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## Linking Trophic Interactions in Above- and Below-Ground Systems

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## Abstract

This study explored trophic interactions among herbivores, plants, and soil communities. Two experiments were conducted such that the below-ground effects of an above-ground herbivore, as well as the above-ground impacts of soil biota could be examined. The first project investigated the effects of gypsy moth (*Lymantria dispar* L.) herbivory on soil communities associated with oak trees (*Quercus rubra* L.). The goal of this study was to compare soil biota from defoliated oak trees with soil communities from undefoliated trees. The abundances of bacteria, bacterivorous nematodes, and herbivorous nematodes were compared between a defoliated sampling site and an undefoliated site in Jefferson Township, NJ. Neither the bacterial abundances, nor the abundances of bacterivorous and herbivorous nematodes differed between the two sites, suggesting that above-ground herbivory does not have implications for soil biota in this system. A second project examined the effects of soil food webs on above-ground ecosystems using an extensive dataset. More specifically, structural equation modeling was used to determine the extent to which herbivorous nematodes and the microbial loop influence *Ammophila arrenaria* L. biomass and carbon allocation through changes in nitrogen availability. Analyses indicated that amoebas, a constituent of the microbial loop, and the associated nitrogen mineralization are responsible for a shift to increased shoot biomass over root biomass. Further, the herbivorous nematode *Criconema* positively affects shoot biomass through mineralization. This suggests that amoebas and *Criconema* indirectly increase carbon allocation to above-ground plant tissues. Together, these two studies reflect the degree to which above- and below-ground systems are connected in different habitats.

MONTCLAIR STATE UNIVERSITY

Linking Trophic Interactions in Above- and Below-Ground Systems

by

Savannah Bennett

A Master's Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

For the Degree of

Master of Science

May 2016

College of Science and Mathematics

Department of Biology

Thesis Committee:

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LINKING TROPHIC INTERACTIONS IN ABOVE- AND BELOW-GROUND  
SYSTEMS

A THESIS

Submitted in partial fulfillment of the requirements

For the degree of Master of Science

by

SAVANNAH BENNETT

Montclair State University

Montclair, NJ

2016

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## **Acknowledgements**

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## List of Figures

Figure 1: This conceptual model describes the broad hypotheses of this study, that above-ground herbivores negatively affect soil organisms through reduced carbon allocation to plant roots. At the same time, above-ground herbivores could also have a positive, direct effect on below-ground communities through frass production and nutrient flux to the soil. In turn, below-ground communities then affect plant growth and biomass allocation. The number one denotes hypotheses tested in the gypsy moth study, and the number two represents hypotheses tested in the soil food web analysis.

Figure 2: Mean bacterial abundances of the undefoliated control site and the gypsy moth defoliation site were not significantly different ( $t = -0.32978$ ,  $n=10$ ,  $P=0.7454$ ,  $df=9$ ), and bars indicate mean  $\pm$  SEM.

Figure 3: Average bacterivorous nematode abundance values were not significantly different between samples taken from the undefoliated control site and the gypsy moth defoliation site ( $t=1.002$ ,  $n=10$ ,  $P=0.3293$ ,  $df=9$ ). Mean plant feeder abundance values were not significantly different between these sites as well ( $t=0.71$ ,  $n=10$ ,  $P=0.4868$ ,  $df=9$ ). Bars indicate  $\pm$  mean SEM.

Figure 4: This hypothetical model depicts how herbivores and the microbial loop may positively influence nitrogen availability, which then shapes plant biomass and resource allocation. It was hypothesized that root herbivory and/or the microbial loop would

indirectly promote shoot biomass, and cause increased carbon allocation to the shoots relative to the roots.

Figure 5: Herbivory significantly affected nitrogen mineralization, which positively influenced shoot biomass. This pathway is represented by dashed arrows. Further, the microbial loop also affected nitrogen mineralization, which negatively impacted root/shoot ratios, and this pathway is represented by solid, bold arrows. There were no significant relationships among the herbivore or microbial loop and root biomass.

Figure 6: Constituents of the microbial loop were separated to determine which organisms had a significant effect on nitrogen availability. Both amoebas and flagellates significantly affected nitrogen mineralization, which then negatively influenced root/shoot ratios ( $\chi^2=0.309$ ,  $P=0.958$ ,  $df=3$ ).

Figure 7: Amoebas had a stronger effect on mineralization than flagellates ( $\chi^2=0.001$ ,  $P=0.961$ ,  $df=1$ ).

Figure 8: *Criconema* significantly influenced mineralization as well. This caused an indirect relationship between *Criconema* and shoot biomass as mineralization had a significant effect on plant shoot biomass ( $\chi^2=0.009$ ,  $P=0.926$ ,  $df=1$ ). Standardized estimate values, depicted above path arrows, indicate that individual relationships in the model were weak despite the strength of the entire model. The  $R^2$  value (above each



response variable) was higher for the relationship between *Criconema* and net nitrogen mineralization than that of mineralization and shoot biomass.

## **I. General Introduction**

Above- and below-ground environments are tightly connected in terrestrial systems, and plants are the primary link between these two habitats (Bonkowski and others 2000; Bonkowski and Brandt 2002; Wardle and others 2004). Below-ground processes and biological communities influence above-ground dynamics. Rhizosphere processes such as the microbial loop, which involves the excretion of ammonium by soil organisms, convert nutrients into useable forms for plants (Bonkowski and others 2000; Bonkowski and Brandt 2002; van der Heijden and others 2008). Likewise, above-ground communities impact soil properties and organisms (Poelman and Dicke 2014; van der Putten and others 2013). Above-ground herbivores, for example, affect plant physiological pathways, ultimately shaping soil nutrient cycling and below-ground communities (Miller-Pierce and others 2010; Vendettouli and others 2014). Invasive herbivores in particular affect the abundance and diversity of soil biota (Gehring and Whitman 1994; Katayama and others 2014; Vendettouli and others 2014).

In order to better understand such interactions, various elements of above- and below-ground systems must be studied across different habitat types (van der Putten and others 2013). These works strive to build upon prior research on above- and below-ground interactions, and enhance current understanding of this complex field by examining the role of above- and below-ground herbivores in two different systems. More specifically, these projects investigated how above-ground insect herbivores impact soil communities, and how below-ground herbivores, as well as the microbial loop, influence plant biomass and growth allocation. In the following text I will describe how components of below-ground systems such as root herbivores and the microbial loop

impact above-ground environments. I will then discuss how above-ground habitats affect below-ground communities, with a focus on exotic invasive insect herbivores.

### *Below-ground systems*

Previous studies indicate that there are several ways in which below-ground ecosystems shape those above-ground, and these effects can be either direct or indirect (Wardle and others 2004). Below-ground systems directly impact above-ground environments through root herbivory (Soler and others 2012). Herbivorous nematodes and other below-ground herbivores consume plant roots, which reduces plant growth and nutritional quality for above-ground herbivores (Bever and others 1997; Soler and others 2012; Steinger and Muller-Scharer 1992; Bezemer and others 2005; van der Putten and others 2013). However, root herbivory can also have positive effects on below-ground communities as it facilitates the movement of carbon from plants to other soil organisms (Soler and others 2012; Yeates 1999; Yeates and others 2009). In some cases, plants exhibit compensatory root growth and nitrogen allocation to the root systems (Steinger and Muller-Scharer 1992). Along with root herbivory, the microbial loop promotes plant growth and function (Bonkowski and others 2000).

### *The microbial loop*

The microbial loop cycles nutrients through soil systems and increases nutrient availability for plants. During this cycle, carbon is released from plant roots into the surrounding rhizosphere, causing increased bacterial abundances. Bacterial feeding nematodes and various protozoa consume these bacteria and excrete ammonium, thereby increasing nitrogen availability for plants, and promoting above-ground plant growth and productivity (Bonkowski and others 2000; Bonkowski 2003; Paul 2014; Yeates 1999;

Yeates and others 2009; van der Heijden and others 2008). Both bacterial feeding nematodes and protozoa are responsible for the process of nitrogen mineralization, where nitrogen is converted from organic forms into inorganic forms that plants can utilize (Bonkowski and others 2000; Paul 2007).

Research suggests that protozoa are particularly important for nitrogen mineralization in that they increase mineralization rates (Bonkowski and others 2000). Protozoa such as amoebas, flagellates, and ciliates also feed exclusively on certain species of bacteria, which promotes the population growth of other taxa, such as nitrifying bacteria (Bonkowski and Brandt 2002; Griffiths 1989). Hence, the microbial loop enhances nutrient cycling, and in doing so, provides additional nutrients for plant roots (Bonkowski and others 2000; Bonkowski and Brandt 2002; Paul 2007; van der Heijden and others 2008). Above-ground herbivores then benefit from an increase in plant growth and nutritional quality (Bezemer and others 2005; van der Putten 2013; Wardle and others 2004). The nutritional quality of a plant drives herbivore performance and survival, which then shapes the performance of higher trophic levels, such as parasitoids that feed on herbivorous insects (Poelman and Dicke 2014).

The diversity of soil biota must be considered when analyzing above- and below-ground interactions because some taxa have a stronger impact on nutrient cycling, and therefore above-ground systems, than others (De Deyne and van der Putten 2005; Wagg and others 2014). For example, the diversity of detritivores has a greater impact on above-ground organisms than the diversity of mycorrhizal fungi (Wardle and others 2004). Detritivore consumption of organic matter increases nutrient mineralization and turnover, and it enables plant nutrient uptake, thus promoting plant growth (Wagg and

others 2014; Wardle and others 2004). Approximately 96% of soil nitrogen is bound within dead plant material, and microorganisms are responsible for converting much of this nitrogen into more useable forms for plants (Bonkowski and others 2000). Mycorrhizae also play a role in enhancing soil nutrient availability (Bonkowski and others 2000). Arbuscular mycorrhizae in particular are important determinants of the rate at which soil nutrient cycling occurs in forest systems because they affect the decomposition rate of leaf litter (Leifheit and others 2015). The presence of certain rhizobacterial organisms can cause plants to produce defensive compounds that alter nutritional quality for above-ground insect herbivores as well (Poelman and Dicke 2014). Soil community structure and composition drive nutrient cycling and promote plant nutritional quality above-ground (Bever and others 1997; Bezemer and others 2005; Ettema 1998; Poelman and Dicke 2014; Wagg and others 2014).

#### *Above-ground systems*

Soil biota, however, are in turn affected by above-ground factors. Both abiotic and biotic factors of above-ground habitats shape those below the soil surface (Wardle 2002; Yeates 1999). Like the below-ground influences on above-ground systems, the ways in which above-ground habitats impact those below-ground can be direct or indirect (Freschet and others 2013; Wagg and others 2014). For example, when leaf litter accumulates on the forest floor and begins to decompose, nitrogen gets cycled into the soil (Freschet and others 2013; Wagg and others 2014). Soil fertility affects anatomical and physiological features of plants such as leaf size and growth rates (Ordonez and others 2009; Wagg and others 2011). Soil fertility also shapes soil community composition, which further affects plant growth (Wagg and others 2011). Above-ground

ecosystems impact soil nutrient cycling, and this has a feedback effect on plants (Wagg and others 2014; Wardle 2002).

Additionally, biotic factors, including plant community dynamics and herbivory, drive productivity and community structure of below-ground systems (Cook-Patton and others 2014; Eisenhauer and others 2012). The abundance and diversity of rhizosphere taxa vary by plant species (Blair and others 1996; Brussaard 1997; Wall and Moore 1999). The diversity of neighboring plants surrounding a rhizosphere community determines the taxa present in that community as well (Cook-Patton and others 2014; Kos and others 2015). In this way, above-ground organisms drive overall below-ground diversity (Cook-Patton and others 2014; Kos and others 2015; Wardle and others 2004). The diversity of below-ground taxa in forest soils can vary among microhabitats because each plant species fosters the development of different soil communities (Eisenhauer and others 2012; Saetre and Baath 2000). Plant species utilize different forms of nitrogen and phosphorus; for example, some plants take in ammonium through their roots as a source of nitrogen, while others primarily use nitrate. As a result, plant roots develop associations with microbes that produce specific forms of nitrogen and phosphorus (Reynolds and others 2003). Previous studies have found that plant taxa also affect the overall diversity of soil nematodes and the diversity of nematode functional feeding groups within a particular community (Eisenhauer and others 2013; Yeates 1999).

#### *Role of herbivory in above- and below-ground interactions*

Furthermore, above-ground herbivores exert both direct and indirect influences on soil systems; herbivory is an important facet of above- and below-ground interactions because herbivores, whether native or exotic, drive community composition below-

ground (De Deyn and others 2007; Vendettuoli and others 2015). Herbivory impacts above- and below-ground interactions, but these effects are context dependent (De Deyn and others 2007; Hamilton and Frank 2001; Krumins and others 2015; McNaughton 1981). Above-ground herbivores, especially invertebrates, consume plant foliage or tree sap, depending on the species. Such feeding can pose a variety of constraints on plant growth and function, including the amount of organic carbon allocated to root tissues. Foliar herbivory can cause a reduction in carbon allocation to plant roots. In this way, foliar herbivory can be a source of top-down control as it regulates rhizosphere bacterial populations (Bezemer and others 2013; Zwart and others 1994). Reductions in carbon allocation to plant roots also cause a decline in arbuscular mycorrhizae, which alters carbon cycling within forest systems (Gehring and Whitman 1994; Metcalfe and others 2014). Similarly, phloem feeding herbivores reduce soil bacteria and nematode abundances through reduced carbon allocation to the roots (Katayama and others 2014; Vendettouli and others 2014). Certain plants, however, exhibit increased growth rates to compensate for lost tissues in response to defoliation, which can ultimately be beneficial to soil organisms (Hawkes and others 2001; McNaughton 1981; Vanderklein and Reich 1999; Wardle and others 2004).

Herbivores have profound impacts on soil organisms associated with grazed plants, some of which are beneficial for soil biota. Frass from insect herbivores becomes incorporated into the soil system as organic matter, increasing soil nitrogen and phosphorus accessible to plants (Kagata and Ohgushi 2012; Lovett and Ruesink 1995). If the nutritional quality of a plant is poor, the nitrogen contents of the insect frass, and therefore the nitrogen input into the soil, will vary (Kagata and Ohgushi 2012). Available

nitrogen concentrations are also increased through inefficient feeding mechanisms where plant material that is not consumed enters the detritus cycle (Krumins and others 2015). Herbivory can essentially be beneficial to plant growth and net primary production because frass and inefficient feeding increase available soil nutrients, and this promotes soil community growth and diversity (Krumins and others 2015; McNaughton and others 1989; Ruess and McNaughton 1987). Thus, above-ground herbivores influence nutrient cycling below-ground (De Deyn and others 2007; Hamilton and Frank 2001; Krumins and others 2015; McNaughton 1981).

#### *Exotic invasive herbivores*

The type of herbivore determines the extent to which plants and below-ground systems are affected by herbivory attacks. Exotic invasive herbivores are gaining interest within the scientific community as recent studies delineate the specific effects of these organisms on above- and below-ground interactions (Holden and Treseder 2013; Vendettuoli and others 2015). In general, influxes of invasive species stem from globalization; the importance of studying these organisms has become apparent as they disrupt natural trophic interactions (Crall and others 2012; Ziska and Dukes 2014). With respect to exotic plants, many previous studies examined invasions in terms of the 'enemy release hypothesis.' According to this hypothesis and related studies, exotic invasive plants tend to thrive and proliferate in novel habitats due to the lack of natural predators or diseases (Elton 2000; Keane and Crawley 2002; Mitchell and Power 2003; Roy and others 2011). Invaded systems have not built up mechanisms to control population growth of these exotic plants. Similarly, insect invasions pose drastic effects on terrestrial systems because they are not effectively controlled by mechanisms such as



plant defenses or predation. Investigating this facet of the enemy release hypothesis is equally important as studying the impact of invasive plants on natural systems (Maron and Vila 2001; Muller and others 2004; Radville and others 2011).

Exotic insect herbivores can be detrimental to plant populations because native plant species have not evolved anatomical and physiological resistances to the invader (Maron and Vila 2001; Muller and others 2004; Radville and others 2011). Repeated, intense grazing exhibited by many invasive insects such as the gypsy moth, *Lymantria dispar* L., the emerald ash borer, *Agrilus planipennis* F., and the spruce aphid, *Elatobium abietinum* W. can lead to extensive tree die-offs (Kenis and others 2008). However, the effects of exotic herbivores on plant communities vary by plant taxa and habitat type (Ingwell and others 2009; Wardle and others 2004). The presence of other herbivores and the intensity of herbivory within a particular system can influence the effect an invasive insect has on a plant (Oesterheld 1992; Preisser and Elkinton 2008; Radville and others 2011). Exotic invasive species, specifically insect herbivores, have a negative impact on the health and survival of native plants (Kegg 1973; Orwig and Foster 1998; Schultz and Baldwin 1982). Native herbivores affect plants as well, but invasive species, like the gypsy moth, graze with an intensity and frequency such that high tree mortality rates occur (Kenis and others 2008). Studies into above below interactions with respect to exotic invasive species would provide new insights into these relationships (Vendettouli and others 2014).

#### *Overall research objectives*

The goal of this project was to explore above- and below-ground interactions using two separate systems. I tested two hypotheses (Figure 1):

1. I hypothesized that above-ground herbivores negatively influence soil biota below through reduced carbon allocation to plant roots. Above-ground herbivores could positively affect below-ground organisms through increased nitrogen availability via frass as well.
2. I also hypothesized that soil communities would positively affect above-ground systems through nitrogen availability for plants.

To explore the effects of above-ground herbivores on soil communities, I conducted a field study in a forested habitat invaded by the gypsy moth (*L. dispar* L.). This study examined the effects of gypsy moth herbivory on soil biota associated with defoliated trees. Then, I analyzed a dataset from a second system to study below-ground effects on above-ground habitats. Using data from a greenhouse experiment with *Ammophila arrenaria* L., I investigated the degree to which herbivorous nematodes and other soil organisms affect nitrogen availability for plant growth and biomass allocation. These studies therefore explored plant-soil interactions between above- and below-ground communities.

## II. Gypsy Moth Study

### *Introduction*

In July 2015, I was able to capitalize on a gypsy moth, *L. dispar* L., outbreak and study the below-ground effects of gypsy moth herbivory in a stand of oak trees (*Quercus rubra* L.). Gypsy moths are an exotic invasive species that hinder the growth and survival of various tree species. In 1869, entomologist Leopold Trouvelet first brought gypsy moths into the United States while researching insects that could be used in the silk industry. Gypsy moths were first introduced in Massachusetts, but have since spread

throughout North America (Liebhold and others 1995). Gypsy moth infestations typically occur in pulse outbreaks, but the severity and timeframe of outbreaks vary by location. The egg sacks and caterpillars are usually found on oak or aspen trees (Eklinton 1990). However, they can be found on almost all tree species during intense outbreaks. Coniferous trees in particular are sensitive to gypsy moth herbivory and can perish after one infestation (Doane and McManus 1981; Lovett and others 2002; Lovett and others 2006). The caterpillars feed on and defoliate the leaves of oak trees, causing reduced growth rates in affected trees (Holden and Treseder 2013). In some areas, pathogens such as the fungus *Entomophaga maimaiga* H.S.S. control gypsy moth populations on a local scale, but this insect continues to threaten forests in the US (Tobin and Whitmire 2005).

Gypsy moth herbivory has short and long term effects on forest habitats. Gypsy moth larval herbivory defoliates individual trees, which opens entire forest canopies (Collins 1961). This does not always result in tree death (Holden and Treseder 2013), and trees will often rebound with a second leaf out the same summer. Reduced leaf quality, altered transpiration rates, and increased water drainage on forest floors are additional impacts of gypsy moth herbivory (Doane and McManus 1981, Kosola and others 2004, Lovett and others 2002). Although they have a negative impact on tree health, gypsy moths can play a positive role in forest ecosystems. Dead caterpillars, along with caterpillar frass, increases soil nitrogen availability, but this spike is quickly immobilized by soil microorganisms (Lovett and Ruesink 1995). In this way, gypsy moth herbivory indirectly increases the abundance of soil microorganisms (Holden and Treseder 2013). Further, small mammals, such as the white-footed mouse (*Peromyscus leucopus* R.), and certain birds, such as cuckoos (Culucidae V.), predate upon gypsy moths (Jones and

others 1998; Thurber and others 1994) with trophic effects moving through the local food web. The indirect effects of this can have implications for forest health and community stability.

### *Goal and hypothesis*

The purpose of this study was to test the hypothesis that gypsy moth herbivory would affect below-ground community structure and composition. More specifically, I hypothesized that abundance values would differ significantly between a highly defoliated site and an undefoliated control site. Abundance values could be lower in the root systems of defoliated oaks if herbivory reduces carbon and nitrogen allocation to the roots. However, herbivory could have the reverse effect on abundances if dead caterpillars and caterpillar feces increase nitrogen concentrations in the soil (Holden and Treseder 2013). Hence, it was predicted that bacterial and nematode abundances would differ between the defoliated and un-defoliated sites, showing that above-ground herbivory affects below-ground organisms.

### *Methods*

Two sampling sites were selected in a forested area of Jefferson Township, NJ. Oak trees at the first sampling site (40°58'16"N, 74°34'21"W) exhibited drastic (75 - 100%) gypsy moth defoliation. The second site (40°57'52"N, 74°35'33"W) was selected to be a control that did not exhibit such defoliation, but this second site had minor signs of defoliation (approx. 10%). At each of the two sampling sites, ten oak trees were selected at random, and a soil sample from the forest floor was obtained from the southwest side of each tree. Samples were brought back to the lab and refrigerated overnight. The following day, a subsample of approximately 0.5g was removed from

each soil sample. Subsamples from each sampling site were combined to obtain the approximate fresh weight of the soil at these sites. The average dry weight of soil from each site was determined as well. These data were used to calculate the average percent soil moisture for each site, which was utilized to determine bacterial and nematode abundances.

Another subsample from each site was collected to determine bacterial abundances. Acridine Orange Direct Counts (AODC) were used to obtain these abundance values (Strugger 1948; Hobbie and others 1977). Using the rest of the soil samples, the bacterivorous and herbivorous nematode abundances were determined. The ten samples from each site were placed individually in coffee filters, which were stabilized with small, plastic baskets. The baskets were subsequently placed on small plates of tap water and left for seventy two hours. During this seventy two hour period, nematodes traveled through the coffee filter and into the surrounding water. These water samples were finally obtained, filtered, and fixed with 1mL of filtered formalin (van Bezooijen 2006). Nematodes were identified by functional feeding group. Bactivorous and herbivorous nematodes were distinguished and counted under a microscope. The abundances of bactivorous and herbivorous nematodes per gram dry weight of soil were calculated.

#### *Data analysis*

Bacterial abundance values, bactivorous nematode abundances, and herbivorous nematode abundance values per gram dry weight of soil were compared between the two sampling sites using t-tests in the statistical software program JMP (SAS Institute, Cary, NC).

### *Outcome*

Bacterial abundances were not significantly different between the defoliated and undefoliated sites ( $t = -0.32978$ ,  $df = 9$ ,  $P=0.7454$ , Figure 2). Similarly, bacterivorous nematode abundance ( $t=1.002$ ,  $P=0.3293$ , Figure 3), and herbivorous nematode abundance ( $t=0.71$ ,  $df=9$ ,  $P=0.4868$ , Figure 3) did not differ significantly between the two sampling sites either.

### *Discussion*

The results of this study suggest that gypsy moth herbivory may not influence the soil communities surrounding oak trees. In this study, bacterial and nematode abundance values did not differ significantly between the defoliated and undefoliated sites. This indicates that gypsy moth does not influence the soil communities associated with oak trees. Based on these results, gypsy moth herbivory does not seem to affect carbon allocation to the roots such that soil communities are affected. Herbivory in this system does not appear to affect soil biota through frass either. The control site in this experiment exhibited slight gypsy moth defoliation, which could have affected the ability to discern differences between the sites. Abundance values from both sites might have been above or below values found in pristine forests without such defoliation. A control site that did not exhibit any signs of defoliation could not be found within the same forested system. An additional control site at another location was not selected because the soil communities could differ naturally from those in the forest of Jefferson Township, which would make the results incomparable. It is also possible that sampling again at a later date would yield different results if it takes a long period of time for the gypsy moth to impact the soil organisms associated with oak roots. Further

experimentation is necessary to better understand the relationship between gypsy moths and soil communities surrounding oak roots, but current data suggest that herbivory does not impact soil biota.

### **III. The Effects of Soil Food Webs on Nutrient Cycling and Plant Growth**

#### *Goal and hypothesis*

The purpose of this study was to determine the relationships between soil communities and plant growth. Interactions among the nematode *Criconema*, bacterial feeding nematodes, protozoa, and plants were explored. More specifically, this study aimed to determine the degree to which root herbivory and the soil microbial loop affect nitrogen availability, and how potential changes in nitrogen availability affect plant biomass. It was hypothesized that herbivory and/or the microbial loop would positively affect plant biomass through mineralization. It was also hypothesized that the abundance of *Criconema* would affect positively plant biomass by increasing nutrient availability. However, it was predicted that there would be a point at which increasing nematode abundance hinders plant growth and function due to herbivory. This project involved analyzing an extensive dataset from an investigation of herbivorous nematode abundances and their feedbacks to production of the dune grass *Ammophila arrenaria* L. This large dataset allowed for analysis that can isolate the effects of the microbial loop versus herbivory on nitrogen flux and plant growth. The following experiment was conducted by Dr. Jennifer Krumins at the Netherlands Institute of Ecology (Wageningen, NL) in 2009.

### *Experimental design*

Dune grass (*A. arenaria* L.) was grown individually in pots of sterilized native dune sand. To test the effects of herbivory and micro-food web trophic activity on nutrient cycling and plant growth, the pots were inoculated with 0, 50, 100, 400, 700, or 1,000 individual nematodes of the genus *Criconea*. The inoculum was prepared from a soil slurry of a stock culture of the nematode. Herbivorous nematode concentration was determined in the slurry such that accurate density inoculation could be done. Nematode extraction was performed using an Oosenbrink elutriator, and nematodes were then fixed in formalin (Ettema 1998). Herbivorous nematode concentration was determined via microscopic counts such that a known concentration of herbivorous nematodes could be added to each treatment pot. Due to their small size, it was inevitable that bacterial feeding nematodes and protozoa would be filtered into the inoculum with the herbivorous nematodes, but the density of these organisms transferred to each pot at inoculation was proportional to the herbivorous nematode density and consistent across each treatment group. The pot microcosms were destructively harvested, and four pots per treatment group were randomly selected. Parameters relating to plant growth and overall health were obtained as well, including dry weights of roots, shoots and therefore, the root/shoot ratios. All soil from the pots was sieved through a coarse (2mm) screen. Data including: nematode abundance and diversity, bacterial abundance, and protozoa abundance and diversity were determined. Bactivorous and herbivorous nematodes were counted and identified by family.

Soil nitrogen was measured as net nitrogen mineralization from the pots after harvest. To determine net nitrogen mineralization, methods were adapted from Kooijman



and others (2009). Soil samples were incubated in plastic bags at 20°C in the dark for six weeks, during which soil moisture was maintained at 12%. Before and after incubation, ammonium concentrations were obtained from the samples. Net nitrogen mineralization was calculated by subtracting the ammonium collected prior to incubation from those obtained after the incubation period (Kooijman and others 2009).

#### *Data analysis*

Using these data, several hypothetical pathways were designed to determine the effect of soil biota on nitrogen availability and plant biomass. Preliminary analyses using the software program JMP indicated that herbivorous nematode abundance does not significantly affect plant biomass (Pearson's Correlation: *Criconema* abundance vs. shoot biomass,  $r=0.0253$ ,  $P=0.832$ ; *Criconema* abundance vs. root biomass,  $r=0.075$ ,  $P=0.5555$ ; *Criconema* abundance vs. root/shoot,  $r=0.1333$ ,  $P=0.2642$ ). Therefore, other relationships among the variables measured were studied. Soil organisms are indirectly linked to plants via nitrogen flux. By consuming plant roots, herbivorous nematodes mineralize nitrogen, which may then promote plant growth or biomass (Bardgett and others 1999; Yeates and others 2009). Similarly, bacterial feeding nematodes and protozoa consume bacteria, and in doing so, also mineralize nitrogen, increasing nitrogen availability to the plants (de Ruyter and others 1993a; de Ruyter and others 1993b; Yeates and others 2009). This analysis examined the degree to which these two processes shape nitrogen mineralization, and how available nitrogen can affect plant growth. Root and shoot biomass values were utilized to determine whether below-ground processes facilitated or hindered plant growth. Root/shoot ratios were also analyzed to determine whether mineralization affected resource allocation to above or below-ground plant tissues.

Structural equation modeling (SEM) was performed in the software program AMOS 23.0 (SPSS IBM) to test the hypothesis that root herbivory or the soil microbial loop has a stronger effect on nitrogen availability, and whether this influences plant growth (Figure 4). SEM is useful in determining the existence of causal relationships among many observed variables in an experimental study (Eisenhauer and others 2015; Shao and others 2015). All variables analyzed in each model were observed variables. SEM involves performing chi square tests to determine whether individual pathways in each model, as well as the entire overall model fit with the observed data. P values obtained based on the  $\chi^2$  values were used to analyze significant relationships among the variables studied, and select models that closely fit with the data (Pugesek and others 2003). A larger P value indicates that the data more closely fits with the tested model. Non-significant models, where data did not fit the hypothesized model, were removed (Veen and others 2010). Further, the standardized coefficients and  $R^2$  values indicate the strength of individual relationships within each model (Grace and Bollen 2005). Standardized coefficients represent how many standard deviations from the mean a response variable is expected to change when the independent variable exhibits a one standard deviation change from the mean (Grace and Bollen 2005).

Several conceptual models were created in AMOS (IBM SPSS), based on the above hypotheses, to describe how soil communities affect nitrogen availability through mineralization, and how nitrogen then affects shoot biomass, root biomass, and root/shoot ratios (Figure 4). For the first general model, it was hypothesized that herbivorous nematodes and the microbial loop would have a positive and indirect influence on plant biomass and/or growth allocation through nitrogen mineralization (Figure 4). This

general model was deconstructed into six individual pathways. Three of these pathways described the role of the microbial loop in indirectly affecting root biomass, shoot biomass, and root/shoot ratios, while the other three pathways depicted the indirect role of herbivorous nematodes on root biomass, shoot biomass, and root/shoot ratios. These six pathways were then further deconstructed such that specific taxa of soil organisms were incorporated into the models (non-significant models not shown here for brevity). In this study, the microbial loop was measured and consisted of amoebas, bacterivorous nematodes, and flagellates. Herbivores included all herbivorous nematode taxa. When performing the data analysis, individual nematode functional groups were separated into separate observed variables in order to discern which species had stronger effects on nitrogen mineralization and plant biomass.

### *Outcome*

SEM analysis suggests that the microbial loop impacted nitrogen availability, which then negatively affected the root/shoot ratios (Figures 5 and 6). The model representing amoebas and flagellates impacting root/shoot ratios through mineralization was significant (Figure 6,  $\chi^2=0.309$ ,  $P=0.958$ ,  $df=3$ ). Further, amoebas had a stronger effect on net mineralization than flagellates (Figure 7,  $\chi^2=0.001$ ,  $P=0.961$ ,  $df=1$ ). This was evident as the strength of the model increased when flagellates were removed from the model. In both models, microbes and net nitrogen mineralization exhibited a weak, negative relationship. Net nitrogen mineralization also had a weak, negative relationship with root/shoot ratios. Other models, such as models that incorporated bacterivorous nematodes, shoot biomass, and root biomass, were not significant. Thus, these data

suggest that amoebas affected nitrogen mineralization, which then impacted root/shoot ratios.

Models incorporating herbivorous nematodes affecting plant biomass through mineralization were significant as well. However, herbivory did not significantly influence net mineralization when all herbivorous nematode taxa were included in the model. *Criconema* was the only organism that significantly affected net nitrogen mineralization, which then affected shoot biomass (Figure 8,  $\chi^2=0.009$ ,  $P=0.926$ ,  $df=1$ ). This is to be expected as *Criconema* was the nematode inoculated into each pot, and therefore was the most abundant herbivore. In this model, *Criconema* exhibited a weak, negative relationship with net nitrogen mineralization. Net nitrogen mineralization had a weak, positive relationship with shoot biomass. The other models incorporating root biomass and root/shoot ratios were not significant, and were consequently removed or not included. Overall, both herbivory and the microbial loop influenced nitrogen availability, but herbivory had a stronger indirect impact on shoot biomass, while the microbial loop, specifically amoebas, impacted root/shoot ratios.

The model incorporating how amoebas and flagellates affect mineralization, which then impacted root/shoot ratios strongly fit with the observed data. This indicates that protozoa indirectly shape plant biomass through mineralization (Figure 6,  $\chi^2=0.309$ ,  $P=0.958$ ,  $df=3$ ). The degree to which bacterivorous organisms such as protozoa and bacterivorous nematodes affect nitrogen mineralization depends on several factors, including the habitat-type and plant species present (de Ruiter and others 1993a). In this study, strength or significance of the model increased even further when bacterivorous

nematodes and then flagellates were removed, suggesting that amoebas have the strongest effect on mineralization and root/shoot ratios (Figure 7,  $\chi^2=0.001$ ,  $P=0.961$ ,  $df=1$ ).

### *Discussion*

Previous research also found that protozoa, and particularly amoebas, are responsible for much of the mineralization in soil communities (de Ruiter and others 1993a). The presence of protozoa can increase net mineralization by consuming bacteria, leading to more available nitrogen for plant roots (Clarholm 1985). This study found a negative relationship between protozoa and net nitrogen mineralization, which does not necessarily indicate that protozoa reduce mineralization rates. Protozoa could cause an increase in mineralization, which would make more available ammonium for nitrification, a process carried out by nitrifying bacteria. Increased nitrification could cause negative net mineralization rates if the ammonium produced via mineralization is used by nitrifying bacteria. This ammonium could also be used by plants. In the presence of increased nutrient concentrations, less energy is needed for root growth and nutrient acquisition (Davidson 1969). Root/shoot ratios would therefore be reduced, as seen in this study, because less energy is allocated for growth of the roots in comparison to shoot growth (Clarholm 1985). Past research has found that nitrogen availability is closely related to plant biomass, specifically root/shoot ratios or allocation to above- and below-ground tissues (Argen and Franklin 2003). The significant models obtained in this study could suggest that net mineralization by amoebas provide ample available nitrogen for *A. arenaria*, which enables increased shoot growth in comparison to root growth.

The model that depicted net mineralization by *Criconema* affecting shoot biomass was also significant (Figure 8,  $\chi^2=0.009$ ,  $P=0.926$ ,  $df=1$ ). This suggests that nitrogen

generated from *Criconema* mineralization affected nitrogen availability for plants, which then impacted shoot biomass. Interestingly, net mineralization by *Criconema* affected shoot biomass, while that of amoebas influenced root/shoot ratios. Similar to the amoebas, *Criconema* could improve nitrogen availability for *A. arenaria*, which could increase energy allocation for shoot growth. Therefore, based on these data, both herbivores and the microbial loop influence *A. arenaria* biomass and growth allocation, but certain groups have a larger influence on net mineralization and plant growth than others. Amoebas indirectly affect root/shoot ratios through mineralization, while herbivorous nematodes indirectly impact shoot biomass. Below-ground systems, in other words, affect plant biomass and carbon allocation.

In all of the significant models, standardized estimate values and  $R^2$  values were relatively low. This suggests that even though the models fit the data, individual relationships in each model were relatively weak. However, this does not necessarily indicate that the models cannot be used to explain the data. Overall stability in many complex food webs stems from weak connections among different trophic levels (O’Gorman and Emmerson 2009; Neutel and others 2002). In other words, stable food webs are comprised of weak interactions that when together, strengthen the entire system (O’Gorman and Emmerson 2009; Neutel and others 2002). This phenomenon has been observed in both terrestrial and marine food webs (O’Gorman and Emmerson 2009; Neutel et al 2002). The integrity of many food webs is maintained by these weak relationships to prevent the system from collapsing when one element of the food web is removed. Results from the SEM analysis suggest that this could apply to plant soil feedbacks as well. Perhaps the significant models consist of weak relationships that

together stabilize the soil community and prevent ecosystem collapse. Based on the SEM analysis, the weak relationships among soil biota, nitrogen availability, and plant biomass could ultimately yield a strong, stable system.

#### **V. General Conclusions:**

Above- and below-ground systems are interdependent environments that are linked by plants and nutrient cycling. Plants connect above- and below-ground habitats by transferring carbon and other nutrients between the shoot and root systems (Wardle and others 2004). The purpose of this project was to investigate the extent to which above- and below-ground systems are connected. The gypsy moth study suggested that there were no significant differences between trees in light versus heavy cases of gypsy moth defoliation. These experiments should be repeated in order to obtain more accurate, reliable data, but this indicates that herbivores may not affect soil communities. The data analysis project revealed that mineralization by both the microbial loop and herbivorous soil nematodes impacts plant biomass and energy allocation. Amoebas indirectly influence root/shoot ratios or the relative amount of energy allocation to above- and below-ground structures. The herbivorous nematode *Criconea* indirectly affects shoot biomass through mineralization. These data as a whole suggest that below-ground organisms affect nutrient cycling, which then shapes plant growth. Data from these two experiments are not directly comparable, but these projects reflect the degree to which above- and below-ground communities are linked in different systems. In sum, this study found that an invasive, foliar herbivore did not affect below-ground diversity, but soil communities impacted plant biomass and carbon allocation.

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## Figures

Figure 1

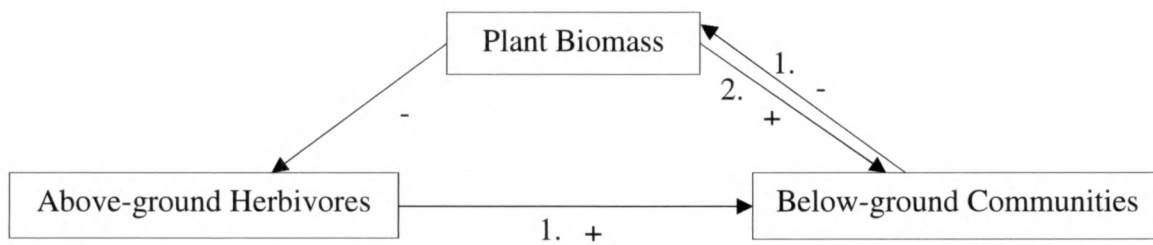


Figure 2

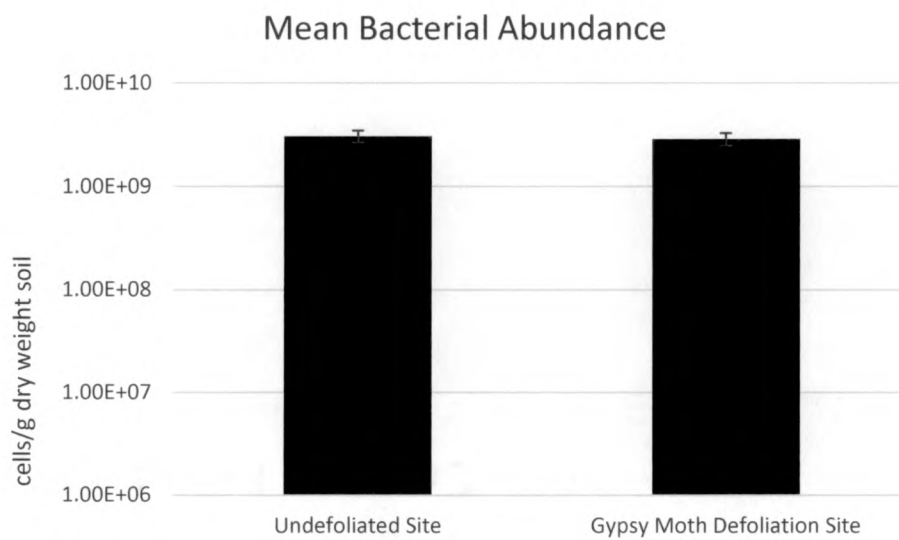


Figure 3

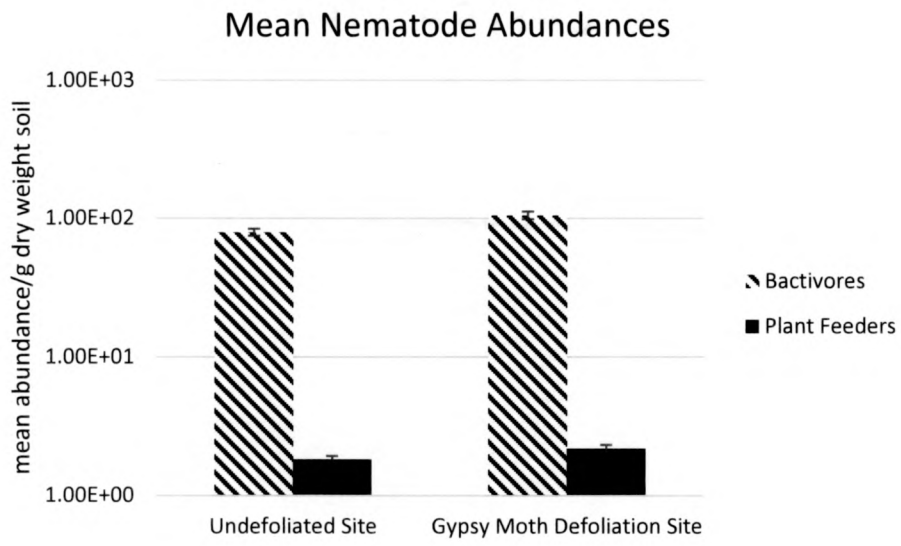


Figure 4

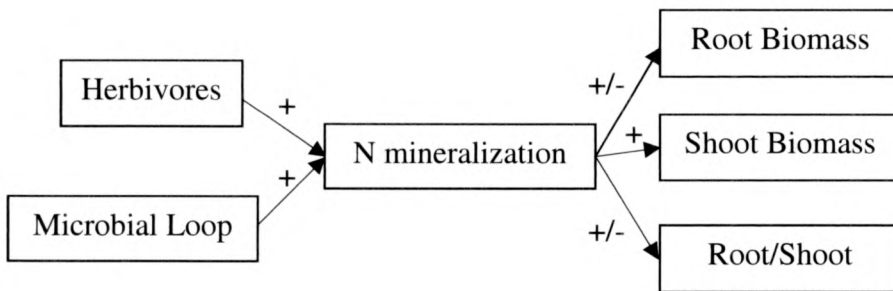


Figure 5

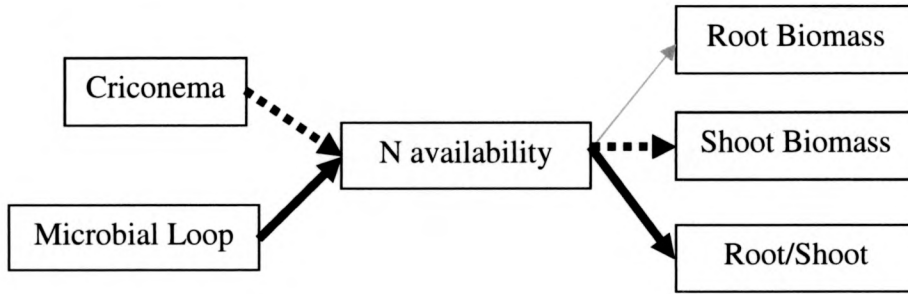


Figure 6

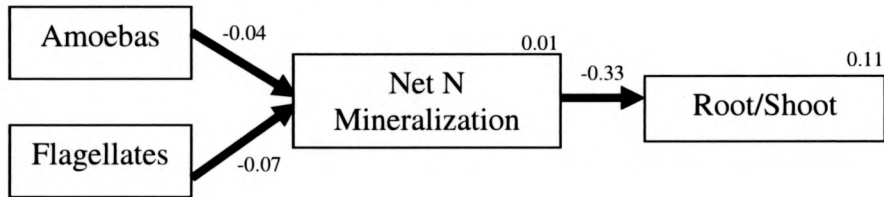


Figure 7

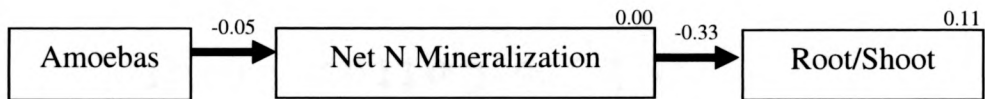


Figure 8

