THE CAUSES AND CONSEQUENCES OF BIODIVERSITY IN MULTITROPHIC COMMUNITIES

by

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ABSTRACT OF THE DISSERTATION

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The work described in this dissertation is linked by the common theme of biodiversity and its relationship to microbially-mediated functions in ecosystems. In the first chapter, I present results from a study where I evaluated the consequences of diversity by manipulating species richness in model aquatic communities. I showed that bacterial abundance remained constant with increasing eukaryotic species richness at low productivity, but significantly declined at high productivity. Furthermore, eukaryotic species richness together with productivity influenced the composition of the bacterial community, and food web diversity and productivity interact to influence bacterial community composition and function. In more diverse food webs, bacterial activity (decomposition) increased despite lower population abundance.

In chapters two and three, I present results from one experiment in which I measured responses of microbial diversity and multiple trophic levels to an environmental perturbation in naturally occurring forest soil food webs from two

geographically different locations. In the second chapter, I showed that diversity of the bacterial and fungal communities (measured by colony and ectomycorrhizal morphotype respectively) responded differently to nitrogen addition depending on geographic context. The composition of the bacterial community differed with nitrogen addition and geographic site, while the composition of the fungal community did not. In chapter three, I evaluated the relative importance of trophic control in the soil micro-food webs from the same two geographic sites (Florida and New Jersey). I found that the FL site supported greater biomass of bacteria and fungi than NJ, and the NJ site supported greater density of measured soil animal groups (collembola, oribatid mites and predatory mites) than FL. I found evidence for top down control by soil animals on microbial biomass, and at the same time, I also found evidence for bottom up control on microbial biomass through limitation of NO_3 and PO_4.

This dissertation demonstrates that microbially mediated-ecosystem functions depend upon trophic interactions with producers, consumers and predators in food webs. Furthermore, it demonstrates that the response of these communities is context dependent. Biotic and abiotic factors play a critical role in shaping a community's diversity, composition and functioning.

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Dedication

I dedicate this dissertation to my husband, Valdis Krumins. His love, encouragement and patience have given me peace and comfort through this work.

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Introduction

The work described in this dissertation is linked by the common theme of biodiversity and its relationship to microbially-mediated functions in ecosystems. In the first chapter, I present results from a study where I evaluated the consequences of diversity by manipulating species richness in model aquatic communities. In the following two chapters, I present results from one experiment in which I measured responses of microbial diversity and multiple trophic levels to an environmental perturbation in naturally occurring forest soil food webs.

Biodiversity can be defined at different levels. The simplest measure of biodiversity, species richness, is defined as the total number of species present in a community. However, diversity can also account for relative abundance of species (evenness) and aspects of functional group representation. Concern over declining biodiversity has been acknowledged for some time (Ehrlich and Wilson 1991), and the causes of declining biodiversity are complex, but include: invasive species, land use change and habitat degradation, climate change, extinction, and pollution (Vitousek et al. 1997, Sala et al. 2000). Research into the causes and consequences of biodiversity is increasing and theory is continuing to expand (Reviewed in: (Kinzig et al. 2001, Loreau et al. 2001, Loreau et al. 2002, Hooper et al. 2005, Duffy et al. 2007, Hector and Bagchi 2007).

At global and local scales, biotic and abiotic factors cause communities to be more or less diverse. Along a latitudinal gradient, biodiversity is highest near

the equator and lowest toward the poles. It is proposed that the latitudinal diversity gradient is due in part to climatic predictability at the equator or age of successional development for lower latitude communities (Morin 1999). A rigorous analysis correlated biotic and abiotic variables with documented latitudinal diversity gradients and found patterns that could be generalized were based on parameters like body size (allometry) or habitat (Hillebrand 2004). At a less than global scale, communities exposed to intermediate levels of disturbance are often more diverse than those that are heavily disturbed (*i.e.*, overgrazing) or those that receive no disturbance (Connell 1978). Productivity, a measure of the rate of energy capture of an ecosystem, also influences diversity. Theory holds that diversity peaks at intermediate productivity (Kassen et al. 2000), because at low productivity energy is insufficient to support large numbers of species, and at high productivity competition will exclude all but the best competitors for resources. Environmental productivity can interact with top-down pressure from consumers to have complex effects on species diversity (Leibold 1996, Jiang and Morin 2005).

Some of the processes that comprise ecosystem functioning include primary and secondary production as well as decomposition and cycling of nutrients and materials. However, functioning can mean different things depending on the context of the study. In general, more diverse communities can be more predictable (McGrady-Steed et al. 1997) and more stable (Ives et al. 2000). However, the response of stability to diversity is complex, and mechanistic studies may help resolve those complex effects (Ives and Carpenter

2007). More diverse communities can be more resilient to perturbations such as temperature extremes (Petchey et al. 1999), and more diverse communities are often more productive (Hector et al. 1999, Tilman et al. 2001). In reality, multiple processes driven by different species operate in whole ecosystems. When considering multiple ecosystem functions simultaneously, the importance of species diversity becomes more apparent. That is, the relative importance of a single dominant species (*i.e.,* the 'sampling effect' discussed below) diminishes with increasing numbers of ecosystem functions under consideration (Hector and Bagchi 2007).

The nature of the relationship between diversity and ecosystem functioning is the subject of continuing debate (Grime 1997, Loreau et al. 2001). The primary arguments focus on the importance of species richness *per se* for particular ecosystem functions (often primary productivity). The debate began when Naeem et al. (1994) and Tilman and Downing (1994) conducted diversity studies in terrestrial systems and observed positive effects of biodiversity on productivity or resistance to perturbations. Their results have been attributed to niche complementarity (Tilman et al. 1997) or to positive interactions among species (Hector et al. 1999). Some argue that having many species in a community ensures community functioning in the event of species loss (Yachi and Loreau 1999). Others attribute such patterns to a 'sampling effect' (Wardle et al. 1997, Wardle 1999b). The sampling effect holds that species richness does not influence ecosystem productivity, but rather, creates a situation where there is a higher probability of having a few highly productive representatives

when more species are present in the community (Huston 1997, Tilman 1997). It is likely that these mechanisms are not mutually exclusive and that a combination of phenomena drives ecosystem responses to diversity, particularly when considering the complexity of different functions that maintain a healthy ecosystem.

Except for two early studies (Naeem et al. 1994, McGrady-Steed et al. 1997), much biodiversity research has focused on the response of primary production to increased plant species richness (*e.g.,* (Tilman and Downing 1994, Hector et al. 1999, Tilman et al. 2001). Subsequent to that, the conceptual framework for biodiversity studies began incorporating multiple trophic levels (Petchey et al. 1999, Petchey et al. 2002). For instance, explorations of diversity loss through species extinctions within food webs revealed important consequences for community structure (Ebenman et al. 2004) and ecosystem functioning (Thebault et al. 2007). Duffy et al. (2007) use a two-dimensional framework to describe how diversity varies within and among trophic levels. 'Horizontal' diversity accounts for species richness within a trophic level, and 'vertical' diversity accounts for richness across multiple trophic levels. By definition, vertical diversity is representative of trophically defined functional diversity in that community. This perspective becomes more important as food web complexity increases, and the nature of vertical and horizontal diversity will likely be different in aquatic and terrestrial habitats (Strong 1992, Polis and Strong 1996).

In both aquatic (Fenchel and Harrison 1976, Sherr et al. 1988, McGrady-Steed et al. 1997, Azam 1998, Tso and Taghon 1999, Krumins et al. 2006) and terrestrial food webs (McNaughton 1976, Clarholm 1994, Coleman 1994, Belovsky and Slade 2000), vertical diversity becomes very important because consumer grazing can stimulate nutrient cycling and material flow. Stimulation of lower trophic levels (*i.e.*, bacteria) occurs when inefficient grazing releases carbon into the environment, or mineralized nutrients are excreted by grazers. In aquatic environments, the well-described microbial loop (Azam 1998) is a key manifestation of this process. The food chain of zooplankton feeding on protozoa, and protozoa feeding on bacteria releases carbon, nitrogen and phosphorus in the water column (due to the stoichiometric relationship between bacteria and higher trophic levels). This creates an available nutrient pool in the upper water column for future uptake by phytoplankton (Azam 1998).

The microbial loop also occurs in soil food webs when protozoa graze on bacteria making nutrients available to plants, soil bacteria, and fungi (Clarholm 1994). In fact, the effects of soil organisms grazing on bacteria have been known for some time (Coleman et al. 1978). Studies testing trophic interactions and microbial grazing by protozoa and nematodes showed that with grazing, microbial biomass declined, but nitrogen cycling increased (Coleman et al. 1983, Ingham et al. 1986). These results were important because they demonstrated the enriching effect of grazing on lower trophic levels in complex soil food webs. Subsequently, the enriching effects of grazing and trophic relations has been

generalized to other functional groups, such as soil microarthropods (Lussenhop 1992).

The positive influence of grazing on nutrient cycling extends across habitats and across varying spatial scales. For example, protozoan grazing on bacteria stimulates bacterial degradation of organic compounds (Fenchel and Harrison 1976, Tso and Taghon 1999), nematode grazing on roots stimulates nutrient cycling in the rhizosphere (Bardgett et al. 1999), soil invertebrate activity affects the diversity and function of lower trophic levels (Lavelle et al. 1997), and wildebeest grazing on the Serengeti plain stimulates primary production of grasses (McNaughton 1976). It is possible that increased consumer diversity will result in a greater diversity of niches for lower trophic levels, thus increasing their potential productivity.

Consumer (vertical) diversity can interact with lower trophic levels to affect ecosystem functioning. For example, in a simple aquatic food web including a mixed assemblage of bacteria and a bactivorous ciliate, grazing by the ciliate increased bacterial diversity and therefore, increased one aspect of ecosystem functioning, decomposition (Jiang and Krumins 2006). Documented cases of cascading trophic effects of consumer diversity in aquatic communities have shown positive (McGrady-Steed et al. 1997) and mixed results (Naeem and Li 1998). Apart from the work of Naeem et al. (1994), there are few manipulative multitrophic biodiversity studies in terrestrial systems, and those studies show mixed responses to biodiversity (Mikola and Setala 1998, Heemsbergen et al. 2004). Soil micro-food webs (*sensu* (Wardle 2002) in particular are highly

complex and naturally diverse (Moore et al. 2004). Below ground feedbacks between organisms that determine species coexistence (reviewed in (Ehrenfeld et al. 2005) and diversity in the plant soil system can be negative (*e.g.,* (van der Putten et al. 2001, Bever 2003) or positive (*e.g.,* (Simard et al. 1997, Klironomos 2002).

Positive interactions among species in soils are a poorly understood component of biodiversity (Wall 2004). However, important work has studied the relationships between plants, soil organisms, and environmental effects that may influence symbioses. Pollution, in the form of nitrogen deposition, can negatively impact the diversity of mycorrhizal fungal symbionts. This result has been found for fungi (Allison et al. 2007), arbuscular mycorrhizae (Johnson 1993, Egerton-Warburton and Allen 2000), and ectomycorrhizae through fertilization studies (Dighton et al. 2004) and across natural nitrogen deposition gradients (Lilleskov et al. 2002, Dighton et al. 2004, Lilleskov 2005). This is potentially important to the health of plant communities, as mycorrhizal diversity is related to plant productivity (Baxter and Dighton 2001, Jonsson et al. 2001) and plant diversity (van der Heijden et al. 1998).

Despite increased understanding of biodiversity in general, little is known about microbial diversity and its influence on ecosystem functioning (Bell et al. 2005) or its place in whole food webs (Cochran-Stafira and von Ende 1998). This is in part due to the difficulty defining a microbial species (Doolittle 1999) and to the assumption that many microbes are functionally redundant (Torsvik et al. 2002). Furthermore, patterns of microbial biogeography (Martiny et al. 2006)

and the spatial relationships of microbes (Franklin and Mills 2003, Green et al. 2004, Horner-Devine et al. 2004) remain poorly understood. Microbial community diversity and functioning may be context dependent. Context dependence is important because not all microbially-mediated ecosystem functions will proceed equally in all habitats. Although microbial community boundaries are difficult to draw, the importance of microbes to trophic relationships and the functional consequences of biodiversity is an important arena of research.

My dissertation evaluates some of the causes and consequences of diversity in microbial communities, focusing on fungi and bacteria in model aquatic food webs and naturally occurring soil food webs. In the first chapter, I show that increased bacterially-mediated decomposition in more diverse food webs is associated with a shift in bacterial community composition. This shift in composition is due to selective pressure from more diverse grazing communities of eukaryotes (Krumins et al. 2006). In the second chapter of this dissertation, I show that simulated nitrogen deposition affects the diversity and composition of bacterial communities differently than fungal communities and that the microbial response depends on a geographical context. In the third chapter of this dissertation, I show that naturally occurring soil food webs can be simultaneously affected by top-down and bottom-up trophic control. Again this response is context dependent and influenced by biogeographic factors.

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Chapter 1

Indirect Effects of Food Web Diversity and Productivity on Bacterial Community Function and Composition

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Summary

1. Previous evidence suggests that bacterially-mediated decomposition of complex organic substrates increases with greater food web diversity. I attempted to identify changes in bacterial community composition and function associated with increased decomposition in more diverse food webs.
2. I used aquatic microcosms where I manipulated productivity with different initial nutrient concentrations. I created a diversity gradient by establishing communities of eukaryotes with 0 (bacteria alone), 1, 2, or 4 microbe species (protists and rotifers) in each of four trophic levels: producers, herbivores, bacterivores and predators. The initial bacterial community was standardized across all treatments. To determine effects of productivity and diversity on the bacterial community, I measured: decomposition, abundance, diversity of colony morphotypes (a measure of composition) and community level physiological profiles (CLPP) (a functional profile based on carbon substrate utilization).

3. Decomposition increased with greater eukaryotic species richness and was not influenced by productivity. Bacterial abundance remained constant with increasing eukaryotic species richness at low productivity, but significantly declined at high productivity. Eukaryotic species richness together with productivity influenced the composition of the bacterial community. However, the CLPP was strongly influenced by productivity and not species richness.
4. Food web diversity and productivity interact to influence bacterial community composition and function. In more diverse food webs, bacterial activity (decomposition) increased despite lower population abundance.

Introduction

The decomposition of organic matter by bacteria is a fundamental process in carbon cycling with potential impacts on community properties including patterns of species diversity (Jiang and Morin 2005), trophic structure (Hairston Jr. and Hairston Sr. 1993), nutrient flux (Harte and Kinzig 1993, Loreau 1994) and stability through increased mineralization of nutrients (Barsdate et al. 1974, DeAngelis 1992) and reduced fluctuations in nutrient availability (DeAngelis 1992). However, the connection between eukaryotic diversity and bacterial functioning remains poorly understood. This connection is important because bacteria play a central role in many ecosystem processes. Given that global

declines in eukaryotic species diversity have occurred (Sala et al. 2000), it is important to explore the indirect effects of declining diversity on bacterial communities. Consumer effects on prey and productivity have been demonstrated in plant-herbivore models (De Mazancourt et al. 1998), and in experiments using microbial communities (Naeem and Li 1997), insect-plant interactions (Belovsky and Slade 2000) and ungulates grazing on the Serengeti grasslands (McNaughton 1976). The relationship between grazing by heterotrophic protozoa and bacterially-mediated decomposition in aquatic systems appears to be broadly similar to the positive effects of grazers on primary production in some terrestrial systems. Decomposition of organic matter (Fenchel and Harrison 1976, Sherr et al. 1982, Tso and Taghon 1999) and total community respiration (Coleman et al. 1978) both increase when protozoa graze on bacteria. The mechanisms causing this increase remain controversial. Grazing can liberate nutrients through bacterial cell lysis and excretion (Barsdate et al. 1974, Fenchel and Harrison 1976). Preferential grazing on larger bacteria can also change the composition of bacterial communities (Simek et al. 1995, Langenheder and Jurgens 2001). These results suggest different possible mechanisms underlying links between bacterial metabolic activity and consumer pressure. Much less is known about how the diversity of all trophic levels within a multitrophic food web can alter bacterial communities and decomposition (see (McGrady-Steed et al. 1997).

Numerous studies suggest that species diversity can influence various ecosystem properties including primary production, temporal stability, and

nutrient uptake (Tilman and Downing 1994, Hector et al. 1999, Tilman et al. 2001). The majority of this research has focused on primary producers and has not examined the effects of consumers (Duffy et al. 2001), or the effects of diversity distributed across an entire food web (Petchey et al. 2002). Within the small sample of studies that manipulated diversity over multiple trophic levels (Naeem et al. 1994, McGrady-Steed et al. 1997, Downing and Leibold 2002) and explored diversity effects on decomposition, only McGrady-Steed et al. (1997) found that an increase in decomposition accompanied increasing eukaryotic species richness. However, they did not identify proximal mechanisms for the response. Because bacterial diversity was not manipulated directly, positive indirect effects of eukaryotic species diversity on bacterially-mediated processes seem plausible.

Indirect effects of eukaryotic diversity on the bacterial processes that drive decomposition could arise from two potentially interdependent mechanisms. First, the more diverse metabolic processes associated with greater eukaryotic species richness might enhance nutrient cycling and promote greater metabolic activity by bacteria (Barsdate et al. 1974, DeAngelis 1992) . Second, a greater diversity of eukaryotic grazers may consume a wider variety of bacterial taxa, causing a change in bacterial community composition and functioning (Langenheder and Jurgens 2001). Here I test these hypotheses by describing effects of eukaryotic species diversity on decomposition and bacterial community composition and functional profiles. Microcosms are ideal for testing these hypotheses as they allow easy manipulation of diversity and provide a tractable

model system to follow complex food web patterns over multiple generations (Gause 1934, Fenchel and Harrison 1976, McNaughton 1988, McGrady-Steed et al. 1997, Naeem and Li 1997, Petchey et al. 2002).

Methods

Microcosm Assembly

Aquatic microcosms were established with two levels of nutrient concentrations to manipulate productivity: 0.7 gl^{-1} of protist pellet (a nutrient source for protist cultivation from Carolina Biological Supply, Burlington, NC) and 1.67 gl^{-1} soil, or 0.07 gl^{-1} protist pellet and 0.167 gl^{-1} soil. Soil came from an organic garden on the Rutgers University campus and was used to supplement the media with trace nutrients and minerals. High productivity and low productivity treatments had total phosphorus concentrations of 145.8 $\mu g\ L^{-1}$ and 25.4 $\mu g\ L^{-1}$ respectively. These concentrations span the mesotrophic to hypereutrophic range (Wetzel 2001). Past research using similar manipulations of nutrient concentrations shows that they produce an increase in the abundance and biomass of organisms that are consistent with increased productivity (Kaunzinger and Morin 1998, Jiang and Morin 2005, Steiner et al. 2005).

On day ten of the experiment, one sterile, dried and pre-weighed wheat seed was added to each microcosm to provide the target substrate for decomposition. Gradients of diversity were created by establishing 0 (bacteria control), 1, 2 or 4 eukaryotic species in each of four trophic levels: primary producers (unicellular algae), algal consumers (protozoa and rotifers),

bacterivores (protozoa) and top predators (protozoa). The 0 eukaryotic diversity treatment was intended to create a control containing only bacteria (although contamination by one unidentified algal species and one small ciliate affected all treatments). The standardized bacteria only control was established by inoculating microcosms with three species of bacteria (*Serratia marcescens, Bacillus cereus* and *Bacillus subtillus*) consumed by all bacterivores in our study plus an inoculum pooled from cultures of all eukaryotic species used in this experiment. Within each diversity treatment level, I established four different compositional combinations (Table 1) to avoid confounding eukaryotic species composition with diversity. Compositional combinations served as replicates for the diversity treatments, and species were randomly drawn from a pool of candidate species at each trophic level. Every treatment and compositional combination was replicated twice, except for the functional profile analysis (see below), which was replicated three times. Starting during the second week of the experiment and continuing weekly thereafter, 10% of the media (by volume) was replaced with fresh and sterile media. Microcosms were incubated at 22°C with 12h light/12h dark, and the experiment was maintained for 40 days.

Sampling

To evaluate the effects of eukaryotic diversity and productivity on bacterial community structure and function, the following variables were measured: decomposition (as % dry weight lost of a wheat seed), bacterial abundance and community structure based on colony morphotypes on R2A agar (Difco Laboratories, Inc., a standard oligotrophic medium frequently used to culture

aquatic environmental bacteria (Franklin et al. 2001, Garland et al. 2001, Muller et al. 2002)), and community level physiological profiles (CLPP) (Garland and Mills 1991). The CLPP method serves as a relative measure of the functional diversity of a bacterial community using Ecolog (Biolog, Hayward, CA) microtiter plates containing an array of 31 different carbon substrates and a water control well. The response of the community to the carbon substrates is determined spectrophotometrically and provides a profile of that bacterial community's metabolic diversity.

To monitor food web diversity, rotifers and protists were sampled every 3-4 days up to day 22 and then every 3-6 days up to the final date of the experiment. Microcosms were first gently mixed and between 900-1500 μl of medium were removed and examined with a dissecting microscope. Rare taxa were enumerated by counting the entire sample volume while abundant taxa were counted in smaller sub-samples of known volume. Algae and small microflagellates were enumerated using a hemocytometer and a compound microscope.

Decomposition and Bacterial Enumeration. Wheat seeds were removed on day 40, dried for 48h at 70°C and weighed to determine mass loss, which was used to calculate percent decomposition. On day 20, bacterial abundance was measured using dilution plating on R2A agar. Microcosm suspensions were diluted tenfold, and plates were inoculated and incubated at room temperature for 48 hours prior to counting.

Colony Morphotype Analysis. Colony morphotype analysis was conducted in a second experiment of similar design. Morphotypes were identified using a colony counter or dissecting microscope. Each morphotype was characterized by size, color, margin, edge and elevation. The experiment was established as outlined above, but diversity treatments were limited to high (at least three species at each trophic level) and low levels (one species at each trophic level), and did not include compositional replicates. Up to fourteen colony types were distinguishable on R2A agar and the relative abundance of the ten present at the end of the experiment were estimated to characterize bacterial community composition. I acknowledge the difficulty in considering all bacterial taxa with culture-based methodology, and I emphasize that this approach provides only a relative comparison of the cultivable bacteria over our treatments (Ovreas and Torsvik 1998, Hughes et al. 2001, Ward 2002). However, prior studies using culture-based methods have effectively captured relative differences among experiment treatments (Garland et al. 2001, Muller et al. 2002). Hence, plate counts can be used as effective proxy measures of relative bacterial diversity and abundance.

Bacterial Functional Profile. CLPP was sampled on day 20 by diluting samples tenfold and inoculated each sample into Ecolog (Biolog Inc. Hayward, CA) microtiter plates. Plates were incubated for 48h in the dark at room temperature. Biolog plates contain a redox dye in each well that indicates metabolism of the substrate by bacteria. The response to the carbon substrates was determined using a spectrophotometer and absorbance at 590 nm; all substrate wells were

blanked against the water control well. Due to order of magnitude differences in the abundance of bacteria between high and low productivity treatments, absorbance data acquired in the CLPP were normalized against the well with the maximum absorbance for each productivity treatment.

Data Analysis

Eukaryotic species richness is presented as 'average realized species richness' rather than as the initial richness in each treatment because not all introduced species persisted throughout the course of the experiment (Table 1). This value was calculated as the mean of eukaryotic richness over the course of the experiment for each replicate. An analysis of covariance (ANCOVA) explored the relationship between average realized species richness and decomposition and bacterial abundance. Productivity was treated as a fixed effect and average realized species richness as a continuous covariate in testing the hypothesis that bacterially-mediated decomposition and abundance increased with increasing food web diversity at both productivity levels.

Principal components analysis (PCA) summarized the bacterial community profiles from both the colony morphotype analysis and the CLPP. The abundance of each colony morphotype was logarithmically transformed prior to analysis. Abundance of the 10 colony morphotypes was treated as a separate variable and the component scores for the productivity and diversity treatments. To test the hypothesis that eukaryotic species richness and productivity altered the metabolic functional profile of the bacterial community, normalized absorbance measures of the CLPP were transformed into binary measures

(1=substrate used and 0=substrate not used) and relationships between the diversity treatments and productivity summarized using PCA. All PCA analyses were followed by a multivariate analysis of variance (MANOVA). Productivity and diversity treatments were fixed factors while the first two component scores were the dependent variables. I conducted all statistical analyses using SAS version 9.1 (SAS Institute, Cary, NC).

Results

Decomposition increased with increasing realized eukaryotic species richness (Fig.1). ANCOVA showed that average realized species richness positively influenced decomposition ($F_{1,51}$=8.44, P<0.01), but productivity ($F_{1,51}$=0.61, P=0.43) had no detectable effect. Eukaryotic species diversity ($F_{1,51}$=7.55, P<0.01) and productivity ($F_{1,51}$=4.17, P<0.05) interacted to affect bacterial abundance (productivity X diversity, $F_{1,51}$=10.18, P<0.001, Fig. 2). At low productivity, bacterial abundance showed little change with increasing eukaryotic diversity, while at high productivity, bacterial abundance decreased with increasing eukaryotic diversity (Fig. 2).

Eukaryotic species diversity significantly altered bacterial community composition in the second experiment (Fig. 3 and Table 2, Wilk's Lambda $F_{6,32}$=21.28, P<0.0001, a diversity effect). The high diversity treatments in both high and low productivity treatments produced a different distribution of colony morphotypes than the low diversity treatment or the control. The composition of the bacterial communities in the low diversity treatments and the bacterial control

were more similar in the same productivity level (Fig. 3, Wilk's Lambda $F_{3,16}$=22.12, P<0.0001, a productivity effect) than the same diversity level.

Eukaryotic diversity treatments did not alter the functional profile of the bacterial community measured by the CLPP analyses. However, productivity significantly changed the functional profile of the bacterial communities (Fig.4, Wilks' Lambda $F_{3,68}$=53.9 and P<0.0001). Approximately half of the carbon substrates utilized in the microtiter plates were significantly correlated with variation described by principal component (PC) axis 1 (Table 3).

Discussion

Decomposition increased with increasing species richness in our food webs, a result that is consistent with previous findings in similar systems (Fenchel and Harrison 1976, McGrady-Steed et al. 1997). This result was not influenced by variation in productivity. The absence of a productivity effect was somewhat surprising since metabolic activity at all trophic levels should increase with nutrient availability. For example, Fenchel and Harrison (1976) found an increase in rate of decomposition in media enriched with NO_3^- and PO_4^{-3} presumably because bacteria were previously limited by those nutrients. Instead, I found that interactions between bacteria and the eukaryotes compensated for what would otherwise be a nutrient-limited environment. This conclusion is supported by an increase in wheat seed decomposition despite lower bacterial population size (Fig. 1 and Fig. 2). Thus, despite the fact that a

higher diversity of consumers and producers apparently limited bacterial population sizes, those that remained were more metabolically active.

The patterns seen in our high productivity treatment are analogous to the impacts of increased herbivore diversity on algal biomass observed in other studies (Naeem and Li 1997, Norberg 2000). Our finding suggests that effects of consumer diversity on the abundance of basal species are broadly similar regardless of whether those basal species are autotrophs or heterotrophs. Both producers and decomposers assimilate carbon and inorganic nutrients, but decomposers also mineralize organic matter into CO_2 and inorganic nitrogen and phosphorus making it available to producers and consumers in the food web. Although it was not directly measured in our study, increased material and nutrient flow associated with grazing may have accompanied a greater diversity of species across the entire food web. This is suggested by the decline in bacterial abundance concurrent with an increase in decomposition of the wheat seed (Fig. 1 and Fig. 2).

Theory suggests that a more diverse assemblage of species will more completely exploit a set of available resources (Tilman et al. 1997). Likewise, I expect that a more diverse assemblage of eukaryotic consumers and osmotrophs might utilize more bacterial taxa and dissolved substrates. The resulting increase in material flow and diversity of metabolites may in turn have a positive effect on the metabolic activity of bacterial communities by potentially releasing them from nutrient limitation. Interactions between producers and decomposers (Harte and Kinzig 1993, Naeem et al. 2000, Loreau 2001) and

autotrophs and heterotrophs (Naeem 2001) involve a reciprocal and obligatory exchange of material between these major functional groups. As decomposers are assumed to be carbon limited (Hairston et al. 1960, Harte and Kinzig 1993, Naeem 2001), it is likely that any increase in carbon and nutrient flow associated with the distribution and diversity of eukaryotic species in a food web will facilitate bacterial metabolic activity. In this work, I hypothesized that a greater diversity of metabolites will result from more eukaryotic species in the food web thus maximizing potential for metabolic activity by bacteria. I saw an increase in decomposition (and presumably bacterial metabolic activity) with increasing eukaryotic diversity. However, that change was not accompanied by a change in the metabolic profile (CLPP) of the bacterial assemblage (Fig. 3). Substrate use by bacterial communities did not vary with eukaryotic diversity, but I did observe differences associated with the productivity level of the microcosm. Bacterial communities in high and low productivity treatments were markedly different in the carbon sources they utilized (Table 3), but they did not differ overall in their aggregate activity affecting decomposition. It is interesting that the bacteria utilized a different suite of carbon substrates depending on their environmental productivity level. This may be due to the metabolic state of the bacterial assemblage (i.e. stationary versus growth phase) that is secondary to the metabolic benefits of a diverse food web that I propose here.

Consumer mediated shifts in species composition may influence the whole community's ability to utilize complex carbon sources like wheat seeds, which served as a convenient proxy for other kinds of allochthonous carbon degraded

by aquatic microbial communities. In fact, I hypothesized that a greater diversity of grazers in the food web will utilize a wider variety of bacterial taxa and alter the composition of the bacterial community. In this case, if I assume that resources were plentiful in the high productivity treatments, bacterial abundance was limited by a greater diversity of consumers that were perhaps capable of exploiting more bacterial taxa (Fig. 2). Similar patterns have been observed for relationships between an assemblage of primary producers and their consumers in other studies (Naeem and Li 1997). However, in this experiment, the grazers in high diversity treatments not only consumed more bacteria, but also changed bacterial community composition in more diverse food webs (Fig. 3 and Table 2). This result together with the decline in bacterial abundance suggests that grazing selects for more productive bacteria in more diverse food webs.

Productivity and consumer diversity interact to indirectly increase bacterially-mediated decomposition. This interaction is illustrated by the combined influence of nutrient limitation in low productivity, low diversity food webs and by more pressure on bacterial taxa when diversity is high. The complex interactions between productivity, eukaryotic diversity and bacterial communities may be explained by trophic level interactions. For instance, diversity in multitrophic communities yields increased stability (Steiner *et al.* 2005) and predictability (McGrady-Steed et al. 1997, Morin and McGrady-Steed 2003), and bacterially-mediated decomposition stabilizes communities through regulation of nutrients in the environment (DeAngelis 1992). The combination of bottom-up effects from bacterially-mediated decomposition and top-down effects

from multitrophic diversity may work synergistically to create stable and productive communities. Research and theory have demonstrated that the interactions between functional groups can be complex (Harte and Kinzig 1993, Naeem et al. 2000, Loreau 2001).

I propose that the indirect effects of grazers on decomposition occurred through a combination of two influences: 1. media enrichment associated with heterotroph and autotroph activity leading to increased metabolic activity and 2. selection for a different and more productive bacterial community composition. The implications of this result to biodiversity and ecosystem function research are important. First, the consequences of increased species richness to ecosystem function are not limited to interactions within discrete trophic levels (Duffy et al. 2001, Petchey et al. 2002). Second, bacterial communities and the processes that they control must be studied in the context of complete food webs to avoid missing key interactions with other functional groups (Cochran-Stafira and von Ende 1998). I propose that indirect consequences of altered biodiversity may be as important as direct effects of diversity on function.

Knowledge about the important causes and consequences of biodiversity is growing, but there is still a critical need for research that explores the consequences of changing diversity on multiple trophic levels (Hooper et al. 2005). The difficulty with incorporating bacteria into such multitrophic level studies involves very different scales of sampling (Hughes et al. 2001, Horner-Devine et al. 2003) and different interpretations given to diversity of prokaryotic and eukaryotic diversity (Martiny et al. 2006). Our work did not directly

manipulate bacterial diversity and functioning (see, *e.g.* (Bell et al. 2005). However, I evaluated mechanisms that highlight the potentially important role of bacterial consumers in diversity studies. Such interactions are likely to be important in most food webs where bacteria and eukaryotes interact in potentially complex ways.

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Figure Legends

Figure 1. The relationship between average realized species richness of protists and rotifers and percent decomposition of sterile dried wheat seeds. Productivity level is indicated by fill, and the trend line of all data is displayed for ease of interpreting data. (ANCOVA Species richness effect: $F=8.44$, $P<0.01$, $n=52$)

Figure 2. The relationship between average realized species richness of protists and rotifers and bacterial abundance measured by dilution plating. Productivity level is indicated by fill, and trend lines are displayed for each productivity level for ease of interpreting data. (ANCOVA Species richness effect: $F=7.55$, $P<0.01$, $n=26$; productivity effect: $F=4.17$, $P<0.05$, $n=26$; interaction: $F=10.18$, $P<0.001$).

Figure 3. The separation of bacterial community composition (summarized by the PCA of bacterial colony morphotypes) across high productivity (open symbols), low productivity (closed symbols) and the bacteria only control (circle), low diversity treatment (square), high diversity treatment (triangle). For each diversity treatment symbol $n=4$ and error bars indicate standard deviation.

Figure 4. The separation of bacterial community functional profiles (summarized by the PCA of bacterial carbon substrate utilization) across high productivity (open symbols), low productivity (closed symbols) and the bacteria only control (circle), diversity treatments: level 1 (square), level 2 (triangle), level 4 (diamond).

For each diversity treatment symbol n=3 for the bacterial control and n=12 for the diversity treatments. Error bars indicate standard deviation.

Table 1. Compositional combinations of diversity treatments

		Diversity Treatment Level		
Trophic Level	Organism	1	2	4
Producers	*Ankistrodesmus*		c	a,c,d
	Chlorella	c	a	a,b
	Chrysopsis		b,c	d
	Clamydomonas	b		c
	Cyclotella		a	a,b,c
	Euglena gracilis	d	b,d	a,b,c,d
	Unknown Diatom sp.	a	d	
Herbivores	*Colpidium striatum*	d		b
	Frontonia	b	a	d
	Gastropus		d	
	Lepadella		b	a,c
	Monostyla		c	a,b
	Paramecium aurelia		a	b,c
	Paramecium caudatum	c		a,c,d
	Paramecium tetraurelia		b,d	
	Rotaria		c	b,c,d
	Stylonychia	a		a,d
Bacterivores	*Chilomonas*		b,c	c
	Coleps	d	a	c
	Colpoda cucullus	b	d	b,c
	Colpoda inflate		a	d
	Gastrotrich sp.			a,b
	Loxocephalus		c,d	
	Spirostomum ambiguum			a,d
	Tetrahymena pyriformis			b,c
	Tetrahymena thermophila	c		a,d
	Tillina		b	
	Unknown Protist sp.	a		a,b,d
Top Predators	*Actinosphaerium*		b	a,b,d
	Amoeba sp.		b	a,c
	Blepharisma americanum	b		d
	Didinium			b,d
	Dileptus	d	b	b,d
	Euplotes	c	c	b
	Oxytricha		a,c	
	Stentor coeruleus		d	a,b
	Tetrahymena vorax	a	d	a,c

Compositional combinations a, b, c and d are shown to illustrate which species from each trophic level are represented in the different diversity treatments. Four compositional combinations were used for each diversity treatment level.

Table 2. Correlations between bacterial colony morphotypes and principle component scores

Colony Morphotype	PC1 (18.1% variance)		PC2 (15.0% variance)	
	r	P value	r	P value
small clear	-0.43158	0.035	*	*
butter	-0.78153	<0.0001	*	*
yellow spreader	0.85279	<0.0001	0.42226	0.038
tiny white	-0.68131	0.0002	*	*
super tiny	*	*	*	*
tiny yellow	*	*	-0.46526	0.022
big white	0.42085	0.041	0.73576	<0.0001
medium bright white	0.72438	<0.0001	*	*
tiny orange	0.42732	0.037	*	*
tiny pink	*	*	0.88045	<0.0001

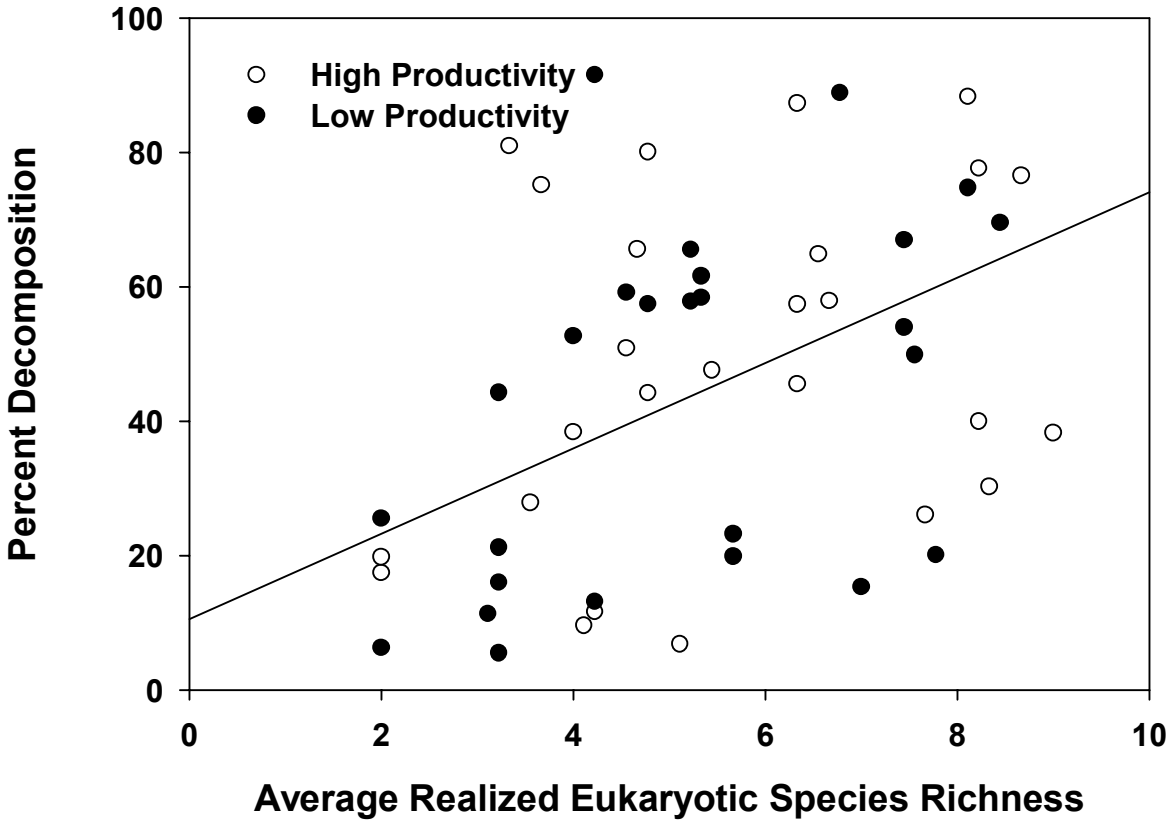
* indicates no significant correlation

Table 3. Correlations between carbon substrates and component scores

Substrate	PC1 (43.1% variance)		PC2 (9.3% variance)	
	r	P value	r	P value
pyruvic acid methyl ester	0.76203	<0.0001	*	*
tween 40	-0.35419	0.0015	*	*
tween 80	*	*	*	*
a-cyclodextrin	*	*	*	*
glycogen	0.83275	<0.0001	0.49639	<0.0001
D-cellobiose	0.76991	<0.0001	*	*
a-D-Lactose	*	*	*	*
B-Methyl-D-glucoside	0.85066	<0.0001	-0.25543	<0.05
D-Xylose	0.46258	<0.0001	*	*
I-erythritol	*	*		
D-Manitol	0.88405	<0.0001	*	*
N-acetyl-D-glucosamine	0.91466	<0.0001	-0.28187	0.0124
D-glucosaminic acid	*	*	-0.23781	0.036
glucose-1-phosphate	0.89144	<0.0001	*	*
D,L-a-glycerol phosphate	0.60573	<0.0001	*	*
D-galactonic acid-g-Lactone	0.80913	<0.0001	-0.32005	0.0043
D-galacturonic acid	*	*	*	*
2-hydroxy benzoic acid	*	*	*	*
4-hydroxy benzoic acid	0.68383	<0.0001	*	*
g-hydroxybutyric acid	0.69685	<0.0001	0.3043	0.0068
itaconic acid	*	*	*	*
a-ketobutyric acid	*	*	*	*
D-malic acid	0.45821	<0.001	0.40878	0.0002
L-argenine	0.37272	0.0008	0.37018	0.0009
L-asparagine	*	*	*	*
L-phenylalanine	*	*	*	*
L-serine	0.25506	0.02	*	*
L-threonine	*	*	*	*
glycyl-L-glutamic acid	*	*	*	*
phenylethylamine	0.68228	<0.0001	0.4579	<0.0001
putrescine	*	*	*	*

* Indicates no significant positive correlation

Figure 1.


High Productivity
Low Productivity
Percent Decomposition
Average Realized Eukaryotic Species Richness
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20
40
60
80
100
0
2
4
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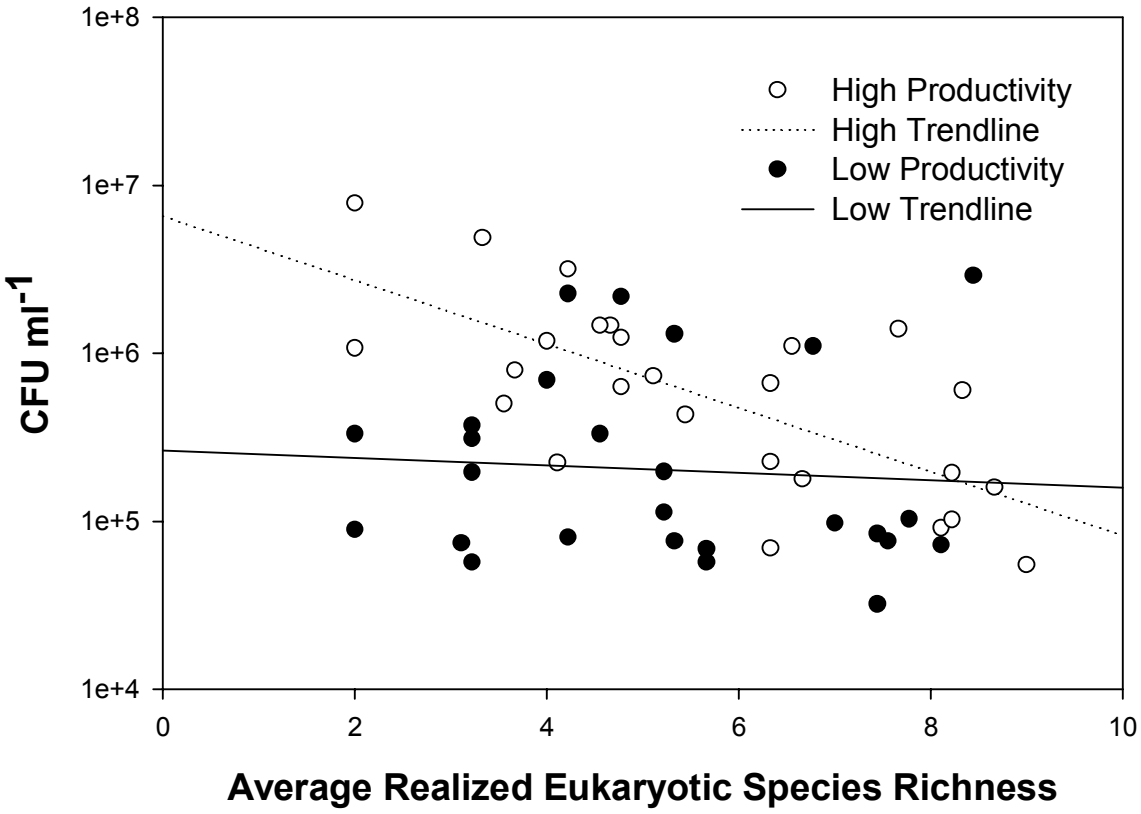
Figure 2.


1e+8
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Low Trendline

Figure 3.

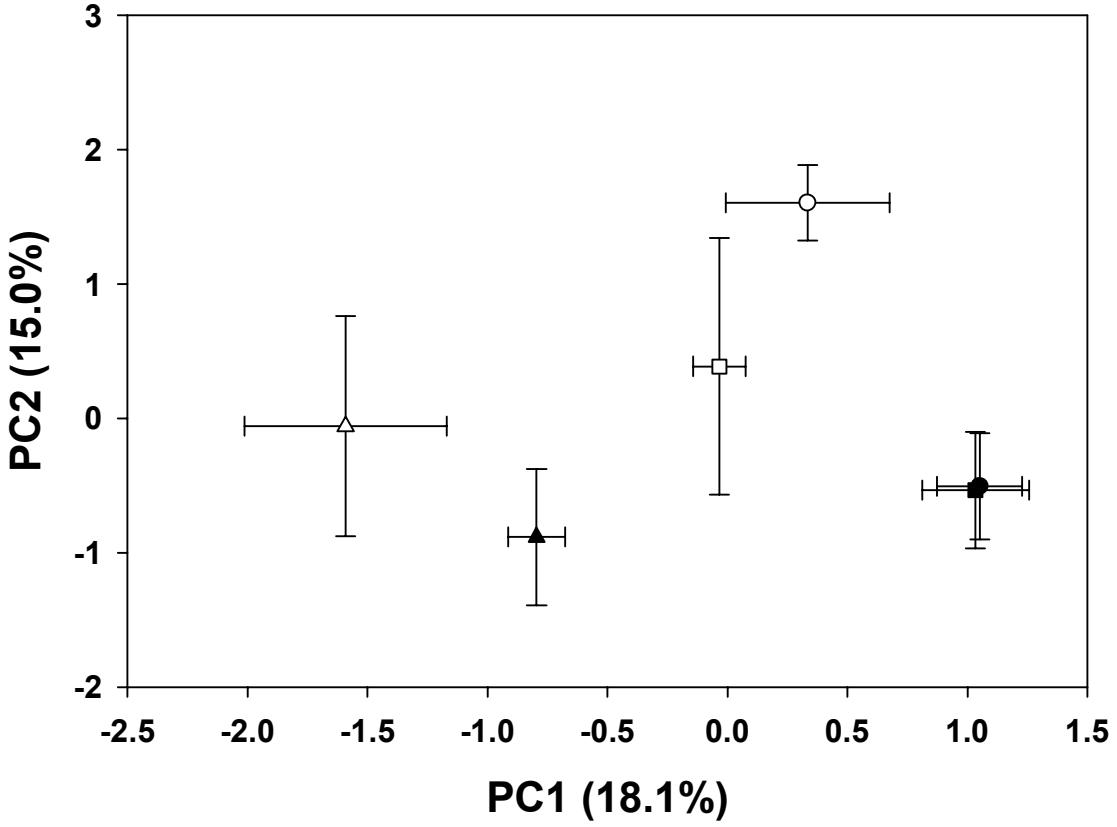
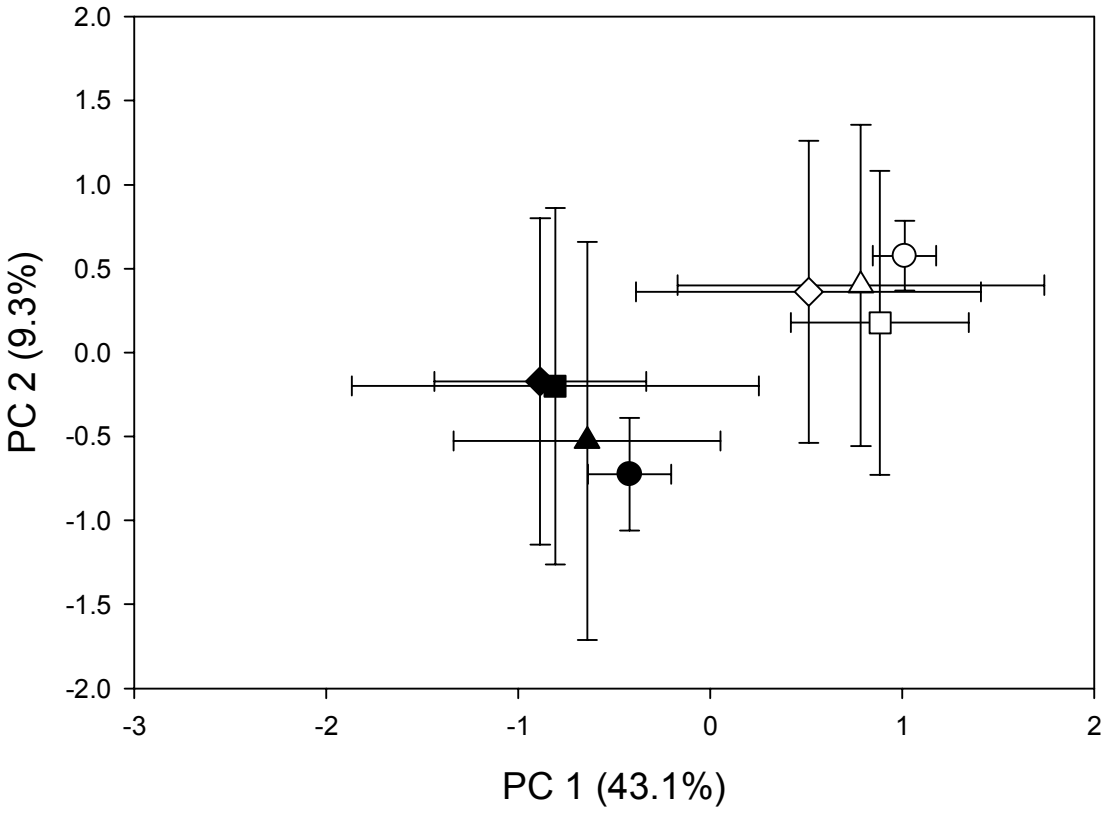


Figure 4.



Chapter 2

Biogeography Affects Soil Microbial Community Response to Simulated Nitrogen Deposition

Abstract

Microbial communities play a pivotal role in soil nutrient cycling, and effects of nitrogen loading on soil fungi and particularly mycorrhizal fungi have been documented. In this experiment, I evaluated the effects of allochthonous nitrogen addition on soil bacteria and fungi in two geographically distinct but structurally similar scrub oak forests, one in Florida (FL) and one in New Jersey (NJ). I applied allochthonous nitrogen as aqueous NH_4NO_3 in three concentrations (0 Kg Ha^{-1} Yr^{-1} (deionized water control), 35 Kg Ha^{-1} Yr^{-1} and 70 Kg Ha^{-1} Yr^{-1}) via monthly treatments over the course of one year. I applied treatments to replicated $1m^2$ plots, each at the base of a reference scrub oak tree (*Quercus myrtifolia in* FL and *Q. ilicifolia* in NJ). I measured microbial community response by monitoring: bacterial and fungal biomass using substrate induced respiration, and several indicators of community composition, including colony and ectomycorrhizal morphotyping and molecular profiling using terminal restriction fragment length polymorphism (TRFLP). Bacterial colony type richness responded differently to nitrogen treatment depending on geographic context, but ectomycorrhizal morphotype richness was not affected by nitrogen or

location. Both experimental sites were dominated by fungi, and FL consistently supported more bacterial and fungal biomass than NJ. Bacterial biomass responded to nitrogen addition, but only in FL. Fungal biomass did not respond significantly to nitrogen addition at either experimental site. The composition of the bacterial community differed between nitrogen treatments and experimental sites, while the composition of the fungal community did not. Our results are surprising in that the bacterial community responded in composition and biomass to allochthonous nitrogen additions while the fungal (including EMF) community primarily did not. Our results imply that bacterial communities may be more sensitive than fungi to intense pulses of nitrogen. However, the response of fungi and bacteria to nitrogen loading may be dependent on geographic context.

Introduction

Soil microbial processes play a critical role in shaping plant community structure and function (Bever et al. 1997, Simard et al. 1997, van der Heijden et al. 1998, Packer and Clay 2000, Baxter and Dighton 2001, Bever 2003). For example, mycorrhizal fungi can help defend a plant against pathogens in experimental systems (Smith and Read 1997), and there is often a direct relationship between mycorrhizal diversity and plant productivity (Baxter and Dighton 2001) or plant diversity (van der Heijden et al. 1998). Energy transfer and metabolic activity in the soil food web hinges on the obligate exchange of carbon and inorganic nutrients between producers, their microbial symbionts and consumers. Mycorrhizae helper bacteria (MHB) can promote the relationship

between mycorrhizal fungi and the host plant by improving root receptivity to the fungus, facilitating fungal growth and improving rhizosphere soil conditions (Garbaye 1994). This response is not universal, and differences in environmental conditions or species composition may reduce the benefits of the mutualism (Jumpponen and Egerton-Warburton 2005).

Nitrogen loading associated with fertilizer use and atmospheric deposition can accelerate the decline of plant diversity and affect the soil organisms in the rhizosphere (Vitousek et al. 1997, Galloway and Cowling 2002). This may have profound influences on nutrient cycling and influence the biotic and abiotic interactions of soil organisms and the environment. Arnolds (Arnolds 1991) first noted the relationship between nitrogen loading and declining soil diversity of ectomycorrhizal fungi (EMF) in Europe. Since that time, multiple field experiments using both natural nitrogen deposition gradients and fertilization manipulations have confirmed shifts in diversity and community composition of mycorrhizae with increasing nitrogen concentration. These studies have found a negative relationship between nitrogen concentration in the soil and diversity of EMF colonizing host trees (Taylor et al. 2000, Lilleskov et al. 2002, Dighton et al. 2004). Some even describe a shift in community composition and the identity of dominant EMF species with the decline in diversity (Lilleskov et al. 2002). Further, this idea has been extended (through molecular profiling) to show that decomposer fungi are sensitive to allochthonous nitrogen input as well (Allison et al. 2007).

Fungal and bacterial communities may respond differently to nitrogen loading depending on their environmental context and site specific soil conditions. The spatial distribution of microbial species and diversity is the subject of debate and comparison to macroorganism patterns (Martiny et al. 2006). Indeed, fungi (Green et al. 2004) and bacteria (Franklin et al. 2000, Franklin and Mills 2003, Horner-Devine et al. 2003) demonstrate local and regional biogeographic patterns. However, very little is known about these factors or the relationship between geographic distribution and function in the environment. This is important because microbes mediate the bulk of biogeochemical processes, particularly nitrogen cycling. Environmental heterogeneity and regional distribution of microbial diversity may cause soil microbial communities to respond differently to nitrogen loading in different locations. Furthermore fungi, particularly mycorrhizal fungi, may be more sensitive than bacteria to allochthonous nitrogen inputs due to their relatively higher C:N and obligate relation with host plants. To date, no studies have simultaneously examined the effects of nitrogen loading on bacterial and fungal communities. Further, this is the first study to evaluate bacterial and fungal response to nitrogen loading under an oak species as opposed to a conifer.

The objectives of this study were: (1) to evaluate the simultaneous response of bacterial and fungal communities to allochthonous nitrogen loading and (2) to evaluate how geographic context and environmental influences interact with the microbial community response to nitrogen loading. I manipulated nutrients by adding NH_4NO_3 in high and low concentration over the

course of one year to replicate experimental plots in Florida (FL) and New Jersey (NJ). I then measured the microbial community response using the following methods: substrate induced respiration (SIR) to determine total microbial biomass (bacterial and fungal), bacterial colony morphotyping, EMF morphotyping, and molecular analysis of bacterial and fungal communities using terminal restriction fragment length polymorphism (TRFLP). The molecular analysis and biomass measures captured both saprotrophic and mycrrorhizal fungi; when discussing these results I use the word 'fungi' to refer to the entire fungal community. The EMF morphotyping only examined the ectomycorrhizal fungi colonizing root tips. Therefore, when discussing these results, I use the acronym EMF to differentiate a subset of the fungal community.

Methods

Site Characteristics

Both experimental sites have dry, low-nutrient, sandy soils. Both sites are fire prone and contain structurally similar scrub oak communities. Prior to starting experiments, I surveyed plant community composition in all plots at each site. Composition was measured as percent cover of each plant within the plot, and the rank order of plants is presented in Table 1. The FL study site is in the NASA Kennedy Space Center/Merritt Island National Wildlife Refuge, an approximately 57,000 ha managed area comprised of brackish estuaries, marshes, scrub oaks, pine forests, and oak/palm hammocks on the Atlantic Coast of central Florida. The research plots are in scrub habitat, adjacent to a

brackish marsh, dominated by *Quercus myrtifolia* with *Serenoa repens* (saw palmetto) in the under story. The NJ site is within the Rutgers University Pinelands Field Station that is part of the greater New Jersey Pinelands Preserve in south-central NJ. The Pinelands includes approximately 304,000 ha of land with heavily restricted development as part of the 445,000 ha NJ Pine Barrens ecosystem. The research plots in NJ are dominated by *Q. ilicifolia* with *Vaccinium angustifolia* (low bush blueberry) in the under story. The two sites are similar in gross vegetation structure and soil types, but experience very different seasonal and climatic influences (Table 1).

Experimental Design and Sampling

This experiment used a 2x3 factorial design with two geographically distinct treatment sites (factor 1) and three different nitrogen addition treatments (factor 2). Over the course of one year beginning in May 2005, I simulated different levels of nitrogen deposition by dispensing aqueous NH_4NO_3 each month in concentrations of 35 Kg Ha^{-1} Yr^{-1}, 70 Kg Ha^{-1} Yr^{-1} and 0 Kg Ha^{-1} Yr^{-1} (deionized water control). I chose these levels of nitrogen as they are comparable to or in excess of levels affecting Europe today (Arnolds 1991). I replicated each treatment combination five times for a total of 15 plots at each treatment site. Each experimental plot measured $1m^2$ and was at the base of a numbered scrub oak tree, *Quercus ilicifolia* in NJ and *Q. myrtifolia* in FL.

I randomly removed three 5cm diameter soil cores from each plot after 12 months of nitrogen additions. I removed each of the three cores for: (1) EMF morphotyping (stored at 4 °C prior to analysis), (2) bacterial colony morphotyping

and biomass measurements using SIR (immediate analysis), and (3) nutrient analysis (immediate analysis). Regarding the second and third core retained for bacterial, SIR and nutrient analyses, I retained the top 10 cm of soil from each core and homogenized the mineral and organic layers. I chose to do this rather than separate organic and mineral horizons because many of the FL plots had a negligible organic layer.

Soil Nutrient Analysis

I measured soil nutrients by collecting the litter, humus and mineral soil fractions from each soil core to a depth of 10cm. I homogenized this material for further analysis. The moisture content was determined by drying soil at 70 °C. I extracted samples from each core using 2.0 M KCl and analyzed for NH_4^+ by ion selective electrode (ISE). I also extracted samples with deionized water (DI) and analyzed for NO_3^- and PO_4^{-3} using the Dionex DX90 ion chromatograph, (Dionex Corp, Sunnyvale CA). I performed all extractions on an approximate 4:1 extractant/ dry wt material basis within 24 hours of sample collection. I performed ISE analysis of NH_4^+ and IC analysis of PO_4^{-3} and NO_3^- according to Standard Methods protocols (Clesceri et al. 1998). I analyzed oven-dried samples for total carbon by infrared CO_2 detection and total nitrogen by N_2 thermal conductivity detection following high temperature combustion using a Leco TruSpec carbon/nitrogen analyzer (Leco Corp., St Joseph MI).

Microbial Community Characterization

Total Bacterial Abundance and Cultivable Bacterial Community Structure I enumerated cultivable bacteria using standard plating techniques on 10%

nutrient agar (Difco Labs, Detroit, MI). I characterized the colony morphotypes that grew after 48 hours at room temperature (~25 °C) by their color, size, margin and elevation. These counts provided a proxy measure of bacterial diversity and composition, and they have been used successfully to capture relative differences in bacterial community structure (Garland et al. 2001, Muller et al. 2002, Krumins et al. 2006). I recognize that only a small fraction of the community is cultivable on solid media (*e.g.* in soils, (Olsen and Bakken 1987), but I can still make useful comparisons of the cultivable bacteria among treatments.

Ectomycorrhizal Community Structure I removed a random and representative sample of root fragments from an intact core designated for EMF analysis and suspended it in water in a gridded petri dish. I characterized the EMF community through direct examination of root tips and ectomycorrhizal morphotyping following the methods of Agerer (Agerer 1987-1999) using a Nikon SMZ dissecting microscope. I counted between 200-400 root tips from each core and quantified the relative abundance of each type.

Bacterial and Fungal Biomass I used a modified SIR method (Beare et al. 1991, Sparling 1995) to separately quantify bacterial and fungal biomass in the soil. I lightly homogenized approximately 13 g of wet soil and placed it into 250 ml media jars. I then treated soil with either 5 ml of 0.064 g ml^{-1} (300 mg) aqueous cyclohexamide in (Sigma Aldrich, St. Louis, MO) to inhibit fungi and

isolate the bacterial community, or 5 ml of 0.013 g ml^{-1} (65 mg) aqueous streptomycin (Sigma Aldrich, St. Louis, MO) to inhibit bacteria and isolate the fungal (eukaryotic) community. I treated another set of soil in jars with DI water (positive control for full microbial activity) or cyclohexamide and streptomycin together (negative control assuming a near sterile jar). For simplicity, I present the results of treated jars and not controls. All treated and control jars were incubated with their antimicrobial compound (or deionized water) for 12 hours at 4ºC. After incubation, I combined an excess of dry glucose (> 300mg, a preliminary dose response experiment determined the saturating mass of glucose) with the soil and attached the jars to an infra-red gas analyzer (Columbus Instruments, Columbus, OH) to measure CO_2 evolution. Under the assumption that respiration and CO_2 evolution correlate with microbial biomass, I calculated bacterial or fungal biomass using the regression equations of Beare et al. (1991) as µg C fungal gdw^{-1} soil or µg C bacterial gdw^{-1} soil. I used the percent moisture of a proximate soil core to calculate dry weight based on the known wet weight of soil added to the jar.

Molecular Microbial Community Characterization Following collection, samples for molecular analysis were stored at -20°C. Later, I extracted whole community DNA from 0.25 g sub-samples using the Ultra Clean Soil DNA Isolation Kit according to their guidelines for maximum yield (MoBio Laboratories, Solana Beach, CA). I analyzed both fungal and bacterial communities for composition differences by amplifying extracted DNA using PCR followed by

terminal restriction fragment length polymorphism (TPFLP) (Liu et al. 1997). Targeting the fungal community, I used a 6FAM (fluorescently labeled) forward primer, ITS1-F (CTTGGTCATTTAGAGGAAGTAA), and an unlabeled reverse primer, ITS4 (TCCTCCGCTTATTGATATGC). These primers amplify the intergenic transcribed spacer region (ITS) of ribosomal DNA and have been used successfully to amplify ascomycete and basidiomycete fungi (Klamer et al. 2002, Allison et al. 2007). Therefore, I assume our molecular profiling captured mycorrhizal as well as saprotrophic fungi. Targeting the bacterial community, I used a 6FAM (fluorescently labeled) forward primer, Bac 27F (AGAGTTTGATCCTGGCTCAG), and an unlabeled reverse primer Bac 1492R (GGTTACCTTGTTACGACTT). These primers amplify the small subunit 16s of ribosomal DNA, and are used extensively to characterize bacterial community structure (*e.g.,* (Blum et al. 2004).

I carried out the bacterial community PCR in 50 µl reactions that included: 1X PCR buffer, 2.0mM $MgCl_2$, 200 µM dNTP (each), 1.0 µM primer (forward and reverse), 0.4 µg μl^{-1} BSA (bovine serum albumin) (Roche Diagnostics, Indianapolis, IN), and 1.25 U DNA polymerase per 50 µl reaction. Unless stated, all PCR reagents were obtained from Applied Biosystems (Foster City, CA). I performed amplification reactions in an MJ Research PTC-200 Thermocycler (Waltham, MA) using the following reaction conditions: initial denaturation at 94°C for 5 minutes followed by 34 cycles of 0.5 minutes at 94°C, 1 minute at 62°C and 2 minutes at 72°C and a final elongation for 3 minutes at 72°C. I carried out fungal community PCR under identical reagent conditions, but within

the 34 cycles, the reaction conditions included an annealing temperature of 50°C for 2 minutes and elongation at 72°C for 3 minutes. The final elongation was held for 5 rather than 3 minutes at 72°C. I validated all PCR reactions on a 1.5% agarose gel.

I digested amplified fungal and bacterial DNA using the restriction enzyme *Hha1* (New England Biolabs, Beverly, MA). I desalted and purified the restriction fragments using the QIAquick Nucleotide Removal Kit (Quiagen, Hilden, Germany) then denatured the fragments at 95°C for 10 minutes prior to electrophoretic analysis. I separated denatured restriction fragments using capillary electrophoresis with an ABI310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Capillary electrophoresis produces an array of multiple terminal fragments of varying length that are detected by their fluorescent marker. Each fragment theoretically represents a unique fungal or bacterial taxa or operational taxonomic unit (OTU). I used Applied Biosystems' GeneScan software to analyze the fragment patterns of each sample and produced a binary array of presence or absence of each OTU in each of our treatment combinations. I established a minimum response threshold of 50 relative fluorescence units for a fragment to be considered an OTU.

Data Analysis

I used a two way analysis of variance (ANOVA) to test for effects of nitrogen treatment and geographic location on: soil nutrients, bacterial colony and EMF morphotype richness, fungal and bacterial biomass, and the

fungal/bacterial biomass ratio (SIR). When appropriate I separated means between nitrogen treatments with a Bonferroni test.

I was able to separate differences in microbial community structure for the following parameters: colony morphotypes of cultivable bacteria, EMF morphotypes and molecular profiles for bacteria and fungi using principle components analysis (PCA). I used a separate PCA for each parameter. The relative abundance of colony morphotypes and EMF morphotypes for bacteria and fungi respectively served as variables for the PCA that separated the communities based on visual morphotype. The presence or absence of OTU served as variables for the PCA that separated bacterial and fungal communities based on molecular profile. I followed all PCA with a multivariate analysis of variance (MANOVA) of the first three component scores to determine significant effects of nitrogen treatment or geographic location. All statistical analyses were conducted in SAS Version 9.1 (SAS Institute, Inc. Cary, NC).

Results

Soil Nutrient Response

Concentrations of extractable soil nutrients were not affected by additions of NH_4NO_3 within either the NJ or the FL sites (Table 2). However, across all plots concentrations of NO_3 are significantly higher in NJ than FL ($F_{1,29} = 10.19$, $P<0.01$), and concentrations of PO_4 are significantly higher in FL than NJ ($F_{1,29} = 24.37$, $P<0.0001$) (Table 2). Therefore, FL soils are nitrogen limited relative to NJ soils, and NJ soils are phosphorus limited relative to FL soils. Although the

higher PO_4 concentrations in the FL soil may have resulted in part from abiotic affects like sea spray and geology, or biotic effects arising from an inability of the microbial community to utilize the PO_4 due to possible nitrogen limitation (*per* Liebig's Law) (Liebig 1840) may be the proximal cause. Total soil carbon, total nitrogen and the ratio of the two (C:N, Table 1) were not significantly different between sample sites or across nitrogen treatments.

Microbial Community Characterization

I found a significant interaction between site and nitrogen treatment affecting bacterial colony morphotypes richness (Fig 1A, $F_{2,24}=3.82$, $P<0.05$). FL supports significantly higher richness of bacterial morphotypes (Fig 1A, $F_{1,29}=33.93$, $P< 0.0001$), and there was no effect of nitrogen treatment on bacterial colony morphotypes. I found no significant interaction between site and nitrogen treatment affecting ectomycorrhizal morphotype richness (Fig. 1B), and there was no significant difference in EMF morphotype richness between FL and NJ or among the nitrogen treatments. These interactions refer to different effects of nitrogen depending on geographic site. Bacterial morphotype richness is lower in nitrogen treated plots than control plots in NJ, but it increases with nitrogen in FL (Fig. 1A). EMF morphotype richness was consistent across sites and nitrogen treatments (Fig. 1B).

I plotted sampling area (assuming each 5 cm diameter soil core removed is equivalent to 19.6 cm^2 of area sampled) versus richness of colony morphotypes (Fig. 2A) and EMF morphotypes (Fig. 2B) described. These results follow logically from Figure 1. The number of bacterial and EMF

morphotypes increased with increasing area sampled, and FL supports a higher richness of bacterial morphotypes than NJ. Our sampling effort may not have been adequate to completely characterize the bacterial and fungal communities in these sites. However, I can still make meaningful comparisons between the sites and treatments.

Both fungal (Fig. 3A, $F_{1,29}$=8.79, P<0.01) and bacterial (Fig. 3B, $F_{1,29}$=18.97, P<0.001) biomass determined by SIR were significantly greater in FL than NJ. Fungal biomass did not respond significantly to nitrogen at either site. In FL, there was a significant difference in bacterial biomass between the low and high nitrogen treatments but not the control (Fig. 3A, $F_{1,29}$=4.63, P<0.05). The fungal to bacterial biomass ratio did not significantly change with nitrogen addition and only showed a non-significant trend (Table 1, $F_{1,29}$=2.96, P=0.098) to be higher in FL than NJ.

Bacterial community composition was significantly different between the two experimental sites. This result was seen both in community characterization of colony morphotypes (Fig. 4A, Wilk's Lamda $F_{1,29}$=8.24, P< 0.001) and molecular fingerprints using TRFLP (Fig. 4B, Wilk's Lamda $F_{1,29}$=3.37, P< 0.05). I found significant effects of nitrogen treatments in the bacterial colony morphotypes in NJ only (Fig. 4A, Wilk's Lambda $F_{2,29}$=2.35, P<0.05), but not in the molecular profiles. Interestingly, fungal community composition was not different at either site or under nitrogen treatments. I found this result through both ectomycorrhizal morphotypes (Fig. 5A) and TRFLP (Fig. 5B).

Discussion

The soil from FL (Schmalzer and Hinkle 1996, Schortemeyer et al. 2000) and NJ (Tedrow 1998) is highly porous, sandy, and known to leach soluble nutrients. Data from greenhouse experiments using native soil from the NJ site shows that additions of NH_4NO_3 (in concentrations comparable to this study) did not result in a change in oak seedling (*Quercus rubra*) biomass relative to controls (unpublished data, J.A. Krumins). I do not believe the nitrogen added in the present study was assimilated by the plants. However, even if it was taken up by plants, indirect effects of the nitrogen on the detrital food web should have been seen as the 'brown world' (detrital based food webs), and 'green world' (producer based food webs) connect in the rhizosphere (Wardle 1999a, Moore et al. 2003, Moore et al. 2004). Furthermore, other work in these systems showed that nitrogen additions did not support a higher density of microarthropod consumers (Krumins et al. In Review). I suspect that our aqueous nitrogen additions quickly leached from the biologically active portion of the soil, before effects on the biota could take place. This conclusion has very important environmental implications. Soluble nitrogen not assimilated into biotic components of soil will be transported to waterways and groundwater where it can lead to eutrophication (Aber et al. 2003, Galloway et al. 2003).

The belief that the majority of the nitrogen likely leached from the system may explain the fact that EMF diversity did not respond to nitrogen additions (Fig. 1B) as opposed to other studies that showed a response (Dighton et al. 2004).

Further, there was no difference in composition of the EMF community (Fig. 5A) or molecular profiles of fungi (Fig. 5 B) with nitrogen concentration or between sites. Porous soil at these sites may explain the lack of fungal response to allochthonous nitrogen additions even though other studies have found an effect of nitrogen additions on EMF morphotype diversity (Dighton et al. 2004), spores of vesicular arbuscular mycorrhizae (VAM) (Johnson 1993) and molecular profiles of fungal communities (Allison et al. 2007). In fact, most of the evidence for an effect of nitrogen deposition on mycorrhizae comes from naturally occurring deposition gradients that have been affecting the environment for extended periods of time (Arnolds 1991, Egerton-Warburton and Allen 2000, Lilleskov et al. 2002, Dighton et al. 2004, Lilleskov 2005). Relative to the time scale of the industrial age and modern nitrogen deposition, our treatments were an intense pulse onto soils known to leach nutrients. Ambient nitrogen deposition is higher in NJ than FL (Table 1). Between site differences may in part be attributable to the press of ambient nitrogen deposition. Significant declines in EMF diversity have been observed across naturally occurring, shallow nitrogen deposition gradients (Dighton et al. 2004). The results of Dighton et al. (2004) contrasted with the findings I present here speak to the importance of time and the long-term effects of even a small amount of nitrogen on a fungal community. Furthermore, our study examines the response of EMF to nitrogen in association with oaks. It is believed that fungi in association with hardwoods may be less sensitive to excess nitrogen (Taylor et al. 2000). The field results of Dighton et al. (2004) were from data collected from mature pitch pine (*Pinus*

rigida) within the NJ Pine Barrens ecosystem, and they found significant differences in EMF morphotype richness.

As opposed to EMF, bacterial colony morphotypes were affected by an interaction between nitrogen concentration and geographic location (Fig. 1A). In FL, colony morphotype richness increased with increasing nitrogen concentration. In NJ, the response was mixed; the lowest diversity of colony morphotypes was found in the low nitrogen treatment and not the control. Interestingly, I found a similar response in FL when bacterial biomass decreased in the low treatment relative to the control, but increased in the high treatment relative to the control (Fig. 3A). This result is difficult to interpret because neither nitrogen treatment was different than the control. Non-linear responses to nitrogen addition may be due to the spatially patchy concentration of soluble nutrients in these plots (see standard error of the mean for NH_4 and PO_4 in Table 2). I think the biomass change was seen in the bacterial community and not the fungal community due to differences in their individual growth patterns. Individual bacteria can access nutrients and divide quickly. Fungi grow more slowly and may not have been able to access soluble nutrients that were quickly leached from the soil.

The divergent responses of bacterial and fungal communities may have a significant impact on the health of forest communities and ecosystem functioning. Bacterial biomass responded to nitrogen addition in FL (Fig. 3A), and bacterial community compositional changed in both FL and NJ (Fig. 4A). Nitrogen addition appears to be differentially affecting bacterial and fungal communities,

and for bacteria, this may depend on their environmental or geographic context. The outcome of diverging bacterial and fungal communities will have a significant impact on functional relations in soil. Changes in the bacterial but not fungal community could alter long standing symbioses between bacteria and EMF (Garbaye 1994), or it could disrupt soil processes like decomposition and nutrient cycling by altering the balance between the fungal and bacterial energy channels in soil (Moore and Hunt 1988).

The number of bacterial and EMF morphotypes increased with each additional plot sampled (Fig. 2) underscoring the highly diverse (Torsvik et al. 2002) and patchy nature (Franklin and Mills 2003) of microbial communities in soil. The incomplete sampling of these communities may have limited my ability to detect differences between the two sites or in the response to the nitrogen treatments. All microbial sampling methods are selective (Hughes et al. 2001). Hence, it is important to view microbial communities through multiple 'lenses' as I have done here. The molecular methods may not have resolved differences in the communities to the extent the microscopic or culture based methods did due to the challenges of amplifying whole community DNA from environmental samples. The NJ samples in particular were difficult to amplify due to high humic content in the soil combined with relatively low microbial biomass. In spite of the sampling variability I encountered in this experiment, meaningful trends in microbial community response (or non-response) emerge. This emphasizes the importance of studying microbial community response to environmental change within the context of different geographic locations.

In conclusion, bacterial and fungal communities responded differently to allochthonous nitrogen inputs. This perhaps reflects their differing stoichiometry, growth rates and ability to acquire nutrients. However it is interesting that bacterial community composition changed with nitrogen addition and the fungal community did not. I think this differential response is due to the limited ability of these soils to retain soluble nitrogen. Bacteria utilize resources and grow faster; they were able to incorporate some of the nitrogen whereas the fungi were not. This was particularly the case at the FL sites where phosphorus is in excess allowing fast growing bacteria to possibly immobilize the nitrogen. The results presented here have important implications for understanding microbial communities and ecosystem health. My results show that geographic context affects the microbial response to environmental perturbations like excess nitrogen loading. In a changing world, microbial communities are likely to respond to environmental perturbation in complex and unpredictable ways.

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Figure Legends

Figure 1. Bacterial colony morphotype richness (A) and ectomycorrhizal morphotype richness (B). All bars represent mean +/- SE and n=5.

Figure 2. Sampling effort versus morphotype accumulation curves for A) colony morphotypes and B) EMF morphotypes from both FL and NJ. Each point represents one soil core within one sampled plot.

Figure 3. Bacterial (A) and fungal (B) biomass as measured by SIR. Different letters over FL bars indicate significant effects of the nitrogen treatments (means separation by the Bonferroni test). All bars represent the mean +/- SE of all treatments at each site and n=5.

Figure 4. PCA plot of bacterial community characterization of colony morphotypes (A) and molecular fingerprints using TRFLP (B). Each symbol represents the mean +/- SE of the component scores and n=5.

Figure 5. PCA plot of fungal community composition characterized by EMF morphotype (A) and molecular fingerprint using TRFLP (B). Each symbol represents the mean +/- SE of the component scores and n=5.

Table 1. Comparison of biotic and abiotic characters from the New Jersy and Florida experimental sites.

	New Jersey Pinelands	Cape Canaveral Florida
Ranked Dominance of Vegetation in Plots	*Quercus ilicifolia* *Q. prinus* *Q. velutina* *Vaccinium angustifolium* *Carex striata* *Q. alba* *Pinus echinata* *Q. coccinia* *Q. stellata* *P. rigida* *Gaylussacia sp.*	*Quercus myrtifolia* *Q. incana* *Serrenoa repens* *Q. chapmanii* *Rhynchospora megalocarpa* *Vaccinium myrsinities* *Ximenia americana* *Aristida stricta* *Tellansia sp.* *Galactia elliota*
Average Depth to O Horizon (cm +/- SE)[a]	3.67 +/- 0.3255	2.47 +/- 0.5259
Average Total C:N of Soil (+/- SE)[b]	61.19 +/- 5.28	76.38 +/- 11.41
Soil Series[e]	Evesboro (mesic, coated lamellic quartzipsamments)	Pomello (sandy, siliceous, hyperthermic oxyaquinc alorthods)
Average Fungal/Bacterial Biomass Ratio (+/-SE)[b]	1.32 +/- 0.015	1.37 +/- 0.028
Rainfall June 2005-May 2006[c]	94.43 cm	132.91 cm
NH_4 Deposition June 05-May 06[c]	4.1 mg l^{-1}	1.71 mg l^{-1}
NO_3 Deposition June 05-May 06[c]	19.18 mg l^{-1}	8.77 mg l^{-1}
Latitude and Longitude	39° 91' N and 74° 53' W	28° 36' N and 80° 40' W
Average Annual Temperature[d]	12.3 °C	22.4 °C

[a] Values are different by a t-test, $P<0.05$

[b] No significant difference

[c] National Atmospheric Deposition Program (NADP) Champaign, IL

[d] National Oceanic and Atmospheric Administration (NOAA)

[e] Web Soil Survey: http://websoilsurvey.nrcs.usda.gov/app/

Table 2. Average soil nutrient concentration after one year of nitrogen additions. Values represent mean +/- se and n=5.

Site	Nitrogen Treatment	NO_3-N (µg g^{-1} soil)	NH_4-N (µg g^{-1} soil)	PO_4-P (µg g^{-1} soil)
Florida	**O Kg Ha^{-1} Yr^{-1}**	0.1714 +/- 0.022	3.3443 +/- 1.96	1.1565 +/- 0.467
	35 Kg Ha^{-1} Yr^{-1}	0.1548 +/- 0.011	0.5231 +/- 0.069	1.445 +/- 0.494
	70 Kg Ha^{-1} Yr^{-1}	0.1484 +/- 0.030	1.6640 +/- 0.683	1.4827 +/- 0.134
New Jersey	**O Kg Ha^{-1} Yr^{-1}**	0.1257 +/- 0.028	2.998 +/- 0.590	0.3128*
	35 Kg Ha^{-1} Yr^{-1}	0.0962 +/- 0.002	1.9537 +/- 0.486	bdl
	70 Kg Ha^{-1} Yr^{-1}	0.1395 +/- 0.016	2.1744 +/- 0.385	bdl

* only one replicate was above detection limit

bdl below detection limit (for PO_4 detection limit = 0.04 mg PO_4-P l^{-1})

Figure 1.

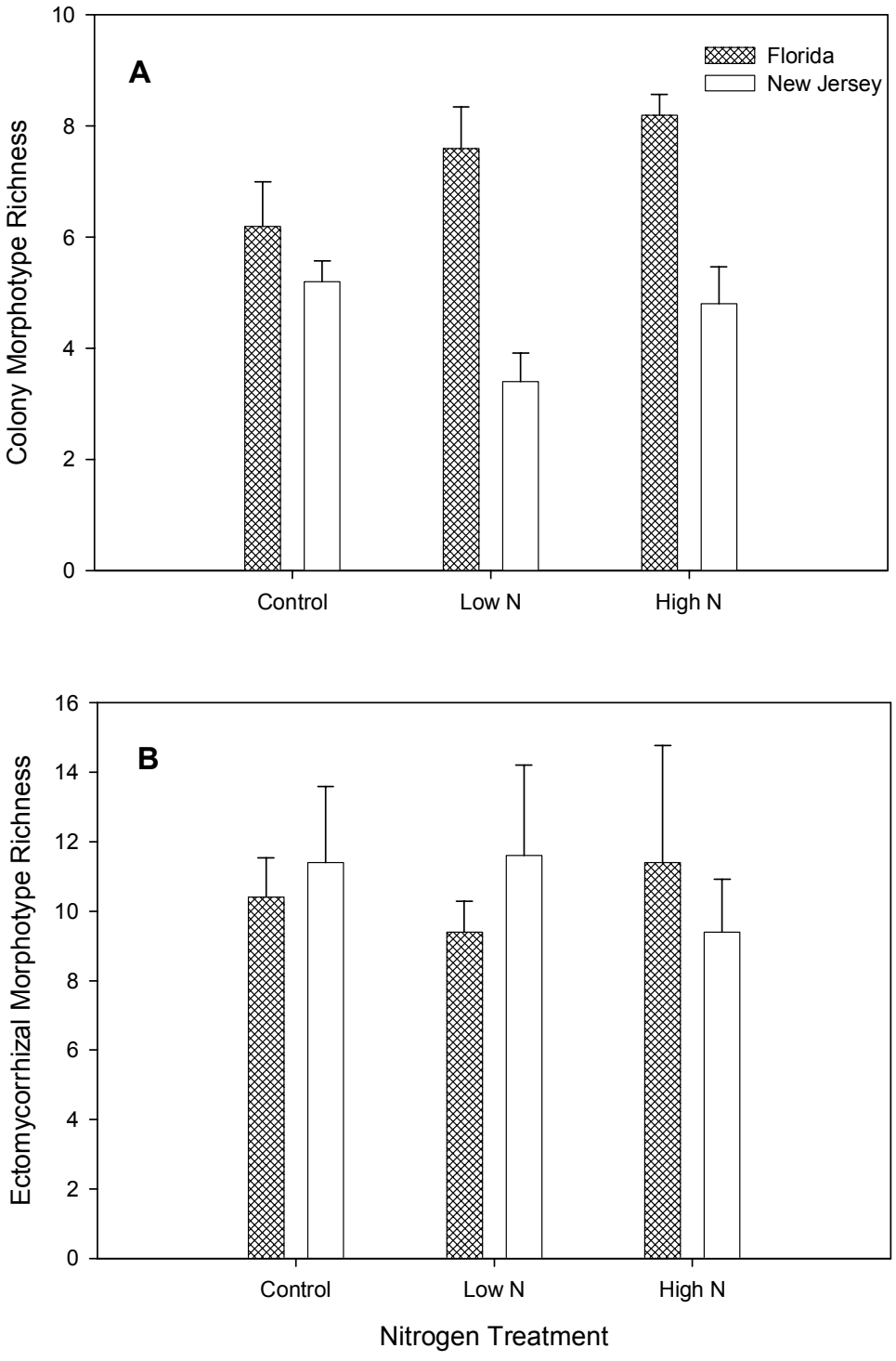

A
Florida
New Jersey
Colony Morphotype Richness
0
2
4
6
8
10
Control
Low N
High N
B
Ectomycorrhizal Morphotype Richness
0
2
4
6
8
10
12
14
16
Control
Low N
High N
Nitrogen Treatment

Figure 2.

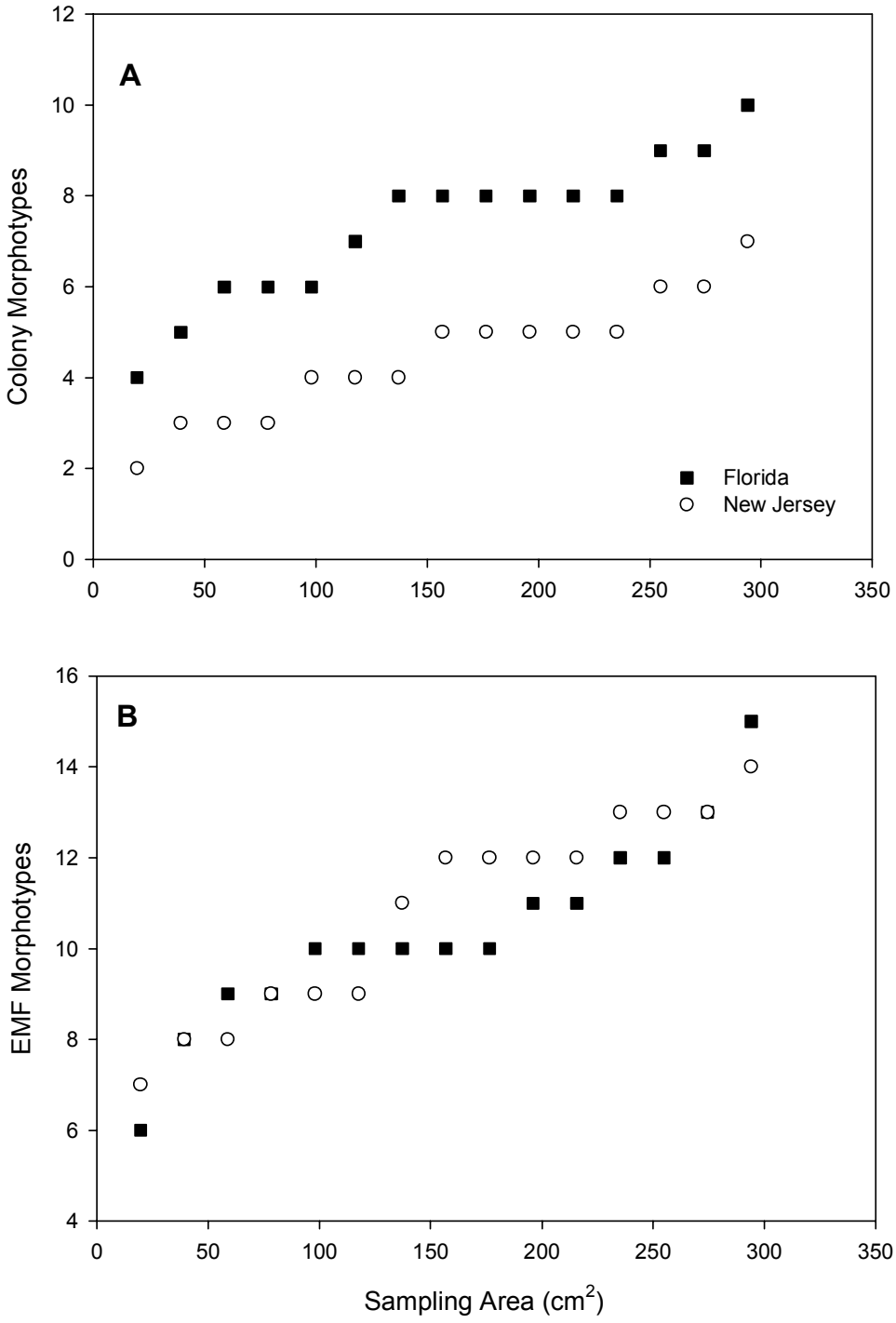

A
Colony Morphotypes
Florida
New Jersey
B
EMF Morphotypes
Sampling Area (cm2)

Figure 3.

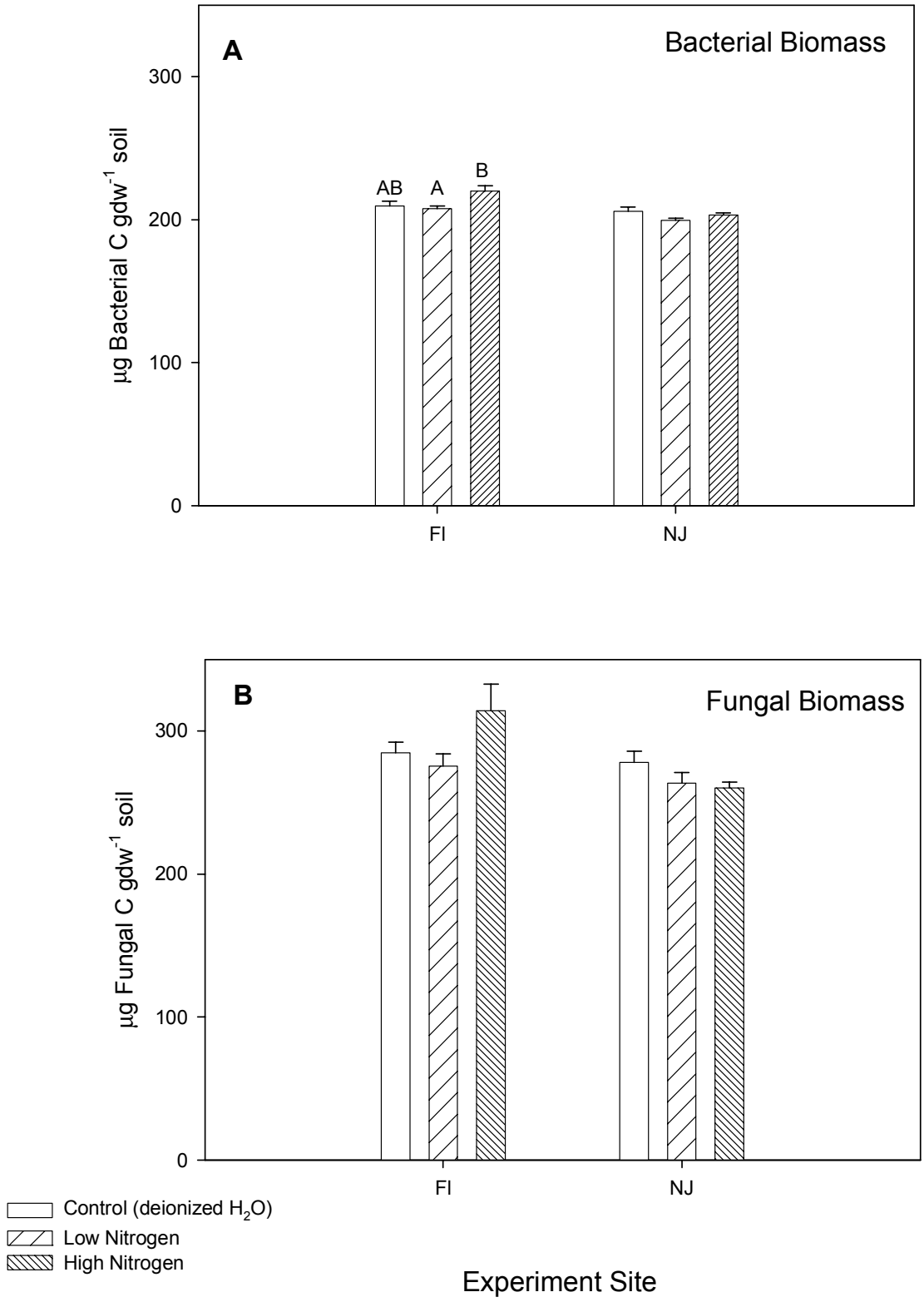

A
Bacterial Biomass
µg Bacterial C gdw-1 soil
300
200
100
0
AB
A
B
FI
NJ
B
Fungal Biomass
µg Fungal C gdw-1 soil
300
200
100
0
FI
NJ
Control (deionized H2O)
Low Nitrogen
High Nitrogen
Experiment Site

Figure 4.

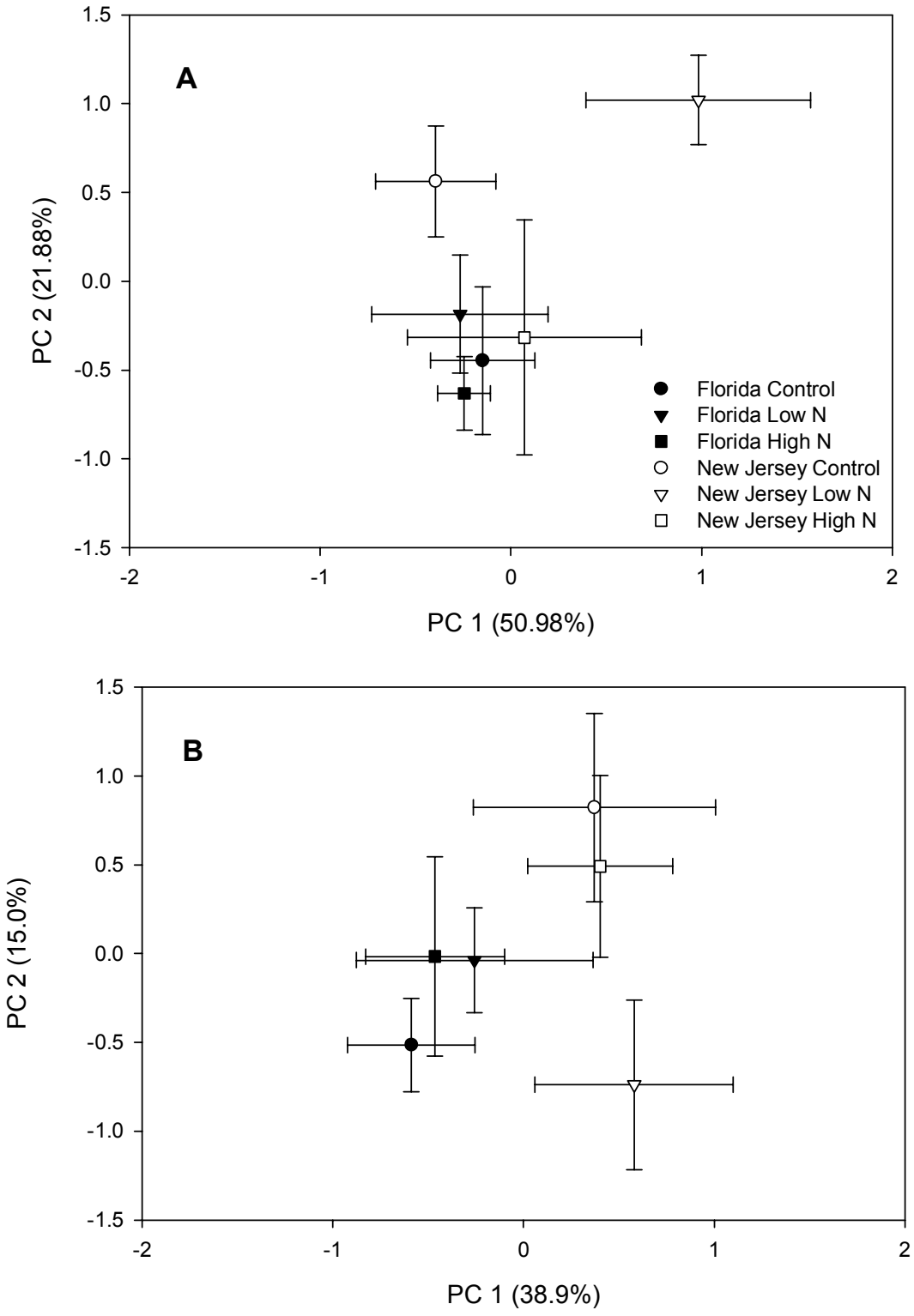
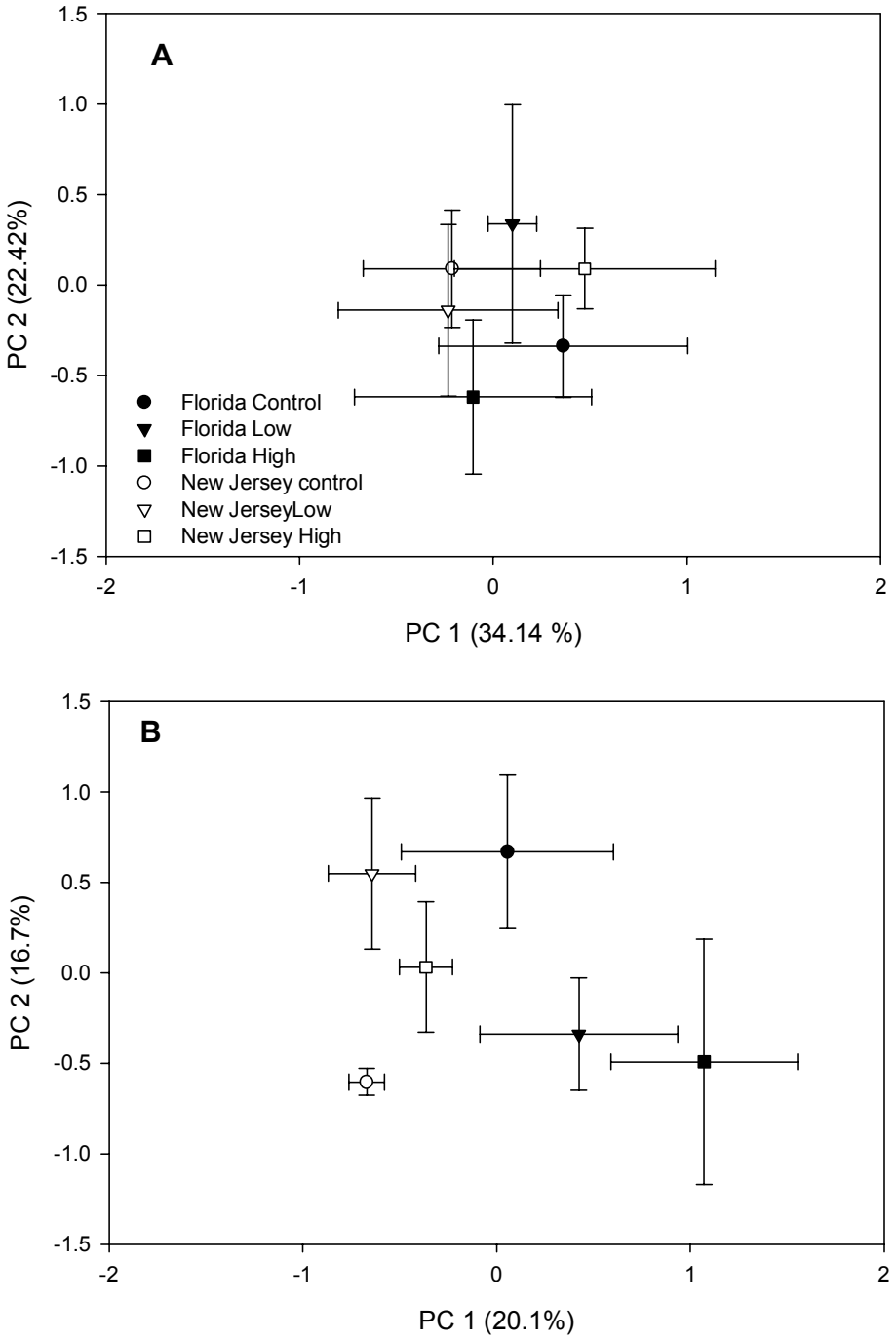

A
PC 2 (21.88%)
PC 1 (50.98%)
Florida Control
Florida Low N
Florida High N
New Jersey Control
New Jersey Low N
New Jersey High N
B
PC 2 (15.0%)
PC 1 (38.9%)

Figure 5.


A
PC 2 (22.42%)
PC 1 (34.14 %)
Florida Control
Florida Low
Florida High
New Jersey control
New JerseyLow
New Jersey High
B
PC 2 (16.7%)
PC 1 (20.1%)

Chapter 3

Biogeography Affects Trophic Control in Soil Food Webs

Abstract

I evaluate the relative importance of trophic control in the soil micro-food web of two geographically distinct but structurally similar scrub oak forests, one in Florida (FL) and one in New Jersey (NJ). I experimentally applied allochthonous nitrogen as 0 Kg Ha^{-1} Yr^{-1} (deionized water control), 35 Kg Ha^{-1} Yr^{-1} and 70 Kg Ha^{-1} Yr^{-1} in monthly increments over the course of one year to replicated $1m^2$ plots situated at the base of a reference scrub oak tree (*Quercus myrtifolia in* FL and *Q. ilicifolia* in NJ). I measured bacterial and fungal biomass as well as density of soil animals including collembola, oribatid and predatory mites. Allochthonous nitrogen did not affect the biomass or density of any of the sampled functional groups at either experimental site. However, the FL site supports a greater biomass of bacteria and fungi than NJ, and the NJ site supports a greater density of all soil animal groups than FL. I correlated microbial biomass and soil animal density with abiotic soil characteristics including, total C, total N, soil moisture, depth of organic horizon, and concentration of soluble nutrients (NO_3, NH_4 and PO_4). I found evidence for top down control by soil animals on microbial biomass. At the same time, I also found evidence for bottom up control because NO_3 and PO_4 positively correlate with microbial biomass. Our results show that top-down and bottom-up control

may be working simultaneously in naturally occurring soil food webs. However, geographic context plays an important role. I found differences in soil food web structure between two forests that have similar above ground biotic structure, but which appear to have very different abiotic drivers.

Introduction

Examples of trophic cascades in soil food webs are rare and are consequently the subject of much debate (Pace et al. 1999, Shurin et al. 2006). It is believed that trophic cascades primarily occur in low-diversity, aquatic communities with linear food chains and algae at the basal trophic level (Strong 1992). In soil food webs, however, bacteria and fungi serve as the basal trophic level. Traditionally, it is believed that detritus is the primary energy source for soil food webs (Moore et al. 2004), but the importance attributed to plant-fixed carbon through root exudates is increasing (Pollierer et al. 2007). This has important implications for top-down versus bottom-up control, as well as feedbacks between plants and soil communities (Wardle et al. 2004). In fact, many argue that neither bottom-up nor top-down trophic control is relevant in soil food webs (*e.g.,* (Bengtsson et al. 1996, Wardle and Lavelle 1997, Moore et al. 2003). Soil food webs are highly reticulated and contain omnivorous nodes that can be misrepresented in traditional linear trophic models (Strong 1992), and they are characterized by complex feedback loops among predators, consumers, decomposing microbes, plant roots and detrital substrates (Moore et al. 2003).

Within reticulated soil food webs, trophic cascades have been demonstrated in the rhizosphere of lupines, where predatory nematodes positively affect plant growth through regulation of root grazing caterpillars (Strong et al. 1996, Preisser 2003). In a coastal food web, Polis and Hurd (1996), described that increasing detrital inputs (bird feces and ocean debris) elevated biomass of top predators (mites and spiders), resulting in a cascade that suppressed biomass of herbivores and secondary consumers. However, for reasons outlined above, examples of trophic control in soils are mixed. In experimental microcosm studies, Mikola and Setala (1998) failed to find evidence of a trophic cascade in a soil micro-food web because compensatory growth by bacteria masked top down control in the food web. Their work helps demonstrate that trophic control can be contextual, and that top-down can become bottom-up when grazing enhances nutrient flow such that the basal trophic level flourishes.

Whether or not a soil food web is subject to top-down or bottom-up control may also depend on environmental factors. Heal and Dighton (1985) describe soil food webs in which increased resource quality leads to longer food chains and greater importance of higher trophic levels. This is in agreement with theory and empirical evidence (Oksanen et al. 1981, Kaunzinger and Morin 1998). However, the nature of the interaction may depend upon environmental circumstances. Soil food webs from extreme Antarctic conditions had higher soil organic matter, yet feeding chains were shorter and were less subject to top down control despite greater resource quality (Heal and Dighton 1985). Also in

the Antarctic, Freckman and Virginia (1997) found short feeding chains. Their communities were very susceptible to disturbance due to the specialization of the few top predatory species (Freckman and Virginia 1997). In the Chihuahuan desert where soil organic content is low, predatory mites significantly increased decomposition by controlling the population size of microbial feeding nematodes and allowing for increased microbial activity (Santos et al. 1981). This result illustrates a conflict in soil food web theory. Santos et al. (1981) report a classic trophic cascade (*sensu* (Hairston et al. 1960) in which the basal trophic level is enhanced by predatory control in a three-level food chain. However, theory (DeAngelis 1992, Zheng et al. 1999, Moore et al. 2003) and empirical evidence (Hedlund and Sjogren Ohrn 1999, Laakso and Setala 1999) suggest that consumer grazing on microbial decomposers can enhance microbial activity by increasing rates of material cycling (Osler and Sommerkorn 2007)(but see (Lenoir et al. 2007). In nature, either mechanism may act depending on biotic and abiotic constraints.

The study presented here had two objectives. First, I wanted to evaluate the relative importance of bottom-up (nutrient addition) and top-down (consumer) control in a naturally occurring soil micro-food web. Second, I wanted to evaluate how biogeography and environmental influences affect trophic control. I conducted this experiment in two geographically distinct but structurally similar forests. I manipulated nutrient inputs by adding allochthonous NH_4NO_3 in high and low concentration over the course of one year to experimental plots in Florida and New Jersey. I then measured biomass changes in microbial groups

(bacterial and fungal), and density changes in consumer (oribatid mites and collembola) and predatory (mesostigmatid and prostigmatid mites) trophic levels. Based on previous studies showing that top-down control is rare in terrestrial food webs because of the presence of multiple omnivorous feeding pathways (Polis and Strong 1996), I initially predicted that the allochthonous nitrogen addition would support increased microbial biomass and result in a greater density of consumers and predators (Oksanen et al. 1981, Kaunzinger and Morin 1998). In particular, due to the relatively lower C:N of bacteria relative to fungi, I predicted a greater increase in fungal biomass that might subsequently favor animals that primarily consume fungi (*e.g.,* collembola and oribatid mites). This research is novel in that, to our knowledge, no one has demonstrated biogeographic influences on trophic control in naturally occurring soil food webs. A vigorous debate surrounds the extent of microbial biogeography (Martiny et al. 2006). This research extends the debate by studying microbial community functioning and trophic control within naturally occurring soil food webs.

Methods

Experimental Design and Sampling

This experiment used a 2x3 factorial design with two geographically distinct treatment sites (factor 1) and three different nitrogen addition treatments (factor 2). The two study sites were at the Kennedy Space Center near Cape Canaveral, Florida (FL) and the Pinelands of New Jersey (NJ). Over the course of one year beginning in May 2005, I simulated different levels of nitrogen

deposition by dispensing aqueous NH_4NO_3 each month in three concentrations of 35 Kg Ha^{-1} Yr^{-1}, 70 Kg Ha^{-1} Yr^{-1} and 0 Kg Ha^{-1} Yr^{-1} (deionized water control). I chose these levels of nitrogen based on responses of mycorrhizal fungi to nitrogen addition in greenhouse experiments (Dighton et al. 2004), and because they are comparable to or in excess of levels affecting Europe (Arnolds 1991). I replicated each treatment combination five times for a total of 15 plots at each treatment site. Each experimental plot measured $1m^2$ and was at the base of a numbered scrub oak tree, *Quercus ilicifolia* in NJ and *Q. myrtifolia* in FL.

I randomly removed three 5cm diameter soil cores from each plot after 12 months of nitrogen additions. I removed each of the three cores for: (1) microarthropod extraction and enumeration, (2) bacterial and fungal biomass measurements using substrate induced respiration (SIR), and (3) soil nutrient analysis. I retained the top five cm (for cores with an organic layer of less than five cm) or the full organic layer of the cores for microarthropod extraction. I retained the top 10 cm of soil from the second and third core for SIR and nutrient analysis, and homogenized the mineral and organic layers. I chose to do this rather than separate organic and mineral horizons because many of the FL plots had a negligible or a highly mixed organic layer. All FL cores were placed in a cooler with cold packs and shipped overnight to NJ for processing.

Site Characteristics

Both experimental sites are characterized by dry, low-nutrient, sandy soils. Both sites are fire prone and contain structurally similar scrub oak communities. Prior to starting experiments, I surveyed plant community composition in all plots

at each site. Composition was measured as percent cover of each plant within the plot, and the rank order of plants is presented in Table 1. The FL study site is in the NASA Kennedy Space Center/Merritt Island National Wildlife Refuge, a 57,000 ha managed area comprised of brackish estuaries, marshes, scrub oaks, pine forests, and oak/palm hammocks on the Atlantic Coast of central Florida. The research plots are in scrub habitat, adjacent to a brackish marsh, dominated by *Quercus myrtifolia* with *Serenoa repens* (saw palmetto) in the under story. The NJ site is within the Rutgers University Pinelands Field Station that is part of the greater New Jersey Pinelands Preserve in south-central NJ. The Pinelands includes 304,000 ha of land with heavily restricted development as part of the 445,000 ha NJ Pine Barrens ecosystem. The research plots in NJ are dominated by *Q. ilicifolia* with *Vaccinium angustifolia* (low bush blueberry) in the under story. The two sites have important similarities in gross vegetation structure and soil types but very different seasonal and climatic influences (Table 1).

Soil Nutrient Analysis

I measured soil nutrients by collecting the litter, humus and mineral soil fractions from each soil core to a depth of 10cm (homogenized for analysis). The moisture content was determined by drying soil at 70 °C. I extracted samples from each core using 2.0 M KCl and analyzed for NH_4^+ by ion selective electrode (ISE). I also extracted samples with deionized water (DI) and analyzed for NO_3^- and PO_4^{-3} using the Dionex DX90 ion chromatograph, (Dionex Corp, Sunnyvale CA). I performed all extractions on an approximate 4:1 extractant/ dry wt material basis within 24 hours of sample collection. I performed ISE analysis of NH_4^+ and

IC analysis of PO_4^{-3} and NO_3^- according to Standard Methods protocols (Clesceri et al. 1998). I analyzed oven-dried samples for total carbon by infrared CO_2 detection and total nitrogen by N_2 thermal conductivity detection following high temperature combustion using a Leco TruSpec carbon/nitrogen analyzer (Leco Corp., St Joseph MI).

Bacterial and Fungal Biomass Measure

I used a modified substrate induced respiration (SIR) method (Beare et al. 1991, Sparling 1995) to separately quantify bacterial and fungal biomass in the soil. I lightly homogenized approximately 13 g of wet soil and placed it into 250 ml media jars. I then treated soil with either 5 ml of 0.064 g ml^{-1} (300 mg) aqueous cyclohexamide in (Sigma Aldrich, St. Louis, MO) to inhibit fungi and isolate the bacterial community, or 5 ml of 0.013 g ml^{-1} (65 mg) aqueous streptomycin (Sigma Aldrich, St. Louis, MO) to inhibit bacteria and isolate the fungal (eukaryotic) community. I treated another set of soil in jars with DI water (positive control for full microbial activity) or cyclohexamide and streptomycin together (negative control assuming a near sterile jar). For simplicity, I present the results of treated jars and not controls. All treated and control jars were incubated with their antimicrobial compound (or deionized water) for 12 hours at 4ºC. After incubation, I combined an excess of dry glucose (> 300mg, a preliminary dose response experiment determined the saturating mass of glucose) with the soil and attached the jars to an infra-red gas analyzer (Columbus Instruments, Columbus, OH) to measure CO_2 evolution. Under the assumption that respiration and CO_2 evolution correlate with microbial biomass, I

calculated bacterial or fungal biomass using the regression equations of Beare et al. (1991) as µg C fungal gdw^{-1} soil or µg C bacterial gdw^{-1} soil. I used the percent moisture of a proximate soil core to calculate dry weight based on the known wet weight of soil added to the jar.

Microarthropod community characterization

I used a MacFadyen air conditioned extractor to sample mites and collembola from the soil cores (MacFadyen 1962, Jonsson et al. 2006). I inverted and placed the top 6 cm of each soil core (no core had an organic horizon deeper than 5 cm) into the extractor cups. I exposed each core to a 50 watt light source for at least 24 hours, and collected the microarthropods in a 75/25 ethanol/glycerol solution in scintillation vials. I stored the preserved samples at 4°C until analysis.

For analysis, I transferred the collected microarthropods to a square gridded petri dish (Fisher Scientific). Using the Nikon SMZ, a variable zoom stereo microscope, I sorted the animals into groups of mites and collembola and mounted them onto glass slides with a glass cover slip using clear fingernail polish to create permanent cells. I then examined the animals more closely using a Nikon Ellipse 80i compound microscope at 100X magnification and counted them in groups of: oribatid mites, mesostigmatid and prostigmatid mites, and all collembola grouped together. Although isolated less frequently, soil animals like symphylids, ants, spiders and other insects were occasionally captured by the extractor. I did not count them as this method was not ideal to quantify their density and results would not be comparable. The enumeration

grouping used is based on the functional attributes of the animal. Oribatid mites are fungal, bacterial and detrital feeders, whereas the prostigmatid and mesostigmatid mites are primarily (but not exclusively) secondary consumers and predators. Collembola consume fungi and root material, although this is not exclusive (Petersen and Luxton 1982, Dindal 1990, Coleman et al. 2004). I analyzed and present all counts as density of the animal group per cm^3 of organic soil.

Data Analysis

I tested for effects of nitrogen treatment and geographic location on soil nutrients, fungal and bacterial biomass, and the fungal to bacterial biomass ratio, and the densities of collembola, oribatid and predatory mites in the soil using a two-way analysis of variance (ANOVA). There were no significant effects of nitrogen addition on the microarthropod community at either site (Appendix 1). There were no significant effects of nitrogen addition on either the fungal or bacterial communities in NJ, and there was only a slight significant increase in bacterial biomass between the low and high NH_4NO_3 treatments in FL (Appendix 1, $F_{2,14}= 4.51$, $P=0.035$). There was no significant effect of nitrogen on fungi in FL (Appendix 1). Therefore, I combined all treatment plots at each study site for further statistical analysis creating a sample size of 15 per site. From the ANOVA, I identify geographic location as the main effect and report significant differences in response between the sites. I calculated Spearman rank correlations between the different trophic group response variables as well as physical and chemical soil properties to evaluate the relative strength of top-

down versus bottom-up control on soil food webs across the two sites. Specifically, I correlated the following: nutrient concentrations (NO_3-N, NH_4-N, PO_4-P), depth of organic horizon, soil moisture, total C, total N as well as biomass of the two microbial groups (bacteria and fungi) and density of the three soil animal groups (collembola, oribatid mites and predatory mites). I did not adjust overall significance levels for multiple comparisons. Based on an $\alpha=0.05$, I would expect 1:20 correlations to be significant by chance alone. I find 17 significant correlations out of 44; it is not likely that our results are spurious (Moran 2003). My conclusions are made knowing that the correlations reflect trends and are not necessarily indicative of cause and effect. I used a t-test to determine if depth of O-horizon and the C:N ratio differed between the two sites. All statistical analyses were conducted in SAS Version 9.1 (SAS Institute, Inc. Cary, NC).

Results

Soil Nutrient Analysis

Concentrations of extractable soil nutrients were not affected by additions of NH_4NO_3 within either the NJ or the FL sites (Table 2). However, across all plots concentrations of NO_3 are significantly higher in NJ than FL ($F_{1,29} = 10.19$, $P<0.01$), and concentrations of PO_4 are significantly higher in FL than NJ ($F_{1,29} = 24.37$, $P<0.0001$). Therefore, FL soils appear to be more nitrogen limited than NJ soils. Although the higher PO_4 concentrations in FL may have resulted in part from abiotic affects like sea spray or geology, biotic effects arising from an inability of the microbial community to utilize the PO_4 due to nitrogen limitation

(*per* Liebig's Law) (Liebig 1840) may be the proximal cause. Total soil carbon, total nitrogen and the ratio of the two (C:N) were not significantly different between sample sites or across nitrogen treatments (Table 1). The depth of organic horizon was significantly higher in NJ than FL (Table 1, $t=2.38$, $p<0.05$).

Microbial Biomass and Animal Density

Density of predatory mites (Fig. 1a, $F_{1,29}=7.91$, $P<0.01$), collembola (Fig. 1b, $F_{1,29}=37.07$, $P<0.0001$) and oribatid mites (Fig. 1c, $F_{1,29}=12.47$, $P<0.01$) were all significantly greater in NJ than in FL. Both fungal (Fig 1d, $F_{1,29}=8.79$, $P<0.01$) and bacterial (Fig. 1e, $F_{1,29}=18.97$, $P<0.001$) biomass were significantly greater in FL than in NJ. The fungal to bacterial biomass ratio did not change signficantly with nitrogen addition and only showed a non-significant trend (Table 1, $F_{1,29}=2.96$, $P=0.098$) to be higher in FL than NJ.

Spearman Rank Correlations

The density of soil animals negatively and significantly correlates with both bacterial and fungal biomass (Table 3, see p values therein except the correlation between oribatid mites and fungi where $p<0.10$). Soil NH_4 concentration positively correlates with soil animal densities, and likewise, negatively correlates with biomass of fungi and bacteria (though non-significant, Table 3). Presumably this is due to nitrogen mineralization associated with soil animal grazing on microbes. Bacterial and fungal biomass correlates significantly and positively with dissolved soil nutrients NO_3 and PO_4 (except fungi and NO_3 where $p<0.10$, Table 3). The depth of organic horizon did not correlate significantly with oribatid and predatory mite densities or fungal and

bacterial biomass. There was a non significant positive correlation ($p<0.10$) between depth of organic horizon and density of collembola. This reflects the very low densities of collembola in FL where the organic horizon is shallow relative to NJ (Table 1). Soil moisture and total soil C and N do not significantly correlate with any of the biotic functional groups measured here although there is a positive relation between C and N and fungi and bacteria (Table 3). All three soil animal functional groups correlate negatively and significantly with NO_3 and PO_4. I think this is an artifact of the steep gradient in nutrient concentrations coupled with the steep gradient in soil animal densities between the two sites that is not necessarily a reflection of biological activity (Table 3).

Discussion

My results illustrate an interesting contrast between top-down control (Hairston et al. 1960) and the traditional model that soil food webs are subject to bottom-up or donor control (Strong 1992). I find this result in naturally occurring soil food webs. Both fungi and bacteria appear to be limited by dissolved soil nutrients and to a lesser extent (non-significant) total soil carbon and nitrogen (Table 3). At the same time, microarthropod consumers are negatively correlated with bacterial and fungal biomass. Coupling this with a positive correlation between microarthropods and soil NH_4 concentration (likely associated with mineralization and excretion of waste) suggests that consumption by microarthropods is limiting microbial biomass and releasing mineralized nutrients. However, the patterns of trophic control manifest

differently in the two study sites. Microarthropod densities are significantly higher in NJ than FL, and both fungal and bacterial biomass is higher in FL than NJ (Fig. 1).

It is not clear from this experiment why FL does not support a substantial microarthropod population. Abiotic soil factors may be controlling population size since the prey base, microbial biomass, does not appear to be limiting the microarthropods I measured in this experiment. The virtual lack of collembola in FL may be explained by the shallow organic layer at this site. Though only marginally significant (P=0.07), collembola correlated positively with depth of organic horizon; soils at the FL site may not provide the necessary habitat to support a dense population of collembola. The significantly shallower organic horizon in FL (Table 1) may be due to geographic differences between the sites that influence factors like the physiology and recalcitrance of leaf litter (Hobbie 1992). Indeed, factors like soil organisms, litter quality and the physical/chemical character of soil all interact to develop the soil environment (Swift et al. 1979).

Broad regional climatic variables like annual actual evapotranspiration play a critical role in decomposition of forest litter (Berg et al. 1993). Climate and variability of leaf litter quality have an important influence on the microbial community's ability to decompose plant litter and regenerate organic material in soil. In fact, one study showed that temperature plays an increasingly important role in litter decomposition with decreasing litter quality (Fierer et al. 2005). If FL leaf litter is not being incorporated as soil humus, there will be little habitat for soil animals (Moore et al. 2004). I did not measure the rate of decomposition or fate

of the leaf litter at the FL site, but litter does not accumulate there. However, since fungal and bacterial biomass is higher in FL (Fig. 1), and the soil C:N is higher in FL (Table 1), nutrients may be immobilized there. In NJ, soil C:N is lower, and microbial activity may be part of a wider positive trophic feedback cycle. Ultimately, *in situ* decomposition studies are required to further test this hypothesis.

Other abiotic factors may be affecting the trophic patterns I find in this study. For instance, if nitrogen is limiting in these sites and microbial biomass is subject to bottom-up control, why did I not see a response in either the microbial community or higher trophic levels to allochthonous nitrogen input? The soils from NJ (Tedrow 1998) and FL (Schmalzer and Hinkle 1996, Schortemeyer et al. 2000) are highly porous, sandy and known to rapidly leach soluble nutrients. Data from greenhouse experiments using native soil from the NJ site shows that additions of NH_4NO_3 (in concentrations comparable to this study) did not result in a change in 18-month old oak seedling (*Quercus rubra*) biomass relative to controls (unpublished data, J.A. Krumins). I do not believe the nitrogen in the present study was assimilated by the plants. However, even if it were taken up by plants, indirect effects of the nitrogen on the detrital food web should have been seen as the 'brown world' (detrital based food webs), and 'green world' (producer based food webs) connect in the rhizosphere (Wardle 1999, Moore et al. 2003, Moore et al. 2004). I suspect that our aqueous nitrogen additions quickly leached from the biologically active portion of the soil. This conclusion has very important environmental implications. Soluble nitrogen not assimilated

into biotic components of soil will be transported to waterways and groundwater where it can lead to eutrophication (Aber et al. 2003, Galloway et al. 2003). However, it affirms the notion that nutrient availability in these soils is dependent on trophic regulation and that abiotic factors, for example attributable to latitude (Seastedt 2000), interact with biotic relationships. The ambient nitrogen deposition levels are higher in NJ than FL (Table 1); this may further explain some of the differences I observe between the sites.

Soil fauna play a critical role in the decomposition, immobilization and mineralization of nutrients in soil (Osler and Sommerkorn 2007). In this study, I suggest that top-down and bottom-up effects work simultaneously in naturally occurring soil food webs. Moore et al. (2003) describe scenarios in which microarthropod grazing (or other soil fauna) stimulates microbial activity and creates a positive feedback loop between consumers and resources. Grazing activity in NJ may stimulate microbial activity thus increasing nutrient mineralization and humic material in soil and lowering soil C:N. In FL, where grazing is limited, microbes may hold more of the biomass and less nutrient mineralization results in a higher soil C:N. Grazing may facilitate decomposition, but only if microbial communities are not limited by low quality substrates.

Given that fungal biomass greatly exceeds bacterial biomass in these soils (Fig. 1 d & e), I can assume that the main path of top-down regulation of litter decomposition and mineralization will be through controls on fungal biomass. Selective grazing by soil animals occurs (Shaw 1988, Ruess and Dighton 1996). Collembolan density is lower in FL than NJ (Fig. 1b), partly explaining the

relatively larger fungal and bacterial biomass in FL. The impact of collembolan grazing pressure on fungal community biomass and composition can be dramatic (Newell 1984). If grazing does indeed cause a positive feedback to microbes, the low density of collembola in FL may have important effects on soil characteristics that ultimately affect soil habitability for microarthropods.

Soil food webs are famously reticulate and omnivory is frequent (Moore and Hunt 1988, Strong 1992). This is particularly the case for the naturally occurring food webs I describe here. The three microarthropod groups I quantified all correlate with each other positively and significantly (Table 3). I would expect a negative correlation between predatory mites and collembola or oribatid mites if they were indeed in a top predator position. This may be explained in a couple of ways. First, I grouped prostigmatid and mesostigmatid mites into the predatory mites, and I grouped all collembola as microbial consumers. This was a coarse categorization around which the boundaries are subjective. Many taxa in those groupings could occupy lower or higher trophic levels (Dindal 1990). Second, many of the mites that were categorized as predators or collembola that were categorized as consumers may in fact be omnivores. Third, I only categorized microarthropods; I did not enumerate other soil fauna, such as nematodes, that play an essential role in soil food webs. Omnivory and reticulated feeding pathways in natural soil food webs will diffuse nutrients moving through the web making trophic boundaries less pronounced (Polis and Strong 1996).

In a changing world, climatic and biogeographic factors will play an increasingly important role in trophic control and soil food web function (Wardle et al. 1998). This may happen if climate change increases plant productivity indirectly affecting soil food web processes (Emmerson et al. 2005). Alternatively, direct abiotic influences such as climate (Wilkinson et al. 2002) and therefore soil moisture (Schimel et al. 1999) may affect bacterial community structure and function. One study found that rainfall amount directly affected predatory spider activity and therefore leaf litter decomposition by altering cascading trophic interactions on decomposers (Lensing and Wise 2006). Although our soil food webs were not influenced by soil moisture (Table 3), the results do suggest that soil food webs may behave differently when they are subject to differing environmental drivers including latitude and likewise climate. It is not surprising that the importance of top-down or bottom-up control is context dependent; this has been established in multiple above ground plant communities (Wardle 2002). For instance, McGlynn et al. (2007) found soil animal density to be limited by phosphorus (specifically soil C:P). This clearly contradicts what I find here; phosphorus concentration is high in FL whereas microarthropod densities are low. It appears that bacteria and fungi in NJ are simultaneously affected by top-down control from microarthropods and bottom-up control from limiting nutrients like NO_3 and PO_4. The soil micro-food web of FL is more easily described by the conventional wisdom that soil food webs are subject to donor control (Strong 1992, Moore et al. 2004), and their relatively greater microbial biomass is the result of a release from consumptive pressure

by microarthropods. Leaf litter quality may play a role in the nature of trophic control in these food webs, and climatic and environmental factors are likely working in concert with biotic processes (Bengtsson et al. 1996).

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Figure Legends

Figure 1. Relative density of a) predatory mites, b) collembola and c) oribatid mites from both the FL and NJ sites as #animals cm^{-3} of organic soil. Biomass of d) fungi and e) bacteria from both the FL and NJ sites as µg C gdw^{-1} soil as determined by SIR. All bars represent mean +/- se and n=15.

Table 1. Comparison of biotic and abiotic characters from the New Jersy and Florida experimental sites.

	New Jersey Pinelands	Cape Canaveral Florida
Ranked Dominance of Vegetation in Plots	*Quercus ilicifolia* *Q. prinus* *Q. velutina* *Vaccinium angustifolium* *Carex striata* *Q. alba* *Pinus echinata* *Q. coccinia* *Q. stellata* *P. rigida* *Gaylussacia sp.*	*Quercus myrtifolia* *Q. incana* *Serrenoa repens* *Q. chapmanii* *Rhynchospora megalocarpa* *Vaccinium myrsinities* *Ximenia americana* *Aristida stricta* *Tellansia sp.* *Galactia elliota*
Average Depth to O Horizon (cm +/- SE)[a]	3.67 +/- 0.3255	2.47 +/- 0.5259
Average Total C:N of Soil (+/- SE)[b]	61.19 +/- 5.28	76.38 +/- 11.41
Soil Series[e]	Evesboro (mesic, coated lamellic quartzipsamments)	Pomello (sandy, siliceous, hyperthermic oxyaquinc alorthods)
Average Fungal/Bacterial Biomass Ratio (+/-SE)[b]	1.32 +/- 0.015	1.37 +/- 0.028
Rainfall June 2005-May 2006[c]	94.43 cm	132.91 cm
NH_4 Deposition June 05-May 06[c]	4.1 mg l^{-1}	1.71 mg l^{-1}
NO_3 Deposition June 05-May 06[c]	19.18 mg l^{-1}	8.77 mg l^{-1}
Latitude and Longitude	39° 91' N and 74° 53' W	28° 36' N and 80° 40' W
Avererage Annual Temperature[d]	12.3 °C	22.4 °C

[a] Values are different by a t-test, $P<0.05$

[b] No significant difference

[c] National Atmospheric Deposition Program (NADP) Champaign, IL

[d] National Oceanic and Atmospheric Administration (NOAA)

[e] Web Soil Survey: http://websoilsurvey.nrcs.usda.gov/app/

Table 2. Average soil nutrient concentration after one year of nitrogen additions. Values represent mean +/- se and n=5.

Site	Nitrogen Treatment	NO_3-N (μg g^{-1} soil)	NH_4-N (μg g^{-1} soil)	PO_4-P (μg g^{-1} soil)
Florida	**O Kg Ha^{-1} Yr^{-1}**	0.1714 +/- 0.022	3.3443 +/- 1.96	1.1565 +/- 0.467
	35 Kg Ha^{-1} Yr^{-1}	0.1548 +/- 0.011	0.5231 +/- 0.069	1.445 +/- 0.494
	70 Kg Ha^{-1} Yr^{-1}	0.1484 +/- 0.030	1.6640 +/- 0.683	1.4827 +/- 0.134
New Jersey	**O Kg Ha^{-1} Yr^{-1}**	0.1257 +/- 0.028	2.998 +/- 0.590	0.3128*
	35 Kg Ha^{-1} Yr^{-1}	0.0962 +/- 0.002	1.9537 +/- 0.486	bdl
	70 Kg Ha^{-1} Yr^{-1}	0.1395 +/- 0.016	2.1744 +/- 0.385	bdl

* only one replicate was above detection limit

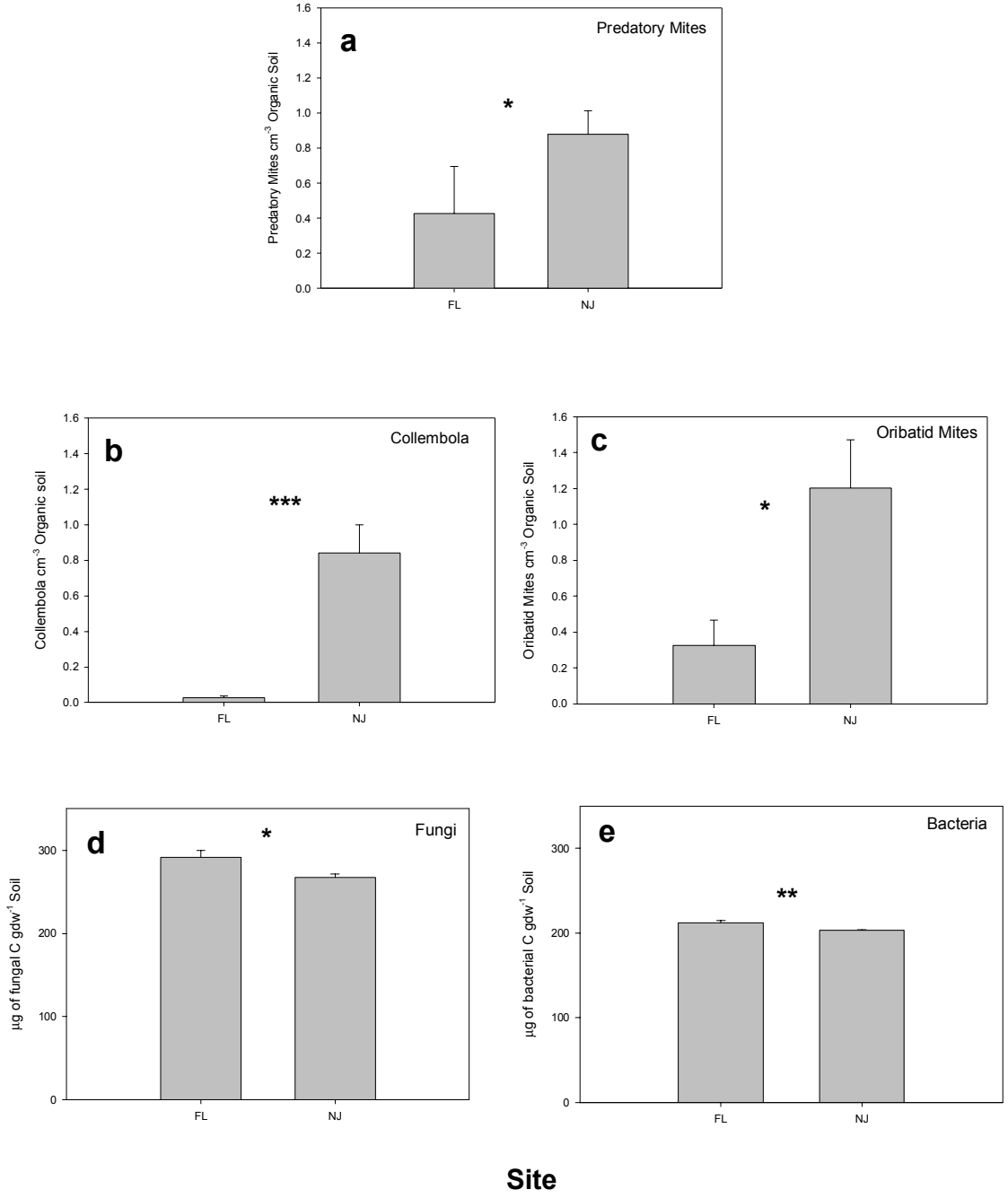
[bdl] below detection limit (for PO_4 detection limit = 0.04 mg PO_4-P l^{-1})

Table 3. Spearman rank correlation matrix of soil animal density, microbial biomass and abiotic soil characteristics. Each value indicates r and n=30.

	Collembola	Oribatid Mites	Predatory Mites	Fungi	Bacteria
NO_3	-0.6111***	-0.4234*	-0.4982**	0.3009	0.4009*
NH_4	0.3075	0.2214	0.2239	-0.2667	-0.3415
PO_4	-0.7976***	-0.5444**	-0.5982***	0.4102*	0.4767*
O depth	0.3353	0.022	-0.1585	0.0289	-0.0405
Collembola	-	0.7647***	0.7491***	-0.4248*	-0.5451***
Oribatid Mites	-	-	0.7921***	-0.3474	-0.3790*
Predatory Mites	-	-	-	-0.4653**	-0.5163**

* indicates P<0.05, ** indicates P<0.01 and *** indicates P<0.001

Figure 1.


a
Predatory Mites
Predatory Mites cm-3 Organic Soil
*
FL
NJ
b
Collembola
Collembola cm-3 Organic soil
***
c
Oribatid Mites
Oribatid Mites cm-3 Organic Soil
*
d
Fungi
µg of fungal C gdw-1 Soil
*
e
Bacteria
µg of bacterial C gdw-1 Soil
**
Site

Conclusions

The work presented in this dissertation builds on important connections between biodiversity, trophic interaction and microbially-mediated functions in ecosystems. Although I conducted the research in very different habitats, model aquatic communities and naturally occurring soil food webs, common themes emerge. This dissertation demonstrates that microbially mediated-ecosystem functions depend upon trophic interactions with producers, consumers and predators in food webs. Furthermore, it demonstrates that the response of these communities is context dependent. Biotic and abiotic factors play a critical role in shaping a community's diversity, composition and functioning.

In the first chapter, I began to resolve one of the mechanisms that explains why bacterially-mediated decomposition increases with greater eukaryotic species richness. Within increasingly complex food webs in either high or low productivity, I showed that bacterial abundance remained constant with increasing eukaryotic species richness at low productivity, but significantly declined at high productivity. Furthermore, eukaryotic species richness together with productivity influenced the composition of the bacterial community. Food web diversity and productivity interact to influence bacterial community composition and function. In more diverse food webs, bacterial activity (decomposition) increased despite lower population abundance.

In the second chapter, I studied the effect of an environmental perturbation (simulated nitrogen deposition) on soil rhizosphere bacterial and

fungal diversity and community composition. Further, I compared the microbial community response across two geographically isolated sites (Florida and New Jersey) to increase generality of the result. I showed that diversity of the bacterial and fungal communities (measured by colony and ectomycorrhizal morphotype respectively) responded differently to nitrogen treatments depending on geographic context. The composition of the bacterial community differed between nitrogen treatments and experimental sites, while the composition of the fungal community did not. My results are surprising in that the bacterial community responded in composition and biomass to nitrogen additions while the fungal (including ectomycorrhizal fungi) community primarily did not. My results imply that bacterial communities may be more sensitive than fungal communities to intense pulses of nitrogen. However, the response of fungi and bacteria to nitrogen may be contextual, varying between geographic locations.

In the third chapter, I present further results from the experiment described in chapter 2. I evaluated the relative importance of trophic control in the soil micro-food webs of the FL and NJ experimental sites. The nitrogen added to the plots in the experiment did not affect the biomass or density of any of the sampled functional groups at either experimental site. I found that the FL site supported greater biomass of bacteria and fungi than NJ, and the NJ site supported greater density of measured soil animal groups (collembola, oribatid mites and predatory mites) than FL. Through Spearman rank correlations of biotic and abiotic soil characteristics, I found evidence for top down control by soil animals on microbial biomass. At the same time, I also found evidence for

possible bottom up control on microbial biomass through correlations between nutrients (NO_3 and PO_4) and soil microbes. My results showed that top-down and bottom-up control may be working simultaneously in naturally occurring soil food webs. Further, this result is emphasized by the difference in food web structure found between two forests that have similar above ground biotic structure, but very different abiotic drivers.

Biodiversity and trophic interactions work simultaneously to affect ecosystem functioning. In this dissertation, I integrate microbial community composition and functioning into biodiversity and food web studies. Historically, few researchers have tackled this because it is difficult to evaluate microbe and macrobe communities in the same context. In using aquatic microcosms, I was able to evaluate bacterial response to the diversity of organisms most closely related to bacterial function, unicellular algae and protozoan consumers. In soil food webs, I was able to evaluate the effects of an environmental perturbation on the microbial community together with the microarthropod consumers in the soil. Microbial communities (bacterial and fungal) are directly or indirectly responsible for all ecosystem functions. This dissertation starts to resolve microbial community composition and diversity as it relates to food webs in aquatic and soil habitats.

Appendix 1. ANOVA responses of microbial biomass and microarthropod density to nitrogen treatments.

Organism Group	Site	$F_{2,14}$	P
Fungi	FL	2.61	0.11
	NJ	2.00	0.18
Bacteria	FL	4.51	0.03*
	NJ	2.48	0.13
Collembola	FL	1.29	0.31
	NJ	0.51	0.61
Oribatid Mites	FL	0.91	0.43
	NJ	1.04	0.38
Predatory Mites	FL	0.78	0.48
	NJ	1.83	0.21

* significant at the $P<0.05$ level.

Curriculum Vita

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1988-1993	The University of Texas at Austin, Bachelor of Arts in History
1993-1996	Veterinary Technician, Austin, TX
1996-1998	Texas State University, Master of Science in Biology
1998-2002	Microbial Ecologist, Dynamac Corporation, Kennedy Space Center, FL

Publications

(Note name change from Jennifer L. Adams to Jennifer Adams Krumins in 2003)

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