

Earthworm communities along a forest-coffee agroecosystem gradient: preliminary evidence supporting the habitat-dependent feeding hypothesis

JOSÉ A. AMADOR^{1*}, KRISTOPHER WINIARSKI² & DAVID SOTOMAYOR-RAMIREZ³

¹Laboratory of Soil Ecology and Microbiology and ²Dept. of Natural Resources Science,
University of Rhode Island, Kingston, Rhode Island, USA

³Dept. of Agroenvironmental Sciences, University of Puerto Rico-Mayagüez Campus,
Mayagüez, Puerto Rico

Abstract: Earthworms often constitute an important component of agricultural foodwebs. As such, knowledge of their feeding preferences is relevant to the sustainable management of carbon and nutrient cycling in agroecosystems. We used natural abundances of stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in earthworms and two of their putative food sources - soil and leaf litter - to test hypotheses regarding the feeding plasticity of earthworm communities of secondary forests (FOR) and coffee (*Coffea arabica* L.) farms with differing management practices (partial shade (SHD) or full sunlight (SUN)) in western-central Puerto Rico. Previous analysis of earthworm abundance indicated that 96 - 100 % of the earthworms in these ecosystems were endogeic, suggesting that their diet should consist primarily of soil across ecosystem types (habitat-independent hypothesis). There were clear differences in food sources utilized by earthworm communities among ecosystem types, as indicated by the magnitude of differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of earthworms and their putative food sources. These results suggest that, despite being made up almost entirely of endogeic species, there may be considerable dietary plasticity among earthworm communities from different ecosystems (habitat-dependent hypothesis). We found the magnitude of differences in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between earthworms and their putative food sources differed by habitat type, increasing with greater plant diversity (SUN < SHD < FOR), a pattern associated with feeding at higher trophic levels. Our results provide preliminary support for the habitat-dependent hypothesis to explain the feeding behavior of earthworm communities dominated by endogeic species in common tropical ecosystems with differing habitat complexity.

Resumen: Las lombrices son un componente importante de las redes tróficas agrícolas. Por ello, conocer sus preferencias alimentarias es relevante para la gestión sostenible de los ciclos de carbono y nutrientes en los agroecosistemas. Usando las abundancias naturales de isótopos estables ($\delta^{13}\text{C}$ y $\delta^{15}\text{N}$) en las lombrices y dos de sus supuestas fuentes de alimento - suelo y mantillo - probamos hipótesis relacionadas con la plasticidad alimentaria en comunidades de lombrices de bosques secundarios (FOR) y cafetales (*Coffea arabica* L.) con prácticas de manejo diferentes (sombra parcial [SHD] o pleno sol [SUN]) en el centro oeste de Puerto Rico. Un análisis previo de la abundancia de lombrices de tierra indicó que 96 - 100 % de las lombrices de tierra en estos ecosistemas son endogeas, lo que sugiere que su dieta debería consistir básicamente de suelo en los varios tipos de ecosistemas (hipótesis de la independencia del hábitat). Hubo diferencias claras en las fuentes de alimento utilizadas por las comunidades de lombrices entre tipos de ecosistema, de acuerdo con la magnitud de las diferencias en $\delta^{13}\text{C}$ y $\delta^{15}\text{N}$ de las lombrices y sus supuestas fuentes de alimento. Estos resultados sugieren que a pesar de consistir casi completamente de especies endogeas, podría haber una plasticidad

*Corresponding Author; e-mail: jamador@uri.edu

considerable en las dietas entre comunidades de lombrices de ecosistemas diferentes (hipótesis de dependencia del hábitat). La magnitud de las diferencias tanto en $\delta^{13}\text{C}$ como en $\delta^{15}\text{N}$ entre las lombrices y sus supuestas fuentes de alimento difirieron de manera creciente entre hábitats con una mayor diversidad vegetal (SUN < SHD < FOR), patrón asociado con la alimentación en niveles tróficos superiores. Los resultados apoyan de forma preliminar la hipótesis de la dependencia del hábitat para explicar el comportamiento alimentario de comunidades de lombrices dominadas por especies endógenas en ecosistemas tropicales comunes que difieren en complejidad de hábitat.

Resumo: As minhocas constituem muitas vezes uma componente importante de cadeias alimentares agrícolas. Como tal, o conhecimento de suas preferências alimentares é relevante para a gestão sustentável dos ciclos do carbono e nutrientes nos agro-ecossistemas. Usou-se a abundância natural de isótopos estáveis ($\delta^{13}\text{C}$ e $\delta^{15}\text{N}$) nas minhocas e em duas das suas fontes putativas de alimento - solo e manta morta - para testar hipóteses sobre a plasticidade alimentar em comunidades de minhocas em florestas secundárias (FOR) e cafezais (*Coffea arabica* L.) com diferentes tipos de manejo (sombra parcial (SHD) ou a pleno sol (SUN)) no centro oeste de Porto Rico. Uma análise prévia da abundância de minhocas indicou que 96 - 100 % das minhocas nestes ecossistemas eram endogénicas, sugerindo que sua dieta deve consistir principalmente de solo em todos os tipos de ecossistemas (hipótese de independência do habitat). Havia diferenças claras nas fontes de alimento utilizados pelas comunidades de minhocas entre tipos de ecossistema, de acordo com a magnitude das diferenças nas $\delta^{13}\text{C}$ e $\delta^{15}\text{N}$ das minhocas e as suas fontes alimentares putativas. Estes resultados sugerem que, apesar de consistir quase inteiramente de espécies endogénicas, poderá haver uma plasticidade alimentar considerável entre as comunidades de minhocas de diferentes ecossistemas (hipótese de dependência do habitat). A dimensão das diferenças em $\delta^{13}\text{C}$ com em $\delta^{15}\text{N}$ entre as minhocas e suas fontes alimentares putativas diferem de uma forma crescente entre tipos de habitats com uma maior diversidade da vegetação, (SUN < SHD < FOR), padrão associado com a alimentação em níveis tróficos superiores. Os nossos resultados apoiam de forma preliminar a hipótese de dependência do habitat para explicar o comportamento alimentar de comunidades de minhocas dominadas por espécies endogénicas em ecossistemas tropicais comuns que diferem em complexidade de habitats.

Key words: Endogeic earthworms, *Coffea arabica* L., $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, biodiversity.

Introduction

Earthworms exert an important control on the transformation of organic detritus and soil organic matter in terrestrial ecosystems. Their activities affect the mineralization of carbon and nutrients in soil, thus directly affecting soil C dynamics and nutrient availability (Edwards & Bohlen 1996). In low-input agricultural ecosystems, earthworms are thought to be an important component of soil fertility by helping to retain C through aggregate formation and to release nutrients from soil and detrital inputs (Brown *et al.* 1999). Furthermore, the quality of detrital inputs may be adjusted to manipulate earthworm activity in order to better synchronize nutrient release and crop needs (Tian *et al.* 1997).

Coffee (*Coffea arabica* L.) agroecosystems constitute an important land use and source of income in mountainous tropical areas throughout the world (International Coffee Organization 2012). Coffee is grown under full sunlight or under full or partial shade, and these differences in agricultural management practices affect the population density and biomass of earthworms (Amador *et al.* 2010; Sánchez-de León *et al.* 2006). Quality and quantity of food sources are among the main factors controlling the size and activity of earthworm populations across ecosystems (Edwards & Bohlen 1996), and thus constitute important targets for management practices that promote their activities in soil. In a previous companion study we reported that earthworm population density and biomass were highest for

coffee grown under full sun (SUN), with lower values observed for coffee grown under shade (SHD) and secondary forests (FOR) in western-central Puerto Rico (Amador *et al.* 2010). Neither earthworm biomass nor abundance was significantly related to litter quantity, litter composition or soil properties for any of the ecosystems studied. In addition, Ramos *et al.* (2010) reported differences in the composition of earthworm communities, with a preponderance of endogeic species (96 - 100 % of the community) in all three ecosystems at the same study sites.

There are two broad hypotheses describing the feeding behavior of earthworms. One, which we refer to as the “habitat-independent” hypothesis, suggests that earthworm feeding habits will be true to their functional group, regardless of ecosystem characteristics. This is supported by the work of Briones *et al.* (1999), who used $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of epigeic, anecic and endogeic earthworm species to examine earthworm food sources in temperate grasslands and cornfields in Spain and England. Their results suggested that the feeding behaviors of functional groups are habitat-independent. An alternative hypothesis, which we refer to as the “habitat-dependent” hypothesis, suggests that the feeding habits of earthworms are plastic, so that earthworms within a functional group may consume different food sources depending on what is available in a particular ecosystem. This is supported by the work of Nielson *et al.* (2000) who, in a study of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of epigeic, anecic and endogeic earthworm species in various temperate habitats, found that classification of earthworms into functional groups was not absolute, but depended on habitat. Differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between earthworms and their diet that were larger than expected for a single trophic transfer led Nielson *et al.* (2000) to suggest that a wide range of dietary sources is likely to be reflected in a wider range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within an ecosystem, with differences in isotopic enrichment between earthworms and their diet increasing with greater ecosystem diversity.

The characteristics of earthworm communities in our study sites (described by Ramos *et al.* 2010 and Amador *et al.* 2012), and the differences in plant community structure among ecosystems, provide an opportunity for a tentative evaluation of these two hypothesis. Endogeic earthworms at our study site accounted for 96 to 100 % of the earthworm populations in SHD, SUN and FOR ecosystems (Ramos *et al.* 2010). Endogeic species

are found in upper soil horizons, where they inhabit a large network of sub-horizontal burrows within the top 10 cm of soil, and feed primarily on humified organic matter (Bouché 1977). Because endogeic species constituted the overwhelming majority of the earthworm population in all three ecosystems, our data allow us to test the habitat-independent hypothesis: that the earthworm communities of these ecosystems feed on the same resource - soil - regardless of ecosystem type. Our study sites also represent a clear gradient of plant biodiversity, which is highest for FOR, intermediate for SHD, and lowest for SUN ecosystems (Amador *et al.* 2010). This allows us to test the habitat-dependent hypothesis: that food sources for the earthworms will vary depending on ecosystem, despite the fact that the earthworm communities of these different ecosystems are overwhelmingly dominated by endogeic species.

In the present study we determined the carbon and nitrogen isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of soil, leaf litter and of earthworm communities in SUN, SHD and FOR ecosystems. The level of isotopic enrichment of an organism reflects that of its food sources minus excretion (Fry 2006). Furthermore, the magnitude of the differences in isotope values between consumers and their diet may be used as a broad indicator of the trophic level at which feeding takes place (Fry 2006). In general, differences in enrichment of 1 ‰ and 3.4 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively, are expected for a single-level trophic transfer (Wada *et al.* 1993), although substantial variation in the magnitude of these values has been reported (Tiunov 2007). Three soil orders commonly associated with coffee plantations - Oxisols, Inceptisols and Ultisols - were represented in the study. The data were used to evaluate the habitat-dependent (e.g. Briones *et al.* 1999) and habitat-independent (e.g. Nielson *et al.* 2000) hypotheses in the context of three land uses common in tropical mountainous areas.

Materials and methods

Study sites

The experiment was conducted in coffee-growing and forested areas in the municipalities of Las Marias, Lares and Jayuya in western-central Puerto Rico. Soil order and soil series were the same for all the plots within a municipality, but differed among municipalities (Table 1). Soils had a high clay content (46 to 64 %), were generally acidic (pH 3.6 to 6.2), with a total N content of 3 to 7 g kg⁻¹ soil and a total C content between 26 and 69 g kg⁻¹ soil (Amador *et al.* 2010).

Table 1. Municipality, soil order & series, altitude, and mean annual precipitation and temperature of coffee agroecosystems and secondary forests examined in this study.

Municipality	Soil order (Series)	Altitude (m above sea level)	Mean annual precipitation (mm)	Mean annual temperature (°C)
Jayuya	Oxisol (Los Guineos)	760 - 820	1935	22
Lares	Inceptisol (Anones)	570 - 670	2494	24
Las Marias	Ultisol (Humatas)	270 - 330	1876	25

The coffee plantations in the study had been in production for 8 to 15 years (Avilés-Vázquez 2009). Soil fertility was managed by application of ~250 kg fertilizer ha⁻¹ (12-5-15-3; N-P₂O₅-K₂O-MgO + trace elements) 2 to 3 times per year (López-Rodríguez 2008). Fertilizer inputs were below those recommended by Cooperative Extension agents (Conjunto Tecnológico para la Producción de Café 1999). FOR ecosystems were classified as very humid subtropical (Holdridge 1996), with a canopy dominated by trees that were < 30 years old (Avilés-Vázquez 2009). A more detailed description of the experimental areas, including information on plant species distribution, can be found in Amador *et al.* (2010) and Avilés-Vázquez (2009).

The relative distribution of earthworm species at the sites was reported previously (Ramos *et al.* 2010) as follows: SUN-Ultisol: *P. corethrurus* (91.3 %), *Amyntas rodericensis* (8.7 %); SHD-Ultisol: *P. corethrurus* (97.1 %), *P. spiralis* (2.9 %); FOR-Ultisol: no earthworms found; SUN-Oxisol: *P. corethrurus* (96.9 %), *Pheretimoid* sp. (3.1 %); SHD-Oxisol: *P. corethrurus* (63.3 %), *P. spiralis* (36.7 %); FOR-Oxisol: *P. corethrurus* (46.6 %), *P. melissae* (20.0 %), *Pontoscolex* sp. (35.6 %); SUN-Inceptisol: *P. corethrurus* (96.9 %), *Pontoscolex* sp. (3.1 %); SHD-Inceptisol: *P. corethrurus* (96.0 %), *A. rodericensis* (4.0 %); FOR-Inceptisol: *P. corethrurus* (100 %). The species *P. corethrurus*, *P. spiralis*, *P. melissae* and *A. rodericensis* are all endogeic; thus the earthworm communities at nearly all sites where earthworms were found were confirmed to consist nearly exclusively (96 - 100 %) of endogeic individuals. The exception, FOR-Oxisol, had an earthworm community that included 64.3 % known

endogeic species, with the remainder made up of unspecified *Pontoscolex* sp.. Given the preponderance of endogeic species within the genus *Pontoscolex*, it seems reasonable to expect that at least some of these individuals were endogeic as well.

Sampling

Within each municipality three replicate square plots (20 m x 20 m) were established in October 2006 in two coffee agroecosystems and one forest ecosystem: (i) partial shade coffee (SHD), (ii) full sunlight coffee (SUN), and (iii) secondary forest (FOR). Sites were sampled between 13 November and 6 December 2007, during the peak of the rainy season. Within each replicate 20 m x 20 m plot, one 30 cm x 30 cm subplot was established for sampling of leaf litter, earthworms and soil. The leaf litter was removed from the subplot and placed in a plastic bag. Soil in the subplot was excavated to a depth of 10 cm, placed on a plastic bag, and earthworms sorted by hand and stored in a sealable plastic bag. A number of studies in a variety of tropical ecosystems show that the majority (> 80 %) of earthworms are found in the top 10 cm of the soil profile (Fragoso & Lavelle 1992; González *et al.* 2007; Sánchez-de León *et al.* 2003). In addition, because the soil moisture content was at or above field capacity, we expected the bulk of the earthworms to be found near the surface.

Sample processing

Earthworms were transported to the laboratory on the day they were sampled, where they were placed in water to void their gut, dried on paper towels, and frozen at - 4 °C before shipping in dry ice to Kingston, Rhode Island, USA by overnight courier. Frozen earthworms were dried using a lyophilizer and ground with a mortar and pestle. Soil was dried overnight at 105 °C and ground with a mortar and pestle. Leaf litter was dried at 65 °C for 24 h and ground in a Wiley mill to pass a 40-mesh-sieve. All samples were stored at room temperature in a dessicator prior to analysis.

Isotope analysis

Samples of earthworms (~1 mg), leaf litter (~5 mg) and soil (~65 mg) were placed in 9 x 5 mm tin cups and analyzed using a Carlo-Erba NA 1500 series II elemental analyzer (Thermo Fisher, Wal-

tham, MA) connected to a continuous flow isotope ratio Micromass Optima spectrometer (Micromass, Manchester, UK). The instrument was operated in the dual isotope mode so that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ could be measured simultaneously on the same sample. Urea and powdered dogfish muscle (DORM-2) reference material (National Research Council, Institute for Environmental Chemistry, Ottawa) were used as working standards for carbon and nitrogen analysis. One in five samples was run in duplicate as a check for analytical accuracy and precision. Reference material analyzed over the course of sample analysis was measured with a precision of $\pm 0.3\text{‰}$ for nitrogen and 0.2‰ for carbon.

Isotope natural abundance was calculated using the equation:

$$\delta_{\text{sample}} = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1000\text{‰} \quad (1)$$

where, R_{sample} and R_{standard} are the ratios of heavy/light isotopes of the sample and standard, respectively.

Statistical analysis

A one-way ANOVA was used to examine differences in isotopic enrichment of soil, earthworms and litter within a study site and among ecosystem types, and in isotopic distance between earthworms and their dietary sources. Tukey's Honestly Significant Difference test was used to identify treatment differences ($P < 0.05$). Sigma-Stat, v. 2.03 was used for all statistical analyses (SPSS, Inc.).

Results and discussion

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of litter, soil and earthworms

The $\delta^{13}\text{C}$ content of litter and soil was significantly different within a soil order and ecosystem in all cases except the SHD-Ultisol combination (Fig. 1). Significant differences in the $\delta^{15}\text{N}$ content of litter and soil were observed for the FOR ecosystem in the Oxisol & Inceptisol, the SHD ecosystem in the Oxisol, and the SUN ecosystem in the Inceptisol. Earthworms from the FOR sites had mean $\delta^{15}\text{N}$ values ranging from 4.98 to 7.40 ‰, followed by SUN (5.78 to 7.76 ‰) and SHD (7.40 to 8.90 ‰) (Fig. 1). FOR sites had the lowest mean $\delta^{13}\text{C}$ values for earthworms (-21.11 to -23.41), followed by SHD (-23.27 to -25.22 ‰) and SUN (-24.26 to -24.67 ‰).

Values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of soil, litter and

earthworms in our study are within those previously reported (Hendrix *et al.* 1999; Lachniet *et al.* 2002) for a range of ecosystems in Puerto Rico. In particular, values for FOR and SHD ecosystems in our study were closest to those for high altitude dwarf forest (Hendrix *et al.* 1999), whereas values for SUN ecosystems were closer to those reported for pastures (Lachniet *et al.* 2002).

Evaluation of the habitat-independent hypothesis

If the habitat-independent hypothesis is correct, we expect the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the earthworm communities relative to their putative food sources (soil and litter) to be the same across ecosystems and soil orders. This was clearly not the case (Fig. 1). Earthworms in FOR ecosystems were significantly more enriched in ^{13}C than either soil or litter for all three soil orders. In SHD ecosystems earthworms were significantly more enriched than litter and soil only for the Inceptisol, with no significant difference between earthworms and soil in either the Oxisol or the Inceptisol. By contrast, $\delta^{13}\text{C}$ of earthworms in SUN was not significantly different from that of either litter or soil for any of the three soil orders. Earthworm populations had a significantly higher $\delta^{15}\text{N}$ than that for litter and soil for all ecosystems and soil orders; however, the magnitude of the differences in enrichment was highest for FOR, intermediate for SHD, and lowest for SUN (Fig. 1).

Evaluation of the habitat-dependent hypothesis

Our study sites represent a gradient of plant biodiversity, which is highest for FOR, intermediate for SHD, and lowest for SUN ecosystems (Amador *et al.* 2010). If the habitat-dependent hypothesis is correct, there should be differences in isotopic enrichment between earthworms and their putative food sources (soil and litter) among ecosystems. Furthermore, these differences should become smaller with decreasing habitat complexity (e.g. FOR > SHD > SUN), as suggested by Nielsen *et al.* (2000).

As noted above, we observed a consistent shift in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the earthworm community relative to soil and litter as a function of ecosystem for all three soil orders, with differences in enrichment generally following the order: FOR > SHD > SUN (Fig. 1). These differences point to a shift in food sources as plant biodiversity decreases along the FOR - SHD - SUN gradient.

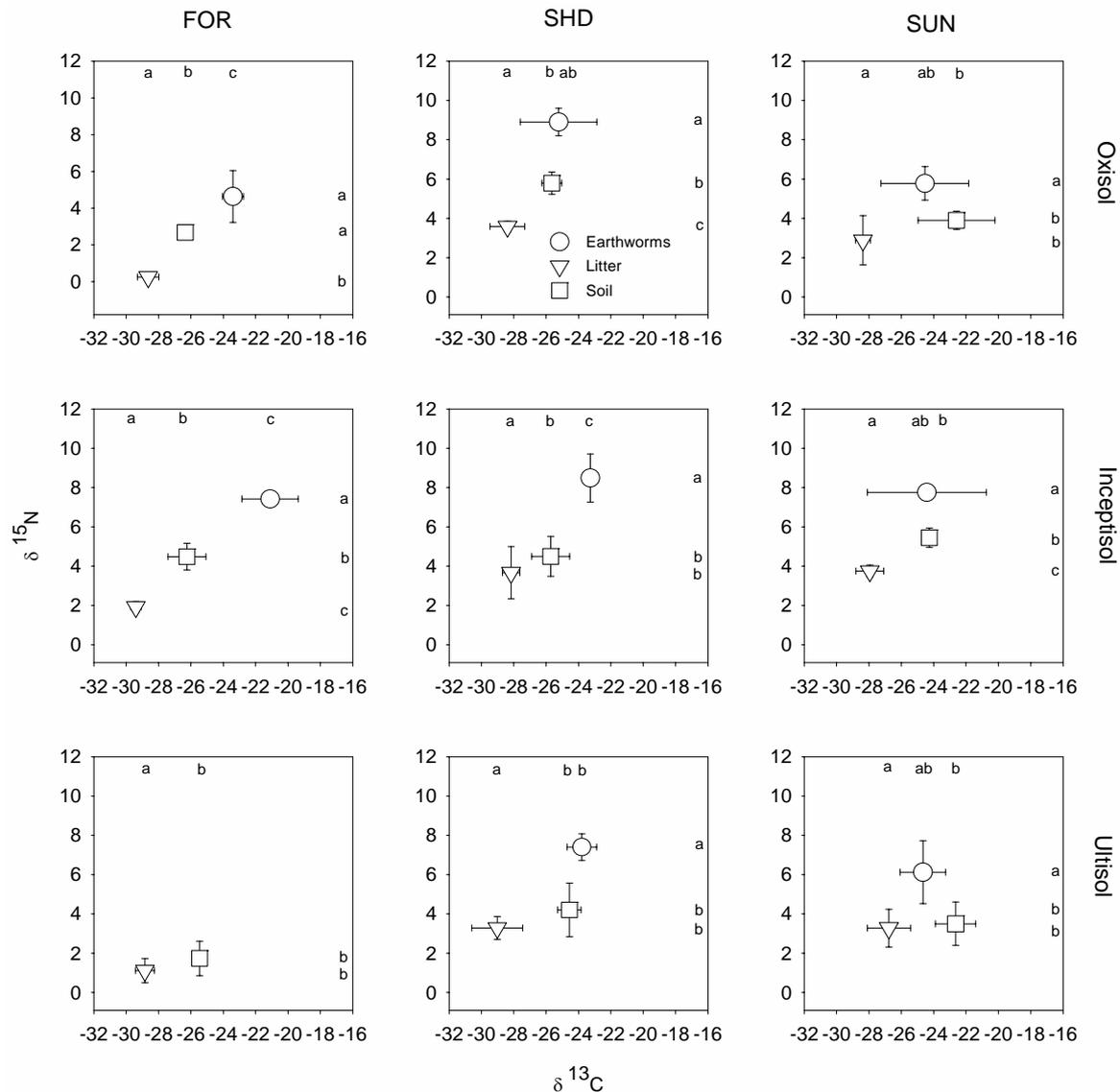


Fig. 1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of earthworms, leaf litter and soil in shade (SHD) and sun (SUN) coffee and forest (FOR) ecosystems in Oxisols, Inceptisols and Ultisols in western-central Puerto Rico. Units for both axis are ‰. Values are means ($n = 3$). Bars represent one standard deviation. Different letters within an axis indicate significant difference ($P < 0.05$).

We pooled the data by ecosystem type to further examine the habitat-dependent hypothesis at a broader scale (Fig. 2). The results show that the $\delta^{13}\text{C}$ of earthworms relative to that of soil and litter decreases consistently as a function of ecosystem, with the highest isotopic difference (Δ) observed for FOR ecosystems, followed by SHD and SUN ecosystems. There were statistically significant differences between FOR and SUN ecosystems in $\Delta \delta^{13}\text{C}$ between earthworms and litter, and earthworms and soil (Fig. 2).

The isotopic difference in $\delta^{15}\text{N}$ between earthworms and litter also differed among ecosystems,

with significant differences observed between SUN and SHD, and SUN and FOR ecosystems (Fig. 2). However, differences in $\delta^{15}\text{N}$ of earthworms relative to soil followed a somewhat different pattern, with significant differences between SHD and SUN, and FOR values between these two.

Our results support the habitat-dependent hypothesis, since the enrichment of earthworm populations in either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ relative to litter and soil differed among ecosystems. The differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ enrichment of earthworms relative to those of the food sources we measured

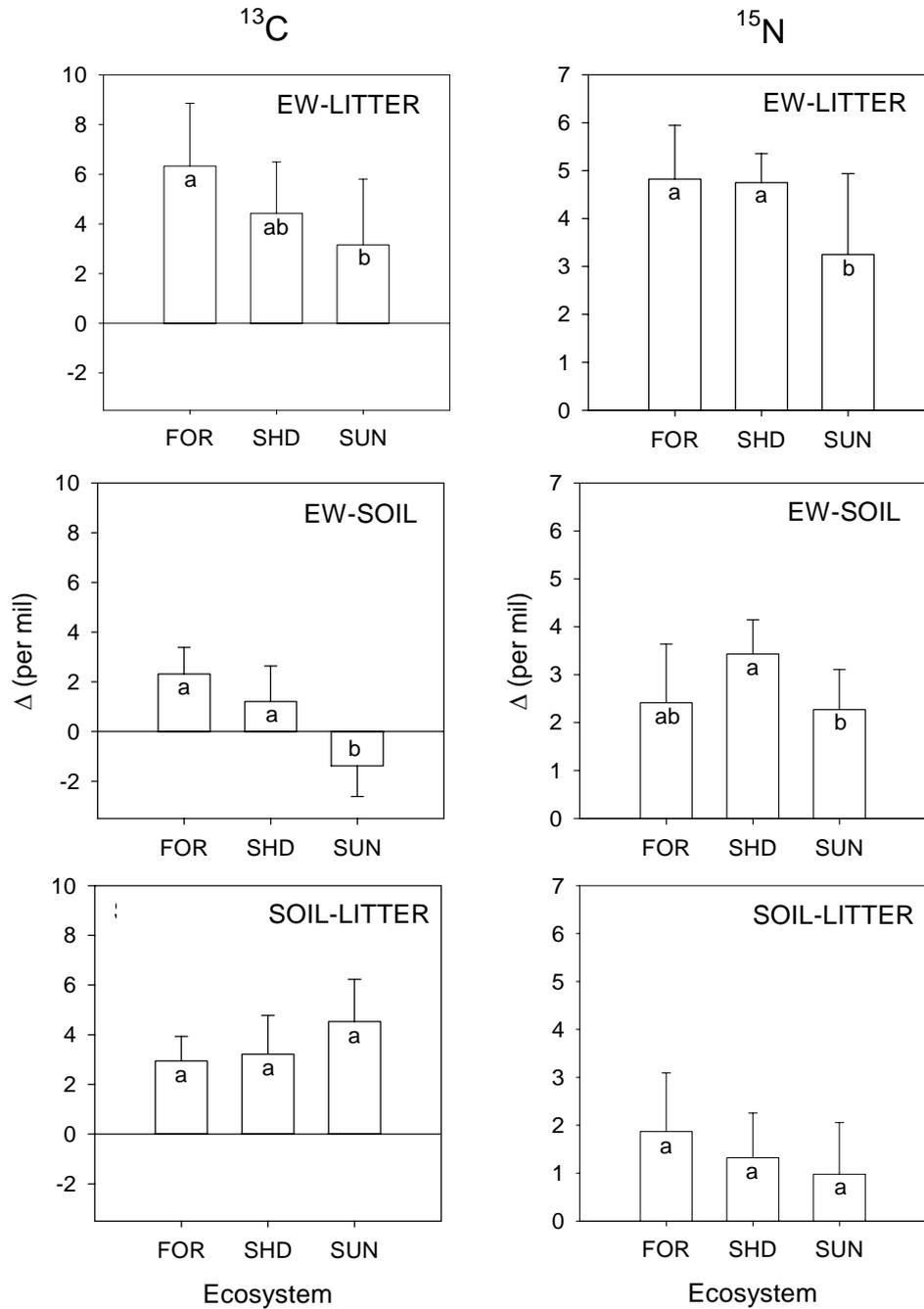


Fig. 2. Difference in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values (Δ) between earthworms and litter (top), earthworms and soil (middle) and soil and litter (bottom) in secondary forests (FOR) and shade (SHD) and sun (SUN) coffee ecosystems in western-central Puerto Rico. Values are means ($n = 9$). Bars represent one standard deviation. Values followed by the same letter were not significantly different ($P > 0.05$).

were higher than expected for a single trophic transfer (e.g. Tiunov 2007; Wada *et al.* 1993). Thus, there must be other sources of food - with higher levels of isotopic enrichment - used by the earthworm communities in FOR and SHD ecosystems, such as fungal biomass (Etchevarria *et al.*

2009), which we did not analyze. A number of studies have shown that earthworms from different feeding groups ingest soil fungi, although their relative importance as a food source is unclear (Curry & Schmidt 2007). Soil from deeper parts of the profile is generally more enriched in

^{15}N (Tiunov 2007). However, the majority (> 80 %) of earthworms in a various tropical ecosystems are found in the top 10 cm of the soil profile (Fragoso & Lavelle 1992; González *et al.* 2007; Sánchez-de León *et al.* 2003), suggesting that feeding on soil from deeper parts of the soil profile may not have been an important factor in our study.

The dominant species in all three ecosystems was *Pontoscolex corethrus* (Ramos *et al.* 2010), an exotic species with demographic characteristics that are typical of the r type, with a rate of reproduction that is generally faster than that of native species, which tend to be typically K strategists in humid tropical areas (Curry 2004). Although it is considered to be strictly geophagous (Lavelle *et al.* 1987), recent studies have suggested that the feeding habits of *P. corethrus* may be fairly plastic. In a field study comparing $\delta^{13}\text{C}$ values of soil, earthworms, and leaves and stems of plants in a sugarcane plantation in Australia - a monoculture situation comparable to coffee grown under full sunlight - Spain *et al.* (1990) suggested that *P. corethrus* derives the bulk of its C from rhizospheric sources. Lachnicht *et al.* (2002), in a feeding study of *P. corethrus* from a rain forest in Puerto Rico using ^{15}N -labeled food resources, suggested that fungal biomass was an important source of N to this earthworm species. The absence of support for the habitat-independent hypothesis, together with the apparently broad range of food sources consumed by *P. corethrus*, suggest that the habitat-dependent hypothesis may better explain the stable isotope enrichment patterns observed for earthworms from different ecosystems. The dietary flexibility reported here for *P. corethrus* has been shown to be an important component of the invasiveness of other exotic earthworms, particularly the epi-endogeic *Amyntas agrestis* (Zhang *et al.* 2010).

Conclusions

Our results lend preliminary support to the "habitat-independent" hypothesis with respect to the food sources utilized by earthworm communities of SUN, SHD and FOR ecosystems dominated by endogeic species. This is reflected in increasing differences in isotopic enrichment between earthworms and their food sources as habitat complexity, and thus the number of trophic levels consumed by earthworms, increases. However, our study represents evaluation of these hypotheses at single point in time. The role of

seasonal changes (e.g. precipitation, temperature, shifts in earthworm community composition and/or food availability) needs to be examined to provide a more rigorous test of the validity of these hypotheses.

Lavelle (1988) has proposed that endogeic species be divided into three subcategories – polyhumic, mesohumic, and oligohumic – based on the type of soil consumed by particular species and the depth at which they are found. Thus, future work on the identity of the various food sources consumed by earthworms in different ecosystems, and their relative contribution to their diet, should yield a more complete picture of the feeding ecology of endogeic earthworm communities as a function of ecosystem type. For example, evaluation of seeds, roots and fungal biomass, and more detailed analysis of soil (e.g. humified vs. non-humified organic matter, microbial biomass) and litter (species, quality, degree of decomposition) as sources of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ will provide a more complete picture of the feeding ecology of these earthworm communities. The latter is particularly important for agroecosystems, since litter quality has been shown to influence the size and activity of tropical earthworm populations (Garcia & Fragoso 2004; Tian *et al.* 1997). Analysis of the feeding plasticity of individual species, particularly those for which information is limited, would also improve our understanding of the role of earthworms in these ecosystems. This information can also be used to inform strategies that integrate the size, structure and function of earthworm communities in the sustainable management of coffee agroecosystems.

Acknowledgements

We are grateful to J. Vega, E. Feliciano, G. López-Rodríguez and M. Matos for technical assistance, all at the University of Puerto Rico-Mayagüez Campus. Use of the stable isotope mass spectroscopy facilities at the U.S. Environmental Protection Agency-Atlantic Ecology Laboratory in Narragansett, Rhode Island, USA is gratefully acknowledged. J. A. thanks the Barreto, Otero and Quiñones families, and C. Añeses for their support and hospitality. This research was funded by a grant to D. S.-R. from the UPR-Mayagüez Campus Atlantea Program and by the authors' personal funds. The study was conducted while J. A. was on sabbatical leave at UPR-Mayagüez.

This is contribution no. 3154 from the Rhode Island Agricultural Experiment Station.

References

- Amador, J. A., C. V. Ramos, M. Alfaro & D. Sotomayor-Ramírez. 2010. Impact of coffee management practices on earthworm populations in Puerto Rico. *Caribbean Journal of Science* **46**: 186-193.
- Avilés-Vázquez, I. 2009. *Fijación Biológica de Nitrógeno y Almacenamiento de Carbono en Agrosistemas de Producción de Café (Coffea arabica L.) en Puerto Rico*. M. S. Thesis, University of Puerto Rico, Mayagüez, Puerto Rico.
- Bouché, M. B. 1977. Stratégies lombriciennes. *Ecological Bulletin* **25**:122-132.
- Briones, M. J. I., R. Bol, D. Sleep, L. Sampedro & D. Allen. 1999. A dynamic study of earthworm feeding ecology using stable isotopes. *Rapid Communications in Mass Spectrometry* **13**: 1300-1304.
- Brown, G. G., B. Pashanasi, C. Villenave, J. C. Patron, B. K. Senapati, S. Giri, I. Barois, P. Lavelle, E. Blanchart, R. J. Blakemore, A.V. Spain & J. Boyer. 1999. Effects of earthworms on plant production in the tropics. pp. 87-147. In: P. Lavelle, L. Brussaard & P. F. Hendrix (eds.) *Earthworm Management in Tropical Agroecosystems*. CAB International, Wallingford, UK.
- Conjunto Tecnológico para la Producción de Café. 1999. Universidad de Puerto Rico, Recinto Universitario de Mayagüez, Colegio de Ciencias Agrícolas, Estación Experimental Agrícola, Río Piedras, Puerto Rico.
- Curry, J. P. 2004. Factors affecting the abundance of earthworms in soils. pp. 91-113. In: C. A. Edwards (ed.) *Earthworm Ecology*. 2nd edn. CRC Press, Boca Raton, FL.
- Curry, J. P. & O. Schmidt. 2007. The feeding ecology of earthworms - A review. *Pedobiologia* **50**: 463-477.
- Edwards, C. A. & P. J. Bohlen. 1996. *Biology and Ecology of Earthworms*. 3rd edn. Chapman & Hall, London.
- Etchevarria, P., D. Huygens, R. Godoy, F. Borie & P. Boerckx. 2009. Arbuscular mycorrhizal fungi contribute to 13 C and 15 N enrichment of soil organic matter in forest soils. *Soil Biology & Biochemistry* **41**: 858-861.
- Fragoso, C. & P. Lavelle. 1992. Earthworm communities of tropical rain forests. *Soil Biology & Biochemistry* **24**: 1397-1408.
- Fry, B. 2006. *Stable Isotope Ecology*. Springer, New York.
- García, J. A. & C. Fragoso. 2004. Influence of different food substrates on growth and reproduction of two tropical earthworm species (*Pontoscolex corethrurus* and *Amyntas corticis*). *Pedobiologia* **47**: 754-763.
- González, G., E. García, V. Cruz, S. Borges, M. Zalamea & M. M. Rivera. 2007. Earthworm communities along an elevation gradient in Northeastern Puerto Rico. *European Journal of Soil Biology* **43**: S24-S32.
- Hendrix, P. F., S. L. Lachnicht, M. A. Callahan & X. Zou. 1999. Stable isotopic studies of earthworm feeding ecology in tropical ecosystems of Puerto Rico. *Rapid Communications in Mass Spectrometry* **13**: 1295-1299.
- Holdridge, L. 1996. *Ecología Basada en Zonas de Vida*. Instituto Interamericano de Cooperación para la Agricultura, San José, Costa Rica.
- International Coffee Organization. 2012. Available online at <http://www.ico.org/index.asp>. (Accessed 5 June 2012)
- Lachnicht, S. L., P. F. Hendrix & X. Zou. 2002. Interactive effects of native and exotic earthworms on resource use and nutrient mineralization in a tropical wet forest soil of Puerto Rico. *Biology and Fertility of Soils* **36**: 43-52.
- Lavelle, P. 1988. Earthworms and the soil system. *Biology and Fertility of Soils* **6**: 237-251.
- Lavelle, P., I. Barois, I. Cruz, C. Fragoso, A. Hernandez, A. Pineda & P. Rangel. 1987. Adaptive strategies of *Pontoscolex corethrurus* (Glossoscolecidae, Oligochaeta), a peregrine geophagous earthworm of the humid tropics. *Biology and Fertility of Soils* **5**: 188-194.
- López-Rodríguez, G. L. 2008. *Mineralización de Nitrógeno en Suelos Bajo Agrosistemas de Producción de Café (Coffea arabica L.) en Puerto Rico*. M. S. Thesis, University of Puerto Rico, Mayagüez, Puerto Rico.
- Nielson, R., B. Boag & M. Smith. 2000. Earthworm $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses suggest that putative functional classifications of earthworms are site-specific and may also indicate habitat diversity. *Soil Biology & Biochemistry* **32**: 1053-1061.
- Ramos, C. V., M. Alfaro, S. Borges & J. A. Amador. 2010. Earthworm taxonomic structure of coffee plantations at three soil associations in Puerto Rico. *Zoology in the Middle East* **S2. 2010**: 125-131.
- Sánchez-de León, Y., E. De Melo, G. Soto, J. Johnson-Maynard & J. Lugo-Pérez. 2006. Earthworm populations, microbial biomass and coffee production in different experimental agroforestry management systems in Costa Rica. *Caribbean Journal of Science* **42**: 397-409.
- Sánchez-de León, Y., X. Zou, S. Borges & H. Ruan. 2003. Recovery of native earthworms in abandoned tropical pastures. *Conservation Biology* **17**: 999-1006.
- Spain, A. V., P. G. Saffigna & A. W. Wood. 1990. Tissue carbon sources for *Pontoscolex corethrurus* (Oligochaeta: Glossoscolecidae) in a sugarcane ecosystem. *Soil Biology & Biochemistry* **22**: 703-706.

- Tian, G., B. T. Kang & L. Brussaard. 1997. Effect of mulch quality on earthworm activity and nutrient supply in the humid tropics. *Soil Biology & Biochemistry* **29**: 369-373.
- Tiunov, A. 2007. Stable isotopes of carbon and nitrogen in soil ecological studies. *Biological Bulletin* **34**: 395-407.
- Wada, E., Y. Kabaya & Y. Kurihara. 1993. Stable isotope structure of aquatic ecosystems. *Journal of Bioscience* **18**: 483-499.
- Zhang, W., P. F. Hendrix, B. A. Snyder, M. Molina, J. Li, X. Rao, E. Siemann & S. Fu. 2010. Dietary flexibility aids Asian earthworm invasion in North American forests. *Ecology* **9**: 2070-2079.

(Received on 07.06.2012 and accepted after revisions, on 08.08.2012)