Oribatid mite communities in soil: structure, function and response to global environmental change

By

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This dissertation is dedicated to my parents. I certainly would not be where I am without their nurture, love and support

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ABSTRACT

Oribatid mite communities in soil: structure, function and response to global environmental change

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Little is known regarding the relative role of stochastic and deterministic forces in the community assembly of soil oribatid mites, which are species-rich and fulfill important roles in ecosystem functioning as detritivores. This dissertation investigates the mechanisms underlying the community assembly of soil oribatid mites and addresses how global environmental change influences their community structure and function. I hypothesized that dispersal limitation was a central mechanism structuring oribatid mite communities, due to their low locomotive activity through the soil. However, a study of the geographic distribution of oribatid mites along a chronosequence in a deglaciated region demonstrates that soil/litter dwelling oribatid mites are not limited by dispersal in their re-colonization of deglaciated areas, as evidenced by an overall high similarity in the species richness and composition of oribatid mites along the chronosequence. In contrast, climatic, biogeochemical and biotic factors explained significant amounts of variation in the species composition of soil oribatid communities, indicating that

environmental filtering and competition for food resource are more important than dispersal limitation in structuring soil oribatid mite communities. Moreover, stable isotope (^{15}N) analysis reveals that over half of soil oribatid mites are fungal feeders and that their trophic positions are stable among diverse environments, indicating a high level of feeding specialization among soil oribatid mites. Feeding specialization and competition for food resources, especially for fungal hyphae, among soil oribatid mites provides a potential mechanism underlying the observed decline in the density of soil oribatids under chronic N deposition. Furthermore, a litterbag experiment reveals that the decline in microarthropod abundance in forest floors is associated with a reduction in the mobilization of newly-added C into fungal biomass. Microcosm experiments further illustrate that a decline in microarthropod abundance alters fungal communities through microarthropod-fungi interactions. Although such changes in fungal communities do not influence subsequent litter decay during the early stages of litter decomposition, changes in the abundance of microarthropods and subsequent feedback on fungal communities may have important influence on the decay of recalcitrant organic matter.

Chapter I

Introduction

Understanding processes that structure biotic communities is a central theme in community ecology (Begon et al. 2006). In niche-based theory, differentiation of ecological traits allows coexistence of competing species by their partitioning of limiting resources and occupancy of different niches (Tilman 2004). In contrast, neutral theory assumes that all individuals are ecologically equivalent in their birth/death rates and that their communities are structured solely by demographic stochasticity and dispersal limitation (Hubbell 2001). In practice, community assembly is determined by both stochastic and deterministic factors (Belyea and Lancaster 1999, Leibold and McPeek 2006). Dispersal limitation determines the pool of potential colonizers that are capable of reaching a location (MacArthur 1967). Abiotic environmental factors restrict species establishment at the site, acting like a filter, or a hierarchy of filters that select for particular traits (Weiher and Keddy 1995). Biotic interactions such as competition and predation operate within constraints imposed by local environmental factors and dispersal limitation to select for a subset of species that actually coexist in the community (Menge and Sutherland 1987). Furthermore, when a community becomes saturated with species that overlap in the use of limiting resources, their populations become smaller and effects of demographic stochasticity are amplified (Lande 1993).

All of these stochastic and deterministic factors may operate simultaneously and interact with one another during community assembly (Strange et al. 1993). For example, the order of species arrival from dispersal may influence the outcomes of species interactions through priority

effects (Figuerola and Green 2002). On the other hand, resident species may alter their habitats to facilitate the colonization of new species (Bruno et al. 2003). Additionally, the relative importance of stochastic and deterministic factors may vary among taxonomic groups and depend on environmental conditions. For instance, some consider that dispersal limitation is negligible in microorganisms, such that "everything is everywhere and environments select" (Martiny et al. 2006). In contrast, in highly disturbed environments, frequent disturbance events may interrupt or diminish the importance of species interactions and their deterministic outcomes in community organization (Ricklefs 1987).

Most studies that have addressed the relative roles of stochastic and deterministic processes as drivers of community assembly have focused on aquatic ecosystems or the aboveground components of terrestrial ecosystems (Strange et al. 1993, Tilman 2004). However, soil animal communities are among the most species-rich components of terrestrial ecosystems (Giller 1996). As such, they hold promise for generating important insights into the ecological forces shaping community assembly. Yet, due to the small body size of most soil animals and the opaque nature of soil, little is known regarding the mechanisms underlying the community structure of soil animals (Coleman 2008).

Meanwhile, anthropogenic activities have been increasingly important in shaping the organization of biotic communities (Sampson and Groves 1989). Global warming has induced irreversible changes in the structures and functions of many plant and animal communities (Root et al. 2003, Memmott et al. 2007). Accumulating evidences also reveals that changes in global climate and land use may have detrimental effects on many groups of soil animals (Blankinship et al. 2011, Eisenhauer et al. 2012). Therefore, understanding the mechanisms underlying the community structure of soil animals is a pressing challenge in a world that is facing increasing

rates of biodiversity loss and ecosystem degradation (Clark 2009). This knowledge will help to predict how soil animals will respond to global environmental changes and the potential consequences of such changes for ecosystem functioning.

This dissertation focuses on an important group of soil animals in many ecosystems, the soil oribatid mites, to investigate the mechanisms underlying their community assembly and address how global environmental change influences their community structure and function. Low locomotive ability and high local diversity have made soil oribatid mites an ideal group of organisms to test general ecological principles underlying community organization. Moreover, their important roles in litter decay as detritivores suggests that any changes in their community structure in response to global environmental change is likely to have further consequences for ecosystem functioning.

In the classic sense of this group (excluding Astigmata), orbatid mites (Oribatida, Acari; also known as beetle mites, armored mites or moss mites) are a suborder of Chelicerata and comprise more than 10,000 named species representing 172 families (Krantz and Walter 2009). Although many are arboreal and a few are aquatic, most oribatid mites inhabit the soil-litter system. The soil habitat is a highly dynamic matrix without continuous inter-connectance among soil pores. Such poor connectance should generate greater obstacles for the movement of soil animals, compared to those in aquatic ecosystems or the aboveground portions of terrestrial ecosystems. Indeed, the maximum active dispersal rate is estimated to be no more than 10 m. $year^{-1}$ for many groups of wingless soil animals including soil oribatid mites (Ghilarov and Perel 1984, Marinissen and Vandenbosch 1992). Consequently, dispersal limitation is likely to be a central mechanism structuring communities of soil oribatid mites.

Despite their low locomotive activity, oribatid mites are often the dominant arthropod

group in highly organic soils of temperate forests. Up to 170 species can coexist in the litter and soil of hardwood forests at a collective density of 300,000 individuals /m^2 (Hansen 2000, Walter and Proctor 2004). Some studies have suggested that many soil oribatid mites have similar feeding habits, with most species apparently consuming a mixture of microbial and plant materials (Scheu *et al.*, 2005). The existence of astonishingly high local diversity, with an apparently low level of food resource specialization, is considered an ecological "enigma" (Anderson 1975a) . However, the feeding behaviors of many oribatid mites specie are actually unknown. Understanding the feeding habits of soil oribatid mites is needed to investigate any niche differentiation in food resource utilization and to address the enigma of their high local diversity.

Soil oribatid mites also perform vital roles during litter decomposition (Scheu et al. 2005) and they affect litter decomposition through three processes: (1) direct litter consumption; (2) litter fragmentation or dispersal of microbial propagules via physical movement and (3) feeding on microorganisms (Lussenhop 1992). Oribatid mites usually exhibit "K-style" life history traits with low reproductive output (1-3 generations per year, 1-6 eggs per clutch) (Walter and Proctor 2004) and a relatively long life cycle, which typically spans one to two years in temperate to boreal regions (Krantz and Walter 2009). The low rate of reproduction and long life cycle suggest that oribatid mites may be sensitive to environmental change (Behan-Pelletier 1999). However, little is known regarding how global environmental changes influence the community structure of soil oribatid mites and their functions in litter decay (Setala 2002).

This dissertation is divided into four primary chapters. Chapter II explores the relative roles of stochastic (dispersal limitation) and deterministic (environmental filtering and biotic interaction) processes as drivers of soil oribatid mite community assembly. Chapter III examines

the trophic structure and trophic dynamics of soil oribatid mites, which provide potential mechanisms underlying their coexistence at high diversity in small areas. Chapter IV investigates how soil oribatid mites (together with other groups of soil microarthropods) respond to chronic N deposition, a pervasive agent of global environmental change; and the consequence of this change on litter decay under field condition. In Chapter V, I established microcosms in the laboratory to further test how changes in oribatid mite communities under chronic N deposition feeds back to influence fungal communities and litter decay.

Chapter II. Stochastic (dispersal limitation) and deterministic (environmental filtering and biotic interaction) processes as drivers of soil oribatid mite community assembly. Soil oribatid mites are a major group of wingless microarthropods in many temperate forests and perform important functions during litter decay (Lussenhop 1992, Hansen 2000) ; nonetheless, little is known about the ecological forces structuring their communities. I hypothesized that dispersal limitation was a central mechanism structuring oribatid mite communities, due to their low locomotive activity through the soil. We tested this hypothesis by studying the geographic distribution of oribatid mites along a chronosequence across a deglaciated region, in which the effect of limited dispersal is expected to be profound. In addition, contemporary ecological factors, such as climate, soil properties and biotic factors, could induce niche partitioning due to environmental filtering and biotic interactions during the assembly of oribatid mite communities along the chronosequence. Therefore, we expected that additional biotic and abiotic factors would combine with dispersal limitation to determine oribatid mite community structure.

Chapter III. Trophic structure and trophic dynamics of soil oribatid mites. High local diversity of soil oribatid mites suggests that intense competitions and differentiation in resource utilization may exist among these oribatid species(Anderson 1975b). However, fine-scale

differences in food resource utilization among soil oribatid mites could go undetected using traditional methods, such as gut content analyses or food choice experiments (Scheu 2002). In this study, I used stable isotope measurements to study the feeding habits of soil oribatid mites. I expected a high degree of trophic differentiation underlying the coexistence of soil oribatid mites. I also investigated the stability of the feeding habits of soil oribatid mites in the face of environmental change, as well as variation among different forest types. If oribatid mites are feeding generalists, I would expect a shift in their feeding habits with changes in food availability under different environments.

Chapter IV. The effects of chronic N deposition on the community structure and function of soil oribatid mites. Anthropogenic nitrogen (N) deposition is a pervasive agent of global environmental change (Vitousek et al. 1997). During the next century, atmospheric nitrogen (N) deposition is projected to more than double (Galloway et al. 2004), potentially slowing litter decomposition by altering microbial community composition and function (Zak et al. 2008, Liu and Greaver 2010). If the flow of energy though detrital food webs is diminished by the slowing of decay under higher rates of atmospheric N deposition, this agent of global change could also negatively impact the abundance and composition of soil fauna. To test this hypothesis, I studied soil oribatid communities in four sugar maple-dominated forests that comprise a long-term N deposition experiment. To examine whether changes in soil oribatid communities could then feed back to influence litter decay, litterbags with ^{13}C-enriched aspen litter were placed in the forest floor in one study site. Litterbags were retrieved four times over a 16-month-study to investigate how litter decay differs between ambient and experimental N deposition treatment.

Chapter V. The consequences of changes in microarthropod communities under chronic N deposition for fungal communities and litter decay. Results from Chapter IV demonstrated

that the abundance of microarthropods declines and the composition of oribatid communities change under chronic N deposition. Because soil microarthropods form essential interactions with soil fungi, any change in microarthropod communities is likely to have further consequences for litter decay via their interactions with the fungal community (Scheu et al. 2005). I hypothesized that the decline in soil microarthropod abundance and the alteration of their species composition under chronic N deposition would further influence litter decay by altering fungal communities. To test this hypothesis, I established 150 microcosms with microarthropods collected from forest floors under ambient and experimental N deposition in a long-term (>17 years) field study. I monitored CO_2 accumulation during a 96-day incubation and characterized fungal communities at the end of the study. I expected that compared to the microarthropod community under ambient N deposition, the lower abundance and different composition of microarthropods under chronic N deposition would alter fungal community composition resulting in lower enzyme activities and slower litter decay.

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Chapter II

Stochastic (dispersal limitation) and deterministic (environmental filtering and biotic interaction) processes as drivers of soil oribatid mite community assembly

Abstract. Soil oribatid mites are a major group of wingless microarthropods in many temperate forests and these organisms perform important functions during litter decay; nonetheless, little is known about the ecological forces structuring their communities. We hypothesized that dispersal limitation was a central mechanism structuring oribatid mite communities, due to their low locomotive activity through the soil. We tested this hypothesis by studying the geographic distribution of oribatid mites along a long-term chronosequence following glacial retreat, in which the effect of limited dispersal is expected to be profound. Our results reveal high local diversity of oribatid mites (46~56 species), but low β diversity (an average of 31% dissimilarity) across the chronosequence. In contrast to our hypothesis that oribatid mite diversity would be the lowest in the youngest site due to dispersal limitation following glacial retreat, we found similar richness and composition of oribatid mite species along the chronosequence. This suggests that passive dispersal might be an overlooked, but prominent, dispersal mode for these forest floor organisms. Interestingly, the degree of dispersal limitation depends on body size, with the subgroup of mites with large body size exhibiting a significant positive relationship between community dissimilarity and geographic distance, a proxy for time since deglaciation. In contrast, the community composition of small-bodied species is similar regardless of geographic distance, suggesting that small-bodied mite species were able to colonize all four study sites more easily than were large-bodied mites. Our study also suggests

that environmental filtering (temperature, litter fall and soil C:N ratio) and competition for food resource (fungal biomass and Collembola abundance) are important determinants of oribatid species composition.

Introduction

The relative roles of stochastic (e.g., dispersal limitation) and deterministic (e.g., environmental filtering and biotic interactions) processes as drivers of community assembly remains an actively debated topic in ecology (Chave 2004, Tilman 2004). Soil animal communities, which contain an astonishing diversity, have been described as "the poor man's tropical forest" (Giller 1996). As such, they hold promise for generating important insights into the ecological forces shaping community assembly. The soil habitat is a highly dynamic matrix without continuous inter-connectance among soil pores. Such poor connectance should generate greater obstacles for the movement of soil animals, compared to those in aquatic ecosystems or the aboveground portions of terrestrial ecosystems. Consequently, dispersal limitation may be a relatively more important ecological force in structuring communities of soil animals.

The importance of dispersal limitation in structuring community assemblages is most profound after severe disturbances such as continental glaciation. For instance, since the end of the Pleistocene glaciation, many forest communities in Eastern North America have gained new species throughout the Holocene, increasing in diversity as forest species expanded their ranges from refuges far to the south (Davis 1983). Whereas some organisms can rapidly expand their geographic distribution following glacial retreat, especially those with high dispersal ability such as plants with winged seeds and flying animals, limited dispersal is probably more important in structuring communities of soil fauna. For example, it is generally accepted that the late Wisconsin Glaciation eliminated most indigenous earthworms in northeastern North America (Gates 1982, Reynolds 2004). The majority of the earthworms now inhabiting soils in Canada and northern regions of the US result from relatively new introductions of European earthworms, which began largely within the last two or three decades (Scheu and Parkinson 1994).

Compared to the systematic research on earthworm distributions (Addison 2009), the distributions of other groups of soil fauna in post-glaciation regions are rarely investigated. Oribatid mites, a major group of wingless microarthropods in the forest floor of many temperate forest ecosystems (Osler and Beattie 1999, Maraun et al. 2007), perform important functions during litter decomposition via litter comminution and selective grazing on microbial communities (Lussenhop 1992). Despite their important roles in ecosystem functioning, oribatid mites exhibit low locomotive activity (Berthet 1964). Based on two *ex situ* experiments of oribatid mite movement through forest soil (Ojala and Huhta 2001, Lehmitz et al. 2012), the maximum active dispersal rate was estimated to be 1-8 m $year^{-1}$, which is comparable to that of 5-10 m $year^{-1}$ for earthworms (Ghilarov and Perel 1984, Marinissen and Vandenbosch 1992). While passive dispersal such as anthropochorous dispersal (via agricultural products, fishing bait etc.) is critical for current earthworm distributions in glaciated regions, it may be less common in soil microarthropods. Because of their low locomotive ability, oribatid mites inhabiting forest floor would advance no more than 80 km over 10,000 years, if they relied solely on their own power (Ojala and Huhta 2001, Lehmitz et al. 2012).

Considering their low dispersal ability, we hypothesized that dispersal limitation was a central mechanism structuring oribatid mite communities. To test this hypothesis, we studied the geographic distribution of oribatid mites along a chronosequence in a deglaciated region of eastern North America, in which we expected the effect of limited dispersal to be most profound. We reasoned that (1) oribatid mite diversity would increase with time since deglaciation, and (2) communities in close proximity would be more similar to one another than would those that are far apart. Furthermore, as the dispersal ability of organisms is highly related to their body size (Finlay 2002), we also expected that small- bodied oribatid mites would experience a lower

degree of dispersal limitation than large-bodied mites. Additionally, contemporary ecological factors, such as climate, soil properties as well as biotic factors, could induce niche partitioning due to environmental filtering and biotic interactions during the assembly of oribatid mite communities. Therefore, we expected that additional biotic and abiotic factors would combine with dispersal limitation to determine oribatid mite community structure.

Materials and Methods

Study sites

Our four study sites span 420 km within Lower and Upper Michigan, USA (Fig. 2.1). These sites are ideal to test our hypothesis regarding the effect of deglaciation on soil fauna communities, because they differ in their ages since deglaciation. We denote the four sites from north to south as site A, site B, site C and site D with ages of 9,500, 11,000, 13,000 and 13,500 years, respectively (Eisenlord et al. 2012; W. Farrand, personal communication). A climatic gradient coincides with the geographic range, in which the northern-most site experiences a lower mean annual temperature (4.54 °C) and the southern-most site experiences a higher mean annual temperature (7.44 °C).

We selected sugar-maple (*Acer saccharum* Marsh.) dominated northern hardwood forest as our study system, because it is a prevalent natural ecosystem in northeastern North America. The overstory ages (through 2011) are similar among sites, ranging from 98 to 104 years (Patterson et al. 2012), ensuring that the chronosequence is not confounded by different successional stages of the sites. Each site contains three 30-m x 30-m plots that are 10- to 150-m apart (Eisenlord et al. 2012). These hardwood forests are underlain by sandy soils that are well-drained sandy typic Haplothords of the Kalkaska series. The understory vegetation consists of mainly sugar maple seedlings (~ 90% of all stems), and the Oi horizon is primarily sugar maple

litter (Patterson et al. 2012). As these sites were selected from a population of 31 candidate sites based on floristic and edaphic similarity (Burton et al. 1991), their shared habitat similarity minimizes those aspects of environmental filtering, which should favor our ability to detect any signal of dispersal limitation.

Sample collection

Forest floor (Oi and Oe/a horizons) samples were collected in May 2011, June 2012 and August 2012. Six forest floor subsamples (10-cm x 10-cm) were collected within each plot on each sampling date for a total of 216 forest floor samples. For the second and third sampling time (June 2012 and August 2012), three additional mineral soil samples were also collected from each plot using 5-cm x 5-cm soil cores (a total of 72 samples of mineral soil).

All the subsamples inside plastic bags were placed in a cooler and transported to the lab within 48 hours. Each subsample was transferred to a modified Tullgren funnel (Crossley and Blair 1991) over 5 days and the extracted microarthropods were stored in 70% ethanol for sorting and identification. Three major groups (Mesostigmata, Collembola and Oribatida) of the extracted microarthropods were enumerated under a microscope. The most abundant group (Oribatida, adults only) was further identified to genus or species, based on the keys written by R. A. Norton and V. M. Behan-Pelletier (unpublished data) for use at the Ohio State University Summer Acarology Program (http://www.biosci.ohio-state.edu/~acarolog/summerProgram/).

Dispersal limitation, environmental filtering and biotic interaction

To determine the relative influence of stochastic (dispersal limitation) and deterministic (environmental filtering and biotic interaction) processes on oribatid mite community assembly, we created four data sets including geographic distance, climatic, biogeochemical and biotic matrices. Because distance between sites overlays time since deglaciation in our

chronosequence, we used distance as a proxy for time to study the role of dispersal limitation in shaping oribatid mite communities. Global positioning system (GPS) coordinates were taken at the center of each sample plot and the geographic distance matrix was calculated as the great circle distance based on the GPS coordinates of the sites.

To estimate potential effects of environmental filtering and biotic interactions on structuring oribatid communities, we created another three matrices: climatic, biogeochemical and biotic matrices (Table 2.1). The climatic matrix included annual mean air temperature (°C) and precipitation (mm) from 1988-2004. All precipitation and temperature data were recorded at each study site; interpolation from the nearest NOAA data was used when study site specific data were unavailable (due to malfunctions of the sensor etc.), using monthly relationships between our site data and that from the nearest NOAA station.

The biogeochemical matrix consisted of characteristics of leaf litter fall, O-horizon and mineral soils that are known to influence the physiology and ecology of oribatid mites (Hasegawa 1997, VanStraalen and Verhoef 1997, Hasegawa 2001, Irmler 2006).These factors included litter fall and its C: N ratio (averaged from year 2001-2011); mass of O-horizon, C: N ratios of Oi-horizon and top 10 cm of forest floor and mineral soil (excluding Oi-horizon), soil organic matter (0-10 cm), soil bulk density (0-10 cm) and soil pH (0-10 cm). The biogeochemical data of O-horizon and upper 10cm of mineral soil were all measured in 2004 except soil pH, which was measured in 2008 (Pregitzer et al. 2008). Foliar calcium (averaged from 1988-2005) is positively correlated with soil calcium concentration; it was included in the biogeochemical matrix, because calcium is vital in the integument development of some oribatid species (Norton and Behan-Pelletier 1991).

The biotic matrix consisted of soil fungal biomass and total microbial biomass measured

as phospholipid fatty acid (PLFA) (measured in 2006, van Dipen et al. 2010). The abundance of Collembola and Mesostigmata (individuals. m^{-2}) in the forest floor were also included (surveyed in May 2011, Gan et al. 2013). Collembola are a subgroup of primitive insects that occupy similar trophic levels as detritivorous oribatid mites (Hishi et al. 2007). Mesostigmata are a subgroup of mites generally with larger body size than Oribatida and are predators of Collembola and sometimes oribatid larvae (Koehler 1999). Therefore, the biotic matrix represents groups of organisms with potential biotic interactions with oribatid mites including food availability, competition and predation.

We use long-term averages of the above climatic, biogeochemical and biotic factors whenever possible, because oribatid mites tend to have long life cycles compared to other microarthropods, with up to 5 years of developmental time from egg to adult and up to 2 years of adult survival (Norton 1994). All of the climatic and litter fall data are archived and publicly accessible on the web http://www.webpages.uidaho.edu/nitrogen-gradient/Archived_data.htm . We recognize the obvious limitation that some of the microbial PFLA and biogeochemical data were collected during different periods than our faunal surveys, and acknowledge that some parameters may have changed over time. However, we include these analyses here because they represent the best data available, and in the hope that differences among sites (the focus of our analyses) have remained relatively stable over time. We nonetheless recommend caution in their interpretation.

Data analysis

For each sampling date, we compiled the species composition of oribatid mites from each plot in three ways: abundance, incidence, and presence/absence matrices. The raw abundance of a species is the number of individuals summed over all subsamples (6 subsamples of forest floor

and 3 subsamples of mineral soil per plot). To down-weight the dominant species, we converted the raw abundance data into an incidence matrix. The incidence score is 1 if the species was only encountered once in one of the subsamples; and the maximum score is 6 for forest floor or 3 for mineral soil if the species was found in all the subsamples in that plot. We initially separated forest floor and mineral soil communities for analysis because they may respond differently to environmental factors. On the other hand, as vertical migration between forest floor and upper mineral soil is likely to occur (Luxton 1981), we also combined species from forest floor and mineral soil to represent an overall litter/soil dwelling oribatid mite community. Additionally, combining these two horizons ensures adequate sampling effort within each site to estimate species composition at the site level.

To investigate whether the species richness of oribatid mites varied with land history after deglaciation, rarefaction curves were generated from the raw abundance data, with 9 data points (3 plots x 3 sampling dates) from each site (forest floor and soil combined). Ninety-five % confidence intervals were calculated for each rarefaction curve to assess any overlap between sites. Secondly, we used non-Metric Multidimensional Scaling (nMDS) to visualize how the species composition varied among sampling horizons (forest floor vs. mineral soil), study sites (n = 4) and sampling dates (n =3). The species composition of each site was the sum of results of three plots at that site. Prior to nMDS, the raw abundance and incidence matrices were each converted to a relative abundance matrix by first dividing by the site sum and then taking the square root transformation as the Hellinger transformation (Legendre and Gallagher 2001). We did not transform the presence/absence matrix before nMDS. Analysis of similarity (ANOSIM) was performed to provide statistical comparisons following nMDS. For both nMDS and ANOSIM, the Bray-Curtis dissimilarity index was used, as it provides a realistic measurement of

dissimilarity between biological communities (Bray and Curtis 1957).

Mantel tests were used to examine if there was any correlation between oribatid mite communities and geographic distance, climatic, biogeochemical or biotic factors. For each of the three oribatid mite community matrices mentioned above (raw abundance, incidence and presence/absence), we summed the species composition of three sampling dates from the same site into one matrix to ensure adequate sampling and to eliminate any seasonal effect. The abundance and incidence matrices at the site level were transformed with Hellinger transformation before Mantel tests were applied. For the Mantel tests, the Bray-Curtis dissimilarity index was used to calculate the dissimilarity of oribatid mite communities between sites. Climatic, biogeochemical and biotic data sets (Table 2.1) were each converted to a dissimilarity matrix by calculating the Euclidean distance after z-transformation. Spearman's rank correlation coefficient (ρ) was used in the Mantel tests and significance level was tested using a permutation method (permutation times = 2000). Partial Mantel tests were also applied if oribatid mite communities had significant association ($P < 0.05$) with more than one explanatory data set (geographic distance, climatic, biogeochemical and biotic factors) to control for covariance between any two explanatory matrices. Scatterplots between community dissimilarity and geographic distance, climatic, biogeochemical and biotic dissimilarities between sites (6 pairs for each Mantel test) were generated to visualize the Mantel tests results.

To determine whether the relationship between community dissimilarity and geographic distance (or climatic, biogeochemical and biotic dissimilarity) from the above Mantel tests was influenced by body size, we divided the communities into two sub-groups based on their body sizes. The values of the body sizes were taken from the literature as the averages of adult body lengths. A histogram of the body sizes of all species from all four study sites (79 spp.) was

generated and the midpoint (0.45mm) was used to divide the community into two subgroups. There are 40 species in the small-bodied group with their body sizes ⩽ 0.45 mm and 39 species in the large-bodied group with their body sizes > 0.45mm. Mantel tests were performed separately on these two sub-groups for all three community matrices with four explanatory data sets as above.

Although the Mantel test is valid for investigating the relationship between community dissimilarity and geographic distance or environmental dissimilarity, it usually underestimates the variance partitioned by explanatory factors (Legendre et al. 2007). We therefore used redundancy analysis (RDA) to further test which environmental factor explains most of the variation in original community composition (rather than the dissimilarity matrix as in the Mantel test) as suggested by Legendre et al. (2007). RDA was used instead of Canonical Corresponding Analysis (CCA) because of the relatively small environmental gradient in our study. Due to our small sample size (n=4), we applied RDA to each climatic, biogeochemical and biotic factor separately. For the combined community from both forest floor and mineral soil, all environmental factors in Table 2.1 were used. When analyzing the communities from forest floor and mineral soil separately, the biogeochemical and biotic factors that were not relevant for that horizon were excluded. We also repeated RDA with the small-bodied and large-bodied subgroups of mites. As we did not have enough statistical power to test the interaction terms in the RDA analysis, Principle Component Analysis (PCA) of all environmental factors was provided to assess any correlation between the explanatory factors. Furthermore, for any environmental variable with significant explanatory power under the RDA analysis, we performed simple regression between its Euclidean distances (after z-transformation) and geographic distance to investigate any spatial autocorrelation of the environmental factor. If we

detected significant spatial autocorrelation, we then compared a simple regression with only environmental dissimilarity to a multiple regression with both environmental dissimilarity and geographic distance, and used Akaike's Information Criterion (AIC) to select the better model.

The species rarefaction curves were generated using EstimateS (Colwell 2006). Multivariate analysis including nMDS, ANOSIM, RDA and PCA were performed in package VEGAN (Oksanen et al. 2012) in R 2.15.1 (R Development Core Team 2012).

Results

Multivariate analyses of species composition using raw abundance, incidence and presence/absence matrices in general yield identical trends with varying significance levels. We have chosen to present the results derived from the incidence data set, as it is likely to be least biased by down-weighting both the dominant species (over-represented in the raw abundance data) and the rare species (over-represented in the presence/absence data).

Overall, we collected and examined 11,101 adult oribatid mites from 79 species with 8 singletons. In contrast to our expectation, the species richness of oribatid mites did not decline northward as sites decreased in time since deglaciation; instead, the total number of oribatid species at each site was similar, ranging from 46 (Site C) to 56 (Site B and Site D). The saturation of the rarefaction curves (Fig. 2.2) illustrates that we had sampled sufficiently to estimate total species richness at each of the 4 sites. While site D had much lower oribatid abundance compared to other sites, its species richness (56 spp.) was comparable to other sites (Fig. 2.2). The overlap of the 95% confidence intervals of the rarefaction curves (not shown, for clarity) indicated that there was no significant difference in species richness among sites.

However, species composition varied among study sites ($R^2 = 0.33$, $P = 0.001$) and differed between forest floor and mineral soil ($R^2 = 0.18$, $P = 0.001$), but remained similar among

sampling dates ($R^2 = 0.09$, $P = 0.223$) (Fig. 2.3.). Among the 5 most dominant species at each site, three species were shared including *Scheloribates pallidulus* (17.2% ~ 27.4%, relative abundance), *Oppiella nova* (9.5% ~ 16.0%) and *Suctobelbella* sp2 (7.5 % to 16.5%). However, some species, such as *Eueremaeus nemoralis*, was dominant at one site (8.1% at site C) but rare at others (~ 0.1 % at sites A and B). Furthermore, the species composition of oribatid mites collected from the mineral soil clearly differed from that in the forest floor (Fig. 2.3.). However, this difference was primarily driven by the lower species richness in mineral soil (50 spp.), which were a subset of the same species found in the forest floor (77 spp.) except two rare species (*Microtritia* sp. and *Nanhermannia* sp.). All species information is provided in Appendix Table A2.1.

When considering all oribatid species, a Mantel test between community dissimilarity and geographic distance revealed that oribatid communities at close proximity were not more similar to one another than were those far apart (Fig. 2.3, $\rho = 0.6$, $P > 0.05$). In contrast, there was a positive association between oribatid mite community composition and the climatic matrix (Fig. 2.3, $\rho = 0.83$, $P = 0.04$) while the associations with biogeochemical and biotic matrices were not significant (Table 2.2, $\rho = -0.65, 0.49$; $P > 0.1$).

When we divided the species into two subgroups based on their body size, we found that the communities of large-bodied oribatid species became more dissimilar as geographic distance increased (Fig. 2.4, $\rho = 0.94$, $P = 0.035$), whereas the community composition of small-bodied oribatids remained similar regardless of distance (Fig. 2.4, $\rho = 0.48$, $P > 0.1$). Associations between oribatid mite communities and biogeochemical and biotic matrices remained insignificant for either small- or large-bodied subgroups; the positive correlation between oribatid mite community dissimilarity (all species) and climatic dissimilarity mentioned above

was retained as marginally significant (Table 2.2, ρ = 0.89, $P = 0.08$) in the small-bodied subgroup but disappeared in the large-bodied subgroup (Table 2.2, ρ = 0.6, $P = 0.12$). Furthermore, the positive correlation between the dissimilarity of large-bodied mites and geographic distance remained significant after controlling for the covariance with climatic factors (partial Mantel, ρ = 0.92, $P = 0.043$). Because distance is a surrogate for time in our study, these results suggest that dispersal limitation is a factor influencing large, but not small-bodied oribatid mites. Analysis of the community in the forest floor showed the same results as that of the combined community, while the dissimilarity of community in the mineral soils did not have associations with geographic distance or any environmental dissimilarity for either size groups (Table 2.2).

While we found a positive association between oribatid mite community dissimilarity and climatic dissimilarity, redundancy analysis revealed that it was temperature in the climatic matrix that contributed significantly to the variation in species composition among sites (Table A2.2, $R^2 = 0.41$, $P = 0.04$). Because temperature overlay with the chronosequence in our study, we compared the simple regression between community dissimilarity and temperature only (adjusted $R^2 = 0.74$, $P = 0.02$) to the multiple regression with both temperature and geographic distance (adjusted $R^2 = 0.71$, $P = 0.07$). The lower AIC score for the first model (-31 vs. -30) suggested that temperature itself was a significant factor explaining variation in the community of oribatid mites. Furthermore, when we applied RDA to small- and large-bodied mites separately, the same pattern remained although the contribution from temperature was only marginal in the large-bodied group (Table A2.2, $R^2 = 0.42$, $P = 0.09$)

Although Mantel tests did not reveal any association between community dissimilarity and biogeochemical or biotic dissimilarity matrices, RDA illustrated that the amount of litter fall

and the abundance of Collembola contributed substantially to variation in oribatid mite communities in the forest floor (Table A2.2, R^2 = 0.46, 0.43, P = 0.08, 0.09). Furthermore, species composition of oribatid mites in the mineral soil was influenced by soil C:N ratio (top 10cm including OeOa horizon, R^2 = 0.42, *P* = 0.04) and soil fungal biomass (R^2 = 0.43, P = 0.08). The influence of soil fungal biomass is more apparent for the small-bodied group in mineral soil (R^2 = 0.52, P = 0.02). While we did have enough statistical power to test the interaction between temperature and fungal biomass, PCA indicated that there was no significant correlation between these factors (Appendix Fig. A2.1). Furthermore, the pairwise dissimilarity (between sites) of these explanatory factors did not correlate with geographic distance, indicating that they are not confounded by spatial autocorrelation in structuring oribatid mite communities in our study.

We further identified the species with the strongest responses to each environmental factor by looking at the first three species with the highest and lowest species score from the significant RDA results. Due to our small sample size (n = 4), we only reported the species scores from RDA with $P < 0.05$ to reduce the Type I error. As showed in Fig. 2.5a, species *Eu. nemoralis*, *Epidamaeus longitarsalis* and *Eremobelba* sp. were associated with high temperature whereas species *Oppia nitens*, *Eniochthonius crosbyi* and *Ceratozetes gracilis* were more likely to occur under low temperature. For soil-inhabiting species, *Fuscozetes fuscipes*, *Oribatella* sp1 and *Eremobelba* sp. responded positively to soil C:N ratio, while *Rhysotritia ardua*, *Xylobates prionotus* and *Nothrus* sp1were associated with low soil C:N ratio (Fig. 2.5b). Furthermore, small-bodied species inhabited in soil including *O. nitens*, *Quadroppia* sp. and *Xylobates. oblongus* were positively associated with soil fungal biomass while *Fosseremus americanus*, *Tryhypochthonious* sp. and *Eremobelba* sp. were associated with low fungal biomass (Fig. 2.5c).

Discussion

We hypothesized that oribatid mite diversity would be the lowest in the youngest site due to dispersal limitation following glacial retreat; however, we did not find any evidence to support this hypothesis at a spatial scale of 400 km. There are two possible explanations, the first of which involves different colonizing routes following glacial retreat. The last glaciation in our study region, known as the Wisconsin advance, reached its maximum extent in Indiana and Ohio approximately 18,000 years ago (Prest 1969). While the ice sheets generally retreated from south to north, the recolonization by biota could occur by different routes depending on their refuge areas and their dispersal modes (Davis 1983). For instance, while maples moved into the northern United States from the west following the retreat of ice, beech moved northward east of the Appalachians, expanding westward across the lower Great Lakes region (Davis 1983). For oribatid mites, it is possible that re-colonization occurred from west of areas of southwest North Dakota, which were ice-free during the last glacial maximum (Clayton and Moran 1982). The existence of multiple routes to recolonization has the potential to blur any clear spatial diversity pattern generated by dispersal limitation.

A second possible explanation for our observed result is that despite low rates of locomotion on the forest floor, oribatids may possess other dispersal modes enabling rapid re-colonization of deglaciated areas. The existence of unexpected long-distance dispersal events, such as dispersal on drift ice and drift wood, have been important mechanisms shaping flora among the isolated Arctic islands following glacial retreat (Coulson et al. 2000, Johansen and Hytteborn 2001). An example of long-distance dispersal in oribatid mites is through phoresy, whereby an organism “hitches” a ride by clinging to the body of another, usually much bigger organism, and dispersing when a new suitable habitat is reached (Houck and OConnor 1991).

Phoresy in oribatids is rare compared to that in its parasitic sister group, Astigmata mites (Houck and OConnor 1991), but there have been observations of phoresy by oribatid mites on harvestman and passalid beetles (Norton 1980). A large number of oribatid mites (146 spp.) have also been recovered from the plumage of a diverse group of raptors and Antarctic birds (Lebedeva and Krivolutsky 2003, Lebedeva and Lebedev 2007). Furthermore, although forest floor oribatid species are rarely dispersed by wind (Karasawa et al. 2005), Lehmitz et al. (2011) reported that about 10% of wind-dispersed oribatid species belonged to species able to live in the soil, suggesting wind as a potential dispersal mechanism for litter/soil dwelling oribatids mites. Rapid recolonization of oribatid mites into recently emerged nunataks (ice-free land in glacial areas) also suggests the existence of long-distance dispersal (Ingimarsdóttir et al. 2012).

Passive dispersal i.e. via water, animals or wind as mentioned above, could be an overlooked, but prominent, dispersal mode for litter or soil dwelling oribatid mites in determining their community structure. Our finding is consistent with this idea, wherein there was a strong signal of dispersal limitation from the oribatid subgroup with large body size, but not for those with a small body size (Fig. 2.4). While one may expect a stronger effect of dispersal limitation from soil-inhabiting species, we did not observe any relationship between community dissimilarity and geographic distance for oribatid mites in mineral soil for either body size (Table 2.2). The lack of such relationship is likely due to the lower sampling effort in mineral soil. Nevertheless, the average dissimilarity of small-bodied species among sites was lower than that of large-bodied mites (Fig. 2.4, 27% vs. 41% of Bray-Curtis dissimilarity); and the same result applied in forest floor (31% vs. 43%) and mineral soil (24% vs. 55%). This suggests that small-bodied mite species are able to colonize all four study sites easily and are more similar in their species composition, whereas species with large body sizes are more

restricted in their movement across the chronosequence. Such difference between body size may be partially due to the fact that one of our study sites (site A) is located in a different peninsula from the other three sites (Fig. 2.1). The water between these two peninsulas may function as a dispersal barrier especially for the large-bodied species, which may be less likely to disperse passively than will small-bodied species.

A similar pattern has been found in land snails on the Pacific islands, in which very small snail genera form 67% of Pacific island snail fauna in contrast to 27% of continental fauna (Vagvolgyi 1975). This provides indirect evidence of a dispersal advantage for small species during aerial oversea dispersal. Small organisms are not only easier to transport, but they also generally exhibit larger population sizes that may increase the probability of dispersal (Van der Gucht et al. 2007). It has even been suggested that there exists a threshold value of body size (1 mm) under which species are ubiquitous dispersers and less likely to be geographically restricted (Finlay 2002). Adult body lengths in oribatid mites encompass this threshold, ranging from about 0.15 mm to more than 2.00 mm, with most species not exceeding 1 mm. However, it would be unrealistic to conclude that oribatids are not dispersal limited solely based on their small body size, as many species, even though less than 1 mm in length (such as those in the large-bodied group in our study), do not reach the high local density that is required for ubiquitous dispersal. The dispersal-competition trade-off between small-bodied species and large-bodied species (Ellwood et al. 2009) could be well applied to oribatid community assembly, contributing to their high local diversity.

The possible existence of unexpectedly high dispersal ability, especially for species with small body size, may have homogenized oribatid mite community composition (an average of 31 % dissimilarity), producing a low β diversity in our study. Oribatid mites also have a low

species turn-over rate at a continental scale, as indicated by low slopes of their species-area relationships on islands and continents (Maraun et al. 2007). Similar results indicating a lack of dispersal limitation come from a study in mangrove forests in which species composition of oribatid mites was affected more by microhabitat diversity than geographic distance between islands (Karasawa and Hijii 2004). In contrast, unexpectedly high β diversity (~ 60% dissimilarity) of oribatid mite communities has been observed in patchy environments (canopy suspended soil) in temperate forests (Lindo and Winchester 2008). Furthermore, several other studies have revealed strong evidence for dispersal limitation among soil oribatid mites (Lindo and Winchester 2009, Caruso et al. 2012, Ingimarsdottir et al. 2012). The discrepancy between these studies and our results could arise from differences in the spatial scales of the studies (Freestone and Inouye 2006). The studies mentioned above were conducted at local scales spanning from 500 meters (Caruso et al. 2012) to 56 km (Lindo and Winchester 2009), whereas our study spanned over 400 km and a chronosequence around 10,000 years. High species turnover and community heterogeneity can occur at a small spatial scales due to intraspecific aggregation or species-specific microhabitat associations, and any dispersal limitation observed at this scale is likely to be confounded by unmeasured fine-scale environmental variables that are spatially structured (Dray et al. 2006). On the other hand, our study sites along the chronosequence were selected for similar edaphic and floristic characteristics to minimize environmental heterogeneity (Burton et al., 1991), which should favor our ability to detect any signal of dispersal limitation. Therefore, the similar species richness and the low β diversity among sites provide strong evidence for the conclusion that soil oribatid mites are not severely limited by dispersal to recolonize the deglaciated regions at a time scale of 10,000 years.

The quick recovery of soil oribatid mites in deglaciated areas is not restricted to our study

sites, but appears to apply to other deglaciated areas in North America. Based on a preliminary analysis of the Catalogue of Oribatida (Acari) of Continental United States and Canada (Marshall et al. 1987), the number of oribatid species recorded in deglaciated areas (Northeast of USA and most parts of Canada) is only slightly lower than that recorded in ice free regions of the southern part of North America (642 vs. 872 species). Furthermore, there are 249 species that only occur in deglaciated areas, and 490 species restricted to ice-free regions. This pattern suggests that soil oribatid mites can disperse rapidly enough to establish a sizeable species pool in deglaciated areas and generate latitudinally-based differences in species composition. It should be noticed that the re-colonization of oribatid species into deglaciated regions has been occurring over the last 10,000 years. The lack of dispersal limitation at this time scale does not exclude the possibility of dispersal limitation at a shorter time scale. As a matter of fact, studies have found that the diversity of soil oribatid mites did not recover in no-tillage farming or abandoned old fields within decades (Siepel 1991, Adl et al., 2006). Nevertheless, the rapid recovery of oribatid mites following glacial retreat is notably faster than other groups of soil fauna such as earthworms, which is at the other extreme of body size among soil fauna. It will be interesting to investigate how dispersal limitation influences the distribution of other groups of soil fauna with intermediate body size (e.g., Isopoda and Enchytraeidae) in deglaciated regions.

Our results suggest that environmental filtering and biotic interactions operate during community assembly of oribatid mites. While correlational, we found strong associations between oribatid communities (forest floor and mineral soil combined) and air temperature in our RDA analysis (Fig 5c). Air temperature was highly correlated with soil temperature in our study ($r^2 = 0.99$), and soil temperature has differential effects on the developmental rates of different oribatid species (Ermilov and Locnyska 2008). Moreover, different oribatid species

have distinct ranges of freeze and heat tolerance (Siepel 1996). It will be interesting to test whether the species with strong association with high temperature in our study (e.g., *E. nemoralis*, *E. longitarsalis* and *Eremobelba* sp.) have low freeze tolerance. Furthermore, we also have evidences for a substantial influence of litter fall (Table A2.2) and soil C: N ratio (Fig. 2.5c) on oribatid communities in the forest floor and mineral soil respectively. The amount of litter fall may represent initial resource input and soil C: N ratio could be an indirect measurement of resource quality. Overall, this suggests that temperature, litter input and soil C: N ratio may serve as an environmental filter to select oribatid mites best adapted to the local environment.

It might seem surprising to find no association between oribatid community structure and precipitation or other biogeochemical properties such as soil pH, which have been shown to influence oribatid communities in previous studies (VanStraalen and Verhoef 1997, Irmler 2006, Nielsen et al. 2010). However, our four study sites are all sugar maple forests, selected based on their similarity in plant communities and soil properties. This necessarily results in a narrow range of precipitation and biogeochemical properties among sites. The similarity of such environmental factors among our study sites could select for similar communities of mites and could partly contribute to the low β diversity we observed.

Soil fungal biomass explained a significant proportion of the variation in the small-bodied oribatid species in mineral soil, suggesting that food availability is important in structuring oribatid mite communities (Fig. 2.5c). At the same time, the abundance of Collembola in the forest floor also seemed to influence the community structure of oribatid mites living in the same horizon. As most oribatid species and Collembola rely at least partially on fungal hyphae as a food resource (Siepel and De Ruiter-Dijkman 1993, Maraun et al. 2003), oribatid mites and Collembola may compete for food resources. Differences in food availability

(i.e., fungal hyphae) could alter the competitive relationships among different oribatid species and thus change their relative abundance. Interestingly, while one species (*X. oblongus*) with a strong association with fungal biomass is found to feed primarily on fungal hyphae as a secondary decomposer, another species (*O. nitens*) is believed to be a predator or scavenger, relying on live/dead animal tissue (Gan et al. submitted manuscript). However, it should be noted that we only had measurements of soil fungal biomass in 2006 and the Collembola abundance (in 2011) was highly variable (Table 2.1). Nevertheless, these results suggest that both fungal biomass and Collembola abundance have the potential to influence the composition of oribatid communities by altering their competitive relationship for food resources.

In conclusion, despite their low locomotive activity, our results reveal a high local diversity of oribatid mites (46~56 spp.), but low β diversity (an average of 31% dissimilarity) across a long-term chronosequence. The lack of a clear spatial pattern in overall oribatid mite community composition across our study sites suggests that passive dispersal might be an overlooked but prominent dispersal mode by which oribatid mites recolonize deglaciated areas. Such passive dispersal may be more effective for species with small body sizes, whereas the effect of dispersal limitation is more pronounced for species with large body sizes. Our study also suggests that environmental filtering (temperature, litter fall and soil C:N) and competition for food resources (fungal biomass and Collembola abundance) contribute to determining oribatid species composition. Understanding the interactions among dispersal, environmental filtering and competition for food resources as driving forces of oribatid community assembly may help to predict how soil fauna will respond to global environmental change. Increases in temperature and changes in precipitation regimes, which are likely to occur in many regions, could have direct consequences for oribatid species composition as well as other soil organisms

(Eisenhauer et al. 2012; Nielsen et al. 2010). Additional drivers of environmental change such as atmospheric nitrogen deposition (Zak et al. 2008) that influence soil fungal communities, could have indirect effects on oribatid communities that would further feedback to alter ecosystem processes (Gan et al. 2013).

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Table 2.1. Climatic, biogeochemical and biotic properties (mean ± SE) of four hardwood sites sampled in our study.

Factors	Site A	Site B	Site C	Site D
Climatic				
Air Temperature (°C)	4.5 ± 0.22	5.9 ± 0.21	6.7 ± 0.21	7.4 ± 0.21
Precipitation (mm)	850 ± 27	880 ± 23	905 ± 22	836 ± 25
Biogeochemical				
Litter fall ($g.m^{-2} y^{-1}$)	414 ± 19.7	370 ± 11.1	515 ± 19.6	552 ± 27.0
Litter C:N	72.6 ± 6.0	52.5 ± 4.0	55.6 ± 3.7	66 ± 5.6
Litter Calcium ($mg.g^{-1}$)	12.4 ± 0.33	16.3 ± 0.30	13.1 ± 0.20	14.3 ± 0.22
O_mass ($g.m^{-2}$)	819 ± 108	2037 ± 519	2001± 372	2513 ± 301
Oi_C:N	40.2 ± 3.4	26.3 ± 0.8	24.7 ± 0.6	29.6 ± 1.0
Top 10 cm C:N †	13.1 ± 0.1	16.4 ± 0.8	14.6 ± 1.1	15.3 ± 0.9
SOM 0-10 cm ($g.m^{-2}$) ‡	4388 ± 570	4709 ± 805	3480 ± 424	2346 ± 526
Bulk density ($g.cm^{-2}$)	1.11 ± 0.16	0.96 ± 0.1	1.17 ± 0.04	0.91 ± 0.12
Soil pH	4.5 ± 0.17	4.7 ± 0.07	4.4 ± 0.07	4.6 ± 0.25
Biotic[c]				
Collembola ($individuals.m^{-2}$)	83 ± 38	257 ± 29	806 ± 286	2872 ± 2432
Mesostigmata ($individuals.m^{-2}$)	739 ± 2111	389 ± 96	1072 ± 195	650 ± 148
Fungi PLFAs ($nmol\ g^{-1}$ soil)	18.1 ± 1.69	10.4 ± 0.68	12.8 ± 3.39	10 ± 1.6
Total PLFAs ($nmol\ g^{-1}$ soil)	94.3 ± 6.7	63 ± 5.5	87.9 ± 23.2	61.7 ± 9.2

Notes: Multi-year measurements include: climatic data (1988-2004), litter fall (2001-2011), litter C:N (2002-2007), litter calcium (1988-2005). Biogeochemical data on O horizons and mineral soil (except soil pH) were measured in 2004 and are available from Pregitzer *et al.* 2008. Soil pH was measured in 2008. Collembola and Mesostigmata were collected in O horizon in 2011. Soil fungal and total microbial PLFAs were measured in 2006 and are available in van Diepen *et al.* 2010.

† Top 10 cm C:N is measurement of top 10 cm of OeOa horizon and mineral soil after removal of Oi horizon.

‡ SOM 0-10 cm is soil organic matter from upper 0-10 cm of mineral soil excluding O horizon.

Table 2.2. Results from Mantel/partial Mantel test with Spearman's rank correlation coefficient (ρ) of dissimilarity matrices between oribatid mite communities and four categories of environmental factors.

Horizons	Forest floor + Mineral soil			Forest floor			Mineral soil		
Body size	All	Large	Small	All	Large	Small	All	Large	Small
Spatial	0.6 •	0.94 *	0.48	0.49	0.71 *	0.09	0.31	0.31	0.6
Spatial (climatic)†	0.32	0.92 *	0.01	0.2	0.66 *	-0.19	0.32	0.32	0.73
Climatic	0.83 *	0.16	0.88 •	0.66 *	0.32	0.43	0.08	0.08	-0.03
Biogeochemical	-0.09	0.37	-0.03	0.14	0.26	-0.14	0.09	0.09	0.66 •
Biotic	-0.14	0.09	-0.09	0.14	0.09	0.37	0.03	0.03	0.83 •

Note: • $P < 0.1$; * $P < 0.05$.

† Partial Mantel test with climatic dissimilarity as covariate.

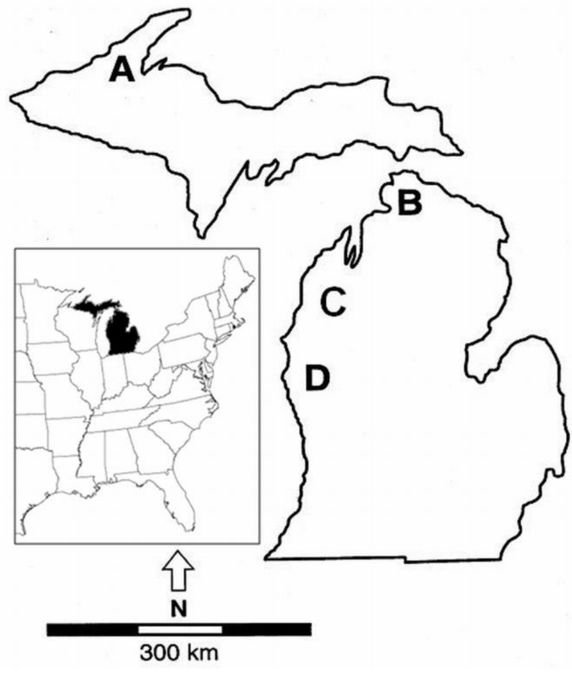

Figure 2.1. Forest sites composing a long-term chronosequence following glacial retreat. The southernmost site D is the oldest (13500 years), whereas the northern-most site A is the youngest (9500 years).

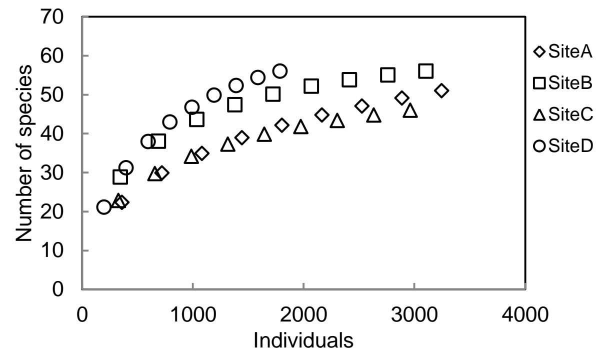

Figure 2.2. Total oribatid species richness (forest floor and mineral soil combined) in four study sites illustrated through rarefaction analysis. The data used for each site consisted of three replicate plots from three sampling dates.

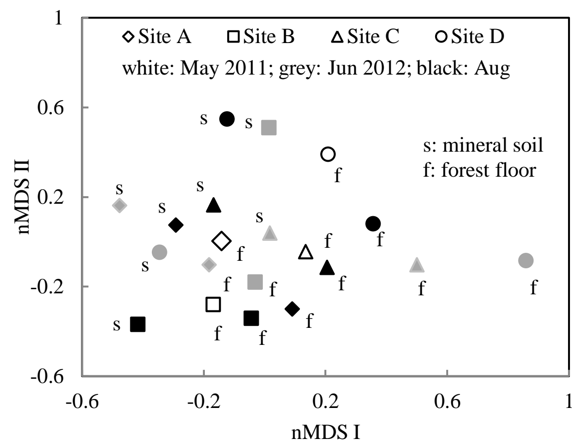

Figure 2.3. non-Metric Multidimensional Scaling (nMDS) illustrated that the species composition of the oribatid community varied among study sites and horizons, but did not differ among sampling dates. Stress value = 0.14. ANOSIM: site $R^2 = 0.33$, $P = 0.001$; horizon $R^2 = 0.18$, $P = 0.001$; season $R^2 = 0.09$, $P = 0.22$. Incidence data (after Hellinger transformation) were used in this analysis.

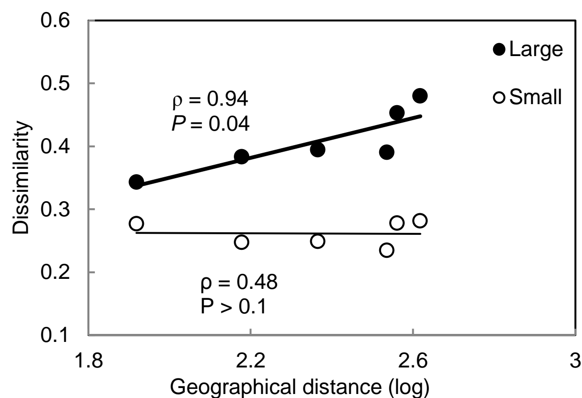

Figure 2.4. The relationship between oribatid community dissimilarity and geographic distance depends on body size. (a) Species with adult body length > 0.45 mm; (b) species with adult body length ⩽ 0.45mm. Species from forest floor and mineral soil are combined for this analysis. ρ indicates the Spearman's rank correlation coefficient between two dissimilarity matrices. *P* values indicate the significance of Mantel tests between the community dissimilarity matrices and the geographic distance matrix from four study sites. Incidence data (after Hellinger transformation) were used in this analysis.

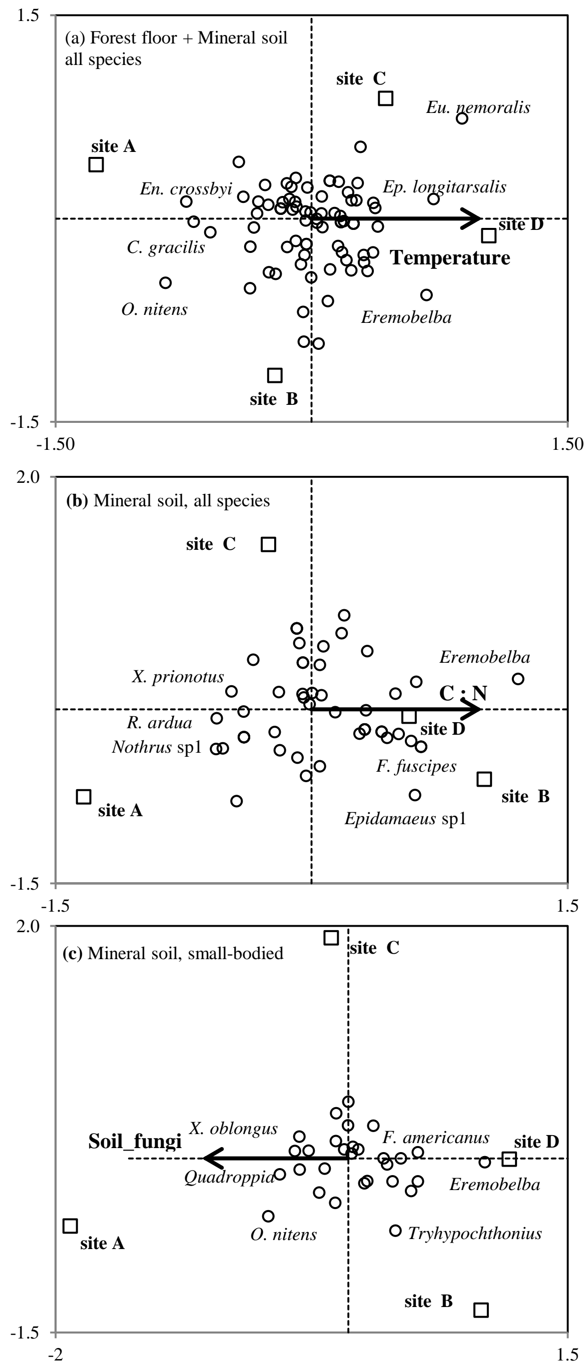

Figure 2.5. Oribatid species with strongest responses to (a) temperature, (b) soil C: N ratio and (3) soil fungal biomass from significant RDA results. Temperature (R^2 = 0.41, P = 0.04), soil C:N ratio (R^2 = 0.42, P = 0.04) and soil fungal biomass (R^2 = 0.54, P = 0.02) contributed substantially to the variation of oribatid mite communities in our study.

Appendix

Table A2.1. Species composition of oribatid mites in our study sites. Incidence data are shown in this table. The values are the total number of times that a species was encountered from a total of 54 subsamples from forest floor and 18 subsamples from mineral soil.

Horizons	Forest floor				Mineral soil			
Species	Site A	Site B	Site C	Site D	Site A	Site B	Site C	Site D
Achipteria sp.	1	6	0	0	0	0	0	0
Adrodamaeus sp.	3	0	3	8	0	1	0	0
Allosuctobelba sp.	4	3	4	0	1	1	2	1
Anachipteria howardi	0	4	0	1	0	0	1	0
Archiphthiracarus sp1	4	3	2	0	0	0	0	0
Archiphthiracarus sp2	2	0	0	0	0	0	0	0
Archiphthiracarus sp3	3	0	0	0	0	0	0	0
Archoplophora sp.	5	11	0	1	1	1	0	0
Atropacarus sp.	3	4	0	0	0	2	1	1
Autogneta sp.	5	2	0	2	0	0	0	2
Banksinoma sp.	1	4	0	0	0	2	0	2
Carabodes polyporetes	6	6	12	3	1	0	0	0
Carabodes sp1	1	4	0	1	0	0	0	0
Carobodes sp2	0	0	1	0	0	1	0	0
Cepheus cepheiformis	0	1	0	0	0	0	0	0
Ceratozetes gracilis	24	9	2	0	5	4	2	2
Chamobates cuspidatus	3	4	10	3	0	0	3	0
Cultroribula sp.	1	17	1	4	1	4	1	1
Eniochthonius crosbyi	36	26	13	0	7	3	8	3
Epidamaeus sp2	0	0	0	2	0	0	0	0
Epidamaeus sp3	0	0	0	5	0	0	0	0
Epidamaeus longiseta	1	2	3	2	0	0	0	0
Epidamaeus longitarsalis	0	1	5	9	0	0	2	2
Epidamaeus sp1	1	18	0	1	1	7	0	3
Eremaeus translamellatus	0	21	2	0	0	2	0	0
Eremobelba sp.	0	19	8	4	0	11	5	8
Eremulus sp.	0	0	0	4	0	1	0	1
Eueremaeus nemoralis	2	2	40	19	0	1	6	0
Euphthiracarus sp1	0	1	0	2	0	1	0	0
Euphthiracarus sp2	0	0	0	2	0	0	0	0
Fosseremus americanus	1	16	5	0	0	3	2	2
Fuscozetes fuscipes	0	19	0	0	0	3	0	1
Galumna sp1	7	3	1	9	0	0	0	0
Galumna sp2	1	0	0	4	0	0	0	0
Graptoppia foveolata	3	0	2	2	5	5	1	4

Heminothrus sp.	1	0	0	0	0	0	0	0
Lepidozetes sp.	21	11	8	9	2	1	3	2
Lucoppia cf. apletosa	0	0	0	2	0	0	0	0
Microtritia sp.	0	0	0	0	0	1	0	0
Multioppia c.f. carolinae	18	7	5	19	4	9	7	9
Nanhermannia sp.	0	0	0	0	0	0	3	0
Nothrus silvestris	18	2	7	2	5	1	1	1
Nothrus sp1	1	0	0	0	0	0	0	0
Ommatocepheus sp.	1	2	2	0	0	0	2	0
Oppia nitens	21	16	0	1	5	2	0	0
Oppiella nova	37	35	25	29	14	12	12	12
Oribatella calcarata	0	4	1	1	0	3	0	1
Oribatella sp1	0	0	1	0	0	0	0	0
Oribatula tibialis	28	33	45	28	1	4	10	1
Parachipteria sp.	2	6	31	2	0	0	3	0
Parapyroppia monodactyla	3	7	0	1	0	0	0	0
Peloribates sp.	0	0	0	1	0	0	0	0
Pergalumina sp.	3	1	0	0	0	0	0	0
Phthiracarus sp1	18	10	3	1	1	2	0	0
Phthiracarus sp2	3	3	0	0	0	0	0	0
Pilogalumna sp.	0	11	4	2	0	1	0	1
Platynothrus sp.	0	0	1	0	1	0	0	0
Podopterotegaeus sp.	4	5	2	1	8	4	7	5
Porobelba parki	2	10	13	7	1	0	3	2
Propelops sp.	1	0	0	0	0	0	0	0
Protokalumna sp.	0	0	0	1	0	0	0	0
Provertex sp.	0	4	0	2	0	0	1	1
Pyroppia sp1	0	2	0	0	0	0	0	0
Quadroppia sp.	3	0	0	0	2	0	0	0
Rhysotritia ardua	12	30	17	13	0	1	3	1
Scheloribates pallidulus	53	45	49	49	11	13	16	11
Siculobata sp.	0	1	2	0	0	0	0	0
Suctobelbella sp1	18	22	20	6	2	3	4	3
Suctobelbella sp2	31	29	22	15	11	13	15	13
Tectocephus spp.	26	29	38	6	4	5	7	5
Tryhypochthonious sp.	0	1	0	0	0	1	0	4
Xylobates oblongus	37	22	19	12	15	7	12	10
Xylobates prionotus	0	0	0	1	0	0	0	0
Xylobates sp1	0	0	0	1	1	0	0	0
Xylobates sp2	1	0	0	0	0	0	0	0
Xylobates sp3	0	0	1	3	0	0	0	0
Zygoribatula frisiae	0	0	0	1	0	0	1	0
Zygoribatula sp1	0	0	3	0	0	0	0	0

Zygoribatula clavata	1	0	2	0	0	0	0	0
Total richness	49	51	41	48	25	36	30	31

Table A2.2. Redundancy analyses of oribatid communities with each climatic, biogeochemical and biotic factor. For the combined community from both forest floor and mineral soil, all environmental factors were used. For community from forest floor (or mineral soil), the biogeochemical and biotic factors which are not relevant for that horizon were excluded.

Horizons	Forest floor + Mineral soil			Forest floor			Mineral soil		
Body size	All	Large	Small	All	Large	Small	All	Large	Small
Temperature	0.41 *	0.42 •	0.40 *	0.43	0.44	0.41	0.37	0.27	0.44
Precipitation	0.27	0.25	0.28	0.28	0.25	0.31	0.27	0.38	0.18
Litter fall	0.38	0.41	0.35	0.46 •	0.48	0.44	…	…	…
Litter C:N	0.30	0.31	0.28	0.28	0.28	0.27	…	…	…
Litter Ca	0.32	0.31	0.33	0.27	0.28	0.25	…	…	…
O_mass	0.39	0.38	0.40	0.36	0.39	0.35	…	…	…
Oi_C:N	0.36	0.37	0.34	0.30	0.33	0.27	…	…	…
Top 10 cm C:N	0.33	0.32	0.34	0.26	0.28	0.25	0.42 *	0.31	0.50 •
Soil pH	0.33	0.32	0.33	…	…	…	0.41	0.46	0.37
SOM 0-10 cm	0.37	0.33	0.36	…	…	…	0.23	0.19	0.25
Bulk density	0.30	0.26	0.35	…	…	…	0.39	0.35	0.43
Collembola	0.35	0.33	0.37	0.43 •	0.40 •	0.47 •	…	…	…
Mesostigmata	0.33	0.33	0.33	0.29	0.31	0.28	…	…	…
Soil fungi	0.37	0.35	0.39	…	…	…	0.43 •	0.28	0.54 *
Soil microbes	0.33	0.29	0.37	…	…	…	0.43	0.30	0.52 •

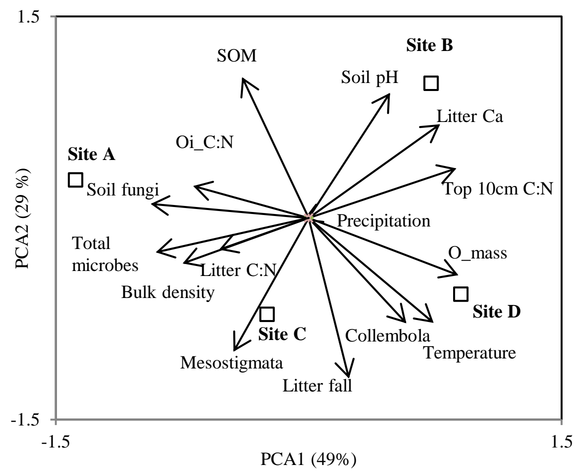

Figure A2.1. Principle Component Analysis (PCA) of all climatic, biogeochemical and biotic factors used in our study.

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Chapter III

Trophic structure and trophic dynamics of soil oribatid mites

Abstract. A key issue in ecology is the degree to which trophic structure within communities responds to environmental change. Organisms with generalist diets are more flexible in their feeding habits than are specialists, and may suffer less in a changing environment. Soil fauna fulfill crucial ecosystem functions in terrestrial ecosystems and many are thought to have generalized diets. They may therefore be buffered from negative effects of environmental change. Here, we used ^{15}N isotope analysis to study trophic differentiation among 91 species of oribatid mites and their responses to chronic atmospheric N deposition. Combining our own measurements with published data, we established that the trophic positions of mite species were remarkably stable within and among forests, as well as between ambient and experimental N deposition. Trophic stability indicates a higher than expected level of feeding specialization, which may foster diversity, but limit the ability to switch food resources in a changing environment.

Introduction

Soil animal communities are among the most species-rich components of terrestrial ecosystems (Giller 1996). In one square meter of soil, there may reside ~200 species of arthropods and up to ~1000 species of soil animals (Anderson 1975a). However, studies of the feeding biology of soil animals have revealed that many exhibit surprisingly similar behavior, with most apparently consuming a mixture of microbial and plant materials; accordingly, they have been classified as non-specialized feeders (Scheu et al. 2005). The existence of astonishingly high local diversity, with a low level of food resource specialization, is considered an ecological "enigma".

To explain the high diversity of soil animal communities, there has been speculation about finer-scale differences in food resource utilization among these generalist decomposers, which could go undetected using traditional methods, such as gut content analyses or food choice experiments (Anderson 1975a). For instance, it is difficult to use gut content analyses to distinguish between what is ingested by soil animals and what is actually assimilated as food, unless egestion is also studied (Scheu 2002). Similarly, observations of litter feeding do not exclude the possibility that the soil animals actually target microorganisms or other animals that colonize or dwell on the leaf litter. In this case, soil animals should be characterized as bacterial/fungal feeders (microbivores) or predators, rather than saprotrophic feeders that consume litter itself (Coleman et al. 2004).

In contrast to gut content analysis or food choice experiments, stable isotope ratios reflect the long-term trophic relationships of animals and are a powerful tool in evaluating the trophic structure of animal communities (Minagawa and Wada 1984, Scheu and Falca 2000). In general, there is discrimination against ^{15}N during catabolism, leading to an accumulation of ^{15}N in organisms relative to their food resource (Minagawa and Wada 1984). Reviews of different food

webs have demonstrated that there is an average increase of 3.4‰ of ^{15}N with each trophic level, although the enrichment levels may vary among different taxonomic groups or developmental stages (Post 2002).

Measuring the trophic structure of soil animals would not only provide insight into potential niche differentiation underlying their coexistence, but also substantially improve our ability to predict effects of global environmental change on soil food web structure and dynamics. If many soil fauna are indeed dietary generalists, they may be buffered to a greater extent from environmental change than are dietary specialists. Recent meta-analyses indicate that chronic atmospheric N deposition, a pervasive agent of global change, can reduce microbial biomass (-20%) and inhibit litter decay in many forests (Knorr et al. 2005, Liu and Greaver 2010), thereby decreasing the flow of energy through soil food webs (Gan et al. 2013). Reduced energy flow from the microbial community could alter the trophic structure of soil food webs, especially if microbivorous soil animals switch their diets to other food sources. The generalist feeding habits ascribed to most soil animals suggest that such a switch is plausible (Behan and Hill 1978, Maraun et al. 2003, Scheu et al. 2005), but we have a limited understanding of how a reduced flow of energy, the result of chronic N deposition, may affect the trophic structure of soil food webs.

Our study focused on the trophic structure of soil oribatid mites, a major group of wingless microarthropods in many ecosystems; up to 170 species can coexist in the litter and soil of hardwood forests (Hansen 2000). Recent studies of their $\delta^{15}N$ reveal that they occupy more than four trophic levels, which may, in part, contribute to their high local diversity (Schneider et al. 2004). However, estimating trophic differentiation among oribatid mites based on ^{15}N analysis requires additional empirical work (Maraun et al. 2011). By obtaining measurements of

^{15}N from oribatid mites dwelling in the forest floor and combining information from previous ^{15}N studies, the first aim of our study was to investigate the trophic structure of an oribatid mite community and determine the frequency of saprotrophic feeders compared to those with other feeding habits. We expected to find a low frequency of saprotrophic species, assuming a lack of coevolution between consumers and dead plant material. Secondly, we investigated the stability of the trophic structure of soil oribatid mites in the face of environmental change, specifically chronic nitrogen deposition as well as variation among different forest types. Previous research at our field sites has revealed that chronic experimental N deposition has reduced plant litter decay and accelerated organic matter accumulation in forest floor and surface mineral soil (Zak et al. 2008). At the same time, microbial biomass has been reduced by 18% under experimental N deposition (DeForest et al. 2004). The lower microbial biomass and the slowing of litter decomposition has reduced the flow of energy into the detrital food web, reducing the abundance of microarthropods by 41% and shifting species composition within the oribatid mite community (Gan et al. 2013). We expected that, following the decrease in microbial biomass and slowing of litter decay under chronic nitrogen deposition (Zak et al. 2008), oribatid mites would feed more on plant litter leading to a decline in their trophic position from higher to lower trophic levels.

Materials and methods

Site description

We collected soil oribatid mites from a long-term study of experimental N deposition consisting of four sugar maple (*Acer saccharum*)-dominated northern hardwood forests in the Great Lakes region of North America. These four sites are denoted as Site A (46:51N; 88:52W), Site B (45:32N; 84:51W), Site C (44:22N; 85:49W) and Site D (43:40N; 86:08W) spanning from north to south in the state of Michigan, USA. The sites are floristically and edaphically matched (>80%

sugar maple on sandy soils), but they differ in climate along a north-south latitudinal gradient. These hardwood forests are underlain by slightly acid soils (pH 4.41 – 4.70) that are well-drained sandy typic Haplothords of the Kalkaska series. Within each study site, six 30-m x 30-m plots were established in 1994; 3 plots receive ambient N deposition and the remaining 3 plots receive an additional 30 kg NO_3^--N ha^{-1} y^{-1}. The additional NO_3^- is delivered over the growing season in six equal applications of solid $NaNO_3$ pellets; an additional 10-m wide buffer surrounds each plot, and it also receives the experimental treatments.

Oribatid mite collections

Forest floor (including Oi and Oe/a horizons) samples were first collected in late May 2011 as in Gan *et al.* (2013). Within each plot, a 10-cm x 10-cm PVC frame was randomly placed on the forest floor, and any organic substrate above the mineral soil was collected and placed into a plastic bag. At each site, a total of 6 samples were collected from each plot receiving either ambient (n = 3) or experimental N deposition (n = 3), resulting in a total of 144 samples (4 sites x 6 plots x 6 samples). All of the samples were transported to the lab in coolers and placed on modified Tullgren funnels within 48 hours to extract microarthropods (Crossley and Blair 1991). After the 5-day extraction, litter was placed in a 60 °C oven for 24 hours for subsequent determination of dry mass. A second microarthropod collection was conducted in early June 2012 in all four study sites, but from ambient N plots only (4 sites x 3 plots x 6 samples = 72 samples total).

The extracted microarthropods were preserved in 70% ethanol, which does not influence the $\delta^{15}N$ of soil animals (Fábián 1998). The major group, oribatid mites, were enumerated under a microscope and further identified to genus or species based on the keys written by R.A. Norton and V.M. Behan-Pelletier (*unpublished data*) for use at the Ohio State University Summer

Acarology Program.

Stable isotope analysis

Dominant oribatid mite species from each site were removed from ethanol and placed into pre-weighed tin capsules. Ten to 150 individuals for each species were composited to generate enough mass for stable isotope analysis, which also ensured that we had a representative sample of individuals from a particular species. The tin capsules with oribatid mites were weighed again, after they were oven-dried at 60 °C for 24 hours, to obtain the dry weight of mites. Each species composite ranged from 0.40 mg to 1.50 mg. For each study site, we selected the dominant species to ensure enough mass for isotope analysis. In total, we were able to analyze 23 species of oribatid mites, each with 1-4 composite replicates from the ambient N plots at our four study sites. In addition, nine of the 23 species were also sufficiently abundant in the experimental N deposition plots for analysis. These 9 species were paired for comparisons with the same species from adjacent ambient N plots, with 5 species pairs from Site A, 3 pairs from Site C and 1 pair from Site B from the same sampling trip (May 2011).

A total of 24 ground litter samples were also analyzed for ^{15}N abundance, which we used as background to adjust the $\delta\ ^{15}N$ of mites. The litter samples collected on May 2011 were oven dried at 60 °C for 24 hours, following microarthropod extraction. The dried litter from each N deposition treatment (ambient vs. elevated) was composited and homogenized for each site. A subsample of 5 g from each of the litter composites was ground. Two replicates (5 mg) from the ground samples, together with mite samples collected in June 2012, were sent for stable isotope analysis at the Stable Isotope Facility at the University of California, Davis. The mite samples collected in May 2011 were analyzed in the Terrestrial Ecology Stable Isotope Lab at the University of Michigan. In both facilities, the $^{15}N/^{14}N$ ratios of animals and litter were

determined by a coupled system of an elemental analyser (UC Davis: NA 1500, Carlo Erba, Milan; U of Michigan: NC2500, CE Elantech, NJ) and stable isotope mass spectrometer (UC Davis: MAT 251, Thermo Finnigan, CA; U of Michigan: Delta Plus, Thermo Finnigan, CA).

The natural abundance of stable isotopes was expressed using δ notation (‰) and calculated as $\delta_{sample} = (R_{sample} - R_{standard}) / R_{standard}$, where R_{sample} and $R_{standard}$ are the heavy/light isotope ratios of the sample and standard. In the facility at UC Davis, two laboratory standards (Nylon 5 and Glutamic acid) were interspersed among the samples and the standard deviation of the laboratory standards ranges from 0.20 to 0.21‰. In the facility at the University of Michigan, six laboratory standards (keratin, caffeine, NBS tomato leaves, NBS bovine liver, corn flour and rice flour) were interspersed among the samples and the regression ($r^2 = 0.9999$) between their known and measured ^{15}N values was used to calibrate that of the samples. The final values were calibrated as relative to an internal standard (atmospheric N_2).

Calibration of the trophic positions of oribatid mites

Because the $\delta^{15}N$ for the basal food resources varied among study sites, we calibrated the trophic positions of oribatid mites separately at each site, based upon the $\delta^{15}N$ of the litter in which the oribatid mites were collected. First, we chose the lowest $\delta^{15}N$ of the litter as a base line (-3.26‰ from the ambient plots of Site D for convenience. Then, the $\delta^{15}N$ values of mites ($\delta^{15}N_{raw}$) from different plots and sites were calibrated by subtracting the difference between the $\delta^{15}N$ values of the litter ($\delta^{15}N_{litter}$) and -3.26‰, such that the calibrated $\delta^{15}N$ values of mites $\delta^{15}N_{calibrated} = \delta^{15}N_{raw} - (\delta^{15}N_{litter} - (-3.26‰))$. We assigned oribatid mite species into different trophic groups based on their calibrated $\delta^{15}N$ values: lichen feeders (lycophages), saprotrophic feeders, bacterial/fungal feeders and predators/scavengers.

Saprotrophic feeders are not enriched in their $\delta^{15}N$ by 3.4‰ above that of litter (Vanderklift and Ponsard 2003). When animals feed on low protein diets (such as litter), the dietary protein is reserved for body composition and maintenance, rather than catabolized for energy (Gannes et al. 1997). As such, the ^{15}N of their diet is maintained in body tissue, instead of an accumulation of the heavier isotope during catabolism. Therefore, we set the $\delta^{15}N$ for saprotrophic feeders to vary around those of their resource ($\delta^{15}N$ of litter +/- 1.7‰) similar to the approach used by (Illig et al. 2005). Lichens in general exhibit distinctly low $\delta^{15}N$ and lichen feeders maintain those values in their bodies. Therefore, we categorized any oribatid mite species with a $\delta^{15}N$ at least 1.7‰ lower than that of litter as a lichen feeder. The upper limit of the $\delta^{15}N$ of saprotrophic feeders, 3.4‰, would designate the upper boundary for fungal/bacterial feeders, and species with $\delta^{15}N$ higher than that would be designated as predators (live animals) or scavenger (dead animals).

Review of previous studies of trophic positions of soil oribatid mites

To determine the prevalence of saprotrophic feeders among soil oribatid mites, and to compare the stability of trophic structure among additional field sites, we summarized the $\delta^{15}N$ of oribatid mites from published studies to enhance the sample size. In total, there were 7 publications with measurements of $\delta^{15}N$ of oribatid mites (Maraun et al. 2011), and we excluded two studies that focused on bark-living oribatid mites (Erdmann et al. 2007, Fischer et al. 2010), resulting in 5 studies with data for oribatid mites dwelling in forest floors, including Scheu and Falca 2000, Schneider et al. 2004, Illig et al. 2005, Pollierer et al. 2009 and Maraun et al. 2011.

From these studies, we used the average $\delta^{15}N$ of each oribatid species and also that of the leaf litter collected from the associated sampling location. To make those measurements comparable among studies, we only included the adults of litter-dwelling oribatid mites, even

though it has been suggested that developmental stage (juveniles vs. adults) or dwelling depth (litter vs. mineral soils) has little effect on trophic positions (Scheu and Falca 2000). To allow comparison of δ^{15}N among oribatid mite species from different studies, we adjusted the δ^{15}N of the animals by correcting for the differences between the δ^{15}N of the litter in our study (-3.26‰) and those in the literature. We assigned oribatid mites into trophic positions based on their calibrated δ^{15}N; these included lichen feeders, saprotrophic feeders, fungal/bacterial feeders and predators/scavengers. The resulting trophic affiliations did not differ from the conclusions presented in the original literature.

Statistical analysis

We used two-way ANOVA (site and treatment, type I sum of squares) to assess any difference in the δ^{15}N of forest floor from our four study sites with different N deposition levels. Each δ^{15}N of forest floor was averaged from the two subsamples from the same ground sample. The forest floor of the experimental N deposition plots at Site B was subjected to a ^{15}N labeling experiment conducted in 1998 and is highly enriched (Zak et al. 2004); we thus treated its δ^{15}N as a missing value in the two-way ANOVA analysis. We also used two-way ANOVA (site and species, with type II sum of squares for site) to test whether the trophic positions of oribatid mites varied among different study sites, after removing the variation among different species. A student's paired *t*-test was used to compare the δ^{15}N of oribatid mites (after calibration) under ambient and experimental N deposition.

We then combined our measurements with data from previous studies. We were interested in whether the δ^{15}N of oribatid mite species differed among measurements from different authors. Therefore, we used two-way ANOVA with Species and Author as main effects to analyze within-species variation in δ^{15}N measurements of the same oribatid species from

different authors after controlling for the variation among species (Species was entered first into the model with type II sum of squares for Author). All of the above statistical analysis was performed in software R 2.15.1 (R Development Core Team 2012).

Results

^{15}N of forest floor

The natural abundance of ^{15}N in the forest floor of the ambient plots was similar among sites, ranging from -3.26 to -1.77‰ ($F_{3,2}$ = 4.06, P = 0.20). As expected, the forest floor in the experimental N deposition plots at Site B was highly enriched in ^{15}N (5.18‰), as a consequence of a ^{15}N labeling experiment conducted at this site in 1998 (Zak et al. 2004). When we excluded Site B from the analysis, we still found a slight increase (an average of 1‰) in ^{15}N in forest floor under chronic nitrogen deposition ($F_{1,2}$ = 17.9, P = 0.051). This likely arises from added NO_3^- -N, which is enriched compared with litter with a 0 $\delta^{15}N$.

Trophic positions of oribatid mites

The calibrated $\delta^{15}N$ of 23 oribatid mite species (ambient plots only, a total of 43 measurements) formed a continuum ranging over 9‰, with *Parachipteria sp.* being the lowest (- 3.54‰), whereas the highest was *Emerobelba sp.* (6.11‰, Fig. 3.1). Two-way ANOVA revealed that the $\delta^{15}N$ values of oribatid mites did not vary between sampling years ($F_{1, 19}$ = 0.23, P = 0.63) or among sampling sites (Fig. 3.2, $F_{3, 17}$ = 1.24, P = 0.32). For subsequent analyses, we treated the samples from different years or sampling sites as replicates, resulting in 1- 4 replicates for each species.

Based on an increase of 3.4‰ per trophic level and the base line level at -3.26‰, we have designated 3 species as saprotrophic feeders (-4.96 to – 1.56‰), 13 species as

fungal/bacterial feeders (-1.56 to 1.84‰) and 6 species as predators/scavengers (1.84 to 5.24‰) that feed on dead or live animal tissues or any food item that has a high δ^{15}N value. We also had one species *Eremobelba.* sp. with an extremely high δ^{15}N (6.11‰), which we therefore designated as a secondary predator (Fig. 3.1).

Response to chronic nitrogen deposition

We were able to collect 9 species with paired measurements from plots receiving ambient and experimental N deposition. A Student's paired *t* test revealed that the trophic levels of oribatid mites did not differ between N deposition treatments (Fig. 3.2, t = - 1.17, P = 0.27). The differences in δ^{15}N between the N deposition treatments were smaller than 1‰ for most species, except *Oppiella nova* (-2.62‰; Fig. 3.2).

Review of the trophic positions of oribatid mites

Combining our own measurements with previously published data, we could examine a total of 91 litter-dwelling oribatid mites. They formed a continuum ranging from -7.11 to 6.11‰ (see Table A3.1 in Appendix). Out of these 91 oribatid species in the forest floor, there was only 1 species with a distinctly negative δ^{15}N (*Carabodes labyrinthicus*), and it was therefore categorized as a lichen feeder. Additionally, one species expressed a δ^{15}N two trophic levels above that of the saprotrophic feeders, and it was therefore categorized as a secondary predator (*Eremobelba.* sp., Fig. 3.1). Over half of the species (55%) were ascribed to the second trophic level as microbivores and only 22% of the species were designated as litter feeders. The remaining 21% of the species were categorized as predators/scavengers (Fig. 3.3). There were 16 species with measurements from more than one study (a total of 46 measurements with 2-5 replicates for each species). Two-way ANOVA of the 16 species revealed that δ^{15}N of the same

species did not differ among studies ($F_{5,25} = 0.15$, $P = 0.96$, Fig. 3.4). A complete list of oribatid species with their trophic positions can be found in Appendix T1.

Discussion

Trophic structure of soil oribatid mites

In contrast to the idea that most soil oribatid mites feed on dead plant material, the occurrence of saprotrophic feeders was low, comprising 13% of species in our study and 22% when we combined our data with that available in the literature (Fig 3.3.). The percentage of lichen feeders was also low, probably due to the scarcity of lichen in the forest floor of these temperate forests. This is in contrast to the high occurrence of lichen feeders on tree bark or in the forest floor of coniferous forests with a high abundance of lichen (Erdmann et al. 2007). Over half of the oribatid mites were categorized as fungal/bacterial feeders. Considering their chewing mouthparts and the observation of fungal hyphae in their guts (Anderson 1975b, Kaneko 1988), oribatid mites are more likely to be fungal feeders than bacterial feeders, which generally require tube-like mouthparts to ingest bacterial cells (Yeates et al. 1993). Oribatid mites in the second trophic level could theoretically feed on saprotrophic mites at a lower trophic position; however, orbatid mites are usually heavily sclerotized, making predation by other oribatids unlikely (Peschel et al. 2006). The oribatid mites that were categorized as predators are more likely to feed on other less-defended soil animals such as fungal-feeding Collembola or bacterial-feeding nematodes. They may also acquire their high δ^{15}N from feeding on dead animals as scavengers. It is uncertain whether *Eremobelba.* sp. acquired its high δ^{15}N by scavenging on dead animals or by preying upon other predators; the latter would make it a secondary predator. Nevertheless, almost 80% of oribatid mite species exhibited δ^{15}N much higher than that of litter, suggesting that litter is not their major food resource. Using a different approach with ^{13}C-labeled plant leaf and root

litter, Pollierer et al. (2007) came to a similar conclusion. In their study, leaf litter contributed little as a carbon source for soil animals, and the great majority of soil animals acquired carbon from roots or root-derived carbon resources, especially ectomyccorrizal fungi.

If the majority of the oribatid mites rely on live food items (i.e., fungi hyphae or animal tissue), then biotic interactions between oribatid mites and their food resources are a potential driver of high oribatid diversity. In particular, over half of the oribatid mites were categorized as fungivores. Considering the vast diversity of fungi in the forest floor, specialized feeding by oribatid mites on certain groups of fungi may help to maintain the coexistence of oribatid species with similar trophic positions. Indeed, soil oribatid mites have distinct preferences when offered different fungal diets (Mitchell and Parkinson 1976, Schneider and Maraun 2005). On the other hand, soil-borne fungi have a plethora of defensive traits against fungivores, including the formation of crystalline structures outside fungal hyphae as well as the production of toxic secondary metabolites (Böllmann et al. 2010). These observations suggest that fungi and fungivorous oribatid mites have the potential to co-evolve in a manner similar to that observed in plant-herbivores interactions aboveground (Thompson 1994). Such close evolutionary interactions may provide the key for understanding the high diversity of both fungi and fungivores in soil habitats.

Understanding the trophic structure of orbatid mite communities not only helps to explain their high local richness, but also has implications for ecosystem functioning. For instance, the contribution of litter feeders to soil respiration could be trivial; however, fungal feeding by orbatid mites could regulate fungal community composition as well as the mineralization of N from ingested fungal hyphae (McGonigle 1995, Crowther et al. 2011). Moreover, other groups of soil animals such as Collembola and Enchytraeidae also derive a majority of their diet from fungi

(Didden 1993, Maraun et al. 2003). Overall, fungivores comprise between 21 and 76% of the soil fauna biomass in both natural and managed ecosystems (McGonigle 1995). Modeling suggests that fungivores may consume 86% of net fungal production in woodland soil (McBrayer et al. 1974), although empirical studies of fungal consumption by soil animals are rare. Gut content analysis has found that fungal hyphae in the guts of soil oribatid mites constitute 3% of the standing fungal biomass in an aspen forest (Mitchell and Parkinson 1976). Considering the low proportion of active hyphae in soil (Kjøller and Struwe 1982), and their consumption by other fungivorous animals, the proportion of net fungal production consumed by soil animals could be substantial. By influencing fungal communities though grazing, fungivores could have important consequences for ecosystem processes such as litter decay (Gan et al. 2013) and C storage (Clemmensen et al. 2013).

Stability of the trophic positions of oribatid mites from different environments

We expected that fungal-feeding oribatid mites would switch to consuming litter under chronic N deposition, in response to reduced fungal biomass and the accumulation of organic matter under experimental N deposition (Zak et al. 2008). However, the $\delta^{15}N$ of oribatid mites categorized as fungal feeders under ambient N deposition did not decline significantly under experimental N deposition treatments. The exception was *O. nova,* which displayed a 2.6‰ decline in $\delta^{15}N$ under chronic N deposition. This marked decline suggests that *O. nova* switched trophic positions from a fungal feeder under ambient N deposition (0.53‰) to a saprotrophic feeder under chronic N deposition (-1.88‰). It should be noted that *O. nova* is the most cosmopolitan species of all oribatid mites and often cited as a pioneer and dominant species in highly disturbed systems (Marshall et al. 1987; Siepel 1996). It reproduces asexually and has the most rapid development time of any species under study (Haenko 1988). Such ecological flexibility and

rapid ontogeny suggests that *O. nova* is more of a generalist than the other oribatids that we studied. However, we were only able to obtain a single measurement of $\delta^{15}N$ from *O. nova* in each N deposition treatment, due to its small body size (0.3 mm), which produced a limited biomass for stable isotope analysis. More replicated measurements will be needed in order to confirm that this decline in $\delta^{15}N$ represents a true dietary shift. In contrast to *O. nova*, most oribatid mites are characterized by "K-selected" life history traits including slow development, low rates of reproduction, and long adult life (Norton 1994). These traits may constrain their ability to respond to environmental change.

The general lack of change in the trophic positions of oribatid mites under chronic N deposition suggests that their trophic structure is relatively stable, despite changes in their food resources (fungal biomass and organic matter) under chronic N deposition. Two further lines of evidence in our study indicate stability in the trophic positions of soil oribatid mites across larger spatial scales. First the $\delta^{15}N$ of oribatid mites did not differ significantly among the four sugar-maple forests that we sampled (Fig. 3.1). These four sites vary in annual average temperature from 4.7 ^{o}C at Site A (northern site) to 7.6 ^{o}C at Site D (southern site), a temperature differential that approximates typical scenarios of global climate change. Second, our literature survey revealed similar trophic positions for the same oribatid species collected from different ecosystems (beech *vs.* sugar-maple forest, Fig. 3.4). Our results are consistent with previous findings that the $\delta^{15}N$ of oribatid mites vary little with soil depths in the same forest (Scheu and Falca 2000) or among different forests (Schneider et al. 2004). Similarly, Erdmann et al. (2007) found that oribatid species from the bark of different trees (coniferous *vs.* broad-leaf trees) shared very similar trophic position, despite differences in the abundance and composition of their potential food resources (lichens, algae and fungi etc.) between these two types of tree bark. By

combining data of our own with data from previous studies, we provide more conclusive evidence that soil oribatid mites express stability in their trophic positions at large spatial scales.

Stability in the trophic positions of oribatid mites is unexpected because oribatid mites are often considered generalists that change their diets based on the food resources that are available (Behan and Hill 1978, Maraun et al. 2003, Scheu et al. 2005). However, the stability in their trophic positions within and among ecosystems, and their lack of response under chronic N deposition, indicate that oribatid mites may be more specialized than previously thought. Schneider et al. (2004) have suggested that niche differentiation among different trophic groups may, in part, contribute to the high diversity of soil oribatid mites. We have evidence to suggest that differentiation within trophic groups may be another important mechanism in maintaining their local diversity. For example, while both categorized in the second trophic level as microbivores, *Galumna lanceata* (0. 82 +/- 0.53‰, mean +/- 1SD) consistently exhibited a higher $\delta^{15}N$ than did *Carabodes femoralis* (-0.83 +/- 0. 24‰) without any overlap in their trophic positions. Likewise, litter feeding *Steganacarus magnus* (- 1.96 +/- 0. 65‰) had a consistently higher $\delta^{15}N$ than did *Platynothrus peltifer* (-4.0 +/- 0. 47‰) in the same trophic group. We recognize the small number of replicates available to us for some species, but these species-specific $\delta^{15}N$ within trophic levels suggest that oribatid species may feed selectively and consistently on food items with different ^{15}N abundances, thereby acquiring the $\delta^{15}N$ of their food resources.

Variation in $\delta^{15}N$ within trophic groups resulted in a continuum of trophic positions of oribatid mites, rather than discrete trophic levels. Such continuous distribution of trophic positions is, in fact, rather common in soil animal communities (Schneider et al. 2004; Chahartaghi et al. 2005), which contrasts with stepwise enrichment of ^{15}N in distinct trophic

levels in some aquatic ecosystems (Minagawa and Wada, 1984). The variation in ^{15}N in the same trophic group of mites may reflect different food resources with varying δ^{15}N in soils, such as differences between fungal groups (saprotrophic or mycorrhizal fungi) or different parts (chitin *vs.* protein) of fungi (Hobbie et al. 2012). Alternatively, continuous change in δ^{15}N may reflect diet mixing, whereby mites are feeding on more than one trophic level. While selective feeding and omnivory are both widespread in oribatid mites (Walter 1987), the use of a single stable isotope is unable to separate these two mechanisms. Nevertheless, the stability of their isotopic trophic positions suggests that oribatid mites feed consistently on specific food items or specific mixtures.

We caution that most of the available ^{15}N measurements of oribatid mites were from temperate forests (and mountain rain forest from Illig et al. 2005); studies from other ecosystems (grasslands, boreal forests and tundra) are sorely needed for comparisons of the consistency of trophic positions of oribatid mites from different ecosystems. However, at least in temperate hardwood forests, the high occurrence of fungivores and the stability of their trophic positions under different environments, suggests a degree of specialization that helps to resolve the enigma of their high local diversity. Moreover, the apparent inflexibility of their diets in the face of environmental variability suggests that oribatid densities may suffer under environmental change, with concomitant effects on ecosystem processes. Indeed, a marked decline in the density of soil oribatid mites under chronic N deposition has been documented in our previous work (Gan et al. 2013). While the degree of dietary specialization in other groups of soil animals is not well known, the negative effects of environmental change on many groups of soil animals suggests that they may generally have a limited ability to accommodate a quickly-changing environment (Blankinship et al. 2011). The change in abundance and community structure of soil

animals is likely to have further consequences for ecosystem functioning, including carbon cycling (Gan et al. 2013).

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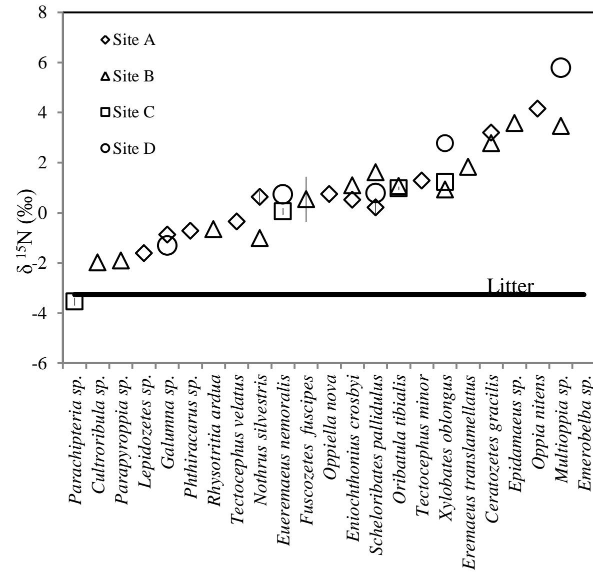

Figure 3.1. Dominant oribatid mite species in the forest floor of four hardwood forests occupy more than three trophic levels. δ^{15}N values of mites were calibrated based on differences between ^{15}N levels of substrates from that of the ambient N treatment of Site D (- 3.26‰). Two-way ANOVA (type II sum of square) indicated that the same species from different sites did not differ in their δ^{15}N values ($F_{3, 17}$ = 1.24, P = 0.32). Diamond: Site A; triangle: Site B; square: Site C; circle: Site D. Error bar represents 1 SD when there were replicated measurements.

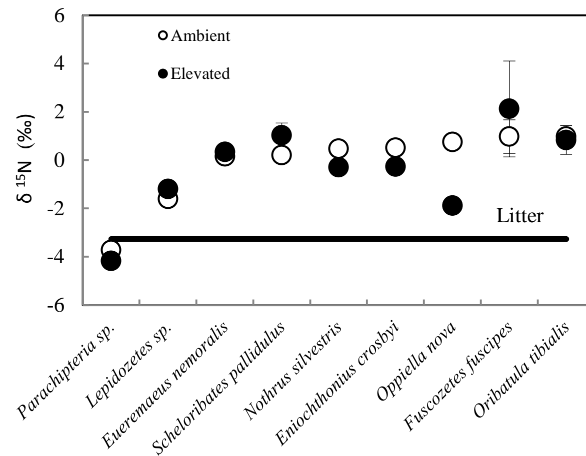

Figure 3.2. Trophic positions of oribatid mites (indicated by their δ^{15}N) in the forest floor did not differ between ambient (empty) and elevated (solid) N deposition plots. δ^{15}N values of mite were calibrated based on differences between δ^{15}N of substrates from that of the ambient N treatment of Site D (- 3.26‰). Error bar represents 1 SD when there were replicated measurements. Student's *t* test (paired): t = - 1.17, P = 0.27.

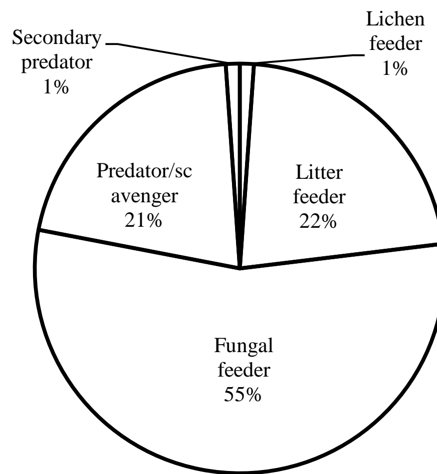

Figure 3.3. The proportions of different feeding habits among 91 oribatid mite species from combining current measurements with previously published studies.

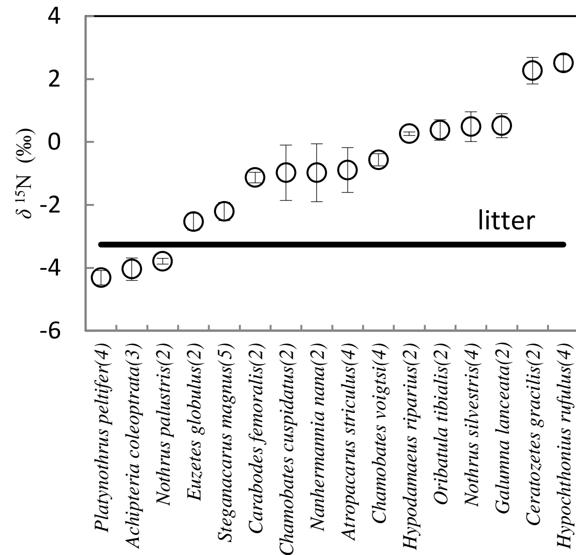

Figure 3.4. Trophic positions of oribatid mites (indicated by δ^{15}N) show little variation among different studies (two-way ANOVA with type II sums of squares, $F_{5,25} = 0.15$, $P = 0.96$). δ^{15}N values of mites were calibrated based on differences between δ^{15}N of substrates from that of the ambient N treatment of Site D (- 3.26‰). Error bar represents 1 SE. The number in parenthesis after the species name indicates the number of studies with measurement for that species.

Appendix

Table A3.1. Trophic positions and possible food resources of oribatid mites from our own samples and from other published studies, as indicated by their average $\delta^{15}N$ value (standard deviation inside parenthesis if there were measurements from more than one study). The $\delta^{15}N$ values were calibrated by setting that of the basal food resource at -3.26 ‰.

Species	$\delta^{15}N$ values	Trophic level†	Food resources	Data sources‡
Carabodes labyrinthicus	-7.44	-1	Lichen	b
Platynothrus peltifer	-4.01(0.46)	1	Predominantly litter,	d, e
Carabodes marginatus	-3.8	1	partly fungi	b
Achipteria coleoptrata	-3.74(0.61)	1		b, d, e
Parachipteria sp.	-3.54	1		f
Rostrozetes ovulum	-3.44	1		c
Nothrus palustris	-3.38(0.21)	1		a, b, e
Tectocepheus spp.	-3.36	1		e
Tectocepheus spp.	-3	1		e
Liacarus spp.	-2.8	1		b
Euzetes globulus	-2.23(0.38)	1		b, d
Liacarus xylariae	-2.16	1		e
Hermannia gibba	-2.01	1		d
Oribatella spp.	-2	1		b
Cultroribula sp.	-1.98	1		f
Steganacarus magnus	-1.90(0.64)	1		a, b, d, e
Parapyroppia	-1.9	1		f
Adoristes spp.	-1.8	1		b
Phthiracaridae *sp.*	-1.76	1		e
Rhysotritia duplicata	-1.7	1		b
Lepidozetes sp.	-1.61	1		f
Phthiracaridae	-1.5	2	Predominantly	e
Hermannia sp.	-1.26	2	fungi, partly litter	e
Eupelops sp.	-1.16	2		e
Galumna sp.	-1.09	2		f
Eupelops plicatus	-0.9	2		b
Carabodes coriaceus	-0.86	2		e
Carabodes femoralis	-0.83(0.24)	2		b, e
Malaconothrus spp.	-0.8	2		b
Phthiracarus sp.	-0.72	2		f
Chamobates cuspidatus	-0.68(1.24)	2		b, e
Nanhermannia nana	-0.68 (1.30)	2		b, c
Damaeus riparius	-0.66	2		e
Rhysotritia ardua	-0.65	2		f
Atropacarus striculus	-0.59(1.42)	2		a, b, e
Hermannobates monstruosus	-0.56	2		c
Tectocephus velatus	-0.35	2		f
Chamobates voigtsi	-0.26(0.39)	2		a, b, e
Cepheus dentatus	-0.2	2		a
Damaeus clavipes	0.04	2		b
Galumna sp.	0.09	2		d
Nanhermannia coronata	0.1	2		b

Eueremaeus nemoralis	0.29	2		f
Xenillus sp.	0.39	2		c
Paradamaeus clavipes	0.4	2		b
Notophthiracarus ecophylus	0.44	2		c
Damaeus onustus	0.44	2		d
Scheloribates spp.	0.5	2		b
Fuscozetes sp.	0.54	2		f
Hypodamaeus riparius	0.57(0.07)	2		a, b
Oribatula tibialis	0.68(0.46)	2		b, f
Scheloribates pallidulus	0.7	2		f
Damaeus clavipes	0.74	2		e
Porobelba spinosa	0.74	2		e
Oppiella nova	0.75	2		f
Nothrus silvestris	0.79(0.94)	2		a, b, e, f
Eremulus sp.	0.79	2		c
Eniochthonius minutissimus	0.8	2		b
Eniochthonius crosbyi	0.8	2		f
Galumna lanceata	0.82(0.53)	2		b, e
Ptyctima	0.89	2		c
Hemileius initialis	1.25	2		b
Tectocephus minor	1.28	2		f
Galumna spp.	1.4	2		b
Scheloribates sp.	1.49	2		c
Uracrobates incertus	1.59	2		c
Xylobates oblongus	1.64	2		f
Beckiella acuta	1.79	2		c
Eremaeus translamellatus	1.84	2		f
Tritegeus bisulcatus	1.84	2		e
Oppiidae/Suctobelbidae	2	3	live/dead animal tissue	b
Pergalumna silvatica	2.09	3		c
Amerus polonicus	2.14	3		e
Pantelozetes paolii	2.14	3		e
Pergalumna sura	2.19	3		c
Pilogalumna spp.	2.2	3		b
Galumna sp.	2.29	3		c
Spatiodamaeus verticillipes	2.34	3		d
Ceratozetes gracilis	2.56(0.60)	3		e, f
Tectocepheus sarekensis	2.79	3		c
Hypochthonius rufulus	2.81(0.50)	3		a, b, d, e
Epidamaeus n.sp.	2.99	3		c
Oppiidae	3.04	3		e
Epidamaeus sp.	3.58	3		f
Oppia nitens	4.15	3		f
Hypochthonius luteus	4.24	3		e
Amerioppia sp.	4.49	3		c
Multioppia sp.	4.63	3		f
Amerus troisii	4.69	3		b
Eremobelba sp.	6.11	4	Secondary predator	f

†We denote the trophic levels as follow: lichen feeder: -1; saprotrophic feeder: 1; fungal feeder: 2 and predator/scavanger: 3.

‡Data sources: a, Scheu and Falca 2000; b, Scheneider *et al.* 2004; c, Illig *et al.* 2005; d, Pollierer

et al. 2009; e, Maraun *et al.* 2011; f, current study.

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Chapter IV

The effects of chronic N deposition on the community structure and function of soil oribatid mites

Abstract During the next century, atmospheric nitrogen (N) deposition is projected to more than double, potentially slowing litter decomposition by altering microbial community composition and function. If the flow of energy though detrital food webs is diminished by the slowing of decay under higher rates of atmospheric N deposition, this agent of global change could also negatively impact the abundance and composition of soil fauna. To test this hypothesis, we studied soil faunal communities in four sugar maple-dominated forests that comprise a long-term N deposition experiment. To examine whether changes in soil faunal communities could then feed back to influence litter decay, litterbags with ^{13}C-enriched aspen litter were placed in the forest floor in one study site. The microbial community within the litterbags was characterized using PLFA analysis. Overall, long-term experimental N deposition reduced the abundance of microarthropods (ambient = 7844 individuals/m^2 *vs.* experimental N deposition = 4357 individuals/m^2; $P = 0.004$). We attribute this overall decline partly to the reduced energy flow entering the detrital food web that has been documented in previous work in our system. While there was no difference in microarthropod species richness between N deposition treatments, there was a shift in community composition within the most abundant group (Oribatida), indicating species-specific responses to N deposition. Experimental N deposition reduced the number of microarthropods colonizing litterbags by 41% (P =0.014). This was associated with a reduction in ^{13}C mobilization from leaf litter into fungal biomass. Overall, this study

demonstrates that chronic N deposition has a detrimental effect on the soil detritus food web, and that the negative effect may feed back to influence litter decay and ecosystem functioning.

Introduction

Global changes in climate and land use are affecting biodiversity in many ecosystems (Wolters et al. 2000), and this has triggered extensive research into the consequences of these anthropogenic forces on ecosystem functioning (Chapin et al. 1998, Diaz et al. 2006). The majority of experiments have focused on plants and animals living aboveground (Hughes 2000, Walther et al. 2002, Wu et al. 2011), whereas comparably less is known regarding belowground responses (Wardle et al. 1999, Blankinship et al. 2011). Most research into soil biotic responses to global change has focused on microbial communities, whereas the responses of soil fauna at higher trophic levels, such as nematodes, mites and springtails, are less understood, but clearly mediate important functions that have ecosystem-level implications (Osler and Sommerkorn 2007, Sackett et al. 2010). For example, soil fauna can alter the physical environments of the soil through litter comminution and soil bioturbation (Lussenhop 1992) or regulate the activity and population dynamics of the microbial community through grazing (Moore et al. 1988, Lussenhop 1992, Hassall et al. 2006). Moreover, significant links between soil fauna communities and decomposition rates have been observed in the field (Neher et al. 2012).

Anthropogenic nitrogen (N) deposition is a pervasive agent of global environmental change (Vitousek et al. 1997). Over the past 150 years, atmospheric N deposition has increased from 0.05- 0.10 g N m^{-2} yr^{-1} prior to 1880 to 1.50-2.00 g N m^{-2} yr^{-1} in the early 1990s across large areas of the Northern Hemisphere; this rate is projected to double during the next century (Galloway et al. 2004). Atmospheric N deposition can stimulate net primary productivity in N-limited terrestrial ecosystems, thereby increasing ecosystem carbon (C) storage, albeit there is considerable debate regarding the extent of this stimulation (Nadelhoffer et al. 1999, Magnani et al. 2007). However, atmospheric N deposition also could increase ecosystem C storage by

slowing plant litter decay and accelerating organic matter accumulation in soil (Carreiro et al. 2000, Frey et al. 2004). Although most studies of N deposition focus on changes in the microbial community (Zak et al. 2011), the few studies that have been conducted suggest that soil fauna at higher trophic levels could also be affected by anthropogenic N deposition (Boxman et al. 1998, Xu et al. 2009, Eisenhauer et al. 2012). Shifts in microarthropod community composition, in response to experimental N deposition, can significantly reduce soil respiration and accelerate nutrient leaching in laboratory microcosms (Heneghan and Bolger 1996). However, our knowledge is incomplete regarding the *in situ* response of soil fauna to anthropogenic N deposition and potential feedbacks that could modify rates of litter decomposition.

Previous studies have reported that anthropogenic N deposition can have either beneficial or detrimental effects on detrital food webs. For instance, Sjursen et al. (2005) found increased microarthropod abundance in a subarctic ecosystem after application of NPK fertilizer, attributing this to the bottom-up effects of higher plant productivity. In contrast, N fertilization can reduce carbon allocation belowground to roots and rhizodeposition (Hogberg et al. 2010), thereby reducing energy availability in the soil environment. Moreover, anthropogenic N deposition can reduce rates of litter decay (Knorr et al. 2005, Zak et al. 2008), which can also reduce the energy entering the detritus-based food web. Both of these belowground responses to anthropogenic N deposition could negatively impact the soil microbial community, as well as higher trophic levels in the soil food web.

Here, we sought to understand the mechanisms by which chronic N deposition has influenced the dynamics of the detrital food web in soil. In a long-term field experiment consisting of four sugar-maple (*Acer saccharum*) forest stands, chronic experimental N deposition has slowed plant litter decay and accelerated organic matter accumulation in forest

floor and surface mineral soil (Pregitzer et al. 2008, Zak et al. 2008, Edwards et al. 2011). The slowing of plant litter decay has resulted from a decline in lignolytic extracellular enzyme activity, originating from the transcriptional down regulation of fungal genes encoding these enzymes (Edwards et al. 2011). At the same time, microbial biomass had been reduced by 18% under experimental N deposition (DeForest et al. 2004). We were interested in determining whether soil fauna at higher trophic levels were also affected negatively by long-term experimental N deposition; we reasoned that a lower microbial biomass and the slowing of litter decomposition could plausibly reduce the flow of energy into the detrital food web. We focused on microarthropod groups involved in important soil processes, such as litter fragmentation and microbial grazing (Collembola, oribatid mites), as well as mesostigmatic mites that, through their predation, are potential regulators of grazer abundance (Coleman et al. 2004). We hypothesized that (1) microarthropod communities are affected negatively by long-term N deposition, due to reduced energy flow entering the detrital food web; and (2) the change in microarthropod communities further feeds back to slow litter decomposition. We tested these hypotheses by quantifying microarthropod populations in forest floors receiving ambient and experimental N deposition. Additionally, we placed litterbags containing ^{13}C-labeled leaf litter in one of the forest stands to determine whether chronic N deposition has compromised the ability of microarthropod communities to colonize and degrade new litter.

Methods

Site description and experimental design

This long-term study of experimental N deposition consists of four sugar maple (*Acer saccharum*)-dominated northern hardwood forests in the Great Lakes region of North America (Burton et al. 1991). The sites are floristically and edaphically matched (>80% sugar maple on

sandy soils), but they differ in climate along a north-south latitudinal gradient (Fig. 4.1). Within each study site, six 30-m x 30-m plots were established in 1994; 3 plots receive ambient N deposition and the remaining 3 plots receive ambient plus 30 kg NO_3^--N ha^{-1} y^{-1}. The additional NO_3^- is delivered over the growing season in six equal applications of solid $NaNO_3$ pellets; a 10-m wide buffer surrounds each plot, and it also receives the experimental treatments. More detailed descriptions of the study site can be found in Zak et al. (2008)

Microarthropod survey

Forest floor litter was collected in June 2009 in site B as an initial survey of microarthropod populations. Within each plot, a 10-cm x 10-cm PVC frame was placed randomly on the forest floor, and the O_i and Oe horizons were collected and placed in a plastic bag. A total of 6 samples were collected from each plot receiving either ambient (n = 3) or experimental N deposition (n = 3). All of the samples were transported to the lab in coolers and placed in modified Tullgren funnels within 48 hours (Crossley and Blair 1991) to extract microarthropods. After the 5-day extraction, litter was placed in a 60 °C oven for 24 hours for the subsequent determination of dry mass. A second microarthropod survey was conducted in May 2011 in all four study sites using the same sampling and extraction scheme.

The extracted microarthropods were preserved in 70% ethanol, and three major groups (Mesostigmata, Collembola and Oribatida) were enumerated under a microscope. For the second survey, the most abundant group (Oribatida) were further identified to genus or species level based on the keys written by Norton & Behan-Pelletier (unpublished) for use at the Ohio State University Summer Acarology Program (http://www.biosci.ohio-state.edu/~acarolog/summerProgram/).

Litterbag experiment

Litterbags (20 cm x 20 cm) were constructed using a layer of 1-cm polyester mesh to form the top of the bag and 1-mm fiberglass mesh on the bottom. The larger top mesh allowed for the free movement of invertebrates into the litter bags, and also reduced potential changes in microclimate created by the litterbags (Bradford et al. 2002). To quantify the active microbial community that relied on the litter inside the litterbags, 20 g of ^{13}C-enriched aspen litter (*Populus tremuloides*) was placed in each litterbag, representing new litter entering forest floor. We used aspen to produce ^{13}C-enriched leaf litter, because sugar maple, the dominant species in our sites, is a slow growing species that would not produce sufficient quantities of leaf litter for this experiment. The ^{13}C-enriched aspen litter was produced by pulse labeling propagated ramets of one aspen genotype. Labeling was conducted in a field chamber during the 2008 growing season, and naturally senesced and abscised leaves were collected during autumn. The labeling chamber was similar to that used by Mikan et al. (2000).

The constructed litterbags were transferred into the field in individual plastic bags. In total, 96 litterbags with aspen litter were placed in plots receiving ambient (n = 3) and experimental N deposition (n = 3) in site B during June 2009. In addition, 16 empty litter bags were placed on the forest floor to control for any external litter input to the litterbags. The Oi horizon was first removed, and the litterbags were secured to the top of the Oe horizon with steel pins; the original Oi horizon was then placed on top of each bag. Three supplementary litterbags were placed in the forest floor, as described above, but were immediately retrieved to estimate any loss during transportation.

Four litterbags from each plot were collected 2, 4, 12 and 16 months after placement in the field. Four empty litterbags were also collected on each date to estimate the mass of any external litter that had infiltrated litterbags. On each collection date, litterbags were placed in

separate plastic bags in coolers and transported to the laboratory within 24 hours. In addition, one soil core sample (5 cm diameter x 5 cm depth) underneath each litterbag was collected. Moreover, one forest floor sample outside the litterbags was collected from each plot. Both the soil and litter samples that were collected from outside the litterbags served as paired controls when comparing the microbial community inside bags (see PLFA analysis below).

In the lab, the litterbags were cut open after the outside was carefully brushed, and any roots that had penetrated the litterbags were removed. The litter was then thoroughly homogenized. Approximately 75% of the field-moist litter was placed in modified Tullgren funnels to extract microarthropods (into 70% ethanol) over 5 days. The remaining litter was freeze-dried for phospholipid fatty acid (PLFA) analysis. The extracted microarthropods were sorted into three major groups including Mesostigmata, Collembola and Oribatid mites; individuals were enumerated under a dissecting microscope and oribatid mites were further identified to species.

After microarthropod extraction, litter was oven dried at 60 ^{o}C and weighed to determine moisture content. The oven-dried litter was further ground into powder and combusted at 500 °C to measure the ash-free dry mass. Five subsamples of the original litter were also combusted to determine the ash-free dry mass of the litter used in the beginning of the experiment. Litter mass loss during the field incubation was corrected for ash content, and mass loss values were expressed on an oven-dry, ash-free basis.

PLFA analysis

Phospholipids fatty acids (PLFAs) were extracted from the contents of each litterbag to determine microbial community composition, biomass and ^{13}C content. Approximately 25% of the field-fresh litter from each litterbag was freeze-dried within 24 hours of collection. The litter

samples on the forest floors outside the litterbags and the soil samples underneath the litterbags were also freeze-dried. About 1 g of freeze-dried litter (or 5 g of soil sample) was subjected to a solution containing 10 mL of CH_3OH, 5 mL of CH_3Cl, and 4 mL of PO_4^{-3} buffer to extract total lipids (White et al. 1979). A 21:0 internal standard (5 pmol) was added to each sample at the beginning of the extraction to determine the extraction efficiency. The extracted PLFAs were then separated by silicic acid chromatography. Isolated polar lipids were further subjected to an alkaline $CHCl_3$-CH_3OH solution to form fatty-acid methyl esters (FAMEs; Guckert et al. 1985). Fatty-acid methyl esters were separated using gas chromatography and quantified using a Finnigan Delta plus mass spectrometer with a GC/C III interface (Thermofinnigan, Bremen, Germany). Alongside the samples, a standard solution containing five common FAMEs (14:0, i15:0, 15:0, cy19:0, 18:1w7t) of known concentrations were analyzed after every fifth sample for quality assurance purposes. The concentration of sample FAMEs was determined by a regression equation based on the standard FAMEs.

A total of 20 PLFA biomarkers were identified and grouped to bacteria, *Actinobacteria* and fungi. The total PLFA abundance (nmol/g litter), after adjustment for extraction efficiency and extracted litter weight, was used as an indication of total viable microbial biomass. The $^{12}C/^{13}C$ ratio for each FAME was used to calculate the ^{13}C content of each group (ug ^{13}C / g litter), which was used to quantify the newly acquired C from the ^{13}C-enriched aspen litter.

Data analysis

The density of microarthropod groups in forest floor was calculated in two ways: the first method combined the abundance of 6 subsamples within each plot and divided the sum by the total collected area (individuals/m^2); The second method employed the sum of the abundance within each plot divided by the total dried weight of the 6 subsamples (individuals/g dried litter).

We also calculated the density-by-area abundance for the microarthropod groups inside the litterbags based on the area of the litterbag (0.04 m^2) and the total dried weight of litter inside. Because the results from these two methods were highly correlated with each other, we present only the results of analyses of density-by-area data.

To test how experimental N deposition influenced the microarthropod community in the forest floor, we first used a Student's *t*-test (unpaired) to analyze the first survey data (site B only). Three-way ANOVA (site, N treatment and taxon group) was used to analyze the density data from the second survey (all four sites). The species richness of the oribatid community was assessed using individual-based rarefaction curves and the 95% confidence zone was used to detect any difference between the N deposition treatments.

The species composition of the oribatid mite communities was represented in a community matrix using three different indices: relative abundance was calculated as the percentage of each species within each plot; presence or absence was the binary conversion from the raw abundance data; incidence of each species was the frequency of finding that species in 6 sub-samples of that plot. The use of incidence data was to scale the original data to 0 (absence) to 6 (occurred in all 6 sub-samples) to prevent bias of the dominant species or the rare species.

Bray-Curtis dissimilarity was calculated for above three community matrices, which were then subjected to non-metric Multidimensional Scaling (nMDS) to visualize any differences in the species composition of oribatid mite communities (Kruskal and Wish 1978; Minchin 1987). To explore relationships between the compositional trends revealed by ordination and different N deposition treatments, we fitted the N deposition levels (ambient = 10 Kg N $ha^{-1}yr^{-1}$, elevated = 40 Kg N $ha^{-1}yr^{-1}$) to the nMDS space by rotational correlation (Dargie 1984), which is similar to a multiple linear regression of the environmental variables on the set of ordination axes.

Statistical differences in community structure were also assessed using Permutational Multivariate Analysis of Variance (PerMANOVA, Anderson 2001). As the three different community matrices yielded similar results for the above multivariate analysis, only the results from the incidence data are shown in the result section.

For the litterbag experiment, repeated measures ANOVA was used to explore the effects of collection date and N deposition treatment on microarthropod density and the microbial PLFA composition inside the litterbags. nMDS was used to visualize the species composition of oribatid communities in the litterbags using the incidence data mentioned above; statistical differences in community composition were assessed by PerMANOVA.

The remaining mass in the litterbags was scaled to percentage of the initial weight, and then fitted to a first order exponential decay equation ($A_t= e^{-kt}$) with A_t as the remaining mass at time t (Jenny et al. 1949). The decay constant *k* was estimated to represent the decay rate for each plot. Student's *t* test was used to compare the difference in decay rate of litterbags under different N deposition treatments.

Repeated measures ANOVA was performed on IBM SPSS Statistics 19. The species rarefaction curves were generated using EstimateS. Multivariate analysis including nMDS and PerMANOVA were performed in package VEGAN in R 2.15.1 (R Development Core Team 2012).

Results

Microarthropod surveys

The initial survey in site B (June 2009) revealed that the density of all three microarthropod groups was reduced by experimental N deposition. Oribatid mite density declined from 9722 ± 442 (mean ± 1 SD) individuals/m^2 under ambient N deposition to 3833 ± 1551 individuals/m^2

under experimental N deposition (P = 0.017); this represented a 60% decline in density. Similarly, Collembola density under experimental N deposition (406 ± 200 individuals/m^2) was only 34% of that under the ambient treatment (1989 ± 362 individuals/m^2; P = 0.044). Finally, the density of Mesostigmata declined from 1289 ± 164 individuals/m^2 under ambient N deposition to 733 ± 116 individuals/m^2 under experimental N deposition (P = 0.011).

For the second survey in May 2011, three-way ANOVA revealed that all three microarthropods groups responded similarly to experimental N addition across all four study sites (Table 4.1; Fig. 4. 2). Consistent with the results from the first survey, experimental N deposition reduced the overall density of microarthropods by 44% across four sites (Fig. 4.2). At the same time, the abundance of Mesostigmata was related positively with the abundance of Collembola and oribatid mites (R^2 = 0.39, $P < 0.01$). Despite the decline in overall density of microarthropods under experimental N deposition, the species richness of the oribatid community was comparable between N deposition treatments (Fig. 4.3), with the number of species slightly lower under experimental N deposition (51 species) compared to that in the ambient treatment (61 species).

However, different oribatid species responded differently to experimental N deposition, resulting in a change in the community composition (Table 4.2, PerMANOVA, P = 0.01). As seen in the nMDS ordination space in Fig. 4.4, oribatid communities from different sites were distinctly different along axis I (PerMANOVA, $P < 0.001$). At the same time, communities under experimental N deposition all clustered lower on axis II, suggesting that experimental N deposition was causing similar changes in species composition (Table 4.2, site x treatment P = 0.514). In addition, fitting the N deposition levels to the ordination space indicates that the elevated N deposition level is associated with mite communities represented at the lower end of

axis II (Fig. 4.4, $R^2 = 0.309$, $P = 0.022$). Oribatid species that were only found (e.g., *Atropacarus striculus*, *Hypochthoninus rufulus*, and *Platynothrus peltifer*) or more abundant (e.g., *Fuscozetes fuscipes*) under experimental N deposition have relatively low scores on axis II. In contrast, species with high scores on axis II are those only present (e.g., *Epidamaeus* spp., *Ommatocepheus* sp. and *Pilogalumna* sp.) or more abundant (e.g., *Tectocepheus velatus*) under ambient deposition. A list of species with their nMDS scores and their association with the N treatments can be found in the supporting material Table A1.

Litterbag results

Aspen litter decayed rapidly, with an average of 17% of initial mass remaining after 16 months. Comparison of the decay constant *k* indicated that there was no difference between mass loss rates of litter under ambient and experimental N deposition (Fig. 4.5, $P = 0.434$).

Similar to the results from the forest floor, the density of microarthropods in the litterbags was lower under experimental N deposition (Fig. 4.6, treatment effect: $P = 0.014$). The populations also varied among the retrieval dates (Table 4.1, time effect $P < 0.001$), wherein oribatid mite densities were greater during the two summer collection dates (August 2009 and June 2010), whereas Mesostigmata and Collembola densities declined through time (Fig. 4.6, time x taxon interaction; $P = 0.016$). There was a positive relationship between the densities of Mesostigmata and Collembola, even after accounting for the time effect (partial regression, time as covariate; $R^2 = 0.51$, $P = 0.021$). Although the number of oribatid species in the litterbags was similar between N deposition treatments (Fig. 4.3), oribatid community composition varied between N deposition treatments (Table 4.2, PerMANOVA treatment effect $P = 0.001$).

PLFA analyses

Total microbial biomass (represented by total PLFA abundance) in the forest floor outside the litterbags and in the soil underneath the litterbags was very similar among 4 retrieval dates and did not differ between the ambient and experimental N deposition treatments (Appendix Fig. A4.1). Similarly, the total microbial biomass inside the litterbags did not differ between the ambient and experimental N deposition treatments (Fig. 4.7a) although the total biomass was slightly lower in the second year (time effect, $P = 0.027$).

Overall, the microbial PLFAs inside the litterbags were highly enriched by ^{13}C, compared to those outside the litterbags. The average ratio of $^{13}C/^{12}C$ of PLFA on the forest floor was 0.012, whereas this ratio inside the litterbags averaged at 0.035. While the total microbial PLFAs were similar, the lower $^{13}C/^{12}C$ ratio led to a significantly lower ^{13}C content of microbial PLFAs under experimental N (Fig. 4.7b, $P = 0.03$); and all three microbial groups (fungi, Actinobacteria and Bacteria) responded similarly (taxon x treatment effect, $P = 0.259$). At the same time, the ^{13}C content of the microbial PLFAs declined over time ($P < 0.001$). Interestingly, the ^{13}C content of soil microbes underneath the litterbags did not increase until the last retrieval date, suggesting a time lag in the mobilization of ^{13}C labeled aspen litter from the litterbags (Appendix Fig. A4.2).

Discussion

After 17 years of experimental N deposition, the abundance of both detritivores (Oribatida and Collembola) and predaceous mites (Mesostigmata) has declined in forest floor, suggesting that a reduction in decay under experimental N deposition has decreased energy flowing through the soil food web. This finding directly contrasts to previous studies, in which positive effects on soil fauna have been found under N fertilization. This discrepancy may arise from the fact that those ecosystems are either young, developing forests (Berch et al. 2006), grasslands (van der Wal et al. 2009) or heathland (Sjursen et al. 2005), in which enhanced productivity under N deposition

has directly increased plant litter production; such an increase would provide additional substrate for soil fauna, thereby increasing energy available to the soil food web (Berch et al. 2009). In our study system, net primary productivity has also increased under experimental N deposition, but has been allocated to stem production, rather than leaf or fine root litter production (Burton et al. 2004, Zak et al. 2011). It appears that equivalent litter production under ambient and experimental N deposition, combined with reduced decay under experimental N deposition has decreased the amount of energy available to fuel organisms at high trophic levels in the soil food web. Our observations, in combination with the studies described above, indicate that changes in plant litter production and its rate of decay can both be important controls of soil fauna communities under N deposition.

Consistent with our observations, detrimental effects of anthropogenic N deposition on soil fauna have been reported from various ecosystems. For example, Eisenhauer et al. (2012) found that the abundance of predatory nematodes and the species richness of nematodes and microarthropods were reduced in a long-term (13-year) grassland study under experimental N deposition. They attributed this decline to decreases in rhizodeposition. Other evidence of the negative effects of anthropogenic N on soil fauna has been observed in boreal spruce forests (Lindberg and Bengtsson 2006), lodgepole pine forests (Berch et al. 2006) and subtropical forest (Xu et al. 2007). However, the amounts of N added in the aforementioned studies were much higher ($\geq$ 100 kg N $ha^{-1}yr^{-1}$) than those in our experiment (30 kg N $ha^{-1}yr^{-1}$); such high levels of N could result in soil acidification, base cation depletion and the mobilization of Al^{3+}, all of which are detrimental to soil fauna (Matson et al. 2002). Such changes in soil chemistry have not occurred in our experiment, because soils are newly developed in calcareous glacial drift; soil pH, salt concentrations, and cations remain unchanged (Zak et al. 2008, Patterson et al. 2012). In

contrast, litter decomposition has slowed due to the inhibition of lignolytic activity, resulting in an accumulation of organic matter in forest floor and surface soil (Pregitzer et al. 2008, Zak et al. 2008). The thickening of forest floor could potentially provide increased living space for soil fauna; however, declines in their densities suggest that these microarthropods are not limited by habitat availability. Rather, the positive relationship between the Mesostigmata and their prey (oribatid mites and Collembola) suggested that the decline in Mesostigmata was due to the decline in their food resources (oribatid mites and Collembola), which in turns was a bottom up effect from the inhibition of microbial activity and litter decomposition under long-term experimental N deposition.

In spite of the decline in overall abundance, we did not observe a change in species richness of the most abundant group (Oribatida) under experimental N deposition (Fig. 3), which contrasts with other studies (Eisenhauer et al. 2012). However, it is difficult to compare the results among studies, because some provide only broad taxonomic resolution (Eisenhauer et al. 2012) and most studies do not quantify sampling effort (i.e., rarefaction analysis), which is crucial to understand whether the majority of species have been encountered. In our study, we did observe fewer species under elevated N deposition; however, rarefaction analysis indicates that species richness should converge between N treatments with additional sampling.

While the total number of oribatid mite species did not differ between the N deposition treatments, species-specific responses resulted in a shift in community composition (Fig. 4.4). Overall, there were 16 species absent under experimental N deposition, whereas five species (*Atropacarus striculus*, *Hypochthoninus rufulus*, *Platynothrus peltifer* and 2 *Xylobates* spp.) occurred exclusively under experimental N deposition. Moreover, it should be noted that species in the same genus can respond differently to experimental N deposition. For instance,

Tectocepheus velatus and *T. minor* are two closely related and morphologically similar species that are often found in temperate regions (Laumann et al. 2007). *T. velatus* is dominant in many ecosystems (Fujikawa 1988, Fujita and Fujiyama 2001), but its relative abundance declined under experimental N deposition. In contrast, the relative abundance of *T. minor* increased under experimental N deposition (Table A4.1). The minor species (*T. minor*) had also been found to dominate *T.velatus* in crop fields, which the authors attributed to the higher migration ability of *T.minor* and its ability to utilize different microhabitats (Fujita and Fujiyama 200). In our case, we are unable to provide an ecological explanation for this response because the autecology of these species (especially *T. minor*) is largely unknown. Changes in energy flow or changes in fungal community composition under experimental N deposition (Edwards et al. 2011) may alter the competitive ability of mites that have different preferences for fungal species (Mitchell and Parkinson 1976). We expect that species that rely more on fungal hyphae in their diets will be more negatively affected by N deposition than those that rely more on detritus or other food items. Indeed, the accumulation of organic matter on the forest floors may even benefit species that feed predominantly on litter. Consistent with this expectation, many of the species detrimentally affected by experimental N deposition (Table A 4.1), including species in the genera *Epidamaeus* (Kaneko 1988), *Eremaeus* (Mitchell and Parkinson 1976), *Carabodes* (Schneider et al. 2004), and *Galumna* (Neena and Haq 1989) feed predominantly on fungal hyphae (Luxton 1972). On the other hand, the three species that only occurred under experimental N deposition rarely feed on fungi. For example, *A. striculus* and *P. peltifer* are primary decomposers which feed predominantly on litter, an insight derived from their $^{15}N/^{14}N$ natural abundance (Schneider et al. 2004) and their lack of chitinase to degrade fungal cell walls (Siepel and De Ruiter Dijkman 1993); *H. rufulus* has been reported to be an omnivore that feeds

mostly on dead animals (Siepel and De Ruiter Dijkman 1993, Schneider et al. 2004). Another omnivore (*F. fuscipes*; Wallwork 1958) also increased in its relative abundance under experimental N deposition.

We expected that reductions in the densities of soil fauna under experimental N deposition would then reduce subsequent litter decomposition, completing a feedback process between litter and soil fauna. Our data are in partial support of this prediction. First, presumably because of their lower densities in natural litter (above), fewer microarthropods colonized our experimental litterbags under experimental N deposition. Various studies have observed that microarthropod communities can accelerate the recovery of fungal communities after disturbance (Maraun et al. 1998a), stimulate extracellular enzyme activity, and increase microbial respiration (Kaneko et al. 1998, Wickings and Grandy 2011). Such simulating effects may arise because microarthropods can: (1) fragment litter, exposing more surface area for microbial activity; (2) disperse fungal spores and hyphae on their body surface; and (3) graze on fungal hyphae and simulate metabolism of microorganisms (Behan et al. 1978, Lussenhop 1992). Therefore, the lower densities of microarthropods under experimental N deposition could result in lower microbial activity that feeds back to reduce litter decay. However, our results provided mixed evidence for this prediction. While we did observe a lower ^{13}C content of microbial PLFAs inside the litterbags, we did not observe any change in total microbial biomass or litter decay rate in litterbags under elevated N deposition. Typical limitations of litterbag experiments (e.g. no control over litter fragments that were lost from the bags) may have reduced the statistical power necessary to detect any difference in decay rates between treatments. Nevertheless, the lower ^{13}C contents of microbial PLFAs may suggest that the ability of the microbial community to utilize new litter was reduced, which may have resulted in part from lower microarthropod activity.

That is, reduced litter fragmentation and a lower surface area for microbial attack may have reduced the amount of litter ^{13}C assimilated by the saprotrophic microbial community. Additionally, changes in the oribatid community could potentially alter the composition of the microbial community by selective feeding (Mitchell and Parkinson 1997; Maraun et al. 1998b) with consequences for mobilization of ^{13}C from the litter.

Ecological Implications

By combining field surveys and a litterbag experiment, we have demonstrated that chronic N deposition has a detrimental effect on a detritus-based food web, which may have diminished the ability of saprotrophic organisms to metabolize fresh litter. This finding argues that soil food webs may not be buffered against global change stressors as previously thought (i.e., elevated CO_2, increased temperature etc.). Functional redundancy has been assumed to be a common feature of soil fauna (Wolters 2001), and it is believed to be the reason why changes in species composition may have little influence on decomposing litter (Liiri et al. 2002). However, there is evidence that environmental stress can produce changes in the abundance and activity of soil fauna with important consequences for nutrient and carbon cycling (Heneghan and Bolger 1996, Briones et al. 2009). For example, some microarthropod groups failed to recover after intense agriculture management, leading to slower organic matter decay in abandoned agricultural areas (Siepel 1991). In our study, the decline in the overall abundance of microarthropods would presumably reduce litter fragmentation for saprotrophic microorganisms potentially contributing to the declining rate of belowground C cycling observed in our system. This suggests a possible feedback mechanism wherein the inhibition of lignolytic activity under experimental N deposition reduces the energy entering the detritus-based food web that detrimentally affects the soil fauna; the decline in soil fauna activity would then feed back to inhibit the ability of

microorganisms to decompose new litter. Our results suggest that future rates of atmospheric N deposition could alter the composition and function of soil food webs, wherein the slowing of microbial decay initiates a cascading negative effect on higher tropic levels culminating in the greater storage of C in soil organic matter.

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Table 4.1. Analysis of Variance (ANOVA) of the density of microarthropods in the forest floors and in the litterbags.

Source of variation	Df	Sum of Squares	*F* value	*P*
Forest floors				
Site	3	2.13	8.29	**< 0.001**
Taxon	2	16.81	98.04	**< 0.001**
Treatment	1	0.8	9.38	**0.004**
Site x Taxon	6	3.52	6.84	**<0.001**
Site x Treatment	3	0.38	1.48	0.233
Taxon x Treatment	2	0.24	1.38	0.262
Site x Treatment x Taxon	6	0.16	0.32	0.926
Residuals	48	4.12		
Litterbags				
Time	3	11.84	27.6	**<0.001**
Taxon	2	5.09	9.25	**0.004**
Treatment	1	2.27	8.23	**0.014**
Time x Taxon	6	2.61	3.05	**0.016**
Time x Treatment	3	1.96	4.57	**0.008**
Taxon x Treatment	2	0.05	0.03	0.913
Time x Taxon x Treatment	6	0.28	0.33	0.919

Note: The forest floor data were pooled from four study sites, and the litterbag data were pooled from four retrieval dates. Three-way ANOVA for forest floor data included site (n = 4), N treatment (n = 2) and taxon group (n = 3). Litterbag data were analyzed using repeated measures ANOVA, with taxon group and N treatment as between subject factors. Densities were calculated as the number of individuals per unit area and were log-transformed before analysis.

Table 4.2. PerMANOVA analysis of the species composition of oribatid mite communities on the forest floor or inside litterbags.

Source of variation	Df	Sum Of Squares	*F* value	R^2	*P*
Forest floor					
Treatment	1	0.19	2.40	0.07	**0.010**
Site	3	1.22	5.13	0.42	**< 0.001**
Treatment x Site	3	0.23	0.97	0.08	0.514
Residuals	16	1.27	0.44		
Total	23	2.92			
Litterbag					
Treatment	1	0.54	3.13	0.11	**0.001**
Time	1	0.69	4.01	0.14	**< 0.001**
Treatment x Time	1	0.27	1.55	0.05	0.140
Residuals	20	3.45	0.70		
Total	23	4.94			

Note: The incidence of the species, calculated as the frequency of encountering that species in the six subsamples within each plot, was used in the community matrix. The forest floors data were from the second microarthropod survey across four sites; the litterbags data were pooled from four retrieval dates. For PerMANOVA analysis, Bray-Curtis index was used and permutation times = 2000.

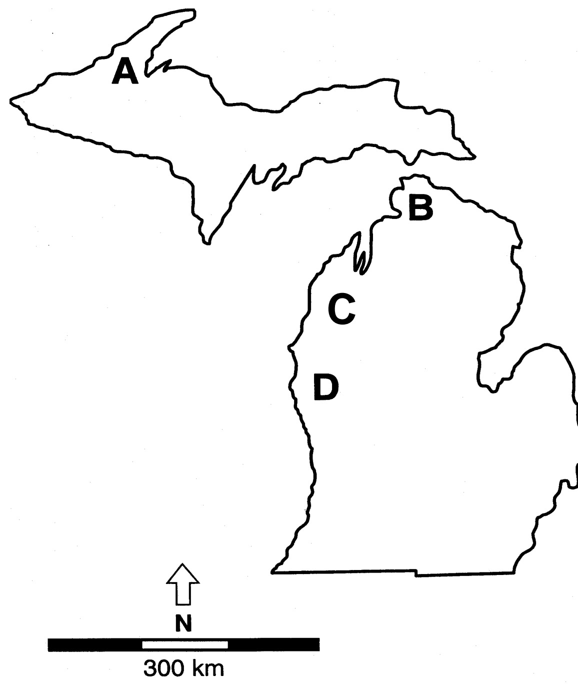

Figure 4.1. Map of four study sites in the Great Lake region in North America.

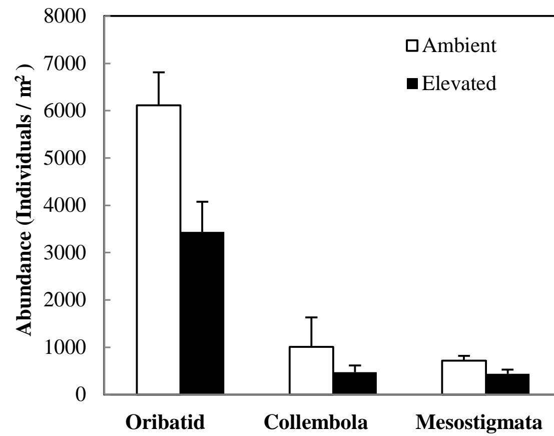

Figure 4.2. Three-way ANOVA indicated that the abundance of Oribatida, Mesostigmata and Collembola in the forest floors was significantly lower under experimental elevated N deposition (P = 0.004). Density data were averaged across four study sites.

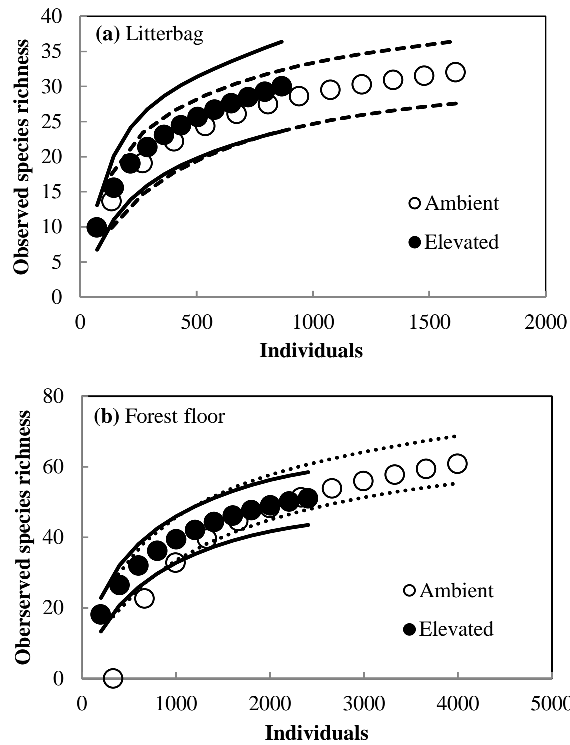

Figure 4.3. The rarefaction curves of oribatid mite communities in the forest floors (a) and inside the litterbags (b). The forest floor data were pooled from four study sites, and the litterbag data were pooled from four retrieval dates. The solid lines are the 95% confidence zones for the rarefaction curve of ambient plots; the dashed lines are 95% confidence zones for the rarefaction curve of elevated N deposition plots. Replicate times = 500.

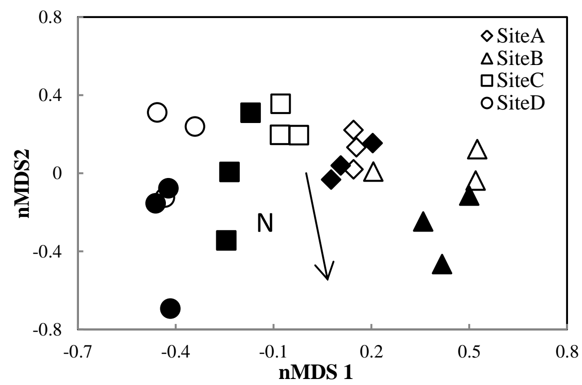

Figure 4.4. The nMDS ordination of the oribatid mite communities on the forest floor. The arrow indicates the direction of increasing N deposition (R^2 = 0.309, P =0.022). Symbols: site A: diamond; site B: triangle; site C: square; site D: circle. Ambient deposition plots: open; Elevation deposition plots: closed.

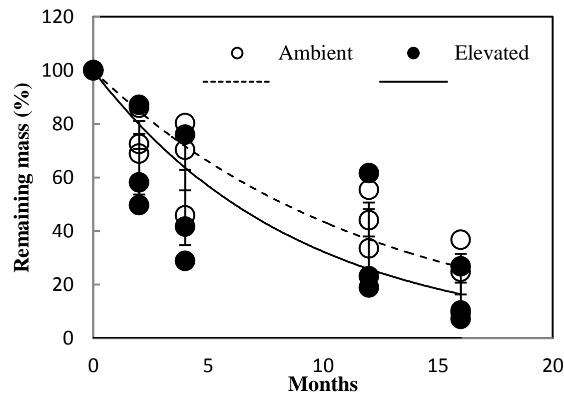

Figure 4.5. The mass loss rates of aspen litter inside the litterbags did not differ between the ambient plots and the elevated N deposition plots ($P = 0.434$). The lines are best fit to the first order exponential decay equation on the average of the ambient deposition (open, dashed lines) and the elevated treatment (closed, solid lines).

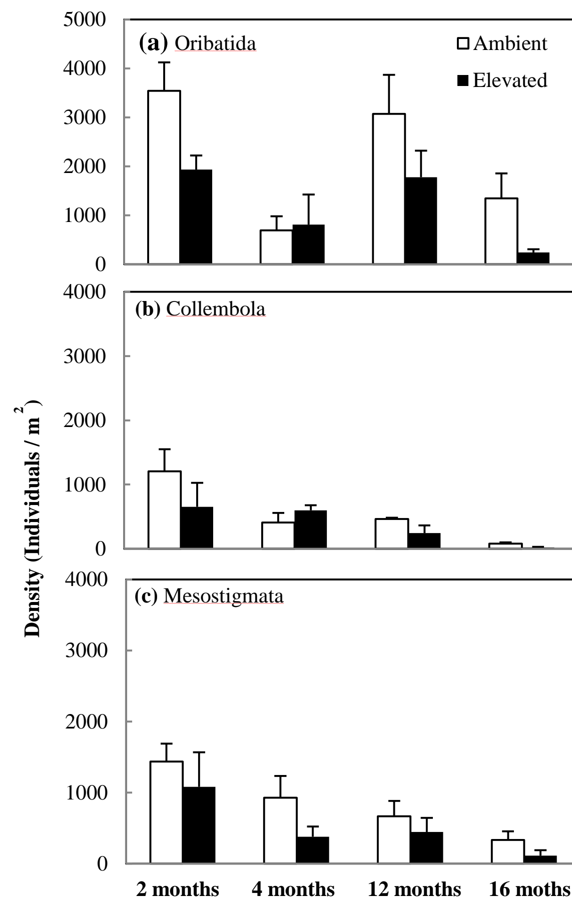

Figure 4.6. The abundance of microarthropods including Oribatida (a), Collembola (b) and Mesostigmata (c) inside the litterbags. Repeated measures ANOVA indicated a decrease in their density in litterbags under elevated N deposition (treatment effect, $P = 0.014$).

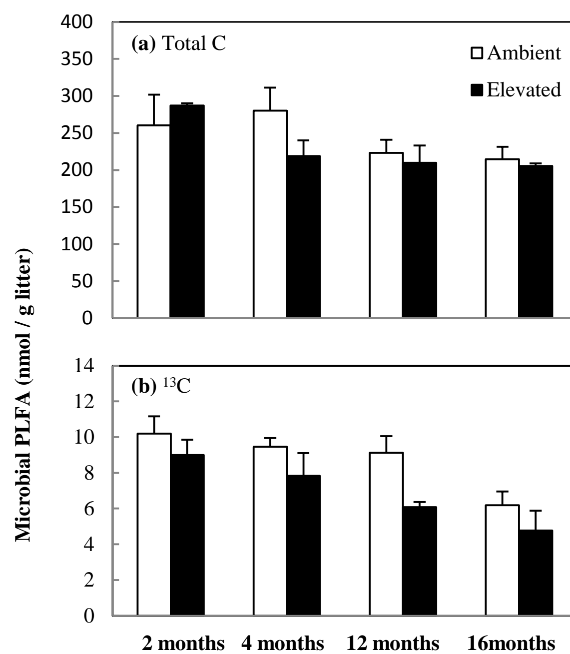

Figure 4.7. Total C (a) and ^{13}C content (b) of microbial PLFAs inside the litterbags. While the total microbial biomass did not differ between the N treatments (a, $P = 0.56$), the ^{13}C content declined over time ($P < 0.001$) and was consistently lower in the N deposition treatment (b, $P = 0.03$).

Appendix

Table A4.1. Species scores from nMDS analysis using the incidence data of oribatid mite communities.

Species	nMDS1	nMDS2	Presence
Ommatocepheus sp.	-0.03	0.90	A
Pilogalumna sp.	-0.15	0.74	A/E
Epidamaeus sp2	-0.85	0.67	A/E
Xylobates sp1	-0.85	0.67	A
Adrodamaeus sp.	-0.85	0.60	A
Eremulus sp.	-0.85	0.60	A
Oribatella sp2	-0.23	0.59	A/E
Parachipteria sp.	-0.02	0.58	A/E
Epidamaeus longitarsalis	-0.58	0.46	A/E
Peloribates sp.	-0.08	0.41	A/E
Tectocepheus velatus	0.07	0.37	A/E
Provertex sp.	-0.23	0.36	A
Carabodes polyporetes	-0.05	0.35	A/E
Eueremaeus nemoralis	-0.39	0.34	A/E
Epidamaeus longiseta	-0.39	0.33	A
Epidamaeus bakeri	0.02	0.28	A/E
Galumna sp1	0.59	0.27	A
Fosseremus americanus	0.59	0.25	A/E
Carabodes sp1	0.36	0.23	A
Galumna sp2	-0.95	0.22	A/E
Suctobelbella sp1	0.20	0.17	A/E
Eremaeus translamellatus	0.70	0.17	A
Euphthiracarus sp1	-0.27	0.16	A
Heminothrus sp.	0.00	0.15	A/E
Autogneta sp.	-0.20	0.13	A/E
Chamobates cuspidatus	0.20	0.12	A/E
Oribatula tibialis	0.07	0.12	A/E
Pyroppia sp1	0.93	0.11	A
Suctobelbella sp2	-0.01	0.10	A/E
Cultroribula sp.	0.65	0.05	A/E
Archiphthiracarus sp.	0.10	0.05	A/E
Anachipteria howardi	0.28	0.04	A/E
Rhysotritia ardua	-0.10	0.04	A/E
Xylobates oblongus	-0.04	0.04	A/E
Oppia nova	-0.14	0.03	A/E
Cepheus cepheiformis	0.96	0.01	A

Euphthiracarus sp2	-0.90	0.01	A
Xylobates prionotus	-0.90	0.01	A
Oribatella calcarata	0.00	0.00	A/E
Podopterotegaeus sp.	0.52	-0.01	A/E
Protokalumna sp.	-0.72	-0.02	A/E
Scheloribates pallidulus	-0.10	-0.02	A/E
Parapyroppia monodactyla	0.56	-0.03	A/E
Allosuctobelba sp.	0.07	-0.03	A/E
Graptoppia foveolata	-0.14	-0.03	A/E
Multioppia sp.	-0.20	-0.04	A/E
Lepidozetes singularis	0.03	-0.06	A/E
Nothrus silvestris	-0.01	-0.07	A/E
Eniochthonius crosbyi	0.31	-0.08	A/E
Archoplophora sp.	0.61	-0.09	A/E
Epidamaeus sp1	0.39	-0.14	A/E
Achipteria sp.	0.88	-0.15	A/E
Xylobates sp3	0.07	-0.16	E
Pergalumina sp.	0.32	-0.17	A/E
Eremobelba sp.	0.17	-0.18	A/E
Siculobata sp.	0.86	-0.25	A/E
Phthiracarus sp.	0.16	-0.26	A/E
Tectocepheus minor	0.46	-0.27	A/E
Ceratozetes gracilis	0.36	-0.31	A/E
Oppia nitens	0.35	-0.33	A/E
Platynothrus peltifer	0.07	-0.38	E
Nothrus sp1	0.08	-0.41	A
Fuscozetes fuscipes	0.79	-0.44	A/E
Hoplophorella sp.	0.13	-0.51	A
Atropacarus striculus	0.68	-0.54	E
Hypochthoninus rufulus	0.73	-0.99	E
Xylobates sp2.	0.70	-1.21	E

Note: The table was sorted by the axis II score. Species with a high axis II scores are more likely to be found in ambient plots; species with more negative axis II scores are more likely to be found in the experimental N deposition plots. A: species present in ambient deposition plots; E: Species present in elevated deposition plots.

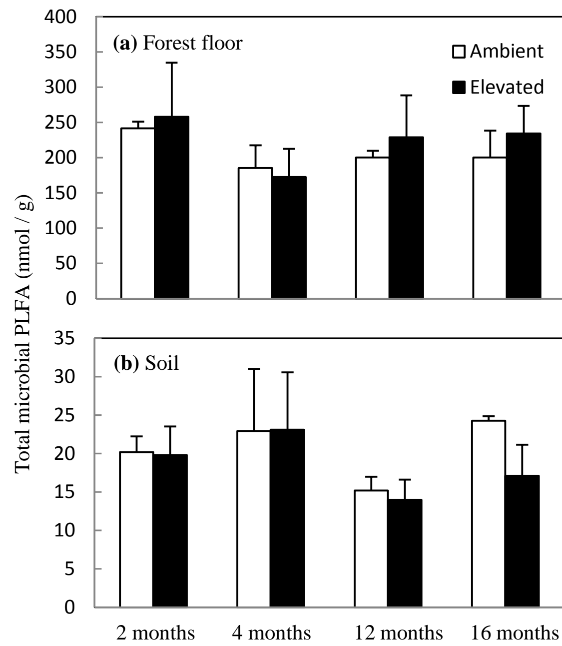

Figure A4.1: Total biomass of microbial PLFAs outside litterbags on the forest floor (a) and in the soil underneath the litterbags (b) did not change over time ($P > 0.1$) or under experimentally elevated N deposition ($P > 0.1$).

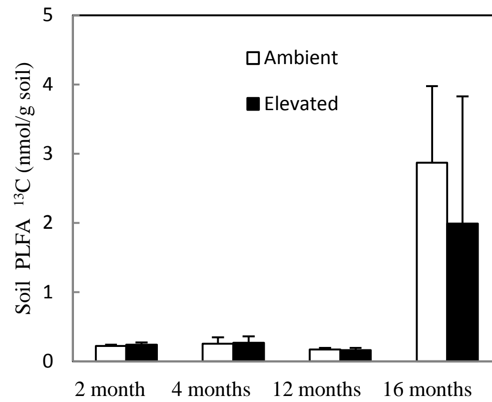

Figure A4.2: The ^{13}C content of soil microbial PLFAs underneath the litterbags increased greatly by the last retrieval date ($P < 0.001$) but did not differ between N deposition treatments ($P = 0.57$).

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Chapter V

The consequences of changes in microarthropod communities under chronic N deposition for fungal communities and litter decay

Abstract. Chronic N deposition can have detrimental effects on many groups of soil animals, including microarthropods. However, little is known about whether such changes in soil microarthropod communities in turn alter ecosystem processes. We hypothesized that declines in soil microarthropod abundance and changes in their species composition under chronic N deposition would inhibit plant litter decay by altering fungal communities. To test this hypothesis, we established 150 microcosms with microarthropods collected from forest floors under ambient and experimental N deposition in a long-term (>18 years) field study. We monitored CO_2 accumulation during a 96-day incubation and characterized fungal communities at the end of the study. Overall, decay rate was lower in litter collected from experimental N deposition plots regardless of microarthropod treatments. In contrast, the total pool of metabolized C was highest when litter was inoculated with microarthropods of the same origin as the litter, suggesting that soil microarthropods may have acclimated to changes in microbial community composition and function under experimental N deposition. Our study also revealed that fungal communities differed between litters from ambient and experiment N deposition plots. However, within each litter type, fungal communities also varied with different levels of microarthropod abundance. This suggests that any changes in the abundance of microarthropods under global environmental change may feed back to influence fungal communities. Although changes in fungal communities under different microarthropod treatments did not influence the

decay of labile organic matter in our study, changes in the abundance of microarthropods and subsequent effects on fungal communities may influence the decay of recalcitrant organic matter.

Introduction

Soil animal communities are among the most species-rich components of terrestrial ecosystems (Giller 1996). They represent one of the greatest reservoirs of global biodiversity and fulfill crucial ecosystem functions in nutrient and carbon cycling (Mikola et al. 2002, Decaens et al. 2008). Numerous studies have demonstrated that soil animals, including protozoa, nematodes, microarthropods, enchytraeids, millipeds and earthworms etc., typically enhance litter decomposition as the result of litter comminution and their effects on microbial activities (Petersen and Luxton 1982, Filser 2002, Huhta 2007, Blouin et al. 2013). However, accumulating evidences reveals that global changes in climate and land use are affecting biodiversity of soil animals in many ecosystems (Blankinship et al. 2011, Eisenhauer et al. 2012). Although neutral or positive responses have been observed, many studies have reported a reduction in the abundance and diversity of soil animals under global warming and intensified land use (Adl et al. 2006, Briones et al. 2007, Blankinship et al. 2011). Such detrimental effects on soil animals have aroused concerns that changes in soil animal communities might alter ecosystem processes (Wolters et al. 2000, Decaens et al. 2008).

Whether the ecosystem functions mediated by soil animals are buffered against global environmental stressors depends in part on the extent of functional redundancy in soil animal communities (Bardgett 2002). Because a large number of soil animals appear to be trophically equivalent, functional redundancy is sometimes thought to be a common feature of soil biota (Andrén et al. 1995); others have questioned the validity of functional redundancy in the absence of empirical evidence (Behan-Pelletier and Newton 1999). A growing number of studies have stressed the importance of key species of soil animal, and overall species composition, rather than biodiversity *per se* on ecosystem functioning (Wolters 2001, Bardgett 2002). For example,

initial reductions in soil species richness resulting from global change are unlikely to alter C dynamics, unless particularly influential species are lost (Nielsen et al. 2011). Therefore, changes in species composition and the subsequent alteration of important species interactions in soil are likely to be the main biotic control of ecosystem function in response to environmental change (Bardgett 2002).

Soil microarthropods are among the most important groups of soil animals that form essential interactions with soil microorganisms and therefore are vital during litter decomposition (Scheu et al. 2005). Soil microarthropods affect litter decomposition through three processes: (1) direct litter consumption; (2) litter fragmentation or dispersal of microbial propagules via physical movement and (3) feeding on microorganisms (Lussenhop 1992). The contribution of direct litter consumption to litter decay is thought to be trivial, because the metabolic activity of soil animals is lower than that of saprotrophic bacteria and fungi (Petersen and Luxton 1982). In contrast, the interaction between microarthropods and microorganisms is believed to have more profound effects on litter decay (Petersen and Luxton 1982), because selective foraging by soil animals can alter microbial community composition and subsequently rates of litter decay. This suggests that any change in microarthropod community elicited by a changing environment is likely to affect litter decay via their interactions with saprotrophic microbial communities.

Atmospheric nitrogen (N) deposition is a pervasive agent of global change and many studies have documented that chronic N deposition is detrimental to soil animals especially microarthropods (Eisenhauer et al. 2012). The negative effects of N deposition on microarthropods could occur by altering the soil chemical environment such as soil acidification, base-cation depletion and Al^{3+} mobilization, all of which are detrimental to soil animals (Matson et al. 2002). On the other hand, atmospheric N deposition can reduce microbial biomass and

inhibit litter decay (Liu and Greaver 2010), which could negatively affect microarthropods through bottom-up effects (Nielsen et al. 2011). For example, the inhibition of litter decay, without additional litter input, can decrease the flow of energy through soil food webs, which can cascaded up to reduce the abundance of microarthropod populations and alter their community composition (Gan et al. 2013). Moreover, the detrimental effects of chronic N deposition on microarthropods appear to be widespread across ecosystem types (Lindberg and Persson 2004, Berch et al. 2006, Xu et al. 2009). However, little is known regarding how declines in microarthropod abundance and changes in their community composition under N deposition feed back to influence microbial communities and subsequent litter decay.

In a previous study, we found that a decline in microarthropod abundance in the forest floors was associated with a reduction in the mobilization of newly-added C into fungal biomass, which was likely due to reduced litter fragmentation from fewer microarthropods under chronic N deposition. (Gan et al. 2013). The reduction in litter comminution would reduce the amount of litter surface exposed for microbial attack, which would ultimately inhibit litter decay. In the current study, we are interested in other mechanisms by which microarthropods influence litter decay, especially the interactions between microarthropods and saprotrophic microorganisms. We focus on fungal communities because two major groups of soil microarthropods, Collembola and oribatid mites, are believed to rely on fungal hyphae as their main food resource (Maraun et al. 2003). Furthermore, Collembola and oribatid mites appear to be selective feeders, thereby altering the competitive relationships among fungal species and their community composition (Maraun et al. 1998, Schneider et al. 2005, Crowther et al. 2011a). We hypothesized that changes in microarthropod communities under chronic of N deposition would inhibit litter decay by altering fungal communities. To test this hypothesis, we established microcosms with

microarthropods collected from forest floors under ambient and experimental N deposition in a long-term field study (Zak et al. 2008). We measured enzyme activities, fungal communities and overall litter decay under different microarthropod treatments. We expected that compared to the microarthropod community under ambient N deposition, the lower abundance and different composition of microarthropods under chronic experimental N deposition would alter fungal community composition resulting in lower enzyme activities and slower litter decay.

Materials and methods

Sites description and microcosms set up

The ecosystem effects of atmospheric N deposition are studied in four forest stands in the Great Lakes region of North America (Burton et al. 1991). The sites are sugar maple (*Acer saccharum*)-dominated and underlain by slightly acid soils (pH 4.41 – 4.70) that are well-drained sandy typic Haplothords of the Kalkaska series. Within each study site, six 30-m x 30-m plots were established in 1994; 3 plots receive ambient N deposition and the remaining 3 plots receive an additional 30 kg NO_3^--N ha^{-1} y^{-1}. The additional NO_3^- is delivered over the growing season in six equal applications of solid $NaNO_3$ pellets; an additional 10-m wide buffer surrounds each plot, and it also receives the experimental treatments.

Ten 10-cm x 10-cm forest floor samples (O-horizon) were randomly collected from each plot receiving ambient and experimental N deposition in one study site (Pellston, MI) in 2011. Samples from each plot were placed in plastic bags and transported to lab in coolers. Samples were combined and homogenized within N deposition treatments for use in experiments. A subsample from each litter composite was frozen at -80 °C for later analysis of the initial fungal community. A second subsample was stored at 4 °C as intact substrate for later use. The remaining composited litter was defaunated by microwaving for 5 minutes in an 800-watt

microwave, which kills most animals but not necessarily bacteria or fungi (Huhta et al. 1989).

A total of 150 microcosms were constructed using clear Perspex cylinders (6 cm in diameter and 5 cm in height, U.S. Plastic Corp) with Nytal nylon screen (mesh width 200 um) attached to the bottom with rubber bands. The microwaved litter from ambient or experimental N deposition was cut into ~1cm pieces and each type of litter was divided into 70 subsets. Cutting the litter into small pieces served to minimize any differential effects of litter comminution by microarthropods among treatments so that we could focus here on direct effects of microarthropods on microbes. Each litter subset (~3 gram, dried weight) was placed into a microcosm. In addition, 5 subsets of unmicrowaved litter from ambient and experimental N deposition were also placed into microcosms. All microcosms were then placed into l-L Mason jars. A layer of wet paper towel was placed in the bottom of each Mason jar. A plastic cup was also placed inside the Mason jar as a stand for the microcosm to avoid its direct contact with the wet paper towel. The mouth of the Mason jar was loosely wrapped by Parafilm. The use of Parafilm and wet paper towel, which was frequently re-wetted during incubation, was to maintain 100% humidity inside the Mason jars.

Since microwaving the litter likely killed some groups of microorganisms, we reintroduced the microbial community by mixing 200 g of unmicrowaved litter (from either ambient or experimental N deposition, composited separately) with 1000 mL of DI water and then filtering the suspension through 100 µm mesh. The use of this mesh size was to intercept most of the soil animals while allowing microorganisms and microfauna (protozoa and small nematodes) to pass through. Four mL of the litter suspension was added to each microcosm, matching the source of the suspension to the N deposition treatment of the microcosm. In total, we had 70 microcosms with microwaved litter and a reintroduced microbial community, and 5

microcosms with unmicrowaved litter (with its initial microbial and faunal community) for both ambient and experimental N deposition treatments. The microcosms were placed in a 20 °C incubator for a week to allow the re-establishment of microbial communities before we introduced microarthropods.

Microarthropods reintroduction

Additional forest floor samples were collected to harvest microarthropods from the ambient and experimental N deposition plots. As before, litter was composited and homogenized within ambient and experimental N deposition treatments. Six grams (wet-weight) of either ambient or N deposition litter was placed in a modified Tullgren funnel (Crossley and Blair 1991) to directly extract microarthropods into a single microcosm. The amount of litter for microarthropod extraction was comparable to that in the microcosm to create a density of microarthropods that resembled field condition. We used a factorial design such that for each litter type, 30 microcosms with microwaved litter were inoculated with microarthropods from litter under ambient N deposition and another 30 microcosms were inoculated with microarthropods from litter from the experimental N deposition treatment. The remaining 10 microcosms with microwaved litter, and the 5 microcosms with unmicrowaved litter, did not receive added microarthropods, and served as no-microarthropod controls and intact incubations for each litter type. The use of intact incubation controls addressed how our manipulation process (defaunation by microwaving and reintroduction of microbial and microarthropod communities) affected the results of our experiments.

To characterize the initial microarthropod communities, we extracted an additional 6 subsamples from each litter type; microarthropods were extracted directly into ethanol for later identification. Additionally, we had previously obtained data on microarthropod communities

under ambient and experimental N deposition (18 subsamples from each N treatment) from the same study site in a previous study (Gan et al. 2013). We combined data from these two samplings to represent the initial microarthropod communities before introducing them into the microcosms.

Respiration measurement

We measured respiration in the microcosms beginning 4 days after the reintroduction of the microarthropods. The CO_2 concentrations inside the microcosms were thereafter measured twice a week during the first 40 days and then once a week for the remaining days for a total of 96 days. On the day before each scheduled measurement date, the Mason jars were removed from the incubator. Parafilm was removed from the Mason jars for 2 hours to equalize CO_2 concentrations with the ambient atmosphere. The Mason jars were then resealed with lids containing septa for later headspace gas sampling. After 24 hours, the headspace gas (25 mL) of the sealed Mason jars was removed and analyzed using a gas chromatograph equipped with a Porapak Q column with a thermal conductivity detector (Trace 2000, Thermo Quest, CA). The column was maintained at 55 °C during the analysis and He was used as a carrier gas.

After sampling of the headspace gas, the lids of the Mason jars were removed for 2 hours to equalize CO_2 with ambient atmosphere. The jars were then loosely wrapped with Parafilm and placed back in the incubator until the next measurement date.

Enzyme activities

To characterize enzyme activities during the incubation (day 55) without destroying the microcosms, all microcosms were extracted with 12 mL of deionized water and a vacuum of 9.96 kPa (field capacity), yielding 8-12 mL leachate from each microcosm. The leachate was frozen at -20°C for later measurements of enzyme activities. We measured the activities of enzymes that

are responsible for the degradation of three common compounds in decaying litter including chitin (N-acetylglucosaminidase), cellulose (cellobiohydrolase and β-glucosidase) and lignin (peroxidase and phenol oxidase). Preliminary measurements of 12 samples indicated that there was little to no peroxidase or phenol oxidase activity. Therefore, these two enzymes were dropped from further measurements. For the remaining three enzyme measurements, 8 ml of leachate from each microcosm was mixed with 10 ml of 50 mM acetate buffer to adjust the pH to around 5. Activities of the three non-ligninolytic enzymes were fluorometrically measured in 96-well plates by mixing the buffered leachate with methylumbelliferone (MUB)-linked model substrates. The details of enzyme analyses can be found in Saiya-Cork et al(2002).

Microarthropod communities

At final harvest (day 96), litter from each microcosm was homogenized and weighed separately. Approximately 0.5 g from each microcosm was flash frozen in liquid N_2 and then stored at -80 °C for fungal T-RFLP analysis. The remainder of the litter was weighed again and placed in a modified Tullgren funnel to extract microarthropods. After the 5-day extraction, litter was placed in a 60 °C oven for 24 hours to determine the remaining dry mass.

The extracted microarthropods were preserved in 70% ethanol, and three major groups (Mesostigmata, Collembola and Oribatida) were enumerated under a microscope. The most abundant group (Oribatida, >80% of total microarthropods) was further identified to genus or species level based on the keys written by R.A. Norton & V.M. Behan-Pelletier (unpublished) for use at the Ohio State University Summer Acarology Program (http://www.biosci.ohio-state.edu/~acarolog/summerProgram/).

T-RFLP of fungal community

Community DNA was extracted from 0.1 g of the frozen litter sample (at harvest) using MoBio

PowerMax DNA kits, following the manufacturer's instructions. Sixteen microcosms from the 30 microcosms of each litter-microarthropod treatment were randomly selected for DNA extraction. All of the no-microarthropod microcosms (10 for each litter type) and intact samples (5 for each litter type) were also extracted for DNA. In addition, 10 DNA extractions from fresh ambient and experimental N deposition litter were also included to characterize the initial fungal communities before the microcosm experiments, yielding to a total of 114 samples. Success of DNA extraction was confirmed by 1% agarose gel electrophoresis with gel red staining. Extracted DNA was stored at -80° C before further analysis.

We targeted fungal ITS sequences for PCR amplification using the fungal-specific primers ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) with the forward primer fluorescently labeled with 6-FAM (6-carboxyfluorescein). These primers span approximately 420 to 825 bp of the fungal ribosomal operon, including part of 18S rDNA, all of ITS1, 5.8S, and ITS2, and part of 28S rDNA (Manter and Vivanco 2007). PCR reactions were performed in 25 μL reaction mixtures contained 1.25 units of Taq DNA polymerase (APEX), 2.5 μL of 10X reaction buffer (Apex), 0.2 mM dNTP's (LifeTechnology), 0.2 uM of each PCR Primer (IDT), 0.4mg/ μL of purified bovine serum albumin (New England BioLabs), and 1 μL of template DNA. The PCR amplification was conducted in a thermocycler as follows: 94°C for 2 min followed by 32 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min and a final extension at 72°C for 2 min. PCR products were visualized with electrophoresis on 1% agarose gels stained with gel red. For each sample, products from two replicated PCR reactions were pooled together and purified for downstream applications using MoBio UltraClean PCR clean-up kits.

We digested the clean PCR products by separately using *Hae*III and *Hinf*I restriction endonucleases (New England Biolabs) in a 20 μL reaction volume. Restriction digests were

performed in 100 μL PCR tubes (Molecular BioProducts) containing 5U of enzyme, 2 μL of 10X reaction buffer, and 30-70 ng of purified PCR product. Restriction digests were incubated at 37 °C for 4 h before enzyme activities were terminated by denaturation at 80 °C for 20 minutes. Reactions were then diluted with 200 μL TE buffer, and purified/concentrated using Microcon Ultracel YM-30 centrifugal filter devices. Reactions were checked for DNA restriction products by 1% agarose gel electrophoresis stained with gel red.

Restriction products were loaded into barcoded ABI Prizm 96-well optical reaction plates (Applied Biosystems); each well contained 2 μL of purified digested DNA, 0.03 μL of MapMarker 1000 X-rhodamine (ROX) labeled DNA internal length standard (BioVentures, Inc.), and Hi-Di formamide (Applied Biosystems) to a total volume of 13 μL. Each sample was sequenced twice for replicate measurements. Sample plates were submitted to the University of Michigan DNA Sequencing Core, where 6-FAM labeled DNA restriction fragments were separated and quantified by capillary gel electrophoresis on an Applied Biosystems Model 3730xl DNA Analyzer.

Terminal restriction fragment (TRF) patterns in the resulting electropherograms were aligned and peaks were screened based on standards with known sizes using GeneMarker 1.6 Software (SoftGenetics, State College, PA). Peaks lower than 75 bp or higher 600 bp were excluded to avoid contamination from excess primers or incomplete digest. Peaks that contributed less than 1% of the total fluorescence from each measurement were deleted. The remaining peaks from all samples from each enzyme digest were assigned to TRF classes using GelCompare II (Applied Maths, Austin, Texas). TRF classes that appeared only once from all profiles were discarded as artifacts. For each sample, only the TRF classes that were present in both replicated measurements were kept for further analysis. The resulting profiles were then

used to generate a binary (presence/absence) matrix indicating the distribution of TRF classes from our 114 samples.

To identify the fungal taxa that were associated with the TRF classes, we compared the sizes of our TRF classes to the theoretical digest of known fungal sequences occurring in our field study (J., Hasset unpublished data, Entwistle et al. In print). All of these sequences can be retrieved from Genebank with access numbers GU174269-GU174233 and KC701765-KC701965 separately. These fungal sequences have been assigned to fungal taxonomic groups (family or genus) based on their sequence similarity with those of known taxonomic identity in Genebank. We assigned a TRF to a certain taxonomic group only when the TRF class appeared in the theoretical digests by both enzymes *Hae*III and *Hinf*I.

Data analysis

We first subtracted the CO_2 concentration (μL /L) of ambient atmosphere from that measured in the headspace of each microcosm to calculate net CO_2 accumulation (μg CO_2) over a 24-hour period. We used this value as the average respiration rate in between two measurement dates to calculate CO_2 accumulation over time. The CO_2 accumulation was normalized by the initial dry weight of the litter in the microcosm, and then fit to a first-order exponential model $A_t = A_0 (1- e^{-kt})$. The two parameters A_0 and k were estimated using a non-linear least squares regression. A_0 indicates the total respired C (ug CO_2/ g C), reflecting the metabolized substrate pool over the course of the incubation period (time = t). k is the first-order rate constant, indicating the proportion of the total metabolized substrate pool that is under decayed per unit time. We also calculated the mass loss in each microcosm. Therefore, we had 3 indices (actual mass loss, A_0 and k) to represent different aspects of the decay process. Simple linear regressions were performed among these 3 indices to address their relationships.

We compared the decay process in microcosms with microwaved litter (n = 140) to those with unmicrowaved litter (n = 10) using a students' t test. Within microwaved litter, we further used two-way ANOVA (Litter, Microarthropod and their interaction) to assess how different litter types and microarthropod treatments influenced litter decay in the microcosms. The main factor Litter consisted of two litter types: litter from ambient or experimental N deposition; and the main factor Microarthropod consisted of three different microarthropod treatments: microarthropods from ambient plots, microarthropods from experimental N deposition plots, and no-microarthropod controls. Similar two-way ANOVAs were applied to the enzyme activities during microcosm incubation and the microarthropod abundance at the end of the incubation. Tukey HSD *post hoc* multi-comparisons were applied to any significant terms from the two-way ANOVAs. Simple linear regressions were performed to assess whether litter decay was correlated with enzyme activities or microarthropod abundance.

For the binary matrix of fungal TRF classes, we used non-metric dimensional scaling (nMDS) to visualize the fungal communities in both unmicrowaved litter (before and after incubation) and incubated microwaved litter. For the second nMDS, we only included the microwaved litter to investigate how fungal communities in the microcosms differed among litter types and microarthropod treatments. Multivariate ANOVA based on dissimilarity (Adonis, The PerMANOVA procedure in the Vegan Package in R) was performed to provide tests of statistical significance for nMDS. We also summed the occurrence of each TRF class across each treatment to compare the frequency of their occurrence. The frequency of their occurrence, divided by the number of samples measured for each treatment, was further used to calculate the similarity between two treatments. The similarity was calculated by 1- dissimilarity (Bray-Curtis index) and represented the percentage of shared TRFs between two communities.

To investigate how the different oribatid communities may have influenced the fungal communities in microcosms, simple regressions were performed between the similarity of fungal communities (calculated as above) and the similarity of oribatid communities in each experimental treatment (2 litter types x 3 Oribatid treatments = 6 treatments total). We only included the pair-wise similarities within the same litter type to exclude possible confounding effects from different litter types. The similarity of oribatid communities was calculated by 1-dissimilarity (Bray-Curtis index). We used two indices of Oribatid community structure. The presence/absence of oribatid species in each treatment was used to calculate the similarity in species composition. Additionally, the abundance of oribatid species in each treatment was used to calculate similarity in abundance.

All the statistical analyses were performed in R 2.15.1 (R Development Core Team 2012), with multivariate analyses including nMDS and perMANOVA performed in package Vegan (Oksanen et al. 2007).

Results

Litter decay of microcosms

As expected, total mass loss within microcosms was positively corrected with the metabolized substrate pool A_0 ($r^2 = 0.34$, $P < 0.001$). A_0 and k were negatively corrected ($r^2 = 0.58$, $P < 0.001$), indicating that litters with a high metabolized substrate pool decayed at a slower rate than did those with a low metabolized substrate pool. Overall, the decay rates (k) were comparable ($P > 0.05$) between the incubations of microwaved and unmicrowaved litter; however, the metabolized substrate pool (A_0) was higher in microwaved litter than in the unmicrowaved litter ($t = 2.46$, $P = 0.025$). We further compared the decay of microwaved litter with different litter types and microarthropod treatments. The decay rates (k) were consistently higher in litter from

ambient N deposition than that from experimental N deposition, regardless of the microarthropod treatment (Fig. 5.1, $F_{1, 134} = 9.06$, $P = 0.003$). In contrast, the metabolized substrate pool (A_0) depended on both litter type and microarthropod treatment (Fig. 5.1, $F_{2, 134} = 2.92$, $P = 0.057$). While Turkey HSD *post hoc* multiple comparisons did not identify any significant comparison, it seems that biota (microarthropods and microorganisms) from ambient N deposition metabolized more substrate when given litter from ambient N deposition, whereas biota from experimental N deposition metabolized more substrate when provided with litter from experimental N deposition treatment (Fig. 5.1).

Enzyme activities

Three enzyme activities during incubation differed among litter types and microarthropod treatments. First, BG activity depended on both litter type and microarthropod treatment (Litter x fauna, $F_{2, 125} = 5.85$, $P = 0.003$). Turkey HSD *post hoc* multi-comparison indicated that BG activity was lowest when litter was inoculated with microarthropods of the same origin; and highest in the no-microarthropod control (Fig. 5.2a). In contrast, CBH activity was consistently higher in litter from the experimental N deposition treatment, regardless of microarthropod treatment (Fig. 5.2b; Litter, $F_{1, 125} = 18.13$, $P < 0.001$). For NAG activity, there was only a marginal effect from microarthropod treatment (Fauna, $F_{1, 125} = 2.95$, $P = 0.06$) and was highest in the no-microarthropod control (Fig. 5.2c). Overall, there was a tendency that the enzyme activities were higher in microcosms without microarthropods for all three enzymes (Fig. 5.2). Furthermore, there were no correlations between enzyme activities and metabolized substrate pool (A_0, $r^2 = 0.02 \sim 0.06$), decay rate (k, $r^2 = 0.02 \sim 0.10$), instantaneous respiration rates before and after enzyme measurement ($r^2 = 0.01 \sim 0.07$) or total microarthropod abundance at harvest ($r^2 = 0.01 \sim 0.03$).

Microarthropod communities

As predicted from previous work (Gan et al. 2013), before the start of the experiment, the abundance of microarthropods in litter from the experimental N deposition treatment was 38% lower than that in litter from the ambient N deposition treatment. Additionally, chronic N deposition reduced the species richness of oribatid mites (34 vs. 41 spp.) and altered their species composition. For example, *Eremaeus translamellatus* had a relative abundance of 2. 4% in litter from ambient N deposition but was totally absent under chronic experimental N deposition. In contrast, the relative abundance of *Fuscozetes fuscipes* was 5.6% under ambient N deposition but this number increased to 15.5% under chronic experimental N deposition. Overall , the five most dominant species in litter from the ambient N deposition treatment were: *Scheloribates pallidulus* (16.5%, proportion of total abundance), *Suctobelba* sp1 (8.9%), *Oppiella nova* (8.8%), S*uctobelba* sp2 (8.4%) and *Tectocepheus* spp. (7.7%). In litter under chronic N deposition, they were *Scheloribates pallidulus* (18.4%), *F. fuscipes* (15.5%) *Suctobelba* sp2 (12.5%), *Tectocepheus* spp. (11.1%) and *Suctobelba* sp1 (7.8%).

However, most oribatid species did not survive the environmental conditions of the microcosms. At harvest, there were only 1-2 oribatid species inside each microcosm, with *O. nova* and *S. pallidulus* being the dominant species with their relative abundances averaging 71% and 16% respectively. The abundance of microarthropods at harvest maintained the same pattern as that before the incubation, in that the abundance of microarthropods was higher in litter from ambient N deposition compared to that from experimental N deposition (Fig. 5.3, Fauna, $F_{2, 134}$ = 22.59, $P < 0.001$).

T-RFLP of fungal community

The total number of TRF classes and the average number per microcosm from *Hinf*I digest were

lower than that from *Hae*III digest (41 vs. 47 and 10 vs. 13 respectively.). This indicates that *Hinf*I was a more conservative enzyme than *Hae*III. However, multivariate analysis of the peak profiles from these two enzyme digests showed similar results. Therefore, only the results from *Hae*III are presented here.

Among 47 TRF classes from *Hae*III digest across all samples, we were able to assign 10 TRF classes to fungal taxonomic groups based on theoretical digests of known sequences. While taxonomy associations inferred from TRF sizes are not guaranteed, we have high confidence that these taxonomic identities are likely correct, because the sequences have been found in our study sites and they appeared in the theoretical digests from both enzymes. The occurrences of these 10 TRF classes represented 17% of the total TRF occurrences and their occurrences among litter types and microarthropod treatments can be found in Table 5.1.

Overall, the first nMDS (not shown) indicated that fungal communities in unmicrowaved litter differed before and after incubation (an average of 56% similarity, Table 5.2). Secondly, the unmicrowaved litter (both before and after incubation) had different fungal communities from those in microwaved litter with later reintroduction of microorganisms (an average of 48% similarity, Table 5.2). This indicated that both manipulation and incubation processes had altered the fungal communities in microcosms. For example, one TRF class, Hygrophoraceae in Euagarics (Size 97), was only present in the initial intact litter but disappeared in the microcosms after incubation (Table 5.1). However, the total number of TRF classes in the unmicrowaved litter was similar to that in microwaved litter (45 vs. 44), indicating that a majority of the fungal communities were able to re-colonize the microwaved litter at the end of the incubation.

Within microwaved litter, the composition of fungal TRF classes varied among litter types and microarthropod treatments (PerMANOVA $F_{2, 78} = 1.88$, $P = 0.03$). First, the fungal

community in litter from ambient N deposition was distinguished from that in litter from experimental N deposition treatment, as demonstrated by the first axis of the second nMDS ordination (Fig. 4). From the assigned TRF classes, size class 171 (Pezizomycotina: Leotiomycetes: Helotiales: Sclerotiniaceae) was absent in litter from ambient N deposition but appeared 4 times in litter under experimental N deposition (Table 5.1). Within litter from ambient N deposition, the fungal communities with microarthropod inoculations were more similar to each other (87% similarity) than to the no-microarthropod control (an average of 80% similarity) (Fig. 5.4, Table 5.2). For example, one TRF class (Size 155) was present in both microarthropod treatments but absent in the no-microarthropod control. Interestingly, Size 155 was assigned in Pezizomycotina: Sordariomycetes: Corycipitaceae: Cordyceps and most of the species in this genus are known as parasites associated with insets. In contrast, for litter from experimental N deposition treatment, the fungal community in the no-microarthropod control was similar to that in microcosms inoculated with microarthropods collected from experimental N deposition. Additionally, fungal communities from these two microarthropod treatments were separated from those in microcosms inoculated with microarthropods from ambient N deposition (Fig. 5.4).

Interestingly, simple regressions revealed that the similarities of fungal communities within each litter type were positively related to the similarities in the abundance of oribatid mites ($R^2 = 0.59$, $P = 0.08$) but not species composition ($R^2 = 0.14$, $P = 0.46$) (Fig. 5.5). For instance, within litter from experimental N deposition, the lowest similarity of fungal community occurred between litter with microarthropods from ambient N deposition and that with no-microarthropod control (Fig.5. 4). At the same time, there was a large difference (thus low similarity) in microarthropod abundance between these two treatments (Fig. 5.3).

Discussion

We were interested in how the decline in soil microarthropod abundance and alteration in their species composition reported under chronic N deposition (Gan et al. 2013) might feed back to influence fungal community structure and litter decay. We initially expected that differences in microarthropod communities would result in different fungal communities through microarthropod-fungi interactions. However, our results revealed that fungal communities in microcosms differed between litter types but that the effects of different microarthropod treatments were weaker. Within each litter type, fungal communities under different microarthropod treatments had an overall high degree of similarity, with an average of 83% of Bray-Curtis similarity index (Fig. 5.4, Table 5.2). Simple regressions revealed that fungal communities within each litter type were similar when there were similar numbers of oribatid mites in the microcosms, whereas there was little influence of oribatid species composition. However, relatively few mite species survived until the end of the experiment and the majority of of the individuals (71%) that did survive belonged to the single species, *O. nova*, which is the most cosmopolitan species of all oribatid mites and often cited as a pioneer and dominant species in highly disturbed systems (Marshall et al. 1987, Siepel 1996).The convergence of oribatid mite communities by the end of the incubation period limits our ability to evaluate the impacts of oribatid community composition on fungal community composition. However, fungal communities seemed to be affected by microarthropod abundance, albeit explaining less than 11% of overall fungal similarity that ranged from 76% to 87% similarity (Fig. 5.5). Although the dominant species *O. nova* is believed to be a feeding generalist and flexible in feeding on plant litter and fungal hyphae (Gan et al. In revision), a high abundance of microarthropods (mostly *O. nova*) was associated with lower occurrences of some fungal species (e.g., S473,

Pezizomycotina: Dothidaeles) and higher occurrences of others (e.g. S134, Pezizomycotina: Helotiales in litter from experimental N deposition) (Table 5.1). This suggests that, similar to the effects of herbivore grazing on plant communities (Olff and Ritchie 1998), different levels of microarthropod abundance may impose different grazing intensities on fungal communities and alter fungal relative abundance through selective feeding.

Fungal community structure at the end of incubation did not correlate with the pattern of litter decay under different microarthropod treatments. While we expected litter decay to be slower with microarthropods from the experimental N plots and slowest without microarthropods, our results did not support this hypothesis. First, decay rate (*k*) was lower in litter from experimental N deposition regardless of microarthropod treatment (Fig. 5.1a). This was probably due to the distinct fungal communities between litter types, which is consistent with the field studies that report that experimental N deposition had altered fungal communities and slowed litter decay (Liu and Greaver 2010, Edwards et al. 2011). Second, the influence of different microarthropod treatments on the metabolized substrate pool (A_0) depended on litter type, wherein A_0 was highest when litter and microarthropods were of the same origin (Fig. 5.1b). This so called “home-field advantage” has been demonstrated in other studies wherein litter decays more rapidly when placed in its original habitat or when inoculated with soil biota of the same origin (Gholz et al. 2000, Strickland et al. 2009). In contrast, Ayres et al. (2006) did not find any evidence of higher litter decay in the presence of indigenous soil biota. It should be noted that soil biota in the aforementioned study only included microorganisms and probably some microfauna (Protozoa and small nematodes). This may suggest that home-field advantage is less likely to occur unless major groups of soil animals such as microarthropods are present as well.

The presence of home-field advantage in our study suggests that after 18 years of experimental N deposition, soil microarthropods may have acclimated to changes in microbial community composition and function under experimental N deposition. Despite of a slower decay rate (*k*), change in microbial and microarthropod communities under chronic experimental N deposition could metabolize similar total amounts of labile substrate as do soil biota under ambient N deposition. This result was consistent with previous a study which reported similar metabolized substrate C pools between ambient and experimental N deposition (Thomas et al. 2012). This may indicate that there is a high degree of functional redundancy among microorganisms and microarthropods in their ability to degrade labile organic matter, albeit there are significant changes in the species composition of both microbial and microarthropod communities under chronic experimental N deposition (Zak et al. 2008, Edwards et al. 2011, Ramirez et al. 2012, Gan et al. 2013). Functional redundancy in degrading labile organic compounds during early stages of litter decomposition may not be surprising, given the fact that many soil organisms can produce enzymes for degrading labile organic compounds (Nannipieri et al. 2002). In contrast, only certain groups of specialized microorganisms have the ability to degrade recalcitrant organic matter such as lignin (Nannipieri et al. 2002, Osono 2007). Field studies have clearly demonstrated that the ability of fungi to degrade recalcitrant organic matter is compromised by chronic N deposition (Carreiro et al. 2000, Knorr et al. 2005). Furthermore, some studies have shown that the presence of soil animals can also influence the decay of recalcitrant organic matter (Scheu 1993, Osono 2007). Therefore, it will be interesting for future studies to address how microarthropods interact with fungal communities to influence the decay of recalcitrant compounds, as the rate and extent of their metabolism could be inhibited under N deposition with significant implications for total soil C storage (Whittinghill et al. 2012).

Alternatively, the home-field advantage that we observed in the metabolized substrate C pool may reflect different grazing intensities in the microarthropod treatments. Fungal respiration can exhibit a parabolic relationship with grazing intensity, wherein fungal respiration increases with increasing microarthropod grazing until a peak level, beyond which fungal respiration is inhibited (Hanlon 1981a). While the presence of grazing can stimulate fungal activity by the removal of senescent tissues or the mobilization of nitrogen and phosphorus in feces or other excretions (Hanlon 1981b, Ingham et al. 1985, Lussenhop 1992), high grazing intensity can reduce fungal activity when tissue loss cannot be compensated for by regrowth (Setälä and Huhta 1991, Lenoir et al. 2007). While it is difficult to determine the optimal grazing intensity, it is possible that microbial biomass and the microarthropod abundance have been co-adapted to an optimal level in the two treatments that resembled field conditions, i.e., both microorganisms and microarthropods from the same level of N deposition (either ambient or experimental). The incubation of microorganisms with microarthropods from different field conditions will result in a too high (i.e, microorganisms from experimental N deposition with microarthropods from ambient N deposition) or too low (i.e., microorganisms from ambient N deposition with microarthropods from experimental N deposition) grazing intensity.

One mechanism by which soil animals affect litter decomposition is through their influence on the enzymes produced by microbial communities (Lussenhop 1992). In contrast to some studies in which invertebrate grazing has stimulated fungal enzyme production (Crowther et al. 2011b), the addition of microarthropods in our study seemed to inhibit enzyme activities at least during some part of the incubation, evidenced by the overall higher enzyme activities in litter without microarthropods. However, we cannot easily quantify the effects of microarthropods on fungal enzyme production, as the enzyme activities included both fungal and

bacterial production. Assuming that the fungal enzyme activity was proportional to the overall enzyme activity, it is possible that the grazing intensity by microarthropods was sufficiently high to reduce the enzyme production from fungal community. This is a possible scenario, because we initially simulated a field density of microarthropods at the beginning of the experiment, but the fungal community may not have recovered to resemble field conditions during the first half of the incubation. On the other hand, we did not observe any relationships between enzyme activities and overall respiration or instantaneous respiration (either before or after the enzyme measurements). This makes us cautious about the interpretation of the enzyme results, which are indicative of maximum enzyme potential when given excess amounts of substrate at optimal conditions, both of which may not be realized in the microcosms (Baldrian 2009).

To summarize, different levels of grazing intensity by microarthropods may have influenced the fungal community and their enzyme production. However, all three of these components were likely to be changing over the period of incubation, making it difficult to link their activities at certain time points to overall litter decay inside the microcosms. Moreover, the bacterial community is also an important contributor in the early stages of litter decay (Strickland and Rousk 2010), and the inclusion of information about the bacterial community and total microbial biomass may provide a more thorough understanding of the mechanisms underlying the overall litter decay patterns that we observed in the microcosms.

Conclusions

Overall, we could not support the hypothesis that a decline in the abundance of microarthropods and the alteration of oribatid mite communities under experimental N deposition inhibits litter decay. Instead, litter with microarthropods of the same origin exhibited the highest metabolized substrate pool, which was comparable between the ambient and experimental N deposition

treatments. This may indicate a high degree of functional redundancy among microorganisms and microarthropods, which could buffer changes in the species composition of both microbial and microarthropod communities under chronic N deposition, at least during the early stages of litter decomposition. Further study should prioritize whether microarthropods interact with the fungal community to inhibit the decay of recalcitrant compounds, which is likely to occur under chronic N deposition and has significant implications for total soil C storage (Whittinghill et al. 2012). On the other hand, our results revealed that fungal communities varied with microarthropod abundance. Given the fact that global environmental changes are altering the abundance of many groups of soil animal, our study stresses the importance of understanding trophic interactions between microbial communities and soil animals under different densities, to fully understand potential effects of global environmental change on microbial communities and litter decomposition.

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Table 5.1. Selected TRF classes from *Hae*III digest with their possible taxonomic associations and the percentage of occurrences (out of total sample measured for each treatment) in the microcosms with different litter types and microarthropod treatments. The occurrences of these 10 TRF classes represented 20% of the total occurrences of all TRF classes (47) from all samples.

TRF class[a]	Possible taxonomic associations			Occurrences in microcosms					
	Subphylum	Order	Family/Genus	Litter types[b]	Unmicrowaved[c]		Microwaved [d]		
					Before (10)[e]	After (5)	A (16)	N (16)	None (10)
S88	Pezizomycotina	Chaetothyriales	Herpotrichiellaceae	A	80%	80%	50%	69%	50%
				N	60%	80%	38%	25%	40%
S93	Pezizomycotina	Botryosphaerales	Microdiploidea	A	80%	60%	0%	6%	0%
				N	40%	20%	0%	0%	0%
S97	Agaricomycetes	Hygrophoraceae	Hygrocybe	A	70%	0%	0%	0%	0%
				N	20%	0%	0%	0%	0%
S134	Pezizomycotina	Helotiales	Hyaloscyphaceae	A	100%	80%	63%	50%	50%
				N	100%	100 %	88%	44%	30%
S155	Pezizomycotina	Corycipitaceae	Cordyceps	A	80%	0%	6%	38%	0%
				N	70%	40%	31%	44%	40%
S171	Pezizomycotina	Helotiales	Sclerotiniaceae	A	60%	40%	0%	0%	0%
				N	60%	20%	19%	6%	0%
S204	Pezizomycotina	Hyalodendriella		A	70%	0%	38%	25%	20%
				N	70%	0%	25%	25%	10%
S446	Agaricomycetes	Cortinariaceae	Cortinarius	A	100%	20%	6%	0%	30%
				N	100%	80%	6%	0%	0%
S473	Pezizomycotina	Dothideales		A	0%	20%	44%	25%	60%
				N	0%	20%	44%	94%	60%
S581	Agaricomycotina	Corticiales	Corticiaceae	A	80%	0%	13%	19%	10%
				N	40%	0%	13%	6%	0%

[a]. The number indicates the size (bp) of the restricted fragment.
[b]. There were two litter types: litter collected from forest floors under ambient or experimental N deposition.
[c]. Before: unmicrowaved litter before incubation; after: unmicrowaved litter after incubation.
d. A: microwaved litter inoculated with microarthropods extracted from litter under ambient N deposition; N: microwaved litter inoculated with microarthropods extracted from litter under experimental N deposition; None: microwaved litter without microarthropod inoculation.
[e]. Number inside parenthesis indicates the number of microcosms with analyses of fungal T-RFLP within each litter and microarthropod treatment combination.

Table 5.2. Similarity of fungal communities with different litter types and microarthropod treatments. The similarity was calculated by 1- Bray-Curtis index of dissimilarity and represents the percentage of shared TRFs between two communities. The data matrix for this calculation was the sum of the occurrence of each TRF class for each litter and microarthropod combination divided by the number of samples measured for that treatment.

Litter/Microarthropod treatments[a]			Litter from ambient N deposition					Litter under experimental N deposition			
			Unmicrowaved		Microwaved			Unmicrowaved		Microwaved	
			Before	After	A	N	None	Before	After	A	N
Litter from ambient N deposition	Unmicrowaved	After	0.52	1							
	Microwaved	A	0.31	0.56	1						
		N	0.32	0.57	0.87	1					
		None	0.29	0.56	0.8	0.8	1				
Litter from experimental N deposition	Unmicrowaved	Before	0.81	0.54	0.38	0.39	0.34	1			
		After	0.59	0.72	0.49	0.5	0.46	0.61	1		
	Microwaved	A	0.42	0.7	0.77	0.75	0.74	0.51	0.62	1	
		N	0.38	0.63	0.71	0.71	0.7	0.42	0.6	0.79	1
		None	0.38	0.64	0.71	0.7	0.72	0.45	0.62	0.76	0.84

[a]. There were two types of litter: litter collected from forest floors under ambient or experimental N deposition; and five types of microarthropod manipulation: Before: unmicrowaved litter before incubation; After: unmicrowaved litter after incubation. A: microwaved litter inoculated with microarthropods extracted from litter under ambient N deposition; N: microwaved litter inoculated with microarthropods extracted from litter under experimental N deposition; None: microwaved litter without microarthropod inoculation.

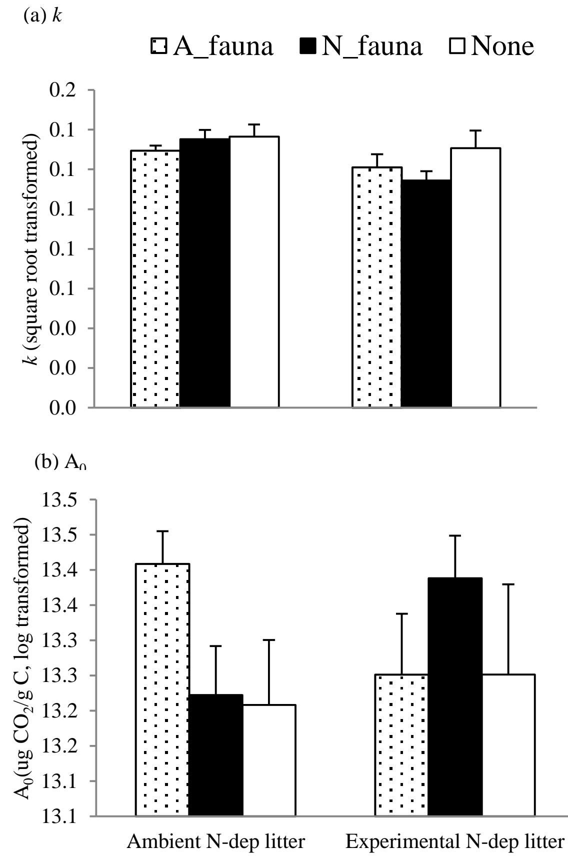

Figure 5.1. Litter decay in the 96-day incubation. (a) Decay rate (*k*) was lower in microcosms with litter from experimental N deposition (Litter, $F_{1,134} = 9.07$, $P = 0.003$) and (b) total respired C (A_0) depended on both litter type and microarthropod treatment (Litter x Fauna, $F_{2,134} = 2.9$, $P = 0.045$). Pattern filled, A_fauna: microarthropods collected from litter from ambient N deposition; Solid filled, N_fauna: microarthropods collected from litter from ambient N deposition; Empty, None: no microarthropods.

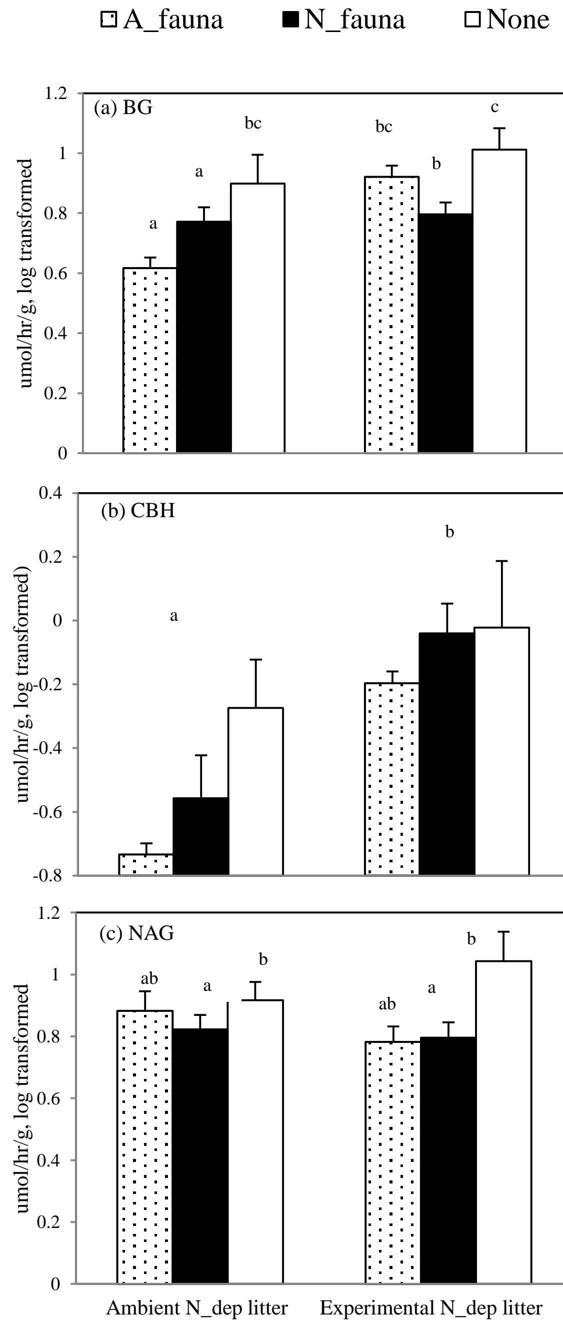

Figure 5.2. Enzyme activities in the leachate from the microcosms on day 55 of the 96-day incubation (a) β-glucosidase (BG) varied among litter types and microarthropod treatments (Fauna x Litter, $F_{2, 134} = 5.9$, $P = 0.003$); (b) Cellobiohydrolase (CBH) was higher in N-deposition litter (Liter, $F_{1, 134} = 18.1$, $P < 0.001$) and (c) acetylglucosaminidase (NAG) was higher in the no-microarthropod control (Fauna, $F_{2,134} = 3.0$, $P = 0.05$) . BG and CBH are cellulose-degrading enzymes and NAG is chitin-degrading enzyme. a ,b and c indicate different levels from Tukey *post-hoc* multi-comparisons. The meanings of legends are similar to Fig. 1.

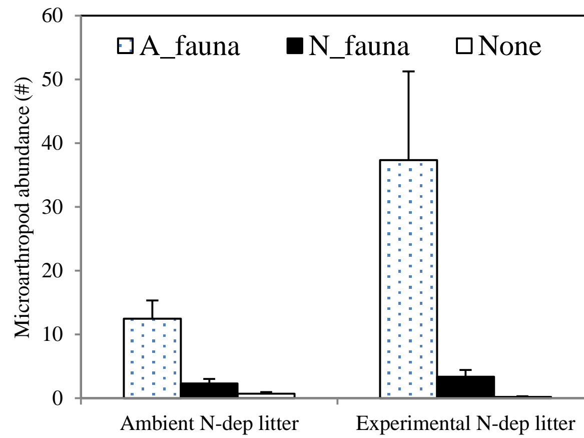

Figure 5.3. The average number of microarthropods in the microcosms at harvest remained higher in the ambient microarthropod treatment than the N-deposition microarthropod treatment (Fauna, $F_{2,\,134} = 24$, $P < 0.001$). The meanings of legends are similar to Fig. 1

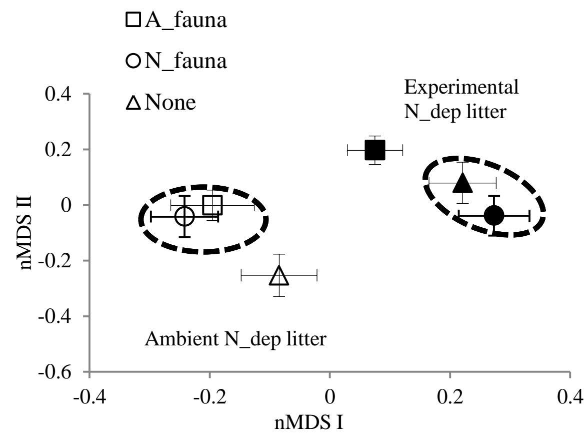

Figure 5.4. Fungal communities, visualized by nMDS of TRF classes from *Hae*III digest, varied between litter types and microarthropod treatments (PerMANOVA, Litter x Fauna, $F_{2, 78} = 1.88$, $P = 0.03$). nMDS: stress = 0.14, dimensions = 3. Empty symbols: litter from ambient N deposition; Filled symbols: litter from experimental N deposition. Square, A_fauna: microarthropods collected from litter from ambient N deposition; Circle, N_fauna: microarthropods collected from litter from ambient N deposition; Triangle, None: no microarthropods. Dashed circle represents similar communities within each litter type.

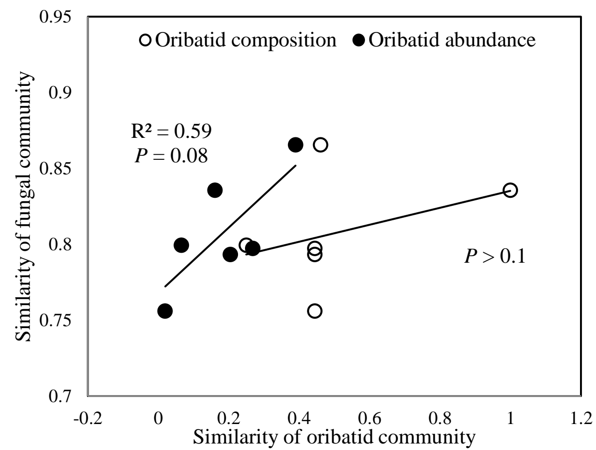

Figure 5.5. Similarities between fungal communities are positively related to the similarities of oribatid abundance (filled circles) but not oribatid species composition (open circles). The similarities were calculated by 1- dissimilarity (Bray-Curtis index). The presence/absence of oribatids was used for the calculation of similarity in species composition; and the abundance of oribatid species was used for the calculation of similarity in abundance.

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Chapter VI

Conclusions

Understanding processes that structure biotic communities is a central theme in community ecology (Begon et al. 2006). It is widely acknowledged that both stochastic and deterministic forces including dispersal limitation, environmental filtering and biotic interactions are important in community assembly (Belyea and Lancaster 1999, Leibold and McPeek 2006). However, little is known regarding the relative role of stochastic and deterministic forces in the community assembly of soil animal communities, which are among the most species-rich components of terrestrial ecosystems (Giller 1996). Understanding the mechanisms underlying the structure of soil animal communities is a pressing challenge in a world that is facing increasing rates of biodiversity loss and ecosystem degradation (Clark 2009). This knowledge will help us to predict how soil animals may respond to environmental changes and the potential consequences of such changes for ecosystem functioning.

In this dissertation, I focused on an important group of soil animals, the soil oribatid mites, to investigate mechanisms underlying their community assembly and to address how global environmental change influences their community structure and function. I first investigated how dispersal limitation, environmental filtering and biotic interactions influenced the diversity and composition of oribatid mite communities across a glacial chronosequence. To address if there is any niche differentiation in food resource utilization among soil oribatid mites, I studied the trophic structure and trophic dynamics of these soil organisms under different environmental conditions. Furthermore, I investigated how soil oribatid mites (together with

other groups of soil microarthropods) respond to chronic N deposition, a pervasive agent of global environmental change. I then established a litterbag experiment in the field and a microcosm experiment in the laboratory to examine whether changes in oribatid communities under chronic N deposition feeds back to influence litter decay. Below I summarize the main findings from my four primary chapters.

Chapter II. Stochastic (dispersal limitation) and deterministic (environmental filtering and biotic interaction) processes as drivers of soil oribatid mite community assembly. I hypothesized that dispersal limitation was a central mechanism structuring oribatid mite communities, due to their low locomotive activity through the soil. I tested this hypothesis by studying the geographic distribution of oribatid mites along a chronosequence across a deglaciated region, in which the effect of limited dispersal is expected to be profound.

However, this study demonstrates that soil/litter dwelling oribatid mites are not limited by dispersal in their re-colonization of deglaciated areas. This is supported by three lines of evidence including similar species richness along the chronosequence, a lack of relationship between overall community dissimilarity and geographic distance, and a large species pool of oribatid mites in deglaciated regions. However, large-bodied oribatid species seem to be affected by dispersal limitation. This is supported by the significant relationship between the community dissimilarity of large-bodied species (but not small-bodied species) and geographic distance. The community composition of large-bodied species is also more dissimilar (41% dissimilarity) among sites than that of small-bodied species (27% dissimilarity). Furthermore, compared to the slow recovery of earthworms in deglaciated areas, the rapid recovery of oribatid mites further supports the conclusion that soil animals with large body size are more affected by dispersal limitation than are small-bodied animals. Our study also reveals that environmental filtering

(temperature, litter fall and soil C:N ratio) and competition for food resources (fungal biomass and Collembola abundance) contribute to determining oribatid species composition.

Chapter III. Trophic structure and trophic dynamics of soil oribatid mites. High local diversity of soil oribatid mites suggests that intense competition and differentiation in resource utilization may exist among these oribatid species (Anderson 1975). In this study, we used ^{15}N isotope analysis to study trophic differentiation among oribatid mites and to examine how chronic atmospheric N deposition may alter their trophic structure. Our findings have provided three novel insights into the trophic structure and trophic dynamic of soil oribatid mites.

First, by combining our own measurements and published data, our study is the first to clearly demonstrate that the majority of soil oribatid mites feed on fungal hyphae and animal tissue rather than dead plant material. Feeding on dead plant material is a long-held misconception due to an inability using traditional gut content analysis to identify the actual food assimilated. Second, I also find that the trophic positions of soil oribatid mites are surprisingly stable within and among forests, and between ambient and experimental N deposition. This indicates a high level of feeding specialization among soil oribatid mites, which counters another misunderstanding, that soil animals are generalists, enabling them to switch diets based on food availability. Finally, specialized feeding on live organisms illustrates the potential for co-evolution between soil oribatid mites and their food resources. Such co-evolution may help to explain the coexistence of high numbers of soil oribatid mite species in small areas. The specialized diet of these soil animals means that their densities will likely suffer in the face of environmental change, as will the important roles that they play in ecosystem processes such as litter comminution.

Chapter IV. The effects of chronic N deposition on the community structure and function of soil oribatid mites. I investigated the influence of anthropogenic nitrogen deposition on soil oribatid mites and asked if those changes could then feed back to influence ecosystem functioning. Accumulating evidence suggests that soil animals are sensitive to multiple environmental stressors (Blankinship et al. 2011, Eisenhauer et al. 2012). However, no study to date has explicitly explored whether such changes could compromise ecosystem processes such as litter decay.

Overall, this study demonstrates that long-term experimental N deposition has reduced the abundance of microarthropods (Oribatid mites, Collembola and Mesostigmata) by 44%. This overall decline is attributed partly to inhibition of microbial activity and the reduced energy flow entering the detrital food pathway under chronic N deposition. While there is no difference in microarthropod species richness between N deposition treatments, there is a shift in community composition within the most abundant group (Oribatida), indicating species-specific responses to N deposition. Experimental N deposition also reduces the number of microarthropods colonizing litterbags by 41%, and this is associated with a reduction in ^{13}C mobilization from leaf litter into fungal biomass. Overall, this study demonstrates that chronic N deposition has a detrimental effect on soil oribatid mites, and that the negative effect may feed back to influence litter decay and ecosystem functioning.

Chapter V. The consequences of changes in microarthropod communities under chronic N deposition for fungal communities and litter decay. Results from Chapter IV demonstrated that the abundance of microarthropods was significantly reduced and the composition of oribatid communities was altered under chronic N deposition. Results from Chapter IV also revealed that the decline in microarthropod abundance in forest floors reduced litter fragmentation. The

reduction in litter comminution might reduce the amount of litter surface exposed for microbial attack, which would ultimately inhibit litter decay. In this study, I was interested in other mechanisms by which microarthropods influence litter decay, especially the interactions between microarthropods and saprotrophic fungi.

Overall, results from the microcosm experiment reveal that changes in microarthropod communities influenced fungal communities but not litter decay. First, the total metabolized C pool is highest when litter is inoculated with microarthropods of the same origin as the litter, and is comparable between ambient and experimental N deposition treatments. This suggests that after 18 years of experimental N deposition, soil microarthropods may have acclimated to changes in microbial community composition and function, at least for labile organic matter during the early stages of litter decay. This study also demonstrates that fungal communities are primarily distinguished by different litter types. However, within each litter type, fungal communities vary with microarthropod abundance. This suggests that any changes in the abundance of microarthropods under global environmental change are likely to feed back to influence fungal communities and possibly the decay of recalcitrant organic matter.

Synthesis. This dissertation has provided insights into the structure and function of soil oribatid mite communities and how they respond to global environmental change. The first conclusive finding from this dissertation is that dispersal limitation does not severely affect the overall soil oribatid mite communities. However, other neutral forces such as demography stochasticity might be important in the assembly of oribatid mite community in environments with frequent and unpredictable disturbances (Maraun et al. 2003). While it is important to test these stochastic forces in community structure, some criticize that it is hard to relate the neutral view of biodiversity to the real world problems (Clark 2009). Instead, it is of greater importance, to test a

set of boarder factors that are actually affecting community structure and ecosystem function, especially in a world facing increasing rate of loss of biodiversity and other global environmental changes (Clark 2009).

This dissertation also illustrated that deterministic forces, i.e., environment filtering and biotic interactions are more important than dispersal limitation in structuring soil oribatid mite communities. Competition for fungal hyphae and niche differentiation in food resource utilization may contribute to explain the enigma of soil oribatid diversity. Competition for food resources as a strong force in shaping oribatid mite community is also demonstrated by manipulative experiments and other stable isotope studies (Anderson 1978a, Schneider et al. 2004, Pollierer et al. 2009). Other studies further stress the importance of competition for space and microhabitats among oribatid mite species (Anderson 1978b, Ferreira and Silva 2001). Furthermore, accumulating evidences suggest that the high diversity of soil animals is in part contributed to the extremely heterogeneous habitats in soils (Irmler 1998, Bardgett 2002, Vanbergen et al. 2007, Nielsen et al. 2010). On the other hand, while controls from predators are important in the community assembly of other groups of soil animals such as nematodes (Hyvonen and Persson 1998), most oribatid mites are heavily sclerotized to defend against predators and therefore likely to live in enemy-free space (Peschel et al. 2006). Nevertheless, this dissertation research, together with the aforementioned studies, indicate that similar to many animals living aboveground, soil oribatid mites are regulated in a predictable fashion by forces underlying their niche differentiation.

Caveats and future directions. The results documented in this dissertation strongly support a central role of deterministic forces, especially competition for food resources, in the structuring of soil oribatid mite communities. In contrast, there is little evidence for the role of dispersal

limitation in shaping overall oribatid communities. However, this dissertation focused on the re-colonization of soil oribatid mites into deglaciated areas, which has been occurring for over 10,000 years. The lack of dispersal limitation at this time scale does not exclude the possibility of dispersal limitation at shorter time scales. As a matter of fact, studies have found that the diversity of soil oribatid mites does not recover over decadal time spans in no-tillage farming or abandoned old fields (Siepel 1991, Adl et al., 2006). Nevertheless, the recovery of oribatid mite communities following glacial retreat is notably faster than that of other groups of soil fauna such as earthworms, which are at the other extreme of body size among soil fauna. It will be interesting to investigate how dispersal limitation influences the distribution of other groups of soil fauna with intermediate body sizes (e.g., Isopoda and Enchytraeidae) in deglaciated regions. The study of the distribution of soil animals at multiple study sites in both deglaciated and ice-free regions would provide further information regarding refuges during glaciation and the routes of their re-colonization.

This dissertation also reveals a high degree of stability in the trophic positions of oribatid mites based on ^{15}N analysis. However, there are some intrinsic limitations associated with stable isotope analysis. For example, it is nearly impossible to identify omnivores using solely ^{15}N data, because the stable isotope signals can be a mixture from two or more food sources. Also, results from ^{15}N analysis did not have sufficient resolution to detect subtle changes in food items that occur within the same trophic levels (e.g., fungal hyphae from different fungi species). Further studies should use additional methods such as fatty acid analysis or molecular fingerprints of gut contents to detect more subtle trophic relationships of soil oribatid mites (Scheu 2002, Chamberlain et al. 2004).

Strong competition for food resources and feeding specialization among soil oribatid

mites suggest that they are likely to respond to global environmental changes in a predictable manner. This dissertation only addresses how atmospheric N deposition affects the community structure and functions of soil oribatid mites in hardwood forests. Future studies with comparative experiments on the responses of soil oribatid mites from contrasting ecosystems (e.g. grasslands vs. forests) to multiple agents of environmental change (e.g. N deposition & global warming) will deepen our knowledge of the mechanisms underlying how soil oribatid mites respond to environmental changes.

Despite these caveats and limitations, the work described in this dissertation adds to our understanding of the mechanisms underlying the community structure and functions of soil oribatid mites. The knowledge that soil oribatid mite communities are primarily governed by deterministic forces helps us to predict the influence of global environmental changes on their community structure and ecological functions.

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