INVASION BY THE NON-NATIVE EARTHWORM *AMYNTHAS AGRESTIS* (OLIGOCHAETA: MEGASCOLECIDAE): DYNAMICS, IMPACTS, AND COMPETITION WITH MILLIPEDES

by

BRUCE ALLEN SNYDER

(Under the Direction of PAUL F. HENDRIX)

ABSTRACT

Over the past several decades, invasive species have become one of the largest issues in the field of ecology. Invasive earthworms have received much attention, especially in northern North America, where native earthworms were extirpated by Pleistocene glaciations and the more recent earthworm invasions have altered Northern Forests significantly. These invasions were primarily by European species and invasions elsewhere in the continent by other earthworm taxa have received less attention. We studied the impacts of invasion by Asian earthworms in the genus *Amynthas* in a field study and two laboratory studies. *Amynthas agrestis* invasion in the Great Smoky Mountains National Park was found to be a dynamic process on monthly time scale. This invasion altered soils by decreasing the depth of partially decomposed organic horizons and increasing soil aggregation. A significant decrease in millipede abundance and species richness was also associated with the invasion, which suggested competitive interactions between the epigeic earthworm and epigeic millipedes.

To further examine this interaction, two microcosm studies were performed. The first study used ^{13}C enriched Red Oak and Eastern Hemlock litter to assess millipede (*Pseudopolydesmus erasus*) and earthworm (*Amynthas corticis*) food preference. Negative effects of earthworms on millipede ^{13}C assimilation were observed as was a mitigation of earthworms' impacts on soil aggregation by millipedes. *A. corticis* caused significant soil aggregation throughout microcosms and increased respiration rates relative to millipede and control treatments.

The second microcosm experiment examined millipedes' (*Sigmoria ainsliei*) and earthworms' (*Amynthas agrestis*) reliance on fresh versus partially decomposed litter as a food resource and whether there was direct competition for these resources. These taxa were found to compete for partially decomposed material and millipedes relied on this resource for survival.

The results of these studies suggest that Asian earthworm invasion in the southern Appalachian Mountains poses a threat to the millipede species endemic to this region. To assist land managers in conservation of this vital resource, a preliminary checklist of millipedes of the Great Smoky Mountains National Park has been compiled. This assembles the knowledge of millipede diversity and species distributions within the Park for the first time.

INDEX WORDS: Invasive species, Competition, Soil structure, C dynamics, Great Smoky Mountains National Park, Earthworm, Oligochaeta, Millipede, Diplopoda, *Amynthas*, *Pseudopolydesmus*, *Sigmoria*

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BRUCE ALLEN SNYDER

B.S., University of Wisconsin - Green Bay, 2002

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BRUCE ALLEN SNYDER

Major Professor:	Paul F. Hendrix
Committee:	Mark A. Bradford Mac A. Callaham C. Ronald Carroll David C. Coleman

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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CHAPTER 1

INTRODUCTION

This dissertation is the product of four years of research into various aspects of the invasion biology of Asian earthworms in North America. To preface the following chapters, I wish to provide a brief history of this research and explain how each study is related.

The main concepts in the dissertation stem from the field study (Chapter 3) conducted in the Great Smoky Mountains National Park (GSMNP). Keith Langdon (GSMNP Inventory & Monitoring Coordinator) first brought the occurrence of then-unidentified earthworms in GSMNP to Paul Hendrix's attention in 2003. These specimens were identified as *Amynthas agrestis* (Goto and Hatai 1899).

I first visited GSMNP in October 2004 during my first semester at the University of Georgia. It was readily apparent by early 2005 that extensive work would be necessary to determine the extent of the invasion. Throughout the summer of 2005 I explored the region of the Park near Lake Chilhowee and delineated several invasion fronts. Fieldwork in 2005 set the stage for the remainder of my studies and was greatly aided by Paul Hendrix, Mac Callaham, and several assistants.

The GSMNP fieldwork found distinct invasion fronts, which allowed us to study the progression of the invasion and how fauna in newly invaded areas were impacted by invasion. Some additional aspects of *A. agrestis* invasion biology were investigated by undergraduate students: Russ Richardson studied *A. agrestis* temperature and moisture tolerances in the laboratory (in press at Southeastern Naturalist); Steven Rostkowski measured the activity of macroinvertebrates at the field site during 2006 (manuscript in preparation); and Chris Hansen conducted a preliminary lab study on *A. agrestis* food preferences.

Chapter 2 consists of a review paper (submitted to Soil Biology & Biochemistry but not accepted) of interactions between earthworms and other soil organisms. Preliminary data from the field study suggested to us that millipedes might be negatively impacted by *A. agrestis* invasion, and we explored the mechanisms for this interaction with two microcosm studies. The first of these (Chapter 4) was designed with Bas Boots (Wageningen University, The Netherlands) and used ^{13}C as a tracer to examine competition and litter species preference. *Amynthas agrestis* was not available at the beginning of this experiment and we instead used the congener *A. corticis*, another Asian, invasive earthworm that has become widespread in North America.

The second microcosm experiment (Chapter 5) was designed with more data from the field study, preliminary data from the first microcosm study, and earthworm culturing advice from Chris Lowe (University of Central Lancashire, Preston, UK). We assessed food preferences of L and FH (F and H combined) organic horizons (recently fallen leaf litter versus partially decomposed organic material, functionally defined by particle size). Additionally, this study differed from the previous microcosm study in its greater duration, use of a larger millipede species, and collection of mortality and biomass data throughout the experiment. Termination of the experiment was to take place when all millipedes and earthworms had perished, but as of this writing (28 March 2008) several individuals remain alive. As a consequence, millipede and earthworm survival to 25 March 2008 were analyzed and presented.

Millipedes are very diverse in the Park, and our field study also discovered several undescribed millipede species. Our field and microcosm studies all suggest that earthworms compete with millipedes. If *A. agrestis* invasion was to spread throughout GSMNP, this could pose a threat to GSMNP millipede diversity. Great Smoky Mountains National Park staff were concerned about this possibility and were interested in documenting millipede diversity in conjunction with the efforts of the All-Taxa Biodiversity Inventory. Further, the field of millipede taxonomy suffers from the widespread phenomenon that there are few new taxonomists. All of these factors led to the final study of the dissertation (Chapter 6), a checklist of millipede species known thus far from within GSMNP. This draws on my interest and experience in taxonomy and provides a link between our newfound understanding of *A. agrestis* invasion and the broader context of GSMNP resource management.

CHAPTER 2

INTERACTIONS OF EARTHWORMS WITH OTHER SOIL ORGANISMS, A REVIEW

Abstract

Earthworms (Oligochaeta: Opisthopora) are found in most terrestrial ecosystems worldwide. Their ubiquity and large size range allows them to interact with soil fauna of all sizes. Their presence as non-native invasive species, especially in North America, has created impetus for understanding their effects on entire ecosystems, and furthered our understanding of earthworm ecology. Herein I review recent literature about earthworm interactions with plants, vertebrates, and all kinds of soil fauna. This review builds on the foundation provided by Brown (1995), with an increased focus on macrofauna. I conclude that the size of the organism relative to the earthworm influences the earthworm's net effect on organism abundance and diversity. Earthworms have direct negative effects and occasional indirect positive effects on smaller organisms, including microbial biomass, protozoa, nematodes, and microarthropods. Most known interactions with similar-sized or larger animal organisms involve predation on the earthworm; competitive and mutualistic interactions with other taxa exist but are relatively unstudied. Additionally, the effect of earthworms on soil community composition is little understood. How earthworms influence soil faunal communities and how these changes affect soil processes mark a new frontier in soil ecology.

Keywords: Earthworm; Oligochaeta; Annelida; interaction; effects; diet; microarthropod; macrofauna; predation; invasive earthworms

Introduction

As one of the dominant soil organisms in temperate and tropical ecosystems, earthworms (Oligochaeta: Opisthopora) can have significant effects on ecosystem processes (Edwards, 2004). The actions of earthworms also affect soil flora and fauna. Over a decade ago, Brown (1995) reviewed what was known about these earthworm interactions and the underlying mechanisms. This review builds on Brown's foundation by incorporating recent literature and emphasizing interactions with macrofauna. Brown (1995) concluded with suggestions for future research, and many of these topics are discussed below.

In recent years, many studies have focused on non-native earthworm invasions, primarily in previously earthworm-free regions of North America (see Hendrix, 2006). Earthworm invasion research has contributed greatly to the knowledge of earthworm ecology in general, and earthworm interactions in particular. This review incorporates applicable information from the earthworm invasion literature, and provides a framework for future studies of earthworm interactions in the context of invasions. The synergy between invasive plants and invasive earthworms (Heneghan et al., 2007) provides further evidence of the importance of earthworm invasion ecology to the broader field of invasion biology.

Herein, earthworm-organism interactions are categorized by organism size, using the classification of Swift et al. (1979). Plants are also soil organisms, and consequently have their own section. Not included in this review are the myriad interactions between earthworm species or individuals. The final section is devoted to interactions with vertebrates, which, in addition to plants, form another major linkage between the belowground earthworms and the aboveground ecosystem component (Wardle, 2002).

Review

Plants

As the existence of earthworm-plant interactions have been known for hundreds of years, many reviews exist on this subject. A few are briefly mentioned here. Lee (1985) provided a thorough review of the relationship between earthworms and plant growth beginning at the time of Darwin. The vast majority were positive interactions (i.e. improvement of plant growth) and reflected only lumbricid earthworms. Sections were devoted to earthworms' effects on soil fertility, production of growth stimulants, and seeds. Earthworm actions that negatively affected plants were discussed under the headings "Relationships with Plant Pathogens" and "Earthworms as Plant Pests."

Brown et al. (1999) summarized what was known about earthworm-plant interactions in the tropics. All of the research discussed was conducted after 1985. They discussed mechanisms of these interactions, spatial and temporal scales of the experiments, and the importance of earthworm density in evaluating effects. Brussaard (1999), in a review of earthworm-plant interactions, also suggested that more focus needs to be placed on understanding the mechanisms behind the interactions.

Brown et al. (2004) provided another thorough review of earthworm's effects on plants. They began with a historical review, commencing 100 years before Lee's (1985), and then dug deeply into the mechanisms behind these effects, expanding beyond the concepts in Brown (1995) and Brown et al. (1999). They listed seven mechanisms of earthworm-plant interactions: alteration of populations of beneficial microorganisms, alteration of populations of plant pests, production of substances which affect plant growth, damage to living plants, seed interactions, changes in soil structure, and changes in soil nutrient availability. They conclude with research needs and suggestions for future experiments.

Scheu (2003) reviewed the earthworm-plant interaction literature over the period 1949-2002, highlighting the methods used for these studies, and categorizing where they have been published. He noted that most studies focus on agricultural systems, and concluded that an ecological - rather than agricultural - perspective needs to be adopted to see beyond the production value of the earthworm-plant interaction and into multiple trophic levels.

This increased emphasis in the area of multi-trophic interactions stems from correlations between earthworm presence and aphid populations. Wurst and Jones (2003) found that earthworm activity decreased aphid populations and that a change in plant quality was not the cause. Wurst et al. (2003) suggested that instead of the nitrogen pathway, the presence of earthworms somehow changed the makeup of the secondary compounds that were produced by the plant, and that these had a role in the palatability of the plant to the herbivore. These ideas were expanded by Wurst et al. (2004), who found that the presence of endogeic lumbricid earthworms increased N-concentration and secondary compound content of *Plantago lanceolata* seedlings. A decrease in aphid reproduction was correlated with the increase in N-concentration.

The aforementioned reviews suggested that the vast majority of earthworm effects on plants were positive. This was contradicted by some recent work on non-native invasive earthworms in systems previously devoid of earthworms. Gundale (2002) found decreases in the population of a rare fern in Minnesota, USA due to non-native earthworms reducing the litter layer. Earthworm invasion led to decreased diversity and abundance of understory vegetation in these forests (Hale et al., 2005; Hale et al., 2006). Multiple mechanisms may be at work in these situations, such as microhabitat modification (e.g. alteration of the forest floor, Burtelow et al., 1998) or direct feeding (e.g. decrease in fine roots, Fisk et al., 2004). In addition to these direct effects, earthworms may affect plants indirectly through removal of O-horizons, decreases in soil N and P, and reductions in mycorrhizae (Frelich et al., 2006). Considering the whole system, Frelich et al. (2006) suggest that as the detrimental effects of earthworm invasion begin to reduce plant numbers, herbivore browsing continues at the same level and this contributes to forest decline.

However, not all studies of invasive earthworms have found negative effects of earthworms on plants; Welke and Parkinson (2003) found positive and neutral effects of non-native *Aporrectodea trapezoides* on Douglas-fir seedlings. Additional research in the North American Boreal forest has shown that the coverage of a given plant species may decrease, increase, or be unaffected by earthworm invasion (Eisenhauer et al., 2007).

Microfauna and microflora

A wide array of studies relating earthworms to microbial functioning have been conducted in recent years, far too many to discuss them all in great detail (reviewed in McLean et al., 2006). Many were addressed in Brown (1995) and major topics and points of controversy are discussed below. In particular, the net effect of earthworms on microbial biomass has been a topic of controversy in the literature (Groffman et al., 2004).

Net effects and mechanisms

Scheu et al. (2002) found that an earthworm's effect on microbial functioning was related to the earthworm's functional group; endogeic earthworms decreased microbial biomass while other groups did not. However, epigeic and anecic earthworms have been found to decrease microbial biomass in several other studies (McLean and Parkinson, 1997a; Zhang et al., 2000; Eisenhauer et al., 2007). Species diversity within a functional group was also found to be important. The mechanisms behind these relationships are currently unknown. The negative effect of earthworms on microbial activity may have been caused by competition for food between earthworms and microbes (Scheu et al., 2002). Positive effects of earthworms on microbial communities have been proposed to be caused by selective feeding (Brown and Doube, 2004) or by the activation of dormant bacteria during passage of the earthworm gut (Fischer et al., 1995).

Hendricksen (1997) found that the presence of earthworms increased microbial respiration in soil under dung, but not in the dung itself. Multiple mechanisms for the increase were suggested: earthworms increased the availability of carbon and water; bacteria passing through the gut were activated; or earthworm activity enhanced mineralization of N and P. Somewhat similar to the latter two mechanisms, Devliegher and Verstraete (1997) introduced the important concepts of GAP (gut-associated processes) and NEP (nutrient-enrichment processes). They suggested that GAP were responsible for decreases in microbial biomass and activity (by digesting microbes, opposite of Hendricksen's second mechanism) and that NEP increased microbial biomass and activity by incorporating organic matter into the soil. Both of these processes were found to increase nutrients (except N) in the soil. Overall, NEP outweighed GAP resulting in a net increase in microbial biomass and activity (Devliegher and Verstraete, 1997). Although potentially useful, few studies have explained changes in microbial biomass with the NEP-GAP framework.

Microbial biomass was found to decrease in the presence of native North American earthworms (Lachnicht and Hendrix, 2001). With this decrease, there was an increase in N-mineralization and a decrease in microbial N, suggesting that earthworms caused a shift of N from microbial to mineral pools. In considering an earthworm invasion, McLean and Parkinson (1997a) also found that a high earthworm density decreased microbial biomass in the litter layer, probably due to a decrease in organic matter. Further studies supported this finding, and also found a decrease in microbial biomass in humus, mineral soil, and middens (Eisenhauer et al., 2007). Another study on non-native earthworm invasion found decreased microbial biomass in the forest floor, increased microbial biomass in the mineral soil, and net increases in microbial biomass and respiration (Groffman et al., 2004). Therefore, the net effect of earthworm invasion on microbial communities continues to be debated (Hendrix and Bohlen, 2002; McLean et al., 2006).

Microbial community composition

Earthworms not only affect microbial biomass but can also alter microbial community composition. This difference has been demonstrated between earthworm-created structures and bulk soil using several methods, including phospholipid fatty acid analysis (Enami et al., 2001), molecular and culture methods (Furlong et al., 2002), and BioLog plates (Amador and Görres, 2007). Mixing of the soil from different microhabitats, the addition of microbes from the earthworm gut, and changes to microbial habitats are all proposed mechanisms behind these community changes (Amador and Görres, 2007).

Bacterial to fungal ratios are sometimes altered by earthworms. Earthworms may change the ratio in favor of the bacteria (Scheu and Parkinson, 1994; Savin et al., 2004), in favor of the fungi (Zhang et al., 2000), or cause no significant shift (Lachnicht and Hendrix, 2001). Further studies are necessary to explore the mechanisms behind these differences, although it has been suggested that destruction of fungal hyphae plays a large role (McLean et al., 2006).

Microbial functioning

Earthworms may alter the rate at which microbes metabolize substrates. Zhang et al. (2000) and Li et al. (2003) found that earthworms increased microbial respiration per unit biomass while McLean and Parkinson (1997b) found a decrease. These differences may be due to changes in the bacterial to fungal ratio, since bacteria have a higher respiration per unit biomass than fungi (McLean et al., 2006).

Protozoa

A few studies have examined the interactions of earthworms and protozoa. Miles (1963) found that some earthworms required consumption of protozoa in order to mature. Earthworms were capable of digesting protozoa and actually showed preference for soil with many amoebae (Bonkowski and Schaefer, 1997). The presence (and therefore probable ingestion) of protozoa caused an increase in earthworm weight gain (Bonkowski and Schaefer, 1997). In contrast to what would have been expected, Hyvöen et al. (1994) found that earthworms had no effect on amoeba or flagellates. This result may have been due to the rapid reproductive capabilities of protozoa.

Mesofauna: Nematodes

Effects on soil mesofauna by earthworms have been less documented than effects on microbes or plants. These animals are certainly small enough to be accidentally ingested by earthworms, and circumstantial evidence suggests that this occurs. Dash et al. (1980) and Piearce and Phillips (1980) both found nematodes in earthworms' upper digestive tract but not in lower portions of the digestive tract. While Dash et al. worked with tropical earthworms and Piearce and Phillips with *Lumbricus terrestris*, both reached the conclusion that earthworms were digesting nematodes. Nematode populations declined when earthworms were introduced into New Zealand pastures (Yeates, 1981). One genus of root-feeding nematodes did not decline, likely due to an increase in root production. A number of pathways were suggested for the overall decline: accidental ingestion, changes in soil structure, decrease in fungal biomass, or competition for bacteria.

Senapati (1992) found that the introduction of earthworms actually increased nematode populations. Organic contributions (i.e. mucus or waste products) and alteration of abiotic conditions were suggested as the cause of this change. A slight increase in nematode density was found in *L. terrestris* middens, but this was not statistically significant (Maraun et al., 1999). An increase was found in burrow walls, where nematode numbers were greatly increased relative to soil outside of the drilosphere (Görres et al., 1997).

Hyvöen et al. (1994) demonstrated that earthworms had a negative effect on nematodes: significantly more nematodes were present in earthworm-free treatments. Enchytraeid worms did not affect nematodes and this led to the conclusion that the size of the earthworms relative to the size of the nematodes could be a reason nematodes were ingested. Competition for food resources could also have been a cause of the nematode population decline (Hyvöen et al., 1994). These same mechanisms were suggested by Domínguez et al. (2003), who found a negative effect of earthworms on bacterivore and fungivore nematodes.

Ilieva-Makulec and Makulec (2002) found that the introduction of earthworms initially decreases nematode numbers. Both the number of nematode functional groups and the numbers of species within those groups affected this interaction. This effect was different on different nematode feeding guilds, with bacterial feeders most affected (Ilieva-Makulec and Makulec, 2002). A logical conclusion can be formed from this information: bacterial feeders are most affected because they will be found where the most bacteria are found (i.e. on fresh organic materials) and earthworms readily and preferentially (Lee, 1985) consume these materials.

Mesofauna: Microarthropods

The presence of earthworms may increase or decrease the density and diversity of microarthropods (Acari and Collembola). Hamilton and Sillman (1989) found that a microhabitat containing earthworm middens had a different density of microarthropods than nearby soil without middens. The results were inconsistent: microarthropod density increased in a mowed field and a woodlot in spring, while it decreased in a woodlot in fall. These differences were caused by seasonal changes in earthworm feeding (causing the disturbance and restructuring of the middens), variable soil moisture content, and differences in the stage of decomposition of leaf fragments (Hamilton and Sillman, 1989). Likewise, in a mull soil some microarthropods were in higher abundance in *L. terrestris* middens while some were in lower abundance (Maraun et al., 1999). Microarthropods in association with an *Octolasion tyrtaeum* invasion decreased in abundance and diversity, but were unaffected by *L. terrestris*, and even increased the abundance in litter associated with *L. terrestris* middens (Eisenhauer et al., 2007). While it has been proposed that the negative impacts of earthworms on microarthropods are due to ingestion, it does not appear that earthworms are actually predators of microarthropods, but instead that consumption is inadvertent (Gutiérrez López et al., 2006).

Composition and total abundance of microarthropods were similar with and without earthworms in laboratory and field microcosms of Adejuyigbe et al. (2006). Decomposition was increased in the presence of both groups, relative to either one alone (Adejuyigbe et al., 2006). In a field study, earthworm presence decreased microarthropod diversity in FH and B_m horizons, increased diversity in the L horizon, and decreased abundance in the FH horizon (McLean and Parkinson, 2000). This somewhat contradicted previous mesocosm experiments, in which oribatid diversity increased in earthworm-added treatments, and abundances of groups of mites either increased or decreased (McLean and Parkinson, 1998). These changes were likely due to the disturbance of earthworm activity and earthworms' removal of the F and H layers.

Wickenbrock and Heisler (1997) examined the effects of earthworms on Collembola and the mechanisms responsible. They found that some Collembola benefited from earthworm burrows while others competed with earthworms directly for detritus. Earthworm burrows probably benefit Collembola because they provide ventilation and water flow into the soil; burrows contain a mucus lining that is quickly colonized by microbes and stabilizes the burrow. This provides food and a suitable microhabitat for some collembolan species. Loranger et al. (1998) showed that earthworm density was correlated with microarthropod density and diversity. They highlighted three similar mechanisms to explain this pattern: availability of food resources, improved air circulation, and water availability. Marinissen and Bok (1988) found that the mean sizes for several Collembola were all larger in plots with earthworms than in plots without, and suggested that while food resources may be important, pore size distribution and architecture were also important.

Some Collembola are actually attracted to earthworms (Salmon and Ponge, 2001); earthworm mucus secretions and/or urine likely have an odor that, over a short distance, attracted Collembola. Collembola were also found to have a direct trophic relationship to earthworms by actually drinking mucus and/or urine (Salmon and Ponge, 2001). Similar attraction has been documented with some other insects (see below).

Mesofauna: Enchytraeidae

Enchytraeidae appear to be affected by earthworms in much the same way as other mesofauna. While there has been little research into this interaction, they were usually negatively impacted, except in a few cases where they were positively impacted by earthworm-created structures (Migge-Kleian et al., 2006). Fewer enchytraeids were found in earthworm-inoculated plots in reclaimed land (Górny, 1984) and in laboratory mesocosms with earthworms (Haimi and Bouchelham, 1991). Huhta and Viberg (1999) found a decrease in the abundance of the enchytraeid *Cognettia sphagnetorum* due to competition with an earthworm. Earthworm biomass only decreased slightly in the presence of the enchytraeid. However, enchytraeids can have a significant impact on earthworms, increasing earthworm mortality (Haukka, 1987).

Earthworm burrows are known to have positive influences on enchytraeids. Earthworm burrows were found to attract enchytraeids and were thought to provide a preferred food source (Dózsa-Farkas, 1978). Earthworm middens had twice the abundance of mesofauna compared to surrounding soil (Schrader and Seibel, 2001).

Macrofauna

Very little is known about the effects earthworms have on other soil macrofauna. In North America, no studies had examined interactions between North American native earthworms and other soil-dwelling invertebrates (James, 1995). Brown's (1995) review included five studies on interactions with macrofauna, only two of which considered other soil dwelling taxa: isopods and millipedes were attracted to earthworm middens (Szlavecz, 1985); and the size of the snail *Helix aspera* increased due to earthworm presence, probably because the earthworms positively influenced a legume fed on by the snail (Thompson et al., 1993).

The other three studies found that (a) corn rootworm utilizes earthworm burrows for oviposition when natural crevices are rare, thereby influencing the rootworm's distribution (Kirk, 1981); (b) earthworms negatively affected populations of the spotted tentiform leafminer, a pest of apples in the eastern United States and Canada, and two associated parasitic wasps (Laing et al., 1986); (c) earthworm mucus was found to contain a kairomone that causes females of the fly *Coenosia trigrina* to oviposit near earthworm populations (Morris and Pivnick, 1991). The fly larvae feed on earthworms, and the adults feed on dipteran pests of crops. A number of other insects may also feed on earthworms (discussed below).

Earthworms have been found in close association with red wood ants (Laakso and Setälä, 1997). The microhabitat created by the ants was suggested to be favorable to earthworms, and further experiments found that earthworm mucus repels the ants, allowing the earthworms to reside in close associataion without being attacked. Laakso and Setälä (1997) suggested that the earthworms consume excess bacteria and fungi from the nest area and the relationship is somewhat mutualistic. However, abundance of two earthworm species was not affected by wood ant density in a field mesocosm experiment, even though earthworm numbers differed in nest mounds (Laasko, 1999).

Earthworms and millipedes may also interact in many ecosystems. Bonkowski et al. (1998) found earthworms were greatly benefited by the presence of millipedes and that the combination of the two taxa increased the removal of surface litter dramatically. Earthworm effects on millipedes in this situation were not reported. Conversely, in the context of non-native earthworm invasions these taxa may compete. Earthworms and millipedes have similar food requirements and occupy similar microhabitats. Snyder et al. (in review) found that millipede abundance and diversity decreased in *Amynthas agrestis* invaded soils. The mechanisms behind this interaction are currently being investigated.

Invertebrate Predators

Known arthropod predators of earthworms include spiders (Sivinski and Forrest, 1983; Nyffeler et al., 2001), ants (Lee, 1985; Yamaguchi and Hasegawa, 1996), carabid beetles (Mitchell, 1963; Lukasiewicz, 1996; Symondson et al., 2006), staphylinid beeltes (Edwards and Bohlen, 1996), dermapterans and mole crickets (Sivinski and Forrest, 1983). According to Edwards and Bohlen (1996), centipedes and other cryptic predators consume earthworms, but they cite no studies that confirm this occurs.

One genus of earthworms, *Agastrodrilus*, are known to feed upon earthworms (Lavelle, 1983). Several species of slugs and leeches are earthworm predators (Edwards and Bohlen, 1996). Earthworms are consumed by several planarian predators (Jennings, 1959), two of which are known to be invasive (Blackshaw, 1997; Ducey et al., 1999). Slugs (Pallant, 1972) and harvestmen (Halaj and Cady, 2000) are known to consume earthworms as scavengers, rather than predators.

Megafauna: Vertebrates, Predation, and Parasitism

Earthworms are eaten by “hundreds of species” of animals (Macdonald, 1983; Curry, 1998). The importance of this to earthworm populations and to predator diets is not clear, and little research has been done from the earthworm’s point of view. Brown (1995) states that earthworms are probably not a big part of the predators’ diet and no empirical evidence exists that earthworm populations are significantly affected by predation. Quantitative data are lacking on this topic (Lee, 1985). Likewise, parasites of earthworms are common and diverse, yet our knowledge of the true effect of these parasites on earthworm populations is lacking. Studies of invasive earthworm populations from native habitats compared to non-native habitats may shed light on this topic. Currently, earthworm parasites are known from the Acari (Oliver, 1962), Platyhelminthes (Edwards and Lofty, 1977), Nematoda (Poinar, 1978), Protozoa (Purrini, 1983), and Diptera (larvae) (Morris and Pivnick, 1991; Edwards and Bohlen, 1996).

Amphibians and Reptiles

Most amphibians and reptiles will consume earthworms that are surface-active at night (Macdonald, 1983). Earthworms are a significant part of the diet for some species of snakes, toads, frogs, lizards, and salamanders (Maerz et al., 2005). Snakes and lizards are known to respond to chemosensory cues of prey earthworms (Wang et al., 1988; Cooper and Habegger, 2001). Frequent consumption of earthworms suggests that earthworms have a positive effect on amphibians and reptiles, but Migge-Kleian et al. (2006) cite unpublished data from several sources that show invasive earthworms to be detrimental. They suggest that this may be due to a temporal change in food resource dynamics, or habitat modification by removal of the litter layer.

Birds, Mammals, and Monotremes

Earthworms are also known to be a significant part of the diets of many birds (reviewed in Macdonald, 1983). Mammals known to consume earthworms include: mice, moles, shrews, hedgehogs, weasels, raccoons, badgers, foxes, and pigs (Macdonald, 1983; Lee, 1985). Earthworm setae even leave permanent marks on teeth of moles, providing clues to their inclusion in mammal diets (Silcox and Teaford, 2002). The long-beaked echidnas (order Monotremata, *Zaglossus* spp.) are adapted to feed on earthworms. They have a long snout and a tongue with a groove containing backward-pointing spines (Griffiths, 1978). Lastly, some human cultures have earthworms as a significant part of the diet (Paoletti et al., 2003).

Conclusions

Earthworms are ubiquitous in their distribution and impacts in soil systems. The scale at which earthworms affect other biota stretches across of several orders of magnitude, from microbes 1 µm long, to vertebrates 2 m long, to plants over 30 m tall. The relative size of the organism and the earthworm often has a great deal to do with the net effects of earthworms on abundance and diversity. Microfauna are much smaller than earthworms, and are usually directly negatively affected through consumption or competition. Yet, they can be indirectly affected in a positive way. Likewise, protozoa have only been found to be negatively affected. The mesofauna, nematodes, mites, and collembola, are slightly larger on average, but are still much smaller than all earthworms. The same pattern applies; earthworms usually have negative direct effects on the mesofauna but sometimes have indirect positive effects, such as within structures that they build.

Organisms around the same size as or larger than earthworms include the invertebrate macrofauna and vertebrates. The vast majority of known interactions are predator/prey interactions. Therefore, the interactions are directly, trophically positive to the organism and negative to the prey earthworm. Lesser-studied interactions may show positive or negative effects of earthworms. More research is needed in this area.

Relative size of soil fauna and earthworms appears to determines interactions: organisms smaller than earthworms are usually negatively affected by earthworms, while those larger are almost always positively affected. However, relative size of plants to earthworms has little impact on plant-earthworm interactions. Earthworm effects on plants are more complicated, because they can occur through direct and indirect pathways simultaneously. Ecosystem management, the ecological grouping of the earthworm, and many site-specific factors all contribute to the directionality of earthworm effects on plants.

It is difficult to summarize the effects of earthworms on soil faunal community composition, because research into this topic is just beginning. This marks one of the new frontiers in soil ecology. Through ongoing research, we are beginning to understand how soil communities are put together, what individual species' roles are, and how a change in composition affects the functioning of the system (Wardle, 2002). The next step is to learn how changes in earthworm community structure can affect floral and faunal community structure, and how this in turn affects ecosystem functioning.

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CHAPTER 3

COMPETITIVE DISPLACEMENT OF NATIVE MILLIPEDES BY INVASIVE EARTHWORMS IN THE GREAT SMOKY MOUNTIANS NATIONAL PARK, USA[1]

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Abstract

Non-native European and Asian earthworms have invaded numerous locations across North America. European earthworms especially are well-known to cause dramatic changes in ecosystems in northern, formerly glaciated portions of the continent. However, less is known about the impacts of earthworm invasions in the southeastern United States, and this is particularly true for invasive Asian earthworms. An invasion of *Amynthas agrestis* (Megascolecidae) discovered along a disturbed roadside bordering the Great Smoky Mountains National Park provided an opportunity to study invasion dynamics, impacts on other detritivores, and effects on soil properties. A distinct invasion front was delineated in 2005 and 54 plots were arrayed across this front. From April - October of 2006 and 2007, earthworms and millipedes were sampled bimonthly, and the invasion front position was monitored monthly. The invasion front was dynamic on this time scale, frequently moving in one direction in some locations and in the opposite direction in other locations. Invasion spread was closely linked to climatic factors of temperature and moisture as measured in the air and soil, and was limited by drought in late 2006 and 2007. Mechanisms controlling the ability of *A. agrestis* to successfully spread are not fully understood. Soil samples collected at the conclusion of the study showed that persistent *A. agrestis* occupation increased A horizon soil aggregation and reduced the thickness of Oe/Oa horizon material, but did not affect A horizon microbial biomass, A horizon C:N, Oi horizon thickness, or mass of Oi and Oe/Oa horizons. Native earthworm abundance was positively associated with this invasion. Millipede species richness and density were greatly reduced by *A. agrestis* invasion, possible due to direct competition for food resources (Oe/Oa material). Continued invasion of non-native earthworms poses a threat to millipede populations.

Key words: *Amynthas*, earthworm, invasion dynamics, invasive species, competition, millipede, Diplopoda, Great Smoky Mountains, biodiversity, soil aggregation, invisible disturbance

Introduction

Invasive earthworms are a global problem and can cause considerable changes to ecosystems (Bohlen et al. 2004a,b, Hendrix 2006, Hendrix et al. in press). In North America, the vast majority of what is known about earthworm invasions comes from studies conducted in previously glaciated regions where no native earthworms are present and where European earthworms (family Lumbricidae) have been widely introduced. Invasion by non-lumbricid earthworms is a more recently documented phenomenon (Steinberg et al. 1997, Burtelow et al. 1998, Callaham et al. 2003a) and the effects of these invasions are poorly understood.

We studied one such invasion in the southeastern glacial refugium of North America, where native earthworms remain today. The primary invasive earthworm was *Amynthas agrestis* (Goto and Hatai 1899) (Megascolecidae), a pheretimoid earthworm native to southeast Asia. Several species of *Amynthas* and the closely related genus *Pheretima* were documented in the central (Illinois) and southeastern (Mississippi) United States by the 1890’s (Garman 1888, Gates 1937, National Museum of Natural History public communications). Approximately 15 species have established and spread widely across the eastern United States (Reynolds and Wetzel 2004). *Amynthas agrestis* has been known in the United States since 1953 (Gates 1953) and was documented in the study region in the 1970's (Reynolds 1978). Burtelow et al. (1998) stated that "little is known about *Amynthas* beyond the physical zoological description." While this assessment remains mostly true, much has come to light recently, including some information on temperature and moisture tolerances for certain species (Fragoso et al. 1999, Richardson et al. unpublished manuscript).

Studies on the introduction and invasion biology of *Amynthas* spp. are rare but invasions by *A. agrestis* have been documented in the southern Appalachian Mountains (Callaham et al. 2003a). It is suspected that most *Amynthas* species, like many other earthworms, were transported by humans in soil of potted plants and continue to be dispersed with horticultural plants, through earthmoving activities, and by anglers using *Amynthas* as fishing bait (Gates 1958, Callaham et al. 2003a). Once introduced, *Amynthas* spp. can have significant impacts on soil ecosystems. In forests in New York, USA, *A. gracilis* increased soil N-mineralization and nitrification, reduced organic horizon organic matter, increased microbial biomass, and increased surface soil aggregation (Steinberg et al. 1997, Burtelow et al. 1998).

Earthworm invasion theory predicts that a disturbance is required to negatively impact or entirely extirpate native earthworm populations before non-native earthworms are able to invade (Kalisz and Wood 1995). Thus, direct competition between native and non-native earthworms is expected to occur rarely, if ever. However, coexistence of native and non-native earthworms has been documented in some disturbed sites (Callaham et al. 2003b, Hendrix et al. 2006) and is predicted to occur at intermediate levels of disturbance (Hendrix et al. in press), although it is unclear if co-occurrence persists. Native earthworms are present in our study site, yet *Amynthas agrestis* seems to be able to invade undisturbed portions of the forest away from the disturbed roadside where the invasion likely began.

Based on what little was known about *A. agrestis*, our hypotheses were first that the population of *A. agrestis* would invade the forest at a steady pace. Second, *A. agrestis* invasion would decrease the abundance and species richness of the millipede community and decrease the abundance and species richness of the earthworm community. Third, invasion would reduce the thickness and mass of organic horizons (both Oi and Oe/Oa) and increase soil aggregation in the A horizon. Finally, we hypothesized that *A. agrestis* would decrease microbial biomass and increase the proportion of C contained in the A horizon relative to soils without *A. agrestis*.

Methods

Site description and experimental design

The Great Smoky Mountains National Park (GSMNP) encompasses most of the highest peaks of the southern Appalachian Mountains, and spans the border between the states of Tennessee and North Carolina, USA. The west end of the Park is bordered by US Highway 129; for 3.8 km this road is flanked on the east by GSMNP and on the west by the Chilhowee Reservoir, a dammed portion of the Little Tennessee River. The Chilhowee Dam was closed in 1957 and the dam project relocated ~5 km of US Highway 129 to its present location. Recreational opportunities, including fishing with live bait, are permitted on this reservoir. While fishing is allowed in the Park, live bait is not permitted.

Local topography drains to the reservoir and features alternating valleys and ridges with tall (70-120 m) bluffs that abut the road. During initial surveys, invasive earthworms (specifically *Amynthas* sp.) were found in every suitable habitat (i.e. areas that were not vertical rock faces) along this stretch of road. Invasion fronts were delineated in several small watersheds to find a suitable location to set up sampling plots.

We delineated the earthworm invasion front during the spring and summer of 2005 by searching through leaf litter and at the soil surface for *Amynthas* individuals, which were recognizable due to their thrashing defense behavior. Earthworms were left where they were found. Hand searching proceeded from the roadside into the forest, 5-10 m at a time, until no *Amynthas* could be found. The search then proceeded in 1 m increments from the last known *Amynthas* location for ~5 m. When a location was reached without *Amynthas*, it was electroshocked (see below) for three minutes to determine that no *Amynthas* individuals had burrowed into the soil. Several earthworms were taken during this period and all were determined to be *Amynthas agrestis*.

Valley vegetation was dominated by *Acer* spp., *Quercus* spp., *Liquidambar styraciflua*, and *Liriodendron tulipifera*, while the more xeric ridges were dominated by white pine (*Pinus strobus*). In general, the area was classified as mesic-xeric oak-dominated climax forest (Whittaker 1956). Ridge soils were a complex of moderately deep Junaluska and deep Brasstown series soils, fine-loamy, mixed, subactive, mesic Typic Hapludults. Valley soils were a complex of shallow Cataska series and moderately deep Sylco series soils, which are loamy-skeltal, mixed, active (Sylco) or semiactive (Cataska), mesic Typic Dystrudepts (USDA 2007).

We selected two adjacent valleys with a distinct invasion front; five parallel transects were placed across the invasion front at randomly chosen locations. Each transect consisted of nine 3 x 3 m plots placed 3 m apart (Fig. 3.1), centered on the invasion front. In 2007 we anticipated the need for additional plots beyond the invasion front in the western valley and therefore three additional plots were placed on these three transects. Due to limitations in the number of leaf litter samples that could be processed we concurrently discontinued sampling the three sets of plots furthest behind the invasion front on these three transects.

Soil sampling

Soil cores (6 cm diameter, 15 cm depth) and ~100 g additional A horizon soil were collected from each plot at the end of the study (October 2007). Upon return to the lab, the O horizon was separated into Oi and Oe/Oa (a combination of Oe and Oa) horizons. The thickness and air-dry mass of these horizons were measured. Samples of air-dry A horizon soil were analyzed for C and N content on a Carlo Erba NA1500 CHN Combustion Analyzer (Carlo Erba, Milan, Italy). To characterize the distribution of water-stable aggregate size classes in each plot, ~50 g of air-dry soil was wet-sieved (Six et al. 2000). Rocks larger than 2 mm were removed by hand before weighing the sample. Soil aggregate fractions were categorized by size-class into POM (floatable particulate organic material), >2000 μm, 2000-250 μm, and 250-53 μm. The <53 μm fraction contained very little material and was excluded from the analysis. Microbial biomass was measured by chloroform fumigation-extraction (Vance et al. 1987) using a K_{ec} of 0.38.

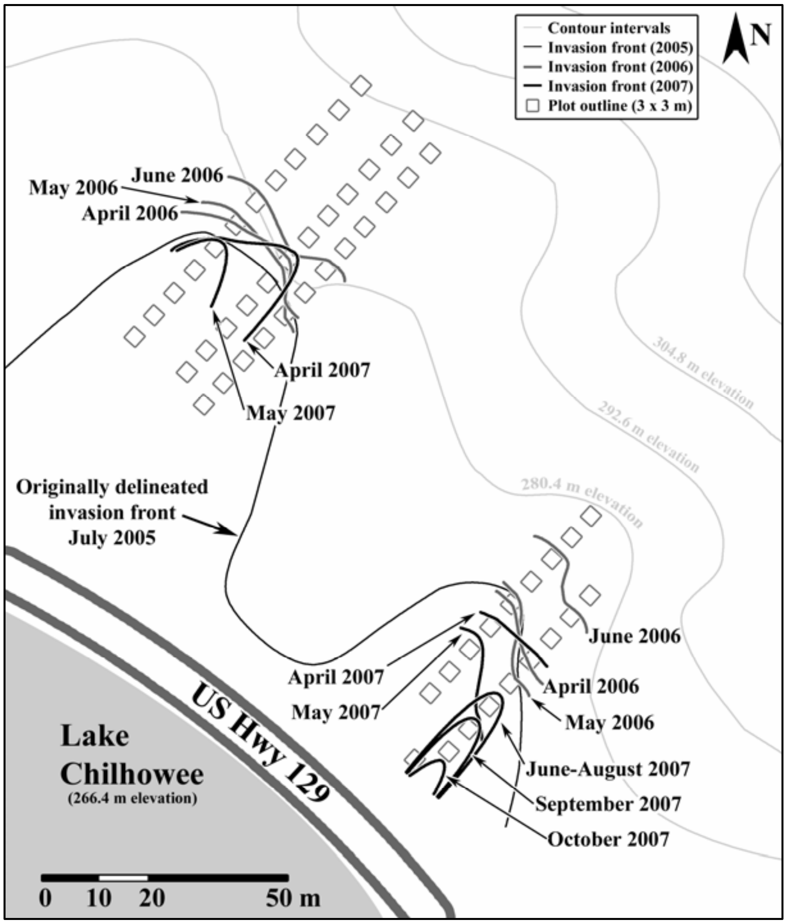

Fig. 3.1. Map of study site and invasion front dynamics of *Amynthas agrestis*. Invasion front locations were determined by a combination of bimonthly electroshocking within plots and monthly leaf-litter searches. No earthworms were found during July - October 2006 due to equipment failure and low soil moisture levels. Plot locations based on field measurements and GPS locations, contour intervals based on the Calderwood Quadrangle USGS 7.5 minute series topographic map.

Sampling of fauna

We monitored invasion front status monthly and sampled earthworms and millipedes bimonthly from April - October 2006 and 2007. No sampling occurred during winter months as cold weather limited soil fauna activity. As *A. agrestis* was reliably found by hand searching during delineation, hand searching through the litter layer was used to monitor the status of the invasion front. Searching was performed in between (not within) the plots and transects to minimize disturbance. Data from earthworm electroshocking were compiled to assess the status of the invasion front in sampling months.

Earthworms were sampled via the octet electroshocking technique (Schmidt 2001) at a randomly chosen location within each plot. The electroshocking method was the only suitable choice since it involved minimal soil disturbance; chemical extraction methods were not feasible because they could have altered soil moisture, and in turn affected earthworm movement. Through June 2006 we used a commercially available electroshocker (DEKA 4000 W, DEKA Gerätebau, Marsberg, Germany) at 300, 350, 400, 500, and 600 V for 2 minutes each. This machine failed during July 2006 and as a result we were unable to collect earthworms during August and October 2006. During 2007 we used a similar instrument (Lachnicht et al. unpublished manuscript); field observations indicated this produced a comparable sample. This second octet device was not automated and so opposing pairs of probes (four probes total) around the octet were activated manually at 300 V for 2.5 min each, then at maximum voltage (350-450 V) for 2.5 min each. All earthworms responding to electroshocking were preserved in 5% formalin and identified to the lowest taxonomic level possible.

Millipede abundance was measured by sampling with the leaf litter collection/Berlese extraction technique (Snyder et al. 2006). Litter was sampled at a randomly chosen location in each plot by placing a 30 cm diameter frame on the ground and collecting the organic soil horizons within the frame. This litter was placed into a canvas bag and kept cool until it was returned to the lab. Litter was transferred onto a Berlese funnel, where it was extracted for 72 h into 70% ethanol. Millipede species richness was estimated for extracted material in combination with a 0.5 person-hour timed hand collection (Snyder et al. 2006). For each sampling date, all earthworm and millipede sampling was completed within a 24 h period.

Environmental data collection

Edaphic conditions were monitored on-site using two HOBO data loggers each equipped with two temperature and two dielectric soil moisture sensors, one set placed at the interface of the O and A horizons (mineral soil surface) and one set at 10 cm depth in the mineral soil. One data logger was placed in the western valley at 269 m elevation and one was located on the ridge top at 284 m elevation. Elevations of the data loggers and the center of each plot were measured with rod and transept from a nearby National Geodetic Survey benchmark. Additional climate data (precipitation and air temperature) were obtained from a National Park Service air quality monitoring station at Cades Cove, 16 km northeast of the study site, 561 m elevation.

Statistical analysis

Because of the dynamic behavior of the invasion front, an index was created to describe the amount of time each plot was impacted by earthworms. A plot that was ‘invaded’ at a sampling time, based on the invasion front location, was scored 1, for a possible total ranging from 0 (never occupied by *A. agrestis*) to 11 (always occupied by *A. agrestis*). The relationships between elevation and millipede species richness and original plot position were investigated with Spearman correlation. Invasion impacts on soil properties were tested with linear regression analysis and impacts of *A. agrestis* invasion on millipedes and earthworms were tested with repeated measures GLM, with sample date (*n* = 8) as the repeated measure. Only plots that were sampled in both 2006 and 2007 could be included in the repeated measures analysis (*n* = 36). Edaphic condition data, logged every 15 min, and air temperature, logged every hour, were averaged for the month preceding invasion front monitoring. These averages were individually regressed against the net movement of the invasion front at each transect. Data analysis was performed with SAS (Version 9.1, SAS Institute, Cary, NC, USA).

Results

Invasion front movement

The *Amynthas agrestis* invasion front was far more dynamic than expected (Fig. 3.1). The invasion front generally moved into the forest during early 2006 (at a rate of 12 m/y) and receded to lower elevations (toward the road) during all of 2007. The severe drought which impacted the region in late 2006 and 2007 contributed to a decline in earthworm activity and the ability of the invasion to proceed into the forest or even to maintain its position. Invasion front dynamics were primarily related to climatic conditions (temperature and moisture, Fig. 3.2). Aerial and edaphic conditions were good predictors of the net change in invasion front position (Table 3.1).

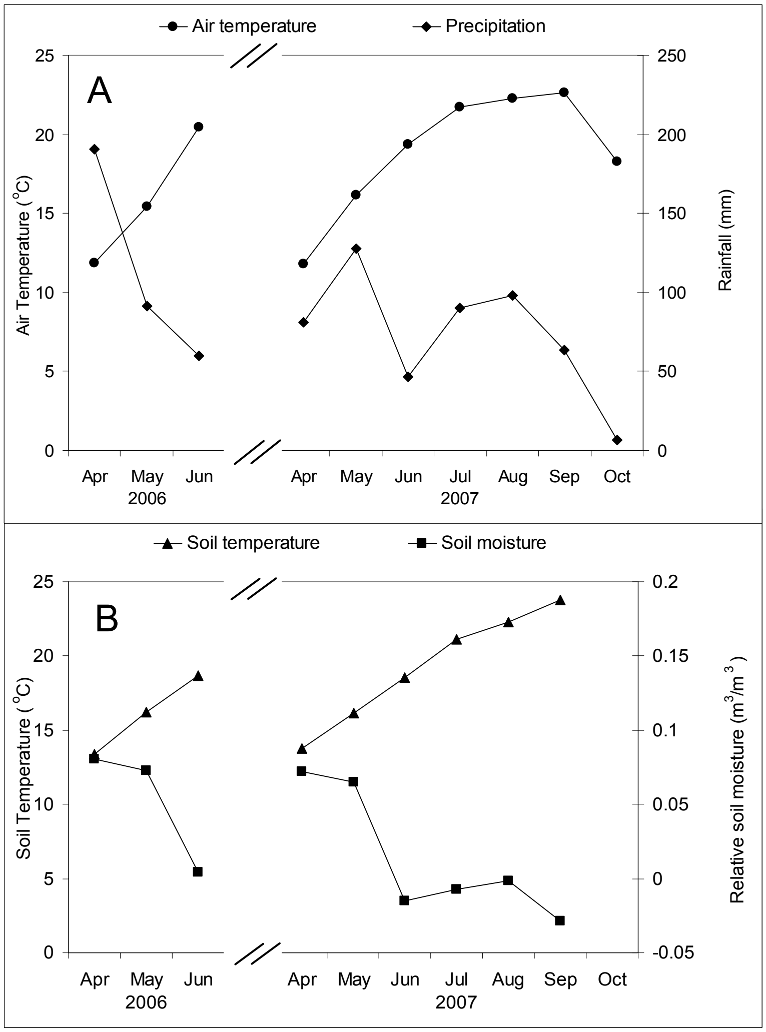

Fig. 3.2. Environmental conditions during the month previous to *A. agrestis* sampling, including (A) air temperature and precipitation recorded at the Cades Cove weather station and (B) soil temperature and relative soil moisture measured at 10 cm depth on the ridge top at the field site. Soil moisture was measured with an uncalibrated dielectric sensor, therefore moisture values are relative and not absolute.

Table 3.1. Results of linear regressions of climatic and edaphic data against net invasion front distance (measured since 2005).

Independent Variable	R^2	*P*
Monthly precipitation	0.1302	0.0100
Air temperature	0.2229	0.0005
Ridge soil surface temperature	0.2788	0.0002
Ridge soil surface moisture	0.1955	0.0043
Valley soil surface temperature	0.4409	<0.0001
Valley soil surface moisture	0.1863	0.0172

Due to equipment failure no earthworms were sampled during August and October 2006, but searches of the leaf litter in July - October 2006 revealed earthworms only in the portion of the valley which was the lowest elevation and thus had highest surface soil moisture (data not shown in Fig. 3.1). *Amynthas agrestis* could also be found only in these wettest locations during July - October 2007 even via electroshocking. During June and August 2007 *A. agrestis* was collected in only one plot and October 2007 sampling produced no earthworm specimens. However, the positions of the invasion front were determined in part by searches outside of the plots (Fig. 3.1). The location of the western plots did not incorporate this valley bottom, but *Amynthas* individuals were visually confirmed in the valley bottom these periods.

Soils

Amynthas invasion had impacts on soil structure, but little impact on chemical or biological characteristics. Soil microbial biomass and soil C:N were unaffected by *Amynthas* invasion (data not shown). Plots which were invaded by *A. agrestis* for longer amounts of time were subject to increased A horizon soil aggregation (Fig. 3.3) and a reduction in the thickness of some organic horizons (Fig. 3.4). Invasion increased soil aggregates in the >2000 μm size class ($P = 0.0035$) with a corresponding decrease of 250-53 μm aggregates ($P = 0.0011$). No change was observed in particulate organic matter ($P = 0.0795$) or 2000-250 μm soil aggregates ($P = 0.0836$). The O horizon was divided into Oi (recently fallen litter) and Oe/Oa (partially decomposed litter) horizons. Oi horizon thickness did not change significantly due to *A. agrestis* invasion ($P = 0.0785$). The thickness of the Oe/Oa horizon was reduced by *A. agrestis* ($P = 0.0010$) yet the mass of this layer did not decrease significantly ($P = 0.1506$).

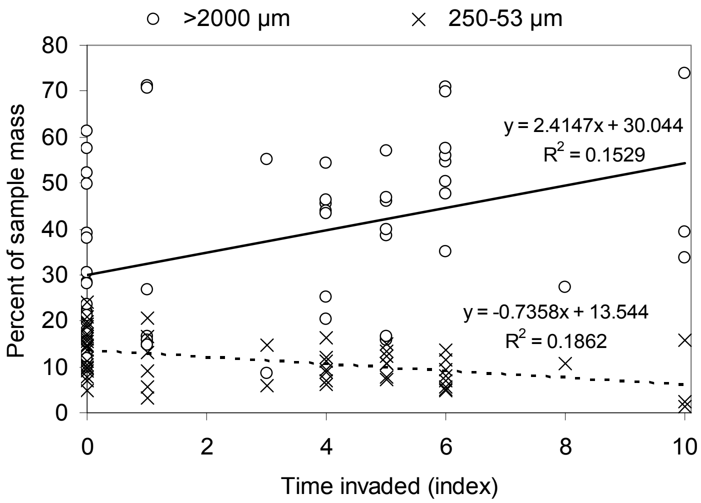

Fig. 3.3. Regression of percent water-stable aggregates in the >2000 µm size class (solid line, ●) (*P* = 0.0035) and 250-53 µm (dotted line, ×) (*P* = 0.0011) against *Amynthas agrestis* invasion (index of time invaded).

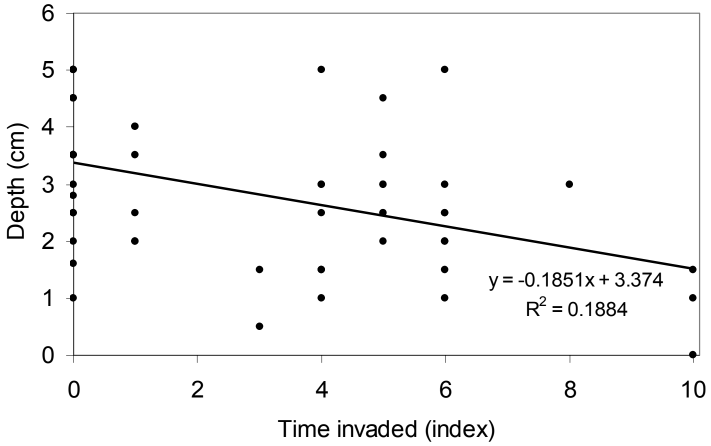

Fig. 3.4. Regression of Oe/Oa horizon thickness (cm) in October 2007 against *Amynthas agrestis* invasion (index of time invaded) (*P* = 0.0010).

Earthworms

Several other species of earthworms were found at the study site, including native *Diplocardia* spp. and *Bimastos* spp., invasive Asian *Amynthas corticis*, and invasive European Lumbricidae (*Aporrectodea* sp., *Dendrobaena octaedra*, *Eiseniella tetraedra*, *Lumbricus rubellus*, and *Octolasion tyrtaeum*). Lumbricid abundance was not affected by *A. agrestis* invasion (Table 3.2, $P = 0.1260$). *Amynthas corticis* was found almost exclusively in one moist valley bottom and therefore it was strongly associated with *A. agrestis* in the statistical analysis ($P < 0.0001$, data not shown). Native earthworm abundance was slightly positively associated with *A. agrestis* invasion (Fig. 3.5, Table 3.2, $P = 0.0331$).

Millipedes

Over the course of the study, millipede species richness varied between 1 and 14 species per plot; 29 species were identified from the study site in total. Millipede species richness was significantly negatively related to *A. agrestis* invasion (Fig. 3.6A, Table 3.2, $P = 0.0009$). Millipede species richness (accumulated over all sampling dates) was not significantly correlated with plot elevation ($P = 0.0709$). Millipede density was similarly negatively impacted (Fig. 3.6B, Table 3.2, $P = 0.0013$) by *A. agrestis* presence.

Discussion

Invasion dynamics

The *Amynthas agrestis* invasion front did not move continuously in one direction, as we had anticipated it would. Instead, the monthly movement was dynamic, with portions of the front moving forwards while others remained static or moved backwards.

Table 3.2. Results of repeated measures GLM analyses.

Source	df	SS	MS	*F*	*P*
Millipede Species Richness					
Time invaded	7	78.129	11.161	4.97	0.0009
Error	28	62.858	2.2449		
Millipede Density					
Time invaded	7	53733.8	7676.3	4.74	0.0013
Error	28	45365.1	1620.2		
Lumbricidae Density					
Time invaded	7	13.424	1.9177	1.80	0.1260
Error	28	29.770	1.0632		
Native Earthworm Density					
Time invaded	7	13.372	1.9102	2.61	0.0331
Error	28	20.500	0.7321		

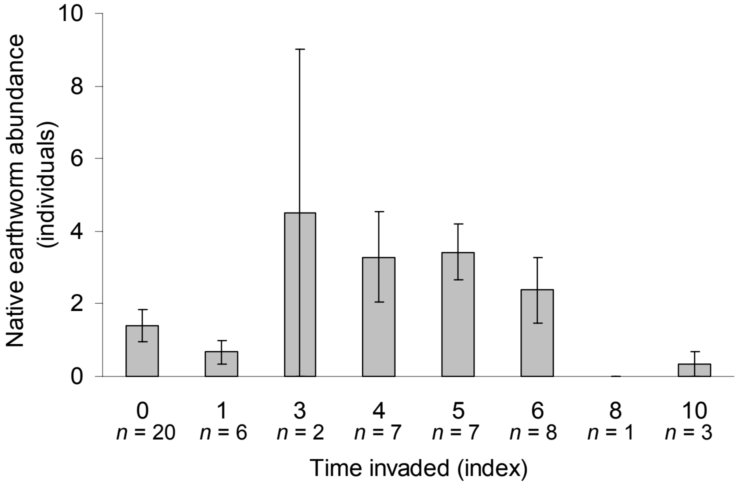

Fig. 3.5. Native earthworm (*Diplocardia* spp. and *Bimastos* spp.) abundance in relation to *Amynthas agrestis* invasion (index of time invaded). Data represents the mean (± 1 SE) of the net number of individuals collected during the 8 sample dates. Mean density of native earthworms across all dates and plots was 1.2 individuals/m^2.

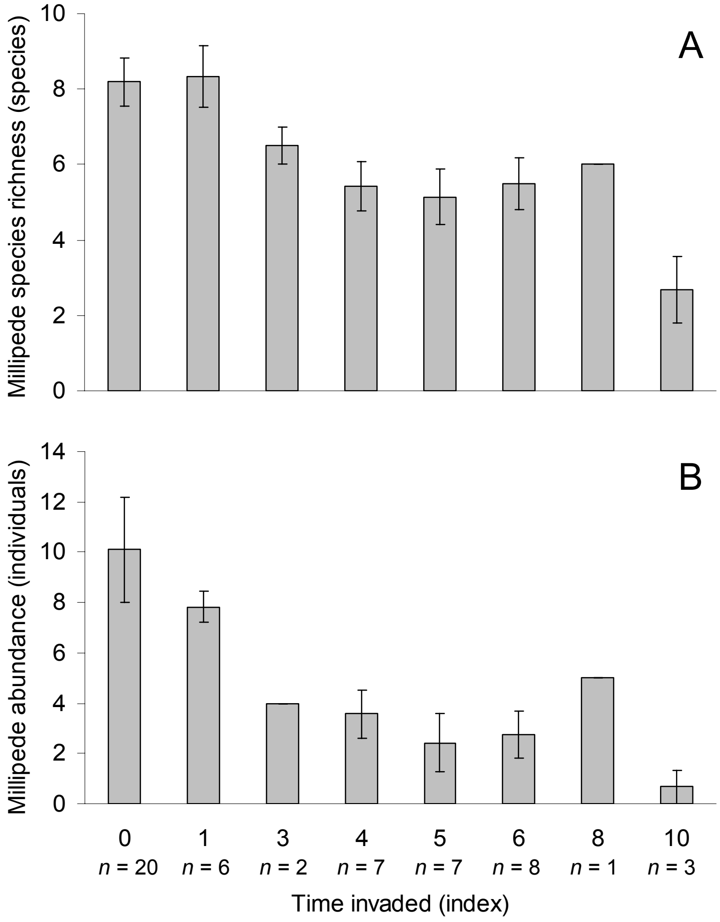

Fig. 3.6. Millipede abundance (A) and species richness (B) in relation to *Amynthas agrestis* invasion (index of time invaded). Abundance data represents the mean (± 1 SE) of the net number of individuals collected during the 8 sample dates. Mean density of millipedes across all dates and plots was 21.1 individuals/m^2. Species richness data represents the mean (± 1 SE) of the species richness accumulated during the study.

Over longer time scales the invasion clearly responded to seasonal and yearly changes in climate observed in soil temperature and moisture conditions. While we observed high densities of *A. agrestis* in 2005, this was during a period of typical precipitation. The original (2005) pattern of *A. agrestis* invasion coincided to a great degree with the topographic variability at the site, but we expect this is primarily due to climatic factors. Indeed, the movement of the invasion front predominantly reflected edaphic conditions at the site. The invasion ceased moving into the Park as a drought began in late 2006 and finally receded to the wettest portions of the study site throughout 2007, as the drought continued. Moisture is clearly a major factor in the success of these earthworms, yet their environmental tolerances are only beginning to become known through carefully controlled laboratory studies (Richardson et al. unpublished manuscript).

Amynthas agrestis *impacts*

Amynthas agrestis invasion caused significant changes in the soil ecosystem that affected epigeic habitat and fauna. Impacts of *A. agrestis* were limited to the soil surface. Only the top few centimeters of mineral soil exhibited increased aggregation, but there were no effects of invasion at deeper soil depths. Native earthworms were found in low densities and the few species for which ecological strategies have been identified are primarily endogeic (James and Cunningham 1989, Callaham and Hendrix 1998), therefore, native *Diplocardia* spp. may have been little impacted because they have little niche overlap with *A. agrestis*. The slight increase observed in native earthworm abundance in invaded plots may have been due to processing of litter by epigeic *A. agrestis*, which may have increased the availability of food resources for endogeic native earthworms. This has been observed with epigeic millipedes and endogeic earthworms (Bonkowski et al. 1998) in European beech forests.

Millipedes, which are primarily epigeic (Hopkin and Read 1992) were more likely to be affected by the rapid removal of the Oe/Oa horizon. Significant declines in millipede richness and abundance were seen with *A. agrestis* invasion. An alternate explanation for millipede decline could lie with an environmental or topographic gradient to which both millipedes and earthworms responded. However, millipede richness was not correlated with elevation, and we would have expected to see more millipedes at higher moisture levels (i.e. lower elevations), but the opposite was observed.

The most likely explanation for the decline in millipede richness and density is competitive exclusion due to *A. agrestis* invasion. Although competitive interactions between these taxa have not yet been described in the literature, there is mounting evidence that *Amynthas* spp. and millipedes compete for food resources (Snyder et al. unpublished manuscript). Invasion of *A. agrestis* and subsequent Oe/Oa horizon removal at larger spatial scales may extirpate entire millipede populations, and thus pose a formidable challenge for millipede conservation in the southern Appalachian Mountains.

Invasion of undisturbed habitat

Invasive earthworms are well known to be successful in disturbed habitats (Kalisz and Dotson 1989), but reports of invasion of undisturbed habitats are less common (see Hendrix et al. 2006). The current paradigm in earthworm invasion theory is that an ecosystem needs to be subjected to a disturbance, reducing or eliminating native earthworm populations, before non-native earthworms are able to invade (Kalisz and Dotson 1989, Kalisz and Wood 1995). In our study area, *A. agrestis* seemed to be able to invade undisturbed portions of the forest away from the disturbed roadside where the invasion presumably began.

Although the study site showed no signs of obvious disturbance beyond an occasional piece of refuse, many “invisible” disturbances (sensu Kalisz and Wood 1995) could have affected this site over the last 200 years: natural fires are known to have occurred in 1988 and 1999 (M. Taylor and B. Nichols, National Park Service personal communication), the area was probably logged before it became a National Park, humans may have entered the site frequently from nearby settlements, and edge effects may exist due to the adjacent road and reservoir. These disturbances could have contributed to the ability of *A. agrestis* to successfully establish in this location.

Whether these factors influenced *A. agrestis* establishment success does not explain what has limited the invasion spread. Human activity, logging, and fires would have disturbed soil much further into the forest than *A. agrestis* has invaded at our study site. The edge effect hypothesis could explain this distribution, and Kalisz and Dotson (1989) suggested that invasive earthworms are typically found only within 50 m of severely disturbed areas (e.g. a frequently mowed roadside). However, the invasion front we monitored went far beyond this distance and our initial survey found other valleys which were invaded at least 500 m from the road.

Amynthas agrestis invasion could have been facilitated by other invasive, non-native earthworm species. An early disturbance may have allowed European Lumbricidae to invade, and the ongoing impacts of these species allow *A. agrestis* to invade. This could be similar to the invasive earthworm succession reported in the northern hardwood forests (Hale et al. 2005) and could be an example of invasional meltdown (Simberloff and Von Holle 1999). However, this also does not explain the distribution of *A. agrestis* because Lumbricidae were found throughout the plot array.

Several explanations, which are not mutually exclusive, could help us understand why this invasion has not spread into all suitable habitat (Kinlan and Hastings 2005). First, climatic factors could slow invasion spread temporarily, particularly in marginal habitat. In this case, the progression and recession of the invasion front occurs as microhabitat conditions change suitability on short time scales. Second, this invasion could be in a lag-phase, where an established invasion has an initial slow spread. This phenomenon has been observed in many invasions yet the underlying mechanisms are not fully understood (Crooks 1995, Suarez et al. 2001). Not enough is known about the origin of this invasion to confirm or deny this hypothesis. Third, the biotic resistance hypothesis (Elton 1958) has been little tested in earthworm invasions. If *A. agrestis* was in competition with millipedes for food resources, this might produce sufficient resistance to slow invasion spread. Lastly, invaders frequently experience predator and parasite release when invading a new habitat (Torchin et al. 2003). However, new predators and parasites may also begin to take advantage of the new food or host, and this has been suggested for earthworm predators, such as salamanders (Maerz et al. 2005). Preliminary evidence of internal parasites in invasive earthworms from the study site (B. A. Snyder personal observations) and increased carabid beetle activity near the invasion front in 2006 (S. C. Rostkowski unpublished data) could suggest that this is occurring. Multiple factors influence the ability of *A. agrestis* invasion to spread and a long-term, large-scale study will be needed to disentangle these factors.

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CHAPTER 4

COMPETITION BETWEEN INVASIVE EARTHWORMS (*AMYNTHAS CORTICIS*, MEGASCOLECIDAE) AND NATIVE NORTH AMERICAN MILLIPEDES (*PSEUDOPOLYDESMUS ERASUS*, POLYDESMIDAE): EFFECTS ON CARBON CYCLING AND SOIL STRUCTURE[1]

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[1] Snyder, B.A., B. Boots, and P F. Hendrix. To be submitted to *Soil Biology & Biochemistry*.

Abstract

Invasive earthworms can have significant impacts on C dynamics through their feeding, burrowing, and casting activities, including the protection of C in microaggregates and alteration of soil respiration. European earthworm invasion is known to affect soil micro- and mesofauna, but little is known about impacts of invasive earthworms on other soil macrofauna. The presence of Asian earthworms (*Amynthas* spp.) is increasingly being reported in the southern Appalachian Mountains in southeastern North America. This region is home to a diverse assemblage of native millipedes, many of which share niches with earthworm species. This situation suggests that there is potential for earthworm-millipede competition in areas subject to *Amynthas* invasion.

In a laboratory microcosm experiment, we used two ^{13}C enriched food sources (red oak, *Quercus rubra,* and eastern hemlock, *Tsuga canadensis*) to assess food preferences of millipedes (*Pseudopolydesmus erasus*), to determine the effects of millipedes and earthworms (*Amynthas corticis*) on soil structure, and to ascertain the nature and extent of the interactions between earthworms and millipedes. Millipedes consumed both litter species and preferred red oak litter over eastern hemlock litter. Mortality and growth of millipedes were not affected by earthworm presence during the course of the experiment, but millipedes assimilated much less ^{13}C in all litter treatments when earthworms were present.

Fauna and litter treatments had significant effects on soil respiration. Millipedes alone reduced CO_2 efflux from microcosms relative to no fauna controls, whereas earthworms alone and together with millipedes increased respiration, relative to the no fauna treatment. CO_2 derived from fresh litter was repressed by the presence of macrofauna. The presence of red oak litter increased CO_2 efflux considerably, compared to hemlock litter treatments.

Millipedes, earthworms, and both together reduced particulate organic matter. Additionally, earthworms created the significant shifts in soil aggregates from the 2000-250 and 250-53 µm fractions to the >2000 µm size class. Earthworm-induced soil aggregation was lessened in the 0-2 cm layer in the presence of millipedes. Statistically significant millipede impacts on ^{13}C in aggregates were limited the top 2 cm of the microcosms; earthworms created ^{13}C aggregates throughout the microcosm.

Our results suggest that invasion of ecosystems by *Amynthas corticis* is unlikely to be limited by available litter species and these earthworms are likely to compete directly for food resources with native millipedes. Invasion could cause a net loss of C at the ecosystem scale due to increased respiration rates, but this may be offset in the long term by C protected in soil aggregates.

Keywords: Millipede, earthworm, *Pseudopolydesmus*, *Amynthas*, competition, isotope, carbon dynamics, aggregation, soil structure, invasive species

Introduction

Earthworm invasions in North America have been observed for over a hundred years (e.g. Eisen, 1900; Smith, 1928). The presence of non-native earthworms in disturbed sites has been documented throughout much of North America (e.g. Reynolds et al., 1974; Reynolds, 1978). However, earthworm invasion of relatively undisturbed habitats has also been recorded. Callaham et al. (2003) recently observed the presence of Asian earthworms (*Amynthas* spp.) in the southern Appalachian Mountains. While the extent and impacts of these invasions are only now becoming clear, many of the ecological impacts of other earthworm invasions are well documented (Bohlen et al., 2004a; Hendrix, 2006).

It is well known that earthworms can have significant impacts on many aspects of ecosystem functioning, including the carbon cycle. For instance, *Aporrectodea caliginosa* casting activity has been shown to increase C sequestration in soil micro- and macroaggregates of southern Appalachian Piedmont soils (e.g. Bossuyt et al., 2005). Incorporation of organic matter into the soil by earthworms can play a major role in C-cycling, but it is clear that these effects may vary with time, earthworm species, soil type, and C availability (Bohlen et al., 2004a; McLean et al., 2006).

In locations where earthworms are not present, millipedes are often the dominant detritivores (Hopkin and Read, 1992), however, very little is known about their ecosystem-level effects. Millipedes function as fragmenters of organic material and are important to the primary decomposition of all forms of detritus (Ausmus, 1977; Hopkin and Read, 1992; Rawlins et al., 2006). Millipede fecal pellets can account for a significant percentage of organic soil layers (up to 39% of standing litter, Dangerfield and Milner, 1996) and because of their chemical and physical nature can serve as hotspots for microbial activity (Anderson and Bignell, 1980).

Litter consumption by millipedes is a significant pathway for C movement (Rawlins et al., 2006). Millipedes assimilate little of the C they consume (Anderson and Bignell, 1982; Bonkowski et al., 1998; Toyota et al., 2006) although considerable variation is reported in the literature (<10-83%). Undigested C is deposited in fecal pellets where it is subject to increased microbial activity and C loss through respiration (Anderson and Bignell, 1980; Maraun and Scheu, 1996), but C remaining is subsequently protected in fecal pellet-derived aggregates (Toyota et al., 2006).

Millipedes share some niche requirements with earthworms, as they both live in and feed on leaf litter and soil. Millipede species are known to differentiate niches by utilizing different microhabitats (O'Neill, 1967; Enghoff, 1983) and different positions within a particular soil horizon (Geoffroy, 1981). Spatial niche partitioning occurs between millipedes and other detritivores (Davis and Sutton, 1977), but interactions between earthworms and millipedes have been little studied and are dependent on the ecological strategies of the species involved. In European Beech forests, endogeic earthworms (*Octolasion lacteum*) benefited from the presence of millipedes, and preferentially fed on their fecal pellets (Bonkowski et al., 1998). Although epigeic and anecic earthworm interactions with millipedes have not been demonstrated, these species all consume leaf litter and are likely to compete for this resource.

Given the increasing documentation of *Amynthas* spp. in the southern Appalachian Mountains, we hypothesized that millipedes in this region are likely to face competition from these invading earthworms. To our knowledge earthworm-millipede competitive interactions have not been examined. Such competitive interactions could have consequences not only for millipede populations, but also for C dynamics at the ecosystem and broader scales. The aim of this study was to document the effects of an invasive earthworm species (*Amynthas corticis*) on the feeding behavior of millipedes

(*Pseudopolydesmus erasus*) and the impacts of these species on C flow in laboratory micrcosms.

Methods

Microcosm setup

Experimental methodology was essentially the same as detailed in Boots et al. (this issue), and is only briefly reviewed here. Soil, FH-layer material, and non-^{13}C enriched leaf litter were collected from Coweeta Hydrologic Laboratory (North Carolina, USA, 35˚2'20"N, 83˚27'10"W). Soil and FH material were both air dried and sieved (soil 2 mm, FH 4 mm) to remove large aggregates, roots, and macrofauna. Litter was air dried and sorted to species (*Quercus rubra,* red oak, or *Tsuga canadensis,* eastern hemlock). Isotopically enriched litter was produced by pulse-labeling oak and hemlock seedlings with ^{13}C enriched CO_2 in a growth chamber. Both ^{13}C labeled and unlabeled oak leaves were shredded to pass a 4 mm sieve to provide a homogeneous food source.

Each microcosm consisted of a 15 cm tall PVC tube, 10.4 cm in diameter, closed on the bottom with 1 mm fiberglass mesh and capped with a perforated plastic lid. Each microcosm contained 10 cm of soil, covered with 2.5 g of FH material, and a mass of litter based on annual litterfall at the collection site (2.50 g oak and/or 0.34 g hemlock). Six litter treatments were used: labeled oak (Oak+), labeled hemlock (Hem+), unlabeled oak (Oak-), unlabeled hemlock (Hem-), labeled oak with unlabeled hemlock (MixOak+), and unlabeled oak with labeled hemlock (MixHem+).

Earthworms [Megascolecidae: *Amynthas corticis* (Kinberg 1867)] were extracted from a residential yard (Athens, Georgia, USA) using the octet electroshocking technique. Four earthworms were used per microcosm (mean mass 0.865 g each). Millipedes [Polydesmidae: *Pseudopolydesmus erasus* (Loomis 1943)] were hand collected from the Coweeta field site. *Pseudopolydesmus erasus* is found throughout the southern Appalachian Mountains (Hoffman, 1999). For each treatment, one replicate received one large (mean mass 0.156 g) and three replicates received two small (mean mass 0.045 g each) *P. erasus*, due to a limitation in number of millipedes available. Faunal treatments included millipede only (Millipede), earthworm only (Earthworm), both millipede and earthworm (Both), and no fauna (None). There were four replicates of each fauna and litter treatment combination for a total of 24 treatments applied to 96 experimental units.

The incubation lasted 28 d. Litter was moistened two weeks prior to the addition of fauna and was maintained by misting the surface of each microcosm with de-ionized water. Earthworms and millipedes were kept in wet paper towels for 24 h prior to introduction to microcosms to allow gut voiding, and each individual was weighed. Soil moisture at the beginning of the experiment was 60% of field capacity, and the temperature was maintained at 18˚C.

Incubation and analysis

Gas samples for $^{13}CO_2$ analysis were collected weekly using specially designed cuvettes fitted with septa, a gastight syringe, and 10 ml Exetainer vacuum tubes (details of analysis in Boots et al., this issue). Samples were analyzed on a Thermo-Finnigan Delta Plus Isotope Ratio Mass Spectrometer (Thermo Scientific, Bremen, Germany). At the end of the incubation, microcosms were destructively sampled by removing any remaining surface litter and the fauna, and separating the soil into 0-2 cm and 2-10 cm depth layers. Fauna were allowed to void their guts for 24 h, after which they were weighed and freeze dried for further analysis.

Soil from microcosms was air dried and a subsample was wet-sieved to separate macro- and microaggregates (Six et al. 2000). Fractions were categorized by size class into POM (floatable particulate organic material), >2000 µm, 2000-250 µm, and 250-53 µm. The <53 µm fraction contained very little material and was excluded from the analysis. All other fractions were analyzed for total C, total N, C:N, and ^{13}C on a Carlo Erba NA1500 CHN Combustion Analyzer (Carlo Erba, Milan, Italy) coupled to a Thermo-Finnigan Delta Plus Isotope Ratio Mass Spectrometer via a Thermo-Finnigan Conflo III Interface Device (Thermo Scientific, Bremen, Germany).

Statistical analysis

Mortality data were arcsine transformed before analysis. Millipede mortality and biomass change; millipede C, N, and ^{13}C content; and soil aggregate proportions, C content, and ^{13}C content were analyzed with a general linear model (SAS PROC GLM). Soil respiration data (C and ^{13}C) were tested with repeated measures GLM, with time as the repeated measure. All statistical analyses were performed using SAS (Version 9.1, SAS Institute, Cary, NC, USA).

Results

Millipede mortality and growth

Millipede mortality was slightly, but not significantly, higher in treatments with earthworms than without (39.6% relative to 33.3%). Litter treatments did not significantly affect millipede survival. Surviving millipedes gained mass in almost all replicates and treatments; however, growth was not affected by litter treatments or by earthworms.

Food preference

Millipedes consumed and assimilated both oak and hemlock litter-derived C (Fig. 4.1). Litter treatment effects on millipede ^{13}C assimilation were significant (*P*<0.0001). Hemlock derived C was consumed at a lower rate than oak. In the mixed litter treatments, millipedes consumed both litter species, and therefore exhibited less ^{13}C assimilation than in the associated individual litter treatment (cf. Oak+ to MixOak+). Less ^{13}C was assimilated by millipedes when earthworms were present (*P*=0.0029), regardless of litter treatment. Changes in *A. corticis* ^{13}C assimilation were observed in the presence of millipedes (*P*=0.029, Fig. 4.1).

Soil respiration

CO_2 and $^{13}CO_2$ evolution decreased throughout the experiment in all treatments (*P*<0.0001). Faunal treatment (Fig. 4.2a, *P*=0.0002) and litter treatment (Fig. 4.3a, *P*<0.0001) both significantly affected total CO_2 flux. Millipede treatments consistently had the lowest CO_2 flux, treatments with both fauna had the highest flux (except at week 1) and the earthworm and no fauna treatments were intermediate. MixOak+, MixHem+, and Oak+ showed consistently higher CO_2 flux than Hem+, Hem-, and Oak-.

CO_2 derived from litter ($^{13}CO_2$) was also significantly affected by faunal treatment (*P*=0.0137) and by litter treatment (*P*<0.0001). The no fauna treatment had the highest $\delta^{13}CO_2$ flux throughout the experiment (Fig. 4.2b). Millipede alone and earthworm alone treatments initially showed the lowest $^{13}CO_2$ respiration. However, during the incubation $^{13}CO_2$ efflux from these treatments decreased less than the both

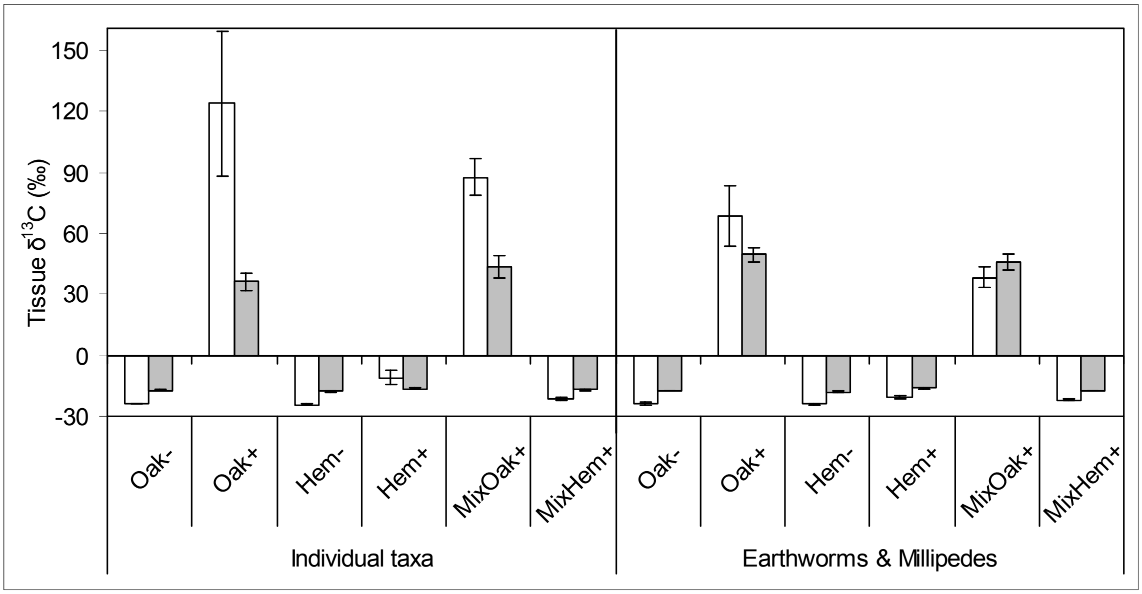

Fig. 4.1. Mean of δ^{13}C (‰) in millipede (open bars) and earthworm (shaded bars) tissue at the end of the experiment. Error bars indicate standard error.

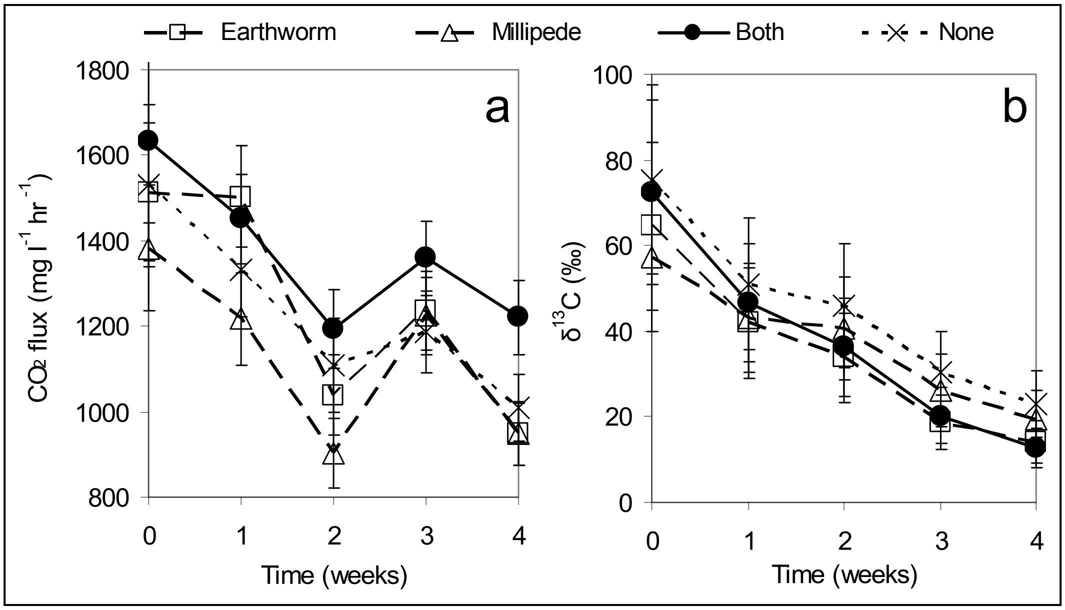

Fig. 4.2. Total soil respiration (a) and $\delta^{13}CO_2$ (b) by faunal treatment: Both (solid line, ●), Millipede (dashed line, Δ), Earthworm (dashed line, □), None (dotted line, ×). Error bars indicate standard error.

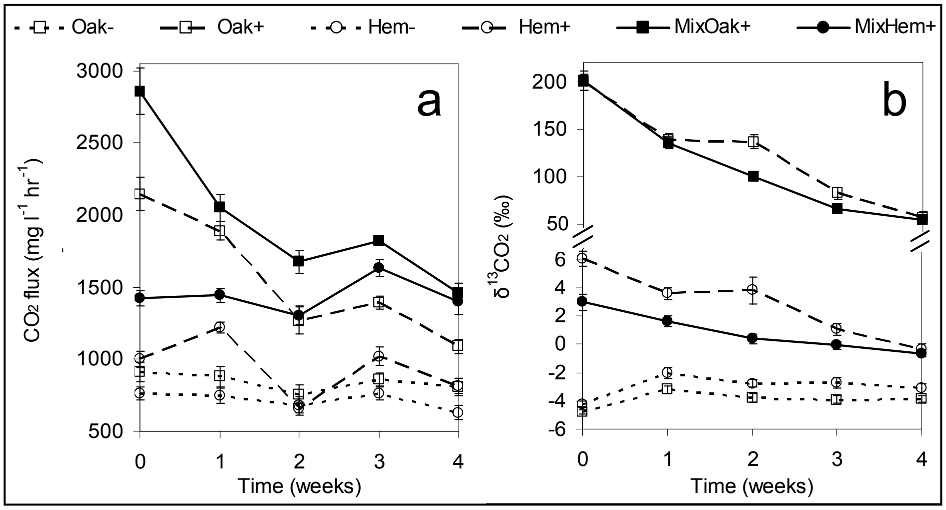

Fig. 4.3. Total soil respiration (a) and $\delta^{13}CO_2$ (b) under Oak- (dotted line, □), Hem- (dotted line, ○), Oak+ (dashed line, □), Hem+ (dashed line, ○), MixOak+ (solid line, ■), and MixHem+ (solid line, ●). Error bars indicate standard error.

fauna treatment, and ended the incubation at an intermediate level. Respiration of $^{13}CO_2$ in the both fauna treatment decreased the greatest amount during the incubation.

Flux of $^{13}CO_2$ was clearly separated by litter treatments that included labeled oak (Oak+, MixOak+), labeled hemlock (Hem+, MixHem+), and only unlabeled litter (Oak-, Hem-) (Fig. 4.3b). Within these groupings, Hem- was more enriched than Oak- and individual labeled litter treatments respired more $^{13}CO_2$ than mixed litter.

Soil aggregation

In the 0-2 cm layer, earthworms, millipedes, and both fauna significantly affected the percentage of aggregates in all size fractions ($P<0.0001$ for each fraction, Fig. 4.4a). More POM remained in the no fauna treatment relative to those treatments with fauna. The analysis revealed a significant interaction term between litter and fauna treatments ($P=0.0118$): Oak+, MixOak+, and Hem- treatments without fauna had significantly more POM at the end of the experiment than almost every other treatment combination. It is unclear why the labeled oak litter and the unlabelled hemlock litter would have this effect. Also, effects of litter on aggregation were observed in the >2000 µm fraction ($P=0.0175$): less soil aggregation occurred in the Oak- treatment. Earthworms shifted aggregate size distribution, creating more aggregates in the >2000 µm fraction and correspondingly reducing the amount of soil in the 2000-250 and 250-53 µm fractions. Earthworms alone produced more >2000 µm aggregates than earthworms together with millipedes ($P<0.0001$).

Faunal treatments also had significant effects on the percentage of all aggregate fractions in the 2-10 cm layer ($P<0.0001$ for each fraction, Fig. 4.4b). Again, the presence of earthworms, millipedes, or both fauna decreased the amount of POM remaining.

Earthworm presence shifted soil aggregate size to the >2000 µm fraction from smaller fractions. The same litter effect was observed (*P*=0.0381) in this fraction as in the 0-2 cm layer; Oak- had more aggregates in the 2000-250 µm fraction (*P*=0.0002).

Soil C and ^{13}C

Percent C in soil aggregate fractions was generally not affected by litter or fauna treatment combinations. However, in the 0-2 cm layer C content was lower in the 250-53 µm fraction of treatments with earthworms relative to those without (*P*<0.0001, data not shown). Conversely, in the 2-10 cm layer earthworm activity increased C in the POM and >2000 µm fractions (*P*<0.0001).

Incorporation of ^{13}C into soil aggregates supports patterns observed for earthworm and millipede tissue ^{13}C and faunal effects on aggregates. Fauna and litter treatments both had significant effects (*P*<0.0001) on aggregate ^{13}C content. Little ^{13}C was incorporated into aggregates in Hem+ treatments. In Oak+ units, significantly more litter-derived organic matter was removed (decrease in POM, Fig. 4.5) in earthworm and both fauna treatments and ^{13}C increased in the >2000 µm fraction. The MixOak+ treatment showed a non-additive effect of earthworm and millipede POM removal. Under Oak+ and MixOak+, all faunal treatments showed an increase in ^{13}C incorporation into the 2000-250 and 250-53 µm fractions. Patterns of ^{13}C incorporation in the 2-10 cm layer were similar to those of 0-2 cm, except that POM showed no ^{13}C enrichment (*P*=0.09). Millipede and no fauna treatments did not affect ^{13}C in the 2-10 cm layer in any soil aggregate fraction (data not shown).

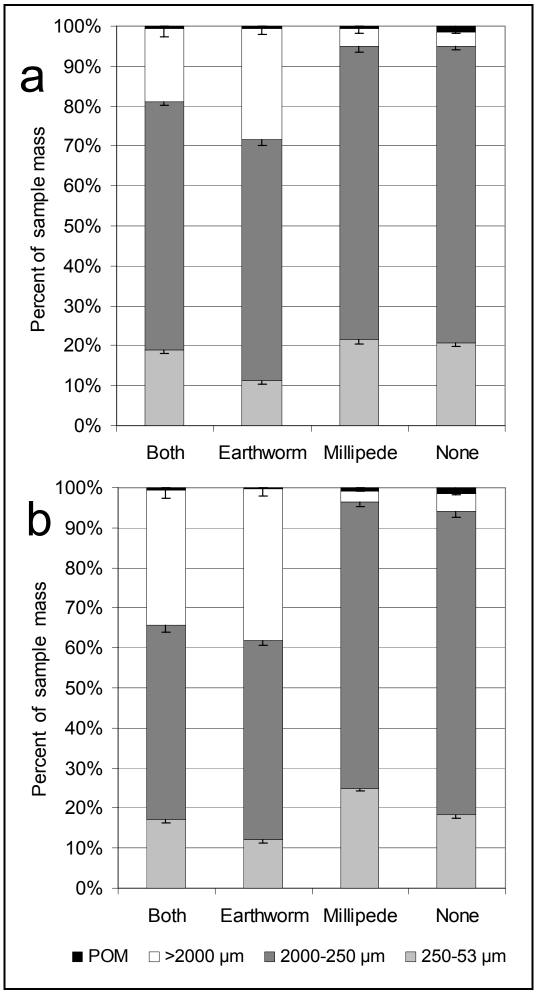

Fig. 4.4. Aggregate size distribution in 0-2 cm (a) and 2-10 cm (b) layers. Error bars indicate standard error.

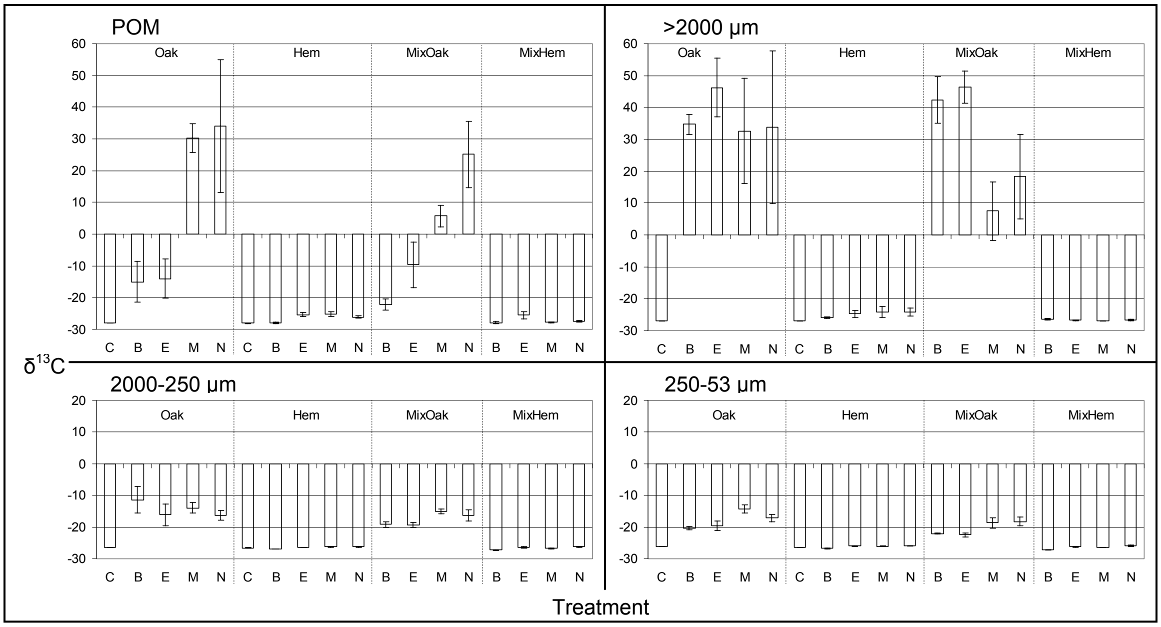

Fig. 4.5. Soil aggregate $\delta^{13}C$ (0-2 cm layer) under treatments with ^{13}C labeled litter. Faunal treatments were earthworms and millipedes (B), earthworms alone (E), millipedes alone (M), and no fauna (N). Values ($\delta^{13}C$) for unlabeled litter treatments (C) are shown for comparison and represent the mean of all faunal treatments (B, E, M, N). Error bars indicate standard error.

Discussion

Food preference and competition

Millipedes consumed both red oak and eastern hemlock litter, even when both litter species were mixed. Oak was clearly the preferred species, as evidenced by greater ^{13}C assimilation (Fig. 4.1). Alternatively, the mass of each litter species played a strong role in millipede ^{13}C assimilation, since a greater mass of oak litter than hemlock litter was provided. However, since the mass of each litter in the microcosms simulated conditions from Coweeta Hydrologic Laboratory, this demonstrated that *Pseudopolydesmus erasus*, given resources similar to those available in a southern Appalachian ecosystem, would obtain most of its C from oak, rather than hemlock.

Millipede ^{13}C assimilation was reduced when earthworms were present (across all litter treatments), strongly suggesting that *Amynthas corticis* directly competes for food resources. Indeed, *A. corticis* also preferred oak (Boots et al., this issue). However, greater ^{13}C assimilation by *A. corticis* was seen in the presence of millipedes, suggesting that *A. corticis* is the better competitor, or at least that these two species interact such that *P. erasus* suffers but *A. corticis* benefits. Preferential earthworm consumption of millipede fecal pellets, in addition to leaf litter, could be a mechanism for this interaction (cf. Bonkowski et al., 1998).

The earthworm-millipede interaction did not lead to competitive exclusion during the course of our experiment, but slightly increased millipede mortality in earthworm treatments suggests that this may occur during a longer-term incubation or in natural ecosystems (Snyder et al., unpublished observations) where annual litter fall does not exceed annual litter consumption by *A. corticis*.

Contributions of millipedes and earthworms to soil structure

Millipedes did not contribute significantly to soil aggregation even though fecal pellets from similarly sized species would have been found in the 2000-250 µm size fraction (Paulusse and Jeanson, 1977). However, this experiment utilized one relatively small millipede species, whereas a natural community of would contain multiple species of different sizes (e.g. 2 mm to 10 cm in length, Snyder et al. 2006) which would produce a variety of fecal pellets sizes, and therefore different sized aggregates.

The presence of *A. corticis* resulted in creation of significantly more macroaggregates, as observed in prior studies (e.g. Burtelow et al., 1998). However, less earthworm-associated aggregation was observed when millipedes were present, suggesting that millipedes could mitigate some of the effects of earthworm invasion on soil characteristics. Several potential mechanisms could cause this reduction in >2000 µm aggregates. Since reduction in macroaggregates occurred throughout the microcosm, and millipedes had little effect below the mineral soil surface (as demonstrated by soil aggregate ^{13}C), aggregate reduction is probably due to a competitive interaction for suitable food resources. Earthworms assimilated more ^{13}C in the presence of millipedes, so a reduction in litter derived food resources is not a probable mechanism. Consumption of millipede fecal pellets by earthworms (Bonkowski et al., 1998) would have provided *A. corticis* with an additional food resource containing ^{13}C, albeit with a lower δ^{13}C, because millipedes had already assimilated some ^{13}C. This possibility is consistent with millipede, earthworm, and soil ^{13}C data. Not only was there less >2000 µm aggregation in the both fauna treatment relative to the earthworm treatment, but these aggregates tended to be less enriched in ^{13}C. An earthworm diet that incorporated millipede fecal pellets, which contain partially digested litter, would have less organic matter, which is known to affect the water stability of aggregates (Six et al., 2002).

Implications for earthworm invasion dynamics

When *Amynthas corticis* is introduced to new habitats, our data shows it is capable of utilizing fresh organic matter resources and effectively competes for these resources with native decomposer fauna. Spatial and temporal distribution of resources is known to affect invasion establishment success (Theoharides and Dukes, 2007). Our data suggest that *A. corticis* can assimilate C from both deciduous and coniferous resources. Therefore, upon introduction to most southern Appalachian forests, which consist of a mixed mosaic of multiple litter species (e.g. Whittaker, 1956), *A. corticis* is likely to find suitable food resources. The ability of *A. corticis* to utilize resources from a variety of litter sources increases the likelihood of successful establishment and spread.

Widespread invasion by these earthworms has implications not only for millipede populations but also for carbon dynamics at the ecosystem scale. A net loss of C due to *A. corticis* activity in soil was observed in this microcosm study (Boots et al., this issue), but there seems to be little mitigation of this effect in the presence of millipedes. Earthworm effects were strong and extensive regardless of millipede presence. However, the duration of this incubation was short and there was a large difference in biomass between millipedes and earthworms.

Implications of millipede-earthworm interactions for ecosystem level processes

CO_2 efflux data suggest that systems with millipedes have lower respiration rates than those without. Depressed litter respiration rates due to millipedes have occasionally been shown (Maraun and Scheu, 1996). Because respiration rates begin lower, C loss via respiration could increase more due to earthworm invasion than previously thought (Bohlen et al., 2004b; McLean et al., 2006). However, high initial rates in the earthworm treatment declined after several weeks (Fig. 4.2). There were synergistic effects of earthworms and millipedes such that CO_2 evolution remained high throughout the experiment in the treatment with both fauna. Whether elevated respiration rates at the end of the experiment represented a new constant level or a temporary fluctuation is not a point we were able to address with this experimental design, but this may point to a mechanism by which forest floor ecosystems could suffer a net loss of C, and should be addressed in future, longer-term laboratory and field studies.

While there was a net loss of C due to earthworm activity in the short term (Boots et al., this issue), there was also a net movement of C within the soil. Litter-derived C was translocated to deeper horizons and to micro- and macroaggregates due to biological activity, mainly that of the earthworm, *A. corticis*. However, millipedes caused shifts in carbon as well, particularly from POM, possibly due their ability to fragment litter and thus increase the availability of this resource to the microbial community. Further leaching and utilization of fecal pellets by other organisms (i.e. *A. corticis* and microorganisms) could lead to enhanced translocation of litter derived C into water-stable aggregates and respired to the atmosphere.

Carbon in water-stable aggregates can persist in soil ecosystems for tens to hundreds of years (Six et al., 2002). Long-term sequestration of soil C in earthworm-created aggregates has the potential to offset the short-term losses caused by earthworm invasion. However, when millipedes and earthworms were together in microcosms more C was respired, less aggregation occurred, and less C was contained in aggregates, relative to earthworms alone. This suggests that earthworm invasion of ecosystems inhabited by millipedes could have greater losses of C due to earthworm-millipede interactions. Long-term C dynamics could further be altered by extirpation of millipedes due to competitive exclusion by earthworms.

Conclusions

Soil macroinvertebrate detritivores have been shown to significantly affect soil C over a short time-scale. *Amynthas corticis* and *Pseudopolydesmus erasus* differed greatly in their impacts on C dynamics and soil structure in microcosms, yet preferred and competed for similar food resources. Impacts of *A. corticis* on soil structure were more extensive: macroaggregates were formed throughout the soil profile, whereas *P. erasus* only had such impacts in the surface layers. Earthworm impacts on soil aggregation were also far more intensive but were reduced somewhat through their interaction with millipedes, possibly due to resource competition or a change in resource use. However, impacts of earthworms and millipedes together on soil respiration were larger and more sustained than either earthworms or millipedes alone. *Amynthas* invasion is expected to cause a short-term net loss of soil C but a long-term perspective is needed to truly assess the overall effects of soil fauna on soil C cycling.

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CHAPTER 5

INVASIVE EARTHWORM (*AMYNTHAS AGRESTIS*, MEGASCOLECIDAE) COMPETITION WITH NATIVE MILLIPEDES (*SIGMORIA AINSLIEI*, XYSTODESMIDAE)[2]

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[2] Snyder, B.A., M A. Callaham, Jr., C.N. Lowe, S.C. Rostkowski, K.J. Seader, and P.F. Hendrix. To be submitted to *Soil Biology & Biochemistry*.

Abstract

The invasive exotic earthworm *Amynthas agrestis* (Goto and Hatai 1899) has recently been documented invading relatively undisturbed forests of the southern Appalachian Mountains in the southeastern United States. This epigeic earthworm has been shown to decrease the depth of organic soil horizons, which are vital to the maintenance of soil biodiversity. Field data has indicated that millipede richness and abundance are decreased in locations where *A. agrestis* has invaded. To investigate the mechanisms behind these effects, earthworms (*A. agrestis*, Megascolecidae) and millipedes (*Sigmoria ainsliei*, Xystodesmidae) were placed into 1 l microcosms constructed with 500 g soil and either L (15 g L horizon material), FH (15 g F and H horizon material), or L/FH (7.5 g L and 7.5 g F and H material) litter treatments. Soil respiration was measured weekly. Microcosms were destructively sampled and reconstructed with the same fauna and treatment every four weeks to assess faunal biomass change and mortality. At each destructive sampling date, millipedes and earthworms were recovered and weighed. Additionally, soil, remaining litter, and material processed by the fauna were collected for future analysis. Soil from microcosms which contained earthworms were wet-sieved (1.4 mm) to assess cocoon production.

Millipede mortality was greatly hastened in treatments that did not have FH horizon material, and within all litter treatments millipedes tended to survive longer without the presence of *A. agrestis*. Earthworm mortality tended to be more rapid in FH and L/FH treatments when millipedes were present. However, earthworms in the L treatment tended survive longer in the presence of millipedes. Earthworm biomass increased in FH and L/FH treatments (until week 8) but decreased from the beginning of the experiment under the L treatment. Many more cocoons were produced under FH and L/FH than under L. Interestingly, *A. agrestis* did not reproduce in the presence of *Sigmoria ainsliei*, with rare exception.

Soil respiration rates increased due to both earthworms and millipedes. Litter treatment also impacted CO_2 efflux rate: respiration was highest in L treatments, lowest in FH, and intermediate in L/FH treatments.

Results from this study suggest that the invasive earthworm *Amynthas agrestis* may directly compete with the millipede *Sigmoria ainsliei* for food resources, particularly the smaller particle material in the FH horizons of the forest floor. However, millipedes increased *A. agrestis* survival when only an L horizon was available. It appears that the millipedes may offer some biotic resistance to the invasion as we observed diminished earthworm reproductive capacity in the experimental units where earthworms and millipedes both were present.

Key words: Millipede, earthworm, *Sigmoria*, *Amynthas*, competition, respiration, carbon dynamics, invasive species

Introduction

Non-native earthworm invasion is a global phenomenon in which invasive species that originate from every continent (except Antarctica) are invading every continent (except Antarctica) (Hendrix et al., in press). In North America, earthworms of Asian origin (the genera *Amynthas*, *Metaphire*, *Pheretima*, and *Pithemera*) have recently been documented in the northeastern (Steinberg et al., 1997; Burtelow et al., 1998; Bohlen et al., 2004a,b) and southeastern (Callaham et al., 2003; Snyder et al., in review) regions of the United States, although these earthworms have been known in North America since the early 20th century (Garman, 1888; Gates, 1937).

Earthworm invasion can significantly alter forest ecosystems. Physical changes to the forest floor through consumption of organic horizons, mixing of organic and mineral horizons, and burrowing and casting activities can impact biogeochemical cycling (Bohlen et al., 2004a,b,c). Loss of soil C due to earthworm invasions has been observed in Minnesota, USA (Alban and Berry, 1994) and New York, USA (Bohlen et al. 2004c). Soil C loss can occur through two pathways: leaching of dissolved organic carbon and respiration of CO_2.

In New York, USA, Fisk et al. (2004) found no change in total soil respiration due to earthworm invasion. However, this measure included respiration from roots; root biomass decreased in earthworm-invaded plots. If root respiration decreased, heterotrophic organism respiration may have increased, but this hypothesis has not been tested (Fisk et al., 2004). However, in some earthworm invasions, decreased respiration in organic and mineral soil horizons has been shown. Decreased basal respiration was shown in *Lumbricus terrestris* and *Octolasion tyrtaeum* invaded sites in Alberta, Canada (Eisenhauer et al., 2007). The relationship between earthworm invasion and soil respiration is complex and appears to be idiosyncratic.

Little is known about how millipedes impact organic and mineral soil respiration. Maraun and Scheu (1996) found that millipedes (*Glomeris marginata*) increased or decreased respiration of Beech litter and this varied seasonally: basal respiration increased in February and May and decreased in August and November. Snyder et al.

(unpublished observations) found that *Pseudopolydesmus erasus* depressed respiration in a microcosm experiment.

Earthworm invasion also impacts soil fauna communities through direct competition and through significant alteration of soil profile and structure (Bohlen et al., 2004b,c; Frelich et al., 2006). Although much is known about the interactions of invasive earthworms with soil micro- and mesofauna, less is known about interactions with detritivorous macrofauna, such as millipedes (Migge-Kleian et al., 2006). Bonkowski et al. (1998) found that earthworms benefited from consuming millipede (*Glomeris marginata*) fecal pellets in a European Beech forest. However, in a microcosm experiment, millipedes were negatively affected by earthworms (*A. corticis*), but earthworms may have similarly consumed millipede fecal material (Snyder et al., unpublished observations).

Amynthas gracilis invasion in forests of New York, USA was found to reduce organic horizon organic matter (Steinberg et al., 1997; Burtelow et al., 1998). Similarly, invasion of *A. agrestis* in the Great Smoky Mountains, USA, Snyder et al. (in review) found that millipedes were negatively affected by *A. agrestis* invasion, and that invasion reduced the depth of the FH horizon (a combination of the F and H horizon), which millipedes rely on for food and in which they reside. To further explore the mechanisms behind this interaction, we performed a microcosm experiment to test whether these two taxa directly competed for food resources found in the L or FH horizons, and whether earthworms and millipedes required these resources to survive.

Methods

Millipedes and earthworms were collected from the Great Smoky Mountains Institute at Tremont (Blount Co., Tennessee), within the Great Smoky Mountains National Park (GSMNP) in early June 2007. All individuals were found by manually searching through leaf litter. Earthworms and millipedes were kept separate during transport to the laboratory. The two taxa were also stored separately until the beginning of the experiment in containers with soil and litter from the collection site.

Microcosms consisted of 1 l transparent plastic containers with perforated snap-on lids. Each microcosm received 500 ± 5 g of soil mixed with 70 ± 5 g tap water. Soil was a commercially acquired ultisol from a recently cleared forest in Clarke Co., GA. Soil was air dried and 4.75 mm sieved to remove large aggregates and rocks. Litter was previously collected from GSMNP and defaunated via Berlese extraction for 72 h, followed by air-drying if necessary. Dominant tree species at the litter collection site were *Acer* spp., *Quercus* spp., *Liquidambar styraciflua*, *Liriodendron tulipifera*, and *Pinus strobus* (Snyder et al., in review).

Litter treatments were functionally defined by particle size: litter was 4.75 mm sieved to separate unfragmented leaves (L horizon) from fragmented and partially decomposed organic matter (FH, combined F and H horizons). Large rocks, twigs, seeds and nuts were discarded. Organic layer treatments were L (15 ± 0.1 g of L horizon), L/FH (7.5 ± 0.1 g each of L and FH horizon), or FH (15 ± 0.1 g of FH horizon). Litter was misted with tap water when microcosms were constructed. Fauna treatments consisted of either no fauna, two *Amynthas agrestis* individuals (mean biomass 0.88 g each), one male *Sigmoria ainsliei* (mean biomass 2.26 g), or both *A. agrestis* and *S.*

ainsliei. Individuals were randomly assigned to treatments with 6 or 7 replicates for a total of 76 microcosms. All microcosms were kept in the dark at 20°C and misted with tap water after each respiration measurement (described below). Incubation began in June 2007 and continued until all millipedes and earthworms died. Data reported herein incorporate mortality through 11 April 2008 (1 millipede and 5 earthworms alive).

Microcosms were destructively sampled every four weeks to collect soil, fecal material, and remaining litter for future analysis. After destructive sampling, new microcosms were constructed and the surviving fauna was weighed and placed into the new microcosms. Earthworms were rinsed in tap water to remove soil prior to weighing. A new microcosm was not constructed if all earthworms and millipedes had died. Soil from treatments that included earthworms was wet-sieved through a 2 mm sieve to assess cocoon production. After the first collected cocoons were found to be slightly larger than 2 mm in diameter, a 1.4 mm sieve was employed to ensure cocoon capture.

Respiration rates were measured on each microcosm using an EGM-4 infrared gas analyzer (PP Systems, Amesbury, Massachusetts, USA) connected via a customized microcosm lid, prior to analysis. CO_2 buildup within each microcosm was ventilated by fanning the open top of the microcosm with a plastic lid before attaching the modified lid for analysis. CO_2 concentrations were recorded every 1 s for 4 min on a CR23X micrologger (Campbell Scientific, Inc., Logan, Utah, USA). Respiration was assessed weekly during the first nine months of the experiment and additional measurements were taken 3 d after reset during months 2-4. Respiration rates were calculated from these data by plotting CO_2 concentration against time. This line was characterized by three portions: a steeply sloped section as the EGM-4 equilibrated to the concentration of CO_2

in the microcosm, a linear portion, and an asymptotic portion wherein CO_2 concentration was governed by laws of diffusion. The slope of linear portion of the line represented the respiration rate (ppm CO_2 s^{-1}), and at least 60 s of data were fitted with a trend line ($R^2 >$ 0.97, almost always > 0.99) to calculate the slope. This data was used to calculate mean monthly respiration rates (the mean of all CO_2 measurements between microcosm reconstructions). During month 2, data from 3 d and 7 d measurements were lost therefore means for this month were based on three measurements.

Respiration data (five months of incubation) were analyzed with a repeated measures mixed model in SAS (Version 8, SAS Institute, Cary, NC, USA) using an unstructured covariance structure and the Satterthwaite method for estimation of denominator degrees of freedom. Biomass data were analyzed with a repeated measures general linear model with SAS (Version 9.1). Millipede and earthworm survival data were analyzed with the Cox proportional hazards regression model (Grambsch and Therneau, 1994) in R (R Development Core Team, 2007). Millipede and earthworm data were found to fit the assumptions of the proportional hazards model. Interaction terms between litter and fauna treatments in the millipede analysis were not significant, and the data was subsequently analyzed without the interaction terms. Likelihood ratio tests determined that neither model fit the data better than the other.

Results

Survival and Growth

Millipedes lived a mean time of 130 d ($n = 37$) during the experiment. Survival time significantly decreased in L relative to FH ($P < 0.0001$). However, L/FH and FH were not significantly different (*P* = 0.073). Earthworm presence did not significantly impact millipede survival time (*P* = 0.092). However, without *A. agrestis* presence, millipedes survived 16.4% longer in L/FH and 19.4% longer in FH (Fig. 5.1). Overall, millipedes survived 45.7% longer in L/FH treatments and 54.6% longer in FH treatments relative to L.

Time to first earthworm mortality and time to second earthworm mortality for each microcosm were analyzed separately. Mean time to first *A. agrestis* mortality was 105 d (*n* = 39) and to the second mortality was 134 d (*n* = 36) from the initiation of the experiment. In the regression model of first earthworm mortality, there was a significant interaction between L and millipede presence (*P* = 0.038). In the analysis of time to second earthworm mortality, this interaction term was significant (*P* = 0.0018) as was L (relative to FH, *P* = 0.0065) and millipede presence (*P* = 0.0083). Earthworms, without the presence of millipedes, survived significantly longer in FH than in L treatments (Fig. 5.2). However, when millipedes were present *A. agrestis* survived longest in L treatments and the shortest amount of time in L/FH treatments. Additionally, earthworms survived longer without millipedes (relative to with millipedes) in FH and L/FH treatments, but survived longer with millipedes in the L treatment. Mean earthworm survival time differed less than 5% between all litter treatments. In microcosms with both earthworms and millipedes, an earthworm outlived the millipede in every FH replicate, every L/FH replicate and 4 of 7 L replicates.

Millipede biomass did not differ between treatments at the beginning of the experiment (*P* = 0.6645) or at the last measurement before mortality (*P* = 0.8087). Millipede biomass exhibited a net increase for almost all individuals (Fig. 5.3A). There

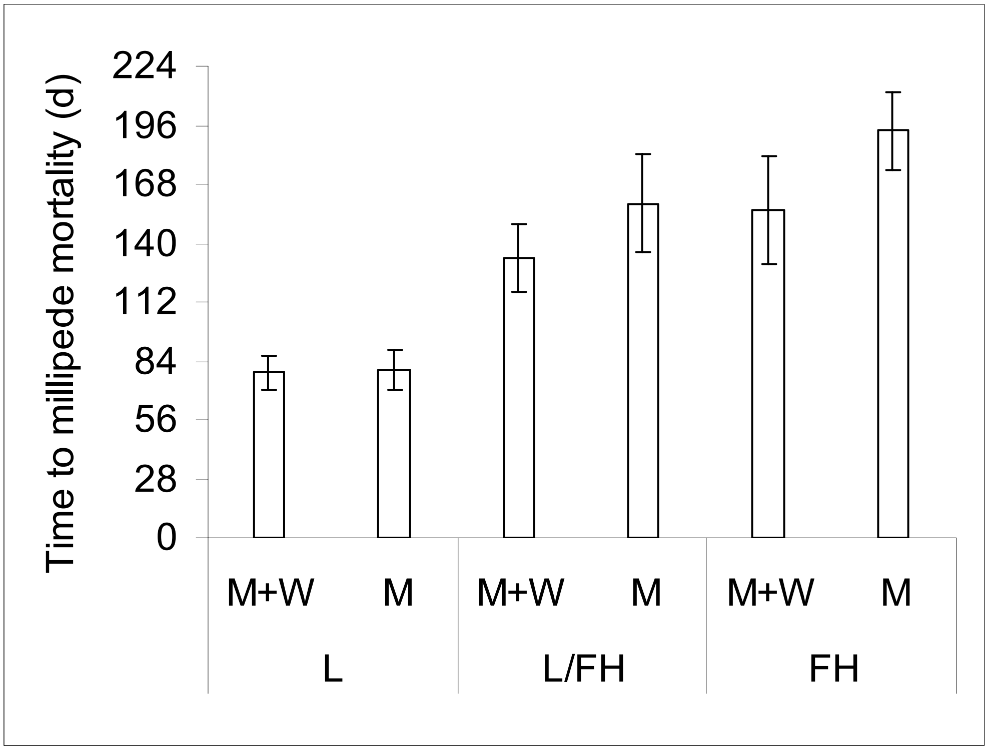

Fig. 5.1. Mean survival (± SE) of *Sigmoria ainsliei* from initiation of the incubation with (M+W) and without (M) earthworms. Litter treatments were litter (L), litter and FH material (L/FH), and FH only (FH).

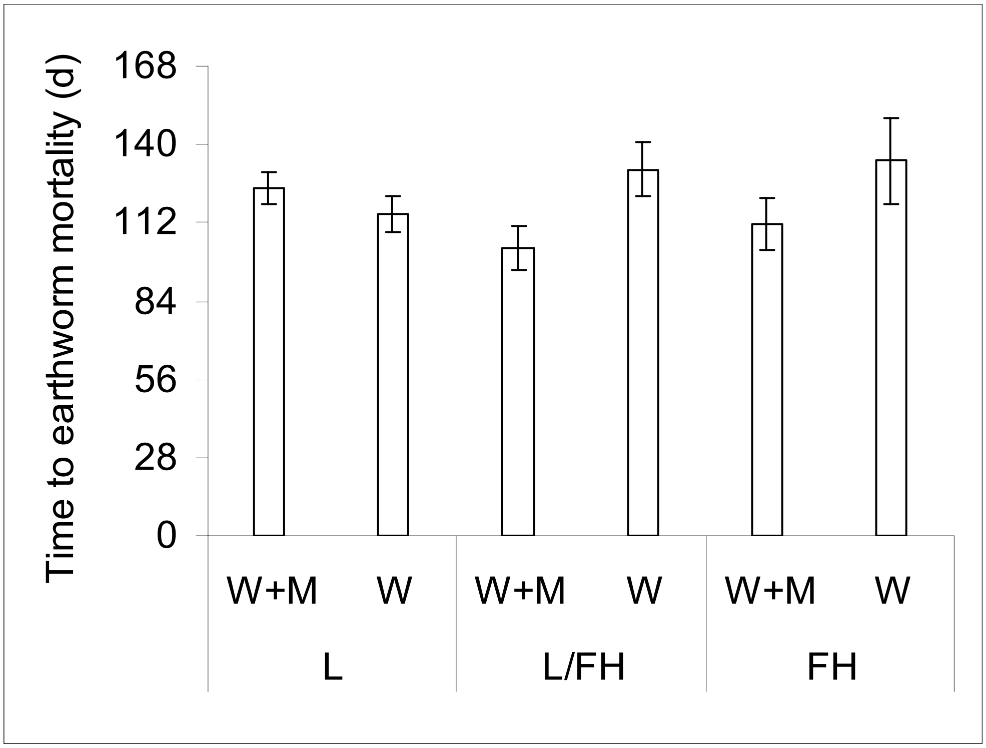

Fig. 5.2. Mean survival (± SE) of *Amynthas agrestis* from initiation of the incubation with (W+M) and without (W) millipedes. Litter treatments were litter (L), litter and FH material (L/FH), and FH only (FH).

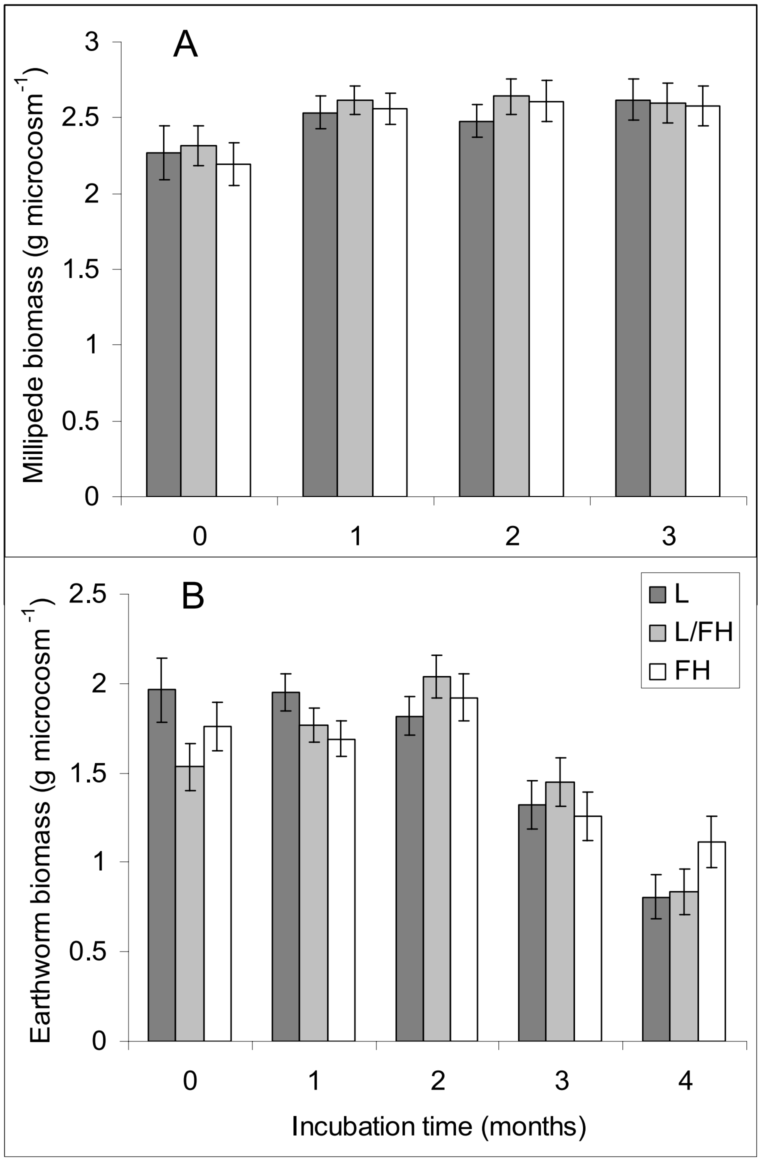

Fig. 5.3. Mean biomass (± SE) of millipedes (A) and earthworms (B) in different litter treatments through month 3 (millipedes) or 4 (earthworms) of the incubation.

were significant differences between litter treatments but earthworms did not impact millipede biomass (Table 5.1, analyzed through 12 weeks). Millipede biomass increased significantly more in FH relative to L, but neither were different than L/FH.

Earthworm biomass did not differ between treatments at the beginning of the experiment (*P* = 0.4399). Earthworm biomass decreased for almost all individuals after 4 weeks of incubation, although it did not differ at the start of the incubation. A significant impact of litter, but not of millipede, treatments was observed in net biomass changes through week 16 (Table 5.2, Fig. 5.3B). *Amynthas agrestis* had a significantly higher biomass in L/FH over FH and L treatments.

Earthworm cocoon production

Cocoons were produced beginning in the fourth month and in every subsequent month of incubation. Cocoons were produced in microcosms that began a month with either one or two earthworms, but cocoon number was not significantly different due to this factor (*P* = 0.900). Only one cocoon (of 88 total, through 1 April 2008) was produced in a millipede treatment during a time when the millipede was alive. Seven cocoons were produced in millipede treatments after millipede mortality. Cocoons production was not impacted by litter treatment (*P* = 0.3977). Total cocoon production and the proportion of microcosms producing cocoons increased towards the end of the incubation (Fig. 5.4).

Respiration

Mean monthly respiration rates varied considerably during the course of the incubation (Fig. 5.5). The mixed model analysis found significant differences (overall model $\chi^2 = 106.86$, $P < 0.0001$) between treatments (Table 5.2). Presence of earthworms

Table 5.1. Results of repeated measures GLM analyses of net biomass change since initiation of the incubation. Biomass was measured every four weeks, millipedes data were analyzed until week 12 and earthworms until week 16. Significant *P*-values at α=0.05 indicated by an asterisk.

Source	df	SS	MS	*F*	*P*
Millipede Biomass					
Fauna treatment	1	0.00303	0.00303	0.10	0.7518
Litter treatment	2	0.28873	0.14436	4.87	0.0167*
Fauna * Litter	2	0.02898	0.01449	0.49	0.6191
Error	24	0.71089	0.02962		
Earthworm Biomass					
Fauna treatment	1	0.17700	0.17700	0.23	0.6381
Litter treatment	2	9.44239	4.72119	6.06	0.0077*
Fauna * Litter	2	2.12425	1.06212	1.36	0.2756
Error	23	17.9123	0.77878		

Table 5.2. ANOVA table for mixed model analysis of mean monthly respiration rates through incubation month 5. Significant *P*-values at α=0.05 indicated by an asterisk.

Fixed Effects	Numerator df	Denominator df	*F*	*P*
Earthworm	1	63.4	13.05	0.0006*
Millipede	1	63.4	6.28	0.0148*
Earthworm*Millipede	1	63.4	0.14	0.7130
Litter treatment	2	63.4	30.67	<0.0001*
Earthworm*Litter	2	63.4	5.71	0.0052*
Millipede*Litter	2	63.4	0.26	0.7683
Earthworm*Millipede*Litter	2	63.4	1.18	0.3125

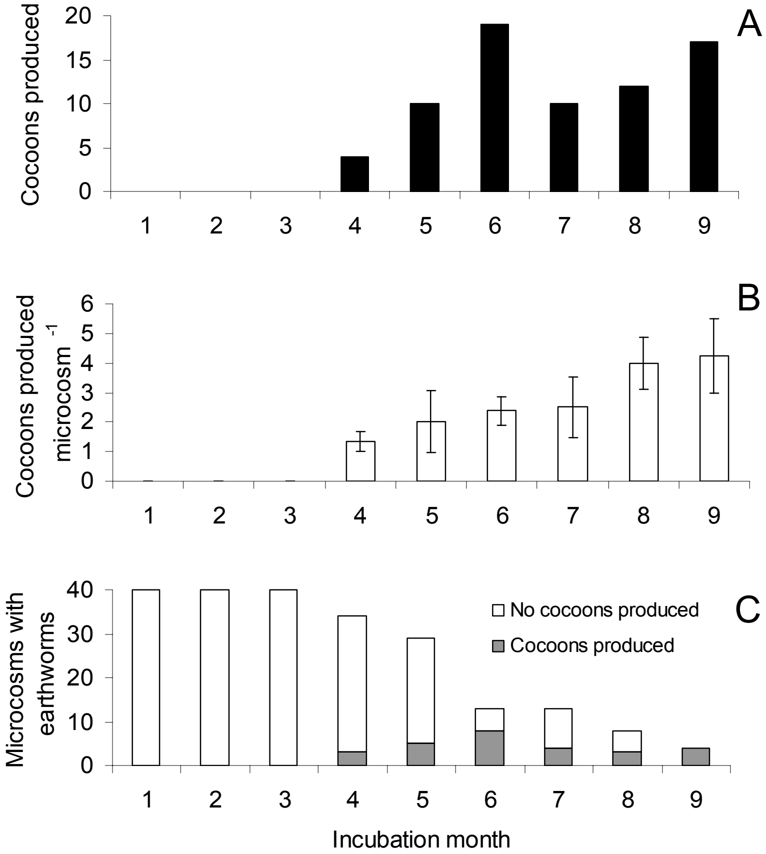

Fig. 5.4. Cocoon production by *Amynthas agrestis* over the duration of the incubation. Data presented as (A) total number of cocoons produced, (B) mean (± SE) number of cocoons produced per microcosm, and (C) number of earthworm-containing microcosms that did (shaded) and did not (open) produce microcosms.

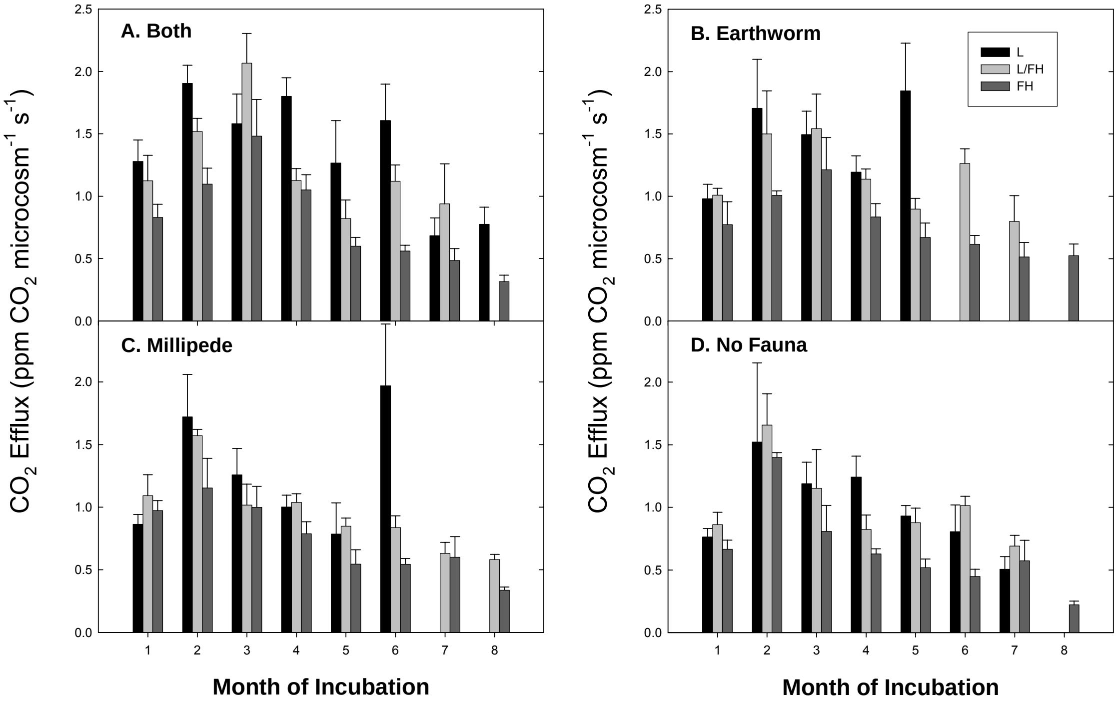

Fig. 5.5. Mean monthly respiration rate (± SE) during the first eight months of incubation in (A) earthworm and millipede, (B) earthworm alone, (C) millipede alone, and (D) no fauna treatments. Litter treatments shown are litter only (L, black bars), litter and FH horizon material (L/FH, light gray bars), and FH only (FH, dark gray bars).

and presence of millipedes were both significant factors, but millipedes and earthworms did not interact to impact CO_2 efflux.

Respiration rates from all litter treatments were significantly different (L/FH and FH, $P < 0.0001$; L/FH and L, $P = 0.0062$; L and FH, $P < 0.0001$). CO_2 was generally respired at the highest rate from the L treatment, at the lowest rate from the FH treatment, and at intermediate rates from L/FH (Fig. 5.5). The model also included a significant earthworm-litter treatment interaction term, suggesting that the differences between L, L/FH, and FH treatments were much larger in the presence of earthworms. Almost all combinations of earthworm and litter treatments were significantly different, except: L/FH with earthworms and L without earthworms; L/FH with earthworms and L/FH without earthworms; L without earthworms and L/FH without earthworms; and FH without earthworms and L/FH without earthworms.

Discussion

Field data has suggested (Snyder et al., in review) that *Amynthas agrestis* and *Sigmoria ainsliei* may directly compete for food resources (specifically partially decomposed F and H horizon material). Our data indicate that *S. ainsliei* does benefit from feeding on FH material: millipede survival time decreased greatly without FH material. *Amynthas agrestis* also aggressively consumed FH material. We observed a trend in L/FH and FH treatments that *S. ainsliei* survived a shorter amount of time when *A. agrestis* was present, but this trend was not significant in our analysis. Interestingly, when both species were present, millipedes almost always died first: the invasive earthworms appeared to outcompete millipedes and eventually exclude them.

Amynthas agrestis also benefited from FH material, in terms of survival time, in treatments without millipedes. However, in the presence of millipedes, *A. agrestis* survived longest in L treatments. Earthworms appeared to benefit from millipede presence in L treatments, probably through consumption of litter that had been processed by millipedes, as has been seen in other studies (Bonkowski et al., 1998; Snyder et al., unpublished observations). Earthworms were also observed to burrow into the soil and may have consumed soil in addition to organic materials.

Respiration

Respiration rate data did not provide any evidence that earthworms and millipedes interacted. Both fauna increased respiration rates but there was no statistical interaction between their impacts on respiration rate. In litter treatments, relative values of respiration rates were exactly opposite of our expectations: FH material is far more fragmented than L and due to larger surface area should have produced higher CO_2 efflux rates (Fig. 5.5). However, FH particles settled to a layer on the soil surface while the structure of L prevented this from occurring, and thus L may have had more exposed surface area.

CO_2 efflux data was highly variable within and between months. One explanation of the variability in respiration rate data is that soil may not have fully air-dried before use, causing moisture content of the soil to be variable between incubation months. Microcosms could only exchange air with the outside atmosphere through small holes in the lid. Moisture in saturated soil would not have been able to drain or exit the microcosm through any other pathway. Redoxomorphic features were observed in the soil during the first few months of incubation, which suggests that some portions of the soil had reached anoxic conditions. Saturated soil could have limited microbial activity and hence respiration from soil and adjacent litter that may have absorbed moisture. This mechanism would have affected FH treatments more than L due to litter structural properties, and may have contributed to the observed differences in respiration rates.

Conclusions and future perspectives

Our data did lend support to the hypothesis that earthworms and millipedes compete directly for partially decomposed leaf material, but were not conclusive in this respect. Greater cocoon production in the absence of millipedes supports the competition hypothesis and suggests that millipedes may provide some biotic resistance to invasion. Mortality of the living earthworm and millipede individuals should further support our conclusions. By including more replicates, future studies could improve their statistical power and compensate for the natural variability in earthworm and millipede mortality. Additionally, initiating treatment conditions on younger individuals may exhibit greater responses. Maintenance of laboratory cultures (Lowe and Butt, 2005) will be a critical step in our ability to perform more of these experiments.

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CHAPTER 6

A PRELIMINARY CHECKLIST OF THE MILLIPEDES (DIPLOPODA) OF THE GREAT SMOKY MOUNTAINS NATIONAL PARK, USA[1]

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[1] Snyder, B.A. Submitted to *Zootaxa*, 20 March 2008.

Abstract

Great Smoky Mountains National Park, the most visited National Park in the United States, is home to a wide diversity of millipede species. A preliminary list of these species is provided, based on literature records and new collections from the All-Taxa Biodiversity Inventory and the author's research. Currently, the Park's fauna consist of 63 species (one of which contains two subspecies) in 21 families and all 10 orders known from North America; including at least five new state records and 18 new Park records. In the near future this list will likely see the addition of several undescribed species and the addition of species that are currently known to occur near the Park, but have never been reported from within the Park's boundary.

Key Words: Appalachian Mountains, ATBI, North Carolina, Tennessee, biodiversity, inventory, GSMNP

Introduction

Investigations into the millipedes of the Great Smoky Mountains National Park (GSMNP) began as part of a research project on non-native earthworm invasion into the Park. This research found that millipedes were detrimentally affected by the earthworms (Snyder *et al.* in review), and it became increasingly important to know which species were present in the Park. As no summary of GSMNP millipedes had ever been complied, and several apparently undescribed species were among the material collected from this research, it was appropriate to create a checklist of GSMNP millipedes, which will both provide a resource for Park management and facilitate the description of new species.

GSMNP is located on the border of North Carolina and Tennessee, USA. The Park covers 210,875 ha of rugged terrain varying between 256 and 2024 m. Millipedes are an important resource in GSMNP; they provide valuable ecosystem services and the colorful xystodesmids are a visible part of the soil fauna to visitors. However, ecosystems in GSMNP are under pressure from invasive species (including the millipedes *Ophyiulus pilosus* and *Oxidus gracilis*), air pollution, and human impacts: GSMNP is the most visited National Park in the USA (over 9 million visits in 2006).

Methods

The species checklist was first complied from existing literature records (references below). Asterisks (*) indicate a type location. Specific locations of each species were recorded. Second, specimens from the author's research were determined and these records were added to the species list. Included are specimens collected by the citizen science project "Millipede March," organized by the staff of the Great Smoky Mountains Institute at Tremont. Finally, a significant portion of the specimens from the All-Taxa Biodiversity Inventory (ATBI) pilot sampling (Parker & Bernard 2006) were determined and new records and locations were added to the checklist. Because of the topographic heterogeneity in GSMNP, specimens which could only be given generic placement are reported below, but only where these specimens identify a new location for the genus or where species concepts are in need of study (e.g. *Narceus* sp.).

A comprehensive search of museum collections was beyond the scope of this research, but all determined specimens from the GSMNP museum in Gatlinburg, TN have been included. This checklist follows the order and taxonomy of Hoffman (1999a), with references to the works of Shelley (1981, 1986) where controversy on generic placement exists.

Specimens from the ATBI pilot sampling were collected by a variety of researchers, and full specimen records will be available on the GSMNP-ATBI database (http://dlia.org/atbi/science/atbi_database.shtml) upon completion of determination of the pilot sampling and structured sampling materials. The design of the ATBI sampling strategy was detailed by Parker and Bernard (2006). Records that refer to "Chilhowee" were collected by the author and field assistants acknowledged below from a site along US Hwy 129/Lake Chilhowee, 35° 33.0' N 83° 60.5' W, 269–291 m elevation, by hand collecting from the organic horizon or leaf litter collection with Berlese extraction (see Snyder *et al.* in review). Specimens from Chilhowee and ATBI currently reside with the author and will be deposited in the GSMNP museum collection in Gatlinburg, TN.

Results

Checklist of the millipedes of the Great Smoky Mountains National Park

Order Polyxenida Lucas 1840

Family Polyxenidae Lucas 1840

Polyxenus prob. *lagurus* (Linnaeus 1758) **NEW PARK RECORD**

Taxonomic note: Nearctic polyxenid species-level determinations are based on the number of sensory cones on the 6th antennomere. This character is variable (e.g. *P. lagurus* has 4–6 cones while *P. fasciculatus* has 7–17), and few individuals were available to solidify the species determination.

Tennessee: *Blount Co.*: Chilhowee, 17 June 2006, 19 Aug 2006, 10 Aug 2007. **NEW STATE RECORD?**

Order Glomerida Leach 1815

Family Glomeridae Leach 1815

Onomeris australora Hoffman 1950

North Carolina: *Haywood Co.*: GSMNP, no specific location (Shelley 2000a).

Onomeris sp.

Taxonomic note: This appears to be an undescribed species of *Onomeris* and its description will be postponed for a revision of the genus.

Tennessee: *Blount Co.*: Chilhowee, 22 Apr 2006, 17 Jun 2006, 19 Aug 2006, 14 Oct 2006, 10–11 Aug 2007.

Order Polyzoniida Gervais 1844

Family Hirudisomatidae Silvestri 1896

Octoglena gracilipes (Loomis 1971) **NEW PARK RECORD**

North Carolina: *Haywood Co.*: ATBI, Albright Grove, 13 Dec 2000–17 Jan 2001, 19 Jun–6 Jul 2001, 16 Oct–6 Nov 2001, 6–19 Nov 2001, 4–21 Dec 2001, 12 May–15 Jun 2002.

Tennessee: *Blount Co.*: Chilhowee, 17 Jun 2006, 19 Aug 2006, 14 Oct 2006, 18 Jun 2007, 10 Aug 2007, 20 Oct 2007.

Tennessee: *Sevier Co.*: ATBI, Porters Creek Trail, 31 Jul 1997; ATBI, Twin Creeks, 27 Nov–12 Dec 2000, 15 Oct–5 Nov 2001, 5 Nov–5 Dec 2001, 5–17 Dec 2001, 12 Jan–4 Feb 2002, 30 May–21 Jun 2002.

Family Polyzoniidae Newport 1844

Petaserpes cryptocephalus (McNeill 1887)

Tennessee: *Sevier Co.*: Elkmont Campground (Shelley 1997).

Petaserpes rosalbus (Cope 1879)

North Carolina: *Swain Co.*: Clingmans Dome (Shelley 1997).

Tennessee: *Blount Co.*: Chilhowee, 22 Apr 2006, 14 Oct 2006, 18 Jun 2007, 20 Oct 2007.

Tennessee: *Sevier Co.*: 10 mi. S Gatlinburg, "The Loop" (as *Polyzonium bivirgatum*, Loomis 1943); along Alum Cave Trail, off TN Hwy. 71 [also US 441], N side of Newfound Gap (as *Polyzonium bivirgatum*, Hoffman 1950a); Greenbrier Campground, Cherokee Orchard, Chimneys Campground (Shelley 1997).

Petaserpes sp.

North Carolina: *Swain Co.*: Mt. Collins (Shelley 1997).

Tennessee: *Blount Co.*: Cades Cove (Shelley 1997); Chilhowee, 22 Apr 2006, 17 Jun 2006, 19 Aug 2006, 14 Oct 2006, 14 Apr 2007, 10 Aug 2007, 20 Oct 2007.

Tennessee: *Sevier Co.*: Sugarlands (Shelley 1997); ATBI, Twin Creeks, 5–17 Dec 2001; Twin Creeks area, 18 Feb 1986, CR Parker.

Order Spirobolida Bollman 1893

Family Spirobolidae Bollman 1893

Taxonomic Note: The following two species have been variously synonymized and split throughout the last century. It was suggested that they be referred to as "*Narceus americanus/annularis* complex" (Shelley *et al.* 2006), although Shelley 2000a only recognized one species. Both types (whether they are species, subspecies, or morphs) can be found in GSMNP; for convenience these are listed separately, but are considered to be one species in the summary.

Narceus americanus (Palisot de Beauvois 1817)

GSMNP, no specific location (Keeton 1960).

North Carolina: *Swain Co.*: ATBI, Ravensford, 1–8 Aug 2001, det. Shelley 2001.

Tennessee: *Blount Co.*: Chilhowee, 19 Aug 2006; Tremont, Middle Prong Trail, 10 July 2007, "Millipede March" participants.

Narceus annularis (Rafinesque 1820)

GSMNP, no specific location (Keeton 1960).

Tennessee: *Blount Co.*: Chilhowee, 14 Oct 2006; Tremont, Middle Prong Trail, 10 July 2007, "Millipede March" participants.

Tennessee: *Sevier Co.*: Gatlinburg and Greenbrier Cove (as *Spirobolus orophilus*, Chamberlin 1943a).

Narceus sp.

Tennessee: *Blount Co.*: Chilhowee, 17 June 2006, 14 Apr 2007, 18 Jun 2007.

Tennessee: *Sevier Co.*: ATBI, Goshen Prong, 17 Sept–22 Oct 2001, 23 May–7 Jun 2002.

Order Spirostreptida Brandt 1833

Family Cambalidae Bollman 1893

Cambala annulata (Say 1821) **NEW PARK RECORD**

Tennessee: *Blount Co.*: Chilhowee, 14–15 Apr 2007.

North Carolina: *Swain Co.*: ATBI, Ravensford, 9–16 Oct 2001, 16–23 Oct 2001, 23–30 Oct 2001, 28 Dec 2001, det. Shelley 2001.

Cambala hubrichti Hoffman 1958 **NEW PARK RECORD**

North Carolina: *Haywood and Swain Cos.*: no specific location, [in GSMNP?] (Shelley 2000a).

Tennessee: *Cocke Co.*: ATBI, Snakeden Ridge, 4–19 Jun 2001, 19 Jun–2 Jul 2001

Cambala sp.

Tennessee: *Sevier Co.*: LeConte Creek, side channel upstream of old mill dams, 22 July 1986, CR Parker.

Order Julida Leach 1815

Family Nemasomatidae Bollman 1893

Orinisobates nigrior Chamberlin 1943

North Carolina: *Haywood Co.*: Big Creek Campground (Enghoff 1985).

North Carolina: *Swain Co.*: Appalachian Trail at Ekaneetlee Gap (Enghoff 1985); Clingmans Dome (Reeves 2001).

Tennessee: *Cocke Co.*: ATBI, Albright Grove, 1–13 Dec 2000, 4–21 Dec 2001

Tennessee: *Sevier Co.*: GSMNP, Gatlinburg* (as *Nemasoma nigrius*, Chamberlin 1943a); Gatlinburg, Rt. 441, Buckeye Nature Trail; Chimneys Picnic Area, 5 mi E Gatlinburg (Enghoff 1985); Clingmans Dome (Reeves 2001); ATBI, Clingmans Dome, 24 Oct–8 Nov 2001, 24 Nov–18 Dec 2001; ATBI, nr. Rocky Spur Branch, 8 Apr 1995.

Family Zosteractinidae Loomis 1943

Ameractis chirogona Enghoff 1982

Tennessee: *Sevier Co.*: 4.6 mi S Gatlinburg* (Enghoff 1982); ATBI, Porters Creek Trail, 31 Jul 1997; ATBI, Goshen Prong, 9 May 2001.

Family Okeanobatidae Verhoeff 1942

Okeanobates americanus Enghoff 1979

Tennessee: *Sevier Co.*: Greenbrier Cove (Enghoff 1979).

Family Parajulidae Bollman 1893

Ptyoiulus coveanus Chamberlin 1943

North Carolina: *Haywood Co.*: ATBI, Cataloochee, 15–30 Nov 2000, 30 Nov–15 Dec 2000, 7–21 Dec 2001; ATBI, Purchase Knob, 15–30 Nov 2000, 5–19 Jul 2001, 21 Nov–7 Dec 2001, 7–21 Dec 2001

Tennessee: *Cocke Co.*: ATBI, Albright Grove, 1–13 Dec 2000, 19 Jun–6 Jul 2001, 21 Jul–2 Aug 2001, 4–21 Dec 2001, 21 Jan–9 Feb 2002, 12 May–15 Jun 2002

Tennessee: *Sevier Co.*: Greenbriar [*sic*] Cove* (Chamberlin 1943a); ATBI, ATBI, Goshen Prong, 10–28 Nov 2000, 28 Nov–11 Dec 2000, 21 May–7 Jun 2001, 7–18 Jun 2001, 22 Oct–9 Nov 2001, 5–12 Nov 2001, 12 Nov–5 Dec 2001, 18 Dec 2001–17 Jan 2002, 17 Jan–10 Feb 2002, 23 May–7 Jun 2002, 7–20 Jun 2002; Indian Gap, 5–17 Jul 2001, 24 Nov–18 Dec 2001, 10 May–19 June 2002, 3–18 Jul 2002; ATBI, Twin Creeks, 24 Oct–6 Nov 2000, 5 Nov–5 Dec 2001, 14 Jan–4 Feb 2002, 4–11 Feb 2002, 11–27 Feb 2002, 30 May–21 Jun 2002.

Ptyoiulus impressus (Say 1821) **NEW PARK RECORD**

North Carolina: *Haywood Co.*: ATBI, Cataloochee, 19 Oct–15 Nov 2000, 21 Nov–7 Dec 2001, 7–21 Dec 2001; ATBI, Purchase Knob, 21 Nov–7 Dec 2001, 7–21 Dec 2001, 15–30 Jan 2002.

North Carolina: *Swain Co.*: ATBI, Ravensford, 1–8 Aug 2001, 8–15 Aug 2001, 15–22 Aug 2001, 9–16 Oct 2001, 23–30 Oct 2001, 30 Oct 2001, 20–28 Dec 2001, 28 Dec 2001, det. Shelley 2001.

Tennessee: *Blount Co.*: Chilhowee, 22 Apr 2006, 17 Jun 2006, 19 Aug 2006, 14 Oct 2006, 14–15 Apr 2007, 18 June 2007, 10–11 Aug 2007, 19–20 Oct 2007.

Tennessee: *Sevier Co.*: ATBI, Brushy Mountain, 23 Oct–6 Nov 2000, 6–27 Nov 2000, 27 Nov–12 Dec 2000, 7–19 Dec 2001; ATBI, Goshen Prong, 5–18 Dec 2001; ATBI, Twin Creeks, 24 Oct–6 Nov 2000, 27 Nov–12 Dec 2000, 12 Dec 2000–16 Jan 2001, 5 Nov–5 Dec 2001, 5–17 Dec 2001.

Ptyoiulus sp.

Tennessee: *Blount Co.*: Tremont, 16 April 2007, 29 May 2007, 31 July 2007, "Millipede March" participants; ATBI, Cades Cove 21 Oct–5 Nov 2001, 3–17 Dec 2001.

Tennessee: *Sevier Co.*: ATBI, Porters Creek Trail, 31 Jul 1997; ATBI, Indian Gap, 5–17 Jul 2001, 10 May–19 June 2002;

Teniulus parvior Chamberlin 1951

Tennessee: *Cocke Co.*: ATBI, Albright Grove, 19 Jun–6 Jul 2001, 6–21 Jul 2001, 16 Oct–6 Nov 2001

Tennessee: *Sevier Co.*: Gatlinburg, grassy bald and spruce–fir* (Chamberlin 1951).

Teniulus setosior Chamberlin 1951

Tennessee: *Sevier Co.*: Gatlinburg, oak–chestnut and grassy bald* (Chamberlin 1951).

Teniulus sp.

Tennessee: *Sevier Co.*: ATBI, Andrews Bald, 13–29 Nov 2000, 17–31 Jul 2001; ATBI, Twin Creeks, 16–30 Jul 2001.

Uroblaniulus atlantus (Chamberlin 1946) **NEW PARK RECORD**

Tennessee: *Blount Co.*: Chilhowee, 14 Oct 2006, 14–15 Apr 2007, 10 Aug 2007, 20 Oct 2007.

Tennessee: *Sevier Co.*: ATBI, Andrews Bald, 3–17 Jul 2001, 17–31 Jul 2001.

Uroblaniulus dixinus Chamberlin 1951

Tennessee: *Sevier Co.*: Gatlinburg* (Chamberlin 1951).

Uroblaniulus exul Chamberlin 1951

Tennessee: *Sevier Co.*: Gatlinburg, cove hardwoods* [as interpreted by Hoffman (1981)] (Chamberlin 1951); ATBI, Twin Creeks, 21 Jun–6 Jul 2001.

Uroblaniulus fumans (Chamberlin 1943)

Tennessee: *Cocke Co.*: ATBI, Albright Grove, 12 May–15 Jun 2002.

Tennessee: *Sevier Co.*: GSMNP, Gatlinburg; and Greenbriar [*sic*] Cove (as *Saiulus fumans*, Chamberlin 1943a)*; ATBI, Goshen Prong, 14–30 Jul 2001, 23 May–7 Jun 2002, 7–20 Jun 2002, 9–17 Jul 2002; ATBI, Indian Gap, 10 May–19 Jun 2002; ATBI, Twin Creeks, 5 Nov–5 Dec 2001, 30 May–21 Jun 2002.

Uroblaniulus sp.

North Carolina: *Swain Co.*: ATBI, Bunches Creek, 1 Sep 1993; ATBI, Ravensford, 9–16 Oct 2001, det. Shelley 2001; Enloe Creek Trail, 17 Aug 2007, BA Snyder.

Tennessee: *Cocke Co.*: ATBI, Snakeden Ridge, 16 Jan–13 Feb 2002.

Tennessee: *Sevier Co.*: ATBI, Clingmans Dome, 6–25 Jun 2001; old growth forest nr. Rocky Spur Branch, 8 Apr 1995, CR Parker; ATBI, Porters Creek Trail, 31 Jul 1997.

Family Julidae Leach 1814

Ophyiulus pilosus (Newport 1843) **NEW PARK RECORD**

North Carolina: *Swain Co.*: ATBI, Ravensford, 30 Oct 2001, 20–28 Dec 2001, det. Shelley 2001.

Tennessee: *Blount Co.*: ATBI, Cades Cove, 19 Nov–3 Dec 2001.

Order Platydesmida DeSaussure 1860

Family Andrognathidae Cope 1869

Andrognathus corticarius Cope 1869

Tennessee: *Blount Co.*: no specific location [in GSMNP?] (Gardner 1975); Chilhowee, 17 Jun 2006, 19 Aug 2006, 14 Oct 2006, 14 Apr 2007.

Brachycybe lecontii Wood 1864 **NEW PARK RECORD**

Tennessee: *Blount Co.*: Chilhowee, 17 Jun 2006, 19 Aug 2006, 14 Oct 2006, 14–15 Apr 2007, 18 June 2007, 11 Aug 2007.

Tennessee: *Cocke Co.*: ATBI, Snakeden Ridge, 5–21 Jun 2002.

Brachycybe petasata Loomis 1936

North Carolina: *Swain Co.*: GSMNP, no specific location, published distribution includes "Great Smoky Mountains in general" (Shelley 2000a); Bunches Creek, 29 Aug 1995, S Boehn; Rhododendron thicket along Enloe Creek Trail, 16 Aug 2007, BA Snyder.

Tennessee: *Blount Co.*: Gregory's Cave, Cades Cove (Causey 1959); Chilhowee, 19 Aug 2006, 14 Oct 2006, 14 Apr 2007, 18 June 2007, 10–11 Aug 2007.

Tennessee: *Cocke Co.*: ATBI, Snakeden Ridge, 9 May 2001

Tennessee: *Sevier Co.*: Chimneys Campground (Loomis 1943); along Alum Cave Trail, off TN Hwy 71 [also US 441], N. side of Newfound Gap (Hoffman 1950a); Newfound Gap; The Sinks (Gardner 1975); old growth forest nr. Rocky Spur Branch, 8 Apr 1995, CR Parker.

Order Callipodida Bollman 1893

Family Abacionidae Shelley 1979

Abacion magnum (Loomis 1943)

North Carolina: *Haywood Co.*: ATBI, Cataloochee, 19 Oct–15 Nov 2000; ATBI, Purchase Knob, 10 Sept–19 Oct 2001; Backcountry campsite 39, 26 June 2005, BA Snyder; Purchase Knob Road, 22 July 2007, “Millipede March” participants.

North Carolina: *Swain Co.*: ATBI, Ravensford, 8–15 Aug 2001, 9–16 Oct 2001, det. Shelley 2001; Enloe Creek Trail, 17 Aug 2007, BA Snyder; intersection of Hyatt Ridge and Enloe Creek Trails, 18 Aug 2007, BA Snyder, J Love, J Lloyd, M Matzko, K Voorhis, R Voorhis, B Taylor.

Tennessee: *Blount Co.*: Chilhowee, 22 Apr 2006, 17 Jun 2006, 18 June 2007, 20 Oct 2007; Tremont, 29 May 2007, “Millipede March” participants.

Tennessee: *Cocke Co.*: ATBI, Albright Grove, 12 May–15 Jun 2002, 6–19 Nov 2001; ATBI, Snakeden Ridge, 19 Jun–2 Jul 2001, 2–12 Jul 2001, 5–21 Jun 2002.

Tennessee: *Sevier Co.*: Chimneys Campground Rt. 71 between Newfound Gap and Gatlinburg (as *Spirostrephon highlandensis*, Hoffman 1950a)

Delophon georgianum Chamberlin 1943

North Carolina: *Swain Co.*: Deep Creek 4 mi N. Bryson City, Smokemont, (Shelley 1979); ATBI, Ravensford, 1–8 Aug 2001, 30 Oct 2001, det. Shelley 2001.

Tennessee: *Blount Co.*: Chilhowee, 22 Apr 2006, 17 Jun 2006, 19 Aug 2006, 14 Oct 2006, 14–15 Apr 2007, 18 June 2007, 11 Aug 2007, 19–20 Oct 2007; Tremont, 31 July 2007, “Millipede March” participants.

Tennessee: *Sevier Co.*: Elkmont, 10.1 mi S Gatlinburg, 4.6 mi S Gatlinburg, Chimneys Picnic Area 7.3 mi S Gatlinburg, Newfound Gap (Shelley 1979); ATBI, Goshen Prong, 19 Jun–2 Jul 2001, 2–17 Jul 2001, 7–20 Jun 2002; ATBI, Twin Creeks, 6–27 Nov 2000, 5 Nov–5 Dec 2001, 30 May–21 June 2002.

Delophon sp. (**Note:** I have no reason to believe this is not *D. georgianum*)

Tennessee: *Cocke Co.*: ATBI, Snakeden Ridge, 16 Oct–6 Nov 2001.

Order Chordeumatida Koch 1847

Family Striariidae Bollman 1893

Striaria columbiana Cook 1899 **NEW PARK RECORD**

Tennessee: *Sevier Co.*: ATBI, Twin Creeks, 5 Nov–5 Dec 2001, 5–17 Dec 2001.

NEW STATE RECORD

Striaria granulosa Bollman 1888 **NEW PARK RECORD**

Tennessee: *Blount Co.*: Chilhowee, 14 Oct 2006.

Striaria zygoleuca Hoffman 1950

North Carolina: *Haywood Co.*: ATBI, Purchase Knob, 7–21 Dec 2001; Backcountry campsite 39, 26 June 2005, BA Snyder.

Tennessee: *Blount Co.*: Chilhowee, 14 Oct 2006, 11 Aug 2007.

Tennessee: *Sevier Co.*: Chimneys Campground, Rt. 71 between Newfound Gap and Gatlinburg (Hoffman 1950a); ATBI, Goshen Prong, 9 May 2001.

Family Cleidogonidae Cook 1896

Cleidogona inexpectata Hoffman 1950

Tennessee: *Blount Co.*: Cades Cove Campground (Shear 1972); Tremont, 31 July 2007, “Millipede March” participants.

Tennessee: *Sevier Co.*: Chimneys Campground, Rt. 71 between Newfound Gap and Gatlinburg* (Hoffman 1950a).

Cleidogona margarita Hoffman 1950

North Carolina: *Haywood Co.*: ATBI, Cataloochee, 19 Oct–15 Nov 2000; ATBI, Purchase Knob, 15–31 Jan 2002.

North Carolina: *Swain Co.*: above Batsaw Branch, 15 Aug 2007, intersection of Hyatt Ridge and Enloe Creek Trails, 18 Aug 2007, BA Snyder, J Love, J Lloyd, M Matzko, K Voorhis, R Voorhis, B Taylor.

Tennessee: *Cocke Co.*: ATBI, Albright Grove, 14 Nov–1 Dec 2000, 16 Oct–6 Nov 2001, 19 Nov–4 Dec 2001; ATBI, Snakeden Ridge, 16 Oct–6 Nov 2001.

Tennessee: *Sevier Co.*: Chimneys Campground, Rt. 71 between Newfound Gap and Gatlinburg* (Hoffman 1950a); ATBI, Andrews Bald, 24 Oct–8 Nov 2001; ATBI, Brushy Mountain, 10–25 Nov 2001; ATBI, Clingmans Dome, 1–30 Aug 2002; ATBI, Goshen Prong, 25 Oct–10 Nov 2000, 12 Nov–5 Dec 2001; ATBI, Indian Gap, 24 Oct–8 Nov 2001, 24 Nov–18 Dec 2001, 1–30 Aug 2002; ATBI, Twin Creeks, 27 Nov 2000–6 Jan 2001, 5 Nov–5 Dec 2001.

Cleidogona medialis Shelley 1976 **NEW PARK RECORD, NEW STATE RECORD**

Tennessee: *Sevier Co.*: ATBI, Goshen Prong, 11 Dec 2000–15 Jan 2001, 12 Nov–5 Dec 2001, 18 Dec 2001–17 Jan 2002; ATBI, Twin Creeks, 27 Nov–12 Dec 2000, 5 Nov–5 Dec 2001.

Cleidogona nantanhala Shear 1972 **NEW PARK RECORD**

Tennessee: *Blount Co.*: Chilhowee, 14 Oct 2006.

Cleidogona sayana (Bollman 1893)

Tennessee: *Sevier Co.*: 10 mi S. Gatlinburg "The Loop" (as *Nemasoma sayanum*, Loomis 1943). [Assigned to *Cleidogona* by Hoffman 1999b.]

Cleidogona tallapoosa Shear 1972 **NEW PARK RECORD, NEW STATE RECORD**

Tennessee: *Blount Co.*: ATBI, Cades Cove, 19 Oct–2 Nov 2000, 7–28 Nov 2000, 5–19 Nov 2001.

Pseudotremia fracta fracta Chamberlin 1951

Tennessee: *Sevier Co.*: Gatlinburg, cove hardwoods* [as interpreted by Hoffman 1981] (as *Pseudotremia fracta*, Chamberlin 1951); West side of Indian Gap; between Gatlinburg and Newfound Gap (as *Pseudotremia cottus*, Shear 1972).

Tennessee: *Blount Co.*: The Sinks; Big Poplar Trail, Cades Cove; Gregory's Cave, Cades Cove (as *Pseudotremia cottus*, Shear 1972); US Hwy. 129 and Tabcat Creek, 17 May 2007, BA Snyder. **Note:** the latter record, based on one male specimen, is geographically between records of *P. f. fracta* and *P. f. nantahala*, and exhibits traits of both subspecies.

Pseudotremia sp.

North Carolina: *Haywood Co.*: ATBI, Purchase Knob, 21 Dec 2001–15 Jan 2002.

Family Trichopetalidae Verhoeff 1914

Trichopetalum montis Chamberlin 1951

Tennessee: *Sevier Co.*: Gatlinburg, grassy area and spruce–fir* (Chamberlin 1951).

Trichopetalum uncum Cook & Collins 1985 **NEW PARK RECORD**

Tennessee: *Blount Co.*: Chilhowee, 14 Oct 2006, 14Apr 2007.

Trichopetalum sp.

North Carolina: *Haywood Co.*: ATBI, Purchase Knob, 5–19 Jul 2001.

Tennessee: *Cocke Co.*: ATBI, Albright Grove, 19 Jun–6 Jul 2001; ATBI, Snakeden Ridge, 9 May 2001, 5–21 Jun 2002.

Tennessee: *Sevier Co.*: ATBI, Andrews Bald, 17–31 Jul 2001; ATBI, Brushy Mountain, 21 Jul–5 Aug 2001; ATBI, Goshen Prong, 17 Jan–10 Feb 2002; ATBI, Indian Gap, 10 May–19 Jun 2002; ATBI, Rainbow Falls Trail, 4 May 1999; ATBI, Twin Creeks, 15 May 2001, 15 Oct–5 Nov 2001, 14 Jan–4 Feb 2002.

Family Conotylidae Cook 1896

Conotyla aeto Shear 1971 **NEW PARK RECORD, NEW STATE RECORD, SOUTHERNMOST RECORD FOR THE GENUS**

North Carolina: *Haywood Co.*: ATBI, Purchase Knob, 15–30 Nov 2000.

Order Polydesmida Leach 1814

Family Xystodesmidae Cook 1895

Tribe Aphelorini Hoffman 1980

Apheloria montana (Bollman 1887) **NEW PARK RECORD**

Tennessee: *Blount Co.*: Chilhowee, 17 Jun 2006, 11 Aug 2007.

Deltotaria brimleii brimleii Causey 1942

Taxonomic Note: *D. brimleardia* Causey 1950 was considered a junior synonym of *D. b. brimleii* by Shelley (1986) but was resurrected by Hoffman (1999a) as *D. brimleii brimleardia*.

North Carolina: *Haywood Co.*: near Cataloochee School, 12 mi NW Waynesville (Shelley 1986); Big Fork Ridge, Caldwell Fork, and Rough Fork Trails, 26 June 2005, BA Snyder (det. Shelley & Snyder 2007).

North Carolina: *Swain Co.*: Park entrance near Deep Creek Campground, 2 mi NE Bryson City (Shelley 1986).

Tennessee: *Cocke Co.*: Cosby Picnic Area (Shelley 1986).

Tennessee: *Sevier Co.*: Ramsey Prong [Trail?] (as *Deltotaria brimleardia**, Causey 1950a); Ramsey Prong [Trail?], Gatlinburg (Shelley 1986).

Deltotaria brimleii intergrades

Tennessee: *Blount Co.*: Tremont, Middle Prong Trail, 10 July 2007, "Millipede March" participants.

Tennessee: *Sevier Co.*: Chimneys Picnic area, 7.3 mi S Gatlinburg (Shelley 1986).

Falloria bidens (Causey 1942)

Taxonomic Note: placed into genus *Sigmoria*, subgenus *Falloria* (Shelley 1981, 1986)

Tennessee: *Sevier Co.*: Chimneys Campground (as *Apheloria bidens*, Causey 1942)*; Porter Creek Flats (Causey 1950a). Several locations along US Hwy. 441, between Gatlinburg and Chimneys Picnic Area, including Sugarlands Nature Trail and Park Headquaters; Little River Trail, 0.1 mi east of Elkmont; Roaring Fork Nature Trail and Cherokee Orchard Road; Junglebrook area on Cherokee Orchard Road (Shelley 1981).

Falloria prolata Shelley 1986

Taxonomic Note: placed into genus *Sigmoria*, subgenus *Falloria* (Shelley 1986).

Tennessee: *Sevier Co.*: Ramsay Cascade Parking area, Greenbrier section*; Greenbrier entrance road, 1.8 mi E of junction with Hwy. 73; Roaring Fork nature trail, between 3rd and 4th bridges (Shelley 1986).

Falloria ainsliei (Chamberlin 1921)

Taxonomic Note: placed into genus *Sigmoria*, subgenus *Falloria* (Shelley 1981, 1986).

Tennessee: *Blount Co.*: Hwy 73 at crossing of Little River [in GSMNP?] (Shelley 1986); Great Smoky Mountains Institute at Tremont, 19 June 2007, BA Snyder, MA Callaham, CN Lowe, SC Rostkowski; Tremont, 29 May 2007, 10 July 2007, 31 July 2007, “Millipede March” participants.

Tennessee: *Sevier Co.*: Little Greenbrier School; along Hwy. 73, 0.6 mi N of Metcalf Bottoms Picnic Area (Shelley 1986).

Falloria fumimontis (Shelley 1981)

Taxonomic Note: placed into genus *Sigmoria*, subgenus *Falloria* (Shelley 1981, 1986).

Tennessee: *Blount Co.*: Abrams Creek Campground*; along Little River on Hwy. 73, 4 mi. W Townsend [in GSMNP?]; along trail to log cabin near Abrams Falls, Cades Cove area (Shelley 1981); Abrams Creek Campground, 20 June 2007, B.A. Snyder.

Falloria lyrea (Shelley 1981)

Taxonomic Note: placed into genus *Sigmoria*, subgenus *Falloria* (Shelley 1981, 1986).

North Carolina: *Swain Co.*: 20.5 mi. WNW Bryson City, along Twenty Mile Creek (Shelley 1981).

Tennessee: *Blount Co.*: Parsons Bridge Road, 0.1 mi N of junction with US Hwy. 129*; ravine 1 mi. E Calderwood, along US Hwy. 129; US Hwy. 129 at Tabcat Creek (Shelley 1981); Chilhowee, 17 Jun 2006, 19 Aug 2006, 14–15 Apr 2007, 18 June 2007, 11 Aug 2007, 20 Oct 2007.

Falloria translineata (Shelley 1981)

Taxonomic Note: placed into genus *Sigmoria*, subgenus *Falloria* (Shelley 1981, 1986).

North Carolina: *Swain Co.*: US Hwy. 441 just below Collins Creek, ~6 mi. N Cherokee; 18.8 mi. NW Bryson City, Appalachian Trail N of Fontana Dam (Shelley 1981).

Tennessee: *Blount Co.*: 5.6 mi. SE Townsend, Rich Mountain Road, just inside Park boundary*; along road to Cades Cove 1.0 mi. S of junction with Hwy. 73, at West Prong Little River; Cades Cove, along Vista Trail, near campground (Shelley 1981); nr. Gregory’s Cave, 21 Jun 2007, BA Snyder; Tremont, 26 June 2007, “Millipede March” participants.

Falloria tuberosa (Shelley 1981)

Taxonomic Note: placed into genus *Sigmoria*, subgenus *Falloria* (Shelley 1981, 1986).

North Carolina: *Swain Co.*: Smokemont Campground*; 4.4 mi. NW Smokemont, along unnumbered road, 3.3 mi. NW junction with US Hwy. 441; 2.3 mi. NW Smokemont, along Collins Creek at picnic area; E Smokemont along Towstring Road, 1 mi. N junction with US Hwy. 441; S Smokemont, vicinity of Mingus Mill, 0.1 mi. W junction with US Hwy. 441 (Shelley 1981).

Sigmoria nantahalae Hoffman 1958

Taxonomic Note: placed into genus *Sigmoria*, subgenus *Falloria* (Shelley 1981, 1986)

North Carolina: *Swain Co.*: along Forney Creek, ~12 mi. NW Bryson City, 8.0 mi. W Park entrance (Shelley 1981).

Sigmoria rubromarginata (Bollman 1888)

North Carolina: *Haywood Co.*: Big Creek Campground (Shelley 1981).

North Carolina: *Swain Co.*: 2 mi. NE Bryson City, at entrance to Deep Creek area, along county road 1339, 1.2 mi. W junction county road 1375; Smokemont Campground; 8.5 mi. NE Cherokee, along Park road, 14.9 mi. W Heintooga Overlook (Shelley 1981); ATBI, Ravensford, 1–8 Aug 2001, det. Shelley 2001 (as *S. rubromarginata rubromarginata*).

Tennessee: *Cocke Co.*: Cosby Picnic Area; along Indian Camp Creek, W Cosby (Shelley 1981).

Tennessee: *Sevier Co.*: along Rhododendron Creek in Greenbrier, 1.8 mi. S junction Hwy. 73 (Shelley 1981).

Family Xystodesmidae Cook 1895

Tribe Rhysodesmini Brolemann 1916

Boraria deturkiana (Causey 1942)

North Carolina: *Swain Co.*: GSMNP, no specific location (Shelley 2000a); above Batsaw Branch, BA Snyder, J Love, J Lloyd, M Matzko, K Voorhis, R Voorhis, B Taylor, 15 Aug 2007; Enloe Creek Trail, K Voorhis, 17 Aug 2007.

Tennessee: *Blount Co.*: Mt. Squires on state line above Cades Cove (Hoffman 1965).

Tennessee: *Cocke Co.*: ATBI, Albright Grove, 22 May–8 Jun 2001, 8–19 Jun 2001, 19 Jun–6 Jul 2001, 12 May–15 Jun 2002, 20 Jul–1 Aug 2002.

Tennessee: *Sevier Co.*: Clingmans Dome (as *Aporiaria deturkiana*, Causey 1942)*; Rainbow Falls Trail, Greenbrier Cove, Double Spring Gap (Causey 1950a); Chimneys Campground, Clingmans Dome, Gatlinburg, Newfound Gap (Hoffman 1965); ATBI, Andrews Bald, 3–17 Jul 2001, 17–31 Jul 2001; ATBI, Brushy Mountain, 3–18 Jun 2002; ATBI, Clingmans Dome, 6–25 Jun 2001, 25 Jun–3 Jul 2001, 10 May–19 June 2002, 1–30 Aug 2002; ATBI, Indian Gap, 28 May–7 Jun 2001, 5–17 Jul 2001, 10 May–19 June 2002; ATBI, Goshen Prong, 7–18 Jun 2001, 2–17 Jul 2001, 17–30 Jul 2001, 22 Oct–9 Nov 2001, 23 May–7 Jun 2002, 7–20 Jun 2002.

Boraria infesta (Chamberlin 1918)

Taxonomic Note: Hoffman (1999a) suggested *Aporiaria brunnior* as a synonym of *B. infesta*. Taxonomy within this genus needs further review.

Tennessee: GSMNP*, no specific location (as *Aporiaria brunnior*, Chamberlin 1943b).

Boraria stricta (Brolemann 1896)

Tennessee: *Blount Co.*: Mt. Squires E of Cades Cove (Hoffman 1965).

Tennessee: *Sevier Co.*: Greenbriar [*sic*] Cove (as *Aporiaria fumans*, Chamberlin 1943b); west side of Indian Gap (Hoffman 1965).

Cherokia georgiana georgiana (Bollman 1889)

Tennessee: *Sevier Co./* **North Carolina:** *Swain Co.*: Newfound Gap (as *Mimuloria georgiana*, Loomis 1943); trail to Siler's Bald [which trail?] (as *Mimuloria georgiana*, Causey 1950a).

Tennessee: *Blount Co.*: Cades Cove, 1 mi E of Ranger Station (Hoffman 1950b); Chilhowee, 17 Jun 2006, 18 June 2007, 11 Aug 2007.

Tennessee: *Sevier Co.*: Near Chimneys (as *Cherokia georgiana*, without subspecies, Hoffman 1950a); Rainbow Falls Trail, Indian Gap, Greenbriar Cove, Double Spring Gap, Bullhead Trail (as *Mimuloria georgiana*, Causey 1950a).

Cherokia georgiana ducilla (Chamberlin 1939)

North Carolina: *Swain Co.*: Heintooga Ridge 8 mi NE of Smokemont; Balsam Mountain Campground (Hoffman 1950b); intersection of Hyatt Ridge and Enloe Creek Trails, 18 Aug 2007, BA Snyder, J Love, J Lloyd, M Matzko, K Voorhis, R Voorhis, B Taylor.

Tennessee: *Blount Co.*: Tremont, 26 June 2007, 10 July 2007, "Millipede March" participants.

Tennessee: *Sevier Co.*: Clingmans Dome; W side of Indian Gap; Chimneys Campground; 5 mi S Gatlinburg; Andrews Bald, 14 June 2007, JP Love; ATBI, Indian Gap, 10 May–19 Jun 2002, 3–18 July 2002; ATBI, Goshen Prong, 7–18 Jun 2001, 2–17 Jul 2001, 17–30 Jul 2001, 17 Jul–5 Aug 2001, 23 May–7 Jun 2002, 7–20 Jun 2002; ATBI, Twin Creeks, 21 Jun–2 Jul 2001, 15 Oct–5 Nov 2001, 30 May–21 Jun 2002.

Cherokia georgiana intergrades

North Carolina: *Haywood Co.*: Backcountry campsite 39, 26 June 2005, BA Snyder.

North Carolina: *Swain Co.*: Smokemont (Hoffman 1950b).

Tennessee: *Blount Co.*: Chilhowee, 20 Oct 2007.

Erdelyia saucra Hoffman 1962 **NEW STATE RECORD(TN), NEW PARK RECORD**

North Carolina: *Haywood Co.*: Purchase Knob Road, 22 July 2007, “Millipede March” participants.

Tennessee: *Sevier Co.*: ATBI, Clingmans Dome, 10 May–19 Jun 2002.

Gyalostethus monticolens (Chamberlin 1951)

Tennessee: *Sevier Co.*: Gatlinburg Cove* (as *Boraria monticolens*, Chamberlin 1951).

Family Xystodesmidae Cook 1895

Tribe Nannarini Hoffman 1964

Nannaria scutellaria Causey 1942

North Carolina: *Swain Co.*: GSMNP, no specific location (Shelley 2000a); ATBI, Cataloochee, 19 Oct–15 Nov 2000, 15–30 Nov 2000, 21 Nov–7 Dec 2001, 7–21 Dec 2001, 28 Jan–14 Feb 2002.

Tennessee: *Cocke Co.*: ATBI, Albright Grove, 19 Jun–6 Jul 2001.

Tennessee: *Sevier Co.*: near Chimneys* (Causey 1942); Greenbrier Cove, Double Spring Gap, Ramsey Prong, Cherokee Orchard, Chimneys, "On island near Two–Mile Branch along highway" [near Park Headquarters] (Causey 1950a,b); Chimneys Campground (Hoffman 1950a); ATBI, Goshen Prong, 25 Oct–10 Nov 2000, 10–28 Nov 2000, 21 May–7 Jun 2001, 17 Sept–22 Oct 2001, 22 Oct–9 Nov 2001, 12 Nov–5 Dec 2001,17 Jan–10 Feb 2002; ATBI, Twin Creeks, 15 Oct–5 Nov 2001, 5 Nov–5 Dec 2001.

Family Sphaeriodesmidae Humbert & DeSaussure 1869

Desmonus earlei Cook 1898 **NEW PARK RECORD**

Tennessee: *Blount Co.*: Chilhowee, 22 Apr 2006, 19 Aug 2006, 14 Oct 2006, 14 Apr 2007, 18 June 2007, 10–11 Aug 2007.

Tennessee: *Sevier Co.*: GSMNP, no specific location (*Desmonus sp.* juveniles, Causey 1958). **Note:** This record was deleted by Shelley (2000b) because the specimens could not be found in the Florida State Collection of Arthropods and *Desmonus* had not previously been found in the Smokies. As the previous record was deemed invalid, the Blount Co. specimens constitute a new GSMNP record.

Family Paradoxosomatidae Daday 1889

Oxidus gracilis (C. L. Koch 1847)

Note: Known to be widespread in the eastern United States (Hoffman 1999a).

North Carolina: *Haywood and Swain Cos.*: part of known distribution [in GSMNP?] (Shelley 2000a).

North Carolina: *Swain Co.*: ATBI, Ravensford, 1–8 Aug 2001, 8–15 Aug 2001, 15–22 Aug 2001, 9–16 Oct 2001, 16–23 Oct 2001, 23–30 Oct 2001, 30 Oct 2001, 28 Dec 2001, det. Shelley 2001.

Tennessee: *Blount Co.*: Tremont, 19 & 22 Jun 2007, BA Snyder; along Little River Road, Hwy. 73, 35˚40.205’N 83˚40.714’W, 20 Jun 2007, BA Snyder.

Family Polydesmidae Leach 1815

Pseudopolydesmus canadensis (Newport 1814)

North Carolina: *Haywood Co.*: ATBI, Purchase Knob, 5–19 Jul 2001, 19 Jul–2 Aug 2001.

North Carolina: *Swain Co.*: Enloe Creek Trail, 17 Aug 2007, BA Snyder.

Tennessee: *Blount Co.*: Lumber Ridge Trail, 13 Aug 2004, GT Watters & B Kasson.

Tennessee: *Cocke Co.*: ATBI, Albright Grove, 14 Nov–1 Dec 2000, 1–13 Dec 2000, 22 May–8 Jun 2001, 19 Jun–6 Jul 2001, 12 May–15 Jun 2002; ATBI, Snakeden Ridge, 5–21 Jun 2002.

Tennessee: *Sevier Co.*: 10 mi S. Gatlinburg "The Loop" (as *Polydesmus branneri*, Loomis 1943); Chimneys Campground Rt. 71 between Newfound Gap, Gatlinburg (as *Dixidesmus branneri*, Hoffman 1950a); ATBI, Brushy Mountain, 3–18 Jun 2002; ATBI, Goshen Prong, 7–18 Jun 2001, 2–17 Jul 2001, 17 Jul–5 Aug 2001, 23 May–7 Jun 2002, 7–20 Jun 2002; ATBI, Twin Creeks, 27 Nov–12 Dec 2000, 21 Jun–6 Jul 2001, 15 Oct–5 Nov 2001, 5 Nov–5 Dec 2001, 12 Dec 2000–16 Jan 2001, 2–14 Jan 2002, 14 Jan–4 Feb 2002, 4–14 Feb 2002, 30 May–21 June 2002.

Tennessee: *Sevier Co./* **North Carolina:** *Swain Co.*: Newfound Gap (as *Polydesmus branneri*, Loomis 1943); Clingmans Dome, 9 Oct 2005, M Strickland.

Pseudopolydesmus erasus (Loomis 1943)

North Carolina: *Swain and Haywood Cos.*: no specific location [in GSMNP?] (Shelley 2000a).

North Carolina: *Haywood Co.*: ATBI, Purchase Knob, 5–19 Jul 2001; Backcountry campsite 39, BA Snyder, 26 June 2005.

Scytonotus australis Hoffman 1962

North Carolina: *Haywood Co.*: ATBI, Purchase Knob, 31 Jan–14 Feb 2002.

North Carolina: *Swain Co.*: 0.5mi N of Oconoluftee Ranger Station, Mingus Mill Creek (Shelley 1993). ATBI, Ravensford, 30 Oct 2001, 20–28 Dec 2001, det. Shelley 2001.

Tennessee: *Cocke Co.*: ATBI, Snakeden Ridge, 16 Oct–6 Nov 2001.

Tennessee: *Sevier Co.*: Gatlinburg [in GSMNP?] (Shelley 1993).

Scytonotus australis x *virginicus michauxi* hybrid

Tennessee: *Sevier Co./* **North Carolina:** *Swain Co.*: Clingmans Dome summit (Shelley 1993).

Scytonotus granulatus (Say 1821)

Tennessee: *Blount and Sevier Cos.*: no specific location [in GSMNP?] (Shelley 1993).

Tennessee: *Cocke Co.*: ATBI, Albright Grove, 14 Nov–1 Dec 2000.

Scytonotus virginicus michauxi Hoffman 1962

North Carolina: *Swain Co.*: GSMNP, no specific location [in GSMNP?] (Shelley 1993).

Tennessee: *Cocke Co.*: ATBI, Snakeden Ridge, 16 Jan–13 Feb 2002.

Tennessee: *Sevier Co.*: Rainbow Falls Trail; 2 mi NNW of Newfound Gap (Shelley 1993).

Scytonotus sp.

Tennessee: *Sevier Co.*: ATBI, Porters Creek Trail, 31 Jul 1997; ATBI, Twin Creeks, 15 May 2001, 30 May–21 Jun 2002.

The above list represents the current state of knowledge on millipede biodiversity and distribution within GSMNP. To provide a more complete picture of the fauna, taxa deemed likely to inhabit GSMNP are presented in Table 1. These taxa have not been recorded from the Park, but have been either been recorded near the Park or in similar habitats to those found within the Park. Additional sampling may reveal their presence.

Table 1. Millipede taxa likely to inhabit, but not yet recorded from, the Great Smoky Mountains National Park.

Taxon	Known Range
Polyzoniida: Polyzoniidae	
Petaserpes lateralis (Shelley 1976)	Known from similar montane habitats ~150 km to the northeast.
Chordeumatida: Cleidogonida	
Cleidogona jocassee Hoffman 1950	Known from Haywood and Swain Cos., NC, outside GSMNP (Shear 1972).
Chordeumatida: Trichopetalidae	
Trichopetalum cornutum Cook & Collins 1895	GSMNP falls within the known range (Hoffman 1999a).
Trichopetalum lunatum Harger 1872	GSMNP falls within the known range (Hoffman 1999a).
Chordeumatida: Branneriidae	
Branneria carinata (Bollman 1888)	Described from Jefferson Co., TN; widespread across the southeastern US (Shear 1972).
Polydesmida: Macrosternodesmidae	
Chaetaspis sp.	Known from Haywood and Swain Cos., NC (see Shelley 2000a). Specimens labeled this in the GSMNP museum appeared to be immature *Scytonotus* sp.
Polydesmida: Xystodesmidae	
Brachoria spp.	Several species are known from eastern Tennessee.
Falloria leucostriata (Shelley 1981)	Known from Cocke and Sevier Cos., TN, but outside of GSMNP (Shelley 1981, Hoffman 1999a).

Discussion

GSMNP has a very diverse millipede fauna, possibly the most diverse of any area of similar size in North America. The fauna currently consists of 63 species, one of which is divided into two subspecies, in 21 families, from all 10 North American orders. These counts are tenuous because the taxonomy underlying many of these taxa is badly in need of revision. Interestingly, most of these species were recorded from areas greatly affected by human impact (e.g. campgrounds, picnic areas, roadsides, and trails). Very little collecting has been done in the backcountry, and almost none has been done off of trails, where disturbance-sensitive millipedes might remain undiscovered.

The ecology of North American millipedes is poorly understood. Data from the GSMNP millipede specimens could contribute greatly to our understanding of millipede ecology by allowing an analysis of the habitat associations of these species. In addition, species with distributions bordering the Park but not found within GSMNP might provide insight to the mechanisms underpinning millipede biogeography and ecology.

A few species (e.g. those in Table 1) may be added to the species list with continued collection and determination of previously collected specimens. Additionally, the GSMNP fauna is anticipated to expand as several undescribed species have been discovered but have not yet been described. Undescribed species from GSMNP exist in the genera *Scoterpes* and *Trichopetalum* (W.A. Shear, personal communication). The author has specimens of *Onomeris*, *Striaria*, and *Cleidigona* which are not referable to species at this time and are likely undescribed. A second species of *Nannaria* is known from GSMNP but cannot be assigned to a species or described without clarifying the identity of *N. tennesseensis* (Bollman 1889), for which a male type specimen does not appear to exist. Description of some of these species may be delayed until generic revisions can be completed.

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CHAPTER 7

CONCLUSION

Amynthas agrestis invasion was observed to be a highly dynamic process that might be controlled by multiple factors. Temperature and moisture were important factors affecting invasion progress in the Great Smoky Mountains National Park. However, most of the current hypotheses about invasion spread have not been explicitly tested for earthworm invasions.

These studies suggested that *Amynthas* spp. invasion altered ecosystem biogeochemistry and impacted populations of other epigeic detritivores through direct food competition. *Amynthas* spp. were shown to primarily consume partially decomposed leaf litter ("FH") and greatly increased soil aggregation through casting and burrowing activities. During these processes, C from leaf litter was incorporated in aggregates and microbial activity of the soil was usually enhanced. However, changes in microbial biomass were not detected in the field, and not measured in the laboratory.

Millipedes also preferred FH-horizon material and this appears to lead to direct competition for this food resource. Millipedes also frequently reside in or near this material; removal of this material by *Amynthas* spp. could be a form of habitat modification that millipedes could not tolerate. Disentangling the impacts of habitat modification from those of food competition was not possible.

Amynthas spp. benefited from millipede's litter processing when resources were limited to low-quality sources (e.g. fresh litter rather than partially decomposed FH material). However, when resources were more palatable, *Amynthas* spp. were unaffected by millipede activity.

Millipedes did not benefit from this interaction. Species richness and abundance were decreased in *Amynthas*-invaded areas relative to *Amynthas*-free conditions. Microcosm studies supported these results, showing trends towards more rapid millipede death in the presence of *A. agrestis*. Additionally, earthworms consumed FH material rapidly, and FH was found to be critical for millipede survival.

Several promising avenues of future research could contribute to our understanding of the myriad impacts of *Amynthas* spp. invasion. Long-term monitoring of the active invasion front in the Great Smoky Mountains National Park would further our understanding of which factors control invasion spread. Additionally, this spread could be modeled with some basic information about *Amynthas* biology. Our field observations indicated an annual lifecycle, but this has not been demonstrated in the literature. Quantitative studies of aspects of *A. agrestis* biology (e.g. dispersal, reproductive abilities), which are relevant to invasion biology, are almost entirely lacking. Finally, these invasions pose a threat to the southern Appalachian Mountains' high millipede diversity. Invasion control techniques need to be investigated to attempt to limit the spread of invasive earthworms with limited impacts on native fauna.