MECHANISMS AND IMPACTS OF EARTHWORM INVASIONS ON NATIVE EARTHWORM SPECIES AND SOIL NUTRIENT DYNAMICS

by

CHING-YU HUANG

(Under the Direction of Paul F. Hendrix)

ABSTRACT

Earthworm invasions have become an important issue lately due to their significant impacts on bioitic communities and ecosystem functions, especially native earthworm populations and nutrient cycling. However, the details of these impacts and potential mechanisms are still unclear. For native earthworms, habitat disturbance and invasive earthworms have been suggested to be the main reasons for their disappearance and decreasing diversities. A field experiment in a successional forest of Puerto Rico, representing different degrees of disturbance, suggested that changes in soil characteristics and vegetation types from habitat disturbances had no impacts on native *Estherella* spp. population. The ability of *Estherella* spp. to re-colonize in the pasture was as good as in the secondary and mature forests. Therefore, habitat disturbance appears not to be the main reason to elucidate the decrease of native earthworm species. A laboratory study that manipulated Puerto Rican soils and earthworms found no competitive interactions between native and invasive earthworm species even for earthworms with similar niches or under reduced litter resources. However, earthworm impacts on soil nutrient dynamics were observed to vary between different earthworm species. These differential impacts were mainly determined by how earthworms interact with microbial populations from different microhabitats, in particular the rhizosphere and detritusphere. A model was developed in this study to project the potential impacts of earthworm invasions on soil carbon dynamics over long-term time scales in areas where peregrine earthworms have invaded or have yet to invade. Results of sensitivity analysis suggested that species characteristics of earthworms (assimilation and production efficiency) can not only affect their own populations but also significantly influence their invasion patterns and impacts on soil carbon dynamics. Also, simulation results suggested that the impacts of earthworm invasions can be affected by invasion history, invasive earthworm assemblages, and pre-invasion ecosystem conditions.

INDEX WORDS: Earthworm invasions, Invasive earthworms, Native earthworms, STELLA model, Carbon cycling, Puerto Rican soils, Competitive interactions, Stable isotopes.

MECHANISMS AND IMPACTS OF EARTHWORM INVASIONS ON NATIVE EARTHWORM SPECIES AND SOIL NUTRIENT DYNAMICS

by

CHING-YU HUANG

B.S., National Cheng-Kung University, Taiwan, 2000

M.S., National Cheng-Kung University, Taiwan, 2002

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2008

MECHANISMS AND IMPACTS OF EARTHWORM INVASIONS ON NATIVE EARTHWORM SPECIES AND SOIL NUTRIENT DYNAMICS

by

Ching-Yu Huang

Major Professor:	Paul F. Hendrix
Committee:	Miguel L. Cabrera Mac A. Callaham, Jr. C. Ronald Carroll David C. Coleman Grizelle González

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2008

ACKNOWLEDGEMENTS

This research is based upon work supported by the National Science Foundation (NSF) under Grant No. 0236276 to Dr. P. F. Hendrix (UGA), Grant No. 0218039 to Dr. G. González (Institute of Tropical Ecosystem Studies), Taiwan Study Abroad Scholarship (台灣教育部留學獎學金), and Graduate School Dissertation Completion Award (UGA).

I want to thank my committee: Paul Hendrix, Grizelle González, Dave Coleman, Miguel Cabrera, Mac Callaham and Ron Carroll for their support and helpful comments on my research. Paul has been a wonderful advisor. I am lucky having him as my major professor. Special thanks to Grizelle, who helped me a lot when I was in Puerto Rico. I am particularly grateful to Jian-Hong Chen, Becky Ball, Bas Boot, Sofia Arce-Flores, Jimmy Blackmon, Jake Richardson, Bob Potter, Caroline Collins, Jessica Maddox, Tom Maddox and the Analytical Chemistry Lab, International Institute of Tropical Forestry staff and researchers, and Sabana Field Research Station staff for their help with my field and laboratory work.

Thank to my friends who always make me laugh and look after me: Becky Ball, Sofia Arce-Flores, Justin Welch, Kyle Wickings, Krista Jacobsen, Will Duncan, John Kominoski, Scott Connelly, Yolima Carrillo, Andrew Mehring, Mike Strickland, Jimmy Blackmon, Bruce Snyder, Tom Maddox, Dac Crossley, and my Taiwanese “mafia” (especially Jenny Hwang, Guo-Jing Weng, Pai-Tsang Chang). I can not express enough thanks to my family for being so supportive all the time.

TABLE OF CONTENTS

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Earthworms are one of the most important soil invertebrates and play a significant ecological role in detrital food webs of terrestrial ecosystems. Their direct consumption of leaf litter, soil organic matter and microbial populations makes nutrients available for above-ground plant growth. Earthworm's casting and burrowing activities change soil structure (porosity and aggregation), stimulate microbial activities and affect soil nutrient cycling (Lee 1985, Edwards 1998, Lavelle et al. 1999). At least 3700 earthworm species have been described in the world, and earthworm taxonomists are expecting this number to increase with future field surveys, especially in tropical regions (Reynolds 1994, Lavelle and Lapied 2003). Despite increasing numbers of described earthworm species, there is limited information about their ecology and life history (Reynolds 2004). Most of the earthworm species are only recorded in the systematic and taxonomic literature with supplemental biological and ecological information. Furthermore, native earthworm populations have been reported to face extinction threats recently, mostly from human activities (Kalisz and Wood 1995, Lavelle and Lapied 2003).

Potential mechanisms for decline and extirpation of native earthworm species could be attributed to two factors: habitat disturbance (especially anthropogenic-derived) and competitive exclusion by peregrine earthworm species (Hendrix et al. 2006). Habitat disturbance can dramatically change local environments in physical, chemical, and biological ways, including break-down of soil structure, changes in resource availability, and disruption of plant population or community equilibrium (White and Pickett 1985). Along with the improvement of transportation and industrial techniques in past centuries, habitat disturbances mostly came from anthropogenic activities, i.e., land-use changes and human habitation. Anthropogenic-driven habitat disturbance often comes with higher frequency and intensity of destruction, and its detrimental impacts can be more permanent on ecosystem functions than naturally-driven ones. For example, land-use management (e.g., logging and cultivation) causes sharp fluctuations of soil physical environment (soil temperature/moisture and soil structures) and affects soil nutrient cycling by permanent removal of above-ground vegetation (González et al. 1999, Huang et al. 2006). In the meantime, continuous agricultural activities for crop production keep habitats homogeneous. Earthworms, which complete whole life cycles in the soil, are strongly affected by these disturbances on edaphic factors (such as soil temperature and moisture, resource availabilities, soil structure) (Lee 1985, Fragoso et al. 1999, Dlamini and Haynes 2004). Dlamini and Haynes (2004) found that earthworm biomass, community composition and diversity were influenced by land use changes. For native earthworm species with much narrower climatic and edaphic plasticity, abrupt soil environment changes can have more significant effects on their population dynamics and community composition than on peregrine earthworms (Fragoso et al. 1999). Kalisz and Wood (1995) suggested that habitat disturbance could extirpate or reduce native earthworm populations in the disturbed areas. Therefore, habitat disturbance may help to explain the distribution of native earthworms limited to natural and undisturbed ecosystems from current earthworm distributions and surveys (Fragoso et al. 1999, Lapied and Lavelle 2003). Nevertheless, studies investigating how exactly habitat disturbance affects native earthworm populations are still lacking. It is also interesting to know whether native earthworms can maintain their population in disturbed habitats for conservation purposes.

Habitat disturbance is not the only reason for the disappearance of native species (Kalisz and Wood 1995, Hendrix et al. 2006). Several papers have shown that some native earthworm populations in disturbed habitats coexisted with peregrine earthworm species (Kalisz 1993, Kalisz and Wood 1995, Fragoso et al. 1999). Invasion of peregrine earthworms is suggested to be another factor for the loss of native earthworms (Hendrix et al. 2006, Winsome et al. 2006). Peregrine earthworm species have invaded all over the world a long time before the early 1900s reports (e.g., Eisen 1900, Beddard 1912), but not until recently has there been significant research on the extent or impacts of these invasions. For example, successful invasion of European lumbricids earthworms in temperate forests of North America have been reported to cause the disappearance of forest floor, changes in soil nutrient dynamics, and effects on local biota diversity (McLean and Parkinson 1997, Hendrix and Bohlen 2002, Bohlen et al. 2004a, Hale et al. 2005, Frelich et al. 2006). A pan-tropical earthworm (*Pontoscolex corethrurus*) has been introduced into many tropical countries, and it has had significant influences on soil structures, soil nutrient cycles, and litter decomposition (Pashanasi et al. 1992, Pashanasi et al. 1996, Hallaire et al. 2000, Liu and Zou 2002, Lapied and Lavelle 2003). Asian invasive earthworms, particularly *Amynthas* spp., have invaded into remote forests of North and South America, which are refuges for native earthworms (Callaham et al. 2003, García and Fragoso 2003, Snyder et al. 2006). Apart from those impacts on soil physical and chemical environments, how invasive earthworms affect native earthworm species have yet to be completely understood. Direct competitive pressure from invasive earthworms is often suggested as a potential mechanism for extirpation of native earthworm population, especially in relatively undisturbed forests or remote areas (Kalisz 1993). In the meantime, invasive earthworms have been suggested to have better resource utilization efficiency, environmental plasticity, and demographic characteristics (i.e. higher reproduction rates and parthenogenesis ability) (Lavelle et al. 1987, Fragoso et al. 1999). In this case, invasive earthworms can possibly harm native earthworm species populations through competitive exclusion.

Several studies have observed and investigated competitive interactions between native and invasive earthworm species. Hendrix et al. (1999) suggested that there could be competition for food resources between exotic *P. corethrurus* and native *Estherella* spp. by observing their stable isotope (^{13}C and ^{15}N) natural abundance. Lachnicht et al. (2002) found that exotic *P. corethrurus* utilized different nitrogen resources when incubated alone or with native *Estherella* sp. in a microcosm study with Puerto Rican soils. Winsome et al. (2006) documented the inter-specific competition observed between native *Argilophilus marmoratus* and exotic *Aporrectodea trapezoids* and further implied this competition could prevent the natives from re-colonizing pastures dominated by exotic *A. trapezoids*. Nevertheless, relationships between different earthworm species may not only be limited to competitive but could also include facilitative and neutral interactions.

Earthworms can utilize a variety of food resources, including leaf litter, soil organic matter, rhizosphere substrates, microbial populations, animal excrements and debris (Lee 1985, Edwards 2004). In general, earthworms are categorized into three ecological groups, epigeic, endogeic and anecic, based on the type of food they mainly consume (Bouché 1977). Epigeic earthworms consume leaf litter and the colonized microbial population on it, and they mostly inhabit litter layers. In contrast, endogeic earthworms burrow in the deep soil and use soil organic matter as their main food resource. Anecic earthworms utilize fragmented leaf litter and soil organic matter, and they build burrows into deep soils. Different ecological groups of earthworms represent different feeding strategies and distribution patterns along soil vertical columns. When peregrine earthworms arrive at a new area beyond their native or natural biogeographical boundaries and begin to establish their populations, potential interactions (facilitation or competition) with native earthworms are expected to occur. Earthworm ecological groups provide a good way to test the hypothesis about competition or facilitation relationships between native and exotic earthworms. Intensive competition possibly occurs when both native and exotic earthworms utilize similar resources (same niche). However, there has been no study manipulating niche similarity and dissimilarity for native-exotic earthworm interactions.

Earthworm feeding strategies also affect their impacts on soil nutrient dynamics differentially. Epigeic earthworms may have less direct effect on soil microbial populations but significant influence on litter decomposition as compared to endogeic and anecic earthworms. Endogeic/anecic earthworms can affect soil microbial populations through casting and burrowing activities. Also, the excretion of mucus by earthworms alters the microbial communities living in gut passage and in their casts and burrows (Mummey et al. 2006). These actions can significantly influence soil C sequestration, soil microbial communities and biogeochemical cycling. Lachnicht et al.

(2002) found that *P. corethrurus* induced higher mineralization rates but this effect was reduced by the interaction of *Estherella* sp.. Beyond the native-exotic population interactions, it is also interesting to investigate their impacts of soil nutrient dynamics regarding both feeding strategies and their interactions.

Anthropogenic activities have contributed to earthworm invasions in the past decades. Human inhabitation, transportation, and global commerce assist peregrine earthworms to cross impassable barriers beyond their immigration abilities. At regional scales, habitat disturbance from human activities help peregrine earthworms to establish their populations in newly invaded areas. Invasive earthworms often come with the occurrence of disturbance and vice versa according to field observations. In this case, the decrease of density and diversity of native earthworms might result from either habitat disturbance, exotic earthworms or both. Therefore, there is a need to examine whether habitat disturbance or competitive pressure from invasive earthworms is the key factor that excludes native earthworms from their habitats.

Earthworms are considered as ecosystem engineers not just because of their direct contribution to soil processes (e.g. litter consumption) but also because of their indirect influence on soil environment and soil biota. Their impacts on ecosystem functions are determined based on not only single species effects but also how they interact with the others. The influences and intermixed interactions on soil processes are important but hard to evaluate one by one. Recently, ecological modeling techniques have provided a useful tool to integrate this complicated network of processes and interactions. For soil food webs, some ecological models have been developed to investigate soil processes. For example, soil organic matter model (SOMM) was developed to simulate organic matter dynamics in soil ecosystems. Nevertheless, soil invertebrates were integrated into a single category without considering their different ecological functions in soil food webs (Chertov and Komarov 1997). Brussaard (1998) noted that there is a need to address ecological interaction between different soil fauna guilds in soil food web models. Fu et al. (2000) developed a model with major soil invertebrate groups and soil micro-organisms to monitor their response on carbon dynamic in an agroecosystem. In their model, the idea of "super organism" representing the whole soil food web was used to process organic matter to simplify the model diagram, and therefore potential contributions from each of the soil invertebrate groups on organic matter dynamic were not singled out (Fu et al. 2000). So far, there is no single paper dealing with ecological contributions of specific soil invertebrate group on soil processes. From litter decomposition, to microbial populations, to mineralization, the complexity of interactions among soil processes calls for an ecological modeling concept which can comprehensively include biotic and abiotic interactions and soil nutrient cycling occurring in soil ecosystems. Earthworms by their importance in detrital food webs can be a good exemplar to develop soil nutrient dynamic models emphasizing special contributions of soil fauna.

Puerto Rico, a Caribbean Island, represents a long history of human disturbance from land-use management in the past decades. Many forests were converted to pastures and cultivated lands in the early 1900's. However, many pastures have been gradually converted to grasslands or secondary forests after the abandonment of agricultural practices due to economic shifts from agriculture to industry in the late 1940s (Birdsey and Weaver 1987). The processes of forest regeneration in Puerto Rico represent a gradient of habitat disturbance (from pasture to mature forests) and they have been monitored for management and conservation purposes (Aide et al. 1995, Zimmerman et al. 1995, Pascarella et al. 2000). The composition of earthworm communities has been observed to change along with vegetation successional stages in Puerto Rican forests (Zou and González 1997, Sánchez-de León et al. 2003). Native earthworm species were only found in the forests with less total habitat disturbance. Zou and González (1997) showed that native *Estherella gatesi* existed only in successional forests older than 15 years following secondary succession of abandoned tropical pastures in the Luquillo Mountains. Five native earthworm species were only discovered in the mature secondary forests of a chronosequence from old tropical pastures to young and mature forests in the Cayey Mountains of Puerto Rico (Sánchez-de León et al. 2003). In contrast, invasive earthworms (mainly *Pontoscolex corethrurus*) have shown dominance in the disturbed pastures of Puerto Rico, but the density of *P. corethrurus* decreased along with advanced successional stage (Sánchez-de León et al. 2003). Degree of disturbance (pasture and plantation management practices) and possibly competitive interactions between native and exotic earthworm species may determine this distribution pattern in regenerated forests of Puerto Rico. Lower degree of habitat disturbance in mature forests, which means improvement and resilience of habitat characteristics (i.e. litter and/or root biomass, soil temperature and moisture, and microbial biomass), may provide a better soil environment for re-colonization of native earthworm species and to increase earthworm diversities during forest regeneration (González et al. 1996, Zou and González 1997). Meanwhile, the dominance of invasive earthworms in pastures may impede the ability of native species to colonize pastures through the competition for available resources (Sánchez-de León et al. 2003). These mixed effects of habitat disturbance and competitive interaction may be the mechanisms affecting current distribution patterns of native and invasive earthworms in Puerto Rican forests.

DISSERTATION FOCUS

This dissertation examines potential mechanisms for the disappearance and decrease of native earthworm species in Puerto Rico, especially emphasizing habitat disturbance and competitive interactions with invasive earthworms. At first, I tested the hypothesis that habitat disturbance could impede the re-colonization ability of native earthworm, *Estherella* spp. (Chapter two). A field experiment was conducted along a chronsequence of successional forests to evaluate whether changes of habitat characteristics from secondary succession have any impacts on native *Estherella* spp. populations. The results showed that sustainability (survivorship and growth) of native *Estherella* population was maintained well in disturbed pastures as well as in less-disturbed young and mature forests during the first six months of introduction. This suggested that the exclusion of native *Estherella* earthworms in disturbed pastures might be due to biotic factors instead of habitat disturbance, particularly competition pressure from invasive earthworms (Huang et al. 2006).

Next, a full-factorial laboratory experiment was designed to investigate potential interactions between native and exotic earthworms and their interactions on soil nutrient dynamics with (dis)similar niche combinations under reduced vs excess litter resource availability (Chapter three). The experiment was set up with soil mesocosms using Puerto Rican forest soils. Three Puerto Rican earthworm species (exotic epi-endogeic

Pontoscolex corethrurus; native epigeic *Estherella* spp.; native endogeic *Onychochaeta borincana*) were collected from the field and adapted for this experiment. ^{13}C- (litter) and ^{15}N-labeled (grass roots) materials were applied to evaluate the feeding strategies of earthworms and to trace the nutrient dynamics. Recently, stable isotopic techniques have improved the knowledge of important mechanisms and energy flow pathways in ecosystem studies (Coleman and Fry 1991, Lajtha and Michener 1994). For instance, stable isotopes (i.e. ^{13}C and ^{15}N) can be used to trace prey-predator interaction, food-preference choice and soil nutrient dynamics. For earthworms, isotopic method helps to clarify and investigate their feeding strategies. Even though earthworm ecological groups are often used to indicate their possible food utilization strategies, no direct evidence is provided for most cases. Earthworm ecological groups are identified based on soil layers where they are found and their body pigmentation and behavior. Several studies have documented that earthworms have flexible feeding behaviors, in particular when preferred food resources are limited. Lachnicht et al. (2002) found that *P. corethrurus* utilized different food resources as coexisting with other earthworms. Hence, the application of stable isotopic method can help to understand flexibility of earthworm feeding behaviors, especially under the conditions as encountering competition or lacking of preferred food resources. In this chapter, consistent feeding behaviors of all three earthworm species were observed during the 22-day mesocosm experiment, even when litter was reduced. Different earthworm species did affect soil carbon and nitrogen dynamics in different ways.

In chapter four, I attempted to model potential influences of earthworms on soil carbon dynamics in terrestrial ecosystems generally. This model not only included potential effects from earthworm's comminuting and casting activities but also the impacts of earthworm invasion on forest floor organic matter, microbial populations, and soil organic matter. The field data from Arnot forest, New York (Bohlen et al. 2004b, Fisk et al. 2004, Groffman et al. 2004), were adapted for model calibration. This model can not only be applied to understand the potential impacts of earthworms on soil carbon cycles in ecosystems but also to earthworm invasions. Results from this model suggested that impacts of earthworm invasions on terrestrial ecosystems may vary depending on invasion history, earthworm species composition, and ecosystem types.

References Cited

Aide, T. M., J. K. Zimmerman, L. Herrera, and M. Rosario. 1995. Forest recovery in abandoned tropical pastures in Puerto Rico. Forest Ecology and Management 77:77-86.

Beddard, F. E. 1912. Earthworms and their allies. Cambridge University Press, London.

Birdsey, R. A., and R. W. Weaver. 1987. Forest area trends in Puerto Rico. Research Note SO-331, USDA Forest Service, Washington, D. C., USA.

Bohlen, P. J., P. M. Groffman, T. J. Fahey, M. C. Fisk, E. Suárez, D. M. Pelletier, and R. T. Fahey. 2004a. Ecosystem consequences of exotic earthworm invasion of north temperate forests. Ecosystems 7:1-12.

Bohlen, P. J., D. M. Pelletier, P. M. Groffman, T. J. Fahey, and M. C. Fisk. 2004b. Influence of earthworm invasion on redistribution and retention of soil carbon and nitrogen in northern temperate forests. Ecosystems 7:13-27.

Bouché, M. B. 1977. Strategies Lombriciennes. Pages 122-132 *in* U. Lohm and T. Persson, editors. Soil organisms as components of ecosystems. Proc. 6th Int. Coll. Soil Zool., Ecological Bulletin, Stockholm.

Brussaard, L. 1998. Soil fauna, guilds, functional groups and ecosystems processes. Applied Soil Ecology 9:123-135.

Callaham, M. A., Jr., P. F. Hendrix, and R. J. Phillips. 2003. Occurrence of an exotic earthworm (*Amynthas agrestis*) in undisturbed soils of the southern Appalachian mountains, USA. Pedobiologia 47:466-470.

Chertov, O. G., and A. S. Komarov. 1997. SOMM: A model of soil organic matter dynamics. Ecological Modelling 94:177-189.

Coleman, D. C., and B. Fry, editors. 1991. Carbon isotope techniques. Academic Press, San Diego, California.

Dlamini, T. C., and R. J. Haynes. 2004. Influence of agricultural land use on the size and composition of earthworm communities in northern KwaZulu-Natal, South Africa. Applied Soil Ecology 27:77-88.

Edwards, C. A. 2004. Earthworm ecology. CRC Press, Boca Raton.

Eisen, G. 1900. Researches in American Oligochaeta, with especial reference to those to the Pacific coast and adjacent islands. Proceedings of the California Academy of Sciences, 3rd Series, Zoology 2:85-276.

Fisk, M. C., T. J. Fahey, P. M. Groffman, and P. J. Bohlen. 2004. Earthworm invasion, fine-root distributions, and soil respiration in North temperate forests. Ecosystems 7:55-62.

Fragoso, C., J. Kanyonyo, A. Moreno, B. K. Senapati, E. Blanchart, and C. Rodríguez. 1999. A survey of tropical earthworms: taxonomy, biogeography and environmental plasticity. Pages 1-26 *in* P. Lavelle, L. Brussaard, and P. Hendrix, editors. Earthworm management in tropical agroecosystems. CABI Publishing, New York.

Frelich, L. E., C. M. Hale, S. Scheu, A. R. Holdsworth, L. Heneghan, P. J. Bohlen, and P. B. Reich. 2006. Earthworm invasion into previously earthworm-free temperate and boreal forests. Biological Invasions 8:1235-1245.

Fu, S., M. L. Cabrera, D. C. Coleman, K. W. Kisselle, C. J. Garrett, P. Hendrix, and D. A. Crossley. 2000. Soil carbon dynamics of conventional tillage and no-till agroecosystmes at Georgia Piedmont-HSB-C models. Ecological Modelling 131:229-248.

García, J. A., and C. Fragoso. 2003. Influence of different food substrates on growth and reproduction of two tropical earthworm species (*Pontoscolex corethrurus* and *Amynthas corticis*). Pedobiologia 47:754-763.

González, G., X. Zou, and S. Borges. 1996. Earthworm abundance and species composition in abandoned tropical croplands: comparison of tree plantations and secondary forests. Pedobiologia 40:385-391.

González, G., X. Zou, A. Sabat, and N. Fetcher. 1999. Earthworm abundance and distribution pattern in contrasting plant communities within a tropical wet forest in Puerto Rico. Caribbean Journal of Science 35:93-100.

Groffman, P. M., P. J. Bohlen, M. C. Fisk, and T. J. Fahey. 2004. Exotic earthworm invasion and microbial biomass in temperate forest soils. Ecosystems 7:43-54.

Hale, C. M., L. E. Frelich, P. B. Reich, and J. Pastor. 2005. Effects of European earthworm invasion on soil characteristics in Northern hardwood forests of Minnesota, USA. Ecosystems 8:911-927.

Hallaire, V., P. Curmi, A. Duboisset, P. Lavelle, and B. Pashanasi. 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, P. F., G. H. Baker, M. A. Callaham Jr, G. A. Damoff, C. Fragoso, G. González, T. Winsome, and X. Zou. 2006. Invasion of exotic earthworms into ecosystems inhabited by native earthworms. Biological Invasions 8:1287-1300.

Hendrix, P. F., and P. J. Bohlen. 2002. Exotic earthworm invasions in North America: ecological and policy implications. BioScience 52:801-811.

Hendrix, P. F., S. L. Lachnicht, Callaham, M. A., and X. Zou. 1999. Stable isotopic studies of earthworm feeding ecology in tropical ecosystems of Puerto Rico. Rapid Communications in Mass Spectrometry 13:1295-1299.

Huang, C.-Y., G. González, and P. F. Hendrix. 2006. The re-colonization ability of native earthworm, *Estherella* spp., in Puerto Rican forests and pastures. Caribbean Journal of Science 42:386-396.

Kalisz, P. J. 1993. Native and exotic earthworms in deciduous forest soils of Eastern North America. Biological Pollution: the control and impact of invasive exotic species. Proceedings of a Symposium held at the University Place Conference Center, Indiana University-Purdue University, Indianapolis, October 25 & 26, 1991:93-100.

Kalisz, P. J., and H. B. Wood. 1995. Native and exotic earthworms in wildland ecosystems. Pages 117-126 *in* P. F. Hendrix, editor. Earthworm Ecology and Biogeography in North America. Lewis Publishers, Boca Raton, FL.

Lachnicht, S. L., P. F. Hendrix, and 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.

Lajtha, K., and R. H. Michener. 1994. Stable isotopes in ecology and environmental science. Blackwell Scientific Publications, Boston.

Lapied, E., and P. Lavelle. 2003. The peregrine earthworm *Pontoscolex corethrurus* in the east coast of Costa Rica. Pedobiologia 47:471-474.

Lavelle, P., I. Barois, I. Cruz, C. Fragoso, A. Hernandez, A. Pineda, and 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.

Lavelle, P., L. Brussaard, and P. Hendrix, editors. 1999. Earthworm Management in Tropical Agroecosystems. CABI Publishing, New York.

Lavelle, P., and E. Lapied. 2003. Endangered earthworms of Amazonia: an homage to Gilberto Righi. Pedobiologia 47:419-417.

Lee, K. E. 1985. Earthworms, their ecology and relationships with soils and land use. Academic Press, New York.

Liu, Z. G., and X. M. Zou. 2002. Exotic earthworms accelerate plant litter decomposition in a Puerto Rican pasture and a wet forest. Ecological Applications 12:1406-1417.

McLean, M. A., and D. Parkinson. 1997. Changes in structure, organic matter and microbial activity in pine forest soil following the introduction of *Dendrobaena octaedra* (Oligochaeta, Lumbricidae). Soil Biology and Biochemistry 29:537-540.

Mummey, D. L., M. C. Rillig, and J. Six. 2006. Endogeic earthworms differentially influence bacterial communities associated with different soil aggregate size fractions. Soil Biology and Biochemistry 38:1608-1614.

Pascarella, J. B., T. M. Aide, M. I. Serrano, and J. K. Zimmerman. 2000. Land-use history and forest regeneration in the Cayey mountains, Puerto Rico. Ecosystems 3:217-228.

Pashanasi, B., P. Lavelle, J. Alegre, and F. Charpentier. 1996. Effect of the endogeic earthworm *Pontoscolex corethrurus* on soil chemical characteristics and plant growth in a low-input tropical agroecosystem. Soil Biology and Biochemistry 28:801-810.

Pashanasi, B., G. Melendez, L. Szott, and P. Lavelle. 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 Biology and Biochemistry 24:1655-1659.

Reynolds, J. 1994. Earthworms of the world. Global Biodiversity 4:11-16.

Reynolds, J. W. 2004. The status of earthworm biogeography, diversity, and taxonomy in North America revisited with glimpses in the future. Pages 63-74 *in* C. A. Edwards, editor. Earthworm Ecology. CRC Press, Boca Raton, FL.

Sánchez-de León, Y., X. Zou, S. Borges, and H. Ruan. 2003. Recovery of native earthworms in abandoned tropical pastures. Conservation Biology 17:999-1006.

Snyder, B., P. F. Hendrix, and M. A. Callaham. 2006. An upstart in iconic territory: Effects of the invasive exotic earthworm *Amynthas agrestis* (Annelida:Megascolecidae) on native taxa. 86th Ecological Society of America Annual Meeting, Memphis, Tennessee. August 2006.

White, P. S., and S. T. A. Pickett. 1985. The ecology of natural disturbance and patch dynamics. Academic Press, Orlando, Florida.

Winsome, T., L. Epstein, P. F. Hendrix, and W. R. Horwath. 2006. Competitive interactions between native and exotic earthworm species as influenced by habitat quality in a California grassland. Applied Soil Ecology 32:38-53.

Zimmerman, J. K., T. M. Aide, M. Rosario, M. Serrano, and L. Herrera. 1995. Effects of land management and a recent hurricane on forest structure and composition in the Luquillo Experimental Forest, Puerto Rico. Forest Ecology and Management 77:65-76.

Zou, X., and G. González. 1997. Changes in earthworm density and community structure during secondary succession in abandoned tropical pastures. Soil Biology and Biochemistry 29:627-629.

CHAPTER TWO

THE RE-COLONIZATION ABILITY OF A NATIVE EARTHWORM SPECIES, ESTHERELLA SPP., IN PUERTO RICAN FORESTS AND PASTURES[1]

---

[1] Huang, C-Y, G. González and P. F. Hendrix. 2006. *Caribbean Journal of Science*. 42: 386-396. Reprinted here with permission of Caribbean Journal of Science, University of Puerto Rico, Mayagüez.

Abstract

Earthworms have significant influences on soil processes in terrestrial ecosystems. However, populations of some native earthworm species are decreasing or disappearing due to human activities such as habitat disturbance and introduction of exotic earthworm species. Habitat disturbance can cause sharp changes in soil physical structure and nutrient cycling, which may reduce native earthworm populations prior to the invasion of exotic earthworms. The purpose of this study is 1) to investigate habitat disturbance as a key process in the decline or extirpation of native earthworms, and 2) to measure the ability of native earthworms to re-colonize disturbed areas. We hypothesized that habitat disturbance will reduce the population of native earthworms and impede their re-colonization in those perturbed areas. We set up 48 soil mesocosms in three field sites representing different degrees of disturbance (abandoned pasture, young and mature forests) in the Cayey Mountains of Puerto Rico. Three individuals of the native earthworm species, *Estherella* spp., were inoculated into each soil core to evaluate their re-colonization ability by measuring survivorship, growth rates and reproduction. We found that, in the absence of exotic earthworm species, the survivorship and growth rates of *Estherella* spp. population in the pasture was not significantly different than that from the young and mature forests during the first six months of re-colonization process. Our results suggest that habitat disturbance (changes in vegetation and soil properties) may not have significant influences on native earthworm (*Estherella* spp.) populations. Alternatively, we propose that biotic factors, such as competitive exclusion of native earthworms by exotic earthworms, may have much considerable effects on retarding their re-colonization and/or causing the disappearance of native earthworm population in disturbed areas.

Key words: exotic earthworm; invasion; Puerto Rico; soil aggregation; succession; survivorship; tropics

Introduction

Earthworms play a significant role in soil food webs. Casting and burrowing can change soil structure (porosity and aggregation) and stimulation of microbial activities can increase nutrient availability for plant growth (Lee 1985, Lavelle et al. 1999, Edwards 2004). A total of 3627 earthworm species has been described and this number is expected to increase by 68 species per year with more field surveys, particularly in tropical regions (Reynolds 1994, Fragoso et al. 1997). However, the diversity of native earthworms is declining along with the invasion of exotic earthworm species that have been introduced widely due to human activities (González et al. 2006). Lavelle and Lapied (2003) found that many native earthworm species are in danger of extinction or have already disappeared in Amazonian areas that are now colonized by exotic species.

The disappearance or decline of native earthworm populations are caused mostly by human activities, especially habitat disturbance and the introduction of exotic earthworm species. Kalisz and Dotson (1989) found that populations of native earthworms in the Appalachian Mountains of Kentucky, USA, were distributed far from severely disturbed areas, whereas exotic earthworms were always near land clearance, agricultural cultivation, and human habitations. Habitat disturbance (e.g. logging and cultivation) may cause sharp fluctuations in soil physical conditions (e.g. soil temperature, moisture and structure) and in soil nutrient cycling as vegetation types and litterfall input are altered. In disturbed habitats, these physical-chemical changes can cause the decline of native earthworm populations prior to invasion by exotic earthworms, so that exotics can occupy vacant habitats without interacting with native earthworm species (Kalisz 1993, Kalisz and Wood 1995). Interactions between native and exotic earthworms, especially competition for resources, may be a determining factor in the decline of native species in relatively undisturbed area (Kalisz 1993, Kalisz and Wood 1995). Yet, it is still unclear how significantly these two mechanisms affect the diversity of native earthworm species at a given site. Human-caused disturbance can affect the suitability of soil as a habitat for native earthworms, thereby degrading a basic requirement for sustaining native earthworm populations, while competitive intensity for finite resources between native and exotic earthworms may change the performance of native earthworm populations in their original habitats after invasion of exotic earthworms. Understanding the impacts of habitat disturbance on native earthworm species should be a priority for biodiversity conservation, and it should be a first step before considering the potential consequences of native and exotic interactions.

Due to soil degradation and/or changes in economic strategies, many tropical pastures have been gradually converted to grasslands or secondary forests following the abandonment of agricultural practices (e.g. Brazil, Buschbacher 1986). Puerto Rico, a Caribbean Island, is a typical example representing the history of land-use changes and forest regeneration in the past decades. Forests in Puerto Rico were converted to pastures and cultivated land in the early 1900's. By the late 1940's, because the island shifted from an agricultural to an industrial economy, the abandonment of agricultural lands had occurred across the whole island (Birdsey and Weaver 1987). Forest cover of the island has increased from 5% to over 30% (Birdsey and Weaver 1987).

The successional processes of forest regeneration in Puerto Rico have been widely investigated for management and conservation purposes (Aide et al. 1995, Zimmerman et al. 1995, Pascarella et al. 2000). Interestingly, the composition of earthworm communities, which mostly disappeared in the pastures, has also changed along with vegetation succession. An earthworm survey following secondary succession of abandoned tropical pastures in the Luquillo Mountains found that native *Estherella gatesi* existed only in successional stages older than 15 years (Zou and González 1997). Sánchez-de León et al. (2003) discovered that five native earthworm species were only found in the mature secondary forests of a chronosequence of old tropical pastures (pastures, young and mature forests) in the Cayey Mountains of Puerto Rico. Therefore, degree of disturbance may determine the distribution of native earthworms among successional stages and management practices in regenerated forests. Lower degrees of habitat disturbance may correlate with a greater opportunity for re-colonization by native earthworm species. Habitat characteristics such as litter and/or root biomass, soil properties (water and nutrient content), and microbial biomass may contribute to a diverse earthworm community during forest regeneration (González et al. 1996, Zou and González 1997). Meanwhile, exotic earthworm species may affect the re-colonization by native species, since the exotic earthworm, *Pontoscolex corethrurus*, was distributed in all successional stages in these old tropical pastures in Puerto Rico. Sánchez-de León et al. (2003) proposed that competition between native and exotic earthworms may cause the disappearance of native earthworms in the disturbed areas. These combined effects may be the mechanisms retarding current re-colonization of native earthworms.

In this study, we investigated whether habitat disturbance (changes in soil properties and vegetation) is a key mechanism for current re-colonization processes of the native earthworm, *Estherella* spp., in a chronosequence of regenerated forests in Puerto Rico. We also determined how *Estherella* spp. affects soil aggregation after colonization of the soil. Our hypothesis is that habitat disturbance will impede the re-colonization of native earthworms (*Estherella* spp.) by decreasing their survivorship, growth rate, and reproduction.

Materials and methods

Study site

The study area is located in the Sierra de Cayey Mountains of southeastern Puerto Rico. All sites are 600–700 m above sea level. We chose three sites (pasture, young and mature secondary forests) on a chronosequence of naturally regenerated forest to include different degrees of habitat disturbance. The pasture site, abandoned 1-2 years before the study, had supported intense land use activities such as: cattle tramping and forest clearance, for the past few years. The young and mature secondary forests have steadily recovered from the impacts of human disturbances, and represent intermediate and slight perturbation, respectively. Grass species are dominant in the pasture site, while woody species become more prevalent in the young and mature secondary forests (Pascarella et al. 2000, Sánchez-de León et al. 2003).

A previous earthworm survey in this area had discovered exotic *Pontoscolex corethrurus,* which was widespread over all the sites (active pasture, young and mature secondary forests) (Sánchez-de León et al., 2003). They also only found a native earthworm community composed of *Borgesia sedecimestae*, *Estherella* sp., *Onychochaeta borincana*, *Neotrigaster rufa*, and *Trigaster longissimus* in the mature secondary forest (Sánchez-de León et al., 2003).

Materials and methods

During this study, we measured litterfall, litterfall carbon/nitrogen content, soil physical characteristics, and microbial biomass to examine the differences among the sites.

On August, 2004, four blocks were chosen (at least 3 m apart) within each of study sites, and four soil cores (soil samples contained within PVC tubes) were collected randomly (30 cm apart) from each of four blocks at each site. We collected intact soil cores (diameter = 11 cm, length = 30 cm) by inserting a PVC tube into the ground after the removal of the surface litter. This method allowed for the collection of physically undisturbed soil samples. All soil cores (n = 48) were put in a freezer (-30 ^{o}C) for 48 hours to eliminate earthworms. We covered the bottom and top of each soil core with 1-mm mesh screen to prevent the migration of earthworms (see below). Soil cores were replaced into exact sites from where they were collected.

Native earthworms, *Estherella* spp., were collected from the Bisley experimental watersheds, which is located at the northeast side of the Luquillo Mountains. The vegetation in the Bisley experimental watersheds includes primary and secondary tabonuco forests (*Dacryodes excelsa*) (Scatena, 1989). Fresh weights of earthworms were recorded. A subsample of earthworms was used to void their gut for 24 hours to calibrate the weight of earthworms minus gut contents [Gut-voiding weight (g) = - 0.0253 + 0.999 Fresh weight (g)]. Three individuals of *Estherella* spp. (mean ± S.D. = 3.17 ± 0.4 g) were inoculated into each of the soil cores in the field.

On October 31, 2004, we gathered initial soil data by sampling one soil core from every block at each site before inoculating with native earthworms. Every two months, one soil core from every block at each site was sampled (Core sample), and another soil sample (diameter = 5 cm, length = 30 cm) was also collected 1 m apart from the core sample within each block (Field sample).

At each sampling date, litterfall was collected in four baskets (57 × 43 cm in size) which were randomly set up at each block. Varied amounts of litter gathered from field litter baskets (see below) were added into the rest of soil cores in the field based on the area of soil cores (95 cm^2) (e.g. pasture/young/mature= 0.12/0.90/0.88 g dry litter per core in March, 2005).

Litterfall and field soil data were collected at all the sites during the experimental period for determination of habitat characteristics (Table 2.1). Litterfall was recorded after air-drying for 48 hours. Field and Core samples were analyzed for soil water content and pH (0-5 and 5-10 cm deep). Ten grams of soil from each sample were oven-dried at 105 ^{o}C for 48 h to determine soil water content. Soil pH was measured using a 1:2 ratio of dried soil and deionized water (Hendershot et al. 1993). Subsamples of litter and soil were finely ground for total carbon (C) and nitrogen (N) analysis. Total C and N in the soil samples (20 mg) and the litter samples (5 mg) were determined by dry combustion in a Carlo Erba model 1500 C/N analyzer.

Earthworms harvested from the experimental cores were used to calculate survivorship and vertical distribution of *Estherella* spp.. We also calculated biomass growth rates (%) of earthworms by using the mean final weight of the worms (gut voided for 24 hours) divided by mean initial weight per core.

The distribution of soil aggregates was analyzed for both Core and Field samples by using a wet-sieving apparatus described in Beare et al. (1994). Three aggregate classes (>2000 μm, 250-2000 μm, and 53-250 μm) were measured in this study. Fifty grams of dried soil were evenly distributed on top of stacked 2000-μm and 250-μm sieves and wetted for 8 minutes with deionized water prior to wet-sieving. Then the stacked sieves were oscillated vertically within deionized water for 5 minutes (31 oscillation cycles per minute). Following the oscillations, the water column was drained through a 53-μm sieve. Soil aggregates with sieves were transferred to aluminum pans then air-dried. Soil aggregates distribution (%) was calculated on a dry weight basis for each size class.

Statistic analysis

All analyses were performed by using SAS software (version 9.1, SAS Institute Inc. USA). Data representing characteristics of the study sites (litterfall and soil data) were analyzed as one way factorial design by the GLM procedure to compare the difference among habitats. Soil pH and microbial biomass data were tested by the GLM procedure for the differences among habitats and treatments (Field and Core samples). If significant, Tukey (HSD) multiple comparison method was used. A logistic procedure was applied to analyze the vertical distribution and survivorship of earthworms because of the discrete responses. The distribution pattern of soil aggregates was tested by MANOVA of ANOVA procedure. The differences of earthworm growth rates among site and time factors were compared by repeated-measures GLM procedure. The significance level for all tests was set at $\alpha = 0.05$.

Results

Site characteristics

Litterfall input in the pasture was significantly the lowest ($F_{site\ 2,\ 21} = 14.9$, $p < 0.0001$; Table 2.1). Litter C was similar among all sites ($F_{site\ 2,\ 45} = 0.74$, $p = 0.48$; Table 2.1), while the litter from the mature secondary forest had the lowest litter nitrogen (N) ($F_{site\ 2,\ 45} = 4.3$, $p = 0.02$; Table 2.1) and highest C/N ratio ($F_{site\ 2,\ 45} = 4.8$, $p = 0.01$; Table 2.1). Soil pH in the mature forest was significantly lower than those in the young forest and pasture in the top 10 cm of soil (0-5 cm: $F_{site\ 2,\ 33} = 40.3$, $p < 0.0001$; 5-10 cm: $F_{site\ 2,\ 33} = 15.8$, $p < 0.0001$; Table 2.1). Soil water content of the top 0-10 cm in the pasture was about 2- 2.5 times higher than those in the young and mature forests (0-5 cm: $F_{2,\ 45} = 58.2$, $p < 0.0001$; 5-10 cm: $F_{2,\ 45} = 76.2$, $p < 0.0001$; Table 2.1). Soil C and N did not significantly differ among habitat types, except that soil N was lower at 5-10 cm soil in the mature secondary forest ($F_{site\ 2,\ 47} = 8.9$, $p = 0.0005$; Table 2.1). Soil C/N was higher in mature forest than in the pasture, suggesting an increase with successional stage (0-5 cm: $F_{site\ 2,\ 44} = 32.5$, $p < 0.0001$; 5-10 cm: $F_{site\ 2,\ 47} = 11.0$, $p < 0.0001$; Table 2.1). Microbial biomass N was significantly higher in the pasture than in the young and mature forests

(0-5 cm: $F_{site\ 2,\ 45}$ =14.6, $p < 0.0001$; 5-10 cm: $F_{site\ 2,\ 45}$ =6.7, $p = 0.003$; Table 2.1). However, microbial C and C/N were similar among all sites ($p > 0.05$; Table 2.1).

Soil water content of the top 10 cm soil in the Core samples of the young and mature forests were 2.5 and 2 times higher than those in the Field soil samples (t-test, $p < 0.0001$; data not shown), but not significantly different in the pasture ($p > 0.1$; data not shown). However, soil C, N and C/N did not significantly differ between the Core and Field samples at top 10 cm soil in all habitats (0-5 cm: $F_{1,\ 17}$ =1.27, 2.03 and 0.07 for C, N and C/N data, respectively; all $p > 0.1$), as well as soil pH ($F_{1,\ 17}$ =1.5, $p > 0.1$) at the end of the experiment.

Soil microbial biomass C in the field mesocosms [Core samples; 0-5 cm (mean ± S.E): 1459.7 ± 140, 571.4 ± 101, 605.2 ± 124 ug/g dry soil ; 5-10 cm (mean± S.E): 1088.1 ± 127, 638.63 ± 80, 257.2 ± 44 ug/g dry soil in the pasture, young and mature forests, respectively] was significantly lower than in the Field samples for the top 10 cm soil at all sites (in average 75 %, 42 %, 32% of the field data in the pasture, young and mature forests, respectively; F test and Tukey comparison, $p < 0.0001$). Soil microbial biomass N in the Core samples [0-5 cm (mean ± S.E): 153.9 ± 16.35, 101.2 ± 10.51, 83.0 ± 11.51 ug/g dry soil ; 5-10 cm (mean ± S.E): 119.4 ± 10.78, 79.0 ± 10.10, 46.9 ± 5.77 ug/g dry soil in the pasture, young and mature forests, respectively] was also lower compared to the Field samples (in average 74 %, 61 %, 45% of the field data in the pasture, young and mature forests; F test and Tukey comparison, $p < 0.0001$). However, soil microbial biomass C and N in the Core samples showed the similar patterns among all sites as the Field samples. Lower soil microbial C/N of young and mature secondary forests were found in the Core samples [microbial C/N (mean ± S.E): 9.85 ± 0.61, 5.70 ±

0.74, and 7.95 ± 1.16 in the pasture, young and mature forests, respectively] than those in the Field samples at 0-5 cm soils ($F_{1, 82}$ =6.5; p = 0.01), but no significant difference at 5-10 cm of the soils [Core samples: microbial C/N (mean ± S.E): 9.09 ± 0.72, 8.43 ± 0.66, and 7.52 ± 2.19 in the pasture, young and mature forests, respectively; $F_{1, 82}$ =1.6; p = 0.20].

Earthworm population

The survivorship of *Estherella* spp. in the pasture was not significantly different than those from the young and mature forests (n = 36, χ_2 = 1.12, p = 0.57; Fig. 2.1A). *Estherella* spp. had higher mortality at later samplings (March and May) in all sites (χ_2 = 6.65, p = 0.04; Fig. 2.1A). No significant difference in the growth rate of earthworms was found among the different habitats (n=25, $F_{site\ 2, 7}$ = 0.19, p = 0.84; $F_{time\ 2, 7}$ = 0.69, p = 0.53; Fig. 2.1B). Highly individual variations in growth rates (wide error bars in Fig. 2.1B) was observed. The total abundance of *Estherella* spp. harvested during the experiment in the pasture, young and mature forests were 23, 29, and 22 worms, which represented 64, 81, and 61% of total worms that were inoculated in each habitat, respectively. Most *Estherella* spp. were found in the top 10 cm of the soils, especially 5-10 cm (n=108, χ_3 = 18.8, p = 0.0003; Fig. 2.2). There was no significant difference among site and time factors (n=108, $\chi_{site, 2}$ = 0.09, p = 0.96; $\chi_{time, 2}$ = 0.66, p = 0.72; Fig. 2.2). No cocoon was found in all the Core samples during the experimental period.

Earthworm effects on soils

Estherella spp. significantly changed distribution patterns of macro- and microaggregates from the beginning (October, 2004) to the end (May, 2005) of the experiment ($F_{\text{time } 3, 16} = 4.1$, $p = 0.02$; initial and final data in Fig. 2.3A and 2.3B). In the presence of *Estherella* spp., the percentages of larger macro-aggregates (> 2000 µm) in the Core samples showed a decline over the experimental time, from 80.1 to 63.2 % and from 71.4 to 66.4 % in the young and mature forests, respectively (Fig. 2.3A), which is in contrast to a rising trend observed in Field samples (increased from 68.8 to 78.9 % and from 56.1 to 74.3 % in the young and mature forests, respectively; Fig. 2.3B).

Discussion

Habitat disturbance is defined as any major event that alters resource availability and/or causes changes in the physical environment (Chapin III et al. 2002). In this study, we chose three habitat types representing a chronosequence of successional stages as sites differing in the degree of disturbance due to cattle ranching. In the pasture, we found the general characteristics of grass-domination, low litterfall input, and higher soil pH and microbial biomass (Aide et al. 1995, Guariguata and Ostertag 2001, Chapin III et al. 2002, Pregitzer and Euskirchen 2004, Bautista-Cruz and del Castillo 2005). Not only habitat characteristics change along succession, but also the composition and abundance of native and exotic earthworm communities varied within this chronosequence. Most native earthworms in Puerto Rico, such as *Estherella* spp., are distributed in mostly undisturbed areas (dwarf forests and tabonuco forests), and less disturbed habitats (later successional stages: naturally regenerated young and mature secondary forests)

(González et al. 1996, Zou and González 1997, Hendrix et al. 1999, Sánchez-de León et al. 2003, Sánchez-de León and Zou 2004). In contrast to the more limited distribution of native earthworms, the pantropical earthworm, *Pontoscolex corethrurus*, has expanded to the pastures, successional forests, tree plantations, and undisturbed forests in Puerto Rico (González et al. 1996, Zou and González 1997, Hendrix et al. 1999, Sánchez-de León et al. 2003). The mechanisms to explain this unequal distribution of native and exotic earthworm communities might be related to the changes of habitat characteristics due to habitat disturbance. González et al. (1996) proposed that soil water content, phosphate availability, root and microbial biomass contributed to the difference in earthworm abundance and composition between plantation and natural secondary forests. Zou and González (1997) suggested that changes in chemistry of litter biomass, rather than soil water content and soil pH, altered earthworm density and diversity along the successional gradient. Our field mesocosm experiment did not support these contentions, as the survivorship and growth rates (re-colonization ability) of the native earthworm, *Estherella* spp., were not significantly different among the sites that represented different habitat characteristics. Based on these data, it is fair to say that the re-colonization ability of *Estherella* spp. in the pasture was as good as in the young and mature forests, at least in the short-term. The soil environment in the pasture (lower litterfall input, higher soil water content and higher pH) did not prevent the survival of *Estherella* spp., even though the field mesocosm method (PVC tubes) caused artificial effects on soil properties such as increased soil water content and reduced microbial biomass C and N in the Core samples of all habitats. Interestingly, the reduction of food resource (litter input) in the pasture did not retard the survivorship and growth of the native *Estherella* spp.. Previous research has suggested that plant fine roots and soil microbial biomass might provide additional food resources for earthworms (Lee 1985, González et al. 1996). Wright (1972)'s laboratory study found that *Lumbricus terrestris* used bacteria as food resource, and he suggested that bacteria could be important in its diet. Fraser et al. (2003) speculated that the reduction of fine root mass observed in one treatment of a laboratory experiment with the presence of the earthworm (*Aporrectodea caliginosa*) partly resulted from the direct feeding by the earthworm. At our pasture site, higher soil microbial biomass C and N, and more plant fine roots (Sánchez-de León et al. 2003) indirectly supports this possibility. The growth and survivorship of *Estherella* spp. in the pasture may be due to the utilization of soil microbial and root biomass as alternative food resource instead of surface litter. More field and controlled experiments are needed to specify the allocation and importance of different food resources (litter, microorganisms, and fine root) in earthworm's diets.

The mechanisms controlling the recovery of native earthworm populations also include biotic factors, particularly interactions with exotic earthworms (González et al. 1996, Sánchez-de León et al. 2003, Sánchez-de León and Zou 2004). The successful survival of *Estherella* spp. in our field mesocosm experiment may be due to the lack of competition pressure from exotic earthworm species, *P. corethrurus*. Hendrix et al. (1999) suggested a potential inter-specific competitive relationship between *Estherella* sp. and *P. corethrurus* at a tabonuco forest by observing the completely overlapping ^{15}N enrichment of these two species. Winsome et al. (2006) conducted field and laboratory experiments in a California grassland, and found that native earthworms (*Argilophilus marmoratus*) performed well both in the nutrient-amended and unamended habitats, but performed poorly when co-existing with an exotic earthworm (*Aporrectodea trapezoides*). They further concluded that the exclusion of native *A. marmoratus* from the pastures might be due to the inter-specific competition with exotic *A. trapezoides*, rather than changes in soil properties (nutrient amendment) or physical disturbance. In Puerto Rico, Zou and González (1997) found that the densities of *P. corethrurus* were as high as 831 and 403 individuals/m^2 (at 25 cm depth of soil) in a pasture and grass-vine-fern site, respectively, whereas less than 141 individuals/m^2 were collected in the shrub-small tree and forest sites of Luquillo Mountains. Sánchez-de León et al. (2003) documented a similar gradient of *P. corethrurus* densities along with successional stages in Cayey Mountains, decreasing in the order 244.4, 151.1, and 52.4 individuals/m^2 (at 25 cm depth of soil) in the pasture, young and mature forests, respectively. This density trend implies potentially stronger competitive pressure from *P. corethrurus* to native earthworm species in the pastures. Competitive exclusion by exotic *P. corethrurus* may result in relatively low abundance of the native earthworm species (0.9 - 14.2 individuals/m^2 in Sánchez-de León et al. 2003) in the mature forests and/or the complete disappearance of the native earthworm communities in the pasture sites.

Although *Estherella* spp. populations performed (survivorship and growth rates) equally well in all three habitats in this study, we found that their survivorship decreased during the first six months of the experiment. It is unlikely that the dry season of the year (from January to April) resulted in the loss of individuals (Brown et al. 1983), because soil water content in the core samples remained higher than in the field. This decline in the population may be partly due to the cost of adapting to a new and/or restrained environment in the soil mesocosms.

Earthworms can have significant effects on both macro- and micro-aggregate formation and stability through their casting and burrowing activities (Marinissen and Dexter 1990, Ketterings et al. 1997, Jongmans et al. 2001, Fraser et al. 2003, Bossuyt et al. 2004). The aging and drying-rewetting cycles of earthworm casts increase the stability and formation of micro-aggregates in the casts and protect soil organic matter from rapid decomposition (Shipitalo and Protz 1988). Fraser et al. (2003) discovered that an endogeic species, *Aporrectodea caliginosa*, increased soil aggregate size (mean weight diameter-millimeter), but had little effect on aggregate stability. Bossuyt et al. (2004) found that the same species (*A. caliginosa*) enhanced the formation of stable micro-aggregates (53-250 μm) through their casting activities. Higher percentages of carbon (^{13}C) storage within large macro-aggregates after only 12 days of incubation suggested that earthworms rapidly incorporated fresh residues into micro-aggregates as the soils passed through their guts. In our study, the observation of fresh casts and burrows within the Core samples indicated high activities of *Estherella* spp. during the experimental period. The casting and burrowing activities of *Estherella* spp. seemed to inhibit the formation of large macro-aggregates (> 2000 μm) at the end of the experiment, compared to soil aggregate data in the field. Besides, the increased formation of larger macro-aggregates (> 2000 μm) in the field soil may have resulted from high densities of *P. corethrurus* distributed in all sites [see above, 52.4 - 244.4 individuals/m^2 at same study area in Sánchez-de León et al. (2003)]. The differential effects on soil aggregation by earthworms imply that the impact of earthworms on soil aggregate distribution is species dependent. For example, endogeic earthworm species, e.g., *A. caliginosa* and *P. corethrurus*, live in the soil and consume organic residues and soil, while epigeic and anecic species feed on surface litter and inhabit litter or upper soil layer (Lee 1985, Edwards 2004). Different ecological groups of earthworms may have differential effects on soil aggregates because of their utilization of different food and space resources. This relative inhibitory effect on soil macro-aggregates by *Estherella* spp., which is in contrast to the facilitation effect by the endogeic *P. corethrurus*, may be due to its identity as an epigeic and anecic species by the dark pigmentation and its inhabitation of top soils (Hendrix et al. 1999, Sánchez-de León et al. 2003, Sánchez-de León and Zou 2004). Our preliminary observation suggests that the influences on soil aggregate distribution and formation by earthworms depend on their feeding behavior and/or the soil environment they inhabit.

Conclusions

In this study, successional status and/or conversion of forest to pasture did not impede the survival of native earthworms (*Estherella* spp.) in disturbed areas (pasture site) in the absence of exotic earthworm species. The lack of re-colonization of native earthworms (*Estherella* spp.) into pastures may be explained by low propagule pressure/introduction opportunities of native earthworms and/or as a consequence of competition by the exotic worm, *P. corethrurus*. We suggest that changes in vegetation and soil properties resulting from habitat disturbance reduce native earthworm populations (*Estherella* spp.), but can not prevent their survival or cause their extinction. Biotic factors, particularly competitive interactions with exotic earthworms, may have significant effects on the populations of native earthworms (*Estherella* spp.). Further research is needed to determine if the competitive relationship with exotic earthworm has additive impacts with habitat disturbance on the native earthworm communities.

Acknowledgements

We are grateful to the staffs at the Sabana Field Research Station and the International Institute of Tropical Forestry (IITF) for their help in various ways. We also thank Dr. David Kissel, the Soil Plant and Water Laboratory and the University of Georgia, for helping us with the soil transportation and sterilization process. This research was supported by National Science Foundation grant number 0236276 to the University of Georgia Research Foundation, Inc.; Graduate Student Research Prize, Center for Biodiversity and Ecosystem Processes, University of Georgia; grant DEB-0218039 from the NSF to the Institute of Tropical Ecosystem Studies, University of Puerto Rico, and the USDA Forest Service, IITF as part of the LTER program in the Luquillo Experimental Forest. We also thank three anonymous reviewers for commenting on an earlier version of this manuscript.

References Cited:

Aide, T. M., J. K. Zimmerman, L. Herrera, and M. Rosario. 1995. Forest recovery in abandoned tropical pastures in Puerto Rico. Forest Ecology and Management 77:77-86.

Bautista-Cruz, A., and R. F. del Castillo. 2005. Soil changes during secondary succession in a tropical montane clouds forest area. Soil Science Society of America Journal 69:906-914.

Beare, M. H., P. F. Hendrix, and D. C. Coleman. 1994. Water-stable aggregates and organic matter fractions in conventional- and no-tillage soils. Soil Science Society of America Journal 58:777-786.

Birdsey, R. A., and R. W. Weaver. 1987. Forest area trends in Puerto Rico. Research Note SO-331, USDA Forest Service, Washington, D. C., USA.

Bossuyt, H., J. Six, and P. F. Hendrix. 2004. Rapid incorporation of carbon from fresh residues into newly formed stable microaggregates within earthworm casts. European Journal of Soil Science 55:393-399.

Brown, S., A. E. Lugo, S. Silander, and L. Liegel. 1983. Research history and opportunities in the Luquillo Experimental Forest. General Technical Report SO-44, USDA Forest Service, Southern Forest Experiment Station, New Orleans, LA.

Buschbacher, R. 1986. Tropical deforestation and pasture development. BioScience 36:22-28.

Chapin III, F. S., P. A. Matson, and H. A. Mooney. 2002. Principles of Terrestrial Ecosystem Ecology. Springer-Verlag, New York. 472 pp.

Edwards, C. A. 2004. Earthworm Ecology. CRC Press, Boca Raton. 441 pp.

Fragoso, C., G. G. Brown, J. C. Patron, E. Blanchart, P. Lavelle, et al. 1997. Agricultural intensification, soil biodiversity and agroecosystem function in the tropics: the role of earthworms. Applied Soil Ecology 6:17-35.

Fraser, P. M., M. H. Beare, R. C. Butler, T. Harrison-Kirk, and J. E. Piercy. 2003. Interactions between earthworms (*Aporrectodea caliginosa*), plants and crop residues for restoring properties of a degraded arable soil. Pedobiologia 47:870-876.

González, G., C.-Y. Huang, X. Zou, and C. Rodríguez. 2006. Earthworm invasions in the tropics. Biological Invasions 8:47-56.

González, G., X. Zou, and S. Borges. 1996. Earthworm abundance and species composition in abandoned tropical croplands: comparison of tree plantations and secondary forests. Pedobiologia 40:385-391.

Guariguata, M. R., and R. Ostertag. 2001. Neotropical secondary forest succession: changes in structural and functional characteristics. Forest Ecology and Management 148:185-206.

Helmer, E. H. 2004. Forest conservation and land development in Puerto Rico. Landscape Ecology 19: 29-40.

Hendershot, W. H., H. Lalande, and M. Duquette. 1993. Soil reaction and exchangable acidity. *In* M. R. Carter ed. Soil Sampling and Methods of Analysis. pp. 141-146. CRC Press, Boca Raton, FL.

Hendrix, P. F., S. L. Lachnicht, Callaham, M. A., and X. Zou. 1999. Stable isotopic studies of earthworm feeding ecology in tropical ecosystems of Puerto Rico. Rapid Communications in Mass Spectrometry 13:1295-1299.

Jongmans, A. G., M. M. Pulleman, and J. C. Y. Marinissen. 2001. Soil structure and earthworm activity in a marine silt loam under pasture versus arable land. Biology and Fertility of Soils 33:279-285.

Kalisz, P. J. 1993. Native and exotic earthworms in deciduous forest soils of Eastern North America. Biological Pollution: the control and impact of invasive exotic species. Proceedings of a Symposium held at the University Place Conference

Center, Indiana University-Purdue University, Indianapolis, October 25 & 26, 1991:93-100.

Kalisz, P. J., and D. B. Dotson. 1989. Land-use History and the occurrence of exotic earthworms in the mountains of Eastern Kentucky. American Midland Naturalist 122:288-297.

Kalisz, P. J., and H. B. Wood. 1995. Native and exotic earthworms in wildland ecosystems. *In* P. F. Hendrix ed. Earthworm Ecology and Biogeography in North America. pp. 117-126. Lewis Publishers, Boca Raton, FL.

Ketterings, Q. M., J. M. Blair, and J. C. Y. Marinissen. 1997. Effects of earthworms on soil aggregate stability and carbon and nitrogen storage in a legume cover crop agroecosystem. Soil Biology and Biochemistry 29:401-408.

Lavelle, P., L. Brussaard, and P. F. Hendrix, editors. 1999. Earthworm Management in Tropical Agroecosystems. CABI Publishing, New York. 300 pp.

Lavelle, P., and E. Lapied. 2003. Endangered earthworms of Amazonia: an homage to Gilberto Righi. Pedobiologia 47:419-417.

Lee, K. E. 1985. Earthworms, Their Ecology and Relationships with Soils and Land Use. Academic Press, New York. 411 pp.

Marinissen, J. C. Y., and A. R. Dexter. 1990. Mechanisms of stabilization of earthworm casts and artificial casts. Biology and Fertility of Soils 9:163-167.

Pascarella, J. B., T. M. Aide, M. I. Serrano, and J. K. Zimmerman. 2000. Land-use history and forest regeneration in the Cayey Mountains, Puerto Rico. Ecosystems 3:217-228.

Pregitzer, K. S., and E. S. Euskirchen. 2004. Carbon cycling and storage in world forest: biome patterns related to forest age. Global Change Biology 10:2052-2077.

Reynolds, J. 1994. Earthworms of the world. Global Biodiversity 4:11-16.

Sánchez-de León, Y., and X. Zou. 2004. Plant influences on native and exotic earthworms during secondary succession in old tropical pastures. Pedobiologia 48:215-226.

Sánchez-de León, Y., X. Zou, S. Borges, and H. Ruan. 2003. Recovery of native earthworms in abandoned tropical pastures. Conservation Biology 17:999-1006.

Scatena, F. N. 1989. An introduction to the physiography and history of the Bisley experimental watersheds in the Luquillo Mountains of Puerto Rico. General Technical Report SO-72, USDA Forest Service, Southern Forest Experiment Station, USA.

Shipitalo, M. J., and R. Protz. 1988. Factors influencing the dispersibility of clay in worm casts. Soil Science Society of America Journal 52:764-769.

Winsome, T., L. Epstein, P. F. Hendrix, and W. R. Horwath. 2006. Competitive interactions between native and exotic earthworm species as influenced by habitat quality in a California grassland. Applied Soil Ecology 32:38-53.

Wright, M. A. 1972. Factors governing ingestion by the earthworm *Lumbricus terrestris* with special reference to apple leaves. Annuals of Applied Biology 70:175-188.

Zimmerman, J. K., T. M. Aide, M. Rosario, M. Serrano, and L. Herrera. 1995. Effects of land management and a recent hurricane on forest structure and composition in the Luquillo Experimental Forest, Puerto Rico. Forest Ecology and Management 77:65-76.

Zou, X., and G. González. 1997. Changes in earthworm density and community structure during secondary succession in abandoned tropical pastures. Soil Biology and Biochemistry 29:627-629.

Table 2.1. Habitat characteristics, including litterfall, soil properties, and microbial biomass C and N, at three study sites representing different degrees of disturbance in the Cayey Mountains of Puerto Rico. Data are mean ± standard error.

Variables	Sites		
	Pasture	Young forest	Mature forest
Litterfall			
Litterfall (g/m^{2} per month)	7.56 ± 2.04^{a}	59.33 ± 4.87^{b}	57.45 ± 5.55^{b}
Carbon (%)	43.44 ± 1.56^{a}	44.48 ± 0.74^{a}	43.40 ± 0.50^{a}
Nitrogen (%)	1.06 ± 0.04^{ab}	1.20 ± 0.04^{b}	1.02 ± 0.05^{a}
C/N	41.13 ± 1.15^{ab}	37.78 ± 1.15^{a}	44.34 ± 1.90^{b}
Soil			
pH			
0-5 cm	4.76 ± 0.02^{b}	4.64 ± 0.02^{b}	4.28 ± 0.06^{a}
5-10 cm	4.69 ± 0.07^{b}	4.61 ± 0.04^{b}	4.30 ± 0.04^{a}
Water content (%)			
0-5 cm	94.7 ± 5.67^{b}	37.6 ± 2.52^{a}	47.5 ± 3.10^{a}
5-10 cm	90.2 ± 5.04^{b}	34.9 ± 2.18^{a}	42.7 ± 2.26^{a}
Carbon (%)			
0-5 cm	4.49 ± 0.02^{a}	4.76 ± 0.29^{a}	5.12 ± 0.29^{a}
5-10 cm	3.58 ± 0.21^{a}	3.84 ± 0.30^{a}	3.26 ± 0.15^{a}
Nitrogen (%)			
0-5 cm	0.38 ± 0.02^{a}	0.37 ± 0.02^{a}	0.34 ± 0.01^{a}
5-10 cm	0.32 ± 0.02^{b}	0.29 ± 0.01^{b}	0.24 ± 0.01^{a}
C/N			
0-5 cm	11.9 ± 0.25^{a}	12.8 ± 0.26^{a}	15.2 ± 0.35^{b}
5-10 cm	11.3 ± 0.15^{a}	12.9 ± 0.59^{b}	13.7 ± 0.23^{b}
Microbial biomass			
Carbon (ug/g dry soil)			
0-5 cm	1834.8 ± 110^{a}	1626.3 ± 146^{a}	1450.0 ± 135^{a}
5-10 cm	1551.6 ± 134^{a}	1298.8 ± 100^{a}	1152.1 ± 152^{a}
Nitrogen (ug/g dry soil)			
0-5 cm	201.4 ±5.88^{b}	162.3 ± 7.82^{a}	158.9 ± 4.37^{a}
5-10 cm	167.1 ± 12.10^{b}	133.1 ± 6.78^{a}	125.2 ± 5.56^{a}
C/N			
0-5 cm	9.1 ± 0.43^{a}	9.8 ± 0.61^{a}	9.1 ± 0.77^{a}
5-10 cm	9.6 ± 0.86^{a}	10.0 ± 0.79^{a}	9.0 ± 1.01^{a}

Note: Data on soil properties and microbial biomass were collected in October (2004), January, March, and May, 2005, while litterfall data were from January, March, and May, 2005. Common letters indicate no significant difference between habitats [Tukey (HSD) multiple comparison method; α = 0.05].

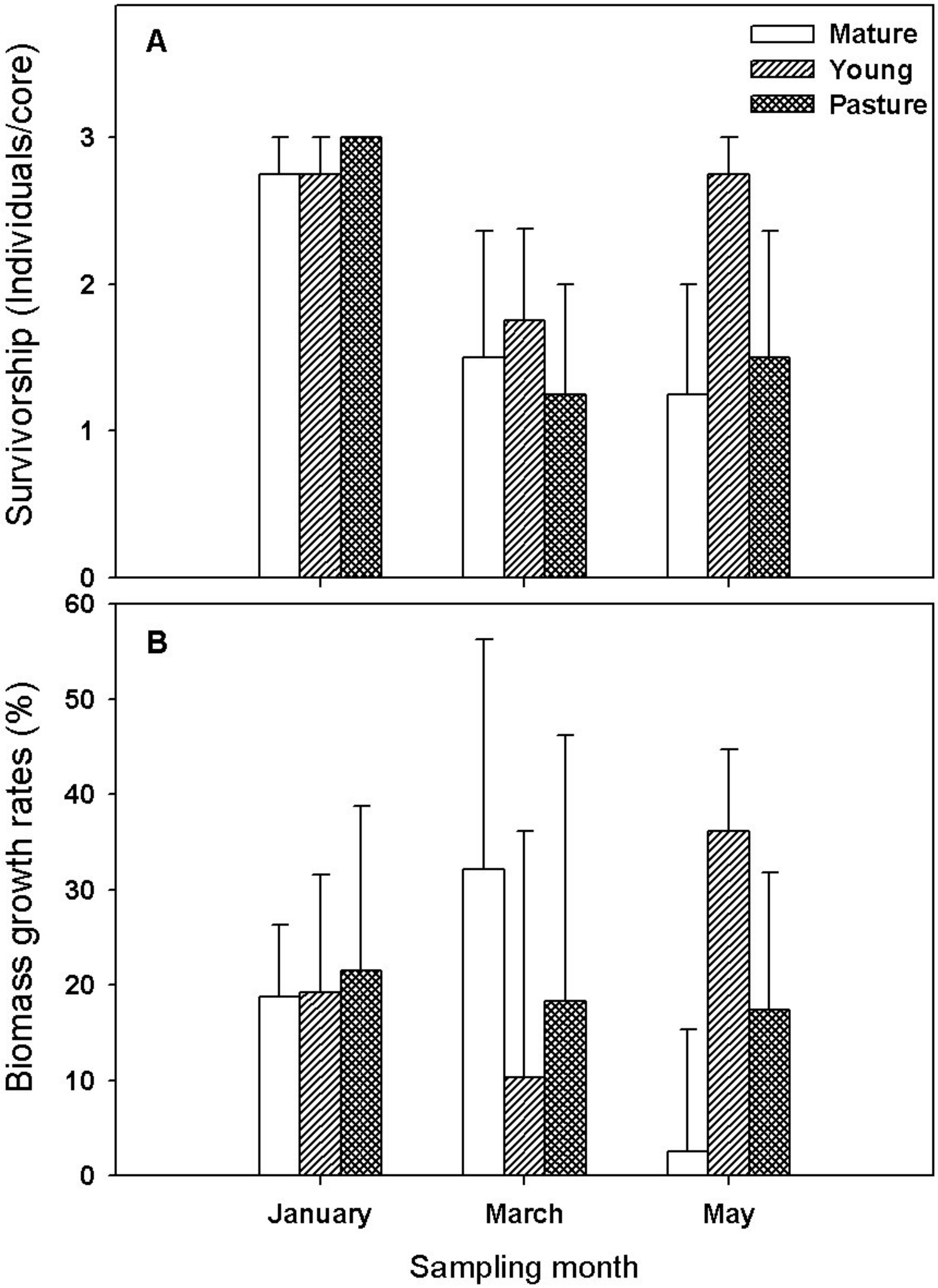

Figure 2.1. Population dynamics of native earthworms, *Estherella* spp., during the experimental period (January, March, and May, 2005). A) Survivorship (mean ± S.E.) and B) percentage of biomass growth rate (mean ± S.E.) of native earthworms, *Estherella* spp., in three study sites representing different degrees of habitat disturbance in the Cayey Mountains of Puerto Rico.

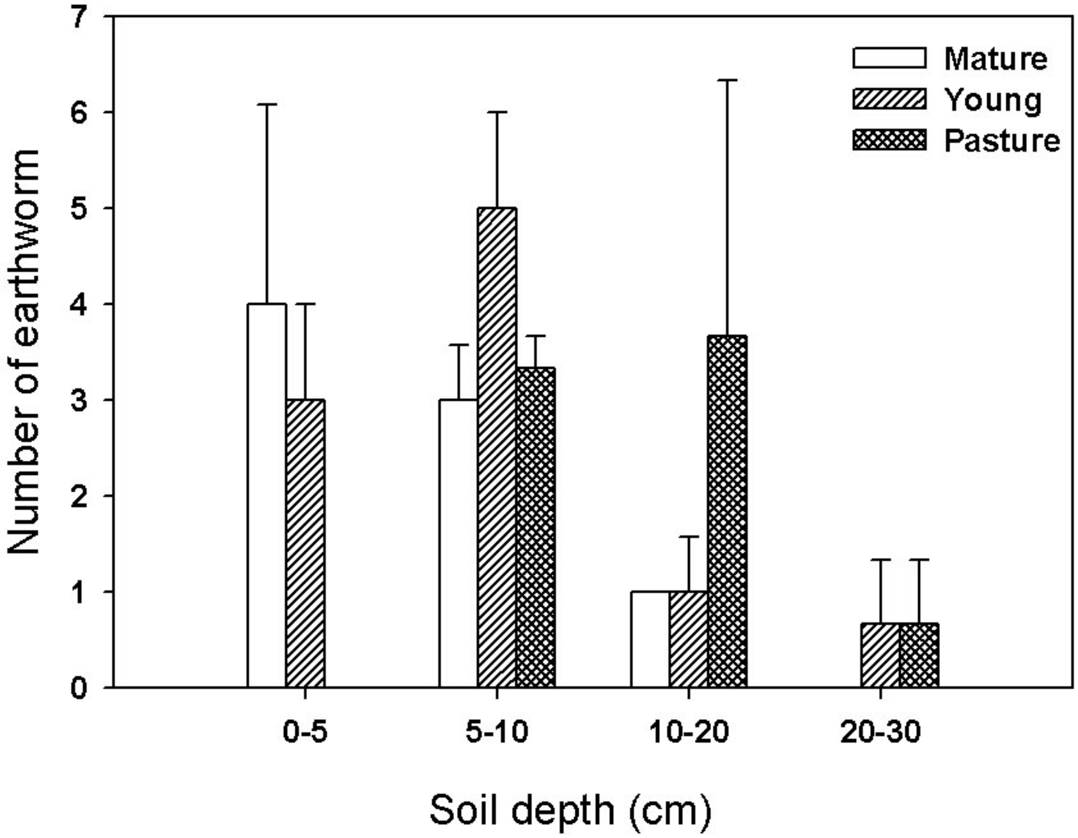

Figure 2.2. Vertical distribution (mean ± S.E.) of native earthworms *Estherella* spp., in the top 30 cm of the soil at three study sites representing different degrees of habitat disturbance in the Cayey Mountains of Puerto Rico.

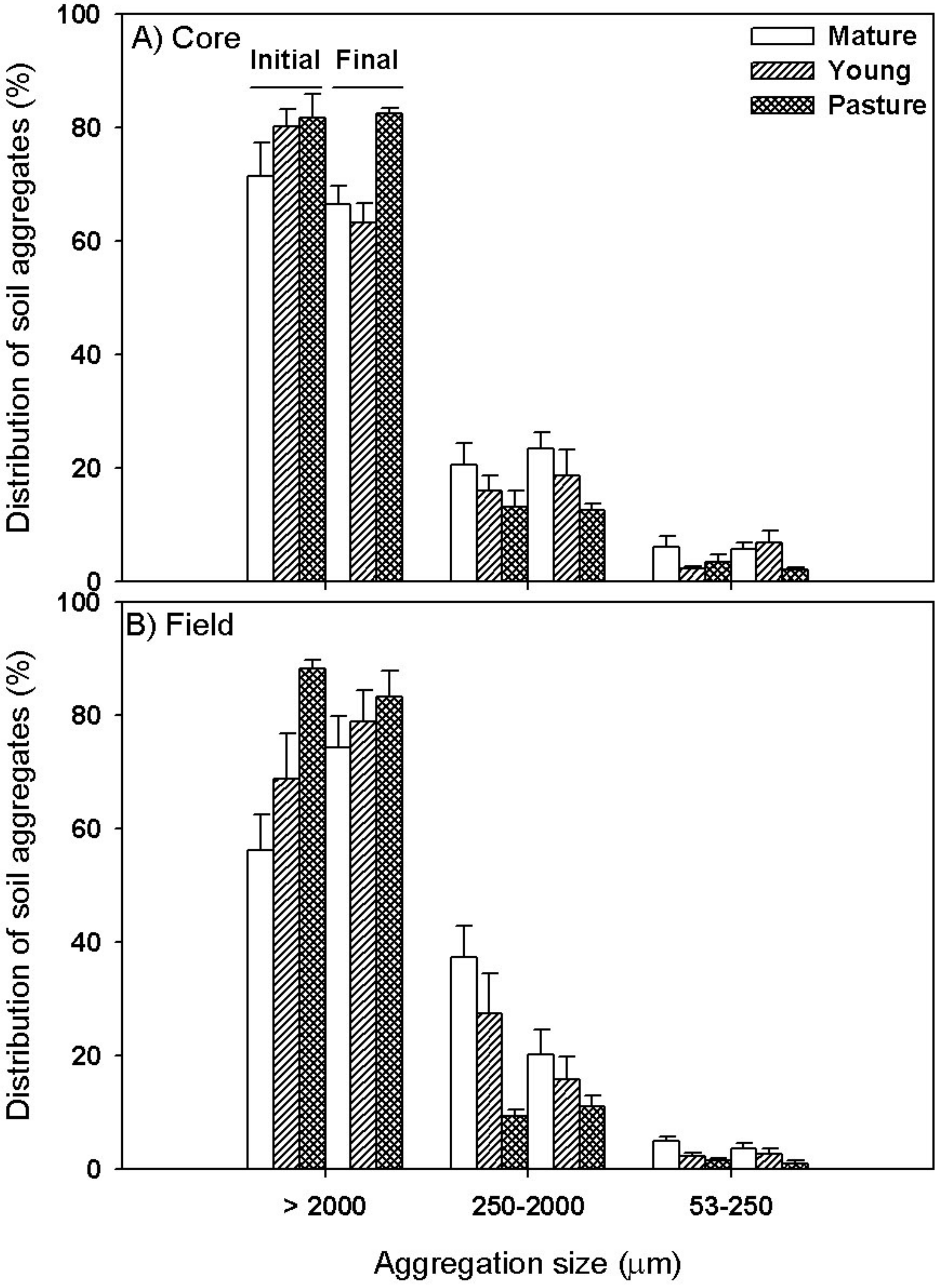

Figure 2.3. Effects of the native earthworm, *Estherella* spp., on soil aggregate distribution (mean ± S.E.) in A) Core with *Estherella* spp. and B) field soil samples at three study sites representing different degrees of habitat disturbance in the Cayey Mountains of Puerto Rico. Initial and final data were collected on October, 2004 and May, 2005, respectively.

CHAPTER THREE

INTERACTIONS BETWEEN NATIVE AND INVASIVE EARTHWORMS AND THEIR IMPACTS ON NUTRIENT DYNAMICS IN PUERTO RICAN SOILS

Introduction

Peregrine earthworms have spread all over the world along with the advance of human transportation and commerce in the past centuries. The impacts of peregrine earthworms are variable depending on their species characteristics, invasion histories, and invaded habitat characteristics (abiotic and biotic). For example, Pleistocene glaciations are thought to have eliminated all native earthworm species, if they had ever existed, in most temperate zones of northern continents (Tiunov et al. 2006). Non-native earthworms have been introduced into these worm-free temperate forests over past several decades; European lumbricids are main invasive earthworms colonizing these areas. The successful invasions of lumbricids might be due to not only the similar climate regime from where they originated but also food availability and the lack of local biotic resistance (especially detritivores) in worm-free areas. In these temperate forests, invasive earthworms have altered forest floor structure, soil properties, plant communities, and nutrient cycling, especially in North America (Groffman et al. 2004, Hale et al. 2005, Frelich et al. 2006). Earthworm invasions often relate to human activities, therefore exotic earthworms are found mostly in disturbed areas, such as human inhabitation and agricultural lands, with exclusion of native earthworms. In this case, Kalisz and Wood (1995) suggested the mechanisms for earthworm invasions as follows: 1) severe habitat disturbance; 2) reduction or elimination of native earthworms; 3) introduction of invasive earthworms; 4) occupation of released niches by invasive earthworm species.

Recently, invasive earthworms have been found to coexist with native earthworm populations in some remote and non-fragmented forests (Kalisz 1993, Kalisz and Wood 1995, Callaham et al. 2003). This has brought attentions of soil ecologists to the potential interaction between native and exotic earthworms. Field observations and experiments suggested that the decline of native earthworms might result from competitive relationships with invasive earthworms in the invaded areas (Lachnicht et al. 2002, Lavelle and Lapied 2003, Winsome et al. 2006).

Whether the impact of exotic earthworms is negative, neutral, or beneficial to native earthworm populations is still ambiguous. Potential native-exotic earthworm interactions are much more complicated than we might expect, because several factors are involved. First, the relationship between native and exotic earthworm species may vary depending on feeding strategies of earthworm species. Earthworms can use leaf litter, soil organic matter, and soil microbial biomass as energy sources. Generally, earthworms are divided into three ecological groups, epigeic, endogeic, and anecic, based on their utilizations on space and food resources (Bouché 1977). Epigeic earthworms mainly consume leaf litter (and microbial populations colonizing on it) and inhabit the litter layer; while endogeic earthworms occupy mineral soils and use soil organic matter as their main food resources. Anecic earthworms utilize mainly leaf litter but with the ability to build burrows deep in the soil. Different ecological groups of earthworms represent different feeding behaviors and life characteristics. When invasive earthworms invade into new areas inhabited by native earthworms, they may either occupy vacant or unused resources (niche) from native earthworms and other detritivores or directly compete with native earthworms for available resources. Competitive exclusion is commonly observed and expected between species using the same resources (niche over-lapping) in ecosystems. In the case of invasive earthworms, extinction of native earthworms may occur because of intense competition from aggressive invasive earthworms as they invade. Lavelle and Lapied (2003) had found that native earthworm populations were disappearing in some Amazonian areas where invasive earthworms had invaded. On the other hand, facilitation may possibly occur when some earthworms make the resources available for the others to use. Both competitive and facilitative interactions with exotic earthworms can affect native earthworm populations, and further alter local earthworm community structure. The native-exotic relationship can be evaluated and manipulated based on the niche characteristics of earthworm species. Some studies have investigated inter-specific interactions between non-native earthworms (Abbott 1980) and between native and exotic earthworms (Lachnicht et al. 2002, Winsome et al. 2006). Abbott (1980) concluded that competitive relationships between *Eisenia foetida* and *Microscolex dubius* might be due to toxic interference or different digestive enzymes. Winsome et al. (2006) found that competitive exclusion from invasive *Aporrectodea trapeziodes* could be the explanation for the inability of native *Argilophilus marmoratus* to re-colonize in pastures. However, there is no study manipulating direct comparisons of similar or dissimilar niches of earthworm species. Also, this native-exotic interaction could be affected by ecosystem attributes, such as resource availability. Resource in term of quality and quantity may influence the potential outcomes. Research results from Winsome et al.

(2006) showed opposite consequences of native and exotic relationships in comparing rich- and poor-resource ecosystems. In resource-rich habitats having exotic *A. trapezoides*, the competitive interaction could prevent native *Ar. marmoratus* from colonization; while the competition from native earthworms could impede exotic *A. trapezoides* from expanding its range into less productive grasslands. Beyond that, flexible behaviors of earthworms also need to be considered in evaluating this native-exotic interaction. When faced with limited resources in the soils, flexibility of feeding behaviors of either native or exotic earthworms could become decisive to the final consequences in the field. Some earthworms, especially peregrine species, have been reported to have superior ability for utilizing different resources. For example, *P. corethrurus* has been reported to have high assimilation efficiency on a variety of food resources (Lavelle et al. 1987). Lachnicht et al. (2002) found that exotic *P. corethrurus* in Puerto Rico used different resources when incubated with, as compared to without, native *Estherella* sp.. Therefore, the interaction between native and exotic earthworm species is basically species- and ecosystem-dependent. More researches considering variable earthworm combinations, resource availability, and earthworm flexible feeding strategies are urgently needed.

Earthworms may also have differential effects on soil nutrient cycling through different feeding behaviors and life characteristics. Epigeic earthworms may have more significant influences on soil nutrient dynamics. Their comminution and digestion of litter substrate can not only determine the amount of nutrient input (mostly carbon and nitrogen) but also the distribution of microbial populations, in addition to soil nutrient cycling. On the other hand, endogeic/anecic earthworms can have effects on soil carbon dynamics through facilitating macro- and micro- aggregate formation (Bossuyt et al. 2004, Mummey et al. 2006). They incorporate fresh C into macro-aggregates and protect C within micro-aggregates from microbial decomposition. Also, the excretion of mucus from earthworm guts alters the microbial population in the casts (Mummey et al. 2006). The consumption by earthworms of microbial populations can inhibit microbial activities and microbial biomass, while earthworm casts and linings of burrows enhance microbial activities (Edwards 2004). Even the actions from both epigeic and endogeic/anecic earthworms can potentially affect soil nutrient dynamics differently; experimental comparison between epigeic and endogeic/anecic earthworms and the native-exotic interaction is still poorly studied. Hence, there is a need to recognize and emphasize this difference.

In Puerto Rico, exotic *P. corethrurus* is distributed across different types of habitats, while native earthworms are only found in less disturbed mature forests (González et al. 1996, Sánchez-de León et al. 2003). *P. corethrurus* had shown a pattern of decreasing densities (from 273.7 to 66.6 ind./m^2) along a chronosequence of regenerated forests in Puerto Rico. Habitat disturbance and competitive interactions are hypothesized as two main mechanisms to impede re-colonization of native earthworms in disturbed areas, i.e. pastures (González et al. 1999, Sánchez-de León et al. 2003). Huang et al. (2006) re-introduced native *Estherella* spp. into a chronsequence of successional forests to see the impacts of habitat characteristics on native earthworm population. They found that the survivorship and growth rate of *Estherella* spp. in pastures were as good as in secondary and mature forests with different litter resources and varied soil characteristics among these field sites. This indicated that *Estherella* population has the ability to recolonize in disturbed pastures in the absence of exotic earthworms. Therefore, this leads to the assumption that competitive relationships between exotic *P. corethrurus* and native earthworms might be the main reason for the absence of native earthworms in the pastures and for the distribution patterns of *P. corethrurus* (Huang et al. 2006).

In this study, a full-factorial experiment was designed to investigate the relationship between native and exotic earthworms, in particular earthworm ecological groups and resource availability, in Puerto Rican forest soils. My hypotheses are: 1) Intensive competitive relationships will be observed in the treatments with niche-overlapping earthworm combination; 2) The competitive interactions will be stronger when food resources are limited.

Methods and Materials

Experimental materials

In this study, the experiment was conducted in a laboratory at Sabana field station in Luquillo, Puerto Rico. PVC pipe tubes (11 cm in diameter) were cut into 20-cm lengths and the bottoms sealed with 1-mm mesh window screen. Experimental soil was collected from the forests located at Bisley watershed area and separated into three different depths, 0-5, 5-10, and 10-15 cm deep. Bisley experimental watershed is located northeast of the Luquillo Mountains. Vegetation at the site includes primary and secondary tabonuco forests (*Dacryodes excelsa*) (Scatena, 1989). The soils are clayey and well weathered Ultisols. All soils were air-dried for 48 hrs then sieved through a 5-mm mesh size sieve to exclude plant roots, rocks and earthworms.

Litter preparation

Seedlings of *Tabebuia heterophylla*, a dominant plant species in Puerto Rico, were incubated in the green house. For the labeling procedures, *Tabebuia* seedlings were incubated in a growth chamber with pulse injection of 99 atom % $^{13}CO_2$ during daytime; *Tabebuia* seedlings incorporated ^{13}C into leaf tissue through photosynthesis cycles. ^{13}C-labeled *Tabebuia* leaves ($\delta^{13}C$ varied from 385 to 804 ‰) were collected after labeling procedures and cut into ca. 1 cm^2 piece. For ^{15}N-labeled material, the dominant grass species, *Andropogon glomeratus*, were planted in each soil mesocosm. During the experiment, ^{15}N-urea (see ”Experiment design”) was applied to grass leaves to label grass roots and root-derived substrates (the rhizosphere) with ^{15}N (Schmidt and Scrimgeour 2001).

Three earthworm species from Puerto Rico were chosen for this experiment. Two native species, *Estherella* spp. and *Onychochaeta borincana*, were collected from Bisley experimental forest and a riparian forest in Almirante Norte, respectively, in Puerto Rico. *Estherella* spp. is classified as epigeic by its dark pigmentation on the back and its occupation of litter and upper soil layers. *O. borincana* is believed to be endogeic because of its pale coloration (living in the deep soil). One invasive earthworm species, *Pontoscolex corethrurus*, was chosen to compare with native earthworms. *P. corethrurus* is the dominant peregrine earthworm distributed all around Puerto Rico and its density can be as high as 244.4 /m^2 in the top 10 cm of pasture soil (Sánchez-de León et al. 2003). *P. corethrurus* is classified as epi-endogeic based on previous researches (Hendrix et al. 1999, Lachnicht et al. 2002), which makes it a good candidate for niche comparison and feeding behavior observations in this study. *P. corethrurus* was collected from the pasture sites nearby Sabana Field Station. Gut contents of all earthworms were voided for

24 hours before introduction into soil mesocosms. Their fresh biomass was recorded before the introduction and used as the initial biomass data.

Experimental design

Earthworm niche experiment

In November 2006, soil mesocosms were set up with 15 cm deep soils collected from the field. Three *A. glomeratus* grass plants (ca. 8 cm tall) were transplanted into each soil mesocosm (except control treatments) and the grass leaves were brushed with 2 atom % ^{15}N-urea solution (following Schmidt and Scrimgeour 2001) every day during the experiment. ^{13}C-labeled *Tabebuia* litter (3.7 grams dry weight, calculated based on field litterfall and forest floor data, 912.5 g litter m^{-2} year^{-1} and 335 g litter m^{-2}; respectively)(Zou et al. 1995) was applied to the soil surface of each soil mesocosm (except control treatments). The purpose of applying both litter and grass roots in the soil mesocosms was to provide two different types of resources (detritusphere and rhizosphere, respectively) for earthworms. Based on the ^{13}C and ^{15}N enrichment in earthworm tissue at the end of experiment, it would help to trace earthworm food utilization. Each soil mesocosm was watered with 35 mL of water every day (calculated and modified from field rainfall data: average annual precipitation 3720 mm; Scatena 1989) to maintain soil moisture. The experiment was designed as a full-factorial experiment with 1) control treatments; 2) single earthworm species; 3) two earthworm species with similar or dissimilar niches; 4) three earthworm species combination. Control treatments (earthworm-free) included soil mesocosms with soil only (S; no litter and grass), *Tabebuia* litter only (L), *A. glomeratus* grass only (G), and both litter and grass (Control). For earthworm treatments, earthworms were introduced into the rest of soil mesocosms (with both litter and grass) according to assigned experimental treatments. Earthworm treatments included: single species treatments- *O. borincana* only (O), *Estherella* spp. only (E), and *P. corethrurus* only (P); two species treatments- *Estherella* and *P. corethrurus* (E+P), *Estherella* and *O. borincana* (E+O), and *O. borincana* and *P. corethrurus* (O+P); three species: *Estherella* and *P. corethrurus* and *O. borincana* (E+P+O). Four soil mesocoms were assigned to each control and earthworm treatments as experimental replicates. Due to size variation within individual earthworms and between earthworm species, total earthworm biomass was considered for earthworm inoculation instead of earthworm number. Average fresh weight of *Estherella* spp. was 5.3 ± 0.5 g mesocosm^{-1} for E treatment; 4.2 ± 0.6 g mesocosm^{-1} for E+O; 3.2 ± 0.4 g mesocosm^{-1} for E+P; and 2.2 ± 0.2 g mesocosm^{-1} for E+O+P. For *O. borincana*, average fresh weight introduced in each mesocosm was 4.9 ± 0.6 g mesocosm^{-1} for O treatment; 3.6 ± 0.5 g mesocosm^{-1} for E+O; 2.7 ± 0.6 g mesocosm^{-1} for O+P; and 2.2 ± 0.4 g mesocosm^{-1} for E+O+P. Average fresh weight for *P. corethrurus* was 2.0 ± 0.3 g mesocosm^{-1} for P treatment; 1.5 ± 0.3 g mesocosm^{-1} for E+P; 1.4 ± 0.1 g mesocosm^{-1} for O+P; and 1.2 ± 0.1 g mesocosm^{-1} for E+O+P. The earthworms were introduced into the soil mesocosms following the order of *O. borincana*, *Estherella* spp., and *P. corethrurus*.

Food treatment experiment

Another set of soil mesocosms were assigned to food treatment experiment. The installation of soil mesocosms were completely the same with those for earthworm niche experiment except litter amount. Three level of ^{13}C-labeled *Tabebuia* litter (half, regular and double amount) was adopted for this food experiment. The amount of half and double litter treatments were calculated based on the regular litter amount (3.7 grams dry weight; see above). This food experiment were only applied on two earthworm treatments: *O. borincana* and *P. corethrurus* (O+P); three species: *Estherella* sp. and *O. borincana* and *P. corethrurus* (E+O+P) due to the expected stronger competitions among all earthworm treatments (the former: both endogeic earthworms; the latter: three earthworm species). Each treatment had four replicates for this experiment.

Experimental methods

The experiment was run for 22 days. At day 3, 11, and 21 of the experiment, soil carbon dioxide evolution was collected by using the alkali absorption technique (Liu and Zou 2002). At each sampling, a PVC chamber (10 cm tall and 5 cm wide) was inserted 1 cm into the soil surface of each mesocosm. A scintillation vial containing 10 ml of 1 mol/L NaOH solution was placed inside each chamber, then the chamber was sealed with plastic wrap and aluminum foil on the top. Twenty-four hours later, the alkali solution was removed from each chamber and sealed. Five control vials were kept closed during the 24-hr incubation, except opened twice at the beginning and the end of incubation to expose them to ambient air. To each sample was added 2 mL of 1 mol/L $BaCl_2$ in the laboratory. Total CO_2 trapped by alkali solution was determined and calculated by titration with 1 mol/L HCl to reach pH neutral point (phenolphthalein endpoint). $BaCO_3$ precipitation from each sample was air died and packed in tin capsules for ^{13}C analysis. The $\delta^{13}C$ value is calculated based on the measure isotope ratios between the samples and the standard:

$$\delta^{13}C\ (‰) = [(R_{sample} - R_{standard}) / R_{standard}] \times 10^3$$

where $\delta^{13}C$ unit is the parts per thousand and R is the mass ratio of the sample and standard (Coleman and Fry 1991). At the end of the experiment (day 22), soil mesocoms were destructively sampled to collect final data. Soil was separated into three soil depths, 0-5, 5-10, and 10-15 cm. A set of 10 grams soil was weighed and oven-dry at 105 °C for 48 hrs for soil dry weight. Subsamples of soils were ground fineness and weighed into tin capsules (ca. 20 mg) for total soil carbon (C) and nitrogen (N) and isotopes (^{13}C and ^{15}N) by dry combustion on a Carlo Erba NA1500 CN analyzer. The fumigation-extraction method (Jenkinson and Powlson 1976) was used to measure soil microbial biomass. Two sets of 20 g 0-5 cm soils from each sample were weighed as control (un-fumigated) and fumigated samples for soil microbial biomass analysis. Soil samples were extracted with 60 mL of 0.5 mol/L potassium sulfate (K_2SO_4) solution (3:1 solution to soil mass ratio) and the extracts were filtered through Whatman 42 filter paper. Total microbial biomass C and ^{13}C was analyzed from K_2SO_4 extracted samples using OI analytical TIC/TOC analyzer coupled to a Thermo-Finnigan Delta Plus Isotope Ratio Mass Spectrometer (IRMS). Dissolved inorganic N (DIN; NH_4^+-N and NO_3^--N) was analyzed for non-fumigated K_2SO_4 extract by Alpkem nitrogen antoanalyzer. Persulfate digestion method was applied to a subsample of K_2SO_4 extract to obtain microbial nitrogen (Microbial N) data (Cabrera and Beare 1993). Microbial N was calculated from the difference between total persulfate nitrogen from fumigated and non-fumigated samples. Total persulfate nitrogen from fumigated samples was used to determine total dissolved nitrogen (TDN). $\delta^{15}N$ data for each portion (DIN, Microbial N and TDN) was obtained by running the samples through isotope diffusion method (Stark and Hart 1996). For DIN (K_2SO_4)

extracts, KCl was added along with MgO and Devarda's alloy to increase the ionic strength of the solution. For microbial N and TDN (persulfate digests) samples, 10 M NaOH was added to raise pH (>13) of the solution instead. Pairs of glass filter disks (Whatman GF/D) were prepared by baking in a muffle furnace at 500°C for 4 hrs. They were acidified with 35 μL 2M H_2SO_4 and then wrapped with Teflon tape. The Teflon-filter packages were incubated in the solutions for 6 days. After the incubation, the packages were dried with concentrated H_2SO_4 for at least 48 h, then packed in silver capsules (Costech Analytical Technologies, Inc.) for dry combustion on a Carlo Erba NA1500 CN analyzer for total N and ^{15}N data.

Tabebuia litter was carefully picked up and oven-dried at 60 °C for 48 hrs. The litter samples were ground and a subsample of 0.5 g litter was burned at 550 °C for 4 h to get ash-free dry matter (AFDM) data. The data was used to calculate litter decomposition rates.

The number of earthworms that survived during the experimental period was recorded for calculation of earthworm survivorship. All earthworms collected at the end of experiment were put into separate containers for 24 h to void their gut contents. Final fresh weight was gathered after gut-voiding. Earthworms were killed by dipping in boiling water for 3 s. One third of earthworm body (tail part) was cut, the gut removed, and rinsed with deioned water. Earthworm tissue was freeze-dried and ground in the laboratory. Two mg of earthworm tissue was weighed and packed into tin capsule and analyzed by dry combustion on a Carlo Erba NA1500 CN analyzer for ^{13}C and ^{15}N.

Statistical analysis

For the niche experiment, which was completely randomized design, the differences of all responses, including litter remaining mass, earthworm tissue ^{13}C and ^{15}N, soil respiration (C-CO$_2$ and ^{13}C- CO$_2$), microbial biomass C/^{13}C and N/^{15}N, dissolved inorganic nitrogen and total dissolved nitrogen between earthworm treatments were analyzed by GLM procedure in SAS statistical software. For the food experiment, GLM (two-way) was adopted to compare the differences of all collected responses between earthworm treatments and food treatments. If significant, Tukey's method was applied for the comparisons between treatments. The significance level was set as $\alpha=0.05$.

Results

Earthworm niche experiment

Litter decomposition

The main effect of earthworm on litter decomposition was not statistically significant (Fig. 3.1; p= 0.08), but for analysis on single-species effect, litter decomposition was significantly slower in the presence of *Estherella* spp. as compared to the control (p= 0. 04). Among *Estherella* spp. treatments, litter decomposition was slower when *Estherella* spp. coexisted with other earthworm species than *Estherella* spp. only treatment (p= 0. 03).

Earthworm populations

In total, the survivorship of *Onychochaeta borincana* was significantly lower (71.8 ±25.0 %) than the other two earthworm species (*Pontoscolex corethrurus*: 96.9±

8.3 %; *Estherella* spp.: 93.8± 13.0 %) ($p = 0.0003$). For each single species, there was no significant difference in survivorship whether they were cultured alone or with other earthworm species (all $p > 0.05$). This showed that each earthworm species could survive well when incubated alone as well as with other earthworm species. On the other hand, *P. corethrurus* was the only earthworm species that reproduced in this mesocosm experiment, and a total of 8 juveniles of *P. corethrurus* were found during the 22-day experiment. The biomass changes of earthworms were not significantly different between earthworm treatments for each earthworm species (data not shown).

Soil $\delta\,^{13}C$ and ^{15}N enrichment from earthworm treatments (Fig. 3.2 and 3.3; -25.93 and 6.45 ‰, respectively) as compared to natural abundance of soil ^{13}C and ^{15}N data (Soil only; Fig. 3.2 and 3.3; -27.86 and 4.46 ‰, respectively) indicated that the isotopically labeled materials did provide a good indicator of food utilization by earthworms. All three earthworm species showed stronger ^{13}C enrichment in their body tissue as compared to soil ^{13}C background data (Fig. 3.2). Earthworms might incorporate ^{13}C-labled *Tabebuia* litter or its derived resources into their body tissue. For single earthworm species, no significant difference of earthworm ^{13}C signature between treatments with single species or mixed earthworm treatments was found ($p>0.05$). This indicated that each earthworm species kept consistent feeding behavior even when coexisting with other earthworms which may compete for the same food resources. However, food partitioning was observed among these three earthworm species. *Estherella* spp. showed less ^{13}C enrichment as compared to other two species, *O. borincana* and *P. corethrurus* (all $p< 0.01$; Fig. 3.2). The latter two might have utilized more litter (or its derived resources) than *Estherella* spp. did. In addition, *P. corethrurus* juveniles did not show significant ^{13}C enrichment as compared to soil ^{13}C background (Fig. 3.2).

Consistent feeding behaviors of each earthworm species on rhizosphere resources were also observed based on their body tissue ^{15}N enrichment patterns. Each earthworm species showed similar ^{15}N enrichment whether cultured alone or with other earthworms (Fig. 3.3; $p>0.05$). However, *O. borincana* showed much stronger ^{15}N enrichment than *Estherella* spp., *P. corethrurus* and background soil ($p< 0.05$; Fig. 3.3). This indicated that endogeic *O. borincana* might have utilized plant roots and rhizodeposition as food resources. The hatched *P. corethrurus* juveniles showed particular ^{15}N enrichment in their tissue after the 22-day experiment (Fig. 3.3).

Microbial biomass carbon and soil respiration

Soil microbial biomass carbon from earthworm treatments was averaged from 340.1 to 532.1 ug C g^{-1} soil and was slightly lower as compared to the control treatment (599.6 ug C g^{-1} soil). However, there was no significant difference between earthworm and control treatments (Fig. 3.4; $p=0.9$). The δ ^{13}C signature in microbial biomass was similar among control (no earthworm treatment; -30.6± 0.8 ‰) and earthworm treatments (average: -30.1 ± 0.5 ‰) ($p=0.9$).

Soil respiration C-CO_2 at day 3, day 11, and day 22 were not significantly different between the control and earthworm treatments ($p> 0.05$; data not shown). Cumulative soil respiration C-CO_2 curves showed a trend of increasing soil carbon emission in the *O. borincana* treatment and lower soil carbon evolved from the *P. corethrurus* treatment. However, there was no statistically significant difference due to wide variance between samples ($p > 0.05$; Fig. 3.5). For ^{13}C, soil respiration ($^{13}C-CO_2$) showed no significant difference between the control and earthworm treatments at day 3 and day 11 samples. However, the control and *P. corethrurus* treatments had higher $^{13}C-CO_2$ evolved than the others at day 21 ($p=0.01$; Table 3.1).

Microbial biomass nitrogen and soil nitrogen dynamics

Earthworms had significant impacts on soil dissolved inorganic nitrogen (DIN) ($p=0.03$; Fig. 3.6A), but not on microbial biomass nitrogen ($p > 0.05$; Fig. 3.6B) or soil total dissolved nitrogen (TDN) ($p > 0.05$; Fig. 6C). There was higher DIN in the *Estherella* spp. and *O. borincana* treatments as compared to the control and the other earthworm treatments (Fig. 6a). The $\delta\ ^{15}N$ value in DIN was observed to average higher in the treatments with *Estherella* spp. and *O. borincana*, however, they were not significantly different between control and earthworm treatments, neither for microbial nitrogen or TDN (Table 3.2). Interestingly, both *Estherella* spp. and *O. borincana* might facilitate rhizosphere microbial population activity by using rhizosphere resources (root exudates and plant roots), and therefore enhance soil nitrogen mineralization.

Food manipulation experiment

Litter decomposition

Litter decomposition rates were not significantly different between earthworm treatments (with *P. corethrurus* and *O. borincana* –O+P or all three earthworm species-E+O+P), which represent different degrees of competition ($p=0.27$; Fig. 3.7). However, litter decomposition rates were higher in treatments with double amounts of litter

($p=0.03$; Fig. 3.7). No interaction between earthworm and food treatments was found ($p> 0.05$).

Earthworm populations

Earthworm ^{13}C enrichment data showed similar patterns as observed from the earthworm niche experiment. All three earthworm species showed stronger ^{13}C enrichment in their tissue as compared to soil ^{13}C background (Fig. 3.8A and 3.8B). *P. corethrurus* and *O. borincana* had similar ^{13}C enrichment in their body tissue when cultured together even under varied amounts of litter treatments (all $p> 0.05$; Fig. 3.8A). With the presence of all three earthworm species, *O. borincana* showed highest ^{13}C enrichment, followed by *P. corethrurus* and *Estherella* spp., in particular in double litter treatment ($p< 0.0001$; Fig. 3.8B). The pattern was especially significantly in double-litter treatment ($p=0.02$; Fig. 3.8B). *O. borincana* appeared to use more ^{13}C-labeld litter or its derived resources when there was more litter available. *Estherella* spp. showed less ^{13}C enrichment in this case (Fig. 3.8B). *Pontoscolex corethrurus* juveniles hatched during the 22-day experiment showed strong ^{13}C enrichment only in double-litter treatment with *O. borincana* and *P. corethrurus* (Fig. 3.8A).

On the other hand, there was no significant difference in ^{15}N enrichment of earthworm tissue between food treatments (Fig. 3.9A and 3.9B; $p>0.05$). However, *O. borincana* showed significantly higher ^{15}N enrichment than *Estherella* spp., *P. corethrurus* and the soil ($p< 0.05$; Fig. 3.9A and 3.9B). The hatched *P. corethrurus* juveniles showed slight ^{15}N enrichment in their tissue after the 22-day experiment (Fig. 3.9A and 3.9B).

Microbial biomass carbon and soil respiration

Among food treatments, soil microbial biomass carbon averaged from 312.0 to 462.6 ug C g^{-1} soil for E+O+P and from 378.6 to 483.4 ug C g^{-1} soil for O+P earthworm treatment. However, there was no significant difference between earthworm and food treatments (Fig. 3.10; all p> 0.5). No significant difference was found in ^{13}C signature of microbial biomass between earthworm treatments and food treatments ($\delta^{13}C$: mean± S.E.; double: E+O+P= -28.0 ± 2.2, O+P= -32.1 ± 3.8; regular: E+O+P= -29.1 ± 1.2, O+P= -28.8 ± 1.9; half: E+O+P= -32.0 ± 0.8, O+P= -32.0 ± 1.9) (all p>0.5).

Soil respiration C-CO_2 was not different between different sampling dates and food treatments (data not shown; p> 0.05). Cumulative soil respiration C-CO_2 curves showed a trend of lower soil C emission from half-litter and regular-litter food treatments with all three earthworm species, effects were not reach statistically significant because of wide variance between samples (p> 0.05; Fig. 3.11). Soil respiration ^{13}C-CO_2 did show significant enrichment in double-litter treatments (Table 3.3); the higher ^{13}C-CO_2 evolved from double-litter treatments might be due to enhanced microbial activities related to faster litter decomposition.

Microbial biomass nitrogen and soil nitrogen dynamics

Earthworms had no significant impacts on dissolved inorganic nitrogen (DIN) (p=0.1; Fig. 3.12A), microbial nitrogen (p>0.05; Fig. 3.12B) or total dissolved nitrogen (TDN) (p>0.05; Fig. 3.12C) in the soil. Food treatment also did not significantly affect microbial nitrogen and TDN (Fig. 3.12B and 3.12C; p>0.05). However, there was higher DIN in the half-litter treatment as compared to double- and regular-litter treatments (Fig.

3.12A; p=0.01). The ^{15}N enrichment was not significantly different between the food and earthworm treatments for DIN or microbial nitrogen. However, ^{15}N enrichment was significantly lower in double-litter treatments than the half-and regular-litter ones in TDN (Table 3.4).

Discussion

Earthworm feeding behaviors are quite variable even beyond their main ecological groups (epigeic, endogiec, and anecic groups). Their feeding strategies can be studied directly from gut content analysis and stable isotopic studies (Judas 1992, Hendrix et al. 1999, Neilson et al. 2000). In particular, natural abundance of stable isotopes has been used to understand the feeding ecology of earthworms in the field (Schmidt et al. 1997, Hendrix et al. 1999, Neilson et al. 2000). Hendrix et al. (1999) discovered that even the same earthworm species could be enriched with differential degrees of ^{13}C and ^{15}N (natural abundance) as from different earthworm communities and ecosystem types. Natural abundance of stable isotopic signatures in earthworms may vary depending on earthworm life stages, earthworm community composition, site characteristics, and available food resources (Schmidt et al. 1997, Hendrix et al. 1999, Neilson et al. 2000). Because natural abundance of stable isotopes is affected by many factors mentioned above, the evaluation on earthworm feeding behaviors is challenging. Recently, stable isotope-labeled materials have been applied to trace food utilization by earthworm species, in particular to study earthworm interactions. Lachnicht et al. (2002) observed interactions between native *Estherella* spp. and invasive *P. corethrurus* in Puerto Rican soils by adding ^{13}C-labeled glucose into the soil and ^{15}N-labeled litter materials on the soil surface. They found that *Estherella* spp. and *P. corethrurus* did show different food use patterns in mixed assemblages (with both earthworm species) as compared to single-species treatments in their 19-day microcosm experiment. *P. corethrurus* acquired more litter resources (higher ^{15}N enrichment) in co-incubated with *Estherella* spp., which used mostly mineral soil and soil organic matter (higher ^{13}C enrichment). In the present study, each earthworm species kept its feeding strategies consistent whether it was incubated alone or with other earthworm species. This suggested no competition for food resources among epigeic *Estherella* spp., endogeic *O. borincana*, and epi-endogeic *P. corethrurus* in the 22-day mesocosm experiment. The reasons to explain this difference between this study and Lachnicht et al. (2002) might be due to different food resources adopted for the experiments. In this study, live *A. glomeratus* grass provided a sustaining rhizosphere environment for earthworms and microorganisms during the course of the experiment instead of one-time application with ^{13}C-glucose solution into the soil as used in Lachnicht et al. (2002). The continuous supply of ^{15}N-labeled live roots, root exudates and rhizodeposition might have relieved the competitive pressure between earthworms observed in Lachnicht et al. (2002). In addition, consistent earthworm feeding behaviors from the present food treatment experiment also indicated that even reduced (half) amounts of litter compared to field conditions still caused no competitive pressure between earthworms in this 22-day mesocosm experiment. Hence, food resource availability (plenty of litter and living grass roots) may relieve the competitive pressure between earthworms in this short term mesocosm study.

In the present study, ^{15}N-labeled rhizosphere and ^{13}C-labeled detritusphere contributed to ^{13}C- and ^{15}N-enrichment of soil in the earthworm mesocosms (Fig. 3.3). Less δ^{13}C-enrichment observed in epigeic *Estherella* spp. (δ^{13}C signature ranged from -21.05 ‰ to -25.6 ‰) suggested that it might feed less on litter as compared to the other two endogeic earthworm species, in particular *O. borincana* (δ^{13}C ranged from -9.88 ‰ to -23.59 ‰) (Fig. 3.2). *Estherella* spp. might have used rhizosphere resources, i.e. soil/microbial carbon, which was observed by Lachnicht et al. (2002). Lachnicht et al. (2002) found that juvenile and sub-adult *Estherella* spp. preferentially used mineral organic carbon as the main resource. *Estherella* spp. might prefer to use easily-accessible food resources. On the other hand, endogeic *O. borincana* showed a strong preference for utilization of grass roots, root exudates, and rhizosphere microorganisms in this study. Endogeic earthworms have been reported to aggregate in the root zones, such as corn plants and sugar cane (Spain et al. 1990, Binet et al. 1997) for consumption on living root fragments or in response to enhanced microbial activities or other microorganism populations (such as protozoa and nematodes) in the rhizosphere (Edwards 2004). Also, endogeic *O. borincana* and epi-endogeic *P. corethrurus* may have directly digested litter fragments or unintentionally consumed mineral and microbial carbon derived from ^{13}C litter. Epi-endogeic *P. corethrurus* was found to be able to access both litter-derived and plant root resources with no specific preferences, which is supported by previous literature (Lavelle et al. 1987, Lachnicht et al. 2002). The efficient use of litter and soil organic resources by *P. corethrurus* is attributed to its mutualistic digestion systems associated with the microflora, which contributed to higher cocoon production and efficient parthenogenesis of this invasive species (Lavelle et al. 1987). In addition, juveniles of *P. corethrurus* that hatched during the experiment utilized both rhizosphere and litter-derived resources, the usage of one resource more than the other depending on where the individual worms occurred. The ^{13}C- and ^{15}N signature patterns in earthworm populations indicated that each earthworm species has its preferences for food resources, even without competitive pressure. This also suggests that earthworm feeding strategies, which are beyond the definition of earthworm ecological groups, may be mostly determined by availability of resource quality and quantity in the habitats (Neilson et al. 2000).

The impacts on soil nutrient dynamics observed from different earthworm species were also related to their feeding behaviors and activities. In the present study, *O. borincana* enhanced soil carbon and nitrogen mineralization (the increase of soil respiration ($C\text{-}CO_2$) and DIN in the soils). This effect from *O. borincana* might be attributed to enhanced soil microbial biomass and microbial activities through its feeding and burrowing behaviors. However, the lack of effects on microbial biomass by *O. borincana* suggested that this earthworm species facilitated C- and N- mineralization through microbial activities rather than microbial biomass. *O. borincana* may have enhanced the turnover rates of both rhizosphere and detritusphere microbial population, and therefore C- and N-mineralization. However, *O. borincana* might have more influences on microbial populations in the rhizosphere than in the detritusphere due to $\delta\ ^{13}C$ (in soil respiration) and $\delta\ ^{15}N$ signature (in DIN) patterns. Meanwhile, this effect seemed to be reduced in the presence of the other two earthworm species. *Estherella* spp. also increased N-mineralization; slower litter decomposition and higher ^{15}N enriched in DIN and microbial nitrogen suggested that this species might have more impacts on rhizosphere microbial populations rather than on microbial populations in the detritusphere. *P. corethrurus* had effects on litter decomposition and nitrogen mineralization but not as strongly as compared to the other two earthworm species. Therefore, even though no significant competition and interactions were observed between these earthworm species, the impacts of earthworms on microbial populations and soil nutrient dynamics varied between different earthworm species.

Earthworm impacts on soil nutrient dynamics and the potential mechanisms on mineralization processes may depend mainly on earthworm activities and their interaction with microbial populations. Earthworms have been reported to affect mineralization processes through changing microbial biomass and activities, and these impacts could be positive, neutral, or negative (Bohlen et al. 2002, Li et al. 2002, Edwards 2004, Eisenhauer et al. 2007, Sheehan et al. 2007). Earthworms can enhance microbial biomass and growth rates through their mucus secretion along burrows and in cast depositions (Bohlen et al. 2002, Li et al. 2002) while they can also decrease the total microbial biomass but make it become more metabolically active (Wolters and Joergensen 1992). However, net impacts on microbial biomass and activities by earthworms are still controversial in the current literature. Earthworm ecological grouping can give a general idea about their potential impacts on microbial populations and soil nutrient dynamics but provide no details for the mechanisms. For example, Li et al. (2002) found that earthworms resulted in an increase in microbial respiration in invaded areas (Scheu and Parkinson 1994, Zhang et al. 2000), but no effects on soil respiration were found by Fisk et al. (2004). Mclean and Parkinson (1997) observed the opposite pattern with the decrease of microbial respiration per unit of biomass by earthworm invasions.

Meanwhile, for microbial biomass the net effects by earthworms are probably a combination of reduction effects from gut passages (Callaham and Hendrix 1998, Lachnicht and Hendrix 2001) and stimulation due to mucus secretion and increasing resource availability (Burtelow et al. 1998, Groffman et al. 2004). Based on isotopic ^{13}C and ^{15}N patterns from this study, the earthworms seemed to regulate soil nutrient distribution and dynamics by how they interact with microorganisms in different microhabitats, especially the rhizosphere and the detritusphere. In the detritusphere, earthworms can enhance microbial activities by their litter consumption activities, while earthworms may graze on microbial population or compete for rhizodeposits in the rhizosphere. In this study, endogeic *O. borincana* was found to affect both microbial populations in the rhizosphere and in the detritusphere. Epigeic *Estherella* spp. affected rhizosphere microbial population more than in detritusphere. In this case, measurements of overall microbial populations without separating different groups (i.e. detritusphere and rhizosphere) could confound those impacts from single earthworm species or mixed earthworm communities. Groffman et al. (2004) found that earthworm invasions in north temperate forests caused a decrease of microbial biomass in forest floor but an increase in the mineral soils, which resulted in a total increase of microbial biomass in the soil profile. The facilitative effects on specific microbial populations from a single earthworm species may be diluted by the suppression of other microbial populations from the same species or other earthworm species. This may explain the divergent outcomes of earthworm-microbe interactions from current studies. Our lack of separation of rhizosphere and detritusphere microbial populations in this study failed to answer this. However, this study suggested that application of ^{13}C and ^{15}N materials can not only help us to understand feeding strategies of earthworms but also how earthworms interact with microbial populations in different microhabitats. Differential impacts from earthworms on separate microbial populations should be considered for future earthworm-microbe interaction studies.

In this study, no significant competitive interaction among different earthworm species was found, even in the earthworm treatments with niche similarity and reduced amount of litter availability. The competitive interactions between the earthworms may be determined by resource availability and flexibility of feeding strategies of earthworms. Meanwhile, different earthworm species did have preferences on food resources beyond their ecological category definitions. Based on their feeding behavior and activities, earthworms can have differential impacts on soil nutrient dynamics in different microhabitats, especially in rhizosphere and detritusphere. The application of stable isotopic labeled materials on earthworm studies can not only provide more information on earthworm feeding behaviors but also help to explain the potential mechanisms on earthworm-microbe-mineralization interaction in the future studies.

References Cited:

Abbott, I. 1980. Do earthworms compete for food? Soil Biology and Biochemistry 12:523-530.

Binet, F., V. Hallaire, and P. Curmi. 1997. Agricultural practices and the spatical distribution of earthworms in maize fields. Relationships between earthworm abundance, maize plants and soil compaction. Soil Biology and Biochemistry 29:577-583.

Bohlen, P. J., C. A. Edwards, Q. Zhang, R. W. Parmelee, and M. Allen. 2002. Indirect effects of earthworms on microbial assimilation of labile carbon. Applied Soil Ecology 20:255-261.

Bossuyt, H., J. Six, and P. F. Hendrix. 2004. Rapid incorporation of carbon from fresh residues into newly formed stable microaggregates within earthworm casts. European Journal of Soil Science 55:393-399.

Bouché, M. B. 1977. Strategies Lombriciennes. Pages 122-132 *in* U. Lohm and T. Persson, editors. Soil organisms as components of ecosystems. Proc. 6th Int. Coll. Soil Zool., Ecological Bulletin, Stockholm.

Burtelow, A. E., P. J. Bohlen, and P. M. Groffman. 1998. Influence of exotic earthworm invasion on soil organic matter, microbial biomass and denitrification potential in forest soils of the northeastern United States. Applied Soil Ecology 9:197-202.

Callaham, M. A., and P. F. Hendrix. 1998. Impact of earthworms (Diplocardia: Megascolecidae) on cycling and uptake of nitrogen in coastal plain forest soils from northwest Florida, USA. Applied Soil Ecology 9:233-239.

Callaham, M. A., P. F. Hendrix, and R. J. Phillips. 2003. Occurrence of an exotic earthworm (*Amynthas agrestis*) in undisturbed soils of the southern Appalachian mountains, USA. Pedobiologia 47:466-470.

Coleman, D. C., and B. Fry, editors. 1991. Carbon isotope techniques. Academic Press, San Diego, California.

Edwards, C. A. 2004. Earthworm ecology. CRC Press, Boca Raton.

Eisenhauer, N., S. Partsch, D. Parkinson, and S. Scheu. 2007. Invasion of a deciduous forest by earthworms: changes in soil chemistry, microflora, microarthopods and vegetation. Soil Biology and Biochemistry 39:1099-1110.

Fisk, M. C., T. J. Fahey, P. M. Groffman, and P. J. Bohlen. 2004. Earthworm invasion, fine-root distributions, and soil respiration in North temperate forests. Ecosystems 7:55-62.

Frelich, L. E., C. M. Hale, S. Scheu, A. R. Holdsworth, L. Heneghan, P. J. Bohlen, and P. B. Reich. 2006. Earthworm invasion into previously earthworm-free temperate and boreal forests. Biological Invasions 8:1235-1245.

González, G., X. Zou, and S. Borges. 1996. Earthworm abundance and species composition in abandoned tropical croplands: comparison of tree plantations and secondary forests. Pedobiologia 40:385-391.

González, G., X. Zou, A. Sabat, and N. Fetcher. 1999. Earthworm abundance and distribution pattern in contrasting plant communities within a tropical wet forest in Puerto Rico. Caribbean Journal of Science 35:93-100.

Groffman, P. M., P. J. Bohlen, M. C. Fisk, and T. J. Fahey. 2004. Exotic earthworm invasion and microbial biomass in temperate forest soils. Ecosystems 7:43-54.

Hale, C. M., L. E. Frelich, P. B. Reich, and J. Pastor. 2005. Effects of European earthworm invasion on soil characteristics in Northern hardwood forests of Minnesota, USA. Ecosystems 8:911-927.

Hendrix, P. F., S. L. Lachnicht, Callaham, M. A., and X. Zou. 1999. Stable isotopic studies of earthworm feeding ecology in tropical ecosystems of Puerto Rico. Rapid Communications in Mass Spectrometry 13:1295-1299.

Huang, C.-Y., G. González, and P. F. Hendrix. 2006. The re-colonization ability of native earthworm, *Estherella* spp., in Puerto Rican forests and pastures. Caribbean Journal of Science 42:386-396.

Jenkinson, D. S., and D. S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil -V. A method for measuring soil biomass. Soil Biology and Biochemistry 8:209-213.

Judas, M. 1992. Gut content analysis of earthworms (Lumbricidae) in a beechwood. Soil Biology and Biochemistry 24:1413-1417.

Kalisz, P. J. 1993. Native and exotic earthworms in deciduous forest soils of Eastern North America. Biological Pollution: the control and impact of invasive exotic species. Proceedings of a Symposium held at the University Place Conference Center, Indiana University-Purdue University, Indianapolis, October 25 & 26, 1991:93-100.

Kalisz, P. J., and H. B. Wood. 1995. Native and exotic earthworms in wildland ecosystems. Pages 117-126 *in* P. F. Hendrix, editor. Earthworm Ecology and Biogeography in North America. Lewis Publishers, Boca Raton, FL.

Lachnicht, S. L., and P. F. Hendrix. 2001. Interaction of the earthworm *Diplocardia mississippiensis* (Megascolecidae) with microbial and nutrient dynamics in a subtropical Spodosol. Soil Biology and Biochemistry 33:1411-1417.

Lachnicht, S. L., P. F. Hendrix, and 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., I. Barois, I. Cruz, C. Fragoso, A. Hernandez, A. Pineda, and 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.

Lavelle, P., and E. Lapied. 2003. Endangered earthworms of Amazonia: an homage to Gilberto Righi. Pedobiologia 47:419-417.

Li, X., M. C. Fisk, T. J. Fahey, and P. J. Bohlen. 2002. Influence of earthworm invasion on soil microbial biomass and activity in a northern hardwood forest. Soil Biology and Biochemistry 34:1929-1937.

Liu, Z. G., and X. M. Zou. 2002. Exotic earthworms accelerate plant litter decomposition in a Puerto Rican pasture and a wet forest. Ecological Applications 12:1406-1417.

McLean, M. A., and D. Parkinson. 1997. Changes in structure, organic matter and microbial activity in pine forest soil following the introduction of *Dendrobaena octaedra* (Oligochaeta, Lumbricidae). Soil Biology and Biochemistry 29:537-540.

Mummey, D. L., M. C. Rillig, and J. Six. 2006. Endogeic earthworms differentially influence bacterial communities associated with different soil aggregate size fractions. Soil Biology and Biochemistry 38:1608-1614.

Neilson, R., B. Boag, and M. Smith. 2000. Earthworm delta13C and delta15C analyses suggest that putative functional classifications of earthworms are site-specific and may also indicate habitat diversity. Soil Biology and Biochemistry 32:1053-1061.

Sánchez-de León, Y., X. Zou, S. Borges, and H. Ruan. 2003. Recovery of native earthworms in abandoned tropical pastures. Conservation Biology 17:999-1006.

Scatena, F. N. 1989. An introduction to the physiography and history of the Bisley experimental watersheds in the Luquillo Mountains of Puerto Rico. General Technical Report SO-72, USDA Forest Service, Southern Forest Experiment Station, USA.

Scheu, S., and D. Parkinson. 1994. Effects of an invasion of an aspen forest (Alberta, Canada) by *Dendrobaena octaedra* (Lumbricidae) on plant growth. Ecology 75:2348-2361.

Schmidt, O., and C. M. Scrimgeour. 2001. A simple urea leaf-feeding method for the production of ^{13}C and ^{15}N labelled plant material. Plant and Soil 229:197-202.

Schmidt, O., C. M. Scrimgeour, and L. L. Handley. 1997. Natural abundance of ^{15}N and ^{13}C in earthworms from a wheat and a wheat-clover field. Soil Biology and Biochemistry 29:1301-1308.

Sheehan, C., L. Kirwan, J. Connolly, and T. Bolger. 2007. The effects of earthworm functional group diversity on earthworm community structure. Pedobiologia 50:479-487.

Spain, A. V., P. G. Saffigna, and A. W. Wood. 1990. Tissue Carbon sources for *Pontoscolex corethrurus* (Oligochaeta: Glossoscolecidae) in a sugarcane ecosystem. Soil Biology and Biochemistry 22:703-706.

Stark, J. M., and S. C. Hart. 1996. Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitorgen-15 analysis. Soil Science Society of America Journal 60:1846-1855.

Tiunov, A. V., C. M. Hale, A. R. Holdsworth, and T. S. Vsevolodova-Perel. 2006. Invasion patterns of Lumbricidae into the previously earthworm-free areas of northeastern Europe and the western Great Lakes region of North America. Biological Invasions 8:1223-1234.

Winsome, T., L. Epstein, P. F. Hendrix, and W. R. Horwath. 2006. Competitive interactions between native and exotic earthworm species as influenced by habitat quality in a California grassland. Applied Soil Ecology 32:38-53.

Wolters, V., and R. G. Joergensen. 1992. Microbial carbon turnover in beech forest soils worked by *Aporrectodea caliginosa* (Savigny) (Oligochaeta: Lumbricidae). Soil Biology and Biochemistry 24:171-177.

Zhang, B.-G., G.-T. Li, T.-S. Shen, W. J.-K., and Z. Sun. 2000. Changes in microbial C, N, and P and enzyme activities in soil incubated with the earthworms *Metaphire guillelmi* or *Eisenia fetida*. Soil Biology and Biochemistry 32:2055-2062.

Zou, X., C. P. Zucca, R. B. Waide, and W. H. McDowell. 1995. Long-term influence of deforestation on tree species composition and litter dynamics of a tropical in Puerto Rico. Forest Ecology and Management 78:147-157.

Table 3.1. $\delta^{13}C$ signature in soil respiration C-CO_2 from different earthworm treatments at day 3, day 11, and day 22 during the 22-day mesocosm experiment with Puerto Rican soils. Control treatment = soil mesocosms without earthworms. Capital letters (E, O, and P) represent treatments with different earthworm species. E= *Estherella* spp.; O= *Onychochaeta borincana*; P= *Pontoscolex corethrurus*; E+O= both *Estherella* spp. and *O. borincana* assemblage; E+P= both *Estherella* spp. and *P. corethrurus* assemblage; O+P= both *O. borincana* and *P. corethrurus* assemblage; E+O+P= *Estherella* spp., *O. borincana* and *P. corethrurus* assemblage. Value is shown as mean (S.E.). GLM shows the statistical results from general linear model analysis. Different letters indicate significant difference among earthworm treatments on the sampling date (Tukey, $p < 0.05$).

	Earthworm treatment								
Sampling date	Control	E	O	P	E+O	E+P	O+P	E+O+P	GLM
$\delta^{13}C$									
Day 3	72.5 (13.7)	86.8 (3.0)	85.5 (15.0)	81.4 (12.5)	107.1 (13.9)	93.5 (7.5)	73.5 (4.3)	81.6 (12.5)	p=0.45
Day 11	85.2 (9.9)	121.1 (10.8)	71.9 (7.8)	95.4 (9.1)	84.0 (18.0)	88.8 (8.4)	99.9 (8.6)	78.9 (18.4)	p=0.19
Day 22	107.5 (5.6)^{a}	91.7 (9.3)^{b}	94.3 (1.6)^{b}	104.9 (10.6)^{a}	91. 3 (3.4)^{b}	100.2 (8.5)^{ab}	71.1 (3.6)^{b}	81.8 (4.5)^{b}	*p=0.01*

Table 3.2 $\delta^{15}N$ signature in dissolved inorganic nitrogen (DIN), microbial nitrogen (Microbial N) , and total dissolved nitrogen (TDN) from different earthworm treatments in the 22-day mesocosm experiment with Puerto Rican soils. For abbreviation see Table 3.1. Value is shown as mean (S.E.). GLM shows the statistical results from general linear model analysis (significant level α=0.05).

	Earthworm treatment								
Sampling date	Control	E	O	P	E+O	E+P	O+P	E+O+P	GLM
$\delta^{15}N$									
DIN	22.3 (1.7)	28.8 (5.6)	23.0 (3.4)	23.5 (3.1)	32.7 (6.3)	23.2 (3.4)	40.7 (12.1)	30.5 (5.9)	p= 0.31
Microbial N	12.3 (2.6)	12.7 (3.1)	8.5 (1.7)	13.0 (2.4)	15.7 (2.0)	12.5 (0.6)	13.2 (1.3)	9.4 (0.1)	p= 0.24
TDN	10.8 (1.5)	15.4 (3.2)	12.0 (1.0)	13.1 (1.9)	17.1 (2.6)	12.7 (0.9)	16.3 (3.1)	13.2 (1.7)	p= 0.43

Table 3.3. $\delta^{13}C$ signature in soil respiration C-CO_2 from different food treatments (Half, Regular, and Double) in *O. borincana* and *P. corethrurus* (O+P) assemblage, and *Estherella* spp., *O. borincana* and *P. corethrurus* (E+O+P) assemblage in the 22-day mesocosm experiment with Puerto Rican soils. Value is shown as mean (S.E.). GLM shows the statistical results from general linear model analysis. Different letters indicate significant difference among food treatments on the sampling date (Tukey, $p < 0.05$).

Food treatment	Half		Regular		Double		
Sampling date	O+P	E+O+P	O+P	E+O+P	O+P	E+O+P	GLM
$\delta^{13}C$							
Day 3	62.0 (9.8) ^{a}	81.2 (2.2) ^{a}	73.5 (4.3) ^{a}	81.6 (12.5) ^{a}	101.9 (11.5) ^{b}	113.2 (10.2) ^{b}	Worm p=0.1 Food *p=0.002* W×F p=0.8
Day 11	66.5 (7.7) ^{a}	53.3 (5.3) ^{a}	99.9 (8.6) ^{b}	78.9 (18.4) ^{b}	93.1 (8.9) ^{b}	97.5 (8.8) ^{b}	Worm p=0.3 Food *p=0.007* W×F p=0.5
Day 21	40.0 (6.9) ^{a}	57.4 (12.2) ^{a}	71.1 (3.6) ^{b}	81.8 (4.5) ^{b}	130.8 (16.3) ^{c}	114.2 (4.2) ^{c}	Worm p=0.1 Food *p<0.0001* W×F p=0.1

Table 3.4. $\delta^{15}N$ signature in dissolved inorganic nitrogen (DIN), microbial nitrogen (Microbial N) , and total dissolved nitrogen (TDN) from different food treatments (Half, Regular, and Double) in *O. borincana* and *P. corethrurus* (O+P) assemblage, and *Estherella* spp., *O. borincana* and *P. corethrurus* (E+O+P) assemblage in the 22-day mesocosm experiment with Puerto Rican soils. Value is shown as mean (S.E.). GLM shows the statistical results from general linear model analysis. Different letters indicate significant difference among food treatments on the sampling date (Tukey, $p < 0.05$).

Food treatment	Half		Regular		Double		
	O+P	E+O+P	O+P	E+O+P	O+P	E+O+P	GLM
$\delta^{15}N$							
DIN	43.7 (3.2)	35.3 (8.4)	40.7 (12.1)	30.5 (5.9)	28.5 (4.7)	22.8 (2.4)	Worm p=0.1 Food p=0.1 W×F p=0.89
Microbial N	10.0 (3.4)	14.9 (2.1)	13.2 (1.3)	9.4 (0.1)	12.2 (2.9)	6.0 (0.8)	Worm p=0.5 Food p=0.3 W×F p=0.06
TDN	17.4 (1.9) ^{a}	18.8 (3.1) ^{a}	16.3 (3.1) ^{ab}	13.2 (1.7) ^{ab}	12.8 (3.1) ^{b}	8.8 (0.5) ^{b}	Worm p=0.1 Food *p=0.002* W×F p=0.8

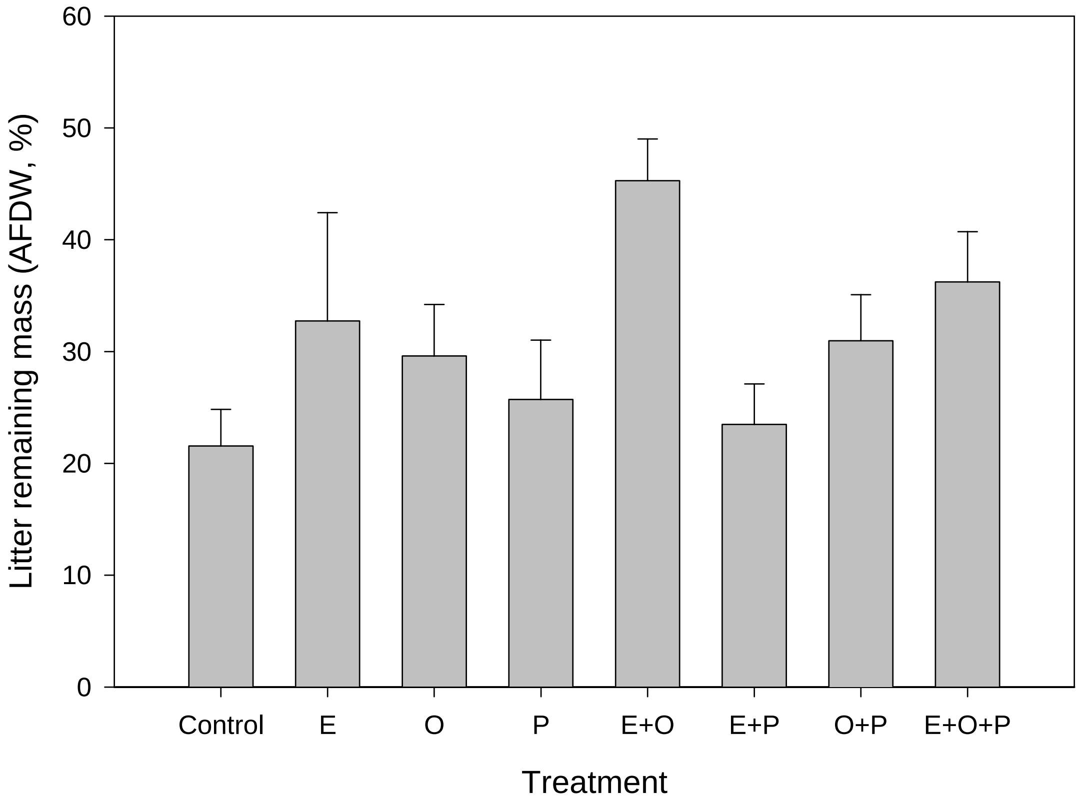

Figure 3.1. Litter mass remaining (AFDM, %) from different earthworm treatments in the 22-day mesocosm experiment with Puerto Rican soils. Control treatment = soil mesocosms without earthworms; Capital letters (E, O, and P) represent treatments with different earthworm species. E= *Estherella* spp.; O= *Onychochaeta borincana*; P= *Pontoscolex corethrurus*; E+O= both *Estherella* spp. and *O. borincana* assemblage; E+P= both *Estherella* spp. and *P. corethrurus* assemblage; O+P= both *O. borincana* and *P. corethrurus* assemblage; E+O+P= *Estherella* spp., *O. borincana* and *P. corethrurus* assemblage.

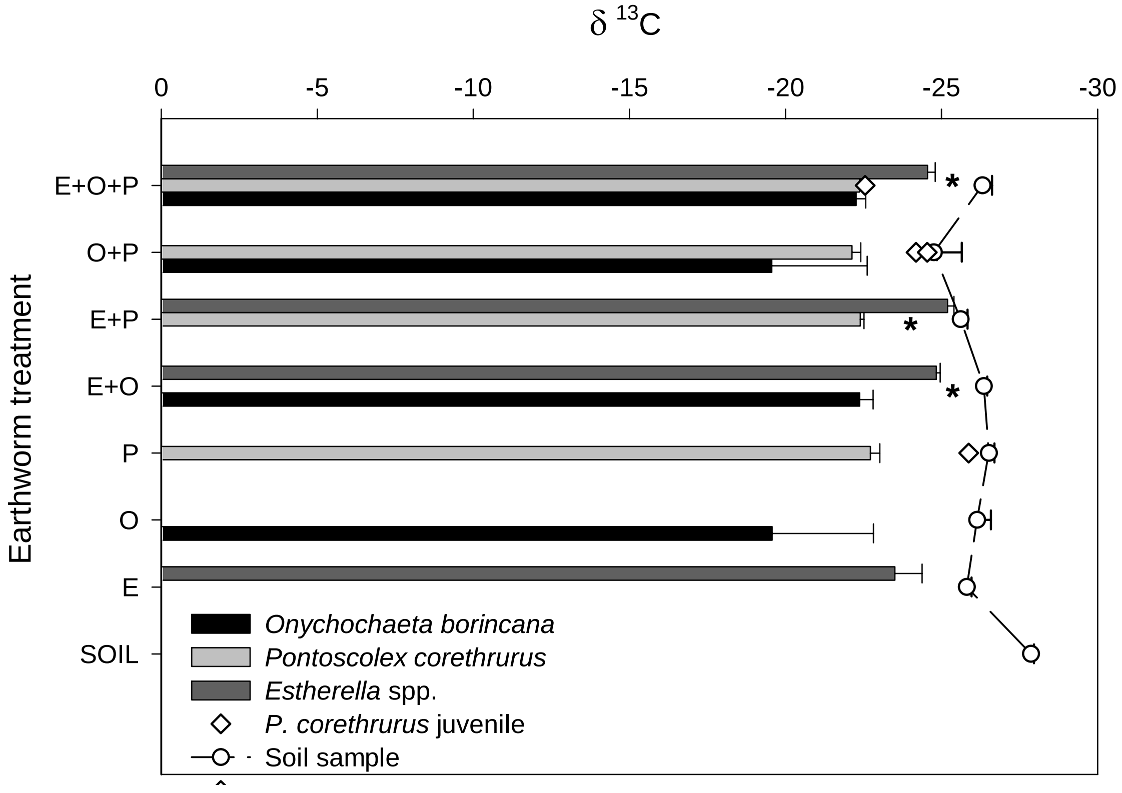

Figure 3.2. $\delta^{13}C$ enrichment in earthworm tissue (bars), *Pontoscolex corethrurus* juveniles (diamond symbol) and the soils (open circle with dashed line) from different earthworm treatments in the 22-day mesocosm experiment with Puerto Rican soils. “SOIL” treatment indicates the experimental mesocosms without ^{13}C- and ^{15}N-labeled materials or earthworms. Asterisks indicate significant differences in $\delta^{13}C$ enrichment between earthworm species within the treatment. Value is shown as mean ± S.E. For abbreviations see the descriptions in Figure 3.1.

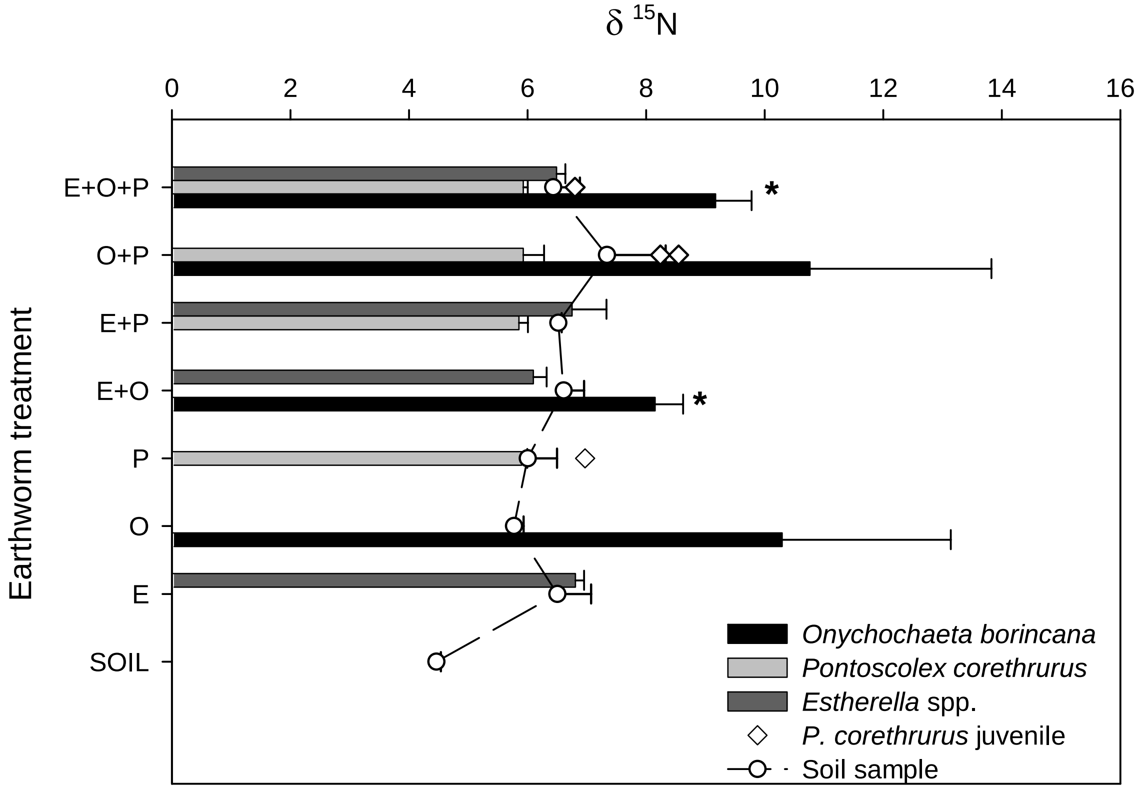

Figure 3.3. δ^{15}N enrichment in earthworm tissue (bars), *Pontoscolex corethrurus* juveniles (diamond symbol) and the soils (open circle with solid line) from different earthworm treatments in the 22-day mesocosm experiment with Puerto Rican soils. "SOIL" treatment indicates the experimental mesocosms without ^{13}C- and ^{15}N-labeled materials and earthworms. Asterisks indicate significant differences in δ^{15}N enrichment between earthworm species within the treatment. Value is shown as mean ± S.E. For abbreviations see the descriptions in Figure 3.1.

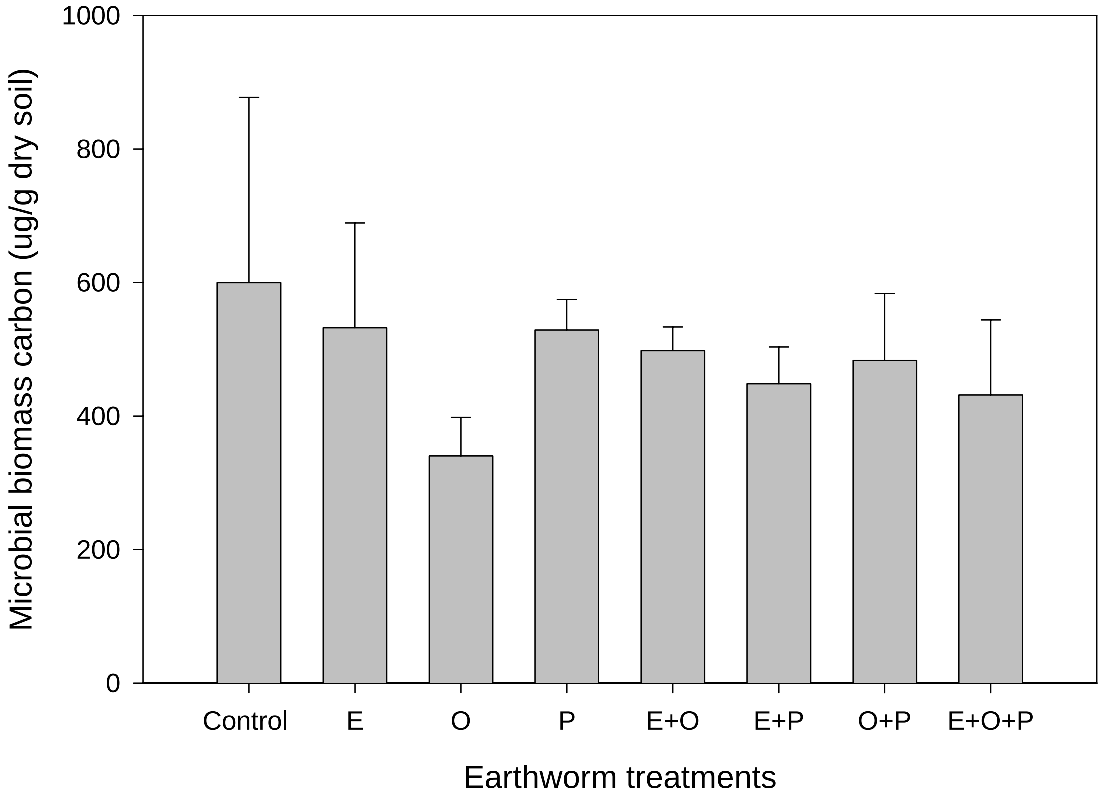

Figure 3.4 Average microbial biomass carbon between different earthworm treatments in the 22-day mesocosm experiment with Puerto Rican soils. For abbreviations see Figure 3.1.

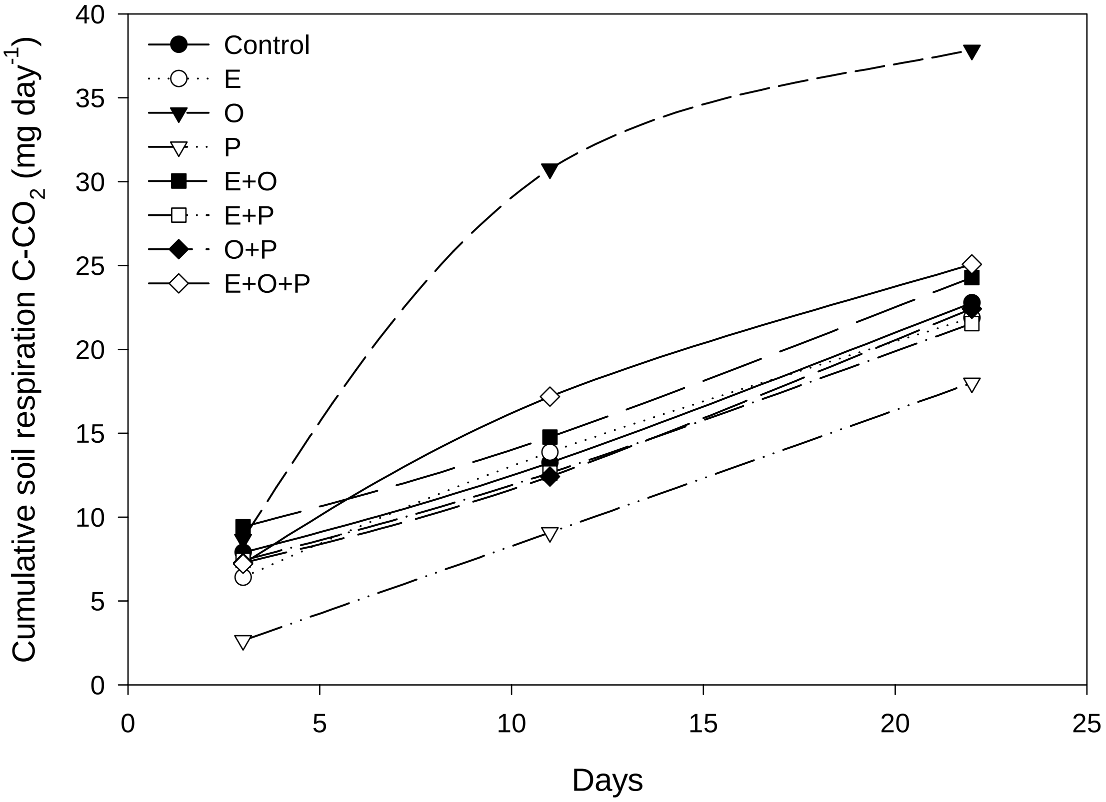

Figure 3.5.Cumulative soil respiration C-CO$_2$ from different earthworm treatments at day 3, day 11, and day 22 during the 22-day mesocosm experiment. Control treatment = soil mesocosms without earthworms. For abbreviations see Figure 3.1.

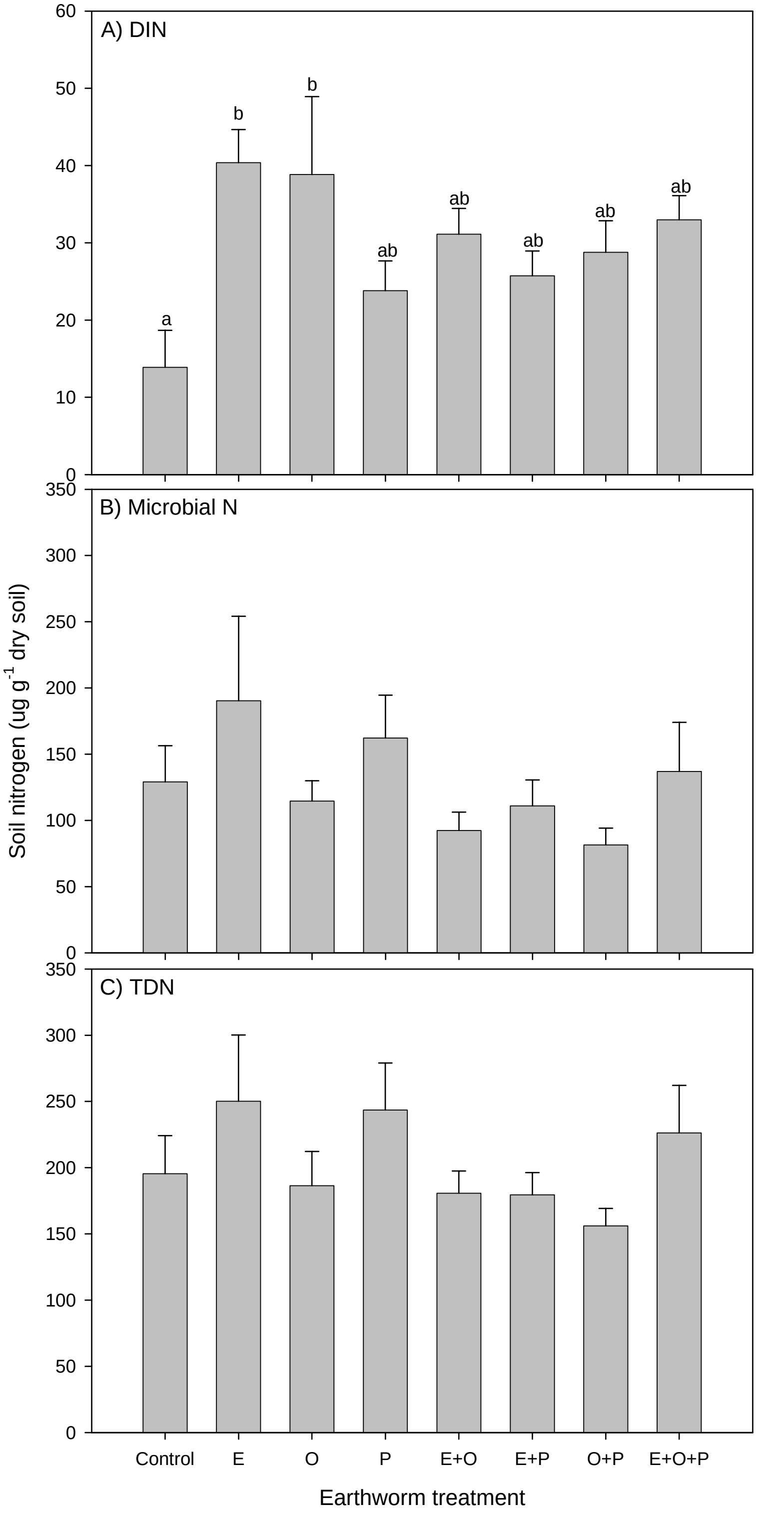

Figure 3.6. Net changes of soil nitrogen content in A) dissolved inorganic nitrogen (DIN), B) microbial nitrogen (Microbial N) , and C) total dissolved nitrogen (TDN) between different earthworm treatments in the 22-day mesocosm experiment with Puerto Rican soils. Different letters in DIN data represent significant difference between earthworm treatments (Tukey, $p< 0.05$). For abbreviations see Figure 3.1.

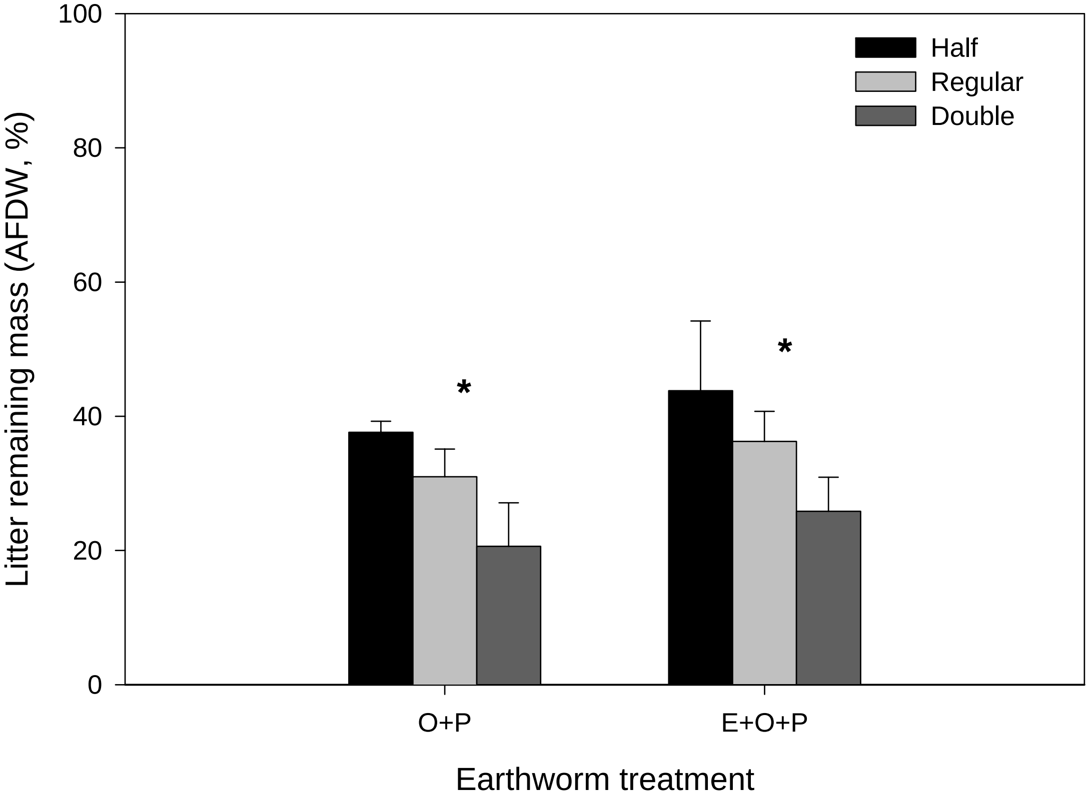

Figure 3.7. Litter mass remaining (AFDM, %) from different food and earthworm treatments in the 22-day mesocosm experiment with Puerto Rican soils. Food treatments are represented by different color of bars: half food treatment= black bar; Regular food treatment= light gray bar; double food treatment= dark gray bar. Two earthworm treatments are indicated as O+P= both *O. borincana* and *P. corethrurus* assemblage; E+O+P= *Estherella* spp., *O. borincana* and *P. corethrurus* assemblage. Asterisks indicated significant difference in litter mass remaining among different food treatments (Tukey, $p< 0.05$). Value is shown as mean ± S.E.

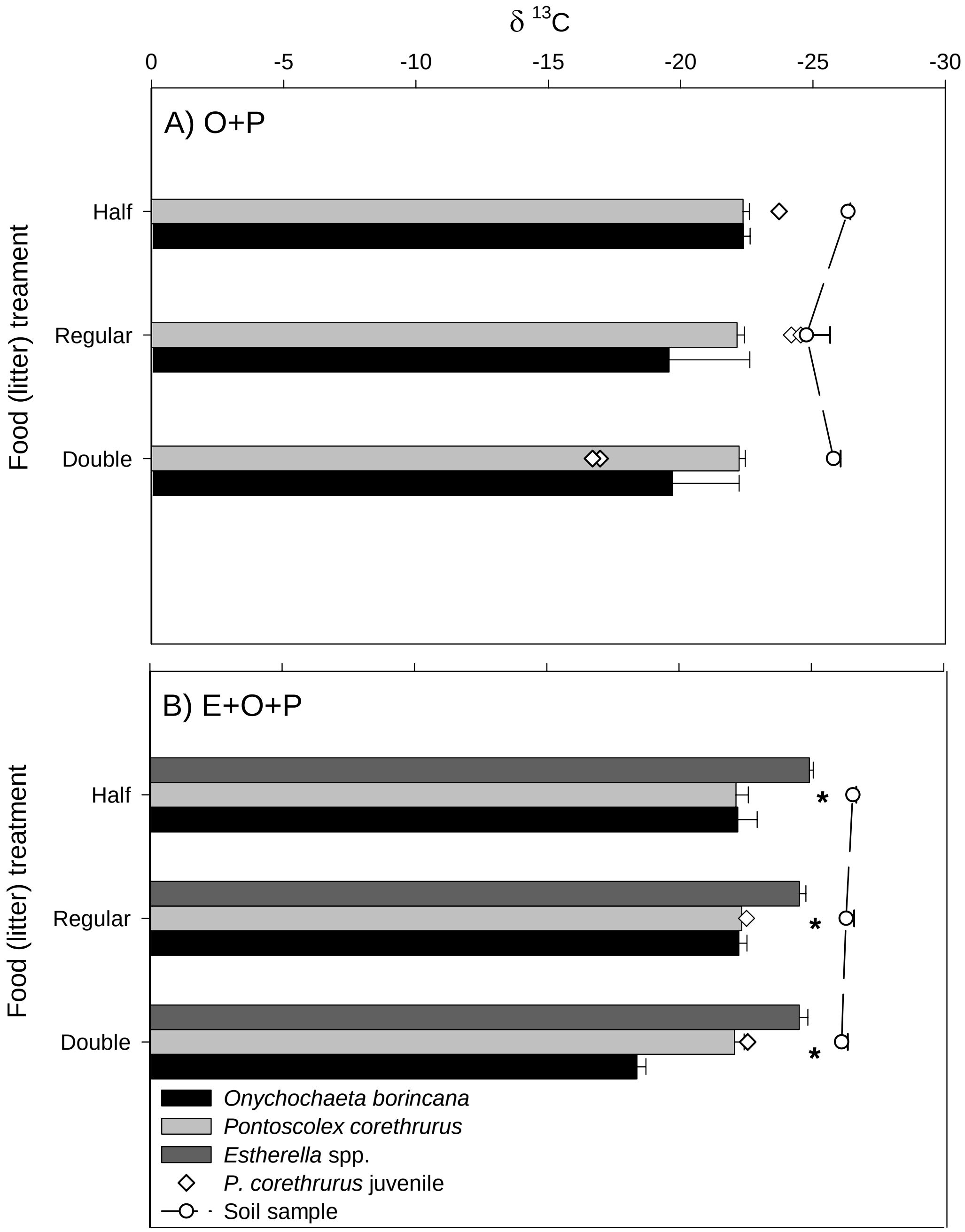

Figure 3.8. δ^{13}C enrichment in earthworm tissue (bars), *Pontoscolex corethrurus* juveniles (diamond symbol) and the soils (open circle with dashed line) from different food treatments in A) *O. borincana* and *P. corethrurus* (O+P) assemblage, and B) *Estherella* spp., *O. borincana* and *P. corethrurus* (E+O+P) assemblage in the 22-day mesocosm experiment with Puerto Rican soils. Asterisks indicate significant differences in δ^{13}C enrichment between earthworm species within the food treatment. Value is shown as mean ± S.E.

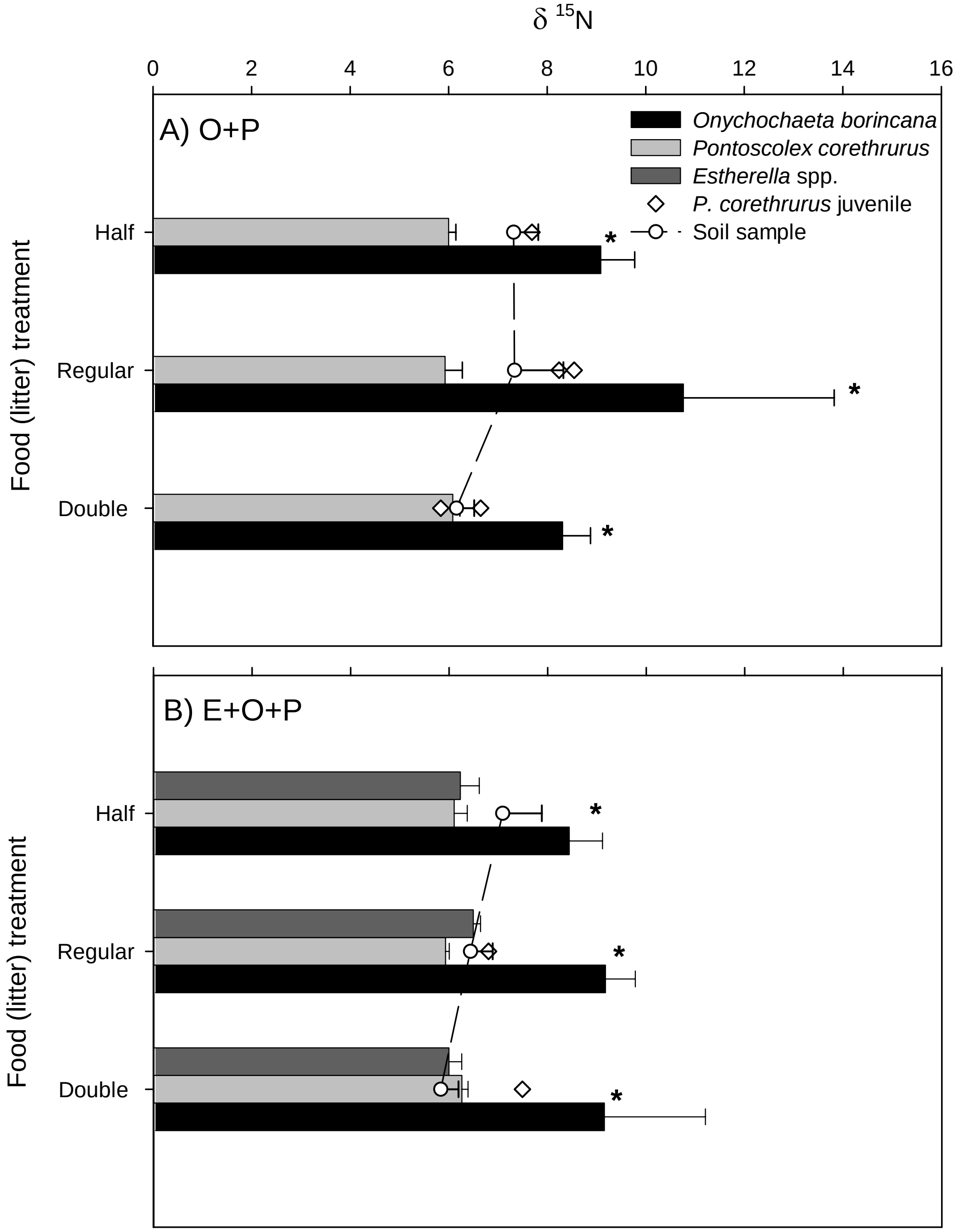

Figure 3.9. δ^{15}N enrichment in earthworm tissue (bars), *Pontoscolex corethrurus* juveniles (diamond symbol) and the soils (open circle with dashed line) from different food treatments in A) *O. borincana* and *P. corethrurus* (O+P) assemblage, and B) *Estherella* spp., *O. borincana* and *P. corethrurus* (E+O+P) assemblage in the 22-day mesocosm experiment with Puerto Rican soils. Asterisks indicate significant difference in δ^{15}N enrichment between earthworm species within the food treatment. Value is shown as mean ± S.E.

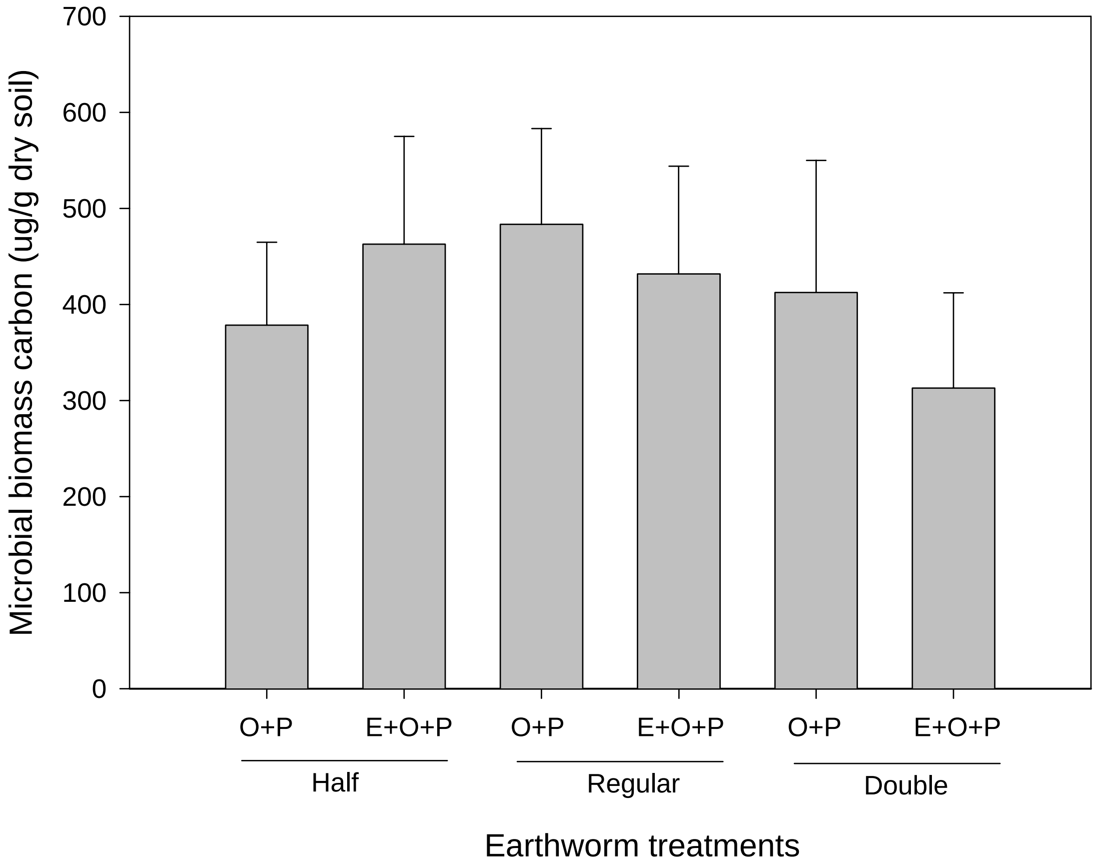

Figure 3.10. Average soil microbial biomass carbon between different food treatments (Half, Regular, and Double) in *O. borincana* and *P. corethrurus* (O+P) assemblage, and *Estherella* spp., *O. borincana* and *P. corethrurus* (E+O+P) assemblage in the 22-day mesocosm experiment with Puerto Rican soils.

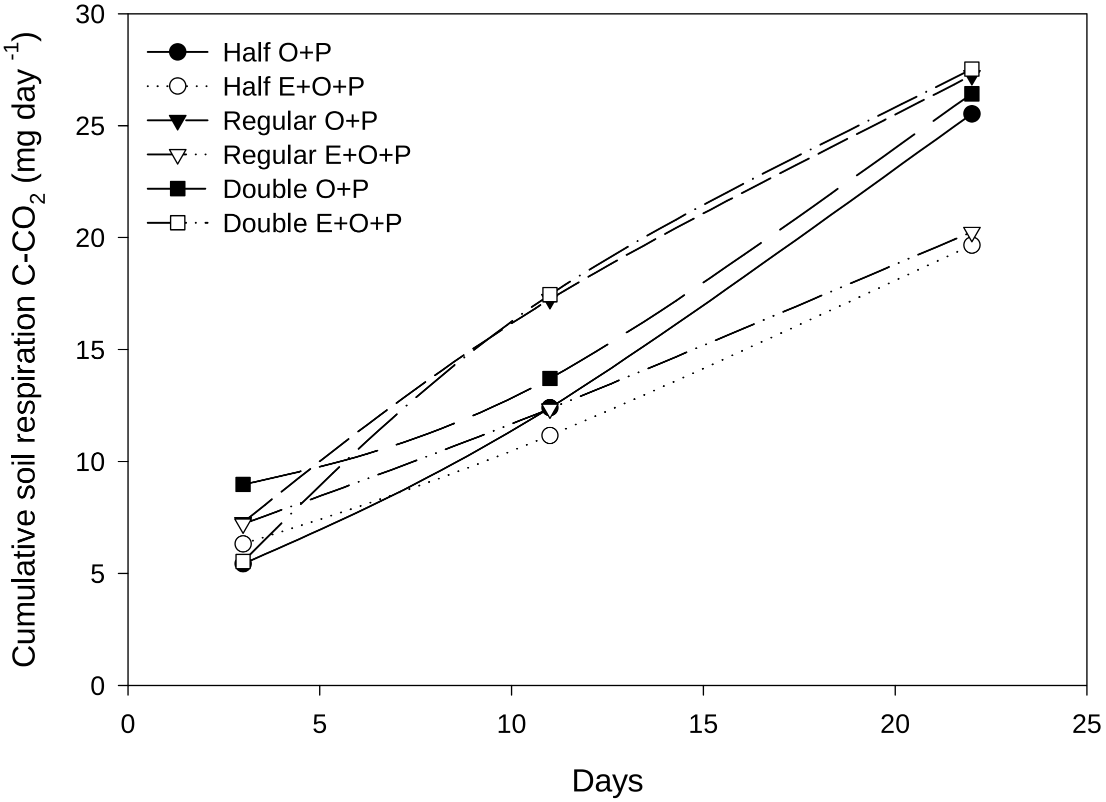

Figure 3.11. Cumulative soil respiration C-CO$_2$ from different food treatments in *O. borincana* and *P. corethrurus* (O+P) assemblage, and *Estherella* spp., *O. borincana* and *P. corethrurus* (E+O+P) assemblage at day 3, day 11, and day 22 during the 22-day mesocosm experiment with Puerto Rican soils.

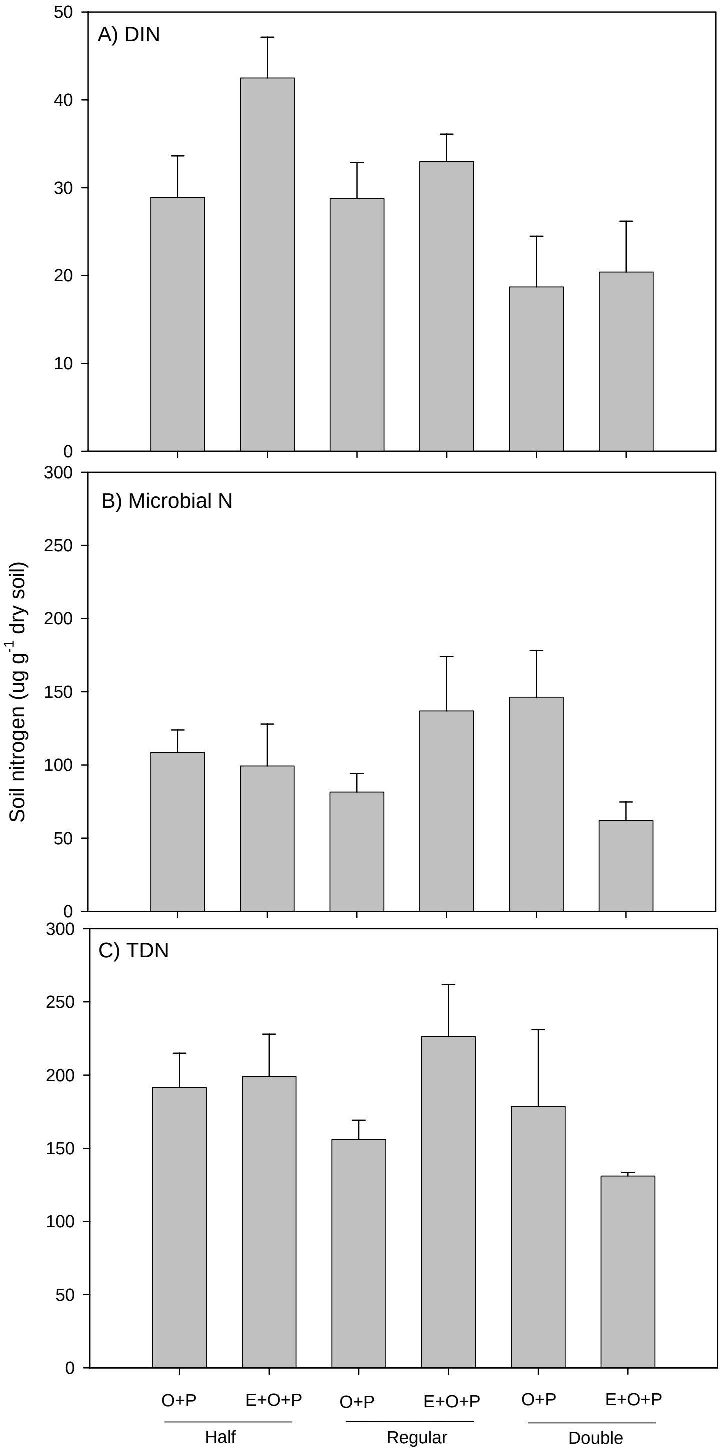

Figure 3.12. Net changes of soil nitrogen content in A) dissolved inorganic nitrogen (DIN), B) microbial nitrogen (Microbial N) , and C) total dissolved nitrogen (TDN) between different food treatments (Half, Regular, and Double) in *O. borincana* and *P. corethrurus* (O+P) assemblage, and *Estherella* spp., *O. borincana* and *P. corethrurus* (E+O+P) assemblage in the 22-day mesocosm experiment with Puerto Rican soils.

CHAPTER FOUR

A SIMULATION MODEL FOR EARTHWORM INVASIONS: PREDICTION AND EVALUATION OF THE IMPACTS OF INVASIVE EARTHWORM SPECIES ON SOIL CARBON CYCLING

Introduction

Life on the earth relies on all biospheric processes, which include not only energy flow through food webs in above-ground ecosystem but also soil processes underneath our feet. It is these individual processes and their interweaving interactions that establish and maintain ecosystems. However, it is not always easy to integrate these pieces together into a whole picture. Recently, improved model development along with advanced computer techniques provide a unique and efficient way to integrate whole concepts and to understand complete structure of the ecosystems. Modern model applications enable us to treat these complicated ecosystem interactions at the same time, and also disentangle their contribution separately (Moore et al. 1996, Muller 1999).

Soils, as a major component of the biosphere, play an essential role in both above- and below-ground ecosystems. Soil-vegetation-atmosphere interfaces include numerous physical, chemical and biological processes (Coleman et al. 2004). Various models have been developed to simulate dynamics within producer food webs (Pimm et al. 1991, Pimm 2002) as well as in detrital food webs (Hunt et al. 1987, de Ruiter et al. 1994a, de Ruiter et al. 1994b, Moore et al. 1996, Moore et al. 2004). These models have focused on trophic structures and nutrient/energy fluxes basically. Hunt et al. (1987) developed a model of the detrital food web showing nitrogen transformation in a shortgrass prairie. Nitrogen flow started from substrate pools (i.e. plant shoots and roots), then went up through decomposer and their predator populations (extend to predatory mites only) and eventually nitrogen recycled back to soil pools for plants to use. Moore et al. (1996) tried to link and integrate the knowledge from field and microcosm studies to model application on the detritus food web. No simulation of specific soil macro-fauna and their potential impacts on ecosystem function was considered in their models.

Some models have been developed to evaluate decomposition processes and the impacts of detrital food webs on nutrient cycling. The soil organic matter model (SOMM) was elaborated to simulate organic matter dynamics in soil ecosystems. SOMM incorporated the kinetic parameters from soil biota to reflect the activities of soil decomposer groups (Bacteria, Arthropoda and Oligochaeta), yet did not put soil invertebrates into separate pools (Chertov and Komarov 1997). Brussaard (1998) noted that there is a need to put efforts into food web models, addressing the ecological interactions in the soil. The ecological interactions include not only trophic relationships but non-trophic ones (abiotic-biotic interactions). Fu et al. (2000) developed a model to monitor the responses of major soil invertebrate groups and soil micro-organisms to carbon dynamics in an agroecosystem. However, the authors used the idea of “super organism” for whole soil food web to process the organic matter to simplify the model diagram, and the potential contributions from each soil invertebrate groups on organic matter dynamic were combined together (Fu et al. 2000). Differential impacts from soil invertebrates and especially effects of dominant groups on soil nutrient cycling were not considered in their model. Brussaard et al. (2007) projected that a conceptual model addressing soil biophysical interactions is necessary and urgently needed. The effects of soil biota on soil physical and chemical structures are essential parts for understanding of soil development and biogeochemical cycles.

Earthworms were called by Aristotle, back in classic Greek times, “the Intestines of the Earth” for their tremendous effects on soil turnover. Not until the late 1800s, did Charles Darwin (1881) bring attention to the importance of earthworms on soils in his published work “The Formation of Vegetable Mould through the Action of Worms” (Coleman et al. 2004). Nowadays, earthworms have been recognized as one of the most important invertebrates in the soil, and have become well-known as ecosystem engineers among soil fauna in terrestrial ecosystems (Hastings et al. 2007). These soil-dwelling worms have a significant influence on soil physical structure, litter decomposition process, nutrient cycling and plant productivities in the terrestrial ecosystems (Lee 1985, Lavelle et al. 1999, Edwards 2004). Their activities, including comminuting litter, feeding, casting and burrowing, can increase the soil porosity, stimulate microbial activities, and release the nutrients from litter to be available for plant growth (Lee 1985, Spiers et al. 1986, Lavelle et al. 1999). Generally, the soil volume which is influenced by earthworm activities has been termed as the “drilosphere”.

The formation and development of the drilosphere can vary considerably among different earthworm species. Earthworms can be categorized into three ecological groups: epigeic, endogeic and anecic, depending on food resources they utilize and their vertical distribution patterns in soil (Bouché 1977). Epigeic earthworms consume leaf litter and the colonized microbial populations on it, and they mostly inhabit litter layers. In contrast, endogeic earthworms burrow in the deep soil and use soil organic matter as their main food resource. Anecic earthworms utilize fragmented leaf litter and soil organic matter, and they build burrows into deep soils. Based on these diverse feeding strategies and distribution patterns, the drilosphere can be developed differentially by different earthworm species. Endogeic earthworms are expected to have stronger and longer impacts on soil physical properties. Their intensive burrowing and casting activities affect the distribution of soil pores (porosphere), soil aggregates (aggregatusphere) and soil water infiltration capacity (Edwards 2004). Additionally, endogeic earthworms have effects on soil carbon dynamics as well. Mucus secretion in the earthworm guts, burrow linings and casts can alter the microbial activities and communities and change nutrient cycling (Edwards and Bohlen 1996, Edwards 2004, Mummey et al. 2006). Epigeic earthworms are believed to have less effect on soil physical structure, i.e. no effect on soil aggregation by epigeic *Eisenia fetida* (Hamilton and Dindal 1989). However, breaking down of litter substrate into small organic matter fractions to serve as a binding agent by epigeic earthworms can possibly enhance soil aggregate formation. Huang et al. (2006) found epigeic *Estherella* spp. had opposite effects, as compared to endogeic earthworms (*Pontoscolex corethrurus*), on soil aggregate distribution in Puerto Rican soil. For soil nutrient dynamics, epigeic earthworms may have more significant influences. Their comminution and digestion of litter substrate can determine not only the amount of nutrient input (mainly carbon and nitrogen) but also the distribution of microbial populations which further enhance soil nutrient cycling. Therefore, the actions of both epigeic and endogeic/anecic earthworms can potentially develop different impacts on microbial populations and nutrient cycling in the drilosphere.

Recently, “species invasion” has become an important issue, because invasive species can completely change the communities and functions of ecosystems (Parker et al. 1999, Mack et al. 2000). Invasive earthworms have been introduced into different terrestrial ecosystems over past decades. When invasive earthworms invade into new areas beyond their biogeographical boundaries, they cause significant impacts on local biota and ecosystem functions. Since the early 1900s, peregrine earthworm species have been introduced by human activities (including horticultural plant imports, habitat disturbance, and land-use changes, etc) and the distribution of invasive earthworms seems to extend to overlap the range of native earthworms (Fragoso et al. 1999, Hendrix and Bohlen 2002). Lavelle and Lapied (2003) indicated that many native earthworm species are in danger of extinction or have already disappeared in Amazonian areas that are now colonized by invasive species. On the other hand, the invasion of peregrine earthworms could not only cause the change of earthworm communities but have an impact on nutrient cycling, especially carbon and nitrogen dynamics (Hendrix and Bohlen 2002, Bohlen et al. 2004a, Bohlen et al. 2004b). For example, Pleistocene glaciations had eliminated all native earthworm species, if they ever existed, in the north temperate forests of North America. European lumbricids are the main invasive earthworms colonizing in these worm-free areas, since European settlements in the 1700s. In these worm-free temperate forests in New York, invasive earthworms caused the loss of forest floor organic matter, the alteration of soil microbial activities, and the changes of plant root and soil nutrient distributions (carbon, nitrogen, and phosphorous) in the soil profiles (Bohlen et al. 2004a, Bohlen et al. 2004b, Fisk et al. 2004, Groffman et al. 2004, Suárez et al. 2006). However, the impacts from earthworm invasions on soil nutrient cycling seem to vary depending on invasive earthworms themselves and other factors (i.e. invasion history and ecosystem characteristics). To manipulate all of these factors in a field experiment requires robust experimental design and substantial grant support. Furthermore, it is even more challenging to investigate long-term monitoring on earthworm invasions. Therefore, there is no such study providing comprehensive information of current earthworm invasions.

In this study, I developed a model to simulate the potential influences of the earthworms on soil carbon dynamics. Important components of below-ground ecosystems (forest floor litter, microbial population biomass, and soil organic matter) are included in this model. This model takes account not only direct consumption of earthworms but also indirectly casting activities for evaluation on earthworm invasions. In addition, this model considers time scales (decades) longer than those available observations in the literature.

Model structure and descriptions

This model was constructed based on a forest ecosystem and most of background data and model parameters were collected from the observation or experimental results in literature. The model has a daily time step for years of simulation by using Euler's integration method in the software package STELLA 8.1. All flows in the model are first-order kinetics. Main variables of the model are specified in Table 4.1.

Conceptual diagram of the model is shown in Figure 4.1. The model can be divided into two main components: aboveground and belowground ecosystems. The state variables aboveground include forest floor, litterfall input and carbon dioxide released from the microorganism and the earthworm, while microbial populations, soil carbon, and earthworm pools are belowground. The unit for each pool is presented as g C m^{-2} and fluxes are g C m^{-2} d^{-1}.

Forest floor litter (FF)

Forest floor litter (FF) presents organic matter accumulated on the soil surface. Litterfall (LF) provides external energy input into this model and the litterfall goes into the forest floor pool. The litterfall is designed to be evenly distributed between day 250 to 300 of a 365-day cycle to simulate the pulse of litterfall input in autumn. Forest floor organic matter is consumed mainly by earthworms and microorganisms. Consumption rates on litter by earthworms (CL_W) are determined depending on their feeding strategies (see *Earthworm pool* section). The amount of litter utilized by microbial populations (CL_M) was calculated from litter decomposition. Carbon dynamics of forest floor litter can be represented as:

$FF(t) = FF(t - dt) + (LF - CL_{W\text{-}Epi} - LM_{CAST} - CL_M - LS_{CAST} - LS) * dt$

Soil carbon pool (S)

All organic forms of carbon (except microbial populations and earthworms) in the soil are categorized into soil carbon pool (S). Carbon input for soil carbon pool includes organic matter that the earthworm and the microorganism have consumed but can not assimilate (WS_{CAST} and MS_{CAST}, respectively). In this model, the soil carbon pool is also designed as the main food resource for earthworms and microbial populations (CS_W and CS_M, respectively). The soil carbon pool is shown as follows:

$S(t) = S(t - dt) + (MS_{CAST} + DS_M + WS_{CAST} + DS_{W\text{-}Epi} + DS_{W\text{-}Endo} + LS_M - CS_{W\text{-}Endo} - LS_{CAST} - CS_{W\text{-}Epi} - CS_M) * dt$

<u>Microbial biomass pool</u> (MP)

The microorganisms utilize forest floor litter, soil carbon, earthworm debris and the nutrients in earthworm's casts (CL_M, CS_M, DW_M, and $WM_{CAST,}$ respectively) for energy production. Forest floor litter (decomposition) and soil carbon are the main resources for microbial population growth. Researchers have pointed out that earthworm casting activities can enhance microbial mineralization processes, because earthworms make nutrients more accessible for microbial populations after organic matter passes thought earthworm's gut. Also, there is more carbon in earthworm casts as compared to nearby soils which enhance microbial mineralization process (Edwards 2004). In this model, I incorporate the facilitating effects of earthworm cast depositions on microbial activity to simulate the situation. The rest of non-assimilated nutrients from earthworm consumption is hypothesized to be contained in the casts, and then is utilized by the microorganisms. Carbon flow through earthworm casts from litter and soil carbon consumption (LM_{CAST} and SM_{CAST}, respectively) to microbial biomass pool can be shown as:

$LM_{CAST} = (CL_W / AL_W \times (1 - AL_W)) \times A_W$

$SM_{CAST} = (CS_W / AS_W \times (1 - AS_W)) \times A_W$

Carbon outflow from microbial populations includes respiration loss (R_M) and death (D_M; goes to its own microbial biomass pool and the soil carbon pool) and grazing impacts by earthworms (CM_W). The assimilation and production efficiency of the microorganisms are set to be 0.9 and 0.23-0.26, respectively, in this model (Table 4.2). The dynamics of microbial populations can be shown as:

$MP(t) = MP(t - dt) + (LM_{CAST} + CL_M + SM_{CAST} + DM_W + CS_M - R_M - CM_W - D_M - MS_W) * dt$

Earthworm pool (E)

Earthworms can consume forest floor litter, microbial biomass, and soil carbon (CL_W, CM_W, and CS_W, respectively). The relative proportions of consumption among these food resources vary between different ecological groups of earthworms (epigeic, endogeic and anecic groups; Table 4.2). In this model, epigeic and anecic earthworms are hypothesized to mainly feed on forest floor litter (70%) and less on microbial populations (30%), when leaf litter resource is not limited. When leaf litter is below a certain level (in this model, 1 % of initial stock), I hypothesized that epigeic and anecic earthworms would ease their consumption on leaf litter (reduce from 70% to 50%) and switch to utilize soil carbon (20% of total energy production) to simulate the flexibility of earthworm feeding strategies. On the other hand, endogeic earthworms consume soil carbon for 70% of their energy production, and the rest (30 %) from microbial populations (Table 4.2). Respiration (R_W) and death (D_W) are the carbon losses from earthworm pool. Dynamics of epigeic earthworm population can be represented as:

$W_{Epi}(t) = W_{Epi}(t - dt) + (CL_{W\text{-}Epi} + CM_{W\text{-}Epi} + CS_{W\text{-}Epi} + WP_{Epi}\text{-}Pulse - R_{W\text{-}Epi} - DS_{W\text{-}Epi} - DM_{W\text{-}Epi}) * dt$

Assimilation efficiencies (A) of earthworm species are listed in Table 4.2. Assimilation efficiency varied among different earthworm species, temperature and food quality (Edwards and Bohlen 1996). Assimilation efficiency has been reported as 2-18% for some endogeic species, while assimilation efficiencies for *Lumbricus terrestris* (anecic species; litter-feeding species) was measured as 30-70% (Edwards and Bohlen 1996). However, assimilation efficiencies of *Allolobophora rosea* (endogeic earthworms) in a beechwood and a mixed community of Megascolecidae and Eudrilidae from a savanna were 0.9-1.3% and 8.9%, respectively (Table 6.3 in Edwards and Bohlen 1996). In this model, assimilation efficiency of the earthworm on leaf litter (detritivore; AL_W), soil organic matter (AS_W) and the microorganisms (microvore; AM_W) are set to 2%, 1% and 30 %, respectively (Table 2). These values are reasonable for soil detritivores and microvores (Fig. 18.22 in Begon et al. 1996).

Carbon dynamics by epigeic/anecic earthworm consumption is represented as:

$CL_{W\text{-}Epi} = CRL_{W\text{-}Epi} \times W_{Epi} \times AL_{W\text{-}Epi}$ // from litter consumption

$CM_{W\text{-}Epi}$ = IF (FF>0.01×Initial FF) THEN ($CL_{W\text{-}Epi}$ /0.7/ $AL_{W\text{-}Epi}$ *0.3* $AM_{W\text{-}Epi}$) ELSE ($CL_{W\text{-}Epi}$ /0.5/ $AL_{W\text{-}Epi} \times 0.3 \times AM_{W\text{-}Epi}$) // from the microorganisms

$CS_{W\text{-}Epi}$ = IF (FF > 0.01*Initial FF) THEN ($CL_{W\text{-}Epi}$/0.7*0.3) ELSE ($CL_{W\text{-}Epi}$/0.5*0.3) // from soil organic matter

Carbon dynamics by endogeic earthworm's consumption:

$CS_{W\text{-}Endo} = CRS_{W\text{-}Endo} \times W_{Endo} \times AS_{W\text{-}Endo}$ // from soil organic matter

$CM_{W\text{-}Endo} = CS_{W\text{-}Endo}/0.7*0.3$ // from the microorganisms

Natural death of microbial (D_M) and earthworm (D_W) populations

The populations of microorganisms and earthworms are regulated mostly by their natural death. The microbial population is controlled by earthworm predation and their own natural death. Earthworm populations are only considered by their natural death and the predation of earthworms is neglected to simplify the model. In the field, this is probably also true for earthworm invasions. Peregrine species are believed to be released from predation pressure when they invade into a new habitat (Sax et al. 2005). For the death of earthworms and microorganisms, I have adopted the “minimum population” protection mechanism from Fu et al. (2000). The concept suggested that the death rates of earthworms and microorganisms are proportional to their own population sizes, but the death rates go to zero as their populations reach a minimum size (W_{Min} and MP_{Min}, respectively; Table 4.2). Mean natural death rates (g C day^{-1}) adopted from Fu et al. (2000) are 0.0137 and 0.00328 for the earthworms and the microorganisms, respectively. The minimum populations for epigeic, anecic and endogeic earthworms are set for 25, 25, and 20 g earthworm fresh weight m^{-2}.

$D_M = 0.00328 \times MP \times MAX(0, 1-MP_{Min}/MP)$

$D_W = 0.01370 \times W \times MAX(0, 1-W_{Min}/W)$

Respiration carbon output

The carbon outflow of this model is the production of carbon dioxide (CO_2) from respiration of earthworms and the microorganisms.

$R_M = (DM_W + LM_{CAST} + CL_M + SM_{CAST} + CS_M) \times (1-P_M)$

$R_W = (CL_W + CM_W + CS_W) \times (1-P_W)$

Temperature regulation of earthworm populations

Earthworm populations are sensitive to annual and daily temperature fluctuation. In this study, I build the temperature module in the model to simulate temperature influences on earthworm populations, in particular their feeding activities. A yearly temperature cycle is simulated by using SINWAVE function in the Stella software package. Maximum and minimum average temperature values and the range between them are set for the upper and lower peaks and amplitude of sinwave in the yearly cycle.

A relationship is created for earthworm consumption rates on soil and leaf litter carbon for endogeic and epigiec/anecic earthworm groups, respectively, under the influence of temperature. Based on the literature, maximum consumption rates of earthworms are set for their preferred temperature ranges, for example, 15-20 ^{o}C for European epigeic/anecic and 10-15 ^{o}C for European endogeic earthworms (Edwards and Bohlen 1996). Within their preferred temperature range, earthworms have maximum consumption rates on soil organic matter or leaf litter to simulate earthworm feeding activities affected by temperature. The consumption rates gradually decline when the temperature rises over or falls below the preferred temperature range for earthworms. Therefore, feeding activities of earthworms are regulated by daily temperature fluctuation. The temperature-earthworm relationships are listed as follows:

Temp-W_{Endo} = GRAPH (Temp, Consumption Efficiency)

(-4.00, 0.00), (-2.00, 0.00), (0.00, 0.00), (2.00, 0.1), (4.00, 0.4), (6.00, 0.6), (8.00, 0.8), (10.0, 1.00), (12.0, 1.00), (14.0, 1.00), (16.0, 0.95), (18.0, 0.85), (20.0, 0.75), (22.0, 0.7)

Temp-W_{Epi} = GRAPH (Temp, Consumption Efficiency)

(-4.00, 0.00), (-2.00, 0.00), (0.00, 0.00), (2.00, 0.1), (4.00, 0.2), (6.00, 0.4), (8.00, 0.65), (10.0, 0.75), (12.0, 0.85), (14.0, 0.95), (16.0, 1.00), (18.0, 1.00), (20.0, 1.00), (22.0, 0.95)

Model Calibration

Field data from Arnot forest, New York, were chosen for calibration of this model (Bohlen et al. 2004a, Groffman et al. 2004). Arnot forest in Central New York is a maple-dominated forest with no history of agricultural activity. Average temperature is 22 ^{o}C in the summer and -4 ^{o}C in the winter. Average annual rainfall is 100 cm. Soils in Arnot forest are categorized as acidic Dystrochrepts with well-developed forest floor (ca. 4 cm thick) and an acidic mineral horizon (Bohlen et al. 2004a). Since logging occurring in the late nineteen century, minimal disturbance has occurred in Arnot forest. However, European earthworms have probably invaded in the temperate forests of North America since the 1700s and continue today (Frelich et al. 2006). From the earthworm survey from Bohlen et al. (2004a), six European earthworm species have been found inhabiting in the Arnot forest. Comprehensive research on earthworm impacts on ecosystem processes have been conducted in Arnot forest since the late 1990s. In Arnot forest, three pairs of worm-free (non-invaded reference plot; earthworm density < 2 individuals m^{-2}) and worm-invaded (earthworm density > 150 individuals m^{-2}) plots were carefully selected to avoid differences in soils, vegetation, and topography among plots (Bohlen et al. 2004a). Impacts of European earthworm invasion in Arnot forest were investigated and evaluated by the difference between paired worm-free and worm-invaded plots. Bohlen et al. (2004b) documented the impacts of invasive earthworms on soil carbon and nitrogen dynamics in Arnot forest. Fisk et al. (2004) found no effect of invasive earthworms on soil carbon emission and fine-root nitrogen concentration. Earthworm invasions in Arnot forest have been found to increase microbial biomass carbon and soil respiration in the mineral soils but not nitrogen mineralization and nitrification (Groffman et al. 2004). Therefore, Arnot forest was chosen for model calibration for its comprehensive background information on earthworm communities and ecosystem carbon stocks (forest floor, microbial population, and nutrient dynamics). In particular, field data from paired worm-free and worm-invaded sites in three plots of Arnot forest provide more precise and complete comparisons.

This model was calibrated in two phases: 1) an equilibrium (steady status) ecosystem was established first without earthworms; 2) then earthworm populations are introduced into the equilibrium ecosystem and carbon dynamics observed in the ecosystem. In the first phase, the model was run with field data from worm-free plots and parameters from literature. Then literature parameter values were adjusted to keep the ecosystem at "steady status" in terms of stable forest floor stock, soil carbon, and microbial population in each plot of Arnot forest. Litterfall data, 235 g C m^{-2} y^{-1}, was adopted from Fisk et al. (2004) for each plot. All carbon stocks (forest floor, microbial biomass, and soil carbon) from worm-free plots were adopted from field data of Arnot forest (Bohlen et al. 2004b, Fisk et al. 2004, Groffman et al. 2004). Litter decomposition is attributed completely to microorganism activities. The decay rates of litter disappearance (k) ranged from -0.43 to -0.55 for oak and sugar maple litter in Arnot forest (Suárez et al. 2006). However, the decay rates from Suárez et al. (2006) included the loss of fragmented litter particles which was translocated into mineral soils but have not been utilized by the microorganisms. Hence, decomposition rates of litter due to microbial activities in this model were set between 7-12 % of the forest floor carbon stock every year, instead of using decay rates, and the value was adjusted accordingly to maintain ecosystem equilibrium in each plot. Consumption rate by the microorganisms on soil organic matter was hypothesized as 2.2 % of the soil carbon pool every year as a default value and modified thereafter to the keep ecosystem at "steady status" (equilibrium) in the first phase before introducing earthworms into the ecosystem. This steady state of ecosystem stocks (forest floor litter, soil carbon, and microbial population data from worm-free plot) was used as the initial condition before adding earthworm communities into the model (Table 4.3). After the ecosystem achieved steady state, earthworms were introduced in the model at a one-time pulse with population size of 0.01 g C m^{-2}. Composition of earthworm communities introduced into the model varied accordingly to the field survey from each plot of Arnot forest. Epigeic *Lumbricus rubellus* and endogeic *Octolasion tyrtaeum* were the dominant earthworm species in worm-invaded plot 1 and plot 2 (Bohlen et al. 2004a)(Table 4.3), while epigeic *L. rubellus*, anecic *Lumbricus terretris*, and endogeic *Aporrectodea tuberculata* were the three main earthworm species inhabiting worm-invaded plot 3 (Bohlen et al. 2004a)(Table 4.3). Maximum consumption rates on litter and soil organic matter by earthworms were gathered from the literature. Maximum litter consumption by epigeic/anecic earthworms were adopted based on the experimental data of *Lumbricus terrestris* (0.08 mg $gram^{-1}$ earthworm fresh weight day^{-1}). The amount of microbial population and soil organic matter consumed by epigeic/anecic earthworms was calculated accordingly based on food allocation mentioned earlier (see *Earthworm pool* section, Table 4.2) (Edwards and Bohlen 1996). Soil consumption rates by *Aporrectodea caliginosa* (200 mg soil $gram^{-1}$ earthworm fresh weight day^{-1}) from the literature were used to calculate total soil carbon digested by endogeic earthworms (Edwards and Bohlen

1996). Also, the amount of microbial population consumed by endogeic earthworms was derived from food allocation as well (Table 4.2). For simulation of flexible feeding behaviors observed in the earthworms, epigeic earthworms would change their food preference and proportions as their main food resource became limited (when forest floor litter level fell below 1% of initial stock) (Table 4.2). The model was run for 72 years for the calibration and the simulation data was compared to the field data from worm-invaded plots (Table 4.3).

The calibration results showed that the simulation data fit the field data fairly well. Simulation results on invasion time have shown that it took longer for plot 3 to reach the field collected data than plot 1 and plot 2 of Arnot forest (Table 4.3 and and Fig. 4.2-4.5). This may be due to different earthworm communities and different pre-invasion forest floor litter, soil carbon pool, and microbial populations among different plots. This suggested that the impacts of invasive earthworms may depend on invasion histories, pre-invasion ecosystem conditions, and invasive earthworm assemblages. Earthworm invasions with more epigeic and anecic earthworm species may have more significant influences on forest floor organic matter pools than endogeic earthworm based on model simulation. Invasive endogeic earthworm species affect soil organic matter dynamics in a significant way but slowly, and this might be due to the feeding biology of endogeic earthworm species.

Comparisons of different functional groups of earthworms

To single out the impacts of epigeic/anecic and endogeic earthworm species on ecosystem processes, the model was simulated by introduction of epigeic or endogeic earthworm species separately (Fig. 4.6 and 4.7). The model was run for 72 years by using pre-invasion conditions of plot 3 in Arnot forest. Results showed that the introduction of endogeic earthworms has a slow influence on both forest floor and soil carbon pool (Fig. 4.6). It took ca. 57 years for endogeic earthworm species to establish and maintain a stable earthworm population (Fig. 4.8A). Therefore, its impacts on forest floor and soil carbon pool significantly occurred after 52 years of invasions (Fig. 4.6A). The invasion of endogeic earthworms decreased the microbial population and their respiration at the beginning of the invasion but then enhanced both of them after the earthworm population started to act on forest floor and soil carbon pool (Figure 4.6B and C).

Interestingly, the invasion of epigeic earthworms showed completely different patterns as compared to that of endogeic earthworms. It only took epigeic earthworms ca. 8 years to reach a maximum and stable population in the forest ecosystems (Fig. 4.8B). Epigeic earthworm species began to significantly destruct forest floor litter after ca.7 years of invasion but slightly increase the soil carbon pool (Fig. 4.7A). At the same time, epigeic earthworms also enhanced the microbial population and microbial respiration (Fig. 4.7B and 4.7 C). The microbial population and respiration was raised dramatically at first then dropped back to initial levels where they remained afterward. The impacts of invasive earthworms on ecosystem function, in particular carbon dynamics, are strongly dependent on their feeding strategies (Figure 4.6 and 4.7).

Sensitivity analysis

A sensitivity analysis was done for the parameters of this model, including assimilation and production efficiency of earthworms and microbes (Table 4.4). The value of each parameter was set ± 10 % of its default value. Only one parameter value was changed for each time of sensitivity analysis while keeping other parameters at the default values. Results of sensitivity analysis showed that microbial assimilation efficiency had significant impacts on soil carbon pool and microbial respiration, while its production efficiency affected only its own population size after 72 years of model simulation. Earthworm production efficiency also influenced their population size as well. Earthworm assimilation efficiencies on soil organic matter and litter had more impacts on the time for endogeic and epigeic earthworms to reach stable population, respectively (Table 4.4).

Discussion

Invasive earthworms have caused significant impacts on local populations, community structure, and ecosystem functions. They have altered forest floor structure, soil texture, plant communities, and nutrient cycling (Groffman et al. 2004, Hale et al. 2005, Frelich et al. 2006). Studies have also pointed out that current disappearance and decrease of native earthworm species may be partly a result of invasive earthworms, especially competitive pressure (Lavelle and Lapied, 2003; Hendrix et al. 2006). However, evidence for these impacts of invasive earthworms are published in different literature and scattered around different journals. To date, there have been no studies that integrate all information to give a whole picture of how exactly invasive earthworms affect ecosystem functions from litter decomposition, to soil microbial population, and to other earthworm species. In the mean time, current studies of earthworm invasions are mostly one-time field survey or laboratory experiments. The lack of invasion history and invasion patterns, insufficient information about invasion earthworm assemblages, and the difficulty of separating environmental factors have made evaluations of earthworm invasions and comparisons between different studies very challenging.

The occurrence of earthworm invasions has often been related to human activities. While human activities are possibly traceable, there are still difficulties to trace back the timeline and the pathway of peregrine earthworm invasions. Also, it is difficult to evaluate the impacts if there are only taxonomic lists of invasive earthworms and one-time earthworm biomass and density data available. The life cycles and population dynamics of invasive earthworms are valuable to understand their colonizing stages in the invaded areas. Whether invasive earthworms are newly introduced or have invaded for a long time affect the evaluations on their influences in ecosystems. Earthworm invasion is much more complicated than we expect because of different assemblages of invasive earthworms and ecosystem-specific characteristics. It is impossible to make a general conclusion and pattern to apply for all earthworm invasion events. The composition of invasive earthworms, especially earthworm ecological groups, is decisive for the magnitude and degree of the impacts on ecosystem functions. Vegetation types, soil properties, and climate factors can play a role in selecting invasive earthworm assemblages and determining the consequences through resource availability and climate fluctuation (i.e. temperature range).

Few models have been developed for earthworms on their impacts on litter consumption and nutrient cycling (Barot et al. 2007, Hobbelen and van Gestel 2007). Hobbelen and van Gestel (2007) ran a dynamic energy budget model to predict the effects of temperature and food on litter consumption by earthworms to Cu pollution.

This model can be applied to the risk assessment of heavy metal pollution by using earthworm as a model detritivore. Barot et al. (2007) ran a model of limiting nutrient cycles following three pathways: without earthworms, with earthworm trophic (direct) effects, and with earthworm nontrophic (indirect) effects. Their simulation results indicated that the presence of earthworms could increase primary production through trophic and non-trophic effects because of the conservation of nutrients in ecosystems (Barot et al. 2007). They also emphasized the necessity of evaluating earthworm impacts on nutrient balance in ecosystems. However, their model focused on nutrient fluxes mainly with no particular emphasis on nutrient pool dynamics.

Frelich et al. (2006) have suggested that soil nutrient dynamics and changes caused by earthworm invasions may possibly cause tree-ring signature due to the changes of vegetation successional trajectory and productivity. They recommended that this may be a good way to date the time of earthworm invasion. However, the earthworm model in this study has provided a more direct way on evaluation of invasion time based on earthworm effects on carbon dynamic. This model can not only provide the evaluation of impacts through the integration of ecosystem stocks and carbon flows, but evaluate the invasion history based on different species characteristics of invasive earthworms. Beyond that, the flexibility and operation of the model can also help us to separate out the influences for single earthworm species and their interactions.

Simulation results from the model have shown that ecosystem carbon dynamics varied depending on different ecological groups of earthworms. From the comparison of simulation results between epigeic and endogeic earthworms, epigeic earthworm species have significant impacts on forest floor pools in a very short time period after invasions.

Shorter generation time, higher fecundity, and higher consumption on litter organic matter may make epigeic earthworm more aggressive as compared to endogeic earthworms, especially in the case of invasive earthworms (Barrios et al. 1999). However, current ecological categories of earthworm feeding behaviors are too general to apply to earthworm ecology studies in the field (Hale et al. 2005). For example, Hale et al. (2005) suggested that even *Dendrobaena octaedra* and *Lumbricus rubellus*, identified as epigeic groups, did have different effects due to the latter having more burrowing and casting activities. Among invasive earthworm species, the epigiec earthworm (*L. rubellus*) is a rapid and large impact invader; the anecic earthworm (*Lumbricus terrestris*) has slow but large impacts; while epigeic earthworm, *D. octaedra* is a slow engineer with small impacts for forest floor habitats as compared to the former two species (Hale et al., 2005; Frelich et al., 2006). Therefore, invasive earthworm assemblages significantly determine the consequences and responses of ecosystems. Calibration results from Arnot forest showed that different earthworm assemblages did cause differential outcomes on carbon dynamics. From simulation results of plot 3, the addition of one more epigeic/anecic earthworm species into the ecosystem eliminated forest floor litter in a shorter time and altered carbon dynamics in a different way.

On the other hand, simulation results from the model also suggested that pre-invasion ecosystem conditions and invasion history could be important as well. Pre-invasion condition of ecosystems (i.e. carbon and nitrogen pools) is critical for each earthworm invasion event. Groffman et al. (2004) suggested that site history (in their case: forest floor thickness due to agricultural cultivation) can strongly affect the nature and extent of impacts on microbial pools from earthworm invasions. Resource availability in the invaded ecosystem may determine the maximum of invasive earthworm species it can carry, the composition of earthworm assemblages and dominant earthworm species. In the case of Arnot forest, the model was able to calculate invasion history due to comprehensive field data from the literature, except the time point when invasive earthworms arrived and established the populations. The simulation model gave rough but comparable invasion times among these three plots. European earthworms might have invaded into Plot 3 first and then extended into Plot 2 and Plot 1. Or invasion events in Plot 3 were different from the ones in Plot 2 and Plot 1. However, more information on human activities (i.e. land-use managements and settlement history) is needed to adequately address these hypotheses.

In the meantime, details of resource utilization and life characteristics of single earthworm species, as well as the potential interactions between earthworm species will be extremely valuable for making precise evaluation of invasion time. The pulse function built into the earthworm model can be used to simulate single or multiple invasion events at different time points by different earthworm species. Frelich et al. (2006) suggested the invasion order of invasive earthworm species may result in completely opposite consequences on ecosystem functions, in particular above-ground plant communities. They suggested that the early invasion of *L. rubellus* followed by *L. terrestris* may result in more devastating impacts on plant communities than the reverse order of invasion in north Minnesota temperate forests. This earthworm model could be applied to test this hypothesis and the results used to compare with field data.

Sensitivity analysis showed that assimilation efficiency of the microorganisms has significant effect on the soil carbon pool, microbial population and microbial respiration.

Assimilation efficiency of earthworms on food resources affects dynamics of earthworm populations. These results indicate that earthworm assimilation efficiency on their main resources is critical for the magnitude and rapidity of their potential impacts. In the presence of earthworms, microbial populations have more influence on the soil carbon pool but not litter pool. This is probably because their mineralization effects on forest floor litter become less significant as compared to the huge amount of litter consumption by earthworms. Therefore, earthworm assimilation efficiency on different resources is critical to reliable evaluation and prediction of earthworm invasions. More information on species characteristics for single earthworm species and earthworm interactions is much needed, especially for important peregrine and endemic native earthworm species.

This earthworm model can apply both to the ecosystem where peregrine earthworms have invaded or are potentially at risk of invasion. For the invaded forests, invasion history and future invasion patterns (i.e. earthworm population and carbon dynamics) can be predicted based on the existing knowledge of invasive earthworm assemblages and ecosystem conditions. This can help development of conservation and regulation policies to prevent expansion of current invasion and further earthworm invasions. For newly invaded habitats or some areas vulnerable to earthworm invasions, dynamics of invasive earthworm populations and responses of ecosystem functions can be evaluated by earthworm model for different invasional stages. Comparisons between model simulation results and field observations can provide the information on potential mechanisms and assess the interactions and the importance of ecosystem components. Even though this earthworm model is based on forest ecosystems, it can still apply to other type ecosystems. The components of the earthworm model (litterfall, forest floor litter, soil carbon, microbial population, earthworm populations) are all fundamental elements to collect from field researches. Therefore, the earthworm model is useful and simple for application of earthworm invasions in different ecosystems.

Literature Cited:

Barot, S., A. Ugolini, and F. B. Brikci. 2007. Nutrient cycling efficiency explains the long-term effects of ecosystem engineers on primary production Functional Ecology 21:1-10.

Begon, M., J. L. Harper, and C. R. Townsend. 1996. Ecology: individuals, populations and communities. Blackwell Science.

Bohlen, P. J., P. M. Groffman, T. J. Fahey, M. C. Fisk, E. Suárez, D. M. Pelletier, and R. T. Fahey. 2004a. Ecosystem consequences of exotic earthworm invasion of north temperate forests. Ecosystems 7:1-12.

Bohlen, P. J., D. M. Pelletier, P. M. Groffman, T. J. Fahey, and M. C. Fisk. 2004b. Influence of earthworm invasion on redistribution and retention of soil carbon and nitrogen in northern temperate forests. Ecosystems 7:13-27.

Bouché, M. B. 1977. Strategies Lombriciennes. Pages 122-132 *in* U. Lohm and T. Persson, editors. Soil organisms as components of ecosystems. Proc. 6th Int. Coll. Soil Zool., Ecological Bulletin, Stockholm.

Brussaard, L. 1998. Soil fauna, guilds, functional groups and ecosystems processes. Applied Soil Ecology 9:123-135.

Brussaard, L., M. M. Pulleman, E. Ouedraogo, A. Mando, and J. Six. 2007. Soil fauna and soil function in the fabric of the food web. Pedobiologia 50:447-462.

Chertov, O. G., and A. S. Komarov. 1997. SOMM: A model of soil organic matter dynamics. Ecological Modelling 94:177-189.

Coleman, D. C., D. A. Crossley, and P. F. Hendrix. 2004. Fundamentals of soil ecology. 2nd Edition. Elsevier Academic Press, San Diego.

de Ruiter, P. C., J. Bloem, L. A. Bouwman, W. A. M. Didden, G. H. J. Hoenderboom, G. Lebbink, J. C. Y. Marinissen, J. A. de Vos, M. J. Vreeken-Buijs, K. B. Zwart, and L. Brussaard. 1994a. Simulation of dynamics in nitrogen mineralization in the belowground food webs of two arable farming systems. Agriculture Ecosystems and Environment 51:199-208.

de Ruiter, P. C., A.-M. Neutel, and J. C. Moore. 1994b. Modelling food webs and nutrient cycling in agro-ecosystems. Trends in Ecology & Evolution 9:378-383.

Edwards, C. A. 2004. Earthworm ecology. CRC Press, Boca Raton.

Edwards, C. A., and P. J. Bohlen. 1996. Biology and ecology of earthworms. Chapman & Hall, New York.

Fisk, M. C., T. J. Fahey, P. M. Groffman, and P. J. Bohlen. 2004. Earthworm invasion, fine-root distributions, and soil respiration in North temperate forests. Ecosystems 7:55-62.

Fragoso, C., J. Kanyonyo, A. Moreno, B. K. Senapati, E. Blanchart, and C. Rodríguez. 1999. A survey of tropical earthworms: taxonomy, biogeography and environmental plasticity. Pages 1-26 *in* P. Lavelle, L. Brussaard, and P. Hendrix, editors. Earthworm management in tropical agroecosystems. CABI Publishing, New York.

Frelich, L. E., C. M. Hale, S. Scheu, A. R. Holdsworth, L. Heneghan, P. J. Bohlen, and P. B. Reich. 2006. Earthworm invasion into previously earthworm-free temperate and boreal forests. Biological Invasions 8:1235-1245.

Fu, S., M. L. Cabrera, D. C. Coleman, K. W. Kisselle, C. J. Garrett, P. Hendrix, and D. A. Crossley. 2000. Soil carbon dynamics of conventional tillage and no-till agroecosystmes at Georgia Piedmont-HSB-C models. Ecological Modelling 131:229-248.

Groffman, P. M., P. J. Bohlen, M. C. Fisk, and T. J. Fahey. 2004. Exotic earthworm invasion and microbial biomass in temperate forest soils. Ecosystems 7:43-54.

Hale, C. M., L. E. Frelich, P. B. Reich, and J. Pastor. 2005. Effects of European earthworm invasion on soil characteristics in Northern hardwood forests of Minnesota, USA. Ecosystems 8:911-927.

Hamilton, W. E., and D. L. Dindal. 1989. Impact of landspread sewage sludge and earthworms introduction on established earthworms and soil structure. Biology and Fertility of Soils 8:160-165.

Hastings, A., J. E. Byers, J. A. Crooks, K. Cuddington, C. G. Jones, J. G. Lambrions, T. S. Talley, and W. G. Wilson. 2007. Ecosystem engineering in space and time. Ecology Letters 10:153-164.

Heal, O. W., and S. F. MacLean. 1975. Comparative productivity in ecosystems-secondary productivity. Pages 89-108 *in* W. H. van Dobben and R. H. Lowe-McConnell, editors. Unifying concept in ecology. The Hague, Junk.

Hendrix, P. F., and P. J. Bohlen. 2002. Exotic earthworm invasions in North America: ecological and policy implications. BioScience 52:801-811.

Hobbelen, P. H. F., and C. A. M. van Gestel. 2007. Using dynamic energy budget modeling to predict the influence of temperature and food density on the effect of Cu on earthworm mediated litter consumption. Ecological Modelling 202:373-384.

Huang, C.-Y., G. González, and P. F. Hendrix. 2006. The re-colonization ability of native earthworm, *Estherella* spp., in Puerto Rican forests and pastures. Caribbean Journal of Science 42:386-396.

Hunt, H. W., D. C. Coleman, E. R. Ingham, R. E. Ingham, E. T. Elliott, J. C. Moore, S. L. Rose, C. P. P. Reid, and C. R. Morley. 1987. The detrital food web in a shortgrass prairie. Biology and Fertility of Soils 3:57-68.

Lavelle, P., L. Brussaard, and P. Hendrix, editors. 1999. Earthworm Management in Tropical Agroecosystems. CABI Publishing, New York.

Lavelle, P., and E. Lapied. 2003. Endangered earthworms of Amazonia: an homage to Gilberto Righi. Pedobiologia 47:419-417.

Lee, K. E. 1985. Earthworms, their ecology and relationships with soils and land use. Academic Press, New York.

Mack, R. N., D. Simberloff, M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecological Applications 10:689-710.

Moore, J. C., E. L. Berlow, D. C. Coleman, P. C. de Ruiter, Q. Dong, A. Hastings, N. C. Johnson, K. S. McCann, K. Melville, P. J. Morin, K. Nadelhoffer, A. D. Rosemond, D. M. Post, J. L. Sabo, K. M. Scow, M. J. Vanni, and D. H. Wall. 2004. Detritus, trophic dynamics and biodiversity. Ecology Letters 7:584-600.

Moore, J. C., P. C. de Ruiter, H. W. Hunt, D. C. Coleman, and D. W. Freckman. 1996. Microcosms and soil ecology: critical linkages between field studies and modelling food webs. Ecology 77:694-705.

Muller, C. 1999. Modelling soil-biosphere interactions. CABI International, Oxon, UK.

Mummey, D. L., M. C. Rillig, and J. Six. 2006. Endogeic earthworms differentially influence bacterial communities associated with different soil aggregate size fractions. Soil Biology and Biochemistry 38:1608-1614.

Parker, I. M., D. Simberloff, W. M. Lonsdale, K. Goodell, M. Wonham, P. M. Kareiva, M. H. Williamson, B. von Holle, P. B. Moyle, J. E. Byers, and L. Goldwasser. 1999. Impact: toward a framework for understanding the ecological effects of invaders. Biological Invasions 1:3-19.

Pimm, S. L. 2002. Food webs. Chapman & Hall, London.

Pimm, S. L., J. H. Lawton, and J. E. Cohen. 1991. Food web patterns and their consequences. Nature 350:669-674.

Sax, D. F., J. J. Stachowize, and S. D. Gaines, editors. 2005. Species invasions: Insights into ecology, evolution, and biogeography. Sinauer Associates Inc., Sunderland, MA.

Spiers, G. A., D. Gagnon, G. E. Nason, E. C. Packee, and J. D. Lousier. 1986. Effects and importance of indigenous earthworms on decomposition and nutrient cycling in castal forest ecosystems. Canadian Journal of Forest Research 16:983-989.

Suárez, E. R., T. J. Fahey, J. B. Yavitt, P. M. Groffman, and P. J. Bohlen. 2006. Patterns of litter disappearance in a Northern hardwood forest invaded by exotic earthworms. Ecological Applications 16:154-165.

Table 4.1. List of variables used in the Earthworm model.

Variables	Interpretation	Unit
Stock		
FF	Forest floor litter (Organic matter)	g m^{-2}
S	Soil organic carbon	g m^{-2}
MP	Microbial biomass pool	g m^{-2}
W	Earthworm biomass pool	g m^{-2}
Flow		
LF	Litterfall	g m^{-2} day^{-1}
LM_{CAST}	Earthworm casts to microbial pool through litter consumption	g m^{-2} day^{-1}
SM_{CAST}	Earthworm casts to microbial pool through soil consumption	
LS_{CAST}	Earthworm casts to soil pool through litter consumption	g m^{-2} day^{-1}
MS_{CAST}	Earthworm casts to soil pool through consumption on microbes	
LS_M	Litter to soil pool through microbial consumption	g m^{-2} day^{-1}
MS_W	Microbial pool to soil pool through earthworm consumption	g m^{-2} day^{-1}
$CRL_{W\text{-}Epi}$	Litter consumption rate of epigeic earthworms	g m^{-2} day^{-1}
$CRS_{W\text{-}Endo}$	Soil consumption rate of endogeic earthworms	
CS_W/CS_M	Consumption on soil carbon by earthworms/microorganisms	g m^{-2} day^{-1}
CL_W/CL_M	Consumption on litter by earthworms/microorganisms	g m^{-2} day^{-1}
CM_W	Consumption on the microorganisms by earthworms	g m^{-2} day^{-1}
AS_W/ AL_W	Assimilation efficiency of earthworms on soil/litter	g m^{-2} day^{-1}
A_M	Assimilation efficiency of microorganisms	
P_W/P_M	Production efficiency of earthworms/microorganisms	g m^{-2} day^{-1}
$DS_{M/W}$	Dead microbial/earthworm biomass to soil pool	g m^{-2} day^{-1}
DM_W	Dead earthworm biomass to microbial pool	g m^{-2} day^{-1}
R_M	Respiration from microbial population	g m^{-2} day^{-1}
R_W	Respiration from earthworm population	g m^{-2} day^{-1}

Table 4.2. List of parameters used in the Earthworm model.

Parameter	Earthworms Endogeic	 Epigeic	 Anecic	Microbes
Food fraction (%)				
Leaf	0	70(50) *	70(50) *	---
Microbes	30	30	30	---
Soil	70	0(20)	0(20)	---
Assimilation efficiency				
Leaf [a]	0.02	0.02	0.02	0.9
Microbes [b]	0.3	0.3	0.3	0.9
Soil	0.01	0.01	0.01	0.9
Production efficiency [c]	0.4	0.4	0.4	0.23-0.265
Natural death rate (day^{-1}) [c]	0.0137	0.0137	0.0137	0.00328
Minimum population (g C/m^2)	20	25	25	62.8-82.5

* Numbers in the parentheses showed that food fraction of earthworm changes when main food resource (forest floor litter) is limited.

a: Adjusted from Lee (1985)

b: Adapted from Heal and MacLean (1975)

c: Adapted from Fu et al.(2000)

Table 4.3. Comparisons of experimental data and simulation results on carbon stocks in Arnot forest ecosystem, New York.

	Carbon pool			
	Forest floor [a]	Microbial pool [b]	Soil pool [c]	Microbial respiration
Arnot Forest, New York, USA				
Plot 1				
No-worm	2005.6 (2005.6-2005.7)	62.86 (32.7-108.3)	4622.46	1.84 (0.2-3.32)
Worm-invaded	37.14 (0-260)	82.53 (25.0-138.4)	4510.59	2.24 (0.62-3.56)
Stimulation Results (39.5 yrs) [d]	119.5	70.56	4510.60	0.96
Earthworm communities [a]	*Lumbricus rubellus* (epigeic); *Octolasion tyrtaeum* (endogeic)			
Plot 2				
No-worm	1360.3 (1360.2-1360.4)	64.9 (20.2-119.2)	4609.40	1.33 (0.42-2.54)
Worm-invaded	43.8 (0-184)	75.2 (21.8-128.9)	4070.57	1.83 (0.59-3.39)
Stimulation Results (59.5 yrs) [d]	15.71	78.3	4070.58	0.89
Earthworm communities [a]	*L. rubellus* (epigeic); *O. tyrtaeum* (endogeic)			
Plot 3				
No-worm	3540.8 (3540.8-3540.9)	82.5 (29.1-165.3)	7600.01	2.45 (0.68-4.28)
Worm-invaded	75.29 (0-527)	99.6 (29.3-244.4)	3599.65	2.75 (0.61-9.93)
Stimulation Results (>80 yrs) [d]	136.97	80.89	4504.02	1.30
Earthworm communities [a]	*L. rubellus* (epigeic); *Aporrectodea tuberculata* (epigeic); *Lumbricus terrestris* (anecic)			

Note: Litterfall data (235 g C m^{-2} y^{-1}) was adapted from Fisk et al. (2004). Data from no-worm plots was set as initial condition during the model calibration. The simulation period is 40 years. All data are shown as g C m^{-2}, except microbial respiration (g C m^{-2} d^{-1}).

a: Data (Unit g m^{-2}) were adapted from Bohlen et al. (2004a). b: Data were adapted from Groffman et al. (2004).

c: Soil carbon data (upper 12 cm) were adapted from Bohlen et al. (2004b). d: Number in parentheses indicated the simulation time for the simulation data.

Table 4.4. Sensitivity analysis of the parameters in Earthworm model.

Parameter	Microbial biomass	Time for FF to fall below 100 (days)	Soil carbon	Microbial respiration	Time for earthworm population to reach maximum population	
					Endogeic	Epigeic/anecic
Microbial assimilation efficiency (A_M)						
+ 10 %	- 4.47 (5 %)	-34 (0.4%)	-1408 (28.3%)	+1413 (5.8%)	0	0
- 10 %	+ 4.04 (5 %)	+302 (3.7%)	+1551(31.2%)	-1555 (6.4%)	0	0
Earthworm assimilation efficiency						
on soil organic matter (AS_W)						
+ 10 %	-1.3 (1.6 %)	0	-108 (2.2%)	+106 (0.4%)	-1844 (7.9%)	0
- 10 %	+1.73 (2.0 %)	0	+158 (3.2%)	-153 (0.6%)	+2244 (9.6%)	0
on litter (AL_W)						
+ 10 %	+0.1(0.1 %)	-83 (1.0%)	-123 (2.5%)	+93 (0.4%)	0	-335 (5.7%)
- 10 %	+0.1 (0.1 %)	+358 (4.4 %)	+133 (2.7%)	-101 (0.4%)	0	+170 (2.95)
on microbial population (AM_W)						
+ 10 %	+0.05(<0.1 %)	0	-2.6 (<0.1 %)	+3.9 (<0.1 %)	0	0
- 10 %	-0.07 (<0.1 %)	0	+4.9 (<0.1 %)	-3.6 (<0.1 %)	0	0
Earthworm production efficiency (P_W)						
+ 10 %	-0.84 (1.0 %)	-344 (4.2%)	-224 (4.5%)	+242 (1.0%)	-1844 (7.9%)	-335 (5.7%)
- 10 %	+1.41 (1.7 %)	+360 (4.4%)	+303 (6.1%)	-316 ((1.3%)	+2244 (9.6%)	+170 (2.95)
Microbial production efficiency (P_M)						
+ 10 %	+10.3 (12.4%)	-372 (4.5%)	-16 (0.3%)	+12.2(<0.1 %)	0	0
- 10 %	-9.7 (11.7 %)	+398 (4.9%)	+33.9 (0.7%)	-28.2 (0.1 %)	0	0

Note: Results were shown as the difference of simulation responses for 72 years as compared to that from the default parameter value. Number in parenthesis indicates the percentage changed based on the results from default parameters.

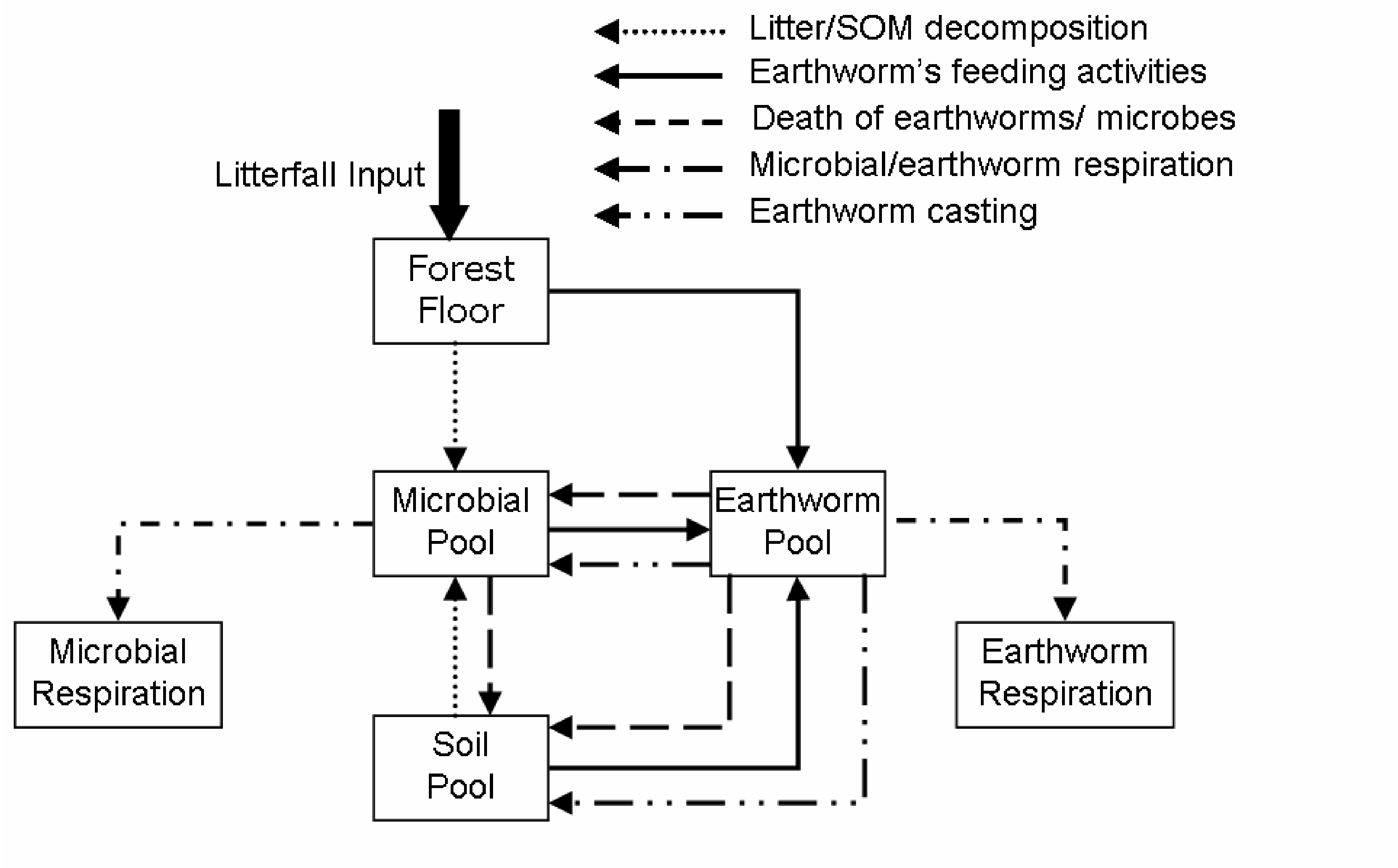

Figure 4.1. Diagram of the Earthworm model representing carbon dynamics in terrestrial forest ecosystem.

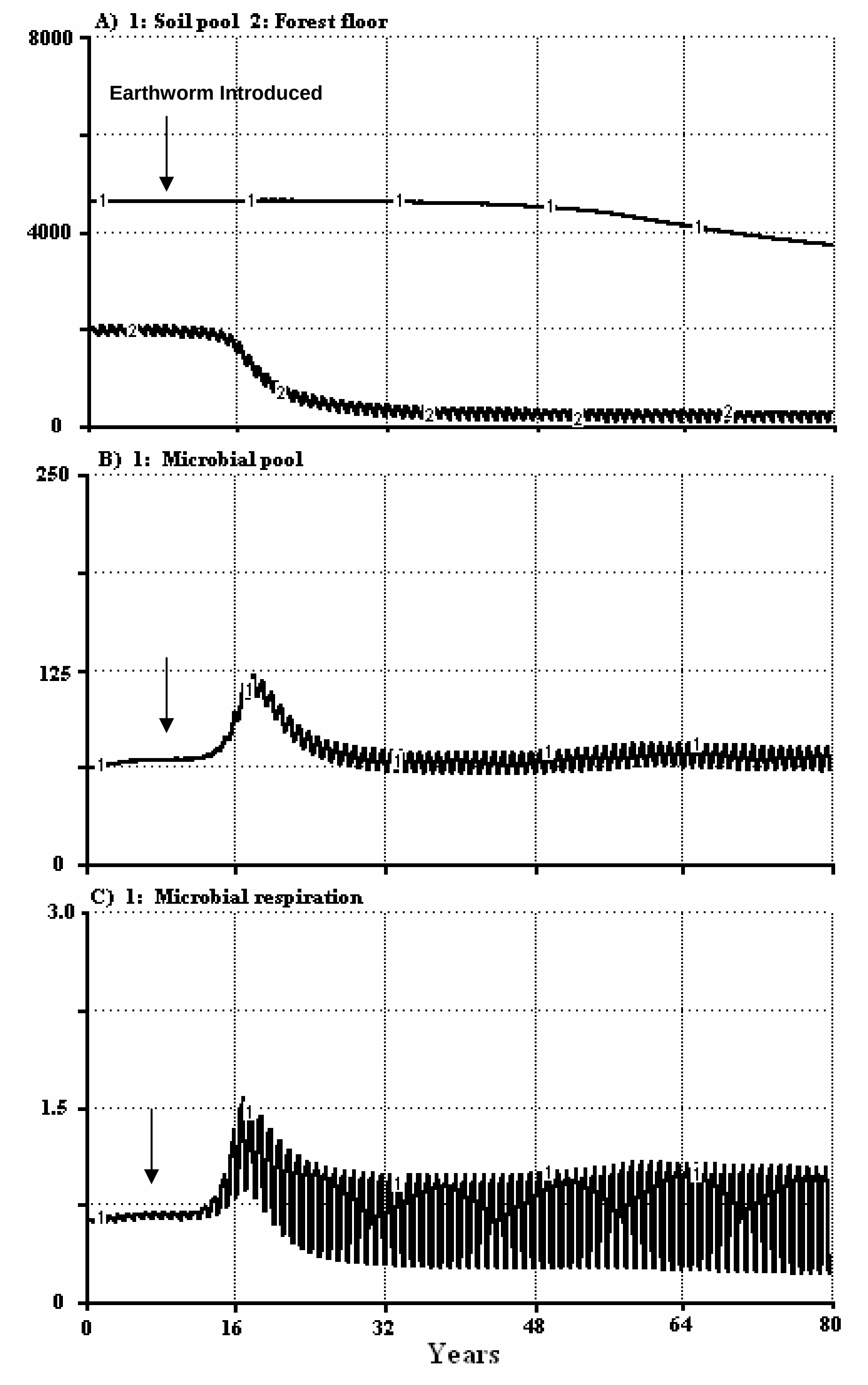

Fig. 4.2. Simulation results of plot 1 on carbon dynamics after earthworm invasion. A) forest floor pool and soil pool; B) microbial population; and C) microbial respiration. The arrow indicated that earthworm population was introduced after 8 years of simulation. The unit for carbon pool is g C m^{-2}.

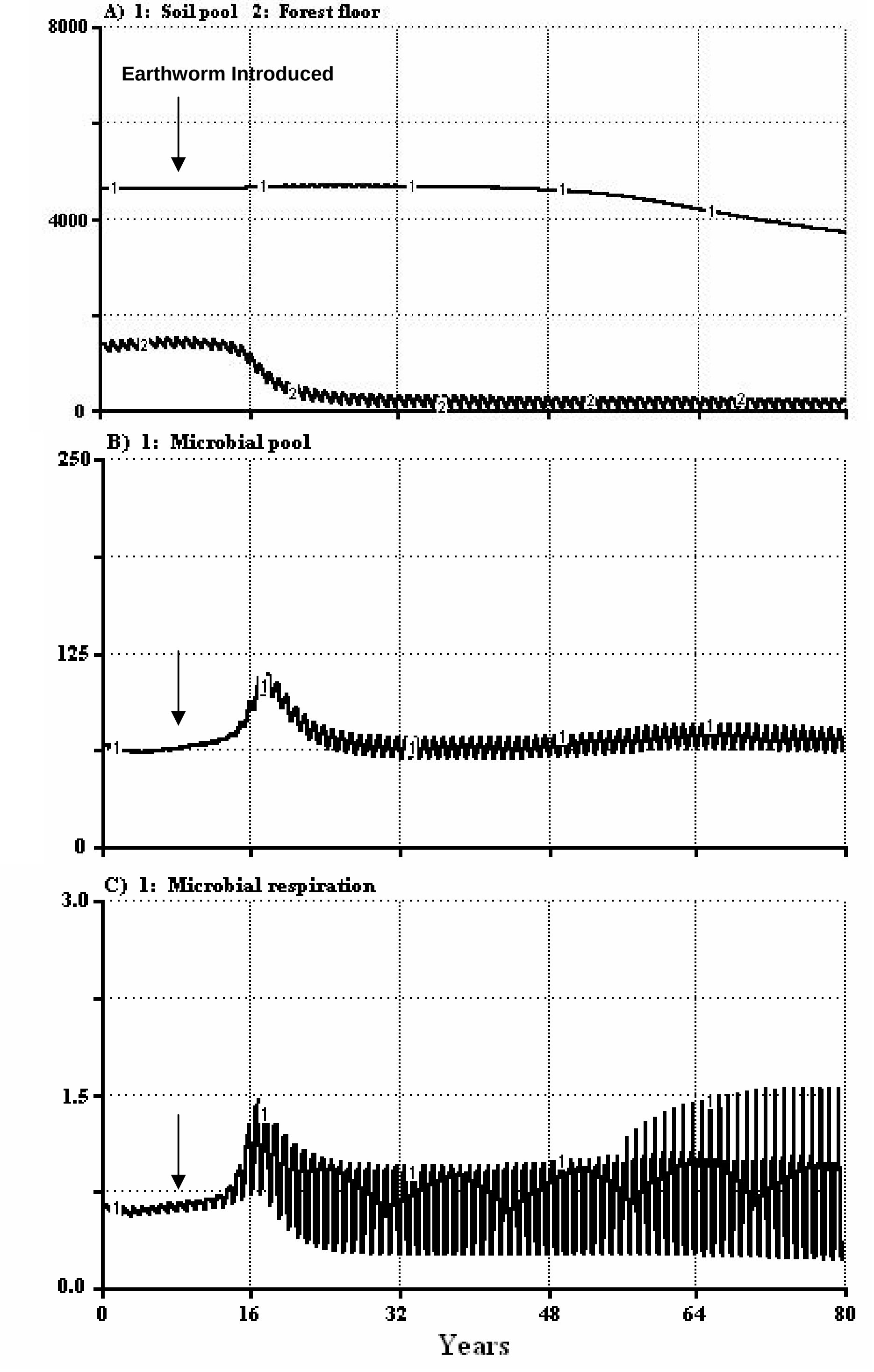

Fig. 4.3. Simulation results of plot 2 on carbon dynamics after earthworm invasion. A) forest floor pool and soil pool; B) microbial population; and C) microbial respiration. The arrow indicated that earthworm population was introduced after 8 years of simulation. The unit for carbon pool is g C m^{-2}.

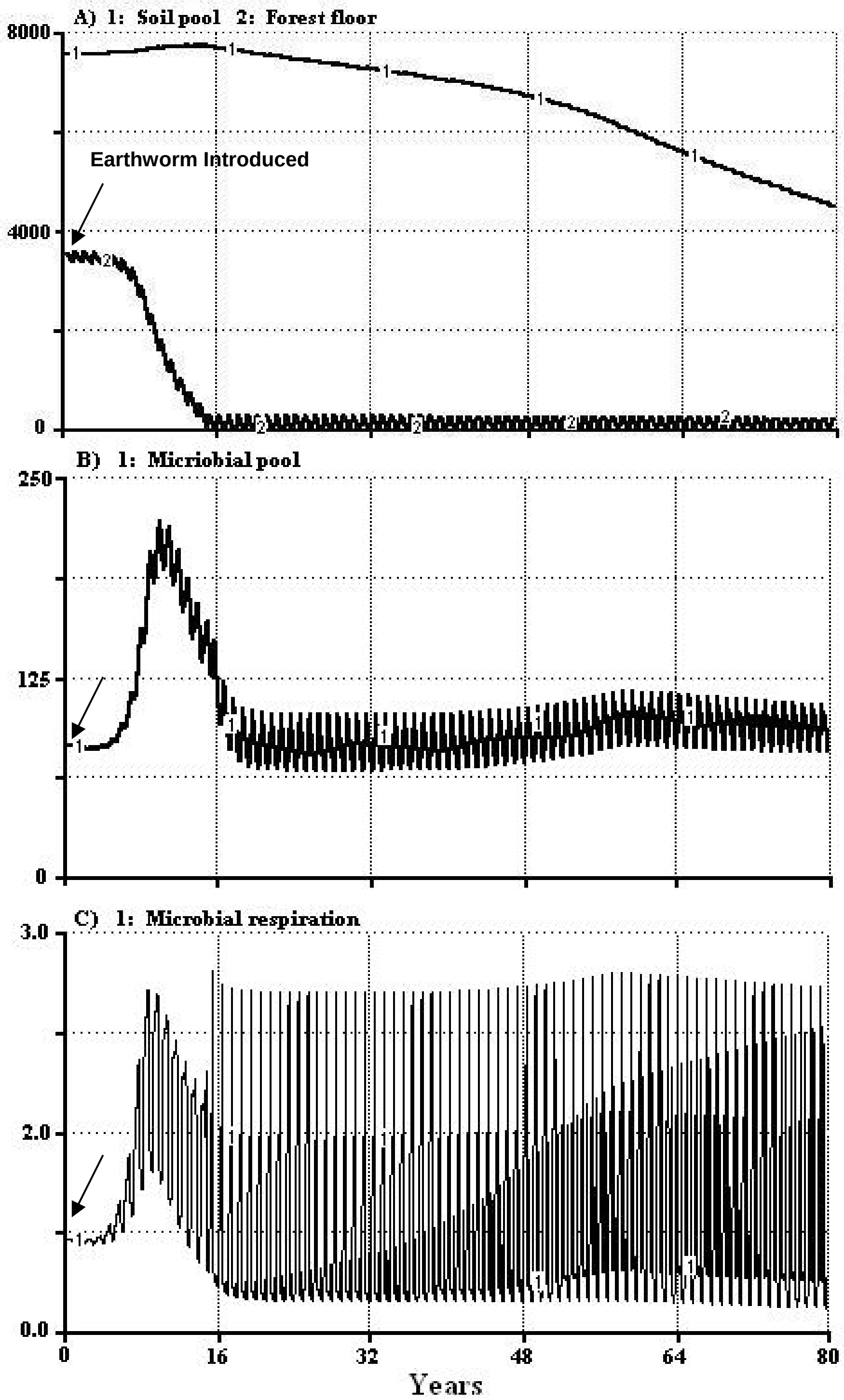

Fig. 4.4. Simulation results of plot 3 on carbon dynamics after earthworm invasion. A) forest floor pool and soil pool; B) microbial population; and C) microbial respiration. Earthworm population was introduced at time 0 of simulation. The unit for carbon pool is g C m^{-2}.

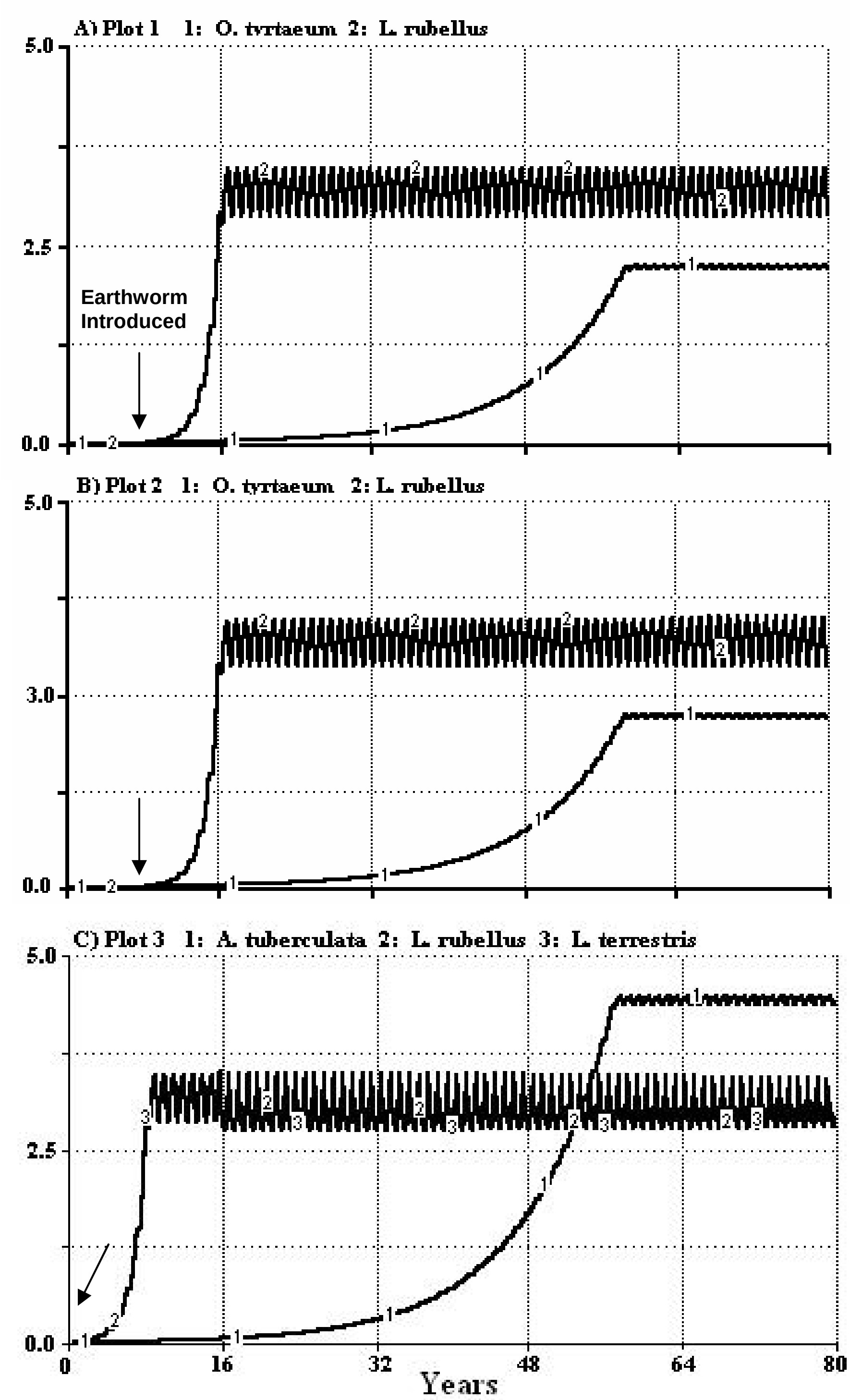

Fig. 4.5. Simulation results of earthworm population dynamics in A) plot 1; B) plot 2; and C) plot 3 after earthworm introduction. Earthworm population was introduced after 8 years of simulation at plot 1 and plot 2 while earthworms were introduced at time 0 of simulation in plot 3. The unit for carbon pool is g C m^{-2}.

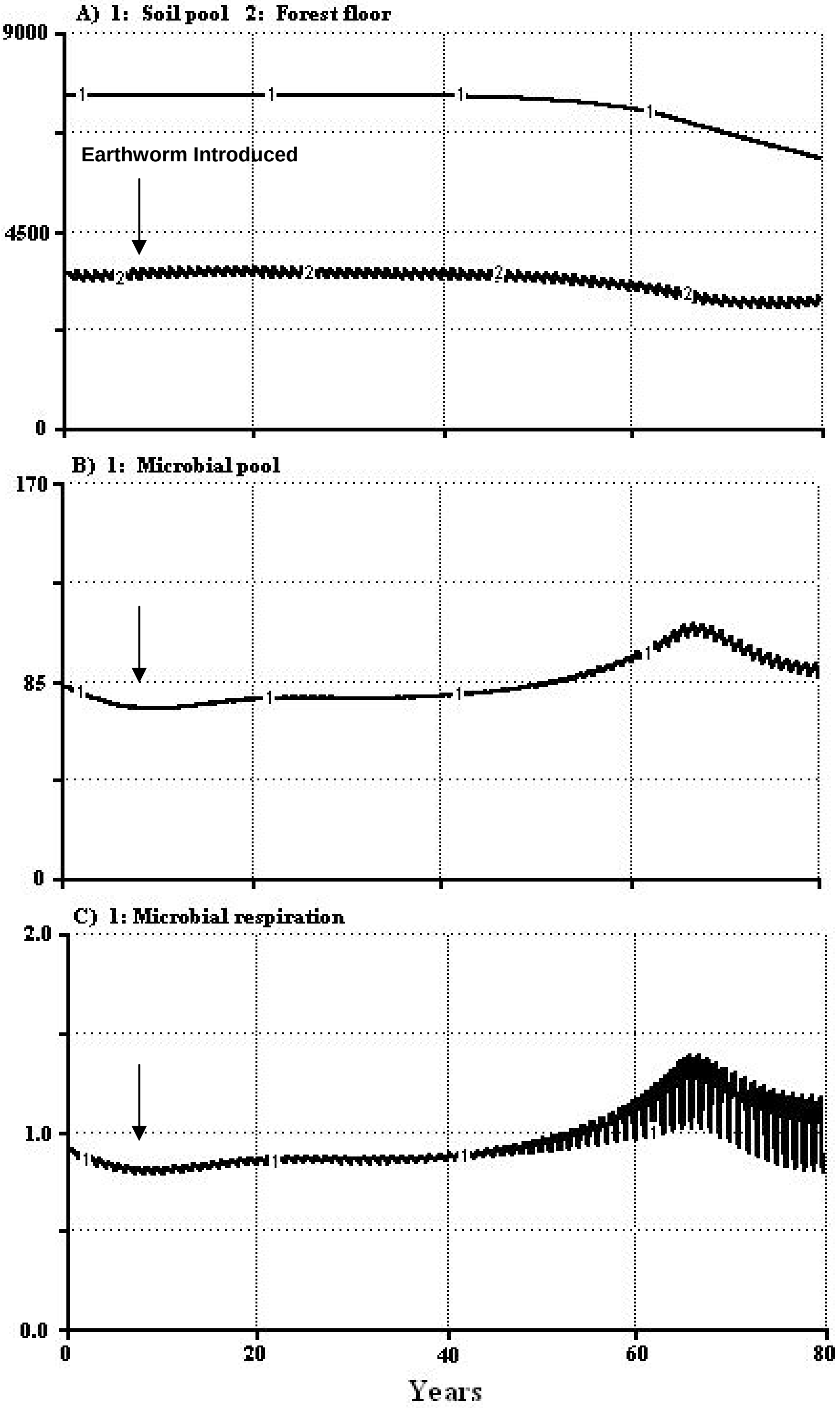

Figure 4.6. Simulation of impacts of endogeic earthworms on carbon dynamics after earthworm invasion. A) forest floor pool and soil pool; B) microbial population; and C) microbial respiration. The unit for carbon pool is g C m^{-2}.

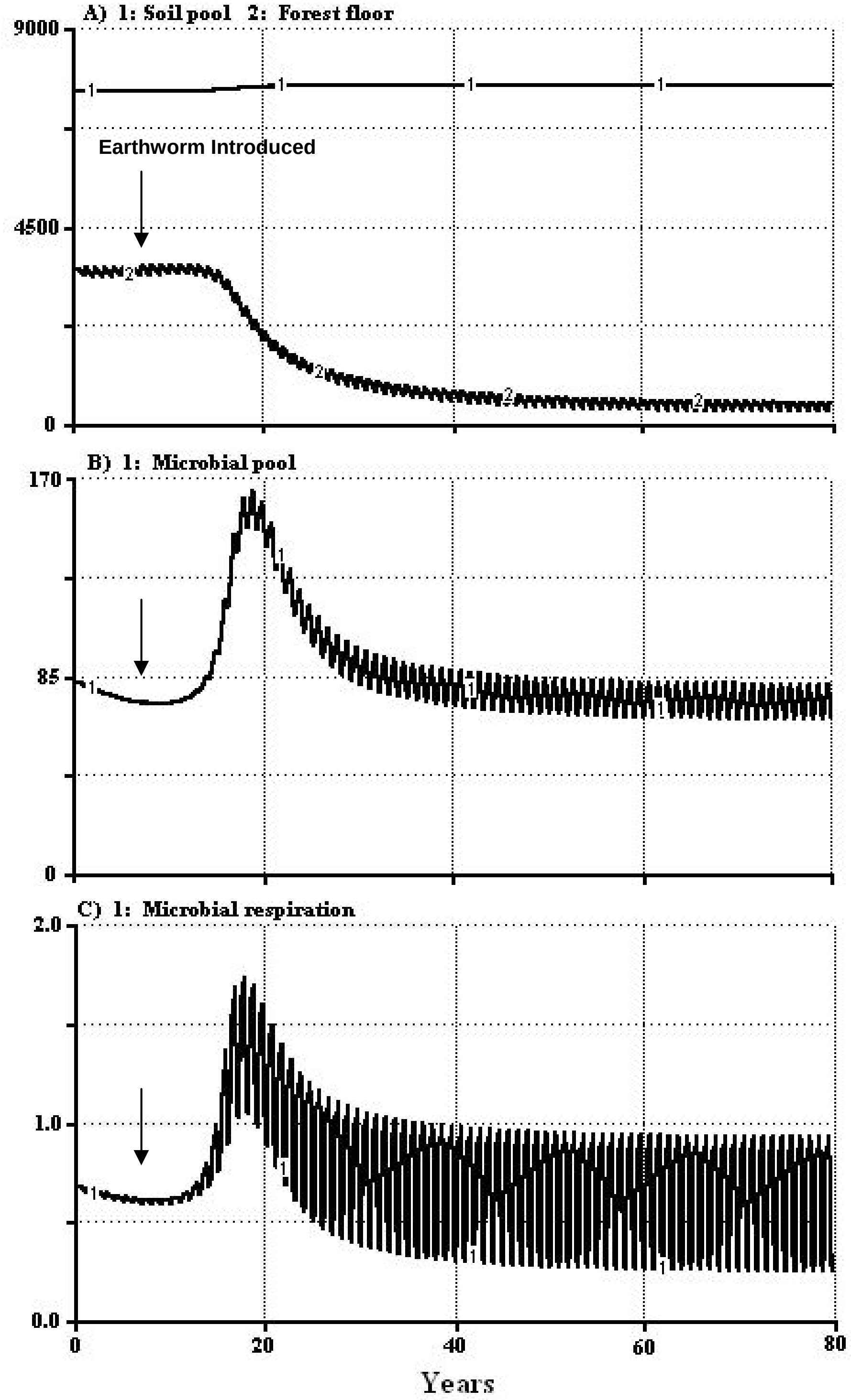

Figure 4.7. Simulation of impacts of epigeic earthworms on carbon dynamics after earthworm invasion. A) forest floor pool and soil pool; B) microbial pool; and C) microbial respiration. The unit for carbon pool is g C m^{-2}.

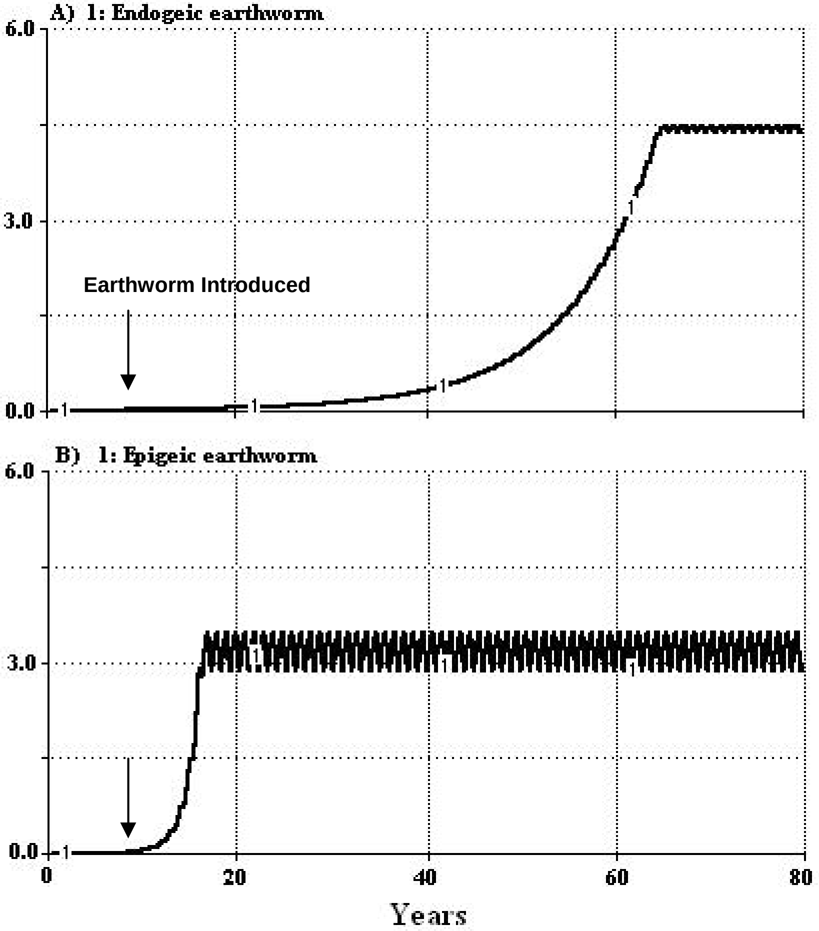

Figure 4.8. Simulation results for population dynamics of A) endogeic earthworms; B) epigeic earthworms for 72 years in the forest ecosystems. The unit for carbon pool is g C m^{-2}.

CHAPTER FIVE

CONCLUSIONS

Recently, decline and extirpation of native earthworm species have been attributed to two factors: habitat disturbance (especially anthropogenic-derived) and competitive exclusion by peregrine earthworm species. In Puerto Rico, the changing composition of native and invasive earthworm communities and their abundance and distribution patterns within successional forests provided an excellent research site to examine the mechanisms of habitat disturbances and native-invasive earthworm competitive relationships. Habitat disturbance could damage native earthworm populations by changing soil characteristics and resource availability where they inhabit, while invasive earthworms could also harm native earthworms through competitive exclusion.

Field experimental results showed that habitat disturbance did not impede the re-colonization ability of native earthworms, *Estherella* spp. (Chapter two). Changes of habitat characteristics along a chronsequence of successional forests had no impacts on re-colonization of native *Estherella* spp. populations. The exclusion of native *Estherella* earthworms in disturbed pastures of Puerto Rico might be due to biotic factors instead of habitat disturbance, particularly competitive pressure from invasive earthworms.

Nevertheless, no competitive interactions between native and invasive earthworm species were observed in a laboratory experiment with similar or dissimilar niche combinations and with reduced or excess resource availability. Sustaining resource availability (live plant roots and abundant litter) in this study may have relieved the competitive pressure between earthworm species observed in previous laboratory literature. Hence, competition pressure may still play a role for the disappearance of native earthworm species, while the competitive intensity may be decided by resource availability. The decreasing abundance and sparse distribution of native earthworm species of Puerto Rico may be attributed to the mixed effects of habitat disturbance (especially sudden environmental destruction at the beginning of habitat disturbance) and competitive pressure from invasive earthworms (for longer time scale). In addition, different earthworms and their interactions did have differential impacts on soil nutrient dynamics. Earthworm ^{13}C- and ^{15}N signature data indicated that earthworms have their feeding behaviors beyond what would be predicted from their ecological categories. Also, ^{13}C and ^{15}N distribution patterns among soil and microbial pools suggested that earthworm impacts on microbial population and mineralization processes may be determined by their interactions with microbial populations in different microhabitats, such as the rhizosphere and detritusphere. Resource availability and flexible feeding strategies of earthworms should be considered for future earthworm interaction studies. Meanwhile, application of stable isotope-labeled material methods can help us to trace earthworm feeding behaviors and to understand earthworm-microbe-mineralization relationships as well.

An earthworm model developed in this study allows for the evaluation of impacts of earthworm invasions on soil carbon cycling over long-term time scales. This earthworm model incorporates not only the effects from earthworm consumption but also their cast deposit impacts on forest floor organic matter, microbial populations, and soil organic matter. Simulation results of this model suggested that impacts of earthworm invasions on terrestrial ecosystems may vary depending on invasion history, invasive earthworm species composition, and pre-invasion ecosystem conditions. Evaluation of invasion history and future invasion patterns (i.e. earthworm population and carbon dynamics) on the invaded forests can help the development of conservation and regulation policies to prevent expansion of current and future earthworm invasions. Comparisons between model simulation results and field observations can provide the information on potential mechanisms and assess the interactions and the importance of ecosystem components under earthworm invasion scenarios. However, more details of species characteristics of peregrine and endemic native earthworm species are urgently needed for precise and robust evaluations and model simulations.

Appendix A. STELLA equations for the earthworm model

Earthworm CO_2 (t) = Earthworm CO_2 (t - dt) + ($R_{W\text{-}Endo}$ + $R_{W\text{-}Epi}$) * dt //Cumulative CO_2 from earthworms

INIT R_W = 0 //Initial earthworm CO_2 is zero

INFLOWS:

$R_{W\text{-}Endo}$ = ($CM_{W\text{-}Endo}$+$CS_{W\text{-}Endo}$)*(1-$P_{W\text{-}Endo}$) // Respiration of endogeic earthworms

$R_{W\text{-}Epi}$ = ($CL_{W\text{-}Epi}$ + $CM_{W\text{-}Epi}$ + $CS_{W\text{-}Epi}$)*(1-$P_{W\text{-}Epi}$) // Respiration of epigeic earthworms

FF(t) = FF (t - dt) + (LF - $CL_{W\text{-}Epi}$ – LM_{CAST} – CL_M – LS_{CAST} - LS_M) * dt //Forest floor litter remaining

INIT FF = 5014.14*0.4 //Initial forest floor litter carbon

INFLOWS:

LF = 235/50*Litterfall_function// Daily litterfall input

OUTFLOWS:

$CL_{W\text{-}Epi}$ = $CRL_{W\text{-}Epi}$*W_{Epi} *$AL_{W\text{-}Epi}$// Litter consumption by epigeic earthworms

LM_{CAST} = ($CL_{W\text{-}Epi}$ / $AL_{W\text{-}Epi}$ *(1- $AL_{W\text{-}Epi}$))*A_M// Earthworm casts to microbial pool from litter consumption by earthworms

CL_M = (FF *0.115/365)*MP/Initial MP* A_M// Litter consumption by the microbes

LS_{CAST} = LM_{CAST} / A_M *(1- A_M)// Earthworm casts to soil pool from litter consumption by earthworms

LS_M = CL_M / A_M *(1- A_M) // Litter to soil pool from litter consumption by the microbes

W_{Epi} (t) = W_{Epi} (t - dt) + ($CL_{W\text{-}Epi}$ + $CM_{W\text{-}Epi}$ + $CS_{W\text{-}Epi}$ + W_{Epi}-Pulse - $R_{W\text{-}Epi}$ - $DS_{W\text{-}Epi}$ - $DM_{W\text{-}Epi}$) * dt// Epigeic earthworm biomass

INIT W_{Epi} = 0// Initial earthworm biomass is zero

INFLOWS:

$CL_{W\text{-}Epi}$ = $CRL_{W\text{-}Epi}$ *W_{Epi}* $AL_{W\text{-}Epi}$// Litter consumption by epigeic earthworms

$CM_{W\text{-}Epi}$ = IF (FF > 0.01*Initial FF) THEN ($CL_{W\text{-}Epi}$/0.7*0.3) ELSE ($CL_{W\text{-}Epi}$/0.5*0.3)// Consumption on the microbes by epigeic earthworms

$CS_{W\text{-}Epi}$ = IF (FF > 0.01* Initial FF) THEN (0) ELSE ($CL_{W\text{-}Epi}$/0.5*0.2)// Soil consumption by epigeic earthworms

W_{Epi}-Pulse = PULSE (0.01, 2920,0)// Introduction of epigeic earthworms

OUTFLOWS:

$R_{W\text{-}Epi}$ = ($CL_{W\text{-}Epi}$+ $CM_{W\text{-}Epi}$ + $CS_{W\text{-}Epi}$)*(1-P_W)// Respiration of epigeic earthworms

$DS_{W\text{-}Epi}$ = $DM_{W\text{-}Epi}$/ A_M *(1- A_M)// Dead epigeic earthworms to soil pool

$DM_{W\text{-}Epi}$ = 0.01370*(W_{Epi})*MAX(0,1-($W_{Epi\text{-}Min}$)/W_{Epi})* A_M// Dead epigeic earthworms to microbial pool

MP(t) = MP(t - dt) + (LM_{CAST} + CL_M + SM_{CAST} + $DM_{W\text{-}Endo}$ + $DM_{W\text{-}Epi}$ + CS_M - R_M - $CM_{W\text{-}Endo}$ - $CM_{W\text{-}Epi}$ - D_M - MS_W) * dt// Microbial biomass pool

INIT MP = 82// Initial microbial biomass

INFLOWS:

LM_{CAST} = ($CL_{W\text{-}Epi}$ / $AL_{W\text{-}Epi}$ *(1- $AL_{W\text{-}Epi}$))*A_M// Earthworm casts to microbial pool from litter consumption by earthworms

CL_M = (FF *0.115/365)*MP/Initial MP* A_M// Litter consumption by the microbes

SM_{CAST} = ($CS_{W\text{-}Endo}$/$AS_{W\text{-}Endo}$ *(1-$AS_{W\text{-}Endo}$)+ $CS_{W\text{-}Epi}$ /$AS_{W\text{-}Epi}$ *(1-$AS_{W\text{-}Epi}$)* A_M// Earthworm casts to microbial pool from consumption on soil pool by earthworms

$DM_{W\text{-}Endo}$ = 0.01370*(W_{Endo})* MAX(0,1-($W_{Endo\text{-}Min}$)/W_{Endo})* A_M// Dead endogeic earthworms to microbial pool

$DM_{W\text{-}Epi}$ = 0.01370*(W_{Epi})*MAX(0,1-($W_{Epi\text{-}Min}$)/W_{Epi})* A_M // Dead epigeic earthworms to microbial pool

CS_M = S *0.022/365*MP/(Initial MP)* A_M// Soil consumption by the microbes

OUTFLOWS:

R_M = ($DM_{W\text{-}Endo}$ +$DM_{W\text{-}Epi}$+ LM_{CAST} + CL_M + SM_{CAST} +CS_M)*(1- P_M)// Respiration of the microbes

$CM_{W\text{-}Endo}$ = $CS_{W\text{-}Endo}$/0.7*0.3// Consumption on the microbes by endogeic earthworms

$CM_{W\text{-}Epi}$ = IF (FF > 0.01*Initial FF) THEN ($CL_{W\text{-}Epi}$/0.7*0.3) ELSE ($CL_{W\text{-}Epi}$/0.5*0.3) // Consumption on the microbes by epigeic earthworm

D_M = 0.00328*MP*MAX(0,1-(MP_{Min}/MP)*(1-A_M))// Death of the microbes

MS_W = ($CM_{W\text{-}Endo}$+ $CM_{W\text{-}Epi}$)/AM_W*(1-AM_W)*(1- A_M)// Microbial pool to soil pool from consumption on the microbes by earthworms

Microbial CO_2 (t) = Microbial CO_2 (t - dt) + (R_M) * dt// Cumulative CO_2 from microbial respiration

INIT Microbial CO_2 = 0// Initial CO_2 from microbial respiration is zero

INFLOWS:

R_M = ($DM_{W\text{-}Endo}$ +$DM_{W\text{-}Epi}$+ LM_{CAST} + CL_M + SM_{CAST} +CS_M)*(1- P_M) // Respiration of the microbes

W_{Endo}(t) = W_{Endo} (t - dt) + ($CS_{W\text{-}Endo}$ + $CM_{W\text{-}Endo}$ + W_{Endo}-Pulse - $R_{W\text{-}Endo}$ - $DM_{W\text{-}Endo}$ - $DS_{W\text{-}Endo}$) * dt// Endogeic earthworm biomass

INIT W_{Endo} = 0// Initial earthworm biomass is zero

INFLOWS:

$CS_{W\text{-}Endo}$ = $CRS_{W\text{-}Endo}$ * W_{Endo} *$AS_{W\text{-}Endo}$// Consumption on soil pool by endogeic earthworms

$CM_{W\text{-}Endo}$ = $CS_{W\text{-}Endo}$/0.7*0.3// Consumption on the microbes by endogeic earthworms

W_{Endo}-Pulse = PULSE (0.01, 2920, 0)// Introduction of endogeic earthworms

OUTFLOWS:

$R_{W\text{-}Endo}$ = ($CM_{W\text{-}Endo}$+$CS_{W\text{-}Endo}$)*(1-P_W)// Respiration of endogeic earthworms

$DM_{W\text{-}Endo}$ = 0.01370*(W_{Endo})* MAX (0,1-($W_{Endo\text{-}Min}$)/W_{Endo})* A_M// Dead endogeic earthworms to microbial pool

$DS_{W\text{-}Endo}$ = $DM_{W\text{-}Endo}$ / A_M *(1- A_M) // Dead endogeic earthworms to soil pool

S (t) = S(t - dt) + (LS_{CAST} + DS_M + MS_{CAST} + $DS_{W\text{-}Epi}$ + $DS_{W\text{-}Endo}$ + LS_M - $CS_{W\text{-}Endo}$ - SM_{CAST} - $CS_{W\text{-}Epi}$ - CS_M) * dt// Soil carbon pool

INIT S = 4622.46// Initial soil stock

INFLOWS:

$LS_{CAST} = LM_{CAST}/ A_M *(1- A_M)$// Earthworm casts to soil pool through consumption on litter by earthworms

$DS_M = 0.00328*MP*MAX(0,1-(MP_{Min}/MP)*(1- A_M))$ //Death of the microbes to soil pool

$MS_{CAST} = (CM_{W\text{-}Endo}+ CM_{W\text{-}Epi})/ AM_W*(1- AM_W)*(1- A_M)$ // Earthworm casts to soil pool through consumption on the microbes by earthworms

$DS_{W\text{-}Epi} = DM_{W\text{-}Epi}/ A_M *(1- A_M)$ // Dead epigeic earthworms to soil pool

$DS_{W\text{-}Endo} = DM_{W\text{-}Endo} / A_M *(1- A_M)$ // Dead endogeic earthworms to soil pool

$LS_M = CL_M/A_M *(1- A_M)$// Litter to soil pool from litter consumption by the microbes

OUTFLOWS:

$CS_{W\text{-}Endo} = CRS_{W\text{-}Endo} *W_{Endo} *AS_{W\text{-}Endo}$// Earthworm consumption on soil pool

$SM_{CAST} = (CS_{W\text{-}Endo}/AS_{W\text{-}Endo}*(1-AS_{W\text{-}Endo})+CS_{W\text{-}Epi}/AS_{W\text{-}Epi} *(1-AS_{W\text{-}Epi}))* A_M$// Earthworm casts to microbial pool from consumption on soil pool by earthworms

$CS_{W\text{-}Epi}$ = IF (FF > 0.01* Initial FF) THEN (0) ELSE ($CL_{W\text{-}Epi}/0.5*0.2$) // Earthworm consumption on soil pool

CS_M = S *0.022/365*MP/(Initial MP)* A_M// Soil consumption by the microbes

$AL_{W\text{-}Epi} = 0.02$// Assimilation efficiency of epigeic earthworms on litter

$A_M = 0.9$// Assimilation efficiency of the microbes

$AM_W = 0.3$// Assimilation efficiency of earthworms on the microbes

$AS_{W\text{-}Endo} = 0.01$// Assimilation efficiency of endogeic earthworms on soil

$AS_{W\text{-}Epi} = 0.01$// Assimilation efficiency of epigeic earthworms on soil

$CRL_{W\text{-}Epi}$ = 0.08* (Temperature Function of Epigeic Earthworms)// Litter consumption rates of epigeic earthworms

$CRS_{W\text{-}Endo}$ = 0.2* (Temperature Function of Endogeic Earthworms)// Soil consumption rates of endogeic earthworms

Litterfall_function = IF (Year_cycle >250 and Year_cycle <300) then 1 else 0

P_W = 0.4// Production efficiency of earthworms

P_M = 0.23// Production efficiency of the microbes

Temp_cycle = ((SINWAVE(26,365)+22)/2)// Daily temperature cycle

Year_cycle = COUNTER(1,365)

Temperature Function of Endogeic Earthworms = GRAPH(Temp, Consumption Efficiency)

(-4.00, 0.00), (-2.00, 0.00), (0.00, 0.00), (2.00, 0.1), (4.00, 0.4), (6.00, 0.6), (8.00, 0.8), (10.0, 1.00), (12.0, 1.00), (14.0, 1.00), (16.0, 0.95), (18.0, 0.85), (20.0, 0.75), (22.0, 0.7)

Temperature Function of Epigeic Earthworms = GRAPH(Temp, Consumption Efficiency)

(-4.00, 0.00), (-2.00, 0.00), (0.00, 0.00), (2.00, 0.1), (4.00, 0.2), (6.00, 0.4), (8.00, 0.65), (10.0, 0.75), (12.0, 0.85), (14.0, 0.95), (16.0, 1.00), (18.0, 1.00), (20.0, 1.00), (22.0, 0.95)