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EFFECT OF INOCULATION WITH THE ENDOGEIC EARTHWORM *PONTOSCOLEX CORETHRURUS* (GLOSSOSCOLECIDAE) ON N AVAILABILITY, SOIL MICROBIAL BIOMASS AND THE GROWTH OF THREE TROPICAL FRUIT TREE SEEDLINGS IN A POT EXPERIMENT

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Summary—Endogeic earthworms *Pontoscolex corethrurus* were inoculated at four different biomasses (i.e. 0, 100, 400 and 800 mg/1.5 kg dry soil) in pots containing seedlings of three tropical fruit trees: *Bactris gasipaes* (peach palm), *Bixa orellana* and *Eugenia stipitata*. After 120 days, significant increases of plant growth were observed in *Bixa* (14–24 times the control value) and *Eugenia* (1.6–2.5) as a result of earthworm inoculation irrespective of the inoculated biomass; an inverse effect (−1.8 to −2.7 times the control) was observed with seedlings of *Bactris*. There was a significant effect of plant species on earthworm growth and significant effects of earthworms on mineralization of nitrogen and microbial biomass accumulation at some dates. Different conditions in the rhizosphere of these species may explain these results. Kinetics of soil variables during the experiment were complex. It is suggested that in some pots an excessive earthworm activity resulted in the sealing of surface with compact casts and further changes in water dynamics which affected both earthworms and microbial communities.

INTRODUCTION

Studies on earthworms carried out in the humid tropics have shown that they improve soil physical structure (measured by increased infiltration and resistance to erosion through the formation of aggregates), activate nutrient release from soil organic matter, and physically protect such organic matter in the structure of their casts (Aina, 1984; Lal, 1987; Blanchart *et al.*, 1990; Barois *et al.*, 1987). As an example of earthworm beneficial effects, inoculation of soils with *Millsonia anomala* in Côte d'Ivoire increased *Panicum maximum* yields 3 times as compared to plant growth on non-inoculated soils (Spain *et al.*, 1992). Such effects may also occur with *Pontoscolex corethrurus*, an endogeic polyhumic earthworm found in the Amazon (Lavelle and Pashanasi, 1989). The objective of this study was to determine the effect of inoculation of the late species on the growth of three tropical fruit trees in the nursery, and to assess the effects of the earthworms on soil nitrogen availability and soil microbial biomass.

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MATERIALS AND METHODS

Seeds of *Bixa orellana* (Bixaceae), *Eugenia stipitata* (Myrtaceae) and the palm tree *Bactris gasipaes* were used for the experiment. The first species produces red seeds which are commonly used as a natural dye for food; *Eugenia* produces a yellow drupe rich in vitamin C; *Bactris* is a peach palm whose fruit is highly appreciated in the Amazon basin.

Following germination, seedlings were transferred to plastic bags (28 × 19 cm) filled with potting media composed of 3 parts soil:1 part sawdust. The soil was obtained from a secondary forest (0–10 cm) and was previously dried in the shade in order to eliminate earthworm cocoons. Approximately 2 weeks after transplanting, the bags were inoculated with different numbers of young *Pontoscolex corethrurus* earthworms.

The appropriate mass of earthworms introduced into the bags was:

(1) 0 mg (control)
(2) 120 mg live weight earthworms (3 individuals)
(3) 400 mg live weight earthworms (10 individuals)

(4) 800 mg live weight earthworms (20 individuals)

After inoculation, the bags were placed in the nursery under an open shed 1.20 m high. Curtains on the sides of the bags prevented sunlight from falling directly on the bags. Seedlings were watered periodically.

Four bags per inoculation treatment and tree species were harvested at days 60, 90 and 120. At each harvest, we measured: overall biomass of seedlings, *Pontoscolex corethrurus* biomass, soil microbial N-biomass (using the ninhydrin method, Amato and Ladd, 1988) and N mineralization in soil incubated for 10 days under ambient conditions in the laboratory (R. A. Dorich and D. W. Nelson, 1980 modified). ANOVAs were performed when necessary using a Fisher test (STATVIEW software).

RESULTS

Plant biomass

Increases in total plant biomass varied with plant species and level of earthworm inoculation (Fig. 1). Maximum growth was observed in the inoculated *Bixa orellana* treatments. Compared to initial values, plant biomass after 120 days was respectively 3.6, 63, 51 and 89 times greater than the initial value in the 0, 120, 400 and 800 mg earthworms/bag treatments. All earthworm treatments significantly differed from the non-inoculated treatment; plant biomass was significantly different in the treatment with the highest inoculated biomass as compared with treatments with lower earthworm biomasses.

A similar pattern was observed with *Eugenia stipitata*. None the less, plant growth had been less important and biomass at day 120 was respectively 5.1, 8.1, 8.0 and 12.6 greater in the non-inoculated treatment and treatments with 120, 400 and 800 mg of earthworm respectively.

Seedlings of *Bactris gasipaes* had a significant negative response to earthworm inoculation: after 120 days the mean biomass per plant had respectively increased by 6.8, 3.7, 2.5 and 3.4 times in treatments inoculated with 0, 120, 400 and 800 mg.

Earthworm biomass

Temporal changes in earthworm biomass over time are given in Fig. 2. In all treatments with 800 mg inoculum, earthworm biomass increased rapidly to high levels, peaked, then fell sharply to levels similar to those in the 400 mg inoculation treatment (in the case of Bixa, to 0).

In Bixa, earthworm biomass had declined to 0 or near 0 in all inoculation treatments by 120 days. In Bactris, earthworm biomass was 2575, 5789 and 7475 mg per bag in the 120, 400 and 800 mg per bag

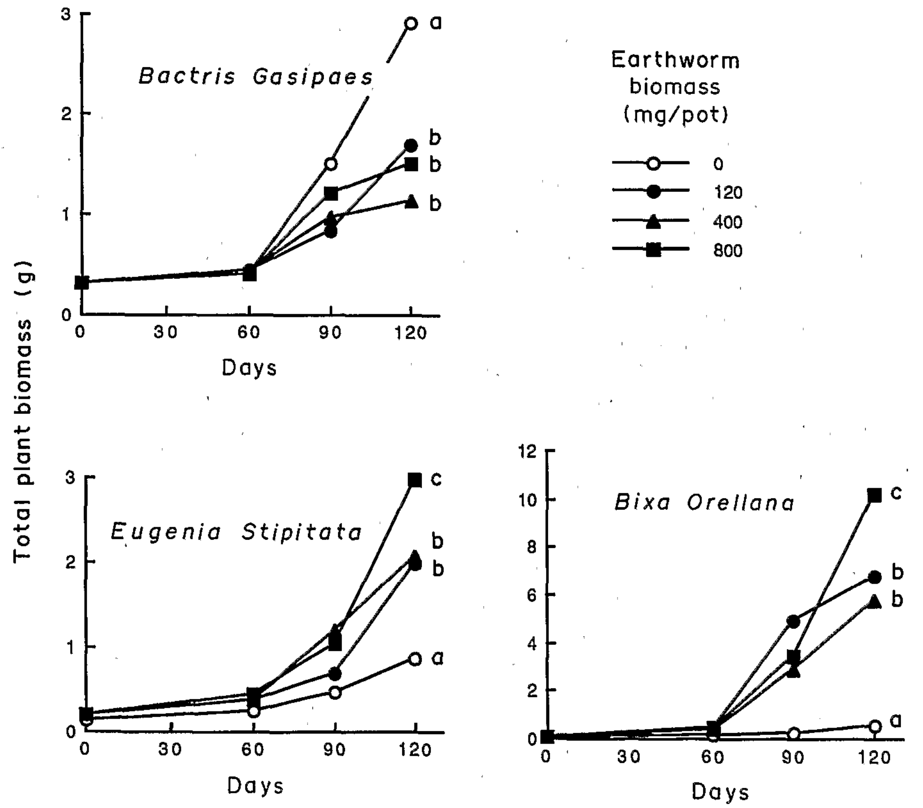

Fig. 1. Total (roots + shoots) biomass accumulation over time by seedlings of three tree species grown in the nursery in relation to the quantity (mg/pot) of *Pontoscolex corethrurus* introduced into each pot.

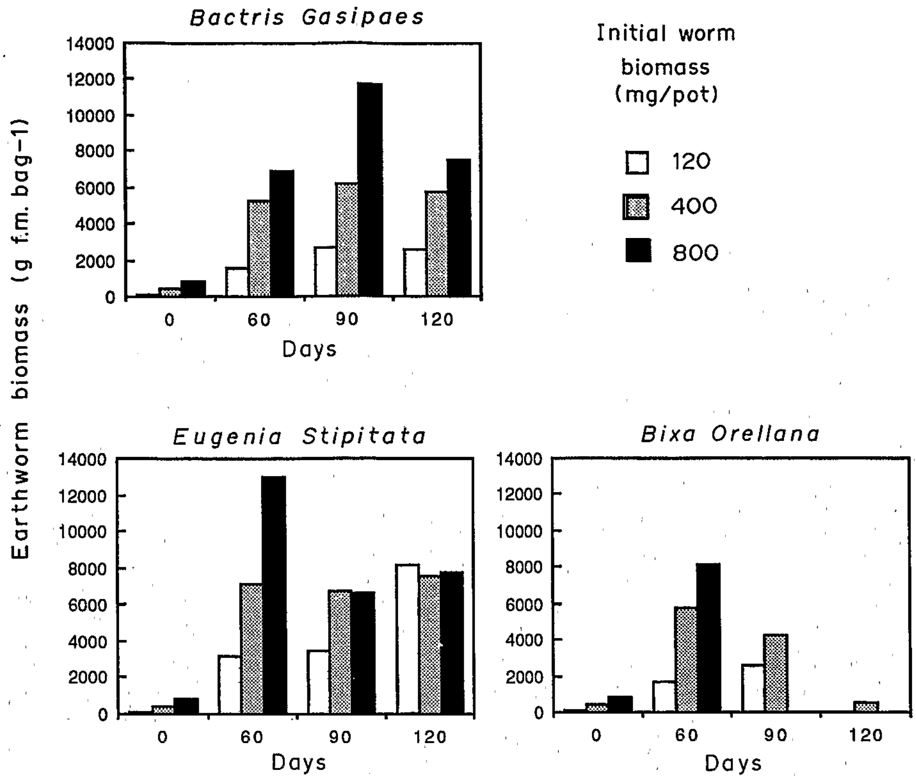

Fig. 2. Temporal changes in biomass of *Pontoscolex corethrurus* in nursery pots containing seedlings of three species of fruit trees.

inoculation treatments, respectively, and was maintained relatively constant in the former two treatments. In Eugenia, earthworm biomass in the 800 mg declined after 60 days. After 120 days, earthworm biomass was similar (*ca* 7700 mg per bag) in all inoculated treatments. This is equivalent to a biomass of *ca* 2t ha^{-1} in natural conditions, i.e. twice as much as biomass measured in good quality pastures at Yurimaguas (Lavelle and Pashanasi, 1989).

Effect on soil properties

Microbial biomass. In all tree species and inoculation treatments, microbial biomass increased during the 0–90 day period then fell to initial levels at 120 days (Fig. 3). Peaks were highest in Bactris (*ca* 60 $\mu g\ g^{-1}$ soil), and were similar in Eugenia and Bixa (i.e. 30–35 $\mu g\ g^{-1}$ soil). At day 90, the difference between 0 and 400 mg inoculation treatments were significant at the 90% level, irrespective of plant species. Other differences were not significant, possibly due to the lack of repetitions.

N mineralization. In all plant species, the amount of ammonia released after 10 days incubation was significantly correlated with earthworm biomass ($P = 0.02$) (Fig. 4). At day 120, there was a significant effect of plant species ($P < 0.0001$), with minimum values associated to Bactris (1.04–1.98 depending on treatments) intermediate values with Bixa (4.5–6.2) and maximum with Eugenia (7.11–9.41). Nitrification was generally low and nitrate concentration was largely inferior to ammonia with one exception (Eugenia treatment T4 at day 90) (Fig. 4). At day 120, treatments with Bactris had significantly lower nitrate contents (*ca* 3 $\mu g\ g^{-1}$) than Eugenia (1.5–7.5 $\mu g\ g^{-1}$) and Bixa (4.5–6.0 $\mu g\ g^{-1}$) ($P < 0.05$).

DISCUSSION

There was a strong positive response of plant growth to earthworm inoculation in Bixa, much less in Eugenia and an inverse response in Bactris. This may be due to physiological differences among the tree species. Direct observation of plants shows that the structure and abundance of the root systems are different. Bixa had long and fine roots. In contrast, Bactris had relatively thick and short roots which would directly explore only a low proportion of the volume of soil and take little advantage of an increased production of nutrients resulting from earthworm activities. Eugenia represented an intermediate situation.

Causes for the different earthworm population dynamics in the various treatments are unclear. In Bixa, abundant surface casting sealed the soil surface and further prevented water to infiltrate. Drying of the soil within the bag presumably caused earthworm mortality. Why the same amount of casting did not occur with the other plant species is unknown.

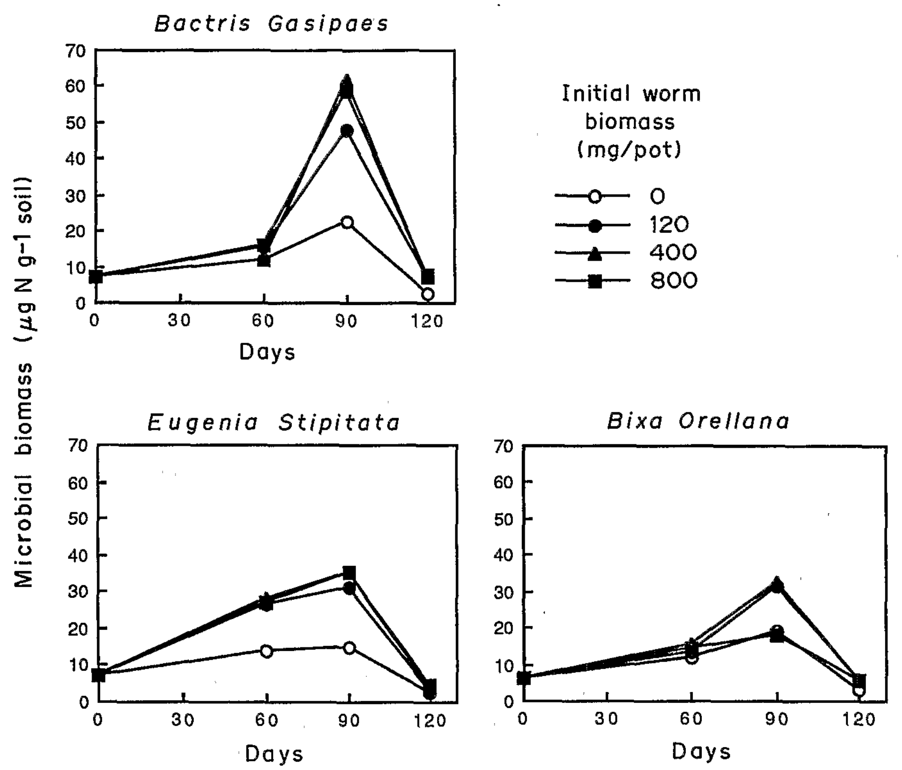

Fig. 3. Effect of different levels of inoculation of *Pontoscolex corethrurus* on microbial biomass in soil obtained from pots containing seedlings of three tree species.

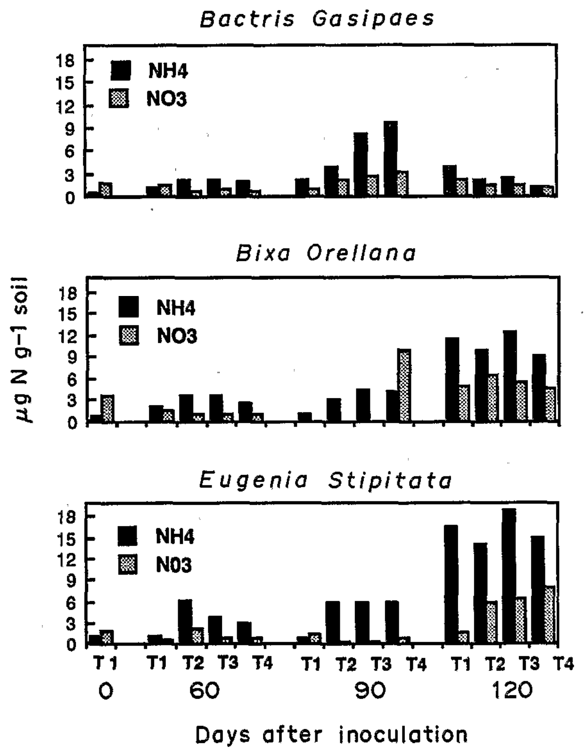

Fig. 4. Nitrogen mineralized from soil of three tropical tree species previously inoculated with different biomasses of *Pontoscolex corethrurus* (10 day laboratory incubations at ambient temperature *ca* 27°C, field capacity). T1 = no worms; T2 = 120 mg earthworm; T3 = 400 mg; T4 = 800 mg.

Perhaps Eugenia and Bactris contributed greater amounts of root exudates and dead roots to the soil, creating a richer medium for earthworm grazing and hence lower requirements for soil ingestion (Lavelle *et al.*, 1983).

The relationship between plant growth response and indices of nutrient availability such as microbial biomass and N mineralization potential was largely inconsistent and complex. Earthworm inoculation generally resulted in increased availability of mineral-N and microbial biomass but the link between this process and plant growth was complex.

These patterns suggest that earthworms had an indirect effect on moisture conditions in the bags in addition to their direct effect on nutrient availability. This may be due to the sealing of the soil surface by worm casts and the overall reduction of microporosity which resulted from the ingestion and further casting of most of the soil during the experiment (Blanchart *et al.*, 1990).

These excessive earthworm effects may have affected microbial biomass at the end of the experiment. This might explain the lack of relationship between microbial biomass and ammonification and nitrification potentials. Within species plant, similar levels of microbial biomass were associated with different ammonification or nitrification potentials, and changes over time in soil microbial biomass were not well-correlated with changes in these potentials. Differences in the N mineralization potential among species and temporal patterns of ammonification and nitrification in relation to earthworm biomass within a given species, suggest that complex temporal interactions between soil moisture and the quantity of organic inputs to the soil from worms and plants are controlling these processes.

CONCLUSION

(1) The inoculation of earthworms had spectacular positive effects on growth of Bixa, still significant effects on Eugenia, but a significant negative effect on Bactris.

(2) Earthworm dynamics varied among plant species and inoculation levels. The production of abundant surface casts in the 800 mg per pot inoculation treatment of Bixa sealed the surface of the soil, resulting in drying of soil within the bags and, presumably, earthworm mortality. None the less, growth of the seedlings was maximum in these treatments. Lower levels of inoculation (120 and 400 mg per bag) in the Bactris and Eugenia treatments appeared to result in stable earthworm populations.

(3) Earthworm and soil microbial biomass appeared to be related at some sampling periods but not others, nor were there consistent relationships between soil microbial biomass and ammonification or nitrification potential. There was a significant correlation between earthworm biomass and N mineralization potential. At day 120, N mineralization potential was significantly lower in Bactris as compared to the other two plant species.

(4) These results suggest that earthworms may affect microbial activities and plant growth through an indirect effect on soil moisture conditions in the bags via surface sealing and soil macroaggregation. In combination with differences in the quality and quantity of organic inputs to the soil from the plants, these factors may be responsible for the lack of clear patterns between earthworm biomass, soil microbial biomass and ammonification or mineralization potential.

The effect of inoculation of the earthworm *Pontoscolex corethrurus* on growth of seedlings is variable. They enhance N mineralization potential and tend to increase microbial biomass. Nonetheless, the response may greatly vary among plant species, probably depending on the structure and function of their root system. Secondly, the initial level of inoculation is important. Earthworm populations build up rapidly in nursery bags. As a result, inoculation of excessive numbers of individuals may have deleterious effects on soil water dynamics if earthworm activity is too intense. One such case has already been reported in sweet potato plantations of Papua New Guinea (Rose and Wood, 1980). The amount of earthworms to introduce should be determined as a function of the feeding quality of the soil, amount of soil in the bags and the expected duration of the growing period. That variable may be calculated from standard measurements of soil ingestion, reproduction and growth of *Pontoscolex corethrurus* made by Lavelle *et al.* (1987) in a variety of temperature and moisture conditions by worms of different weights.

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