Biol Fertil Soils (1992) 14:49–53

Biology and Fertility of Soils

© Springer-Verlag 1992

Nitrogen mineralization and reorganization in casts of the geophagous tropical earthworm *Pontoscolex corethrurus* (Glossoscolecidae)

P. Lavelle[1], G. Melendez[2], B. Pashanasi[2], and R. Schaefer[1]

[1]Laboratoire d'Ecologie des Sols Tropicaux, ORSTOM/Université P. et M. Curie, F-93-Bondy, France
[2]INIAA, Estación experimental San Ramón, Yurimaguas, Loreto, Perú

Received January 13, 1992

Summary. Mineral N concentrations ranged from 133.1 to 167.8 $\mu g\ g^{-1}$ dry soil in fresh casts of the endogeic earthworm *Pontoscolex corethrurus* fed on an Amazonian Ultisol; this was approximately five times the concentration in non-ingested soil. Most of this N was in the form of NH_4^+. N also accumulated in microbial biomass, which increased from a control value of 10.5–11.3 to 67.5–74.1 $\mu g\ g^{-1}$ in fresh casts. During a 16-day incubation, part of the NH_4^+-N was nitrified and/or transferred to the microbial biomass. Total labile N (i.e., mineral+biomass N) decreased sharply at first (ca. 50% in the first 12 h), and then more slowly. The exact fate of this N (microbial metabolites, denitrification, or volatilization) is not known. After 16 days, the overall N content of the casts was still 28% higher than that of the control soil. Incubation of the soil before ingestion by the earthworms significantly increased the production of NH_4^+ in casts. We calculate that in a humid tropical pasture, 50–100 kg mineral N may be produced annually in earthworm casts. Part of this N may be conserved in the compact structure of the cast where the cast is not in close contact with plant roots.

Key words: Earthworm – *Pontoscolex corethrurus* – Nitrogen mineralization – Microbial biomass – Casts – Ultisol

Recent experiments (Spain et al. 1992) have demonstrated that the growth of cultivated tropical plants may be improved in the presence of geophagous earthworms. In a 70-day assay, the total production of *Panicum maximum* significantly increased in parallel with the biomass of *Millsonia anomala*, a common endogeic earthworm species in humid savannas of the Ivory Coast. Maximum dry matter production of 2.8 times the control production was observed with an earthworm biomass equivalent to 750 kg fresh weight ha^{-1}. The plant biomass was also significantly enriched in N (+29% to +116% and +88% to +260%, respectively, in leaves and roots) and P (+58% to +152% in roots; no difference in leaves).

At Yurimaguas, in the Peruvian Amazonia, a similar experiment was conducted for 120 days to assess the effect of *P. corethrurus*, a geophagous earthworm common throughout the humid tropics, on the growth of tree seedlings. The response varied, depending on the tree species; *Bixa orellana* grew 3 times better in the presence of earthworms, *Eugenia stipitata* grew 1.3–1.7 times better, while no significant effect was observed with the palm-tree *Bactris gasipaes*. The different responses by the tree seedlings were probably a result of the different rooting systems, the *Bixa* sp. having a dense, deep rooting system that allowed a much better use of the nutrients released by earthworms compared with the *Bactris* sp., which has a low density of coarse superficial roots. Dramatic effects on N mineralization and microbial biomass were observed, with highly significant increases in these parameters in the presence of earthworms (Pashanasi et al. 1992).

The present work was designed to test the hypothesis that similar improvements in plant growth would result from increased N mineralization during transit through the earthworm gut and in the casts. Previous studies have indicated that earthworm casts always have higher contents of mineral N than the bulk soil (Syers et al. 1979; review in Lee 1985). Nonetheless, these studies used litter-feeding species, and the increased amounts of nutrients in the casts may have simply reflected the original composition of the ingested material.

P. corethrurus is a peregrine species with a high potential for introduction in tropical cropped soils (Lavelle et al. 1987). It is therefore important to evaluate the impact on N cycling of the activities of this earthworm in order to determine the short- and long-term effects on soil fertility (Lavelle and Martin 1992).

In the present study, mineral N (NH_4^+-N+NO_3^--N) contents were measured in casts incubated for different periods (from 0 to 16.5 days) to assess the amount of mineral N available as NH_4^+ and NO_3^- and the dynamics of

Correspondence to: P. Lavelle

ORSTOM Fonds Documentaire
29 OCT. 1993 N° : 38.607 ex 1
Cote : B

Table 1. Characteristics of the soil used in cultures (ultisol) from a secondary forest at Yurimaguas, Peruvian Amazonia

Organic C ($g\,kg^{-1}$)	Total N	pH	P ($mg\,kg^{-1}$)	Ca	Mg	K	CEC	Al saturation (%)
				($\mu mol/kg$)				
16.8	1.33	3.9	13.6	0.20	0.11	0.15	5.55	91.8

Particle size fractions (μm)				
0–2	2–20	20–50	50–100	100–250	250–2000
16.0	17.5	17.2	45.3	1.3	2.7

the N transformations involved (nitrification, reorganization in microbial biomass). The worms that produced the casts were fed on a forest soil which had been previously incubated for 0, 3, 6, and 9 days in order to detect any spontaneous mineralization occurring in the soil and distinguish it from that triggered by earthworm digestion.

Materials and methods

The soil used was taken from the upper 10 cm of an ultisol supporting a 15-year-old secondary forest (Table 1). Ten samples of about 1 kg were taken at random, mixed, and then thoroughly homogenized. The earthworms were taken from a nearby pasture and left for 2 days in the experimental soil to empty their guts of the pasture soil they had previously ingested.

The worms were then cultured for 24 h in boxes filled with 1 kg experimental soil which had been previously moistened at 25% (pF 2) and sieved through a 2-mm mesh. This technique, developed by Lavelle (1975), separates casts produced by geophagous earthworms from non-ingested soil. The quantity of worms in the culture boxes was adjusted to obtain casts of about 50 g (20 g to analyse mineral N and 30 g to measure microbial biomass). After 24 h the worms were removed from the boxes. A droplet of fresh cast was obtained from each worm by gently pressing the posterior extremity of the animal. Casts deposited in the experimental soil were immediately separated from the non-ingested soil (yielding casts that had been incubated for a mean time of 12 h) or were further incubated to obtain total incubation times of 1.5, 2.5, 4.5, 8.5, and 16.5 days. When extracted, the casts were immediately stored at 4 °C until the analyses were performed.

Mineral N and microbial biomass N were analysed using standard methodology recommended by the Tropical Soil Biology and Fertility (TSBF) programme (Anderson and Ingram 1989), comprising a colorimetric method for mineral N and a ninhydrin method for biomass N. For statistical analyses, the SAS package was used.

Results

Overall effect of earthworms

Earthworm digestion had a dramatic effect on N mineralization (Fig. 1), with highly significant differences between controls and casts for total mineral N ($P<0.0015$), NH_4^+ ($P<0.025$), and NO_3^- ($P<0.03$). Biomass N was also significantly different, although to a lesser extent ($P<0.062$; Table 2):

Effect of incubation time (Table 3)

The length of incubation of the casts had a strong effect on the contents of NH_4^+-N, NO_3^--N, and their sum. The microbial biomass N also responded significantly (Table 3). The NH_4^+ concentration was greatest in fresh casts with a mean value of 114.7 $\mu g\,g^{-1}$, i.e., 18.5 times the control value. The concentration decreased regularly with increasing times of incubation, but tended to stabilize after 8 days at a value 3.3 times greater than the control.

The NO_3^- concentration was greater in the fresh casts than in the control soil, but only by 52%. After a decrease in 0.5-day casts, the NO_3^- concentration increased regu-

Table 2. Changes in NH_4^+, NO_3^-, total mineral N and microbial biomass N with time of incubation

Treatment	Time pre-incub. (days)	Time incub. (days)	NH_4^+	NO_3^-	Total min ($\mu g\,g^{-1}$)	Microbial biomass N
C	0.0	0.0	6.1	20.0	26.1	11.3
C	3.0	0.0	5.7	21.6	27.3	10.5
C	6.0	0.0	6.5	27.8	34.3	10.9
C	9.0	0.0	6.7	29.1	35.8	10.5
L	0.0	0.0	110.8	22.4	133.1	68.4
L	3.0	0.0	119.7	40.1	159.8	73.7
L	6.0	0.0	106.8	41.2	148.0	67.5
L	9.0	0.0	121.6	46.2	167.8	74.1
L	0.0	0.5	32.3	19.1	51.3	21.8
L	3.0	0.5	34.5	25.8	60.2	25.2
L	6.0	0.5	48.2	42.2	90.4	22.7
L	9.0	0.5	74.3	34.5	108.7	27.9
L	0.0	1.5	39.0	34.2	73.2	22.5
L	3.0	1.5	46.9	37.3	84.2	30.5
L	6.0	1.5	57.0	32.0	89.0	26.2
L	9.0	1.5	61.0	45.6	106.6	30.6
L	0.0	2.5	27.5	40.1	67.6	19.4
L	3.0	2.5	23.5	34.9	58.4	18.5
L	6.0	2.5	40.1	38.9	78.9	22.3
L	9.0	2.5	45.9	40.7	86.7	27.3
L	0.0	4.5	22.7	42.3	65.0	16.9
L	3.0	4.5	14.9	42.6	57.9	17.4
L	6.0	4.5	28.3	38.7	67.0	19.7
L	9.0	4.5	43.5	44.9	88.5	25.3
L	0.0	8.5	14.5	44.7	59.2	15.6
L	3.0	8.5	14.2	43.7	57.9	17.1
L	9.0	8.5	31.1	47.6	78.7	21.0
L	0.0	16.5	16.0	71.1	87.1	19.2
L	3.0	16.5	13.7	49.8	63.5	16.1
L	6.0	16.5	23.7	56.4	80.1	17.9
L	9.0	16.5	28.8	49.5	78.3	18.8

N expressed per g dry soil; pre-incub., pre-incubation; incub., incubation; min, mineral; C, control soil; L, earthworm casts

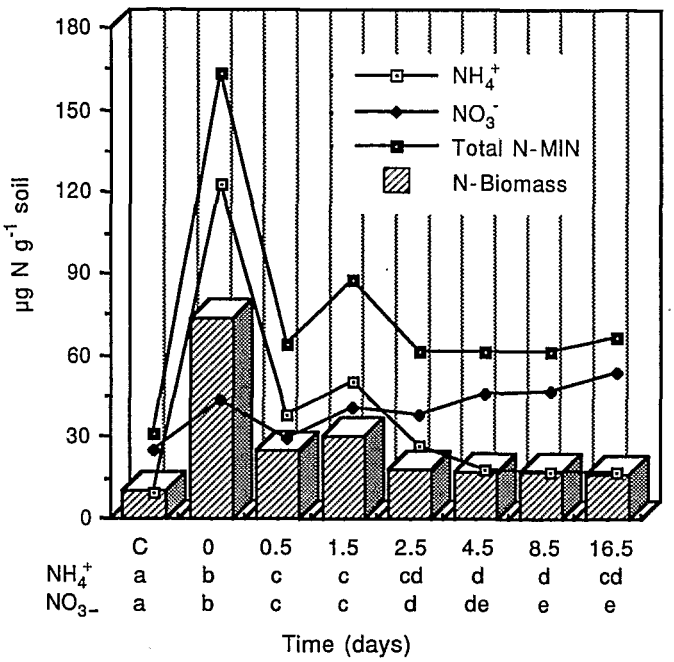

Fig. 1. Changes over time in mineral N (*N-MIN*) and microbial biomass N in casts of *Pontoscolex corethrurus*. *C*, control non-ingested soil. Different letters indicate significantly different values ($P<0.05$)

larly, reaching a maximum value of 56.7 µg g^{-1} (2.3 times control) in casts incubated for 16.5 days.

Total mineral N was greatest in fresh casts (152.2 µg g^{-1}, 4.9 times control), and decreased to values between 66 and 77 (more than twice as much as in the control), with some tendency to increase again after 16.5 days. Microbial biomass N also increased sharply in the fresh casts (71 µg g^{-1}, 6.6 times control) and then decreased rapidly, reaching an equilibrium value of 18 µg g^{-1} after 8 days of incubation.

Effects of pre-incubation

Pre-incubation increased the accumulation of mineral N (Table 4). After 9 days the maximum increase was 26 µg g^{-1} (+33%), which is negligible compared to the effect of earthworm digestion. However, this increase was 2.6 times greater than that observed in the control soil (+9.7 µg g^{-1}, but comparable when expressed as a percentage (+37%). Changes in microbial biomass N were limited to a small increase of 22% after 9 days.

Table 3. Mean mineral N and microbial biomass N in control soil and earthworm casts as a function of time of incubation

	Time of incubation (days)	NH_4^+	NO_3^-	Total min N	Microbial biomass N
		(µg g^{-1})			
Control	0–9	6.2	24.6	30.8	10.8
Casts	0.0	114.7a	37.5ab	152.2a	71.0a
	0.5	47.3b	30.4a	77.7b	24.4bc
	1.5	51.0b	37.3ab	88.2b	27.4b
	2.5	34.2c	38.7ab	72.9bc	21.9cd
	4.5	27.4cd	42.1b	69.6c	19.8de
	8.5	21.1d	45.8b	66.9c	17.9e
	16.5	20.6d	56.7c	77.3bc	18.0e

n = 4 samples, N expressed per g dry soil, values within columns followed by a different letter are significantly different at $P<0.05$

Table 4. Effect of pre-incubation time on mineral N accumulation and microbial biomass N in earthworm casts aged 0–16.5 days

Pre-incub. time (days)	NH_4^-	NO_3^-	Total min N	Microbial biomass N
	(µg g^{-1})			
0	37.5a	39.1a	76.7a	26.3a
3	38.2a	39.2a	77.4a	27.7a
6	46.9b	42.4a	89.3b	28.4a
9	58.0c	44.1a	102.2c	32.2b

n = 7 samples. Pre-incub., pre-incubation. For other explanations, see Table 3

Discussion

Considerable amounts of mineral N were found in casts of *P. corethrurus*. The NH_4^+ content was at a maximum in fresh casts (152.2 µg g^{-1}, i.e., 4.9 times as much as in the non-ingested soil) and then decreased, sharply at first in the 2 days following deposition, and more gently afterwards. After 16.5 days of incubation the NH_4^+ concentration was stabilized at ca. 20 µg g^{-1} (Fig. 1). NO_3^- accumulated slowly, reaching a maximum value of 56.7 µg g^{-1} after 16.5 days. Unlike NH_4^+, the NO_3^- concentration, did not seem to have reached a plateau by that time and further accumulation appeared likely. High N levels also accumulated in the microbial biomass, with a peak at 71 µg g^{-1} on day 0, followed by a sharp decrease to an equilibrium value of ca. 20 µg g^{-1} after 4 days.

Between day 0 and 0.5, the sum of mineral N plus biomass N decreased from 223.3 to 112.1 µg g^{-1}. This sharp decrease may perhaps be attributed to NH_4^+ losses by volatilization, although the slightly acid conditions of the casts do not seem to favour such a process, and denitrification of NO_3^-. It is also possible that the day 0 sample was not comparable to those of casts further separated from the soil after 24 h. Fresh casts were obtained by gently pressing the posterior part of the animal and they may have differed from casts the animal would have expelled if not submitted to that stress. Reabsorption of assimilable C and N present in the gut may have been incomplete, and some of the assimilable N may have been transformed into NH_4^+ during dessication of the samples. This would explain the high concentrations of NH_4^+-N and microbial biomass N measured in casts taken directly from the worm. Furthermore, fresh casts extruded in the soil may rapidly lose part of their assimilable N by diffusion into the surrounding soil. This hypothesis has not been given much attention until now because of the compact strucuture of the casts and their relative physical isolation from the surrounding soil. Further studies should compare casts deposited on the soil surface with casts deposited beneath the surface and fresh casts taken directly from the worm to clarify this point.

An accumulation of NH_4^+ in fresh earthworm casts has been observed several times. NH_4^+ concentrations of 60–80 $\mu g\, g^{-1}$ have been reported in fresh casts of *P. corethrurus* by Barois et al. (1987). Comparable accumulations have also been reported in earthworm casts in temperate regions (Syers et al. 1979), whereas lower concentrations of 25 $\mu g\, g^{-1}$ dry soil have been measured in fresh casts of the endogeic tropical species *Millsonia anomala* (P. Lavelle and S. Martin, unpublished data).

This accumulation is a result of (1) excretion of NH_4^+ through endonephridia, whose lumen opens into the gut, and (2) mineralization of soil organic matter, realized in the middle and posterior part of the gut by ingested soil microflora using the intestinal mucus produced in the anterior gut as a substrate (Barois and Lavelle 1986).

Selection by the worm of organic particles may increase the amount of soil organic matter actually ingested, so that the content of mineral NH_4^+ in fresh casts may have originated from the mineralization of larger amounts of soil organic matter than would be apparent from analyses of the soil associated with the casts.

The accumulation of nitrates during incubation seems to be attributable to nitrification of NH_4^+. A similar decrease in the NH_4^+ concentration in casts followed by the accumulation of NO_3^- has also been reported in surface casts of undetermined temperate endogeics (Syers et al. 1979). In contrast, in casts of the African geophagous species *Millsonia anomala*, the NH_4^+ content had decreased after 8 days to control levels, and no NO_3^- had accumulated (S. Martin and P. Lavelle, unpublished data). In this African soil, incubation never results in the production of NO_3^- (Abbadie 1983), demonstrating that the N dynamics of aging casts are no different from those currently observed in the bulk soil.

The amount of NO_3^- formed during the present experiment (19.2 $\mu g\, g^{-1}$), however, was much less than the amount of NH_4^+ which disappeared (94.1 $\mu g\, g^{-1}$) from day 0. As indicated, most of the losses occurred during the first 12 h following deposition. After that time, total losses of mineral N plus biomass N were limited to 27 $\mu g\, g^{-1}$.

Microbial biomass N, which had increased from 10.8 to 71.0 $\mu g\, g^{-1}$ in fresh casts, decreased afterwards, and 53 $\mu g\, g^{-1}$ had been lost from this compartment after 16.5 days. Thus, at the end of the experiment, casts aged 16.5 days had accumulated 95.3 $\mu g\, g^{-1}$ dry soil of labile N in the form of NH_4^+ (+14.4 $\mu g\, g^{-1}$), NO_3^- (+32.1 $\mu g\, g^{-1}$) and microbial biomass N (+7.2 $\mu g\, g^{-1}$. This represents 2.3 times the amount contained in the control soil.

Finally, on-going N mineralization in the soil had significant effects on the assimilable N found in casts. Both mineral N and biomass N increased in casts when the soil had been previously incubated, and the increase was greater than the amount of assimilable N released during incubation. This probably means that part of the organic N contained in the soil had been transformed into simpler compounds, which were further mineralized or incorporated into the microbial biomass during transit through the earthworm gut.

Conclusion

Digestion of soil organic matter by *P. corethrurus* first resulted in the release of large amounts of NH_4^+ (114.7 $\mu g\, g^{-1}$ dry soil) and a sudden accumulation of N in the microbial biomass (71 $\mu g\, g^{-1}$). During the next 2–4 days, the NH_4^+ concentration and biomass N decreased rapidly, and tended to stabilize at respective values of 3.3 and 1.7 times the control soil concentrations. Nitrification was intense during the incubation of casts and 20.4% of the N lost from the NH_4^+ and biomass compartment was converted to NO_3^-. The fate of the 74.9 $\mu g\, g^{-1}$ that disappeared from the labile pool is not known. Under the conditions of a humid tropical pasture, endogeic earthworms may ingest several hundreds of tonnes of soil $ha^{-1}\, year^{-1}$. As a result, a minimum amount of 50–100 kg of mineral N is released into the soil.

The production of such large amounts of mineral N and its synchrony in time and space with the nutritional needs of plants may explain why plant growth may be so greatly enhanced in the presence of earthworms, especially in soils where N is a limiting factor for plant growth (Spain et al. 1992; Pashanasi et al. 1992). If earthworms are active in the rhizosphere of these plants, a real spatial and temporal synchrony may occur between the release of mineral N and its use by the plant, leading to a very efficient use of nutrients (Swift 1986). Plants will gain the most benefit from N mineralization in casts if the casts are deposited in contact with their roots. Where casts are not in close contact with plant roots their compact structure allows them to retain labile N for some time, mainly in the form of NO_3^- and microbial biomass N. Nonetheless, possible losses of mineral N from freshly deposited casts, through denitrification (Elliott et al. 1990) or volatilization should be investigated.

Acknowledgments. This research was supported by the Commission for the European Community (DGXII/STD2 programme). The help of the administrative and technical staff of Estación experimental San Ramón and support from the Direction are acknowledged. We also thank Misión Carolina del Norte at Lima for administrative and logistic assistance and the TSBF programme for scientific support.

References

Abbadie L (1983) Contribution à l'étude de la production primaire et du cycle de l'azote dans les savanes de Lamto (Côte d'Ivoire). Ph. D. Thesis, Paris VI, Publication du Laboratoire de Zoologie ENS

Anderson JM, Ingram J (1989) Tropical soil biology and fertility handbook of methods. CAB International, Wallingford, UK

Barois I, Lavelle P (1986) Changes in respiration rate and some physicochemical properties of a tropical soil during transit through *Pontoscolex corethrurus* Glossoscolecidæ, Oligochæta). Soil Biol Biochem 18:539–541

Barois I, Verdier B, Kaiser P, Mariotti A, Rangel P, Lavelle P (1987) Influence of the tropical earthworm *Pontoscolex corethrurus* (Glossoscolecidæ) on the fixation and mineralization of nitrogen. In: Omodeo P, Bonvicini AM (eds) On earthworms. Mucchi, Bologna, Italy, pp 151–158

Elliott PW, Knight D, Anderson JM (1990) Denitrification in earthworm casts and soil from pasture under different fertilizer and drainage regimes. Soil Biol Biochem 22:601–605

Lavelle P (1975) Consommation annuelle de terre par une population

naturelle de vers de terre (*Millsonia anomala* Omodeo, Acanthodrilidæ, Oligochæta) dans la savane de Lamto (Côte d'Ivoire). Rev Ecol Biol Sol 12:11–24

Lavelle P, Martin A (1992) Small-scale and large-scale effects of endogeic earthworms on dynamics of organic matter of moist savanna soil. Soil Biol Biochem (in press)

Lavelle P, Barois I, Cruz C, Hernandez A, Pineda A, Rangel P (1987) Adaptative strategies of *Pontoscolex corethrurus* (Glossoscolecidæ, Oligochæta), a peregrine geophagous earthworm of the humid tropics. Biol Fertil Soils 5:188–194

Lee KE (1985) Earthworms: Their ecology and relationships with soils and land use. Academic Press, New York

Pashanasi B, Melendez G, Szott L, Lavelle P (1992) Effect of inoculation with the endogeic earthworm *Pontoscolex corethrurus* (Glossoscolecidæ) on N availability, soil microbial biomass, and the growth of three tropical fruit tree seedlings in a pot experiment. Soil Biol Biochem (in press)

Spain AV, Lavelle P, Mariotti A (1992) Preliminary studies of the effects of some tropical earthworms on plant growth. Soil Biol Biochem (in press)

Swift MJ (1986) Tropical soil biology and fertility (TSBF): Inter-regional research planning workshop. Report of the third Workshop of the decade of the Tropics/TSBF program. Biol Int 13:1–68

Syers JK, Sharpley AN, Keeney DR (1979) Cycling of nitrogen by surface-casting earthworms in a pasture ecosystem. Soil Biol Biochem 11:181–185