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## Interactive effects of native and exotic earthworms on resource use and nutrient mineralization in a tropical wet forest soil of Puerto Rico

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**Abstract** Investigation of single or mixed assemblages of native *Estherella* sp. and exotic *Pontoscolex corethrurus* from a rain forest in Puerto Rico was undertaken to understand resource use patterns, and linkages with C and N mineralization in a 19-day incubation. Resource use was explored with addition of  $^{15}\text{N}$ -enriched leaf litter and  $^{13}\text{C}$ -enriched glucose to reconstructed organic and mineral soil horizons. Juvenile *Estherella* sp. became at least 6.06‰ more enriched in  $^{13}\text{C}$  than sub-adult *Estherella* sp. or adult *P. corethrurus*. Sub-adult *Estherella* sp. became >3.6‰ enriched in  $^{13}\text{C}$  over *P. corethrurus*.  $\delta^{15}\text{N}$  acquired by *P. corethrurus* was greater by 0.83–1.56‰ in the mixed-species than the single-species assemblages.  $\delta^{15}\text{N}$  of sub-adult *Estherella* sp. was enriched by 0.73–0.81‰ over juvenile *Estherella* sp. in the single-species assemblage. Net N immobilization occurred in the organic layer of all  $^{15}\text{N}$ -enriched treatments. Net N mineralization in mineral soil layers was significantly greater in microcosms with *P. corethrurus* than in those containing only *Estherella* sp.. Cumulative respiration was greatest in *P. corethrurus* assemblages, however, assemblages with only *Estherella* sp. released more  $^{13}\text{C}$  in respiration. *P. corethrurus* assimilated different N resources when incubated with, as compared to without, native *Estherella* sp..  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures acquired by assimilation of  $^{13}\text{C}$  and  $^{15}\text{N}$  differed

by species, developmental stage, and competitive interactions. The results showed that alone, exotic *P. corethrurus* induced higher-mineralization rates than native *Estherella* sp., but that the interaction of exotic and native species impinged on resource use by *P. corethrurus*, reducing the effect of the exotic species on C and N mineralization. Invasion of exotic *P. corethrurus* may change the mineralization potentials of C and N and their biogeochemical cycling in soils.

**Keywords** Neotropical earthworms · Stable isotopes · Soil respiration · Nutrient cycling · Competition

### Introduction

There is concern that introduced earthworm species out-compete and replace natives (Stebbing 1962; Kalisz and Wood 1995; Fragoso et al. 1999), causing changes in the composition and structure of the biotic community and compromising soil function (Beare et al. 1995; Lavelle et al. 1997). In North American systems, invasive species have had impacts on soil structure, nutrient cycling, and native populations (Alban and Berry 1994; Steinberg et al. 1997; Burtelow et al. 1998; Groffman and Bohlen 1999). Twenty-nine endemic and 11 exotic species of Oligochaeta have been catalogued on the island of Puerto Rico, (Fragoso et al. 1995; Borges 1996). Among introduced taxa, *Pontoscolex corethrurus* (Müller 1856) has been found in all types of undisturbed native and secondary forests (González et al. 1996; Borges and Alfaro 1997; Zou and González 1997; Hendrix et al. 1999a). Zou and González (1997) found that *P. corethrurus* persisted along gradients of secondary succession after initial agricultural disturbances.

There is little information as to whether the introduction and persistence of *P. corethrurus* has had any impact on soil fertility or native species in the natural systems of Puerto Rico. Manipulation of *P. corethrurus* populations to manage or improve soil fertility in agricultural systems (Lavelle et al. 1987, 1997; Fragoso et al. 1999) has

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experimentally produced positive effects by restructuring soil and soil aggregates (Barois et al. 1993; Zund et al. 1997; Hallaire et al. 2000) and by increasing N availability and/or plant growth (Pashanasi et al. 1992, 1996; González and Zou 1999). Hendrix et al. (1999a) surveyed endemic-exotic species assemblages in disturbed and undisturbed habitats within the Luquillo Experimental Forest and analyzed the  $^{13}\text{C}$  and  $^{15}\text{N}$  content of earthworm tissue and plant and soil resources. Overlapping  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic signatures suggested that potential interspecific competition over resources existed between native *Estherella* sp. and exotic *P. corethrurus* found in a disturbed secondary forest community. However, distinct  $\delta^{15}\text{N}$  signatures between native species and *P. corethrurus* found in an undisturbed high elevation dwarf forest indicated partitioning of N resources. Community patterns and nutrient cycling in endemic-exotic earthworm assemblages are little studied. The present study was conducted to investigate the impact of *P. corethrurus* on soil C and N dynamics, and to determine the potential for competitive interaction with a native species from a Puerto Rican rain forest.

Single-species and mixed species assemblages of native *Estherella* sp. and exotic *P. corethrurus* were used to investigate resource use, nutrient turnover, and the potential for changes due to competitive pressures. To determine the effects of both endemic and exotic species of earthworms on soil processes, nutrient turnover was investigated by measuring C and N mineralization in laboratory microcosms. Patterns of resource use were investigated with isotopically enriched materials,  $^{15}\text{N}$ -enriched leaf litter and  $^{13}\text{C}$ -enriched glucose, applied to surface organic and subsurface mineral soil horizons, respectively. Given that exotic species are believed to increase nutrient cycling rates, net mineralization of C or N was expected to be greater with activity of *P. corethrurus* than with *Estherella* sp. or than in microcosms lacking earthworm activity.

## Materials and methods

Soil and earthworms were collected within the Luquillo Experimental Forest at the El Verde Experimental Research Station (18°20'N, 65°49'W) in northeastern Puerto Rico. The study area is a tabonuco (*Dacryodes excelsa*) forest exposed to disturbance caused by landslides, tree falls, tropical storms and hurricanes (Waide and Reagan 1996). Organic (O) layer forest floor and mineral (A) layer soil were collected along a hill slope and both sieved through a 5-mm mesh. Two earthworm species, *P. corethrurus* and *Estherella* sp., were collected from the same hill slope, sorted and left 24 h in containers with moist paper towels to void their guts.

Fifty-three microcosms were constructed within bottom-sealed 1-l containers. In each, 40 g O-layer and 450 g A-layer soil (wet weight, respectively at 260% and 77% gravimetric moisture) were used. Two initial treatments were established for a pre-incubation period of 5 days. Thirteen containers were established as reference treatments (RT) and received 10 ml deionized water applied to mineral soil. The other 40 microcosms were established as amendment treatments (AT), which received  $^{13}\text{C}$ - and  $^{15}\text{N}$ -enriched materials.  $^{13}\text{C}$ -enriched material was applied in 10 ml of a 10% glucose solution (10 atom %) added to the wet A-layer soil in each microcosm. Ten milliliters of the glucose solution provided 2.2 mg glu-

cose  $\text{g}^{-1}$  wet soil and 93  $\mu\text{g}^{13}\text{C}$ . The glucose amendment rate was chosen as the minimum amount necessary to stimulate maximum microbial activity determined with the substrate induced respiration method modified from Anderson and Domsch (1973).  $^{15}\text{N}$ -enriched material was applied in 1.5 g  $^{15}\text{N}$ -labeled eucalyptus leaf ( $\delta^{15}\text{N}=970\text{‰}$ ) cut into ca. 1-cm<sup>2</sup> sections and mixed with native O-layer material in each AT microcosm. To allow time for microbial assimilation of the  $^{13}\text{C}$ -enriched glucose into biomass or by-products, microcosms were pre-incubated for a 5-day period consistent with Webster et al. (1997). After pre-incubation, four randomly chosen replicates from each RT and AT were sampled for initial (day 0) measurements. An additional three treatments were established at day 0 by reassigning 27 of the remaining 36 AT microcosms. Three earthworm treatments were established: (1) a *Pontoscolex* assemblage (PA) with four adult individuals of *P. corethrurus*; (2) an *Estherella* sp. assemblage (EA) with one juvenile and one sub-adult *Estherella* sp.; and (3) a mixed species assemblage (MA) with two adult *P. corethrurus* and one sub-adult *Estherella* sp. The individuals of *Estherella* sp. were distinguished by size; sub-adults were larger by size and weight than juveniles and were non-clitellate. All five treatments (AT, RT, PA, EA, MA) established at day 0 had nine replicates.

Three replicates were selected from each treatment to measure soil respiration for the duration of the experiment. Soil respiration was measured in 24-h periods on five different dates: day 0, day 4, day 10, day 14 and day 19. Respired  $\text{CO}_2$  was trapped in 10 ml NaOH. Two 5-ml sub-samples of the alkali trap were precipitated with  $\text{BaCl}_2$ , and unreacted NaOH was titrated to phenolphthalein end point with 1 N HCl in the first sub-sample.  $\text{CO}_2\text{-C}$  produced over 24 h was calculated from the end-point titration volume and corrected for blanks (Stotzky 1965). The  $\text{BaCO}_3$  precipitate formed in the second sub-sample was freeze-dried and stored for  $^{13}\text{C}$  analysis. C mineralization was obtained as the cumulative mg  $\text{CO}_2\text{-C}$  produced over the entire 19-day incubation, interpolating for days between measurements.

Three replicates of each treatment were destructively sampled on days 6, 14 and 19 after pre-incubation (day 0). Microcosms were deconstructed by organic (O) and mineral (A) layers. The A-layer was further divided into top and bottom halves, A<sub>1</sub> and A<sub>2</sub>, respectively, to examine more localized effects. Soils were stored at 4°C, for up to 2 days, until extraction. Soil sub-samples for each layer were extracted with 0.5 M  $\text{K}_2\text{SO}_4$  at 2:1 solution to wet soil mass ratio and filtered through Whatman no. 42 ashless filters. A second sub-sample of soil was fumigated with 40 ml alcohol-free chloroform in atmosphere under vacuum for 48 h and extracted to determine microbial biomass C (Vance et al. 1987). Total inorganic N ( $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}+\text{NO}_2^-\text{-N}$ ) from the non-fumigated sub-sample extracts was measured on an Alpkem autoanalyzer (Keeney and Nelson 1982). Net N mineralized was calculated as the total inorganic N on the sample date minus the initial total inorganic N measured on day 0 and expressed in milligrams per gram total soil N of the later sample date. Dissolved organic C (DOC) from fumigated and non-fumigated sample extracts was measured on a Shimadzu total organic C analyzer. Microbial C released by fumigation was calculated as the difference in total DOC between fumigated and non-fumigated sample extracts corrected for blanks.

Earthworms were removed from microcosms and allowed to clear guts for 24 h on moist paper towels, weighed fresh then killed by dipping for 1 s in boiling water. The body region posterior to the clitellum was dissected, cleaned of remaining materials, frozen, and freeze-dried for stable isotope analysis. For *P. corethrurus* only the intestinal region between the clitellum and the last ten segments was used. The intact body segments remaining of both species were preserved for identifications.

Total soil C and N and isotopic  $\delta$  signatures ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) of organic and mineral soil were determined by dry combustion on a Carlo Erba CN analyzer in line with a Finnigan Delta C isotope ratio mass spectrometer (accuracy of 0.1‰). Isotopic signatures in delta notation ( $\delta$ ) are expressed as the proportion of the ratio of heavier isotope to lighter isotope of sample versus the ratio of reference standards (‰) and relative to Pee-Dee Belemnite for  $^{13}\text{C}$  (Eq. 1) and atmospheric N concentration for  $^{15}\text{N}$  (Eq. 2).

$$\delta^{13}\text{C} = \left[ \left( \frac{{}^{13}\text{C} : {}^{12}\text{C}_{\text{sample}}}{{}^{13}\text{C} : {}^{12}\text{C}_{\text{standard}}} \right) - 1 \right] \times 1000 \quad (1)$$

$$\delta^{15}\text{N} = \left[ \left( \frac{{}^{15}\text{N} : {}^{14}\text{N}_{\text{sample}}}{{}^{15}\text{N} : {}^{14}\text{N}_{\text{standard}}} \right) - 1 \right] \times 1000 \quad (2)$$

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of freeze-dried earthworm tissue and  $\text{BaCO}_3$  precipitate were also obtained as above. Soil and earthworm tissue were ground into a fine powder with a ball mill prior to analysis.

Statistical analyses were performed with SAS (Statistical Analysis Systems 1998). Pre-incubation data were analyzed by two-way ANOVA for differences between RT and AT and soil layers. Due to the strong variability between soil layers and complex changes over time, transformations could not be applied consistently to stabilize normality or variance of the data collected after earthworm additions. Therefore, only one-way effects were analyzed. Isotopic signatures of earthworm tissue were analyzed within date and also within treatment. For each soil layer, treatment effects were analyzed within date. Differences in cumulative soil respiration among treatments on the final date were analyzed.  $\delta^{13}\text{C}$  of soil respiration was analyzed within individual dates first to establish a significant difference between RT and enriched treatments and again across the enriched treatments only. Post-hoc multiple comparisons among treatments were tested with Student-Newman-Keuls' method (SNK). A significance level of  $\alpha=0.05$  was used for all tests.

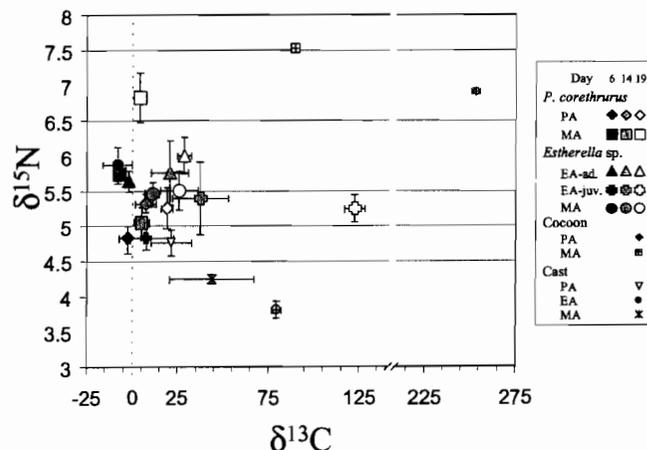
## Results

### Earthworm dynamics

#### Activity

Over the incubation, fungal hyphae proliferated in the O-layer of most enriched microcosms, but never in the RT. Fungal growth on individual sampling days was most pronounced in the AT with lessening proliferation in earthworm treatments in the order of EA>MA>PA. Burrowing and casting behavior of *P. corethrus* and *Estherella* sp. differed within single-species and mixed-species assemblage treatments. Cast production was observed in all earthworm treatments. In treatments with *P. corethrus*, casts of mineral materials were found at the interface between the O- and  $A_1$ -layers and those containing O material were found in the  $A_2$ -layer of PA treatments and in the  $A_1$ -layer of the MA treatment. Casting and burrowing activity of *Estherella* sp. adults was most apparent in the lower  $A_2$ -layer while activity of juveniles was apparent in the upper  $A_1$ -layer where smaller burrows were found. Only casts appearing to be of mineral material were observed in EA treatments.

Initial total fresh earthworm weights per microcosm averaged  $2.00 \pm 0.05$  g (range 1.92–2.08 g) for PA,  $2.13 \pm 0.22$  g (range 1.86–2.58 g) for EA, and  $2.53 \pm 0.35$  g (range 2.12–2.95 g) for MA. Surviving earthworms from the PA gained  $0.14 \pm 0.06$  g fresh weight per individual, however, three *P. corethrus* adults died on each of the last two sampling dates. *P. corethrus* adults from MA gained an average fresh weight  $0.12 \pm 0.9$  g per individual, and all adults survived. All sub-adult and juvenile *Estherella* sp. individuals survived through the incubations. Sub-adult *Estherella* sp. from the EA had an average loss of  $0.29 \pm 0.18$  g fresh weight, and there was no change in the weight of individuals from the MA ( $0.00 \pm 0.20$  g per



**Fig. 1**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (‰) of earthworms, casts and cocoons, by sample day and treatment (mean  $\pm$  SE;  $n=3$ ). Earthworms: day 6 (black), day 14 (gray), and day 19 (white); casts and cocoons: day 19 only. Please note the break in the x-axis. PA *Pontoscolex corethrus* assemblage, EA *Estherella* sp. assemblage, EA-ad. *Estherella* sp. sub-adults, EA-juv. *Estherella* sp. juveniles, MA mixed species assemblage

individual). From the EA, juvenile *Estherella* sp. averaged a gain of  $0.10 \pm 0.06$  g per individual.

#### Isotopes

Earthworms were grouped together by their  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures on the first 2 sampling days but exhibited enrichment in either  $^{13}\text{C}$  or  $^{15}\text{N}$  on the final day (Fig. 1). *P. corethrus* from the MA obtained the greatest enrichment of  $^{15}\text{N}$  by the nineteenth day of incubation, while juvenile *Estherella* sp. obtained the greatest enrichment of  $^{13}\text{C}$  (Fig. 1).  $\delta^{13}\text{C}$  signatures of all earthworm tissues, initially negative, increased at least 10‰ to positive values over the 19-day incubation. Between days 6 and 19, tissue  $\delta^{13}\text{C}$  was significantly increased in juvenile and sub-adult *Estherella* sp. from EA and in *P. corethrus* tissue from both PA and MA (Table 1). On all days, juvenile *Estherella* sp. were enriched in  $^{13}\text{C}$  at least 6.06‰ over all other earthworm signatures; this enrichment was significant on day 19 (Table 1). Sub-adult *Estherella* sp. were enriched in  $^{13}\text{C}$  by >3.6‰ over *P. corethrus* on days 14 and 19. The  $\delta^{15}\text{N}$  signatures did not exhibit a specific trend but varied over time in all earthworms and ranged from 4.42‰ to 6.67‰.  $\delta^{15}\text{N}$  of *P. corethrus* from the MA significantly increased with incubation and was significantly higher than in other earthworms, except EA sub-adults, by 0.83–1.56‰ on day 19 (Table 1). In the EA,  $\delta^{15}\text{N}$  of sub-adult *Estherella* sp. was consistently more enriched than that of juvenile *Estherella* sp. by 0.73–0.81‰ across all days. *P. corethrus* from the MA were generally more enriched in  $^{15}\text{N}$  and less enriched in  $^{13}\text{C}$  than individuals from PA.

Earthworms were enriched in  $^{13}\text{C}$  at 6 days of incubation with  $\delta^{13}\text{C}$  values >12‰ over all treatment O-layers or 17‰ over RT A-layers.  $\delta^{13}\text{C}$  of earthworms was gen-

**Table 1** Results of one-way ANOVA and multiple comparison tests (Student-Newman-Keuls, SNK) for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of earthworm tissue, casts, and cocoons. Within a row, different capital letters indicate significant differences within a species across dates, by SNK at

$P < 0.05$ . Within a column, different lowercase letters indicated significant differences across earthworm species or treatments, by SNK at  $P < 0.05$ . PA *Pontoscolex corethrus* assemblage, EA *Estherella* sp. assemblage, MA mixed species assemblage

Treatment		$\delta^{13}\text{C}$						$\delta^{15}\text{N}$					
Species	$F_{\text{row}}$	Day 6	Day 14	Day 19	Cast	Cocoon	$F_{\text{row}}$	Day 6	Day 14	Day 19	Cast	Cocoon	
PA	<i>P. corethrus</i>	8.08*	Aa	ABa	Ba	a	a	1.67 NS	Aa	Aa	Aa	a	a
EA	<i>Estherella</i> sp.					b						b	
	Juvenile	21.5**	Aa	Aa	Bb			0.52 NS	Aab	Aa	Aa		
	Sub-adult	6.15*	Aa	Aa	Ba			0.34 NS	Aab	Aa	Ab		
MA					ab	b						c	b
	<i>P. corethrus</i>	5.17*	Aa	Ba	Ba			13.42**	Ab	Aa	Bb		
	<i>Estherella</i> sp.	4.76	Aa	Aa	Aa			0.91	Aab	Aa	Aa		
	sub-adult	NS						NS					
		$F_{\text{column}}$	0.68	2.78 NS	66.72***	5.66*	3108.9***		3.58*	0.28	6.31**	16.53**	174.62***
			NS							NS			

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , NS not significant at  $P = 0.05$

**Table 2** ANOVA of mean initial concentrations ( $\pm$ SE;  $n=4$ ) of total soil C,  $\delta^{13}\text{C}$ , total soil N,  $\delta^{15}\text{N}$ , microbial biomass C (MBC), and inorganic N, for reference treatment (RT) and amendment treatment

(AT), sampled prior to earthworm additions, by organic (O) and mineral ( $A_1$  and  $A_2$ ) layers. Within a variable, means followed by different letters are significantly different by SNK at  $P < 0.05$

Variable	Treatment	Soil layer			Treatment	Layer	Treatment $\times$ layer
		O	$A_1$	$A_2$			
Total soil C %	RT	27.84 (1.61) a	4.38 (0.04) b	4.30 (0.06) b	0.54 NS	334.15***	0.77 NS
	AT	25.80 (1.84) a	4.62 (0.04) b	4.40 (0.06) b			
$\delta^{13}\text{C}$	RT	-28.12 (0.02) a	-27.16 (0.05) a	-27.00 (0.13) a	2284.41***	460.00***	445.90***
	AT	-16.03 (2.31) b	110.02 (4.06) c	125.32 (4.24) d			
Total soil N %	RT	1.57 (0.08) a	0.40 (0.00) c	0.40 (0.00) c	3.33 NS	340.53***	3.84*
	AT	1.35 (0.09) b	0.42 (0.01) c	0.41 (0.00) c			
$\delta^{15}\text{N}$	RT	2.09 (0.04) a	4.04 (0.07) c	4.04 (0.02) c	121.31***	82.40***	121.15***
	AT	24.26 (2.00) b	3.99 (0.17) c	4.10 (0.02) c			
MBC mg g <sup>-1</sup> soil C	RT	5.28 (0.56) a	10.90 (0.19) c	10.50 (0.45) c	9.25**	63.24***	3.45 NS
	AT	6.22 (0.29) b	11.15 (0.78) d	13.8 (0.94) d			
Inorganic N mg g <sup>-1</sup> N	RT	8.05 (0.28) a	4.19 (0.33) c	3.70 (0.29) cb	324.36***	104.10***	8.66**
	AT	3.06 (0.18) b	0.89 (0.12) d	0.68 (0.29) d			

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , NS not significant at  $P = 0.05$

erally 55% less enriched with respect to A-layers of AT, PA, EA and MA treatments. By day 6, earthworm  $\delta^{15}\text{N}$  was 2–3% higher than that of the RT O-layer and 1–1.3% higher than that of the A-layers of all treatments.  $\delta^{15}\text{N}$  was much lower in earthworms than in the  $^{15}\text{N}$ -labeled O-layers of AT, PA, EA and MA treatments.

#### Casts and cocoons

Casts collected on day 19 were generally enriched in  $^{13}\text{C}$  but consistently depleted in  $^{15}\text{N}$  relative to tissue signatures (Fig. 1).  $\delta^{13}\text{C}$  of casts from EA was significantly greater than those from PA, but neither were different from those of MA, whereas  $\delta^{15}\text{N}$  of casts was significantly different among all three assemblages (Table 1).  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of casts were in the range of, or depleted relative to, A-layer signatures. Cocoons, produced only by *P. corethrus*, were enriched in  $^{13}\text{C}$  and  $^{15}\text{N}$  relative to earth-

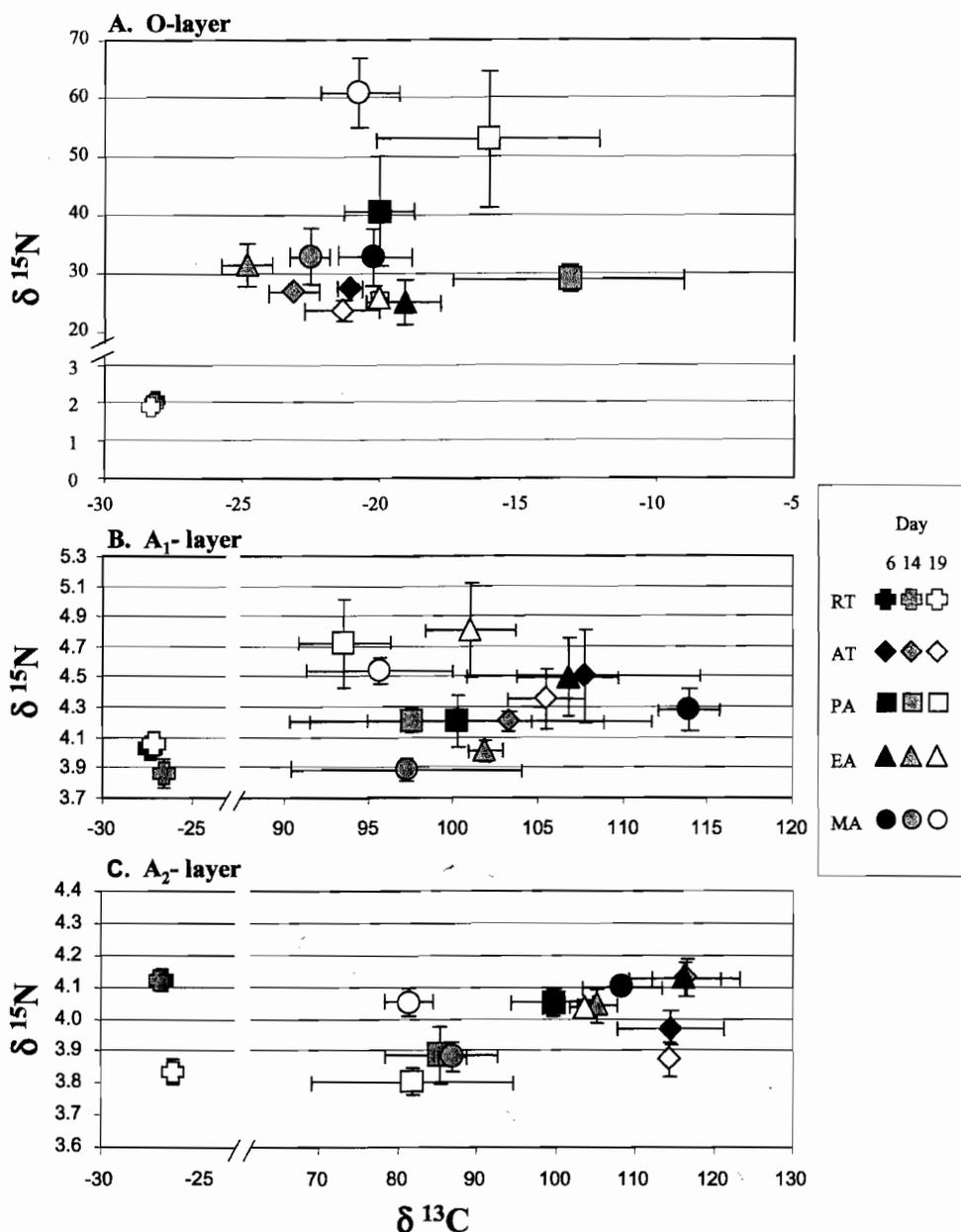
worm tissues (Fig. 1).  $\delta^{13}\text{C}$  of PA cocoons were 150% enriched over MA cocoons, however  $\delta^{15}\text{N}$  was 0.7% lower; both differences were significant (Fig. 1, Table 1).

#### Soil dynamics

##### Initial conditions

Concentrations of total and extractable C and N of organic and mineral soil were assessed before earthworm additions (Table 2). In both treatments, total C and N were significantly greater in the O-layer than in the A-layers. Within soil layers, the addition of enriched materials did not significantly affect total C or N, however, on average both were decreased in the O-layer and increased in the A-layers (Table 2). Prior to addition of glucose and leaf litter,  $\delta^{13}\text{C}$  of the mineral soil averaged  $-27.1 \pm 0.09\%$  and  $\delta^{15}\text{N}$  averaged  $4.0 \pm 0.08\%$ . In the RT,  $\delta^{13}\text{C}$  was simi-

**Fig. 2**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (‰) of soil by treatments and sample day within the **A** O-layer, **B**  $A_1$ -layer and **C**  $A_2$ -layer (mean $\pm$ SE;  $n=3$ ). Day 6 *black*, day 14 *gray*, day 19 *white*. Please note the breaks in both the x- and y-axes. RT Reference treatment, AT amendment treatment; for other abbreviations, see Fig. 1



lar across all layers, however  $\delta^{15}\text{N}$  was significantly lower in the O-layer than in the A-layers. Addition of enriched materials to the AT did significantly increase  $\delta^{15}\text{N}$  of the O-layer and  $\delta^{13}\text{C}$  of all three soil layers (Table 2). Initial microbial biomass C (MBC) was significantly lower in the O-layer than in the A-layers of both RT and AT, however, inorganic N was significantly greater in the O-layer (Table 2). Within layers, MBC was significantly greater in AT, however, inorganic N was significantly greater in the RT (Table 2).

#### Isotopes

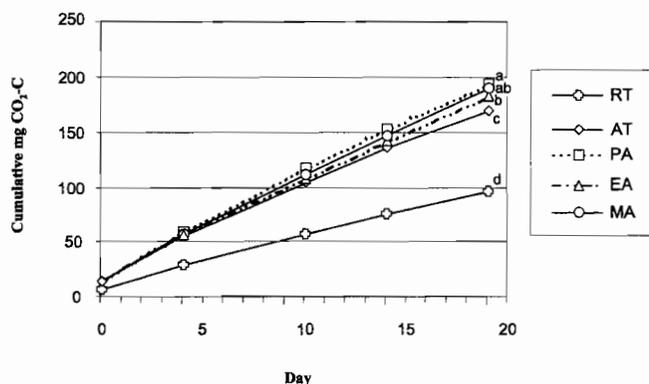
With incubation, the combined  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures showed that PA and MA treatments diverged from all

other treatment signatures across all soil layers (Fig. 2). There were several significant treatment effects for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  that only reflected the expected difference between the RT and the four enrichment treatments (Table 3). An increase in  $\delta^{13}\text{C}$  in the O-layer was apparent in the PA, however, this shift was significantly different from all other treatments on day 14 (Fig. 2a, Table 3). At 19 days,  $\delta^{15}\text{N}$  of the O-layer was significantly increased in both PA and MA over all other treatments (Table 3). In the  $A_1$ -layer a shift in  $\delta^{15}\text{N}$  by 19 days set the three earthworm treatments apart from all other treatments and dates, however, signatures were not significantly different (Fig. 2b, Table 3). In the  $A_2$ -layer, both PA and MA were set apart by  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  from other treatments at 14 and 19 days (Fig. 2c). On day 14, the only significant difference in  $\delta^{15}\text{N}$  was between PA and RT; for  $\delta^{13}\text{C}$ , PA

**Table 3** Results of one-way ANOVA and multiple comparison tests (SNK) for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of soil, by date within layer. Within a row, different capital letters indicate significant differences by SNK at  $P < 0.05$ . For abbreviations see Tables 1 and 2

ANOVA factor		$\delta^{13}\text{C}$				$\delta^{15}\text{N}$						
$F_{\text{row}}$		RT	AT	PA	EA	MA	$F_{\text{row}}$	RT	AT	PA	EA	MA
Day 6	O-layer	12.10***	A	B	B	B	8.45**	A	B	B	B	B
	A <sub>1</sub> -layer	135.59***	A	B	B	B	0.98 NS	A	A	A	A	A
	A <sub>2</sub> -layer	128.34***	A	B	B	B	2.57 NS	A	A	A	A	A
Day 14	O-layer	8.09**	A	A	B	A	20.51***	A	B	B	B	B
	A <sub>1</sub> -layer	94.99***	A	B	B	B	4.51*	A	A	A	A	A
	A <sub>2</sub> -layer	209.48***	A	B	C	B	4.33*	A	AB	B	AB	AB
Day 19	O-layer	4.85*	A	AB	B	AB	16.43***	A	B	C	B	C
	A <sub>1</sub> -layer	418.11***	A	B	B	B	1.89 NS	A	A	A	A	A
	A <sub>2</sub> -layer	89.52***	A	B	C	BC	7.90**	A	A	A	B	B

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , NS not significant at  $P = 0.05$



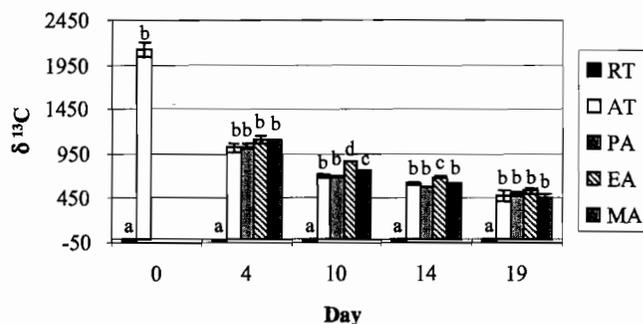
**Fig. 3** Cumulative  $\text{CO}_2\text{-C}$  in ( $\text{mg day}^{-1}$ ) by treatment over 19 days of incubation (mean  $\pm$  SE,  $n = 4$  day 0 else  $n = 3$ ). Lowercase letters indicate significant differences among treatments in final cumulative  $\text{CO}_2\text{-C}$  on day 19 [Student-Newman-Keuls' method (SNK),  $P < 0.05$ ]. For abbreviations see Figs. 1 and 2

and MA together were significantly different from AT and EA, and all were different from RT (Table 3). On day 19,  $\delta^{15}\text{N}$  was significantly higher in the EA and MA over the other treatments; for  $\delta^{13}\text{C}$ , PA and MA together were significantly different from AT, and all were different from RT (Table 3).

#### Mineralization

Cumulative soil respiration in all enriched treatments was greatly stimulated over the unenriched RT (Fig. 3). Respiration rate was similar in enriched treatments through day 4, then diverged among treatments after 10 days of incubation and continued at the highest rate in PA. After day 4, production and accumulation of respired  $\text{CO}_2\text{-C}$  appeared fairly constant within all treatments. Cumulative  $\text{CO}_2\text{-C}$  after 19 days was significantly different among treatments (Fig. 3).

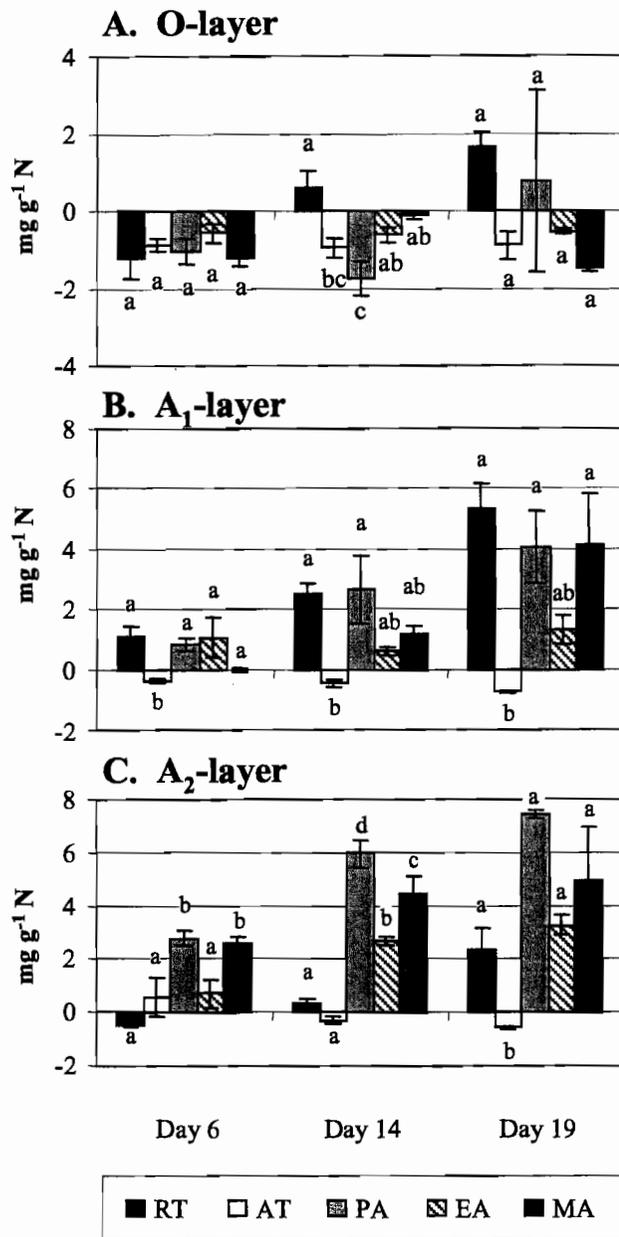
The  $\delta^{13}\text{C}$  of respired  $\text{CO}_2\text{-C}$  from enriched treatments was much greater than that in RT (Fig. 4). The  $\delta^{13}\text{C}$  signature of respired  $\text{CO}_2\text{-C}$  from the enrichment treatments decreased over the incubation period from an initial high



**Fig. 4**  $\delta^{13}\text{C}$  (‰) of respired  $\text{CO}_2\text{-C}$  collected by sample date comparing treatments over days elapsed from the addition of earthworms (mean  $\pm$  SE,  $n = 4$  day 0 else  $n = 3$ ). Lowercase letters represent significant differences among treatments within a sample date (SNK,  $P < 0.05$ ). For abbreviations see Figs. 1, 2 and 3

on day 0.  $\delta^{13}\text{C}$  of respiration from EA was significantly greater than that of the other enriched treatments on the tenth and fourteenth days of sampling (Fig. 4).  $\delta^{13}\text{C}$  values of mineralized C from RT were constant at  $-26.01 \pm 1.50$ ‰ over all the sampling dates.

Net mineralization or N immobilization on days 6, 14, and 19, determined as the difference from total mineral N on day 0, depended on soil layer and treatment (Fig. 5). Over the 19-day incubation, net N immobilization increased in the AT in all soil layers, however net N mineralization increased over time in all soil layers of RT and in the A<sub>1</sub>- and A<sub>2</sub>-layers of the earthworm treatments (Fig. 5). In the O-layer, net N immobilization was measured in all treatments, however, mineralization occurred only in RT and PA; differences among treatments were significant only on day 14 (Fig. 5a). In the A<sub>1</sub>-layer for all sampling dates, net N immobilization was measured only in the AT (Fig. 5b). Net N differences in the A<sub>1</sub>-layer were not significant among the RT and earthworm assemblages on any day, however RT and PA were significantly different from the AT on all dates (Fig. 5b). Net N mineralization in the A<sub>2</sub>-layer was significantly greater in PA and MA over other treatments on day 6 and day 14, however, on day 19, net N immobilization in the



**Fig. 5** Net change in mineralized N ( $\text{mg NO}_3^- \text{-N/NO}_2^- \text{-N+NH}_4^+ \text{-N g}^{-1}$  total N) by treatment and sample date for **A** O-layer, **B** A<sub>1</sub>-layer, and **C** A<sub>2</sub>-layer soil (mean  $\pm$  SE,  $n=3$ ). Different letters within a soil layer and sample date represent significant differences among treatments (SNK,  $P < 0.05$ ). For abbreviations see Figs. 1, 2 and 3

AT was significantly different to all other treatments in which net mineralization was measured (Fig. 5c).

## Discussion

### Earthworm resource use

In the present study, resource use by native and exotic earthworm species was investigated by tracking changes in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of earthworms, cocoons, casts and soil.

Earthworms assimilated sufficient  $^{13}\text{C}$ , in the first 6 days of incubation, to increase  $\delta^{13}\text{C}$  by 15‰ over the field population signature,  $\delta^{13}\text{C}$  ( $-26$  to  $-23.5$ ‰) of these species (Hendrix et al. 1999a). The increase indicated preferential assimilation of the “new”  $^{13}\text{C}$ -enriched materials in the soil likely incorporated into microbial components during the pre-incubation period. Preference of new C by *Millsonia anomala*, was demonstrated by  $\delta^{13}\text{C}$  signatures most similar to the  $\delta^{13}\text{C}$  of recent organic inputs (Martin et al. 1992b). Greater incorporation of newer C was found in other populations of earthworms collected from areas where shifts between C3 and C4 vegetation had caused traceable changes in soil  $\delta^{13}\text{C}$  (Martin et al. 1992a). Earthworm  $\delta^{13}\text{C}$  did increase with incubation time as both species continued to assimilate components with more of the recently added  $^{13}\text{C}$ . At natural abundance levels, earthworm  $\delta^{13}\text{C}$  was 1–3‰ more enriched than dietary sources (Spain et al. 1990; Martin et al. 1992a; Schmidt et al. 1997; Spain and Le Feuvre 1997; Schmidt 1999; Hendrix et al. 1999a, 1999b; Neilson et al. 2000). However,  $\delta^{13}\text{C}$  of the earthworms in this study was not as enriched as the soil due to insufficient time for complete turnover of tissue C. Enrichment of consumer tissue was expected because of fractional loss of lighter  $^{12}\text{C}$  in respiration with an increased concentration of  $^{13}\text{C}$  with the degree of processing (Boutton 1996).

The extent of  $^{13}\text{C}$  and  $^{15}\text{N}$  assimilation by earthworms from enriched sources was related to developmental stage (juveniles, sub-adults, adults). The greater incorporation of  $^{13}\text{C}$  by juvenile *Estherella* sp. was evidently due to production of new biomass, linked to greater intake of labeled resources. Immature *P. corethrurus* examined by Lavelle et al. (1987) ingested more soil and grew more in relation to total biomass than adults. Loss of weight in sub-adult *Estherella* sp. during incubation from reduced intake or lower assimilation efficiency limited incorporation of newly derived C. The lower  $\delta^{13}\text{C}$  found in *P. corethrurus* than in either sub-adult or juvenile *Estherella* sp. was affected by its reproductive phase, since the  $^{13}\text{C}$  and  $^{15}\text{N}$  acquired apparently were allocated to production of cocoons which were enriched by >71‰ in  $^{13}\text{C}$  and up to 0.7‰ in  $^{15}\text{N}$  relative to parent tissue.

Earthworm  $\delta^{15}\text{N}$  was only up to 3.0‰ enriched relative to the A-layer suggesting N resources were mainly assimilated from the mineral layer rather than the labeled organic layer. However,  $\delta^{15}\text{N}$ , differing by 0.83–1.59‰ between *P. corethrurus* and *Estherella* sp. at the end of incubation, supported potential trophic level differences in feeding within the A-layer. Trophic differences were established among earthworm species where  $\delta^{15}\text{N}$  signatures were significantly separated by 1.5–3.8‰ and 2.4–4.5‰ (Schmidt et al. 1997). Humic feeders were separated from humic formers with  $\delta^{15}\text{N}$  signatures that differed significantly by 0.8–3.6‰ in six different earthworm communities (Neilson et al. 2000). Single trophic transfers, usually indicated by a 1–2.5‰ enrichment of  $^{15}\text{N}$  in consumer tissue with respect to dietary sources, result from fractionation and loss of  $^{14}\text{N}$  in by-products or wastes as material is processed (Nadelhoffer and Fry

1994). Differences observed between species or development stages could be due to assimilation of similar resources at different stages of processing or decomposition, as determined with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of adults and juveniles of *Aporrectodea longa* (Schmidt 1999). Cortez et al. (1989) also associated the accumulation of  $^{14}\text{C}$  and  $^{15}\text{N}$  from labeled residue in earthworm biomass with degree of microbial decomposition of the material. Juvenile *Estherella* sp. remained slightly less enriched in  $^{15}\text{N}$  than *Estherella* sp. sub-adults or *P. corethrurus*, suggesting the use of material that had undergone less microbial processing or trophic transfers.

Differences in assimilation of  $^{15}\text{N}$  during the incubation suggested that *P. corethrurus* exhibited plasticity in the use of  $^{15}\text{N}$  components in response to interactions with *Estherella* sp. sub-adults. Cast and tissue signatures for *P. corethrurus* suggested increased assimilation of resources derived from the  $^{15}\text{N}$ -labeled material. Direct feeding on labeled material was not observed, however, the lack of fungal hyphae in the O-layer of *P. corethrurus* treatments, compared to other treatments, suggested consumption of fungal biomass. Fungal biomass, which can naturally translocate  $^{15}\text{N}$  as found with mycorrhizal fungi (Högberg et al. 1996), may have been a source of enrichment. Interactions between *P. corethrurus* and sub-adult *Estherella* sp. clearly resulted in stratification of N resource use in the MA, which lead to greater  $^{15}\text{N}$  incorporation into *P. corethrurus* in this treatment. Differences in resource use were expected with *P. corethrurus* since this species has exhibited wide climatic and edaphic tolerances (Fragoso et al. 1999) as well as nutritional plasticity (Hendrix et al. 1999a).

#### Effects of earthworms on soil and mineralization

Shifts in soil  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  during the incubation were supported by visible turnover and reworking of O- and A-layers due to earthworm activity. Structural changes in soil were expected with the activity of *P. corethrurus* (Zund et al. 1997; Hallaire et al. 2000). Incorporation of comparatively enriched casts increased O-layer  $\delta^{13}\text{C}$  in the PA, however,  $\delta^{13}\text{C}$  of A-layers, more highly enriched than casts, decreased over the course of incubation. EA casts, although highly enriched in  $^{13}\text{C}$ , had little impact on the A-layers. Increased  $\delta^{15}\text{N}$  in the O-layer was most obvious in PA and MA treatments where *P. corethrurus* preferentially removed native organics obviously leaving behind the  $^{15}\text{N}$ -enriched leaf material. However,  $\delta^{15}\text{N}$  of casts did not correlate with shifts in  $\delta^{15}\text{N}$  in all soil layers. Shifts in  $\delta^{15}\text{N}$  may have been driven more by nitrogenous waste released externally and not in casts, fungal translocation of  $^{15}\text{N}$ , or microbial mineralization of N.

The addition of glucose obviously stimulated soil respiration, however, earthworm activity was an added stimulus for greater release of  $\text{CO}_2\text{-C}$ . The decrease in  $\delta^{13}\text{C}$  of respired  $\text{CO}_2\text{-C}$ , after an initial spike (day 0), in all enriched treatments was likely due to increased immobilization of  $^{13}\text{C}$  into microbial by-products with time (Webster

et al. 1997). The presence of juvenile *Estherella* sp. likely lead to the greater  $\delta^{13}\text{C}$  of respiration because turnover rates of  $^{13}\text{C}$ -enriched "new" components may have been greater. Although the addition of glucose stimulated microbial respiration it caused N immobilization, where initially in the AT, microbial biomass C was increased and available inorganic N was decreased. C mineralization remained linked to the N immobilization in the AT.

Greater mineralization of both C and N in earthworm treatments could be due to casting activity. Lavelle et al. (1989) attributed direct effects on C and N cycling to digestion processes and cast production of earthworms. The feeding activity of *P. corethrurus* resulted in increased or continued microbial biomass and activity in casts after soil was passed through the gut and excreted (Barois 1992; Barois and Lavelle 1986). Microbially mediated N mineralization and increased nitrification have been observed in aging casts produced by *P. corethrurus* and other earthworms (Lavelle et al. 1992; Lavelle and Martin 1992). Barois and Lavelle (1986), Barois (1992) and Barois et al. (1993) have determined that microbial activity, stimulated in the digestive tract of *P. corethrurus*, biodegrades and humifies ingested soil organic matter. Although stimulation of N mineralization in casts has not been shown directly for *Estherella* sp. it has been shown in several other species of tropical native and exotic earthworms (Barois et al. 1999). The continuation of microbial activity in casts was suggested as a cause for greater nutrient mineralization rates observed in bulk soil turned over into casts. In the present study, C and N mineralization were stimulated in the presence of both species of earthworms, although the exotic *P. corethrurus* contributed to greater mineralization.

In conclusion, we investigated the interactions between native and exotic earthworms as measured by resource utilization and nutrient mineralization. When combined, the activity of both earthworm species was spatially separated, and it appeared that *Estherella* sp. and *P. corethrurus*, respectively, excluded each other from bottom and surface layers. *P. corethrurus* assimilated different resources when incubated with, as compared to without, native *Estherella* sp. sub-adults, thus influencing the differences observed in net mineralization. Our data suggest that *P. corethrurus* might occupy its own ecological niche rather than compete directly with at least one native species in this system. Before *P. corethrurus* is introduced to improve soil structure and fertility in the management of agricultural systems (Lavelle et al. 1987, 1989, 1997; Fragoso et al. 1999), further investigation is required to determine the impacts of this in the longer term and at greater spatial scales than those examined in the present study.

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## References

- Alban DH, Berry EC (1994) Effects of earthworm invasion on morphology, C and nitrogen of a forest soil. *Appl Soil Ecol* 1: 243–249
- Anderson JPE, Domsch, KH (1973) Quantification of bacterial and fungal contributions to soil respiration. *Arch Mikrobiol* 93:113–127
- Barois I (1992) Mucus production and microbial activity in the gut of two species of *Amyntas* (Megascolecidae) from cold and warm tropical climates. *Soil Biol Biochem* 24:1507–1510
- Barois I, Lavelle P (1986) Changes in respiration rate and some physicochemical properties of a tropical soil during transit through *Pontoscolex corethrurus* (Glossoscolecidae, Oligochaeta). *Soil Biol Biochem* 18:539–541
- Barois I, Vilemin G, Lavelle P, Toutain F (1993) Transformation of the soil structure through *Pontoscolex corethrurus* (Oligochaeta) intestinal tract. *Geoderma* 56:57–66
- Barois I, Lavelle P, Brossard M, Tondoh J, Martinez M, Rossi JP, Senapati BK, Angeles A, Fragoso C, Jimenez JJ, Decaens T, Lattaud C, Kanyonyo J, Blanchart E, Chapuis L, Brown GG, Moreno A (1999) Ecology of earthworm species with large environmental tolerance and/or extended distributions. In: Lavelle P, Brussaard L, Hendrix P (eds) *Earthworm management in tropical agroecosystems*. CABI, Wallingford, pp 57–85
- Beare MH, Coleman CC, Crossley DA Jr, Hendrix PF, Odum EP (1995) A hierarchical approach to evaluating the significance of soil biodiversity to biogeochemical cycling. *Plant Soil* 170:5–22
- Borges S (1996) The terrestrial oligochaetes of Puerto Rico. *Ann NY Acad Sci* 776:239–248
- Borges S, Alfaro M (1997) The earthworms of Bano de Oro, Luquillo Experimental Forest, Puerto Rico. *Soil Biol Biochem* 29:231–234
- Boutton TW (1996) Stable C isotope ratios of soil organic matter and their use as indicators of vegetation and climate change. In: Boutton TW, Yamasaki S (eds) *Mass spectrometry of soils*. Dekker, New York, pp 47–82
- Burtelow AE, Bohlen PJ, Groffman PM (1998) Influence of exotic earthworm invasion on soil organic matter, microbial biomass and denitrification potential in forest soils of the northeastern United States. *Appl Soil Ecol* 9:197–202
- Cortez J, Hameed R, Bouche MB (1989) C and N transfer in soil with or without earthworms fed with <sup>14</sup>C- and <sup>15</sup>N-labelled wheat straw. *Soil Biol Biochem* 21:491–497
- Fragoso C, James SW, Borges S (1995) Native earthworms of the north neotropical region: current status and controversies. In: Hendrix PF (ed) *Earthworm ecology and biogeography in North America*. Lewis, Boca Raton, Fla. pp 67–115
- Fragoso C, Kanyonyo J, Moreno A, Senapati BK, Blanchart E, Rodríguez C (1999) A survey of tropical earthworms: taxonomy, biogeography and environmental plasticity. In: Lavelle P, Brussaard L, Hendrix P (eds) *Earthworm management in tropical agroecosystems*. CABI, Wallingford, pp 1–26
- González G, Zou X (1999) Earthworm influence on N availability and the growth of *Cecropia schreberiana* in tropical pasture and forest soils. *Pedobiologia* 43:824–829
- 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
- Groffman PM, Bohlen PJ (1999) Soil and sediment biodiversity: cross-system comparisons and large-scale effects. *Bioscience* 49:139–148
- Hallaire V, Curmi P, Dubois A, Lavelle P, Pashanasi B (2000) Soil structure changes induced by the tropical earthworm *Pontoscolex corethrurus* and organic inputs in a Peruvian ultisol. *Eur J Soil Biol* 36:35–44
- Hendrix PF, Lachnicht SL, Callahan MA Jr, Zou X (1999a) Stable isotopic studies of earthworm feeding ecology in tropical ecosystems of Puerto Rico. *Rapid Commun Mass Spectrom* 13:1295–1299
- Hendrix PF, Callahan MA Jr, Lachnicht SL, Blair JM, James SW, Zou X (1999b) Stable isotopic studies of resource utilization by nearctic earthworms (*Diplocardia*, Oligochaeta) in subtropical savanna and forest ecosystems. *Pedobiologia* 43:818–823
- Högberg P, Högbom L, Schinkel H, Högborg M, Johansson C, Wallmark H (1996) <sup>15</sup>N abundance of surface soils, roots and mycorrhizas in profiles of European forest soils. *Oecologia* 108:207–214
- Kalisz PJ, Wood HB (1995) Native and exotic earthworms in wildland ecosystems. In: Hendrix PF (ed) *Earthworm ecology and biogeography in North America*. Lewis, Boca Raton, Fla. pp 117–126
- Keeney DR, Nelson DW (1982) Nitrogen – inorganic forms. In: Page AL, et al. (eds) *Methods of soil analysis*. Part 2. Chemical and microbiological properties, 2nd edn. Agronomy monographs 9. ASA, Madison, Wis. pp 643–693
- Lavelle P, Martin A (1992) Small-scale and large-scale effects of endogeic earthworms on soil organic matter dynamics in soils of the humid tropics. *Soil Biol Biochem* 24:1491–1498
- Lavelle P, Barois I, Cruz I, Fragoso C, Hernandez A, Pineda A, and Rangel P (1987) Adaptive strategies of *Pontoscolex corethrurus* (Glossoscolecidae, Oligochaeta), a peregrine geophagous earthworm of the humid tropics. *Biol Fertil Soils* 5:188–194
- Lavelle P, Barois I, Martin A, Zaidi Z, Schaefer R (1989) Management of earthworm populations in agro-ecosystems: a possible way to maintain soil quality? In: Clarholm M, Bergstrom L (eds) *Ecology of arable land: perspectives and challenges*. Kluwer, Dordrecht, pp 109–122
- Lavelle P, Melendez G, Pashanasi B, Schaefer R (1992) Nitrogen mineralization and reorganization in casts of the geophagous tropical earthworm *Pontoscolex corethrurus* (Glossoscolecidae). *Biol Fertil Soils* 14:49–53
- 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
- Martin A, Balesdent J, Mariotti A (1992a) Earthworm diet related to soil organic matter dynamics through <sup>13</sup>C measurements. *Oecologia* 91:23–29
- Martin A, Mariotti A, Balesdent J, Lavelle P (1992b) Soil organic matter assimilation by a geophagous tropical earthworm based on <sup>δ13</sup>C measurements. *Ecology* 73:118–128
- Nadelhoffer KJ, Fry B (1994) Nitrogen isotope studies in forest ecosystems. In: Lajtha K, Michener H (eds) *Stable isotopes in ecology and environmental science*. Blackwell, Oxford, pp 22–44
- Neilson R, Boag B, Smith M (2000) Earthworm <sup>δ13</sup>C and <sup>δ15</sup>N analyses suggest that putative functional classifications of earthworms are site-specific and may also indicate habitat diversity. *Soil Biol Biochem* 32:1053–1061
- Pashanasi B, Melendez G, Szott L, Lavelle P (1992) 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. *Soil Biol Biochem* 24:1655–1659
- Pashanasi B, Lavelle P, Alegre J, Charpentier F (1996) Effect of the endogeic earthworm *Pontoscolex corethrurus* on soil chemical characteristics and plant growth in a low-input tropical agroecosystem. *Soil Biol Biochem* 28:801–810
- Schmidt O (1999) Intrapopulation variation in C and nitrogen stable isotope ratios in the earthworm *Aporrectodea longa*. *Ecol Res* 14:317–328
- Schmidt O, Scrimgeour CM, Handley LL (1997) Natural abundance of <sup>15</sup>N and <sup>13</sup>C in earthworms from a wheat and a wheat-clover field. *Soil Biol Biochem* 29:1301–1308
- Spain A, Le Feuvre R (1997) Stable C and N isotope values of selected components of a tropical Australian sugarcane ecosystem. *Biol Fertil Soils* 24:118–122.
- Spain AV, Saffigna PG, Wood AW (1990) Tissue C sources for *Pontoscolex corethrurus* (Oligochaeta: Glossoscolecidae) in a sugarcane ecosystem. *Soil Biol Biochem* 22:703–706
- Stebbins JH (1962) Endemic-exotic earthworm competition in the American Midwest. *Nature*, 196:905–906

- Steinberg DA, Pouyat RV, Parmelee RW, Groffman PM (1997) Earthworm abundance and nitrogen mineralization rates along an urban-rural land use gradient. *Soil Biol Biochem* 29:427-430
- Stotzky G (1965) Microbial Respiration. In: Black CA, Evans DO, Ensminger LE, White JL, Clark FE, Dinauer RC (eds) *Methods of soil analysis. Part 2. Chemical and microbiological properties*. ASA, Madison, Wis. pp 1550-1572
- Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. *Soil Biol Biochem* 19:703-707
- Waide RB, Reagan DP (1996) The rainforest setting. In: Reagan DP, Waide RB (eds) *The food web of a tropical rain forest*. University of Chicago Press, Chicago, Ill. pp 1-16
- Webster EA, Chudek JA, Hopkins DW (1997) Fates of  $^{13}\text{C}$  from enriched glucose and glycine in an organic soil determined by solid-state NMR. *Biol Fertil Soils* 25:389-395
- Zou X, González G (1997) Changes in earthworm density and community structure during secondary succession in abandoned tropical pastures. *Soil Biol Biochem* 29:627-629
- Zund PR, Pillai-McGarry U, McGarry D, Bray SG (1997) Repair of a compacted Oxisol by the earthworm *Pontoscolex corethrurus* (Glossoscolecidae, Oligochaeta). *Biol Fertil Soils* 25:202-208