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Alteration of soil chitinolytic bacterial and ammonia oxidizing archaeal community diversity by rainwater redistribution in an epiphyte-laden *Quercus virginiana* canopy

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ABSTRACT

Forest canopy structure controls the timing, amount, and chemical character of precipitation supply to soils through interception and drainage along crown surfaces (primarily as throughfall). Yet, few studies have examined forest canopy structural connections to soil microbial communities, and none have measured how throughfall affects microbial nitrogen (N) functions. Maritime *Quercus virginiana* Mill. (southern live oak) forests on St Catherine's Island (GA, USA) provide an ideal venue to study this interaction as its throughfall patterns are spatially heterogeneous due to the arboreal epiphyte, *Tillandsia usneoides* L. (Spanish moss), and its edaphic conditions are relatively homogeneous. To test the hypothesis that throughfall patterns alter soil microbial community N-function, we examined soil microbial community N-functional (ammonia oxidizing and chitinolytic) genes, soil chemistry/texture, and throughfall amounts/chemistry for points along a canopy coverage continuum: large canopy gaps (0%), bare *Q. virginiana* canopy (50–60%), and *Q. virginiana* canopy hosting heavy *T. usneoides* (>=85%) over a typical growing season (Mar–Sep 2014). Denaturing Gradient Gel Electrophoresis (DGGE) and quantitative Polymerase Chain Reaction (qPCR) analyses were used to assess changes in the diversity and abundance, respectively, of soil chitinolytic bacterial and ammonia oxidizing archaeal genes. Significant differences in throughfall water and solute delivery (Na^+, Cl^-, PO_4^{3-}, SO_4^{2-}, K^+, Ca^{2+}, NO_3^-, NH_4^+) were found to alter soil sodicity and salinity. Diversity of chitinolytic bacterial and ammonia oxidizing archaeal communities significantly differed across cover classes and negatively correlated to soil salinity, soil Na^+ concentration, and throughfall Cl^-, SO_4^{2-}, and PO_4^{3-} concentrations. Results suggest throughfall can alter patterns in the soil microbial community's N-functional gene diversity.

1. Introduction

Soil microbial communities are the primary driver for several processes essential to the introduction and transformation of inorganic nitrogen (N) in soils. As the primary source of N introduction in forest ecosystems is biological N fixation, N (particularly inorganic N) oftentimes acts as a limiting factor to net primary production (Lebauer and Treseder, 2008). Once fixed, inorganic N forms—primarily ammonium (NH_4^+) and nitrate (NO_3^-)—are found in small concentrations compared to organic N pools (Bormann et al., 1977). Plant uptake of inorganic and organic N forms is difficult as both are susceptible to removal by leaching and volatilization (Lebauer and Treseder, 2008). While research indicates that dissolved organic N can be directly taken up by plants in N-limited systems, NH_4^+ and NO_3^- are considered the primary N-source for plants (Harrison et al., 2007). Thus, production of these inorganic N forms by microbial communities, is essential to forest N availability and forest health (van der Heijden et al., 2008). This complex balance makes forest N cycling a focal point between biogeochemistry and microbial ecology. For these reasons, studies are needed to evaluate what mechanisms control the spatiotemporal availability of N-functional soil microbial communities and their N turnover to improve our understanding of N cycling within forest soils.

Many forest canopy functions alter soil conditions, in turn,

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affecting microbial community structure and function, including soil temperature and insolation (Gömöryová et al., 2009), litterfall (Sayer, 2006), atmospheric deposition (Corre et al., 2007), and soil moisture (Coenders-Gerrits et al., 2013). Rainwater redistribution by the canopy affects several major drivers of soil microbial community spatial patterns, yet has received limited research attention (Rosier et al., 2015). When rainwater contacts forest canopies, it is redistributed to the soil surface primarily by throughfall (rain that reaches soils by dripping from canopy surfaces or through gaps). Temporally-persistent canopy structures can result in consistent throughfall patterns (Keim et al., 2005) capable of creating storm-based pulses in soil moisture (Coenders-Gerrits et al., 2013) and altering soil solution chemistry (de Schrijver et al., 2004). Throughfall patterns also vary on a similar scale, 5–6 m (Keim et al., 2005), to soil microbial community patterns (Saetre and Bååth, 2000). Results from few studies including throughfall heterogeneity as a potential influence on soil microbial community composition indicate throughfall-related moisture and solute flux dynamics may impact soil bacterial and fungal community structure (Wilkinson and Anderson, 2001; Nemergut et al., 2010; Rosier et al., 2015). While these studies demonstrate that soil microbial community structure may be connected to throughfall, the impact of throughfall on soil microbial community functionality (i.e., N cycling) has yet to be addressed.

N-functional gene analysis is a standard method of assessing soil microbial community N functionality as these genes produce enzymes required for N cycling, including mineralization (chitinase) and nitrification (ammonia monooxygenase) (Terahara et al., 2009). This analysis does not directly measure soil enzymes' presence; however, it has been shown to be a good estimator of N activity (Wankel et al., 2011). To assess the connection between throughfall and soil microbial community N-functionality, we sampled throughfall and soils in an old-growth southern live oak (*Quercus virginiana* Mill.) forest hosting the epiphyte *Tillandsia usneoides* L. (Spanish moss). This forest has a natural canopy cover continuum that increases solute leaching and wash-off while decreasing throughfall amount (Gay et al., 2015). Three N-functional genes were selected for analysis: bacterial *chiA* (mineralization) as well as bacterial, AOB, and archael, AOA, *amoA* (nitrification). *ChiA* is a group A bacterial chitinase gene that is highly conserved across bacteria, accounting for 70–80% of soil chitin degradation (Terahara et al., 2009). Multiple pathways exist for N mineralization, yet *chiA* was chosen because (1) chitin is a dominant source of organic N for soils, even in some woodlands (e.g., Lindsay et al., 2010), and (2) release of N during plant biomass decomposition is regulated by a greater complexity of functional genes requiring specialized equipment (e.g., Geochip 5.0 high throughput microarray used by Cong et al., 2015). AOA *amoA* and AOB *amoA* genes produce the subunit A of NH_3 monooxygenase (*amoA)*, the enzyme that facilitates NH_3 oxidation, the first and rate-limiting step in nitrification (Chen et al., 2013).

Our hypotheses were that throughfall from contrasting canopy covers will alter *chiA*, AOA *amoA*, and AOB *amoA*: (1) community structure and diversity, and (2) gene abundance. We evaluated these hypotheses by: measuring throughfall amount and chemistry; determining physicochemical parameters of soils receiving throughfall; and characterizing structure (via PCR-DGGE) and abundance (via qPCR) of N-functional genes. By addressing these hypotheses our research established a potential link between changes in environmental factors (i.e., alteration of throughfall chemistry) and shifts in soil microbial N-functionality. This is of critical importance as deposition of reactive N has tripled in the past century due to anthropogenic activities (fossil fuel burning, fertilization) (Galloway et al., 2008). Changes in soil-N pools can alter several ecosystem services (soil-C storage, nutrient cycling, and soil structure) (Cusack et al., 2011), posing a possible global risk per recent global change projection models (Perveen et al., 2014).

2. Materials and methods

2.1. Study site description

Samples were collected on St. Catherine's Island (SCI, Fig. 1), a barrier island, south of Savannah, GA (31°38′23″N, 81°9′59″W). SCI's 30-year mean annual precipitation ranges from 750 to 1200 mm with temperatures ranging between 3 and 35 °C (GA Office of the State Climatologist, 2012). The study site is within an interior maritime forest primarily populated by *Quercus virginiana* Mill. (southern live oak), accounting for 86% of trees with a diameter at breast height (dbh) ≥ 20 cm. The *Q. virginiana* canopy is bestrewn with a bromeliad epiphyte, *Tillandsia usneoides* L. (Spanish moss). Variation in canopy structure provides 3 discrete canopy types: gap canopy (Gap, 0% coverage), bare canopy without bromeliad cover (−B, 50–70% coverage), and canopy with heavy bromeliad coverage (+B, >85% coverage). Cover percentage was determined using a convex spherical densiometer. Litter layer thickness and quality was similar across the site. The soil at the site is of the Foxworth series that is moderately well-drained with highly permeable fine sand as the substrate and a low, 0–3% slope (NRCS-WSS, 2014). SCI is well-suited for testing our hypotheses as many of the factors controlling soil microbial community structure in forests are relatively constant at the plot scale (Rosier et al., 2015).

2.2. Throughfall monitoring and chemical analysis

Throughfall samples and meteorological data were collected to determine canopy alterations to precipitation inputs, rates, and ion enrichment (Feb–Sep 2014). For throughfall, 21 manual collectors were split into 10 for −B, 10 for + B, and 1 for Gap. Spatial and statistical design of gauge locations are described in Rosier et al. (2015) and Gay et al. (2015). Care was taken to ensure only 2–3 layers of canopy were above the collectors. Rain and throughfall were collected using high density polyethylene (HDPE) funnels (0.33 m diameter) connected to opaque HDPE collection jugs (4 L). Glass wool was used as a filter for any large debris and was replaced every 2 months. Throughfall and rain volumes were measured within 48 h of any storm >0.2 mm rainfall after a minimum 8 h dry period. Storm duration and precipitation amount were measured in a nearby 0.5 km^2 clearing using a tipping bucket rain gauge, model TR-525I (Texas Electronics, Dallas, TX, USA). Water samples were taken for 1–2 events per month for ion analysis. Throughfall gauges were triple rinsed with ultrapurified water (Barnstead GenPure xCAD Water Purification System; Thermo Scientific, USA) once a week and after each rain event. Sampling bottles were cleaned with acidified GenPure water (pH = 2), then triple rinsed with sample before filling. Water samples were filtered immediately after collection to 0.2 μm and stored at 4 °C until ion analysis at Georgia Southern University. Ion chromatography was done using Dionex DX600 (Thermo Scientific, USA) per manufacturer-recommended techniques for concentration of major cations (Ca^{2+}, Mg^{2+}, K^+, Na^+, NH_4^+) and anions (SO_4^{2-}, Cl^-, NO_3^-, PO_4^{3-}).

2.3. Soil sampling, physicochemical measurements and DNA extraction

Soil samples were collected with a 2.22 cm soil corer every 2 months resulting in 4 total samplings in 2014: March, May, July, and September. Samples were collected using a blocked design with 5 grouping areas (Fig. 1). Grouping areas were absent of significant