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INTERACTIONS AMONG TOP-DOWN REGULATORS IN A TEMPERATE FOREST FLOOR ECOSYSTEM: EFFECTS ON MACROFAUNA, MESOFAUNA, MICROBES AND LITTER DECAY

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DEDICATION

I dedicate my dissertation to Dr. Nelson Hairston, Sr. whose research has inspired my own. Nelson Hairston designed and conducted a five year study to assess competition as the mechanism for the observed narrow zone of vertical overlap among large *Plethodon* in the Appalachians. He began his legendary salamander removal study on April 23, 1974, the day that I was born. I was so impressed by Hairston's work, that on the same day in 2003 I began my own predator removal field study to examine potential interactions (competitive and predatory) among ecologically similar, but taxonomically divergent, predators occupying temperate forest floor food webs. It was after reading Hairston et al. 1960 that I decided to consider these interactions in the context of food web dynamics, a perspective that seems to be required if we are to understand community structure and function.

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CARI-ANN M. HICKERSON

ABSTRACT

High species diversity and complexity of forest-floor food webs present a challenge for understanding the role of species interactions (e.g. competition and predation) as regulatory mechanisms for ecosystem processes such as decomposition and nutrient cycling. In particular, we understand very little about the roles of forest-floor predators in regulating diversity and abundance of lower trophic levels and ecosystem processes. However, ecological theory and several studies suggest that interactions among intraguild predators (IGP) may be important controls of diversity and abundance of organisms and detritus in lower trophic levels within food webs. A key prediction is that interactions among predators weaken trophic cascades. My research examined this prediction by characterizing interactions among predators and examining their effects on lower trophic levels within the forest-floor food web of Northeast Ohio.

The results of the laboratory microcosm studies, in combination with several previous studies, suggest that the effects of removal treatment on intraguild predators, especially centipedes, spiders, carabid beetles, and salamander, were not the result of intraguild predation, but were more likely to have been the result of non-consumptive competitive interactions (NCEs). Predator removal from open, unrestricted field plots

resulted in changes in the abundances of several groups of predators and macrodetritivores. Additionally, I found that predator manipulation affected composition of microflora within the soils at my field site. The mechanisms for this effect remain uncertain but may be indicative of antibiotic interactions within the soil through bacteria dispersed through skin secretions and feces of predators, particularly salamanders.

This work contributes significantly to a growing body of evidence indicating that territorial predators, such as *P. cinereus*, which are constrained to spatially fixed microhabitats, can be strong regulators of guild members and lower trophic levels. My results also support an important role for top-down, predator-mediated regulation of species composition in a forest-floor, detrital food web, a system that is commonly thought to be regulated primarily through bottom-up effects of organic matter supply, i.e, leaf litter. Further, this is the only study of which I am aware that attempted, and detected, significant top-down, predator-mediated effects at multiple trophic levels, including the microbial level, in the complex food web of the temperate forest-floor.

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INTRODUCTION/SUMMARY

The focus of my dissertation research has been to determine how generalist predators in forest floor communities interact with one another and to assess the importance of those interactions in regulating population and community structure. Specifically, I have attempted to examine the mechanisms responsible for the observed abundance and distribution of organisms occupying the terrestrial detrital food web of temperate forests by using a combination of field and laboratory studies to address the following hypotheses: 1) Interspecific competition and/or intraguild predation (IGP) occurs among major categories of generalist predators: salamanders, centipedes, spiders and carabid beetles. 2) These predators initiate trophic cascades that limit densities of organisms that are involved in leaf litter decomposition. 3) Trophic cascades indirectly affect rates of leaf litter decay which is the basal resource within the web.

Significance and Background

Early studies of food web dynamics used simple models to predict patterns and outcomes of distribution and abundance of species in various systems. For example, Hairston and Hairston (1993) noted that, historically, models assumed that links in food chains were equal in value, and that interactions among organisms were either assumed to exist where consumers share resources, or ignored if the interactions were non-consumptive in nature. Over the past 50 years ecologists have begun to appreciate that many factors influence the abundance and distribution of organisms in communities. For example, Hairston et al. (1960) concluded that interspecific competition must necessarily occur among producers, carnivores and decomposers because their own resources limit them. We now understand "multi-channel" omnivory is widespread in food webs that have an array of predators feeding at several trophic levels (Polis, 1998). It is insufficient to look at linear, single factor food webs if we are to understand what is happening in nature. Thus, a combination approach of studying biotic interactions (e.g. competition, predation) and the abiotic factors (e.g. soils, pH, temperature, moisture) that influence them is necessary for determining the distribution and dynamics of populations and their places in communities. Polis (1991, 1994) argued that food webs are central in community ecology and suggested that food web studies conducted in the absence of knowledge about competition and predation tell us little about how community structure and ecosystem function are maintained. For example, competition and intraguild predation can attenuate the effects on prey and rates of nutrient input to the system (Paine 1980, Polis et al. 1989) and it these kinds of non-consumptive effects that have largely been ignored in food webs research.

Salamanders as regulators of detrital food webs

There is an extensive body of research suggesting that terrestrial salamanders in the family Plethodontidae are important in the regulation of invertebrate communities and decomposition of organic material on the forest floor (Burton and Likens 1975, Hairston 1987, Rooney et al. 2000, Wyman 1998, Walton 2005, Walton and Steckler 2005). For instance, these salamanders can be extremely abundant and have been estimated to consume more than one complete turnover of invertebrates annually (Hairston 1987). This impact may exceed that estimated for forest floor spiders (Moulder and Reichle 1972). Therefore, plethodontid salamanders are expected to be important in determining community structure in the detrital based forest floor food web.

Interestingly, there are many predatory, forest floor invertebrates that are ecologically similar to, and have the potential to interact with salamanders, thereby altering community composition and food web stability by adding complexity to the system. For example, large centipedes are similar to the salamander, *Plethodon cinereus* in the following ways: 1) they have large biomasses (Lewis 1981); 2) they occupy similar microhabitats (Shelley 2002, Hickerson et al. 2004); 3) they are generalist predators (Roberts 1956) and have similar foraging tactics (Jaeger and Barnard 1981, Formanowicz and Bradley 1987); 4) they are similar in size to small terrestrial plethodontids; and 5) they show some degree of parental care in the form of egg brooding (Lewis 1981). Such similarities among salamanders and large forest floor invertebrates provide the rationale for studying competition and predation among forest floor predators.

Intraspecific interactions

One way terrestrial woodland salamanders compete for resources is through interference competition and territoriality (see Mathis 1995 for a review). Terrestrial salamanders forage on the forest floor in leaf litter while conditions are wet but will move beneath rocks and logs when conditions become dry. For salamanders, territoriality is adaptive because moisture and prey can become concentrated under cover objects. Therefore, territories beneath rocks and logs allow salamanders' access to the surface to forage for longer periods, and they become refugia for isolated prey populations as the forest dries. Extensive laboratory and field evidence suggests that the red-backed salamander, *Plethodon cinereus,* is territorial (Gergits and Jaeger 1990, Horne and Jaeger 1988, Jaeger 1981, Jaeger et al.1982), including populations in Ohio (Deitloff et al 2008). Individuals of *P. cinereus* exhibit site tenacity, advertise their presence in an area, and are able to expel intra- and interspecific intruders from that area. Although many studies in general have been conducted that investigate predator interactions, most are limited in focus on within species interactions, as described above.

Interspecific interactions

More recently, researchers have begun to consider the potential interactions that may occur in nature among distantly related, but ecologically similar organisms. For example, two studies examined behavioral interactions between individuals of *Plethodon cinereus* and predatory invertebrates, a carabid beetle, *Platynus tenuicollis* (Gall et al. 2003) and a centipede, *Scolopocryptops sexspinosus* (Hickerson et al. 2004). Beetles were more aggressive when tested as residents compared to when tested as intruders, suggesting that *Platynus tenuicollis* is territorial. Salamanders responded to odors of, and encounters with beetles and centipedes in a similar way as they respond to conspecific intruders, showing increased levels of aggression compared to controls. Salamanders exhibited antipredator responses to beetles in some trials (Brodie et al. 1979). Gall et al. (2003) suggested that if aggressive behavior by beetles can be explained in a territorial context and if the behavior works to exclude salamanders, then interactions with beetles may be important in determining salamander distributions on the forest floor. Similarly, if the attempted predation by beetles and the antipredator response of *P. cinereus* indicate a predator-prey relationship, then intraguild predation (IGP) may be an important mechanism for interactions between these two species. Centipedes never attempted to bite salamanders but negative co-occurrence in the field suggests that competition may be a mechanism for interactions between salamanders and centipedes (Hickerson et al. 2004).

Intraguild predation (IGP)

Intraguild predation (IGP) is common in food webs and has the potential to add significant complexity to food web interactions (Polis 1991). IGP occurs when potential competitors also eat each other and can be categorized as symmetrical (looping; Polis et al. 1989), in which species A and B are mutual predators of one another, or asymmetrical in which species A always preys on species B. IGP in each of these categories can be influenced by ontogenetic changes in size and resulting vulnerability to predation. It is often assumed that symmetrical IGP occurs as a result of ontogenetic reversal of predation, such that adults of species A eat juveniles of species B and adults of B eat juveniles of A (Polis et al. 1989). With regard to salamander and centipede interactions, it is further assumed that because both groups are generalist predators that experience large changes in size through ontogeny, and because centipedes are venomous, the likelihood of symmetrical IGP is high. However, a recent study investigating interactions between juveniles of *P. cinereus* and adult centipedes reports no evidence for predation on juvenile salamanders by larger, adult centipedes (Anthony et al. 2007). Similar results were reported by Ducey et al. (1999). They examined trophic interactions between small terrestrial vertebrates (salamanders and small snakes) and an introduced predatory flatworm. Despite the larger size of predators used in the experiment, flatworms were rejected as suitable prey in most cases. Rubbo et al. (2001, 2003) paired wolf spiders (*Gladicosa pulchra*) with juvenile spotted salamanders (*Ambystoma maculatum*) and with juvenile ground skinks (*Scincella lateralis*) and found predation on both salamanders and skinks by the spiders. Lack of predation on juveniles of *P. cinereus* in the Anthony et al. study may be due to the production of noxious skin secretions in the salamander (Brodie

1977), or to differences in prey handling between centipedes and spiders. Centipedes kill large prey through venom injection before using their mandibles to macerate the prey item (Lewis 1981). Spiders often begin the digestion process externally via venom and regurgitated stomach enzymes (Foelix 1996). It is possible that centipedes are unable to chew through the sticky secretions of the salamander. The previous examples provide evidence that it is difficult to predict if IGP occurs among predators despite size asymmetries.

Research investigating how IGP affects trophic cascades in food webs is important because it shows how complex interactions among guild members at the consumer level can potentially alter the strength of the cascade at lower trophic levels (Holt and Polis 1997). Studies have suggested that IGP can dampen top-down effects in some systems (Finke and Denno 2002 & 2003, Halaj and Wise 2002). Finke and Denno (2002) found evidence in the laboratory, and field, that IGP can affect herbivore density differently depending on the structural complexity of the microhabitat. In simple habitats, aggressive behavior and IGP on mirids by spiders relaxed predation on herbivore leafhoppers. Complex habitats offered refuge for mirids, increasing the combined effects of both predators on herbivore suppression.

The goal of my dissertation work was to investigate the synergistic relationship between competition and IGP among forest floor predators as a driving force in detrital food webs using a dual field and laboratory approach. Because plethodontid salamanders are important regulators of detrital food webs and because other large invertebrate predators appear to be ecologically similar to salamanders, understanding how competition and predation affect interactions among these organisms seems a logical part of determining which mechanisms are most important in driving community structure. Furthermore, including various age classes of organisms is important because shifts in prey utilization throughout development can affect the types and strengths of interactions (Chen and Wise 1999).

For the past 60 years, or more, ecologists have been increasingly interested in how species interactions affect ecosystem function (e.g. Paine 1966), and in the past 35 years, studies that examine mechanisms of interaction such as competition, predator/prey interactions, mutualisms etc. have become more rigorous with regard to experimental design (Hurlbert 1984). There is a continuum with regard to the ways in which investigators have historically approached studies of species interactions involving salamanders and other predatory invertebrates. Along this continuum there is increasing strength with which inferences can be made (e.g. from simple laboratory studies to complex field studies) and decreasing control of variables that might affect the outcome. Therefore it is important to adopt a dual laboratory and field approach for investigating species interactions. Bruce (2008) compiled and summarized the extensive literature on IG interactions and population regulation in plethodontid salamanders. In Bruce's concluding remarks he reminds us that in 1973 Nelson Hairston, Sr. outlined a program of ecological research to investigate relationships within *Plethodon* and despite many experimental studies since then, we are still in need of long-term time series studies to evaluate population growth rates and density in conjunction with manipulative studies in open, unenclosed field plots. In my dissertation I have attempted to approach the ecological questions from multiple scales. I have conducted tightly controlled laboratory studies to examine behavioral interactions, and I have projects with high levels of realism in unrestricted field plots to address questions concerning species interactions and the presence of trophic cascades. The following briefly describes the structure of chapters one through four of my dissertation.

My dissertation is broken into four chapters that fall under the large umbrella that is predator interactions. I have attempted to study two somewhat more narrow topics contained within that broad research area. Chapters one and two focus on native and introduced centipedes because they have the potential to influence food web function and structure. I have examined how two species interact with one another and also how they interact with small, juvenile terrestrial salamanders. The interactions addressed are competition and predation. Chapters three and four are the focus of the bulk of my data collection and dissertation work. In these chapters, I present data from a 4.5 year predator removal field experiment designed to test for the presence of trophic cascades in which suppression of predator density indirectly affects basal trophic levels. The interactions among predators and their effects on macrodetritivores are presented in chapter three. The effects on the meso- and microfauna and flora are presented in chapter four.

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CHAPTER I

EDGE EFFECTS AND INTRAGUILD PREDATION IN NATIVE AND INTRODUCED CENTIPEDES: EVIDENCE FROM THE FIELD AND LABORATORY MICROCOSMS

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ABSTRACT

Human alteration of habitat has increased the proportion of forest edge in areas of previously continuous forest. This edge habitat facilitates invasion of exotic species into remaining fragments. The ability of native species to resist invasion varies and may depend on intrinsic variables such as dispersal and reproductive rates as well as external factors such as rate of habitat change and the density of populations of introduced species in edge habitat. We examined the distributional and competitive relationships of two members of the class Chilopoda, *Scolopocryptops sexspinosus*, a centipede native to the eastern US, and *Lithobius forficatus*, an exotic centipede introduced from Europe. We found that *L. forficatus* was most abundant in edge habitat and *S. sexspinosus* was most abundant in the interior habitat at our field sites. Although *L. forficatus* was present in habitat interiors at 11 of 12 sites, there was no correlation between fragment size and numbers of *L. forficatus* in interior habitat. The native centipede was rarely found occupying fragment edges. We used laboratory microcosms to examine potential competitive interactions and to indirectly assess prey preferences of the two species. In microcosms both species consumed similar prey, but the native centipede, *Scolopocryptops sexspinosus*, acted as an intraguild predator on the introduced centipede. Native centipedes were competitively superior in both intra- and interspecific pairings.

Our results suggest that intraguild predation may aid native centipedes in resisting invasion of introduced centipedes from edge habitat.

INTRODUCTION

Two major threats to global biodiversity are human induced habitat alteration and the introduction of non-native species. The most obvious negative consequences of habitat alteration occur when certain habitats required of species are destroyed and those species are then lost from a community. Biodiversity also suffers when non-native species expand their ranges and populations at the expense of native species (Gurnell et al 2004; Rooney et al 2004) resulting in homogenization or the replacement of native species by invasive exotics (McKinney and Lockwood 1999; Olden et al 2004). Habitat alteration and the introduction of non-native species have the potential to act synergistically because habitat alteration often favors the growth and spread of non-native species. For example, a consequence of habitat alteration in forested areas is the production of edge habitats along areas of cleared forest (Didham et al 1996). As forest is cleared for agriculture and development, habitat is created along the forest edge, and this type of disturbed habitat supports the spread of introduced species (Laurance and Yensen 1991; Hill and Curran 2001).

Once introduced species become established, they have the potential to interact with native species in a number of different ways. They may compete directly with native species for contested resources. For example, in the Eastern United States, the Old World paper wasp, *Polistes dominulus*, is displacing the native *P. fuscatus* through exploitive competition (Gamboa et al 2002; Gamboa et al 2004). The introduced common house gecko, *Hemidactylus frenatus*, is displacing native gekkonids on many tropical islands through a combination of exploitation and interference competition (Case et al 1994; Petren and Case 1998). Intraguild predation, the killing and eating of prey species by a predator that can also utilize the resources of those prey (Polis et al 1989), is also likely to play a role in some of these interactions. In laboratory trials, adults of *Hemidactylus frenatus* preyed upon juveniles of a native competitor (Bolger and Case 1992) and the well documented displacement of the green anole (*Anolis carolinensis*) by the introduced brown anole (*Anolis sagrei*) in the southeastern United States may be mediated by predation of juvenile green anoles by adult brown anoles (Gerber and Echternacht 2000). Recent studies have focused on the role that invasive species play in forest floor ecosystems. For example, introduced earthworms have been shown to influence colonization rates by mycorrhizal fungi (Lawrence et al 2003) and are associated with the decline of a rare fern (Gundale 2002). Introduced detritivores such as isopods (Kalisz and Powell 2004) and millipedes (Griffin and Bull 1995) have successfully invaded forest ecosystems, but the long term effects of these invasions remain unclear. The introduction of predators, such as the terrestrial flatworm *Bipalium adventitium*, also illustrates how exotic species may alter food webs. *Biplaium* is a voracious earthworm predator that is apparently distasteful to and is avoided by vertebrate predators (Ducey et al 1999). It has the potential to exert top-down forces on detrital food webs by reducing numbers of earthworms which could affect the rate of soil formation and leaf litter decomposition. Introduced predators, especially those that occupy the upper levels of trophic pyramids, have the greatest potential to alter food web structure by out-competing native predators (Vitousek 1990).

Centipedes are top predators in terrestrial detrital food webs. They have large biomasses (Lewis 1981), are generalist predators, and are potentially important in determining community composition of the detrital macro- and mesofauna. Several species of centipedes are of European origin (Williams and Hefner 1928; Shelley 2002) and one introduced species, *Lithobius forficatus,* are often associated with areas of human impact (Lee 1980) and are found under debris in edge habitats (Auerbach 1951).

We examined the interactions between *L. forficatus* and a native species of centipede, *Scolopocryptops sexspinosus* through a three-part experiment. Field data were collected on distribution and abundance of these two species in forest edge and forest interior habitats. We hypothesized that the introduced centipede, *L. forficatus*, would be more abundant at the forest edge than in the interior of the fragments because colonization by exotic species is positively correlated with human disturbance. In a microcosm experiment, we used changes in centipede mass over a nine week period to assess the competitive ability of each species. We hypothesized that differences in competitive ability would be reflected in differential mass gain. Finally, we assessed the effects that each species had on diversity and abundance of leaf litter invertebrates in microcosms.

MATERIALS AND METHODS

Field distribution - edge habitat use

We collected field data 19 – 29 May 2003 from 12 forested sites in northeast Ohio (Fig. 1). Sites were visited in random order. Forest fragment boundaries were delineated on topographic maps using Terrain Navigator 5.0 and the geographic center of each fragment was identified and located using a GPS unit. One hundred cover objects (rocks, logs, and bark > 25 cm^2) were turned at each site, 50 along the fragment edge, and 50 in the fragment interior. We defined edge habitat as a 10 m deep strip at the forest edge and interior habitat as the area surrounding the fragment center in a 15 m radius. A total of 1200 cover objects were sampled. We recorded numbers of *Lithobius forficatus* and *Scolopocryptops sexspinosus* found beneath each cover object for both habitat types. Species identifications were determined using Shelley (2002) for *Scolopocryptops* and Williams and Hefner (1928) for *Lithobius*. We excluded small specimens of *Lithobius* from analysis because of the difficulty in keying these individuals to species. Specimens were collected, preserved and brought back to the lab for identification before being placed in the invertebrate collection at the Cleveland Museum of Natural History.

Comparisons of the abundance of *Scolopocryptops sexspinosus* in edge habitat versus interior were made using two-tailed, paired t-tests ($\alpha = 0.05$). Comparisons of the abundance of *Lithobius forficatus* in edge habitat versus interior were made using two-tailed, Wilcoxon signed-ranks test because the data failed to meet the assumptions of parametric statistics ($\alpha = 0.05$). We explored the relationship between fragment size and centipede abundance in forest interiors with multiple regression analysis (Zar 1999). Statistical analyses were done using SPSS for Windows, version 11.5.

Microcosms – change in centipede mass and effects on invertebrate communities

Adult centipedes (*Lithobius forficatus* and *Scolopocryptops sexspinosus*, N = 36 of each species) and leaf litter used in the microcosm experiment were collected on 29

September and 1 October 2003 from a forested site (Lake County) in northeastern Ohio (41° 35' 56" N; 81° 21' 22" W) and brought to the laboratory. We mixed leaf litter thoroughly by shaking and by transferring portions among two large garbage bags. Leaf litter was then used to create 46 microcosms (Ziploc® boxes, 20 cm long x 12 cm wide x 6 cm deep) containing 110 g wet mass of leaf litter. The leaf litter in microcosms approximated litter depth in the field. After every fourth microcosm was created, a litter sample was set aside to assess initial (pre-experiment) litter invertebrate abundance and diversity (N = 12).

We measured and weighed centipedes prior to placing them in microcosms (mean mass and length of *Lithobius* = 0.100 g, SE = 0.006 and 23.41 mm, SE = 0.478, mean mass and length of *Scolopocryptops* = 0.228 g, SE = 0.007 and 40.77 mm, SE = 0.568). Size asymmetries were reduced by randomly pairing centipedes within three size classes to lessen the potential competitive advantage of larger size. Centipede pairs were then assigned to one of three experimental treatment microcosms. Treatment one consisted of boxes with two individuals of *Lithobius* (intraspecific pairs, L/L, N = 12). Treatment two consisted of boxes with one *Scolopocryptops* and one *Lithobius* (interspecific pairs, S/L, N = 12). Treatment three consisted of boxes with two individuals of *Scolopocryptops* (intraspecific pairs, S/S, N = 12). The fourth treatment was a control to assess litter abundance and diversity in the absence of centipede predators (post-experiment, N = 12). Microcosms were maintained at 10° C ± 1° C on a 12-h light/12-h dark photoperiod.

Contents of all microcosms were emptied weekly into a large stainless steel tray and hand sifted until the two experimental centipedes were located. Controls were treated similarly to experimental treatments. Mass was recorded for each centipede in experimental treatments and all contents (litter, inverts etc.) were placed back into the microcosms. Each microcosm was dampened with spring water (approximately 1.5 ml) before being placed back in the environmental chambers. Microcosms were moved each week among rack positions within the chamber to reduce positional effects. The experiment ran nine weeks (1 October - 28 November).

Focal centipedes were removed from all microcosms and invertebrates were extracted from the microcosm litter at the end of the nine week experiment to examine the effect of the two centipede species on the invertebrate community and to indirectly assess any differences in the diets of *L. forficatus* and *S. sexspinosus*. Invertebrates were separated from leaf litter by Berlese extraction into 70% ethanol. Berlese funnels were run for four days and invertebrates were separated from ethanol by pouring flask contents over paper coffee filters. Invertebrates were counted and identified to order. Invertebrates were not extracted from litter in treatment two (interspecific pairs) because of a high occurrence of predation on *L. forficatus* reduced the sample size.

We used changes in centipede body mass to determine if one species was a better competitor in microcosms than the other. We reasoned that in a closed system with limited resource availability, individuals would be more likely to lose mass when compared with competitive dominants. To assess whether a species was a stronger intra- or interspecific competitor in laboratory microcosms, we compared the frequency of individuals that lost weight in intraspecific pairings to the frequency that lost weight in interspecific treatments. Here, we predicted that competitive dominants would be less likely to lose mass in interspecific trials. To assess the intensity of competition within intraspecific trials we recorded instances where centipedes gained mass at the expense of their box mate and compared these frequencies across intraspecific treatments. We predicted that in competitively dominant species intense competition would prevent both individuals from gaining mass. We used two-tailed chi-square tests for these comparisons.

We analyzed the effect of intraguild predation on mass gain using a one–tailed, Mann–Whitney U test where we compared the change in mass of *Scolopocryptops* that ate *Lithobius* (N = 7) to a random sample of non–predatory *Scolopocryptops* (N = 7) in the weeks that a *Lithobius* was consumed. To directly assess the effect of intraguild predation on mass gain, we compared mass of individual *Scolopocryptops* before and after eating box-mates (*Lithobius*) in interspecific treatments using a one-tailed, Wilcoxon signed ranks test. We used one-tailed tests for these analyses because we predicted that intraguild predators would gain rather than lose weight.

Pre- (N = 12) versus post-experimental controls (N = 9; three replicates were lost due to experimenter error) were compared to quantify changes in the invertebrate community over the nine week period in the absence of adult centipedes. We compared total numbers of each taxon using two-tailed Mann-Whitney U tests. A three way comparison of the abundance of common taxa was conducted between post-experimental control, and the two intraspecific treatments (L/L and S/S) using a Kruskal Wallis test. Taxa selected for analysis were those that occurred frequently enough in samples that there were relatively few zero cases. No Berlese funnel extractions were conducted on the interspecific treatment (S/L) due to intraguild predation and a resulting small sample size. All statistical analyses were conducted with SPSS for Windows, version 11.5 with alpha set at 0.05.

RESULTS

Field distribution - edge habitat use

Mean numbers of *Lithobius forficatus* differed significantly in interior compared to edge habitats (Table 1). Sixty-four *L. forficatus* were located in edge habitat and only 33 in fragment interiors. *Scolopocryptops sexspinosus* was found in fragment interiors significantly more often than in edge habitat. Twenty-three *S. sexspinosus* were collected from forest interiors and only four from fragment edges. There was no relationship between fragment size and numbers of either species in fragment interiors (Fig. 2; *L. forficatus*, $R^2 = 0.024$, $P = 0.633$; *S. sexspinosus*, $R^2 = 0.0037$, $P = 0.85$).

Microcosms – change in centipede mass and effects on invertebrate communities

The mean mass of *S. sexspinosus* remained consistently higher in the interspecific treatment compared to the intraspecific treatment (Fig 3a). For *Lithobius*, the reverse was true (Fig. 3b). By week eight, 27% of surviving *Scolopocryptops* lost mass when paired with *Lithobius* (Fig. 4a) and 52% lost mass when paired with conspecifics (Fig. 4b). However, treatment had no statistically significant effect on mass lost by *Scolopocryptops* ($X^2 = 1.30$; df = 1; $P = 0.3$). In contrast, significantly more *Lithobius* lost mass when paired with *Scolopocryptops* than when paired with conspecifics ($X^2 = 6.67$; df = 1; $P < 0.025$). By week eight, 67% of surviving *Lithobius* lost mass when paired with *Scolopocryptops* (Fig. 4c) but only 14% lost mass when paired with conspecifics (Fig 4d).

In intraspecific pairings, we recorded instances where paired centipedes either gained mass, lost mass, or one gained and one lost mass. In 6 of 9 surviving pairs of *Lithobius,* both centipedes gained mass or showed no change and in only three cases did a *Lithobius* gain mass while its box mate lost mass ($X^2 = 6.0$; df = 2; P = 0.05). However, in 8 of 11 pairs of *Scolopocryptops*, one centipede gained mass while its box mate lost mass and in only one instance did both centipedes gain mass ($X^2 = 6.26$; df = 2; P = 0.04).

Mortality rates differed by treatment ($X^2 = 22.89$; $P < 0.001$; week 5); the highest rates were observed in interspecific pairings. In interspecific trials, seven of 12 *Lithobius* were killed and consumed by *Scolopocryptops*; a single *Scolopocryptops* died but was not consumed by the *Lithobius* with which it was paired. Individuals of *Scolopocryptops* were significantly heavier (mean increase of 0.017 g or 6.8%) the week following consumption of *Lithobius* (n = 7; paired Wilcoxon signed ranks test; P = 0.018) and *Scolopocryptops* that ate *Lithobius* (n = 7) tended to gain more mass than those that did not (n = 5; Mann-Whitney U test; P = 0.073). We assumed that mortality was due to predation because no deaths occurred for 10 weeks post-experiment when centipedes were held separately.

Eighteen common taxa were identified in the four treatments and a total of 10148 invertebrates were counted (Table 2). Regardless of treatment, microcosms were dominated numerically by mites, Collembola, and Coleoptera (Table 2). We compared numbers of invertebrates in leaf litter at the beginning of the experiment (pre-control) to the numbers at the end of the experiment (post-control) in the absence of adult centipede predators. At the end of the nine week experiment there were fewer mites (Mann-Whitney U test, P = 0.011) and fewer larval beetles (P = 0.042) in post-control microcosms compared to pre-controls (Table 2). Remaining comparisons were made between the post-control and centipede treatments. We found significantly more small centipedes and spiders in controls than in either experimental treatment (Figs. 5a and 5b). Similar numbers of pseudoscorpions were found in control and *Lithobius* treatments, but there were significantly fewer pseudoscorpions in *Scolopocryptops* treatments compared to control and *Lithobius* treatments (Fig. 5c).

DISCUSSION

Land use by humans in recent decades has resulted in an increase in forest fragmentation. One result of such fragmentation is an increase in edge habitat that often influences the distribution and abundance of species (Didham et al 1996). Rapid anthropogenic habitat alteration has the potential to cause a reversal in the competitive advantage that previously well adapted native species have over non-native species, and in extreme cases can drive native species to extinction (Petren and Case 1998; Byers 2002). We used two common species of forest dwelling centipedes, one native and one introduced by humans, to examine the effects of forest edge on species interactions.

At our field sites, *Lithobius* was more abundant in edge habitat than interior habitat and was 16 times more abundant in edge habitat than was *Scolopocryptops sexspinosus*. Even within forest interiors, *Lithobius* outnumbered *Scolopocryptops,* but to a much lesser degree. Our findings are consistent with Auerbach (1951) who noted that *L.*

forficatus is associated with areas of human disturbance, and with the findings of Summers and Uetz (1979) who found *L. forficatus* to be most abundant in clear-cut habitats. Similarly, in a survey of North American woodland centipedes by Lee (1980), *L. forficatus* was only found along the edge habitat created by road cuts. Likewise, in German forests, the abundance of *L. forficatus* decreased with increased distance to forest edges (Frund et al 1997). In contrast to the distribution of *L. forficatus*, we found the native centipede, *Scolopocryptops sexspinosus*, to be most abundant in forest interiors and rarely occupying microhabitats in forest edge. Most workers have described *S. sexspinosus* as a deciduous forest or moist pine forest species (Lee 1980; Shelley 2002) but we know of no other studies that have established its lack of abundance in edge habitat. There are several possible explanations for the observed distributions of *L. forficatus* and *S. sexspinosus* at our field sites. Nonrandom spatial patterns can result from differences in microhabitat use (Lee 1980, Blackburn et al 2002), competition for contested resources (Hairston 1980; Blackburn et al 2002, Hickerson et al 2004), predator avoidance (Murray et al 2004), and intraguild predation (Suutari 2004). Our use of microcosms allowed us to indirectly assess use of shared resources and the potential role of intraguild predation in interactions of these two species.

In our microcosms, 7 of 12 *L. forficatus* were preyed on by *S. sexspinosus* in interspecific pairings. Thus we view *S. sexspinosus* as an intraguild predator of *L. forficatus*. Predation on guild members is a potentially costly behavior because of the risk of physical injury resulting from capturing and subduing prey that are similar in size and fighting ability. An additional cost may be incurred by transfer of pathogens to intraguild predators that prey on phylogenetically similar guild members (Pfennig 2000). These fitness costs are similar to those incurred by cannibalistic species (Dawkins 1976; Polis 1981; Elgar and Crespi 1992; Pfennig et al 1998). One way these costs can be offset is through the immediate nutritional benefit of consumption (Polis et al 1989). Although we interpret the observed increase in mass of *S. sexspinosus* that preyed on *L. forficatus* as an example of a direct benefit of intraguild predation, it is not clear if the behavior is adaptive in this context. *Lithobius forficatus* is a relatively new introduction to North America so there may be no reason to expect populations of *Scolopocryptops* to respond adaptively. Alternatively, native species of *Lithobius* have probably coexisted with *Scolopocryptops* over long periods. If *Scolopocryptops* responds similarly to *L. forficatus* as it does to native *Lithobius* then the observed behavior may be an example of an exaptation (sensu Gould and Vrba 1982).

In a recent study, Snyder et al (2004) examined interactions between native and introduced species of ladybird beetles (Coccinelidae) and found that in laboratory microcosms, intraguild predation played a significant role in competitive outcomes. In contrast with our results, native ladybird beetles were at a significant disadvantage, as both larvae and adults, in interactions with introduced species. The reported declines in native ladybird abundance (Day et al 1994; Brown and Miller 1998) may be mediated by intraguild predation in these species (Obrycki et al 1998). Not all introduced species cause declines in their native counterparts. For example Bolger et al (2000) found that native spiders and carabid beetles increased in abundance with increasing fragment age, despite increases in exotic species in these habitat fragments. Our results were similar in that native centipedes did not appear to decline with decreasing fragment size. Instead, we found that *Scolopocryptops* coexists with *Lithobius* in forest interiors.

Holt and Polis (1997) suggest that intraguild predators are more likely to coexist if the intraguild prey (*L. forficatus* in our study) is a superior exploitative competitor for shared prey resources. Thus in our system we might expect coexistence only if *S. sexspinosus* (the intraguild predator) is an inferior exploitative competitor. Otherwise theory would predict the eventual competitive exclusion of *L. forficatus* through intraguild predation and competition. We have only indirect evidence for shared prey from our microcosm study. Centipedes, regardless of species, consumed smaller predators present in their microcosms as indicated by a significant reduction in spiders and small centipedes. Additionally, no statistical differences in species composition of prey remaining in microcosms were detected, with the exception of a reduction in the number of pseudoscorpions in the *Scolopocryptops* treatment. Thus centipedes in our microcosms appear to be entering into competition for limited resources. However, in contrast with the predictions of Holt and Polis (1997), interactions that occurred within microcosms suggest that *S. sexspinosus* is a stronger competitor (both intra- and interspecifically) than is *L. forficatus*. In our study, *L. forficatus* lost mass when paired with *S. sexspinosus* and more *S. sexspinosus* lost mass when paired with conspecifics than when paired with *L. forficatus*.

In forest fragments, *Lithobius* is not confined to edge habitat, though we did find significantly fewer *Lithobius* in fragment interiors. Thus, *Lithobius* apparently can coexist with the competitively dominant *Scolopocryptops,* despite serving as intraguild prey for this species. Temperate forest floor centipedes have historically been thought of as predators on other small soil dwelling invertebrates and insects, but Lewis (1965) found that plant material in the form of dead leaf fragments, fungal hyphae, rootlets and spores made up approximately 50% of the diet of *L. forficatus* during the winter and spring months. The remaining gut contents consisted of common arthropods such as aphids, Collembola, and mites. We hypothesize that access to resources at lower trophic levels by *Lithobius* increases the likelihood of coexistence between it and its intraguild predator through reduction in competition for detrital mesofauna.

In edge habitat, *Lithobius* was sixteen times more abundant than was *Scolopocryptops*, yet *Scolopocryptops* preys on *Lithobius* and appears to be competitively dominant in laboratory microcosms. Edge habitat can promote the proliferation of shade intolerant plant species, alter microclimate, light regimes, moisture levels and facilitate exotic species invasions (Laurance and Yensen 1991; Hill and Curran 2001). Additionally, exotic species can substantially change aspects of their environment (e.g. soil development, nutrient cycling, hydrology, and primary or secondary productivity) and may intensify the effects of other invaders causing ecosystem level changes (Vitousek and Walker 1989; Vitousek 1990). Thus, edge habitat can change so drastically as to become inhospitable to native species, while becoming ideal habitat for introduced species. Indeed, we found populations of *Lithobius* in this altered habitat to be 2 – 3 times as dense as *Scolopocyptops* populations in forest interiors. Janzen (1983) described how influx of weedy species from fragment edges and the surrounding habitat could result in local extinction of competitively dominant species in fragment interiors. Though we found no evidence for such an effect in our study, the failure to find a significant relationship between fragment size and abundance for introduced centipedes in interior habitat suggests that *Lithobius* can disperse from edge habitat where it is abundant. We hypothesize that intraguild predation by *Scolopocryptops* serves to maintain its competitive footing in forest interiors despite continual influx of *Lithobius* from the surrounding edge habitat.

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Figure Legend

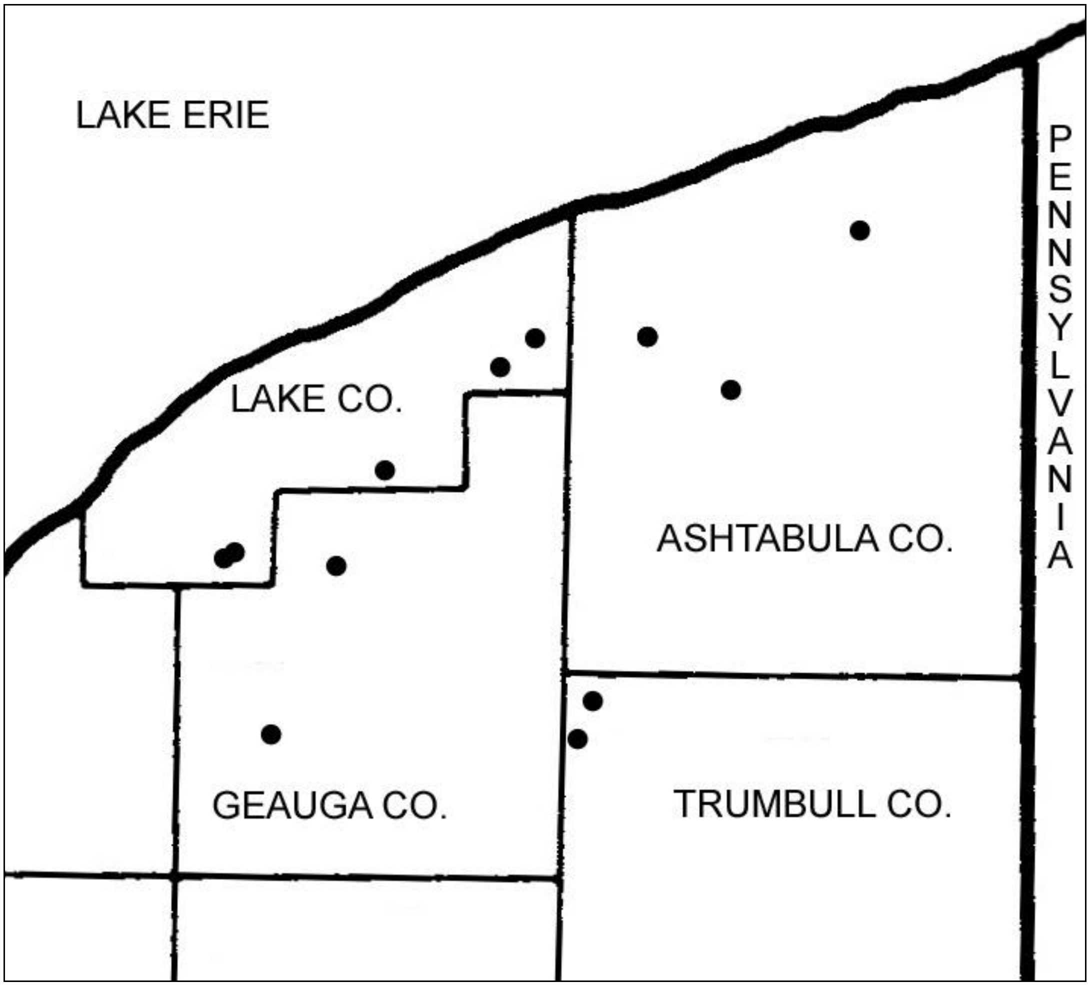
Figure I - 1. Spatial distribution of the 12 forested field sites in northeastern Ohio. Sites were visited 19 – 29 May 2003 in random order.

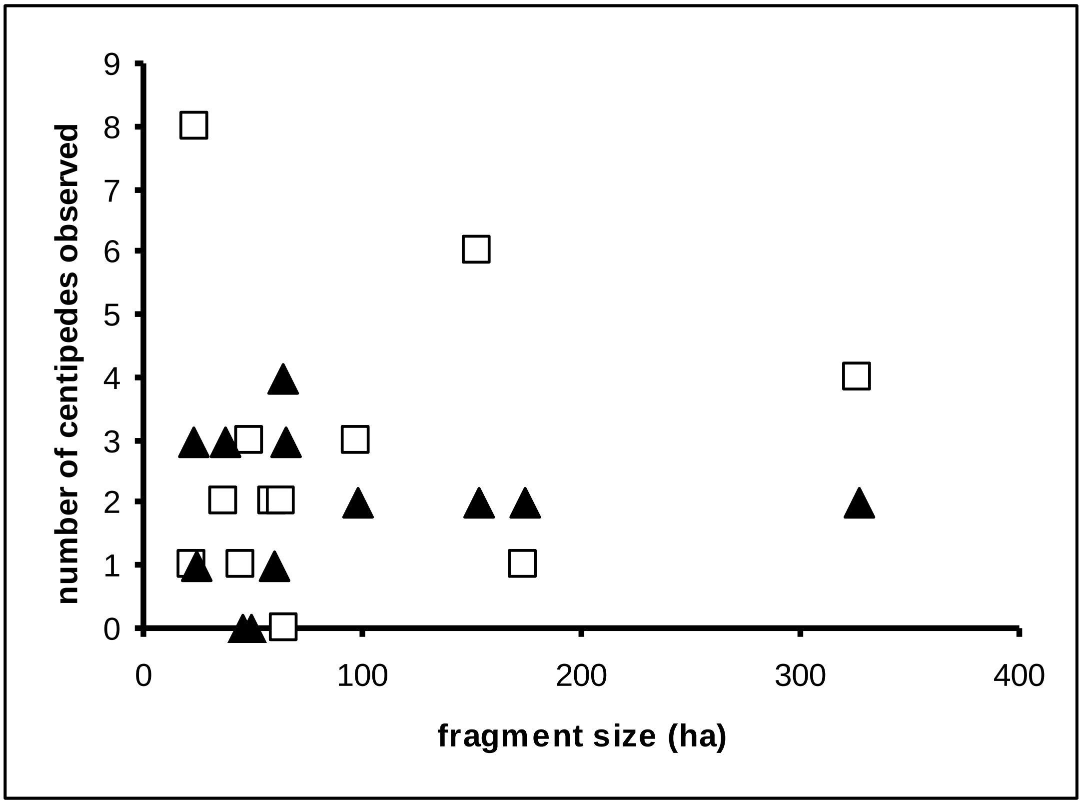
Figure I - 2. Numbers of centipedes observed in forests fragments of varying size. Centipedes (*Lithobius* - squares and *Scolopocryptops* - triangles) were observed in forest interiors during constrained searches. No significant effect of forest fragment size on abundance of either species was detected.

Figure I - 3. Mean mass of *Scolopocryptops* (a) and *Lithobius* (b) centipedes in intraspecific (squares) and interspecific (circles) pairings. Bars indicate SE. Centipedes were paired in microcosms containing leaf litter. The centipedes were weighed weekly.

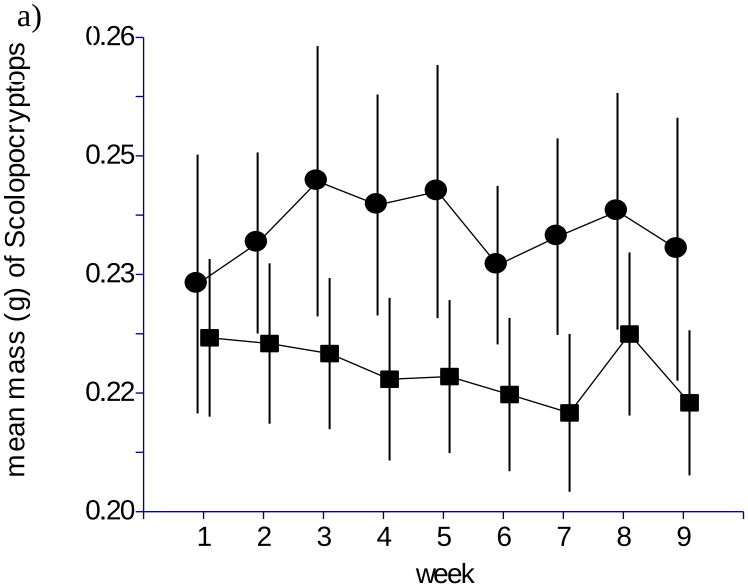
Figure I - 4. Equal probability plots illustrating mass gain and loss in the four microcosm treatments. Points above the line indicate a mass gain by the end of the experimental period. (a) S/L treatment where the majority of *Scolopocryptops* gained mass when paired with *Lithobius*. (b) S/S treatment where approximately equal numbers of *Scolopocryptops* lost and gained mass when paired with conspecifics. (c) L/S treatment where the majority of *Lithobius* lost mass when paired with *Scolopocryptops*. (d) L/L treatment where the majority of *Lithobius* gained mass when paired with conspecifics.

Figure I - 5. Mean numbers of invertebrates in the three treatments at the end of the nine week microcosm experiment. (a) Mean number of juveniles or smaller centipede species in each treatment. (b) Mean numbers of spiders in each treatment. (c) Mean number of pseudoscorpions in each treatment. Lowercase letters above bars indicate statistical differences at P = 0.05.


LAKE ERIE
LAKE CO.
ASHTABULA CO.
GEAUGA CO.
TRUMBULL CO.
PENNSYLVANIA

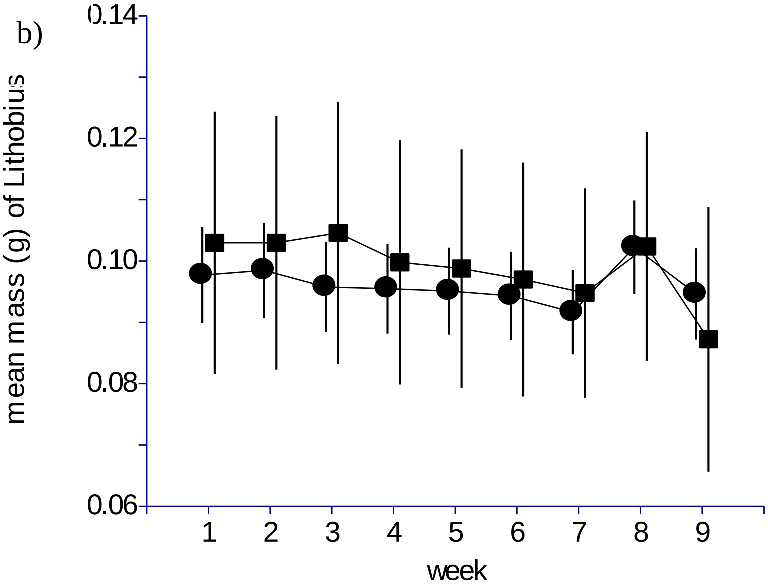

number of centipedes observed
0
1
2
3
4
5
6
7
8
9
0
100
200
300
400
fragment size (ha)

a)



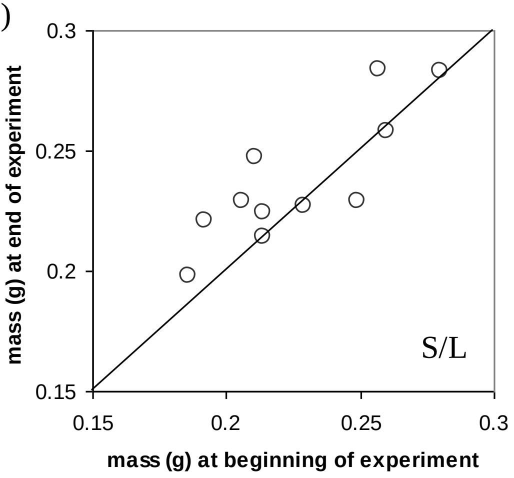
mean mass (g) of Scolopocryptops
0.26
0.25
0.23
0.22
0.20
1
2
3
4
5
6
7
8
9
week

b)

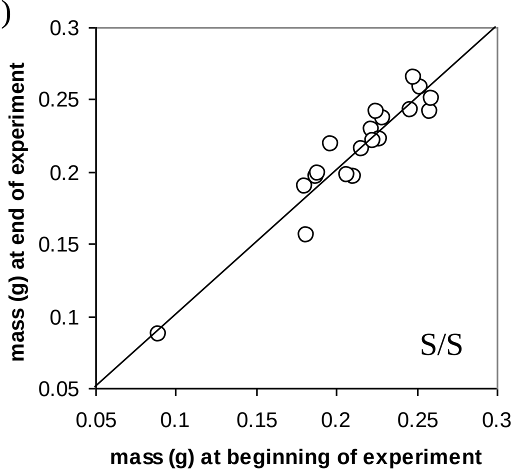


mean mass (g) of Lithobius
0.14
0.12
0.10
0.08
0.06
1
2
3
4
5
6
7
8
9
week

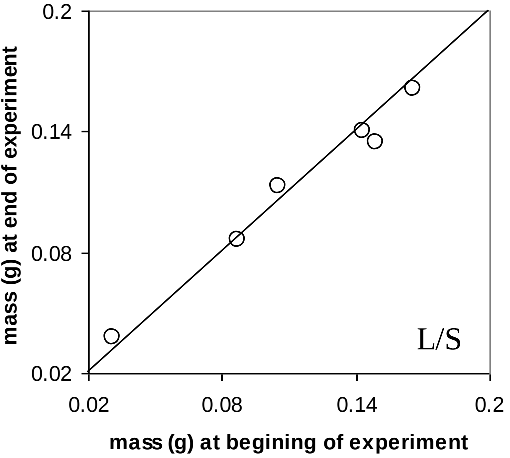
a)


0.3
0.25
0.2
0.15
0.15
0.2
0.25
0.3
mass (g) at end of experiment
mass (g) at beginning of experiment
S/L

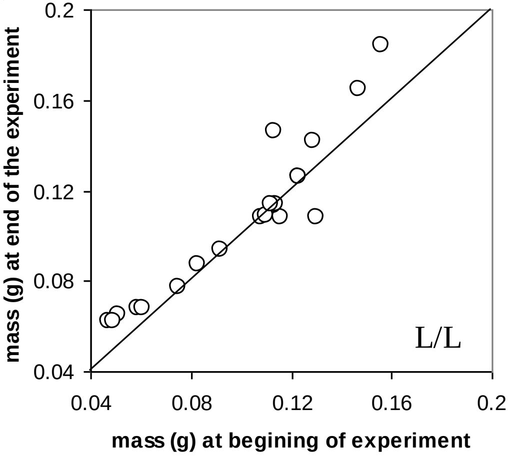
b)

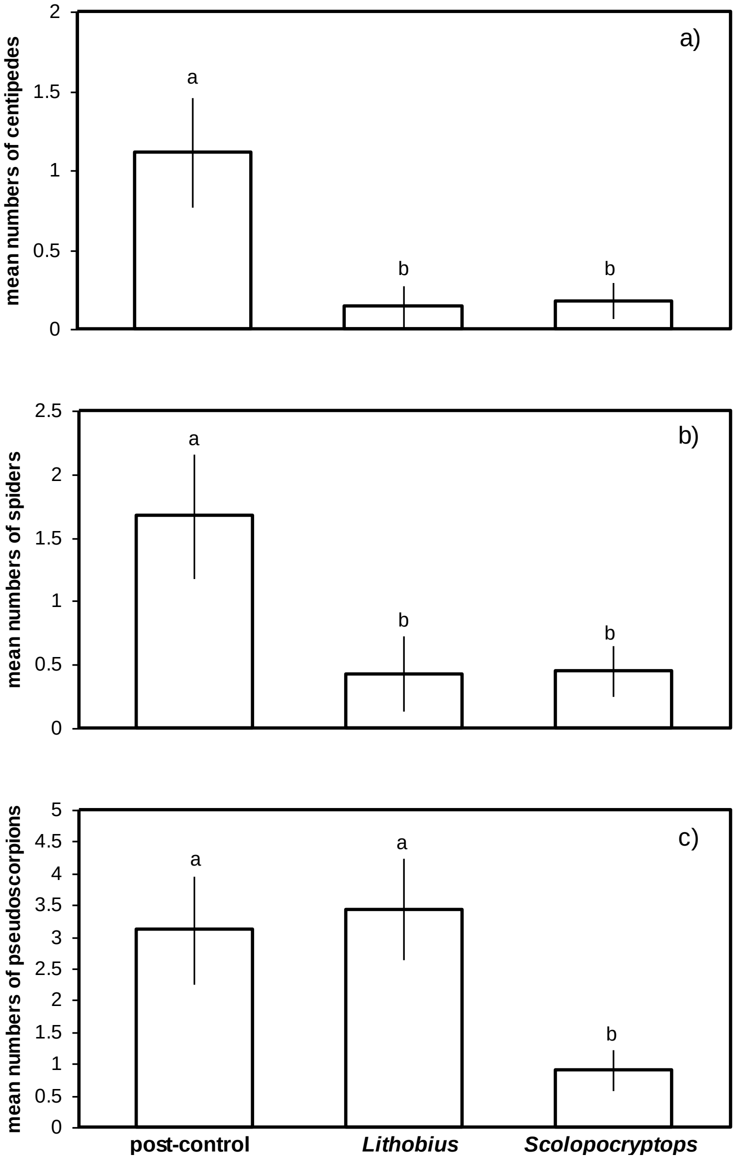

0.3
0.25
0.2
0.15
0.1
0.05
0.05
0.1
0.15
0.2
0.25
0.3
mass (g) at end of experiment
mass (g) at beginning of experiment
S/S

c)


0.2
0.14
0.08
0.02
0.02
0.08
0.14
0.2
mass (g) at end of experiment
mass (g) at begining of experiment
L/S

d)


0.2
0.16
0.12
0.08
0.04
0.04
0.08
0.12
0.16
0.2
mass (g) at end of the experiment
mass (g) at begining of experiment
L/L



a)
mean numbers of centipedes
0
0.5
1
1.5
2
a
b
b
b)
mean numbers of spiders
0
0.5
1
1.5
2
2.5
a
b
b
c)
mean numbers of pseudoscorpions
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
a
a
b
post-control
Lithobius
Scolopocryptops

Table I - 1. Mean (SE) numbers of centipedes observed in edge and interior habitats of the 12 forests sampled. Two-tailed tests.

	Interior	Edge	test statistic	P
Lithobius	2.75 (0.66)	5.33 (1.18)	2.10 (Z)	0.036
Scolopocryptops	1.92 (0.35)	0.33 (0.19)	4.18 (T)	0.002

Table I - 2. Mean (SE) numbers of invertebrates in control and experimental treatments.

	Control		Predator	
Taxa	Pre	Post	Lithobius	Scolopocryptops
Nematoda	0.42 (0.19)	0.33 (0.24)	0.14 (0.14)	0 (0)
Gastropoda	0.25 (0.18)	0.22 (0.15)	0 (0)	0.27 (0.14)
Oligochaeta	1.00 (0.56)	0.44 (0.34)	0 (0)	0 (0)
Isopods	2.67 (0.79)	3.67 (0.96)	1.14 (0.56)	2.09 (0.68)
Symphyla	0 (0)	0.11 (0.11)	0.14 (0.14)	0.27 (0.14)
Diplopoda	0.58 (0.37)	1.00 (0.37)	0.43 (0.30)	0.90 (0.28)
Chilopoda	0.75 (0.35)	1.11 (0.35)	0.14 (0.14)	0.18 (0.12)
Pseudoscorp	4.25 (0.71)	3.11 (0.86)	3.43 (0.81)	0.91 (0.34)
Acari	142.67 (18.99)	66.67 (19.19)	108.14 (20.39)	109.55 (21.23)

Table I - 2 (continued).

Aranae	2.25 (0.57)	1.67 (0.50)	0.43 (0.30)	0.45 (0.21)
Collembola	13.75 (3.58)	7.56 (1.56)	6.71 (1.61)	11.09 (3.99)
Hemiptera	0.58 (0.23)	0.67 (0.24)	0.57 (0.30)	0.36 (0.15)
Thysanoptera	0.67 (0.19)	1.11 (0.59)	0.43 (0.20)	0.09 (0.09)
Pscoptera	0.58 (0.23)	0 (0)	0 (0)	0 (0)
Coleoptera (adult)	6.25 (1.19)	5.11 (0.89)	5.86 (1.16)	5.00 (1.11)
Coleoptera (larv)	3.67 (0.72)	1.89 (0.92)	4.43 (1.69)	6.36 (2.26)
Hymenoptera	1.67 (0.54)	1.00 (0.33)	1.71 (0.47)	0.91 (0.46)
Diptera (adult)	0.42 (0.19)	0.22 (0.15)	0 (0)	0.36 (0.15)
Diptera (larv)	1.67 (0.53)	1.33 (0.69)	2.29 (0.68)	2.64 (1.29)
Lepidoptera (larv)	1.42 (0.38)	0.44 (0.24)	0.14 (0.14)	0.27 (0.14)
Unidentified larvae	0.67 (0.14)	1.67 (0.78)	1.14 (0.26)	0.73 (0.36)

CHAPTER II

RESPONSES OF JUVENILE TERRESTRIAL SALAMANDERS TO INTRODUCED AND NATIVE CENTIPEDES

A version of Chapter 2 previously published as:

Anthony, C.D., Hickerson, C. A. M. and Venesky, D. M. 2007. Responses of juvenile terrestrial salamanders to introduced (*Lithobius forficatus*) and native centipedes (*Scolopocryptops sexspinosus*). *Journal of Zoology* 271: 54–62.

ABSTRACT

When introduced species invade ecosystems, alterations in community structure can emerge from the competitive and predatory interactions that occur between introduced and native guild members. Because a number of recent studies have shown that large predatory invertebrates can both compete with, and prey on, small vertebrates and because introductions of non-native species may play a role in amphibian declines, the effects of introduced (*Lithobius forficatus*) and native (*Scolopocryptops sexspinosus*) centipedes on juveniles of the red-backed salamander (*Plethodon cinereus*) were examined. In laboratory arenas, juvenile salamanders exhibited submissive behavior in response to the odors of both species of centipede. There were no significant differences in salamander response to the two centipede odor treatments, but compared to controls, juveniles of *P. cinereus* spent significantly more time in escape and in a flattened submissive posture when presented with native centipede odors. Despite significant size differences between centipedes and juvenile salamanders, no predation of salamanders by either species of centipede occurred in any pairings. Juveniles exhibited more chemosensory behavior toward native centipedes and toward their odors and they exhibited marked reductions in aggressive posturing when centipedes were present. Field and laboratory data suggest that juveniles of *P. cinereus* and centipedes were negatively associated. In laboratory trials, the native centipede excluded juvenile salamanders from cover objects and we found fewer instances of co-occurrence in the field than expected. These studies are the first to examine the behavioral interactions between juveniles of *P.*

cinereus and invertebrate predators, one introduced and one native, of eastern deciduous forest-floor food webs.

INTRODUCTION

Plethodontid salamanders are important components of the forest-floor food webs of eastern temperate forests of North America. Of particular importance are small-bodied and numerically abundant species, such as the red-backed salamander (*Plethodon cinereus*), because their small body mass (usually less than 1 gram) allows them to exploit prey species that are not suitable prey for other vertebrates (Pough, 1983). This ability, combined with exceptionally large population size and biomass (Burton & Likens, 1975), makes them important regulators of below ground food webs. Indeed, a growing body of evidence suggests that the presence or absence of salamanders in forest-floor food webs can have far reaching effects on invertebrate abundance and leaf litter decomposition (Wyman, 1998; Walton & Steckler, 2005).

Given the important roles that amphibians play in ecosystem function, ecologists have expressed concern over the global declines of these species (Alford & Richards, 1999; Houlahan, Findlay & Schmidt, 2000; Lannoo, 2005). A number of potential causes of the global amphibian decline have been identified including climate change, emerging disease, habitat loss, ultra-violet radiation, environmental toxins, and introduced species (see cites in Green, 2003). Recent evidence (Highton, 2005) indicates that salamanders of the genus *Plethodon* are experiencing declines throughout the Eastern United States but it is unclear which, if any, of the above causes play a role in the decline of populations of this genus. Although recent studies have examined the effects of introduced predators on aquatic amphibian populations (Kats & Ferrer, 2003), few researchers have examined the effects of introduced species on terrestrial salamander populations (Ducey et al., 1999; Maerz et al., 2005).

Despite their perceived importance in detrital food webs and the potential negative effects that introduced predatory invertebrates have on terrestrial salamanders, we know little of the specific behavioral interactions that occur among these species. Forest dwelling centipedes and red-backed salamanders have similar food and habitat requirements and likely compete for resources such as prey and space on the forest-floor (Hickerson, Anthony & Wicknick, 2004). Cover objects, such as rocks and logs, provide isolated patches of moisture and associated prey during dry spells between periods of rainfall (Jaeger, 1981). Thus, when the forest-floor dries, salamanders may enter into competition with other guild members. Larger centipedes, such as the two species examined in this study, may act as intraguild predators on one another. In laboratory microcosms, *Scolopocryptops* preyed on *Lithobius* (Hickerson, Anthony & Walton, 2005) and large individuals of either species have the potential to act as intraguild predators on juvenile red-backed salamanders.

Lithobius forficatus, a non-native centipede introduced to the Eastern United States from Europe in the 1800s, is associated with human disturbance (Lee 1980) but has invaded some forested areas (Frund, Balkenhol & Ruszkowski, 1997; Hickerson, et al., 2005). This species reaches 26 mm total length (Williams & Heffner, 1928), approximately twice the size of a neonate red-backed salamander. *Scolopocryptops sexspinosus* is a native resident of forest-floor habitats in the Eastern United States and reaches 69 mm total length (Shelley, 2002). Adult red-backed salamanders in the region of this study average 40.5 mm snout to vent length (SVL)(Pfingston & Downs, 1989). Both *Lithobius* and *Scolopocryptops* are venomous and are capable of delivering painful bites (Williams & Hefner, 1928). Because red-backed salamanders are territorial (Mathis et al., 1995), simply fleeing from dangerous competitors may not be an adaptive solution to an encounter. Instead, territorial owners are expected to use behavioural displays in an attempt to expel invertebrate intruders. Previous studies, using *adult* red-backed salamanders, support this hypothesis. In laboratory trials, male red-backed salamanders exhibit aggressive behaviour toward carabid beetles (Gall, Anthony & Wicknick, 2003) and centipedes (Hickerson et al., 2004). We are aware of only two studies that have addressed how juvenile salamanders interact with predatory invertebrates (Ovaska & Smith, 1988; Rubbo et al., 2003) and it is unknown how juveniles of red-backed salamanders (*P. cinereus*) interact with native and introduced predatory invertebrates. In this study we addressed the following questions: 1) Do adult centipedes exclude juveniles of *P. cinereus* from cover objects in the field or in the lab? 2) Do juveniles of *P. cinereus* respond differently to the odors of, or to laboratory encounters with, either species of centipede? 3) Do adult centipedes of either species prey on juveniles of *P. cinereus*?

MATERIALS AND METHODS

Co-occurrence under artificial cover objects (ACOs) in the field

During the months of April through December 2004, we examined the degree of co-occurrence of juvenile salamanders and centipedes under artificial cover objects (ACOs) in the field. We sampled an array of 72 ACOs for the presence of salamanders and centipedes. The array was constructed as part of a larger study examining the interactions of salamanders and large invertebrates in forest-floor food webs. The entire array consisted of 288 ACOs and included removal treatments that were not sampled for this study. The ACO array was assembled in early April 2004 and sampling began two weeks after ACOs were laid down. We used large (30.5 x 30.5 cm) ceramic tiles as ACOs and visited the site approximately every two weeks through early December of 2004. We turned each cover object and recorded the numbers of adults (> 32 mm SVL) and juveniles (< 22 mm SVL) of *Plethodon cinereus*, the numbers of adults of *Scolopocryptops sexspinosus* (> 35 mm), and the numbers of lithobiomorph centipedes over 15 mm total length. We determined whether cover objects yielded salamanders and/or centipedes at any time during the study and assessed the degree of co-occurrence with chi-square tests of independence. For the analysis, we assigned each of the 72 cover objects to one of six categories based on whether salamanders, centipedes, or salamanders and centipedes were found under an ACO during any visit. Thus, if an ACO produced a centipede during visit one and a juvenile salamander during visit 12, it was designated as a shared cover object. This is a conservative approach that over estimates the degree of co-occurrence between salamanders and centipedes. In generating the expected distribution, we assumed that the occurrence of each category type was equally probable. Thus the expected distribution was simply the number of ACOs examined divided by the number of categories used in each analysis.

General methods for laboratory trials

Specimens were collected from three adjacent counties in northeastern Ohio, USA. We collected juveniles of *Plethodon cinereus* in October of 2004, from mature beech maple forest in northern Summit County. Adults of *Lithobius forficatus* were collected from a residential area in Cuyahoga County and adults of *Scolopocryptops sexspinosus* were collected from a mature beech maple forest in Lake County. We housed salamanders and centipedes individually in plastic chambers (17 x 11 x 4.5 cm for salamanders; 24 x 16.5 x 6 cm for centipedes) on leaf litter under a natural photoperiod at 16.7 ± 1.1 C. All specimens were fed *Drosophila hydei ad libitum.* Centipedes were denied food for 4 days prior to testing. Salamanders and centipedes were weighed periodically throughout the study and no individuals lost mass during the study.

Odor discrimination

In December 2004, we examined the responses of juveniles of *P. cinereus* to the odors of adult centipedes. We exposed juveniles of *P. cinereus* (mean SVL ± SE = 20.1 ± 0.55 mm; mean mass ± SE = 0.143 ± 0.011 g, *n* = 15) to 3 substrate odors in separate trials. We used plastic petri dishes (1.5 cm x 14 cm dia) lined with 15 cm dia Ahlstrom® qualitative filter paper for our experimental arenas. Salamanders were carefully placed on damp circular filter paper on which an adult *Scolopocryptops,* an adult *Lithobius,* or no centipede had previously resided for 5 days. We allowed salamanders to freely interact with the substrate and any associated odors for 15 minutes. In experimental treatments (centipede odors), centipedes were removed from substrates less than 60 sec prior to exposure of the salamander to the substrate. We presented salamanders with odor treatments in a randomized block design where each salamander was exposed to each odor on a different day and equal numbers of salamanders were tested on each odor each day. No salamander was tested more than once in each 5-day period. To avoid temporal bias in the data, treatments and controls were evenly dispersed across test dates and observers (Hurlbert, 1984). During data collection, we made every effort to disguise the treatment type from data recorders.

We used the software package EVENT-PC (James C. Ha, University of Washington) to record the frequency and duration of the following behaviors of juveniles of *P. cinereus* when exposed to the 3 odor treatments. These behaviors were modified from Jaeger (1984) and Hickerson et al. (2004). Flattened (FLAT) – considered a submissive posture, the entire ventral surface of the body and the chin is in contact with the substrate. Front trunk raised (FTR) – considered a resting posture. All trunk raised (ATR) – considered an aggressive posture, the legs are extended such that the head, trunk and sometimes tail are lifted off of the substrate. Nose tapping the substrate (NTS) – considered an investigative behavior, contact of the nasolabial cirri to the substrate. Nose rubbing the substrate (NTR) – considered an investigative behavior, the snout is held to and sometimes rubbed on the substrate for several seconds at a time. Immobility (IMMOBILE) – considered an antipredator behavior. Escape behavior (ESCAPE) – defined as circling the periphery of the chamber while pressing the snout or body against the outer edge of the Petri dish. Sustained escape (SUST ESCAPE) – defined as the longest interval of escape per trial. Escape behavior can be considered either submissive (Wise & Jaeger, 1998) or as an antipredator behavior. Comparisons among treatments were made using 2-tailed paired *t* tests (where the data met the assumptions of parametric statistics) or Wilcoxon signed-ranks tests, a nonparametric equivalent. We reduced alpha to 0.025 because each data set was used twice in each analysis. We employed 1-tailed tests when analyzing time spent in escape because a previous study (Hickerson et al., 2004) indicated that adults of *P. cinereus* exhibited increases in escape when exposed to the odors of centipedes.

Behavioral interactions between salamanders and centipedes

In January and February 2005, we explored the potential for aggression and intraguild predation between juvenile salamanders and adult centipedes in laboratory arenas. We paired juveniles of *P. cinereus* (mean SVL = 19.3 ± 0.31 (SE) mm; mean mass = 0.116 ± 0.003 (SE) g, *n* = 30) with adults of *L. forficatus* (mean total length (TL) = 27.9 ± 0.45 (SE) mm; mean mass = 0.146 ± 0.007 (SE) g, *n* = 30) and with adults of *S. sexspinosus* (mean TL = 43.4 ± 0.56 (SE) mm; mean mass = 0.282 ± 0.010 (SE) g, *n* = 30) in separate trials. We minimized mass differences between paired animals by sorting animals by mass and then randomly pairing within each of 5 mass classes. In pairings, salamander SVL was always shorter than centipede TL ((mean ± SE) difference for *Lithobius/Plethodon* pairs = 8.6 ± 0.54 mm; for *Scolopocryptops/Plethodon* pairs = 23.9 ± 0.61 mm). Salamanders were always lighter in mass than *Scolopocryptops* ((mean ± SE) difference = 0.16 ± 0.01 g) and lighter than *Lithobius* in 25 of 30 trials ((mean ± SE) difference = 0.04 ± 0.007 g). We paired each salamander with a centipede of each species in random order. Eight to 10 days passed between pairings and equal numbers of salamanders were paired with *Lithobius* first and *Scolopocryptops* first. Centipedes were not used more than once in this experiment, but some of the salamanders used in the odor experiment were reused. We were careful not to pair salamanders with individual centipedes that they had experienced odors from in the previous experiment. To avoid temporal bias in the data, equal numbers of *Lithobius* and *Scolopocryptops* trials were run on each test day (Hurlbert, 1984). Observers collected data from approximately equal numbers of each trial type, but it was not possible to conduct these trials in a blind design because the species identity of the centipede was conspicuously apparent.

Salamanders and centipedes were tested in circular arenas (as described in the first experiment) on damp (unmarked) filter paper. We carefully placed each salamander and centipede (*Scolopocryptops* or *Lithobius*) into the arena and covered each animal with an opaque habituation dish (5.5 cm dia). After a 5 min acclimation period the dishes were lifted and the trial was started immediately after it was apparent that the salamander was aware of the presence of the centipede (indicated by the salamander turning its head toward the centipede, or the centipede moving across the salamander's forward field of vision).

We used the software package EVENT-PC to record frequency and duration of behaviors of salamanders and centipedes during each 15 min trial. For salamanders, we recorded the same behaviors as listed above in experiment 1 (FLAT, FTR, ATR, NTS, NTR, IMMOBILE, ESCAPE, SUST ESCAPE) and the following additional behaviors. Nose tapping the centipede (NTC) – contact of the nasolabial cirri to the centipede. (Hickerson et al., 2004). Move toward (MT) – salamander approaches the centipede in a direct path that would result in contact if the movement were to continue. Flipping (FLIP) – rapid twisting or writhing that carries the salamander away from the centipede in a salutatory manner (Brodie, 1977). Comparisons between the two treatments were made using 2-

tailed paired t-tests (where the data met the assumptions of parametric statistics) or Wilcoxon signed-ranks tests, a nonparametric equivalent.

Competition for artificial cover objects in the laboratory

In March 2005, we examined the frequency in which juveniles of *P. cinereus* co-occurred with adults of either centipede species under cover objects in experimental arenas. We used small (7.5 x 5.25 x 0.9 cm) ceramic tiles as cover objects. A 1 cm length of 0.6 cm dia surgical tubing was used as a shim to raise one short end of the tile. The tile and shim were placed on a single sheet of 15 cm dia filter paper in a 22 cm square experimental arena. We dampened the filter paper with 5 ml of spring water prior to introducing the animals. This provided three microhabitats within the arena: under cover and on damp filter paper (6.3% of chamber); on damp filter paper only (22 % of chamber); and on the dry surface of the chamber (71.7% of chamber). As in the previous experiment, salamanders and centipedes were randomly paired within size classes to minimize size differences. In pairings, salamander SVL was always shorter than centipede TL ((mean ± SE) difference for *Lithobius/Plethodon* pairs = 8.3 ± 0.32 mm); for *Scolopocryptops/Plethodon* pairs = 23.8 ± 0.58 mm). Salamanders were always lighter in mass than centipedes ((mean ± SE) difference = 0.16 ± 0.009 g) for *Scolopocryptops/Plethodon* pairs and 0.027 ± 0.003 g) for *Lithobius/Plethodon* pairs). Each salamander (*n* = 28) was randomly placed into each of 3 treatments: A control treatment where no centipede was present and two experimental treatments where a *Lithobius* or a *Scolopocryptops* was present. Salamanders were not tested more than once in any 4-day period and, although the same centipedes and salamanders from the previous experiment were used, salamanders were not re-paired with individual centipedes that they had interacted with in previous experiments.

We introduced a juvenile *P. cinereus* and either an adult *Lithobius* or an adult *Scolopocryptops* simultaneously into the arenas between 16:00 and 17:00. Salamanders and centipedes were allowed to interact until 10:30 the following day when we recorded the location of each animal within the chamber as well as the number and location of salamander fecal pellets. We predicted that, in the control treatment, salamanders would be free to use the cover object but that in the presence of centipedes they would be excluded from cover. For each treatment, the position of the salamander at the end of trial was scored as either under cover (+) or not under cover (-) and a sign test was employed to determine if salamander position was influenced by centipede presence. We used a G-test of independence to determine if fecal pellet location was influenced by centipede presence. Here we reasoned that salamanders would be less likely to deposit pellets under cover if they were excluded by centipedes from using cover objects. We employed 1-tailed tests where we had evidence from field data (this study) and from a laboratory pilot study on artificial cover object use that indicated that centipedes and juvenile salamanders were unlikely to share cover objects.

RESULTS

Co-occurrence under artificial cover objects (ACOs) in the field

We visited the field site 15 times from April through December 2004. Sixty-eight of the 72 ACOs produced either salamanders, centipedes or both at sometime during the study.

Thus the ACOs provided suitable cover. Individuals of *Plethodon cinereus* were active under cover objects during 13 of 15 visits and centipedes were found during 14 of 15 visits (Fig. 1). A juvenile salamander co-occurred at the same time with a centipede (a lithobiid) in only one instance during the 8-month sampling period. We placed each of the 72 ACOs into one of six categories based on whether salamanders and/or centipedes were present or absent during the sampling period. This way, potential bias from multiple sampling of the same individuals over time could not affect the sample size or which category an ACO was assigned to. Categories were defined as follows: (1) ACOs that yielded neither centipedes nor juvenile salamanders but did yield adult salamanders (n = 26 ACOs); (2) ACOs that yielded juvenile salamanders but no centipedes (n = 8 ACOs); (3) ACOs that yielded *S. sexspinosus* and juvenile salamanders (n = 1 ACO); (4) ACOs that yielded lithobiids and juvenile salamanders (n = 6 ACOs); (5) ACOs that yielded *Scolopocryptops* and no juvenile salamanders (n = 10 ACOs), and (6) ACOs that yielded lithobiids and no juvenile salamanders (n = 23 ACOs). Three ACOs that yielded neither centipedes nor salamanders of any species or size class were excluded from the analysis. We found that more cover objects housed adult salamanders, but not juveniles or centipedes, than expected (category one: Chi-square test; $\chi^2_5 = 40.0$, $P < 0.001$). When we partitioned these cover objects out from the analysis, we found significantly more cases of non-native centipedes occurring alone (category six: Chi-square test; $\chi^2_4 = 28.04$, $P < 0.001$). The native centipede never co-occurred with juvenile salamanders, but this result was not statistically significant (category three: Chi-square test; $\chi^2_3 = 7.16$, $P = 0.072$). We over estimated the degree of co-occurrence between juvenile salamanders and centipedes by combining independent observations of individuals throughout the duration of the experiment. We interpret the lack of co-occurrence of juvenile salamanders and centipedes under these conservative restrictions as evidence that these species are negatively associated on the forest-floor.

Odor discrimination experiment

We found little evidence that juveniles of *P. cinereus* detect substrate odors of the introduced centipede, *L. forficatus*. In no instances did salamanders behave differently on *Lithobius* substrates compared to controls (Table 1, Fig. 2). In contrast, when on *Scolopoctyptops* substrates juveniles of *P. cinereus* spent significantly more time in sustained escape behavior (Wilcoxon signed-ranks test; t = 2.11, n = 15, P = 0.011, one-tailed; Fig. 2b) and in FLAT (Wilcoxon signed-ranks test; t = 1.97, n = 15, P = 0.010; two-tailed; Fig. 2a) compared to control substrates. Salamanders tended to spend more time in ESCAPE (paired t-test; t_{14} = 2.01, P = 0.032, one-tailed; Fig. 2c) when on *Scolopocryptops* substrates, compared to controls, as well. No significant differences in salamander behavior were detected between centipede odor treatments (Table 1, Fig. 2).

Behavioral interactions between salamanders and centipedes

We observed no instances of intraguild predation in behavioral pairings. Centipedes occasionally chased and appeared to bite salamanders and in several cases salamanders bit centipedes, but no injuries were observed. In no cases were bites by any species held for extended periods and we have no evidence that bitten salamanders were envenomated by centipedes. When paired with centipedes, salamanders spent a large portion of the trial in escape behavior, usually climbing to the top edge of the Petri dish at some point during each trial. We did not observe this climbing behavior in the odor trials. Compared to the odor trials, salamanders spent approximately ten times as much time in escape behavior when centipedes were present and approximately one third as much time in the aggressive posture ATR when centipedes were present (Table 2). The submissive behavior FLAT was observed in only three trials. We detected few differences in salamander behavior between centipede treatments. Juveniles of *P. cinereus* spent significantly more time in NTR (Wilcoxon signed-ranks test; t = 2.17, $n = 30$, $P = 0.03$, two-tailed; Fig. 3a) and in ATR (Wilcoxon signed-ranks test; t = 2.45, $n = 30$, $P = 0.014$, two-tailed; Fig. 3b) when paired with native centipedes, but salamanders exhibited similar levels of other behaviors in both treatments (Table 2).

Competition for artificial cover objects in the laboratory

Scolopocryptops and *Lithobius* were found under cover in the experimental chambers in 100% and 82.14% of trials respectively. There was a weak, but significant effect of the presence of the native centipede on cover use by juvenile *P. cinereus*. When paired with *Scolopocryptops*, juvenile salamanders were significantly more likely to be found either out from under the cover object (14.3% on the filter paper; 7.1% in the dry portion of the chamber) or only partially under the cover object (21.4% of salamanders) (sign test; statistical $n = 14$, $P < 0.05$, one-tailed; Fig. 4) and there were significantly fewer pellets found under cover objects in this treatment compared to the control and *Lithobius* treatments (G-test of independence; G = 36.13, $n = 28$, $P < 0.001$; Fig. 5). No effect of introduced centipede presence on cover object use by salamanders was detected (sign test; statistical $n = 7$, $p > 0.5$, one-tailed). Despite the negative effect of centipede presence on cover object use by salamanders, by the end of the experiment salamanders shared cover objects with native and introduced centipedes in 60.7% and 53.5% of trials respectively. As in the behavioral pairings, we observed no evidence of intraguild predation between the species tested, even with the extended length (at least 17.5 h) of the trials.

DISCUSSION

In laboratory arenas, juvenile salamanders exhibited submissive behavior when exposed to the odors of both species of centipedes, but we detected no significant differences in their responses to centipede odor treatments. Juveniles of *Plethodon cinereus* spent significantly more time in escape behavior and in a flattened (submissive) posture when presented with native centipede odors. Inability to detect odors of introduced centipedes may be due to lack of an innate avoidance of non-native species or lack of exposure to non-native centipedes. At our field sites, the non-native species was rare compared to the native species, thus there may have been few chances for learned avoidance of introduced centipedes by juvenile salamanders. These results are consistent with those of Murray, Roth & Wirsing (2004) who argued that predator avoidance behavior tended to be learned, not innate, in several Western North American amphibian species. Alternatively, lithobiid centipedes may not pose a predatory threat to juveniles of *P. cinereus.*

Despite significant size and mass differences between centipedes and salamanders, we found no evidence of intraguild predation of juvenile salamanders by either species of centipede in any laboratory pairings. Previous studies pairing large wolf spiders

(*Gladicosa pulchra*) with juvenile spotted salamanders (*Ambystoma maculatum*) and with juvenile ground skinks (*Scincella lateralis*) reported significant predation on both vertebrate species (Rubbo et al., 2001; 2003). Our centipedes were treated similarly to the spiders used in the studies by Rubbo and colleagues. Centipedes were denied food prior to testing and they were paired with salamanders in small, structurally simple arenas. Lack of predation in our study could be a result of noxious skin secretions of the salamanders (Brodie, 1977) or differences in prey handling between spiders and centipedes. Centipedes kill large prey through venom injection before using their mandibles to macerate the prey item (Lewis, 1981). Spiders often begin the digestion process externally via venom and regurgitated stomach enzymes (Foelix, 1996). It is possible that spiders are able to effectively avoid adhesive and/or noxious skin secretions by breaking down the secretion prior to ingestion. Centipedes, however, may not prey on salamanders because they may be unable to insert their forcipules, or chew through the adhesive skin secretions of salamanders. Because our centipedes were denied food prior to testing, continued to feed and gain mass in the months following testing, and in one experiment were held with their potential vertebrate prey overnight, we are confident that these two species of centipede do not consume juvenile red-backed salamanders.

Intraguild predation (IGP) is an important behavioral attribute of food webs and it has the potential to add significant complexity to food web interactions. IGP can be categorized as symmetrical (looping, Polis, Myers & Holt, 1989), in which species A and B are mutual predators of one another, or asymmetrical in which species A always preys on species B. IGP in each of these categories can be influenced by ontogenetic changes in size and resulting vulnerability to predation. IGP can also be influenced by prey handling and antipredator behavior of IG predators and prey. It is often assumed that symmetrical IGP occurs as a result of ontogenetic reversal of predation, such that adults of species A eat juveniles of species B and adults of B eat juveniles of A (Polis et al., 1989). With regard to salamander and centipede interactions, it is further assumed that because both groups are generalist predators that experience large changes in size through ontogeny, and because centipedes are venomous, the likelihood of symmetrical IGP is high. However, we found no evidence of predation by adult centipedes on juvenile salamanders and we caution that researchers should not assume symmetrical IGP is occurring based simply on differences in body size and trophic membership. Ducey et al. (1999) reported similar results when examining trophic interactions between small terrestrial vertebrates (salamanders and small snakes) and an introduced predatory flatworm. Despite the larger size of predators used in the experiment, flatworms were rejected as suitable prey in most cases.

Juvenile salamanders exhibited increased ATR toward native centipedes and exhibited more chemosensory behavior toward native centipedes and their odors. The aggressive behavior ATR has been well studied in adult *Plethodon* (Mathis, 1995), and Hickerson et al. (2004) concluded that ATR functions as a threat display when exhibited by adults of *P. cinereus* toward native centipedes. The function of ATR in juvenile behavior is less clear. In our trials, the time spent in this behavior decreased drastically when centipedes were present compared to the odor trials where they were not. In the presence of centipedes, juvenile salamanders shifted from ATR to escape behavior or to immobility. If ATR in juveniles is a threat display that functions in a competitive context, then the lack of ATR exhibited toward non-native centipedes may be due to inexperience

(Murray et al., 2004) or it may result from decreased niche overlap between *L. forficatus* and red-backed salamanders. *Lithobius forficatus* is known to be omnivorous during periods of reduced prey availability (Lewis, 1965) and the species occurs more commonly in disturbed habitats (Lee, 1980) than does *P. cinereus*.

Our field and laboratory data suggest that juveniles of *P. cinereus* and centipedes avoid one another. In laboratory trials, the native centipede excluded juvenile salamanders from cover and we found fewer instances of co-occurrence of these two species in the field than expected. Hickerson et al. (2004) reported similar responses by adults of *P. cinereus* toward native centipedes in the field. Fecal deposition patterns by juvenile salamanders, in our shared cover object experiment, are consistent with this result. Juveniles deposited pellets under cover objects when placed in arenas alone and when paired with introduced centipedes, but they deposited pellets exclusive of cover when paired with native centipedes. This suggests that salamanders were either unable to access space beneath cover, or they were marking an area exclusive of the cover object when paired with native centipedes. Adults of *P. cinereus* deposit pheromones on fecal pellets (Simons, Felgenhauer & Thompson, 1999) and territory intruders use pellets to gain information regarding competitive abilities of territory holders (Mathis, 1990). The function of fecal pellet placement, and or marking, by juvenile salamanders is unknown, but juveniles of *P. cinereus* do possess active postcloacal glands (Simons, Jaeger & Felgenhauer, 1995), the source of territorial pheromones in red-backed salamanders (Simons & Felgenhauer, 1992).

These studies are the first to examine behavioral interactions between juveniles of *P. cinereus* and introduced and native invertebrate predators. As amphibian populations experience global population declines (Lannoo, 2005), it is increasingly important to strive to understand the factors responsible for those declines. We found no evidence that centipedes (introduced or native) prey on terrestrial salamanders, but we cannot discount negative competitive effects from such species. Predatory macrofauna such as spiders, beetles, and centipedes reach large biomasses (Lewis, 1965; Scheu et al., 2003; Wise & Chen, 1999) and likely interact strongly with intermediate vertebrate predators such as red-backed salamanders. Recent behavioral studies indicate that salamanders recognize and respond to some of these invertebrate predators in predictable ways (Gall et al., 2003; Hickerson et al., 2004; this study). We are currently conducting field removals of forest-floor predators in hopes of providing a clearer picture of the complex interactions that occur within forest-floor food webs.

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Table II - 1. Behaviour of juveniles of *Plethodon cinereus* in odor treatments

Behaviour	Control	Lithobius	Scolopocryptops
	n = 15	*n* = 15	*n* = 15
FLAT	3.1 (2.4)	10.0 (6.5)	25.8 (13.3)
FTR	513.1 (63.2)	429.6 (80.0)	400.6 (72.5)
ATR	177.3 (38.7)	233.6 (50.4)	202.5 (50.6)
IMMOBILE	742.1 (39.1)	782.1 (25.6)	725.9 (29.4)
ESCAPE	32.6 (12.6)	78.0 (34.2)	105.2 (39.4)
SUST ESCAPE	10.2 (3.6)	23.7 (9.5)	37.3 (13.1)
NTS	18.6 (4.5)	15.6 (5.4)	18.4 (4.1)
NTR	34.3 (11.1)	13.3 (4.1)	36.0 (11.3)

Juvenile salamanders were exposed to control (no odor) and experimental (centipede odor) substrates. All behaviors were timed (seconds) with the exception of nose tap substrate (NTS), which was recorded as a frequency. Values are means (SE). See text for descriptions of the behaviors.

Table II - 2. Behaviour of juveniles of *Plethodon cinereus* when paired with native and introduced centipedes

Behaviour of salamander	*Lithobius*	*Scolopocryptops*	Test statistic	*P*
	n = 30	*n* = 30		
FTR	206.1 (45.4)	129.8 (26.3)	*T* = 0.99	0.33 ns
ATR	49.9 (16.0)	92.1 (23.7)	*T* = 2.45	0.014 *
MT	63.3 (11.0)	53.9 (8.9)	*t* = 0.76	0.46 ns
IMMOBILE	834.6 (7.7)	818.7 (7.9)	*t* = 1.53	0.14 ns
ESCAPE	469.3 (48.5)	532.0 (36.2)	*t* = 1.03	0.31 ns
SUST ESCAPE	219.4 (33.0)	261.9 (34.6)	*t* = 0.85	0.41 ns
NTS	2.7 (0.63)	4.1 (0.80)	*t* = 1.39	0.17 ns
NTR	0.31 (0.15)	1.84 (0.83)	*T* = 2.17	0.03 *
NTC	1.0 (0.26)	1.1 (0.25)	*t* = 0.26	0.79 ns
FLIP	0.47 (0.19)	0.40 (0.14)	*T* = 0.61	0.54 ns

Juveniles of *Plethodon cinereus* were paired with either an adult of *Lithobius forficatus* (the introduced centipede) or an adult of *Scolopocryptops sexspinosus* (the native centipede). Nose tap substrate (NTS), nose tap centipede (NTC), flipping by the salamander (FLIP) were recorded as frequencies. All other behaviors were timed. Values are means (SE). See text for descriptions of the behaviors. * indicates significance at alpha = 0.05.

Figure Legend

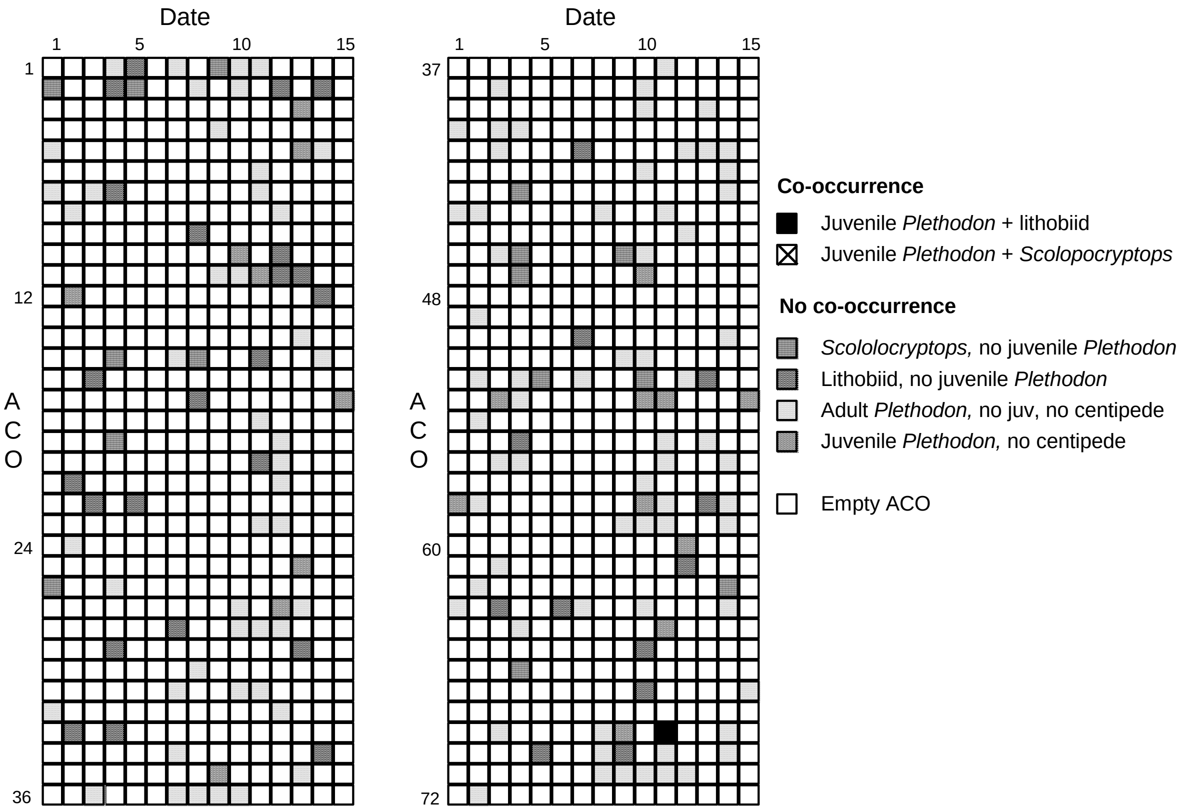
Figure II - 1. Occurrence of salamanders and centipedes under the 72 artificial cover objects (ACOs) used in the field study. The dates (1 – 15) correspond to approximately bi-monthly observations made from April to December 2004. Salamanders and centipedes co-occurred if they utilized the same ACO at anytime during the study. For example, ACO #10 was considered a shared cover object because it was utilized by a juvenile salamander on observation date 10 and by a lithobiid centipede on date 12. One case of simultaneous co-occurrence of a juvenile salamander and a lithobiid is indicated by the filled square. There were no cases of juvenile salamanders simultaneously sharing a cover object with a *Scolopocryptops.*

Figure II - 2. Behaviour of juvenile salamanders when exposed to controls (n = 15), odors of introduced (*Lithobius*, n = 15), and odors of native (*Scolopocryptops*, n = 15) centipedes. Mean time spent in (a) the submissive posture FLAT, (b) sustained escape, and (c) escape behaviour by salamanders exposed to the three odor treatments. Different letters above bars indicate statistically significant differences.

Figure II - 3. Responses of juvenile salamanders when paired with introduced (*Lithobius*, *n* = 30) and native (*Scolopocryptops, n* = 30) centipedes. Juvenile salamanders spent significantly more time in investigative and aggressive behaviors when paired with native centipedes. Mean time spent in (a) the investigative behavior nose tap rub (NTR) and (b) the aggressive posture all-trunk raised (ATR) by salamanders. Different letters above bars indicate statistically significant differences.

Figure II - 4. Location of salamanders in laboratory arenas in the cover object experiment. Juvenile salamanders were less likely to be found completely under the cover object when paired with native (*Scolopocryptops, n* = 28) centipedes. When paired with the introduced (*Lithobius*, *n* = 28) centipedes, cover object use by salamanders did not differ significantly from controls (*n* = 28).

Figure II - 5. Location of fecal pellets left by juvenile salamanders in the laboratory cover object experiment. Juveniles were placed in arenas alone or with either a native (*Scolopocryptops*) or introduced (*Lithobius*) centipede. After spending the night in arena, juvenile salamanders were significantly less likely to deposit fecal pellets under cover objects when paired with native (*Scolopocryptops*) centipedes.


Date
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37
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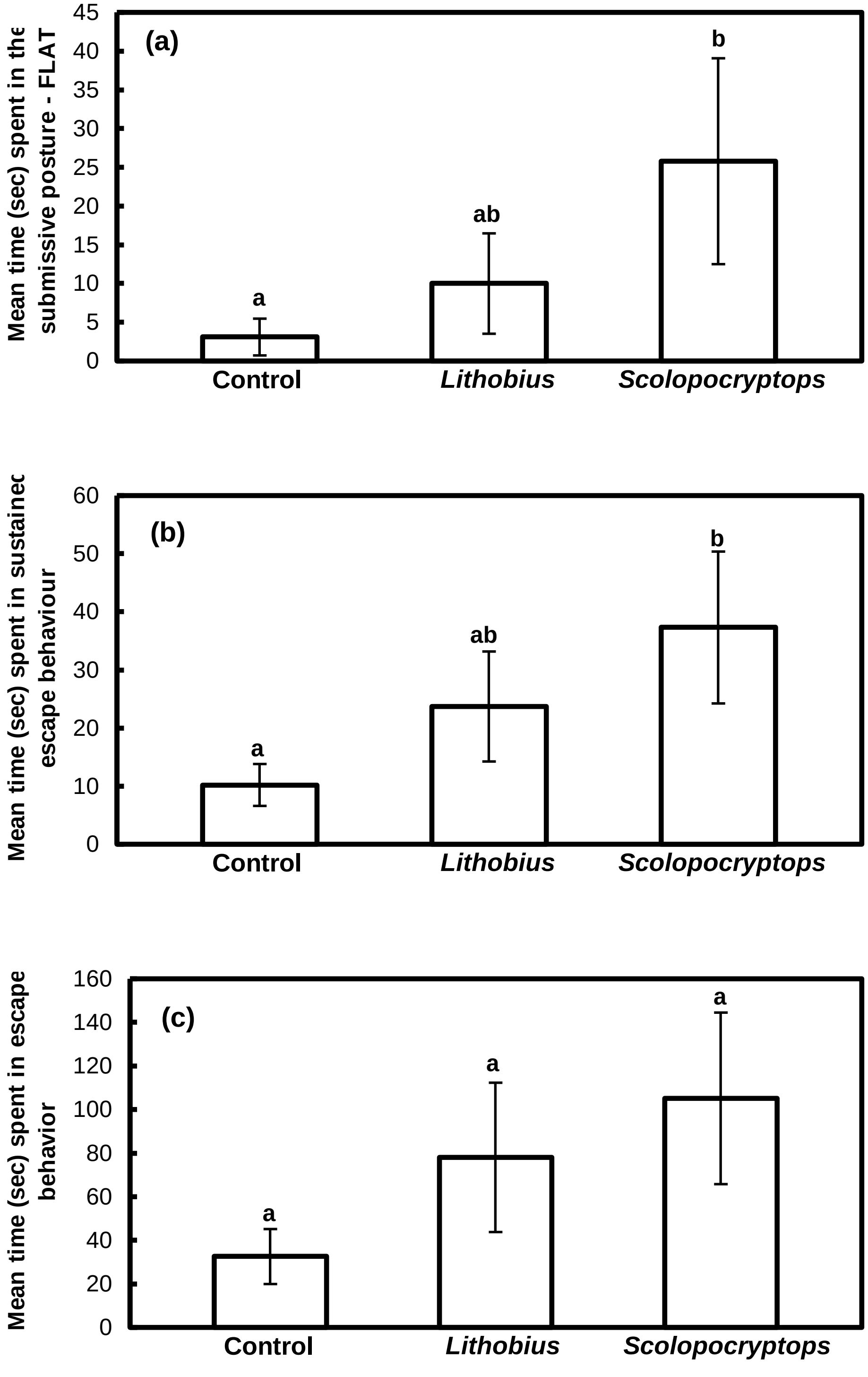
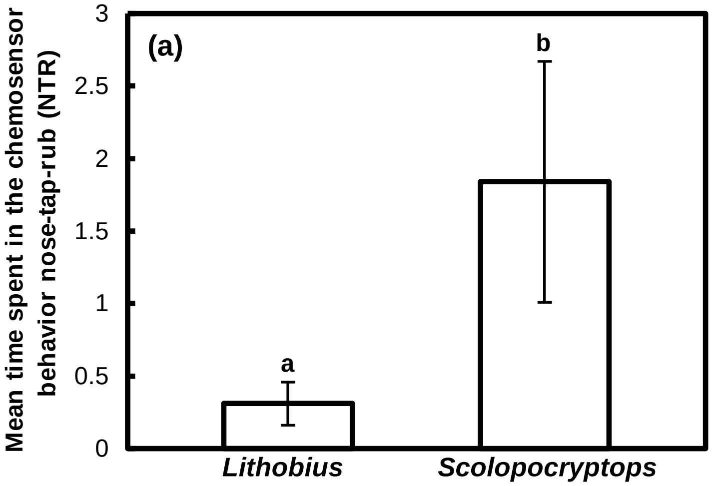
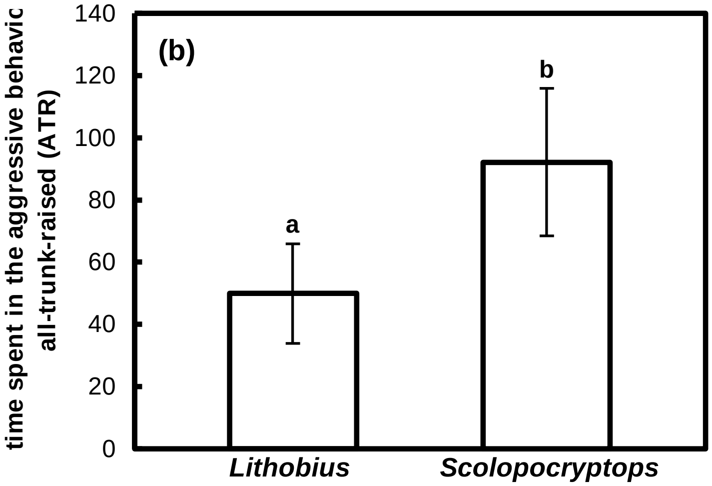
60
72
A
C
O
Co-occurrence
Juvenile *Plethodon* + lithobiid
Juvenile *Plethodon* + *Scolopocryptops*
No co-occurrence
Scololocryptops, no juvenile *Plethodon*
Lithobiid, no juvenile *Plethodon*
Adult *Plethodon,* no juv, no centipede
Juvenile *Plethodon,* no centipede
Empty ACO

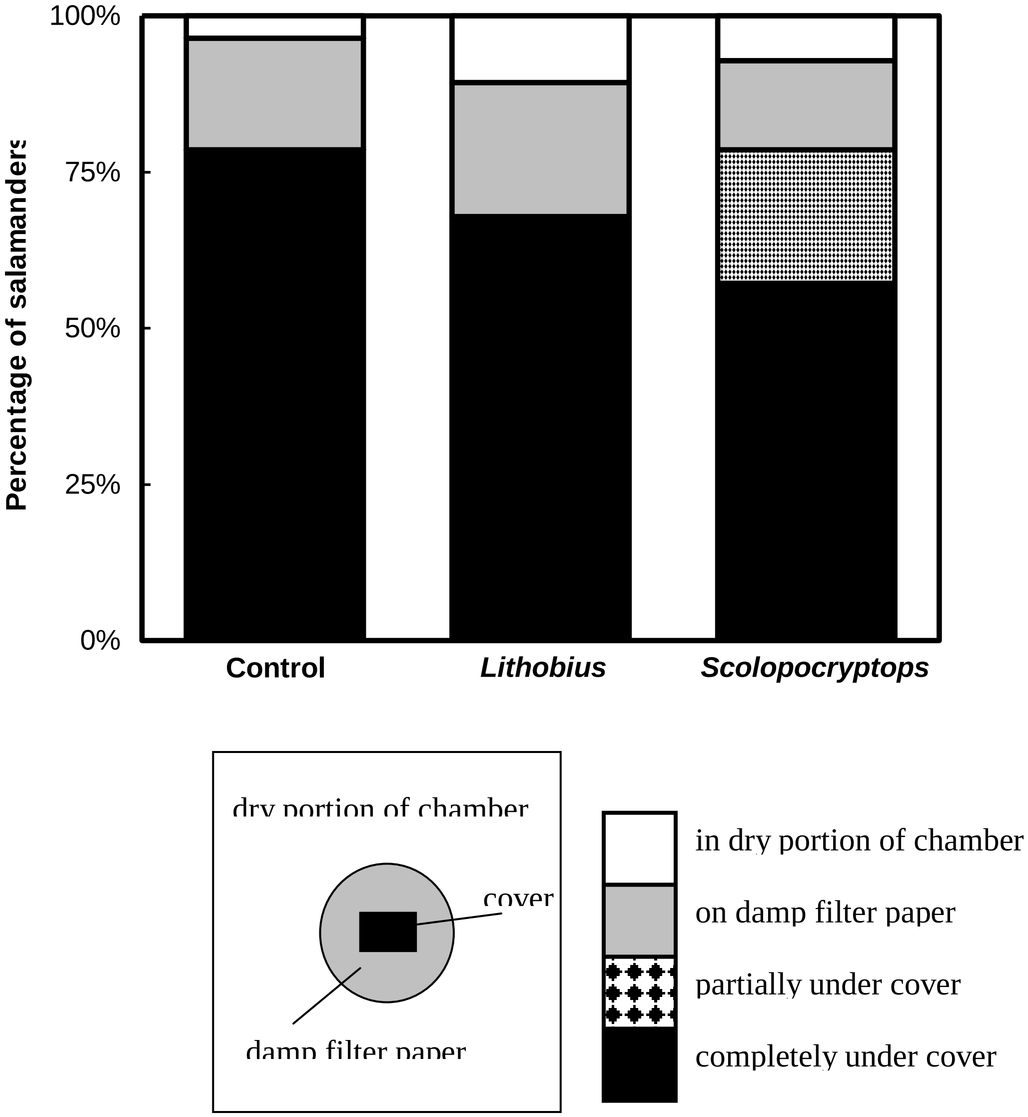

(a)
Mean time (sec) spent in the submissive posture - FLAT
0
5
10
15
20
25
30
35
40
45
a
ab
b
Control
Lithobius
Scolopocryptops
(b)
Mean time (sec) spent in sustained escape behaviour
0
10
20
30
40
50
60
a
ab
b
Control
Lithobius
Scolopocryptops
(c)
Mean time (sec) spent in escape behavior
0
20
40
60
80
100
120
140
160
a
a
a
Control
Lithobius
Scolopocryptops

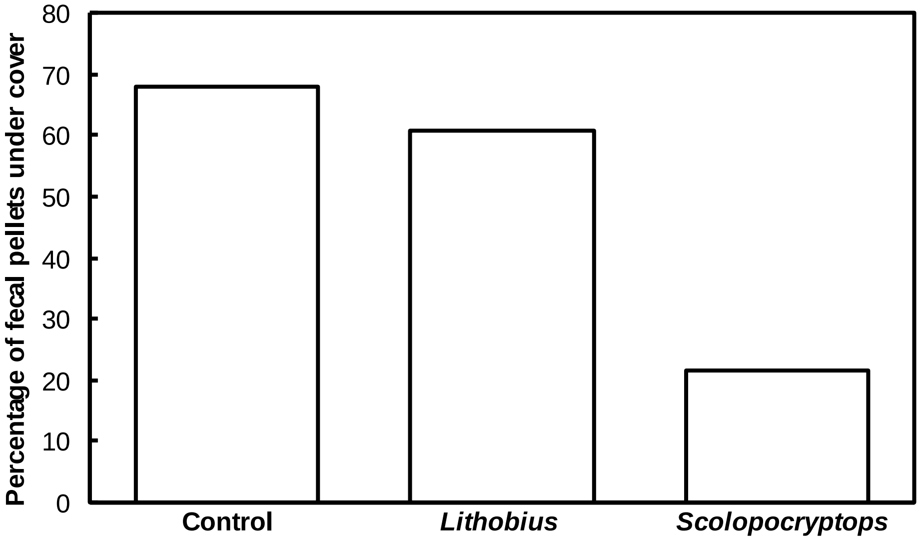


Mean time spent in the chemosensor behavior nose-tap-rub (NTR)
3
2.5
2
1.5
1
0.5
0
(a)
a
b
Lithobius
Scolopocryptops



time spent in the aggressive behavic all-trunk-raised (ATR)
140
120
100
80
60
40
20
0
(b)
a
b
Lithobius
Scolopocryptops


100%
75%
50%
25%
0%
Percentage of salamanders
Control
Lithobius
Scolopocryptops
dry portion of chamber
cover
damp filter paper
in dry portion of chamber
on damp filter paper
partially under cover
completely under cover


Percentage of fecal pellets under cover
80
70
60
50
40
30
20
10
0
Control
Lithobius
Scolopocryptops

CHAPTER III

NON-TROPHIC INTERACTIONS AMONG LARGE PREDATORS IN A FOREST-FLOOR DETRITAL FOOD WEB

Chapter 3 previously formatted for and submitted to *Ecology* (in revision):

Hickerson, C. A. M., Anthony, C.D., and Walton, B. M. Non-trophic interactions among large predators in a forest-floor detrital based food web.

ABSTRACT

Recent studies have suggested that non-consumptive effects (NCEs) may be just as important as direct consumption in determining trophic structure in food webs. In territorial species that defend areas beneath rocks and logs on the forest-floor, the effect of predators on their prey may be strengthened as a result of predators and prey interacting in a structurally simple microhabitat. Additionally, aggressive behavior exhibited toward heterospecifics by territorial residents may result in non-consumptive trophic cascades within food webs. We manipulated predator abundance (salamanders, carabid beetles, spiders, centipedes and flatworms) in an open field plot design in an effort to detect the effects of these predators on each other and on their potential prey. We removed 5672 predators during the 3.5 year study, effectively reducing the number of predators in removal treatments relative to the control. Our treatments resulted in top-down effects. Removal of salamanders resulted in an increase in the number of spiders and decrease in the number carabid beetles and some macrodetritivores. Centipede removals resulted in an increase in the number of salamanders, carabid beetles, ant colonies, and some macrodetritivore taxa, but spiders decreased in this treatment. We also detected an increase in the number of ant colonies in the treatment in which multiple predator species were removed. Removal treatments resulted in significant increases in species diversity and the largest gains were in treatments where all predators were removed. We infer that some of the treatment effects we detected resulted from competitive interactions (NCEs) among top-predators and not via intraguild predation. A growing body of evidence, our data included, suggests that territorial predators

constrained to spatially fixed microhabitats, may strongly regulate the abundances of guild members and of species in lower trophic levels. During wet periods these effects may be dampened if predators forage more widely on the forest floor.

INTRODUCTION

The relationships between direct consumption and the non-consumptive effects (NCEs) of predators on prey are crucial to understanding the relative strengths of these interactions and how they operate within food webs at the population and community level (Huang and Sih, 1990, Werner 1991, Schmitz et al. 1997, and Peckarsky et al. 2008). Polis (1991) suggested that food web studies conducted without consideration for competition (i.e. NCE) and predation (direct consumption) tell us little about how community structure and ecosystem function are maintained. For example, competition and intraguild predation can attenuate the effects on prey and rates of nutrient input to the system (Polis et al. 1989). We used a territorial salamander to examine the relative importance of direct predation and NCEs on invertebrate guild members and macrodetritivores in a temperate forest floor food web.

There is a growing body of evidence that suggests that terrestrial salamanders in the family Plethodontidae are important regulators of invertebrate communities and decomposition of organic material on temperate forest floors (Burton and Likens 1975, Hairston 1987, Rooney et al. 2000, Wyman 1998, Walton 2005, Walton and Steckler 2005). These salamanders can be extremely abundant (Mathis 1991) and have been estimated to consume more than one complete turnover of invertebrates annually

(Hairston 1987). This impact may exceed that estimated for forest floor spiders (Moulder and Reichle 1972). Therefore, plethodontid salamanders are expected to be important in determining community structure in the detrital web of temperate forests.

There are many predatory, forest floor invertebrates that are ecologically similar to, and have the potential to interact with salamanders thus theses organisms can alter community composition and food web stability by adding complexity to the system. For example, large centipedes are similar to the salamander, *Plethodon cinereus* in the following ways: 1) they have large biomasses (Lewis 1981); 2) they occupy similar microhabitats (Shelley 2002, Hickerson et al. 2004); 3) they are generalist predators (Roberts 1956) and have similar foraging tactics (Jaeger and Barnard 1981, Formanowicz and Bradley 1987); 4) they are similar in size (Hickerson et al. 2004); and 5) they show some degree of parental care in the form of egg brooding (Lewis 1981). Such similarities among salamanders and relatively large invertebrates provide the rationale for studying interactions among distantly related species that co-occur in forest floor microhabitats.

One way terrestrial woodland salamanders compete for resources is through interference competition and territoriality (reviewed in Mathis et al. 1995). Terrestrial salamanders forage on the forest floor in leaf litter while conditions are wet but will move beneath rocks and logs when conditions become dry (Jaeger 1980, Gabor and Jaeger 1994). For salamanders, territoriality is adaptive because moisture and prey can become concentrated under cover objects. Therefore, territories beneath rocks and logs allow salamanders’ access to the surface to forage for longer periods, and they become refugia for isolated prey populations as the forest dries.

Recent studies have examined aggressive interactions that may occur in nature among distantly related, but ecologically similar organisms. For example a number of studies have examined responses of territorial salamanders to predatory forest floor invertebrates (carabid beetles (Gall et al. 2003), centipedes (Hickerson et al. 2004, Anthony et al. 2007), and spiders (Figura 2007, unpublished MS thesis)). In all cases, salamanders exhibited territorial or aggressive behavior toward these invertebrates (or their odors), and spatial data from these studies suggest negative co-occurrence beneath cover objects in the field. Interspecific aggression could result from competitive interaction or from intraguild predation (IGP) in which territorial residents attempt to drive off potential predators. IGP is common in food webs and has the potential to add significant complexity to food web interactions (Polis 1991). It is often assumed that symmetrical IGP occurs as a result of ontogenetic reversal of predation, such that adults of species A eat juveniles of species B and adults of B eat juveniles of A (Polis et al. 1989). With regard to interactions between salamanders and large predatory arthropods, it is further assumed that the likelihood of symmetrical IGP is high because both groups are generalist predators that experience large changes in size through ontogeny. However, recent studies investigating interactions between juveniles of *Plethodon cinereus* and adult centipedes (Anthony et al. 2007) and spiders (Figura 2007, unpublished MS thesis) report no evidence for predation on juvenile salamanders by larger, adult arthropods. This example indicates that it is difficult to predict if IGP occurs among predators despite size asymmetries.

Although these studies suggest that the relationships between *P. cinereus* and large arthropod predators is competitive (Hickerson et al. 2004, Anthony et al. 2007) rather than predatory, the ways in which these interactions function within the intricate forest-floor food web is unclear. Because terrestrial salamanders are important regulators of detrital food webs and because other large invertebrate predators appear to be ecologically similar, understanding the role of non-consumptive interactions among these organisms seems a logical part of determining which mechanisms are most influential in structuring the community. For example, Peckarsky et al. (2008) illustrate how inclusion of data on NCEs in classic textbook predator/prey data sets (e.g. lynx-snowshoe hair cycles) can alter our original understanding of population and community dynamics.

Despite the growing pool of literature that suggests terrestrial salamanders are important regulators of invertebrate abundance and leaf litter decomposition rates, there are relatively few long-term experiments in terrestrial habitats that examine how behavioral interactions among predators influence the ecology of detritivore prey. We conducted a 3.5 year, predator removal study in unrestricted, open field plots to evaluate whether the removal of one or more predators would affect the distribution and abundance of those predators, and to evaluate the relative importance of those interactions in regulating species occupying other trophic levels within the terrestrial detrital web. Based on previous laboratory and field studies (Gall et al. 2003, Hickerson et al. 2004, Hickerson et al. 2005, and Anthony et al. 2007, Figura 2007, unpublished MS thesis) we predicted that red-backed salamanders, *P. cinereus*, would influence the abundance of large arthropod predators through non-consumptive behavioral interactions, and that those NCEs would translate to lower trophic levels in the detrital food web. We also predicted that we would detect these effects most strongly beneath cover objects that serve as territories for these salamanders.

MATERIALS AND METHODS

On 12 and 13 April, 2004 we placed 288 artificial cover objects (ACOs) on the forest-floor in the Cuyahoga Valley National Park (CVNP), Summit county, Ohio (41° 13' 46.62" N, 81° 31' 7.77" W). The field site is mixed deciduous forest that is dominated by *Acer saccharum* (Sugar Maple), *Fagus grandifolia* (American Beech), *Liriodendron tulipifera* (Tulip Poplar), and *Quercus rubra* (Red Oak) and lies on a north/northeast facing slope (elevational range 260 – 271 m). We used white ceramic floor tiles measuring 30 x 30 cm as ACOs. The ACOs were arranged in 32 arrays; each was separated by approximately two meters and covered a 20 x 40 m area. Each of the 32 arrays consisted of a cluster of nine ACOs separated by one meter, all of which received the same treatment application. Each array was systematically assigned to one of four treatments (n = 8, Fig. 1); 1) no removals/controls (NR), 2) salamander removal (SR), 3) centipede removal (CR), and 4) all predator removal (PR). Predators removed from the PR treatment included salamanders, centipedes, spiders, carabid beetles, and predatory flat worms (*Bipalium*).

Data collection began on 23 April 2004 and continued through 20 November 2007. The field site was visited every two weeks, except for winter months, through the end of 2005, and weekly beginning in spring 2006 through the duration of the study (total of 98 visits). During each visit we hand-turned ACOs, counted and identified macrofauna from beneath each, and removed predators from the appropriate treatments. Arrays were visited in random order to remove any temporal bias in sampling. Macroinvertebrates recorded at our field site were centipedes (Lithobiomorpha, Scolopendromorpha and

Geophilomorpha), spiders (the largest and most abundant were amaurobiids), beetles (Carabidae), numbers of ant colonies, flatworms (*Bipalium*), millipedes, isopods, slugs, snails, crickets, earthworms and hemipterans. Invertebrates from removal treatments were hand caught and preserved in 70% ethanol. At our field site the salamander, *P. cinereus*, made up 99.5% of the total number of observed salamanders. Only rarely did we see other salamander species (*P. glutinosus, Notophthalmus viridescens* and *Eurycea bislineata*). Salamanders in removal arrays were relocated across barriers (streams or roads) so that they were unable to move back into the arrays (Marsh et al. 2007).

During every other visit we measured and recorded abiotic variables at the center ACO of each array (32 points evenly spaced throughout the site) to determine if there was variation in the abiotic environment at the microhabitat scale that might influence the distribution and abundance of the occupants of the forest-floor detrital web. Abiotic measurements included ground surface temperature, soil temperature, air temperature, percent humidity, percent soil moisture, and soil pH. We used an infrared temperature sensor (Cole-Parmer Instrument Company, Illinois, USA) to measure surface temperature of the ground under the ACO. Soil moisture and pH were taken at 10 cm below the soil surface with a Kelway soil moisture meter (94302, Forestry Suppliers, Inc, Mississippi, USA). We used an Oakton digital max./min. thermohygrometer to measure air temperature (°C) and percent relative humidity.

Statistical analyses

We used a general linear model MANOVA to test the effectiveness of the treatment manipulations, differences in the measured abiotic variables, and top-down and lateral effects of predators among years (dates) and ACO treatments. In our model, treatment and date were fixed factors. To assess our effectiveness in removing various predators, we compared the number of salamanders, spiders, centipedes, and carabid beetles in the control arrays (NR) to the appropriate removal arrays (SR, PR or CR). We calculated Shannon's diversity index for all treatments. We used two-tailed t-tests to determine if diversity differed in the control treatment compared to each of the removal treatments. Removed taxa were not included in the calculation of diversity in removal treatments or in the control.

Interaction strengths were calculated to examine the effects of each predator taxon on the others and on taxa at lower trophic levels. Interaction strength was calculated as the natural logarithm of the ratio of density of the potentially affected taxon within control arrays (NR) over the density of the affected taxon within the focal predator removal array. For example, the effect of centipedes on spiders was calculated by dividing the mean number of spiders on each sampling day in CR arrays by the mean number in NR arrays (Wootton and Emmerson 2005). The means were compared over the 98 sampling days. Similar calculations were made to examine the effect of SR and CR on carabid beetle abundance. To examine the effect of predators on other groups of invertebrates in the food web we compared numbers of invertebrates, represented by eight taxa, in control arrays (NR) to the appropriate predator removal treatments (SR, PR or CR). Rare taxa that made up less than 1% of the total number of individuals counted were excluded from the analyses. Abiotic measurements were compared among all treatments over time. All data were log-transformed, $\log_{10}(x+1)$ to improve adherence to normality.

RESULTS

We detected significant increases in Shannon's Index of Diversity in all removal treatments relative to controls. Increases in diversity were the highest in treatments in which all predators were removed (8.2%, $T = 2.40$, $P = 0.016$) followed by treatments in which only salamanders were removed (6.3%, $T = 2.34$, $P = 0.020$) and treatments in which only centipedes were removed (3.5%, $T = 2.24$, $P = 0.026$).

Effectiveness of treatment applications

We removed a total of 2575 salamanders from SR and PR arrays, 1454 spiders from PR arrays, 1056 centipedes from CR and PR arrays and 587 carabid beetles from PR arrays over the 3.5 year study. Despite the open plot design of our experiment, we were effective in significantly reducing numbers of most predators in removal treatments compared to controls (Fig. 2). By the end of the experiment, we reduced total salamander abundance by 28% (Fig. 2a) and adult salamander abundance by 47% in salamander removal arrays compared to control arrays. Spiders (Fig. 2b) and carabid beetles (Fig. 2c) were reduced in predator removal (PR) arrays by 18% and 31% respectively. The abundance of centipedes was reduced by 7% in centipede removal arrays compared to controls, and the difference was most prominent in 2007 (Fig. 2d). We found no differences in abiotic measures among treatments.

Intraguild predator interactions

Salamanders and centipedes had negative effects on one another and opposite effects on spiders and carabid beetles (Fig. 3). Salamanders had a strong negative effect on spiders while centipedes had a positive effect on spiders (Fig 3a). There were significantly more spiders in SR arrays (mean = 3.04/array) and fewer in CR arrays (mean = 1.93/array) compared to control (NR) arrays (mean = 2.27/array, Table 1). Salamanders had a positive effect on carabid beetle abundance, while centipedes had a negative effect on carabid beetles (Fig. 3b). There was a significant decrease in the abundance of carabid beetles in SR arrays (mean = 0.94) and an increase in CR arrays (mean = 1.40/array) compared to controls (mean = 1.09/array, Table 1).

Predator effects on other macrofauna

Three detritivore taxa (millipedes, slugs and isopods) and numbers of ant colonies appeared to be positively associated with salamanders and negatively associated with centipedes (Table 1). This relationship is most evident when we compared arrays with the most salamanders (CR; mean salamanders = 2.68/array) to those with the fewest (SR and PR; mean salamanders = 1.64/array). Here, differences were significant for each of these groups of invertebrates (Table 2). Millipedes (mean = 8.23/array), isopods (mean = 25.46/array) and slugs (mean = 2.22/array) were most abundant in centipede removal arrays and least abundant in SR arrays (mean = 6.17/array, Fig 4, Table 2). The number of ant colonies increased over the 3.5 years in controls and were most numerous in arrays where all large predators were removed (PR, mean = 0.519/array) compared to control arrays (mean = 0.329/array).

DISCUSSION

Intraguild predator interactions

Our data support the hypothesis that removal of top predators results in changes in the abundance of intermediate arthropod predators within the forest-floor food web. Interactions were complex and often involved multiple species such that removals positively affected some taxa but negatively affected others. Our ability to test more specific hypotheses is limited by the types of removals conducted, but in light of previous pair-wise studies of salamanders and large predatory invertebrates (Gall et al. 2003, Hickerson et al. 2004, Anthony et al. 2007, Figura 2007, unpublished MS thesis), we argue that interference competition (a behaviorally mediated effect), rather than predator-prey interactions (IGP) may be the best explanation for our findings. Our data indicate both direct (2 predator) and indirect (3 predator) non-consumptive effects are operating in this system. For example, the negative relationship between salamander abundance and spider and centipede abundances may be the result of direct effects in which we observed an increase in number of spiders and centipedes in which salamanders were removed. Alternatively there could have been an indirect effect of increased numbers of centipedes in salamander removal arrays resulting in a positive effect on spider abundance. No studies have explored the potential for a positive relationship between centipedes and spiders but evidence from previous studies suggest negative associations between salamanders and these two predators (Hickerson et al. 2004, Anthony et al. 2007, Figura 2007, unpublished MS thesis) so it is likely that the increased numbers of spiders and centipedes in salamander removal (SR) arrays are a direct result of the treatment application. Another recent study suggested that spider abundance increased in field exclosures in which birds and mammals were excluded. Dunham (2008) reported that spiders were 2.3 times more abundant in predator exclosures compared to control exclosures, a result that mirrors ours. We agree with Dunham who points out the difficulty in discerning whether the observed increase in spider abundance in her predator exclosures was the result of reduced predation by birds and mammals (a direct trophic link), or reduced interspecific competition in the absence of predators (birds and mammals) for macro-invertebrate prey (an indirect behaviorally mediated effect).

We found an increased number of salamanders and carabid beetles in centipede removal (CR) arrays compared to controls. Again it is impossible to say whether our observed abundances were the result of direct interactions (removal of centipedes led to increases in beetle abundance), or indirect interactions (removal of centipedes led to increased numbers of salamanders, and in turn increased numbers of beetles). The existing evidence on beetle salamander interactions indicates that the salamander, *P. cinereus*, and the carabid beetle, *Platynus tenuicollis*, are mutually territorial (Gall et al. 2003) and therefore the most likely scenario would be the direct effect of centipede removal on beetles. It is most probable that centipedes have a negative effect on beetles, suggestive of IGP or competition for prey.

There are very few data sets on gut contents of predatory invertebrates like centipedes, spiders and beetles. However, data on stomach contents of *P. cinereus* reveals that spiders, centipedes and carabid beetles do not make up a significant proportion of the diet (Jaeger 1990, Maglia 1996, Adams and Rohlf 2000, and Anthony et al. 2008). For example, Maglia (1996) reported that spiders made up only 1.6% of the total invertebrates by number in the diet of 172 *P. cinereus* from Tennessee, and Anthony et al. (2008) found that centipedes, spiders and carabid beetles combined made up only 1.2% of 489 prey items taken from 81 salamanders at our field site. Studies on asymmetrical IGP and predator diets, like those described above, provide further support for behaviorally mediated interactions as the mechanism driving changes in the abundance of predators in this forest-floor web rather than direct consumption.

Predator effects on other macrofauna

We found that predators affected the distribution and abundances of macrodetritivores at our field site. Few studies have examined the effects of predation on macrodetritivores in terrestrial food webs but salamander predation has been shown to decreased larger detritivores and decreased rates of litter decomposition (Wyman 1998). Walton and Steckler (2005) reported similar decreases in macrodetritivores in mesocosms, but increased abundance of mesofauna such as Collembola. Our results differ from both of these studies because we found significantly more millipedes, slugs and isopods in open field plots with the highest salamander abundance (centipede removals). The difficulty in making overriding statements about how predators affect and are affected by lower trophic levels in detrital food webs may be related to differences in experimental methods, including experiment duration, seasonal differences, the taxa considered and scale of the experiment (mesocosm versus field enclosures versus open field plots; Walton 2005). In previous studies conducted in laboratory mesocosms and field enclosures, predators (salamanders) were enclosed with their prey. With few alternatives and little refuge for prey, predators were able to have significant negative effects, even on taxa that are not typically important diet items (i.e., millipedes, spiders, centipedes). In our open field design, predators were free to forage optimally on preferred prey species, and these species were free to recolonize plots as they became depleted. Resident predators that exclude guild members via behavioral mechanisms may generate a net benefit for other arthropods that are not normally included in the diets of territorial residents.

Centipedes and spiders are most often classified as polyphagous predators (Wise 1993, Foelix 1996, Toft and Wise 1999, and Lewis 2008). Studies on the natural diets of these generalist predators are few, but research does suggest that prey quality is important for growth and reproduction of spiders and that some spiders do show preferences for high quality prey items (Toft and Wise 1999). Although most spiders are generalists, there are some that appear to specialize on woodlice (Řezáč and Pekár 2007) and gastropods (reviewed in Nyffeler and Symondson 2001). Our results suggest that centipedes, spiders or both prey heavily on macrodetritivores. We observed reduced numbers of macrodetritivores in the arrays with the most spiders (salamander removals) and increased numbers in centipede removal arrays. These results also imply that salamanders probably are not feeding on the large detritivore taxa given that macrodetritivores are most abundant in the arrays with many salamanders. At our field site, it is most likely the case that spiders have been released from competitive interactions with salamanders allowing them to prey upon macrodetritivores in salamander removal treatments and that the reduction in centipedes in CR arrays allowed for the invasion of macrodetritivores from the surrounding habitat.

Another interesting result from our study was the observed increase in numbers of ant colonies in the predator removal (PR) and centipede removal (CR) arrays compared to control arrays (NR). Sanders and Platner (2007) manipulated the abundance of ants and spiders in an above ground grassland web and found that the abundance of the ant, *Formica cunicularia*, was significantly higher in spider removal plots. Their study also revealed that higher densities of ants negatively affected the abundance and biomass of web building spiders in their field enclosures, but had no effect on the presence of wandering spiders. Because we restricted sampling to beneath ACOs, most of the spiders removed from our arrays were those that construct webs that line burrows and cover the openings at the ground surface, or attach webs directly to the underside of the cover objects, so we did not quantify the abundance of above ground web builders. Sanders and Platner (2007) found a weak overall effect when a single predator group was removed, but when both ants and wandering spiders were removed from experimental plots the abundance of web building spiders increased. They argued that a highly diverse predator guild may stabilize the entire system, which could explain the attenuated trophic cascade observed when removing only one predator. Our measures of species diversity indicate a similar pattern in which diversity of remaining taxa increased with increasing numbers of predator taxa and individuals removed.

It is thought that top-down trophic cascades should be weakened in leaf litter habitats that are structurally complex and provide refugia for prey (Finke and Denno 2002 and 2003, Halaj and Wise 2002). However, in systems where the microhabitat is centered around structurally simple areas beneath cover objects (rocks and logs) that serve as territories for predators (salamanders, and perhaps centipedes, spiders and beetles) top-down effects may become localized, and are likely to occur because prey are more exposed to predators that do not forage broadly in space during all times of the year.

Recently there has been discussion about food web stability occurring through fast and slow energy channels that are linked by predators. Mobile predators couple strong and weak interaction chains through prey switching between chains based on prey density (Rooney et al 2006). In forest ecosystems that experience periods of moisture, species that have strict moisture requirements (amphibians and arthropods) may move from one energy channel to another during wet periods but may be restricted from doing so during dry periods between rains. As a lungless salamander, *P. cinereus* is confined to moist environments and so the localized effects of this predator should be most pronounced during dry periods when prey are trapped within territories under cover objects. We predict that in more mesic environments, or during wetter seasons, the effects will be weaker because salamanders are free to forage widely for invertebrate prey in the surrounding leaf litter and on vegetation. During such periods the role of terrestrial, territorial salamanders may be more like that described by Rooney et al. (2006) where they regulate prey in fast chains before moving from those depleted chains to the chains that have experienced some degree of recovery.

Conclusions

This study is the first of its kind to examine interactions among top predators within detrital based forest-floor food webs. By focusing on non-consumptive effects, we have found evidence that territorial predators exert strong top-down effects within their territories. This suggests that trophic cascades can be localized in space and we predict that territorial predators are more likely to generate strong top-down effects than are widely foraging species. It also suggests that territorial interactions among predators are important factors influencing spatial variability in community composition and trophic interactions.

Early studies of food web dynamics and community structure used simple models to predict patterns and outcomes of distribution and abundance of species in various systems. Hairston Jr. and Hairston Sr. (1993) noted that, historically, models assumed that links in food chains are equal in value, and interactions among organisms were either ignored or assumed to exist where consumers share resources. Over the past 50 years ecologists have begun to appreciate that complex factors influence community structure. Ecologists now commonly consider complex interactions in webs, such as the role of non-consumptive effects in regulating trophic interactions at various levels along the connected chains. Additionally, researchers are beginning to understand the importance of temporal and spatial variation, and that such variation may help to stabilize community structure and may be important for ecosystem resilience.

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Table 1. Mean changes in abundance of predators, detritivores, and ant colonies in removal treatments relative to controls. SR = salamander removals, CR = centipede removals, PR = all predators removed (salamanders, spiders, carabid beetles, and centipedes).

Treatment		Salamanders	Spiders	Carabid Beetles	Centipedes	Millipedes	Isopods	Slugs	# ant colonies
SR	mean change	-0.64	0.77	-0.41	0.02	-0.9	-7.63	-0.22	0
	SE	0.01	0.01	0.01	0.01	0.02	0.03	0.01	0.04
	F_3	71.53	87.11	24.43	2.46	11.35	13.44	8.18	14.62
	P	<0.000	<0.000	0.545	0.319	0.905	0.325	0.870	0.995
CR	mean change	1.04	-0.34	0.31	-0.05	1.16	1.36	0.49	0.2
	SE	0.01	0.01	0.01	0.01	0.02	0.04	0.02	0.04
	F_3	71.53	87.11	24.43	2.46	11.35	13.44	8.18	14.62
	P	0.092	0.003	<0.000	0.998	0.357	0.996	0.248	<0.000
PR	mean change	-0.64	-0.41	-0.34	-0.07	0.51	-9.52	-0.22	0.19
	SE	0.01	0.01	0.01	0.01	0.01	0.04	0.01	0.03
	F_3	71.53	87.11	24.43	2.46	11.35	13.44	8.18	14.62
	P	<0.000	<0.000	0.008	1.00	0.985	0.079	0.898	0.002

Notes: F values are between-subjects effects in a GLM MANOVA. Dunnett T3 was used as a post-hoc test because of unequal variance among groups. Statistically significant P values are bold. Marginally significant P values are underlined.

Table III - 2. Mean changes in abundance of detritivores and ant colonies in arrays with the most salamanders (CR) relative to arrays with the fewest salamanders (SR).

	millipedes	isopods	slugs	# of ant colonies
mean change	+2.60	+6.27	+0.71	0.20
SE	0.02	0.03	0.01	0.04
F_3	11.35	13.44	8.18	14.62
P	**0.033**	**0.014**	**0.014**	**<0.000**

Notes: F values are between-subjects effects in a GLM MANOVA. Dunnett T3 was used as a post-hoc test because of unequal variance among groups. Statistically significant *P* values are bold.

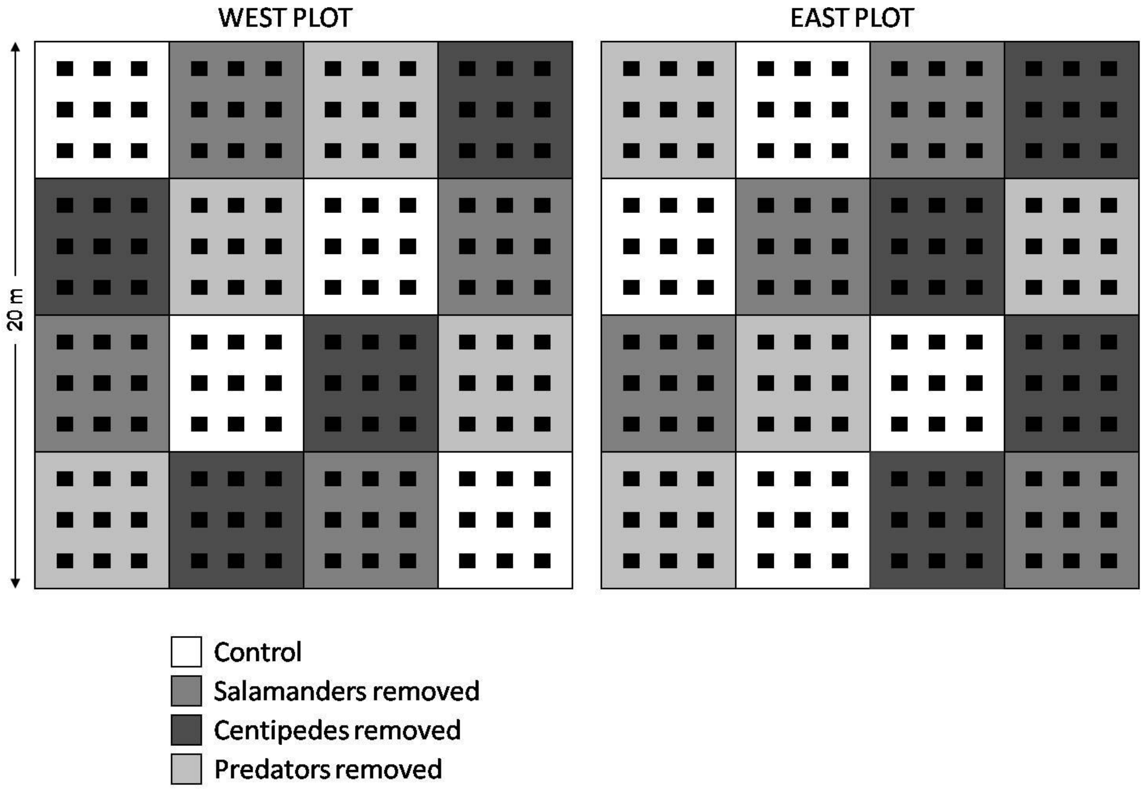
Figure Legend

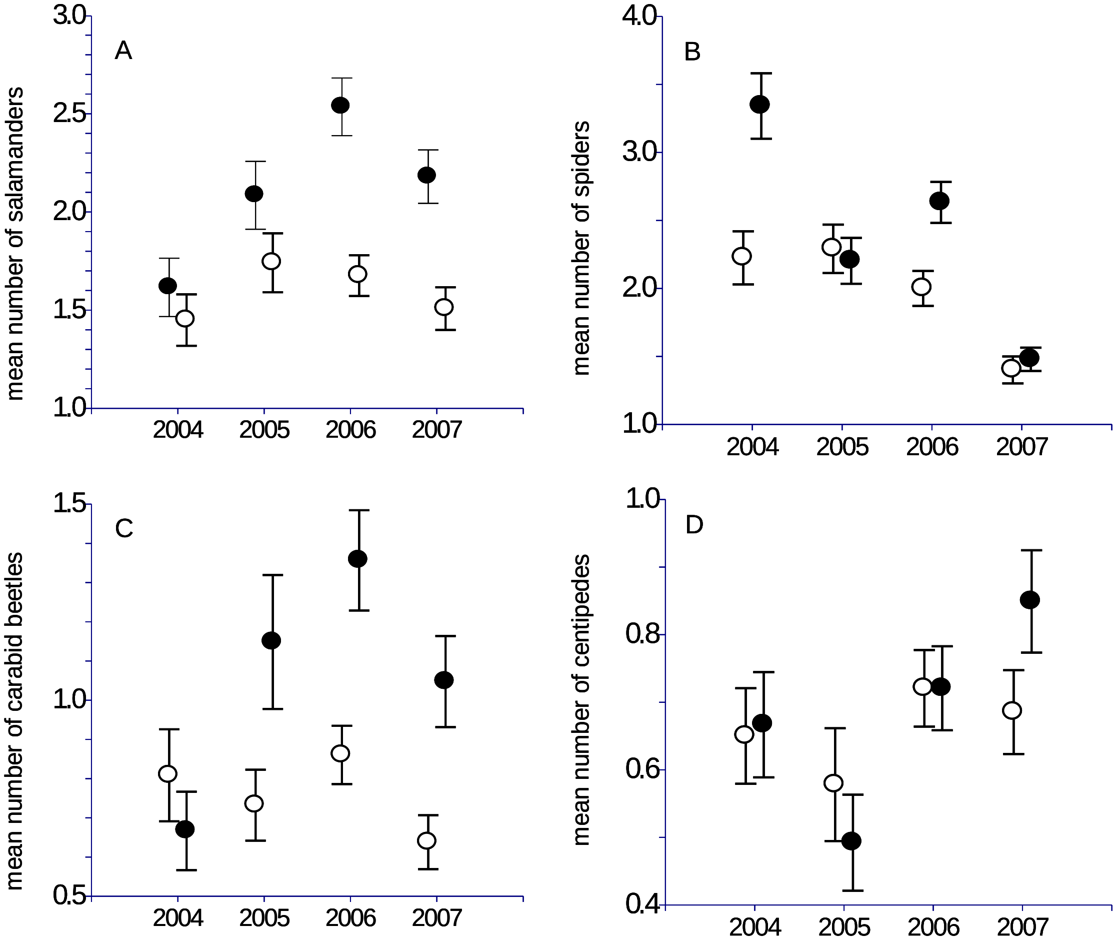
Figure III - 1. Schematic of the experimental design showing the artificial cover object (ACO) placement among the four treatments (NR - control = no animals removed, SR = all salamanders removed, CR = all centipedes removed and PR = arrays where we removed carabid beetles, spiders, centipedes, salamanders and the flatworm, *Bipalium*). 288 ACOs (small black squares) were divided into east and west plots and placed into four blocks containing one of each of the four treatments. Each treatment array contained nine ACOs spaced one meter apart, and there were four arrays per block.

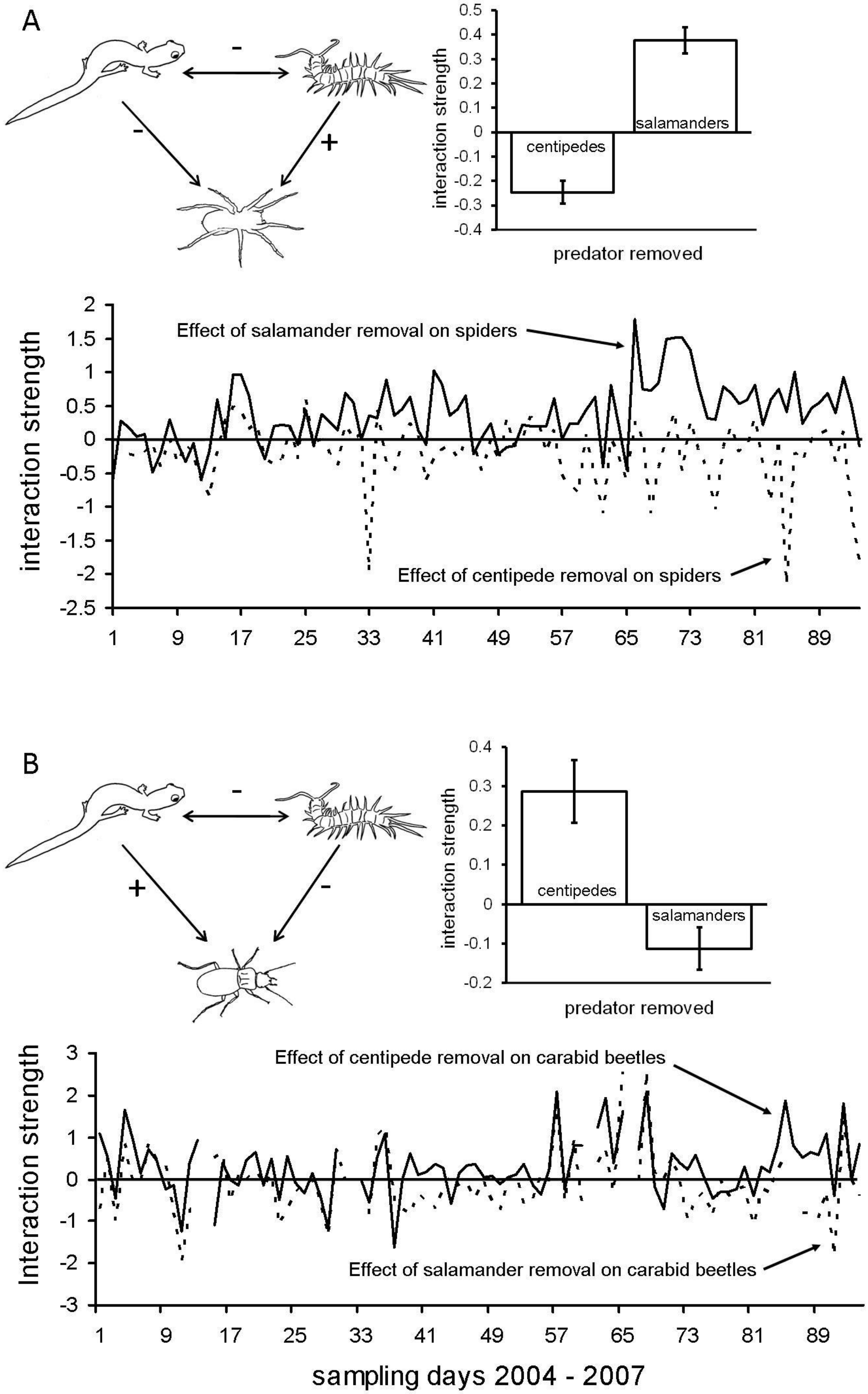
Figure III - 2. Mean numbers per replicate (N=8) of predators observed under control treatment ACO's (black circles) compared to respective removal treatment ACO's (open circles) over the 3.5 year study. (A) Mean number of salamanders (mean = 2.28. *SE* = 0.01) in controls (NR) compared to salamander removals (SR) (mean = 1.64, *SE* = 0.01, F_3 = 71.53, ***P* <0.000**). (B) Mean number of spiders (mean = 2.27, *SE* = 0.01) in controls compared to predator removals (PR) (mean = 1.86, *SE* = 0.01, F_3 = 87.11, ***P* <0.000**). (C) Mean number of carabid beetles (mean = 1.09, *SE* = 0.01) in controls compared to predator removals (PR) (mean = 0.75, *SE* = 0.01, F_3 = 24.43, ***P* = 0.008**. (D) Mean number of centipedes (mean = 0.71, *SE* = 0.01) in controls compared to centipede removals (CR) (mean = 0.66, SE = 0.01, F_3 = 2.46, *P* = 0.998. *F* values are between subjects effects in a GLM MANOVA. Dunnett T3 test was used as a post-hoc test because of unequal variance among groups. NR = control arrays. Statistically significant *P* values are bold.

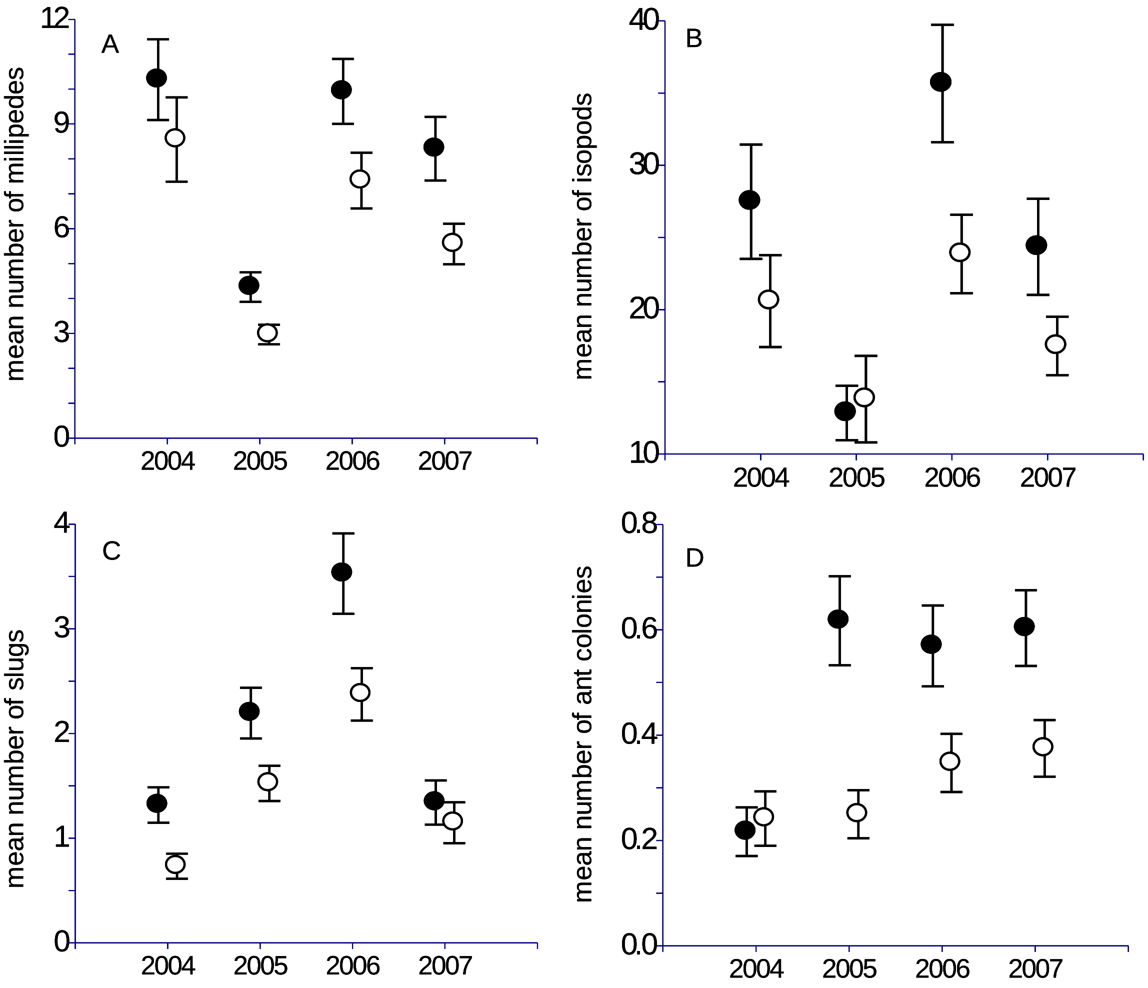
Figure III - 3. The effect of salamanders and centipedes on spider abundance (A), and carabid beetle abundance (B). Connections among members of the predator guild show the directions of the interactions. The bar graphs show the mean strength and direction of salamander and centipede removal treatments on the two predators. The line graphs show the strength and direction of salamander and centipede removal treatments on the two predators over the 94 sampling days (2004 – 2007).

Figure III - 4. Mean numbers per replicate (N=8) of important macrofauna observed under ACOs with the most salamanders (CR, black circles) compared to ACOs with the fewest salamanders (SR, open circles) over the 3.5 year study. Mean numbers of millipedes (A), isopods (B), slugs (C) and ant colonies (D) in controls (CR) compared to salamander removals (SR).


WEST PLOT
EAST PLOT
20 m
Control
Salamanders removed
Centipedes removed
Predators removed


A
mean number of salamanders
3.0
2.5
2.0
1.5
1.0
2004
2005
2006
2007
B
mean number of spiders
4.0
3.0
2.0
1.0
2004
2005
2006
2007
C
mean number of carabid beetles
1.5
1.0
0.5
2004
2005
2006
2007
D
mean number of centipedes
1.0
0.8
0.6
0.4
2004
2005
2006
2007


A
-
-
+
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
interaction strength
centipedes
salamanders
predator removed
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
interaction strength
Effect of salamander removal on spiders
Effect of centipede removal on spiders
1
9
17
25
33
41
49
57
65
73
81
89
B
-
+
-
0.4
0.3
0.2
0.1
0
-0.1
-0.2
interaction strength
centipedes
salamanders
predator removed
3
2
1
0
-1
-2
-3
Interaction strength
Effect of centipede removal on carabid beetles
Effect of salamander removal on carabid beetles
1
9
17
25
33
41
49
57
65
73
81
89
sampling days 2004 - 2007


A
mean number of millipedes
0
3
6
9
12
2004
2005
2006
2007
B
mean number of isopods
10
20
30
40
2004
2005
2006
2007
C
mean number of slugs
0
1
2
3
4
2004
2005
2006
2007
D
mean number of ant colonies
0.0
0.2
0.4
0.6
0.8
2004
2005
2006
2007

CHAPTER IV

TOP-DOWN EFFECTS OF PREDATORS ON MESOFAUNA, MICROBES, AND LEAF LITTER DECOMPOSITION IN OPEN FIELD PLOTS IN A TERRESTRIAL FOREST FLOOR COMMUNITY

Chapter 4 formatted for submission to *Pedobiologia*:

Hickerson, C. A. M., Anthony, C.D., and Walton, B. M. Top-down effects of predators on mesofauna, microbes and leaf litter decomposition in open field plots in a terrestrial forest floor community. in prep for *Pedobiologia*

ABSTRACT

Trophic cascades are thought to be common in terrestrial systems but most have been observed in aboveground webs. Of the relatively few studies in which researchers have considered pathways in terrestrial detrital food webs, the results are extremely variable and the effects of predator density on different groups of detritivores differ, as do the effects on leaf litter decomposition. Although the results differ among studies, they are similar in that none address the trophic level linking Collembola densities and rates of litter decomposition -- the microbes. We explored how the manipulation of forest floor predator densities, through predator removal, affects the densities of microbivores (e.g. Collembola), microbes (fungi and bacteria) and rates of leaf litter decomposition in open field plots in a mixed deciduous forest in NE Ohio. We hypothesized that the presence of predators would initiate top-down trophic cascades limiting densities of some prey taxa and affecting microbial biomass, and thus impacting indirectly the rate of leaf litter decomposition. There were no statistically significant effects of our treatment applications on leaf litter decay. However, litter in multiple predator removal arrays tended to decompose fastest and lose the most mass, followed by the single predator removal treatments, and finally the control which decomposed the slowest and lost the least mass. There were significantly more gamasid mites in centipede removal arrays, and the biomass of some bacterial phospholipids fatty acids (PLFAs) was lower in centipede removal arrays compared to the control. The ratio of fungal to bacterial PLFAs was highest in centipede removal arrays compared to control arrays. Overall diversity was lowest in the treatment with the most salamanders and highest in the two treatments in

which salamanders were removed although results were not significant. Previous work suggests web complexity may weaken trophic cascades. Biodiversity, spatial and, temporal heterogeneity are characteristics of temperate forest floor webs that may attenuate top-down cascades. Additionally, species interactions like competition and intraguild predation (IGP) add further complexity to food web dynamics. Despite such complexity, we found strong effects of predator density manipulation in unenclosed field plots. More work will be required in open field plots to test the effect of experimental manipulation of the numbers of species (or trophic levels) and the degree of habitat complexity to better predict the strength of trophic cascades in detrital forest floor webs.

INTRODUCTION

Trophic cascades occur when predation changes the abundance, biomass or productivity of populations or communities across two or more trophic links (Pace et al., 1999). Although often considered more common in aquatic ecosystems, several analyses have indicated that top-down trophic cascades are common in terrestrial systems (Pace et al., 1999; Schmitz et al., 2000; Halaj and Wise, 2001). Most previous trophic cascade studies focused on grazing, aboveground food webs. For instance, Beard et al. (2003) documented top-down effects of amphibian predators on herbivorous invertebrates and herbivory. However, much less is known about the relative strength of top-down versus bottom-up forces within belowground systems, even though most global primary production eventually enters detrital food webs (O’Neill and Reichle, 1980; Swift et al., 1979). Theory suggests that high species diversity, omnivory, intraguild predation, habitat complexity, non-consumptive interactions among predators, long chain length and high web connectance should attenuate top down trophic cascades (Scheu and Setälä, 2002; Wardle, 2002). Despite having many of the above mentioned characteristics, studies on terrestrial forest-floor webs have documented effects of predators on various trophic levels including litter invertebrate composition, and in some cases litter decay and nutrient cycling rates (Kajak, 1995, 1997; Wyman, 1998; Lawrence and Wise, 2000, 2004; Beard et al., 2002, 2003; Wise, 2004; Walton, 2005a 2005b; Walton et al. 2006; Dunham 2010, 2008; Hickerson et al., submitted).

Forest Floor Food Webs

Decaying plant material makes up the base of forest floor food webs and is the primary energy source in these systems (Fig. 1). Soil microbes (fungi and bacteria) act as primary decomposers, and microbivores, which are principally fungivores (Collembola; Chen et al., 1996 and orabatid mites; Coleman et al., 2004), act as secondary decomposers of detritus, although some microbivores also may serve as primary decomposers (Scheu and Falca, 2000). In addition to small arthropod fungivores (Collembola; Chen et al., 1996 and Acari; Coleman et al., 2004), macro-arthropod detritivores contribute to the breakdown of litter by serving as leaf fragmenters (Diplopoda and Isopoda). A wide range of both primary and secondary predators such as salamanders, spiders, gamasid mites, centipedes, and carabid beetles prey upon the fungivores and detritivores.

Much of the research examining terrestrial detrital webs examines the role of spider predation on fungivore density and leaf litter decomposition. For example, Lawrence and Wise (2000) found that the presence of spiders decreased densities of

Collembola and rates of straw litter decomposition relative to the spider removal treatment. These findings are similar those of Kajak and Jakubczyk (1977). The faster rate of litter decomposition in the removal treatment occurred presumably through fragmenting action brought about by high Collembola densities. More recent studies found similar effects of spider predation on Collembola abundance (Lawrence and Wise, 2004; Wise, 2004), but the opposite effect of spiders on litter decomposition (Lawrence and Wise, 2004), suggesting that high Collembola densities, caused by removing spider predators, may have over-exploited microbial populations thereby decreasing decomposition rates. Moya-Laraño and Wise (2007) examined the effect of ants on Collembola, among other groups, and found that densities of tomocerid Collembola increased in high density ant treatments in open field plots. Dunham (2010) investigated how an African rain forest understory community has responded to a non-native fire ant, *Wasmannia auropunctata* and to explore the possible pathways of interaction between grazing and detrital subsystems. They found strong negative effects of the ants on Collembola and leaf chewing herbivores, but slight positive effects on sap feeding herbivores. They also found that litter mass lost was negatively affected by top-down cascade possibly involving trophic and/or trait-mediated effects of ants on Collembola and litter consuming detritivores.

Other studies have focused on the effects of terrestrial plethodontid salamanders, *Plethodon cinereus*, on macrodetritivores, microbivores and litter decay. Wyman (1998) found salamander predation decreased larger detritivores such as millipedes, fly larvae, beetle larvae and mollusks, and decreased rates of litter decomposition. Rooney et al. (2000) found that the presence of salamanders in field enclosures increased numbers of

Collembola. Walton (2005) found that salamanders can produce significant reductions in mesofaunal detritivores, in excess of 30% for some taxa, in field plots, but this effect varies seasonally. Finally, Walton and Steckler (2005) and Walton et al. (2006) reported that predation by the salamander, *P. cinereus*, reduced numbers of large detritivores in laboratory microcosms, but increased abundance of mesofauna such as Collembola. Walton and Steckler (2005) hypothesized that salamander predation increased numbers of mesofauna by reducing macro-detritivore competitors and/or by subsidizing microbial growth with salamander feces. Walton and Steckler (2005) found no effect of salamander presence on rates of litter decomposition.

The difficulty in making overriding statements about how predators affect and are affected by lower trophic levels in detrital food webs is apparent (Table 1). It is possible that differences in experimental methods, including experiment duration, seasonal differences, the taxa considered and scale of the experiment (mesocosm versus field enclosures versus open field plots) are responsible for some of the inconsistency in the literature that make it difficult to compare results among studies (Walton, 2005). For example, of the above-mentioned studies on the effects of salamander predation, those that used enclosures (either field or laboratory mesocosms) found strong negative effects of salamanders on macrodetritivores and positive effects on microbivores, but differing litter decay rates. In contrast, the single study showing negative effects of *P. cinereus* on microbivores, i.e., Walton (2005), was conducted in the field using open plots.

Although the results differ among studies that examine top down effects in temperate forest floor webs (Table 1), few address the trophic level linking Collembola densities and rates of litter decomposition -- the microbes (Fig.1, but see Johnson et al.

2005). Mesofaunal microbivores are known to be abundant in temperate forest floor systems (Swift et al., 1979) and are therefore probably important regulators of microbes. Additionally, ecologists are increasingly recognizing the important role that soil microbes play with regard to ecosystem function, and tools are readily available to measure microbial community composition (Drenovsky et al., 2008).

We explored how the manipulation of forest floor predator densities, through predator removal, affects the densities of leaf fragmenters (macrodetritivores), mesodetritivores (e.g. Collembola), microbes (fungi and bacteria) and rates of leaf litter decomposition in open field plots in a mixed deciduous forest in NE Ohio. We hypothesized that the presence of predators would limit the strength of top-down trophic cascades initiated by predators. Assessing the effect of predators on lower trophic levels and rates of decomposition is one way to gain a better understanding of the strength of top down trophic cascades in terrestrial detrital webs and provides the link that ties together litter decay rates and microbivore abundance.

MATERIALS AND METHODS

Predator Removal

On 12 and 13 April, 2004 we placed 288 artificial cover objects (ACOs) on the forest-floor in the Cuyahoga Valley National Park (CVNP), Summit County, Ohio (41° 13′ 46.62″ N, 81° 31′ 7.77″ W). The field site is mixed deciduous forest that is dominated by *Acer saccharum* (Sugar Maple), *Fagus grandifolia* (American Beech), *Liriodendron tulipifera* (Tulip Poplar), and *Quercus rubra* (Red Oak) and lies on a north/northeast facing slope (elevational range 260 – 271 m). We used white ceramic floor tiles measuring 30 x 30 cm as ACOs. Artificial cover objects (ACOs) have been used successfully in previous studies to sample salamanders (Davis 1997, Houze and Chandler 2002). The ACOs were arranged in 32 arrays; each was separated by approximately two meters and covered a 20 x 40 m area. Each of the 32 arrays consisted of a cluster of nine ACOs arranged in three rows with 1 m spacing between tiles, all of which received the same treatment application. Each array was systematically assigned to one of four treatments (n = 8) 1) no removals/controls (NR); 2) salamander removal (SR); 3) centipede removal (CR); and 4) all predator removal (PR). Predators removed from the PR treatment included salamanders, centipedes, spiders, carabid beetles, and predatory flat worms (*Bipalium*).

Data collection began on 23 April 2004 and continued through November 2008. The field site was visited every two weeks, except for winter months, through the end of 2005, and weekly beginning in spring 2006 through the duration of the study (total of 98 visits). During each visit we hand-turned ACOs, counted and identified macrofauna from beneath each tile, and removed predators from the appropriate treatments. Arrays were visited in random order to remove any temporal bias in sampling.

Leaf litter decomposition from leaf bags

We examined the indirect effect of predator manipulations on the rate of leaf litter disappearance using leaf bags with known amounts of mixed canopy litter placed in our arrays in early spring. We collected and oven dried (60° C) for 3 days mixed leaf litter from our field site on 21 March 2007. We used mixed litter, rather than one species of leaves, to ensure that invertebrates and microbes experienced a natural microhabitat. We constructed 160 leaf bags (5 for each of 32 arrays) from black tulle fabric (2 mm mesh). The mesh size was large enough that both meso- and some macrofauna could enter and exit the leaf bags. Each bag was made from a square foot of mesh, rolled around 10 g of oven dried leaf litter and secured on both ends with zip strips. On 6 April 2007 five bags were randomly positioned, and secured with a thin metal stake, in a row above the center ACO in each array. Bags were left in place for just over 6 months before the first bag was pulled. One bag was removed at random from each array on 19 October 2007, 8 April 2008, 13 June 2008, 6 September 2008 and 5 November 2008. After invertebrates were removed from leaf bags, the litter bag samples were placed in a drying oven at 60º C, dried to constant weight, and re-weighed to obtain the percent litter mass loss over time and to calculate rates of disappearance.

Invertebrates in leaf bags

The effect of the various predator removal treatments on invertebrate (mesofauna and macrofauna) abundance was evaluated by examining invertebrates inhabiting the leaf bags after they were removed from the field site on the five previously mentioned sampling dates. We used Berlese extraction into 70% ethanol to separate invertebrates from leaf bag samples. Extractors were run for 72 hours. Invertebrates were then identified to taxonomic category and counted. We used blind protocols for litter bag invertebrate sampling and identification to minimize potential investigator bias. Data are presented as density per g dry leaf mass.

PLFA analysis

Phospholipids are major components of cell membranes and their polar heads and ester-linked side chains (FA) vary in composition between eukaryotes and prokaryote (fungi versus bacteria) and also among prokaryotic groups (Gram + versus Gram -). These compounds rapidly degrade upon cell death making them good indicators of living organisms in soils (Vestal and White, 1989). PLFA can supply information about specific fatty acids that act as biomarkers of certain functional groups. The sum of all PLFAs provides a proxy for total microbial biomass (minus Archael biomass), and the number of PLFAs detected provide a rough diversity estimate. PLFA is an effective method because it provides a snapshot of the living microbial community (Drenovsky et al., 2004) and it is useful for detecting broad changes among treatments (Bossio and Scow, 1998).

On one day in May, July and October of 2006, three sub-sample soil cores (10.0 cm depth) from around the center ACO were taken from each replicate of our four treatment groups. Arrays were evenly disturbed to avoid uncontrolled effects in other experiments. The three subsamples from each array were immediately combined, homogenized, placed in 50 ml centrifuge tubes and placed on ice for transport to the ultra cold freezer (-20°C) at John Carroll University. Soil removal instruments were sterilized with isopropyl alcohol between each array. Frozen soil samples (32 arrays x 3 dates = 96 samples) were shipped to the Scow Soil Microbial Ecology Lab at the University of California, Davis for blind phospholipids fatty acid analysis (PLFA). PLFA analysis was not possible for one of the 96 soil samples (May SR, N = 7).

Statistical analysis

To test the null hypothesis that predator density manipulations would not influence leaf litter decomposition rate, invertebrate abundance, or the soil microbial community (as represented by PLFA biomarkers from soil samples) we used a GLM MANOVA for all response variables (including litter decay rates, the most abundant invertebrates, total number of PLFAs, total bacterial PLFAs, total fungal PLFAs, fungal :bacterial ratios, total invertebrate density, and diversity indices for invertebrates and PLFAs) to examine the effects of two factors (treatment and month) as well as the interaction between the two. When MANOVA results were significant at $P < 0.05$, differences among means were assessed using univariate tests. We used Shannon's diversity index for both mesofauna taxa in our leaf bags and PLFAs in soil samples.

Principal components (PCs) of the PLFA data were used to decrease the dimensionality of the microbial data set and to minimize the likelihood of a type I error by analyzing each fatty acid separately. PCs were also used to identify PLFAs that responded to treatment manipulations in similar ways. PCs with eigenvalues > 1 were retained for GLM MANOVA. Tests of individual PLFAs were limited only to those that loaded heavily on PCs that were significantly affected by our treatments.

We used an exponential decay model to estimate the rate of litter decay. The decomposition coefficient (k) was estimated with the equation $y = e^{-kx}$, where y is the original mass remaining, e is the natural log and x is the time given in weeks. The higher the k value, the faster the decomposition rate. Over the duration of the study four leaf bags gained weight and were removed from the analysis (3 from SR and 1 from CR). Two of the 160 invertebrate samples were lost to experimenter error (1 from CR one from PR). Only the invertebrate taxa and PLFAs that made up greater than 1% of the total for each sample were analyzed. Invertebrate data were $\log_{10}$ (x+1) transformed.

RESULTS

Predator manipulations

Removal treatments (CR, SR and PR) had an effect on the predator community at our field site. We were able to significantly decrease focal predators in all treatments except centipede removals. Centipedes were reduced in the centipede removal (CR) treatment by only 7% (Table2).

Leaf litter decomposition from leaf bags

After 20 months 30% of the original mixed litter from leaf bags had disappeared, but predator manipulations had no significant effect on mass loss (Table 3, Fig. 2a). However there was a trend for the rate of litter decay to be fastest in the PR treatment followed by SR, CR and control respectively (Table 3, Fig. 2b). The presence of predators seemed to have slowed the decomposition process, although this pattern is not statistically significant ($F_3 = 0.680$, $P = 0.573$, Table 3). The removal of single predators had no effect on the rate of decay but when multiple predators were removed the rate of litter loss tended to be faster (Fig. 2).

Invertebrates in leaf bags

We counted and identified over 29,000 individual invertebrates belonging to 25 invertebrate taxa from 160 leaf bags (Table 4). Mites (Acari) and springtails (Collembola) made up 89% of the total invertebrates by number. Collembola were slightly more abundant than mites (48% and 41% respectively). Of the 25 taxa identified, only the most abundant (>1% of the total) were used in our statistical analysis. Total invertebrate abundance and diversity did not differ among treatments in our leaf bags. Overall we found no significant effect of treatment manipulations, but date had a strong effect on the invertebrate abundances in our leaf bags (Table 5). Of the 10 individual taxa analyzed, only gamasid mites were affected significantly by our treatment application and they differed between the centipede removal (mean = 7.50 per g dry litter) and control treatments (mean = 4.89 per g dry litter, Table 4, Fig. 3a). There were no treatment effects on the various groups of abundant Collembola but there were significant differences by date (Fig. 3b-f). We measured species diversity of invertebrates from the leaf bags and found no significant differences among treatments and the control ($F_3 = 1.62$, $P = 0.187$). The centipede removal treatment was the least diverse (Shannon's diversity index = 1.82) and the SR and PR had the highest diversity with equal indices (Shannon's diversity index = 1.90).

PLFA analysis

The number of PLFAs (a proxy for diversity) did not differ in treatments compared to controls (Tables 6 and 7). Mean microbial biomass, the numbers of fungal PLFAs and bacterial PLFAs were highest in the control and lowest in the centipede removal plots (Table 6), although this trend was not statistically significant (Table 7).

The fungal to bacterial ratio increased in the centipede removal treatment relative to the control (Table 6 and 7, Fig. 4a) and differed between May (mean = 0.057, SE = 0.039) and July (Fig. 4b, mean = 0.070, SE = 0.038, $F_3 = 3.12$, $P = 0.049$). We calculated Shannon's diversity index for PLFAs in each soil sample. There were no differences in mean diversity among experimental treatments and the control ($F_3 = 0.70$, $P = 0.556$), but there were differences among seasons. Diversity was highest in summer (mean = 3.13) followed by spring (mean = 3.09) and then fall (mean = 3.06; $F_2 = 17.05$, $P < 0.001$).

There were nine principal components (PCs) that had eigenvalues > 1 and these made up 83% of the total variation in PLFAs detected from our soil samples (Table 8). The factor scores from these nine PCs were retained as dependent variables in our MANOVA. Independent fixed factors included treatment (NR, CR, PR, and SR), date (spring, summer, and fall) and plot orientation (east and west). We found an overall significant effect of treatment, month and plot orientation but failed to detect significant two or three way interactions (Table 9). PCs 2, 6 and 9 were significant in our univariate analysis and accounted for 13% of the total variance in our data. PC 2 and was a descriptor of seven PLFAs that had factor loading greater than 0.5 (Table 8). The value of PC 2 differed significantly among treatments and was lowest in the CR treatment (Fig. 5). Analyses for the individual PLFAs that loaded strongly on PC 2 showed similar responses (Table 10). For example, 18:1ω7t and 15:0 3OH were also lowest in the CR treatment. PC 2 also differed slightly by month (Fig. 5b) and plot orientation (Fig. 5c), although these trends were not statistically significant (Table 10). PC 2 was higher in all treatment arrays in the east plot compared to the west plot except CR arrays (Fig. 5c). The individual PLFA 18:1ω7t responded similarly with regard to plot orientation (Table

10). PLFA 15:0 3OH also differed by sampling month and plot orientation (Table 10). PLFA 15:0 3OH was lowest in CR arrays in May and October but did not differ among arrays in July. PLFA 15:0 3OH also responded to treatment manipulations differently in east compared to west plots. In both east and west plots 15:0 3OH was lowest in the CR treatment but this pattern was statistically significant only in the east plot (Table 10). PC 6 accounted for 3% of the variation and was defined primarily by one individual PLFA, 16:0 3OH. The value of PC 6 differed by treatment and marginally by plot orientation but there was no significant interaction (Table 10, Fig. 6). PC 9 was responsible for 2% of the variation in the PLFA data and defined most strongly by PLFA 14:0 2OH. PC 9 differed significantly by treatment, date and plot and there was a significant treatment x date interaction (Table 10, Fig. 7). Further inspection of PLFA 14:0 2OH revealed that this PLFA was present in only nine of 96 soil samples and of those nine six were centipede removal arrays.

DISCUSSION

Predator effects on mesofauna

We examined the effect of predator removal from open field plots in a temperate forest floor food web to determine if manipulation of single and multiple predator densities would translate to a trophic cascade that would be detectable at multiple levels within the web (i.e. mesofauna, microbes and the level of the basal resource, leaf litter). There were strong treatment effects on intraguild predators (Table 2 and see Hickerson et al., submitted) and macrofaunal detritivores quantified from beneath artificial cover objects (ACOs) in the arrays (see Hickerson et al., submitted), but there was no significant effect of the treatment applications on mesofauna invertebrate taxa quantified from leaf bags with the exception of one taxon, gamasid mites. These predatory mites were more abundant in centipede removal arrays (CR) compared to controls (NR).

Top-down effects on mesofauna abundance may have been weak on most taxa in our litter bags for four reasons 1) large predators were unable to enter the bags 2) the microhabitat in the litter may have been spatially complex relative to the area beneath the ACOs 3) web structure (i.e. microhabitat complexity, omnivory, species diversity etc.) may have effectively attenuated or weakened the effect of predator manipulation on litter bag invertebrates and 4) replacement of one predator by a different, but functionally equivalent predator may result in insignificant suppression of mesofauna.

Given that the mesh size of our leaf bags was 2 mm it would have been unlikely that most adult macrofauna could get into the bags. In some cases it may have been possible for larval forms to enter the leaf bags and become trapped if they metamorphosed while in the bag but large predators (e.g. salamanders and spiders) were probably unable to enter the bags. Kampichler and Bruckner (2009) conducted a meta-analysis of literature from the 1960’s to the end of 2005 on litter bag studies investigating the role of microarthropods on decomposition. They suggested that the effects on decomposition reported in the literature are cumulative effects of the true microarthropod effect plus mesh size effects. When the data were corrected for the estimated mesh size effect the results revealed negative effects of microarthropods on decomposition, a result opposite to the widely accepted idea that microarthropds have a positive effect on litter decay. The authors concluded that after 40 years of litter bag studies our knowledge is still limited on the matter. Since our experiment was not designed to include a comparison of the effect of different mesh sizes we do not know whether our treatment applications in the surrounding field array affected interactions within the litter bags. It is possible that the microhabitat within the litter bags differed substantially from that beneath the ACOs.

We quantified macropredators and macrodetritivores from beneath ACOs defended as territories by *Plethodon cinereus*. It was within this very specifically defined microhabitat that we expected interactions among the macrofauna to occur at least during periods when prey resources are limited in the surrounding forest floor. It is however possible that the strong effects found under ACOs were attenuated in the surrounding leaf litter because of microhabitat complexity that exists in forest litter but is reduced beneath cover objects on the forest floor. Trophic cascades are expected to be weak both in complex habitats, and in complex webs, relative to more simple ones (Polis and Strong 1996; Pace et al., 1999). For example, Polis and Strong (1996) argued that omnivory resulting in increased connectance within diverse food webs may dissipate trophic cascades because of the increased number of potential pathways in the web. As a result, the removal of predators could be unpredictable and non-repeatable in natural communities. Polis and Strong (1996) discouraged researchers from thinking about webs in the context of "trophic levels connected in a single linear chain." They suggested that such oversimplifications make it impossible to understand community dynamics. We can imagine that such complexities would be magnified in terrestrial, detrital webs in temperate forests where the microhabitat in leaf litter is heterogeneous and may provide refuge to many members of the forest floor community.

Finally, if consumer trophic levels are occupied by many generalist predators with broad and extensively overlapping diets it is possible that our manipulation of one or just a few predators may result in little overall effect on mesofauna. For example, in arrays in which salamanders were removed (SR), there were significant increases in spider abundance. If spiders and salamanders share prey taxa, and consume similar amounts, there may be very little effect of the treatment application on mesofauna abundance. Further studies that assess diet overlap and functional equivalence of various predators would be necessary to tease apart these potential connections.

Predator effects on microbes

Although we found very little effect of predator removal on mesofauna abundance in leaf litter bags from our field plots, we did find a significant effect of our predator manipulations on the microbial community. Although diversity and biomass of the microbes in our soil samples as measured by summing total, fungal and bacterial phospholipid fatty acids (PLFAs) were not affected, we did observe highly significant effects of treatment, season and plot orientation on newly constructed variables (principal components) from our data reduction analysis. At our field site salamanders were most abundant in the centipede removal (CR) treatment (N = 2105), followed by the control (NR, N = 1784), and then the salamander removal (SR) and multiple predator removal (PR) treatments (N = 1288 and 1287 respectively). Given that we were only able to decrease centipedes in CR arrays by 7% it seems most likely that the high densities of salamanders in CR arrays may be an important factor affecting more basal trophic levels. For example, there were significantly more gamasid mites in CR arrays and lower biomass of specific bacterial PLFAs compared to control (NR) arrays. The ratio of fungal to bacterial PLFAs was highest in CR arrays compared to NR arrays and overall species diversity was lowest in the CR treatment and highest in the two treatments in which salamanders were removed (SR and PR). These results suggest that top predators like the salamander, *Plethodon cinereus,* may have negative cascading effects on some bacterial microbes through indirect channels.

P. cinereus densities at our field site were significantly higher in the CR arrays compared to controls and the some of the microflora PLFA biomarkers were depressed in CR arrays. One mechanism potentially responsible for this effect could be the presence of strong interactions between soil bacteria and the cutaneous bacteria that reside on the epidermis of *P. cinereus*. Species of bacteria that live on the skin of terrestrial, direct developing frogs and salamanders that brood their eggs have the ability to inhibit the growth of fungal pathogens that attack embryos (Austin, 2000). These cutaneous bacteria are specific to them and somewhat different than the microbial community found in the soil (Austin, 2000; Culp et al., 2007).

Given that the fungi in our soil samples were relatively unaffected by our treatment manipulations it is possible that interactions (e.g. competition) among/between soil and cutaneous bacteria from salamander skin were responsible the high fungal:bacterial ratio and the suppression of some specific bacterial PLFAs in CR arrays. Recall that we observed significantly more *P. cinereus* in CR arrays relative to controls. Long term use of territories centered on cover objects should concentrate and distribute salamander specific microflora via skin secretions and fecal marking. Therefore, *P. cinereus* may cause different microbial assemblages within territories that are perhaps emphasized through competition mediated by antibiotic properties of cutaneous microbes. This possibility could be explored further by designing experiments that would compare soil microbial assemblages in salamander territories to the soil microbial communities in microhabitats not occupied by salamanders.

Predator effects on litter decay

Although litter disappearance rates were statistically unaffected by our treatment applications, the trend was such that leaf litter in multiple predator removal arrays decomposed fastest and loss the most mass, followed by the single predator removal treatments (CR and SR) and the control, which decomposed the slowest and lost the least mass. These results were not statistically significant but the trend of a faster decay rate in the multiple predator removal treatments may have been the result of a slight positive, indirect effect of predator removals on mesofauna abundance under cover objects in those arrays, a microhabitat that was not assessed for mesofauna in our study.

Our study illustrates the importance of species diversity and functional redundancy in stabilizing ecosystems. We removed single (CR and SR) and multiple (PR) predators from open field plots and saw strong effects within the predator guild, on macrofaunal detritivores, and numbers of ant colonies. We saw only very weak effects on mesofauna and litter decay. It is possible that the dominant species of predators changed and affected some groups, but trophic level function did not.

The observed effects on the soil microbial community demonstrate the subtle, indirect effects that predators can have within ecosystems, in addition to direct consumption. We recognize that with our experimental design distinctions cannot be made between direct and indirect effects but our results show significant effects at multiple trophic levels within this web, including the microbial level. This is the only study that we are aware of that has detected changes in the abundance of organisms at the microbial level from predator manipulated open field plots in an eastern deciduous forest floor food web. We hypothesize that terrestrial, territorial salamanders may be important determinants of spatial variability in microbial communities and microhabitats within forests.

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Table IV - 1 Summary of relevant studies examining the effects of predation on detritivores, microbivores and litter decay in temperate deciduous forest floor webs. Arrows indicate the density increase or decrease in the presence of the predators.

authors	year	predator	macrodetritivores	microbivores	litter decay
Wyman	1998	salamanders	↓	------	↓
Lawrence & Wise	2000	spiders	------	↓	↓
Rooney *et al.*	2000	salamanders	------	↑	------
Lawrence & Wise	2004	spiders	------	↓	↑
Wise	2004	spiders	------	↓	------
Walton	2005	salamanders	no change	↓	------
Walton & Steckler	2005	salamanders	↓	↑	no change
Walton *et el.*	2006	salamanders	↓	↑	------
Moya-Laraño & Wise	2007	ants	------	↑	------
Dunham *et al.*	2010	invasive ants	↓↑ (macrofauna, plant herbivores)	↓	no change

Note: Dashed lines represent unmeasured variables.

Table IV - 2. The effectiveness of predator removal. Mean (SE) per sampling date and total percent reduction for each predator group in control arrays compared to the appropriate removal arrays.

Predator removed	mean # in NR	mean # in removal	% reduction	F_3	P
salamanders	2.28 (0.01)	1.64 (0.01)	28	71.53	**<0.000**
spiders	2.27 (0.01)	1.86 (0.01)	18	87.11	**<0.000**
carabid beetles	1.09 (0.01)	0.75 (0.01)	31	24.43	**=0.008**
centipedes	0.71 (0.01)	0.66 (0.01)	7	2.46	=0.998

Note: F values are between-subjects effects in a GLM MANOVA. Dunnett T3 was used as a post-hoc test because of unequal variance among groups. NR = control arrays. Statistically significant *P* values are bold.

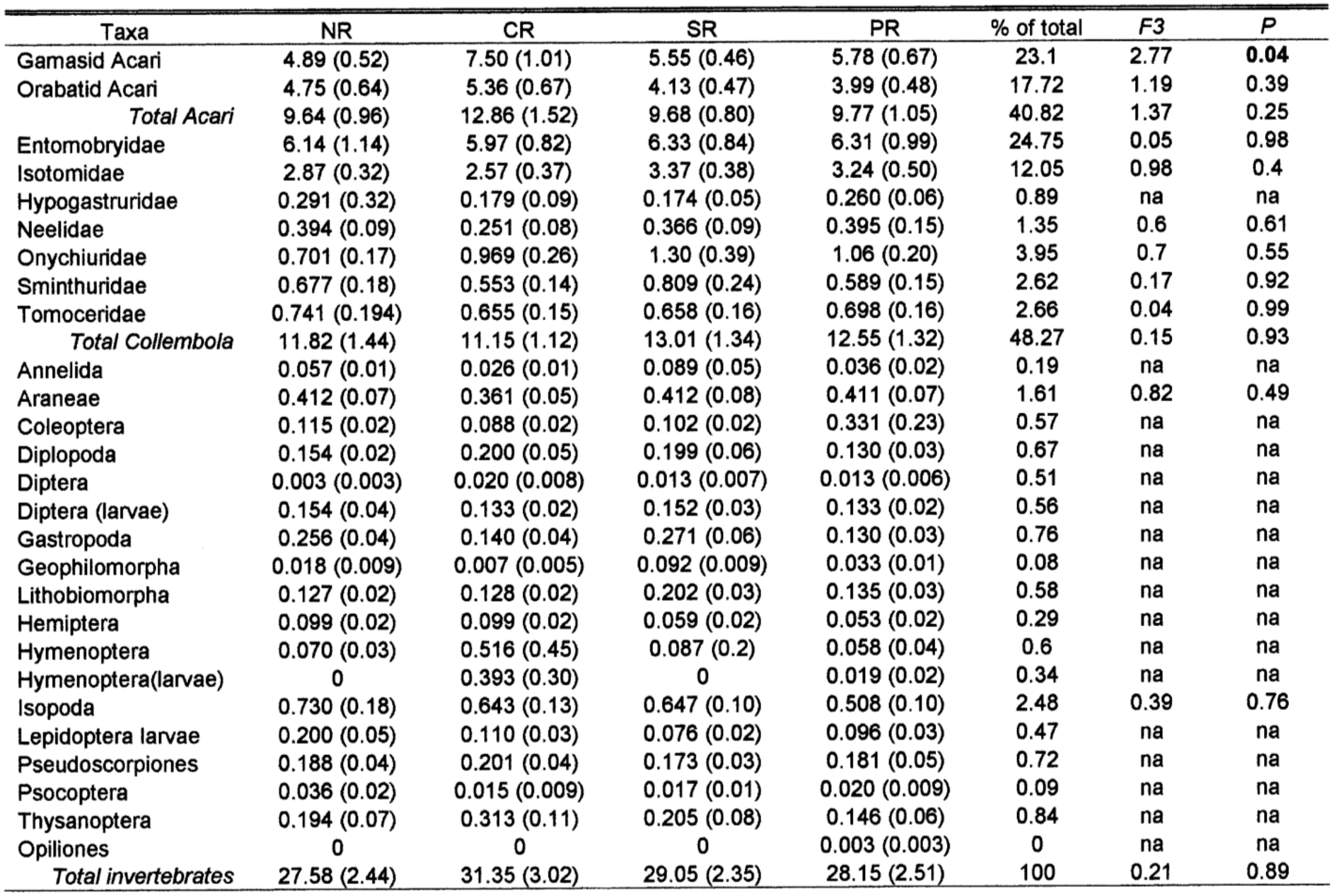
Table IV - 3. Mean mixed deciduous leaf litter remaining from five replicates per treatment on each of five sampling dates. Means are percentages of original mass*

Treat.	Oct. 07	Apr. 08	Jun. 08	Sep. 08	Nov. 08	mean (trts.)
NR	87.04	78.01	72.88	72.86	67.02	75.56
CR	82.95	79.51	72.63	73.28	63.78	74.43
SR	82.54	78.21	71.27	64.35	70.58	73.39
PR	85.43	74.00	68.72	64.08	66.58	71.76
mean (dates)	84.49	77.43	71.38	68.64	66.99	

Note: There were no treatment effects on litter decomposition ($F_3 = 1.36$, $P = 0.257$). Sampling date had a significant effect on litter decay ($F_4 = 17.75$, $P < 0.000$).

* Original mass of each leaf bag was 10 g

Table IV - 4 Mean (SE) density g^{-1} dry leaf litter of invertebrates in four treatments (N=32) at our field site. Effects of predator manipulation treatments are given for the ten most numerically dominant groups of invertebrates.



Taxa	NR	CR	SR	PR	% of total	*F3*	*P*
Gamasid Acari	4.89 (0.52)	7.50 (1.01)	5.55 (0.46)	5.78 (0.67)	23.1	2.77	**0.04**
Orabatid Acari	4.75 (0.64)	5.36 (0.67)	4.13 (0.47)	3.99 (0.48)	17.72	1.19	0.39
Total Acari	9.64 (0.96)	12.86 (1.52)	9.68 (0.80)	9.77 (1.05)	40.82	1.37	0.25
Entomobryidae	6.14 (1.14)	5.97 (0.82)	6.33 (0.84)	6.31 (0.99)	24.75	0.05	0.98
Isotomidae	2.87 (0.32)	2.57 (0.37)	3.37 (0.38)	3.24 (0.50)	12.05	0.98	0.4
Hypogastruridae	0.291 (0.32)	0.179 (0.09)	0.174 (0.05)	0.260 (0.06)	0.89	na	na
Neelidae	0.394 (0.09)	0.251 (0.08)	0.366 (0.09)	0.395 (0.15)	1.35	0.6	0.61
Onychiuridae	0.701 (0.17)	0.969 (0.26)	1.30 (0.39)	1.06 (0.20)	3.95	0.7	0.55
Sminthuridae	0.677 (0.18)	0.553 (0.14)	0.809 (0.24)	0.589 (0.15)	2.62	0.17	0.92
Tomoceridae	0.741 (0.194)	0.655 (0.15)	0.658 (0.16)	0.698 (0.16)	2.66	0.04	0.99
Total Collembola	11.82 (1.44)	11.15 (1.12)	13.01 (1.34)	12.55 (1.32)	48.27	0.15	0.93
Annelida	0.057 (0.01)	0.026 (0.01)	0.089 (0.05)	0.036 (0.02)	0.19	na	na
Araneae	0.412 (0.07)	0.361 (0.05)	0.412 (0.08)	0.411 (0.07)	1.61	0.82	0.49
Coleoptera	0.115 (0.02)	0.088 (0.02)	0.102 (0.02)	0.331 (0.23)	0.57	na	na
Diplopoda	0.154 (0.02)	0.200 (0.05)	0.199 (0.06)	0.130 (0.03)	0.67	na	na
Diptera	0.003 (0.003)	0.020 (0.008)	0.013 (0.007)	0.013 (0.006)	0.51	na	na
Diptera (larvae)	0.154 (0.04)	0.133 (0.02)	0.152 (0.03)	0.133 (0.02)	0.56	na	na
Gastropoda	0.256 (0.04)	0.140 (0.04)	0.271 (0.06)	0.130 (0.03)	0.76	na	na
Geophilomorpha	0.018 (0.009)	0.007 (0.005)	0.092 (0.009)	0.033 (0.01)	0.08	na	na
Lithobiomorpha	0.127 (0.02)	0.128 (0.02)	0.202 (0.03)	0.135 (0.03)	0.58	na	na
Hemiptera	0.099 (0.02)	0.099 (0.02)	0.059 (0.02)	0.053 (0.02)	0.29	na	na
Hymenoptera	0.070 (0.03)	0.516 (0.45)	0.087 (0.2)	0.058 (0.04)	0.6	na	na
Hymenoptera(larvae)	0	0.393 (0.30)	0	0.019 (0.02)	0.34	na	na
Isopoda	0.730 (0.18)	0.643 (0.13)	0.647 (0.10)	0.508 (0.10)	2.48	0.39	0.76
Lepidoptera larvae	0.200 (0.05)	0.110 (0.03)	0.076 (0.02)	0.096 (0.03)	0.47	na	na
Pseudoscorpiones	0.188 (0.04)	0.201 (0.04)	0.173 (0.03)	0.181 (0.05)	0.72	na	na
Psocoptera	0.036 (0.02)	0.015 (0.009)	0.017 (0.01)	0.020 (0.009)	0.09	na	na
Thysanoptera	0.194 (0.07)	0.313 (0.11)	0.205 (0.08)	0.146 (0.06)	0.84	na	na
Opiliones	0	0	0	0.003 (0.003)	0	na	na
Total invertebrates	27.58 (2.44)	31.35 (3.02)	29.05 (2.35)	28.15 (2.51)	100	0.21	0.89

Note: na = rare taxa that were not statistically analyzed.

Table IV - 5. MANOVA results for comparisons of mesofauna from leaf bags among treatments (CR, SR, PR, NR), and dates (Oct 07, Apr 08, Jun 08, Sep 08, Nov 08)

Pillais Trace	df	*F* - statistic	*P* - value
treatment (T)	3	1.12	0.192
sampling date (D)	4	10.52	**<0.0001**
T x D	12	0.709	0.997

Note: Significant results are bold.

Table IV - 6 Mean PLFAs detected in soil samples from the control (NR) and predator removal treatments (CR = centipede removal, SR = salamander removal and PR = all predator removal) during the three sampling dates. Overall treatment means are bold. PLFA concentrations are given in nmolg^{-1} soil.

Treat.	Month	# PLFAs	Total Biomass	All Fungi [a]	All Bacteria [b]	Fungal: Bacterial Ratio[c]
NR	May	52.25	107.99	14.36	28.60	0.044
	July	49.13	111.96	16.77	29.51	0.058
	Oct	49.13	100.42	15.85	28.88	0.058
	Overall	50.17	106.79	15.66	29.00	0.053
CR	May	50.25	93.76	14.39	22.94	0.060
	July	52.13	101.12	15.93	26.35	0.090
	Oct	47.63	83.85	13.37	22.58	0.061
	Overall	50.00	92.91	14.56	23.96	0.070
SR	May	53.14	109.21	17.47	28.17	0.057
	July	51.63	100.46	15.43	25.94	0.065
	Oct	48.25	91.80	13.67	24.57	0.056
	Overall	51.07	100.49	15.52	26.23	0.059
PR	May	51.63	97.49	14.01	25.04	0.065
	July	46.88	99.04	14.72	25.79	0.066
	Oct	50.38	102.17	17.38	28.29	0.068
	Overall	49.63	99.57	15.37	26.37	0.066

[a] Sum of 16:1ω5c, 18:3ω6,9,12c, 18:1ω9c, 18:2ω6,9c (Vestal and White1989, Potthoff *et al*. 2006)

[b] Sum of 15:0i, 15:0a, 15:0, 16:0i, 16:0a, 17:0, 17:0i, 17:0a, 17:0c, 19:0c (Potthoff *et al*. 2006)

[c] 18:2ω6c/15:0i, 15:0a, 15:0, 16:0i, 16:0a, 17:0, 17:0i, 17:0a, 17:0c, 19:0c (Bossio and Scow 1998)

Table IV - 7 ANOVA results for PLFA variables in soil samples from the control (NR) and predator removal treatments (CR = centipede removal, SR = salamander removal and PR = all predator removal).

	Treatment (T)		Month (M)		T x M interaction	
	F_3	P	F_2	P	F_6	P
PLFA variables						
# PLFAs (diversity)	0.52	0.668	4.68	**0.012**	2.05	<u>0.068</u>
Total Biomass	1.60	0.196	1.45	0.241	0.55	0.768
All Fungi	0.36	0.784	0.28	0.760	1.49	0.193
All Bacteria	2.19	<u>0.095</u>	0.45	0.638	0.59	0.734
Fungi:Bacteria	3.04	**0.033**	3.12	**0.049**	1.18	0.419

Note: Significant results are bold. Marginally significant results are underlined.

Table IV - 8. Principal components analysis (PCA) on PLFA data resulted in nine PC's with
eigenvalues greater than one. These PC's were retained for multivariate analysis.

PC	eigenvalue	% variance	cumulative % variance
1	27.39	49	49
2	4.36	8	57
3	3.38	7	63
4	2.46	4	68
5	2.11	4	72
6	1.95	3	75
7	1.64	3	78
8	1.43	3	81
9	1.16	2	83

Note: PCs significantly affected by treatment in the overall MANOVA are bold.

Table IV - 9. MANOVA results for comparisons of PC's computed from original PLFA's among treatments (CR, SR, PR, NR), months (May, July, October) and plot orientation (east/west).

	df	*F* - statistic	*P* - value
treatment (T)	3	2.22	**0.001**
sampling date (D)	2	10.32	**<0.0001**
plot orientation (P)	1	3.13	**0.004**
T x D	6	0.921	0.635
T x P	3	1.38	0.11
T x D x P	6	0.805	0.836

Note: Significant results ($P < 0.05$) are shown in bold.

Table IV - 10. Univarite tests for comparisons among treatments, sampling dates and plot orientation. Principal components (PCs) are from phospholipid fatty acids (PLFAs) with eigenvalues greater than one and were affected by independent variables. Individual PLFAs are a subset of those that loaded heavily on PC 1.

	indep. variables	df	*F*	*P*
	PC 2			
treatment (T)		3	3.15	**0.03**
sampling date (D)		2	2.46	0.09
plot orientation (P)		1	3.59	0.062
T x D		6	1.05	0.403
T x P		3	1.35	0.265
T x D x P		6	0.74	0.62
	PC 6			
treatment (T)		3	4.19	**0.009**
sampling date (D)		2	0.76	0.473
plot orientation (P)		1	3.79	0.056
T x D		6	0.5	0.803
T x P		3	0.21	0.891
T x D x P		6	0.08	0.998
	PC 9			
treatment (T)		3	4.42	**0.007**
sampling date (D)		2	6.09	**0.004**
plot orientation (P)		1	4.67	**0.034**
T x D		6	4.13	**0.001**
T x P		3	1.63	0.191
T x D x P		6	1.38	0.235
	18:ω7t			
treatment (T)		3	7.02	<**0.001**
sampling date (D)		2	1.34	0.268
plot orientation (P)		1	16.8	<**0.001**
T x D		6	1.1	0.374
T x P		3	1.62	0.192
T x D x P		6	1.8	0.112

15:0 3OH			
treatment (T)	3	3.06	**0.034**
sampling date (D)	2	3.17	**0.048**
plot orientation (P)	1	3.97	0.05
T x D	6	1.41	0.222
T x P	3	0.25	0.861
T x D x P	6	1.13	0.355
14:0 2OH			
treatment (T)	3	7.63	<**0.001**
sampling date (D)	2	6.68	**0.002**
plot orientation (P)	1	6.95	**0.01**
T x D	6	6.86	<**0.001**
T x P	3	1.28	0.289
T x D x P	6	4.38	**0.001**

Note: Significant results ($P < 0.05$) are shown in bold; marginally significant results ($0.10 > P > 0.05$) are underlined.

Figure Legend

Figure IV - 1. A simplified terrestrial detritus-based food web. Oval represents trophic level not measured in studies looking at the effects of predators (salamanders, spiders, ants) on detritivore abundance. Dashed lines represent indirect links. Rectangles represent commonly measured variables.

Figure IV - 2. The effect of predator density manipulations on mixed leaf litter decomposition. Mean mass remaining (A), and rates of litter disappearance (B) in the three treatments and the control over 80 weeks. Open squares = controls, NR; open circles = centipede removals, CR; closed circles = salamander removals, SR and closed squares = all predator removals, PR.

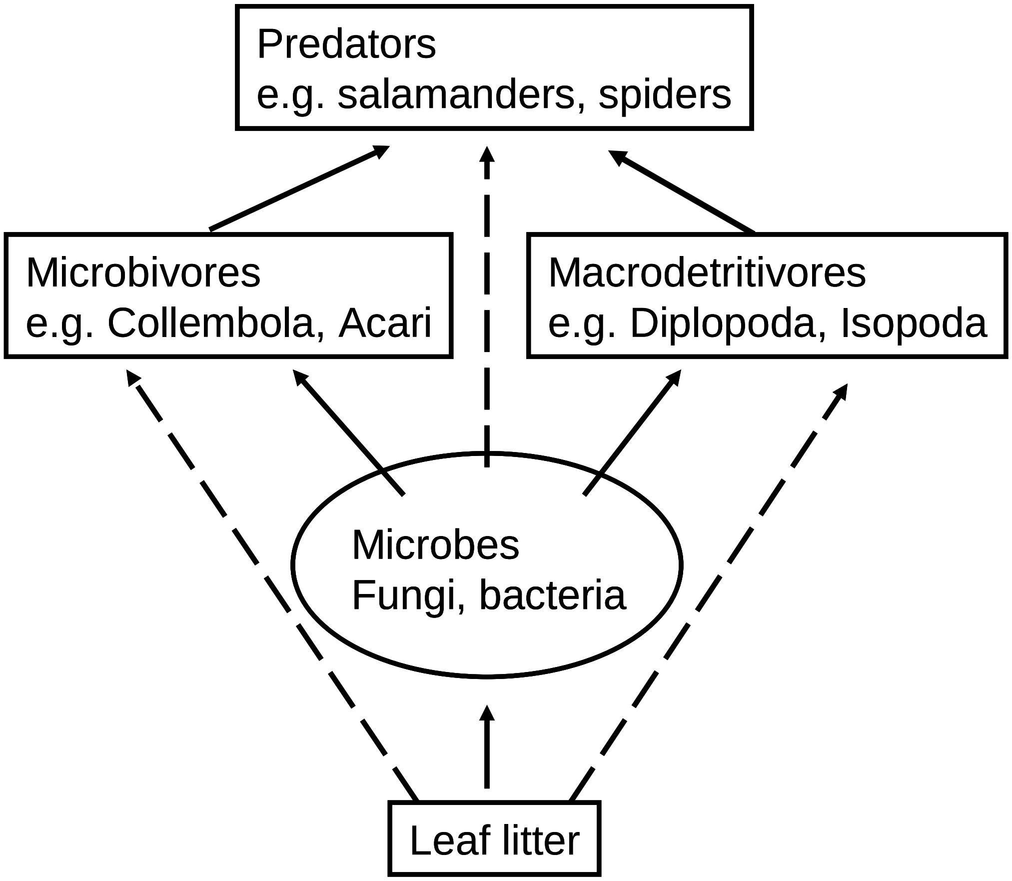
Figure IV - 3. Density of select taxa of mesofauna in extracted from leaf bags on five dates. Leaf bags were from open field plots in each of three treatments and the control (N=8). (A) gamasid mites, (B-F) various Collembola families; (B) isotomids, (C) onychiurids, (D) neelids, (E) tomocerids and (F) sminthurids. Open squares = controls, NR; open circles = centipede removals, CR; closed circles = salamander removals, SR and closed squares = all predator removals, PR.

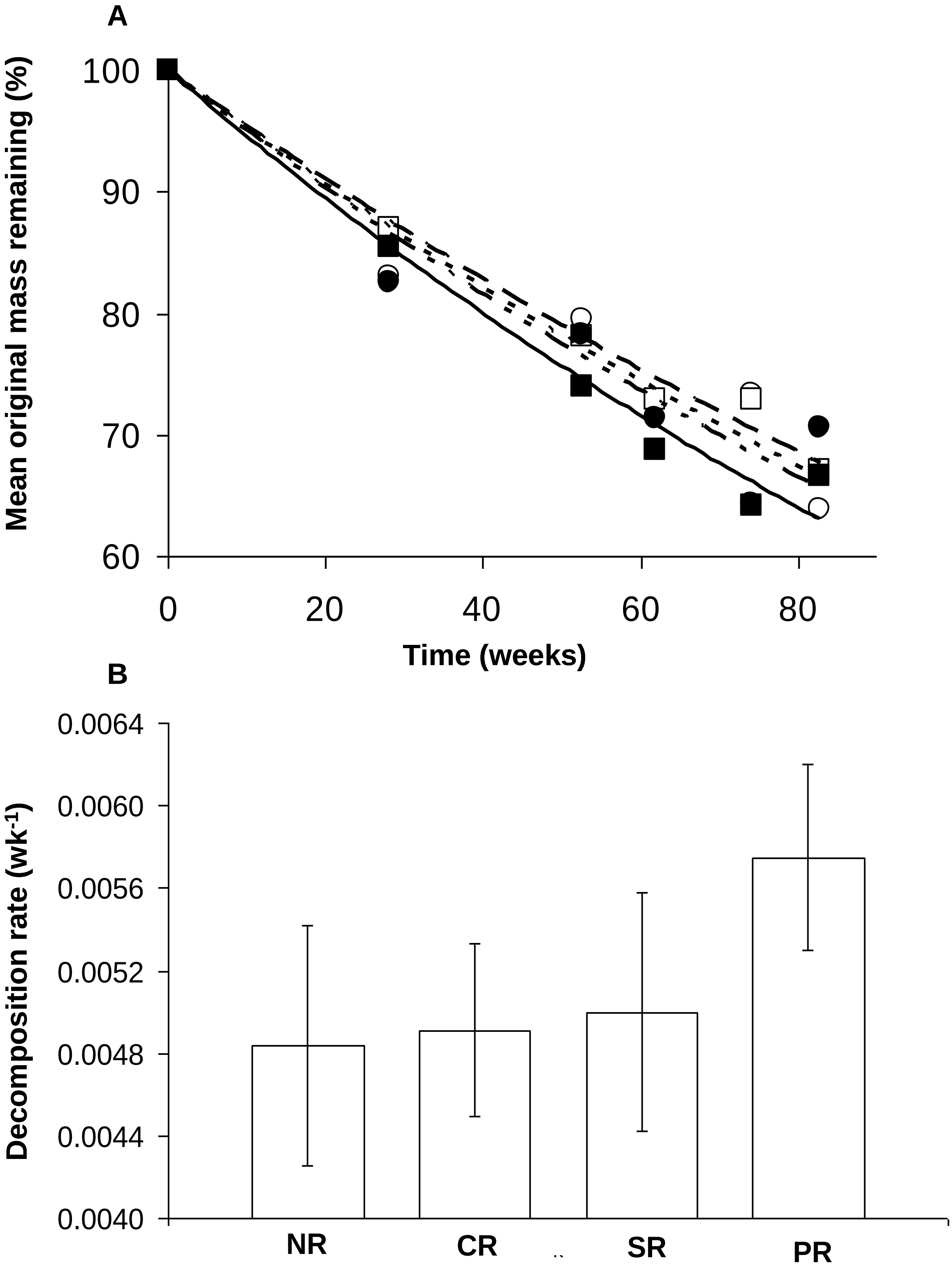
Figure IV - 4. (A) fungal:bacterial ratio in each treatment. CR = centipede removal – open circles, NR = control (nothing removal) – open squares, PR = all predator removal – closed squares, and SR = salamander removal – closed circles. (B) fungal:bacterial ratio plotted as a function of sampling month.

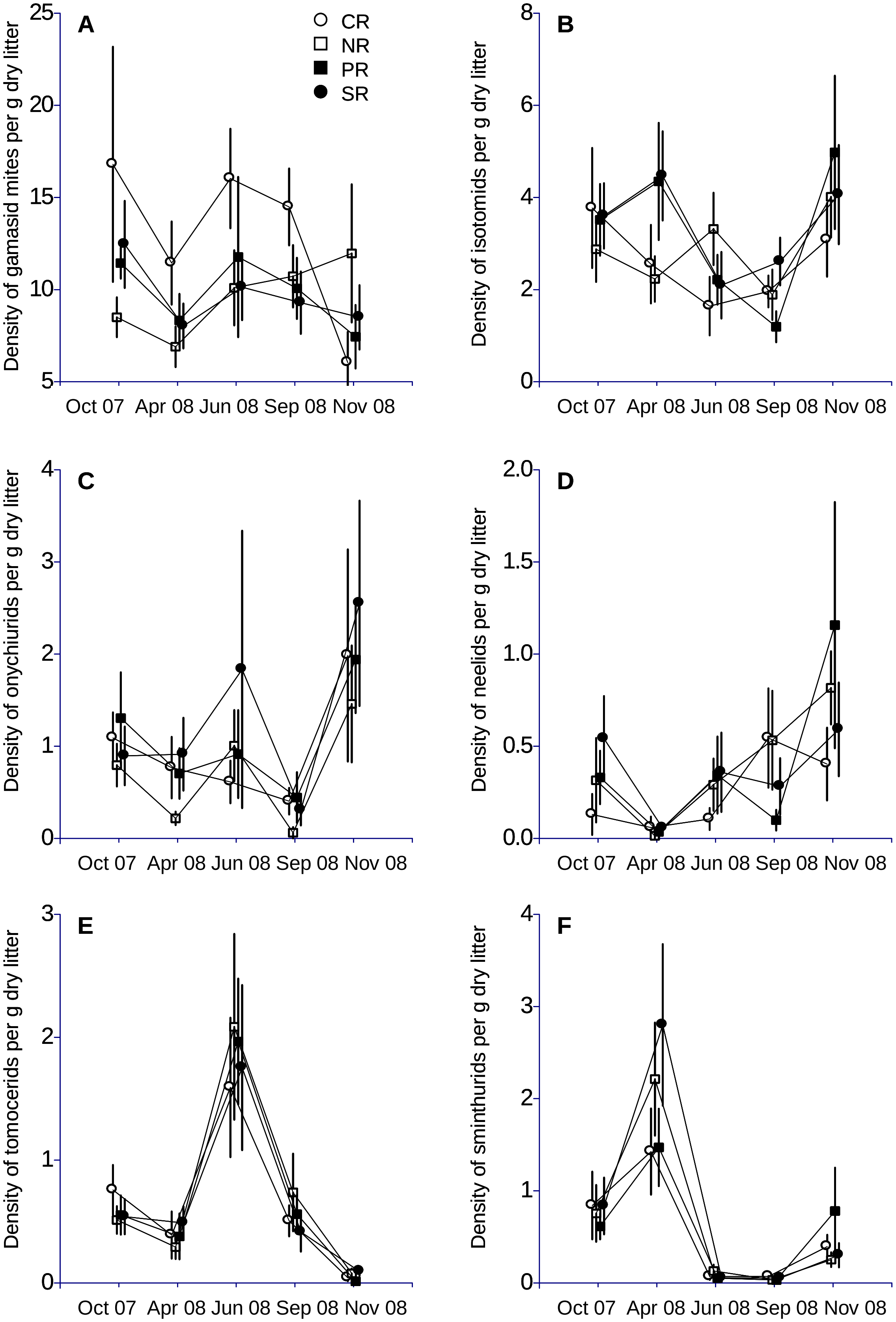
Figure IV - 5. Principle component 2 plotted across (A) treatments and as a function of (B) month and (C) plot orientation. CR = centipede removal – open circles, NR = control (nothing removal) – open squares, PR = all predator removal – closed squares, and SR = salamander removal – closed circles.

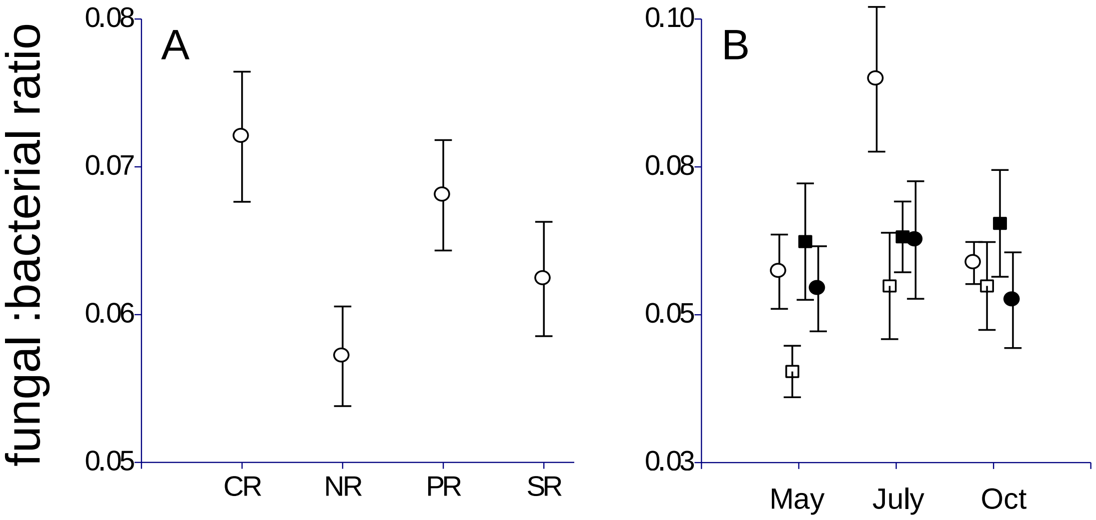
Figure IV - 6. Principle component 6 plotted across (A) treatments and as a function of (B) month and (C) plot orientation. CR = centipede removal – open circles, NR = control (nothing removal) – open squares, PR = all predator removal – closed squares, and SR = salamander removal – closed circles.

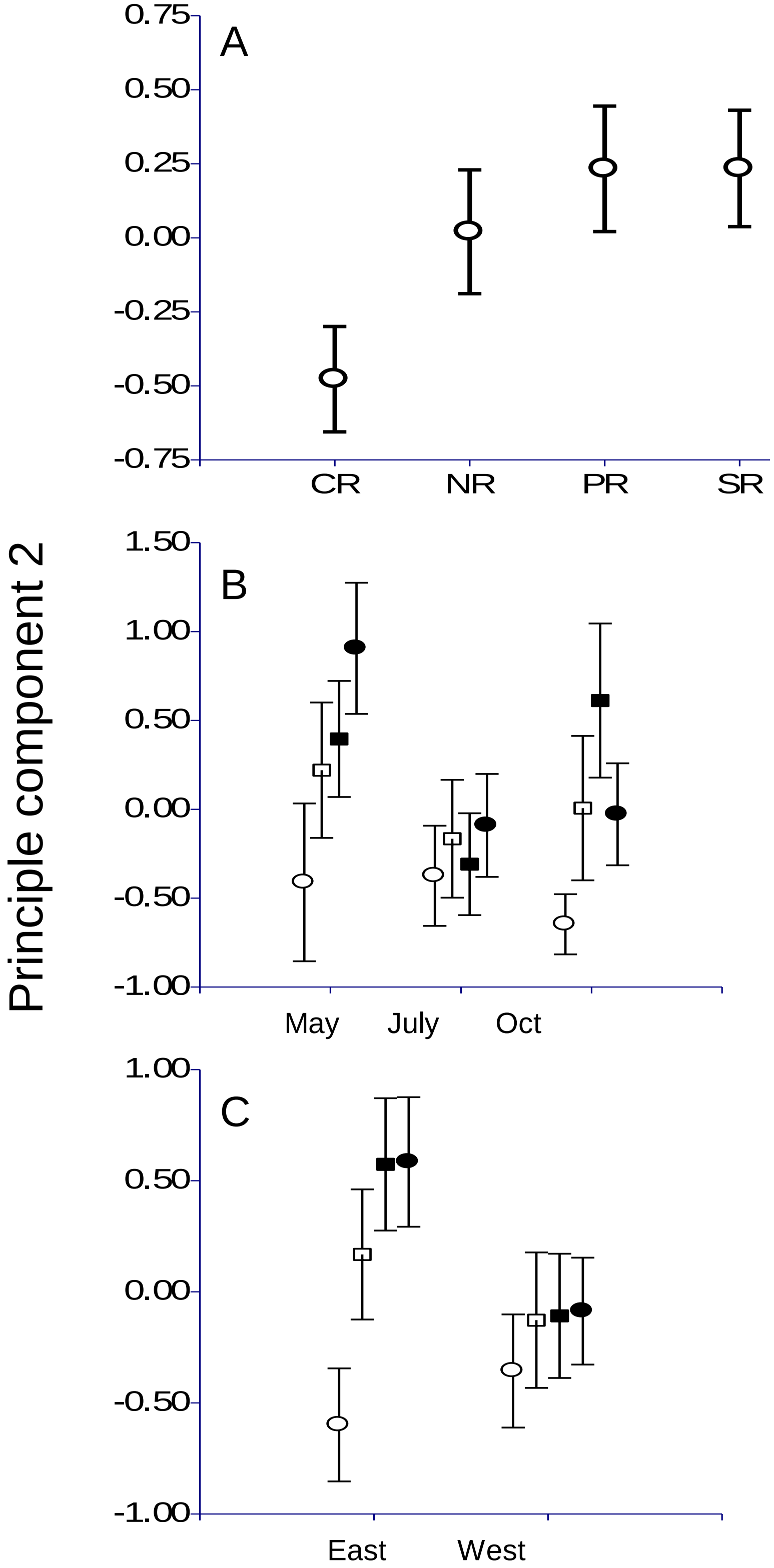
Figure IV - 7. Principle component 9 plotted across (A) treatments and as a function of (B) month and (C) plot orientation. CR = centipede removal – open circles, NR = control (nothing removal) – open squares, PR = all predator removal – closed squares, and SR = salamander removal – closed circles.


Predators
e.g. salamanders, spiders
Microbivores
e.g. Collembola, Acari
Macrodetritivores
e.g. Diplopoda, Isopoda
Microbes
Fungi, bacteria
Leaf litter

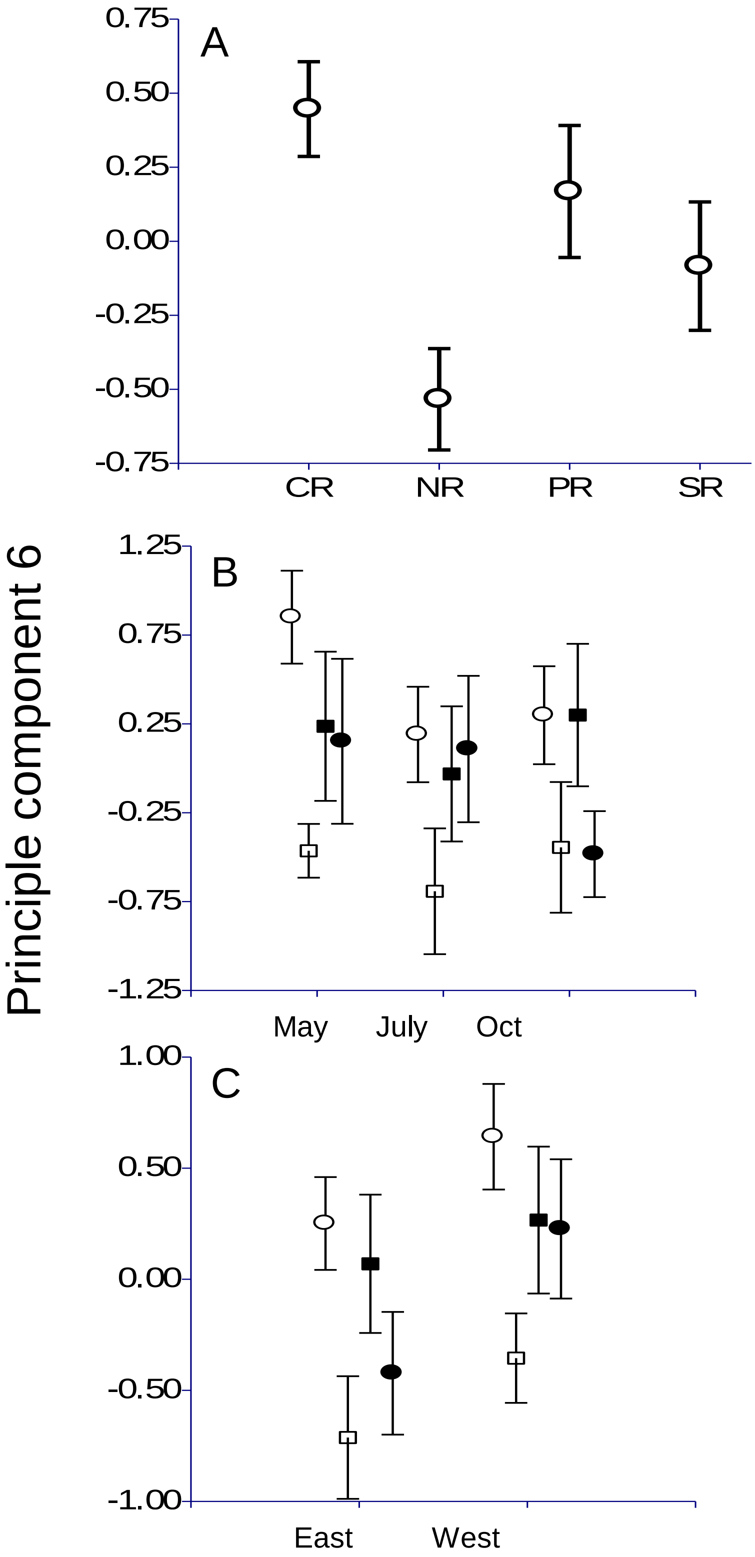

A
Mean original mass remaining (%)
100
90
80
70
60
0
20
40
60
80
Time (weeks)
B
Decomposition rate (wk^{-1})
0.0064
0.0060
0.0056
0.0052
0.0048
0.0044
0.0040
NR
CR
SR
PR

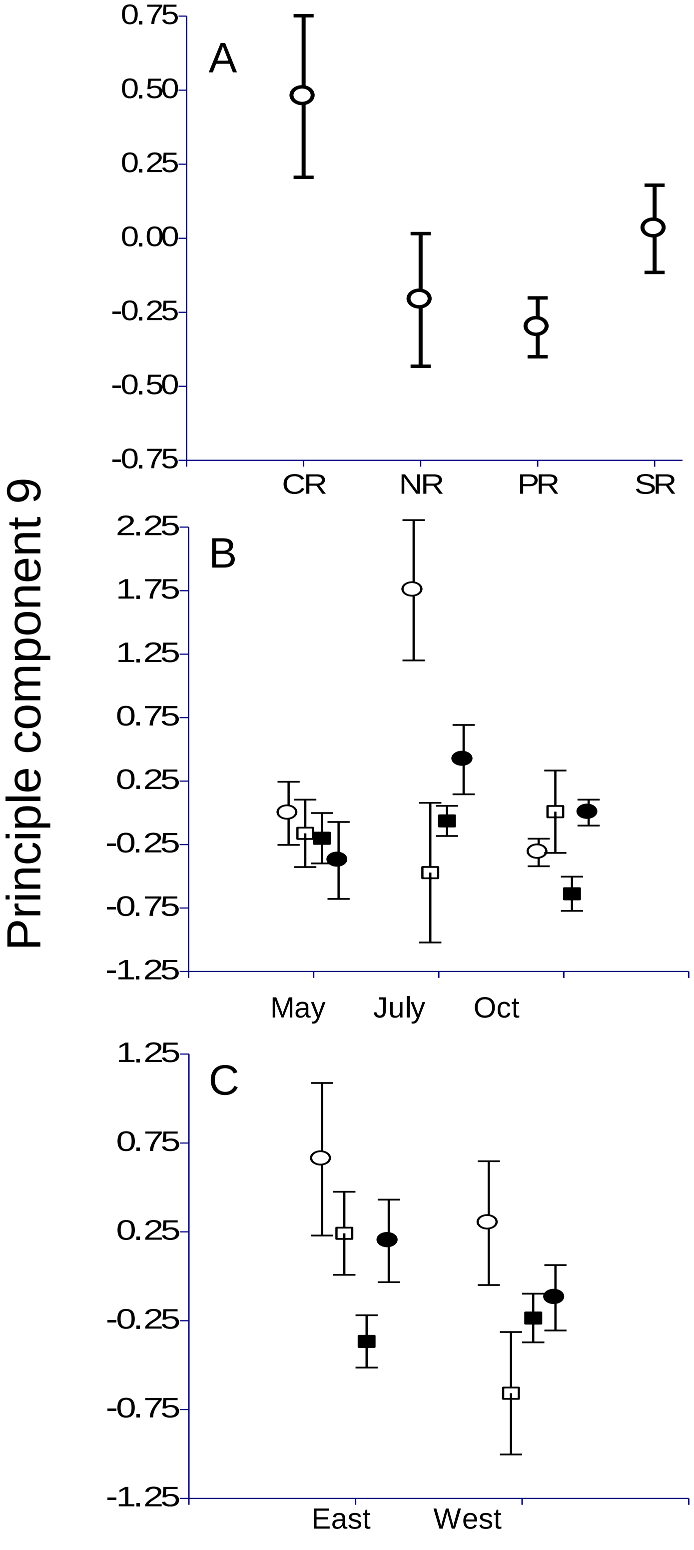

A
B
C
D
E
F
CR
NR
PR
SR
Density of gamasid mites per g dry litter
Density of isotomids per g dry litter
Density of onychiurids per g dry litter
Density of neelids per g dry litter
Density of tomocerids per g dry litter
Density of sminthurids per g dry litter
25
20
15
10
5
8
6
4
2
0
3
1
2.0
1.5
1.0
0.5
0.0
Oct 07
Apr 08
Jun 08
Sep 08
Nov 08


fungal :bacterial ratio
A
0.08
0.07
0.06
0.05
CR
NR
PR
SR
B
0.10
0.08
0.05
0.03
May
July
Oct



Principle component 2
A
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
CR
NR
PR
SR
B
1.50
1.00
0.50
0.00
-0.50
-1.00
May
July
Oct
C
1.00
0.50
0.00
-0.50
-1.00
East
West


Principle component 6
A
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
CR
NR
PR
SR
B
1.25
0.75
0.25
-0.25
-0.75
-1.25
May
July
Oct
C
1.00
0.50
0.00
-0.50
-1.00
East
West


Principle component 9
A
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
CR
NR
PR
SR
B
2.25
1.75
1.25
0.75
0.25
-0.25
-0.75
-1.25
May
July
Oct
C
1.25
0.75
0.25
-0.25
-0.75
-1.25
East
West