did not detect important rhizosphere nutrient (in this case of $^{15}N$) assimilation by this species, indicating that it is probably more of a bulk soil organic matter feeder, and also does not feed on the soil microbial biomass.

The present experiment highlights the important role of earthworms in stimulating plant production, including leguminous grain crops, in nutrient poor soils. The way in which earthworms affect plant growth are numerous and often multiple mechanisms operate simultaneously. By producing burrows covered with nutrient rich mucus and castings that act as nutrient mineralization hot-spots, or foci of increased nutrient availability within soils, earthworms can modify root distribution and density, and hence plant nutrition and yields. When active in the rhizosphere, an even greater enhancement of plant yields may be expected, since their beneficial activities are in closer contact with plant roots. Further experiments should assess the extent of rhizosphere activities of different earthworm species, as well as look into additional mechanisms of plant growth effects, including physical, chemical and especially, microbiological, where interactions with earthworms may be particularly important (Brown *et al.*, 2000e).

Photo 1. Vue d'un pot utilisé dans l'experimentation avec les harricots (chap. 3).

Photo 2. Vue d'un pot avec litière superficielle et une plante de maïz dans le experiment avec l'apportation de litière marqué avec $^{15}N$ (chap. 4).

# CHAPITRE 4

# EARTHWORM (*PONTOSCOLEX CORETHRURUS*: GLOSSOSCOLECIDAE) EFFECTS ON MAIZE PRODUCTION AND DISTRIBUTION OF $^{15}$N FROM LABELLED RESIDUES[§]

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## SUMMARY

Earthworm (*Pontoscolex corethrurus*) effects on maize production was assessed under greenhouse conditions using both large and small pots, residues and four different harvest dates (37, 54, 131 and 183 d). A clayey Andosol rich in C and N, taken from a tropical rainforest with predominance of $C_3$-type plants ($\delta^{15}N = 6.0‰$; $\delta^{13}C = -27.3‰$) and surface-applied $^{15}$N-labelled residues (3.68 atom% $^{15}$N; $\delta^{15}N = 9123‰$) were used to trace changes in earthworm $\delta^{13}$C and the fate of N derived from the residues. Laboratory cultures of *P. corethrurus* produced castings to study nutrient enrichments over uningested soil. In the large experiment, maize grain production was slightly lower (without residues) and higher (with residues) than local yields in low-input systems. Recovery of the $^{15}$N from residues in the plants was high (>40% on average), indicating a rapid recycling of the residue N into growing plants. In the absence of maize plants, most (>70%) of the residue $^{15}$N was recovered in the soil; $\delta^{15}$N signatures were higher than background (unlabeled soil) signatures and than in soil from pots with plants, throughout the 30 cm pot depth. Earthworms decreased significantly the residue N and $^{15}$N stocks but had little effects on plant production parameters or nutrient uptake. Soil enrichment in the castings and soils from the pots was also lower than expected (when compared with other, especially C- and N-poor soils), except for mineral N and exchangable Ca and K. The only significant effects of earthworms on plants were a lower above-ground biomass and higher root densities in treatments with earthworms and residues at the final harvest, and higher root densities in treatments with *P. corethrurus* only (no residues) at 131 d. The number of large VAM spores was significantly increased by earthworm presence, although no differences were found in root infection by VAM. The small effects of earthworms on maize under these experimental conditions are likely due to the soil's richness, resulting in a greater independence of the plants for nutrients. The presence of plants, on the other hand, had a significant effect on earthworm nutrition; after 183 d, about 8% of tissue C was derived from maize, and the presence of plants increased the fraction of N derived from residues in worm tissues by almost 25 times (up to nearly 1%). Residues increased earthworm survival rates from 26% to 85%. Bulk soil $\delta^{13}$C increased at the end of the experiment, probably due to the large rhizodeposition typical of maize culture. *P. corethrurus*

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[§] Submitted to *Soil Biology and Biochemistry* appears to derive impotant proportions of its diet, particularly N, from plants, associated with rhizosphere feeding activities.

## INTRODUCTION

The role of earthworms in modifiying soil characteristics and plant production is by now well recognized. Recent reviews (Blakemore and Temple-Smith, 1995; Brown *et al.*, 1999; chap. 2), describe how the many experiments undertaken since the beginning of the 20th century, have led to generally positive effects on plant production, especially the above-ground portions. In tropical regions, shoot yields were mostly enhanced in C-poor, sandy and moderately acid soils, and perennial plants had the highest growth promotions (Brown *et al.*, 1999; chap. 2). Most of the experiments with maize have been performed in the tropics: average shoot and root biomass increases due to earthworms were 13% (not significantly different than controls), while grain yield increases were >3x higher (42%) (Brown *et al.*, 1999; chap. 2). Moderate (20-40%) and agriculturally important (>40%) grain production increases (maize+other grain crops), respectively, were obtained with earthworm biomasses above 17 and 32 g $m^{-2}$ (using a curvilinear relationship). The pantropical geophagous endogeic earthworm *Pontoscolex corethrurus* was shown to be a particularly good promoter of plant production in a variety of instances, although under certain conditions, its activity could also lead to decreased yields (Brown et al., 1999; chap. 2).

Despite this apparent wealth of knowledge of earthworm effects on plant production, detailed descriptions of the mechanisms for observed earthworm effects on plants are rare. Earthworms modify many of the soil properties which can determine plant growth rates and characteristics, and thus several mechanisms may be operating at the same time. It is believed that the extent of earthworm interaction with the rhizosphere may be an important mechanism, yet few experiments have addressed this issue. Brown *et al.* (2000a; chap. 3) found few relationships between two tropical earthworms and common bean (*Phaseolus vulgaris*) rhizosphere while Spain *et al.* (1990) and Spain and Le Feuvre (1997) have proposed the preference of the *P. corethrurus*, for sugarcane rhizosphere as a habitat for their feeding activities. Other earthworm species (particularly some lumbricids) may feed on roots (Carpenter, 1985; Cortez and Bouché, 1992; Sackville-Hamilton and Cherret, 1991), although preferential rhizophagy (unlikely) and subsequent effects (if any) on above-ground biomass have yet to be observed. Perhaps earthworms feed on dying roots thus aiding plant sanitation, and maybe this root herbivory even causes compensatory below-ground growth, although this has also yet to be documented. In any case, what has been most apparent is that presence of earthworms generally leads to an enhancement of shoot:root ratios, supporting the hypothesis that plants are investing more energy in above-ground (especially fruit or grain) growth (Brown *et al.*, 1999; chap. 2). This generally occurs when plants are healthier and able to absorb more essential elements and water from the soil (Fitter, 1985; Lavelle and Spain, 1999).

Thus, given the high potential of *P. corethrurus* for affecting plant yields, and its possible preference of rhizosphere-C as nutrient source, the following experiments were set up to:

1) assess *P. corethrurus* activity in maize rhizosphere and the consequent effects on maize above and belowground production;

2) determine the increase of plant available nutrient content in *P. corethrurus* casts and worm-worked soil;
3) study the role of *P. corethrurus* in maize residue-derived N translocation into the soil and growing plants;
4) determine the amount of C derived from growing maize plants, and the amount of N from residues assimilated by the earthworms.

Several harvest dates and two pot sizes were used to assess temporal changes in the effect of earthworms on plants and difference in container size. Natural abundance $^{13}C$ labelling and $^{15}N$ isotope enrichment were used to trace changes in earthworm tissue and soil $\delta^{13}C$ and $\delta^{15}N$, and effects on plant production parameters.

## MATERIALS AND METHODS

### $^{15}N$-labelled maize residue production

A sandy loam nutrient-poor Alfisol, was collected from the A horizon (0-10 cm) of an abandoned maize field used for a previous experiment (Patrón *et al.*, 1994), at the Centro de Investigaciones Costeras "La Mancha", Veracruz, sieved at 2 mm, and stored in burlap bags. After mixing well, 8 kg (equivalent oven dry weight) soil were placed in four 20 l plastic buckets, watered to field moisture capacity (12% $H_2O$) and the buckets placed in a greenhouse at the Jardin Botánico of the Instituto de Ecología, A.C. The equivalent to 156 kg ha$^{-1}$ of $^{15}N$-labelled (7.5 g of 10.5 atom% $^{15}N$ $KNO_3$) and 52 kg ha$^{-1}$ of unlabled ($^{14}N$ DAP = di-ammonium phosphate) N-fertilizer were applied in three separate and equal doses (15, 35 and 55 d). A total of 52 kg ha$^{-1}$ P and 418 kg ha$^{-1}$ K were also addded to each pot. Certified maize seeds (variety V-530) were purchased locally and four seeds planted in each bucket. After 15 d, the smaller plants were removed, leaving only the largest. Plant heights and ambient maximum and minimum temperatures were recorded periodically. Plants were watered as needed, and after 98 d the maize shoots from each pot were harvested, dried (60°C), weighed, broken into 5-15 cm long pieces and mixed thoroughly with one another. The residues were placed in brown paper bags and stored until used for the large experiment (below).

### Soil sampling and processing

Soil naturally low in $^{13}C$ was taken from a tropical rainforest at the Estación de Biología Tropical "Los Tuxtlas", located near the Gulf of Mexico in the Southern part of Veracruz (18°35' N and 95°04' W), at an altitude of about 180 m. The vegetation is predominantly of the $C_3$ photosynthesis type (Estrada *et al.*, 1985). This is an endangered ecosystem that supports many native plant and animal species including some eight endemic earthworms (Fragoso, 1997), as well as the peregrine *P. corethrurus*. Given the heavy rainfall (>4500 mm year$^{-1}$), the soil is never dry, the earthworms are active throughout the year and the clay mineralogy, especially of the surface horizon, indicates intense influences on soil properties and processes resulting from their activities (D. Dubroeucq, pers. comm.; Annexe 9). The soil is a dark red-brown (ferric) deep (>2 m) Andosol with several buried horizons, due to previous volcanic ash depositions. Several soil physico-chemical properties are given in Table 1 (Annexe 8). The texture of the surface horizon is clayey with an equally high silt content (~40%), and rich in organic matter (9.3%), N (0.46%), and several major plant nutrients. Surface litter was removed and the underlying topsoil (0-10 cm; $A_1$+$A_2$ horizons) collected in May and September 1994, stored in burlap bags, and later sieved at 5 and 2 mm. In this process all rocks, large roots, native earthworms and other large soil organisms (primarily ants, termites and diplopods) were removed.

Table 1. Selected chemical properties of the A horizon of an Andisol from the tropical rainforest at the Estación de Biología Tropical "Los Tuxtlas, Veracruz, Mexico, and their changes after passage through the intestines (casts) of *P. corethrurus* (data from the present experiment and from Barois *et al.*, 1999; Hernández, 1999). Fertility classes determined according to Landon (1991): n.d.= not determined; Med = Medium; Adeq = Adequate. Values accompanied by different letters within the same row are significantly different at $P<0.05$. † = Significant difference at $P<0.1$.

| | Forest soil | Fertility class | *P. corethrurus* casts |
|---|---|---|---|
| C (%) | 5.76b | Med | 6.80a |
| $\delta^{13}C$ (‰) | -27.3 | n.d. | n.d. |
| N (%) | 0.51b | High | 0.59a |
| $\delta^{15}N$ (‰) | 6.0 | n.d. | n.d. |
| $NH_4$ (ppm) | 48.6b | Med | 152.5a |
| $NO_3$ (ppm) | 134.2b | High | 472.6a |
| C:N | 11.4a | Adeq | 11.6a |
| pH ($H_2O$) | 5.87a | Med | 5.79a† |
| pH (KCl) | 5.3 | n.d. | n.d. |
| Exch. Al (meq $100g^{-1}$) | 0.13a | Low | 0.12a |
| CEC (meq $100g^{-1}$) | 30.3b | High | 38.3a |
| K (meq $100g^{-1}$) | 0.56b | High | 0.76a |
| Ca (meq $100g^{-1}$) | 23.4a | High | 30.6a† |
| Mg (meq $100g^{-1}$) | 6.0a | High | 6.3a |
| Na (meq $100g^{-1}$) | 0.21a | n.d. | 0.16b |
| P-resin (ppm) | 18.6a | Adeq | 18.7a |
| P-bray (ppm) | 6.7a | Low | 6.2a |
| Zn (ppm) | 10.1b | Adeq | 10.9a |
| Cu (ppm) | 5.0a | Adeq | 4.9a |
| Mn (ppm) | 46.7a | Adeq | 48.2a |
| Fe (ppm) | 303a | High | 283a |
| Sand (%) | 20.7a | n.d. | 17.3b |
| Silt (%) | 37.4a | n.d. | 31.8b |
| Clay (%) | 41.9b | n.d. | 50.9a |

## Earthworm sampling

*P. corethrurus* is not very abundant at the "Los Tuxtlas" station, and only about 120 individuals were recovered, enough for the experiment using $\delta^{13}C$ labelling. It is not native to the forest but has invaded from the neighboring cattle pastures and cropping systems (esp. maize) that are replacing the forest. The remaining earthworms for the large pot experiment ($^{15}N$-labelling) were therefore taken from a well studied $C_4$ grass pasture at 800 m above sea-level at Plan de las Hayas, Veracruz (Lavelle *et al.*, 1981). Earthworms (*P. corethrurus*) for the small pot extperiment were taken from an abandoned maize field at the Centro de Investigaciones Costeras "La Mancha" (19°35' N and 96°23' W). All earthworms were brought to the laboratory and placed for several weeks in the forest soil in large plastic containers before using them for the experiment.

## Large pot experiment (Photo 2)

The sieved soil was mixed vigorously and the equivalent to 12.5 kg (oven dry weight) packed to a bulk density of approximately 0.8 g $cm^{-3}$, in 20 l white plastic buckets and watered to slightly above field capacity (pF 2.0; 55% $H_2O$). Certified maize seeds (variety V-530) were purchased locally and planted at the rate of 3 seeds $bucket^{-1}$. Earthworms were dried with paper towels and approx. 9-10 *P. corethrurus* (150 ind. $m^{-2}$) weighing in total about 4 g (60 g $m^{-2}$; gut contents included), were placed on the soil surface in the evening. Any earthworms not found to enter the soil overnight were replaced the following morning. Buckets were established with and without earthworms to assess their effect on maize production. To measure the amount of C derived from maize plant presence, treatments were set up with earthworms from the forest, but without maize plants. To study the effect of earthworms on $^{15}N$ re-distribution, 9 g (equiv. to 1.4 t $ha^{-1}$) of $^{15}N$-labelled residues (3.12% N; 3.68 atom %$^{15}N$; $\delta^{15}N = 9123‰$) from the previous experiment were placed on the surface of each pot (Photo 2).

A total of 40 buckets were used for the following treatments:

1. Forest soil with (PONTO) or without (CONT) *P. corethrurus* from the same forest (Los Tuxtlas), and planted with maize (14 replicates each);
2. Forest soil with *P. corethrurus* only (3 replicates) and no maize plants (PONTO-NP);
3. Forest soil with (PONTO+R) or without (CONT+R) *P. corethrurus* and $^{15}N$-labelled maize residues, and planted with maize (4 replicates each);
4. Forest soil with *P. corethrurus* only (1 replicate) plus $^{15}N$-labelled maize residues, but no maize plants (PONTO+R-NP).

All the buckets were placed in a random order in a clear plastic-roofed greenhouse at UNCADER (Unión Nacional de Capacitación y Desarollo Rural), in the nearby city of Coatepec, Veracruz. An example of a bucket with earthworms and residues, with a brief description of some harvesting methods is shown in Photo 2 and Figure 1. A thermometer was placed 8 cm up from the bottom of one CONT bucket treatment and the soil and ambient air temperatures recorded every few days. Plant heights and health were also monitored every few days. After 20 d all extra germinated maize plants were removed, leaving only one plant for further measurements. After 37 d, and at flowering (131 d), 3 replicates of the PONTO and CONT treatments were harvested, and one replicate each of PONTO-NP buckets taken down. After 183 d, when most maize plants had reached maturity, the remaining pots of all treatments were harvested. At each harvest date, the pots were cut in half and soil bulk density, moisture, $NO_3$ and $NH_4$-N (131 and 183 d only) and pH (KCl and $H_2O$), as well as plant height, root density, root and shoot biomass, earthworm wet and dry weights and numbers and $\delta^{13}C$ of body tissues (PONTO) were measured. At the final harvest, additional measurements included number of leaves, total ear, grain and cob biomass, residue weight remaining and the $^{15}N$ contents of each plant part, earthworm tissue (PONTO+R and PONTO+R-NP), and soil at six different depths (0-1, 2-3, 3-6, 6-9, 15-20, and 25-30 cm).

Roots were separated by manually sorting through the soil. At the final harvest, a small amount of roots from five pots in which plants were still alive, in the presence ($n = 2$) or absence ($n = 3$) of *P. corethrurus*, was preserved in FAP to study vesicular-arbuscular mycorrhizal (VAM) infection using the standard method of Phyllips and Hayman (1970). Root density was assessed following the method in Brown *et al.* (2000c; chap. 5).

To prepare earthworm, plant and soil samples for $^{13}C$ analyses, each material was dried for 48 h at 60°C, ground manually in a mortar, passed through a 0.02 mm sieve, and stored in plastic vials. Isotopic analyses were performed on a high-precision (± 0.05‰) MICROMASS SiRA10 ($\delta^{13}C$) and OPTIMA ($^{15}N$; precision 0.5‰) mass spectrometers both coupled to a Carlo Erba NA 1500 elemental CHN analyser at the Laboratoire de Biogéochimie Isotopique at the University of Paris VI (Mariotti, 1991) and on a MICROMASS EA-CF Isochrom dual isotope ratio ($^{13}C$, $^{15}N$) mass spectrometer, coupled to a Carlo Erba 1108 CHNS-O elemental analyser at the Universidad Autónoma de Madrid (precision for $^{13}C$ and $^{15}N$ = 0.15‰ and 0.2‰, respectively). Several enriched $^{15}N$ samples were also analysed on a Finnigan Delta S mass spectrometer, coupled to a SCA C:N elemental analyser (precision 1% relative; 0.3‰ for natural abundance) at the Service Central d'Analyse (CNRS), Vernaison, France.

Soil bulk density was obtained by inserting a round metal cylinder (5.2 cm diam., 5 cm deep) into the soil. Mineral-N ($NO_3$-N & $NH_4$-N) was obtained colorimetrically following TSBF (Tropical Soil Biology and Fertility) methodology (Anderson and Ingram, 1993) at the two final harvest dates. pH was determined using a 1:5 ratio of soil:water placed into a beaker, shaken intermitently for 30 min, and measured 1 h after the last shaking. At the final harvest 20 g soil samples were taken from five CONT and four PONTO pots to quantify the number of large (retained by a >150 µm sieve) and small (>40 µm) VAM spores (Gedermann and Nicholson, 1963).

### Small Pot Experiment

Small pots with approximately 0.9 kg (equivalent oven dry weight) of soil, moistened to 66% $H_2O$, were placed in the greenhouse at the Jardín Botánico of the Instituto de Ecología, A.C. Two earthworms (*P. corethrurus*; rate equivalent to 220 individuals $m^{-2}$) were added with a biomass equivalent to 32 g $m^{-2}$. Four replicates of the control (no earthworms) and earthworm treatments were established. Three maize seeds (variety H-90) were planted and throughout the 54 d of the experiment plant heights, ambient temperatures and presence of earthworm casts were noted. At harvest, the plant above ground portion was cut at the soil surface, the pot overturned and the soil removed to extract living earthworms and roots. Plant shoots and roots were dried (60°C) for 48 h and weighed. Earthworms were counted and weighed (fresh).

### Statistical Analyses

The number of replicates was generally sufficient for traditional parametric statistics (ANOVA's; Fisher's Protected LSD), which were performed on the package SUPERANOVA® (Abacus Concepts). In a few cases, only single replicates were available, and comparisons of means were not possible. When present, these cases are indicated (with n).

## RESULTS

### $^{15}$N-labelled maize residue production

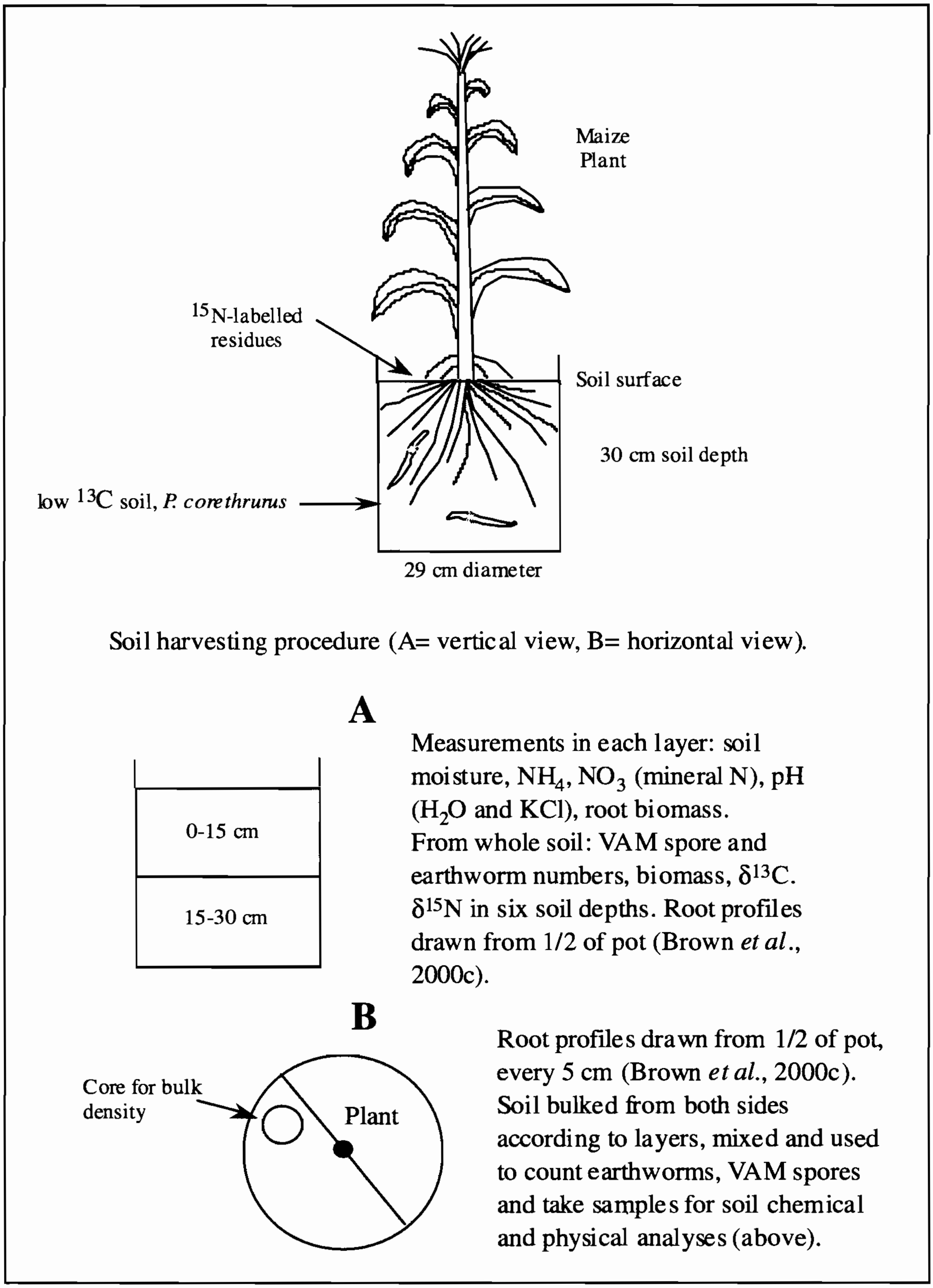
Conditions for maize growth were generally good, with average maximum and minimum temperatures of 34 and 15°C, respectively (absolute max 38°C, min 4°C). After 90 days the maize plants had already flowered, reaching maximum heights of almost 2 m, and small ears were beginning to form. The above ground production averaged 5 t ha$^{-1}$ dry matter (33.4 g plant$^{-1}$), and the recovery of $^{15}$N fertilizer applied was about 33%. Isotopic labelling of the plant shoots reached 3.68 atom %$^{15}$N.

### Large Pot Experiment

Over the 183 d experimental period, temperature ranges in the greenhouse were extreme (Fig. 2), warming up during the day-time to consistently over 40°C, and at night cooling to around 15°C (aboslute min. 7°C). Many clear days caused the greenhouse to heat excessively and, because no extracting fans were present, temperatures reached up to 56°C. Soil temperatures measured at various times during the day, however, did not exceed 32°C. Nevertheless, plant growth under these conditions is not ideal, so the results given below indicate the reaction of plant and earthworm parameters to harsh conditions.

#### *Plant parameters*

Early in the growth cycle (the first month), P deficiencies became apparent in most plants, and in a few plants was maintained throughout the growing season. This is common in volcanic soils, where solution and labile-P are scarce due to the high surface-binding areas of the clay minerals found in these soils (halloysite in this case). K deficiency was also common in many of the plants, despite high available K soil levels. N deficiency was observed in a only a few plants (high soil mineral-N contents). Pests, particularly ants (*Dorymyrmex* ca. bicolor) and aphids became an increasing problem after 80 d. Two aphid-infested plants also suffered from rampant fungal growth that covered the leaves. Yields of these maize plants were also affected when, after 160 d, the plants died.



Soil harvesting procedure (A= vertical view, B= horizontal view).

Measurements in each layer: soil moisture, $NH_4$, $NO_3$ (mineral N), pH ($H_2O$ and KCl), root biomass. From whole soil: VAM spore and earthworm numbers, biomass, $\delta^{13}C$. $\delta^{15}N$ in six soil depths. Root profiles drawn from 1/2 of pot (Brown *et al.*, 2000c).

Root profiles drawn from 1/2 of pot, every 5 cm (Brown *et al.*, 2000c). Soil bulked from both sides according to layers, mixed and used to count earthworms, VAM spores and take samples for soil chemical and physical analyses (above).

Figure 1. Example of the experimental bucket (20-liter capacity) using forest soil from "Los Tuxtlas," *P. corethrurus* and *Zea mays*. Harvesting procedures for the vertical (A) and horizontal (B) planes are also displayed.

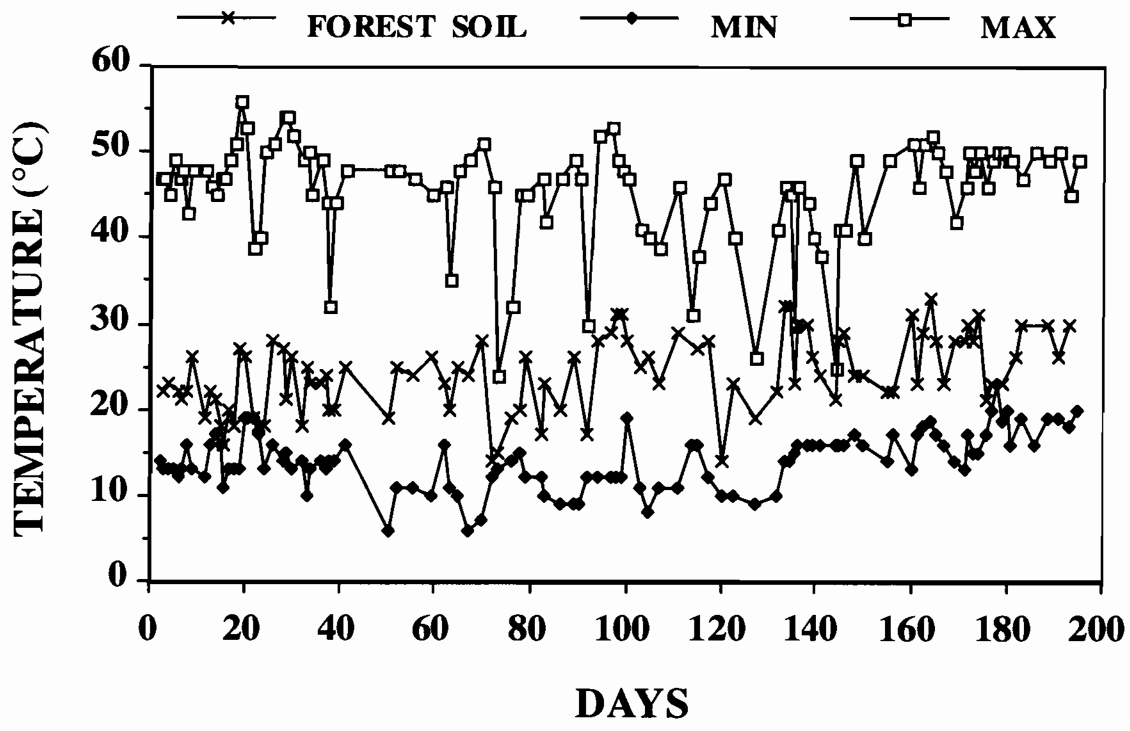


Figure 2. Maximum and minimum ambient temperatures (°C) of the greenhouse at UNCADER, Coatepec, Ver., and in the Forest soil (at 25cm depth) throughout the experimental period (183d).

Table 2. Biomass (g pot$^{-1}$) of the different parts of the maize plant, and shoot:root ratios of plants after 183 d in the presence (PONTO; PONTO+R) or absence (CONT; CONT+R) of inoculated *P. corethrurus* and surface-applied maize residues. R = residues.

| Plant Parameter | n[1] | CONT | n | PONTO | n | CONT+R | n | PONTO+R |
|---|---|---|---|---|---|---|---|---|
| Stover | 8 | 75.0 | 8 | 72.8 | 4 | 89.5 | 4 | 69.9 |
| Whole ear | 8 | 38.8 | 7 | 40.8 | 4 | 44.4 | 2 | 32.0 |
| Grain | 6 | 16.5 | 7 | 20.2 | 3 | 22.7 | 2 | 10.1 |
| Cob | 7 | 6.4 | 7 | 6.9 | 4 | 8.2 | 2 | 10.2 |
| Roots | | | | | | | | |
| A (0-15 cm) | 8 | 14.3 | 8 | 16.3 | 4 | 15.4 | 4 | 10.3 |
| B (15-30 cm) | 8 | 12.7 | 8 | 15.4 | 4 | 14.3 | 4 | 9.0 |
| Total | 8 | 30.0 | 8 | 31.7 | 4 | 29.7 | 4 | 19.3 |
| Shoot:Root | 8 | 5.1 | 8 | 4.5 | 4 | 6.0 | 4 | 5.3 |

1. Number of plants with a particular plant parameter.

Average heights of the maize plants during the 183 d experimental period are shown in Figure 3. The CONT+R treatments had the tallest maize plants, throughout most of the period beyond 110 d. Up to 120 d (beginning of the flowering period), the PONTO+R treatment followed closely the heights of CONT+R. After 120 d the PONTO+R treatments followed the growth of PONTO and CONT treatments more than the CONT+R. At the very end of the growth cycle, the PONTO+R treatment performed very poorly. Heights of PONTO and CONT plants were very similar throughout the growing cycle. No significant effects of earthworms were observed on tassling or flowering dates.

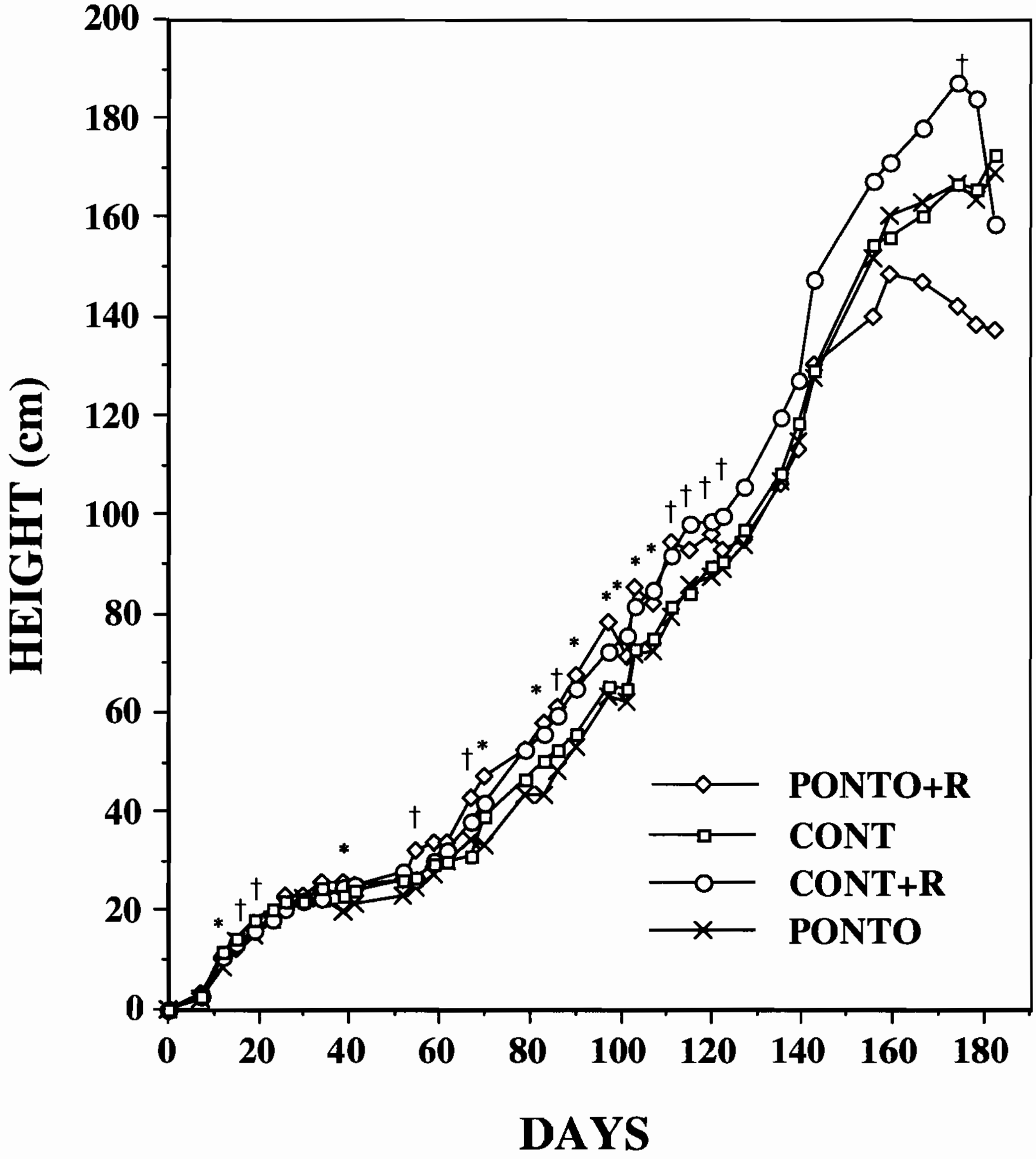


Figure 3. Average heights of the maize plants grown in forest soil in control (CONT), control + maize residues (CONT+R), *P. corethrurus* (PONTO), and *P. corethrurus* + maize residues (PONTO+R) treatments over the experimental period (* = significant differences between tallest and shortest plants at $p<0.05$; † = $p<0.1$).

Above-ground plant biomass of the different treatments is shown in Figure 4, and weights of the distinct plant components (stover, ears, grain, cobs and roots), as well as the shoot:root ratios are given in Table 2. Above-ground biomass was significantly greater in CONT+R than PONTO+R treatments, although no significant differences between treatments were obtained for any of the other plant components at any of the dates, due to the very high variability of the results. Root density (from Brown *et al.*, 2000c; chap. 5) was significantly higher in the horizontal cuts due to *P. corethrurus* presence at 131 and 183 d. Contrastingly, no differences in overall vertical root density were detected due to earthworms, despite a trend for higher values at all dates with and without residues. Root density increased slightly from 131 d to 183 d without residues, but when residues were applied, vertical root density in both treatments and horizontal density in CONT+R were significantly lower than in the absence of residues (Brown *et al.*, 2000c; chap. 5).

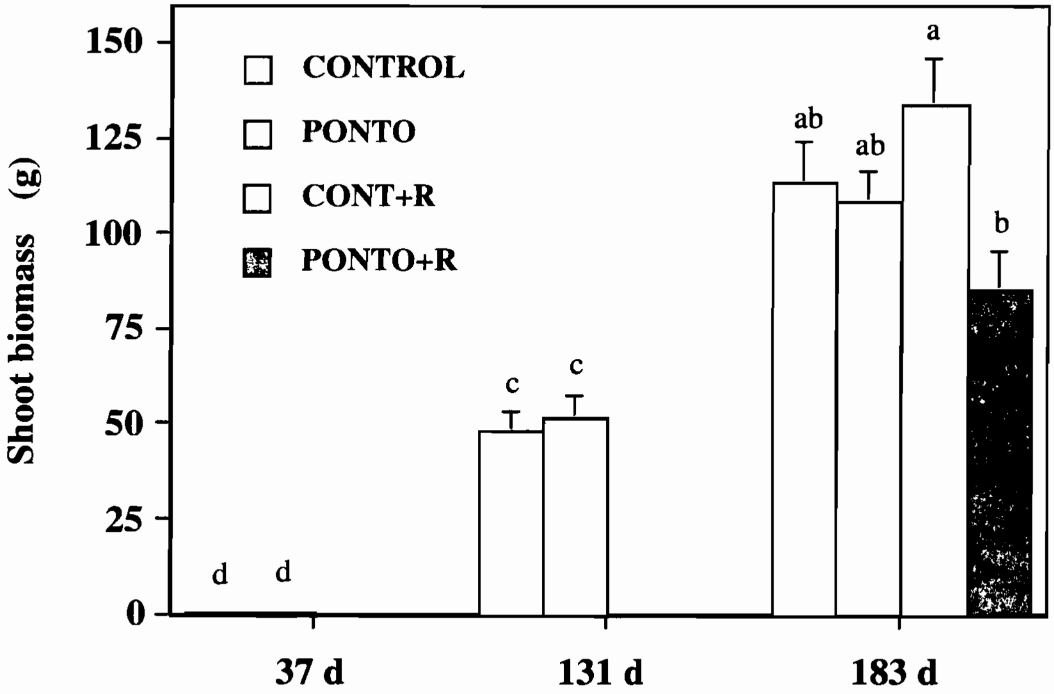


Figure 4. Total shoot biomass of maize grown in the presence and absence of earthworms (*P. corethrurus*) and surface-applied maize residues at each harvest date (37, 131 and 183 d). (Values shown are means + SE bars). Bars with the same letter are not significantly different at P<0.05. Treatment abbreviations as in Fig. 3.

Earthworms induced a significant reduction in the amount of surface residues present, their N content (Table 3) and the percentage of the original $^{15}N$ remaining (Fig. 5). The %N of the plant shoots and cobs had a trend for higher values with earthworms, and the %N in grain was significantly higher than in CONT+R treatments. The atom %$^{15}N$ enrichment of the different plant parts were not significantly affected by *P. corethrurus* presence. The RCU (Real Coefficient of Utilization of $^{15}N$ = % recovery of original $^{15}N$ applied) was calculated using equation (1) for the different plant parts:

$$\text{RCU}(\%) = \frac{\text{Npp}}{\text{Nr}} * \frac{\text{Epp}}{\text{Er}} * 100 \qquad (1)$$

Where Npp is the total N content and Epp is the isotopic (atom %$^{15}$N) excess (E) of the different plant parts (pp), Nr is the total N content and Er is the isotopic excess of the original $^{15}$N-labeled maize residues (r). RCU of the different plant parts was not significantly different due to earthworms although a trend for higher recoveries in CONT+R treatments was observed (Fig. 5). Total $^{15}$N recovery was greatest in the PONTO+R-NP treatment, with a value close to 100%, while significantly lower recoveries were encountered in CONT+R and PONTO+R pots with. Earthworms (PONTO+R) resulted in a significantly lower recovery compared with CONT+R treatments.

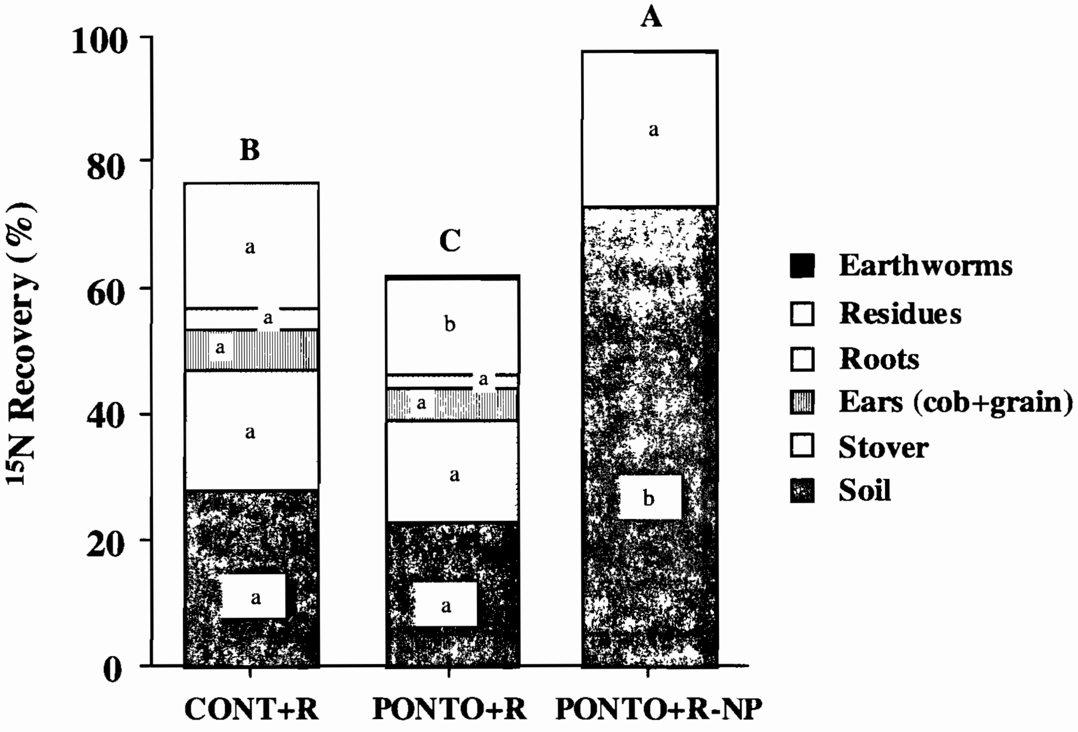


Figure 5. Recovery of $^{15}$N derived from the labelled maize residues in each of the different plant parts, residues, earthworms and soil pools in the presence or absence of earthworms (*P. corethrurus*) and growing maize plants. CONT+R = control + maize residues; PONTO+R = *P. corethrurus* + maize residues; PONTO+R-NP = *P. corethrurus* + maize residues and no maize plants. Different capital letters mean significant differences in total recovery at P< 0.05, and different lower-case letters mean significant differences for the different components measured at P< 0.05.

Only five pots were studied for VAM colonization of roots (these were pots where plants were still living and green at harvest). In the presence of earthworms, VAM colonization was 92.2% and in their absence, 90.5%. However, the low number of pots, and the drying out and death of plant shoots and roots at and before harvest does not permit a generalization of these results. In single replicates from the first harvest (37d) the treatment with earthworm had a colonization of 52% while that without had only 14%.

Table 3. Elements of the N budget within the plant, the residues remaining on the surface, and *P. corethrurus* tissues, after 183 d maize growth in the presence (PONTO+R) or absence (CONT+R) of earthworms and maize plants (-NP) and surface-applied $^{15}$N-labelled maize residues (R). Values accompanied by different letters within the same row are significantly different at $P<0.05$.

| TREATMENT | n[1] | CONT+R | n | PONTO+R | n | PONTO+R-NP |
|---|---|---|---|---|---|---|
| RESIDUES | | | | | | |
| Mass (g pot$^{-1}$) | 4 | 3.88a | 4 | 3.71a | 1 | 4.39a |
| %N | 4 | 2.07a | 4 | 1.76b | 1 | 2.17a |
| Atom %$^{15}$N | 4 | 2.79a | 4 | 2.57a | 1 | 2.76a |
| SHOOTS | | | | | | |
| %N | 4 | 1.54a | 4 | 1.66a | | nd[2] |
| Atom %$^{15}$N | 4 | 0.48a | 4 | 0.49a | | nd |
| Ndfr (%) | 4 | 3.4a | 4 | 3.6a | | nd |
| COB | | | | | | |
| %N | 3 | 0.51a | 2 | 0.83a | | nd |
| Atom %$^{15}$N | 3 | 0.16a | 2 | 0.49a | | nd |
| Ndfr (%) | 3 | 1.0a | 2 | 3.7a | | nd |
| GRAIN | | | | | | |
| %N | 3 | 1.96a | 2 | 2.92b | | nd |
| Atom %$^{15}$N | 3 | 0.50a | 2 | 0.50a | | nd |
| Ndfr (%) | 3 | 3.8a | 2 | 3.9a | | nd |
| EARTHWORM | | | | | | |
| %N | | nd | 11 | 9.3a | 9 | 8.7a |
| Atom %$^{15}$N | | nd | 11 | 0.40a | 9 | 0.37b |
| $\delta^{15}$N (‰) | | nd | 11 | 96.0a | 9 | 11.1b |
| Ndfr (%) | | nd | 3 | 0.9 | 1 | 0.3 |
| RCU (%) | | nd | 3 | 0.17 | 1 | 0.05 |

1. n = number of replicates; 2. nd = not determined

*Soil parameters*

In treatments receiving forest soil and maize plants, no differences between treatments were found for any of the soil chemical parameters measured on any of the dates at either of the depths sampled (Table 4). On the other hand, the number of large VAM spores recovered in pots with *P. corethrurus* was more than twice (n = 67.3) that found without earthworms (n = 30.6). The number of small spores was much higher than the large ones in CONT (average >1000), but the effect of earthworms on the number of these spores was not determined. Earthworm casts collected in laboratory cultures (Hernández, 1999) had similar C:N, pH, exchangable Al, Mg and Ca, resin and Bray-P, total Fe, Cu and Mn contents, but higher Total C, CEC, exch. K, % clay, total and mineral-N ($NO_3$ and $NH_4$), and lower echx. Na, % sand and silt (Table 1). Largest increases were observed in $NO_3$-N (3.5x higher) and $NH_4$-N (3x higher).

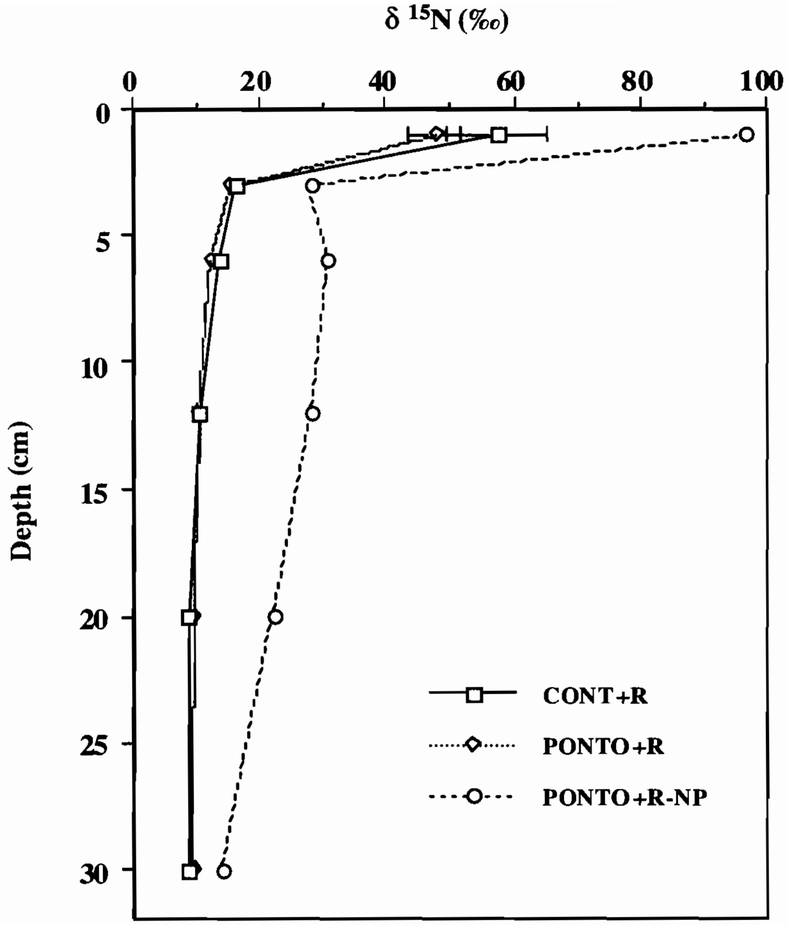


Figure 6. $\delta^{15}N$ signatures (means ± SE bars) of the bulk soil pool at different depths of pots in the presence and absence of *P. corethrurus* (PONTO) or maize plants (-NP), with surface-applied $^{15}N$-labeled maize residues (R). Treatment abbreviations as in Fig. 5.

The presence of plants caused a dramatic decrease of $^{15}N$ recovery from the soil (Fig. 5) due to plant uptake. Throughout the six depths sampled, when plants were absent soil $\delta^{15}N$ signatures were always higher (Fig. 6). On the other hand, no difference was observed in soil $\delta^{15}N$ at different depths due to earthworms, despite lower values in most cases. At depths beyond 6 cm, $\delta^{15}N$ signatures were similar to those of unlabeled soil when plants were present, but when plants were absent, $\delta^{15}N$ signatures were still higher than unlabeled soil even at 30 cm. Thus, plants were taking up the $^{15}N$ released by the residues, and the earthworms were responsible for accelerating the transfer of $^{15}N$ from the residues into the soil (Table 3).

Table 4. Soil pH, available N ($NO_3$, $NH_4$) and total mineral N in the upper (A = 0-15 cm) and lower (B = 15-30 cm) horizons of buckets with (PONTO) or without (Control) addition of *P. corethrurus* and surface-applied maize residues after 37, 131 and 183 d of maize culture in a clayey Andosol from Los Tuxtlas, Veracruz, Mexico

| TREATMENT | | pH ($H_2O$) | | | $NH_4$ (ppm) | | | $NO_3$ (ppm) | | | Mineral-N | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 37 d | 131 d | 183 d | 37 d | 131 d | 183 d | 37 d | 131 d | 183 d | 37 d | 131 d | 183 d |
| CONT | A | 5.9 | 6.0 | 6.2 | | 22.0 | 49.3 | | 151 | 87.7 | | 173 | 123 |
| | B | 5.9 | 5.7 | 6.2 | n.d. | 27.7 | 29.5 | n.d. | 183 | 124 | n.d. | 210 | 151 |
| PONTO | A | 5.9 | 5.9 | 6.2 | | 48.1 | 22.1 | | 145 | 66.0 | | 193 | 87.9 |
| | B | 5.9 | 5.8 | 6.2 | n.d. | 28.1 | 17.3 | n.d. | 166 | 104 | n.d. | 194 | 119 |
| CONT+R | A | | | 6.0 | | | 26.3 | | | 69.5 | | | 95.9 |
| | B | n.d. | n.d. | 6.3 | n.d. | n.d. | 9.1 | n.d. | n.d. | 87.3 | n.d. | n.d. | 96.4 |
| PONTO+R | A | | | 6.0 | | | 32.1 | | | 55.0 | | | 100 |
| | B | n.d. | n.d. | 6.2 | n.d. | n.d. | 21.9 | n.d. | n.d. | 131a | n.d. | n.d. | 153 |

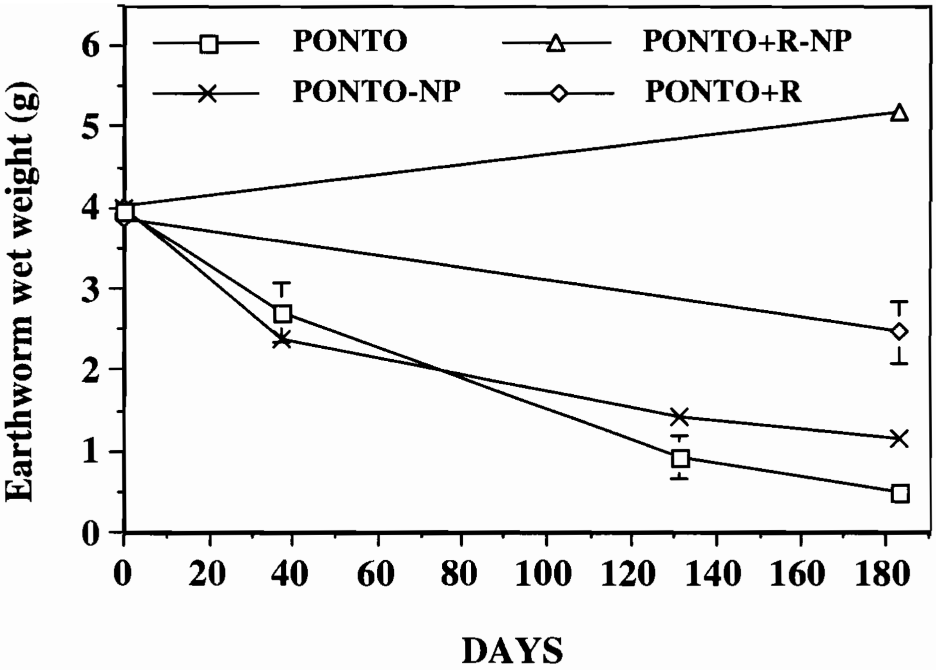


Figure 7. Biomass (means ± SE bars) of *P. corethrurus* in the presence and absence of maize plants and residues at each harvest date (37, 131 and 183d). PONTO = *P. corethrurus* + maize plants; PONTO-NP = *P. corethrurus* and no maize plants; PONTO+R = *P. corethrurus* + maize residues and maize plants; PONTO+R-NP = *P. corethrurus* + maize residues and no maize plants.

*Earthworm parameters*

Despite the high soil and ambient temperatures *P. corethrurus* reproduced on several occasions (particularly in pots with no plants). When plants were present there appears to have been a competition between the earthworms and plants for soil $H_2O$. *P. corethrurus* are generally most active when soil is well above field capacity (Lavelle *et al.*, 1987), so their activities were mostly limited to the bottom 10 cm of the buckets, where the soil was more humid. When plants were not present, not only were they found to be active over a greater portion of the bucket soil area but also to maintain higher biomass at most sampling dates (Fig. 7). When residues were placed in the maize treatments (PONTO+R), a significantly higher biomass was obtained after 183 d than when no residues and both with (PONTO) and without plants (PONTO-NP) were present. Despite the high organic matter content of the forest soil *P. corethrurus* lost considerable weight (except when residues were applied), following an exponential curve very similar for treatments with and without maize, respectively. The coefficients of these curves (second order polynomial regressions) were r = 1.0 for PONTO and r = 0.98 for PONTO-NP treatments.

When in presence of maize plants *P. corethrurus* maintained a significant increase in $\delta^{13}C$ (linear regression $r^2$= 0.95; $p<0.03$), even when feeding in soil with very low $\delta^{13}C$ content ($C_3$), indicating that these worms were feeding on and assimilating higher $\delta^{13}C$ maize plant-derived C

($C_4$) (Fig. 8). A calculation of the percentage of maize-derived C in the diet of *P. corethrurus* at the 183 d harvest was thus made using the following formula (Martin *et al.*, 1992a):

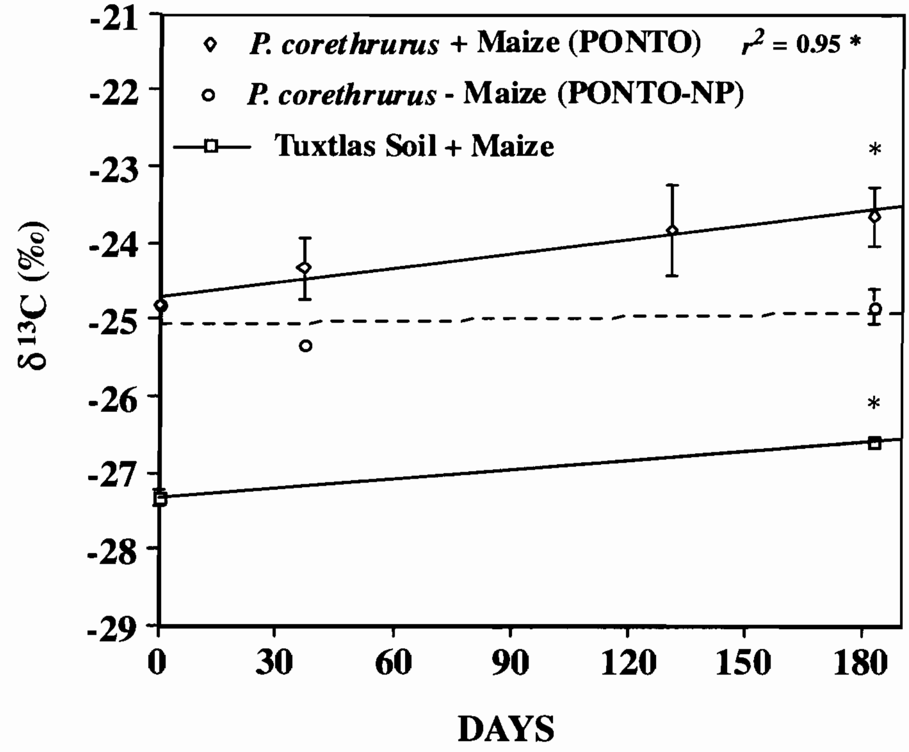


Figure 8. $\delta^{13}C$ signatures (means ± SE bars) of the Tuxtlas forest soil in presence of maize plants and of *P. corethrurus* tissues in the presence (PONTO) and absence of maize (PONTO-NP) at each harvest date (37, 131 and 183d). Asterisks (*) denote significant differences between initial (0 d) and final (183 d) values at $P<0.05$. Statistically significant linear regression r value is also displayed.

$$F = (100) * \frac{\delta - \delta_1}{\delta_2 - \delta_1} \qquad (2)$$

Where F is the percentage of C derived from the second material, $\delta_1$ is the $\delta^{13}C$ value of earthworms living in the original soil ($C_3$ forest), $\delta_2$ is the $\delta^{13}C$ signature of earthworms living in soil under $C_4$ plants (maize, in this case) and $\delta$ is the $\delta^{13}C$ signature of earthworms living in the mixed environment ($C_3$ and $C_4$). The exact value of $\delta_2$ could not measured and was assumed to be similar to that of *P. corethrurus* found under long-term continuous sugarcane ($\delta^{13}C$ = 9.9‰; mean value taken from Spain *et al.*, 1990 and Spain and Le Feuvre, 1997), another $C_4$ crop. Thus, after 183 days, F was calculated to be 7.8%, respectively. Bulk soil $\delta^{13}C$ signatures also increased significantly, from -27.3‰ to -26.6‰ at the end of the experiment at the 15-30 cm depth in treatments with residues. The fraction of SOM C (s) derived from the maize ($F_s$) was calculated using $\delta$ as the $\delta^{13}C$ of the soil OM at 183 d, $\delta_1$ as the original soil $\delta^{13}C$, and $\delta_2$ as the

$\delta^{13}C$ signature of dead soil organic matter coarser than 2 mm ($\delta^{13}C$ = 12.8‰), derived from maize (Balesdent and Balabane, 1996). Thus $F_s$ was calculated as 5.0%.

When $^{15}N$-labelled residues were added to the surface of the pots planted with maize, *P. corethrurus* had a higher %N in their tissues as well as a significantly greater $\delta^{15}N$ (96‰) than in the absence of maize ($\delta^{15}N$ = 11.1‰) (Table 3). This latter value, although significant, was only slightly greater than background (unlabelled) earthworms ($\delta^{15}N$ = 7.0‰). The recovery of $^{15}N$ in earthworm tissues (RCU) from the surface residues applied was calculated using the N content of the earthworms and their atom% excess in equation (1). RCU by earthworms was small (compared to that in the plants), although >3 times higher in the presence of maize plants (0.17%) than in their absence (0.05%; Table 3). Using a procedure similar to that for $^{13}C$, the percentage of maize residue-derived N in *P. corethrurus* (Ndfr) tissues was calculated using Equation (3).

$$\text{Ndfr}(\%) = (100) * \frac{\text{Ew (p, np)}}{\text{Er}} \qquad (3)$$

Where Ew is the isotopic (atom %$^{15}N$) excess of the earthworm tissue (w) in the presence (p) or absence (np) of maize plants and Er is the isotopic excess of the original $^{15}N$-labeled surface-applied maize residues after 183 d. With maize plants, Ndfr in earthworm tissues was 0.98%, while without plants, it was only 0.04%. Thus, the plants contributed to increase the N-derived from the residues in earthworm tissues, increasing this fraction by almost 25 times.

### Small Pot Experiment

Plant growth in treatments in presence and absence of *P. corethrurus* was similar, and heights throughout the trial as well as final shoot and root biomass at harvest were not significantly different between the treatments. No earthworms were recovered at harvest. Presumably, all had died within the pots or escaped to the outside. Shoot biomass was similar to that harvested at 37 d in the large pot experiment (about 0.8 g), but root biomass was about 4 times lower (0.4-0.5 g).

## DISCUSSION

*Zea mays* or maize, a native Mexican plant, is the primary food crop of this country, and plays an important role in family nutrition throughout the tropics. In low-input agroecosystems such as those found in the Los Tuxtlas region of Veracruz, maize cultivation is primarily for self-subsistence. Under these systems, external inputs are rarely used and plant density is low, resulting in low grain yields, generally under 1.5 t $ha^{-1}$ (Paré *et al.*, 1994). In the present experiment, P-availability was low, although soil and N content were high, and the concentration of other nutrients adequate. P deficiencies and parasites afected several plants and high ambient temperatures plus a late planting date altered flowering and maturity, all of which had a negative impact on plant yields. The plants grew slowly, reaching maturity 2 months after normal maturation time (120 d). Grain yields, nevertheless, did not appear to be greatly reduced compared to the regional average (above). An extrapolation of the average grain biomass in the highest yielding treatment (CONT+R) to a 1 ha basis (using 90,000 plants $ha^{-1}$) resulted in a 2 t

$ha^{-1}$ grain production, slightly higher than the local average, while production in the PONTO+R (0.9 t $ha^{-1}$) treatment was lower than the average (Paré *et al.*, 1994). Despite this difference of almost two-fold, the small number of replicates including maize residues (n = 4), in addition to the death of several of these plants before the end of the experiment made it difficult to detect statistical differences between these treatments.

In humid tropical regions such as around Los Tuxtlas, where temperature and moisture regimes are stable throughout the year, biological systems of soil regulation become increasingly important (Lavelle, 1984). Furthermore, in low-input agroecosystems, OM management and the activities of the soil biota such as earthworms play an essential role in the formation and conservation of stable soil physical structure, in nutrient cycling and availability to plants (Lavelle *et al.*, 1998; Swift and Sanchez, 1984). Their role decreases, however, as the natural fertility of the soil, or its OM content increases (Brown *et al.*, 1999; chap. 2). The high OM content of the forest soil in the present experiment, a greater independence of plants for nutrients and the insignificant effects of earthworms on soil parameters in the pots were probably the main factors responsible for the null effects of earthworms on plant production.

In the presence of residues, earthworms reduced above ground biomass, and a significant negative relationship was found between shoot biomass percent increase and the final earthwom biomass of treatments (with and without residues; n = 9 pots) with live individuals at the final harvest ($r = 0.85$; $p< 0.01$). Previous experiments using *P. corethrurus* have observed mostly positive effects on plant biomass, although neutral and slightly lower (though not significant) yields have also been reported (Patrón *et al.*, 1994; Patrón, 1993; Pashanasi *et al.*, 1996; unp. data; Spain *et al.*, 1992). In a C-rich soil at El Cielo, Mexico, a trend for lower grain production with earthworms and incorporated residues was observed in the field (Patrón, 1993). In the present experiment, the negative effect of earthworms on plant production in the PONTO+R treatment may be due to negative effects on VAM root colonization (not measured in this treatment) or to some other negative influences on soil biological or physical properties. Negative impacts of *P. corethrurus* on various plants have been observed mostly in clayey soils such as that used in this experiment (Brown et al., 1999; chap. 2; Barros, 1999; Rose and Wood, 1980). Furthermore, *P. corethrurus* can both increase or decrease VAM infection of roots, depending on the plant and soil treatment (Brown *et al.*, 2000d; chap. 6; Ydrogo, 1994). Precise mechanisms behind these negative effects require further investigation.

In the present experiment, the only plant parameters positively affected by earthworms were grain %N (increased) in PONTO+R, and horizontal root density to 30 cm depth at 131 d in PONTO and at the final harvest (increased) in PONTO+R (Brown et al., 2000c; chap. 5). Thus, even though earthworms had no effect on root biomass and a negative effect on shoots, they increased the root density and the number of smaller roots, those primarily involved in nutrient uptake. However, this increase had no effect on total N uptake (despite higher grain N). Earthworms and roots may compete for water, and perhaps the greater root densities in presence of earthworms indicates a plant response to competition (Brown, 2000b; chap. 1).

Passage of soil through *P. corethrurus* guts is known to stimulate primarily soil Ca (Kale and Krishnamoorthy, 1980), mineral N (Lavelle *et al.*, 1992) and P (Chapuis-Lardy *et al.*, 1998; López-Hernández *et al.*, 1993) availabilities in their castings, and these trends were confirmed, except for P. If other P forms (organic, inorganic, total) had been measured, perhaps differences would have been found. Andosols pose a particular problem for plant P availability, especially after drying, when irreversible changes occur to the soil. The soil in the present experiment was not completely air-dried before use, but sufficient water was lost so that some of its properties may have been altered. P deficiencies were observed in many plants, but these did not impede maturity or even grain production at harvest. The constant supply of $H_2O$, the high biological activity in this OM rich soil, the extensive root growth in the pots and the interaction with VAM (in pots without residues, high root colonization was found) probably helped the plants partly overcome these deficiencies.

*P. corethrurus* has been implied to affect surface applied sugarcane residue decomposition by burying the plant material with surface casts (Spain *et al.*, 1990). As a geophagous (soil feeding) polyhumic endogeic species, *P. corethrurus* is considered an ecosystem engineer (Lavelle, 1997) with minor (indirect) effects on litter transformation, but major effects on soil physical properties and OM cycling (Blanchart *et al.*, 1999; Villenave *et al.*, 1999). The presence of *P. corethrurus* in the present experiment did not affect the maize residue biomass remaining. However, the ideal conditions for *P. corethrurus* present under these residues resulted in a significant reduction in the amount of N and the recovery of $^{15}N$ in the residues. Thus, *P. corethrurus* was accelerating the transfer of residue-derived N (and $^{15}N$) into other pools. In the presence of the litter-processing species such as *Lumbricus rubellus*, such phenomena could be expected (e.g., Brown *et al.*, 1998a), however, with geophagous species, this is less likely. *P. corethrurus* in the present case may have been accelerating the biological turnover of the litter-derived N by decreasing the soil fungal network involved in N-immobilization, and by promoting a soil microbial "priming effect" (Lavelle and Gilot, 1994) by extensive vertical and horizontal burrowing in the pots and through the liberation of mucus (assimilable C) and N-rich castings throughout the soil.

Few experiments have addressed the effects of earthworms on N recovery from labelled residues in the tropics. When Gilot-Villenave *et al.* (1996) inoculated the geophagous species *Millsonia anomala* into a nutrient-poor sandy loam Alfisol in Ivory Coast, and incorporated $^{15}N$-labelled maize residues into the top 10 cm, after 56 d of maize culture the earthworms had no effect on the decomposition of the residues. However, the recovery of $^{15}N$ within the soil, and the amount in the plants (RCU) were significantly affected by *M. anomala*. In the present experiment, the amount recovered in the soil and by the plant above-ground portions was slightly, but not significantly, lower with *P. corethrurus*. Less $^{15}N$ (23%) was recovered in the soil, since residues were not incorporated (as with *M. anomala*). However, little $^{15}N$ remained in the residues (15%), and much more was taken up by the maize with *P. corethrurus* compared with *M. anomala*, probably because of the longer growth period (183 d vs. 70 d). The higher $\delta^{15}N$ signatures in the soil throughout the 30 cm pot depth when plants were absent and *P. corethrurus* present also show the role of earthworms in $^{15}N$ movement into the soil, and the importance of plants as N sinks. The lower total $^{15}N$ recoveries in pots with earthworms may have been due to higher denitrification rates of the mineralized residues.

Patrón *et al.* (1994) and Patrón (1993) found an important role of residue applications in *P. corethrurus* survival under maize cultures. In the present experiment biomass maintenance and survival rates when residues were placed on the soil surface were much higher than in their absence probably due to the more favorable soil microclimate conditions. In the absence of residues, both with and without maize plants, biomass recoveries were around 15-30% of that applied, even though the soil was rich in clay, silt and OM. Previous laboratory studies on the growth of this species (Hernández *et al.*, unp. data; Annexe 10) showed good growth and survival in this soil. In the present experiment, plant $H_2O$ uptake and high soil temperatures may have limited earthworm activity and growth, especially without residues.

Little is known of the role of plants in *P. corethrurus* diets. After 6 months under maize, despite the low survival rates in PONTO treatments, $\delta^{13}C$ signatures of *P. corethrurus* increased with time, confirming that this species was assimilating and accumulating $C_4$-C in their tissues. This C could be derived from dead or dying maize roots, or more likely, from rhizosphere soil or rhizobiota with higher C and $\delta^{13}C$ contents. *P. corethrurus* is known to select smaller (clay) particles, richer in C (Barois *et al.*, 1999). In the present study, the first time that the amount of C in earthworm tissues derived from growing plants is estimated, about 8% of the C came from the maize after 6 months. The fraction of N from surface residues found in worm tissues was also greatly enhanced (by almost 25 times) by maize plant presence. This seems to denote a more important role of plants for the N nutrition of earthworms than previously thought. Perhaps this role is even more important than for C, as the high increase in $^{15}N$ in worm tissues due the maize seems to imply. If this is the case, it may be due to the C-rich nature of the soil used in the present experiment, a greater independence of the earthworms from growing plants for their C needs, lower C assimilation efficiencies, or to a more C-conservative behavior of these earthworms.

As observed in previous studies (e.g., Martin et. al, 1992a;b; Neilson *et al.*, 1998; Spain *et al.*, 1990; Spain and Le Feuvre, 1997), $\delta^{13}C$ signatures of initial Los Tuxtlas *P. corethrurus* tissues were several units higher (about 2.5‰) than the soil OM they were feeding on. $\delta^{15}N$ signatures were similar (diff. not signif.) in *P. corethrurus* tissues (7.0‰) and bulk soil (6.0‰). When present, differences in earthworm tissue $\delta^{15}N$ and $\delta^{13}C$ signatures compared to bulk soil may be due to: stepwise enrichment through microbial food webs (Spain and Le Feuvre, 1997); selection of specific particle sizes (Barois *et al.*, 1999) of soil fractions which contain different $\delta^{15}N$ and $\delta^{13}C$ signatures than those of the bulk soil (Balesdent, 1991; Martin *et al.*, 1992a;b); $N_2$ fixation in the earthworm gut (Barois *et al.*, 1987); preferential ingestion of residue or plant tissue decomposition products (Spain and Le Feuvre, 1997); feeding on particular plant residues (Schmidt *et al.*, 1997) or specific plant parts with different $\delta^{15}N$ and $\delta^{13}C$ signatures (Martin and Lavelle, 1992; Nielson *et al.*, 1998); differences in feeding habits of the different earthworm ecological categories (Martin *et al.*, 1992a; Schmidt *et al.*, 1997); congregating and feeding under a particular plant species' rhizosphere within a diverse plant community (Boettcher and Kalisz, 1991; Schmidt *et al.*, 1997); seasonal changes in soil or plant $\delta^{15}N$ and $\delta^{13}C$ and feeding habits of earthworms (Nielson *et al.*, 1998; Schmidt *et al.*, 1997); preferential C discrimination in digestion (as in microorganisms) which favors assimilation of higher $\delta^{13}C$ materials.

The improved plant biomass and earthwom survival rates with residues in the present experiment highlight the importance of OM management as an important aspect of low-input agriculture. Despite the low amount of residue-derived N ($^{15}N$) as a proportion of total N in plant tissues (~3% in shoots and grain), the high percentage of the N from the litter taken up by the plant (RCU) shows that the residues are important in plant N nutrition even during the first crop cycle. The recoveries (RCU) observed here are smaller than those observed with mineral fertilizers (Balabane and Balesdent, 1992), but besides the nutritional aspect, residues also offer a source of OM for the soil foodweb, ameliorate soil microclimate, promote soil structure stabilization, protect the soil surface from rainsplash erosion, and constitute one of the main OM inputs to the soil. The large fraction of earthworm N derived from the residues, enhanced by a short cycle through the plant uptake of $^{15}N$, demonstrates the important role that both residues and the root system play in earthworm nutrition. Polyhumic endogeic earthworm species, typically associated with the higher OM surface soil, may be extensive rhizosphere feeders. Their survival and maintenance in C and N-poor soils such as in row-crop agroecosystems may also be enhanced by concentrating in the rhizosphere (e.g., Binet *et al.*, 1997; Spain *et al.*, 1990). Further investigation should focus on assessing the preference of various earthworm species (of different ecological categories) for different plant rhizospheres, using a combination of stable isotopes and other available techniques (Brown, 2000a; chap. 7).

Photo 3. Vue du dernier coupe horizontale (15 cm) dans un pot avec harricots et sol de La Víbora (chap. 5). Divers racines sortent des galeries des vers (*P. corethrurus*).

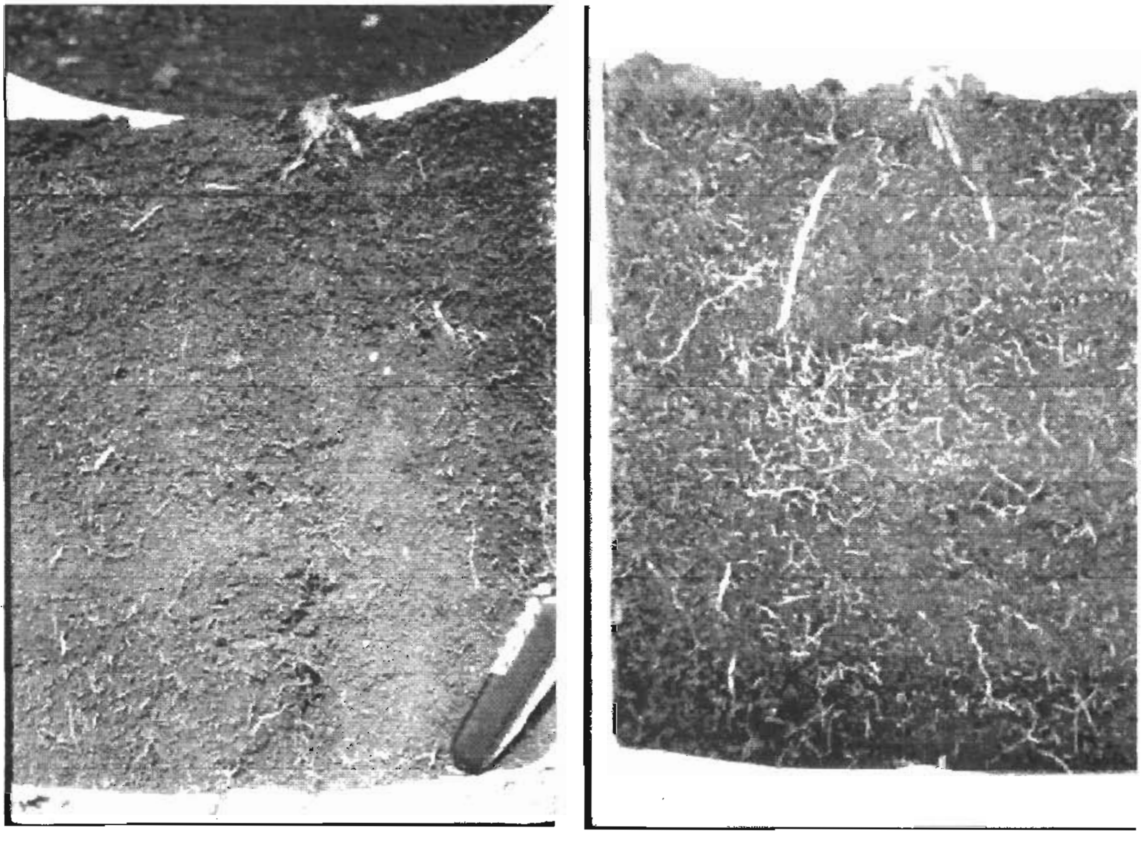


Photo 4. Vue de deux perfiles verticales des pots avec maïz (utilisé dans le chap. 4 pour determiner la biomasse racinaire) et sol de la forêt de Los Tuxtlas, pour estimer la densité des racines (chap. 5). À gauche, échantillonage à 131 jours, à droite, 183 jours.

# CHAPITRE 5

# MODIFICATION OF ROOT DISTRIBUTION AND GROWTH IN POT EXPERIMENTS WITH TWO TROPICAL EARTHWORMS**

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## SUMMARY

Three greenhouse experiments were performed to assess the role of two common tropical geophagous endogeic earthworm species, *Pontoscolex corethrurus* and *Polypheretima elongata*, on root distribution and biomass of several plant species in two soil types, a clayey Andosol and a sandy Alfisol, from Veracruz, Mexico. The equivalent of about 12kg dry soil were placed into 20 l plastic pots and 4 or 7 g (60 and 100 g $m^{-2}$, respectively) earthworms were inoculated to pots planted with common beans (*Phaseolus vulgaris*), *Brachiaria decumbens* pasture grass under four P fertilization regimes (0, 1.6, 8.4 and 10 kg $ha^{-1}$ P) and maize (*Zea mays*) with or without surface residues. At harvest roots and shoots were weighed, the pots cut in half and a transparent plastic sheet (overheads) used to draw root and earthworm structures in vertical and horizontal (every 5 cm) planes. The drawings were scanned, binarized and submitted to image analysis techniques to determine root and worm structure densites. Results showed few effects of earthworms on root biomass, with an increase observed only in beans with *P. elongata* and a decrease in *B. decumbens* with *P. corethrurus* and 10 kg $ha^{-1}$ P. Shoot/root ratios in maize + residues were twice lower in presence than absence of earthworms indicating more relative effort into root production. Root density was generally higher and more evenly distributed both horizontally and vertically in the presence of earthworms. Few relationships were observed between earthworm structure density and plant performance. Root density however, predicted well root biomass in most cases, although few relationships were observed with shoot biomass. These results suggest that earthworms may play a role in enhancing plant resistance to stress, although the induced changes in the root system may not necessarily lead to greater yields. Further studies using both destructive and non-destructive methods are necessary to properly describe the spatial and temporal interactions of earthworm activity and their structures (burrows and casts) with plant roots.

## INTRODUCTION

The role of earthworms in modifiying soil characteristics and plant production is by now well recognized. Over a century ago, Darwin (1881), in his last book, stated that "worm burrows ... greatly facilitate the downward passage of roots of moderate size; and these will be nourished

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** Submitted to *Soil Biology and Biochemistry* by the humus with which the burrows are lined." Since this initial work, much knowledge has been accumulated on earthworm ecology and relationships with plants and soils, however few detailed descriptions on the interactions between earthworms and plant roots are available.

Some earthworm species appear to be very common in the root zone (Rovira *et al.*, 1987; Robertson *et al.*, 1994), possibly indicating a preference of this region for their activities. The higher C content and populations of microorganisms in the rhizosphere may well act as an attractant to earthworms, particularly polyhumic species that feed on regions rich in OM. Furthermore, earthworm activity in plant rhizospheres may be important in fast cycling processes, providing readily-available plant nutrients, especially N (Lee, 1992). Some authors have even reported that earthworms feed on roots (Baylis *et al.*, 1986; Carpenter, 1985; Cortez and Bouché, 1992, Sackville-Hamilton and Cherret, 1988) and rhizosphere soil (Doube and Brown, 1998), although reduction in above-ground biomass has yet to be clearly demonstrated and directly related to this phenomenon. Perhaps some earthworm species feed on dead or dying roots thus aiding in plant sanitation, and maybe this root herbivory even causes compensatory below-ground growth, although this has also yet to be shown.

If earthworm migration to and preference for the rhizosphere is still not clearly defined, conversely, plant root migration into earthworm burrows has been better described, although the origin (and creator) of the original gallery occupied by the root has often been placed into question (Kretzschmar, 1998; Springett and Gray, 1997). That is, whether the earthworms first produced the gallery or whether it was previously created by a root or another organism and then taken advantage of (and expanded) by earthworms is not generally known, particularly in field situations. The answer to this dillema is most likely that both earthworms and roots develop together and adapt their own strategies to cope with the soil environment (Kretzschmar, 1998). Nonetheless, a concentration of roots in earthworm burrows has been commonly observed, particularly in lower (esp. B) soil horizons, where compaction often limits root penetration (Kretzschmar, 1978; Logsdon and Linden, 1992). In this region, the percentage of roots in earthworm burrows versus those outside may be very high (Ehlers *et al.*, 1983; Pitkänen and Nuutinen, 1997).

Both earthworms and roots may thus benefit from each other's presence and activities. The extent of this synergistic interaction and its spatio-temporal dynamics, however are still largely unknown. The few studies performed so far have addressed primarily the overlap of earthworm and natural channels (macropores) with root presence (e.g., Krebs *et al.*, 1994; Kretzschmar, 1978; Pitkänen and Nuutinen, 1997), the possible attraction of roots to earthworm channels (Springett and Gray, 1997) and vice-versa (Hirth *et al.*, 1998; Springett and Gray, 1997). Most of these experiments were performed in temperate regions, with pasture grasses and cereal grains and a limited number of earthworm species. Up to the present, to the author's knowledge, no study has been performed using tropical earthworm species. Therefore, to assess both the temporal and spatial dynamic of earthworm interaction with roots the following experiments were performed using common tropical earthworm and plant species and multiple sample dates.

## MATERIALS AND METHODS

The materials, methods and experimental designs for the three trials performed are detailed in separate publications (Brown *et al.*, 2000a; chap. 3; Brown *et al.*, 2000b; chap. 4; Patrón *et al.*, 1999). Thus we will only briefly state here the main points in the materials and designs used, referring in more detail to the methodology used to quantify and describe root density and distribution in soil.

Two soil types, one a clayey (40% clay, 40% silt, 10% sand) Andosol and the other a sandy (82% sand, 10% clay, 8% silt) Alfisol were collected from the field by removing the top 10 cm. The former, taken from a native-grass pasture located at La Víbora (18°50' N 96°07' W, 35 m alt.), was severely P-limited and had only 0.9% C, 0.1% N and a CEC of 11.7 meq $100g^{-1}$. The latter was taken from a tropical rainforest at the Estación de Biología Tropical "Los Tuxtlas" (18°35' N and 95°04' W, 380m alt.) and had 5.8% C, 0.5% N and a CEC of 30.3 meq $100g^{-1}$. The soils were partly air-dried, sieved at 5 and then 2 mm and stored in burlap bags in the greenhouse.

Two pantropical geophagous endogeic earthworm species, *Pontoscolex corethrurus* and *Polypheretima elongata* were chosen for this study. *P. corethrurus* were taken from a well studied pasture at Plan de las Hayas (Lavelle *et al.*, 1981), from the Los Tuxtlas station and from the Centro de Investigaciones Costeras "La Mancha." *P. elongata* were also collected at the latter site. Both species were placed for several days in the target soils in large plastic tupperwares before using them for the experiment.

About 12 kg (oven dry weight equivalent) soil was packed into 20-liter white plastic pots and watered to field capacity (pF 2.0). The sandy savanna soil required little water and was easily packed to 1.2 g $cm^{-3}$ into the pots while the clayey forest soil due to its andic properties retained much water and was packed at a lower bulk density (0.8 g $cm^{-3}$). Certified seeds of *Phaseolus vulgaris* (black bean), *Zea mays* (maize) and *Brachiaria decumbens* (pasture grass) were purchased locally and planted in their respective pots. Selected pots planted with maize received 9g each (equivalent to 1.36 T $ha^{-1}$) of maize residues applied on the soil surface. Buckets with *B. decumbens* had 0, 1.6 (surface-applied), 8.4 (injected into the root zone) or 10 kg $ha^{-1}$ (injected+superficial) P fertilizer. Earthworms were dried with paper towells and a mean of 9-10 *P. corethrurus* (150 indiv. $m^{-2}$) and 3 *P. elongata* (50 indiv. $m^{-2}$) weighing a total of about 4 g (wet weight, gut contents included; equivalent to 60 g $m^{-2}$), were placed on the soil surface of pots with beans and maize. Buckets with *B. decumbens* received from 9-14 *P. corethrurus* (mean 150 indiv. $m^{-2}$) for a total of about 7g (110 g $m^{-2}$). All the pots were placed in a random order in the greenhouse. The first two experiments were performed at Coatepec, Veracruz while the latter was performed at the Intstituto Nacional de Investigaciones Nucleares (ININ), Salazar, Mexico. Ambient temperatures, plant heights and other parameters (plant health) were recorded every few days. The designs used for the three experiments are briefly shown in Table 1.

Beans were harvested at 97 d, *B. decumbens* at 51 d and maize at 131 and 183 d. At each harvest date plant (height, number of leaves, shoot and root biomass) and earthworm (biomass, number) parameters and soil properties (bulk density, moisture and nutrient contents) were measured according to the methods detailed in Brown *et al.*, (2000a; chap. 3; 2000b; chap. 4), Patrón (1998) and Patrón *et al.* (1999).

### Root and earthworm-structure density calculations

At each harvest date, the plastic pots were cut in half and clear plastic overheads were used to trace roots and earthworm structures (casts and burrows) on the vertical plane, using permanent ink markers of different colors. An example of a vertical cut drawing in a maize treatment with residues and *P. corethrurus* is shown in Figure 1. For beans and maize, additional cuts were performed every 5 cm on the horizontal plane; for beans there were three horizontal planes (5, 10, 15 cm) while for maize there were four (5, 10, 15, 20 cm; 131 d) or five (5, 10, 15, 20, 25 cm; 183 d) planes. Selected pots were chosen from each experiment according to the following criteria: both earthworms and plants were alive at harvest and (for beans and maize) grain was harvested. Thus, for beans, the number of replicates (n) used was n = 5 for *P. corethrurus* and uninoculated controls, and n = 8 for *P. elongata* treatments. For maize+residues n = 2 for *P. corethrurus* and n = 3 for controls and for maize without residues at 131 d, n = 3 for both ± earthworm treatments and at 183 d, n = 4 for both treatments. With *B. decumbens*, n = 4 for 0 and 8.4 kg $ha^{-1}$ P and n = 3 for 1.6 and 10 kg $ha^{-1}$ P, respectively.

Table 1. Brief summary of the experimental designs used in the study of earthworm-plant root interactions.

| Earthworm species | Biomass (g $m^{-2}$) | Soil Type Texture | Plant Species | Harvest (days) |
|---|---|---|---|---|
| *Pontoscolex corethrurus*<br>*Polypheretima elongata* | 56-60 | Alfisol<br>sandy loam | *Phaseolus vulgaris*<br>(Black beans) | 97 |
| *P. corethrurus* | 110 | Alfisol<br>sandy loam | *Brachiaria decumbens* | 51 |
| *P. corethrurus* | 60 | Andisol<br>clay | *Zea mays* (Maize) | 131, 183 |
| *P. corethrurus* | 60 | Andisol<br>clay | *Zea mays* (Maize)<br>+ surface residues | 183 |

The drawings were then scannned, producing a digitized black image which was then transformed into a binary image. On this image, earthworm structures and roots were separated creating two different files. Both files were then separately submitted to an image analysis technique using the shareware program NIH (National Institute of Health, USA) IMAGE which produced a grid of uniformly sized squares (with a definite number of pixels) to reveal the number of black pixels (roots) in each square, thus giving an estimate of the mean root density (mean number of black pixels per square), according to equation 1.

$$\text{mean root density} = \frac{\text{number of black pixels}}{\text{total number of pixels}} \qquad (1)$$

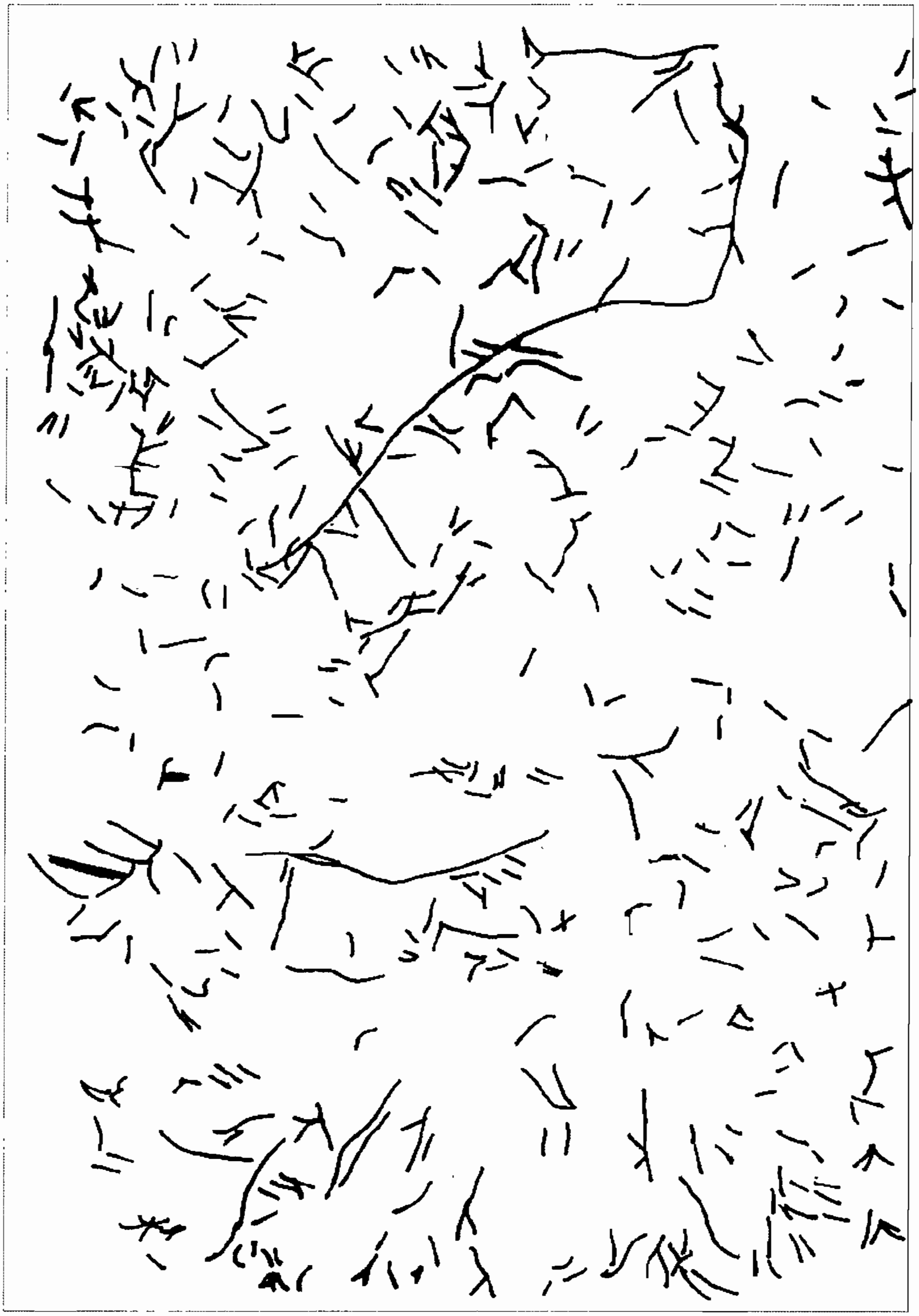

Figure 1. Example of a scanned and binarized vertical root drawing (0-19 cm) from a maize pot with *P. corethrurus*. Original size of the drawing was 554 cm$^2$. Top of the drawing is on left.

## Root distribution measurements

The spatial distribution of roots (aggregated, uniform, random) was then studied in two steps: first of all, the size of the squares used to calculate root density was progressively increased, producing a density function curve, in which the variance ($\sigma^2$) of the number of black pixels in each square was related with the size of the square. The equation describing this function was:

$$\sigma^2 = \frac{K * \text{mean}}{\text{grid}^x} \qquad (2)$$

In which x is a power factor, determining the size of the square grid and related to the shape of the curve (distribution of roots in the drawing), while K is related to the initial 'y axis' variance. As x increases towards 1 (steeper curve), the distribution of roots becomes more heterogeneous, while if it decreases towards 0, the distribution becomes more random (not aggregated) until it becomes uniform (straight line; $x = 0$).

Next, the type of root distribution was studied by developing a series of random images with 200-400 objects of various shapes (blocks, oblong or eliptical; limited to minor/major diameter of figure always <0.1). The images were then subjected to a series of deletions until only 10% of the objects were present. The deletions ranged from 2-20 pixels, and after each deletion the number and percentage of remaining black pixels was calculated, producing a graph with of the number of deletions * the % of objects remaining. The spatial distribution of the objects was considered random when a straight line (no. deletions * % objects) resulted, since this represented an equal probability of finding an object to delete. A polynomial or logarithmic curve shape indicated a spatially dependent distribution (e.g., aggregated), due to an unequal probability of deleting an object. Each of the images of maize and bean plant roots were submitted to an analysis of the spatial distribution of the roots by fitting the curves of deletions by % objects remaining to a linear, logarithmic or polynomial regression. The intercept (s) of the regression equations and the coefficient (*r*) were obtained and compared between treatments. The closest fit to each curve (highest *r*) was assumed to describe the type of distribution (random, uniform or aggregated) of roots in the pots.

## Statistical analyses

Each of the treatment effects (earthworms, fertilizers, residues) on root density both in the horizontal (different depths) and vertical planes were assessed by comparing the means with the appropriate controls using ANOVA. The relationship between root density and various other plant parameters (number of leaves, root, shoot and stover biomass, root/shoot ratios), as well as with the density of earthworm structures was explored using linear regression. The regression coeficients of the different treatments obtained from the distribution calculations were also compared using ANOVA. All analyses were performed using the software package SuperAnova® (Abacus Concepts).

# RESULTS

## Root biomass and density

In the beans, *P. elongata* significantly increased root biomass (Brown *et al.*, 2000a; chap. 3) and density in the vertical plane (Fig. 2), while for *P. corethrurus* these parameters were higher but not significant. On the other hand, mean root density in the horizontal cuts was significantly higher with both earthworm species (Fig. 2). Similarly, at 5 cm significantly more roots were found in both earthworm treatments compared to controls (Fig. 3) although at 10 and 15 cm these differences were absent.

Root density values of *B. decumbens* (Fig. 4) were closer to those of beans than of maize, probably due to the shorter length of the experiment. Fertilization increased mean root density significantly, and the highest densities were obtained in the no-worm treatments with 8.4 and 10 kg ha$^{-1}$ P (Fig. 4). These values were significantly different than those obtained for treatments with earthworms at the same fertilization level. No difference was observed between density with or without earthworms at 0 or 1.6 kg ha$^{-1}$ P.

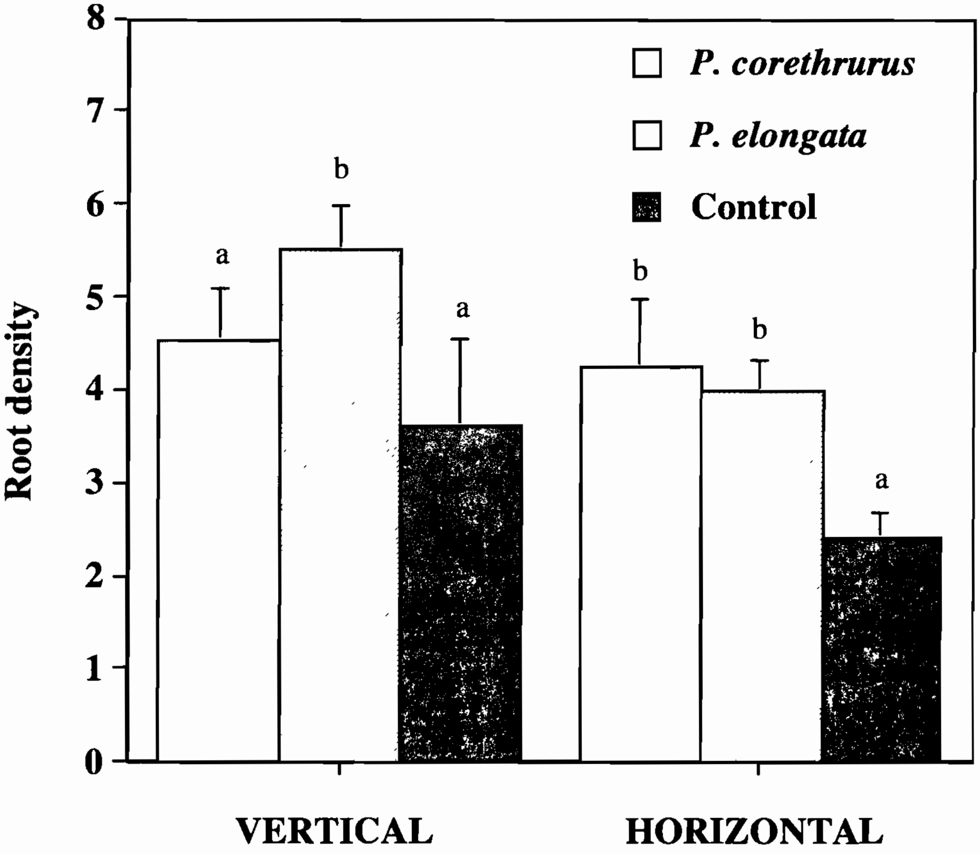

Figure 2. Vertical and horizontal density (means + SE) of bean roots at the final harvest (97 d) in the presence or absence (control) of *P. corethrurus* or *P. elongata*.

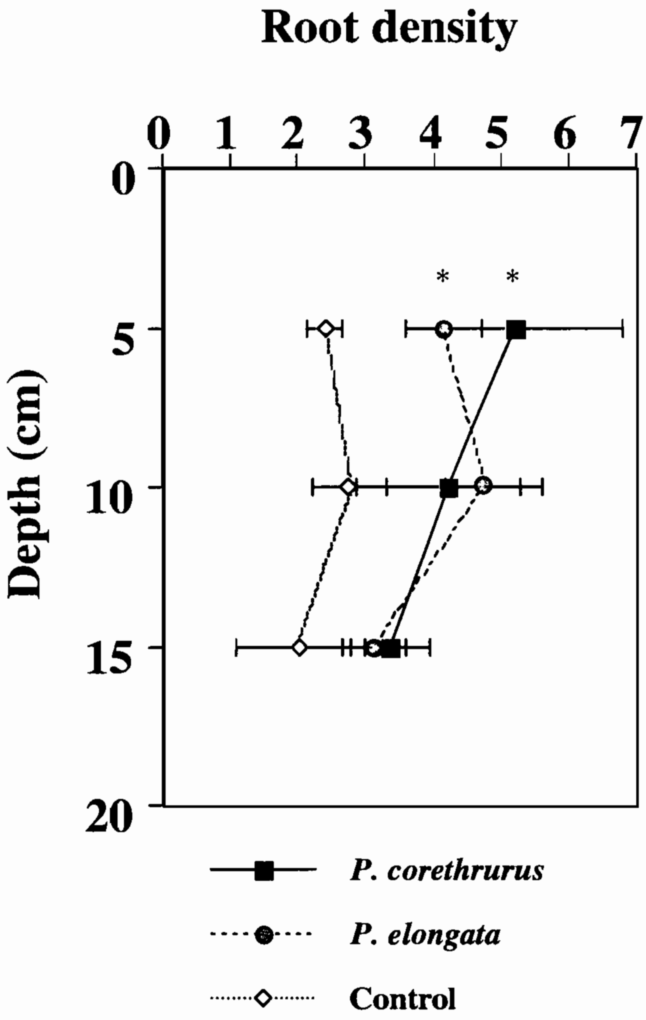

Figure 3. Distribution of *Phaseolus* bean root density (means ± SE bars) with depth, in the presence or absence (control) of *P. corethrurus* or *P. elongata*. Asterisks (*) denote significant differences between earthworm and control treatments at $P<0.05$.

No differences were observed in total root biomass due to the different treatments with maize (Brown *et al.*, 2000b; chap. 4). Shoot/root ratios in plants under maize + residues with earthworms, however, were 50% lower (3.5) than in control treatments (7.4). In the maize without residues, horizontal and vertical density increased slightly from 131 to 183 d (Photo 4; Fig. 5), and at 131 d horizontal density was significantly higher with *P. corethrurus* (Fig. 5). Nevertheless, no significant differences were observed at 183 d between worm and no-worm treatments. In the maize + residues vertical densities were lower than without residues (Fig. 5). Significantly higher horizontal densities were observed in the earthworm treatments but, despite higher values in the vertical plane, these were not statistically significant.

The results of correlations using plant production parameters and mean overall horizontal and vertical root densities (from all treatments combined or earthworm treatments alone) are shown in Table 2. With beans both vertical and horizontal densities were positively correlated with above and below-ground yields, but not correlated with shoot/root ratios. The number of pods per pant was significantly related with root density in the horizontal plane. Earthworm effects were only significant when relating vertical density with root biomass in the presence of *P. elongata*, or when relating horizontal density with root and shoot biomass and the number of pods in treatments with *P. corethrurus*. In pots with *B. decumbens*, correlations between vertical root density with shoot and root biomass and shoot/root ratios were significant using all data and treatments with only *P. corethrurus*.

In maize without residues at 131 d, no significant correlations were observed, except for a positive relationship with root biomass in pots with *P. corethrurus*. At the 183 d harvest, densities were significantly related with root biomass and shoot/root ratios or with the number of leaves (in no residue treatments). Shoot biomass was related with root density in the horizontal planes. No significant effects of *P. corethrurus* on plant parameters other than shoot/root ratios were observed. In the presence of residues, the number of replicates was small (n = 2 and 3), and few relationships with root density were observed; positive with root biomass using horizontal plane data, negative with shoot/root ratios using horizontal data, and positive with grain yield using vertical data.

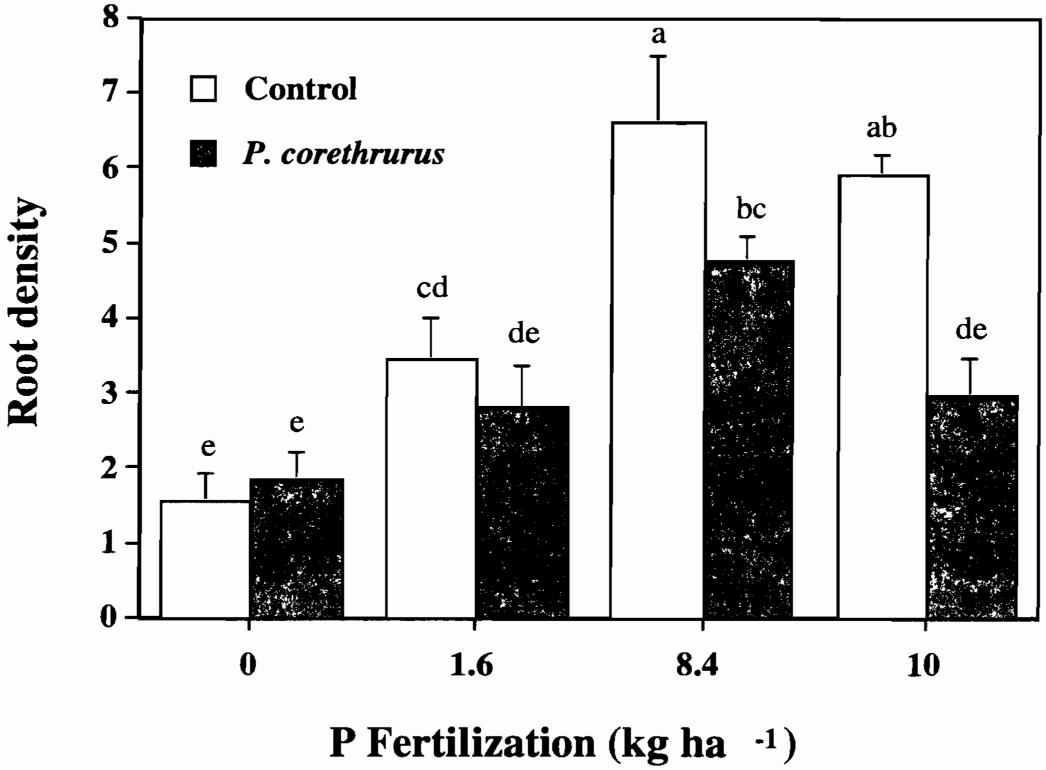

Figure 4. Density (means + SE) of *B. decumbens* roots in the presence or absence (control) of *P. corethrurus* under four P fertilization regimes (0, 1.6, 8.4 or 10 kg ha$^{-1}$).

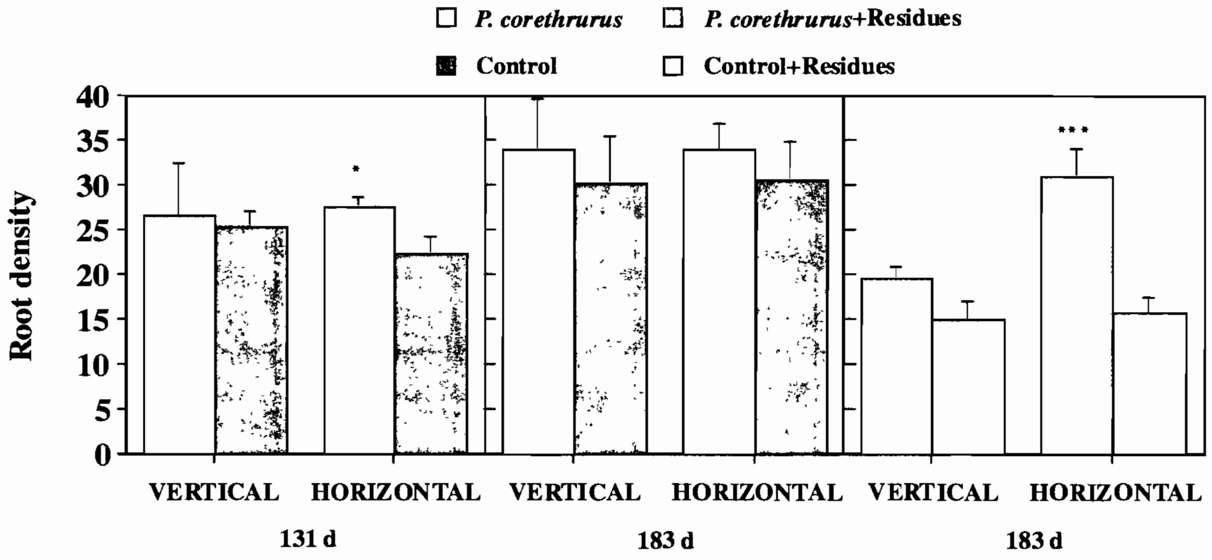

Figure 5. Vertical and horizontal density (means + SE) of maize roots at two harvest dates (131 and 183 d) without residues and with surface-applied maize residues at 183 d in the presence or absence (control) of *P. corethrurus*.

Table 2. Regressions of root densities with plant parameters for beans and *B. decumbens* (A) and for maize (B) using data from all treatments combined or earthworm treatments separately. Statistical significance as follows: *** P<0.001; ** P<0.01; * P<0.05; † P<0.1, ns = not significant, ND = not determined. *P.c.* = *P. corethrurus*; *P.e.* = *P. elongata*.

Table 2A. Beans and *B. decumbens*

| Plant | Parameter | Vertical Density | | | Horizontal Density | | |
|---|---|---|---|---|---|---|---|
| | | All | Earthworms | | All | Earthworms | |
| Treatment | | treatments | *P.c.* | *P.e.* | treatments | *P.c.* | *P.e.* |
| *Phaseolus* beans | Root biomass | 0.64** | ns | 0.79* | 0.42† | 0.89* | ns |
| | Shoot biomass | 0.42† | ns | ns | 0.58* | 0.80† | ns |
| | Shoot/Root | ns | ns | ns | ns | ns | ns |
| | Number of pods per plant | ns | ns | ns | 0.49* | 0.90† | ns |
| *Brachiaria decumbens* | Root biomass | 0.69*** | 0.73** | ND | ND | ND | ND |
| | Shoot biomass | 0.84*** | 0.77** | ND | ND | ND | ND |
| | Shoot/Root | 0.78*** | 0.70* | ND | ND | ND | ND |

Table 2B. Maize with or without residues

| Plant Treatment | Parameter | Vertical Density |  | Horizontal Density |  |
|---|---|---|---|---|---|
|  |  | All treatments | *P. corethrurus* | All treatments | *P. corethrurus* |
| Maize no residues | Root biomass | ns | 1.0* | ns | 0.99† |
| 131 d | Shoot biomass | ns | ns | ns | ns |
|  | Shoot/Root | ns | ns | ns | ns |
| Maize no residues | Root biomass | 0.84** | ns | 0.88** | ns |
| 183 d | Shoot biomass | ns | ns | 0.69† | ns |
|  | Shoot/Root | -0.89** | -0.93† | -0.84** | -0.96* |
|  | Leaf biomass | 0.63† | ns | 0.56* | ns |
|  | Stover biomass | ns | ns | 0.70† | ns |
| Maize+Residues | Root biomass | ns | ns | 0.63*** | 0.61† |
| 183 d | Shoot biomass | ns | ns | ns | ns |
|  | Shoot/Root | ns | ns | -0.99** | ns |
|  | Grain biomass | 0.95* | ns | ns | ns |

### Earthworm structure density

The density of earthworm structures in the different experiments and treatments is given in Table 3. Few earthworm structures were visible in the vertical plane of pots with *B. decumbens*. Similarly, in the 131 d harvest of maize, density of structures was low, although it varied with depth; highest density (7.3) was observed at 15 cm and lowest (0) at 5 cm.

At 183 d without residues, both vertical and mean horizontal densities were smaller than with residues; furthermore, at each horizontal plane, density with residues was higher than without. At the 10 and 15 cm plane, densities were the highest, indicating this region as they preference for activity. The mean obtained from the horizontal cuts was higher than with the vertical in the treatment with residues, perhaps indicating relatively greater vertical activity. With beans, density increased with depth, from a mean of 11.0 and 2.0 at 5 cm with *P. corethrurus* and *P. elongata*, to 30.2 and 18.9, respectively at 15 cm. Mean density in the vertical plane tended to higher values than obtained in the horizontal plane, in presence of both species, indicating relatively greater horizontal activity.

The regressions of earthworm structure densities with root densities and other plant parameters are shown in Table 4. Very few relationships were found, although a few interesting and strong correlation coefficients were observed. For the horizontal structure density, the number of bean grains collected was strongly related with earthworm structure density. Root density in pots with *P. elongata* was also related to earthworm structures, and root biomass, with *P. corethrurus*. In maize without residues at 183 d the biomass of shoots was strongly related to earthworm structure density. Using the vertical data, a strong relationship with root density was observed with maize and no residues at 131 d. Finally, bean pod biomass and number were strongly related to *P. corethrurus* structures.

### Root spatial distribution

Distributions through the pot profile as measured in the horizontal planes at different depths, showed a general trend for homogenization of root density in presence of earthworms, with a more even distribution of roots over depth in maize compared with the no earthworm controls (Fig. 6). An exception was the sharp decrease in density from 5-10 cm in pots with residues applied. At depth, differences between earthworm and control treatments tended to increase.

Roots were often observed in earthworm burrows, particularly with beans (Photo 3). Furthermore, the distribution of maize and bean roots in the pots was never random, following in all cases very closely, a second order polynomial curve when the percentage of objects remaining was regressed against the number of deletions. However, no significant differences between the *r* values for the different experiments and treatments was found. The x (power) factor values of the density function curves for the different treatments always ranged from 0.50 to 0.60. Few differences between treatments with and without earthworms were found in terms of the root distributions, and these were only in the horizontal planes; in the vertical plane, no differences between treatments were observed in any of the three experiments. In the beans, at the 15 cm horizontal plane, root distribution in the control treatments was more heterogeneous (x = 0.57) than with *P. corethrurus* (x = 0.50). Similarly, at 183 d in the maize without residues, the same occurred at the 20 cm horizontal plane (x lower with *P. corethrurus*). In the maize with residues, x values tended towards higher values in the control treatment and the mean horizontal x value was higher (x = 0.58; significant at $p<0.07$) than with *P. corethrurus* (x = 0.56). At the 15 and 25 cm planes, control values were higher (significant at $p<0.08$) than with earthworms, indicating a more heterogeneous root distribution.

## DISCUSSION

Root morphology at a given time results from inherent genetic characteristics (plant species; cultivar) and both past and present soil environmental conditions such as structure, texture, water and nutrient availability, temperature, gaseous composition, microbial and faunal populations and activity, carbon and additional energy inputs (Smucker, 1993). Roots can sense soil water, nutrient and mechanical conditions and send signals (including various plant hormones) to shoots, which can ultimately regulate plant growth (Aiken and Smucker, 1996). Since nutrients and water are supplied by the root system to the shoot, it is the density, distribution and activity of roots which largely determine plant production (Brown and Scott, 1984).

Table 3. Mean (±sd) density of earthworm structures in the horizontal and vertical planes of beans, maize and *B. decumbens* pasture (with different levels of P fertilization) in the presence *P. elongata* or *P. corethrurus* in pot experiments using a clayey or sandy tropical soil. Rows with different letters mean significant differences between treatments at P<0.05. ND=not determined

| Treatment | Beans | | *B. decumbens* (kg ha$^{-1}$ P) | | | | Maize no Res. | Maize no Res. | Maize+Res. |
|---|---|---|---|---|---|---|---|---|---|
| | *P. corethrurus* | *P. elongata* | 0 | 1.6 | 8.4 | 10 | 131 d | 183 d | 183 d |
| Vertical | 36.2a (13.5) | 15.5b (10.4) | 2.1c (2.2) | 1.4c (1.1) | 1.0c (0.5) | 2.3c (1.1) | 2.7c (2.4) | 11.8bc (5.0) | 18.0b (1.26) |
| Horizontal | 18.3b (12.7) | 7.9c (9.0) | ND | ND | ND | ND | 3.3c (4.5) | 10.0c (10.2) | 34.9a (16.7) |

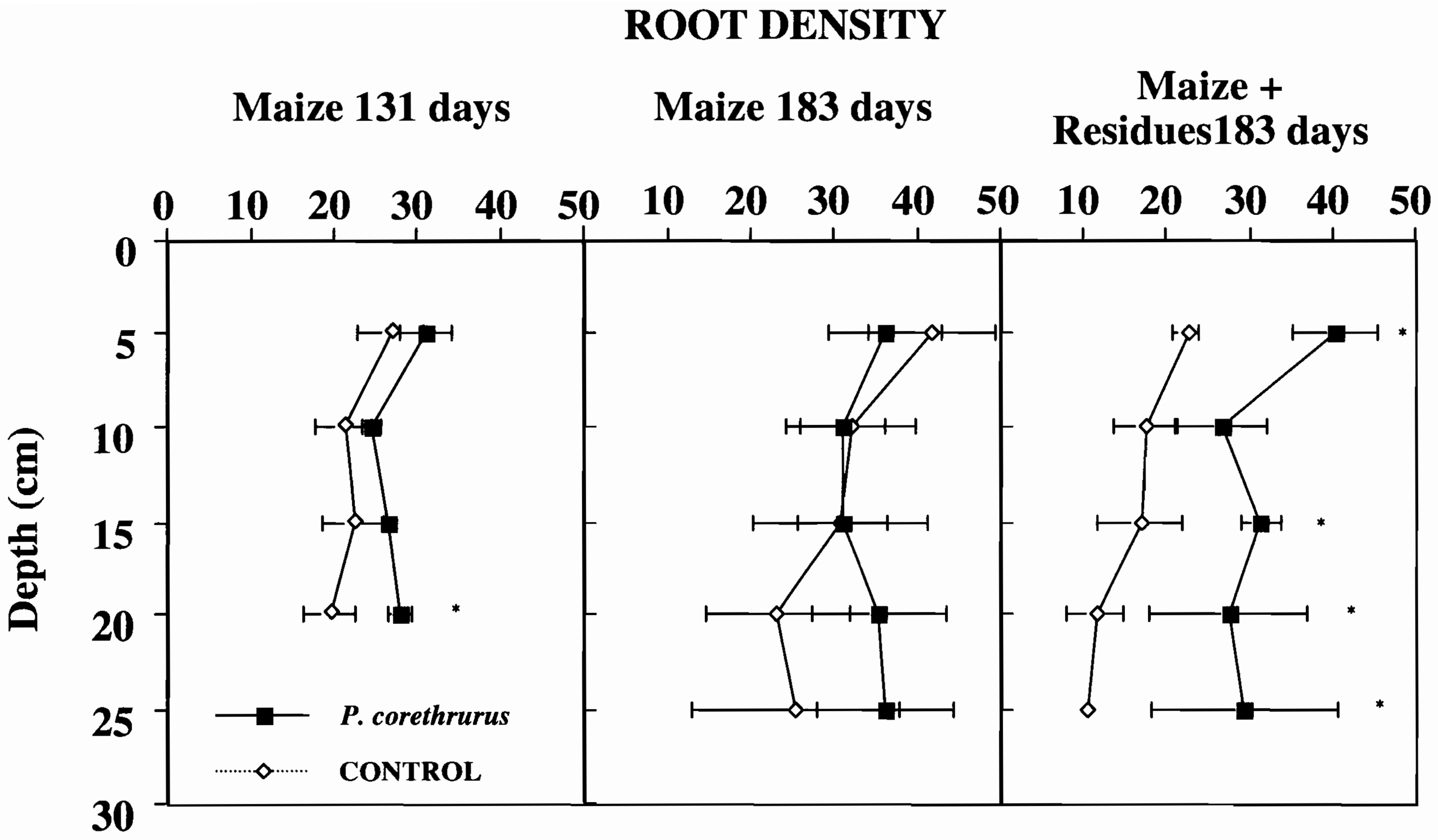

Figure 6. Density (means ± SE bars) distributions of maize roots in horizontal cuts through the pot profile in the presence or absence (control) of *P. corethrurus*, with or without surface-applied maize residues at two harvest dates (131 and 183 d). Asterisks (*) denote significant differences between earthworm and control treatments at $P<0.05$.

Table 4. Results of the regressions between earthworm structure (burrows, casts) density and various plant paramters, in the different experiments. ND = not determined; ns = not significant. *P. coreth.* = *P. corethrurus*.

| Parameter | *Phaseolus* beans | | *Brachiaria decumbens* | Maize no Res. 131 d | Maize no Res. 183 d | Maize +Res. 183 d |
|---|---|---|---|---|---|---|
| | *P. coreth.* | *P. elongata* | | | | |
| *Horizontal* | | | | | | |
| root density | ns | 0.42* | ND | ns | ns | ns |
| root biomass | 0.83† | ns | ND | ns | ns | ns |
| shoot biomass | ns | ns | ND | ns | 0.99* | ns |
| Bean number | 0.71† | 0.99† | ND | ND | ND | ND |
| *Vertical* | | | | | | |
| root density | ns | ns | 0.28† | 0.99† | ns | ns |
| root biomass | ns | ns | ns | ns | ns | ns |
| shoot biomass | 0.96** | ns | ns | ns | ns | ns |
| shoot/root | ns | ns | ns | ns | ns | ns |
| Pod biomass | 0.97* | ns | ND | ND | ND | ND |
| Pod number | 0.94† | ns | ND | ND | ND | ND |

Root maps and spatial analysis of roots in the field have shown that root distributions are seldom uniform (homogeneous) and are often clustered along pedon faces and biopores (Smucker, 1993). These heterogeneous (aggregated) distributions can result from compaction and regions rich in water and nutrients. This spatial clustering results in a lower soil volume exploited by the roots and can increase the potential for greater biotic and abiotic stress (Smucker, 1993). In the present experiment, roots were not uniformly distributed and earthworms had little effects on root distribution, but when significant differences occurred, the roots tended to be slightly more homogeneously distributed in the earthworm treatments. This is despite the production of channels and aggregates within the soil, where roots often concentrate (Ehlers, 1983; Lavelle *et al.*, 1998), and several visual observations of roots within growing in worm channels (Photo 3). This was probably due to the lack of physical impedence (low bulk density values) for roots in the pots, the low amount of roots produced in beans and *B. decumbens* and, in the case of maize, relatively less earthworm-induced increases in soil fertility (in casts) in the Andosol compared with the Alfisol used for beans (Hernández, 1999).

Root systems consisting of mostly fine roots (such as those of grasses), develop greater surface areas and root densities at lower relative C costs to the plant (Eissenstat, 1992) and permit a better utilization of soil resources and resistance to stress. The fibrous root system of maize with extensive lateral branching (many secondary roots) resulted in much higher root densities than with beans or *B. decumbens*. The earlier harvest of the latter plant and the different rooting strategy of the former (taprooted dicot, deeper and fewer roots) are probably partly responsible for these differences. Further factors involved could be the differences in physical properties and fertility of the soils used and in earthworm activity levels. The denser root system may also be related to competition between the plants and earthworms for water or to changes in the soil pore size distribution due to earthworm activities, and the indirect effects of these changes on water availability to plants. The presence of residues in maize reduced root density, particularly in the top 10 cm. This could be due to the higher availability of nutrients leaching from the residues or to greater protection from wetting-drying cycles, which tend to increase root branching (Smucker and Aiken, 1992).

If denser root systems favor plant performance, the increase in root density in the presence of earthworms observed in several of the above experiments would thus seem to imply that these treatments should yield more than the controls. For beans, this appeared to be the case for pots with *P. elongata* although for *P. corethrurus*, despite higher values, these were not statistically significant. In maize, contrary to expected, control treatments generally outyielded those with earthworms. Perhaps in these treatments, the greater investment in roots was to the detriment of shoots. In fact, with residues, shoot/root ratios were twice lower in the presence of earthworms in the treatments used for root dentity estimations. With *B. decumbens,* root biomass increased up to >4 times with 8.4 kg $ha^{-1}$ P compared with the lower fertilization levels (0 and 1.6 kg $ha^{-1}$ P), and no differences were observed in treatments with or without earthworms (Patrón *et al.*, 1999). With 10 kg $ha^{-1}$ P, the treatment without earthworms yielded the same as those with 8.4 kg, while the treatment with earthworms yielded significantly less, with biomass similar to those found with 1.6 kg $ha^{-1}$ P (Patrón *et al.*, 1999). Both earthworms and fertilization decreased VAM infection of roots and these, in addition to observed problems in the water storage in the 10 kg $ha^{-1}$ treatment with earthworms could have been responsible for the lower yields (Patrón *et al.*, 1999). In apple orchards in Holland, van Rhee (1977) found a greater number of small (<0.5 mm diam.) roots in orchards inoculated with earthworms, although few differences in apple yields were observed. These results appear to imply that increased root density due to earthworms does not necessarily coincide with greater yields.

Many experiments however, have reported earthworm-induced increases in root growth and biomass both in field and greenhouse experiments, although roots generally received less attention than the above ground parts in most trials (Brown, 2000b; chap. 1). In no-tillage situations, Edwards and Lofty (1978; 1980) and Springett (1985) showed that root depth distribution depended on the earthworm species present. Anecic species such as *Aporrectodea longa* and *Lumbricus terrestris* increased root biomass at greater depths than shallow burrowing species. This is likely because the former species not only tend to burrow more vertically but also produce deeper channels which roots can follow. Roots can also enter and follow the mostly horizontal burrows produced by geophagous endogeic species, and the probability of encountering these channels is likely to be higher than that of encountering vertical burrows (Tisdall and McKenzie, 1995). More research is needed to determine the reasons why roots chose to follow burrows in some soils and not in others, and which type, size, abundance, distribution and orientation of burrows is best for the growth of a particular plant.

Biological effects such as the interaction of earthworms with plant root pathogens (fungi & bacteria), parasitic nematodes or beneficials such as Rhizobia, VAM and plant growth-promoting rhizobacteria are also important and have begun to receive more attention (Brown *et al.*, 2000d; chap. 6, Brown et al., 2000e; Doube and Brown, 1998). In the present experiments interaction with VAM may have been an important controller determining the effect of earthworms on yields. Further confirmation of this hypothesis is necessary. The role of earthworms in plant growth regulating hormone production also needs more research. The early work of Springett and Syers (1979), showing negative geotropism of roots towards surface-deposited casts of *Lumbricus rubellus* seems to imply that hormones in castings may play an important role in root growth and distribution in soils. However, the absence of this effect with *Aporrectodea caliginosa* casts and the lack of preferential elongation of ryegrass roots into macropores filled with *Aporrectodea rosea* casts (Hirth *et al.*, 1997) illustrate the possible specificity of this phenomenon.

In conclusion, we have shown that earthworms affected both vertical and horizontal root distribution in several instances, tending to create more even distributions. Thus, plants growing in the presence of earthworms may benefit not only from possible changes in soil structure (aggregation, biopores for root elongation), fertility (higher nutrient content in casts) and biological characteristics (populations of pathogens, parasites and beneficials), but also from a greater volume of soil under exploitation by roots, enhancing the plant's resistance to stress. In fact, root density measurements were generally well correlated with root biomass, although in only a few cases was it related with shoot biomass. We are still far from understanding the way in which plant roots and earthworms interact, and what effects this will have on yields. This is largely because even the mechanisms responsible for root branching and the photosynthate costs associated with the production and maintenance of these branches are essentially unknown (Smucker, 1993). Furthermore, there is still much to learn about the impact of earthworms on soil physical, chemical and biological soil properties and the stability, durability and spatial distribution of these effects. Computer Assisted Tomography (CAT-scanning) (e.g., Capowiez *et al.*, 1998; Langmaack *et al.*, 1999), mini-rhizotrons (Springett and Gray, 1997) and other non-destructive descriptive techniques that can be repeated in time, combined with destructive sampling and image analysis are a good start in describing interactions of earthworms and roots (Krebs *et al.*, 1994; Hirth *et al.*, 1997; Springett and Gray, 1994). However, there is also need for even more basic research since earthworm burrowing behavior in different soils, the composition of the linings, the amount of below vs. above-ground castings, the chemical, physical and microbiological characteristics of casts and their changes over time and the amount of overlap of earthworm activity with rhizospheres of different plants are still unknown for many earthworm species, particularly in tropical regions.

Photo 5. Vue générale des pots (25 cm diam., 25 cm haut.) avec *B. decumbens* dans le serre à Xalapa, Veracruz.

# CHAPITRE 6

# THE ROLE OF INTERACTIONS BETWEEN EARTHWORMS (*PONTOSCOLEX CORETHRURUS*), ARBUSCULAR MYCORRHIZAl FUNGI (AMF) AND FERTILIZERS IN *BRACHIARIA DECUMBENS* PASTURE PRODUCTION AND NUTRIENT UPTAKE[††]

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## SUMMARY

To investigate the effect of the interaction of the pantropical geophagous earthworm *Pontoscolex corethrurus* with vesicular-arbuscular mycorrhizae (AMF) on production and nutrient uptake of the tropical pasture grass *Brachiaria decumbens* under different fertilizer regimes, a greenhouse experiment was set up using 15-l plastic pots filled with the equivalent to 10 kg o.d. soil from a nutrient- (esp. N and P) poor pasture near La Víbora, Veracruz. Four fertilizer treatments were studied: no fertilizers (0F), NPK, PK and NK (200N, 100P, 200K; kg ha$^{-1}$), each mixed homogeneously into the soil. For AMF- treatments, soil was irradiated with 100 krads of gamma-ionizing radiation. A total of 16 treatments were investigated, each replicated four times: Fertilizers * ± Worms (60 g m$^{-2}$) * ±AMF. *B. decumbens* harvest (70 d after transplanting) biomass ranged from 0.47 T ha$^{-1}$ with NK-Worm-AMF to 6.5 T ha$^{-1}$ with PK and NPK fertilization. Yields followed the order of importance Fertilizers>AMF>Worms. P was essential for good production, but N was less important, yields being similarly low with 0F and NK and similarly high with PK and NPK. Both earthworms and AMF by themselves affected in a generally positive manner almost all of the measured plant parameters. Their effects, nonetheless varied significantly depending on the fertilizer treatment and if present alone or together. When N was applied, earthworms decreased AMF colonization, indicating possible negative effects of soil turbation or grazing on AMF growth. With PK, the treatment +AMF+Worm had lower yields indicating a possible increased C drain due to higher AMF colonization. In 0F, an important positive interaction of Worm * AMF was observed, with an 84% yield increase and higher AMF root colonization in the 10-20 cm soil layer. The $^{15}N$ analyses of earthworm tissues seemed to indicate activity in *B. decumbens* rhizosphere. The results of this experiment confirm the important role of soil fauna and flora interactions on plant production and the effect of fertilization on these interactions. Much more attention to these interactive edaphic phenomena is necessary, in both natural ecosystems and low-input agroecosystems where external (fertilizer) inputs are few or non-existent, and in high input systems where fertilizers are applied.

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[††] Submitted to *Plant and Soil*

## INTRODUCTION

In Mexico, the state of Veracruz occupies the first place in number of heads of cattle (>5,000,000), 64% of the total area of the state is in pastures and approximately 70% is dominated by native grasses and 30% by introduced pastures with exotic grasses (INEGI, 1997). A great part of these pastures are located near the coast, in soils of poor fertility and low P availability, where the potential benefits of more intimate associations with vesicular arbuscular mycorrhizae (AMF) are greater.

AMF fungi colonize plant roots establishing a symbiosis, where the fungi uses plant C for their metabolism, but at the same time providing the plant with nutrients (mainly P, N and micronutrients) and water extracted from the soil through an extensive net of extramatrical hyphae that acts as a natural extension of the root system. Other benefits of AMF association with plants can be protection against plant pathogens, greater stress tolerance and plant growth-promoting substance production (Ingham and Molina, 1991). AMF fungi also promote soil aggregation (Tisdall, 1991), and serve as food for other soil organisms (Ingham and Molina, 1991; Rabatin and Stinner, 1991; Fitter and Sanders, 1992).

*Brachiaria decumbens* is an excellent AMF host and is highly mycorrhizal-dependent when grown in P-poor soils (Saif, 1987). This grass species is extending its range in Mexico (between 1989 and 1992 more than 100,000 has were sown) due to its high quality forage, the high weight gains of animals grazing these pastures and its excellent adaptability to nutrient-poor soils (Enríquez, 1994; Lascano and Euclides, 1996; Rao *et al.*, 1996).

A great abundance and biomass of soil fauna, especially earthworms, inhabit Mexican pastures, which frequently surpass the weight of the livestock that graze it. The mean abundance and biomass of earthworms in 23 Mexican pastures were 283 ind. $m^{-2}$ (max. 861 $m^{-2}$) and 37 g $m^{-2}$ (max. 83 g $m^{-2}$; fresh weight) (Brown *et al.*, unp. data). *Pontoscolex corethrurus*, an exotic worm to Mexico has colonized many disturbed agroecosystems and is common in the pastures, reaching biomass values up to >30 g $m^{-2}$ fresh weight (Lavelle *et al.*, 1987). This species has been considered of great potential for raising productivity of grasses and perennial plants (dicots) (Brown *et al.*, 1999) and also shown to have an important interaction with AMF (Patrón *et al.*, 1999; Reddell and Spain, 1991; Ydrogo, 1994).

The objective of the present study was to evaluate the interaction between AMF and earthworms in the nutrition and productivity of *B. decumbens*, in a sandy, nutrient-poor soil from the coastal zone of the State of Veracruz, Mexico.

## MATERIALS AND METHODS

### Preparation of soils, earthworms, plants and AMF

Topsoil (700 kg) from the A horizon (0-10cm) of a grazed pasture near La Víbora (18° 53.1 N, 96° 09.1 W, 35 m above sea level) classified as a sandy (81% sand, 10% clay and 9% silt) kanhaplic Haplustalf (USDA) or Haplic Lixisol (FAO) was collected, sieved (2 mm) and air-dried. Selected chemical properties are displayed in Table 1. Half the soil was irradiated with a low dose of gamma radiation (100 krads) at the Institute of Nuclear Sciences (UNAM) in order to eliminate the native AMF population without causing important chemical modifications to the soil (Jakobsen, 1984). Four holes were made in the bottom of each pot and these were covered with a cloth (200 μm) in order to prevent earthworm escape. Each pot received the equivalent to 10 kg soil (oven dry weight), at a bulk density of 1.3 g $cm^{-3}$, and water was added up to field capacity (20% $H_2O$, pF 2.0). A microbial inoculant solution was prepared by adding 200 g non-irradiated soil to 1-l of $H_2O$ and then passed through a 40 μm sieve in order to remove the native AMF spores. The fertilizer combinations were weighed and added to the microbial inoculating solution which was then applied to the soil and intimately mixed until obtaining a uniform moisture.

Approximately 400 earthworms of the species *Pontoscolex corethrurus* were collected at the Center for Coastal Investigations "La Mancha" due to the low density of this species at La Víbora. The worms were externally rinsed with distilled water and one half of the individuals placed in the experimental soil (not irradiated) and the other half in soil previously sterilized in the autoclave (15 bar, 1 h) but re-inoculated with an aqueous solution of native microorganisms (without AMF) and stabilized at least 24 h earlier. In order to assure that the worms didn't have AMF (hyphae or viable spores) in their intestinal tract, after 5 d they were rinsed once more and placed into a new batch of sterilized soil, where they remained until their use. Each pot received about 7 individuals, with total weight of 3 g $pot^{-1}$ (equivalent to 60 g $m^{-2}$): 1 mature, 1 newly born and 5 juveniles.

Seeds of the introduced African grass pasture *Brachiaria decumbens* (purity 98.5%, 75% germination) imported from Brazil were previously germinated in agrolita (sterilized for AMF-treatments), an inert substrate without any nutrients. Seeds for AMF- treatments were surface-sterilized with a 5% bleach for 3 min. Distilled water was added to all the seeds and seedlings until transplanting (18 d). Two days after placing the soil and fertilizers in the pots and inoculating the earthworms, 10 seedlings of *B. decumbens* were transplanted and the soil inoculated with AMF. During the cycle, distilled water was added as necessary (by weighing the pots) to maintain field moisture capacity.

The AMF inoculant was obtained from the pasture at La Víbora, and consisted of roots of the native grasses washed in abundant $H_2O$ in order to remove the soil and cut into pieces of approx. 5 cm length. To the center of each pot 5 g of the fragments were added at 5-10 cm depth. The colonization with native AMF of these fragments was estimated at 78%. Fragments for AMF-treatments were sterilized previously (30 min.) in the autoclave. At the end of the experimental cycle the remaining fragments were removed from the center of the pots before taking samples for soil analysis. The sterilization changed significantly the properties of the inoculant; sterilized roots had a C/N ratio of 37 (1.0% N, 37.1% C) and total P content of 0.04% while the unsterilized roots had a C/ N of 66 (0.66% N, 43.4% C) and Pt of 0.16%.

The experiment consisted of 16 treatments: with and without (±) mycorrhizae, ± earthworms and four fertilizer mixtures; no fertilizers (0F), NK, PK and NPK. The fertilizers were added in the following formulations (and amounts): $NH_4NO_3$ (200 kg $ha^{-1}$ N), $KH_2PO_4$ (100 kg $ha^{-1}$ P) and $KH_2PO_4$ + KCl (200 kg $ha^{-1}$ K). The N fertilizer was doubly labeled ($^{15}NH_4$, $^{15}NO_3$) with 0.76 atom% $^{15}N$ ($\delta^{15}N$ = 1100‰). The pots were placed on tables in the clear-plastic roofed greenhouse (3 x 4 m) completely at random. There were four replicates per treatment, for a total of 64 pots. Four hours of artificial light were provided in the morning, for a total of approx. 13 h daily.

### Parameters studied

The heights of all the 640 plants (tallest stolons only) were measured on 10 occasions (13, 16, 20, 23, 31, 37, 44, 55, 62 70 d after transplanting, d.a.t.). Some earthworm casts from the surface of the soil in pots of each treatment with AMF were collected in order to quantify the number of AMF spores (Gerdemann and Nicholson, 1963). Near the end of the experiment (48 d.a.t.) the number of stolons were counted and the number of days to flowering was noted. At 70 d.a.t. the plants were cut at their base, and each of the 10 plants weighed individually. The number of leaves, stolons and flowers, and the height of the tallest stolon were measured and the plants placed to dry for 48 h at 60°C in order to obtain dry weight. Each pot was weighed and the soil divided in two layers: A = 0-10 cm and B = 10-20 cm. Each layer was also divided into 3 vertical sections, the first to estimate root biomass (manual separation) and percentage of AMF colonization according to Phyllips and Hayman (1970), and the other two for analysis of the soil: number of AMF spores (Gerdemann and Nicholson, 1963), mineral N ($NO_3$ + $NH_4$) according to Anderson and Ingram (1993), total N (micro-Kjeldahl), $^{15}N$ by mass spectrometry (Autonomous University of Madrid), available-P (Bray) and pH $H_2O$ (1:2.5, soil:water). Three to six plants from each pot were fine-ground in order to analyze total N, $^{15}N$ and total P. The earthworms were manually separated from the soil, weighed by layer, and placed in moist filter paper to empty their intestinal contents for 48 h. Once empty, they were sacrificed in water at ± 80°C (3 sec. immersion), dried at 40°C and re-weighed. The worms from the treatments with $^{15}N$ and some treatments without $^{15}N$ (natural abundance) were fine-ground and analyzed for $^{15}N$.

### Statistical Analyses

All the data on plant, earthworm, AMF and soil parameters were entered into spreadsheets and analyzed using ANOVAs and Fisher's Honest LSD (F-tests) with the statistical package SUPERANOVA® (Abacus Concepts) in order to detect significant differences between treatments.

## RESULTS AND DISCUSSION

The environmental conditions in the greenhouse were ideal for the growth *of B. decumbens*, with temperature maxima averaging 33°C and minima 17°C. The following sections contain detailed description of the results of the plant, soil, AMF and earthworm parameters in each treatment.

### Plant Parameters

#### *Height*

In the treatments with P fertilization (PK and NPK) the plants grew quickly, reaching at the end of the experiment (70 d.a.t.) heights between 83 cm in the NPK-AMF-Worm treatment and 92 cm with NPK+AMF+Worm (Figure 1a). Without applied P (0F and NK), the plants grew more slowly and the heights at harvest were between 46 cm in the NK-AMF-Worm treatment and 59 cm with 0F+AMF+Worm (Figure 1b). A 3-way anova revealed significant differences between fertilizer treatments throughout the 70 d plant growth cycle.

As for the effects of the AMF, between 13 and 37 d, we observed a positive effect of their presence on the height of the treatments with P (NPK and PK) and a negative effect when P (0F and NK) was not applied. This response could be due to the lower natural P-availability in the experimental soil and a greater C-drain in the treatments without P while the AMF established their populations in the plant roots. If this were the case, with applied P the plants could compensate the C-drain (Jakobsen, 1991), since there were fewer limitations to their growth (P was the most-limiting nutrient). With applied P, the AMF probably played a positive role in the growth of the plants (see later). After 44 d, no significant effects of AMF on plant height were observed.

Significant effects of earthworms were only observed after 55 d, when the plants in the treatments with NK, NPK and 0F were taller in the presence of earthworms. In contrast, with PK the effect of earthworms on plant height was negative. This could be related to interactions with AMF (e.g., higher root colonization and C-drain; see later). When positive, the effects of earthworms on heights tended to be greater in treatments without AMF. The lag time in order to observe positive or negative effects of earthworms (in comparison with the AMF, see above) on plants is probably due to the presence of a threshold, above or below which the accumulation of earthworms effects on soil physico-chemical and biological properties begin to change the growth of the plants (positive or negatively).

### *Shoot and root biomass*

Shoot and root biomass of all treatments is displayed in Figures 2a and 2b. *B. decumbens* shoot biomass at harvest (70 d.a.t.) ranged from 0.23 g plant$^{-1}$ (0.47 T ha$^{-1}$) in the treatment NK-AMF-Worm to >3 g plant$^{-1}$ (6.5 T ha$^{-1}$) with PK and NPK fertilization (Figure 2a). The production of *B. decumbens* was significantly affected by all three factors (AMF, Worms and Fertilizers), depending on the treatment. The 3-way anova (Table 2) revealed that shoot biomass followed the order of importance Fertilizers>AMF>Worms. Fertilization was most important as for their contribution to the sum of squares. AMF also affected the productivity in most treatments (with $P = 0.06$), but the earthworms, combining all treatments, did not affect shoot production significantly ($P = 0.91$), although in some fertilizer treatments significant effects were observed. This can be seen by observing the interactions, all of which (except the AMF * Worm, with $P = 0.12$) were statistically significant (Table 2).

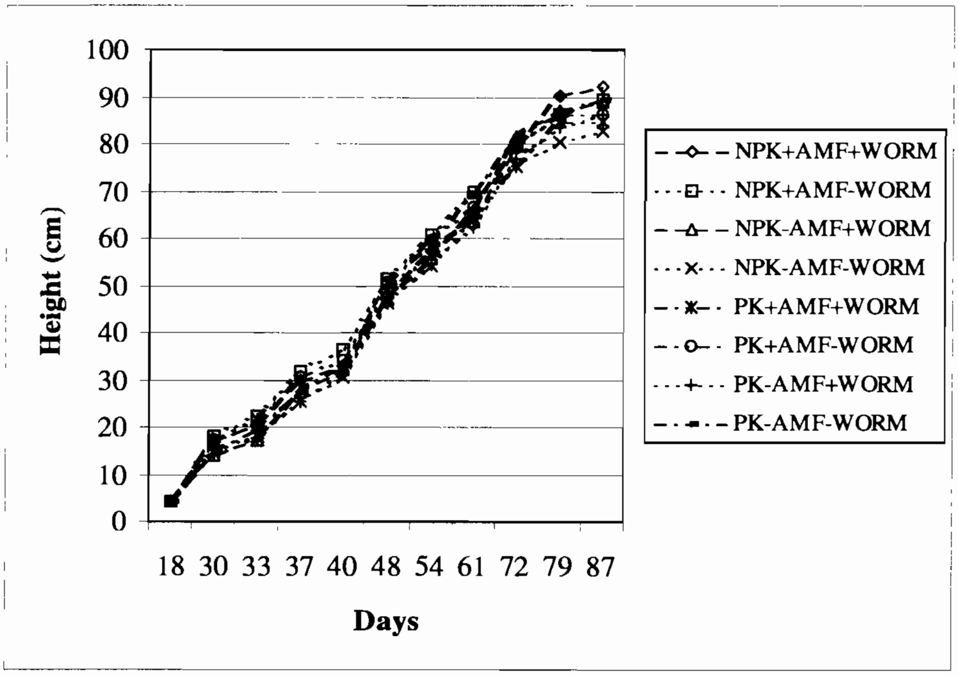
Root biomass, measured in both A (0-10 cm) and B (10-20 cm) layers was always higher (from 1.5 to 4.6 times) in the A layer. Root yields ranged from a minimum of about 0.2-0.3 T ha$^{-1}$ (NK and 0F treatments) up to >1 T ha$^{-1}$ (NPK+AMF+Worm) (Figure 2b). Total biomass (Table 3) ranged from around 0.7 T ha$^{-1}$ (NK-AMF-Worm) to 7.7 T ha$^{-1}$ (NPK+AMF+Worm). The most productive treatments were those that received P (PK and NPK), being the application of N alone (without P, NK treatment) not so important for productivity, resulting in lower yields than without fertilizers (0F). This was probably due to a nutritional imbalance in the plants and the exacerbation of P-deficiency.

Table 1. Selected chemical parameters of the non-irradiated and irradiated soils and the fertility class according to Cotennie (1984); Landon (1991) and Villaseca *et al.* (1995). Values with different letters within the same column indicate significant differences at $P<0.05$.

| Soil | C (%) | N (%) | C/N | pH $H_2O$ | $NO_3$ (mg $kg^{-1}$) | $NH_4$ (mg $kg^{-1}$) | P Bray (mg $kg^{-1}$) | Pt (mg $kg^{-1}$) | Ca (meq $100g^{-1}$) | Mg (meq $100g^{-1}$) | Na (meq $100g^{-1}$) | K (meq $100g^{-1}$) | CEC (meq $100g^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Non irradiated | 1.08a | 0.13a | 8.3a | 4.8a | 10.1a | 27.2a | 7.2a | 81.2 | 7.8 | 3.6 | 0.15 | 0.08 | 11.7 |
| Irradiated | 1.13a | 0.13a | 8.7a | 5.0a | 8.0b | 24.9a | 6.6a | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Fertility class | Poor | Low | Adeq. | S.ac. | n.d. | n.d. | Low | n.d. | Adeq. | High | Adeq. | Def. | Low |

n.d. = not determined; Adeq. = Adequate; S.Ac. = Strongly acid; Def. = Deficient

a. Treatments with P fertilizer



b. Treatments without P fertilizer

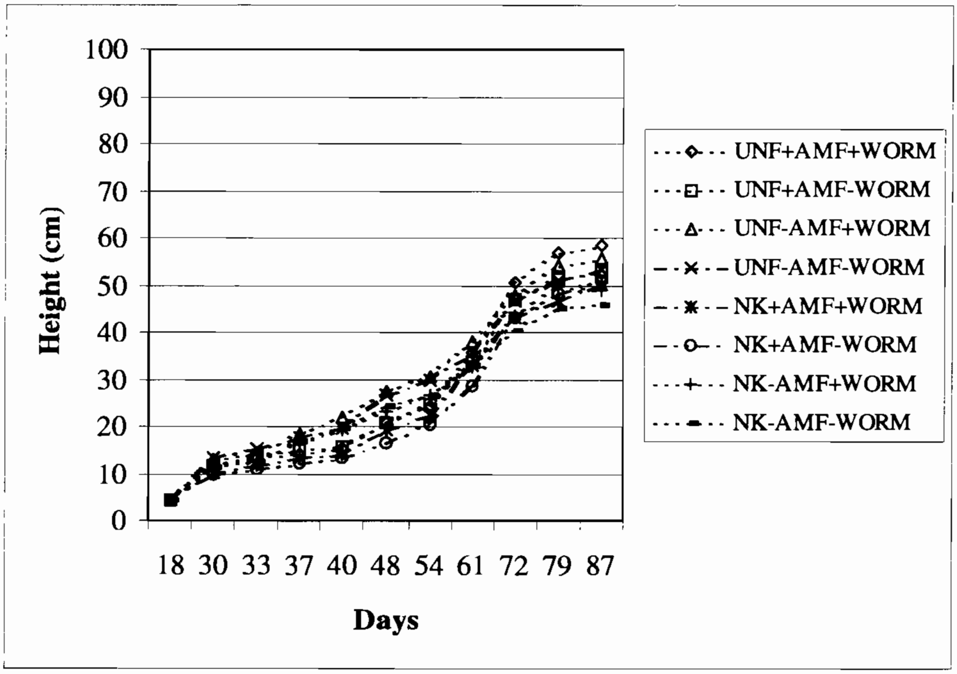
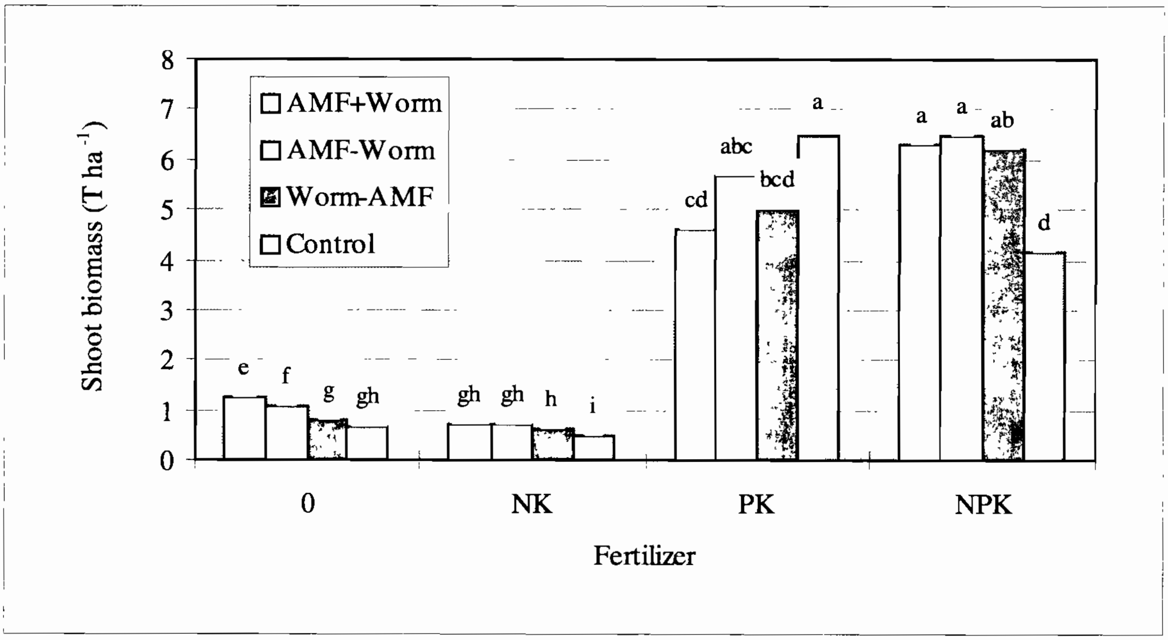


Figure 1. Height of *B. decumbens* plants (mean of tallest stolon, n = 10 pot$^{-1}$) in the different treatments with (a) and without (b) P fertilizers.

a. Shoot Biomass



b. Root Biomass

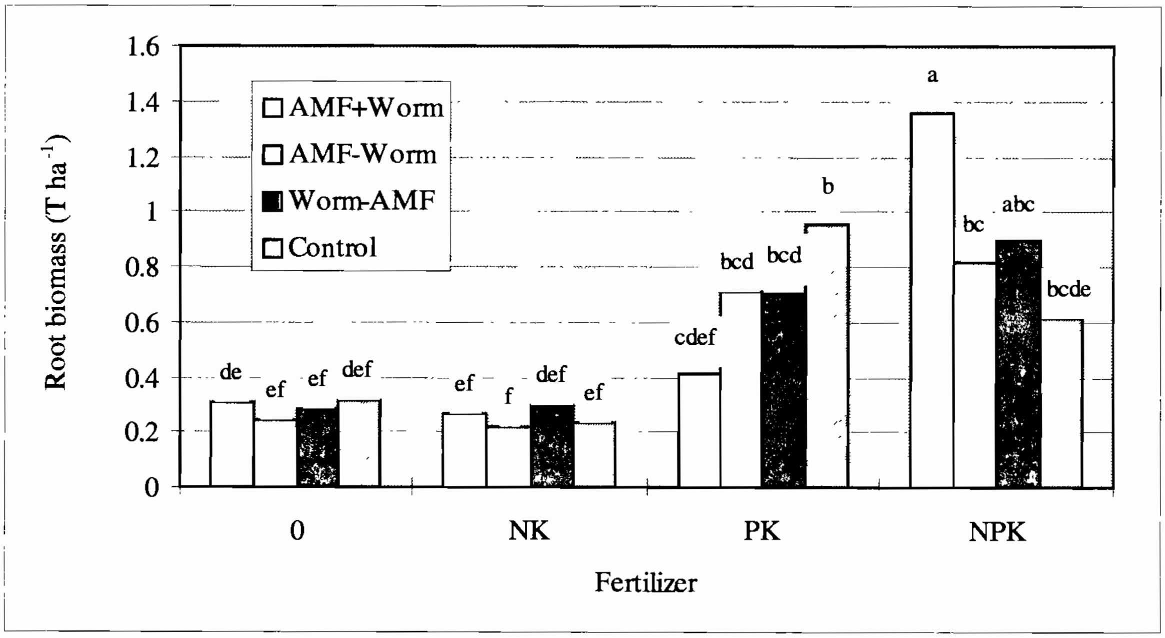


Figure 2. Shoot (a) and root (b) biomass of *B. decumbens* (T ha$^{-1}$) 70 d.a.t. in a sandy, nutrient-poor Alfisol from La Víbora with and without earthworms (*P. corethrurus*), AMF and four fertilizer treatments. Bars with different letters mean significant differences at P<0.05. Control = -AMF-Worm.

Combining all sub-treatments within each fertilizer, the order of productivity (shoot biomass) according to fertilization was NPK≥PK>0F≥NK (Table 2, data for shoots). Root biomass followed the order NPK>PK>0F≥NK. Shoot yields of plants in treatments with NPK and PK were not significantly different, although both were higher than with 0F or NK (differences also not significant). Patr6n *et al.* (1999) also found up to 10 times greater production of *B. decumbens* in the same soil from La Vîbora, with only about 8.4 kg ha$^{-1}$ P injected into the root zone, confirming the important P limitation of the experimental soil. Similar positive responses of *B. decumbens* to P fertilization have been observed by other researchers (e.g., Carneiro *et al.*, 1996; Pastrana, 1994; Rao *et al.*, 1996). The effects of earthworms and AMF and their interactions (with each other and with fertilizers) on shoot and root biomass are described below.

### *Other plant parameters*

The effects of fertilization were similar to those observed with plant biomass for the other plant parameters measured (Tables 3 and 4). Without P fertilization, only the NK+AMF+Worm treatment produced flowers by harvest. The number of stolons (n = 6-8) and leaves (n = 28-34) at harvest were always at least twice higher, and the absorption of P and N by shoots reached >25 times and 10 times higher, respectively, with P fertilization (Tables 3 and 4). In spite of the low natural P-availability and the absence of P fertilization, all the plants had higher total P contents than those considered critical for their development (0.1%; Rao *et al.*, 1996), and the biomass yields were similar to those obtained in other experiments, even without fertilizers (Miles *et al.*, 1996).

Table 2. Results of the 3-way ANOVA and the means ± standard error and standard deviation of the mean for the main treatments, using the data on shoot biomass (T ha$^{-1}$) of *B. decumbens* 70 d.a.t. df = degrees of freedom, SS = sum of squares, sd = standard deviation, se = standard error, P = statistical significance level (F-Test).

| Treatments | df | SS | P |
|---|---|---|---|
| Fertilizer | 3 | 788.2 | 0.0001 |
| AMF | 1 | 2.93 | 0.06 |
| Worm | 1 | 0.01 | 0.91 |
| Fertilizer * AMF | 3 | 14.2 | 0.0007 |
| Fertilizer * Worm | 3 | 21.5 | 0.0001 |
| AMF * Worm | 1 | 2.0 | 0.12 |
| Fertilizer * AMF * Worm | 3 | 8.5 | 0.01 |
|  | Mean | sd | se |
| NF | 0.94 | 0.31 | 0.08 |
| NK | 0.63 | 0.22 | 0.06 |
| PK | 5.50 | 1.88 | 0.49 |
| NPK | 5.72 | 2.07 | 0.55 |
| +AMF | 3.38 | 2.80 | 0.51 |
| -AMF | 3.00 | 2.84 | 0.54 |
| +Worm | 3.09 | 2.69 | 0.52 |
| -Worm | 3.29 | 2.93 | 0.53 |

Table 3. Total plant biomass (T ha$^{-1}$), shoot/root ratios, number of leaves, stolons and flowers and their biomass (g) per *B. decumbens* plant at harvest (70 d.a.t.), the number of stolons at 48 d.a.t. and the number of days to flowering by treatment. Different letters within a same column mean significant differences at $P<0.05$. nf = no flower

| Treatments | | Total Biomass (T ha$^{-1}$) | Shoot/Root ratio | Leaves plant$^{-1}$ | Stolons plant$^{-1}$ (48 d.a.t.) | Stolons plant$^{-1}$ (70 d.a.t.) | Flowering date (d.a.t.) | Flowers plant$^{-1}$ | Biomass flowers (g plant$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| 0F | +AMF+Worm | 1.6d | 4.1efg | 14.0e | 3.1f | 3.2d | n.f. | 0e | 0e |
| | +AMF-Worm | 1.3de | 4.4defg | 13.0e | 2.1g | 3.1d | n.f. | 0e | 0e |
| | -AMF+Worm | 1.1ef | 3.0fg | 8.6g | 1.3hi | 2.1f | n.f. | 0e | 0e |
| | -AMF-Worm | 1.0ef | 2.1g | 7.8g | 1.1hi | 2.1f | n.f. | 0e | 0e |
| NK | +AMF+Worm | 1.0ef | 2.7fg | 11.2f | 1.6h | 2.9d | 70d | 0.03d | <0.01d |
| | +AMF-Worm | 0.9ef | 3.4fg | 10.5f | 1.5hi | 2.5ef | n.f. | 0e | 0e |
| | -AMF+Worm | 0.9ef | 2.1g | 7.7g | 1.4hi | 2.1f | n.f. | 0e | 0e |
| | -AMF-Worm | 0.7f | 2.0g | 6.2h | 1.0i | 1.6g | n.f. | 0e | 0e |
| PK | +AMF+Worm | 5.0bc | 11.9a | 28.2d | 3.9e | 6.5c | 52.8abc | 1.0bc | 0.04bc |
| | +AMF-Worm | 6.4abc | 8.2b | 30.4bcd | 4.9bc | 6.9bc | 51c | 1.8a | 0.07ab |
| | -AMF+Worm | 5.7abc | 7.2bc | 29.5cd | 4.3de | 6.5c | 57ab | 1.0bc | 0.03c |
| | -AMF-Worm | 7.4ab | 7.5bc | 29.8cd | 4.5cd | 6.9bc | 58.8a | 1.0bc | 0.06ab |
| NPK | +AMF+Worm | 7.7a | 5.1cdef | 35.7a | 5.5a | 8.3a | 52bc | 1.2ab | 0.07ab |
| | +AMF-Worm | 7.3ab | 8.1b | 33.9ab | 4.9bc | 7.6ab | 49.8c | 1.8a | 0.08a |
| | -AMF+Worm | 7.1abc | 7.1bcde | 33.2abc | 5.4ab | 7.7ab | 53.5abc | 1.5ab | 0.07ab |
| | -AMF-Worm | 4.8c | 7.1bcd | 28.6d | 4.2de | 6.9bc | 53.8abc | 0.6c | 0.02c |

Shoot/Root ratios (Table 3) ranged from a low of 2.0 (NK-AMF-Worm) up to 11.9 (PK+AMF+Worm), and when all treatments with or without AMF were combined, a significant positive effect of AMF presence was observed (5.8 in AMF+, 4.8 in AMF-). It is well recognized that plants will invest more energy into shoots relative to roots when grown in AMF-inoculated soils (Marschner, 1996) and the present results confirm this observation. As with most other plant parameters, shoot/root ratios were also higher with applied P, although when all fertilizer treatments were combined, they followed the order PK>NPK>0F≥NK (8.5>6.8>3.4≥2.6). When viewed separately, the only significant differences between treatments were found when comparing the treatments with NPK or PK+AMF with and without earthworms (higher with PK and lower with NPK, respectively, due to earthworm inoculation).

### *Effects of AMF, Earthworms and their interactions*

Within each fertilizer treatment, the greatest increment in shoot yields compared with the control was observed in AMF+Worm with 0F (+84%), while in root yields (+121%) it was with AMF+Worm and NPK (Figure 2). In general, except with PK, the treatments +AMF+Worm produced more than the others. The efficiency of the AMF alone was calculated by taking the increment in any measured variable (in %), calculated comparing the treatments +AMF-Worm with the -AMF-Worm (see e.g., Figure 2). The additional effect of the AMF in treatments with earthworms was measured comparing the treatments -AMF+Worm with those +AMF+Worm, while the effect of the earthworms on the efficiency of AMF was obtained comparing the treatments +AMF-Worm with the +AMF+Worm. The effect of the earthworm alone (without interference of AMF), was calculated comparing the treatments -AMF+Worm with the -AMF-Worm. Finally, the effect of the two organisms together was measured comparing the treatments +AMF+Worm with the control of each fertilizer treatment, -AMF-Worm.

Based on these comparisons, AMF alone increased shoot biomass (Figure 2), the number of leaves and stolons (70 d.a.t) in all treatments except PK, reduced the number of days to flowering with PK, increased the biomass of flowers with NPK and shoot P uptake with NK (Tables 3 and 4). Shoot N uptake had a tendency for higher values due to AMF, but the differences were not significant. No significant differences due to AMF were seen in the root biomass.

In soils with low P-availability and strong P-limitations, AMF generally play an important role in plant P-uptake (Marschner and Dell, 1994; Marschner, 1996). The transport of N to the plant by hyphae may also be important for yields, although this phenomenon has received less attention than P (Johansen *et al.*, 1993). Miranda (1996), Rao *et al.* (1993) and Saif (1987) showed the important role of AMF in P and N uptake by *B. decumbens*. The results of this experiment confirm the importance of AMF in *B. decumbens* yields and various other plant parameters, including P uptake under conditions of extreme limitation (NK treatment).

Earthworms alone had no significant effect on root biomass but they increased significantly shoot biomass and the number of leaves with NK and NPK with NPK, the number of stolons (70 d.a.t.) and shoot P uptake with NK , the number of stolons at 48 d.a.t. and the number and biomass of flowers with NPK. However, their activity reduced flower biomass and P uptake with

PK (Tables 3 and 4). Patrón *et al.* (1999) also observed a decrease in shoot and root biomass with aprox. 10 kg ha$^{-1}$ of superficial+injected (to the roots) P in the presence of *P. corethrurus*.

Table 4. Total N and P uptake by shoots (T ha$^{-1}$), N and P use efficiency by shoots and roots (g$^{-1}$ g$^{-1}$) and root N and P uptake activity by *B. decumbens* (mg g$^{-1}$ d$^{-1}$) at harvest (70 d.a.t.) Different letters within a same column mean significant differences at $P<0.05$.

| Treatments | N uptake (T ha$^{-1}$) | Shoot N use eff. (g$^{-1}$ g$^{-1}$) | Root N use eff. (g$^{-1}$ g$^{-1}$) | N uptake activity (mg g$^{-1}$ d$^{-1}$) | P uptake (T ha$^{-1}$) | Shoot P use eff. (g$^{-1}$ g$^{-1}$) | P uptake activity (mg g$^{-1}$ d$^{-1}$) |
|---|---|---|---|---|---|---|---|
| 0F | | | | | | | |
| +AMF+Worm | 36.8c | 34.2defg | 123.9b | 1.9cde | 2.7c | 466b | 0.14ef |
| +AMF-Worm | 33.3c | 31.9efgh | 120.9b | 2.1cde | 2.5cd | 421bc | 0.17ef |
| -AMF+Worm | 25.7cd | 31.4efgh | 108.2bc | 1.5e | 2.2cd | 369bcd | 0.14ef |
| -AMF-Worm | 20.1cd | 33.5def | 73.1cd | 1.1e | 1.8cd | 383bcd | 0.10ef |
| NK | | | | | | | |
| +AMF+Worm | 23.5cd | 30.5fgh | 41.1d | 1.6de | 2.1cd | 350bcd | 0.13ef |
| +AMF-Worm | 24.0cd | 30.2fgh | 43.4d | 2.1cde | 2.4cd | 291d | 0.18de |
| -AMF+Worm | 23.1cd | 27.6gh | 46.7d | 1.4e | 1.8d | 360bcd | 0.10ef |
| -AMF-Worm | 18.3d | 25.6h | 44.8d | 1.4e | 0.8e | 667a | 0.06f |
| PK | | | | | | | |
| +AMF+Worm | 129.0ab | 35.5cdef | 115.4b | 4.8a | 14.2ab | 323cd | 0.58a |
| +AMF-Worm | 115.5b | 49.1a | 181.8a | 2.4bcde | 16.4ab | 347cd | 0.38b |
| -AMF+Worm | 117.3b | 42.3bc | 127.0b | 2.5bc | 13.6b | 367bcd | 0.32bc |
| -AMF-Worm | 137.8ab | 46.5ab | 126.8b | 2.5bcd | 21.7a | 294d | 0.42b |
| NPK | | | | | | | |
| +AMF+Worm | 180.0a | 35.0def | 42.3d | 2.4bcd | 15.4ab | 411bc | 0.21cde |
| +AMF-Worm | 161.5ab | 39.7bcd | 51.3d | 3.2bcd | 18.1ab | 371bcd | 0.35b |
| -AMF+Worm | 151.4ab | 40.0bcd | 69.2cd | 2.8bc | 18.4ab | 333bcd | 0.35bc |
| -AMF-Worm | 109.4b | 38.2cde | 56.1d | 2.9bc | 11.5b | 385bcd | 0.30bcd |

Normally, earthworms (including the species *P. corethrurus*) exert positive effects on plant production, especially on grasses and perennial plants (Brown *et al.*, 1999). This is due to the amelioration of physical and chemical factors limiting root growth, such as increases in the nutrient (esp. mineral N and inorganic P) availability, water holding capacity and porosity in the drilosphere of *P. corethrurus* (Barois *et al.*, 1999; Brown *et al.*, 1999; Chapuis-Lardy *et al.*, 1998; Lavelle *et al.*, 1992; López-Hernández *et al.*, 1993; Patrón, 1998). However, there are occasions (rare) in which earthworms (e.g., *P. corethrurus*) can damage plants thus reducing yields (e.g., Barros *et al.*, 1996; Brown, 2000b), or not have any significant effect (Brown *et al.*, 1999). Some authors (Barros, 1999; Chauvel *et al.*, 1999) have suggested that the negative effects derive from changes in the structure of soil porosity and hydrological complications, but the precise mechanisms of these effects have still not been clearly described and need further investigation. In the present experiment, the negative effects seem to be linked to the AMF colonization of roots (see below).

The additional presence of earthworms in the treatments +AMF (comparison of full treatment with +AMF-Worm) changed little the effect of AMF on the different plant parameters in most fertilization treatments, being the important effects observed in the number of stolons, root biomass and shoot/root ratios with NPK and PK, flowering with NK, and shoot biomass with 0F (Figure 2; Tables 3 and 4). The additional effect of AMF in the treatments with earthworms (comparison complete treatment with -AMF+Worm) was more important with 0F and NK. The only significant changes were found in these treatments: greater number of leaves and stolons with 0F and NK, higher shoot biomass with 0F and earlier flowering, with more and heavier flowers with NK (Tables 3 and 4). Comparing the full treatment with the controls, significant changes (mostly positive) were observed in most of plant parameters without fertilizers (0F) and with application of NK and NPK. In contrast, with PK, many plant parameters were negatively affected; the only significant increase observed was in the shoot/root ratio. Many of these effects seem be linked to AMF * Worm interactions (see below).

*Efficiency of N and P use by the plant*

The efficiency of the use of N and P (the weight of above or below ground biomass produced by the amount of nutrients taken up, in g $g^{-1}$) by the plant and the activity of N and P uptake by the roots (amount of nutrients absorbed per root biomass per day, in mg $g^{-1}$ $d^{-1}$) were calculated using the formulae (Myers *et al.*, 1994; Rao *et al.*, 1996):

$$\text{Efficiency of the use of N or P by the plant} = \frac{\text{g of forage or root biomass produced}}{\text{g of N or P taken up by shoot or root}}$$
(weight/ weight)

$$\text{Daily activity of N or P uptake by the roots} = \frac{\text{g of P or N taken up}}{\text{root biomass}} * \frac{1}{70\ \text{d}}$$

The comparisons between the efficiency of the N use by plants in each treatment (Table 4) were significantly different among the different fertilizer treatments. The highest average of the four treatments within each fertilizer treatment was found with application of PK (43.9 g $g^{-1}$), followed by NPK (38.0 g $g^{-1}$), 0F (32.6 g $g^{-1}$) and the lowest with NK (28.5 g $g^{-1}$). These values are much lower than those obtained by Rao et al. (1996) with another variety of *B. decumbens* (cv. Basalik) fertilized with NK (152 g $g^{-1}$) or NPK (195 g $g^{-1}$) in a nutrient-poor sandy loam soil from the Colombian llanos. These differences could be due to a lower inherent (genetically-based) production capacity of the variety used in the present experiment or differences between the fertility of the Colombian soil and that of La Víbora, since three other species of *Brachiaria* used by Rao *et al.* (1996) also had higher efficiencies than *the B. decumbens* in this experiment. Regarding the effects of earthworms and AMF, significant differences (decreases) were only observed in the efficiency with PK fertilization, when either Worms or Worms+AMF were included. Plants in the PK+AMF-Worm treatment produced significantly more forage relative to the uptake of N than the plants in all the other treatments.

Root N use efficiency was significantly higher than for shoots in all fertilizer treatments (41-182 g $g^{-1}$). This was the case especially when no N fertilizer was applied (0F and PK), indicating a higher relative effort into root production to take up soil N, although this could also be interpreted as more biomass produced with lower N uptake. In treatments with N, no significant difference was observed within treatments. With PK, the highest values were observed, and the treatment +AMF-Worm was significantly different than the other treatments. The higher root N use efficiency compared to the treatment +AMF-Worm could be due to the related to the (lower) root colonization by AMF with earthworms (see later). In treatments with 0F, on the other hand, AMF had a positive effect, which was also enhanced in the treatment +AMF+Worm. This could be due to the mycorrhizal colonization of roots, enhancing a greater root biomass relative to the amount of N taken up (possibly due to the role of AMF in N uptake), and to the generally positive effects of earthworms on N availability (Lee, 1985) in worm-worked soils.

Combining all treatments with and without AMF, N uptake efficiency was significantly ($P<0.01$) greater with (2.5 mg $g^{-1}$ $d^{-1}$) than without AMF (2.0 mg $g^{-1}$ $d^{-1}$), confirming the hypothesis presented above, with 0F. The role of AMF in N uptake has not been as well studied as with P and deserves further attention, particularly in N poor soils. As with root N use efficiency, root N uptake activity (Table 4) was highest in treatments with PK (no N fertilizer). In the treatment PK+AMF+Worm, activity values were significantly higher than in all other treatments. Therefore with PK fertilization, earthworms, in spite of reducing efficiency of N use by the plant (biomass of both shoots and roots produced by N absorbed in AMF+ treatments, see above), increased the uptake of N per g of root, that is, with lower relative root biomass, the uptake activity of plants was higher with *P. corethrurus*. In this case, the response could be due to the higher mycorrhizal colonization of roots with earthworms (see later), inducing more N uptake (despite lower relative root biomass) and/or the greater mineral N availability induced by *P. corethrurus* activity in this soil (Brown *et al.*, 2000a; Barois *et al.*, 1999; Hernández, 1999). This greater N availability would be relatively more important in the PK treatment (where the all the necessary P was available but N was growth-limiting), and in the treatments -AMF.

The efficiency of the use of P by the plants varied between 291 and 667 g $g^{-1}$ (Table 4), being the highest and lowest values observed in the treatment with application of NK. As seen with the values of N-use efficiency, the efficiency of P-use was much lower in this experiment that the values reported by Rao *et al.* (1996) for another variety (cv. Basalik) of *B. decumbens* (948 and 1067 g $g^{-1}$ with NK and NPK, respectively) in Colombia (see above). The efficiency of the P-use in the present experiment was closer to those of another *Brachiaria* species (*B. humidicola,* 422 and 722 g $g^{-1}$ with NK and NPK, respectively; Rao *et al.*, 1996). Combining all the fertilizer sub-treatments, with PK the efficiency was significantly lower (333 g $g^{-1}$) than with NK (421 g $g^{-1}$), 0F (408 g $g^{-1}$) or NPK (381 g $g^{-1}$), although when the treatments were compared individually, the only significant differences observed were between the NK-AMF-Worm and the NK+AMF treatment with and without earthworms, where the efficiencies were much lower. The mycorrhizae in this treatment, therefore, didn't seem to have increased the efficiency of P-use in order to produce more forage. On the other hand, in the treatment without fertilizers (0F), the treatments with AMF had the opposite effect, with a tendency to increase P-use efficiency (440 vs. 375 g $g^{-1}$).

When all the treatments with AMF were combined, a trend for higher root P uptake activity (0.26 mg $g^{-1}$ $d^{-1}$) was observed compared with treatments without AMF (0.22 mg $g^{-1}$ $d^{-1}$), although the difference was not significant ($P<0.07$). Combining all the treatments within each fertilizer application treatment, the activity of P uptake by roots was significantly higher with PK fertilization (0.41 mg $g^{-1}$ $d^{-1}$) than with the other fertilizers (0.29 with NPK and 0.12 mg $g^{-1}$ $d^{-1}$ with NK) and 0F (0.14 mg $g^{-1}$ $d^{-1}$). However, when compared individually (Table 4), the only significant differences observed were a positive effect due to earthworms with NK, and between the PK+AMF+Worm and the remaining treatments with PK. *P. corethrurus* is known to increase

P availability in soils (including the experimental soil; Barois et al., 1999; Hernández, 1999) and the greater uptake in this treatment could be due to higher availability. In the treatment with PK, the positive effect could be due to both earthworms and AMF, which displayed a high root colonization rate (see later) and lower root biomass (Figure 2) in the treatment PK+AMF+Worm.

*Use of the soil P (native+fertilizer) and efficiency of the P fertilizer*

The percentage use of the soil P (native+fertilizer P) and the efficiency of the fertilizer were calculated according to the formulae (Ndikumana and de Leeuw, 1986; Saif, 1987):

$$\%\text{ use of the soil P} = \frac{\text{g of P taken up by the plant shoots}}{\text{g of applied P fertilizer}} * 100$$

$$\text{Efficiency of the P fertilizer} = \frac{\text{difference in the total weight (g) of plants with and without P}}{\text{g of applied P fertilizer}}$$

Table 5. Percentage use of soil P (native+fertilizer P) and efficiency of the P fertilizer (g $g^{-1}$), in the treatments with P at 70 d.a.t. of *B. decumbens*. Treatments with different letters within a same column mean significant differences at P<0.05.

| Treatments | | Use of soil P (%) | Efficiency of the P fertilizer (g $g^{-1}$) |
|---|---|---|---|
| PK | +AMF+Worm | 14.2ab | 46.0a |
| | +AMF-Worm | 16.4ab | 56.7a |
| | -AMF+Worm | 13.6b | 50.2a |
| | -AMF-Worm | 21.7a | 64.9a |
| NPK | +AMF+Worm | 15.4ab | 63.1a |
| | +AMF-Worm | 18.1ab | 64.8a |
| | -AMF+Worm | 18.3ab | 62.1a |
| | -AMF-Worm | 11.5b | 41.4a |

The % use of the soil P (native+fertilizer P) was significantly higher in the PK-AMF-Worm treatment that in the treatments PK-AMF+Worm and NPK-AMF-Worm (Table 5). the causes of the negative effect of the worms with PK are unknown and merit further research. With NPK the effect could be due to the absence of earthworms and/or AMF, whose presence had the tendency to increase the efficiency of P-use in this treatment. The values of % use obtained in the present experiment were generally slightly lower than those reported by Saif (1987) for another variety (cv. Basalik) of *B. decumbens* (24.8%) inoculated with AMF and approached more those of another species of *Brachiaria* (*B. brizantha*, 17.3%). However, the % use in the treatments without AMF in the present experiment were much higher than those observed by Saif (1987) for *B. decumbens* in his experiment (1.6%).

P fertilizer efficiency was high (due to the deficiency of this nutrient in the soil), ranging between 41.4 g $g^{-1}$ (NPK-AMF-Worm) and 64.9 g $g^{-1}$ (PK-AMF-Worm), but the differences between treatments were not significant (Table 5). However, a clear tendency was observed for lower efficiencies in the treatments with PK+AMF+Worm and NPK-AMF-Worm. In the first case, the effect is probably linked to the AMF * Worm interaction and in the second case it may be due to the absence of AMF (see below).

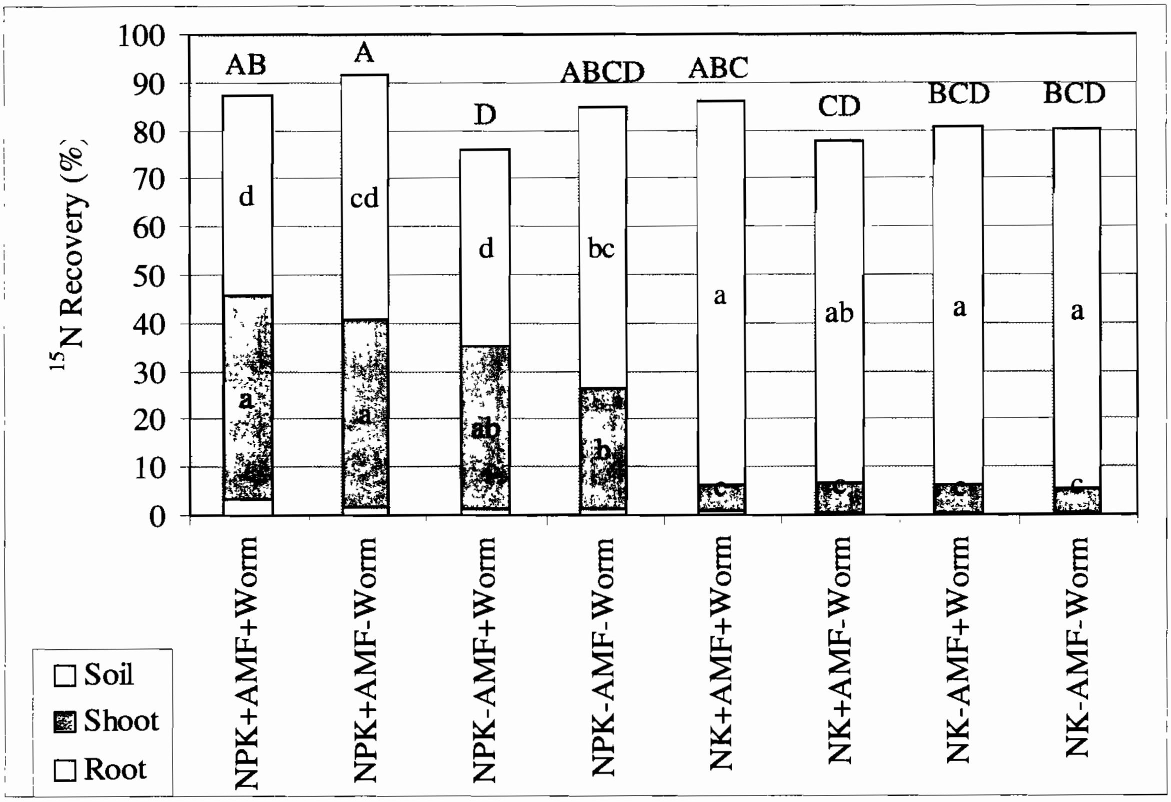


Figure 3. Recovery of the $^{15}$N-labeled fertilizer (0.78 atom% $^{15}NH_4{}^{15}NO_3$, 200 kg ha$^{-1}$ N) in the soil, plant shoots and roots in the different treatments with and without earthworms (*P. corethrurus*), AMF and P fertilizers, 70 d.a.t. *B. decumbens*. Treatments with different lower-case (recovery in shoots and soil) or upper-case (Total recovery) letters mean significant differences at P<0.05.

### *Recovery of the $^{15}$N-labeled fertilizer*

The efficiency of the use or the recovery of the N fertilizer labeled with $^{15}$N was calculated using the values of excess atom% $^{15}$N obtained from the mass spectrometer. Similarly, the percentage of the $^{15}$N remaining from the amount initially applied recovered in the earthworms and the soil at the end of the experiment, were calculated using the following equation:

$$\text{Recovery of the } ^{15}\text{N } (\%) = \frac{(\text{Np, w, s})}{(\text{Nf})} * \frac{(\text{Exc. Atom\% } ^{15}\text{Np, w, s})}{(\text{Exc. Atom\% } ^{15}\text{Nf})} * 100$$

In which Np, w, s are the %N of the plant (p), earthworm (w) and soil (s) and Nf is the %N of the $^{15}$N-labeled fertilizer. The total amounts of fertilizer recovered varied between 76% with NPK-AMF-Worm and 92% with NPK+ AMF-Worm (Figure 3). The $^{15}$N not recovered (8-24%

of the total initially applied) could: a) have been lost in leachates (with $H_2O$ through the bottom of the pot); b) have denitrified and/or volatilized (Mulvaney *et al.*, 1997); c) be in fragments of roots not picked up in the manual separation of low efficiency (unlikely); d) have been immobilized by the root inoculant (high C:N ratio) or the microbial biomass (e.g., in fungi growing in the inoculant fragments) (Urquiaga *et al.*, 1998).

In the plants and earthworms, the recovery of $^{15}N$ is also known as the Real Coefficient of Utilization (RCU) of the N fertilizer. In the treatments with NPK, the RCU ranged from 26.5 to 45.6%, being greatest in the shoot (25.4 to 42.4%) and with a maximum of only 3.2% in the roots (NPK+AMF+Worm) (Figure 3). With NPK, the AMF influenced significantly $^{15}N$ uptake by the plants, but earthworms alone had no significant effect. In general there was no significant additional effect of the presence of earthworms with AMF on the RCU by the plants (probably due to the lower colonization of the roots with AMF in these treatments, see later). With NK the plant only absorbed 5.3 to 7.0% of the fertilizer (4.8 to 6.5% in the shoots and <0.7% in the roots), being the greatest part (>71%) recovered in the soil (Figure 3). There were no significant differences in the RCU of the plants between the treatments with NK.

Miranda (1996) also found higher amounts of $^{15}N$ in *B. decumbens* due to AMF inoculation, but could not distinguish if the increase was due to the AMF or to the higher root biomass in inoculated treatments. Since the root biomass in NPK treatments was not significantly greater with AMF alone, then the AMF were probably responsible for the higher $^{15}N$ uptake by plants in this treatment. As *B. decumbens* (roots) takes up $NO_3$ preferentially (over $NH_4$; Rao *et al.*, 1996), the higher $^{15}N$ recovery in presence of AMF could be due to more $NH_4$ uptake by AMF.

## Soil Parameters

The fertility of the soil used was low, being low and insufficient the amounts of C, N, P-Bray, CEC, and exchangeable K for high production (Table 1). Irradiation of the soil only affected negatively (and significantly) the amount of $NO_3$, although there were indications of a loss in total P content as well (data not shown). Despite careful weighing of pots and watering (as necessary), at harvest the $H_2O$ content of pots was significantly different depending on the treatment. Pots with PK and especially NPK tended to have lower soil moisture values in irradiated soils (-AMF), which always had lower soil moisture than in non-irradiated soils. Soil moisture ranged from a low of 5.1% (NPK-AMF+Worm; A layer, 0-10 cm) up to 15.3% $H_2O$, w/w (NPK+AMF-Worm; B layer, 10-20 cm). It is not known why the irradiated soils generally had less water. It might be an artifact of sampling, and the low values (pF 4.0, plant wilting point in this soil is at 4% $H_2O$) found were probably due to rapid evaporation of $H_2O$ which occurred during the manual sieving for roots and earthworms, when the moisture samples were taken. Soil moisture was always higher in the lower (B) layer than the upper (A) layer.

The cultivation with *B. decumbens* significantly reduced the pH of the initial soil and differences between the treatments were observed (Table 6). In general, treatments with PK had significantly higher pH that the others. N fertilization seems to have reduced the pH of most soils with NK and NPK, although the pH in the treatments without fertilization (0F) were also lower than the initial soil. The values of pH reached critical levels where small pH changes could significantly affect the ability of AMF to colonize plant roots (Abbott and Robson, 1994). The mineralization of soil organic N (nitrification), or N nitrification of N-fertilizers tends to increase soil acidity (Mulvaney *et al.*, 1997), so lower pH values with N fertilizers were expected in this experiment. The increase in $NO_3$ levels in the unfertilized soils compared with the levels in the original soil is evidence of nitrification activity (Table 6). The decrease in total N in some treatments also corroborates this phenomenon.

Table 6. pH, P-Bray, $NO_3$, $NH_4$ and Total N in the soil 70 d.a.t. *B. decumbens* in the presence or absence of earthworms (*P. corethrurus*), AMF and fertilizers. Different letters within a same column mean significant differences at $P<0.05$.

| Treatments | | pH ($H_2O$) | P-Bray (mg $kg^{-1}$) | Total N (%) | $NO_3$ (mg $kg^{-1}$) | $NH_4$ (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|
| 0F | +AMF+Worm | 4.1def | 4.6a | 0.12cde | 39.2cd | 25.8cdef |
| | +AMF-Worm | 4.2cde | 6.6a | 0.19a | 39.6cd | 22.6def |
| | -AMF+Worm | 4.0efg | 6.2a | 0.14cde | 27.3d | 30.6bcde |
| | -AMF-Worm | 4.1cdef | 5.9a | 0.14bcd | 25.6d | 25.2cdef |
| NK | +AMF+Worm | 3.7g | 6.0a | 0.11de | 56.5abc | 41.1b |
| | +AMF-Worm | 4.0defg | 6.2a | 0.11de | 62.1abc | 34.2bcd |
| | -AMF+Worm | 4.0def | 6.6a | 0.12cde | 75.7ab | 61.9a |
| | -AMF-Worm | 4.2cde | 6.9a | 0.11de | 80.3a | 64.2a |
| PK | +AMF+Worm | 4.4abc | 15.3b | 0.15bc | 27.3d | 18.7f |
| | +AMF-Worm | 4.5a | 15.1b | 0.15bc | 27.3d | 22.4ef |
| | -AMF+Worm | 4.5a | 18.6b | 0.17ab | 39.2cd | 37.3bc |
| | -AMF-Worm | 4.5ab | 18.1b | 0.13cde | 39.4cd | 37.3bc |
| NPK | +AMF+Worm | 3.9fg | 15.6b | 0.11e | 45.3cd | 27.9cdef |
| | +AMF-Worm | 4.2bcd | 15.8b | 0.11e | 52.2bcd | 28.3cdef |
| | -AMF+Worm | 4.3a | 15.7b | 0.10e | 22.5d | 26.7def |
| | -AMF-Worm | 4.0defg | 15.2b | 0.11de | 48.3bcd | 32.4bcde |

The greatest amounts of mineral N were found with NK (Table 6), probably due to the lower N absorption by plants in these treatments. The presence of AMF alone significantly reduced the amount of $NH_4$ in soil with NK and PK, although this didn't concomitantly increase (significantly) total N uptake by the plants (Table 3). In the treatments with NK and NPK there was a decrease in total N in comparison with the original Nt and that in the others treatments (Table 6). This could be due to a "priming effect" of the added N fertilizer. The increases in Nt due to earthworm presence in PK and due to AMF with 0F are difficult to explain and could be due to: a) fragments of roots or inoculum in the soil samples; b) $N_2$-fixing activity of free-living $N_2$-fixing microorganisms associated with *B. decumbens* rhizospheres (Boddey and Victoria, 1986; Rao *et al.*, 1996); c) higher rates of nitrogenase activity, $N_2$ fixation and/or greater populations of $N_2$-fixing microorganisms in the drilosophere (Bhatnagar, 1975; Simek and Pizl, 1989); d) extensive AMF growth, which has been associated with increases in soil organic matter contents (Quintero-Ramos et al., 1993).

In the treatments with NK, no significant differences were observed in terms of the amount of $^{15}N$ recovered in soil (71-80%; Figure 3). Conversely, the amount of $^{15}N$ recovered in soil with NPK ranged from 41 to 58%, being higher with NPK-AMF-Worm, and significantly lower due to earthworm activity (Figure 3). The higher losses of $^{15}N$ with earthworms could be due to higher denitrification and/or volatilization rates in their castings (Elliott *et al.*, 1992; Lensi *et al.*, 1992) or more N leaching, commonly observed in soils with earthworm inoculation (Anderson *et al.*, 1983; Haimi and Boucelham, 1991; Knight *et al.*, 1989; Subler *et al.*, 1997). Only on few occasions, immediately after watering, did some water leach through the bottom of the pots, therefore, leaching was probably not the main reason for the observed losses.

Bray-P in soil was slightly lower than the initial level in the treatments without P fertilizer, and increased significantly in the treatments with fertilizer-P (Table 6). Significant differences between the treatments were only observed taking all treatments with or without P, together.

The additional effect of earthworms in the treatments +AMF only changed significantly (reduced) total soil N contents with 0F and pH with NPK (Table 6). This could be due to higher N mineralization rates and to the "priming effect" mentioned above, induced by microbial activation (a phenomenon commonly observed in soils inoculated with earthworms; Blair *et al.*, 1997; Hendrix *et al.*, 1998; Lavelle *et al.*, 1992; Scheu, 1993; Subler *et al.*, 1998). The additional effect of AMF on the treatments with worms significantly decreased the amount of $NH_4$ in soil with NK, PK and NPK, and lowered pH with NK (Table 6). The lower $NH_4$ is probably linked to higher uptake by plants with AMF (Johansen et al., 1993).

## Mycorrhizal population

### *Number of spores*

The number of AMF spores was quantified in the initial irradiated and non-irradiated soil and at the end of the experiment in the A (0-10 cm) and B (10-20 cm) layers and in earthworm castings. The initial irradiated soil had 28.4 spores $g^{-1}$, although the viability of these spores was destroyed by irradiation. In the non-irradiated soil, the initial amount was 50.5 spores $g^{-1}$. At the end of the experiment, *P. corethrurus* castings had an average of 9 spores $g^{-1}$ soil (min. 1.4 $g^{-1}$, max. 18.8 $g^{-1}$), while in the soil the concentration was much greater, varying between 30 and 76 $g^{-1}$ (Table 7). The low number of spores in *P. corethrurus* casts is surprising since this species tends to feed in the zone near roots and therefore be in frequent contact with AMF. However, little is known about the food sources of this earthworm and what passes with the ingested spores in the intestine of *P corethrurus*. They may be destroyed, exiting in lower number than the amount originally ingested, although Reddell and Spain (1991) found a great number of spores in casts of *P. corethrurus* in Australia (140-390 $g^{-1}$) which passed undamaged through their intestinal tract, and were viable to infect sorghum roots. The viability tests carried out with *P. corethrurus* casts from this experiment were null (Trejo *et al.*, unp. data), but this was probably due to the old age of the casts (1.5 years), the low spore number and the limited mass of available casts, and not necessarily to lack of viability.

Many soil factors and the growth cycles of plants can influence the number of AMF spores in soil (Abbott and Robson, 1994). The relationship between soil moisture and the number of spores is generally negative, with spores being more abundant in dry soils, due to interactions with root growth dynamics (Hayman, 1970). The number of spores in the present experiment was greater in the A than the B layer (Table 7), possibly due to the lower soil moisture contents and the lower higher root biomass in the A layer comparison with the B layer.

Table 7. Mycorrhizal root colonization (% fragments with hyphae, vesicles or arbuscules) of *B. decumbens* in the A (0-10 cm) and B (10-20 cm) layers according to the different treatments, and the number of spores $g^{-1}$ soil (extracted from 100 g) in both layers and surface castings of *P. corethrurus* (variable weights) at 70 d.a.t. Different letters within a same column mean significant differences at P<0.05.

| Treatment | Spores in A (0-10 cm) | Spores in B (10-20 cm) | Spores in castings ($g^{-1}$) | Colonization in A (0-10 cm) | Colonization in B (10-20 cm) |
|---|---|---|---|---|---|
| 0F+Worm | 71.8 | 59.6 | 1.4 | 37ab | 66b |
| 0F-Worm | 74.1 | 45.8 | | 44a | 54d |
| NK+Worm | 72.0 | 67.2 | 9.7 | 41a | 55cd |
| NK-Worm | 76.2 | 42.1 | | 44a | 78a |
| PK+Worm | 67.1 | 30.5 | n.d. | 27bc | 83a |
| PK-Worm | 76.0 | 51.6 | | 28bc | 61bcd |
| NPK+Worm | 41.3 | 54.3 | 18.8 | 20c | 63bc |
| NPK-Worm | 60.3 | 49.4 | | 34ab | 79a |

As for fertilization effects, the number of spores in the A layer with NPK were significantly lower than in the other treatments. In the B layer, the PK treatment had fewer spores than the others. The effect of the earthworms on the number of spores was variable: in the A layer, they had a negative effect with NPK and in the B layer, a positive effect with 0F and NK, but negative with PK. In the other treatments the effect was practically nil. The effect with NPK and PK could be due to an interaction with the P fertilizer that was absent in the treatments without P, although it could be also linked to the higher root growth with P and changes in the dynamics of AMF colonization of roots (below).

### *Root colonization*

There was no detectable root colonization in the plants growing in pots with irradiated soil. In the pots with non-irradiated soil inoculated with AMF, the mycorrhizal root colonization varied between 20% (NPK+Worm) and 83% (PK+Worm), although there were important differences according to the layer (A or B) sampled (Table 7). In the A layer, the lowest colonization's were with NPK and PK, confirming the negative effect of P application. Earthworms reduced significantly the infection with NPK. On the other hand, in the B layer, the colonization's were significantly higher with PK+Worm, NK-Worm and NPK-Worm than in the remaining treatments. Earthworms increased colonization with 0F and PK, and decreased it with NK and NPK. The average of the root colonization in both A and B layers (Figure 4) clearly shows the trend for negative effects of earthworms with N application, the positive effect with PK and the absence of effects with 0F.

Pattinson *et al.* (1997) found a negative effect of the temperate lumbricid earthworm *Aporrectodea trapezoides* on root colonization of clover grown in a greenhouse in Australia. The authors attributed the negative effect to possible consumption (grazing) of hyphae or (more likely) to physical damage of earthworm activity (interference) on the mycorrzhizal hyphal net. Soil disturbance such as bio-turbation and invertebrate grazing on hyphae results in disconnection of external with internal AMF mycelia and generally reduces AMF root colonization and efficiency (Fitter and Sanders, 1992; Fitter and Garbaye, 1994; Jasper *et al.*, 1989; 1991). Patrón *et al.* (1999) also observed, in the same soil used for the present trial, a negative effect of *P. corethrurus* on root colonization of *B. decumbens*, fertilized or not with P. Without P, the earthworms decreased by 50% AMF colonization and with aprox. 10 kg ha$^{-1}$ of P injected into the soil, root colonization was practically 0 in both treatments. In the present study both positive and negative effects of *P. corethrurus* on *B. decumbens* root colonization by AMF were observed, depending on the fertilizer treatment. The species of plant used can also affect the AMF * Worm interaction. For example, Ydrogo (1994) found important increases in AMF colonization of roots of three species of ornamental and/or fruit plants due to *P. corethrurus*. This increment was probably responsible for the greater biomass of two species (*Bixa orellana* and *Eugenia stipitata*) in presence of earthworms.

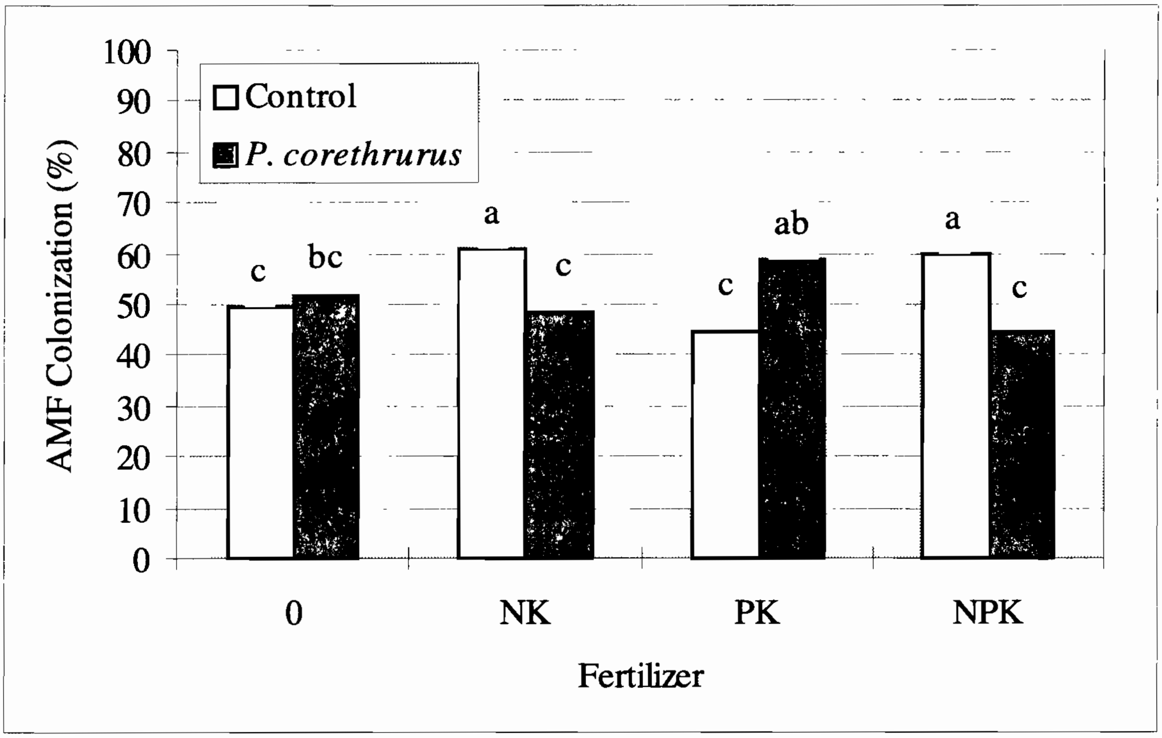


Figure 4. Percentage AMF colonization of *B. decumbens* roots with or without earthworms (*P. corethrurus*) and four fertilizer treatments (mean of both A and B layers). Treatments with different letters mean significant differences at P<0.05.

It is well known that plants depend less than on AMF for their nutrition when P fertilizers are applied and that root colonization is generally reduced by increasing doses of applied P (e.g., Bolan, 1991; Jasper *et al.*, 1979). *B. decumbens* is known to be highly mycorrhizal dependent (Saif, 1987) in P-poor soils. Root colonization of *B. decumbens*, nonetheless has been demonstrated to be both negatively affected and not show any effects when P is added in (high) doses up to 100 kg ha$^{-1}$ (Carneiro, 1996; Rao, 1995). In the present study, the lowest colonization rates were observed with application of NPK and the highest with PK (10-20 cm layer). It is interesting to note that, among the treatments with PK, it was without earthworms and AMF (-Worm-AMF) that the highest yields were obtained (Figure 2). The high rates of colonization in the treatments with PK were probably having a negative and parasitic effect on the plant, in which the greater C-drain of plants with AMF was prevailing over their possible benefits on nutrient uptake (since P was not limiting the growth of these plants) and stress resistance, or the protection against plant pathogens. Since earthworms increased the % AMF root colonization, this could explain the negative effect of their presence (in the +AMF+ Worm treatment) on plant biomass. However, when the values of shoot/root ratios in the different treatments with PK are compared (Table 3), the +AMF+Worm treatment had the highest shoot/root ratio, indicating a certain benefit of the presence of both earthworms and AMF in the amount of the energy deposited in the shoots compared with roots. This is a common phenomenon in soils inoculated with AMF or earthworms (Brown *et al.*, 1999; Marschner, 1996) and is probably related to the ability of both organisms to increase the availability and/or uptake of various nutrients to/by the plants.

In contrast to what was observed with PK, in the treatment +AMF+Worm without fertilizers (0F), the increase in *B. decumbens* root colonization in the B layer due to the presence of earthworms could responsible for higher plant yields. Since P-availability was low and the plants were P-limited, the increase in AMF colonization and the consequent higher N and P uptake (greater but not significant), probably helped overcome any possible negative effects on their internal C dynamics. This effect can be confirmed comparing the shoot/root ratios (Table 3) in 0F treatments. Although not significant, the comparisons +AMF and +Worms showed that both affected positively shoot/root ratios. The plants in the full treatment and +AMF-Worm were investing much more energy into the shoots than roots.

When observing the root colonization of treatments with NPK and NK, the presence of earthworms (+AMF+Worm) resulted in similar increments in the productivity of *B. decumbens* than with AMF alone, indicating that decreases in colonization (due to earthworms) do not always negatively (or positively) affect plant yields (Ingham and Molina, 1991; Jakobsen, 1991). The shoot/root ratios in NPK, nevertheless, indicate a negative effect of earthworms which may be related to their negative effect on AMF colonization of roots; to produce the same shoot biomass in these treatments, the plants had to invest much more energy into the roots. The negative effect of earthworms could be, due to direct effects on their populations, or indirect effects, such as changes in soil properties, in particular, a lower pH in pots with earthworms.

Other factors could be at play in the inter-relationships of mycorrhiza and earthworms:

a) the earthworms could be having a positive role (alone) in plant productivity that counteracts possible negative effects of reduced root colonization by AMF;
b) the earthworms could be "cleaning" the AMF hyphal network of less efficient hyphae, increasing the efficiency of the remaining hyphae (Fitter and Sanders, 1992);
c) low level grazing could be stimulating AMF hyphae proliferation (Fitter and Sanders, 1992; Rabatin and Stinner, 1991);

d) overgrazing could be placing higher C-demands on the plant, reducing AMF efficiency (Fitter and Sanders, 1992; Ingham and Molina, 1991) or plant production;
e) grazing (in this case possibly by earthworms) on AMF hyphae, which can release ammonium or organic N (Coleman *et al.*, 1983);
f) earthworm activities could be changing soil properties that affect AMF populations;
g) the activity of earthworms could be changing microorganism populations (species composition, dominance) in the rhizosphere, for example, increasing organisms antagonistic or beneficial to AMF (e.g., Actinomycetes) or plant growth promoting rhizobacteria populations (Brown, 1995; Ingham and Molina, 1991; Linderman, 1988).

Evidence of the rhizosphere activity of *P. corethrurus* is presented in the following section.

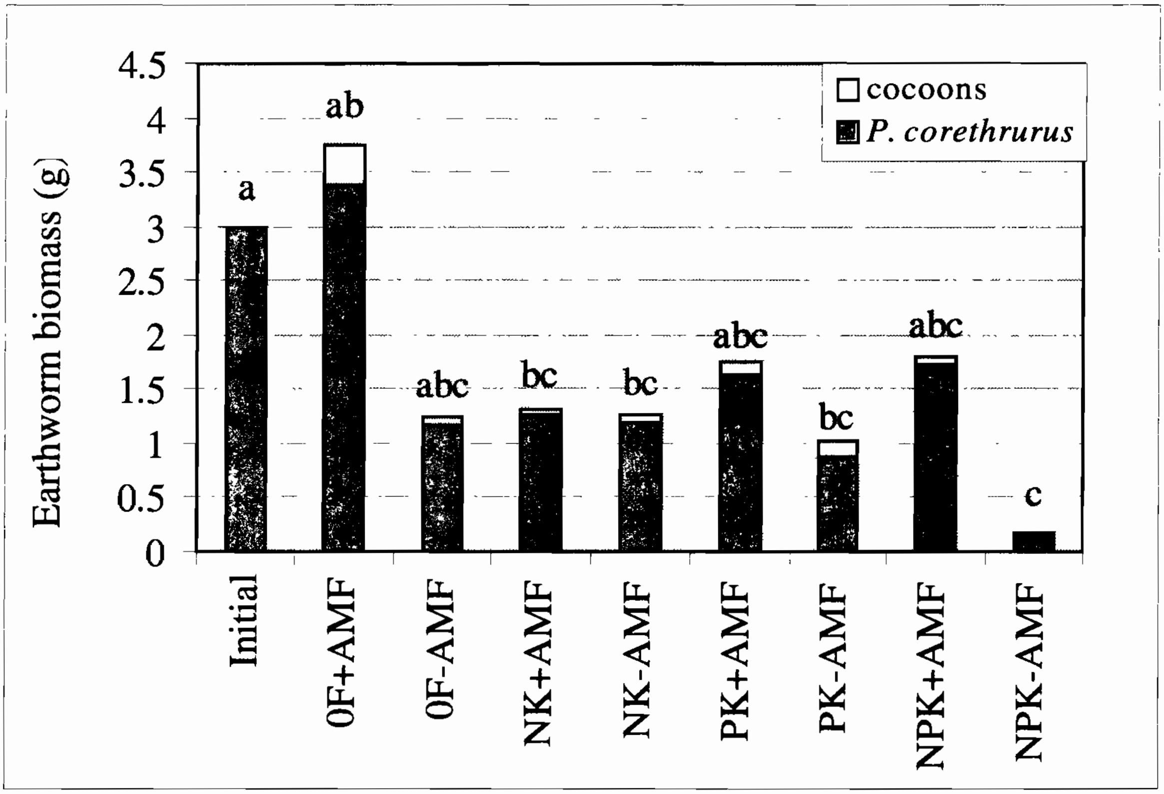


Figure 5. *P. corethrurus* biomass (+cocoon mass; g pot$^{-1}$) in the different treatments with and without AMF and/or fertilizers 70 d.a.t. *B. decumbens*. Treatments with different letters mean significant differences at P<0.05.

## Earthworm population

*P. corethrurus* survival varied from 7% (NPK-AMF) to 70% (0F+AMF) of the inoculated individuals, and the recovered biomass only increased in the 0F+AMF treatment (Figure 5). The survival (P< 0.06) and the biomass (P< 0.12) at harvest was more than twice higher with AMF that in their absence (irradiated soils). Fertilization had negative effects, with lower earthworm recovery (number of individuals) in PK and NPK treatments and greater biomass loss with NPK, NK and PK. However, in all treatments cocoons were recovered, indicating that the environmental conditions permitted reproduction (Figure 5). The survival and growth of *P. corethrurus* in this soil in its natural state (without fertilization) are normally good (Barois *et al.*, unp. data). Several factors could be causing the lower recoveries of earthworms in the fertilized treatments: the competition for $H_2O$ between plant roots and earthworms, the negative effect of N fertilization on soil pH, soil irradiation and its possible effects on soil properties compared with non-irradiated soil (e.g., microorganism populations), besides the absence of AMF (Edwards and Bohlen, 1996; Auerswald *et al.*, 1996).

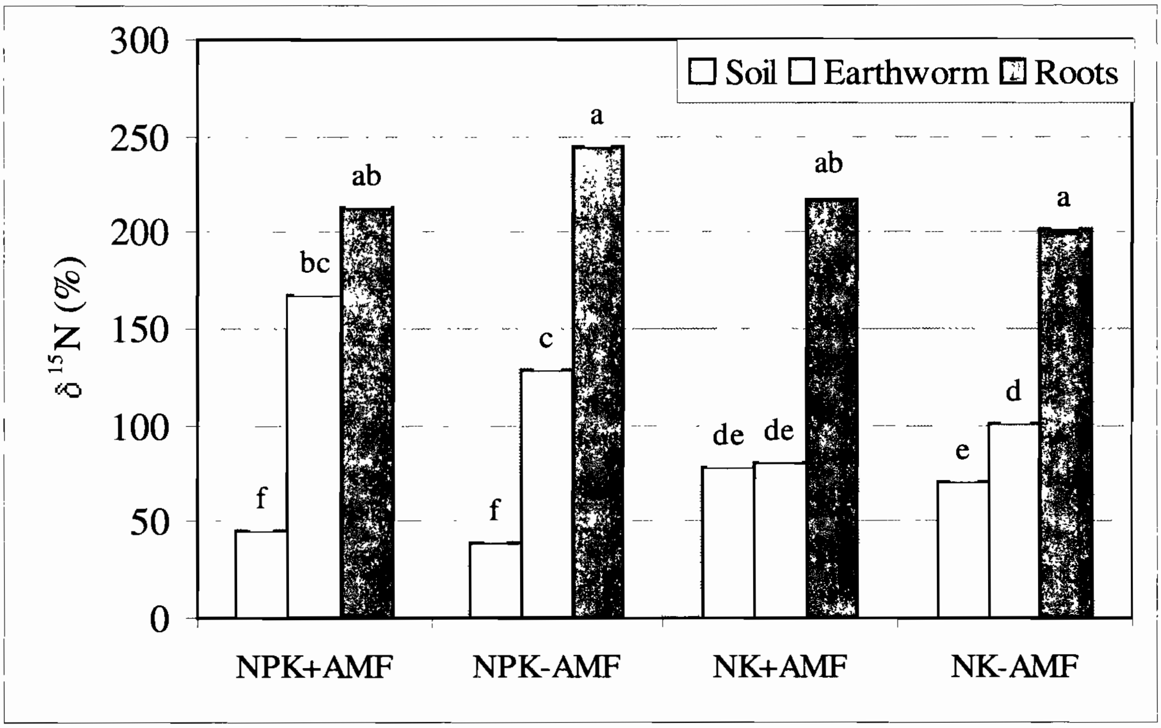


Figure 6. Natural abundance (unlabled) earthworms (*P. corethrurus*) and the isotopic labeling of soil, *B. decumbens* roots and the earthworms 70 d.a.t., in the presence or absence of AMF and a) NK or b) NPK fertilizers. N was applied at the rate of 200 kg ha$^{-1}$ N (0.78 atom% $^{15}NH_4{}^{15}NO_3$, $\delta^{15}N$=1100‰). Columns with different letters are significantly different at P<0.05. Values shown are means +SE bars.

The recovery of $^{15}N$ (RCU) by the earthworms was very small, on average 0.33% with NPK and 0.17% with NK, despite significant increase in tissue $^{15}N$ compared with the initially inoculated earthworms ($\delta^{15}N$ = 9‰; 0.3696 atom% $^{15}N$, natural abundance). With NPK+AMF, the $^{15}N$ labeling of *P. corethrurus* resulted in a $\delta^{15}N$ of 168‰, a value closer to that of *B. decumbens* roots ($\delta^{15}N$ = 213‰) than of soil ($\delta^{15}N$ = 46‰) (Figure 6b). With NPK-AMF the $^{15}N$ signature was also significantly greater in *P. corethrurus* tissues ($\delta^{15}N$ = 129‰) than soil ($\delta^{15}N$ = 39‰), although slightly lower than in the presence of AMF. With NK earthworms reached values of $\delta^{15}N$ closer to that of soil than the roots of *B. decumbens* and the only significant difference was observed in NK-AMF (Figure 6a).

Considering the previous data, and that earthworms cannot assimilate inorganic-N ($NH_4$ or $NO_3$) directly (as opposed to plants and microorganisms), we propose that the N assimilated by the earthworms came mainly from N recycled by the roots and possibly immobilized N assimilated by the microbial biomass (Brown, 2000a). It is not known how much *P. corethrurus* feeds on soil microorganisms, although it has been demonstrated that they can ingest and digest fungi (including AMF), protozoa and nematodes (Barois, 1987; Boyer, 1998; González, 1990; Reddell and Spain, 1991). However, since the biomass of these microorganisms comprises only a small part of the total C and N content of soils (less than 3 to 5%) and is probably insufficient to feed these earthworms, we believe that *P. corethrurus* more likely feeds on fresh soil organic matter and C derived from rhizo-deposition (including sloughed off cells, mucilage and exudates) although the microbial biomass (including AMF) probably constitutes an important nutrient supplement to these earthworms (Brown, 2000a; Doube and Brown, 1998; Spain *et al.*, 1990; Spain and Le Feuvre, 1997).

## CONCLUSIONS

In the nutrient-poor, P-limited sandy soil used in this experiment, P fertilization increased significantly *B. decumbens* yields, with N being less important for production. P fertilization is generally recommended for the planting and maintenance of pastures in the Mexican and American tropics in general (Fenster and León, 1979; Ortiz, 1977). However, it is a rare practice between most ranchers in Mexico. Under the native fertility conditions of unfertilized pastures, AMF and their interactions with earthworms (such as the common species *P. corethrurus*) play an important role in the nutrition and sustainable production of pastures and grasslands. When fertilizers are applied, the natural balance of the edaphic community changes and the interactions between soil organisms can lead to positive (e.g., with N) or negative (e.g., with P) effects on plant yields. Interactions such as these that result in significant effects on the studied parameters (such as plant biomass or soil fertility) are common but little studied (Fitter and Sanders, 1992) due to the difficulty of managing multiple components in a single system. The results of this experiment show the importance of including and studying in more detail the interactions between macrofauna like earthworms and microorganisms such as AMF in traditionally reductionistic and uni-disciplinary studies on the effects of these organisms on soil fertility and plant production. The exclusion of either organism (AMF or earthworms) from each other could lead to considerable under-estimations of their effects (due to their interactions) and erroneous interpretations as to the importance of each organism in the plant-soil system.

# CHAPITRE 7

# CONFIRMATION OF EARTHWORM ACTIVITY IN PLANT RHIZOSPHERES USING STABLE ISOTOPES[‡‡]

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## SUMMARY

Rhizosphere feeding activities of two pantropical geophagous endogeic earthworm species (*Pontoscolex corethrurus* and *Polypheretima elongata*) under three plants (maize, common beans and *Brachiaria decumbens* pasture) were assessed in several greenhouse pot experiments using calculations, based on stable isotope ($^{13}C$ and/or $^{15}N$) technology, of the fraction of C and N recovered in earthworm tissues derived from plant roots, residues and/or fertilizers. *P. corethrurus* was found to be active in the rhizospheres of maize and *B. decumbens*, yet no preferential activity was detected under beans. After six months, the amount of *P. corethrurus* body tissue C derived from maize was estimated at 8%, while the fraction of N derived from surface residues increased almost 25 times in the presence of maize plants than in their absence, indicating that these earthworms may derive important amounts of their dietary C and N needs from plant rhizodeposited resources. Under *B. decumbens*, *P. corethrurus* also seemed to be assimilating N recycled by the plants, since the fraction of N derived from labeled fertilizer was much higher in earthworm tissues than bulk soil, and their $\delta^{15}N$ signatures were closer to those of plant roots than of soil with NPK fertilizers. In contrast, under beans no changes in tissue $\delta^{13}C$ of either *P. corethrurus* or *P. elongata* were observed, and little of the fertilizer derived N was assimilated by *P. elongata* under maize, despite high $^{15}N$-labeling of the soil and plants. Thus under the present experimental conditions, *P. elongata* did not appear to be feeding in maize or bean rhizospheres and probably derived its nutrition mostly from bulk soil organic matter. Although these results provide evidence of rhizosphere activities of *P. corethrurus*, further research is needed to confirm the specificity of and the mechanisms for the observed responses of both earthworm species (and others, of temperate and tropical regions) using a variety of other techniques and plant species. In summary, it appears that not only do growing plants often benefit from earthworm activities, earthworms may also be benefiting more from plant rhizodeposited resources than was previously thought.

## INTRODUCTION

Earthworms can alter plant growth by modifying soil properties at both large and small spatio-temporal scales, from pedogenesis through microbial activity (Brown, 1995; Brown et al, 1999; chap. 2; Lavelle, 1988). Over the short term, a cropping cycle for example, modification of soil in or around the rhizosphere (the thin soil layer surrounding plant roots) may be important for

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[‡‡] Submitted to *Soil Biology and Biochemistry*

earthworms to have significant effects on plant growth, although previous effects of earthworms on soil physical and bio-chemical conditions are also important (e.g., Brown *et al.*, 1998a). Furthermore, nutrients, soil biological or physical conditions must be limiting plant growth to some extent, and earthworms must be able to help reduce these limiting factors for plants to respond positively. Thus, at the rhizosphere level quantification of earthworm activity at both the physical (spatial overalap of earthworms with the rhizosphere) and biochemical scales (such as that detected with stable isotope chemistry) is essential if we are to assess what impact, both direct and indirect, earthworms can have on plant root growth and hence on above-ground yields.

Stable isotopes are a relatively recent addition to ecological and agronomic research involving earthworms. Up to the present, most of the published literature using $^{13}C$ and $^{15}N$ has addressed earthworm effects on C and N cycling in different ecosystems (e.g., Binet *et al.*, 1994; Brown *et al.*, 1998a; Cortez *et al.*, 1989; Curry *et al.*, 1995; Villenave *et al.*, 1999; Hameed *et al.*, 1993; 1994a,b; Jégou *et al.*, 1998; Whalen *et al.*, 1999), although a growing number of experiments have used these isotopes to determine earthworm diets (Martin *et al.*, 1992a,b; Neilson *et al.*, 1998; Schmidt *et al.*, 1997; 1999; Spain *et al.*, 1990; Spain and Le Feuvre, 1997). In the first set of publications, research was devoted primarily to tracing the fate and transfer of $^{13}C$ and $^{15}N$ from plant residues into the soil and drilosphere components (including earthworm tissues), and the estimation of $^{15}N$ losses from labeled earthworms. In the latter experiments, attention was primarily devoted to describing patterns of earthworm food assimilation under field or laboratory situations, identifying major diet components from soil and plant C and N isotope analyses but without specifying exact sources of nutrition. Of these studies, only two (Spain *et al.*, 1990; Spain and Le Feuvre, 1997) associated patterns of C and N assimilation (using $^{13}C$ and $^{15}N$) by earthworms (*Pontoscolex corethrurus*) with rhizosphere activities (in sugarcane).

The present paper details the results of three experiments performed to assess rhizospheric activities of two common tropical geophagous endogeic earthworm species using three plant species, three soil types and the stable isotopes $^{15}N$ and $^{13}C$ to estimate the amounts of root-derived C and N assimilated into earthworm tissues.

## MATERIALS AND METHODS

### Generalities

To determine the effects of earthworms on plant growth, several greenhouse experiments were set up in the region of Xalapa, Mexico. Details of the methodologies employed can be found in Brown *et al.* (2000a; chap. 3), Brown *et al.* (2000b; chap. 4) and Brown *et al.* (2000d; chap. 6). The basic design consisted of plastic pots (25-30 cm diam.) filled with soil, including or excluding earthworms, and planted (or not, depending on the treatment) with three plant species, maize (*Zea mays*), common beans (*Phaseolus vulgaris*) and *Brachiaria decumbens*, a tropical pasture grass (details in Table 1). Three soil types were used, two of them sandy loams (Psamment and Haplustalf) and the other a clay (Andosol). All three soils had some degree of nutrient limitations to plant growth, the Alfisol being the poorest soil utilized (Table 2). The dry weight equivalent of soil used ranged from eight to 12.5 kg, and water was added up to field capacity, and thereafter watered regularly (by weight) to maintain similar water contents in the soil for the plant throughout its growing cycle. Two earthworm species were used, *P.*

*corethrurus* (Glossoscolecidae) and *Polypheretima elongata* (Megascolecidae), both pantropical geophagous endogeic species, known to be important modifiers of soil properties and with potential to affect plant growth (Barois *et al.*, 1999; Brown *et al.*, 1999; chap. 2). *P. corethrurus* is mostly associated with ingestion of richer soil portions (polyhumic) and is often found near the root zone, while *P. elongata* feeds on organic matter (OM) of intermediate quality (mesohumic) (Barois *et al.*, 1999). Earthworms were added at the rate equivalent to about 60 g m$^{-2}$ except for *P. elongata* in the Entisol (276 g m$^{-2}$). The experiments lasted from 70 up to 183 d (maize). To trace natural abundance $\delta^{13}C$ changes, and isotopic $^{15}N$ enrichment of the plant-soil-earthworm compartments, the following experiments and designs were used (Table 1).

Table 1. Brief description of the 3 experiments performed, including the soil types and weights, fertilizer, residue or mycorrhizae presence or absence, the plant type ($C_3$ or $C_4$), the earthworm species and inoculum weight applied, the duration of each experiment and the isotope measurements performed (discussed in the text). n.d. = not determined; ± = presence or absence; *P. corethr.* = *P. corethrurus*.

| | Experiment | | | | |
|---|---|---|---|---|---|
| | 1a | 1b | 2a | 2b | 3 |
| Soil | $C_3$ | $C_3$ | $C_4$-$C_3$ | $C_4$-$C_3$ | $C_4$ |
| Earthworms | ± $C_3$ | ± $C_3$ | ± $C_4$-$C_3$ | ± $C_3$ | $C_4$ |
| Plant | ± $C_4$ (maize) | ± $C_4$ (maize) | ± $C_3$ (beans) | $C_4$ (pasture) | $C_4$ (maize) |
| Residues | None | $^{15}N$-labeled (1.4 Mg ha$^{-1}$) | None | None | None |
| Fertilizers | None | None | None | $^{15}N$-labeled (200 kg ha$^{-1}$) +P or +PK | $^{15}N$-labeled (156 kg ha$^{-1}$) +PK |
| Mycorrhizae | Present | Present | n.d. | Present/ absent | n.d. |
| Duration (days) | 183 | 183 | 97 | 88 | 90 |
| Soil type (weight in kg) | Andosol (12.5) | Andosol (12.5) | Inceptisol (12.5) | Inceptisol (10) | Entisol (8) |
| Earthworm spp. | *P. corethr.* | *P. corethr.* | *P. corethr.; P. elongata* | *P. corethr.* | *P. elongata* |
| Worm live weight (g m$^{-2}$) | 59.4 | 59.4 | 56.1; 60.5 | 61.1 | 276 |
| Surface area of container (m$^{-2}$) | 0.063 | 0.063 | 0.063 | 0.049 | 0.063 |
| Measurements | $\delta^{13}C$ | $\delta^{13}C$; $\delta^{15}N$ | $\delta^{13}C$ | $\delta^{15}N$ | $\delta^{15}N$ |

### Experiment 1a. Maize in a forest soil

Topsoil (Andosol) from the 0-10 cm (A) horizon and earthworms (*P. corethrurus*) of a tropical rainforest ($C_3$ vegetation) at Los Tuxtlas, Veracruz, both known to have low $^{13}C$ contents, were collected and brought to the laboratory. The soil was sieved at 2 mm and treatments with or without (±) earthworms and/or maize ($C_4$ plant) plants were established simultaneously. After 37, 131 and 183 d the maize roots and shoots were harvested, the soil and earthworms removed from the pots, ground to a fine powder and analyzed for $^{13}C$. At 37 and 131 d three replicates, and at 183 d 10 replicates of the ± earthworm treatments with maize plants were harvested, respectively. At each date only one replicate each of the no plant+earthworm treatment was harvested, although earthworms separated from these treatments were generally analyzed individually (n generally $\geq 3$). Further details of the experimental methodologies are presented elsewhere (Brown *et al.*, 2000b; chap. 4).

### Experiment 1b. Maize in a forest soil and $^{15}N$-labeled surface-applied maize residues

*P. corethrurus* collected from a pasture ($C_4$ vegetation) near Plan de las Hayas, Veracruz (there were insufficient earthworms from the Los Tuxtlas site for this experiment), were placed into the forest soil and the $^{15}N$-labeled maize residues obtained in Experiment 3 (3.1% N, 3.68 atom% $^{15}N$; $\delta^{15}N = 9123‰$) added to the surface of pots planted or not planted (±) with maize, at the rate equivalent to 1.4 Mg $ha^{-1}$. Each treatment was replicated four times, except for the pot without plants (n = 1). Earthworms sampled were analyzed individually (n = 2-8 $pot^{-1}$). After 183 d the maize was harvested, the earthworms and residues removed and the different plant parts, earthworms and remaining soil fine-ground and analyzed for $^{15}N$. Other details of the experimental methodologies are available elsewhere (Brown *et al.*, 2000b; chap. 4).

### Experiment 2a. Common beans in a savanna soil

Topsoil from a nutrient poor Alfisol was collected from a grass savanna-derived pasture (with predominance of native $C_4$ grasses) at La Víbora, Veracruz (Table 2), treated similarly to the forest soil (above), and planted with common beans ($C_3$ plant). *P. corethrurus* (low density at the site) and *P. elongata* (absent at the site) were collected elsewhere ($C_4$ pasture at Plan de las Hayas, and $C_4$-$C_3$ fallow at La Mancha, respectively) and added to the surface of the pots the night of seeding. After 30 and 62 d three replicates each, and at 97 d eight to 10 replicates of the ± earthworm treatments with beans were harvested, respectively. Only one replicate each of the no plant+earthworm treatment was harvested at each date. Further details of the experimental methodologies are presented elsewhere (Brown *et al.*, 2000a; chap. 3).

### Experiment 2b. $^{15}N$-labeled fertilizer and mycorrhizae in a savanna soil

Topsoil collected from the pasture at La Víbora was treated similarly as above, and *B. decumbens* transplanted (15 d old seedlings) into pots in the presence or absence (±) of both vesicular arbuscular mycorrhizae (VAM) and/or earthworms (*P. corethrurus*). Treatments involved no fertilization, or the addition of doubly $^{15}N$-labeled $^{15}NH_4{}^{15}NO_3$ (0.76 atom% $^{15}N$; $\delta^{15}N = 1100‰$) at the rate of 200 kg $ha^{-1}$ N, with either K alone (KCl, 200 kg $ha^{-1}$ K) or K + P ($KH_2PO_4$, 100 kg $ha^{-1}$ P) fertilizers. All treatments were replicated four times. After 70 d, the plant shoots and roots were harvested, the earthworms removed, and the soils, plants and earthworms ground and analyzed for $^{15}N$. Further details of the experimental methodologies are given elsewhere (Brown *et al.*, 2000d; chap. 6).

Table 2. Selected physical and chemical characteristics of the soils used for each of the experiments.

| Soil type | C (%) | $\delta^{13}C$ (‰) | N (%) | $\delta^{15}N$ (‰) | C/N | pH ($H_2O$) | CEC (meq $100g^{-1}$) | P-Bray (ppm) | K (meq $100g^{-1}$) | Ca (meq $100g^{-1}$) | Mg (meq $100g^{-1}$) | Sand (%) | Silt (%) | Clay (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Haplustalf | 1.00 | -19.1 | 0.13 | 4.7 | 7.7 | 5.0 | 12.0 | 7.0 | 0.08 | 7.8 | 3.6 | 81.8 | 7.5 | 10.7 |
| Andosol | 5.76 | -27.3 | 0.51 | 6.0 | 11.3 | 5.9 | 30.3 | 6.7 | 0.56 | 23.4 | 6.0 | 20.7 | 37.4 | 41.9 |
| Psamment | 1.65 | -19.4 | 0.11 | 7.0 | 15.7 | 7.9 | 30.9 | n.d. | n.d. | 20.8 | 2.67 | 75.4 | 8.6 | 16 |

### Experiment 3. $^{15}$N-labeled fertilizer and maize

Four pots were filled with about 9 kg of air dry topsoil (Entisol) taken from a fallow plot at La Mancha, Veracruz (Table 2), seeded to maize and 10.5 atom% $^{15}$N-labeled $KNO_3$ added on the soil surface at the total rate of 156 kg ha$^{-1}$ (418 kg ha$^{-1}$ K added) in three separate and equal doses (15, 35 and 55 d). Additional fertilization included 52 kg ha$^{-1}$ of $^{14}$N and 64 kg ha$^{-1}$ P as DAP (di-ammonium phosphate). Earthworms (*P. elongata*) collected at the same site were inoculated after 45 d. At 90 d after planting (45 d later) the plants were harvested, earthworms removed, and plant shoots, earthworms and soil ground and analyzed for $^{15}$N.

### Isotopic and Statistical Analyses

For the above experiments, all materials (plant, soil, earthworms) were dried at 60° C for 48 h and manually ground to a fine powder in a mortar, before $^{15}$N and $^{13}$C analyses. To prevent contamination of earthworm tissues with soil in the intestines, they were placed for 48 h in small plastic containers with moist paper towels to void their guts, after which point they were rapidly (3 sec immersion) killed in boiling water. Generally, enough replicates ($n \geq 3$) were used to permit parametric statistical tests (ANOVAs), but in a few cases, the results reported represent a single analysis of a bulked sample (with $n \geq 4$ for bulking; e.g., $n = 4$ for maize residues from experiment 3 and $n = 2$-$8$ earthworms pot$^{-1}$ without plants in experiments 1 and 2). Results are expressed in delta "per mil" ($\delta$‰), representing the difference of the isotope ratio (R) of the heavier to the lighter isotopes ($^{15}N/^{14}N$ and $^{13}C/^{12}C$) of the sample to that of a standard, obtained from the following equation (1):

$$\delta_{sample} = \frac{R_{sample} - R_{standard}}{R_{standard}} * 1000 \qquad (1)$$

The analyses were performed on a Fisions (Micromass) SiRA10 ($^{13}$C) and Optima ($^{15}$N) mass spectrometers (Manchester, England) both coupled to a Carlo Erba NA 1500 elemental CHN analyzer (Milan, Italy) at the Laboratoire de Biogeochimie Isotopique (LBGCI), Université Paris VI, and on a Micromass VG Isochrom dual isotope ratio ($^{13}$C, $^{15}$N) mass spectrometer, coupled to a Carlo Erba 1108 CHNS-O elemental analyzer at the Laboratório de Isótopos Estables (LIE), Universidad Autónoma de Madrid. Analytical precisions (standard deviation of the mean, sd) on $^{13}$C and $^{15}$N samples were 0.05‰ and 0.5‰, respectively, at LBGCI, and 0.15‰ and 0.2‰, respectively, at LIE. A few enriched $^{15}$N samples were analyzed on a Finnigan Delta S mass spectrometer (Bremen, Germany), coupled to a SCA C:N elemental analyzer (precision 1% relative for enriched samples) at the Service Central d'Analyse (CNRS), Vernaison, France.

## RESULTS

Details of the various plant and soil parameters measures in the above experiments are given in separate publications (Brown *et al.*, 2000a; chap. 3, Brown *et al.*, 2000b; chap. 4; Brown *et al.*, 2000d; chap. 6) and the results discussed here deal only with earthworm survival and stable isotope analyses of the plant and earthworm tissues and bulk soil.

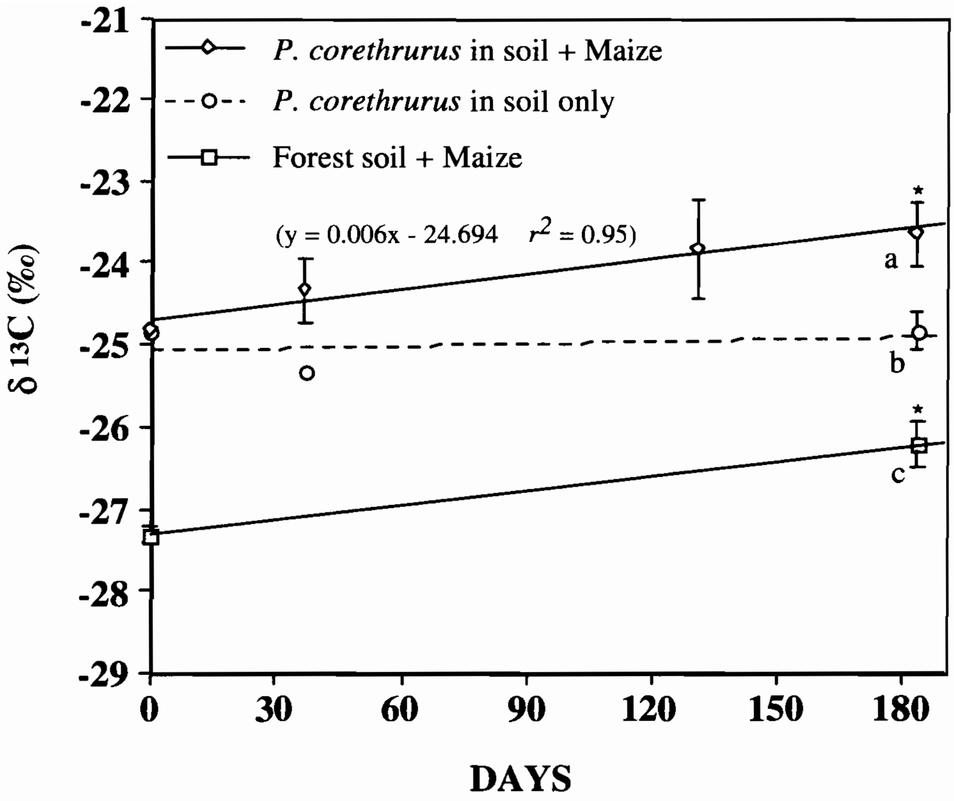

Figure 1. Change in $^{13}C$ content (δ‰) of soil and earthworm (*P. corethrurus*) tissue in the presence and absence of maize ($C_4$ plant), after 37, 131 and 183d of culture in a clayey Andosol from a tropical rainforest ($C_3$ vegetation). Linear regression (equation shown) with earthworms significantly ($P< 0.05$) different in presence of maize than in absence. Asterisk (*) indicates significant ($P< 0.05$) difference between initial and final $\delta^{13}C$, and different letters mean significant ($P< 0.05$) differences between each compartment. Values presented are means + standard error (SE) bars.

### Experiment 1a and 1b

*P. corethrurus* biomass recovered at the final harvest was significantly lower than the inoculated values, and the survival was highly dependent on residue presence. When surface residues were applied, 65% of the individuals were recovered and biomass was maintained at around 80% of the applied, but without residues only 12-13% of the individuals and biomass applied were recovered. Nonetheless, in the latter treatments (without residues), the earthworms found at the final harvest (183 d) showed significantly higher $\delta^{13}C$ content in their tissues (δ = -23.6‰) than the inoculated earthworms (initially applied) and those in the same treatment, but without maize plants (both δ = -24.8‰) (Fig. 1). This indicated that the earthworms were assimilating new $C_4$-C derived from the maize plants. A calculation of the percentage of maize-derived C in the diet of *P. corethrurus* was made using the following formula in Equation (2).

$$F (\%) = (100) * \frac{\delta - \delta_1}{\delta_2 - \delta_1} \qquad (2)$$

Where F is the percentage of C derived from maize in earthworms tissues, $\delta_1$ is the $\delta^{13}C$ signature of earthworms (*P. corethrurus*) living in the original soil ($C_3$ forest), $\delta_2$ is the $\delta^{13}C$ signature of earthworms living in soil under $C_4$ plants (maize) and $\delta$ is the $\delta^{13}C$ signature of earthworms living in the mixed environment ($C_3$ and $C_4$). The exact value of $\delta_2$ could not be measured since no long term sites with continuous $C_4$ vegetation were available in the Los Tuxtlas region. Under long-term continuous sugarcane (another $C_4$ plant) in Australia, $\delta^{13}C$ signatures of *P. corethrurus* tissue were on average -10.8‰ and -9.0‰ (Spain *et al.*, 1990; Spain and Le Feuvre, 1997). For the present calculations, $\delta_2$ was assumed to be similar to that under sugarcane and a mean value of $\delta^{13}C$ = -9.9‰ was used. Using these values, F = 7.8% after 183 d, indicating that about 8% of the earthworm tissue C was derived from maize plants.

As with earthworm tissue, bulk soil OM $\delta^{13}C$ signatures also increased significantly from the initial value of $\delta_1$ = -27.3‰ to $\delta$ = -26.2‰ after 183 d of maize culture (Fig. 1), at the 15-30 cm depth in the pots. Using equation (2) and $\delta_2$ values from the literature ($\delta^{13}C$ = -12.8‰; signature of dead soil organic matter coarser than 2 mm derived from maize) (Balesdent and Balabane, 1996), the fraction of soil C derived from the maize inputs was estimated at F = 7.6%. This value was very similar to that obtained for the earthworms, above.

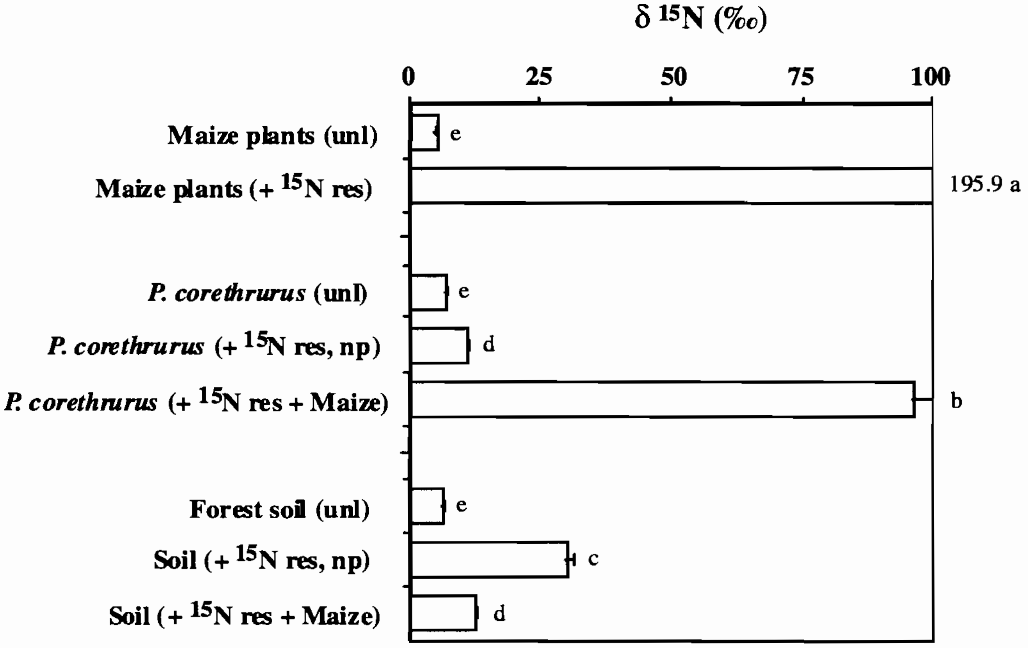

Figure 2. Maize root, earthworm (*P. corethrurus*) tissue and soil natural abundance (unl) $^{15}N$ contents, and their respective changes after six months in the presence and absence (np) of maize plants and $^{15}N$-labeled maize residues on the surface of a clayey Andosol from a tropical rainforest. Different letters mean significant differences at $P< 0.05$. Values shown are means + SE bars.

When $^{15}N$-labeled residues were added to the surface of the pots planted with maize, earthworms induced a significant reduction in the amount of residues present, their N content and the percentage of the original $^{15}N$ remaining (20% without worms, 15% with) (Brown *et al.*, 2000b; chap. 4). Pots with plants were watered more often than those without plants because of higher evapotranspiration in the former, so more $^{15}N$ was probably entering the soil pools by leaching and decomposition of the residues. Nevertheless, after 183 d the amount of $^{15}N$ remaining in the soil was significantly higher in the absence of plants (73%) than in their presence (25%), mostly due to the high plant uptake of $^{15}N$ (Brown *et al.*, 2000b; chap. 4). Despite the lower $^{15}N$ remaining in soil pools with plants, *P. corethrurus* was far more labeled in the presence ($\delta^{15}N = 96‰$) than absence ($\delta^{15}N = 11.1‰$) of maize (Fig. 2). This latter value was only slightly greater than background (unlabeled) earthworms ($\delta^{15}N = 7‰$; natural abundance), indicating that earthworms were deriving more $^{15}N$ from the residues when in the presence of growing maize than when in their absence, independent of the amount in the soil. The percentage of maize residue-derived N in *P. corethrurus* (Ndfr) tissues was calculated using equation (3).

$$\text{Ndfr}\ (\%) = (100) * \frac{\text{Ew (p, np)}}{\text{Er}} \qquad (3)$$

Where Ew is the isotopic (atom $\%^{15}N$) excess (E) of the earthworm (w) in the presence (p) or absence (np) of maize plants and Er is the isotopic excess of the original $^{15}N$-labeled surface-applied maize residues ®. Therefore, in the treatments without plants, Ndfr was 0.04% after 183 d, while in the presence of maize Ndfr was almost 25 times greater, at 0.98%. The recovery of the initially-applied $^{15}N$ in earthworm tissues (Real Coefficient of Utilization, RCU), from the surface residues was also calculated, using equation (4).

$$\text{RCU}\ (\%) = \frac{\text{Nw (p, np)}}{\text{Nr}} * \text{Ndfr} \qquad (4)$$

Where Nw is the total N content of the earthworms in the presence or absence of plants and Nr is the total N content of the original $^{15}N$-labeled maize residues. Thus RCU in the absence of plants was only 0.05%, a value more than three times lower than in the presence of maize plants (0.17%).

## Experiment 2a

Earthworm survival averaged about 69% for *P. corethrurus* and 29% for *P. elongata*. Biomass recovered, however, was only 51% and 16% of the applied for each species, respectively. Laboratory cultures under ideal temperature and moisture conditions in the same soil, also showed much higher survival for *P. corethrurus* than for *P. elongata*, which seemed poorly adapted to the sandy soil (Barois *et al.*, unp. data; voir Annexes 5 et 7). Contrary to trends found under maize in the previous experiment, both earthworm species showed no significant changes in their tissue $\delta^{13}C$ contents when in the presence versus the absence of bean plants, indicating absence of measurable bean root-derived $C_3$-C assimilation (Fig. 3). The only significant differences observed were in P. corethrurus tissues on all three harvest dates.

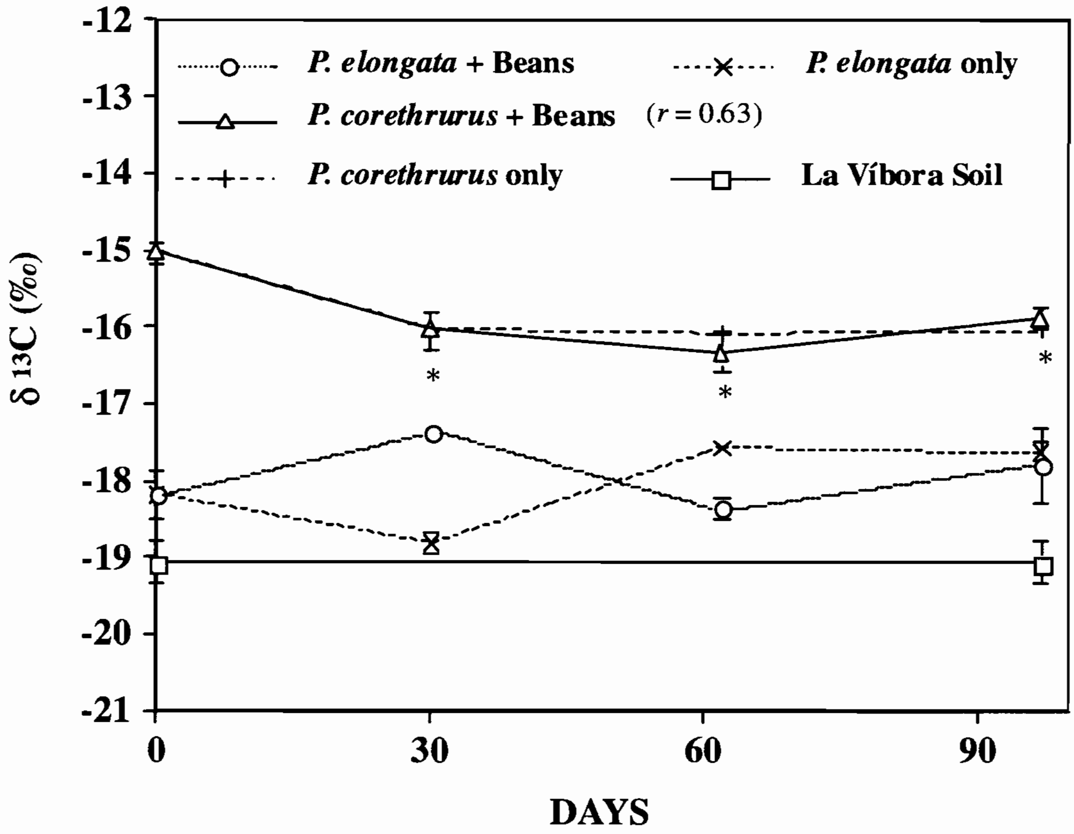

Figure 3. Effect of presence and absence of *Phaseolus* bean plants for 30, 62 and 97 d on $\delta^{13}C$ values of *P. corethrurus* and *P. elongata* body tissues in a savanna soil. Regression equation for *P. corethrurus* + beans shows the trend in tissue $\delta^{13}C$ decrease due to ingestion and assimilation of $^{13}C$-poor resources. Asterisks indicate significant difference from initial $\delta^{13}C$ signature of *P. corethrurus*. Values shown are means + SE bars.

### Experiment 2b

*P. corethrurus* survival was low with NPK fertilization (mean about 20%), increasing with NK (37%) and no fertilizers (48%). Biomass recovered averaged about 40% with NPK and NK fertilization, and 70% with no fertilizers. About twice higher biomass was found in the presence of VAM (70%) than in its absence (33%). The only treatment in which total earthworm biomass increased at the end of the experiment was +VAM and no fertilizers.

When NPK was applied, plants grew more vigorously (greater shoot and root production), assimilated more $^{15}N$ and less $^{15}N$ was recovered in the soil (Brown *et al.*, 2000d; chap. 6). Recovery of the $^{15}N$ fertilizer by earthworms calculated using equation (4) was low (RCU = 0.33% with NPK; 0.17% with NK), despite significant isotopic enrichment of their tissues compared with the initial unlabeled earthworms (9‰; 0.3696 atom% $^{15}N$). Most of the $^{15}N$ applied remained in the soil or was taken up by the plant tissues (RCU = 5-45% in plant; 40-80% in soil) (Brown *et al.*, 2000d; chap. 6). With NPK+VAM, the isotopic labelling of *P. corethrurus* tissues ($\delta^{15}N$ = 168‰) was closer to that found in plant roots ($\delta^{15}N$ = 213‰) than in the soil

($\delta^{15}N$ = 46‰) (Fig. 4b). In the NPK-VAM treatment, δ values in the earthworms ($\delta^{15}N$ = 129‰) were still significantly higher than in the soil ($\delta^{15}N$ = 39‰), but slightly (although not significantly) lower than in the presence of VAM. Conversely, in the NK treatments, plant production was low and $^{15}N$ recovery minimal and most $^{15}N$ remained in soil pools. Earthworm $\delta^{15}N$ values were closer to those of the soil than of the plant roots; the only significant difference (small) was observed in VAM- (Fig. 4a).

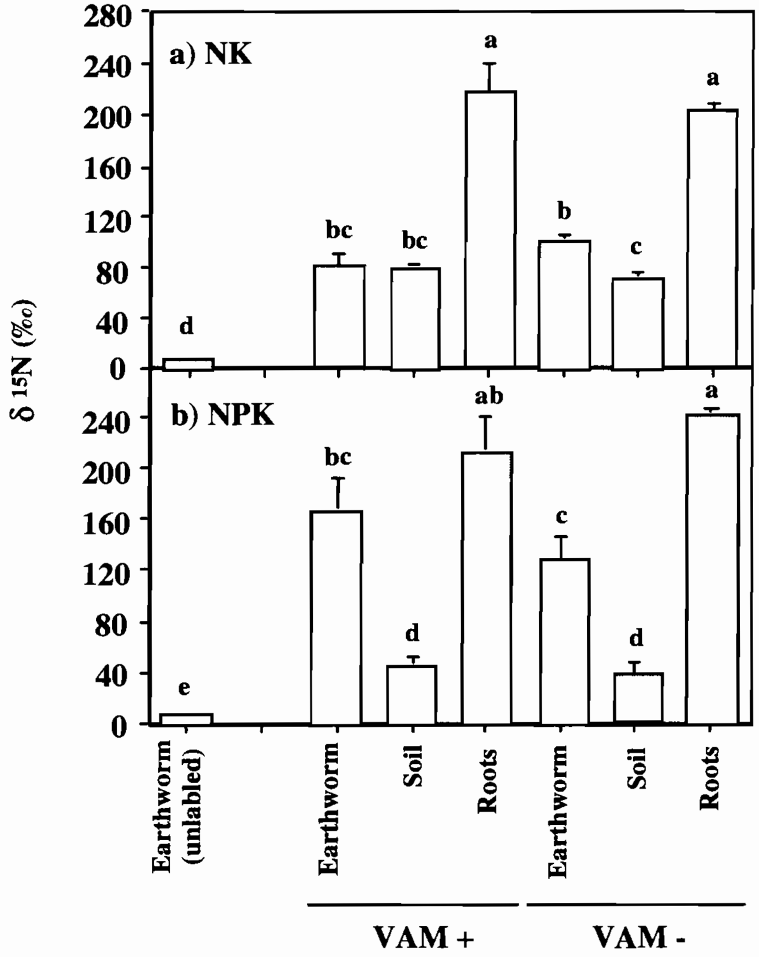

Figure 4. Natural abundance (unlabled) earthworm (*P. corethrurus*) tissue $\delta^{15}N$, and the effect of 88 d culture of *B. decumbens* pasture in the presence (VAM+) and absence (VAM-) of vesicular arbuscular mycorrhizae and fertilization with a) NK or b) NPK, on root, soil and earthworm tissue $\delta^{15}N$ signatures. Fertilizer N was 200 kg ha$^{-1}$ of 0.76 atom% $^{15}N$-labeled $NH_4NO_3$ ($\delta^{15}N$ = 1100‰) Different letters mean significant differences between the components at $P < 0.05$. Values presented are means + SE bars.

The fraction of N in earthworm tissues derived from the fertilizer (Ndff) was calculated by substituting the residues %N and atom% excess $^{15}N$ values with those of the fertilizer and the appropriate values of atom% excess $^{15}N$ and %N of earthworm tissues from the treatments NPK+VAM, NPK-VAM and NK-VAM in equation (3). With NPK+VAM, Ndff was 14.6%; in

NPK-VAM, it was 11.0%. These values were more than three times higher than the Ndff in the soil of the same treatments (3.9% and 3.3%, respectively). With NK-VAM, Ndff was 8.4% in the earthworm tissues, a value only slightly larger than in soil (Ndff = 6.2%).

### Experiment 3

*P. elongata* survival was on average 63%, although weight loss was observed in all the pots, with only 36% of the biomass recovered overall. Mean weight of the worms decreased from 1.26 g worm$^{-1}$ initially, to 0.76 g worm$^{-1}$ after 45 d in the presence of the maize. This means that the earthworms may have been feeding (and thus assimilating) very little throughout the experiment (45 d). In fact, very little of the $^{15}N$ fertilizer applied was recovered in *P. elongata* tissues (RCU < 0.005%), that reached isotopic enrichment values ($\delta^{15}N$ = 23‰) only slightly more than twice higher those of the unlabeled inoculated earthworms ($\delta^{15}N$ = 10‰), despite the very high isotopic labeling of the applied fertilizer (10.5 atom% $^{15}N$). Earthworm tissue $\delta^{15}N$ values were dramatically lower than those in the soil ($\delta^{15}N$ = 1427‰; 0.885 atom% $^{15}N$) and plants ($\delta^{15}N$ = 9123‰; 3.68 atom% $^{15}N$) (Fig. 5).

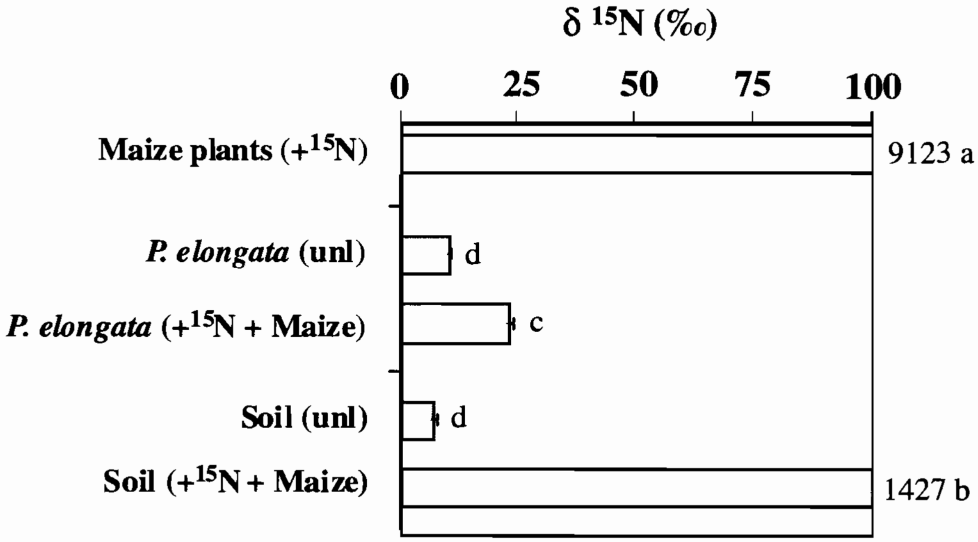

Figure 5. Initial natural abundance (unl) $\delta^{15}N$ values of maize plants, earthworm (*P. elongata*) tissue and soil and the effect of 90 d maize culture fertilized with 150 kg ha$^{-1}$ 10.5 atom% $^{15}N$-labeled $KNO_3$ on $\delta^{15}N$ signatures of the maize and earthworm tissue, and the Entisol from La Mancha, Veracruz. Different letters mean significant differences at $P< 0.05$. (SE bars shown when possible)

## DISCUSSION

Earthworm activities (burrowing, feeding and casting) can deeply influence both directly and indirectly the rhizosphere, a thin soil layer (generally <0.5mm) surrounding plant roots. Thus, there can be important consequences to microbial activity, root growth, mycorrhizal infection and plant production (Brown, 1995; Brown *et al.*, 1999; chap. 2). Indirect effects are related to earthworm-induced changes in soil physical and chemical properties which influence (or limit) root growth, and to soil biological conditions, such as pest or pathogen populations (e.g., nematodes, fungi) and beneficial microorganisms (e.g., plant growth promoting rhizobacteria, biocontrol agents). The direct effects have to do with how much earthworms feed on and are active in the rhizosphere, a matter of much present contention and speculation, which few experiments have addressed.

Several authors have reported higher earthworm abundances close to or associated with plant roots (e.g., Robertson et. al, 1994; Rovira *et al.*, 1987). Others have documented earthworms feeding on live roots (Baylis *et al.*, 1986; Carpenter, 1985; Cortez and Bouché, 1992), although this phenomenon does not seem to be widespread since studies on earthworm crop, gizzard or gut contents in over 30 spp. revealed that roots form a minor component of the ingested materials in most species (Brown *et al.*, 1999; chap. 2). Rhizosphere feeding, on the other hand, has been inferred from visual observations of *Aporrectodea trapezoides* in wheat (Australia) and other lumbricid spp. in a grassland (U.K.) or from stable isotope analyses of *P. corethrurus* tissues under maize in the present experiment (Mexico) or under sugarcane in Australia (Carpenter, 1985; Doube and Brown, 1998; Spain *et al.*, 1990; Spain and Le Feuvre, 1997).

Specific positive responses of earthworms to particular plants (e.g., *P. elongata* attraction to various grass roots) have been observed (Babel *et al.*, 1992; Boettcher and Kalisz, 1991; Kale and Basha, 1975; Westernacher and Graff, 1987) and vice-versa, with most plants responding positively (e.g., producing more biomass) to the presence of particular earthworm species (Brown *et al.*, 1999; chap. 2; Brown, 2000b; chap.1). The identification of the mechanisms for both these effects is a complex task (many mechanisms are still poorly understood) involving highly interactive and probably reciprocal and complementary phenomena within the soil-plant-animal system. Chemostimulants or other attractants, specific microbial communities, soil conditions, rhizodeposited resources and other variables all interact below-ground to produce a particular plant and/or earthworm response. To the author's knowledge few (if any, besides empirical) results are available concerning a possible earthworm attraction to specific roots, and the mechanism(s) involved.

In experiment 1, the higher $\delta^{13}C$ and $\delta^{15}N$ values in *P. corethrurus* tissues in the presence of maize gives evidence of earthworm activity in this plant's rhizosphere since rhizodeposited resources do not normally travel great distances within the soil and are quickly utilized or immobilized by (mostly micro-) rhizobiota or fixed in soil particles (Grayston *et al.*, 1996; Jones, 1998). When these results are plotted using $\delta^{13}C$ and $\delta^{15}N$ values of the different plant, soil and earthworm components, the displacement of earthworm (and soil) isotope values in the presence of maize plants is evident (Fig. 6). Thus, maize plants in the present experiment were not only acting as important N-sinks, taking up residue-derived $^{15}N$ (Brown *et al.*, 2000b; chap. 4), but sources as well, with "leaky" cells or high root turnover rhizodepositing N assimilable by the earthworms. Much of this N was apparently coming in a facilitated transfer from the residues via the plants. The Ndfr of earthworms in the presence of maize (1%) was about 8 times lower than the fraction of C derived from the maize (8%). This is to be expected considering the low residue biomass applied (1.4 Mg $ha^{-1}$; >40% remaining at harvest) compared with the root biomass at harvest (4.8 Mg $ha^{-1}$) (Brown *et al.*, 2000b; chap. 4), its placement (surface vs. below-ground) and the geophagous habits of *P. corethrurus*.

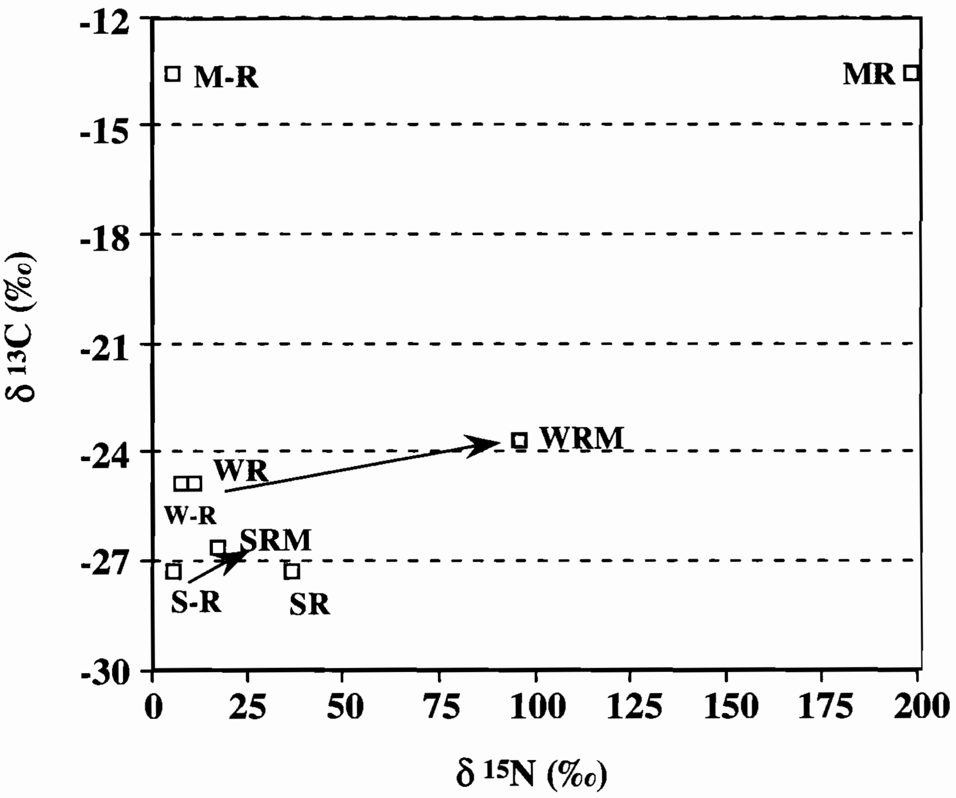

Figure 6. Earthworm (*P. corethrurus*) tissue, maize, and soil $\delta^{15}N$ and $\delta^{13}C$ changes after six months of maize grown in a tropical rainforest soil, in the presence and absence of $^{15}N$-labeled maize residues. M = maize (roots), -R = no residues, R = + residues, W = earthworms, S = soil. Arrows show the effect of residues and maize presence on $\delta^{15}N$ and $\delta^{13}C$ increase in soil and earthworms.

In experiment 1, *P. corethrurus* were also assimilating the newer forms of rhizodeposited C ($C_4$-C) derived from the maize plant roots. This new C was assimilated by almost 8% after six months. Although this implies that *P. corethrurus* derives some of its dietary needs from new plant-C and is therefore feeding in the rhizodeposition zone, this value was not different than the amount of maize-C found in the bulk soil-C. Furthermore, the fraction of earthworm-C derived from maize, calculated using the estimated value of $\delta_2$ = -9.9‰, could have been lower if the real values for earthworms under long term maize (sites non-existant) were discovered to be lower (e.g., $\delta^{13}C$ = -10 to -11‰). Similarly, any difference betweeen the real and the estimated values of the soil $\delta_2$ (-12.8‰) would also result in changes in the calculated F values for the amount of soil-C derived from the maize. Thus, using estimated $\delta_2$ values can lead to important errors depending on the deviation from the true value of $\delta_2$. Three other factors could also be important in explaining the lack of difference between the F values for earthworm tissue and soil OM:

(1) The poor survival and weight loss of *P. corethrurus* in the no residue treatments used for the $\delta^{13}C$ analyses indicates sub-optimal feeding activity. Laboratory cultures of this species under ideal moisture and temperature conditions showed that this soil is a good growth

substrate (Hernández et al., unp. data; Annex 10) but in the present experiment, the high ambient and soil temperatures and possibly competition with the plants for $H_2O$ (greater without residues), decreased their population almost 90%. If the earthworms had been feeding and growing more actively, a greater increase in the tissue $\delta^{13}C$ contents would probably have been observed.

(2) High native C resources in soils (particularly coarse fractions) such as in the forest Andosol used for this experiment (5.8% C), can feed earthworms for several years before being rarified (Villenave *et al.*, 1999). If these sources are preferred by the earthworms, only when they become scarcer will the earthworms shift to feeding on new C sources. If, for example, a C-poor forest soil were used, if some of the coarse OM were removed, or if it were previously cultivated for a few years with $C_3$ plants before switching to $C_4$ plants to exhaust some of the native soil C (but maintain the same $C_3$ soil C signatures), perhaps the switch over to new $C_4$-C would be quicker resulting in a more rapid increase in tissue $\delta^{13}C$ contents.

(3) The quality, digestibility and assimilability of the soil OM vs. the maize derived C were not compared in this experiment although important differences between the two are likely. These might have induced a particular (unmeasured) response in the earthworm tissue $\delta^{13}C$. The similarity of the F values for soil and earthworms in this experiment could indicate the lack of preferential assimilation of new (maize) $C_4$-C by the earthworms. If they had been preferentially assimilating maize derived-C, F would be much higher in earthworm tissues than bulk soil.

Contrary to what was observed above with maize, no difference in the $\delta^{13}C$ signatures due to bean presence could be detected for both earthworm species involved in experiment 2. Several reasons may account for these phenomena:

(a) the time lapse with the beans could have been insufficient to detect changes;
(b) the beans produced less roots (observed) and rhizodeposition (not measured) than maize;
(c) conditions for the earthworms were not ideal for their feeding activities and they both (especially *P. elongata*), lost considerable weight;
(d) *P. elongata* are not preferential bean rhizosphere or rhizobiota feeders;
(e) *P. corethrurus* prefer feeding in and around maize than bean rhizospheres.

The significant decreases in tissue $\delta^{13}C$ observed in *P. corethrurus*+beans compared to inoculated earthworms were probably due to digestion and assimilation of the soil OM (and not root derived-C) lower in $^{13}C$ (Annexe 2).

As with experiment 2a, the results from experiment 3 also seem to imply that *P. elongata* were not very active in the maize rhizosphere and obtained their food mostly from non-rhizosphere soil OM (especially the unlabeled fractions). The large loss in biomass of this species, however, may also mean that they were feeding (and assimilating) very little over the 45 d period. Nonetheless, the absence of measurable rhizosphere (in both beans and maize) activities of this species is further supported by studies on in-vitro gut tissue cultures that show it is able to digest much of its own food (i.e., has a well developed enzymatic apparatus) from intermediate quality soil OM sources (Lattaud *et al.*, 1998). Thus, this earthworm may not need to seek out easily assimilable C from the rhizosphere as actively as *P. corethrurus*, that has a poor enzymatic apparatus and also seems to depend more on an intimate mutualistic relationship with the gut microflora to cover its metabolic energy requirements (Lattaud *et al.*, 1998).

The importance of earthworm interactions with VAM was explored by Brown *et al.* (2000d; chap. 6) and in experiment 2b. Since there were no comparison treatments without plants and the microbial $^{15}N$ pools were not measured, the exact source of *P. corethrurus* assimilated $^{15}N$ could not be pinpointed. However, considering that:

a) *P. corethrurus* probably has a rapid turnover of $^{15}N$ in their tissues (Barois *et al.*, 1987);

b) 40-80% of the applied $^{15}N$ fertilizer remained in the soil;

c) earthworms are unable to assimilate inorganic soil N ($NH_4$, $NO_3$) forms directly (unlike plants and microorganisms),

the higher $\delta^{15}N$ signatures of the earthworms in contrast to those in soil (and their closeness to those of plant roots) seems to imply that the N assimilated by the earthworms came mostly from plant-recycled N and possibly some microbial immobilized N.

These results seem to point to an important and much neglected role of roots and rhizodeposited resources in the nutrition of some earthworm species, exemplified in this case by *P. corethrurus*. This widely distributed (pantropical) and highly adaptable species has relatively high assimilation efficiencies (around 19% for C when feeding on soil only; little is known of the N assimilation efficiencies) (Lavelle *et al.*, 1987) primarily due to an efficient mutualistic digestion system with the ingested microflora (Barois and Lavelle, 1986; Lavelle *et al.*, 1995). Furthermore, this species has also been shown to selectively ingest smaller size soil particles (Barois *et al.*, 1993; 1999) rich in clay and OM and feed on the microbial biomass, ingesting and probably digesting (to a certain extent) both fungi (including VAM) and protozoa (Barois, 1987; González, 1990; Reddell and Spain, 1991). All these factors may be of adaptive value to *P. corethrurus*, aiding in the colonization of and biomass sustenance in C and N-poor soil environments.

The relative importance of different diet components of *P. corethrurus* is unknown. However, since micro-organisms represent only a small fraction (< 3-5%) of the total soil C and N and would probably be insufficient to supply enough of the earthworm's metabolic energy needs (unless feeding in the rhizosphere, where these organisms are more abundant), we can hypothesize that *P. corethrurus* feeds mostly on soil OM, especially relatively fresh C derived from the rhizosphere or plant residues (e.g., leached-C and N, sloughed off cells, root exudates, lysates or mucilage), although the microbial biomass (higher in the rhizosphere than bulk soil) may complement their diets (Brown *et al.*, 2000e; Doube and Brown, 1998; Spain *et al.*, 1990; Spain and Le Feuvre, 1997).

Thus, the presence and persistence of endogeic earthworms, particularly some of the widespread peregrine poly- and meso-humic geophagous species such as *P. corethrurus* and others (e.g., many of the *Amynthas* and Pheretimoid groups, *Dichogaster* spp., *Ocnerodrilus occidentalis*; Fragoso *et al.*, 1999b) in many C- and N-poor soils (particularly in agroecosystems) may be due to their ability to utilize root-derived C and N, over soil C and N. Further investigations of this nature should be undertaken to confirm this hypothesis, as well as the preference of particular rhizospheres by different earthworm species.

The questions -how active are earthworms in plant rhizospheres?- and -what rhizospheres are preferred?- still remain unanswered. We know that the answers depend on the species of earthworm and the plant in question, and possibly even on the microbial community present and soil type (especially its C- and N-richness). Further investigations could combine a range of earthworm and plant species and soil types to determine preference of particular rhizospheres by different earthworm species or ecological categories. These experiments should attempt to relate the results to plant performance and rhizosphere processes, particularly changes in the rhizosphere microbial communities, where interactions with earthworms may be important (Brown *et al.*, 2000e). Sampling of earthworm mucus $^{13}C$ and $^{15}N$ could be used as a non-destructive and rapid method of detecting assimilated rhizodeposited resources (Schmidt *et al.*, 1999). These C and N stable isotopes, here confirmed to be highly useful tools for detecting rhizosphere-derived C and N assimilated by earthworms, could also be combined with radio-isotopes or other technologies (detection of different plant sugars or amino acids, bacterial probes and other bio-markers) to further differentiate sources of tissue C and N, and their relationship to the type of soil, plant or microbial community present. The results obtained would permit a much better estimation of the potential functional implications of earthworm activities in plant rhizospheres.

# CONCLUSIONS DE LA DEUXIÈME PARTIE

Les expériences en pots confirment l'influence importante des vers endogés *P. corethrurus* et *P. elongata* sur les propriétés et processus du sol et sur la production des plantes. Les deux espèces induisent une augmentation de la densité racinaire des haricots, et sont associées, à la dernière récolte, aux plus hautes biomasses des racines et des parties aériennes. Les différences observées dans la biomasse des plantes entre les traitements avec et sans vers augmentent avec le temps, montrant que les effets des vers sur les sols et les plantes sont cumulatifs. Par contre, la biomasse des cosses et des grains n'est pas significativement affectée. Apparemment, dans cette expérience, les vers de terre stimulent plus la production des parties non-reproductives de la plante que celle des parties reproductives. Les raisons n'en sont pas connues, mais peuvent être liées à des effets hormonaux; cette hypothèse nécessite cependant confirmation. Les mycorhizes peuvent être responsables des augmentations de biomasse observées mais la colonisation des racines n'a pas été mesuré dans cette expérience. Aucun effet des vers sur la nodulation racinaire n'a été observé, même dans le cas de l'application d'une solution inoculatrice de *Rhizobium.* Il est difficile de créer des nodulations sur les racines de haricots et aucune espèce présentant des nodules (rhizobia) n'a été trouvée dans le sol utilisé. En Australie, Doube *et al.* (1994a) et Stephens *et al.* (1994f) ont observé des effets significatifs du ver *A. trapezoides* sur la nodulation du trèfle et alfalfa cultivé en pots. On sait par ailleurs que les vers de terre peuvent être un facteur de dissémination de *Rhizobium* dans le sol (Doube *et al.*, 1994b; Madsen et Alexander, 1982; Rouelle, 1983). Cependant, en région tropicale, peu d'informations sont disponibles concernant le rôle des vers dans la propagation de rhizobia et la nodulation des racines. Des recherches sur ce thème doivent être développées, particulièrement au champ.

Le rendement du maïs cultivé dans un sol de forêt riche en matière organique n'est pas plus élevé en présence de *P. corethrurus,* malgré une augmentation de la densité racinaire dans les traitements avec ou sans application de résidus de récolte. Cette observation confirme d'autres résultats obtenus dans des essais au champ avec cette plante au Mexique où en seulement une occasion (cinquième cicle du cultif à La Mancha) des effets significatifs de ce ver sur le rendement ont été observé (Patrón *et al*, données non publiées; Patrón, 1993). L'investissement plus important dans les racines s'est peut-être fait au détriment des parties aériennes. Nous avons également pu constater que les effets des vers tendent à diminuer lorsque le contenu en matière organique augmente (chap. 2) et lorsque la fertilité du sol est élevée (chap. 1). *P. corethrurus* est connu pour favoriser de façon significative la production du maïs dans un sol de forêt tropicale au Pérou; l'augmentation de la production de grains à la première récolte s'échelonne de 31 %, dans le cas d'une culture avec application des résidus de la récolte précédente et engrais vert, à 75 %, lorsque aucun apport organique n'est effectué (Charpentier, 1996; Pashanasi *et al.*, 1996). Les raisons de la diminution de production observée dans la présente étude ne sont pas précisément établies. Cependant, le développement de parasites, la faible augmentation du contenu en nutriments dans les turricules, si on la compare à ce qui peut être observé pour d'autres sols considérés comme moins fertiles, et la plus grande indépendance des plantes vis à vis des éléments nutritifs pourraient être responsables de cette diminution. La différence de colonisation des racines par les mycorhizes n'est pas significative dans les traitements avec ou sans vers (sans doute en raison du faible nombre d'observations). L'effet des mycorhizes, s'il est insensible à la présence des vers de terre, peut avoir masqué leur effet sur le rendement.

L'augmentation de la densité racinaire observée pour le maïs et le haricot, ainsi que l'homogénéisation de la distribution des racines à l'intérieur des pots, indiquent la capacité éventuelle du ver à augmenter le prélèvement des nutriments et la résistance au stress de la plante. La densité racinaire est généralement un bon indicateur de la biomasse des racines, mais elle ne semble pas être reliée à la biomasse des parties aériennes et à la production de grains. Il est ainsi important de noter que des changements dans les caractéristiques du système racinaire ne conduisent pas nécessairement à une augmentation du rendement des grains òu la biomasse aeriènne.

Dans l'expérience combinant mycorhizes et *B. decumbens*, l'activité de *P. corethrurus* est liée au taux de colonisation par les VAM qui varie suivant le traitement fertilisant et la profondeur racinaire. Dans les traitements sans engrais, l'effet des vers de terre sur le rendement des parties aériennes est plus important, ce qui confirme leur rôle dans l'augmentation de la production des plantes dans des sols pauvres en nutriments. Au travers de la forte interaction observée entre les vers et les VAM, les interactions vers-mycorhizes (ou vers-autres organismes symbiotiques) apparaissent comme étant des mécanismes majeurs intervenant dans la croissance et la production de la plante, et dans le prélèvement des éléments nutritifs; ces aspects mériteront une attention particulière dans les recherches futures. Les résultats suggèrent également une combinaison des effets observés dans des expériences étudiant les effets sur le rendement où ces deux organismes étaient testés indépendamment.

Certaines caractéristiques chimiques, telles que la disponibilité de l'azote, sont stimulées par l'activité excrétoire des deux espèces de vers. Ceci a pu être observé pour un alfisol pauvre en nutriments utilisé dans des essais de cultures de haricots, et pour l'Andosol utilisé dans des essais de maïs. Cependant, dans les compartiments du sol du dispositif expérimental, très peu de différences significatives ont été observées entre les pots témoins et les traitements avec vers. Un prélèvement accru par la plante peut avoir compensé une augmentation de la disponibilité en azote, offrant ainsi un résultat apparemment nul.

L'étude menée à l'aide d'isotopes stables sur les interactions vers-rhizosphère confirme l'importance de la rhizodéposition pour la nutrition de *P. corethrurus*. Ecologiquement, cela signifie que cette espèce, et d'autres endogées polyhumiques, peut rechercher des ressources supplémentaires dans la rhizosphère des plantes, et peut même concentrer ses activités de recherche de nourriture dans cette zone, particulièrement dans les sols pauvres en nutriments et en matière organique. Les recherches futures devraient explorer cette possible relation synergique, en étudiant, par exemple, l'importance des nutriments issus des racines dans la nutrition des vers (et l'amélioration de la valeur adaptative que cela leur procure), et le rôle des vers sur la croissance, la biomasse et la distribution spatiale des racines et sur la production des parties aériennes. *P. elongata* n'est apparemment pas un mangeur de racines (pas de C ou de N issus des racines n'a été retrouvé dans ses tissus); ce ver tire son alimentation de la matière organique du sol grâce à son système de digestion mutualiste (association avec la microflore de son intestin) et à son éventail enzymatique très large. D'autres recherches devront être menées pour confirmer cette hypothèse dans le cadre de combinaisons cultures-sols différentes.

Les expérience en pots ont permis d'illustrer les interconnexions, voire la dépendance des effets des vers sur la production des plantes avec le type de sol (et spécialement sa fertilité), l'espèce de vers de terre (en prenant en compte sa stratégie écologique), la présence ou l'absence d'organismes symbiotiques (VAM) et le type de plantes (type de racines, de croissance et cycle de vie).

# CONCLUSIONS GÉNÉRALES

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De nombreux travaux ont montré les effets positifs des vers de terre sur la croissance des plantes (>100 publications) sans que l'on puisse dire que les vers de terre jouent toujours un rôle important dans la production des plantes. Au vu des résultats on peut cependant repondre à la très ancienne question "les vers améliorent-ils la fertilité du sol ou se développent-ils simplement dans les sols fertiles (e.g., Linden *et al.*, 1994; Satchell, 1983) par la première affirmation. Dans les écosystèmes naturels, leur contribution souvent masquée devient apparente lors des changements entrainent une augmentation ou une diminution de leur abondance et/ou de leur biomasse. Malgré le poids des facteurs limitants de nature purement édaphique, dans les écosystèmes naturels et cultivés, l'équilibre atteint par l'evolution à optimisé les interactions entre la faune du sol et la production des plantes. Il est toutefois difficile de mettre en évidence les relations intrinsèques liant l'activité des vers et la production des plantes dans des écosystèmes naturels sans en perturber le fonctionnement. Les résultats de plusieurs essais au champ semblent indiquer une possible synergie entre les vers de terre et les plantes (Brown *et al.*, unp. data; Brown *et al.*, 1999; chap. 2); cette relation pourrait cependant être associée à des facteurs environnementaux (e.g., précipitations) plus qu'à la présence d'un des deux partenaires. D'autres recherches sont nécessaires pour confirmer l'importance de cette relation et mieux cibler les facteurs qui la contrôlent.

Les vers de terre sont des membres importants du réseau trophique du sol. Ce sont aussi des fournisseurs de "services écosystémiques" pour l'Homme; ils facilitent la décomposition de la matière organique, influencent le cycle des nutriments, participent activement à la bioturbation et limitent le développement de maladies et de ravageurs pouvant se développer dans le sol (Brussaard *et al.*, 1997). Dans les agrosystèmes, la réponse des plantes est souvent considérée comme l'ultime marque des "services" rendus par les vers. Ils modifient également l'état physique du sol (via leurs constructions) affectant ainsi sa stabilité et sa structure, et la disponibilité des ressources offertes aux autres organismes, animaux ou végétaux (Jones *et al.*, 1994; Lavelle *et al.*, 1997).

De nombreuses expériences (>150) ont préciséles effets des vers de terre sur la production des plantes (chap. 1 et 2) et globalement justifié la classement des vers comme "organismes bénéfiques" du sol (Boucher, 1990; Macgregor, 1994). Il existe cependant des cas où leurs activités conduisent à une baisse de la production des plantes (chap. 1 et 2). En de rares occasions, les vers peuvent causer des dommages directs aux plantes; dans d'autres cas, les dommages peuvent provenir d'un "accident de biodiversité" (Lavelle, comm. pers.). Ces accidents surviennent lorsqu'un groupe fonctionnel est fortement dominant dans le sol (par exemple, une espèce compactante telle que *P. corethrurus*) et que les autres groupes fonctionnels sont peu présents voire absents; le fonctionnement du sol est alors fortement affecté (notamment dans ses caractéristiques physiques) et les performances des plantes sont diminuées (Barros, 1998; Chauvel *et al.*, 1999; Puttarudriah et Sastry, 1961). Ces accidents semblent être liés aux activités anthropiques qui modifient la structure originelle de la faune du sol et aux modes d'utilisation ou de gestion des systèmes et des sols (et plus particulièrement des sols argileux) qui favorise ça dégradation lorsque les espèces régénératrices de la structure grumeuse du sol sont absentes ou inactives (Lal, 1991).

Bien que les vers de terre puissent affecter directement la croissance des plantes dans certaines circonstances, la plupart des mécanismes responsables de la réponse des plantes sont indirects. Ainsi les vers engendrent des modifications des caractéristiques physiques, chimiques et biologiques du sol: minéralisation accrue de la matière organique, meilleure disponibilité des nutriments, construction de galeries profondes profitant à l'expansion des racines au drainage et à l'aération du sol, changements dans la viabilité des semences et interactions avec les organismes pathogènes ou symbiotiques des plantes. En présence de facteurs physiques limitants, la bioturbation peut significativement stimuler la croissance de la plante. Lorsque les limitations sont nutritionnelles, les effets chimiques sur le sol et les interactions avec les organismes symbiotiques de la plante (particulièrement, les mycorrhizes) deviennent prédominants. Et lorsque les organismes nuisibles ou les parasites limitent la croissance de la plante, ce sont les interactions biologiques et le contrôle biologique qui deviennent prépondérantes. Le travail expérimental réalisé dans cette thèse (chap. 3-7) confirme le rôle régulateur des vers de terre dans la libération des nutriments et leur transfert depuis les résidus. Il confirme également l'importance des interactions avec les mycorhizes, la dépendance entre la biomasse des vers et la réponse des plantes en terme de rendement et la compétition entre les vers et les plantes pour l'eau. Ces expériences mettent également en évidence l'importance de la fertilité initiale du sol, la liaison étroite entre la réponse de la plante, les espèces de ver et de plantes utilisées et le type de sol ainsi que la composante spatio-temporelle des associations ver-plante.

Certains mécanismes expliquant l’effet des vers de terre sur la croissance de la plante sont proposés dans le cadre de ce travail (chap. 1). D'autres mécanismes, bien que déjà validés, nécessitent une étude plus poussée. C’est par exemple le cas pour le rôle des hormones ou autres substances actives dans la réponse des plantes, la contribution directe des vers à la nutrition des plantes (particulièrement pour l'azote), l'interaction avec le populations bactériennes aux environs de la racine (rhizobactéries), de microbes pathogènes pour la plante ou de nématodes parasites. Il ne faut pas oublier non plus les exemples d’effets négatifs de l'activité des vers sur les processus et propriétés du sol ainsi que le phénomène de rhizophagie.

Du fait que racines régulent la croissance de la plante (Aiken et Smucker, 1996) et qu’elles partagent avec les vers de terre le même environnement, on peut supposer qu'il existe une interaction importante entre racines et vers et que cette interaction se répercute sur la biomasse et le développement de chacun. Si cette interaction est positive (synergique), les deux partenaires en tireront un bénéfice mutuel. Cependant, il semble qu'ils entrent le plus souvent en compétition pour l'espace et les ressources, l'un et l'autre tirant tour à tour profit de la situation. Il existe probablement des seuils propres à chaque association plante-sol-ver en dessous ou au dessus desquels les effets positifs ou négatifs de l'un sur l'autre prédominent (chap. 1 et 2).

Dans les expériences en pots (chap. 3-7), *P. elongata* augmente la biomasse des racines de haricots alors que avec *P. corethrurus* l'augmentation n'est pas statistiquement significative malgré une valeur plus importante. Avec le maïs, aucune différence significative dans la valeur de la biomasse racinaire n'est observée entre les différents traitements. Pour *B. decumbens*, un effet positif significatif des vers est observé uniquement dans le cadre du traitement NPK+VAM. Dans l'expérience de Patrón *et al.* (1999), pour un niveau de fertilisation en P de 10 kg ha$^{-1}$, la densité et la biomasse des racines de *B. decumbens* sont plus faibles en présence de *P. corethrurus* , sans doute à la suite de problèmes d'alimentation en eau.

D'un autre côté, la densité des racines horizontales est significativement plus élevée en présence des deux espèces de vers dans la culture de haricots, et aux deux dates de mesure pour le maïs en présence de *P. corethrurus* . La densité des racines aux profiles verticales de ces deux plantes est moins affectée par les activités des vers de terre. La distribution des racines aux profiles horizontales tend à être plus homogène; cette répartition peut augmenter la résistance de la plante au stress (Smucker, 1993). La relation entre la biomasse et la densité des racines est généralement positive pour les trois espèces de plante. Une relation positive entre densité racinaire et biomasse des parties aériennes n'est observée que pour le haricot et pour *B. decumbens*. Pour le maïs, ce qui est bon pour les racines ne l'est pas forcément pour les tiges. En effet, malgré un équilibre fonctionnel entre racine et tige, les facteurs de contrôle de la production ne sont pas les mêmes pour les racines que pour les parties aériennes (Brown et Scott, 1984). Il ressort de ces études, que la biomasse des racines est généralement moins affectée que celle des parties aériennes, et que la densité et la distribution des racines dans le sol peuvent être plus affectée que leur biomasse par les activités des vers de terre.

L'exploration de la relation racine-ver par le biais des isotopes stables montre l'importance des racines de maïs dans la nutrition des vers en dépit de la compétition possible pour l'eau du sol. *P. corethrurus* dérive des racines de maïs 8% du carbone de ses tissus, et en présence de la plante, le $^{15}N$ des résidus de surface est jusqu'à 25 fois plus assimilé par le ver que lorsque la plante est absente. Ainsi, les plantes constituent un lien important entre l'azote des résidus et l'azote assimilé par les vers de terre. Inversement, bien qu'ils n'augmentent pas significativement le prélèvement de $^{15}N$ par la plante, les vers par leurs activités augmentent le transfert d'azote des résidus vers le sol. En présence de *B. decumbens*, *P. corethrurus* semble également prélever plus de $^{15}N$, sans doute du fait de l'activité rhizosphérique, et en particulier par consommation et assimilation de ressources proches de la racine ou d'exsudats racinaires. D'un autre côté, en présence de haricots ou de maïs fertilisé ($^{15}N$), *P. elongata* ne montre aucune augmentation mesurable des contenus en $^{13}C$ et $^{15}N$ dans ses tissus. La spécificité de la réponse des vers de terre aux ressources issues des racines dépend probablement de la fertilité initiale du sol (particulièrement de la disponibilité des ressources nutritives), de l'auto-suffisance et de l'efficacité de son système digestif et de ses habitudes nutritives (dictées par l'appartenance à une espèce et à une catégorie écologique).

L'expérience avec les Mycorhizes Vesiculaires Arbusculaires (VAM)(chap. 6) met en valeur l'importante interaction ver-mycorhize qui pourrait être responsable de beaucoup des effets précédemment observés avec des plantes mycorhizées. Il sera indispensable dans les expériences futures de contrôler ce paramètre afin de mieux cerner les mécanismes responsables des réponses observées. Pour les Légumineuses, la nodulation et le taux de fixation de $N_2$ devra également être estimé afin de confirmer les effets positifs de l'activité des vers sur la plante et l'organisme symbiotique. La dispersion de *Rhizobia* induite par les activités du ver peut profiter aux plantes ayant des difficultés à noduler. Pour les plantes infectées, les effets indirects du ver sur la nodulation, créés par bioturbation ou altération des communautés microbiennes (par exemple, promotion d'espèces rhizobiales infestantes ou symbiotiques, mycorhization), peuvent être importants. Ainsi, plusieurs auteurs ont constaté un plus grand pourcentage de trèfle dans les pâturages en présence de vers (Sears et Evans, 1953; Stebler *et al.*, 1904; Thompson *et al.*, 1993); cette augmentation pourrait être due, entre autres facteurs, aux effets directs et/ou indirects des vers de terre sur l'efficacité de la symbiose rhizobienne (Satchell, 1958).

Cette thèse, en s'intéressant aux interactions ver-racine, a permis de progresser dans la compréhension des mécanismes responsables des réponses des plantes à l'activité des vers de terre. La prochaine étape sera la mise en place de modèles capables de combiner l'information concernant les facteurs limitants du sol et les connaissances écologiques, édaphiques et agronomiques disponibles sur la capacité des vers à améliorer ces facteurs. C'est une étape nécessaire pour prédire efficacement les effets de différentes espèces de vers de terre sur la croissance et la production de différents espèces de plantes selon le type de sol. La gestion de l'activité des vers de terre dans les agrosystèmes requiert un tel outil.

L'équilibre naturel établi entre la faune du sol et la végétation est, dans les agrosystèmes, désorganisé par les pratiques culturales, telles que le labour, la fertilisation, le chaulage et l'applications de pesticides. La perte ou l'amoindrissement des activités de la faune conduisent souvent à une détérioration de la fertilité du sol sur le long terme (particulièrement dans le cas de cultures continues), ce qui augmente la nécessité d' apports extérieurs pour maintenir ou restaurer la productivité. De fait, la dégradation du sol (spécialement sous les tropiques) semble liée à une diminution des activités et de la diversité de la faune du sol (Lal, 1991). Le "second paradigme" de Sanchez (1994) (voir l'introduction générale et l'article de Swift, 1999) et plus particulièrement les conclusions du programme MACROFAUNA (Lavelle *et al.*, 1999) encouragente une gestion du sol biologiquement intégrée, basée sur l'utilisation de la faune du sol, directement ou indirectement, pour maintenir la stabilité et la productivité des agrosystèmes. Les efforts pour compléter ou restaurer la composante "Ver" du sol ont cependant rencontré un succès mitigé, du fait, le plus souvent d'une connaissance incomplète de l'écologie des vers et des mécanismes responsables de la réponse des plantes (Blakemore, 1994).

Les manipulations directes (introduction) et indirectes (gestions des populations) requièrent des changements dans les pratiques culturales pour assurer la survie des vers de terre, qui ainsi peuvent jouer le rôle de moniteurs de la gestion du système (et plus particulièrement, de la fertilité du sol) (Baker, 1998; Buckerfield *et al.*, 1997; Oades et Walters, 1994). Il est, en particulier, essentiel de nourrir ces animaux par une gestion précise des apports organiques (Lavelle *et al.*, in press). A l'heure où une agriculture plus conservatrice (sans labour et avec des intrants organiques) tend à remplacer l'agriculture conventionnelle, où le bénéfice pouvant être tiré de l'équilibre des communautés au sein du sol est enfin perçu, où les problèmes de dégradation des sols au niveau international deviennent de plus en plus critiques, de telles manipulations deviennent plus intéressantes, économiquement et écologiquement.

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# ANNEXES

# ANNEXE 1

## CHAPITRE 2.

Table 1. Summary of all data collected during the Macrofauna Project and other data from the literature on the effects of earthworms on plant production. The earthworm and plant species studied, residues applied, size of plot or pot used and the response of plants (grain, shoot, root and total biomass and shoot:root ratios in presence and absence of earthworms) and earthworms (initial and final biomass, mass difference) are shown.

| Country | Location | Crop | Earthworm species | Residues (kg m$^{-2}$) | Plot size (m$^{2}$) | Grain yield (T ha$^{-1}$) | | | Shoot yield (T ha$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Contr | Worm | % Incr | Contr | Worm | % Incr |
| Ivory Coast | Lamto | Yam | *M. anomala* | 0.25 | 0.72 | | | | 0.72 | 0.96 | 33.79 |
| Ivory Coast | Lamto | Yam | *M. anomala* | 0 | 0.72 | | | | 0.47 | 0.58 | 24.18 |
| Ivory Coast | Lamto | Yam | *M. anomala* | 0.4 | 0.72 | | | | 0.27 | 0.35 | 30.21 |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0 | 1.28 | 3.52 | 3.45 | -2.00 | 6.62 | 5.94 | -10.27 |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0.25 | 1.28 | 3.40 | 3.35 | -1.38 | 6.71 | 6.84 | 1.94 |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0 | 1.28 | 1.09 | 1.16 | 5.71 | 1.95 | 2.04 | 4.62 |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0.63 | 1.28 | 1.28 | 1.03 | -19.51 | 2.25 | 2.07 | -8.00 |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0 | 1.28 | 1.70 | 1.80 | 5.99 | 3.14 | 3.38 | 7.64 |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0.31 | 1.28 | 1.67 | 1.98 | 18.22 | 3.03 | 3.09 | 1.98 |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0 | 1.28 | 1.65 | 1.26 | -23.70 | 3.20 | 2.98 | -6.88 |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0.35 | 1.28 | 1.51 | 1.81 | 20.21 | 3.25 | 3.40 | 4.62 |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0 | 1.28 | 1.23 | 1.30 | 5.70 | 3.09 | 3.84 | 24.27 |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0.5 | 1.28 | 0.94 | 1.24 | 32.50 | 2.87 | 2.91 | 1.39 |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0 | 1.28 | 0.61 | 0.74 | 21.79 | 1.63 | 1.77 | 8.59 |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0.34 | 1.28 | 0.55 | 0.66 | 21.43 | 1.41 | 1.64 | 16.31 |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0.16 | 0.72 | 3.02 | 3.57 | 18.23 | 3.67 | 3.44 | -6.27 |
| Mexico | La Mancha | Maize | *P. corethrurus* | 0 | 0.64 | 2.10 | 2.68 | 27.62 | 19 | 19 | 0.00 |
| Mexico | La Mancha | Maize | *P. corethrurus* | 1.12 | 0.64 | 2.23 | 2.45 | 9.87 | 11 | 12 | 9.09 |
| Mexico | La Mancha | Maize | *P. corethrurus* | 0 | 0.64 | | | | 7.8 | 8.2 | 5.13 |
| Mexico | La Mancha | Maize | *P. corethrurus* | 2 | 0.64 | | | | 9.5 | 9.2 | -3.16 |
| Mexico | La Mancha | Maize | *P. corethrurus* | 0 | 0.64 | | | | 2.1 | 2.2 | 4.76 |
| Mexico | La Mancha | Maize | *P. corethrurus* | 0.92 | 0.64 | | | | 3.1 | 3.3 | 6.45 |
| Mexico | La Mancha | Maize | *P. corethrurus* | 0 | 0.64 | 2.13 | 2.02 | -5.16 | 5.2 | 6.5 | 25.00 |
| Mexico | La Mancha | Maize | *P. corethrurus* | 0.33 | 0.64 | 2.00 | 2.05 | 2.50 | 5.7 | 7 | 22.81 |
| Mexico | La Mancha | Maize | *P. corethrurus* | 0 | 0.64 | 1.00 | 1.43 | 43.00 | 19 | 20 | 5.26 |
| Mexico | La Mancha | Maize | *P. corethrurus* | 1.2 | 0.64 | 1.35 | 1.51 | 11.85 | 14 | 12 | -14.29 |
| Mexico | La Mancha | Maize | *P. corethrurus* | 0 | 0.64 | 0.84 | 1.48 | 76.19 | 7 | 7.7 | 10.00 |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0 | 0.28 | 1.09 | 1.53 | 40.37 | 1.89 | 2.52 | 33.33 |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0.25 | 0.28 | 1.22 | 1.70 | 39.34 | 2.55 | 2.65 | 3.92 |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0.43 | 0.28 | 1.62 | 2.13 | 31.48 | 2.05 | 3.12 | 52.20 |
| Peru | Yurimaguas | Rice | *P. corethrurus* | 0 | 0.28 | 0.77 | 1.57 | 103.9 | 1.28 | 2.13 | 66.41 |
| Peru | Yurimaguas | Rice | *P. corethrurus* | 0.25 | 0.28 | 0.78 | 1.62 | 107.6 | 2.09 | 1.8 | -13.88 |
| Peru | Yurimaguas | Rice | *P. corethrurus* | 0.56 | 0.28 | 0.95 | 1.49 | 56.84 | 1.31 | 2.71 | 106.8 |
| Peru | Yurimaguas | Cowpea | *P. corethrurus* | 0 | 0.28 | 0.84 | 0.85 | 1.19 | 1.23 | 1.16 | -5.69 |
| Peru | Yurimaguas | Cowpea | *P. corethrurus* | 0.21 | 0.28 | 0.91 | 0.78 | -14.29 | 1.28 | 1.86 | 45.31 |
| Peru | Yurimaguas | Cowpea | *P. corethrurus* | 0.52 | 0.28 | 1.24 | 1.22 | -1.61 | 1.52 | 1.69 | 11.18 |
| Peru | Yurimaguas | Rice | *P. corethrurus* | 0 | 0.28 | 0.73 | 1.12 | 53.42 | 1.56 | 2.71 | 73.72 |
| Peru | Yurimaguas | Rice | *P. corethrurus* | 0.12 | 0.28 | 1.02 | 1.53 | 50.00 | 2.35 | 2.6 | 10.64 |
| Peru | Yurimaguas | Rice | *P. corethrurus* | 0.32 | 0.28 | 1.39 | 2.00 | 43.88 | 2.32 | 3.14 | 35.34 |
| Peru | Yurimaguas | Rice | *P. corethrurus* | 0 | 0.28 | 0.86 | 0.71 | -17.44 | 1.39 | 0.98 | -29.50 |
| Peru | Yurimaguas | Rice | *P. corethrurus* | 0.27 | 0.28 | 1.16 | 0.66 | -43.10 | 1.09 | 1.92 | 76.15 |
| Peru | Yurimaguas | Rice | *P. corethrurus* | 0.56 | 0.28 | 1.59 | 0.95 | -40.25 | 1.88 | 1.23 | -34.57 |
| Peru | Yurimaguas | Rice | *P. corethrurus* | 0 | 0.28 | 0.30 | 0.94 | 213.3 | 0.98 | 2.82 | 187.7 |
| Peru | Yurimaguas | Rice | *P. corethrurus* | 0.1 | 0.28 | 1.10 | 1.21 | 10 | 1.82 | 3.22 | 76.92 |
| Peru | Yurimaguas | Rice | *P. corethrurus* | 0.37 | 0.28 | 1.70 | 1.95 | 14.71 | 2.64 | 4.08 | 54.55 |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0.25 | 0.28 | 1.18 | 1.49 | 26.96 | 2.51 | 2.85 | 13.63 |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0.29 | 0.28 | 0.66 | 2.90 | 341.1 | 2.6 | 2.9 | 11.78 |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0.29 | 0.28 | 0.12 | 0.78 | 525.0 | 1.85 | 2.68 | 44.96 |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0.28 | 0.28 | 0.83 | 0.53 | -36.42 | 1.6 | 2.14 | 33.54 |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0.21 | 0.28 | 1.86 | 2.89 | 54.99 | 5.23 | 7.13 | 36.32 |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0.71 | 0.28 | 1.36 | 2.44 | 79.21 | 2.02 | 2.48 | 22.56 |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0.25 | 0.28 | 0 | 0.09 | . | 4.81 | 3.66 | -23.89 |

| Root yield (T ha$^{-1}$) | | | Shoot/Root ratio | | | Total yield (T ha$^{-1}$) | | | Earthworm mass (g m$^{-2}$) | | | Mass |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Contr | Worm | % Incr | Contr | Worm | % Incr | Contr | Worm | % Incr | Initial | Final | Surviv | Diff. |
| 27.08 | 31.81 | 17.44 | 0.03 | 0.03 | 13.92 | 27.80 | 32.76 | 17.86 | 25 | 10.18 | 40.72 | 5.67 |
| 31.67 | 30.56 | -3.51 | 0.01 | 0.02 | 28.69 | 32.13 | 31.13 | -3.11 | 28.5 | 4.5 | 15.79 | 4.27 |
| 3.61 | 5.97 | 65.38 | 0.07 | 0.06 | -21.27 | 3.88 | 6.32 | 62.97 | 30 | 2.58 | 8.60 | 2.7 |
| | | | | | | | | | 27 | 20.23 | 74.93 | 19.7 |
| | | | | | | | | | 27 | 21.58 | 79.93 | 21.5 |
| | | | | | | | | | 27 | 41.42 | 153.4 | 36 |
| | | | | | | | | | 27 | 10.05 | 37.22 | 5 |
| | | | | | | | | | 16 | 31.39 | 196.1 | 21 |
| | | | | | | | | | 16 | 25.93 | 162.0 | 21 |
| | | | | | | | | | 31.39 | 2.53 | 8.06 | 2 |
| | | | | | | | | | 25.93 | 2.04 | 7.87 | -5 |
| | | | | | | | | | 27 | 15 | 55.56 | -2 |
| | | | | | | | | | 27 | 9.21 | 34.11 | -2.5 |
| | | | | | | | | | 16 | 3.24 | 20.25 | 0.8 |
| | | | | | | | | | 16 | 2.26 | 14.13 | 2 |
| 0.26 | 0.25 | -5.26 | 13.91 | 13.76 | -1.06 | 6.95 | 7.26 | 4.40 | 52.1 | 8.4 | 16.12 | 8.5 |
| 0.79 | 0.67 | -15.59 | 24.08 | 28.53 | 18.47 | 21.89 | 22.35 | 2.09 | 35.5 | 8.12 | 22.87 | 7.86 |
| 0.77 | 0.73 | -4.68 | 14.30 | 16.37 | 14.45 | 14.00 | 15.18 | 8.46 | 35.5 | 23.7 | 66.76 | 21.3 |
| 0.54 | 0.60 | 11.33 | 14.50 | 13.69 | -5.58 | 8.34 | 8.80 | 5.53 | 35.5 | 11.5 | 32.39 | 7.71 |
| 0.70 | 0.73 | 3.27 | 13.49 | 12.65 | -6.22 | 10.20 | 9.93 | -2.71 | 35.5 | 19.6 | 55.21 | 9.98 |
| 0.15 | 0.24 | 60.9 | 13.91 | 9.05 | -34.90 | 2.25 | 2.44 | 8.53 | 35.5 | 19.8 | 55.77 | 3.4 |
| 0.30 | 0.33 | 12.1 | 10.44 | 9.91 | -5.06 | 3.40 | 3.63 | 6.95 | 35.5 | 28.1 | 79.15 | -3.6 |
| 0.57 | 0.57 | 0.71 | 9.19 | 11.40 | 24.12 | 7.90 | 9.09 | 15.12 | 35.5 | 10.1 | 28.45 | 1.7 |
| 0.82 | 0.69 | -15.37 | 6.95 | 10.09 | 45.10 | 8.52 | 9.74 | 14.37 | 35.5 | 17 | 47.89 | -5.2 |
| 0.35 | 0.36 | 4.61 | 54.76 | 55.10 | 0.62 | 20.35 | 21.79 | 7.11 | 35.5 | 21.4 | 60.28 | -3.2 |
| 0.53 | 0.50 | -5.13 | 26.62 | 24.05 | -9.65 | 15.88 | 14.01 | -11.76 | 35.5 | 20.3 | 57.18 | -21.6 |
| 0.37 | 0.40 | 8.31 | 18.77 | 19.06 | 1.56 | 8.21 | 9.58 | 16.69 | 35.5 | 38.2 | 107.6 | 9.6 |
| 0.20 | 0.44 | 120.0 | 9.45 | 5.73 | -39.39 | 3.18 | 4.49 | 41.19 | 36 | 27.5 | 76.39 | 27.5 |
| 0.40 | 0.33 | -17.50 | 6.38 | 8.03 | 25.97 | 4.17 | 4.68 | 12.23 | 36 | 35.3 | 98.06 | 35.3 |
| 0.22 | 0.34 | 54.55 | 9.32 | 9.18 | -1.52 | 3.89 | 5.59 | 43.70 | 36 | 32.5 | 90.28 | 32.5 |
| 0.29 | 0.54 | 86.21 | 4.41 | 3.94 | -10.63 | 2.34 | 4.24 | 81.20 | 36 | 47.4 | 131.6 | 47.4 |
| 0.29 | 0.37 | 27.59 | 7.21 | 4.86 | -32.50 | 3.16 | 3.79 | 19.94 | 36 | 42.1 | 116.9 | 42.1 |
| 0.16 | 0.47 | 193.7 | 8.19 | 5.77 | -29.58 | 2.42 | 4.67 | 92.98 | 36 | 81.4 | 226.1 | 81.4 |
| 0.05 | 0.05 | 0.00 | 24.60 | 23.20 | -5.69 | 2.12 | 2.06 | -2.83 | 36 | 26 | 72.22 | 26 |
| 0.04 | 0.04 | 0.00 | 32.00 | 46.50 | 45.31 | 2.23 | 2.68 | 20.18 | 36 | 38.1 | 105.8 | 38.1 |
| 0.07 | 0.04 | -42.86 | 21.71 | 42.25 | 94.57 | 2.83 | 2.95 | 4.24 | 36 | 80.5 | 223.6 | 80.5 |
| 0.28 | 0.50 | 78.57 | 5.57 | 5.42 | -2.72 | 2.57 | 4.33 | 68.48 | 36 | 16.2 | 45 | 16.2 |
| 0.33 | 0.47 | 42.42 | 7.12 | 5.53 | -22.32 | 3.70 | 4.60 | 24.32 | 36 | 24.3 | 67.5 | 24.3 |
| 0.37 | 0.53 | 43.24 | 6.27 | 5.92 | -5.51 | 4.08 | 5.67 | 38.97 | 36 | 23.4 | 65 | 23.4 |
| 0.32 | 0.15 | -53.13 | 4.34 | 6.53 | 50.41 | 2.57 | 1.84 | -28.40 | 36 | 15.3 | 42.5 | 15.3 |
| 0.16 | 0.25 | 56.25 | 6.81 | 7.68 | 12.73 | 2.41 | 2.83 | 17.43 | 36 | 30.3 | 84.17 | 30.3 |
| 0.29 | 0.28 | -3.45 | 6.48 | 4.39 | -32.24 | 3.76 | 2.46 | -34.57 | 36 | 45.8 | 127.2 | 45.8 |
| 0.22 | 0.54 | 145.4 | 4.45 | 5.22 | 17.23 | 1.50 | 4.30 | 186.6 | 36 | 48.3 | 134.1 | 48.3 |
| 0.45 | 0.77 | 71.11 | 4.04 | 4.18 | 3.40 | 3.37 | 5.20 | 54.30 | 36 | 54.3 | 150.8 | 54.3 |
| 0.39 | 0.89 | 128.2 | 6.77 | 4.58 | -32.28 | 4.73 | 6.92 | 46.30 | 36 | 71.4 | 198.3 | 71.4 |
| | | | | | | | | | 70.7 | 35.3 | | |
| | | | | | | | | | 0 | 42.1 | | |
| | | | | | | | | | 0 | 38.1 | | |
| | | | | | | | | | 0 | 24.3 | | |
| | | | | | | | | | 0 | 30.3 | | |
| | | | | | | | | | 0 | 54.3 | | |
| | | | | | | | | | 0 | 35.3 | | |

Table 1. continuation

| | | | Earthworm | Residues | Plot size | Grain yield (T ha$^{-1}$) | | | Shoot yield (T ha$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Country | Location | Crop | species | (kg m$^{-2}$) | (m$^2$) | Contr | Worm | % Incr | Contr | Worm | % Incr |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0.37 | 0.28 | 2.36 | 2.80 | 18.80 | 4.62 | 3.81 | -17.51 |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0.38 | 0.28 | 2.37 | 1.94 | -18.07 | 4.83 | 3.94 | -18.48 |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0.39 | 0.28 | 1.43 | 0.93 | -34.71 | 5.85 | 5.3 | -9.48 |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0.59 | 0.28 | 1.87 | 2.96 | 58.17 | 10.47 | 9.46 | -9.6 |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0.95 | 0.28 | 0.62 | 0.63 | 1.46 | 6.37 | 5.57 | -12.55 |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0 | 1250 | 1.29 | 0.36 | -72.09 | | | |
| Peru | Yurimaguas | Maize | *P. corethrurus* | 0 | 1250 | 0.90 | 0.83 | -7.78 | | | |
| Peru | Yurimaguas | Cassava | *P. corethrurus* | 0 | 1250 | | | | | | |
| Peru | Yurimaguas | Rice | *P. corethrurus* | 0 | 1250 | 1.31 | 1.23 | -6.11 | | | |
| Peru | Yurimaguas | Cowpea | *P. corethrurus* | 0 | 1250 | 0.51 | 0.37 | -27.45 | | | |
| Peru | Yurimaguas | Rice | *P. corethrurus* | 0 | 1250 | 0.53 | 0.51 | -4.16 | | | |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 0 | 0.54 | | | | 1.02 | 2.67 | 161.3 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 21.75 | 0.54 | | | | 1.01 | 2.96 | 192.6 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 0 | 0.54 | | | | 1.43 | 4.21 | 195.3 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 21.75 | 0.54 | | | | 0.98 | 3.00 | 206.4 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 0 | 0.54 | | | | 0.97 | 2.84 | 192.9 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 21.75 | 0.54 | | | | 0.43 | 1.64 | 279.6 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 0 | 0.54 | | | | 0.68 | 2.14 | 215.2 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 21.75 | 0.54 | | | | 0.93 | 4.18 | 351.8 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 0 | 0.54 | | | | 1.12 | 3.70 | 231.4 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 21.75 | 0.54 | | | | 1.23 | 5.10 | 315.5 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 0 | 0.54 | | | | 1.00 | 2.34 | 135.1 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 21.75 | 0.54 | | | | 1.07 | 2.58 | 141.5 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 0 | 0.54 | | | | 1.36 | 4.43 | 226.1 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 21.75 | 0.54 | | | | 1.12 | 2.96 | 164.2 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 0 | 0.54 | | | | 1.09 | 3.14 | 188.2 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 21.75 | 0.54 | | | | 0.46 | 1.60 | 245.5 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 0 | 0.54 | | | | 0.58 | 2.07 | 255.4 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 21.75 | 0.54 | | | | 1.21 | 4.27 | 253.5 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 0 | 0.54 | | | | 1.22 | 3.09 | 152.4 |
| India | Sheikamuldi | Tea | *P. corethrurus et al.* | 21.75 | 0.54 | | | | 1.50 | 5.15 | 242.6 |
| India | Sambalpur | Rice | *D. willsii* | 0 | 4.65 | | | | 15.57 | 16.8 | 8.03 |
| India | Sambalpur | Rice | *D. willsii* | 2.15 | 4.65 | | | | 16.82 | 22.9 | 36.27 |
| India | Sambalpur | Rice | *D. willsii* | 0 | 4.65 | | | | 19.9 | 20.8 | 4.37 |
| India | Sambalpur | Rice | *D. willsii* | 2.15 | 4.65 | | | | 20.55 | 24.8 | 20.73 |
| Australia | Narayen | Grasses | *Diplotrema sp.nov. 1* | 0 | 0.5 | 1.08 | 1.49 | 39.00 | 3.16 | 5.75 | 81.91 |
| Australia | Narayen | Grasses | *P. corethrurus* | 0 | 0.5 | 1.40 | 1.50 | 6.59 | 3.16 | 4.46 | 40.99 |
| Australia | Narayen | Grasses | *A. trapezoides+ E. rosea* | 0 | 0.5 | 1.37 | 1.57 | 14.33 | 3.16 | 4.67 | 47.75 |
| Australia | Narayen | Grasses | *E. eugeniae* | 0 | 0.5 | 1.48 | 1.81 | 22.87 | 3.16 | 5.81 | 83.87 |
| Australia | Narayen | Grasses | *D. affinis + saliens* | 0 | 0.5 | | | | 3.16 | 4.55 | 43.90 |
| Australia | Narayen | Grasses | *D. barwelli + A. minimus* | 0 | 0.5 | | | | 3.16 | 5.96 | 88.43 |
| Australia | Samford | Grasses | *P. corethrurus* | 0 | 0.5 | | | | 5.99 | 8.89 | 48.25 |
| Australia | Samford | Grasses | *A. trapezoides + E. rosea* | 0 | 0.5 | | | | 5.99 | 6.69 | 11.68 |
| Australia | Samford | Grasses | *E. eugeniae* | 0 | 0.5 | | | | 5.99 | 6.44 | 7.41 |
| Australia | Samford | Grasses | *D. affinis+saliens* | 0 | 0.5 | | | | 5.99 | 6.03 | 0.6 |
| Australia | Samford | Grasses | *D. barwelli + A. minimus* | 0 | 0.5 | | | | 5.99 | 8.32 | 38.84 |
| Australia | Samford | Grasses | *A. rodericensis* | 0 | 0.5 | | | | 5.99 | 9.26 | 54.45 |
| Australia | Samford | Grasses | *P. taprobanae* | 0 | 0.5 | | | | 5.99 | 7.51 | 25.36 |
| Brazil | Guarapuava | Beans | *Amynthas sp.* | 0 | 1 | 1.01 | 1.07 | 5.93 | 2.05 | 1.81 | -11.83 |
| Brazil | Guarapuava | Beans | *Amynthas sp.* | 0 | 1 | 1.01 | 1.02 | 0.89 | 2.05 | 2.12 | 3.27 |
| Brazil | Guarapuava | Beans | *Amynthas sp.* | 0 | 1 | 1.01 | 1.10 | 8.70 | 2.05 | 2.04 | -0.23 |
| Brazil | Guarapuava | Wheat | *Amynthas sp.* | 0 | 1 | 1.44 | 1.48 | 2.78 | 3.63 | 4.02 | 10.94 |
| Brazil | Guarapuava | Wheat | *Amynthas sp.* | 0 | 1 | 1.44 | 1.49 | 3.61 | 3.63 | 3.85 | 6.19 |
| Brazil | Guarapuava | Wheat | *Amynthas sp.* | 0 | 1 | 1.44 | 1.58 | 9.38 | 3.63 | 4.25 | 17.28 |
| Brazil | Curitiba | *Mimosa scabrella* | *Amynthas sp.* | 2.23 | 2.70 | | | | 4.87 | 6.97 | 43.21 |
| Brazil | Curitiba | *M. scabrella* | *Amynthas sp.* | 2.23 | 2.70 | | | | 4.87 | 8.20 | 68.52 |
| Brazil | Curitiba | *M. scabrella* | *Amynthas sp.* | 2.23 | 2.70 | | | | 4.87 | 7.26 | 49.25 |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0 | 0.04 | | | | 0.16 | 0.65 | 309.5 |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0 | 0.04 | | | | 0.16 | 0.26 | 65.08 |

| Root yield (T ha$^{-1}$) | | | Shoot/Root ratio | | | Total yield (T ha$^{-1}$) | | | Earthworm mass (g m$^{-2}$) | | | Mass |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Contr | Worm | % Incr | Contr | Worm | % Incr | Contr | Worm | % Incr | Initial | Final | Surviv | Diff. |
| | | | | | | | | | 0 | 42.1 | | |
| | | | | | | | | | 0 | 38.1 | | |
| | | | | | | | | | 70.7 | 24.3 | | |
| | | | | | | | | | 70.7 | 30.3 | | |
| | | | | | | | | | 70.7 | 54.3 | | |
| | | | | | | | | | 1.31 | 1.88 | 143.5 | 3.75 |
| | | | | | | | | | 5.04 | 7.76 | 153.9 | 18.54 |
| 17.10 | 11.50 | -32.75 | | | | | | | 0.96 | 5.53 | 576.0 | 1.9 |
| | | | | | | | | | 3.84 | 7.34 | 191.1 | 7.14 |
| | | | | | | | | | 7.34 | 10.13 | 138.0 | 11.08 |
| | | | | | | | | | 3.84 | 4.3 | 111.9 | 3.23 |
| | | | | | | | | | 14 | | | |
| | | | | | | | | | 14 | | | |
| | | | | | | | | | 0 | | | |
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| | | | | | | | | | 0 | | | |
| | | | | | | | | | 0 | | | |
| | | | | | | | | | 648 | 18.82 | 2.9 | -9.26 |
| | | | | | | | | | 648 | 106.6 | 16.46 | 98.97 |
| | | | | | | | | | 12.9 | 71.25 | 552.3 | 71.25 |
| | | | | | | | | | 12.9 | 116.2 | 901.1 | 116.25 |
| | | | | | | | | | 12.9 | 90 | 697.6 | 90.00 |
| | | | | | | | | | 12.9 | 101.2 | 784.8 | 101.25 |
| | | | | | | | | | 8.00 | 0 | 0 | 0 |
| | | | | | | | | | 164.0 | 0.07 | 0.04 | 0.07 |
| | | | | | | | | | 165.8 | 3.30 | 1.99 | 3.20 |
| | | | | | | | | | | | | |
| | | | | | | | | | 140.0 | 0 | 0 | 0 |
| | | | | | | | | | 6.66 | 0 | 0 | 0 |
| | | | | | | | | | 24.00 | 0 | 0 | 0 |
| | | | | | | | | | | | | |
| | | | | | | | | | 164.0 | 2.90 | 1.77 | 0.70 |
| | | | | | | | | | 165.8 | 0.55 | 0.33 | -1.40 |
| | | | | | | | | | | | | |
| | | | | | | | | | 140.0 | 0.00 | 0.00 | -1.63 |
| | | | | | | | | | 6.66 | 0.00 | 0.00 | -2.05 |
| | | | | | | | | | 24.00 | 0.15 | 0.63 | -1.80 |
| | | | | | | | | | | | | |
| | | | | | | | | | 28.28 | 0.15 | 0.53 | -2.13 |
| | | | | | | | | | 136.0 | 27.57 | 20.27 | 25.33 |
| | | | | | | | | | 30.00 | | | |
| | | | | | | | | | 60 | | | |
| | | | | | | | | | 90.00 | | | |
| | | | | | | | | | 30.00 | 45.81 | 152.7 | 45.81 |
| | | | | | | | | | 60.00 | 61.33 | 102.2 | 61.33 |
| | | | | | | | | | 90.00 | 66.40 | 73.78 | 66.4 |
| | | | | | | | | | 30.00 | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | 60.00 | | | |
| | | | | | | | | | 90.00 | | | |
| 0.14 | 0.11 | -21.43 | 1.13 | 5.86 | 421.2 | 0.30 | 0.76 | 153.7 | 25 | 30 | 120 | 30 |
| 0.14 | 0.07 | -53.57 | 1.13 | 4.00 | 255.5 | 0.30 | 0.33 | 9.24 | 50 | 25 | 50 | 25 |

Table 1. continuation

| Country | Location | Crop | Earthworm species | Residues (kg m$^{-2}$) | Plot size (m$^2$) | Grain yield (T ha$^{-1}$) |  |  | Shoot yield (T ha$^{-1}$) |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  | Contr | Worm | % Incr | Contr | Worm | % Incr |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0 | 0.04 |  |  |  | 0.16 | 0.59 | 273.0 |
| Ivory Coast | Lamto | Maize | *P. corethrurus* | 0 | 0.04 |  |  |  | 0.16 | 0.34 | 112.7 |
| Ivory Coast | Lamto | Maize | *H. africanus* | 0 | 0.04 |  |  |  | 0.16 | 0.20 | 28.57 |
| Ivory Coast | Lamto | Maize | *S. porifera* + *C. zielae* | 0 | 0.04 |  |  |  | 0.16 | 0.30 | 92.06 |
| Ivory Coast | Lamto | *Panicum maximum* | *M. anomala* | 0 | 0.04 |  |  |  | 0.94 | 1.70 | 81.78 |
| Ivory Coast | Lamto | *P. maximum* | *M. anomala* | 0 | 0.04 |  |  |  | 0.94 | 2.36 | 151.7 |
| Ivory Coast | Lamto | *P. maximum* | *M. anomala* | 0 | 0.04 |  |  |  | 0.94 | 2.35 | 150.3 |
| Ivory Coast | Lamto | *P. maximum* | *M. anomala* | 0 | 0.04 |  |  |  | 0.94 | 3.25 | 247.0 |
| Ivory Coast | Lamto | *P. maximum* | *M. anomala* | 0 | 0.04 |  |  |  | 0.94 | 2.72 | 190.2 |
| Ivory Coast | Lamto | *P. maximum* | *M. anomala* | 0 | 0.04 |  |  |  | 6.66 | 10.08 | 51.41 |
| Ivory Coast | Lamto | *P. maximum* | *M. anomala* | 0 | 0.04 |  |  |  | 6.66 | 5.97 | -10.29 |
| Ivory Coast | Lamto | *P. maximum* | *S. porifera* + *C. zielae* | 0 | 0.04 |  |  |  | 0.94 | 1.75 | 86.23 |
| Ivory Coast | Lamto | *P. maximum* | *S. porifera* + *C. zielae* | 0 | 0.04 |  |  |  | 0.94 | 2.94 | 214.0 |
| Ivory Coast | Lamto | Rice | *S. porifera* + *C. zielae* | 0 | 0.053 | 1.24 | 1.09 | -11.99 | 1.55 | 1.66 | 7.10 |
| Ivory Coast | Lamto | Rice | *H africanus* | 0 | 0.053 | 1.24 | 1.28 | 3.19 | 1.55 | 1.42 | -8.39 |
| Ivory Coast | Lamto | Rice | *M. anomala* | 0 | 0.053 | 1.24 | 1.21 | -2.43 | 1.55 | 1.59 | 2.58 |
| Ivory Coast | Lamto | Rice | *M. anomala et al.* | 0 | 0.053 | 1.24 | 1.51 | 21.70 | 1.55 | 1.76 | 13.55 |
| Ivory Coast | Lamto | Peanuts | *S. porifera* + *C. zielae* | 0 | 0.053 | 2.32 | 1.68 | -27.72 | 2.92 | 3.04 | 4.11 |
| Ivory Coast | Lamto | Peanuts | *H. africanus* | 0 | 0.053 | 2.32 | 1.82 | -21.71 | 2.92 | 2.87 | -1.71 |
| Ivory Coast | Lamto | Peanuts | *M. anomala* | 0 | 0.053 | 2.32 | 1.73 | -25.45 | 2.92 | 3.18 | 8.90 |
| Ivory Coast | Lamto | Peanuts | *M. anomala et al.* | 0 | 0.053 | 2.32 | 2.18 | -6.26 | 2.92 | 3.02 | 3.42 |
| Ivory Coast | Lamto | Maize | *S. porifera* + *C. zielae* | 0 | 0.053 | 0.17 | 0.20 | 19.32 | 3.34 | 3.71 | 11.08 |
| Ivory Coast | Lamto | Maize | *H. africanus* | 0 | 0.053 | 0.17 | 0.26 | 54.55 | 3.34 | 3.65 | 9.28 |
| Ivory Coast | Lamto | Maize | *M. anomala* | 0 | 0.053 | 0.17 | 0.42 | 152.2 | 3.34 | 4.66 | 39.52 |
| Ivory Coast | Lamto | Maize | *M. anomala et al.* | 0 | 0.053 | 0.17 | 0.50 | 201.1 | 3.34 | 4.12 | 23.35 |
| Mexico | La Vibora | Beans | *P. corethrurus* | 0 | 0.009 |  |  |  | 0.17 | 0.19 | 8.28 |
| Mexico | La Vibora | Beans | *P. elongata* | 0 | 0.009 |  |  |  | 0.17 | 0.16 | -8.92 |
| Mexico | La Vibora | Beans | *P. corethrurus* | 0 | 0.064 |  |  |  | 0.14 | 0.19 | 33.33 |
| Mexico | La Vibora | Beans | *P. elongata* | 0 | 0.064 |  |  |  | 0.14 | 0.34 | 144.4 |
| Mexico | La Vibora | Beans | *P. corethrurus* | 0 | 0.064 | 0.06 | 0.07 | 25.97 | 0.08 | 0.17 | 112.5 |
| Mexico | La Vibora | Beans | *P. elongata* | 0 | 0.064 | 0.06 | 0.06 | 1.66 | 0.08 | 0.2 | 150.0 |
| Mexico | Los Tuxtlas | Maize | *P. corethrurus* | 0 | 0.064 | 2.58 | 3.16 | 22.42 | 15.84 | 14.19 | -10.42 |
| Mexico | Los Tuxtlas | Maize | *P. corethrurus* | 0.14 | 0.064 | 3.44 | 1.53 | -55.51 | 13.55 | 10.58 | -21.9 |
| Mexico | Los Tuxtlas | Maize | *P. corethrurus* | 0 | 0.009 |  |  |  | 0.94 | 0.81 | -14.61 |
| Mexico | La Vibora | *Brachiaria decumbens* | *P. corethrurus* | 0 | 0.064 |  |  |  | 0.24 | 0.2 | -16.18 |
| Mexico | La Vibora | *B. decumbens* | *P. corethrurus* | 0 | 0.064 |  |  |  | 0.59 | 0.57 | -4.4 |
| Mexico | La Vibora | *B. decumbens* | *P. corethrurus* | 0 | 0.064 |  |  |  | 2.91 | 2.73 | -6.2 |
| Mexico | La Vibora | *B. decumbens* | *P. corethrurus* | 0 | 0.064 |  |  |  | 3.22 | 1.1 | -65.8 |
| Mexico | La Vibora | *B. decumbens* | *P. corethrurus* | 0 | 0.049 |  |  |  | 0.67 | 0.81 | 21.21 |
| Mexico | La Vibora | *B. decumbens* | *P. corethrurus* | 0 | 0.049 |  |  |  | 0.47 | 0.63 | 34.78 |
| Mexico | La Vibora | *B. decumbens* | *P. corethrurus* | 0 | 0.049 |  |  |  | 6.49 | 5.02 | -22.57 |
| Mexico | La Vibora | *B. decumbens* | *P. corethrurus* | 0 | 0.049 |  |  |  | 4.14 | 6.21 | 50.25 |
| Peru | Yurimaguas | *Bixa orellana* | *P. corethrurus* | 0 | 0.036 |  |  |  | 0.148 | 1.870 | 1158 |
| Peru | Yurimaguas | *B. orellana* | *P. corethrurus* | 0 | 0.036 |  |  |  | 0.15 | 1.64 | 1000 |
| Peru | Yurimaguas | *B. orellana* | *P. corethrurus* | 0 | 0.036 |  |  |  | 0.15 | 2.17 | 1357 |
| Peru | Yurimaguas | *B. orellana* | *P. corethrurus* | 0 | 0.036 |  |  |  | 0.80 | 1.64 | 106.7 |
| Peru | Yurimaguas | *B. orellana* | *P. corethrurus* | 0 | 0.036 |  |  |  | 0.80 | 2.23 | 180.4 |
| Peru | Yurimaguas | *Bactris gasipaes* | *P. corethrurus* | 0 | 0.036 |  |  |  | 0.80 | 0.46 | -42.04 |
| Peru | Yurimaguas | *B. gasipaes* | *P. corethrurus* | 0 | 0.036 |  |  |  | 0.80 | 0.31 | -60.74 |
| Peru | Yurimaguas | *B. gasipaes* | *P. corethrurus* | 0 | 0.036 |  |  |  | 0.80 | 0.41 | -48.30 |
| Peru | Yurimaguas | *B. gasipaes* | *P. corethrurus* | 0 | 0.036 |  |  |  | 1.69 | 1.86 | 10.10 |
| Peru | Yurimaguas | *B. gasipaes* | *P. corethrurus* | 0 | 0.036 |  |  |  | 1.69 | 1.70 | 0.71 |
| Peru | Yurimaguas | *Eugenia stipitata* | *P. corethrurus* | 0 | 0.036 |  |  |  | 0.24 | 0.57 | 138.1 |
| Peru | Yurimaguas | *E. stipitata* | *P. corethrurus* | 0 | 0.036 |  |  |  | 0.24 | 0.60 | 150.2 |

| Root yield (T ha$^{-1}$) | | | Shoot/Root ratio | | | Total yield (T ha$^{-1}$) | | | Earthworm mass (g m$^{-2}$) | | | Mass |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Contr | Worm | % Incr | Contr | Worm | % Incr | Contr | Worm | % Incr | Initial | Final | Surviv | Diff |
| 0.14 | 0.21 | 51.79 | 1.13 | 2.76 | 145.7 | 0.30 | 0.80 | 168.9 | 125 | 6.75 | 5 4 | 6.75 |
| 0.14 | 0.10 | -28.57 | 1.13 | 3.35 | 197.7 | 0.30 | 0.44 | 46.22 | 50 | 0 | 0 | 0 |
| 0.14 | 0.06 | -57.14 | 1.13 | 3.38 | 200.0 | 0.30 | 0.26 | -11.76 | 50 | 0.25 | 0.5 | 0.25 |
| 0.14 | 0.18 | 28.57 | 1.13 | 1.68 | 49.38 | 0.30 | 0.48 | 62.18 | 25 | 22 | 88 | 22 |
| 1.62 | 2.67 | 64.79 | 0.58 | 0.64 | 10.31 | 2.56 | 4.38 | 71.01 | 25 | 72 5 | 290 | 72.5 |
| 1.62 | 3.88 | 139.1 | 0.58 | 0.61 | 5.28 | 2.56 | 6.24 | 143.7 | 50 | 107.5 | 215 | 107.5 |
| 1.62 | 3.52 | 117.0 | 0.58 | 0.67 | 15.34 | 2.56 | 5.87 | 129.2 | 75 | 85 | 113.3 | 85 |
| 1.62 | 4.45 | 173.9 | 0.58 | 0.73 | 26.70 | 2.56 | 7.70 | 200.6 | 100 | 127.5 | 127.5 | 127.5 |
| 1.62 | 4.19 | 158.0 | 0.58 | 0.65 | 12.50 | 2.56 | 6.91 | 169.8 | 127.5 | 95 | 74.51 | 95 |
| 3.91 | 7.57 | 93.35 | 1.70 | 1.33 | -21.69 | 10.57 | 17.65 | 66.93 | 41 | 140 | 341.4 | 140 |
| 3.91 | 4.19 | 7.09 | 1.70 | 1.43 | -16.23 | 10.57 | 10.16 | -3.86 | 50 | 117.5 | 235 | 117.5 |
| | | | | | | | | | 12.5 | 36.25 | 290 | 36.25 |
| | | | | | | | | | 25 | 50.75 | 203 | 50.75 |
| 0.40 | 0.84 | 110.9 | 3.89 | 1.98 | -49.22 | 3.19 | 3.59 | 12.61 | 56.5 | 36 | 63.72 | 32.6 |
| 0.40 | 0.31 | -21.33 | 3.89 | 4.53 | 16.45 | 3.19 | 3 02 | -5.49 | 56.5 | 6.4 | 11.33 | 3 |
| 0.40 | 0.63 | 58.77 | 3.89 | 2.52 | -35.39 | 3.19 | 3.44 | 7.64 | 56.5 | 64.2 | 113.6 | 60.7 |
| 0.40 | 0.60 | 49.76 | 3.89 | 2.95 | -24.18 | 3.19 | 3.87 | 21.24 | 56.5 | 76 | 134.5 | 72.6 |
| 0.92 | 1.08 | 18.35 | 3.19 | 2.81 | -12.03 | 6.16 | 5.80 | -5.77 | 56.5 | 19.6 | 34.69 | 19.6 |
| 0.92 | 0.68 | -25.57 | 3.19 | 4.21 | 32.05 | 6.16 | 5.37 | -12.80 | 56.5 | 13.8 | 24.42 | 13.8 |
| 0.92 | 0.89 | -2.89 | 3.19 | 3.58 | 12.14 | 6.16 | 5.80 | -5.80 | 56.5 | 52.6 | 93.1 | 52.6 |
| 0.92 | 0.81 | -11.13 | 3.19 | 3.71 | 16.38 | 6.16 | 6.01 | -2.39 | 56.5 | 32.3 | 57.17 | 32.3 |
| 1.53 | 1.44 | -6.17 | 2.18 | 2.58 | 18.38 | 5.04 | 5.34 | 6.11 | 56.5 | 34 | 60.18 | 34 |
| 1.53 | 1.36 | -11.34 | 2.18 | 2.69 | 23.26 | 5.04 | 5.26 | 4.51 | 56.5 | 12.3 | 21.77 | 12.3 |
| 1.53 | 1.51 | -1.23 | 2.18 | 3.08 | 41.26 | 5.04 | 6.59 | 30.86 | 56.5 | 93.6 | 165.6 | 93.6 |
| 1.53 | 1.17 | -23.43 | 2.18 | 3.52 | 61.09 | 5.04 | 5.79 | 15.00 | 56.5 | 43.2 | 76.46 | 43.2 |
| 0.08 | 0.07 | -8.22 | 2.15 | 2.54 | 17.98 | 0.26 | 0.26 | 3.04 | 58.9 | 29.45 | 50 | 29.45 |
| 0.08 | 0.07 | -13.70 | 2.15 | 2.27 | 5.54 | 0.26 | 0.23 | -10.43 | 54.5 | 8.8 | 16.15 | 8.8 |
| | | | | | | | | | 61.8 | 107 | 173.1 | 107 |
| | | | | | | | | | 62.9 | 48.8 | 77.58 | 65.4 |
| 0.06 | 0.08 | 35.05 | 1.39 | 2.19 | 57.34 | 0.19 | 0.32 | 64.33 | 49.3 | 29.73 | 60.30 | 29.73 |
| 0.06 | 0.11 | 87.77 | 1.39 | 1.85 | 33.14 | 0.19 | 0.37 | 88.33 | 47.9 | 28.3 | 59.08 | 28.3 |
| 4.22 | 4.95 | 17.26 | 3.76 | 2.87 | -23.60 | 22.64 | 22.29 | -1.52 | 58.9 | 16.2 | 27.50 | 16.2 |
| 4.5 | 2.92 | -35.01 | 6 | 5.3 | -11.67 | 20.27 | 12.97 | -36 | 60 | 37.36 | 62.26 | 37.36 |
| 0.52 | 0.41 | -22.17 | 1.8 | 2.02 | 12.22 | 1.47 | 1.21 | -17.3 | 32 | 0 | 0 | 0 |
| 0.04 | 0.04 | 0 | 5.61 | 4.7 | -16.18 | 0.29 | 0.25 | -14.04 | 117 | 91 | 78.27 | 91 |
| 0.16 | 0.19 | 20 | 18.16 | 14.19 | -21.83 | 3.07 | 2.92 | -4.82 | 116 | 73 | 62.9 | 73 |
| 0.05 | 0.07 | 39.22 | 11.59 | 7.96 | -31.33 | 0.64 | 0.64 | 0.78 | 108 | 110 | 101.8 | 110 |
| 0.18 | 0.08 | -58.47 | 17.61 | 14.5 | -17.65 | 3.4 | 1.18 | -65.34 | 117 | 89 | 75.52 | 89 |
| 0.13 | 0.13 | -2.5 | 5 | 7.14 | 42.8 | 0.81 | 0.94 | 16.22 | 60.5 | 25.06 | 41.21 | 25.06 |
| 0.08 | 0.12 | 59.14 | 6.05 | 5.19 | -14.12 | 0.54 | 0.75 | 38.86 | 61.93 | 25.06 | 41.4 | 25.06 |
| 0.39 | 0.73 | 88.31 | 11.58 | 9.53 | -17.67 | 4.53 | 6.94 | 53.26 | 60.91 | 0 | 0 | 0 |
| 0.76 | 0.48 | -37.05 | 10.15 | 10.91 | 7.41 | 7.25 | 5.5 | -24.13 | 58.47 | 20.98 | 36.02 | 20.98 |
| 0.036 | 0.588 | 1529 | 4.12 | 3.18 | -22.73 | 0.18 | 2.46 | 1231 | 3.2 | 0 | 0 | 0 |
| 0.04 | 0.41 | 1033 | 4.12 | 4.00 | -2.87 | 0.18 | 2.04 | 1006 | 10.6 | 14.7 | 138.6 | 14.7 |
| 0.04 | 0.63 | 1640 | 4.12 | 3.44 | -16.30 | 0.18 | 2.79 | 1412 | 21.2 | 0 | 0 | 0 |
| 0.34 | 0.61 | 83.35 | 2.37 | 2.68 | 12.78 | 1.13 | 2.26 | 99.83 | 10.86 | | | |
| 0.34 | 1.05 | 214.5 | 2.37 | 2.11 | -10.84 | 1.13 | 3.29 | 190.5 | 21.7 | | | |
| 0.45 | 0.31 | -30.57 | 1.80 | 1.50 | -16.53 | 1.24 | 0.77 | -37.94 | 3.2 | 68 | 2125 | 68 |
| 0.45 | 0.24 | -45.23 | 1.80 | 1.29 | -28.32 | 1.24 | 0.56 | -55.19 | 10.6 | 153 | 1443 | 153 |
| 0.45 | 0.29 | -35.25 | 1.80 | 1.43 | -20.15 | 1.24 | 0.70 | -43.63 | 21.2 | 197.7 | 932.5 | 197.7 |
| 0.92 | 0.77 | -15.89 | 1.84 | 2.41 | 30.91 | 2.60 | 2.63 | 0.95 | 10.86 | | | |
| 0.92 | 1.07 | 16.77 | 1.84 | 1.59 | -13.76 | 2.60 | 2.77 | 6.36 | 21.7 | | | |
| 0.06 | 0.20 | 236.2 | 4.02 | 2.85 | -29.18 | 0.30 | 0.77 | 157.6 | 3.2 | 217.5 | 6796 | 217.5 |
| 0.06 | 0.18 | 193.0 | 4.02 | 3.44 | -14.58 | 0.30 | 0.78 | 158.8 | 10.6 | 199 | 1877 | 199 |

Table 1. continuation

| | | | Earthworm | Residues | Plot size | Grain yield (T ha$^{-1}$) | | | Shoot yield (T ha$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Country | Location | Crop | species | (kg m$^{-2}$) | (m$^2$) | Contr | Worm | % Incr | Contr | Worm | % Incr |
| Peru | Yurimaguas | *E. stipitata* | *P. corethrurus* | 0 | 0.036 | | | | 0.24 | 0.86 | 258.1 |
| Peru | Yurimaguas | *E. stipitata* | *P. corethrurus* | 0 | 0.036 | | | | 0.95 | 1.11 | 17.10 |
| Peru | Yurimaguas | *E. stipitata* | *P. corethrurus* | 0 | 0.036 | | | | 0.95 | 1.17 | 23.37 |
| India | Sambalpur | Rice | *D. willsii* | 0 | 0.071 | 0.20 | 0.38 | 95 | | | |
| India | Sambalpur | Rice | *D. willsii* | 0 | 0.071 | 0.14 | 0.46 | 230 | 0.86 | 1.03 | 19.77 |
| India | Sambalpur | Rice | *D. willsii* | 0.28 | 0.071 | 0.20 | 0.59 | 200 | 0.89 | 1.37 | 53.93 |
| India | Sambalpur | Tea | *P. corethrurus* | 0 | 0.008 | | | | 2.08 | 2.38 | 14.46 |
| India | Sambalpur | Tea | *P. corethrurus* | 25.5 | 0.008 | | | | 2.38 | 2.68 | 12.63 |
| India | Sambalpur | Tea | *P. corethrurus* | 6.4 | 0.008 | | | | 2.14 | 2.43 | 13.45 |
| India | Sambalpur | Tea | *P. corethrurus* | 31.8 | 0.008 | | | | 2.68 | 3.08 | 14.95 |
| India | Sambalpur | Tea | *P. corethrurus* | 0 | 0.008 | | | | 2.19 | 2.45 | 12.00 |
| India | Sambalpur | Tea | *P. corethrurus* | 25.5 | 0.008 | | | | 2.43 | 3.80 | 56.70 |
| India | Sambalpur | Tea | *P. corethrurus* | 6.4 | 0.008 | | | | 2.28 | 3.30 | 45.05 |
| India | Sambalpur | Tea | *P. corethrurus* | 31.8 | 0.008 | | | | 3.69 | 4.83 | 30.85 |
| Australia | Narayen | Grasses | *D. affinis* | 0 | 0.043 | | | | 3.99 | 5.77 | 44.61 |
| Australia | Narayen | Grasses | *D. afinis* | 0 | 0.043 | | | | 8.58 | 11.16 | 30.04 |
| Australia | Biloela | Sorghum | *P. elongata* | 0 | 0.043 | 2.07 | 4.40 | 112.3 | 15.95 | 24.21 | 51.75 |
| Australia | Biloela | Sorghum | *P. elongata* | 0 | 0.043 | 2.67 | 2.95 | 10.43 | 17.30 | 17.4 | 0.56 |
| Australia | Biloela | Sorghum | *O. occidentalis et al.* | 0 | 0.043 | 2.67 | 3.05 | 13.91 | 17.30 | 15.3 | -11.57 |
| Australia | Biloela | Sorghum | *P. corethrurus* | 0 | 0.043 | 2.67 | 4.05 | 51.30 | 17.30 | 19.72 | 13.97 |
| Australia | Biloela | Sorghum | *A. trapezoides* | 0 | 0.043 | 2.67 | 5.51 | 106.0 | 17.30 | 20.38 | 17.79 |
| Australia | Narayen | Grasses | *D. affinis + saliens* | 0 | 0.043 | | | | 5.58 | 8.93 | 59.85 |
| Australia | Narayen | Grasses | *S. minor* | 0 | 0.043 | | | | 5.58 | 4.01 | -28.20 |
| Australia | Narayen | Grasses | *P. corethrurus* | 0 | 0.043 | | | | 5.58 | 5.26 | -5.79 |
| Australia | Narayen | Grasses | *P. elongata* | 0 | 0.043 | | | | 5.58 | 3.74 | -32.94 |
| Australia | Narayen | Grasses | *P. taprobanae* | 0 | 0.043 | | | | 5.58 | 5.17 | -7.37 |
| Australia | Narayen | Grasses | *E. eugeniae* | 0 | 0.043 | | | | 5.58 | 6.58 | 17.83 |
| Australia | Narayen | Grasses | *A. trapezoides* | 0 | 0.043 | | | | 5.58 | 7.42 | 32.90 |
| Australia | Narayen | Grasses | *M. californica* | 0 | 0.043 | | | | 5.58 | 5.82 | 4.29 |
| Australia | Narayen | Grasses | *F. unicus* | 0 | 0.043 | | | | 5.58 | 6.69 | 19.87 |
| Australia | Narayen | Grasses | *E. saltensis* | 0 | 0.043 | | | | 5.58 | 5.15 | -7.75 |
| Australia | Narayen | Grasses | *D. bruneus* | 0 | 0.043 | | | | 5.58 | 4.43 | -20.57 |
| Australia | Kingaroy | Grasses | *D. affinis + saliens* | 0 | 0.043 | | | | 5.04 | 4.69 | -6.96 |
| Australia | Kingaroy | Grasses | *P. corethrurus* | 0 | 0.043 | | | | 5.04 | 5.48 | 8.72 |
| Australia | Kingaroy | Grasses | *E. eugeniae* | 0 | 0.043 | | | | 5.04 | 4.68 | -7.20 |
| Australia | Kingaroy | Grasses | *A. trapezoides* | 0 | 0.043 | | | | 5.04 | 5.50 | 9.09 |
| Australia | Kingaroy | Grasses | *F. unicus* | 0 | 0.043 | | | | 5.04 | 5.63 | 11.72 |
| Australia | Samford | Grasses | *D. affinis + saliens* | 0 | 0.043 | | | | 3.61 | 6.20 | 71.60 |
| Australia | Samford | Grasses | *P. corethrurus* | 0 | 0.043 | | | | 3.61 | 4.21 | 16.61 |
| Australia | Samford | Grasses | *P. elongata* | 0 | 0.043 | | | | 3.61 | 4.76 | 31.68 |
| Australia | Samford | Grasses | *P. taprobanae* | 0 | 0.043 | | | | 3.61 | 4.30 | 19.00 |
| Australia | Samford | Grasses | *E. eugeniae* | 0 | 0.043 | | | | 3.61 | 3.91 | 8.37 |
| Australia | Samford | Grasses | *A. trapezoides* | 0 | 0.043 | | | | 3.61 | 4.88 | 35.03 |
| Australia | Samford | Grasses | *M. californica* | 0 | 0.043 | | | | 3.61 | 4.12 | 13.97 |
| Australia | Samford | Grasses | *E. saltensis* | 0 | 0.043 | | | | 3.61 | 3.91 | 8.18 |
| Australia | Narayen | Oats | *D. affinis + saliens* | 0 | 0.043 | | | | 9.07 | 10.43 | 14.92 |
| Australia | Narayen | Oats | *S. minor* | 0 | 0.043 | | | | 9.07 | 7.54 | -16.89 |
| Australia | Narayen | Oats | *P. corethrurus* | 0 | 0.043 | | | | 9.07 | 10.00 | 10.20 |
| Australia | Narayen | Oats | *P. elongata* | 0 | 0.043 | | | | 9.07 | 7.53 | -16.94 |
| Australia | Narayen | Oats | *P. taprobanae* | 0 | 0.043 | | | | 9.07 | 10.14 | 11.79 |
| Australia | Narayen | Oats | *E. eugeniae* | 0 | 0.043 | | | | 9.07 | 11.69 | 28.89 |
| Australia | Narayen | Oats | *A. trapezoides* | 0 | 0.043 | | | | 9.07 | 8.91 | -1.82 |
| Australia | Narayen | Oats | *M. californica* | 0 | 0.043 | | | | 9.07 | 8.13 | -10.38 |
| Australia | Narayen | Oats | *F. unicus* | 0 | 0.043 | | | | 9.07 | 9.53 | 5.10 |
| Australia | Narayen | Oats | *E. saltensis* | 0 | 0.043 | | | | 9.07 | 8.59 | -5.33 |
| Australia | Narayen | Oats | *Dig. bruneus* | 0 | 0.043 | | | | 9.07 | 8.72 | -3.85 |
| Australia | Kingaroy | Oats | *D. affinis + saliens* | 0 | 0.043 | | | | 7.63 | 6.54 | -14.27 |
| Australia | Kingaroy | Oats | *P. corethrurus* | 0 | 0.043 | | | | 7.63 | 7.27 | -4.73 |
| Australia | Kingaroy | Oats | *E. eugeniae* | 0 | 0.043 | | | | 7.63 | 6.17 | -19.18 |
| Australia | Kingaroy | Oats | *A. trapezoides* | 0 | 0.043 | | | | 7.63 | 5.81 | -23.78 |
| Australia | Kingaroy | Oats | *F. unicus* | 0 | 0.043 | | | | 7.63 | 6.10 | -20.09 |
| Australia | Samford | Oats | *D. affinis + saliens* | 0 | 0.043 | | | | 6.15 | 6.13 | -0.34 |
| Australia | Samford | Oats | *P. corethrurus* | 0 | 0.043 | | | | 6.15 | 5.63 | -8.48 |

| Root yield (T ha$^{-1}$) | | | Shoot/Root ratio | | | Total yield (T ha$^{-1}$) | | | Earthworm mass (g m$^{-2}$) | | | Mass Diff. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Contr | Worm | % Incr | Contr | Worm | % Incr | Contr | Worm | % Incr | Initial | Final | Surviv | |
| 0.06 | 0.31 | 413.9 | 4.02 | 2.80 | -30.31 | 0.30 | 1.17 | 289.1 | 21.2 | 205 | 966.9 | 205 |
| 0.43 | 0.46 | 7.55 | 2.20 | 2.40 | 8.88 | 1.38 | 1.57 | 14.11 | 10.86 | | | |
| 0.43 | 0.30 | -29.48 | 2.20 | 3.85 | 74.96 | 1.38 | 1.47 | 6.85 | 21.7 | | | |
| | | | | | | | | | 42.4 | 56.6 | 133.4 | 56.6 |
| 0.65 | 0.69 | 6.52 | 1.33 | 1 49 | 12.43 | 1.65 | 2.18 | 32.52 | 42.4 | 63.7 | 150.2 | 63.7 |
| 0.77 | 1.20 | 54.55 | 1.15 | 1 14 | -0.40 | 1.86 | 3.16 | 69.66 | 42.4 | 70.7 | 166.7 | 70 7 |
| 1.35 | 2.08 | 53.70 | 1.54 | 1.14 | -25.53 | 3.43 | 4.45 | 29.93 | 127.3 | 264.8 | 208.0 | 264.8 |
| 1.20 | 2.16 | 80.21 | 1.98 | 1.24 | -37.50 | 3.58 | 4.84 | 35.31 | 127.3 | 383.2 | 301.0 | 383.2 |
| 1.28 | 1.65 | 29.41 | 1.68 | 1.47 | -12.33 | 3.41 | 4.08 | 19.41 | 127.3 | 300.5 | 236.0 | 300.5 |
| 1.61 | 1.58 | -2.33 | 1.66 | 1.95 | 17.69 | 4.29 | 4.65 | 8.45 | 127.3 | 431.6 | 339.0 | 431 6 |
| 1.44 | 1.93 | 33.91 | 1.52 | 1.27 | -16.36 | 3.63 | 4.38 | 20.69 | 127.3 | 99.3 | 78 | 99.3 |
| 1.51 | 3.88 | 15.20 | 1.60 | 0.98 | -38.84 | 3.94 | 7.68 | 94.92 | 127.3 | 163 | 128.0 | 163 |
| 1.65 | 2.91 | 76.52 | 1.38 | 1.13 | -17.82 | 3.93 | 6.21 | 58.28 | 127.3 | 220.3 | 173.0 | 220.3 |
| 2.25 | 2.16 | -3.89 | 1.64 | 2.23 | 36.14 | 5.94 | 6.99 | 17.68 | 127.3 | 314.5 | 247.0 | 314.5 |
| | | | | | | | | | 23.06 | 92.22 | 399.9 | 92.22 |
| | | | | | | | | | 23.06 | 57.64 | 249.9 | 57.64 |
| | | | | | | | | | 325.9 | 274.6 | 84.24 | 274.6 |
| | | | | | | | | | 276.7 | 238.1 | 86.06 | 238.13 |
| | | | | | | | | | 23.45 | 14.07 | 60 | 14.07 |
| | | | | | | | | | 311.8 | 147.7 | 47.37 | 147.74 |
| | | | | | | | | | 262.6 | 35.18 | 13.39 | 35.18 |
| 13.07 | 10.79 | -17.44 | 0.43 | 0.83 | 93.61 | 18.65 | 19.72 | 5.70 | 13.49 | 104.9 | 778.1 | 104.94 |
| 13.07 | 10.19 | -22.06 | 0.43 | 0.39 | -7.87 | 18.65 | 14.20 | -23.90 | 45.34 | 10.32 | 22.76 | 10.32 |
| 13.07 | 9.93 | -24.02 | 0.43 | 0.53 | 24.00 | 18.65 | 15.19 | -18.56 | 49.41 | 111.0 | 224.7 | 111.04 |
| 13.07 | 7.60 | -41.81 | 0.43 | 0.49 | 15.25 | 18.65 | 11.35 | -39.16 | 82.54 | 320.6 | 388.5 | 320.68 |
| 13.07 | 13.44 | 2.85 | 0.43 | 0.38 | -9.94 | 18.65 | 18.61 | -0.21 | 112.7 | 170.4 | 151.1 | 170.48 |
| 13.07 | 9.93 | -24.02 | 0.43 | 0 66 | 55.08 | 18.65 | 16.51 | -11.49 | 99.98 | 67.42 | 67.44 | 67.42 |
| 13.07 | 10.42 | -20.28 | 0.43 | 0.71 | 66.72 | 18.65 | 17.84 | -4.36 | 70.91 | 80.26 | 113.1 | 80.26 |
| 13.07 | 10.07 | -22.95 | 0.43 | 0.58 | 35.36 | 18.65 | 15.89 | -14.80 | 127.8 | 73.87 | 57.77 | 73.87 |
| 13.07 | 12.30 | -5.87 | 0.43 | 0 54 | 27.34 | 18.65 | 19.00 | 1.83 | 181.3 | 38.93 | 21.47 | 38.93 |
| 13.07 | 13.74 | 5.16 | 0.43 | 0.37 | -12.27 | 18.65 | 18.90 | 1.30 | 13.95 | 23.45 | 168.1 | 23.45 |
| 13.07 | 15.02 | 14.95 | 0.43 | 0.30 | -30.90 | 18.65 | 19.46 | 4.31 | 111.6 | 0 | 0 | 0 |
| 9.44 | 7.19 | -23.89 | 0.53 | 0.65 | 22.24 | 14.48 | 11.88 | -18.00 | 13.49 | 52.65 | 390.4 | 52.65 |
| 9.44 | 8.21 | -13.05 | 0.53 | 0.67 | 25.04 | 14.48 | 13.69 | -5.48 | 33.71 | 89.93 | 266.7 | 89.93 |
| 9.44 | 13.07 | 38.42 | 0.53 | 0.36 | -32.96 | 14.48 | 17.75 | 22.54 | 99.98 | 28.73 | 28.73 | 28.73 |
| 9.44 | 8.33 | -11.82 | 0.53 | 0.66 | 23.71 | 14.48 | 13.83 | -4.54 | 51.15 | 69.88 | 136.6 | 69.88 |
| 9.44 | 11.12 | 17.73 | 0.53 | 0.51 | -5.11 | 14.48 | 16.75 | 15.64 | 184.8 | 46.08 | 24.93 | 46.08 |
| 9.86 | 11.16 | 13.21 | 0.37 | 0.56 | 51.58 | 13.47 | 17.36 | 28.86 | 31.62 | 136.3 | 431.0 | 136.30 |
| 9.86 | 10.93 | 10.85 | 0.37 | 0.39 | 5.20 | 13.47 | 15.14 | 12.39 | 49.41 | 141.3 | 286.0 | 141.34 |
| 9.86 | 12.19 | 23.58 | 0.37 | 0.39 | 6.55 | 13.47 | 16.94 | 25.76 | 86.03 | 150.7 | 175.2 | 150.78 |
| 9.86 | 11.07 | 12.26 | 0.37 | 0.39 | 6.00 | 13.47 | 15.37 | 14.07 | 97.65 | 67.77 | 69.40 | 67.77 |
| 9.86 | 14.74 | 49.53 | 0.37 | 0.27 | -27.52 | 13.47 | 18.66 | 38.49 | 101.1 | 104.2 | 103.0 | 104.24 |
| 9.86 | 11.51 | 16.75 | 0.37 | 0.42 | 15.66 | 13.47 | 16.39 | 21.65 | 55.22 | 61.03 | 110.5 | 61.03 |
| 9.86 | 9.81 | -0.47 | 0.37 | 0.42 | 14.51 | 13.47 | 13.93 | 3.40 | 126.7 | 129.4 | 102.1 | 129.44 |
| 9.86 | 9.93 | 0.71 | 0.37 | 0.39 | 7.42 | 13.47 | 13.84 | 2.71 | 13.95 | 4.92 | 35.30 | 4.92 |
| | | | | | | | | | 13.49 | | | |
| | | | | | | | | | 45.34 | | | |
| | | | | | | | | | 49.41 | | | |
| | | | | | | | | | 82.54 | | | |
| | | | | | | | | | 112.7 | | | |
| | | | | | | | | | 99.98 | | | |
| | | | | | | | | | 70.91 | | | |
| | | | | | | | | | 127.8 | | | |
| | | | | | | | | | 181.3 | | | |
| | | | | | | | | | 13.95 | | | |
| | | | | | | | | | 111.6 | | | |
| | | | | | | | | | 13.49 | | | |
| | | | | | | | | | 33.71 | | | |
| | | | | | | | | | 99.98 | | | |
| | | | | | | | | | 51.15 | | | |
| | | | | | | | | | 184.8 | | | |
| | | | | | | | | | 31.62 | | | |
| | | | | | | | | | 49.41 | | | |

Table 1. continuation

| | | | Earthworm | Residues | Plot size | Grain yield (T ha$^{-1}$) | | | Shoot yield (T ha$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Country | Location | Crop | species | (kg m$^{-2}$) | (m$^2$) | Contr | Worm | % Incr | Contr | Worm | % Incr |
| Australia | Samford | Oats | *P. elongata* | 0 | 0.043 | | | | 6.15 | 6.36 | 3.41 |
| Australia | Samford | Oats | *P. taprobanae* | 0 | 0.043 | | | | 6.15 | 6.58 | 7 |
| Australia | Samford | Oats | *E. eugeniae* | 0 | 0.043 | | | | 6.15 | 6.17 | 0.45 |
| Australia | Samford | Oats | *A. trapezoides* | 0 | 0.043 | | | | 6.15 | 6.02 | -2.08 |
| Australia | Samford | Oats | *M. californica* | 0 | 0.043 | | | | 6.15 | 6.46 | 5.03 |
| Australia | Samford | Oats | *E. saltensis* | 0 | 0.043 | | | | 6.15 | 5.87 | -4.54 |
| Australia | Narayen | Sorghum | *Diplotrema sp.nov. 1* | 0 | 0.043 | | | | 7.31 | 7.69 | 5.09 |
| Australia | Narayen | Sorghum | *O. beatrix* | 0 | 0.043 | | | | 7.31 | 7.15 | -2.23 |
| Australia | Narayen | Sorghum | *E. rosea* | 0 | 0.043 | | | | 7.31 | 9.27 | 26.71 |
| Australia | Narayen | Sorghum | *D. barwelli* | 0 | 0.043 | | | | 7.31 | 8.87 | 21.30 |
| Australia | Narayen | Sorghum | *Diplotrema sp.nov. 2* | 0 | 0.043 | | | | 7.31 | 7.74 | 5.88 |
| Australia | Narayen | Sorghum | *A. minimus* | 0 | 0.043 | | | | 7.31 | 7.38 | 0.95 |
| Australia | Narayen | Sorghum | *H. bongeen* | 0 | 0.043 | | | | 7.31 | 10.21 | 39.59 |
| Australia | Samford | Sorghum | *Diplotrema sp.nov. 1* | 0 | 0.043 | | | | 4.38 | 4.21 | -3.98 |
| Australia | Samford | Sorghum | *O. beatrix* | 0 | 0.043 | | | | 4.38 | 1.81 | -58.62 |
| Australia | Samford | Sorghum | *E. rosea* | 0 | 0.043 | | | | 4.38 | 4.37 | -0.27 |
| Australia | Samford | Sorghum | *D. barwelli* | 0 | 0.043 | | | | 4.38 | 4.12 | -6.10 |
| Australia | Samford | Sorghum | *A. minimus* | 0 | 0.043 | | | | 4.38 | 4.93 | 12.47 |
| Australia | Samford | Oats | *P. excavatus* | 0 | 0.043 | | | | 2.51 | 2.48 | -1.21 |
| Australia | Samford | Oats | *E. eugeniae* | 0 | 0.043 | | | | 2.51 | 2.39 | -4.55 |
| Australia | Samford | Oats | *A. rodericensis* | 0 | 0.043 | | | | 2.51 | 2.38 | -4.92 |
| Australia | Narayen | Oats | *Diplotrema sp.nov. 1* | 0 | 0.043 | | | | 3.30 | 4.37 | 32.39 |
| Australia | Narayen | Oats | *O. beatrix* | 0 | 0.043 | | | | 3.30 | 4.09 | 23.94 |
| Australia | Narayen | Oats | *E. rosea* | 0 | 0.043 | | | | 3.30 | 5.23 | 58.45 |
| Australia | Narayen | Oats | *D. barwelli* | 0 | 0.043 | | | | 3.30 | 4.49 | 35.92 |
| Australia | Narayen | Oats | *Diplotrema sp.nov. 2* | 0 | 0.043 | | | | 3.30 | 3.35 | 1.41 |
| Australia | Samford | Oats | *Diplotrema sp.nov. 1* | 0 | 0.043 | | | | 2.76 | 3.05 | 10.27 |
| Australia | Samford | Oats | *O. beatrix* | 0 | 0.043 | | | | 2.76 | 3.40 | 22.90 |
| Australia | Samford | Oats | *E. rosea* | 0 | 0.043 | | | | 2.76 | 2.91 | 5.22 |
| Australia | Samford | Oats | *D. barwelli* | 0 | 0.043 | | | | 2.76 | 2.77 | 0.17 |
| Cameroon | Mbalmayo | Maize | *Unknown* | 0 | 0.059 | | | | 1.244 | 2.193 | 76.29 |
| Cameroon | Mbalmayo | Maize | *Unknown* | 0.5 | 0.059 | | | | 3.25 | 3.71 | 14.06 |
| Marti-nique | St. Anne | *Digitaria decumbens* | *P. elongata* | 0 | 50 | | | | | | |
| Martini-que | St. Anne | *D. decumbens* | *P. elongata* | 0 | 50 | | | | | | |
| Martinique | St. Anne | *D. decumbens* | *P. elongata* | 0 | 50 | | | | | | |
| Martinique | St. Anne | *D. decumbens* | *P. elongata* | 0 | 50 | | | | | | |

| Root yield (T ha$^{-1}$) |  |  | Shoot/Root ratio |  |  | Total yield (T ha$^{-1}$) |  |  | Earthworm mass (g m$^{-2}$) |  |  | Mass |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Contr | Worm | % Incr | Contr | Worm | % Incr | Contr | Worm | % Incr | Initial | Final | Surviv | Diff. |
|  |  |  |  |  |  |  |  |  | 86.03 |  |  |  |
|  |  |  |  |  |  |  |  |  | 97.65 |  |  |  |
|  |  |  |  |  |  |  |  |  | 101.1 |  |  |  |
|  |  |  |  |  |  |  |  |  | 55.22 |  |  |  |
|  |  |  |  |  |  |  |  |  | 126.7 |  |  |  |
|  |  |  |  |  |  |  |  |  | 13.95 |  |  |  |
|  |  |  |  |  |  |  |  |  | 29.08 | 56.51 | 194.3 | 56.51 |
|  |  |  |  |  |  |  |  |  | 68.94 | 36.82 | 53.40 | 36.82 |
|  |  |  |  |  |  |  |  |  | 137.8 | 0 | 0 | 0 |
|  |  |  |  |  |  |  |  |  | 39.87 | 66.60 | 167.0 | 66.60 |
|  |  |  |  |  |  |  |  |  | 23.92 | 43.97 | 183.8 | 43.97 |
|  |  |  |  |  |  |  |  |  | 44.79 | 33.42 | 74.61 | 33.42 |
|  |  |  |  |  |  |  |  |  | 253.2 | 0 | 0 | 0 |
|  |  |  |  |  |  |  |  |  | 27.44 | 0 | 0 | 0 |
|  |  |  |  |  |  |  |  |  | 60.03 | 16.02 | 26.68 | 16.02 |
|  |  |  |  |  |  |  |  |  | 134.8 | 3.13 | 2.32 | 3.13 |
|  |  |  |  |  |  |  |  |  | 42.91 | 25.87 | 60.27 | 25.87 |
|  |  |  |  |  |  |  |  |  | 40.57 | 9.22 | 22.74 | 9.22 |
|  |  |  |  |  |  |  |  |  | 31.28 | 19.26 | 61.54 | 19.26 |
|  |  |  |  |  |  |  |  |  | 96.28 | 13.24 | 13.75 | 13.24 |
|  |  |  |  |  |  |  |  |  | 108.3 | 56.56 | 52.22 | 56.56 |
|  |  |  |  |  |  |  |  |  | 29.08 |  |  |  |
|  |  |  |  |  |  |  |  |  | 68.94 |  |  |  |
|  |  |  |  |  |  |  |  |  | 137.8 |  |  |  |
|  |  |  |  |  |  |  |  |  | 39.87 |  |  |  |
|  |  |  |  |  |  |  |  |  | 23.92 |  |  |  |
|  |  |  |  |  |  |  |  |  | 27.44 |  |  |  |
|  |  |  |  |  |  |  |  |  | 60.03 |  |  |  |
|  |  |  |  |  |  |  |  |  | 134.8 |  |  |  |
|  |  |  |  |  |  |  |  |  | 42.91 |  |  |  |
| 1.08 | 0.59 | -45.44 | 1.15 | 3.73 | 223.1 | 2.32 | 2.78 | 19.78 | 164.1 |  |  |  |
|  |  |  |  |  |  |  |  |  | 164.1 |  |  |  |
|  | n.s.d. |  |  |  |  |  |  |  | 90 | 35.7 | 39.7 | 35.6 |
|  | n.s.d. |  |  |  |  |  |  |  | 90 | 46.6 | 51.8 | 46.4 |
|  | n.s.d. |  |  |  |  |  |  |  | 90 | 32.8 | 36.4 | 32.2 |
|  | n.s.d. |  |  |  |  |  |  |  | 90 | 42.3 | 47.0 | 39.2 |

# ANNEXE 2

## CHAPITRE 3.

Description of the profile of savanna pasture soil at La Víbora, Veracruz.

Profile dug by Michel Brossard and George Brown, on July 27, 1994.

Profile 90cm deep
Temperature was 84°F at all levels probably due to the fairly constant (hot) atmospheric temperature and $H_2O$ being the only conductor of heat down the soil profile.

A horizon:
0-10cm
Sandy texture, massive structure sin any particular aggregation. Lower inferior transition wavy (uneven) and progressive over 3-4cm. The limit is observed by an increase in clay.
High root density (>5 roots $cm^{-2}$). Lots of spots with red oxidized Fe. Roots also with oxidized Fe around them. Roots found going horizontally at the limit (10cm), indicating a limiting bulk density to penetrating the B horizon.
Color (Munsel) = 10YR 4/2 or 5/2 or 5/3 (wet). Brownish beige. The oxidadas patches are 1-2 mm and 2.5 YR 3/6 (wet).
Bulk density is 1.44 - 1.6 g $cm^{-3}$. Mean of 6 samples = 1.54 g $cm^{-3}$. Water contents 14.4 - 18.2%. Mean of 6 samples = 15.9%.

Bt horizon:
10-20 to 50 (55) cm
Bt like horizon, with great increase in clay over the A horizon. Porosity very low, compacted, with slow water movement through horizon. Likely due to the cattle trampling the surface when wet. This compaction limits root penetration. The texture becomes clayier with a feeling of >30% clay. Very few roots, massive structure, very humid (wet) horizon, but with some sub-angular blocky small pedons. Dark color (due to OM?). Spots of oxidacion and concretions of Fe (0.5-1 cm size). Concretions hard but breakable with a pocket knife. Apparent slickensides, probably due to smectite clays. Lower limit with Bg is progressive with increase in clay and the appearance of yellowish subhorizons and spots of oxidation-reduction.
Color = 10 YR 2/2 or 3/2 or 3/1 greyish black (wet). Concretions are black (dark spots associated with roots and some un-associated); color of these is 10YR 3/4 (wet).
Water content 18.5%.

Bg horizon:
From 50-55 cm to about 70cm
Concretion of Fe, increase in clay and small rocks (perhaps alluvial?) and a pseudo-gley type horizon. The limit is very uneven down to 60-70 cm where the lower (BC) horizon begins, with lots of clay, small rocks and a very yellow color. Free water on the surface of pedons. Apparent slickensides, probably due to smectite. This Bg is almost a BC, with many primary, non-weathered minerals. The rocks below are light (pumice-like). Fe concretions in this horizon are hard, but breakable with a knife.

Color = 2.5 Y 4/2 or 5/2 greenish yellow-gray (wet). Drier Bg has same color. The spots are 7.5 YR 4/4 (wet).
Bg water content is 21.7%.

BC horizon:
>70cm, with variable thickness
Shallow horizon, limiting the lower Bg and the C horizon. More obvious below 70 cm. Great variability in $H_2O$ contents, with some areas being very hard, and others softer.
BC water content was 16.2% in one sample and 19.6% in another.
BC is yellowish, 7.5 YR 5/2 or 4/2 (wet). Minerals are abundant, pumice, ashes probably.

Conclusion: Kanhaplic Haplustalf (USDA); haplic Lixisol (FAO) or Sol Ferrugineux Tropicale (ORSTOM)

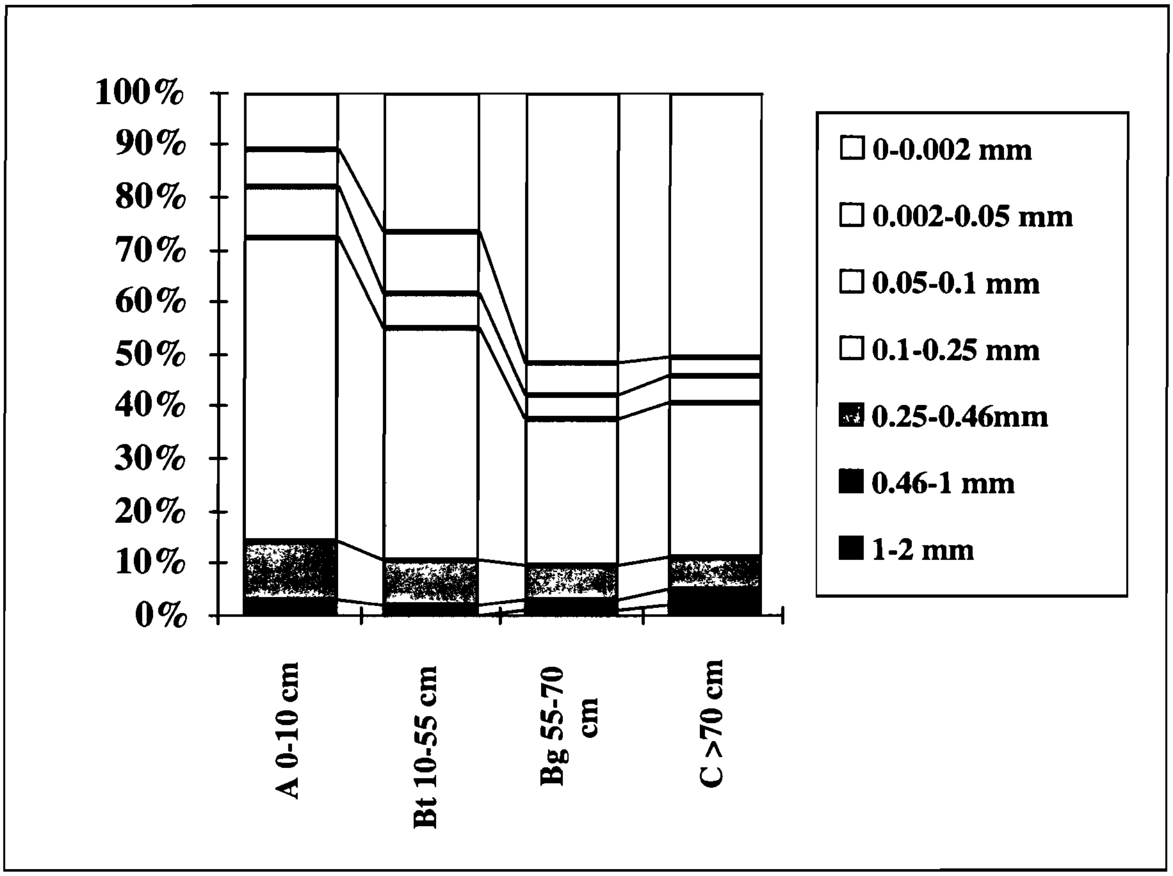


Figure 1. Texture (according to USDA classification) of the horizons from soil profile dug in the pasture at La Víbora, Veracruz (texture obtained as in Gee and Bauder, 1986).

Table 1. Selected physico-chemical properties of the surface and subsurface soil horizons of the pasture paddock at La Vîbora, Veracruz. n.d. = not determined.

| Property | A | $B_1$ | $B_2$ | C |
|---|---|---|---|---|
| Depth (cm) | 0-15 | 15-55 | 55-70 | >70 |
| Sand (%) | 81.7 | 66.7 | 49.6 | 47.7 |
| Silt (%) | 8.1 | 9.0 | 6.0 | 4.0 |
| Clay (%) | 10.2 | 24.3 | 44.4 | 48.3 |
| Texture | loamy sand | sandy clay loam | sandy clay | sandy clay |
| %C | 0.96a | 0.89a | 0.56b | 0.35c |
| %N | 0.13a | 0.03b | 0.03b | 0.02b |
| C:N | 7.6c | 29.1a | 18.8b | 18.9b |
| pH $H_2O$ | 5.01d | 5.88c | 7.25b | 8.55a |
| pH KCl | 3.96d | 4.57c | 5.59b | 7.33a |
| P-Bray (mg kg$^{-1}$) | 6.7a | 8.3a | 3.6b | 7.3a |
| P total (mg kg$^{-1}$) | 61.4 | n.d. | n.d. | n.d. |
| P org (mg kg$^{-1}$) | 16.9 | n.d. | n.d. | n.d. |
| P inorg (mg kg$^{-1}$) | 43.7 | n.d. | n.d. | n.d. |
| P resin (mg kg$^{-1}$) | 15.3 | n.d. | n.d. | n.d. |
| $NO_3$ (mg kg$^{-1}$) | 7.6 | n.d. | n.d. | n.d. |
| $NH_4$ (mg kg$^{-1}$) | 33.1 | n.d. | n.d. | n.d. |
| CEC (meq 100g$^{-1}$) | 11.7cd | 12.1c | 33.6b | 41.2a |
| K (meq 100g$^{-1}$) | 0.08 | 0.04$^{\dagger}$ | 0.40$^{\dagger}$ | 0.07$^{\dagger}$ |
| Ca (meq 100g$^{-1}$) | 7.79c | 8.16c | 22.57b | 31.84a |
| Mg (meq 100g$^{-1}$) | 3.62c | 3.48c | 9.15a | 7.78b |
| Na (meq 100g$^{-1}$) | 0.15c | 0.41b | 1.52a | 1.47a |
| Fe (mg kg$^{-1}$) | 118.7a | 21.6b | 3.2c | 1.8c |
| Mn (mg kg$^{-1}$) | 14.1a | 2.6b | trace | trace |
| Zn (mg kg$^{-1}$) | trace | trace | trace | trace |
| Cu (mg kg$^{-1}$) | 1.2a | 1.4a | 1.0b | 0.9c |

$^{\dagger}$ n=1

Table 2. Soil water contents (%$H_2O$, w/w) at which different pF values were obtained (ORSTOM, Laboratoire de Physique du sol), and the container capacity (determined as in Cassel and Nielsen, 1986) of the topsoil (A horizon, 0-10 cm) from La Vîbora, Veracruz. The pF measurements were performed on air dry soil which may have lost some physical properties.

| Parameter/Description | % $H_2O$ (w/w) |
|---|---|
| pF 0 = saturation | 38.4 |
| pF 2 = Field capacity | 19.2 |
| pF 2.5 | 7.6 |
| PF 3 = sluggish capillary adjustment | 5.5 |
| PF 4.2 = plant wilting point | 4.2 |
| Container capacity | 35.4 |

Table 3. $\delta^{13}$C and $\delta^{15}$N signatures (mean ± standard deviations) of the soil profile, A horizon light OM fractions and earthworm tissue (Gen. nov.1) from the pasture at La Víbora, Veracruz. Numbers accompanied by different letters within the same column indicate significant differences at the $p<0.05$ level). nd = not determined.

| | $\delta^{13}$C (‰) | sd | $\delta^{15}$N (‰) | sd |
|---|---|---|---|---|
| Bulk A horizon | -19.09a | (±0.56) | 4.67d | (±2.48) |
| Fraction <0.1mm | -17.83 | nd | 5.94 | nd |
| Light fraction >0.1mm | -18.82 | nd | 5.16 | nd |
| Light fraction >0.4mm | -19.21 | nd | 4.25 | nd |
| Light fraction >2mm | -19.85 | nd | 4.47 | nd |
| Gen. nov. earthworms | -19.49a | (±0.48) | 7.40c | (±0.29) |
| $B_1$ Horizon | -15.0bc | (±0.32) | 9.27b | (±0.73) |
| $B_2$ Horizon | -15.59b | (±0.51) | 9.51b | (±0.78) |
| C Horizon | -14.91c | (±0.55) | 12.04a | (±1.00) |

Table 4. Percentage light OM (floating and particulate) fractions (LF) and heavy fractions (HF) in the topsoil (0-10cm) horizon from La Víbora, Veracruz. Fractions were gravitationally separated in $H_2O$ after shaking for 17 hours. Values are means of 3 replicates for % LF, while C and N values are results of a composite sample analysis. nd = not determined.

| Particle size (mm) | **Light OM fraction** (%) | LF C (%) | LF N (%) | Heavy fraction (%) | HF C (%) | HF N (%) |
|---|---|---|---|---|---|---|
| >2.0 | 0.10 | 19.33 | 1.38 | 0.13 (gravel) | nd | nd |
| 0.4-2.0 | 0.79 | 32.0 | 2.05 | 6.2 (coarse sand) | nd | nd |
| 0.2-0.4 | 1.00 | nd | nd | 46.4 (medium sand) | nd | nd |
| 0.1-0.2 | 0.74 | 11.34 | 1.0 | 26.3 (fine sand) | nd | 0.64 |
| <0.1 | 0 | nd | nd | 21.0 (very fine sand+silt+clay ) | nd | nd |

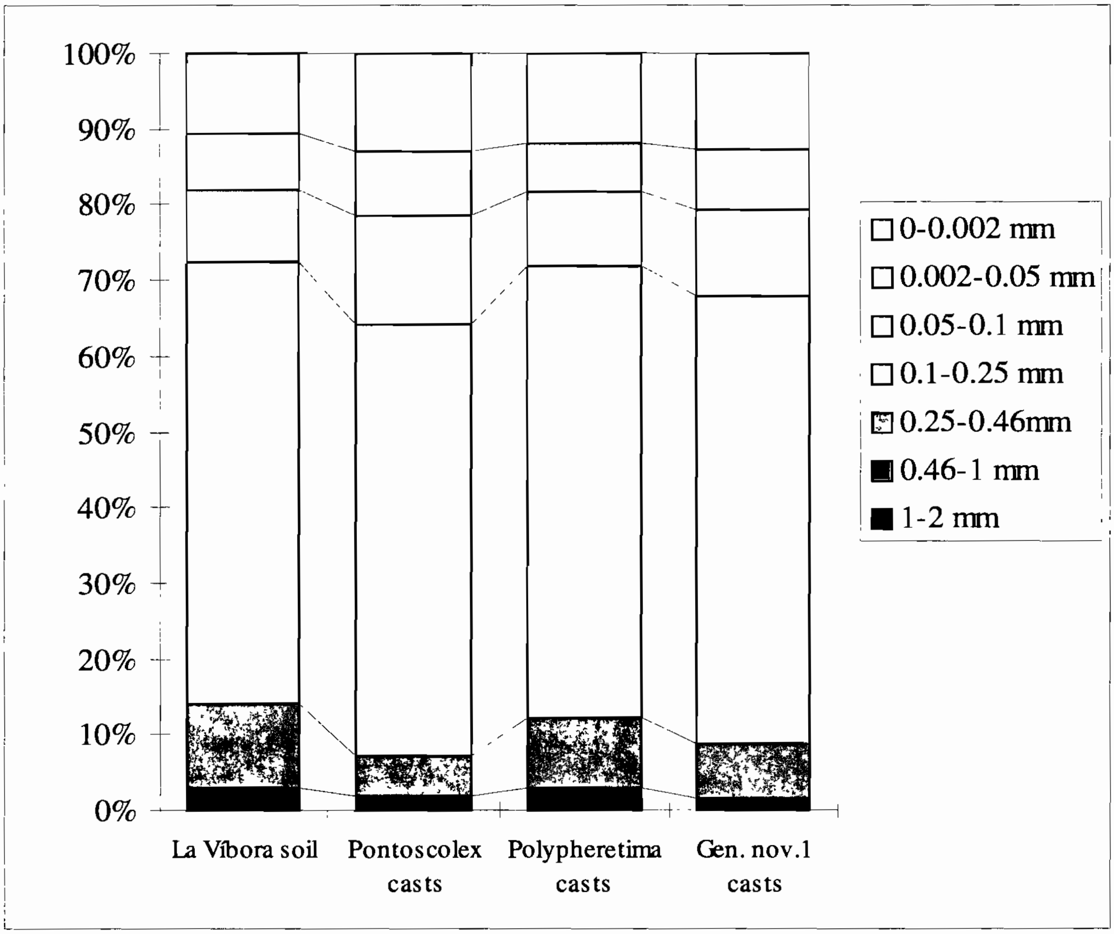


Figure 2. Texture of the topsoil from La Víbora and castings of Gen. nov.1, *Pontoscolex corethrurus* and *Polypheretima elongata* produced in laboratory cultures using the topsoil (0-10cm) horizon.

# ANNEXE 3

## CHAPITRE 3.

Results of the mineralogical analysis (ORSTOM-Bondy) of the soil profile horizons at La Vîbora, Veracruz.

Horizon A: Smectite et/ou interstratifié irrégulier se refermant mal à 10Å au chaffage, pic large (échantillon mal cristallisé). Un peu de minéral à 7Å probablement kaolinite désrdonnée et/ou interstratifié kaolinite-smectite. Un peu de quartz. Un peu de cristobalite.

Horizon B1: Smectite et/ou interstratifié irrégulier se refermant mal à 10Å au chaffage, pic large (échantillon légèrement mal cristallisé). Un peu de minéral à 7Å probablement kaolinite désrdonnée et/ou interstratifié kaolinite-smectite. Un peu de quartz. Un peu de cristobalite.

Horizon B2: Smectite et/ou interstratifié irrégulier se refermant mal a 10Å au chaffage, pic large (échantillon mieux cristallisé). Un peu de minéral à 7Å probablement kaolinite désrdonnée et/ou interstratifié kaolinite-smectite. Un peu de quartz.

Horizon BC-C: Smectite et/ou interstratifié irrégulier se refermant mal a 10Å au chaffage, pic large (échantillon mieux cristallisé). Un peu de minéral à 7Å probablement kaolinite désrdonnée et/ou interstratifié kaolinite-smectite. Traces possibles de goethite. Traces de quartz.

## CHAPITRES 3-7.

Brief description of the earthworm *Pontoscolex corethrurus* (from Sims and Gerard, 1985).

*Pontoscolex corethrurus* (F. Müller)
*Lumbricus corethrurus* (F. Müller, 1856)
*Pontoscolex corethrurus*: Gates, 1972

Length 60-120mm, diameter 4-6mm, segment number 90-212. Dorsal pores absent. Prostomium absent but everted buccal tissue may stimulate a prostomium or proboscis, as segment 1 is retractile. This organ probably helps the earthworm sense its environment and may perform a chemo-sensitive function (Kale et al., 1981). Spermathecal pores paired, minute (seldom seen) in furrows 6/7/8/9 in setal line c. Female pores paired slits on segment 14, located by furrow 14/15 between setal lines ab. Male pores minute (seldom seen) paired in furrow 20/21 nearby to setal line b. Clitellum saddle-shaped over segments (15) 16-17 (18) with the tubercula pubertatis forming (paired) longitudinal bands across segments 19-21 (22) laterally to setal line b.

Setae closely paired anteriorly becoming gradually wider apart on alternate segments until posteriorly the quincunx arrangement is attained (setal couples being alternately closely and widely paired on successive segments); posterior setae modified, stout distally with rows of fine teeth. Setae ab on segments carrying the tubercula pubertatis and adjacent segments, with genital tumescences.

Internally, the oesophagus has 3 pairs of extra-mural (stalked) calciferous glands in segments 7, 8 9. Single pair of testes in segment 11 (metandry) with the seminal vesicles in 12 extending back for several segments.

Distribution: Pantropical, especially in the moist and warmer regions, principally in disturbed ecosystems, although in Southern Mexico, Brazil and Venezuela (center of origin presumed to be in the Guyanan Shield) can be commonly found under primary vegetation.

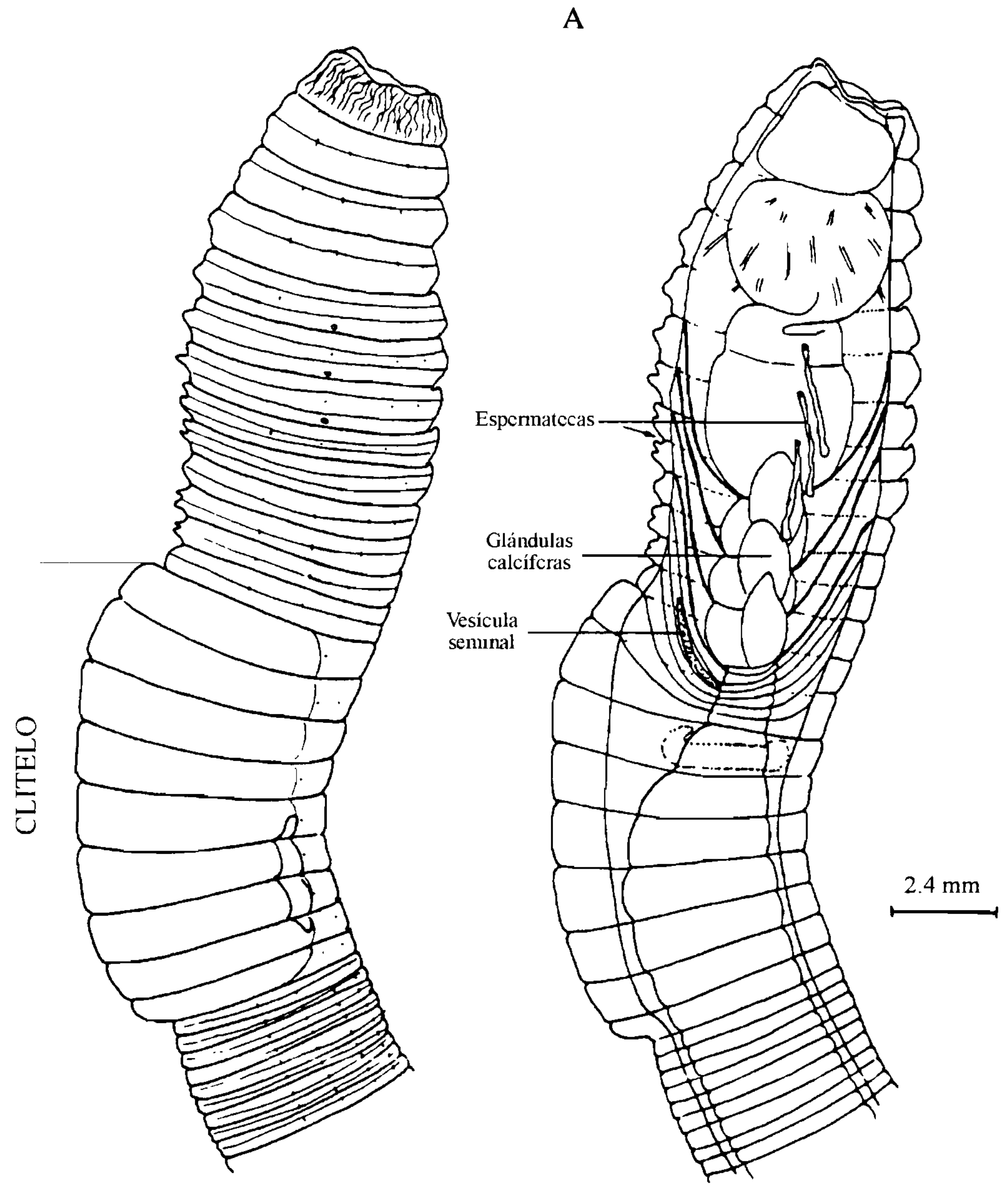
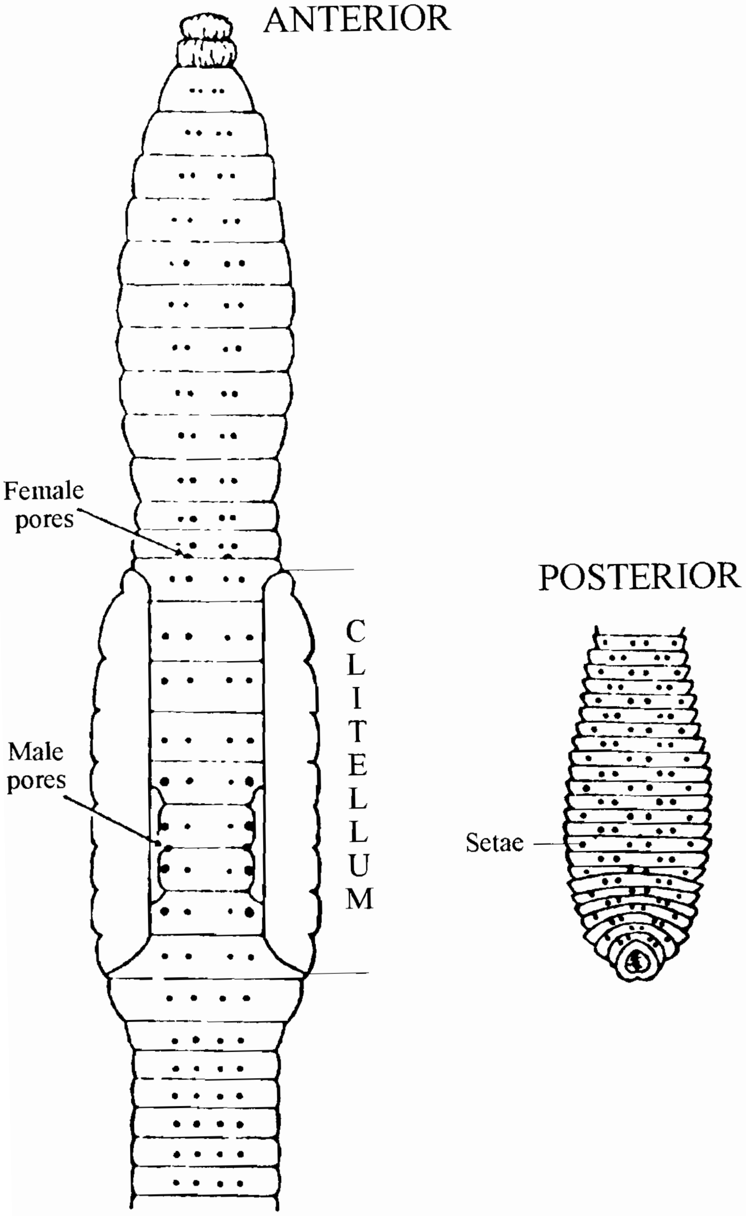


Figure 1. Several views of the external and internal morphology of *P. corethrurus*. A. Lateral view of external and internal structures drawn by Ana Moreno, from Borges (1988). B. Ventral view anteriorly and posteriorly (note distribution of setae) as in Sims and Gerard (1985).

**B**


ANTERIOR
Female pores
Male pores
CLITELLUM
POSTERIOR
Setae

# ANNEXE 5

## CHAPITRE 3.

Table 1. Detailed results of the laboratory cultures using *Pontoscolex corethrurus* earthworms in soil from La Víbora, Veracruz. A. Earthworm biomass; B. Cocoon production; C. Cast production. (Barois, Irisson and Brown, unp. data).

A. Earthworm biomass

| NO. DE CAJA | Parametro | FECHA | | | | | | FECHA | | | | | | FECHA | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 12/6/95 | 26/6/85 | 10/07/95 | 23/07/95 | 7/08/95 | 21/08/95 | 4/09/ 95 | 19/09/95 | 2/10/95 | 23/10/95 | 15/11/95 | 1/12/95 | 13/12/95 | 4/01/96 | 29/01/96 | 13/02/96 | 5/03/96 |
| | | DIAS TRANSCURRIDOS | | | | | | | | | | | | | | | | |
| | | 0 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 | 141 | 164 | 180 | 192 | 214 | 240 | 255 | 276 |
| 1 | PESO DE LOMBRICES | 0,067 | 0,1218 | 0,0778 | 0,16 | 0,215 | 0,347 | | | | | | | | | | | |
| | | 0,085 | 0,142 | 0,2027 | 0,3102 | 0,405 | 0,4215 | 0,2766 | 0,2764 | 0,2946 | 0,2338 | | | | | | | |
| | | 0,095 | 0,1546 | 0,233 | 0,3132 | 0,4417 | 0,4434 | 0,3512 | 0,3703 | 0,3726 | 0,5628 | 0,6225 | 0,1166 | 0,1992 | 0,5074 | 0,443 | 0,3675 | 0 |
| | | 0,106 | 0,1575 | 0,2559 | 0,3297 | 0,4713 | 0,5344 | 0,407 | 0,382 | 0,9088 | 1,1764 | 1,2648 | 0,5388 | 0.4792 | 0,5342 | 0,6539 | 0,6467 | 0,8967 |
| | | 0,116 | 0,182 | 0,262 | 0,3512 | 0,4765 | 0,5985 | 0,7552 | 0,9935 | 1,064 | 1,2662 | 1,5537 | 0,6127 | 0,6208 | 0,6498 | 0,7237 | 0,8489 | 1,4801 |
| 2 | PESO DE LOMBRICES | 0,059 | | | | | | | | | | | | | | | | |
| | | 0,07 | 0,108 | 0,1725 | 0,255 | | | | | | | | | | | | | |
| | | 0,0711 | 0,1372 | 0,18 | 0,287 | 0,3306 | 0,4868 | 0,5945 | 0,659 | 0,9103 | 1,3623 | 1,0763 | 0,8437 | 1,2476 | 0,9214 | 0,9482 | 1,1144 | 1,2865 |
| | | 0,131 | 0,184 | 0,2572 | 0,4429 | 0,3879 | 0,5824 | 0,6502 | 0,8245 | 0,9587 | 1,3672 | 1,1067 | 1,0166 | 1,2921 | 1,2196 | 1,1587 | 1,1498 | 1,5638 |
| | | 0,1476 | 0,24 | 0,3546 | 0,5092 | 0,6389 | 0,7896 | 0,6973 | 0,9384 | 1,214 | 1,3788 | 1,3585 | 1,0328 | 1,3221 | 1,665 | 1,5466 | 1,1475 | 1,146 |
| 3 | PESO DE LOMBRICES | 0,0952 | 0,1205 | 0,1407 | 0,2133 | 0,316 | 0,4108 | 0,5602 | 0,608 | 0,6589 | 0,7605 | 1,0425 | 0,6625 | 0,837 | 0,737 | 0,875 | 0,357 | 0,3236 |
| | | 0,1033 | 0,129 | 0,1513 | 0,2317 | 0,317 | 0,4361 | 0,5783 | 0,6664 | 0,7724 | 0,9682 | 1,0628 | 0,8751 | 1,0855 | 0,8358 | 0,9896 | 0,8009 | 0,878 |
| | | 0,118 | 0,1425 | 0,1724 | 0,2536 | 0,3363 | 0,4619 | 0,6139 | 0,6865 | 0,8121 | 0,99 | 1,0643 | 0,9243 | 1,1149 | 0,9187 | 0,9939 | 0,8561 | 0,9851 |
| | | 0,1217 | 0,1669 | 0,2036 | 0,2593 | 0,3532 | 0,4632 | 0,6344 | 0,7196 | 0,8225 | 1,0428 | 1,1741 | 0,9551 | 1,1982 | 0,9379 | 1,0792 | 0,9593 | 0,994 |
| | | 0,123 | 0,2831 | 0,2093 | 0,3333 | 0,3954 | 0,5536 | 0,7645 | 0,8356 | 0,9884 | 1,486 | 1,2143 | 0,9654 | 1,2873 | 0,9771 | 1,1354 | 0,9566 | 1,1743 |
| 4 | PESO DE LOMBRICES | 0,0595 | 0,0597 | 0,0345 | | | | | | | | | | | | | | |
| | | 0,0611 | 0,097 | 0,1537 | 0,2086 | 0,3336 | 0,4195 | 0,3999 | 0,2382 | 0,2795 | 0,7261 | 0,7229 | 0,9261 | 0,9386 | 0,7802 | 0,982 | 0,9616 | 0,4625 |
| | | 0,0656 | 0,1152 | 0,1811 | 0,2514 | 0,389 | 0,5185 | 0,7293 | 0,8758 | 0,814 | 1,1275 | 0,9903 | 1,0505 | 1,1023 | 0,8102 | 0,9942 | 0,9927 | 0,9516 |
| | | 0,0892 | 0,1606 | 0,2216 | 0,2882 | 0,3911 | 0,5224 | 0,7606 | 0,9595 | 0,8532 | 1,2211 | 1,3551 | 1,2022 | 1,3078 | 0,8874 | 1,0437 | 1,0034 | 1,1716 |
| | | 0,1532 | 0,2602 | 0,3601 | 0,4694 | 0,6042 | 0,685 | 0,7847 | 0,8784 | 0,8685 | 1,1542 | 1,5205 | 1,2394 | 1,321 | 1,0245 | 1,1983 | 1,1803 | 1,525 |
| 5 | PESO DE LOMBRICES | 0,0199 | | | | | | | | | | | | | | | | |
| | | 0,0328 | 0,0346 | 0,0513 | 0,0739 | 0,129 | 0,2279 | 0,1171 | 0,156 | 0,4808 | 0,7838 | 0,6573 | 1,157 | 0,6672 | 0,6761 | 0,6154 | 0,6835 | 0,6953 |
| | | 0,0357 | 0,0742 | 0,0852 | 0,168 | 0,2753 | 0,4621 | 0,3726 | 0,6402 | 0,7067 | 0,9678 | 0,9824 | 0,8892 | 0,9956 | 0,7495 | 0,8436 | 0,966 | 0,8962 |
| | | 0,066 | 0,1157 | 0,1525 | 0,1786 | 0,2756 | 0,4922 | 0,3798 | 0,7769 | 0,8332 | 1,0509 | 1,244 | 0,9904 | 1,1041 | 0,8317 | 0,8797 | 1,0911 | 1,2023 |
| | | 0,0772 | 0,2106 | 0,19 | 0,2906 | 0,3707 | 0,6603 | 0,9455 | 0,8708 | 0,9597 | 1,4696 | 1,3991 | 1,1466 | 1,2051 | 0,9515 | 1,0599 | 1,1136 | 1,1502 |
| | Individuos | 25 | 23 | 23 | 22 | 21 | 21 | 20 | 20 | 20 | 20 | 19 | 19 | 19 | 19 | 19 | 19 | 18 |

Table 1. Continuation

B. Cocoon production

| NO. DE CAJA | PARAMETROS | FECHA | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 12/6/95 | 26/6/95 | 10/07/95 | 24/07/95 | 7/08/95 | 21/08/95 | 4/08/95 | 19/09/95 | 2/10/95 | 23/10/95 | 15/11/95 | 1/12/95 | 13/12/95 | 4/01/96 | 29/01/96 | 13/02/96 | 5/03/96 |
| | | DIAS TRANSCURRIDOS | | | | | | | | | | | | | | | | |
| | | 0 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 | 143 | 164 | 180 | 192 | 214 | 240 | 255 | 276 |
| 1 | PRODUCCION DE CAPULLOS | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 7 | 3 | 0 | 0 | 0 | 0 | 0 | 2 |
| 2 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 6 | 2 | 10 | 3 | 3 | 5 | 2 | 5 |
| 3 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 6 | 2 | 8 | 0 | 4 |
| 4 | | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 2 | 4 | 7 | 4 | 5 | 4 | 4 | 6 | 5 | 4 |
| 5 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 8 | 13 | 8 | 7 | 7 | 0 | 5 | 7 | 4 |
| suma | | | | | | | | 6 | 5 | 18 | 33 | 18 | 25 | 20 | 9 | 24 | 14 | 19 |

C. Cast production (nc = not collected)

| NO. DE CAJA | PARAMETROS | FECHA | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 12/6/95 | 26/6/95 | 10/07/95 | 24/07/95 | 7/08/95 | 21/08/95 | 4/08/95 | 19/09/95 | 2/10/95 | 23/10/95 | 15/11/95 | 1/12/95 | 13/12/95 | 4/01/96 | 29/01/96 | 13/02/96 | 5/03/96 |
| | | DIAS TRANSCURRIDOS | | | | | | | | | | | | | | | | |
| | | 0 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 | 143 | 164 | 180 | 192 | 214 | 240 | 255 | 276 |
| 1 | POIDS DE TURRICULES | | 40,237 | 51,072 | 60,56 | 69,767 | 75,943 | 15,587 | 24,167 | 64,288 | nc | 34,681 | 37,812 | 33,422 | 18,83 | 44,32 | 17,44 | 84,23 |
| 2 | | | 54,002 | 36,163 | 26,915 | 27,879 | 19,152 | 65,733 | 18,472 | 74,196 | nc | 52,8 | 64,181 | 78,723 | 70,898 | 149,07 | 34,192 | 86,043 |
| 3 | | | 45,731 | 28,129 | 50,47 | 51,213 | 80,431 | 39,112 | 32,505 | 52,424 | nc | 41,347 | 46,748 | 8,3588 | 25,863 | 121,45 | 4,957 | 66,712 |
| 4 | | | 29,112 | 19,746 | 93,339 | 29,946 | 74,274 | 114,49 | 18,472 | 27,599 | nc | 53,521 | 79,434 | 98,885 | 32,094 | 72,691 | 25,549 | 36,007 |
| 5 | | | 4,46 | 15,842 | 17,904 | 41,302 | 45,342 | 27,224 | 24,167 | 18,472 | nc | 23,288 | 91,726 | 59,244 | 36,178 | 62,011 | 50,906 | 78,598 |
| | SUMA | | 173,54 | 150,95 | 249,19 | 220,11 | 295,14 | 262,15 | 117,78 | 236,98 | nc | 205,64 | 319,9 | 278,63 | 183,86 | 449,54 | 133,04 | 351,59 |
| | PROM/CAJA | | 34,708 | 30,19 | 49,837 | 44,021 | 59,028 | 52,43 | 23,557 | 47,396 | nc | 41,127 | 63,98 | 55,727 | 36,773 | 89,908 | 26,609 | 70,318 |
| | PROM/IND | | 7,5453 | 6,5631 | 11,327 | 10,481 | 14,054 | 13,107 | 5,8892 | 11,849 | nc | 10,823 | 16,837 | 14,665 | 9,677 | 23,66 | 7,0023 | 19,533 |
| | suma seco | | 143,96 | 125,22 | 206,71 | 182,59 | 244,83 | 217,47 | 97,707 | 196,59 | nc | 170,59 | 265,37 | 231,14 | 152,52 | 372,91 | 110,37 | 291,66 |

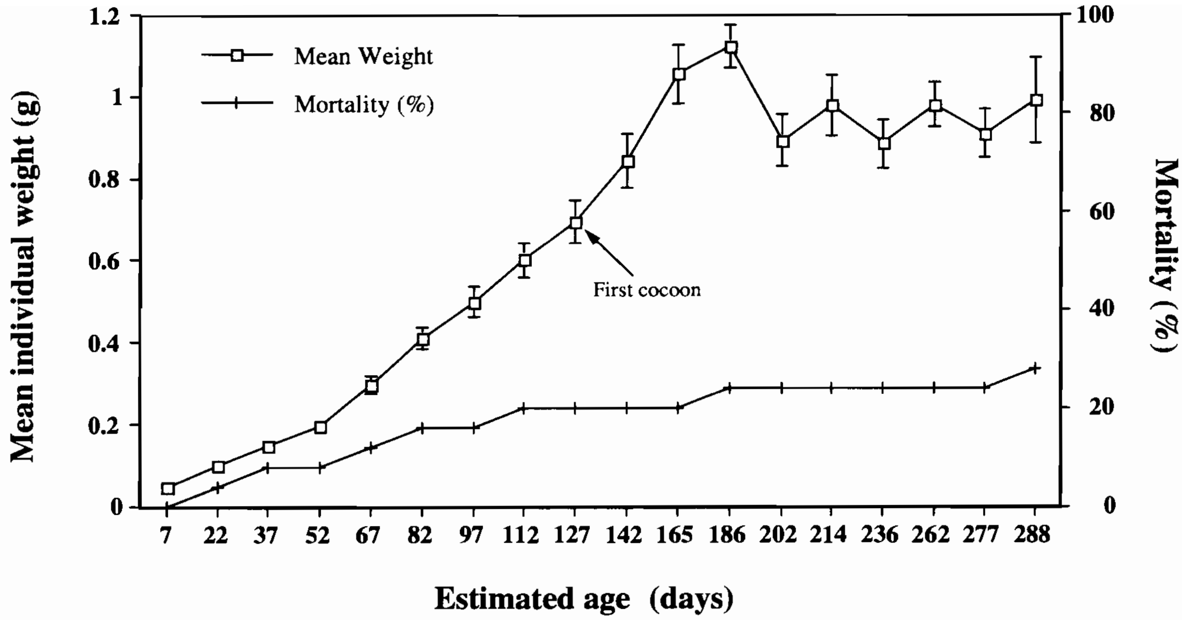


Figure 2. Growth and mortality of *P. corethrurus* in laboratory cultures using soil from La Víbora, Veracruz. (Data from Table 1 was modified to fit estimated ages)

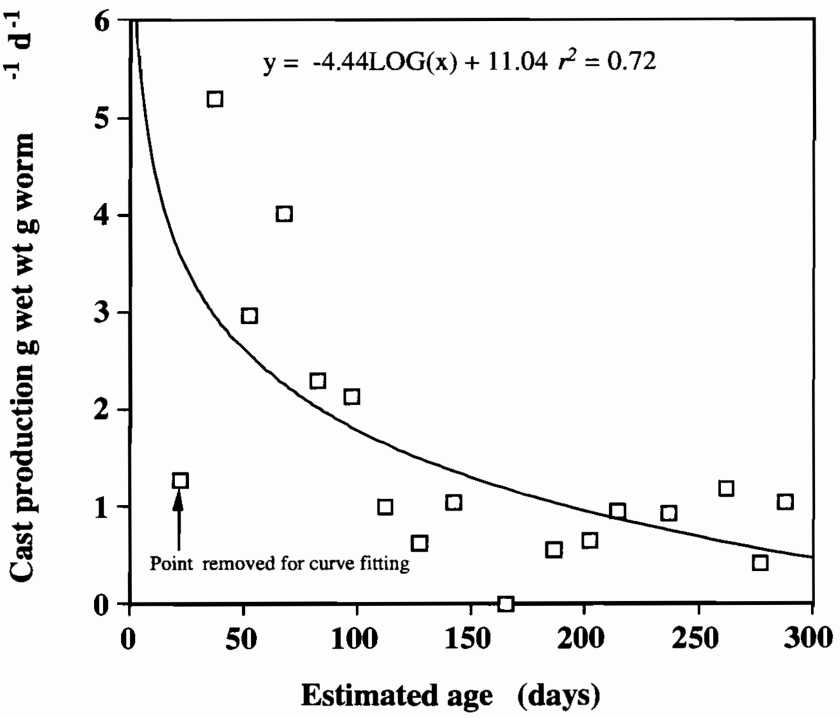


Figure 3. Surface cast production (in g) per weight (g) of *P. corethrurus* earthworms per day, according to the estimated age of the earthworm. Data from the laboratory cultures using soil from La Víbora, Veracruz (Table 1).

# ANNEXE 6

## CHAPITRES 3, 5 et 7.

Brief description of the earthwom *Polypheretima elongata* (from Borges, 1988).

Genera *Polypheretima* Michaelsen (1934)
Quart. J. Microsc. Sci. 77: 15. Type: *Perichaeta stelleri* Michaelsen (1892).

*Polypheretima elongata* (Perrier, 1872)

*Perichaeta elongata* Perrier (1872) Nouv. Arch. Mus. Hist. Nat. Paris 8:124. Type locality, Peru. in Musée de Histoire Naturelle de Paris.
*Metapheretima elongata*, complex of species: Sims and Easton (1972), Biol. J. Linn. Soc. (London) 4: 255.
*Pheretima elongata*: Gates (1972), Trans. Amer. Phil. Soc. 62: 182.
*Metapheretima elongata*: Easton (1976), Bull. Br. Mus. Nat. Hist. (Zool.) 30: 40.
*Polypheretima elongata*: Easton (1979), Bull. Br. Mus. Nat. Hist. (Zool.) 30: 53

Anatomía externa:

Esta especie es de color grisáceo, con la parte anterior color marfil y el clitelo crema, rojizo o amarillento. 213-274 segmentos. 127-225mm de largo; diámetro preclitelar 3.5-5mm, clitelar 3.5-4.5mm y postclitelar 3-5mm. Peso 1.39-2.93g. El prostomio es reducido o ausente. Tiene surcos longitudinales en el prostomio y en el peristomio. Segmentos 1-3 anillación sencilla, segmentos 4-13 y postclitelares trianillados, con excepción de los últimos 10 sencillos.

Poro dorsal en 12/13. Poros nefridiales ausentes (tiene meronefrídios). Poros epermatecales ventrolaterales en 5/6 y/o 6/7. Un poro feminino medioventral en 14. Poros masculinos en 18 y situados en poróforos circulares que descargan en bolsas copulatorias. Clitelo anular en 14, 15 y 16. Es liso, sin quetas ni intersegmentos, ni poros dosales.

Quetas periquetianas regularmente dispuestas alrededor de cada segmento, con un espacio mayor entre las aa y tambien entre las zz. A lo largo de todo el cuerpo, las quetas a y son más largas que las demás, y a partir del segmento 7, las a son más largas aún. 62-77 quetas en 7, 55 quetas en 37, 60-62 quetas entre 51-70. Quetas sexuales ausentes. Marcas genitales presetales comienzan en 19 y pueden estar presentes sucesivamente hasta 24. Son ovaladas, usualmente pareadas y están localizadas más o menos en línea con los poros masculinos.

Anatomía interna:

Faringe hasta 5. Premolleja 7/8 y 9/10. Intestino inicia en 15, con tiflosol simple y lameliforme. Profundidad, una sexta parte del diámetro del lumen del intestino. 5 pares de corazones laterales en 9-13. Holándrica. Embudos seminales y sacos testiculares en 10 y 11. Vesículas seminales en 11 y 12 y pseudoseminales en 13. Ovários y embudos ováricos en 13. Espermatecas piriformes presentes o ausentes en 5 y 6, dependendo del indivíduo.

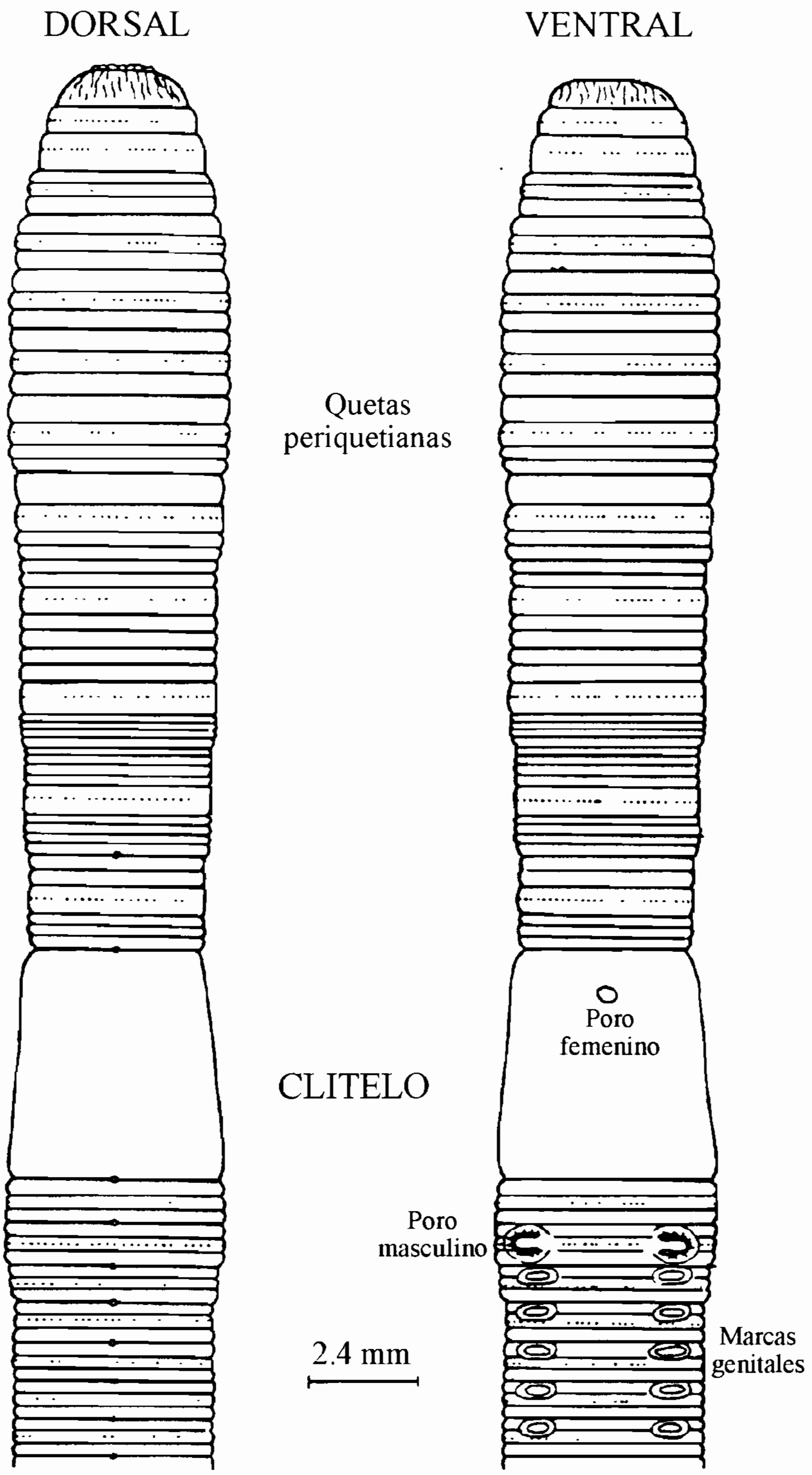


Figure 1. External ventral and dorsal views of *P. elongata* (drawings by Ana Moreno, in Borges, 1988).

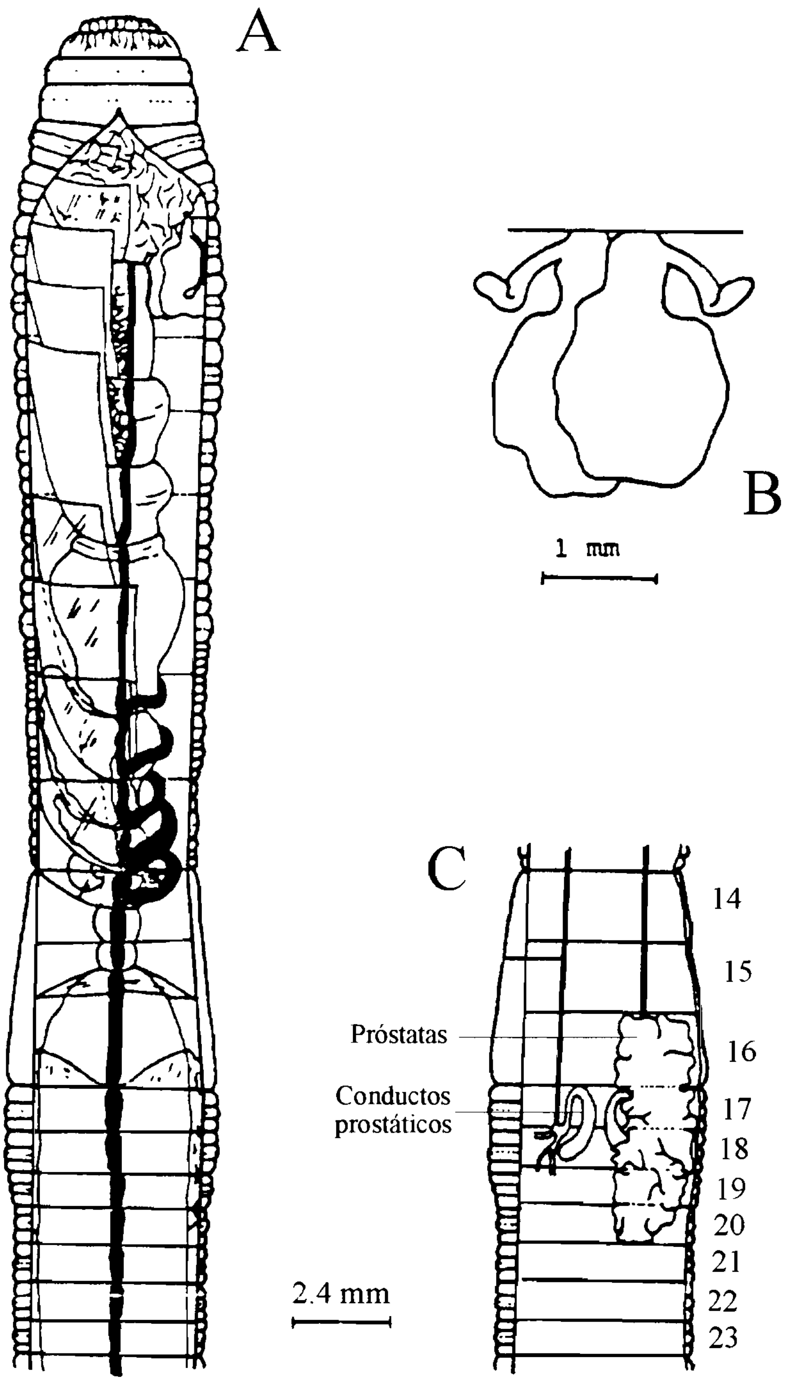


Figure 2. Internal anatomy of *P. elongata*. A. Ventral view, showing circulatory system, crop, gizzards and intestine. B. Detail of two spermatheca. C. Detail of the genitalia in segments 14-23.

# ANNEXE 7

## CHAPITRE 3.

Table 1. Detailed results of the laboratory cultures of *P. elongata* earthworms in soil from La Víbora, Veracruz. A. Earthworm biomass; B. Cocoon poduction; C. Cast production. (Barois, Irisson and Brown, unp. data)

A. Earthworm biomass.

| NO. DE CAJA | Parametro | FECHA | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 12/6/95 | 26/6/85 | 10/07/95 | 23/07/95 | 7/08/95 | 21/08/95 | 4/09/ 95 | 19/09/95 | 2/10/95 | 23/10/95 | 15/11/95 | 1/12/95 | 13/12/95 | 4/01/96 | 29/01/96 | 13/02/96 | 5/03/95 |
| | | DIAS TRANSCURRIDOS | | | | | | | | | | | | | | | | |
| | | 0 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 | 141 | 164 | 180 | 192 | 214 | 255 | 270 | 291 |
| 1 | PESO DE LOMBRICES | 0,017 | | | | | | | | | | | | | | | | |
| | | 0,023 | | | | | | | | | | | | | | | | |
| | | 0,032 | 0,0571 | 0,0696 | | | | | | | | | | | | | | |
| | | 0,049 | 0,0642 | 0,0823 | 0,062 | | | | | | | | | | | | | |
| | | 0,096 | 0,07 | 0,0865 | 0,0945 | 0,0712 | 0,1145 | 0,1444 | 0,2272 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | PESO DE LOMBRICES | 0,0308 | | | | | | | | | | | | | | | | |
| | | 0,0364 | 0,0133 | 0,0193 | | | | | | | | | | | | | | |
| | | 0,0452 | 0,036 | 0,0905 | 0,0642 | 0,0514 | 0,1001 | 0,2191 | 0,1319 | 0,1794 | | | | | | | | |
| | | 0,0673 | 0,0882 | 0,1475 | 0,1275 | 0,1245 | 0,1544 | 0,1729 | 0,1468 | 0,2012 | 0,342 | 0,2594 | 0,2957 | 0,2835 | 0,2671 | 0,2 | | |
| | | 0,0748 | 0,1222 | 0,1665 | 0,1918 | 0,161 | 0,2034 | 0,2191 | 0,3379 | 0,3976 | 0,8088 | 0,588 | 0,5388 | 0,6177 | 0,6403 | 0,5068 | 0,7901 | 0,8825 |
| 3 | PESO DE LOMBRICES | 0,0535 | 0,0697 | | | | | | | | | | | | | | | |
| | | 0,0548 | 0,1018 | 0,0828 | 0,0821 | 0,0865 | 0,1092 | 0,161 | 0,1412 | 0,1673 | | | | | | | | |
| | | 0,0673 | 0,1047 | 0,0919 | 0,1026 | 0,0953 | 0,1512 | 0,2105 | 0,2542 | 0,2843 | 0,5008 | 0,4869 | 0,456 | 0,5451 | 0,5042 | 0,4442 | 0,6435 | 0,6131 |
| | | 0,0681 | 0,1069 | 0,0957 | 0,1463 | 0,1224 | 0,2158 | 0,2532 | 0,404 | 0,462 | 0,8705 | 0,7363 | 0,7465 | 0,8275 | 0,7544 | 0,6293 | 0,8318 | 0,8846 |
| | | 0,0696 | 0,1305 | 0,1525 | 0,1906 | 0,182 | 0,2411 | 0,3152 | 0,4352 | 0,5227 | 0,8822 | 0,7603 | 0,7666 | 0,8753 | 0,767 | 0,6748 | 0,8665 | 0,8945 |
| 4 | PESO DE LOMBRICES | 0,0579 | 0,0954 | | | | | | | | | | | | | | | |
| | | 0,0691 | 0,124 | | | | | | | | | | | | | | | |
| | | 0,0747 | 0,1274 | 0,1362 | 0,1862 | 0,1587 | 0,2128 | 0,2275 | 0,3601 | 0,3482 | | | | | | | | |
| | | 0,0833 | 0,1574 | 0,1409 | 0,1952 | 0,1745 | 0,2238 | 0,256 | 0,428 | 0,4594 | 0,2665 | 0,497 | 0,5093 | | | | | |
| | | 0,0992 | 0,1733 | 0,1997 | 0,2111 | 0,2014 | 0,2389 | 0,2581 | 0,4974 | 0,5053 | 0,4708 | 0,706 | 0,6215 | 0,3614 | 0,3081 | 0,2966 | 0,3527 | 0,515 |
| 5 | PESO DE LOMBRICES | 0,0587 | | | | | | | | | | | | | | | | |
| | | 0,0609 | 0,1285 | 0,1219 | 0,1076 | 0,1105 | 0,1123 | 0,1354 | 0,1794 | 0,1319 | | | | | | | | |
| | | 0,0784 | 0,1368 | 0,1328 | 0,1535 | 0,1597 | 0,117 | 0,2101 | 0,3151 | 0,3795 | 0,3946 | 0,3917 | 0,244 | 0,5845 | 0,5298 | 0,5791 | 0,602 | 0,9826 |
| | | 0,0807 | 0,165 | 0,1455 | 0,1833 | 0,1675 | 0,1765 | 0,3601 | 0,5487 | 0,6542 | 1,0847 | 0,8353 | 0,8678 | 0,7099 | 0,6689 | 0,7633 | 0,7852 | 0,7523 |
| | | 0,1041 | 0,2681 | 0,2594 | 0,2764 | 0,314 | 0,3775 | 0,6038 | 0,8941 | 0,8968 | 1,1192 | 0,9144 | 0,9292 | 0,7933 | 0,7107 | 0,7714 | 0,842 | 1,0343 |
| | No. individuos | 25 | 21 | 18 | 16 | 15 | 15 | 15 | 15 | 14 | 10 | 10 | 10 | 9 | 9 | 9 | 8 | 8 |

Table 1. continuation

B. Cocoon production

| NO. DE CAJA | Parametro | FECHA | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 12/6/95 | 26/6/85 | 10/07/95 | 23/07/95 | 7/08/95 | 21/08/95 | 4/09/ 95 | 19/09/95 | 2/10/95 | 23/10/95 | 15/11/95 | 1/12/95 | 13/12/95 | 4/01/96 | 29/01/96 | 13/02/96 | |
| | | DIAS TRANSCURRIDOS | | | | | | | | | | | | | | | | |
| | | 0 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 | 141 | 164 | 180 | 192 | 214 | 255 | 255 | |
| 1 | PRODUCCION DE CAPULLOS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 2 | | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 3 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 4 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 5 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |

C. Cast production

| NO. DE CAJA | Parametro | FECHA | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 12/6/95 | 26/6/85 | 10/07/95 | 23/07/95 | 7/08/95 | 21/08/95 | 4/09/ 95 | 19/09/95 | 2/10/95 | 23/10/95 | 15/11/95 | 1/12/95 | 13/12/95 | 4/01/96 | 29/01/96 | 13/02/96 | |
| | | DIAS TRANSCURRIDOS | | | | | | | | | | | | | | | | |
| | | 0 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 | 141 | 164 | 180 | 192 | 214 | 255 | 255 | |
| 1 | PRODUCCION DE TURRICULOS | | 5,565 | 2,4795 | 3,6239 | 0 | 2,6522 | 0,4464 | 1,443 | 3,1282 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 2 | | | 8,3127 | 5,6942 | 3,9348 | 4,46 | 3,6291 | 9,6177 | 1,5186 | 10,72 | | 0 | 24,373 | 7,3519 | 7,7708 | 4,245 | 9,177 | |
| 3 | | | 11,387 | 5,486 | 1,3918 | 6,0129 | 5,0155 | 6,7449 | 7,0482 | 8,9592 | | 39,385 | 49,207 | 41,211 | 4,9372 | 6,6631 | 27,35 | |
| 4 | | | 11,165 | 6,5746 | 1,2536 | 15,072 | 3,4207 | 2,5943 | 7,0482 | 14,112 | | 25,527 | 46,289 | 50,835 | 6,5566 | 4,5758 | 2,0287 | |
| 5 | | | 13,666 | 6,9019 | 2,4582 | 8,1708 | 5,2029 | 9,8113 | 25,397 | 28,605 | | 42,347 | 74,66 | 87,482 | 16,237 | 33,133 | 26,168 | |
| | SUMA | 0 | 50,096 | 27,136 | 12,662 | 33,716 | 19,92 | 29,215 | 42,455 | 65,525 | 0 | 107,26 | 194,53 | 186,88 | 35,501 | 48,617 | 64,724 | |
| | PROM/CAJA | 0 | 10,019 | 5,4272 | 2,5325 | 6,7431 | 3,9841 | 5,8429 | 8,491 | 13,105 | 0 | 21,452 | 38,906 | 37,376 | 7,1003 | 9,7234 | 12,945 | |
| | PROM/IND | 0 | 2,3855 | 1,5076 | 0,7914 | 2,2477 | 1,328 | 1,9476 | 2,8303 | 4,6803 | 0 | 10,726 | 19,453 | 20,764 | 3,9446 | 5,4019 | 8,0905 | |
| | suma seco | | 40,243 | 21,799 | 10,172 | 27,084 | 16,002 | 23,469 | 34,105 | 52,637 | 0 | 86,164 | 156,27 | 150,12 | 28,519 | 39,055 | 51,994 | |

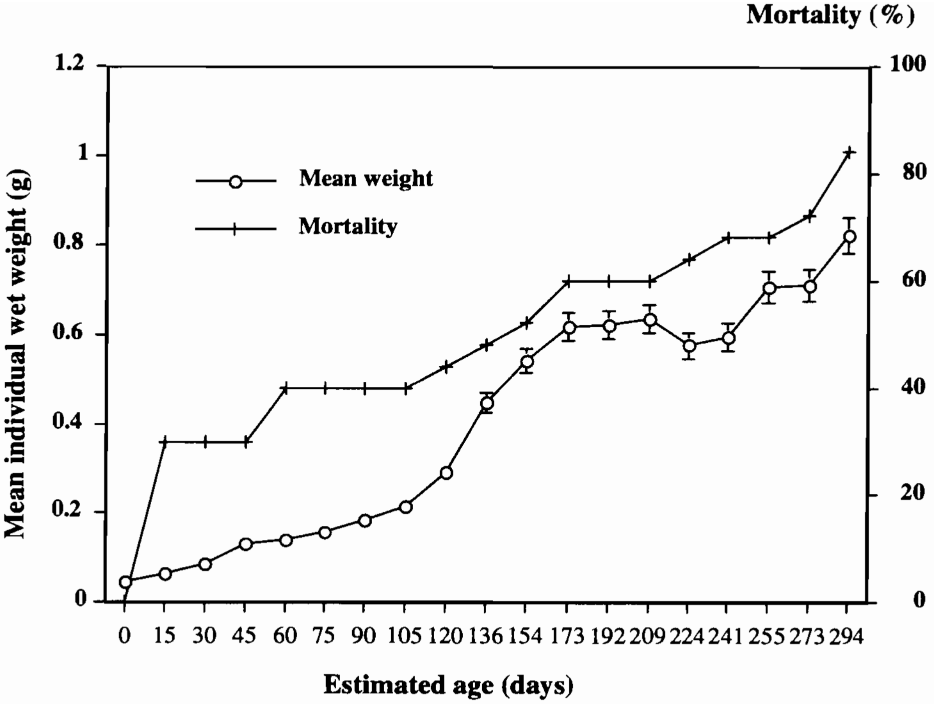


Figure 1. Growth and mortality of *P. elongata* in laboratory cultures using soil from La Víbora, Veracruz. (Data modified from Table 1 to fit estimated ages)

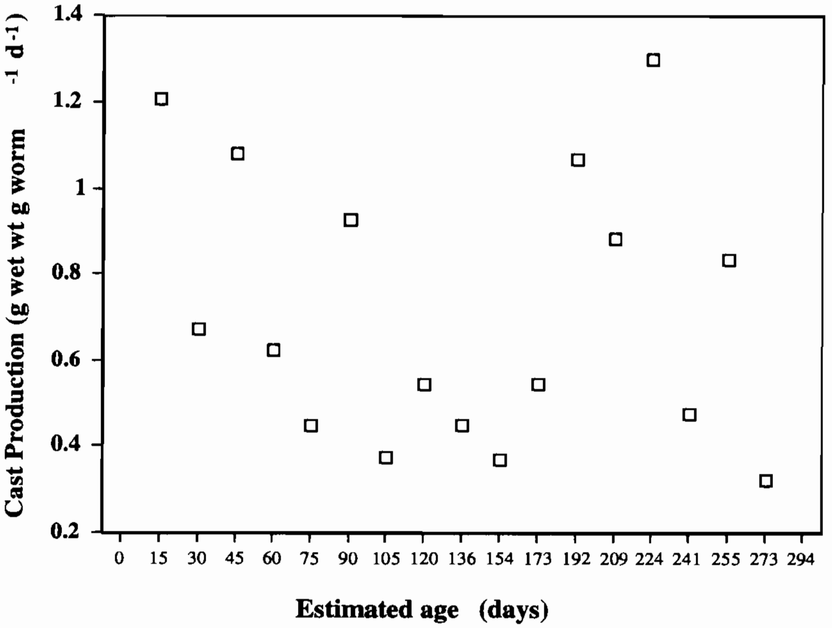


Figure 2. Surface cast production (in g) per weight (g) of *P. elongata* earthworms per day, according to the estimated age of the earthworm. Data from the laboratory cultures using soil from La Víbora, Veracruz (Table 1). No relationship between age and cast production was observed.

# ANNEXE 8

## CHAPITRE 4.

Description of the soil profiles at the Estación de Biología Tropical "Los Tuxtlas," Veracruz.

Profiles dug by Michel Brossard and George Brown, on July 28, 1994.

FOREST PROFILE
Profile 60 cm deep.

General feeling of the soil:
In digging, encountered lots of rocks, some of which broke with the shovel, others not. Superficial layer of leaves was 2.5cm deep, with many termites and ants. These are probably the more important litter processers in this soil (together with millepedes). Encountered an earthworm at 30 cm, which was preserved in a small jar. Abundant white-rot fungi, and very abundant small superficial roots in the top litter layer. Ectomycorrhizae also abundant and easily visible. Roots made cutting through the soil very difficult. Roots travelled vertically up to 20-25 cm and then mostly horizontally as they encountered rocks of a more dense horizon.

Ao (litter) horizon 2.5cm deep.

A1 horizon 6-10 cm deep. Crumbly, very highly aggregated by root and fungal activities. Darker brown color.

A2 horizon clayier, 10-30 (35) cm deep, with less root activity and a higher bulk density. Less aggregated.

A3 or B, at depth of 30-35 cm, where clay content increases drastically, though limit (transition) is gradual. The color of the soil, its feeling and bulk density also change, being more paste-like and with apparently less $H_2O$. At this horizon, roots are still present but rare and in isolated spots. This is probably very a deep layer (we did not reach the end of it), due to accumulation of sediment in this zone from the higher areas. Rocks found in this horizon were from small (a few cm), to very large (>30cm). Most of larger ones were rounded off, without rough edges.

To solve the problem of the deepness of the profile, we went to the roadside and cleared a horizon to get pictures and describe the colors etc. The profile is described as follows:

ROADSIDE PROFILE
Descriptive samples for laboratory analysis taken from the Ao, A1 and B (10-20, 20-30, 30-40, 40-50 cm depths) horizons.

Dacite or andesite are the parent materials. Some primary minerals were present - small and centimetric after 50 cm depth. All the profile has a clayey texture, compacted after 80 cm. Since this profile gets more sun, it is drier than in the woods so not very representative of what would be seen there (since the soil is volcanic in origin and allophanes are abundant, the soil loses many of its original properties after drying).

Ao and A1 Horizon (in total 0-35 to 40 cm deep)
Ao horizon:
0-10cm
Between fresh and dry. Roots very abundant, fine and medium sizes. Underneath the leaves there was lots of fungus, and both VAM and ECM (mycelia visible), plus white-rot fungi. Substructure was granular, very fine, with texture determination dificult due to the abundance of organic matter (probably >10%).
Color 7YR 3/2 (wet). Structure polyhedric or cubic and very friable. Centimetric
51.4% $H_20$. 66% solids. Out of 1639g, 1419= soil 2mm, 178=rocks > 2mm, and 41.5g= roots.
Weight of leaves and organic material on surface in eight replicate 25x25 cm blocks, mean 42.3g = 6.8Mg $ha^{-1}$.
A1 horizon:
10-20cm
Fresh, fine granular structure. Aparently loamy clay. 1 or 2 fine milimetric remains of primary minerals.
Color 7.5YR 3/2 (wet).
45.3% $H_20$, 68.8% solids.
20-30cm
More humid than above horizon. Same texture and structure as 10-20.
Color 10 YR 2/2 (wet).
42.8% $H_20$. 70% solids.

B Horizon:
From 35-40 cm to >2meters deep
30-40cm
Clayey, fresh and humid. Fine porosity very important. Massive structure. Substructure polyhedric angular, with very fine angles. Some bright spots on surfaces indicating free water. Primary minerals were somewhat abundant, yellow, and the size of sands and clean.
Color 7.5 YR 3/4 (wet).
40% $H_20$, 71.4% solids.
40-50cm
Clayey, but drier than above horizon. Fine porosity important. Massive strucure with polyhedric fine substructures. Friable, angular. Minerals the same as above.
Color 5 YR 3/3, 4/3 or 3/4 (wet).
38.6% $H_2O$, 72.2% solids.

Conclusion: Sol Brun Andique ou Sol Brun Socielle à Halloysite?

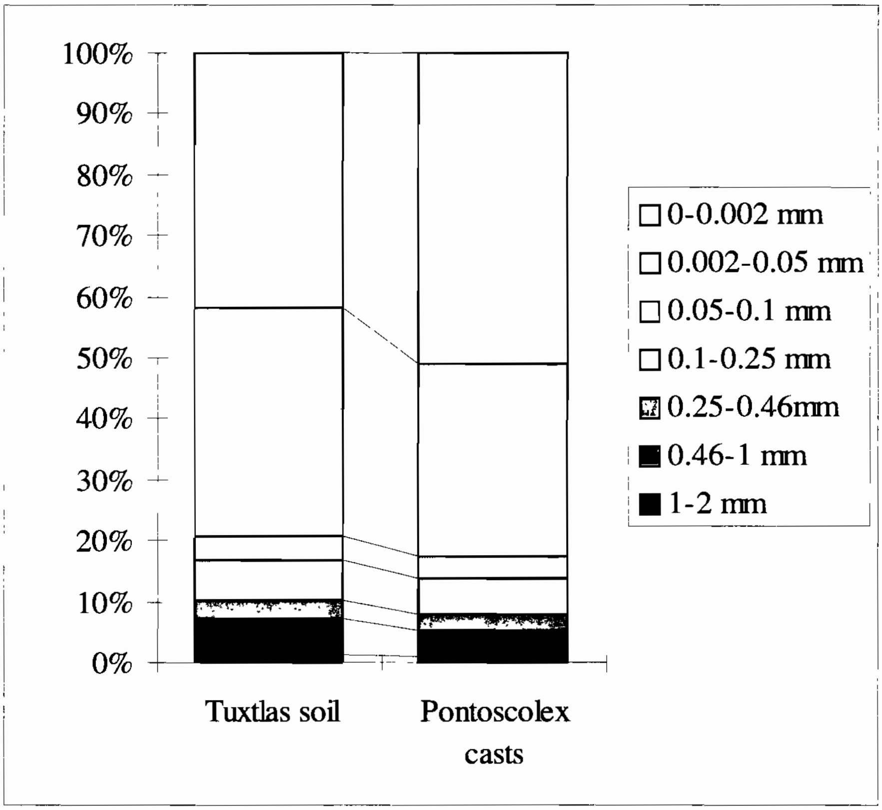


Figure 1. Texture of the topsoil (USDA classification) from the forest at Los Tuxtlas and castings of *Pontoscolex corethrurus* produced in laboratory cultures using the topsoil (texture obtained as in Gee and Bauder, 1986).

Table 1. Soil water contents (%$H_2O$, w/w) at which different pF values were obtained (ORSTOM, Laboratoire de Physique du sol), and the container capacity (determined as in Cassel and Nielsen, 1986) of the topsoil (A horizon, 0-10 cm) from the forest at Los Tuxtlas, Veracruz. The pF measurements were performed on air dry soil which may have lost some physical properties. Field capacity of this soil is probably closer to 80% (near the container capacity).

| Parameter/Description | % $H_2O$ (w/w) |
|---|---|
| pF 2 = Field capacity | 47.2 |
| pF 2.5 | 39.4 |
| PF 3 = sluggish capillary adjustment | 35.4 |
| PF 4.2 = plant wilting point | 31.1 |
| Container capacity | 74.4 |

Table 2. Soil and earthworm (*P. corethrurus*) tissue $\delta^{13}C$ and $\delta^{15}N$ (means ± standard deviations) from the topsoil (A Horizon; 0-10cm) of the tropical rainforest at Los Tuxtlas, Veracruz. nd = not determined

| | $\delta^{13}C$ (‰) | sd | $\delta^{15}N$ (‰) | sd |
|---|---|---|---|---|
| Bulk A horizon | 27.30 | ±0.17 | 6.00 | ±1.68 |
| Fraction >0.1mm | -25.85 | nd | 3.74 | nd |
| Fraction <0.1mm | -26.74 | ±0.29 | 6.41 | nd |
| Light fraction >0.1mm | -26.83 | nd | 4.98 | nd |
| Light fraction >0.2mm | -27.2 | nd | 4.18 | ±0.32 |
| Light fraction >0.4mm | -27.85 | nd | 3.26 | nd |
| *Ponstocolex corethrurus* | 24.82 | ±0.13 | 7.05 | ±0.27 |

Table 3. Percentage light OM (floating and particulate) fractions (LF) and heavy fractions (HF) in the topsoil (0-10cm) horizon from the forest at Los Tuxtlas, Veracruz. Fractions were gravitationally separated in $H_2O$ after shaking for 17 hours. Values are means of 3 replicates for % LF, while C and N values were taken from a composite sample (n=1) of the three separates in each fraction. nd = not determined

| Particle size (mm) | Light OM fraction (%) | LF C (%) | LF N (%) | Heavy fraction (%) | HF C (%) | HF N (%) |
|---|---|---|---|---|---|---|
| >2.0 | 0.34 | nd | nd | 0.91 (gravel) | nd | nd |
| 0.4-2.0 | 2.21 | 35.0 | nd | 13.64 (coarse sand) | nd | nd |
| 0.2-0.4 | 0.68 | 12.1 | 1.77 | 9.57 (medium sand) | nd | nd |
| 0.1-0.2 | 0.43 | 12.9 | 1.26 | 6.71 (fine sand) | 3.84 | 0.55 |
| <0.1ı | 0ı | ndı | ndı | 69.18 (very fine ı sand+silt+clay ) | 5.85 | 0.73 |

# ANNEXE 9

## CHAPITRE 4.

Results of the mineralogical analysis (ORSTOM-Bondy) of the soil profile horizons in the forest at Los Tuxtlas, Veracruz.

Ao Horizon: Halloysite a 7Å et/ou kaolonite désordonnée. Un peu de goethite. Un peu de hématite. Un peu de magnétite. Un peu de quartz.

A1 Horizon (10-20cm): Halloysite a 7Å et/ou kaolonite désordonnée. Un peu de goethite. Un peu de hématite. Un peu de magnétite. Un peu de quartz.

A1 Horizon (20-30cm): Halloysite a 7Å et/ou kaolonite désordonnée. Un peu de goethite. Un peu de hématite. Traces de magnétite.

B1 Horizon (30-40cm): Halloysite a 7Å et/ou kaolonite désordonnée. Un peu de goethite. Un peu de hématite. Un peu de magnétite.

B1 Horizon (40-50cm): Halloysite a 7Å et/ou kaolonite désordonnée. Un peu de goethite. Un peu de hématite. Traces de magnétite.

Interprétation sélon Didier Dubroeucq, ORSTOM (IRD), Bondy:

Los picos de las arcillas en los diagramas (0.73nm y 0.445nm) quedan muy bajos en comparación con el contenido global en arcilla de la muestra. Esto significa que estas arcillas no producen reflecciones claras con los rayos X, debido a su grado cristalino muy bajo y su permanencia en estado desordenado, incluso después de un tratamiento de secado con sedimentación lenta (arcillas orientadas). Las muestras de suelos a simple vista presentan una microagregración bastante desarrollada y probablmente de origen biológica. Parecen ser suelos de bosque tropical.

Estas arcillas son haloisitas tubulares. En la muestra más profunda (del B1 a 40-50cm) aparecen pocas cantidades de minerales interestratificados con esmectita. No aparece ninguna inflexión a 1.0nm característica de la haloisita hidratada. Si las muestras fueron secadas todas en la estufa antes de entregarlas, es normal porque el secado a 80-100°C hace desaparecer el pico a 1.0nm. Pero si solamente han sido secadas al aire (este es el caso de estas muestras) este resultado corresponde más a arcillas microcristalinas y desordenadas. Dichas arcillas son características de la parte superficial bioturbada de los suelos de bosque tropical húmedo que han sufrido un secado climáctico, tal vez por deforestación.

Note: the soil had been air-dried to an extent that may have changed some of the physical properties, thus the statement of Didier regarding the secado climáctico…

# ANNEXE 10

## CHAPITRE 4.

Table 1. Detailed results of the laboratory cultures using *P. corethrurus* earthworms and soil from the forest at Los Tuxtlas, Veracruz (Hernández, Barois and Brown, unp. data). A. Earthworm biomass; B. Cocoon production; C. Cast production; D. Hatchling production (weights).

A. Earthworm biomass

| NO. DE CAJA | Parametro | FECHA | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 15/11/96 | 29/11/96 | 13/12/96 | 20/12/96 | 7/01/97 | 21/01/97 | 04/2/ 97 | 27/02/97 | 13/03/97 | 27/03/97 | 10/04/97 | 24/04/97 | 08/05/97 | 22/05/97 | 05/06/97 | 19/06/97 |
| | | DIAS TRANSCURRIDOS | | | | | | | | | | | | | | | |
| | | 0 | 14 | 28 | 35 | 53 | 67 | 81 | 104 | 118 | 132 | 146 | 160 | 174 | 188 | 202 | 216 |
| 1 | PESO DE LOMBRICES | 0,846 | 0,1668 | 0,6395 | 0,2032 | 0,3943 | 1,0128 | 0,5528 | 0,7028 | 1,0992 | 0,9171 | 0,9138 | 0,5025 | 0,7735 | 0,8539 | 0,4985 | 0,5095 |
| | | 0,213 | 0,865 | 0,2375 | 0,9533 | 0,3937 | 0,5805 | 1,0519 | 0,496 | 0,968 | 0,6477 | 0,6589 | 0,6567 | 0,7975 | 0,6093 | 0,7478 | 0,684 |
| | | 0,235 | 0,2563 | 0,9698 | 0,354 | 0,6083 | 0,4982 | 0,5765 | 0,5253 | 0,5956 | 0,6914 | 0,6511 | 1,0013 | 0,5058 | 0,7123 | 0,4949 | 0,7883 |
| | | 0,179 | 0,4532 | 0,3125 | 0,6977 | 0,8393 | 0,772 | 0,6845 | 0,9911 | 0,6975 | 1,0201 | 1,0436 | 0,8679 | 0,4195 | 0,503 | 0,5784 | 0,4528 |
| | | 0,092 | 0,331 | 0,4075 | 0,4767 | 0,9689 | 1,028 | 0,857 | 0,4461 | 0,6723 | 0,5477 | 0,6186 | 0,4753 | 0,4903 | 0,5315 | 0,8067 | 0,4431 |
| 2 | PESO DE LOMBRICES | 0,1803 | 0,322 | 1,116 | 0,5081 | 0,6594 | 0,7682 | 1,131 | 0,6951 | 0,722 | 0,7514 | 1,1408 | 0,6868 | 0,788 | 0,8404 | 0,6128 | 0,4439 |
| | | 0,5941 | 0,2798 | 0,1231 | 0,3813 | 0,5778 | 0,7088 | 0,3772 | 0,5275 | 0,7475 | 1,0379 | 0,8452 | 0,6055 | 0,503 | 0,6856 | 0,5404 | 0,7417 |
| | | 0,163 | 1,0037 | 0,3939 | 0,4693 | 0,2032 | 1,0902 | 0,7795 | 0,7608 | 1,0623 | 0,7474 | 0,8587 | 0,7206 | 0,6056 | 0,6493 | 0,7598 | 0,5562 |
| | | 0,1407 | 0,2717 | 0,4496 | 0,1489 | 0,5134 | 0,7526 | 0,7287 | 0,7313 | 0,8562 | 0,8416 | 0,9363 | 0,8464 | 0,6047 | 0,5881 | 0,5742 | 0,6245 |
| | | 0,0542 | 0,0867 | 0,3401 | 1,0721 | 1,0704 | 0,3371 | 0,5982 | | | | | | | | | |
| 3 | PESO DE LOMBRICES | 0,3972 | 0,8462 | 0,3613 | 0,8085 | 0,9683 | 1,0022 | 0,9538 | 0,3397 | 0,912 | 0,4608 | 0,7228 | 0,7197 | 0,6398 | 0,625 | 0,7546 | 0,575 |
| | | 0,1179 | 0,6614 | 0,804 | 0,8613 | 0,5857 | 0,3229 | 0,4057 | 0,7946 | 0,9245 | 0,9706 | 0,8993 | 0,9766 | 0,7432 | 0,89 | 0,863 | 0,6049 |
| | | 0,0546 | 0,2683 | 0,7996 | 0,4144 | 0,9785 | 0,9914 | 0,7098 | 0,6518 | 0,7642 | 1,0943 | 0,4456 | 0,8723 | 0,8329 | 0,4282 | 0,6913 | 0,6667 |
| | | 0,334 | 0,1043 | 0,9271 | 0,1737 | 0,8947 | 1,0438 | 0,8779 | 0,6909 | 0,9369 | 0,7509 | 1,0329 | 0,3896 | 0,3467 | 0,7954 | | |
| | | 0,5528 | 0,6003 | 0,1397 | 0,8674 | 0,266 | 0,78 | 1,0272 | 0,6963 | 0,4538 | 0,7313 | | | | | | |
| 4 | PESO DE LOMBRICES | 0,621 | 0,5499 | 0,2396 | 0,8231 | 0,4558 | 0,6554 | 0,6813 | 0,809 | 0,8953 | 0,8471 | 0,6682 | 0,696 | 0,7355 | 0,6319 | 0,6412 | 0,4819 |
| | | 0,41 | 0,8403 | 0,2451 | 0,8003 | 0,82 | 0,9188 | 0,69 | 0,6886 | 0,712 | 0,7176 | 0,582 | 0,6669 | 0,5565 | 0,8703 | 0,6388 | 0,531 |
| | | 0,3594 | 0,1646 | 0,765 | 0,7589 | 0,3952 | 0,8562 | 0,894 | 0,8537 | 0,7629 | 0,4906 | 0,9256 | 0,4425 | 0,4226 | 0,7094 | 0,5485 | 0,4861 |
| | | 0,0912 | 0,6322 | 0,6725 | 0,2931 | 0,8167 | 0,5418 | 0,925 | 0,7001 | 0,8805 | 0,8588 | 0,7949 | 0,7834 | 0,6119 | 0,6713 | 0,7471 | 0,6029 |
| | | 0,0866 | 0,1698 | 0,7895 | 0,2738 | 0,8692 | 0,8586 | | | | | | | | | | |
| 5 | PESO DE LOMBRICES | 0,2602 | 0,5766 | 0,3869 | 0,8676 | 0,941 | 0,7622 | 0,7266 | 0,7948 | 0,8913 | 0,7812 | 0,7972 | 0,7384 | 0,9123 | 0,8822 | 0,8863 | 0,265 |
| | | 0,5542 | 0,2298 | 0,7976 | 0,7601 | 0,551 | 0,7424 | 0,7881 | 0,7256 | 0,2738 | 0,4264 | 0,9349 | 0,7339 | 0,7427 | 0,6953 | 0,7099 | 0,7294 |
| | | 0,1186 | 0,2277 | 0,8643 | 0,34 | 0,7455 | 1,0416 | 1,0876 | 0,6182 | 0,7129 | 0,8236 | 0,6993 | 0,9838 | 0,721 | 0,7313 | 0,6906 | 0,5797 |
| | | 0,2959 | 0,8545 | 0,6211 | 0,3956 | 0,85 | 0,999 | 0,9747 | 0,6032 | 1,0614 | 1,0018 | 0,3076 | 0,3383 | 0,291 | 0,2335 | 0,2239 | 0,59 |
| | | 0,1177 | 0,4087 | 0,3492 | 0,6743 | 0,4913 | 0,6915 | 0,3231 | 0,0412 | 0,6637 | | | | | | | |
| | No. de individuos | 25 | 25 | 25 | 25 | 25 | 25 | 24 | 23 | 23 | 22 | 21 | 21 | 21 | 21 | 20 | 20 |

Table 1. continuation

B. Cocoon production

| NO. DE CAJA | Parametro | FECHA | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 15/11/96 | 29/11/96 | 13/12/96 | 20/12/96 | 7/01/97 | 21/01/97 | 04/2/ 97 | 27/02/97 | 13/03/97 | 27/03/97 | 10/04/97 | 24/04/97 | 08/05/97 | 22/05/97 | 05/06/97 | 19/06/97 |
| | | DIAS TRANSCURRIDOS | | | | | | | | | | | | | | | |
| | | 0 | 14 | 28 | 35 | 53 | 67 | 81 | 104 | 118 | 132 | 146 | 160 | 174 | 188 | 202 | 216 |
| 1 | PRODUCCION DE CAPULLOS | 0 | 1 | 1 | 1 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | | 0 | 0 | 2 | 1 | 1 | 0 | 2 | 1 | 1 | 1 | 0 | 0 | 0 | 3 | 0 | 0 |
| 3 | | 0 | 0 | 1 | 2 | 1 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| 4 | | 0 | 1 | 3 | 0 | 0 | 2 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 |
| 5 | | 0 | 2 | 3 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |

C. Cast production

| NO. DE CAJA | Parametro | FECHA | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 15/11/96 | 29/11/96 | 13/12/96 | 20/12/96 | 7/01/97 | 21/01/97 | 04/2/ 97 | 27/02/97 | 13/03/97 | 27/03/97 | 10/04/97 | 24/04/97 | 08/05/97 | 22/05/97 | 05/06/97 | 19/06/97 |
| | | DIAS TRANSCURRIDOS | | | | | | | | | | | | | | | |
| | | 0 | 14 | 28 | 35 | 53 | 67 | 81 | 104 | 118 | 132 | 146 | 160 | 174 | 188 | 202 | 216 |
| 1 | POIDS DE TURRICULES | 0 | 6,5 | 13,01 | 1,37 | 0 | 9,09 | 4,9 | 7,29 | 4,28 | 7,36 | 18,18 | 13,15 | 32,84 | 17,91 | 19,14 | 36,2 |
| 2 | | 0 | 4,61 | 7,4 | 2,89 | 0 | 6,97 | 5,8 | 8 | 4,24 | 7,76 | 8,98 | 5,61 | 27,54 | 25,49 | 15,7 | 26,98 |
| 3 | | 0 | 3,2 | 16,91 | 1,35 | 0 | 7,45 | 5,4 | 10,55 | 4,11 | 17,37 | 7,01 | 18,25 | 24,06 | 35,83 | 20,63 | 36,16 |
| 4 | | 0 | 3,44 | 8,55 | 5,29 | 0 | 8,33 | 4,53 | 8,66 | 4,97 | 6,86 | 8,74 | 10,73 | 35,58 | 32,48 | 23,59 | 22,34 |
| 5 | | 0 | 6,98 | 9,59 | 3,45 | 0 | 11,69 | 4,18 | 4,98 | 6,07 | 8,98 | 12,55 | 17,25 | 31,58 | 24,98 | 43,56 | 25,36 |
| | SUMA | | 24,73 | 55,46 | 14,35 | 0 | 43,53 | 24,81 | 39,48 | 23,67 | 48,33 | 55,46 | 64,99 | 151,6 | 136,69 | 122,62 | 147,04 |
| | PROM/CAJA | | 4,946 | 11,092 | 2,87 | 0 | 8,706 | 4,962 | 7,896 | 4,734 | 9,666 | 11,092 | 12,998 | 30,32 | 27,338 | 24,524 | 29,408 |
| | PROM/IND | | 0,9892 | 2,2184 | 0,574 | 0 | 1,7412 | 1,0338 | 1,7165 | 1,0291 | 2,1968 | 2,641 | 3,0948 | 7,219 | 6,509 | 6,131 | 7,352 |
| | suma seco | | 6,1825 | 13,865 | 3,5875 | 0 | 10,883 | 6,2025 | 9,87 | 5,9175 | 12,083 | 13,865 | 16,248 | 37,9 | 34,173 | 30,655 | 36,76 |

## Table 1. continuation

### D. Hatchling production (weights)

| NO. DE CAJA | Parametro | FECHA | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 15/11/96 | 29/11/96 | 13/12/96 | 20/12/96 | 7/01/97 | 21/01/97 | 04/2/ 97 | 27/02/97 | 13/03/97 | 27/03/97 | 10/04/97 | 24/04/97 | 08/05/97 | 22/05/97 | 05/06/97 | 19/06/97 |
| | | DIAS TRANSCURRIDOS | | | | | | | | | | | | | | | |
| | | 0 | 14 | 28 | 35 | 53 | 67 | 81 | 104 | 118 | 132 | 146 | 160 | 174 | 188 | 202 | 216 |
| 1 | PRODUCCION de LOMBRICES | 0 | 0 | 0 | 0 | 0 | 0,033 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0,0464<br>0,0508 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | | 0 | 0 | 0 | 0 | 0 | 0 | 0,049 | 0 | 0,0478<br>0,0038 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0,0746 | 0,0443<br>0,0666<br>0,0609<br>0,0481 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | | 0<br>0,000 | 0<br>0 | 0<br>0 | 0<br>0 | 0<br>0 | 0<br>0 | 0<br>0 | 0 | 0,075<br>0,0391<br>0,0459 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | SUMA | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 11 | 0 | 0 | 0 | | | | |
| | PROM/CAJA | 0 | 0 | 0 | 0 | 0 | 0,2 | 0,4 | 0,2 | 2,2 | 0 | 0 | 0 | | | | |
| | PROM/IND | 0,000 | 0 | 0 | 0 | 0 | 0,04 | 0,0833 | 0,0435 | 0,4783 | 0 | 0 | 0 | | | | |

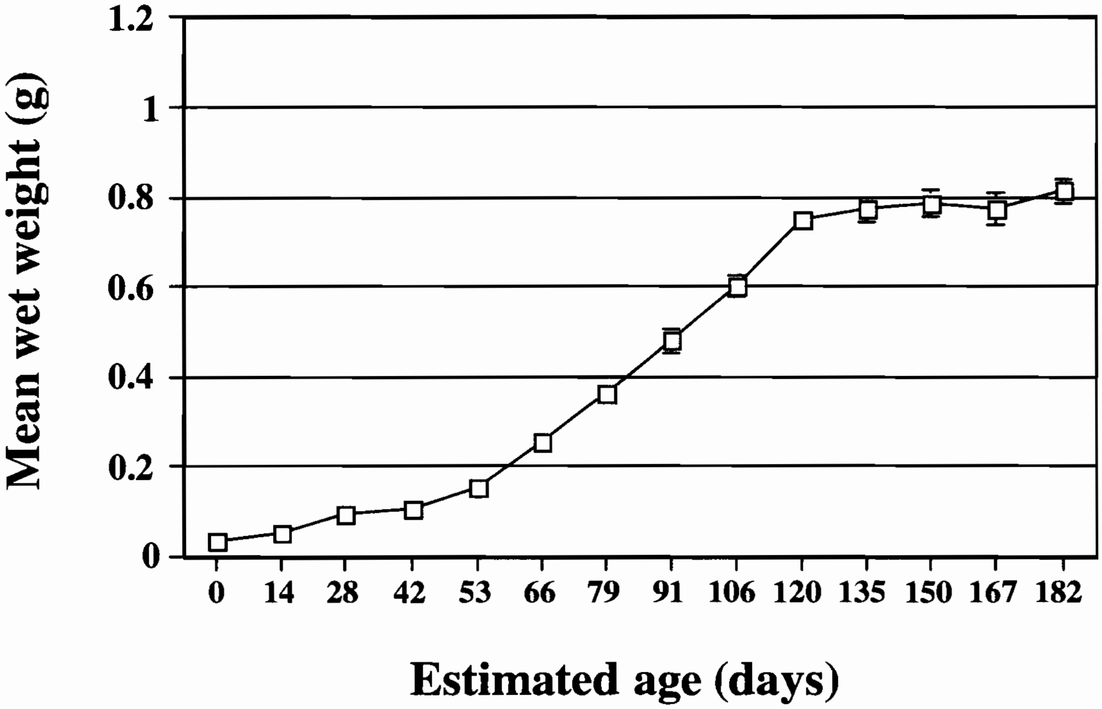


Figure 1. Growth of *P. corethrurus* in the laboratory cultures using soil from the forest at Los Tuxtlas, Veracruz (Data modified from Table 1 to fit estimated ages).