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Soil microbial community response to nitrogen enrichment in two scrub oak forests

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ABSTRACT

Microbial communities play a pivotal role in soil nutrient cycling, which is affected by nitrogen loading on soil fungi and particularly mycorrhizal fungi. In this experiment, we 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). We 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 1 yr. We applied treatments to replicated 1 m^2 plots, each at the base of a reference scrub oak tree (*Quercus myrtifolia in* FL and *Q. ilicifolia* in NJ). We 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 in the different sites, 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 imply that bacterial communities may be more sensitive than fungi to intense pulses of nitrogen in sandy soils.

1. 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 (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

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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).

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. For this reason, we carried out the following experiments in two structurally similar but distinct oak forests.

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. Our work will simultaneously examine the effects of nitrogen loading on bacterial and fungal communities. Further, this work is novel because we evaluate bacterial and fungal response to nitrogen loading in oak forests characterized by oligotrophic, sandy soils as opposed to coniferous stands.

The objective of this study was to evaluate the simultaneous response of bacterial and fungal communities to allochthonous nitrogen loading in two structurally similar but geographically distinct scrub oak forests. The results of this work show that geographic context and environmental influences interact with the microbial community response to nitrogen loading. We manipulated nutrients by adding NH_4NO_3 in high and low concentration over the course of 1 yr to replicate experimental plots in Florida (FL) and New Jersey (NJ). We 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 mycorrhizal fungi; when discussing these results we 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, we use the acronym EMF to differentiate a subset of the fungal community.

2. Methods

2.1. Site characteristics

Both experimental sites have dry, low-nutrient, sandy soils (see bulk densities in Table 1). Both sites are fire prone and contain structurally similar scrub oak communities. Prior to starting experiments, we surveyed plant community composition in all plots at each site. Composition was measured as percent cover of each plant within the each plot; those numbers were summed to create a relative rank of each plant across the entire site. The rank dominance 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*

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 across all plots at each site[a]	*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)[b]	3.67 ± 0.3255	2.47 ± 0.5259
Average total C:N of soil (±SE)	61.19 ± 5.28	76.38 ± 11.41
Average bulk density of soil (±SE)	0.888 ± 0.027	0.748 ± 0.035
Fungal/bacterial biomass ratio (±SE)	1.32 ± 0.015	1.37 ± 0.028
Rainfall June 2005–May 2006[c]	94.43 cm	132.91 cm
NH_4 deposition June 2005–May 2006[c]	4.1 mg l^{-1}	1.71 mg l^{-1}
NO_3 deposition June 2005–May 2006[c]	19.18 mg l^{-1}	8.77 mg l^{-1}
Latitude and longitude	39.958 and −74.628	28.615 and −80.694
Average annual temperature[d]	12.3 °C	22.4 °C
Soil series[e]	Evesboro (mesic, coated lamellic quartzipsamments)	Pomello (sandy, siliceous, hyperthermic oxyaquinc alorthods)

[a] Percent cover was measured for each plant in each plot; these numbers were summed to create a ranked abundance across the site.
[b] Values significantly different by *t*-test ($P < 0.05$).
[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/.