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Effect of earthworm addition on soil nitrogen availability, microbial biomass and litter decomposition in mesocosms

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Abstract The aim of the study was to determine the effect of adding two tropical earthworm species, *Rhinodrilus contortus* and *Pontoscolex corethrurus*, to mesocosms on the availability of mineral N (NH_4^+ and NO_3^- concentrations), soil microbial biomass (bio-N), and the decomposition rates of three contrasting leaf litter species, in a glasshouse experiment. The mesocosms were filled with forest soil and covered with a layer of leaf litter differing in nutritional quality: (1) *Hevea brasiliensis* (C/N=27); (2) *Carapa guianensis* (C/N=32); (3) *Vismia* sp., the dominant tree species in the second growth forest (control, C/N= 42); and, (4) a mixture of the former three leaf species, in equal proportions (C/N=34). At the end of the 97-day experiment, the soil mineral N concentrations, bio-N, and leaf litter weight loss were determined. Both earthworm species showed significant effects on the concentrations of soil NO_3^- ($p<0.01$) and NH_4^+ ($p<0.05$). Bio-N was always greater in the mesocosms with earthworms (especially with *R. contortus*) and in the mesocosms with leaf litter of *H. brasiliensis* ($6 \mu\text{g N g}^{-1}$ soil), the faster decomposing species, than in the other treatments ($0.1\text{--}1.6 \mu\text{g N g}^{-1}$). Thus, earthworm activity increased soil mineral-N concentrations, possibly due to the consumption of soil microbial biomass, which can speed turnover and mineralization of microbial tissues. No significant differences in decomposition rate were found between the mesocosms with and without earthworms, suggesting that experiments lasting longer are needed to determine the effect of earthworms on litter decomposition rates.

Keywords Earthworms · Nitrogen mineralization · Litter decomposition process · Amazonia

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Introduction

In Amazonia, few studies on soil fauna have indicated earthworms as having an important biomass (Lavelle and Pashanasi 1989; Römbke and Verhaagh 1992; Barros 1999; Barros et al. 2001, 2002), or as playing a fundamental role on the litter decomposition process (Luizão 1985; Luizão and Schubart 1987). The geographical distribution of earthworms is mainly limited by macroclimate, but litter quality is a factor that determines their local distribution and activity (González et al. 1996). In both the soil and the litter layer, earthworms can increase by up to five times the number of edaphic microorganisms (Atlavinyte and Lugauskas 1971; Guerra and Asakawa 1981). Thus, interactions between earthworms and microorganisms can change microbial composition and activity, making earthworms important in the regulation of decomposition processes and nutrient recycling (Parle 1963; Fragoso et al. 1997; Lavelle et al. 1997; Wardle and Lavelle 1997; Lavelle 2000). For instance, earthworms can influence N mineralization, either directly, through N release by their metabolic products (faeces, urine, mucus) and dead tissues, or indirectly, through changes caused in soil physical properties, fragmentation of organic material, and through their interactions with other elements of the soil biota (Lee 1985).

Despite being very well known in the temperate zones, limited information is available to date for the tropics on the role of earthworms in the N cycle, partly disclosed only in the last decade. Pashanasi et al. (1992) have shown that the introduction of *Pontoscolex corethrurus* increased significantly the soil microbial biomass-N (bio-N) and mineral-N availability in experiments with seedlings of fruit species in Peru. Other studies in the tropics showed that earthworm populations are highly sensitive to soil management practices (Lavelle and Pashanasi 1989; Decaens et al. 1994, 1999; Fragoso et al. 1997; Barros et al. 2002). A better understanding of earthworm biology and ecology, including relationships with the litter layer and biochemical soil characteristics, is an essential step towards the adoption of ecologically

sound management practices and maintaining the nutrient recycling processes (Jiménez et al. 1998; Decaens et al. 1999).

This study aimed to determine the effects of introducing two earthworm species, common in the region, *Rhinodrilus contortus* and *Pontoscolex corethrurus*, on the availability of soil mineral N (NH_4^+ and NO_3^-), on the soil microbial biomass-N (bio-N), and on litter decomposition rates of leaf species differing in nutritional quality, in mesocosms containing soils from second-growth vegetation.

Material and methods

Two sizes of mesocosms were used for the different earthworm species: mesocosms measuring 20×20×20 cm for the smaller earthworm species *P. corethrurus*, and 30×30×40 cm mesocosms for the larger *R. contortus* earthworms. Both mesocosms were filled with a Xathic Ferralsol soil (Oxisol) collected from 8-year-old second-growth forest (Araujo 2000), located at the EMBRAPA/CPAA Experimental Station, 30 km along the AM-010 Road, in Manaus, Amazonas, Brazil (geographic coordinates: 2°51'S and 59°52'W). This soil had a high content of clay (60%), extreme acidity (pH=4), low concentrations of mineral N (1.6 mg g⁻¹), cation exchange capacity of 0.5 cmol kg⁻¹, and base saturation of 33%. Before filling the mesocosms, the soil samples were air-dried and sieved through a 6-mm mesh in order to remove larger organic matter fragments. The earthworms were collected from plots of primary forest and second growth ("capoeira") at the EMBRAPA station. In the treatments with earthworms, two immature individuals of *R. contortus* were added to each large mesocosm, and four individuals of *P. corethrurus* (three adults and one immature) to each smaller mesocosm. In all the mesocosms belonging to the two size classes (small or large), the biomass of inoculated earthworms by mesocosm was similar at the beginning of the experiment (9.14 g of *R. contortus* and 2.17 g of *P. corethrurus*).

The surface of the mesocosms was covered with freshly fallen leaf litter, also collected at the EMBRAPA station, air-dried, and stored in a dry place. Leaves were cut into 2×2-cm pieces, in order to speed their decomposition in the short period of the experiment. Four treatments were applied, adding leaves of distinct nutritional quality, as defined by their C:N ratios: *Hevea brasiliensis* Muell. Arg.; *Carapa guianensis* Aubl.; *Vismia* sp.; and a mixture of the three species, with equal weights. (*H. brasiliensis* and *C. guianensis* have been used by EMBRAPA to form rows of valuable tree species, growing together with the natural regeneration, after deforesting abandoned and sick rubber-tree plantations). In this study, the *H. brasiliensis* leaves (C:N=27) with a low C:N ratio were considered to be of high nutritional quality, while *Vismia* sp. leaves (C:N=42) with a high C:N ratio were considered to be of low nutritional quality, and the *C. guianensis* leaves (C:N=32) and mixed litter (C:N=34) were considered to be of intermediate nutritional quality (Beck et al. 1998). The tops of the mesocosms were covered by a 1-mm nylon mesh to prevent the earthworms from leaving the boxes. Each of the treatments with earthworms had four replicates; the treatments without earthworms had three replicates, making up a total of 56 mesocosms, besides the controls.

The experiment was carried out from August to November 1999 in a protected (walls in nylon mesh) glasshouse located at INPA-V8 secondary campus, in the city of Manaus (3°88'S; 60°1'O). Soil moisture of the 0- to 10-cm layer was kept between 30 and 42%. The water matrix potential in the upper 10-cm soil layer of mesocosms corresponded to pF=2–2.5. Air temperature and relative humidity in the glasshouse, during the experiment, ranged from 24–31 °C and 38–61%, respectively.

After 97 days, the mesocosms were emptied and all earthworms found were retrieved. All individuals were counted and their fresh weight determined using a precision balance. Estimates of the

proportion of faecal residues present on the soil surface in the mesocosms were obtained by counting the number of faecal pellets present on the surface of each mesocosm at the end of the experiment. The relative proportion (percentage) of faecal material produced by each earthworm species was then calculated, giving an estimate of the activity of the earthworms (Guerra 1988).

In each mesocosm, composite soil surface samples (0–5 cm) were taken using a fine corer (2 cm diameter), and soil analyses were made on fresh soil, immediately after collection. The concentrations of NO_3^- and NH_4^+ were determined after extraction with 1 M KCl (20 g of soil and 150 ml of KCl) (Maynard and Kalra 1993). Measurements were taken using a flow injection system (SFA-2, Burkard Scientific). Bio-N was measured using the fumigation-extraction method (Brookes et al. 1985). Decomposition rates of the litter added to mesocosms were estimated, at the end of the experiment, by the weight loss of the leaves in relation to the initial dry weight.

Multiple analyses of variance (MANOVA) were used to assess the differences between the concentrations of mineral-N and bio-N with and without earthworms, as well as for each of the added earthworm species, and for each litter species. For all analyses, the statistical package SYSTAT 6.0 for Windows (Wilkinson 1996) was used.

Results

Density and biomass of earthworms

Earthworm density and biomass decreased in all treatments at the end of the experiment for the species *R. contortus* (Table 1). The greatest loss of earthworm biomass occurred in the mesocosms receiving the addition of *Carapa guianensis* leaves (0.5 g per mesocosm); the least loss of earthworm biomass was found in the treatment with addition of *Hevea brasiliensis* leaves (3.5 g per mesocosm).

In the mesocosms inoculated with the species *P. corethrurus*, earthworm densities increased and immature individuals were abundant, besides the presence of some cocoons. For this species, the total number of individuals in the mesocosms showed no significant differences between the different leaf types in the litter addition treatments (10.2–11.4 ind. per mesocosm). At the end of the experiment, the earthworm biomass (2.4–3.8 g per mesocosm) was greater than the initial (2.1 g per mesocosm), in all treatments (Table 1).

Earthworm activity

The presence of faecal material on the surface of the mesocosms allowed an estimate of earthworm activity, which was greater in the species *P. corethrurus* than in *R. contortus* (Fig. 1). Both species were more active under the treatment with addition of *H. brasiliensis*, while smaller activities were found under the treatments with additions of *Vismia* and the mixture of leaf species. The species *P. corethrurus* had similar activity in the two latter treatments. The lowest activity was found with the addition of *C. guianensis* leaves, for both earthworm species.

Table 1 Mean number of individuals and mean initial and final biomass of earthworms in the mesocosms ($n=4$). Litter species: *H* *Hevea brasiliensis*; *C* *Carapa guianensis*; *M* mixed litter; *V* *Vismia* sp

Life stage	Individuals/ mesocosm	Final (97 days)				Fresh biomass (g/mesocosm)				
		Initial (H,C,M,V)				Initial (H,C,M,V)				
		H	C	M	V	H	C	M	V	
<i>R. contortus</i>										
Adults	0	0	0	0	0	0	0	0	0	0
Juveniles	2	0.2	0.5	0.5	0.5	9.1	3.5	0.5	1.4	1.7
Cocoons	0	0	0	0	0	0	0	0	0	0
Total	2	0.2	0.5	0.5	0.5	9.1	3.5	0.5	1.4	1.7
<i>P. corethrurus</i>										
Adults	3	3.2	4.0	2.7	3.2	2.0	2.0	2.4	1.9	2.1
Juveniles	1	6.7	5.2	8.2	7.0	0.1	1.9	0.9	0.5	1.7
Cocoons	0	1.2	1.0	0.5	0.2	0	0	0	0	0
Total	4	11.1	10.2	11.4	10.4	2.1	3.9	3.3	2.4	3.8

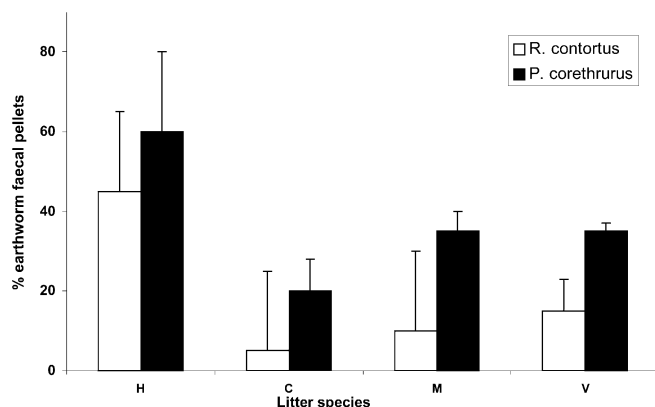


Fig. 1 Relative proportion (%) of amount of faecal material produced by each earthworm species in the mesocosms. Values represent means \pm 1 standard error ($n=4$). *H* *Hevea brasiliensis*; *C* *Carapa guianensis*; *M* mixed litter; *V* *Vismia* sp

Nitrate concentrations

The upper soil layer in the mesocosms with *R. contortus* showed NO_3^- concentrations higher than soil with *P. corethrurus* (Table 2). The presence of *R. contortus* had a significant effect on soil NO_3^- concentrations (ANOVA; $F=20.4$; $p<0.01$). Treatments with addition of *H. brasiliensis* and *Vismia* sp. showed the greatest NO_3^- concentrations ($13 \mu\text{g N g}^{-1}$ for both), followed by the mixture of three leaf species ($10.2 \mu\text{g N g}^{-1}$) and *C. guianensis* ($6.2 \mu\text{g N g}^{-1}$). However, no significant differences ($p>0.05$) were found among the four litter addition treatments. Mesocosms with *P. corethrurus* presented NO_3^- concentrations significantly higher (ANOVA; $F=17.8$; $p<0.01$) than mesocosms without earthworms (Table 2). The concentrations ranged from $4.5 \mu\text{g N g}^{-1}$ under *C. guianensis* to $5.6 \mu\text{g N g}^{-1}$ under *H. brasiliensis*, but the differences between the four litter addition treatments were not significant ($p>0.05$). Nitrate concentrations did not vary significantly in the control treatments.

Ammonium concentrations

Ammonium concentrations in the mesocosms with *R. contortus* showed a similar pattern as for NO_3^- ; thus, addition of *C. guianensis* leaves presented the smallest value ($1.7 \mu\text{g N g}^{-1}$), and the greatest was found in the treatment with *H. brasiliensis* leaves ($4.7 \mu\text{g N g}^{-1}$) (Table 2). However, such differences between litter addition treatments were not significant ($p>0.05$). Mesocosms without *R. contortus* also showed no significant differences among litter addition treatments; soil NH_4^+ concentrations ranged from 1.2 – $2.3 \mu\text{g N g}^{-1}$. Nonetheless, the species *R. contortus* had a general positive and significant effect on soil NH_4^+ concentrations ($F=9.9$; $p<0.01$), when compared to the control. Soil NH_4^+ concentrations in the mesocosms inoculated with *P. corethrurus* ranged from $1.5 \mu\text{g N g}^{-1}$ (*H. brasiliensis*) to $1.9 \mu\text{g N g}^{-1}$ (*Vismia*). Mesocosms without *P. corethrurus* had concentrations ranging from 0.7 (*Vismia*) to $1.4 \mu\text{g N g}^{-1}$ (*H. brasiliensis*), but these differences were not significant ($p>0.05$).

Soil microbial biomass-nitrogen (bio-N)

The greatest values for bio-N were found in the mesocosms with *R. contortus* (Table 2), especially with addition of *H. brasiliensis* ($6 \mu\text{g N g}^{-1}$) and *Vismia* ($1.6 \mu\text{g N g}^{-1}$) leaves. The controls (with no inoculation of *R. contortus*) presented very low values of bio-N, ranging from 0.03 – $0.1 \mu\text{g N g}^{-1}$. Bio-N values in the mesocosms with *P. corethrurus* were greater than in the mesocosms without earthworms (Table 2). The greatest value of bio-N was recorded under the treatment with *H. brasiliensis* ($0.4 \mu\text{g N g}^{-1}$), while the smallest was found under the mixed litter treatment ($0.1 \mu\text{g N g}^{-1}$). The mesocosms without *P. corethrurus* showed bio-N values between 0.05 and $0.1 \mu\text{g N g}^{-1}$. Though consistently greater than the control (without earthworm inoculation), the bio-N values recorded in the treatments with earthworms were not significantly ($p>0.05$) different, neither in the treatment

Table 2 Soil NO_3^- , NH_4^+ and microbial biomass-N (*bio-N*) concentrations ($\mu\text{g N/g}$) in the experiment with ($n=4$) and without ($n=3$) inoculation of *R. contortus* and *P. corethrurus* under different litter addition treatments, 97 days after inoculation. Values are means \pm standard deviation. Different letters *a* and *b* in the lines indicate significant differences between treatments with and without earthworm inoculation (NO_3^- : $p<0.01$), (NH_4^+ : $p<0.05$). Bio-N showed no significant differences between treatments with and without both species of earthworms

Leaf species	Treatment			
	With <i>R. contortus</i>	Without <i>R. contortus</i>	With <i>P. corethrurus</i>	Without <i>P. corethrurus</i>
	$\mu\text{g N-NO}_3^- \text{ g}^{-1}$			
<i>H. brasiliensis</i>	13.0 \pm 1.4 a	5.6 \pm 0.4 b	5.6 \pm 2.0 a	2.5 \pm 2.1 b
<i>C. guianensis</i>	6.2 \pm 1.7 a	4.4 \pm 0.7 b	4.5 \pm 0.7 a	2.8 \pm 0.8 b
Mixed litter	10.2 \pm 3.8 a	5.6 \pm 0.7 b	4.8 \pm 0.9 a	3.5 \pm 0.7 b
<i>Vismia</i> sp.	13.0 \pm 6.1 a	6.3 \pm 2.4 b	5.0 \pm 1.2 a	2.5 \pm 1.5 b
	$\mu\text{g N-NH}_4^+ \text{ g}^{-1}$			
<i>H. brasiliensis</i>	4.7 \pm 1.5 a	1.6 \pm 0.5 b	1.5 \pm 0.5 a	1.4 \pm 0.04 b
<i>C. guianensis</i>	1.7 \pm 1.2 a	1.2 \pm 0.9 b	1.7 \pm 0.4 a	1.3 \pm 0.9 b
Mixed litter	3.1 \pm 1.8 a	2.3 \pm 0.5 b	1.5 \pm 0.8 a	1.2 \pm 0.3 b
<i>Vismia</i> sp.	2.7 \pm 1.2 a	1.3 \pm 0.8 b	1.9 \pm 0.5 a	0.7 \pm 0.3 b
	$\mu\text{g N-bio-N g}^{-1}$			
<i>H. brasiliensis</i>	6.02 \pm 9.3	0.05 \pm 0.04	0.36 \pm 0.5	0.05 \pm 0.04
<i>C. guianensis</i>	0.11 \pm 0.1	0.03 \pm 0.01	0.30 \pm 0.3	0.07 \pm 0.06
Mixed litter	0.50 \pm 0.7	0.04 \pm 0.04	0.09 \pm 0.1	0.09 \pm 0.06
<i>Vismia</i> sp.	1.62 \pm 3.2	0.10 \pm 0.12	0.15 \pm 0.1	0.05 \pm 0.05

Table 3 Dry weight loss (%) of litter in the mesocosms with ($n=4$) and without inoculation ($n=3$) of *R. contortus* and *P. corethrurus* in the litter addition treatments, 97 days after earthworm inoculation.

Values are means \pm standard deviation. (No significant differences were found between treatments with and without earthworms)

Leaf species	Treatment			
	With <i>R. contortus</i>	Without <i>R. contortus</i>	With <i>P. corethrurus</i>	Without <i>P. corethrurus</i>
<i>H. brasiliensis</i>	34.8 \pm 2.6	38.2 \pm 9.3	30.6 \pm 4.8	46.1 \pm 14.4
<i>C. guianensis</i>	26.6 \pm 2.7	24.5 \pm 1.4	22.9 \pm 1.1	31.2 \pm 6.6
Mixed litter	29.0 \pm 3.3	24.2 \pm 2.8	28.9 \pm 7.6	28.7 \pm 3.3
<i>Vismia</i> sp.	18.4 \pm 0.5	15.2 \pm 1.4	15.5 \pm 1.0	17.1 \pm 1.1

with *R. contortus* nor with *P. corethrurus*, presenting a very high data variability among mesocosms.

Leaf litter weight loss

Disregarding the earthworm treatment, the greatest weight losses were always found on the *H. brasiliensis* litter, followed by the mixed litter and the *C. guianensis*; the slowest weight loss occurred in the *Vismia* litter (Table 3). However, the weight loss rates of the mixed litter and *C. guianensis* were not significantly different (Tukey, $p>0.05$). Neither the species *R. contortus* nor the species *P. corethrurus* had significant effect on leaf litter decomposition ($p>0.05$ for both species) during the 97-day-long experiment.

Discussion

The decrease in both density and biomass of *R. contortus* in the larger mesocosms indicates that this species was severely affected by the environmental conditions developed within the mesocosms during the experiment, causing the earthworms to die or to flee. Still very little is known on the biology and ecology of *R. contortus*; information on the appropriate conditions in captivity is

unavailable. They appear to be very mobile animals which do not tolerate disturbances in the soil. Alternatively, their low densities may be a result of insufficient amount of the specific resources they need to survive (Lavelle 2000, personal communication). Apparently, the earthworms did not die or leave the mesocosms in the first weeks of the experiment, considering that faeces were found on the surface and bottom of the mesocosms, as well as distributed in all soil layers, in addition to the galleries within the soil profile. This confirms *R. contortus* as an endogeic and geophagous species (i.e., one that lives and feeds in soil).

On the other hand, the observed increase in *P. corethrurus* densities within the mesocosms indicates that mature individuals of this species are reproducing during the experiment, which means they were not severely affected by the treatment stress. *P. corethrurus* was able to maintain or even increase its biomass within the mesocosms during the whole experiment. This species presents a large geographical distribution and high adaptability to diverse environments (Lavelle et al. 1987; Barros 1999). Very likely because of such adaptability, *P. corethrurus* showed higher activity within the mesocosms (as estimated by the presence of faeces on the soil surface) than *R. contortus*.

In spite of this activity, none of the earthworm species had a significant effect on litter decomposition, indepen-

dent of its nutritional quality. Both earthworm species are geophagous, i.e., soil feeders; thus, they are not directly feeding on litter. However, their possible indirect effect on the decomposition rate of different litter species should not be dismissed; that may occur through the enhancement of the microbial activity, by their faeces and N release. In fact, earthworms were more active in the mesocosms covered with *Hevea brasiliensis* leaves, a treatment in which were also found the highest values of bio-N and NO_3^- . Possibly, the fast decay of the *Hevea* leaves produced soluble compounds which stimulated microbial growth and nitrification activity (Atlavinyte and Lugauskas 1971; Guerra and Asakawa 1981; Barois et al. 1987; Bohlen and Edwards 1995). However, in principle, such nitrification activity could only be carried out by fungi, in view of the strong soil acidity of the substrate used here (pH=4). Under these conditions, heterotrophic nitrification by fungi prevails over bacterial nitrification, which only converts NH_4^+ into NO_3^- when oxygen is present, allowing the chemioautotrophic process to take place (Brookes et al. 1985; Maynard and Kalra 1993). Apparently, all fungi are able to utilize some organic N sources for their nitrification activities (Jennings 1989). The possible stimulation of nitrification and nitrifying organisms remains an open and important issue for future research.

The highest weight loss of *Hevea* leaves at the end of the experiment was likely due to its better nutritional quality and relatively low C/N ratio (C/N=27), allowing a faster decomposition rate (Palm and Sanchez 1990). Among all treatments, the lowest earthworm activity in the mesocosms was found in the treatment with *C. guianensis* leaves, possibly because of its low nutritional quality (C/N=32) and the thickness and hardness of the leaves.

In spite of the decrease in density and biomass of *R. contortus*, observed at the end of the experiment, the apparent death of earthworms did not produce an increase of soil mineral N, nor of bio-N, in the mesocosms. Even in the mesocosms from which the earthworms disappeared or died (thus, being decomposed 'in situ'), the concentrations of mineral N and bio-N were not significantly higher than in the mesocosms where the earthworms remained alive to the end of the experiment.

Most of the mineral N produced during the experiment was found as NO_3^- . The presence of *R. contortus* led to a near-doubling of N-NO_3^- concentrations and increased the bio-N by four- to 14-fold in the treatments with addition of *Vismia* leaves. Increases in the mineral and microbial N concentrations in soil as a result of earthworm presence and activity have been reported in other studies in the tropics (Lee 1985; Barois et al. 1987; Pashanasi et al. 1992). The positive influence of earthworms on N mineralization may have been a result of either the N release in their metabolic products (faeces, urine, mucus) and dead tissues, or the changes caused in soil physical properties, and fragmentation of organic material (Lee 1985). The highest concentrations of N-NH_4^+ were found under *Hevea* leaves, the fastest

decomposing leaf species; however, in all treatments, there were losses of NH_4^+ in relation to the control. Probably higher N mineralization happened due to the decomposition process (highly controlled by fungi), and this stimulated the transformation of N-NH_4^+ into N-NO_3^- in the presence of *R. contortus*. Higher losses of N-NH_4^+ have also been recorded in temperate locations; for instance, Anderson et al. (1985) found substantial increases in N-NH_4^+ losses in microcosms where animals were introduced.

The species *P. corethrurus* also increased mineral-N and bio-N contents within the mesocosms; however, such increases were lower than those produced by *R. contortus*. Probably, this is a result of the highest biomass of *R. contortus*, a species that can eat and process much more soil than *P. corethrurus*, thus causing a higher N turnover.

Other studies have shown partially similar results to those found here. For instance, Bohlen and Edwards (1995) and Blair et al. (1997) found that earthworms increased the concentrations of soil N-NO_3^- , but the bio-N concentrations decreased with an increase in earthworm populations. In contrast to the above-mentioned authors, an increase of the microbial activity, as an indirect effect of earthworms, has been reported by several authors (Scheu 1987; Lavelle 1988; Barros 1999; Decaens et al. 1999). Apparently, earthworms have a variable influence on the microbial biomass and activities, inducing decreases in bio-N and/or in N transformations in some experiments, and increases in others.

The effect of the species *P. corethrurus* on mineral-N and microbial biomass has been studied in the tropics by Barois et al. (1987), who found in a laboratory experiment using pasture soil 2.7 times more N-NH_4^+ in the earthworms' faecal material than in the original soil; N-NO_3^- concentrations also increased, but the differences were not significant. Pashanasi et al. (1992), in pots with soils from second-growth areas, planted with seedlings of three tropical fruit species, found positive correlations between the concentrations of N-NH_4^+ and earthworm biomass, while nitrification was generally low, and the concentrations of N-NO_3^- were smaller than those of N-NH_4^+ ; in all treatments, there was an increase of bio-N. In the present study, however, concentrations of N-NH_4^+ were always smaller than those of N-NO_3^- , suggesting the existence of mechanisms other than autotrophic nitrification occurring in the presence of earthworms. In temperate agrosystems, Blair et al. (1997) also found a significant increase of N-NO_3^- concentration when earthworm population was also increased, despite recording only a modest increase in N-NH_4^+ , and a low bio-N. In a temperate deciduous woodland soil, a 50% increase in the net mineralization was recorded due to the presence of soil animals, which significantly disrupt the time course of microbial process and enhance the turnover of microbial populations, with release of N-NO_3^- from the dead microbial bodies (Anderson et al. 1985).

In the Amazon, few published works report the effects of earthworms on the soil microflora and nutrients. Guerra and Asakawa (1981) showed that the presence of

P. corethrusus, in pots, increased the microbial population over time, while Barros (1999) found a positive correlation between the bio-C and earthworms biomass. In the present study, the presence of both earthworm species increased the soil bio-N, due to the edible substrates (carbon) made available to the microorganisms by the earthworms, by the secretion of cutaneous and intestinal mucus (Barois et al. 1987; Blair et al. 1997). Within the earthworm intestines, the mucus is mixed with the ingested soil, or deposited along the soil galleries (Barois et al. 1987). Thus, earthworm faeces have more organic matter, in a partly digested form which can be easily assimilated by the microorganisms (Shaw and Pawluk 1986; Blanchart et al. 1993; Decaens et al. 1999; Barros et al. 2001).

The effect of earthworms on microbial biomass seems to depend upon many factors, and the experimental design must be taken into account before making generalizations regarding effects of earthworms on soil biogeochemistry. Additionally, attention should be paid to the specific role of fungi in the nitrification process in such acidic soils. Future research should determine both heterotrophic and autotrophic nitrification and should address the question of whether the nitrification and nitrifiers are or are not stimulated by organic compounds, by directly monitoring changes in nitrifiers.

Despite the limitations of the present study, including its short duration, the positive role of earthworms in the N cycle of tropical soils is confirmed, thus being similar to that of earthworms from temperate zones (where most of the cited studies were made). The importance of earthworms for organic-N mineralization and for the control of microbial populations, which makes the mineral-N available in soil, is now demonstrated. Both NH_4^+ and NO_3^- are easily taken up by plant roots, favoring considerably plant growth; thus, earthworms can stimulate the transfer of nutrients from microbial biomass to the plants.

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References

- Anderson JM, Huish SA, Ineson P, Leonard MA, Splatt PR (1985) Interactions of invertebrates, micro-organisms and tree roots in nitrogen and mineral element fluxes in deciduous woodland soils. In: Fitter AH, Atkinson D, Read DJ, Usher MB (eds) Ecological interactions in soil. Blackwell Scientific Publications, Oxford, pp 377–392
- Araujo Y (2000) Oligoquetos sob adição de liteira e sua relação com a disponibilidade de nitrogênio em solos de capoeira na Amazônia central. Master Thesis, INPA/UA, Manaus
- Atlavinyte O, Lugauskas A (1971) The effect of lumbricidae on soil microorganisms. *Ann Zool Ecol Anim Spec Publ* 4:73–80
- Barois I, Verdier B, Kaiser P, Mariotti A, Rangel P, Lavelle P (1987) Influence of the tropical earthworm *Pontoscolex corethrusus* (Glossoscolecidae) on fixation and mineralization of nitrogen. In: Boncini AM, Omodeo P (eds) On earthworms. Mucchi, Modena, pp 151–159
- Barros E (1999) Efeito de la macrofauna sur la structure et les processus physiques du sol de paturages degradés d'Amazonie. These de Doctorat, Université Paris 6
- Barros E, Curmi P, Hallaire V, Chauvel A, Lavelle P (2001) Role of macrofauna in the transformation and reversibility of soil structure of an Oxisol during forest to pasture conversion. *Geoderma* 100:193–213
- Barros E, Pashanasi B, Constantino R, Lavelle P (2002) Effects of land-use system on the soil macrofauna in western Brazilian Amazonia. *Biol Fert Soils* 35:338–347
- Beck L, Höfer H, Martius C, Garcia M, Franklin E, Römbke J (1998) Soil fauna and litter decomposition in primary forest, secondary forest and polyculture systems in Amazonia—study design and methodology (SHIFT Project ENV 52) In: Lieberei R, Voss K, Bianchi H (eds) Proc 3rd SHIFT Worksh, Manaus, Brazil, pp 463–469
- Blair JM, Parmelee RW, Allen MF, McCartney DA, Stinner BR (1997) Changes in soil N pools in response to earthworm population manipulations in agroecosystems with different N sources. *Soil Biol Biochem* 29:361–367
- Blanchart E, Bruand A, Lavelle P (1993) The physical structure of *Millisonia anomala* (Oligochaeta: Megascolecidae) casts in shrub savanna soils (Côte d'Ivoire). *Geoderma* 56:119–132
- Bohlen PJ, Edwards CA (1995) Earthworm effect on N dynamics and soil respiration in microcosms receiving organic and inorganic nutrients. *Soil Biol Biochem* 27:341–348
- Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol Biochem* 17:837–842
- Decaens T, Lavelle P, Jimenez JJ, Escobar G, Rippstein G (1994) Impact of land management on soil macrofauna in the Oriental Llanos of Colombia. *Eur J Soil Biol* 30:157–168
- Decaens T, Rangel AF, Asakawa N, Thomas J (1999) Carbon and nitrogen dynamics in ageing earthworm casts in grasslands of the Eastern Plains of Colombia. *Biol Fert Soils* 30:20–28
- Fragoso C, Brown GG, Patrón JC, Blanchart E, Lavelle P, Pashanasi B, Senapati B, Kumar T (1997) Agricultural intensification, soil biodiversity and agroecosystem function in the tropics: the role of earthworms. *Appl Soil Ecol* 6:17–35
- González G, Zou X, Borges S (1996) Earthworm abundance and species composition in abandoned tropical croplands: comparisons of tree plantations and secondary forests. *Pedobiologia* 40:385–391
- Guerra RT (1988) Ecologia dos Oligochaeta da Amazônia. II Estudo da estivação e da atividade de *Chibui bari* (Glossoscolecidae, Oligochaeta), através da produção de excrementos. *Acta Amazonica* 18:27–34
- Guerra RT, Asakawa N (1981) Efeito da presença e do número de indivíduos de *Pontoscolex corethrusus* (Glossoscolecidae, Oligochaeta) sobre a população total de microorganismos do solo. *Acta Amazonica* 11:319–324
- Jennings DH (1989) Some perspectives on nitrogen and phosphorus metabolism in fungi. In: Boddy L, Marchant R, Read DJ (eds) Nitrogen, phosphorus and sulphur utilization by fungi. Cambridge University Press, Cambridge, pp 1–31
- Jiménez JJ, Moreno AG, Decaens T, Lavelle P, Fisher MJ, Thomas RJ (1998) Earthworm communities in native savannas and man-made pastures of the Eastern Plains of Colombia. *Biol Fert Soils* 28:101–110
- Lavelle P (1988) Earthworm activities and the soil system. *Biol Fert Soils* 6:237–251
- Lavelle P (2000) Ecological challenges for soil science. *Soil Sci* 165:73–86
- Lavelle P, Pashanasi B (1989) Soil macrofauna and land management in Peruvian Amazonia (Yurimaguas, Loreto). *Pedobiologia* 33:283–291
- Lavelle P, Barois I, Cruz I, Fragoso C, Hernandez A, Pineda A, Rangel P (1987) Adaptive strategies of *Pontoscolex corethrusus* (Glossoscolecidae, Oligochaeta), a peregrine geophagous earthworm of the humid tropics. *Biol Fert Soils* 5:188–194

- Lavelle P, Bignell D, Lepage M, Wolters V, Roger P, Ineson P, Heal OW, Dhillon S (1997) Soil function in a changing world: the role of invertebrate ecosystem engineers. *Eur J Soil Biol* 33:159–193
- Lee K (1985) Earthworms: their ecology and relationships with soils and land use. Academic Press, New York
- Luizão FJ (1985) Influência da calagem e adubação orgânica na mesofauna e em algumas propriedades físicas de um Latossolo Amarelo textura argilosa. *Rev Bras Ci Solo* 9:81–84
- Luizão FJ, Schubart HOR (1987) Litter production and decomposition in 'terra firme' forest of the Central Amazonia. *Experientia* 43:259–285
- Maynard DG, Kalra YP (1993) Nitrate and exchangeable ammonium nitrogen. In: Carter MR (ed) Soil sampling and methods of analysis. Lewis Publishers, New York, pp 25–38
- Palm CA, Sanchez PA (1990) Decomposition and nutrient release patterns of the leaves of three tropical legumes. *Biotropica* 22:330–338
- Parle JN (1963) A microbial study of earthworm casts. *J Gen Microbiol* 31:13–22
- Pashanasi B, Melendez G, Szott L, Lavelle P (1992) Effect of inoculation with endogeic earthworm *Ponstoscolex corethrurus* (Glossoscolecidae) on N availability, soil microbial biomass and the growth of three tropical fruit tree seedlings in a pot experiment. *Soil Biol Biochem* 24:1655–1659
- Römbke J, Verhaagh M (1992) About earthworm communities in a rain forest and an adjacent pasture in Peru. *Amazoniana* 12:29–49
- Scheu S (1987) Microbial activity and nutrient dynamics in earthworm casts (Lumbricidae). *Soil Biol Biochem* 5:230–234
- Shaw C, Pawluk S (1986) Faecal microbiology of *Octolasion tyrtaeum*, *Aporrectodea turgida* and *Lumbricus terrestris* and its relation to carbon budgets of three artificial soils. *Pedobiologia* 29:327–339
- Wardle D, Lavelle P (1997) Linkages between soil biota, plant litter quality and decomposition. In: Cadisch G, Giller KE (eds) Driven by nature. CAB International, Wallingford, pp 107–125
- Wilkinson L (1996) SYSTAT 6.0 for Windows. SPSS, Chicago, Illinois