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Trophic stability of soil oribatid mites in the face of environmental change

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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 be affected 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.

1. 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).

Describing 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

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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 and reducing the population size of soil animals especially microarthropods (−44%, Gan et al., 2013). The decline of soil microarthropods in response to reductions in microbial biomass suggests that these animals are limited by food resources. Therefore, reduced energy flow from the microbial community could also 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 three 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 co-evolution 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 positions from higher to lower trophic levels.

2. Materials and methods

2.1. Site description

We collected soil oribatid mites from a long-term study of experimental N deposition consisting of four sugar maple (*Acer saccharum* Marsh.)-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 × 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.

2.2. 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 × 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 × 6 plots × 6 samples). All of the samples were transported to the lab in coolers and each sample was placed on a modified Tullgren funnel within 48 h to extract microarthropods (Crossley and Blair, 1991). After the 5-day extraction, litter was placed in a 60 °C oven for 24 h 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 × 3 plots × 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*) for use at the Ohio State University Summer Acarology Program.

2.3. Stable isotope analysis

Dominant oribatid mite species from each site were removed from ethanol and placed into pre-weighed tin capsules. For each species, 10–150 individuals from the same site and sampling date 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 h, 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 h, 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).

2.4. 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, plus 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).

2.5. 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. We performed a literature search on Web of Science using the keywords 'stable isotope' and 'mite'. In total, there were 8 publications with measurements of $\delta^{15}N$ of oribatid mites, and we excluded three studies that focused on bark-living or moss-living oribatid mites (Erdmann et al., 2007; Fischer et al., 2010; Perdomo et al., 2012), 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).

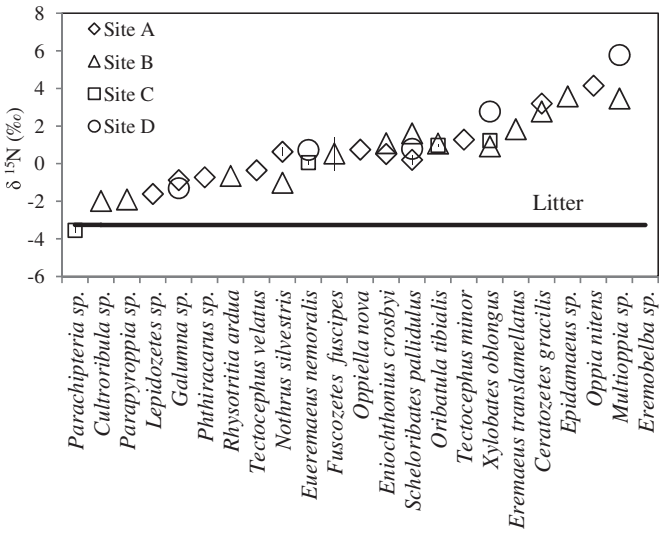

Fig. 1. Dominant oribatid mite species in the forest floor of four hardwood forests occupy more than three trophic levels. $\delta^{15}N$ values of mites were calibrated based on differences between ^{15}N levels of substrates from that of the ambient N treatment of Site D (−3.26‰). Two-way ANOVA (Type II sum of square) indicated that the same species from different sites did not differ in their $\delta^{15}N$ 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.

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 $\delta^{15}N$ among oribatid mite species from different studies, we adjusted the $\delta^{15}N$ of the animals by correcting for the differences between the $\delta^{15}N$ of the litter in our study (−3.26‰) and those in the literature. We assigned oribatid mites into trophic positions based on their calibrated $\delta^{15}N$; 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.

2.6. Statistical analysis

We used two-way ANOVA (site and treatment, Type I sum of squares) to assess any difference in the $\delta^{15}N$ of forest floor from our four study sites with different N deposition levels. Each $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ of oribatid mite species differed among measurements from different authors. Therefore, we used two-way ANOVA with Species and Author as

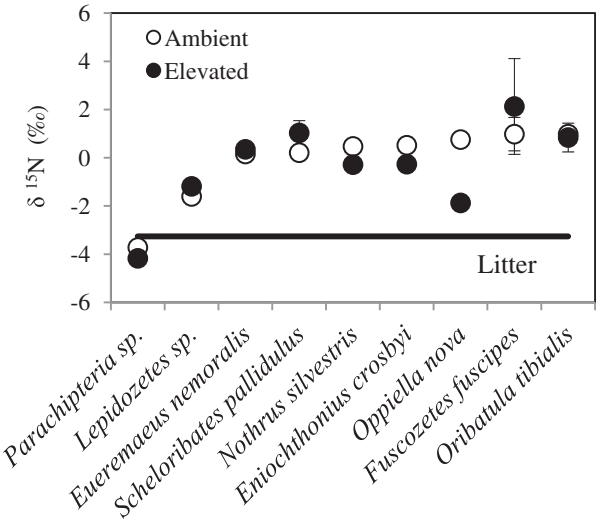

Fig. 2. Trophic positions of oribatid mites (indicated by their $\delta^{15}N$) in the forest floor did not differ between ambient (empty) and elevated (solid) N deposition plots. $\delta^{15}N$ values of mite were calibrated based on differences between $\delta^{15}N$ of substrates from that of the ambient N treatment of Site D (−3.26‰). Error bar represents 1 SD when there were replicated measurements. Student's t test (paired): $t = -1.17$, $P = 0.27$.

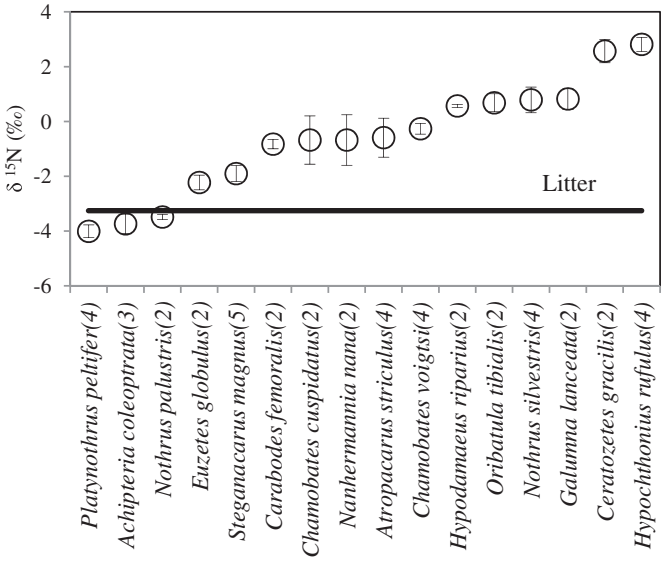

Fig. 3. Trophic positions of oribatid mites (indicated by $\delta^{15}N$) show little variation among different studies (two-way ANOVA with Type II sums of squares, $F_{5,25} = 0.15$, $P = 0.96$). $\delta^{15}N$ values of mites were calibrated based on differences between $\delta^{15}N$ of substrates 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.

main effects to analyze within-species variation in $\delta^{15}N$ 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).

3. Results

3.1. ^{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 $\delta^{15}N$.

3.2. 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 *Eremobelba* sp. (6.11‰, Fig. 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. 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 (1.84–5.24‰) that feed on dead or live animal tissues or any food item that has a high $\delta^{15}N$ value. We also had one species *Eremobelba* sp. with an extremely high $\delta^{15}N$ (6.11‰), which we therefore designated as a secondary predator (Fig. 1).

3.3. 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. 2, $t = -1.17$, $P = 0.27$). The differences in $\delta^{15}N$ between the N deposition treatments were smaller than 1‰ for most species, except *Oppiella nova* (−2.62‰; Fig. 2).

3.4. 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 S1 in Supplementary information). Out of these 91 oribatid species in the forest floor, there was only 1 species with a distinctly negative $\delta^{15}N$ (*Carabodes labyrinthicus*), and it was therefore categorized as a lichen feeder. Additionally, one species expressed a $\delta^{15}N$ two trophic levels above that of the saprotrophic feeders, and it was therefore categorized as a secondary predator (*Eremobelba* sp., Fig.1). Over half of the species (55%) were ascribed to the second trophic level as microbivores and 22% of the species were designated as litter feeders. The remaining 21% of the species were categorized as predators/scavengers. 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 $\delta^{15}N$ of the same species did not differ among studies ($F_{5,25} = 0.15$, $P = 0.96$, Fig. 3). A complete list of oribatid species with their trophic positions can be found in Supplementary Table S1.

4. Discussion

4.1. 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. 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. While oribatid mites in the second trophic level are more likely to be fungal feeders than bacterial feeders considering the common chewing mouthparts and the observation of fungal hyphae in their guts (Anderson, 1975b; Kaneko, 1988), some oribatid mites with modified chelicerae are able to scrape bacterial films or to draw soil suspension including bacteria (Norton and Behan-Pelletier, 2009). Recent fatty acid analyses have revealed that bacterial fatty acids contribute 5–10% of the total fatty acids in four oribatid mites, and this value is higher (19%) in a predatory oribatid mite feeding on bacterial-feeding nematodes (Pollierer et al., 2012). Oribatid mites in the second trophic level could theoretically feed on saprotrophic mites at a lower trophic position; however, oribatid 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 (Heidemann et al., 2011; Pollierer et al., 2012). 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. Further studies on cheliceral morphology may distinguish scavengers from predators as the latter are characterized by chelicerae with low leverage for rapid closing after catching prey (Perdomo et al., 2012). 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 ectomycorrhizal 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 microbivores. 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 oribatid 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 oribatid 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).

4.2. 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 δ^{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 δ^{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 δ^{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 δ^{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 δ^{15}N of oribatid mites did not differ significantly among the four sugar-maple forests that we sampled (Fig. 1). These four sites vary in annual average temperature from 4.7 °C at Site A (northern site) to 7.6 °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). 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, Díaz-Aguilar and Quideau (2013) found that isotopic nitrogen fractionation within mites was not affected by their habitats (spruce vs. aspen) or clear-cutting practices. Furthermore, oribatid species from the bark of different trees (coniferous *vs.* broad-leaf trees) seemed to share 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 (Erdmann et al., 2007). 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 ± 1 SD) 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2013.09.019.

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