INTERSPECIFIC INTERACTIONS BETWEEN NATIVE AND NON-NATIVE FOREST FLOOR DETRITIVORES AND TEMPERATURE: IMPLICATIONS FOR ECOSYSTEM FUNCTIONING

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ABSTRACT

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Macroinvertebrate detritivores directly and indirectly affect ecosystem processes and nutrient cycling, but the effects of detritivore interactions on ecosystem processes remain poorly understood. Furthermore, mounting evidence emphasizes the importance of understanding the effects and implications of environmental disturbance on interactions between soil organisms, including invasive species and climate change.

In this study, we asked: Is there evidence of competitive or complementary interactions between a native detritivore and two non-native detritivores? Additionally, can detritivore species-specific and interspecific interaction effects on soil ecosystem processes and microbial activity, be moderated by temperature?

To answer these questions, we performed a mesocosm experiment which included three detritivore species, a millipede native to North America (*N. americanus*) and two introduced earthworm species (*L. rubellus* and *L. terrestris*). We fully replicated a Simplex mixture design using these species under two temperature treatments, ambient and warmed 3.3°C.

We expected to observe species-specific and complementary effects of the study organisms due to differences in functional traits. Furthermore, we anticipated that temperature would alter species interactions, and warming would exert a disproportionately greater negative effect on surface dwelling millipedes. Overall, we expected invasive earthworm effects to overwhelm the effects of millipede presence.

An interaction between *L. rubellus* and *N. americanus* predicted an increase in litter mass lost and microbial biomass C, indicating a potential complementarity effect. *N. americanus* reduced both NAG and BG enzyme activity. *L. terrestris* reduced NAG and BG activity at warmed temperature, but increased activity at ambient temperature. An interaction effect between *L. rubellus* and *L. terrestris* also predicted a reduction in NAG activity. Earthworm biomass was significantly reduced over the duration of the experiment regardless of temperature treatment. *L. terrestris* significantly increased NH_4^+ leaching, and detritivores did not significantly affect carbon (CO_2) efflux.

There was no evidence to indicate that interspecific interactions between these detritivores are moderated by temperature. However, these results indicate that *N. americanus* may exert some biotic resistance to invasion pressures by *L. rubellus* and *L. terrestris*. Future experiments may consider manipulations of food resources, additional trophic levels, and physical soil characteristics to parse out underlying mechanisms.

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INTRODUCTION

In terrestrial systems, invertebrate detritivores are soil-dwelling animals that obtain nutrients by consuming detritus, which is primarily comprised of plant litter, dead animal material, and feces in soil systems (Coleman, Crossley, and Hendrix, 2004). The consumptive activities of detritivores directly influence ecosystem process rates, including litter decomposition and mineralization, which ultimately affects carbon (C) and nutrient cycling (Bohlen et al., 2004a; Crumsey et al., 2015; Fahey et al. 2013). Detritivores can also exert indirect effects on ecosystem processes by stimulating the activities of microbial decomposers (Dempsey et al., 2013; Groffman et al., 2015).

Millipedes and earthworms are macroinvertebrate detritivores which have been given significant attention in soil literature due to their classifications as litter transformers and ecosystem engineers, respectively (Jouquet et al., 2006; Lavelle et al., 1997; Lavelle et al., 2006). By physically processing litter through shredding, or by chemically altering litter through digestion, millipedes increase substrate availability for microbial colonization (Lavelle et al., 1997). Millipedes have also been shown to dampen the effect of anthropogenic nitrogen (N) deposition by consuming excess fungal growth in upper soil horizons, significantly reducing fungal biomass to what would be expected under natural conditions (Crowther et al., 2015). Comminution of fungal hyphae by millipedes can also alter microbial enzyme activity, and thus indirectly affect decomposition (Crowther et al., 2015). On the other hand, ecosystem engineers are organisms that directly, or indirectly, mediate resource availability to other species by modifying the biotic and abiotic characteristics of the environment they inhabit (Jones, Lawton, and Shachak, 1994). Earthworms humify, or mix soil layers and increase soil porosity through burrowing and consumptive activity (Edwards, 2004). In this way, earthworms can dictate local soil microclimate and facilitate resource inputs into belowground systems, thus creating (Hale, Frelich, and Reich, 2006) or dismantling (Crooks, 2002; Snyder et al., 2010; Snyder et al., 2013) available habitat for plants, microbes, and mesofauna (springtails, mites, nematodes) (Eisenhauer et al., 2009; Eisenhauer et al., 2010; Eisenhauer et al., 2012).

Despite accumulating knowledge on the taxon-specific effects of detritivores on ecosystem processes, current understanding of the importance of detritivore interspecific interaction effects on ecosystem processes remains less resolved (Andriuzzi et al., 2013; De Oliveira, Hättenschwiler, and Handa 2010; Gessner et al., 2010; Vos et al., 2011). However, complementarity effects, moderated by niche differentiation and facilitation mechanisms, seem to drive interspecific interactions among naturally co-occurring soil fauna, and may explain the coexistence of high numbers of soil-dwelling species (Wardle, 2006). Non-additive complementarity effects between detritivores are observed when two or more species interact to enhance ecosystem process rates, for example, litter decomposition (De Oliveira et al., 2010; Hedde et al., 2010; Heemsbergen et al., 2004; Vos et al., 2011; Zimmer et al., 2005). Niche differentiation typically occurs when there is resource partitioning between two or more species and facilitation is observed when one species in a community affects others in such a way that enhances the impact of the affected species on an ecosystem process (Gessner et al., 2010). However, previous studies have had difficulty parsing out niche differentiation and facilitation from detritivore complementarity effects (Heemsbergen et al., 2004; Vos et al., 2011). Furthermore, both mechanisms may occur simultaneously, which illustrates the need to better understand the factors that drive the strength, direction, and mechanisms of detritivore interactions (De Oliveira et al., 2010).

Mounting evidence indicates that organism functional traits are useful in predicting the outcomes of soil fauna interactions (Andriuzzi et al., 2015; Heemsbergen et al., 2004; Hedde et al., 2010). Functional traits are phenotypes of organisms associated with biotic interactions and explain species ecological roles in terms of their effects on ecosystem processes (Cardinale et al., 2002). In the case of detritivores, functional traits include, but are not limited to: consumptive preferences, mouthpart morphology, body size, burrowing behavior, growth rate, offspring production rate, as well as differing nutrient requirements and dietary shifts at various developmental stages (Poorter et al., 2007; Violle et al., 2007; Chapin et al., 1997). Competition for resources may be more prevalent between detritivores that share similar functional traits, but functional trait divergence (as well as spatial and temporal discrepancies in species resource use) may attenuate competitive interactions between detritivores and help explain coexistence via complementarity mechanisms (niche differentiation and facilitation) (Andriuzzi et al., 2015; De Oliveira et al., 2010; Heemsbergen et al., 2004; Sheehan et al., 2006). It is also important to acknowledge that detritivore functional trait-expression (e.g., burrowing behavior) is flexible, and changes in response to environmental variables (Perreault & Whalen, 2006). Furthermore, our ability to predict the outcomes of soil fauna interactions and the impacts of environmental change on communities and ecosystems often depends on evaluating the strength of multiple interacting stressors (Coyle et al., 2017; Vitousek, 1994).

Invasions of non-native macroinvertebrate detritivores have been shown to drive native biodiversity loss under certain contexts, which can have drastic effects on ecosystem processes and stability (Bohlen et al., 2004a). In these cases, invading detritivores change the pool of soil fauna functional traits, and therefore alter natural trophic dynamics and nutrient cycling. In North America, peregrine earthworm species introduced from Europe and Asia are widespread and can rapidly colonize new habitats (Bohlen et al., 2004a). Depending on the site and earthworm species present, introduced worms can accelerate litter decomposition (Bohlen, 1996), reduce forest floor organic matter (Hale et al., 2006), change soil pH, C:N ratios (Bohlen et al., 2004b), and C,N, and P pools (Crumsey et al., 2015; Groffman et al., 2015: Suárez et al., 2004), enhance N losses (leaching and gaseous) (Andriuzzi et al., 2015; Marhan et al., 2015), alter forest floor microbial biomass (Groffman et al., 2015; Li et al., 2002; McLean et al., 2006), increase microbial respiration (Chang et al., 2016; Fisk et al., 2004; Jennings & Watmough, 2016), and increase bacteria to fungi ratios in forest soils (Dempsey et al., 2013; Dempsey, Fisk, and Fahey, 2011). Additionally, by reducing the thickness of forest floor organic layers and increasing bulk soil density, non-native earthworms reduce native understory plant and invertebrate diversity in forest systems, and may accelerate succession of invasive plants (Eisenhauer, 2010; Hale et al., 2006). Despite this knowledge, it is not well understood how invasive detritivores can be expected to indirectly influence soil ecosystem processes by interacting with native detritivores.

Some studies have detected competition for food resources between native detritivores and non-native earthworms (Snyder, Boots, and Hendrix, 2009; Snyder et al., 2013), as well as among invading earthworms themselves (Chang et al., 2016), which may have implications for local community assembly and ecosystem processes. A study by Snyder et al. (2009) found that non-native earthworms and native millipedes competed for similar resources. Furthermore, when both organisms were present in experimental mesocosms, microbial respiration increased, which suggests a potential mechanism for net soil C loss in forest systems. However, in a similar study, Snyder et al. (2013) noted that while native millipede survival was reduced in the presence of non-native earthworms, earthworm reproduction was impeded by millipede presence, which could indicate that native detritivores exert biotic resistance against peregrine earthworm invasions. Studies on naturally co-occurring earthworms and millipedes have also noted trends towards complementarity, specifically by way of niche differentiation, in which millipedes fed primarily on low-quality litter and earthworms fed preferentially on high-quality litter (Hättenschwiler & Gasser, 2005). Therefore, detritivore interactions may be species and context specific.

Soil organisms are highly dependent on soil temperature and moisture (Berry & Jordan, 2001; Collison et al., 2013; Kung, Gaugler, and Kaya, 1991; Orchard & Cook, 1983; Wall & Virginia, 1999), which makes understanding the impacts of climate change on invertebrate-mediated ecosystem processes critical. Elevated soil temperature in climate manipulation experiments has been shown to induce species-specific responses in detritivores (Berry & Jordan, 2001; Coûteaux et al., 2002; Makoto, Arai, and Kaneko, 2014; Rouifed et al., 2010; Uvarov & Scheu, 2004), and functional traits and soil characteristics seem to be useful predictors in determining how detritivores will respond to different types of disturbance (Collison et al., 2013; Crowther et al., 2011; Crumsey et al., 2015; Heemsbergen et al., 2004). Climate change is a major driver of biodiversity loss (Thomas et al., 2004), and therefore presents a significant threat to ecosystem functioning (Cardinale et al., 2012; Loreau et al., 2001). Yet, despite evidence of temperature differentially affecting soil-dwelling organisms, but generally causing an increase in activity and resource use, current knowledge on the ability of climate change variables to dictate detritivore species-specific and interaction effects on ecosystem processes is contradictory and lacking (Barrett et al., 2008; Crumsey et al. 2015, Crowther et al., 2015; Fisk et al., 2004; Ott, Rall, and Brose, 2012; Pelini et al., 2015).

In this study, we sought to unravel the role temperature plays in dictating species-specific effects and interspecific interaction effects between native and non-native detritivores. We asked three questions: (1) Is there evidence of competitive or complementary interactions between a native detritivore and two non-native detritivores? (2) Can detritivore species-specific and interspecific interaction effects on microbial biomass and activity (enzyme activity, respiration) be moderated by temperature? (3) How are soil processes (such as decomposition and leaching) affected by detritivore species identity, interspecific interactions, and detritivore interactions with temperature?

To answer these questions, we performed a mesocosm experiment where detritivore community assemblages were manipulated using three detritivores (two earthworm species, *Lumbricus rubellus* and *Lumbricus terrestris*, and a millipede, *Narceus americanus*) common to North American forests. The detritivores used were selected because they encompass a range of functional traits, specifically in regard to burrowing, feeding, and physiology, and can thus provide the necessary framework to ask questions about the direct and indirect effects of climate change on species interactions and consequences for ecosystem processes within belowground food webs. Additionally, each mesocosm was subjected to one of two temperature treatments: ambient, or warmed 3.3°C above ambient (which is in accordance with predicted temperature changes for the region by 2100) (IPCC, 2013).

L. rubellus and *L. terrestris* are peregrine species introduced to North America from Europe and represent two different feeding guilds (Bohlen et al., 2004a; Curry & Schmidt, 2007). *L. rubellus* is a small, epigeic species that occurs in the upper organic soil horizons and feeds on surface litter (Edwards, 2004). It burrows horizontally and is generally considered to have little effect on bioturbation. *L. terrestris* is a larger, anecic species that resides in permanent vertical burrows which can extend up to 1-2 m deep (Edwards, 2004). *L. terrestris* individuals surface to feed on decomposing litter, or to pull litter into their burrows, and have been found to enhance humification and accelerate transition from mor (low pH and low biological activity) to mull (neutral pH and high biological activity) soils in North American forests (Bohlen et al., 2004b; Coleman et al., 2004). *Narceus americanus* is a litter-dwelling millipede native to North America (O'Neill, 1968). It creates simple burrows in upper organic horizons which exhibit varying morphologies (Bowen & Hembree, 2014). *N. americanus* feeds on litter and moist, decomposing wood in forest systems (O'Neill, 1968).

It is difficult to predict if *N. americanus* will compete with or exhibit complementarity effects with *L. rubellus* and *L. terrestris*, primarily because current knowledge on its specific feeding preferences and interactions with other organisms are poorly understood (O'Neill, 1968). Nevertheless, we anticipate that increasing temperature will increase decomposition processes by increasing the consumptive activities of all detritivore species. However, we expect that sub-surface dwelling earthworms will dominate effects on litter decomposition over surface dwelling millipedes due to greater competitive ability in terms of habitat utilization and physiological resilience to temperature stress. In the warmed scenario, we expect *L. rubellus* to exhibit the highest fitness, outcompeting the other two species due to its generalist feeding pattern and previous evidence which indicates increased fitness under slightly elevated temperatures (Eisenhauer et al., 2014; Perreault & Whalen, 2006; Uvarov, Tiunov, and Scheu, 2011). Studies on the responses of both *L. rubellus* and *L. terrestris* to increased temperature indicate that they alter their burrowing behavior to adjust to fluctuating environmental conditions, but may respond positively to a marginally warmer climate (up to + 5 °C) (Eisenhauer et al., 2014; Perreault & Whalen, 2006; Uvarov et al., 2011). Concurrently, studies on millipedes indicate that life history traits including development time, biomass, and fertility are positively affected by slightly elevated temperature (3.3 °C) (David & Handa, 2010). Despite this, we predict the millipedes will be outcompeted as a combination of previous research which indicates lower fecundity when earthworms are present in soil communities and the reduced ability of millipedes to avoid desiccation by deep burrowing (Snyder et al., 2009; Snyder et al., 2013).

We anticipate enhanced C flux and decomposition rate under increased temperatures as the consumptive activity of detritivores and microbial respiration increases with temperature (Allison, Wallenstein, and Bradford, 2010; David & Handa, 2010; Eisenhauer et al., 2014; Makoto et al., 2014; Ott et al., 2012; Snyder et al., 2009; Uvarov et al., 2011). However, it is more difficult to predict how detritivores will affect overall microbial biomass and activity as this result most likely depends on microbial resource demand as well as how efficiently each detritivore species incorporates C and N into the soil under both temperature scenarios (Groffman et al., 2004).

Previous studies have identified species-specific effects of detritivores on microbial enzyme activity (Crowther et al., 2011; Flegel & Schrader, 2000; Hoang et al., 2016), however, the effects of detritivore interactions on enzyme activity are relatively unknown (Sauvadet et al., 2017). Enzyme activity is expected to increase in warmed treatments and we anticipate that earthworms (particularly *L. terrestris*) will have more significant impacts on enzyme activity and microbial biomass than millipedes, again due to burrowing and soil consumption behavior. Specifically, we expect that earthworms will enhance enzyme activity by stimulating drilosphere microbes. However, it has been shown that millipedes will preferentially graze on fungi when available, which may forecast an increase in enzyme activity or a reduction of both enzymatic activity and microbial C storage (Crowther et al., 2011; Crowther et al., 2015).

Millipedes tend to increase the availability of inorganic N in soils via casting (Cárcamo et al., 2000; Makoto et al., 2014). Earthworms, on the other hand, tend to reduce total N pools in forest soils, but may enhance microbial N carrying capacity. Despite this, earthworm burrowing can exacerbate nitrate leaching, which may negate the effects of millipede presence on soil N availability (Crumsey et al., 2015; Bohlen et al., 2004a). Overall, we expect that N-retention will be reduced in response to the interactive effect of earthworm species and temperature.

METHODS

Study System

Mesocosms were established on a covered porch at the University of Michigan Biological Station (UMBS) in Pellston, MI, USA (45-35.5°N, 84-43°W). UMBS soils (used in this experiment) are acidic (pH 4.8), and classified as mixed, frigid Entic Haplorthods of the Rubicon series (92.9% sand, 6.5% silt, 0.6% clay). Local dominant tree species include bigtooth aspen (*Populus grandidentata*), red maple (*Acer rubrum*), northern red oak (*Quercus rubra*), paper birch (*Betula papyrifera*), Eastern white pine (*Pinus strobus*), and American beech (*Fagus grandifolia*). Average (1979-2010) summer (June-August) temperature and precipitation at UMBS is 17.8 °C and 219 mm respectively (Nave et al., 2011).

Experimental Design

A total of 40 mesocosms were constructed and subjected to one of two temperature treatments, ambient or elevated 3.3°C above ambient (which corresponds to IPCC projections for northern Michigan (IPCC, 2013)), resulting in 20 mesocosms per temperature treatment. Within each temperature treatment, detritivore assemblages comprised of two invasive earthworm species (*L. rubellus* and *L. terrestris*) and a native millipede (*N. americanus*) were introduced to mesocosms following a Simplex design (Cornell, 2011) (Figure 1).

The Simplex design, which has been successfully implemented in other studies on diversity-functioning relationships among soil detritivores (Andriuzzi et al., 2015; O'Hea et al., 2010; Piotrowska et al., 2013; Sheehan et al., 2006; Sheehan et al., 2007; Sheehan et al., 2008), allowed us to manipulate detritivore biomass on a continuous spectrum. Biomass was used as a proxy for species richness and evenness, and explicitly manipulated on the assumption that, in soil systems, biomass is intrinsically linked to an organism's functional impact (Bradford et al., 2002; Bílá et al., 2014; Turnbull et al., 2014; van Geffen et al., 2011). By controlling biomass as a measure of evenness, the Simplex design further allowed us to probe into the strength of species interactions (Petchey & Gaston, 2002; Kirwan et al., 2007).

The purpose of the Simplex design is to assess mixture performances of species combinations including: single species monocultures, 'centroids' where each species contributes equally to total detritivore biomass, and intermediary combinations of biomass (Figure 1). Single species monocultures and centroids were duplicated to account for losses due to mortality, and three detritivore-free mesocosms served as controls under each temperature treatment. Replicating the other species combination treatments was not necessary because they encompass a continuous range of species co-occurrences as opposed to factorial combinations, which allows the data to be analyzed using multiple regression. The Simplex design is advantageous to diversity-functioning studies as it makes possible the ability to differentiate (a) species identity effects from the effects of interspecific interactions as well as (b) interaction effects from biomass effects on ecosystem processes (Kirwan et al., 2007; Kirwan et al., 2009).

Each mesocosm containing detritivores received approximately 6 grams of detritivore biomass (which is comparable to the higher end of total detritivore biomass densities observed at this site, (Crumsey et al., 2014)). Total biomass was relatively consistent across mesocosms with small discrepancies between ideal values dictated by the Simplex design, and actual values (± 1.8% on average, Range: 0.808 g). Discrepancies in biomass by species for each combination were also relatively small (± 4.5% on average). The number of individuals in each bucket was also kept as even as possible (± 1 *N. americanus* individual, ± 1 *L. terrestris* individual, ± 1 to 2 *L. rubellus* individuals) across treatments to reduce variation associated with the number of intra- and interspecific interactions.

Mesocosm Construction and Maintenance

Mesocosms were constructed using 5-gallon plastic buckets (height 14.5", diameter at top 11.9", diameter at bottom 10.33"). A circular hole 6 cm in diameter was cut into the center of the bottom of each bucket and covered with 2 mm mesh to allow for adequate drainage and to prevent organisms from escaping. Funnels were then attached underneath each hole and fixed with removable plastic bottles that were used to collect leachate (Figure 2). The buckets were placed underneath a porch overhang and suspended 3 feet off the ground between wooden planks (Figure 3).

Soil was collected during the second week of May 2016. Litter and surface detritus were raked away before soil was removed in layers by shovel. Collected soil was separated into B, E, and mixed O/A horizons and placed into separate plastic bins by layer for 24 hours. Each layer was then sieved through ¼ inch wire, which removed large rocks and macroinvertebrates, but maintained much of the naturally occurring soil aggregates in each layer. Each layer was homogenized separately using the cone and quarter method (Gerlach et al., 2002). This process involves: piling soil onto a plastic tarp forming a cone shape, raking quartered sections of the pile towards four opposing directions, and shoveling the distributed soil around to other quarters to evenly disperse the soil before reforming the original cone. The cone and quarter method was applied to each layer 3 times, and each layer was visually inspected for residual macroinvertebrates before being added to the mesocosms. Four liters of B horizon were added first to each bucket, followed by four liters of E horizon and finally seven liters of O/A horizon. These proportions deviate slightly from field proportions. More organic O/A horizon was included to support detritivore and microbial populations in the mesocosms. The mesocosms were then allowed stabilize for four weeks prior to start of the experiment. During this time, 200 mL of distilled water was added to each mesocosms weekly to prevent drying and death of microbial communities.

Circles (25.4 cm diameter) were cut out of the accompanying bucket lids so that only the lip remained; this allowed for ease of removal and replacement when measuring respiration. Mesh screening (1 mm) was fitted to cover the frame to allow for opening air exchange, as well as to prevent organisms from entering and leaving the mesocosms.

Mixed diversity litter composed of six species (see *Study System* for species descriptions) from a forest stand burned in 1954 was collected, dried, and stored in trash bags in a climate controlled laboratory prior to the start of the experiment. Litter bags, composed of 10 g dried, leaf litter representative of dominant local hardwood species and 4 g of quaking aspen wood chips, were constructed using fish netting (hole width approximately 12.7 mm). This material was used as it facilitated large detritivore movement through the litter bags.

Litter bags were randomly assigned to buckets, and mesocosms were randomly assigned temperature and detritivore treatments. Heat bulbs (Phillips® 250W 120V) were suspended above the surface of mesocosms to raise air temperature 3.3 °C (± 0.25°C) above ambient at the surface of the soil in the elevated temperature treatments (Figure 4). A single heat lamp was used to warm 4 mesocosms, so mesocosms were clustered into groups of 4 for a total of 10 groups of 4, with 5 groups under each temperature treatment (Figure 3). These groups were treated as blocks in the statistical analyses.

I-Buttons (DS1925, Maxim Integrated, San Jose, CA) were used to monitor temperature changes over the duration of the experiment. I-Buttons were placed in each mesocosm (40 total) at the soil surface at the edge of the bucket furthest from the heat lamps and bucket cluster center. Mesocosm moisture content was monitored using gravimetric methods. The mass of each bucket with soil at field moisture capacity was measured at the start of the experiment and was adjusted weekly using DO water as needed. Volumetric water content of each mesocosm was only recorded at the conclusion of the experiment in order to avoid altering detritivore burrowing and efflux measurements.

Detritivore Assemblages

Detritivores were hand collected from forests surrounding UMBS during late May 2016. *L. rubellus* and *L. terrestris* were retrieved using an electroshock extraction method (Crumsey et al., 2014). Due to difficulties finding mature *L. terrestris* individuals in late Spring, individuals purchased from bait shops were used as supplements. To avoid biases towards using purchased versus collected *L. terrestris* individuals in mesocosms, all *L. terrestris* individuals were pooled in single terrarium and selected randomly when assigned to mesocosms. All detritivores were housed in the climate controlled Lakeside Laboratory (21°C) at UMBS and separated into terrariums by species for two weeks prior to the start of the study. Each terrarium contained soils and litter representative of UMBS forests.

Before being introduced to mesocosms, earthworms were identified to species, and only individuals that appeared healthy were used. Due to difficulties in collecting adult *L. terrestris* with low enough biomass requirements for some Simplex proportions dictated by the design, juveniles were used as necessary. To avoid the possibility of confusing *L. rubellus* juveniles with *L. terrestris*, only juveniles that were observed leaving burrows in habitat types primarily dominated by *L. terrestris* during electroshocking were used in the study on the assumption that these burrows were early indications of anecic burrowing behavior. At the conclusion of the study, the matured juveniles were identified and confirmed as *L. terrestris* in all cases.

The earthworms and millipedes were placed in containers with moist paper towels and allowed to clear their guts for 24 hours prior to being introduced to the mesocosms. The mass of each organism was recorded before being placed on the mesocosm surface soil and allowed to burrow.

Temperature treatments began one week after detritivore treatments to allow for adequate acclimation. Casting, molting, and soil surface mortality were observed and recorded throughout the duration of the experiment.

Carbon Efflux Measurements

Carbon Efflux was measured weekly using a LI-6400 (LICOR, Lincoln, NE). Efflux was calculated using Equation 1, where dc'/dt is rate of change of CO_2 corrected for evaporation, H is the space between the soil surface and where the IRGA covers the mesocosm, and 12.011 is the atomic weight of carbon.

Equation 1.

$$\left(\frac{dc'}{dt}\right) \times H \times 12.011\, g_{/mol} \times \left(\frac{1\, g}{1000\, mg}\right) \times 60_{(\mathrm{sec})} \times 60_{(sec)} \times 24_{(hours)} = C - CO_2\, mg_{/day}$$

$$C - CO_2\, mg_{/day} \times \left(\frac{1\, kg}{1000\, mg}\right) \div Bucket\ Mass_{(kg)} = C - CO_{2\ bucket_{/day}}$$

Nitrogen Leachate Measurements

Bottles of collected leachate were analyzed at the end of the mesocosm stabilization period, at four weeks, and at mesocosm harvest, for a total of three separate collection dates. The day before each collection date, each mesocosm received 500 mL of DO water and bottles were collected 24 hours later. Each sample was acidified with 6N TMG HCl and filtered prior to analysis (Fisherbrand™ Glass Fiber Circles, Whatman GF/F). Ammonium (NH_4^+) concentration was analyzed using the automated phenate method (Bran + Leubbe® AA3). Nitrate (NO_3^-) concentration was determined using IC (ThermoFisher™/Dionex™ Integrion HPIC™); column set AS-11 HC (4 micron), isocratic separation using 30 mM KOH as eluent. Total leachate NH_4^+ and NO_3^- are reported as µg/L and mg/L respectively.

Mesocosm Harvest

The experiment ran for a total of 8 weeks and mesocosms were harvested randomly over a three-day period.

On the first day of harvest, litter bags were removed from the surface of the soil and visually inspected to detect if any of the study organisms were inside the bags. The litter bags were then sealed inside plastic bags to ensure no litter was lost prior to drying. Two soils cores at a depth of 15 cm were taken from the soil surface; one core was extracted from directly beneath the litter bag, and another core was taken randomly around the circumference of the surface soil. When detritivore burrows were present at the soil surface, the second core was targeted on those areas. The reasoning behind this comes from evidence that suggests the chemical and physical properties of detritivore burrows and casts are species-specific and affect microbial community functioning as well as soil ecosystem dynamics (Jégou et al., 2001). Millipede molts were not collected, but recorded, due to similar evidence which suggests that arthropod molts affect microbial community functioning (Cabib, 1987). Soil samples were placed in large plastic bags, homogenized for 5 minutes, and stored in a 4°C refrigerator.

On the second harvest day, half of the mesocosms were destructively sampled to remove surviving organisms. The remaining half of mesocosms were harvested on the third day. The remaining organisms were then placed in containers with moist paper towels for 24 hours to clear their guts after being extracted from the mesocosms. After 24 hours, the final biomass of each organism was recorded to determine change in biomass.

Enzyme Assays

Immediately following collection, soils were placed in sealed plastic bags and stored at 4°C for six days. Soils were then placed into large coolers with ice and transported to the University of Toledo for enzyme analysis. Hydrolytic (β-1,4-glucosidase (BG) and N-acetylglucosaminidase (NAG)) and oxidative (phenol oxidase (POX) and peroxidase (PER)) microbial enzyme activity were measured using fluorimetric methods six days after soil collection following the methods outlined in (Saiya-Cork et al., 2002). The oxidative enzyme levels were found to be below detection limits, and are therefore not reported here.

Enzyme activity is typically used as a proxy for microbial nutrient demand (Schimel & Weintraub, 2003). The main function of BG is to hydrolyze cellobiose (a dissacharide derivative of cellulose) to release glucose for microbial C-acquisition (Sinsabaugh, 2005). NAG exhibits an analogous function, but acts on chitin, hydrolyzing chitobiose (a nitrogenous glucosamine dimer) to monomers for microbial N-acquisition (Sinsabaugh et al., 2008). Enzyme activity is reported as nmol per hour per gram dry soil.

Fluorometrically labelled enzyme substrates were prepared one day prior to assays using 4-methylumbelliferone (MUF) to make 4-MUF-β-D-glucopyranoside and 4-MUF-N-acetyl-β-D-glucosaminide for BG and NAG respectively. The average soil pH of 10 randomly sampled mesocosms (~ 5.3) was used as the reference point for preparing the modified universal buffer (MUB) pH. Approximately 1 g (± 0.1 g) of each sample was added to 125 mL plastic bottles and slurried with ~125 mL of MUB. Each sample then was poured into a wide mouth plastic container and was continuously stirred. While stirring, 200 µL aliquots of each sample was pipetted into 96-well microplates for a total of 16 replicates per sample per assay. Plates were allowed to incubate for 5 hours at 20°C prior to reading. Following the incubation period, fluorescence was measured for each well using a BioTek Synergy HT microplate reader (BioTek Instruments Inc., Winooski VT, USA).

Microbial Biomass C and N

Extractable microbial C (DOC) and N (DON) were determined using the chloroform fumigation-extraction technique outlined in Brookes et al. (1985) and modified by Scott-Denton et al. (2006). Approximately 5 g (± 0.1 g) of each soil sample was added to separate centrifuge tubes and extracted with 25 mL 0.5 M K_2SO_4. Two sets of centrifuge tubes per sample as well as blanks were prepared to account for fumigated and non-fumigated samples. Prepared centrifuge tubes were placed horizontally on a shaker table and allowed to shake for 1 hour. One set of centrifuge tubes was placed under a fume hood with 2 mL ethanol-free chloroform where it was allowed to fumigate for 24 hours. Additionally, both sets of extracts were filtered using Whatman #1 filter paper and diluted at a 1:10 ratio prior to TOC analysis. The diluted extracts were analyzed using a Shimadzu TOC-Vcpn total organic carbon analyzer with a total N module (Shimadzu Scientific Instruments Inc., Columbia, MD, USA). Microbial biomass carbon and nitrogen were determined by subtracting DOC and TN in the unfumigated samples from the DOC and TN in the fumigated samples. Organic C and N (DOC and DON) are reported as µg per gram dry soil.

Litter Mass Lost

Following removal from the mesocosms, litter bags were placed in a drying oven at 70°C for 24 hours. Soil that had adhered to the bags was carefully removed before the mass of the litter was determined.

Statistical Analyses

All analyses were performed using R (R Core Team, 2016) using the 'nlme' package (Pinheiro et al., 2017) unless otherwise mentioned.

General linear models (GLMs) with Wald's *F*-tests were used to analyze the main effects of detritivore species biomass and temperature treatment, as well as interaction effects between detritivore species biomass, and interactions between detritivore species biomass and temperature treatment on microbial enzyme activity (β-glucosidase (BG) and N-acetylglucosaminidase (NAG)), total microbial biomass (C and N), and litter mass lost. A paired t-test was used to analyze the effect of temperature treatment on percent change in biomass for each species as well as overall change in biomass across species. Species biomass was pooled if a mesocosm contained two or more individuals of the same species.

Longitudinal linear mixed effect models (LME) using Wald's Type II Chi-square tests were used to analyze the main effects of detritivore species biomass and temperature treatment, as well as interactions effects between detritivore species biomass, and interactions between detritivore species biomass and temperature treatment on carbon (CO_2) efflux and N (NO_3^- and NH_4^+) leached. Carbon efflux data were standardized by dividing efflux measurements by the total mass of the soil (kg) due to slight discrepancies in soil mass (± 2.5% on average).

Detritivore species biomass and temperature treatment were included in models as continuous variables and a categorical variable respectively. While real-time temperature data were collected using I-Buttons, temperature was treated as a categorical variable during analyses as the original objective was to assess detritivore species effects and interspecific interaction effects between two treatments, ambient and warmed. I-Button data were primarily used to monitor that the temperature treatments were acting as intended. When interactions between detritivore biomass and temperature treatment were detected, we performed post hoc tests using least square means and trends with the R package "lsmeans" (Lenth & Hervé, 2015) to assess significant differences in slope by temperature treatment. Optimal models for both general linear models and mixed effect models were selected using Akaike Information Criterion (AIC). Full models, which included all explanatory variables and possible interactions between explanatory variables, were selectively reduced using the 'step' function beginning with the highest-order interactions. Anovas were then used to compare the fit of newly reduced models to previous models until a model that best explained the data was obtained. In this case, the models with the lowest AIC scores were selected. For the mixed effect models, random effects structures were determined first. In line with the experimental design, mesocosm nested within block was used as the random effect and an auto-regressive correlation structure was used to account for violations of independence of weekly repeated measures in the case of efflux measurements and date in the case of leached NO_3^- and NH_4^+.

Volumetric water content was included in each abovementioned model as a covariate prior to AIC model selection as it was found to negatively correlate with temperature. Collinearity between predictor variables can inflate β in multiple regression models, so the variance inflation factor (VIF) for each variable in each model was assessed using the 'vif' function in the 'car' package in R (Fox et al., 2016). In this case, neither volumetric water content nor any of the other variables were found to significantly inflate the values found for β in the models reported here.

Mean effect sizes (%) for each response variable were calculated by averaging the percentage change in the response predicted by the models at each level of detritivore biomass (g) indicated in the Simplex design. For detritivore interactions with temperature, the response per gram change in detritivore biomass was calculated for each treatment.

The models and p-values indicated by the models were used to estimate mean and standard errors. Models were checked for collinearity between predictor variables, and outliers. An outlier was removed if and only if the data point was determined to be obviously incorrectly measured. Additionally, all models were plotted and residuals were visually inspected to ensure that assumptions of normality and homogeneity were met.

RESULTS

All results are reported considering an alpha of 0.05 for significant observations and an alpha of 0.1 for marginally significant observations as these may also be considered ecologically relevant.

Total detritivore biomass decreased significantly over the duration of the experiment under both temperature treatments (Ambient (A): t = 7.464, df = 16, $p < 0.001$; Elevated (E): t = 7.385, df = 16, $p < 0.001$). Total *N. americanus* biomass did not significantly change under either temperature treatment (A: t = 1.2052, df = 11, $p = 0.253$; E: t = 0.177, df = 11, $p = 0.863$) (Figure 5). Change in biomass was significant under ambient and elevated temperature for both *L. rubellus* (A: t = 4.430, df = 11, $p = 0.001$; E: t = 5.957, df = 11, $p < 0.001$) and *L. terrestris* (A: t = 3.952, df = 11, $p = 0.002$; E: t = 3.959, df = 11, $p = 0.002$) (Figure 5). Additionally, mean detritivore mortality was greatest under ambient temperature for all species tested (Figure 6).

The models predicted a significant interaction between biomass of *N. americanus* and *L. rubellus* on litter mass lost (Table 1, Figure 7), as well as a marginal interaction between *N. americanus* and temperature on litter mass lost (Table 2, Figure 8).

The co-occurrence of *N. americanus* and *L. rubellus* biomass predicted a significant increase in litter mass lost (mean effect size: +1.9% litter mass lost per g of *L. rubellus* and *N. americanus* in parallel) (GLM: $F_{1,26} = 4.37$, $p = 0.046$). Temperature treatment marginally affected *N. americanus* biomass effects on litter mass lost (GLM: $F_{1,26} = 1.04$, $p = 0.071$). In warmed treatments, *N. americanus* biomass predicted a reduction in litter mass lost (mean effect size: -4.5% per g *N. americanus*), while positive effects were noted in the ambient treatment (mean effect size: +2.8% per g *N. americanus*) (t = 3.31, df = 37, p = 0.002) (Figure 8).

L. rubellus biomass marginally decreased microbial biomass carbon (C) (mean effect size: -1.7% microbial C per g *L. rubellus*) (GLM: $F_{1,26} = 3.78$, $p = 0.06$) (Table 1, Figure 9). Comparatively, the interaction between *L. rubellus* and *N. americanus* marginally increased microbial biomass C (mean effect size: +0.3% microbial C per g of *L. rubellus* and *N. americanus* in parallel) ($F_{1,26} = 3.90$, $p = 0.06$) (Table 1, Figure 7). However, it should be noted that an adjusted R^2 of 0 might indicate that the data did not fit the model well. *L. rubellus* biomass also marginally decreased microbial biomass nitrogen (N) (mean effect size: -3.9% microbial N per g *L. rubellus*) (GLM: $F_{1,36} = 3.03$, $p = 0.09$) (Table 1, Figure 9).

N. americanus significantly reduced NAG activity across temperature treatments (mean effect size: -5.3% NAG activity per g *N. americanus*) (GLM: $F_{1,24} = 4.61$, $p = 0.04$) (Table 1, Figure 10). Additionally, the interaction between the two earthworms (*L. terrestris* and *L. rubellus*) marginally reduced NAG activity across temperature treatments (mean effect size: -1.2% NAG activity per g of *L. rubellus* and *L. terrestris* in parallel) (GLM: $F_{1,24} = 4.24$, $p = 0.05$) (Table 1, Figure 7). Furthermore, a significant interaction between *L. terrestris* and temperature was also detected (GLM: $F_{1,24} = 4.75$, $p = 0.039$). Post-hoc least square trends indicated a decrease in NAG activity in warmed treatments with increasing *L. terrestris* biomass (mean effect size: -4% per g *L. terrestris*) and an increase in NAG activity in ambient treatments with increasing *L. terrestris* biomass (mean effect size: +6.2% per g *L. terrestris*) ($t = 3.27$, df = 37, $p = 0.002$) (Table 2, Figure 11).

N. americanus marginally decreased BG activity across temperature treatments (mean effect size: -1% BG activity per g *N. americanus*) (GLM: $F_{1,33} = 3.49$, $p = 0.07$) (Table 1, Figure 10). Additionally, a significant interaction (GLM: $F_{1,33} = 6.16$, $p = 0.02$) between *L. terrestris* biomass and temperature treatment indicated a decrease in BG activity in warmed treatments with increasing *L. terrestris* biomass (mean effect size: -2.8% per g *L. terrestris*) and an increase in BG activity in ambient treatments with increasing *L. terrestris* biomass (mean effect size: +1.5% per g *L. terrestris*) ($t = 2.13$, df = 37, $p = 0.04$) (Table 2, Figure 11).

Mixed effect models detected no discernible effects on nitrate (NO_3^-) leached from mesocosms for any treatments. However, *L. terrestris* biomass predicted a significant increase in the amount of ammonium (NH_4^+) leached (mean effect size +16.1% NH_4^+ leached per g *L. terrestris*) (LME: $X^2 = 7.31$, $p = 0.01$) (Table 1, Figure 12).

There were no significant detritivore effects on carbon (CO_2) efflux. Rather, the best fit models indicated temperature as the significant predictor of efflux (LME: $X^2 = 6.62$, $p = 0.01$).

DISCUSSION

This study was driven by three questions: (1) Is there evidence of competition or complementarity between a native detritivore and two non-native detritivores? (2) Can detritivore species-specific effects and interspecific interaction effects on microbial biomass and activity (enzyme activity, respiration) be moderated by temperature? (3) How are soil processes (such as decomposition and leaching) affected by detritivore species identity, interspecific interactions, and detritivore interactions with temperature?

We detected species-specific effects, interspecific interaction effects, and effects of species interactions with temperature. The interaction between *N. americanus* and *L. rubellus* increased both litter mass lost and microbial biomass C, which could suggest a complementary relationship between a native and non-native detritivore. Furthermore, biomass of *N. americanus* increased over the duration of the experiment, whereas biomass of both *L rubellus* and *L. terrestris* decreased over the course of the experiment, which could hint at some degree of biotic resistance against earthworm invasion by *N. americanus*. Temperature moderated the magnitude and the direction of species-specific effects on microbial and soil process variables. However, temperature did not alter interspecific interaction effects. Overall, the results here provide a framework to further probe into the mechanisms underlying the observed effects of species and temperature treatment interactions on microbes and soil process rates.

Biomass declined significantly over the duration of the experiment and under both temperature treatments for the two earthworms, *L. rubellus* and *L. terrestris*, which was unexpected (Figure 5). Mortality was also high for both species, but more so under ambient temperature, which is congruent with previous studies that indicate increased earthworm fitness under warmer temperatures (Eisenhauer et al., 2014; Perreault & Whalen, 2006; Uvarov et al.,

2011) (Figure 6). Habitat sensitivity to moisture and particle size has been documented in the field for both species (Crumsey et al., 2014), and we suspect that the biomass decline and high mortality observed under both temperature treatments was driven by sensitivity to the low moisture, high sand content of the mesocosm soil (Crumsey et al., 2014). *L. rubellus* and *L. terrestris* exhibit moisture sensitive integuments (Edwards, 2004; Holmstrup, 2001), and considering the high survival and biomass increase observed for the surface dwelling *N. americanus*, it seems likely that the earthworms were more strongly affected by physical soil characteristics. Prior studies assessing interactions between peregrine earthworms and native millipedes have noted some degree of competitive exclusion in favor of earthworms (Chang et al., 2016; Snyder et al., 2009). Despite this, the results here indicate that *N. americanus* may effectively compete with invasive earthworms and exhibit some biotic resistance to earthworm invasion if local soil characteristics do negatively affect the fitness of *L. rubellus* and *L. terrestris.* The results of this study contrast with a previous study that noted decreased millipede fitness in the presence of invasive earthworms (Snyder et al., 2009; Snyder et al., 2011). While not a significant result, *N. americanus* individuals seemed to gain more biomass in the elevated temperature treatment, which is consistent with previous studies that indicate increased millipede fitness under slightly warmer temperatures (+ 2.5–5°C) (David & Handa, 2010).

An interaction between *N. americanus* and *L. rubellus* predicted a significant increase in litter mass lost (Table 1) (Figure 7), indicating a potential complementarity effect between these two species. Complementarity effects are observed when two interacting species exert non-additive effects on metrics of ecosystem processes (i.e., litter mass lost). Complementary relationships are often discussed in plant diversity literature (Cardinale et al., 2007; Loreau & Hector, 2001), but are receiving greater attention in regard to detritivore communities

(Hättenschwiler & Gasser, 2005; Heemsbergen et al., 2004; Kitz et al., 2015; De Oliveira et al., 2010; Zimmer et al., 2005). Detritivores can exert complementarity effects via niche differentiation or facilitation mechanisms, but these mechanisms can be difficult to separate and may even act simultaneously (De Oliveira et al., 2010). Nevertheless, we suspect that the synergistic effects on litter mass lost between *L. rubellus* and *N. americanus* can be primarily explained by niche differentiation and to a lesser extent, facilitation.

Macrofauna effects on litter decomposition are, in part, dictated by litter quality, and therefore the species of litter present (Hättenschwiler & Gasser, 2005). *L. rubellus* is a generalist species and will typically feed on high-quality litter (low C:N) when available (Curry & Schmidt, 2007; Maraun et al., 2003)). However, *L. rubellus* has also been observed to oscillate between a litter and soil-based diet when soil organic matter is high (Curry & Schmidt, 2007). *N. americanus* is also a generalist species (O'Neill, 1968), but much less is known about its specific food preferences. *N. americanus* has been observed consuming wood in the field, and may therefore prefer lower-quality litter material (high C:N, defensive compounds) (O'Neill, 1969). Previous field studies have noted that earthworms enhance the decomposition rate of high-quality litter and millipedes enhance the decomposition rate of low-quality litter (Hättenschwiler & Gasser, 2005). Furthermore, the complementary effect of the detritivores on decomposition in the study by Hättenschwiler and Gasser (2005) became more pronounced as the number of litter species present exceeded six.

In this case, divergence in food preferences, nutrient requirements, and mouthpart morphology between *N. americanus* and *L. rubellus* may explain the enhanced complementary effects on decomposition and allude to the possibility of coexistence between a native and invasive detritivore. This result may also be driven by differences in preference for litter at varying stages of decay as has been observed in other studies of functional trait diversity between detritivores (De Oliveira et al., 2010). However, niche differentiation can also operate through indirect mechanisms. Previous studies have noted that millipedes will selectively consume fungi when it is available, as fungi can be a more palatable and nutrient-rich food source due to generally lower C:N in hyphae (Crowther et al., 2015; Maraun et al., 2003). Furthermore, comminution of fungi can alter fungal nutrient allocation and microbial enzyme activity, which can affect litter decomposition rate.

Another possible explanation for the observed complementarity effect is a facilitation mechanism. Facilitation in decomposer systems can moderated by indirect interactions between soil fauna and microbial communities (Hale, Frelich, and Reich, 2005). For example, invasive earthworm activity is known to facilitate the establishment of non-native plant species (Hale et al., 2006) and increase the ratio of bacteria to fungi in upper soil horizons (Dempsey et al., 2011). In this case, the movement and foraging of *L. rubellus* near the surface of mesocosms could have reduced hyphal growth of saprophagous fungi (Bonkowski, Griffiths, and Ritz, 2000) and therefore influenced *N. americanus* to shift towards a litter-based diet. This dietary shift could at least partially explain the non-additive effects on litter decomposition. This interpretation also lends support to the compensatory feeding hypothesis, which asserts that species will consume larger quantities of poor-quality food sources in order to maintain nutrient stoichiometry and meet metabolic requirements (Catalan, Tamara, and Lardies, 2008; Gessner et al., 2010; Ott et al., 2012). *N. americanus*, in the presence of *L. rubellus*, may have been forced to adopt a diet of poorer-quality materials and therefore had to consume a higher quantity of litter detritus to account for metabolic and nutritional demand. Our findings do not elucidate the mechanisms that are driving the observed responses, but may hint at patterns worth teasing apart.

Future studies may consider explicit manipulations of food sources for these two species (*N. americanus* in particular) to elucidate where the discrepancies lie in terms of resource partitioning.

Interestingly, there was also a significant response of litter mass lost as a result of the interaction between *N. americanus* and temperature treatment (Table 2) (Figure 8). Litter mass lost decreased as millipede biomass increased under warmed temperature, which was unexpected, but could be the result of desiccation avoidance (O'Neill, 1969). Previous studies have noted that millipede litter consumption rate is directly related to millipede size (Bonkowski, Scheu, and Schaefer, 1998). Here, litter mass lost decreased with increasing millipede biomass in the warmed treatments, which may indicate that temperature induced a physiological stress response that disproportionately affected larger *N. americanus* individuals. Volumetric water content was significantly lower in the warmed treatments, and because millipedes lack a waxy cuticle and are therefore vulnerable to water loss, larger *N. americanus* individuals may have burrowed down towards cooler, higher-moisture soil, in an effort to evade metabolic stress (Bailey & Kovaliski, 1993). Millipedes were also observed molting over the course of the experiment, and considering that molting can be an adaptive response to dry conditions (O'Neill, 1969), it is possible that *N. americanus* individuals spent more time in an inactive molting state and not consuming litter in the warmed treatments. There was also a positive, yet non-significant, trend of increasing litter mass lost in the ambient temperature treatment (Figure 8), which may further support the notion that fluctuations of soil temperature and moisture are more problematic for mature, high biomass millipedes.

The litter used in the decomposition bags was representative of the diversity of dominant hardwood tree species in the region. Therefore, the result found here may be more generalizable in terms of complementarity effects between *N. americanus* and *L. rubellus* on decomposition at a larger scale. However, this interpretation should be approached with caution, as other environmental variables, such as moisture (Collison et al., 2013; Coulis et al., 2015; Wall et al., 2008), may influence detritivore-mediated effects on decomposition rate. Further experiments that manipulate abiotic and biotic variables on communities of detritivores that share similar functional traits will be valuable in bettering our understanding of the underlying mechanisms driving temperature effects on detritivore-mediated decomposition.

In addition to the positive interaction effects on litter mass lost, there was a positive interaction effect between *L. rubellus* and *N. americanus* biomass on microbial biomass C (Table 1) (Figure 7). While the underlying mechanism behind this observation remains unclear, it is possible that the increase in microbial C is tied to how these species affect N-inputs to the soil as microbial biomass C acquisition and retention may be constrained by N-limitation (Schimel & Weintraub, 2003). Previous mesocosm studies have noted that millipede frass can significantly increase N mineralization (Anderson & Ineson, 1984; Cárcamo et al., 2000; Makoto et al., 2014), but the opposite has also been observed (Kaneko, 1999). Furthermore, N mineralization can be dependent upon millipede developmental stage (Toyota & Kaneko, 2012) and biomass (Anderson & Ineson, 1984; Bonkowski et al., 1998), with 10 to 15 times greater effects of N mineralization observed in mesocosms that contain high millipede densities (Bonkowski et al., 1998). *L. rubellus* may assist in mixing fecal pellets and other N-rich millipede waste into surface soils, which might release microbial constraints on C acquisition. However, the leachate chemistry indicates that the mesocosms were generally not N-limited, which suggests that the positive interaction between *L. rubellus* and *N. americanus* on microbial biomass C may not operate indirectly through N-pathways.

Millipedes tend to have low assimilation efficiencies, so millipede fecal pellets are often rich in C that is protected in deposited fecal aggregates (Toyota et al., 2006). Furthermore, when millipede density is great enough, fecal pellet deposition can enhance microbial biomass and carbon stocks (Toyota et al., 2006). A previous study noted that invasive earthworms can further lessen native millipede assimilation efficiency by outcompeting millipedes for high quality resources (Snyder et al., 2009). Additionally, earthworms have been observed feeding on millipede feces (Bonkowski et al., 1998). While not directly observed here, earthworm consumption and fragmentation of millipede feces in the mesocosms may have released the carbon locked in fecal pellet aggregates and facilitated increases in microbial biomass C. Furthermore, in mesocosms containing both *L. rubellus* and *N. americanus,* fecal pellets may have been richer in C due to *L. rubellus* outcompeting *N. americanus* for higher quality food sources. We did not directly measure fecal pellet chemistry here, but this information may lend further support to the compensatory feeding hypothesis in the case of *N. americanus*, and the interaction between *L. rubellus* and *N. americanus* on litter mass lost. Despite this, decreases in microbial C due to the interaction between an invasive earthworm species and a native millipede have also been detected (Snyder et al., 2009), but when considering the accumulating evidence that macroarthropods and earthworms exert species-specific effects on soil processes, these results are not generalizable.

Our models also predicted that increasing *L. rubellus* biomass in mesocosms marginally decreased both microbial biomass C and N (Table 1) (Figure 9). Previous studies on the effects of non-native earthworms on microbial biomass in forest systems have yielded idiosyncratic results, with some studies reporting increases in overall microbial biomass (Groffman et al., 2004; Li et al., 2002), and others reporting decreases (Eisenhauer et al., 2011; McLean et al., 2006). These studies collectively suggest that the magnitude and direction of earthworm effects on microbial biomass are complex, and may be mediated by species-specific effects (including species abundance) as well as soil characteristics, specifically C availability in bulk soil, and the component of microbial biomass being measured (McLean et al., 2006).

Hendrix et al. (1998) noted that microbial N was reduced in surface and mineral soil in the presence of *L. rubellus*, but more so at the surface in sandy, coarse-textured soil as opposed to clayey, fine-textured soil, consistent with the findings of this study. *L. rubellus* tends to accelerate microbial N turnover in soils (Hendrix et al., 1998), and the rate of turnover is exacerbated is sandy soils. Documented effects of *L. rubellus* on microbial biomass C are similarly idiosyncratic. Zhang and Hendrix (1995) also noted a decrease in microbial C in addition to the decrease in microbial N in the presence of *L. rubellus*. They attributed this result to a possible disruption of hyphal connections between soil and litter due to the distinctive, epigeic bioturbation effects of *L. rubellus* in the upper soil horizons, which were directly observed in the surface soil of our mesocosms. Hyphae reduction could also be a result of direct consumption as a result of selective foraging (Curry & Schmidt, 2007), and could potentially impede C immobilization and therefore microbial biomass. *L. rubellus* has also been known to increase soil consumption rates when there is a dearth of adequate soil organic matter, which is a characteristic of sandy soils (Bruce & Langdale, 1997). *L. rubellus* biomass did not significantly predict litter mass lost in our models, which may suggest that they were consuming more soil, and potentially microbial food sources, in our mesocosms.

N. americanus significantly depressed enzyme activity (BG and NAG) (Figure 10), which ran counter to our initial hypothesis. Because millipede exoskeletons represent a significant source of chitin (Cabib, 1987), it was expected that millipede molting behavior (observed during the experiment) might increase NAG activity due to an increase of substrate availability. However, a suite of enzymes is involved in microbial N-acquisition (Sinsabaugh, 2005), and could have been more influential predictors of microbial N-demand in this circumstance, but these enzymes were not measured.

The decreases in enzyme activity might be explained by reduced substrate availability (German, Chacon, and Allison, 2011). In a similar manner to earthworm casts, millipede fecal pellets aggregates can protect C from microbial decomposition (Toyota et al., 2006). Previous studies indicate that detritivore-mediated effects on enzyme activity also depend on the food source utilized by litter feeding organisms (Flegel & Schrader, 2000). Oak, a relatively low quality litter with high C:N, was the dominant litter type in the litter bags used in this experiment. Therefore, millipede consumption of the oak-rich litter mixture and subsequent deposition of fecal pellets may have increased overall C recalcitrance in the mesocosms. However, aside from substrate availability, microbial demand for simple forms of C and nutrients dictate enzyme activity (Allison & Vitousek, 2005). The observed decreases in both BG and NAG activity may indicate that *N. americanus* increased C and N mineralization in mesocosms, and subsequently facilitated C and N uptake and immobilization by microbial communities, which might explain the lack of enzyme production. Nevertheless, the results of this study stand in contrast to recent literature which reports increasing enzyme activity in response to increasing millipede density (da Silva et al., 2017).

An alternate explanation for the reduction in enzyme activity in the presence of *N. americanus* might involve the resource use strategies of millipedes. Generally, studies on invertebrate-microbe functional interactions suggest that fungal grazing increases specific enzyme activity, and can ultimately stimulate organic matter decomposition (Crowther et al., 2011a; David, 2014). One previous study found that invertebrate foraging strategy and preferences, presumably driven by functional morphology, moderated enzymatic response to microbial grazing (Crowther et al., 2011). Notably, millipedes in the study by Crowther et al. (2011) significantly reduced NAG activity through selective foraging, which may suggest that *N. americanus* selectively feeds on chitinous substrate found in bacteria and fungi, and processes chitin into more available forms on N during gut passage independent of microbes. Contrarily, Crowther et al. (2011) also reported increases in BG activity in response to millipede grazing. Damage to fungal hyphae may promote or retard production of enzymes depending on the functional traits of both the grazers and the fungal species (Crowther et al., 2011). We did not explicitly measure microbial feeding in this study, but future studies might consider manipulations of both fungi and detritivore species to reveal the underlying mechanisms behind microbial activity and grazing pressure.

A combination of high *L. rubellus* and *L. terrestris* biomass marginally depressed NAG activity (Figure 7). This result contrasts with previous studies that have noted increases in enzyme activity associated with earthworm presence. However, our findings may be attributed to the biotic and abiotic effects of earthworms in soil. Generally, earthworms disrupt hyphal connections through burrowing and consumptive effects (Curry & Schmidt, 2007), resulting in a decrease in the ratio of fungi to bacteria (Dempsey, Fisk, and Fahey, 2011). Burrowing activity can cause lysing of microbial cells and therefore may alter microbial allocation towards enzyme production. Previous studies have noted the presence of NAG activity in the guts of some earthworms (Zhang et al., 1993), which could suggest that earthworms contribute to chitin decomposition processes to simple forms of N independent of microbial communities. Therefore, species-specific discrepancies in gut enzyme concentrations and activity may be useful for predicting species-specific and interspecific interaction effects of earthworms and other detritivores on microbial enzyme production (Lattaud et al., 1998). Food source also affects enzyme activity in earthworm casts (Flegel & Schrader, 2000), so investigating interactions between invasive worms and litter type may also be useful in understanding earthworm impacts on microbial functioning in North American forests.

Another explanation for the interactive effect between *L. rubellus* and *L. terrestris* may come from their combined effects on soil moisture. Soil moisture and temperature were negatively correlated in this study, and soil enzyme activities are highly sensitive to changes in temperature and moisture (Allison & Treseder, 2008). The activities of earthworms can directly affect soil moisture, and earthworms themselves are sensitive to changes in temperature and moisture (Ernst et al., 2009; Perreault & Whalen, 2006). Specifically, *L. rubellus* tends to burrow near the surface and under litter, which maintains soil moisture (Ernst et al., 2009). On the other hand, by creating deep vertical burrows, *L. terrestris* individuals enhance aeration and surface area susceptible to evaporation losses (Ernst et al., 2009). The litter bags used in this experiment did not cover the entirety of the surface soil, and because the soil is sandy and therefore more susceptible to water loss, the combined bioturbative effects of *L. rubellus* and *L. terrestris* may have exacerbated drying at the soil surface by increasing available surface area for evaporation. Previous mesocosm studies show that *L. terrestris* individuals burrow more deeply and explore a greater volume of soil in drier conditions, which creates a positive feedback on soil moisture loss (Perreault & Whalen, 2006). This result may be supported by personal observations during mesocosm harvest, as larger *L. terrestris* individuals were found inactive towards the bottom of the buckets where moisture had collected. Organisms at larger biomass tend to have greater effects in soils (Turnbull et al., 2014), and the combination of these two earthworms and their functional traits related to burrowing may have increased exposed soil surface area and therefore indirectly reduced NAG activity by decreasing moisture, as microbial growth and activity are generally enhanced by moisture availability (Paul, 2014; Xiang et al., 2008). Additionally, these results may corroborate a recent study which concluded that detritivore species identity and community composition have stronger effects when moisture is limiting (Collison et al., 2013). However, temperature and moisture have idiosyncratic effects on specific enzyme activities, including BG and NAG, as enzyme responses may be species-specific to certain fungi (A'Bear et al., 2014). Therefore, we encourage future studies to increase functional resolution to include fungal traits as well, as these may also play an important role in detritivore-mediated effects on microbial activity.

The interactive effect of temperature treatment and *L. terrestris* biomass on enzyme activity presumably operates through a similar mechanism. Both NAG and BG decreased with increasing *L. terrestris* biomass in the warmed temperature treatments, and increased in the ambient treatments (Table 2, Figure 11). Despite this observation, previous studies report increases in BG activity in *L. terrestris* invaded plots (Dempsey et al., 2013) and castings (Kızılkaya & Hepsen, 2004). There is also some evidence which indicates that NAG activity may double in *L. terrestris* burrows when compared with bulk soil (Hoang et al., 2016). Therefore, we suspect that earthworm burrowing traits, in response to temperature and moisture, are driving the observed results. Yet, despite the significance indicated by the enzyme results, the responses of the enzymes to detritivore activity were marginal overall and may suggest that the impact of detritivores on microbial activity is negligible compared to the influence of other variables (i.e. temperature, moisture).

Temperature was the primary driver of carbon (CO_2) efflux in the mesocosms, but other studies have noted contrasting effects of soil fauna on CO_2 respiration in warmed and unwarmed soils (Hedde et al., 2007; Jennings & Watmough, 2016; Maran & Pelini, 2016; Pelini et al., 2015; Snyder, Boots, and Hendrix, 2009; Speratti & Whalen, 2008). In this particular instance, the detritivores in the mesocosms may not have been at high enough densities to significantly affect microbial respiration. Indeed, previous studies have shown a linear relationship between detritivore density on microbial respiration, specifically regarding earthworms (Aira et al., 2008), and millipedes (Kaneko, 1999).

Similarly, no significant changes in nitrate (NO_3^-) were observed despite evidence of increased nitrate lost in the presence of millipedes and earthworms in prior studies (Martens et al., 2001). Millipedes alone were not expected to directly contribute to nitrate leaching as macroarthropods generally do not excrete nitrates as waste (Hopkin & Read, 1992). However, they may indirectly influence nitrate loss through enhanced ammonium (NH_4^+) additions to soil (Kaneko, 1999), and subsequent losses post-nitrification, but this result was not supported by our data. Earthworms, on the other hand, tend to increase nitrate loss through casting and burrowing activity (Bohlen et al., 2004; Bohlen & Edwards, 1995; Marhan et al., 2015; Sheehan et al., 2006), and this effect can be enhanced by temperature (Marhan et al., 2015). Despite these findings, other mesocosm studies have noted negligible or reduced species-specific and interaction effects on nitrate and ammonium leaching in earthworm-dominated communities (Andriuzzi et al., 2015; Butenshoen et al., 2009; Sheehan et al., 2006). Contrasting results from the literature indicates that earthworm effects on nitrogen mobilization are context dependent, and may rely on nitrogen sources as well as physical soil characteristics (Bohlen et al., 2004a).

The only significant effect detected in leachate analyses was the positive relationship between increasing *L. terrestris* biomass and amount of NH_4^+ leached, which supported our initial hypothesis (Table 1) (Figure 12). *L. terrestris* individuals are known to increase soil porosity and drainage due to their distinctive deep, vertical burrowing behavior (Edwards, 2004). This trait, in combination with the tendency of *L. terrestris* to pull surface litter deeper into soils where it can more closely interface with microbial activity, increases N movement and cycling (Bohlen & Edwards, 1995).

Previous mesocosm studies on *L. terrestris* effects on N cycling also report high levels of ammonium in leachate (Sheehan et al., 2006), consistent with our results (Figure 12). Furthermore, Sheehan et al. (2006) note that the increase in ammonium leached from their mesocosms was significantly greater at high biomass of *L. terrestris* and low food sources, which also coincides with the observations of this study. Accumulating evidence indicates that earthworm effects on N leaching depend on earthworm biomass and the quantity and quality of available food (Bohlen et al., 2004a; Costello & Lamberti, 2008; Sheehan et al., 2006), which may suggest that earthworm colonization of North American forests will become more problematic for forest N-retention over time. The increase in leached ammonium from the mesocosms may be attributed to increased competition between *L. terrestris* and microbes for resources, resulting in worms outcompeting microbes for N, and subsequently excreting more ammonium than can be nitrified by the reduced microbial community (Sheehan et al., 2006). Our results and previous studies suggest that, in forested areas mainly dominated by *L. terrestris* biomass at high densities, there is a greater likelihood that ammonium-derived N will be leached from the system faster than it can be nitrified. Moving forward, resolving the extent to which detritivore functional traits and interspecific interactions impact belowground nitrogen mobilization will be increasingly important, as these effects ultimately hold important consequences for forest productivity.

Conclusions and Future Considerations

The goal of this study was not to uncover mechanisms of species interactions *per se*, but rather to reveal species-specific effects and interaction effects between native and non-native detritivores under different warming scenarios. Species roles in ecosystems are moderated by functional traits, but many of these traits are flexible and change in response to environmental stress. Therefore, our understanding of detritivore effects on ecosystem processes will remain inadequate unless we can come to a better understanding of how functional trait flexibility is influenced by climate variables.

Here, we report species-specific effects and interaction effects between native and non-native detritivores on ecosystem processes. Furthermore, it appears temperature has a role in moderating species-specific effects, while the influence of temperature on interaction effects was not apparent. Future experiments focused on interactions between invertebrates and microbial communities might consider explicit manipulations of both detritivore and microbial traits as well as soil characteristics. These types of manipulations will be useful in clarifying and distinguishing between biotic effects of detritivores, and abiotic effects in terms of how specific species and interspecific interactions alter soil characteristics, such as soil moisture and pH. Additionally, including higher trophic levels into experimental manipulations will yield more realistic interpretations of ecosystem functioning, as predators do influence trait expression in prey species (Buchkowski, 2016; Schmitz et al., 2009; Schmitz et al., 2015).

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APPENDIX A: TABLES

Response Variable	Explanatory Variables	*P*
N-acetylglucosaminidase (NAG)	NA	0.042
	LT x LR	0.050
	LT x Temperature	0.039
β-glucosidase (BG)	NA	0.071
	LT x Temperature	0.018
Litter Mass Lost	NA x LR	0.047
	NA x Temperature	0.071
Microbial C (MBC)	LR	0.063
	NA x LR	0.059
Microbial N (MBN)	LR	0.090
Ammonium (NH_4^+) Leached	LT	0.007

Table 1. Summary of the models that best explained soil ecosystem process and microbial response variables, including associated p-values. An "x" denotes an interaction effect. NA, LR, and LT correspond to biomass *Narceus americanus*, *Lumbricus rubellus*, and *Lumbricus terrestris,* respectively.

Response Variable	Explanatory Variables	Trend (*β*)	*t (df = 37)*	*P*
N-acetylglucosaminidase (NAG)	LT x Temperature	Ambient: 2.253 Elevated: -1.297	3.268	0.002
β-glucosidase (BG)	LT x Temperature	Ambient: 1.371 Elevated: -2.525	2.133	0.040
Litter Mass Lost	NA x Temperature	Ambient: 0.093 Elevated: -0.150	3.306	0.002

Table 2. Summary of the least squared means comparison of lines between Ambient and Elevated temperature treatments and species interactions including *β* for each treatment, as well as the associated *t* and *p* values.

APPENDIX B: FIGURES

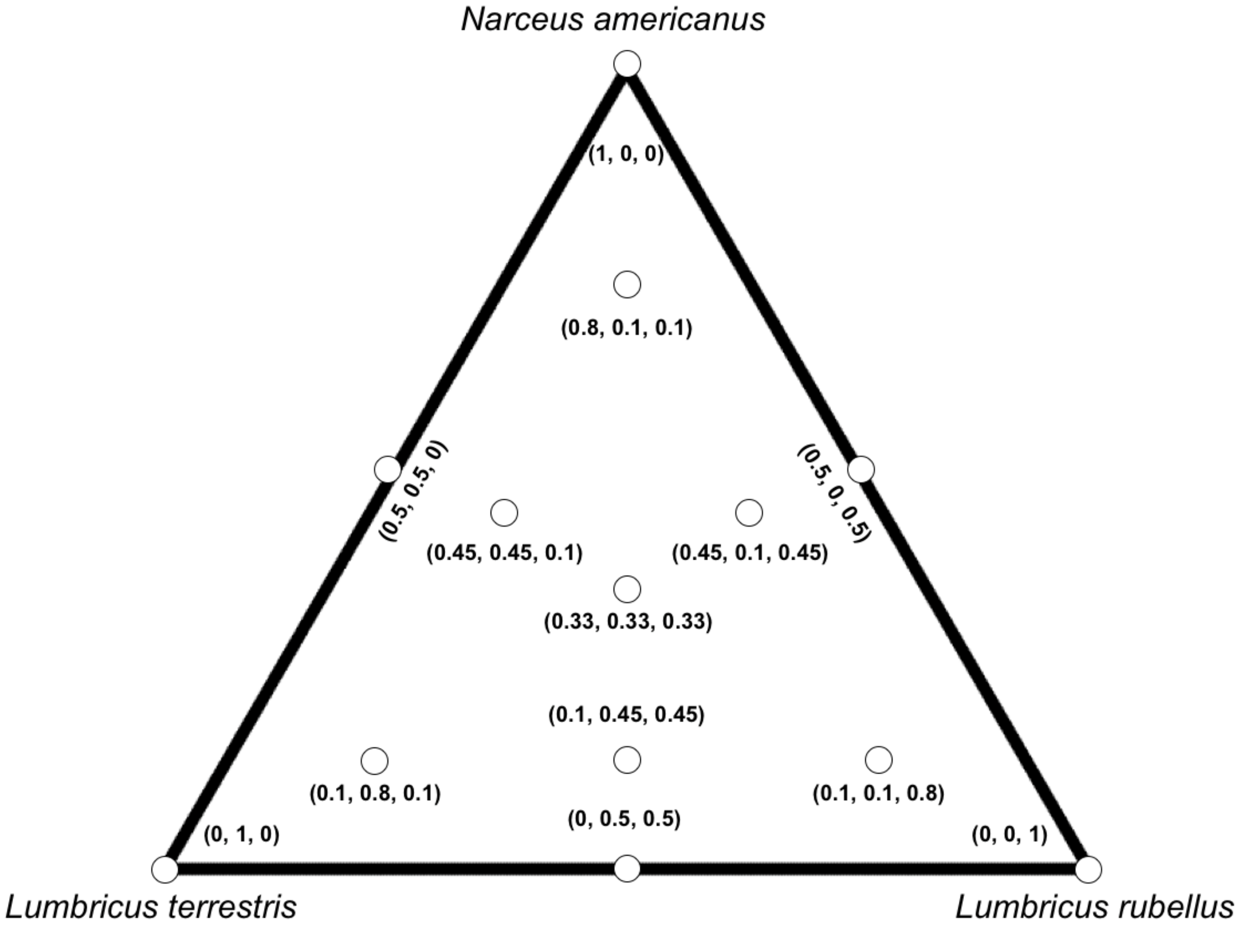

Figure 1. Simplex design used to dictate proportion of biomass represented in each mesocosm. Each corner of the triangle represents single-species monocultures, whereas the other points indicate multi-species proportions. For example, (0.5,0.5,0) indicates that 50% of community biomass will be represented by *L. terrestris*, 0% by *L. rubellus* and 50% by *N. americanus*.

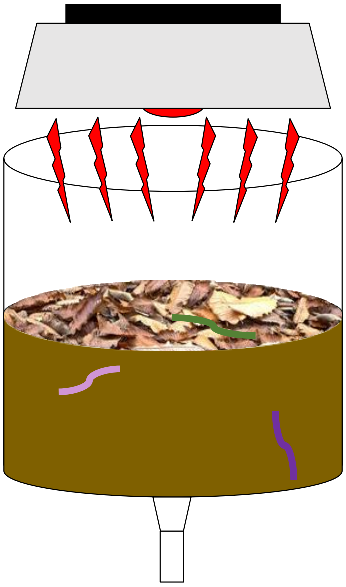

Figure 2. Diagram of mesocosm bucket design under heating treatment, colored lines denote species in a (0.33,0.33,0.33) centroid, collection bottle fixed underneath.

Figure 3. Photo of experimental setup showing warming treatments.

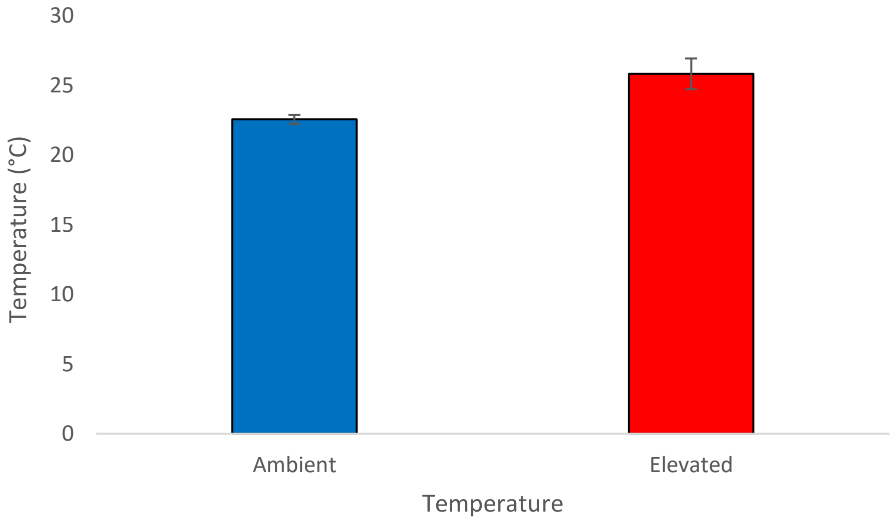

Figure 4. Mean temperature (± standard error) differences between treatments. Ambient mean was 22.6° C (± 0.07°), and elevated was 25.8° C (± 0.25°). These data exclude the stabilization period (four weeks, first week of May to the first week of June).

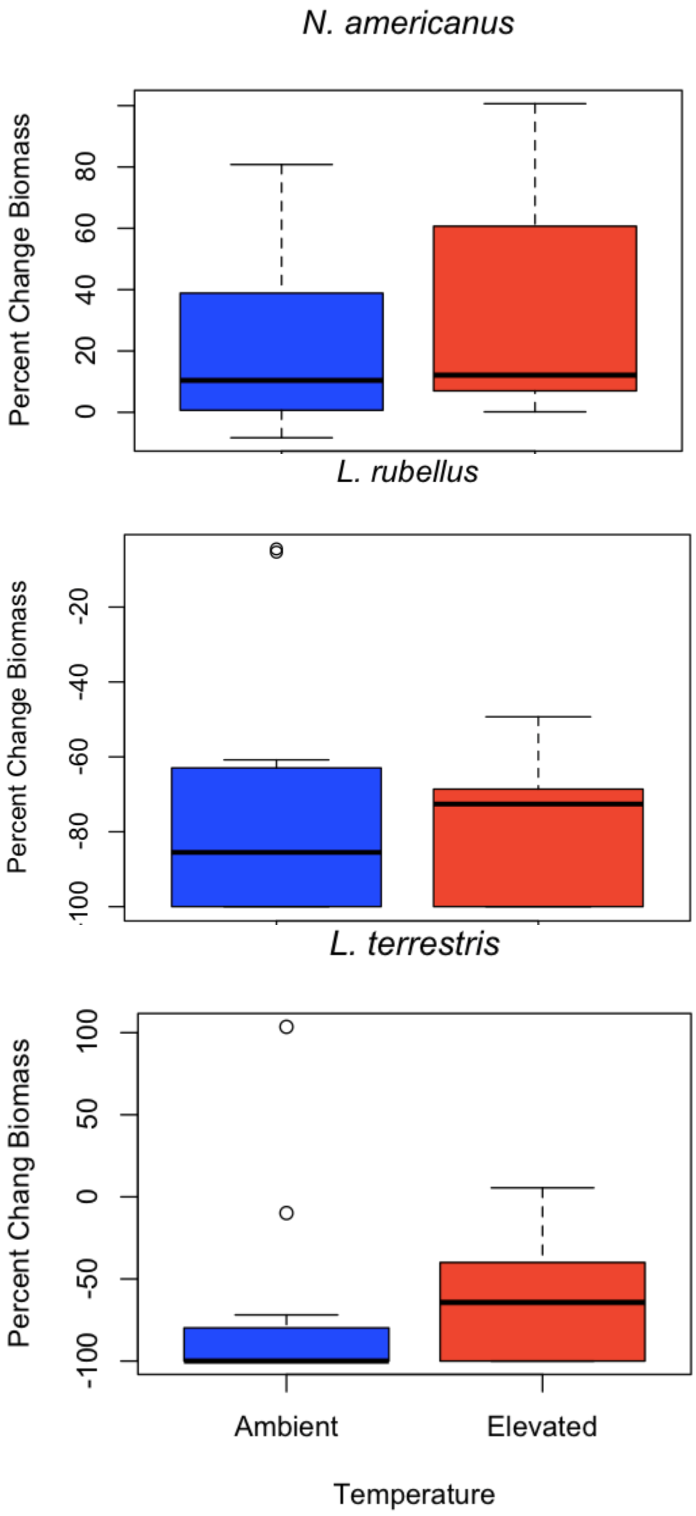

Figure 5. Percent change (%) in biomass (± standard error) between temperature treatments for each species: *N. americanus* (top), *L. rubellus* (middle), *L. terrestris* (bottom).

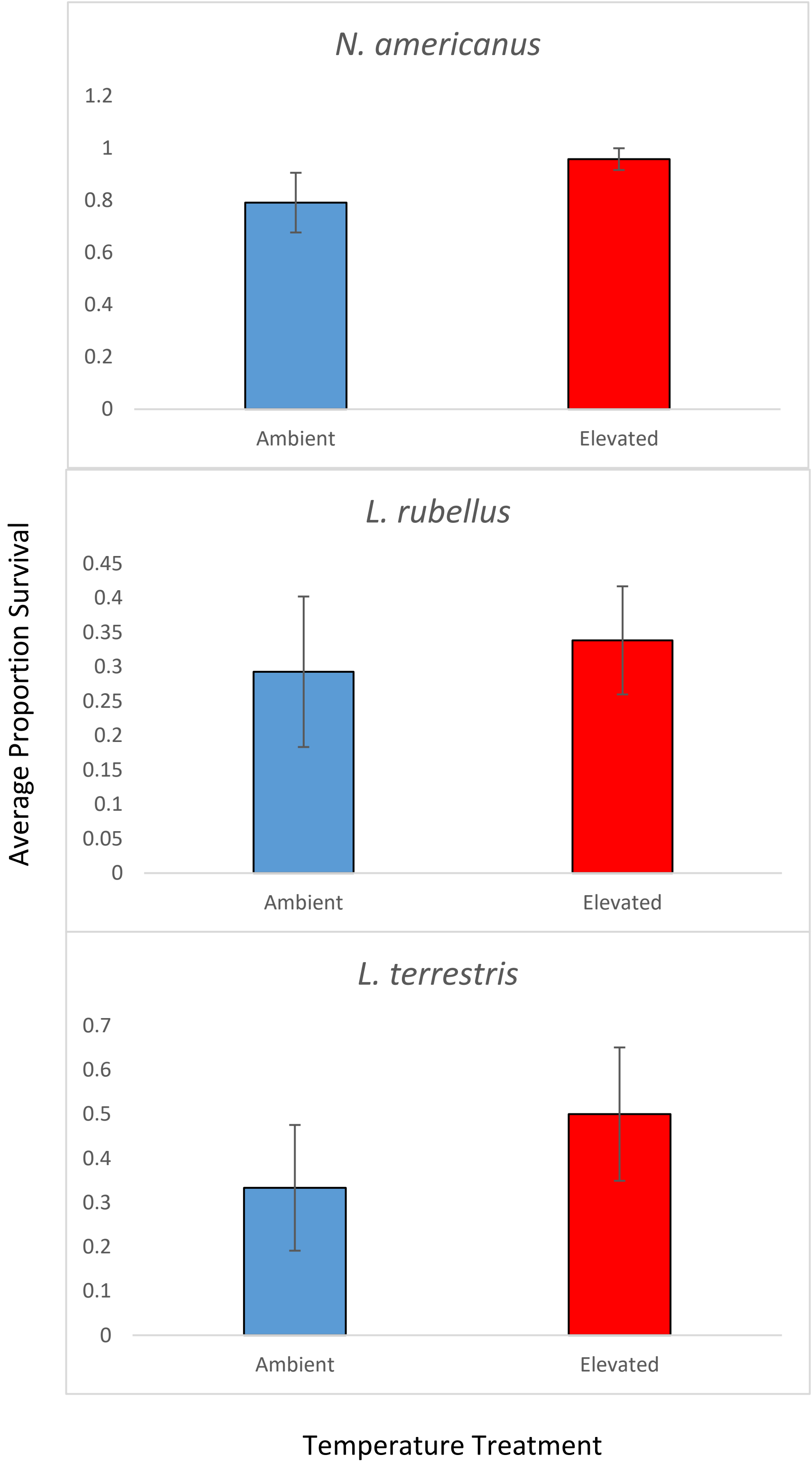

Figure 6. Mortality (expressed as proportion survival) (± SE) under ambient and elevated temperature treatments for each species: *N. americanus* (top), *L. rubellus* (middle), *L. terrestris* (bottom).

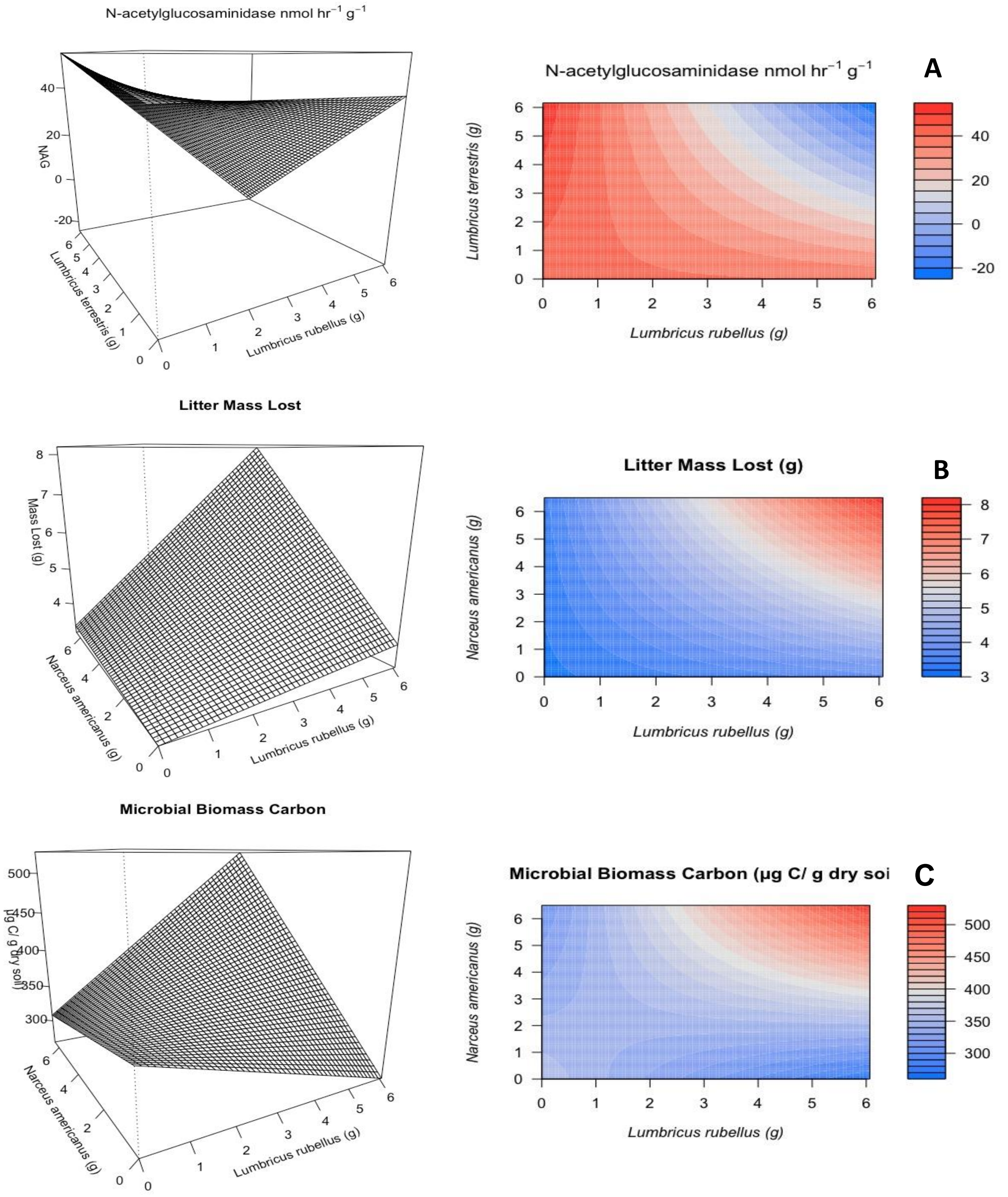

Figure 7. Two-way species interactions between detritivores and effects on ecosystem processes (Table 1). (A) Interaction effect between *L. rubellus* and *L. terrestris* biomass on NAG activity. (B) Interaction effect between *N. americanus* and *L. rubellus* biomass on litter mass lost. (C) Interaction effect between *N. americanus* and *L. rubellus* biomass on microbial biomass C.

Vertical axes of both perspective (left) and image (right) graphs represent z-values, or the response values predicted at biomass increments for both species. Perspective graphs were included to more clearly illustrate how detritivore biomass at each interval explains the response (z), whereas the image graphs were included to more clearly illustrate general trends predicted by the interactions.

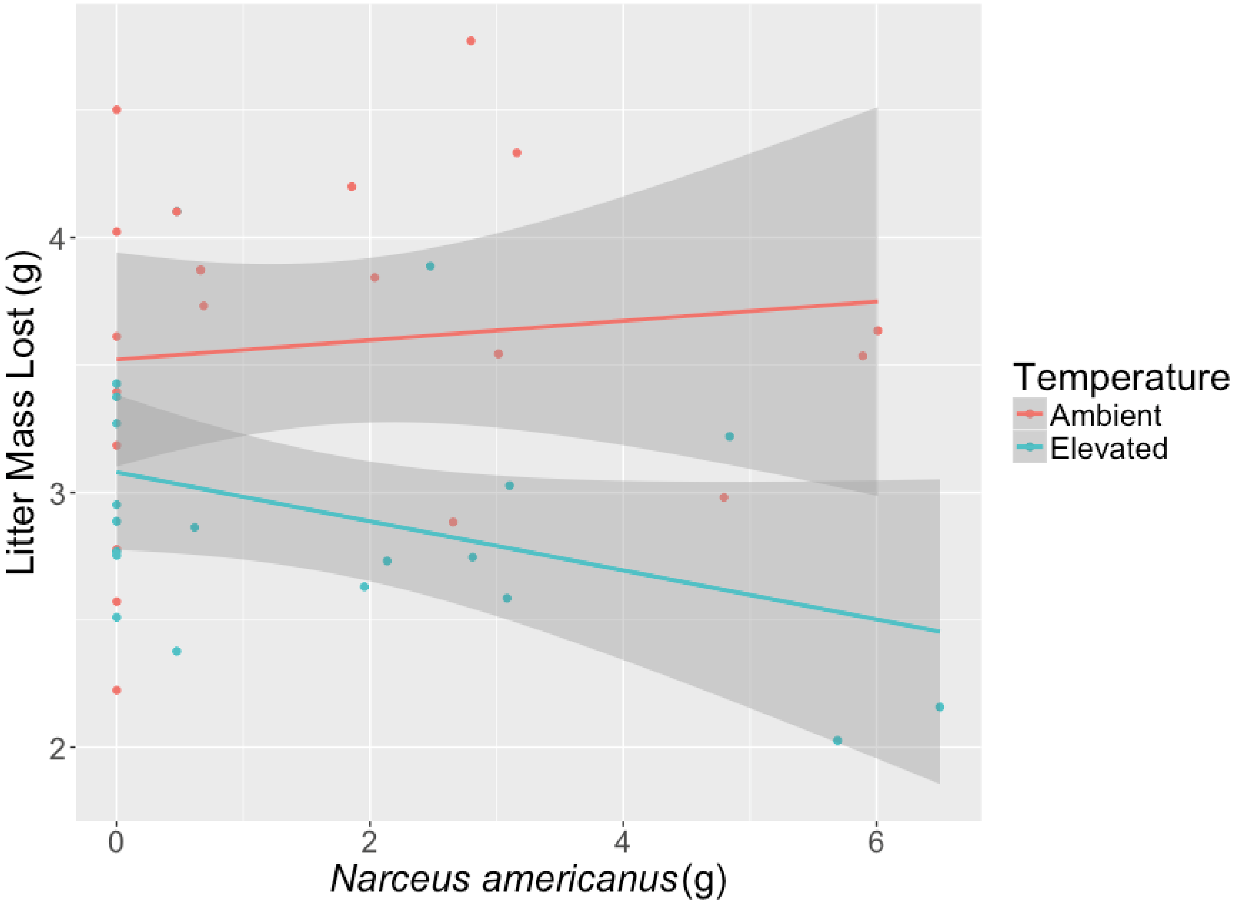

Figure 8. Graph illustrating an interaction effect between *N. americanus* biomass and temperature treatment ($F_{1,26} = 1.0446$, $p = 0.07147$) where litter mass lost was reduced with increasing biomass under warmed temperature and marginally increased with increasing biomass under ambient temperature ($t = 3.306$, $df = 37$, $p = 0.0021$). Gray shading indicates confidence intervals (95%) around the regression line.

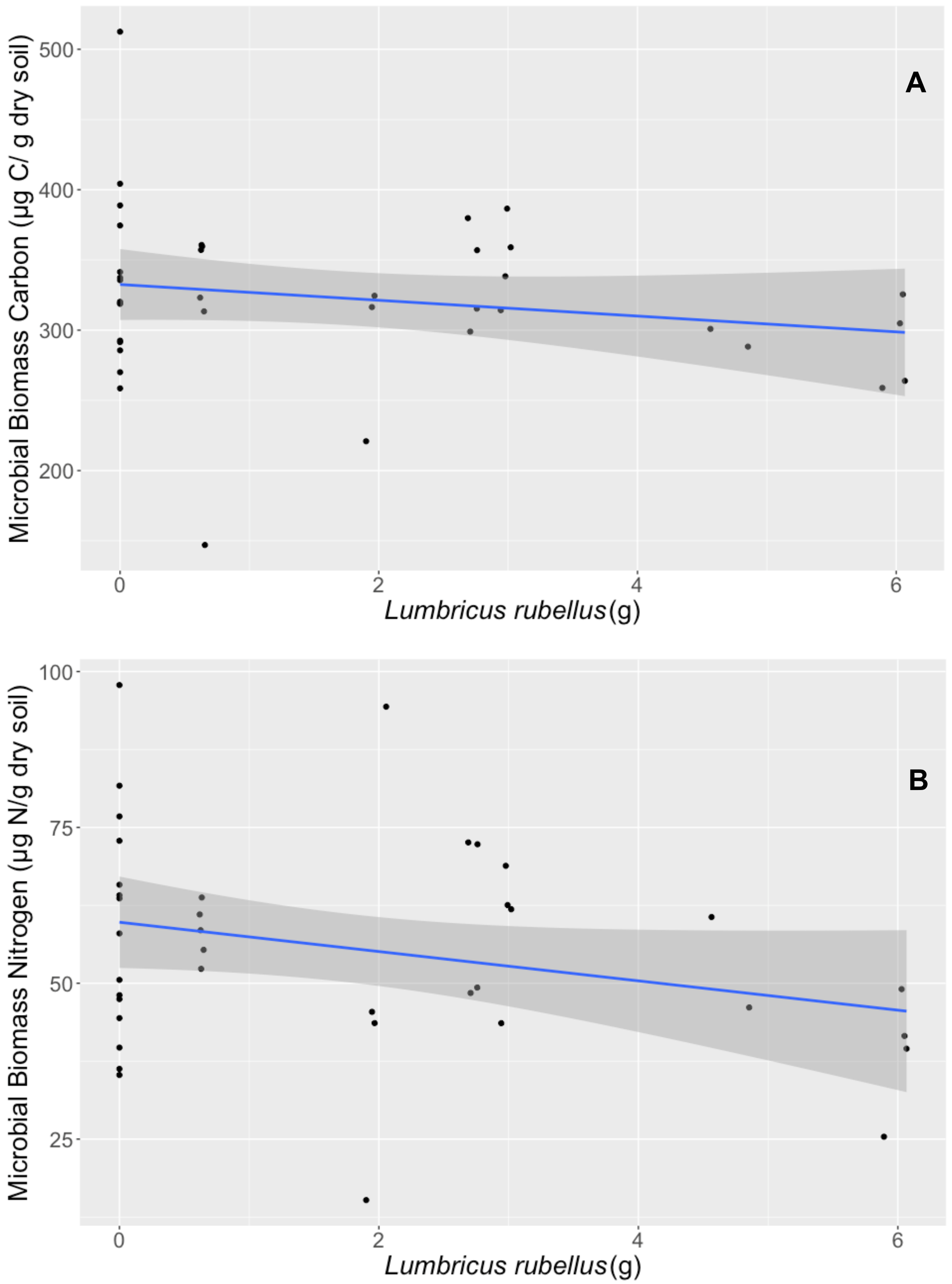

Figure 9. Graphs illustrating the effects of increasing *L. rubellus* biomass on (A) microbial biomass C ($F_{1,26} = 3.7849$, $p = 0.06261$) and (B) microbial biomass N ($F_{1,36} = 3.0284$, $p = 0.09036$). Gray shading indicates confidence intervals (95%) around the regression line.

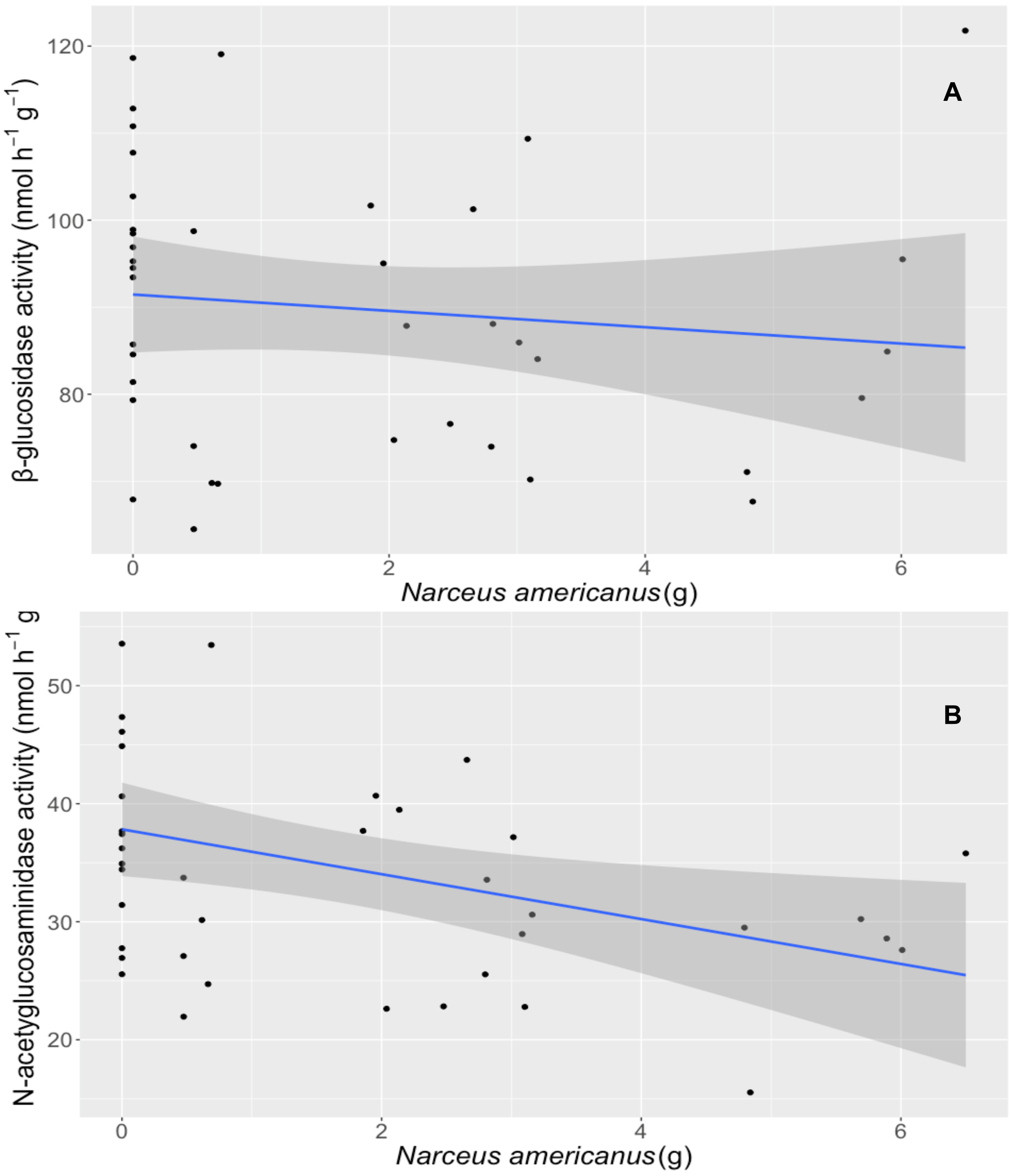

Figure 10. Graphs illustrating the effects of increasing *N. americanus* biomass on (A) NAG activity ($F_{1,24} = 4.6124$, $p = 0.04205$) and (B) BG activity ($F_{1,33} = 3.4856$, $p = 0.07081$). Gray shading indicates confidence intervals (95%) around the regression line.

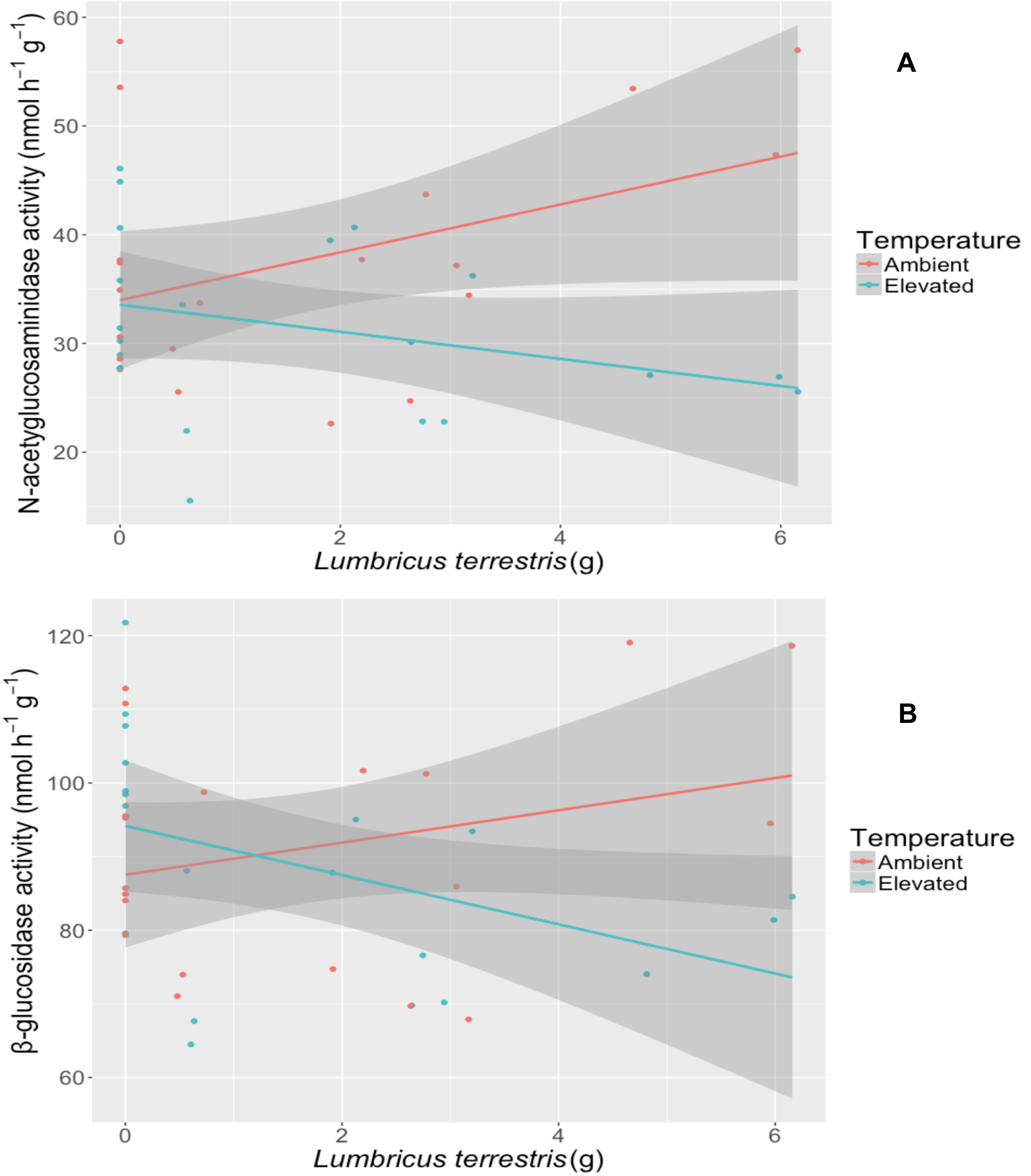

Figure 11. Interactions between detritivore biomass and temperature (Table 1) (Table 2). (A) NAG ($F_{1,24} = 4.75$, $p = 0.039$) and (B) BG ($F_{1,33} = 6.16$, $p = 0.02$) enzyme activity decrease with increasing *L. terrestris* biomass under warmed treatments, and marginally increase with increasing biomass under ambient treatments (NAG ($t = 3.27$, df = 37, $p = 0.002$); BG ($t = 2.13$, df = 37, $p = 0.04$)). Gray shading indicates confidence intervals (95%) around the regression line.

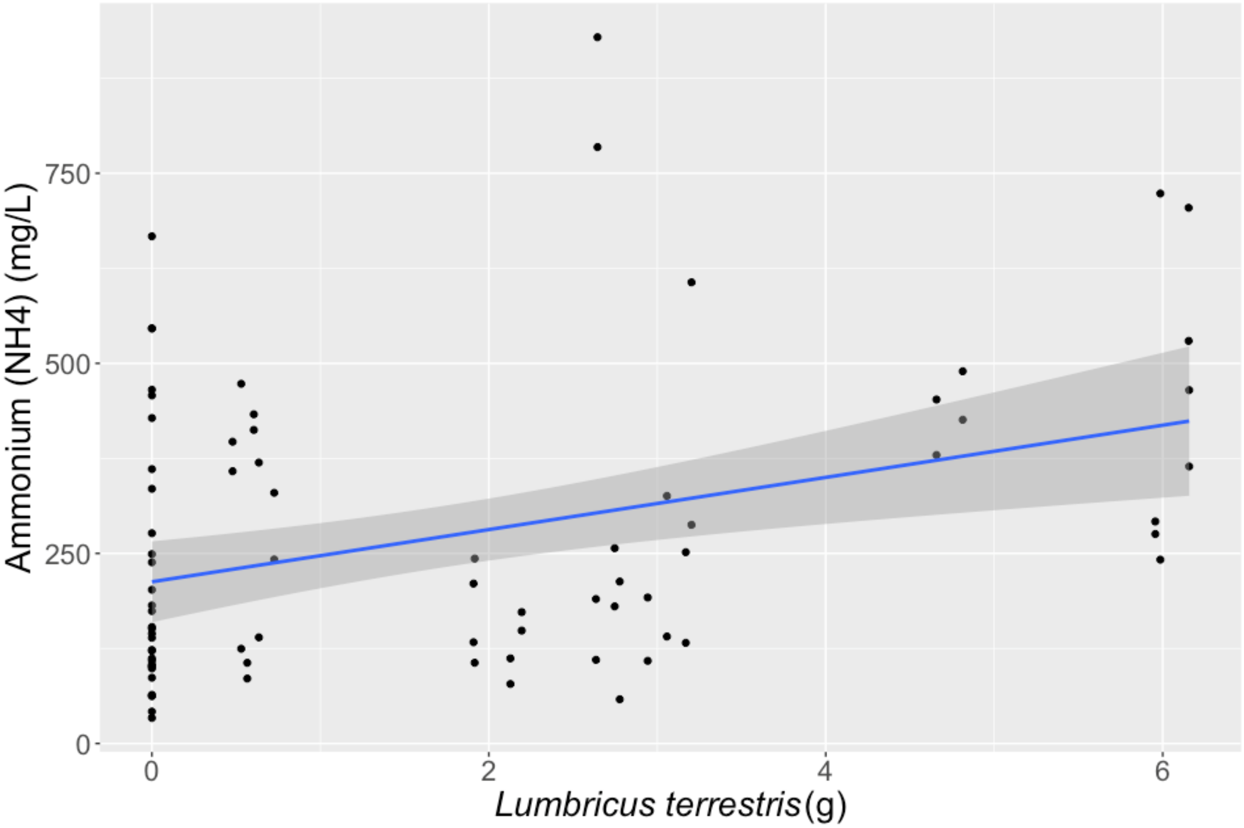

Figure 12. Graph illustrating the effect of increasing *L. terrestris* biomass on NH_4^+ (ammonium) leachate concentrations (Table 1) ($X^2 = 7.3127$, $p = 0.006847$). Gray shading indicates confidence intervals (95%) around the regression line.