Soil Biol. Biochem. Vol. 24, No. 12, pp. 1629–1633, 1992
Printed in Great Britain.

0038-0717/92 $5.00 + 0.00

STIMULATION OF PLANT GROWTH BY TROPICAL EARTHWORMS

A. V. SPAIN,[1]* P. LAVELLE[2] and A. MARIOTTI[3]

[1]Davies Laboratory, CSIRO, Private Mail Bag, Aitkenvale, Queensland 4814, Australia, [2]Laboratoire d'Ecologie, Ecole Normale Supérieure, 46 rue d'Ulm, 75230 Paris Cedex 05 and [3]Université Paris VI, INRA, Laboratoire de Biogéochimie Isotopique, T26 4 Et, 4 Place Jussieu, 75252 Paris, France

Summary—In experiments conducted in the Côte d'Ivoire, increased growth of maize and guinea grass in an infertile, granite-derived soil was associated with the addition of earthworms to the soil in which these plants were grown. Experiments using ^{15}N-labelled *Millsonia anomala* and soil microbial biomass indicated an increased incorporation of N from the microbial biomass into *Panicum maximum* in the presence of earthworms.

INTRODUCTION

It has been recognized for some years that temperate–climate earthworms are capable of stimulating plant growth although little is yet known of the influences of non-lumbricid earthworms on plant production (Lee, 1985). It is thus of considerable importance to know whether tropical earthworms, with their more diverse ecological strategies (Lavelle, 1983), also promote plant growth.

This paper reports the results of four preliminary studies aimed at elucidating the role of selected tropical earthworm species on plant growth. The first experiment assesses the influence of four species of earthworms on the growth of maize (*Zea mays*). The second examines the influence of a series of different masses and two body-size classes of an endogeic earthworm (*Millsonia anomala* Acanthodrilidae) on the growth of *Panicum maximum*, a tropical perennial grass. The third experiment examines the response of *Panicum maximum* to the addition of two masses of eudrilid earthworms, *Chuniodrilus zielae* and *Stuhlmannia porifera*. The final experiment investigates the effects of *Panicum maximum* on the transfer of nitrogen from ^{15}N-labelled *M. anomala* and microbial biomass to *P. maximum*.

STUDY AREA

The studies were conducted at the Station d'Ecologie Lamto (5° 2′ W, 6° 13′ S, elevation 105 m) in the Côte d'Ivoire. Table 1 presents selected properties of the surface 10 cm of the granite-derived, savanna soil (ferralsol, FAO classification) used in the experiments. Average annual rainfall (25 yr of records) at the site is 1228 mm, largely distributed over two wet seasons that extend from March to October with a minimum in August. Average monthly temperature ranges from 25.7°C in August, to 28.8°C in February.

METHODS

Experiment 1. Effects of four earthworm treatments on the growth of maize

The following five earthworm species were selected:

(i) A mixture of unknown proportions of *Chuniodrilus zielae* and *Stuhlmannia porifera*, two small polyhumic endogeic species, probably closely associated with the rhizosphere.
(ii) *Hyperiodrilus africanus*, a pigmented, epi-endogeic species common in West Africa which feeds on soil and litter.
(iii) *Pontoscolex corethrurus* (Glossoscolecidae), an endogeic species common throughout the humid tropical zone but which does not occur at Lamto.
(iv) *Millsonia anomala*, a mesohumic endogeic species which dominates the Lamto savanna earthworm communities and attains 5–6 g live weight at the adult stage.

Shortly after planting, the biomasses (Table 2) of the five earthworm species listed in the above treatments were added to 10 litre plastic containers containing carefully-mixed, 2 mm-sieved soil from the top 10 cm (A horizon) of the profile. The containers were furnished with holes covered with a fine mesh to allow free drainage and prevent the entry or egress of earthworms and plant roots. All except the largest maize seedling was removed from the containers 1 week after emergence. The experiment was conducted as a completely-randomized design with four replicates of each treatment and ran for 12 weeks, from 25 November 1986 to 10 February 1987. At harvest, the oven-dry (105°C) biomasses of the roots and the above-ground parts of the plants were measured

*Present address for correspondence: Queensland National Parks and Wildlife Service, P.O. Box 5391, Townsville, Queensland 4810, Australia.

Table 1. Some properties of the granite-derived surface (0–10 cm) (Riou, 1974)

Property	Level	Property	Level
Clay	7.5%	Carbon	1.09%
Silt	14.0%	Nitrogen	0.076%
Fine sand (<50 μm)	29.4%	P_2O_5	0.18%
Coarse sand (>50 μm)	46.0%	Fe (total)	0.63%
Basic exchangeable cations [cmol $p(^+)$ kg^{-1}]			
Calcium	2.34	Magnesium	0.69
Potassium	0.12	Sodium	0.05

separately and the live biomasses of the earthworms remaining in the pots were also determined.

Experiment 2. The effects of different biomasses and two size groupings of individuals of M. anomala *on the growth of* Panicum maximum

A similar experimental procedure was used as in the previous experiment except that *Panicum maximum* was used as the test plant and three seedlings were permitted to grow in each bucket. A randomized-block layout was used, with each treatment replicated five times. Earthworms were added to the treatments within 3 days of planting. Biomasses of 1–5 g of earthworms were added to the buckets; 1 g is the equivalent of 275 kg ha^{-1} of earthworm biomass in the field or *ca* 144 mg of live earthworm biomass g^{-1} oven-dry (105°C) soil. Each biomass level was represented by both "small" (<0.5 g) and "large" (>1.0 g) earthworms. There were 10 control buckets with no earthworms added.

The experiment was planted on 1 April 1987, the seedlings started to emerge 5 days later and the mature plants were harvested after *ca* 79 days of growth. Tops were harvested by cutting *ca* 5 mm above ground level and root weights were determined by wet sieving and drying a random sample of the replicates to constant weight. A number of the replicates were used to assess treatment effects on soil structure; thus, the number of root samples is smaller than that of the above-ground material. Similarly, N and P concentrations of a subset of the foliage and root samples were determined using a Kjeldahl digest followed by colourimetric estimation.

Experiment 3. The effects of two species of Eudrilidae on the growth of Panicum maximum

The stimulatory effects of a mixed population of unknown proportions of two eudrilid earthworms (*C. zielae* and *S. porifera*) on the growth of *Panicum maximum* were examined using the same methods as for *M. anomala*). Treatment live biomasses of 0.5 and 1.0 g of these earthworms were added to the five replicates.

Experiment 4. Studies on the influence of M. anomala *on the transfer of nitrogen from the soil to* Panicum maximum

In order to examine the mechanisms whereby nitrogen transfers to the plant are influenced by the presence of earthworms, both the microbial biomass of the soil and the earthworms were labelled with ^{15}N. The microbial biomass of the soil was labelled by incubating the soil for a month (27°C, pF 2.5) with ^{15}N-labelled (77.7%) ammonium sulphate (1.67 g) and glucose mixed in such proportions that the C:N ratio was *ca* 15. After 1 month, juvenile specimens of *M. anomala* were introduced into the marked soil and maintained therein for 1 month to effect a labelling.

In the experiments, labelled soil was mixed with unlabelled soil at a ratio of 1:5.8 and the *P. maximum* plants were grown as described for experiment 2. Three combinations were used: labelled soil and unlabelled worms, labelled soil without worms and unlabelled soil with labelled worms. The abundance of ^{15}N was measured using a high precision mass spectrometer with an accuracy of 14^{-4} (Mariotti and Letolle, 1978).

RESULTS

Experiment 1. Effects of four earthworm treatments on the growth of maize

Table 2 presents the number of maize plants surviving to harvest in each treatment, their mean biomasses and the ratios of the root to the above-ground biomass. The maize plants survived poorly in this nitrogen- and phosphorus-deficient soil and those surviving grew indifferently and showed deficiency symptoms.

Neither *Pontoscolex corethrurus* nor *H. africanus* survived and the biomasses of the eudrilid earthworms *C. zielae* and *S. porifera* either remained constant or declined over the course of the experiment. The biomasses of *M. anomala* declined similarly although amongst the replicates in which both plants and earthworms survived to maturity,

Table 2. Experiment 1: the effects of four earthworm treatments on the survival and growth of maize plants ($n = 4$)

Species	Earthworm live biomass added per pot	Plants surviving to harvest	Mean earthworm biomass at harvest (g)	Mean plant biomass at harvest (g)	Ratio root: above-ground biomass
Control	0	1	0	1.19	0.88
C. zielae and *S. porifera*	1	3	0.88	1.93	0.59
H. africanus	2	2	0.01	1.05	0.30
Pontoscolex corethrurus	2	3	0	1.74	0.30
M. anomala	1	2	1.20	3.02	0.17
M. anomala	2	3	1.0	1.30	0.25
M. anomala	5	2	0.27	2.79	0.44

M. anomala biomasses increased slightly. Nonetheless, excluding the smallest surviving plant (which, perhaps because of insect damage, grew only to 18 cm), amongst all replicates where both plants and earthworms survived, there was a significant correlation ($r = 0.811$; $P < 0.01$, $n = 7$ between the natural logarithms of the total dry matter produced by *Panicum maximum* and earthworm biomasses at harvest (Fig. 1). In this same subset of replicates, no significant correlations existed between plant dry matter at harvest and the initial earthworm biomass added. In addition, the ratio root:above-ground biomass was lower where moderate (1–2 g) earthworm biomasses had been added but was greater with higher or lower biomasses.

Experiment 2. The effects of different biomasses and two size groupings of individuals of M. anomala *and on the growth of* Panicum maximum

No significant ($P > 0.05$, F-test) effects of size class of *M. anomala* on the growth of *Panicum maximum* were evident and data for the small and large individuals were combined at each biomass level.

Figure 2 (a)–(c) presents plots of, respectively, the harvested masses of the above-ground parts, the roots and the total masses of harvested materials presented on an oven-dried (105°C) organic matter basis. It is clear that, in all cases, plant biomass increased notably with added earthworm biomass. Regression analyses indicated the following relationships between the oven-dry biomasses of *P. maximum* at harvest and those of *M. anomala* added to the containers at the start of the experiment:

$$\log_e(\text{Ba}) = \underset{(0.13\ \text{SE})}{1.46} + \underset{(0.04\ \text{SE})}{0.20\text{Be}},\ R^2 = 30.0\%,\ n = 57$$

$$\log_e(\text{Br}) = \underset{(0.13\ \text{SE})}{2.09} + \underset{(0.14\ \text{SE})}{0.18\text{Be}},\ R^2 = 33.3\%,\ n = 36$$

$$\log_e(\text{Bt}) = \underset{(0.14\ \text{SE})}{2.53} + \underset{(0.05\ \text{SE})}{0.19\text{Be}},\ R^2 = 33.1\%,\ n = 36$$

Ba is the biomass of *P. maximum* above ground, Br is the root biomass, Bt is the total biomass of the plant and Be is the live biomass (g) of earthworm per container.

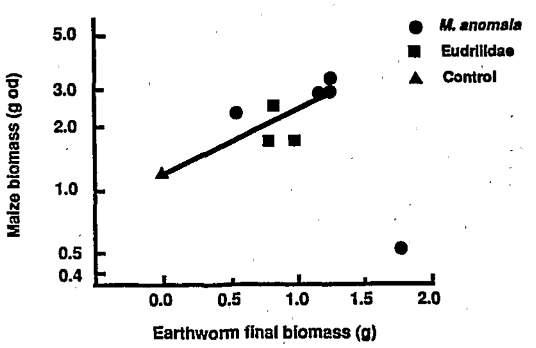

Fig. 1. Harvested total maize biomass in relation to the final live earthworm biomass.

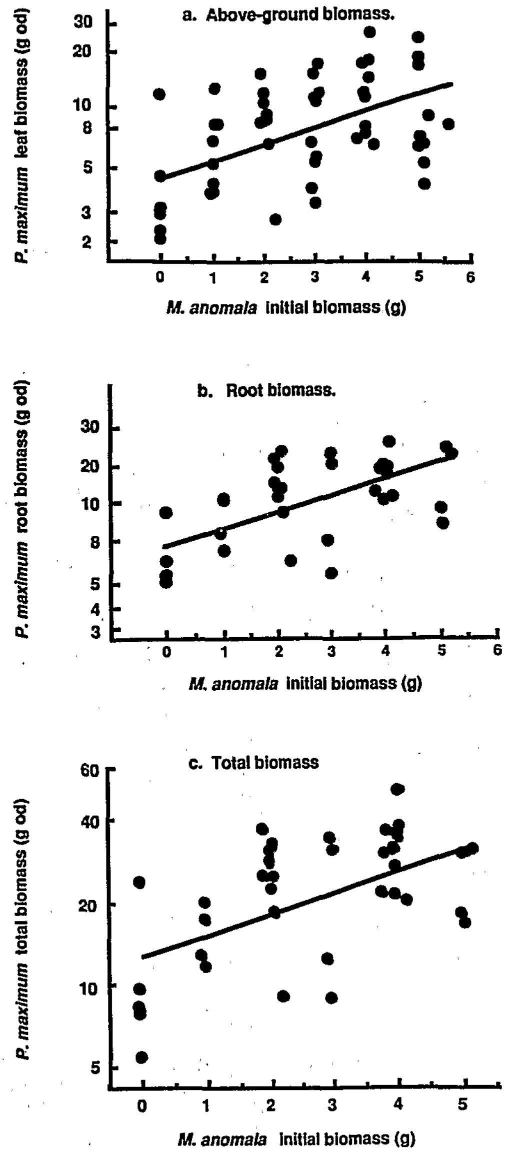

Fig. 2. Harvested biomass of *Panicum maximum* in relation to the initial live biomass of *M. anomala*.

The plots of above-ground, root and total biomass with the biomass of *M. anomala* added suggest a curvilinear relationship, although pure error tests proved non-significant ($P > 0.05$). The ratio of root:above-ground biomass (Fig. 3) increased with added earthworm biomass to a maximum at *ca* 3 g and thereafter diminished.

Over the course of the experiment, earthworm biomass increased in all except the 5.0 g treatments. At harvest, the mean biomasses of *M. anomala* in the five weight categories changed as follows (initial:harvest): 1.01:2.90; 2.04:4.28; 3.00:3.39; 4.00:5.16; 5.11:3.88. There were significant ($P < 0.01$) correlations between the yield of *P. maximum* and the biomass of *M. anomala* remaining at harvest, despite some mortality following storms that flooded certain pots and led to the death of a few

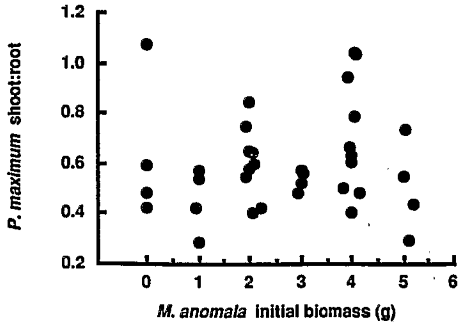

Fig. 3. Ratio of above-ground:root biomass in relation to the initial live biomass of *M. anomala*.

Table 4. Experiment 4. ^{15}N levels in the plants, the soil and the earthworms at the beginning and the end of the experiment. Treatments as in Table 3

Treatment	Material	^{15}N (%) 0 days	^{15}N (%) 67 days
(1)	Soil	0.3685	ND
	M. anomala	1.1502	0.5419
	Panicum maximum		
	Foliage	ND	0.3777
	Roots	ND	0.3715
(2)	Soil	4.3264	ND
	M. anomala	0.3685	1.1323
	Panicum maximum		
	Foliage	ND	0.7600
	Roots	ND	1.0142
(3)	Soil	4.3264	ND
	Panicum maximum		
	Foliage	ND	0.4451
	Roots	ND	0.6513

earthworms. Correlations between earthworm masses at harvest and the natural logarithms of above-ground, root and total biomass of *P. maximum* were, respectively, 0.59 ($n = 36$), 0.52 ($n = 31$) and 0.53 ($n = 31$) and were slightly lower in magnitude than correlations of harvested masses and the initial biomasses of earthworms added.

N and P concentrations in the foliage ranged between 0.71 and 2.04% and were not significantly correlated with either the initial or final masses of *M. anomala* ($n = 11$, $P > 0.05$ for both elements). In the roots, N concentrations ranged from 0.13 to 0.64% and P concentrations between 0.017 and 0.063%; concentrations of both elements were positively correlated (for N, $r = 0.59$, $n = 22$, $P < 0.01$; for P, $r = 0.65$, $n = 22$, $P < 0.01$) with the initial earthworm live biomasses added to the containers.

Experiment 3. The effects of two species of Eudrilidae on the growth of P. maximum

The mean above-ground biomasses of *P. maximum* in each of the treatments at harvest were 3.75 g ($s = 3.01$) for the control, and 6.98 ($s = 4.74$) and 11.77 ($s = 11.71$) for the 0.5 and 1.0 earthworms biomass treatments, respectively. It is clear that the presence of these earthworms led to a significant ($P < 0.025$, Kruskal–Wallis test) increase in the final biomass of *P. maximum* compared with the control treatment. However, there was no significant difference in the final biomass of *P. maximum* between the 0.5 and 1.0 biomass treatments (Wilcoxon–Mann–Whitney test, $P > 0.05$). Over the course of the experiment, eudrilid biomass increased substantially; to an average of 1.45 g in the 0.5 g treatment and 2.03 g in the 1.0 g treatment ($n = 2$ for both treatments).

There were no significant differences in the above ground masses of *P. maximum* in the 1.0 g treatments of the eudrilid earthworms and the *M. anomala* from experiment 2 ($P > 0.10$, Wilcoxon–Mann–Whitney test).

Experiment 4. Studies of the influence of M. anomala *on the transfer of nitrogen from the soil to* P. maximum

Table 3 presents the harvested biomasses of the *P. maximum* plants for all treatments. Total biomass in the treatment with ^{15}N-labelled *M. anomala* was substantially higher than the corresponding treatment in the previous experiments with *M. anomala* although the reason for this is not known. The higher *P. maximum* biomasses in the two remaining treatments may have been due to the N added in labelling the microbial biomass. In the two treatments where they were included, earthworms grew actively throughout the experimental period.

Table 4 presents the levels of ^{15}N in the plants, the soils and *M. anomala* at the start and the end of the experiments. In the treatment comprising labelled earthworms and unlabelled microbial biomass (1), concentrations of ^{15}N in the foliage and roots were only marginally higher than in the unlabelled soil. While this suggests that the nitrogen excreted by *M. anomala* may be directly assimilated by the plant, this requires re-confirmation. The lower ^{15}N concentrations in the earthworms at the end of the

Table 3. Experiment 4: means and standard deviations for the harvested oven-dried biomass (g) of *Panicum maximum* in the treatments using ^{15}N-labelled materials

Treatment	*M. anomala* Initial	*M. anomala* Final	Biomass Foliage	Biomass Roots	Biomass Total
(1) Microbial biomass not labelled, *M. anomala* labelled, $n = 2$	1.64 (0.28)	5.62 (0.71)	40.32 (1.90)	30.26 (9.31)	70.58 (11.17)
(2) Microbial biomass labelled, *M. anomala* labelled, $n = 5$	2.07 (0.10)	4.71 (2.17)	23.89 (10.37)	16.76 (5.72)	40.65 (15.69)
(3) Microbial biomass labelled, no *M. anomala*, $n = 3$	ND	ND	26.63 (11.02)	15.65 (5.41)	42.27 (15.84)

experiment are considered to result from a combination of secretory and excretory losses and dilution due to growth. Where the microbial biomass alone was labelled, both the earthworms and plant parts accumulated ^{15}N and *this was substantially greater in* the plant parts where *M. anomala* was included.

DISCUSSION

The addition of earthworms to the containers in which the test plants were growing led to an increased plant production related to the earthworm biomass added. This growth stimulation occurred at earthworm biomass levels found normally in field situations and is commensurate with that found in temperate climates (Lee, 1985). Further, in the studies described above, both the polyhumic endogeic eudrilid earthworms and the mesohumic endogeic species *M. anomala* proved effective in growth stimulation.

While no statistical evidence exists for departure of the above relationships from linearity, it is suggested that the underlying relationship is curvilinear and that this has been obscured by the variability of the results. Above an added biomass of *ca* 4 g of live earthworms per container, it is likely that the level of growth stimulation declines due to the formation of a solid, poorly-permeable structure that results from excessive earthworm working of these soils (Blanchart *et al.*, 1989, 1990). In addition, earthworms apparently survive less well at these higher biomass levels. A clear maximum value occurs in the relationship between earthworm biomass and the ratio above-ground: root biomass suggesting that a higher above-ground production per unit mass of roots occurs in the presence of earthworms.

While the relationships between plant production and earthworm biomass are statistically significant, the results are highly variable suggesting that other unknown factors also limit productivity. The mechanisms whereby plant growth is stimulated are not clear. Earthworms have been shown in the above experiments to facilitate the transfer of N and P from the soil microbial biomass to the plant and levels in the roots were elevated where *M. anomala* was added to the plant growth containers. P availability to plants is increased in the casts of anecic earthworms (Sharpley and Syers, 1977) and appears also to occur in the endogeic *M. anomala*. Other factors include the hormone-like effects of microbial by-products excreted with the casts (Tomati *et al.*, 1988). The recent finding that viable mycorrhizal propagules are almost ubiquitous in earthworm casts in field situations (Reddell and Spain, 1991) suggests that part of the growth stimulation accredited to earthworms may be due to more rapid and intensive infections by these fungi.

The failure of the maize plants to grow satisfactorily in the infertile Lamto soil demonstrates that the stimulatory effect of earthworms, including materials from the decomposing earthworms, was insufficient to meet the nutrient requirements of the high-performing variety in these and similar soils.

The reasons for the failure of *P. corethrurus* and *H. africanus* to survive in the maize growth experiments is unknown. *P. corethrurus* does not occur in the Lamto area and *H. africanus* is found locally only in coconut plantations where more litter and locally-higher organic matter levels are likely. Even *M. anomala* which is adapted to these soils with their low levels of organic matter, failed to flourish in the absence of productive plants but did so in the presence of an adapted grass species better able to cope with the infertile soils. This emphasizes the need to choose earthworm species carefully in relationship to their ecological strategies and to their tolerance of particular soil and environmental conditions, if these animals are to be successfully employed to increase productivity in tropical agriculture.

Acknowledgements—We gratefully acknowledge the kind assistance of M. Roger Vuattoux, Director of the Station d'Ecologie Lamto, Côte d'Ivoire, and his staff during the course of the present study.

REFERENCES

Blanchart E., Lavelle P. and Spain A. (1989) Effects of two species of tropical earthworms (Oligochaeta : Eudrilidae) on the size distribution of aggregates in an African soil. *Revue d'Écologie et de Biologie du Sol* **26,** 417–425.

Blanchart E., Lavelle P. and Spain A. (1990) Effects of biomass and size of *Millsonia anomala* (Oligochaeta : Acanthodrilidae) on particle aggregation in a tropical soil in the presence of *Panicum maximum* (Gramineae). *Biology and Fertility of Soils* **10,** 113–120.

Lavelle P. (1983) The structure of earthworm communities. In *Earthworm Ecology from Darwin to Vermiculture* (J. E. Satchell, Ed.), pp. 449–446. Chapman & Hall, London.

Lee K. E. (1985) *Earthworms, Their Ecology and Relationships with Soils and Land Use*. Academic Press, Sydney.

Mariotti A. and Letolle R. (1978) Analyse isotopique de l'azote au niveau des abondances naturelles. *Analysis* **10,** 421–425.

Reddell P. R. and Spain A. V. (1991) Earthworms as vectors of viable propagules of mycorrhizal fungi. *Soil Biology & Biochemistry* **23,** 767–774.

Riou G. (1974) Les sols de la savane de Lamto. In *Analyse d'Un Ecosystème Tropical Humide: La savane de Lamto (Côte d'Ivoire). I. Les Facteurs Physiques du Milieu.* N. S. Lamto I, pp. 3–38. Publications du Laboratoire de Zoologie de l'Ecole Normale Supérieure.

Sharpley A. N. and Syers J. K. (1977) Seasonal variation in casting activity and in the amounts and release to solution of phosphorus forms in earthworm casts. *Soil Biology & Biochemistry* **9,** 227–231.

Tomati U., Grapelli A. and Galli E. (1988) The hormone-like effects of earthworm casts on plant growth. *Biology and Fertility of Soils* **5,** 288–294.