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

L. Dean Moore^a, John T. Van Stan II^{b*}, Trent E. Gay^b, Carl Rosier^c, Tiehang Wu^a

[a]{Dept. of Biology, Georgia Southern University, Statesboro, GA, USA}

[b]{Dept. of Geology & Geography, Georgia Southern University, Statesboro, GA, USA}

[c]{Plant and Soil Sciences, University of Delaware, Newark, DE, USA}

[*] Correspondence to: Dr. John Van Stan (jvanstan@georgiasouthern.edu)

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* ($\geq 85\%$) 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.

Key words: Throughfall, ammonia monooxygenase, chitinase, barrier island, soil microbial community, Nitrogen functional genes

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, 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 had 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.). 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 archaeal, 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 NH₃ monooxygenase (*amoA*), the enzyme that facilitates NH₃ 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 750-1200 mm with temperatures ranging between 3-35°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) $\geq$ 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 hours 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 km² 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 (Ca²⁺, Mg²⁺, K⁺, Na⁺, NH₄⁺) and anions (SO₄²⁻, Cl⁻, NO₃⁻, PO₄³⁻).

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 woody debris, overlain by a similar depth and quality of *Q. virginiana* leaf litter (~2-3cm), had a negligible grass understory, and >10 m from the nearest tree trunk to avoid stemflow. Grouping areas were ~25 m² and contained three 1 m² sampling plots beneath each cover type. Each 1 m² block was sampled in 3 random locations and homogenized (Fig. 1). Soil samples were ~100 g each collected from the mineral soil horizon (0-10 cm) and placed into sterile sampling bags. Samples were transported on ice (~8 h at ~4 °C) from the site to where they were stored at -20 °C. Texture, total organic matter (%), cation exchange capacity (CEC), exchangeable soil cation concentrations (K⁺, Mg²⁺, and Ca²⁺), total NO₃⁻, plant-available PO₄³⁻, and pH of each sample were measured by standard methods (Klute, 1986) at A & L Plains Lab (Lubbock, TX, USA). Total dissolved solids (TDS) were determined from saturated soil-pastes (Rhoades et al., 1989). Dry root mass was measured by hand sieving in triplicate for each of the 15 sampling sites. Three 10 cm³ soil samples were taken from random locations within the 1 m² soil collection plots following USDA protocols (National Soil Survey Center, 2014). DNA was extracted from 0.3 g of homogenized soil using PowerSoil® DNA Isolation Kits (Mo-BIO Laboratories, Inc., Carlsbad, CA, USA) following manufacturer protocols. DNA yielded was used for all molecular analyses and stored at -20 °C.

2.4 Quantification of N-functional genes

Quantitative PCR was used to test hypothesis 2 (gene abundance differences with cover type), and was performed using the QuantStudio™ 6 Flex Real-Time PCR System (Life Technologies, Carlsbad, CA, USA) with SYBR-Green I fluorescent dye. All PCR mixtures used recommended 20 µl protocol for SYBR® Premix Ex Taq™ II (Takara Bio Inc., Shiga, Japan) consisting of 10 µl of 2X PCR Master Mix, 0.8 µl of 10 µM forward and reverse primer, 1 µl DMSO, 2 µl of DNA template, and 5.4 µl of nuclease-free water. Primers GA1F and GA1R were used for *chiA* (Williamson et al., 2000), Arch-*amoA*F and Arch-*amoA*R for AOA *amoA* (Francis et al., 2005), and *amoA*-1F and *amoA*-2R for AOB *amoA* (Rotthauwe et al., 1997). The *chiA* PCR cycling program included an initial denaturation step of 10 min at 95 °C; 40 cycles of 1 min at 94 °C, 1 min at 61 °C, 1 min at 72 °C, and a 20 s fluorescence reading step at 82 °C; and a final elongation step at 72 °C. The AOA *amoA* PCR program included 10 min at 95 °C; 40 cycles of 30 s at 95 °C, 1 min at 55 °C, and 1 min at 72 °C; and a final elongation step at 72 °C. AOB *amoA* utilized the same cycling as AOA *amoA*, but with an annealing temperature of 57 °C. Melting curve and agarose gel confirmation analysis was conducted for all reactions. Standards for each primer set were created using the soil extracted DNA. Product bands were excised from agarose gels then extracted and purified using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). The resulting fragments were ligated into TOPO® vectors and cloned into TOP10 competent cells (Life Technologies, Carlsbad, CA, USA) using manufacturer protocols. Plasmids were extracted from the resulting culture using QIAprep Spin Miniprep Kits (Qiagen, Hilden, Germany) and plasmid concentrations were quantified using Nanodrop® ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Ten-fold serial dilutions were used to create standard sets (ranging from 3.0×10^2 to 3.0×10^7). Efficiencies were 85-100% for *chiA* and 90-100% for AOA *amoA* with $r^2 > 0.98$ for all reactions. qPCR measurement of AOB *amoA* was unsuccessful. The inability to amplify AOB *amoA* from acidic, sandy, dry soils (like those on SCI) is preceded (Lu et al., 2012).

2.5 Characterization of N-functioning communities

DGGE was used to test hypothesis 1 (cover will alter soil community structure and diversity), producing one gel per sampling event (Mar, May, Jul, Sep, 2014). DGGE primer sets were the same as those used for qPCR with the exception of *chiA* primers which were GASQF and

GASQR (Williamson et al., 2000). A guanine-cytosine clamp was used on the forward primer of each primer set to stop the separation of double stranded DNA during DGGE (Muyzer et al., 1993). PCR mixtures were 25 μ l, consisting of 12.5 μ l of 2X Promega GoTaq PCR master mix, 0.8 μ l of 10 μ M forward and reverse primers, 1 μ l of DNA template, and 10.5 μ l of nuclease free water. The *chiA* amplification included 5 min at 94 °C; 35 cycles of 1 min at 94 °C, 30s at 61 °C, and 1 min at 72 °C; and 10 min at 72 °C. AOA *amoA* cycling program included 5 min at 95 °C; 35 cycles of 30 s at 95 °C, 1 min at 53 °C, and 1 min at 72 °C; and 10 min at 72 °C. AOB *amoA* amplification parameters were the same as AOA *amoA* with an annealing temperature of 57 °C. Gels were run using a DGGEK-1001 Cipher DGGE Kit (C.B.S. Scientific). All gels were 8% (w/v) acrylamide gels with a linear chemical gradient ranging from 20-50% for AOA *amoA* and 45 to 70% for *chiA* and AOB *amoA* products. Gels were run in 1X Tris-acetate-EDTA (TAE) buffer at 60 °C for 5 h at 150 V for AOA *amoA*, 7 h at 150 V for AOB *amoA*, and 6 h at 200 V for *chiA*. Gels were stained with 1X SYBR Gold Nucleic Acid Gel Stain (Life technologies). Attempts at DGGE analysis for AOB *amoA* were unsuccessful, just as occurred for qPCR.

2.6 Data processing and statistical analyses

Throughfall rates and ion concentrations did not meet normality assumptions and transformation failed to normalize the data. As 1 collector measured rainfall (gap throughfall), sample size was smaller for Gap than -B and +B (e.g., for volumes $n = 29, 289, \text{ and } 290$; and for concentration $n = 14, 140, \text{ and } 139$ respectively). To accommodate the sample size differences, k -sample Kolmogorov-Smirnov tests were used for comparisons between Gap, -B, and +B. Soil chemistry data were normally distributed, allowing for analysis of variance (ANOVA) testing for significance between means. Gelcompare II v.3.0 (Applied Maths, NV, USA) was used to quantify community structural differences/similarities in the AOA *amoA* and *chiA* community DGGE profiles (per hypothesis 1). Banding patterns were normalized and band matching conducted before the quantitative data was exported and analyzed with Primer 6 software (Primer-e, WA, USA). Banding patterns were transformed to presence/absence matrices for analysis to eliminate the effect of band intensity. Presence-absence data was used for an analysis of similarity (ANO-SIM) for each gel. Nonmetric multidimensional scaling (NMDS) was then conducted on the distance matrix. Shannon's diversity (H'), Margalef's species richness (d), and

Pielou's evenness (J') indices were also calculated. Diversity indices data were compiled for each treatment and tested for significance between means using an ANOVA followed by a Tukey-Kramer HSD test. Regression analyses were done for mean Margalef's index values and mean environmental conditions for the 3 coverage types for all 4 samplings, resulting in 12 values. Throughfall data in the regressions were averaged from storms occurring 2 months prior to each sampling. Statistics were calculated using Statistica 12 (Statsoft, OK, USA).

3 Results

3.1 Throughfall beneath each cover type

Throughfall rates decreased from +B, to -B, to Gap across the study period (Fig. 2a). This pattern was observed for nearly all storms (Fig. 2a), resulting in a significant difference between Gap and +B ($D(290) = 0.428$, $p < 0.001$) as well as +B and -B ($D(290) = 0.221$, $p < .001$). Throughfall concentrations of select dry deposited (Cl^-), mixed origin (PO_4^{3-} , Ca^{2+}), and biologically active (K^+) ions significantly increased from Gap to -B to +B (Fig. 2b). Cl^- produced the greatest concentration of any observed ion under +B, over 6 times its gap concentration (Fig. 2b). There was a 2-fold increase between Gap and -B and a 3-fold increase between -B and +B for throughfall Cl^- concentration (Fig. 2b). Ca^{2+} was the next most concentrated in throughfall as under +B, it was more than double the Gap concentration (Fig. 2b). Throughfall K^+ concentrations tripled between Gap and -B, then was followed by a 44% increase in K^+ concentrations between -B and +B throughfall (Fig. 2b). PO_4^{3-} had the lowest throughfall ion concentrations, but produced the greatest increase between groups with a 128 fold increase from Gap to +B (Fig. 2b). Throughfall SO_4^{2-} concentrations (not plotted) steadily increased with increasing cover, significantly differing between Gap and +B as well as -B and +B ($D(20) = 0.5$, $p = 0.03$) with a peak concentration of $89 \mu\text{eq L}^{-1}$ beneath +B. Throughfall NO_3^- concentration significantly decreased from -B to +B (Fig. 2c). NH_4^+ had the lowest concentration under -B and its highest beneath +B (Fig. 2c). As a result, NH_4^+ significantly differed between -B and +B (Fig. 2c).

3.2 Soil physicochemical variability

Soil texture was consistent across coverage types (Table 1). Despite the differences in throughfall chemistry, cover showed very little effect on soil chemical parameters (Table 1). In fact, the only chemical parameter significantly different across groups was the dry deposited sea salt ion Na^+ (Table 1). Na^+ differed significantly ($p < 0.0001$) between groups with a 10% increase from -B to +B and a 15% increase from Gap to +B (Table 1). Additionally, soil pH was found to be significantly different ($p = 0.04$) as pH decreased 12% from Gap to -B (Table 1).

3.3 Soil microbial community N-functioning gene structure and abundance

Significant differences across coverage types were found for Shannon's diversity index (H') for AOA *amoA* and *chiA* (Fig. 3), supporting hypothesis 1. For AOA *amoA* and *chiA*, changing cover from Gap to -B to +B decreased diversity by 26% for AOA *amoA* and 11% for *chiA* (Fig. 3a). The diversity change from Gap to +B was significantly different for both N-functional genes (Fig. 3a). Species richness was significantly different for both groups (Fig. 3b), while Pielou's evenness was not (Fig. 3c), suggesting that species richness drove diversity differences. The largest difference in richness was observed between Gap and +B for AOA *amoA* with a 23% decrease (Fig. 3b). Richness for *chiA* beneath Gap coverage was significantly ~20% greater than +B and -B (Fig. 3b). AOA *amoA* diversity significantly correlated to throughfall Cl^- , SO_4^{2-} , and PO_4^{3-} concentrations, decreasing as ion concentration increased (Fig. 4a). Similar to AOA *amoA*, *chiA* diversity negatively related to Cl^- and SO_4^{2-} with increasing coverage (Fig. 4b). Diversity of *chiA* also inversely related to Na^+ soil content (Fig. 4b). Both N-functional genes produced significantly inverse relationships with soil salinity (total dissolved solids) through a range that spanned an order of magnitude, 22-230 ppm (Fig. 4c). No consistent cover-related clustering in DGGE N-functional gene banding patterns were found (Table 2). Band grouping within treatments were also not observable in NMDS analysis (data not shown). Mean number of bands observed per sampling ranged from 13-28 for AOA *amoA* and 16-38 for *chiA*, not significantly changing throughout the study period. Gene quantities were similar across cover types for AOA *amoA* (interquartile range: 2.8×10^4 – 25.4×10^4 gene copies g^{-1} soil) and *chiA* (interquartile range: 5.7×10^6 – 19.1×10^6).

4 Discussion

Our results support hypothesis 1, suggesting diversity of N-functional soil microbial communities (Fig. 3) can be affected by throughfall supply to soils from heterogeneous cover patterns (Fig. 2) when soil texture, chemistry, and root biomass are comparatively homogenous (Table 1). Despite changes in diversity (Fig. 3a), there were no consistent similarities in community structure beneath cover types (Table 2). Gene abundance of AOA *amoA* and *chiA* communities did not support hypothesis 2 as gene amounts were similar across throughfall conditions (Section 3.3). Similar gene abundance across cover types strengthens the DGGE-derived significant differences in Shannon's diversity index. Capture and transport of sea salt to soils via throughfall from heavy *T. usneoides* cover (Fig. 2) appears to drive soil salinization (Table 1), altering the diversity of N-functional gene communities (Fig. 4). As epiphytes are ubiquitous across forest types (Van Stan and Pypker, 2015), it is likely that forests with similar canopy biometeorological and biogeochemical interactions as observed at SCI exist elsewhere. Thus, canopy influences over throughfall (and, perhaps stemflow) should be considered when assessing environmental factors influencing soil microbial community structure and functional gene patterns.

4.1 Throughfall influence on soil physicochemical conditions

A unique characteristic of our site is the spatially homogeneous edaphic conditions (Table 1). However, increasing soil Na^+ and TDS from Gap to -B to +B cover (Fig. 4) mirrors trends in throughfall salt concentration (Fig. 2b). It is possible that the increasing canopy surface area and roughness created by *T. usneoides* enhances capture of sea salt ocean aerosols. Subsequently, throughfall may wash off significant amounts of Na^+ and Cl^- (Van Stan and Pypker, 2015), increasing soil salinity. Although soil moisture was not measured for this study, contrasting throughfall rates, such as those observed, may create spatiotemporally persistent intra- and post-storm soil moisture patterns when soil texture conditions are homogenous.

4.2 Diversity of N-functioning soil microbial communities

Several trends in throughfall water and solute supply (Fig. 2), their potential production of soil moisture pulses, and alteration to soil salinity beneath the different coverage types (Table 1) were likely mechanisms altering the diversity of soil microbial community N-functional genes

(Figs. 3-4). Differences in diversity appear to be driven by species richness for both communities (Fig. 3b) while evenness plays a minor role but is less defined between groups (Fig. 3c). Previous research suggests increased diversity does not indicate increased functionality of soil communities due to functional redundancies within the soil, however, recent research has shown that diversity has a direct impact on some N cycling processes (e.g., Philippot et al., 2013). Thus, our results could indicate a direct change of N-cycling functionality, though further research efforts are required to validate this conclusion. Chitinolytic bacterial and AOA *amoA* diversity had a strong negative relationship with throughfall and soil salinity (Fig. 4). This suggests that the diversity of chitinolytic and AOA *amoA* bearing soil microbial communities are affected by soil salinity. While salinity is a measure of a broader array of salts, Na^+ and Cl^- likely play a primary role at our sample site due to ocean proximity. General bacterial abundance has been negatively correlated with total soluble salts (Ragab, 1993)—a trend attributed to decreased osmotic potential with increasing salt concentration. The influence of salinity has not been addressed in regard to chitinolytic bacteria, but AOA *amoA* communities (Li et al., 2011) and general bacterial communities (Rath and Rousk, 2015) have shown similar negative correlations with soil salinity. Thus, increasing salinity in throughfall (Fig. 2) and its magnification of sea salt aerosols in soils (Table 1) may limit diversity (Fig. 3) by restricting chitinolytic and AOA *amoA* communities to halophilic groups.

Throughfall SO_4^{2-} concentration also demonstrated a significant negative relationship with selected N-functional genes (Fig. 4). To the knowledge of the authors, increased SO_4^{2-} in soils has not been connected to changes in chitinolytic bacteria or AOA *amoA* communities, though increased SO_4^{2-} is associated with soil acidification and cation loss through SO_4^{2-} leaching (Zhang et al., 2014). Cation losses along with acidification potentially impact several soil conditions affecting bacteria and archaea containing N-functional genes. PO_4^{3-} had a negative relationship with AOA *amoA* diversity (Fig. 4b), suggesting an influence of site conditions as soils are acidic and P content is low (~80-100 ppm) compared to typical amounts in soil (500-2000 ppm) (Vance et al., 2003). Acidic soils are known to contribute to PO_4^{3-} immobilization due to sequestration by Fe, Al, and Ca ions at low pH (Kuramae et al., 2011). Under P-limited conditions, primary productivity of the surrounding plants can increase when soluble P is introduced, increasing N uptake and limiting NH_3 (Su et al., 2015) and potentially pressuring NH_3 -oxidizers. Although soil moisture was not measured in this study, differing throughfall rates

may impact intra- and post-storm soil moisture dynamics (Coenders-Gerrits et al., 2013). Soil drying necessitates an increase in intracellular solutes to prevent plasmolysis (Stark and Firestone, 1995), costing cellular energy and requiring hydration of enzymes (Schimel et al., 2007). Soil drying can also reduce substrate supply by limiting and complicating paths that substrates take to diffuse into cells that can limit microbial growth (Stark and Firestone, 1995). Thus, drying and rewetting by throughfall pulses (particularly from epiphyte-covered canopy) may cause stress to cells if repeated frequently—as seen in other moisture pulse studies (e.g., Schimel et al., 2007).

5 Conclusions

Alterations to the amount and character of rain water reaching forest soils can influence the diversity of soil N-functional (chitinolytic bacterial and NH₃-oxidizing archaeal) communities. Our results show that the dynamics of throughfall water and solute inputs to soils (particularly elevated concentrations of the sea salt ions, Na⁺ and Cl⁻, in forests near the coast) can increase soil sodicity and salinity (total dissolved solids). This throughfall-induced soil salinization effect was most prominent beneath dense epiphyte (*T. usneoides*) cover, likely due to their rough surfaces' enhancing the capture of sea salt aerosols for rainfall to wash off. The diversity of chitinolytic bacterial and NH₃-oxidizing archaeal communities significantly and indirectly correlated with throughfall chemistry, soil Na⁺ concentrations, and soil salinity. However, our results did not show throughfall influence over *chiA* or AOA *amoA* gene abundance or DGGE banding patterns. The connection between throughfall and N-functioning genes represents a significant and novel finding as it introduces a new mechanism that may influence soil N availability and speciation—a factor oftentimes limiting ecosystem-level biogeochemical functions (eg, net primary production). Similar and other effects of throughfall on soil microbial community structure and function may be found in other ecosystems with heterogeneous cover consisting of structures that differentially alter solute concentrations and water inputs.

Acknowledgements

This research was supported by the US National Science Foundation (0841146), Sigma Xi grants-in-Aid of Research, Georgia Southern University Professional Development and Student Government Travel grants and was part of Moore's thesis. We would like to thank Allie, Garret, and Ashley for lab assistance.

References

- Bormann, F.H., Likens, G.E., Melillo, J. M., 1977. Nitrogen budget for an aggrading northern hardwood forest ecosystem. *Science* 196, 981-983.
- Chen, Y., Xu, Z., Hu, H., Hu, Y., Hao, Z., Jiang, Y., 2013. Responses of ammonia-oxidizing bacteria and archaea to nitrogen fertilization and precipitation increment in a typical temperate steppe in inner Mongolia. *Applied Soil Ecology* 68, 36-45.
- Coenders-Gerrits, A., Hopp, L., Savenije, H.H.G., Pfister, L., 2013. The effect of spatial throughfall patterns on soil moisture patterns at the hillslope scale. *Hydrology and Earth System Sciences* 17, 1749-1763.
- Cong, J., Yang, Y., Liu, X., Lu, H., Liu, X., Zhou, J., Li, D., Yin, H., Ding, J., Zhang, Y., 2015. Analyses of soil microbial community compositions and functional genes reveal potential consequences of natural forest succession. *Scientific Reports* 5, 10007.
- Corre, M.F., Brumme, R., Veldkamp, E., Beese, F.O., 2007. Changes in nitrogen cycling and retention processes in soils under spruce forests along a nitrogen enrichment gradient in Germany. *Global Change Biology* 13, 1509-1527.
- Cusack, D.F., Silver, W.L., Torn, M.S., Burton, S.D., Firestone, M.K., 2011. Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests. *Ecology* 92, 621-632.
- de Schrijver, A., Nachtergale, L., Staelens, J., Luyssaert, S., de Keersmaecker, L., 2004. Comparison of throughfall and soil solution chemistry between a high-density corsican pine stand and a naturally regenerated silver birch stand. *Environmental Pollution* 131, 93-105.
- Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., Oakley, B.B., 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proceedings of the National Academy of Sciences* 102, 14683-14688.
- Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., 2008. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* 320, 889-892.
- Gay, T.E., Van Stan, J.T., Moore, L.D., Lewis, E.S., Reichard, J.S., 2015. Throughfall alterations by degree of *Tillandsia usneoides* cover in a southeastern US *Quercus virginiana* forest. *Canadian Journal of Forest Research* 45, 1688-1698.
- Gömöryová, E., Hrivnak, R., Janisova, M., Ujhazy, K., Gomory, D., 2009. Changes of the functional diversity of soil microbial community during the colonication of abandoned grassland by a forest. *Applied Soil Ecology* 43, 191-199.
- Harrison, K.A., Roland B., Bardgett, R.D., 2007. Preferences for different nitrogen forms by coexisting plant species and soil microbes. *Ecology* 88, 989-999.
- Keim, R.F., Skaugset, A.E., Weiler, M., 2005. Temporal persistence of spatial patterns in throughfall. *Journal of Hydrology* 314, 263-274.
- Klute, A., 1986. *Methods of Soil Analysis*. 2nd Edition, Soil Science Society of America, USA, 1358 p.

399 Kuramae, E., Gamper, H., van Veen, J., Kowalchuk, G., 2011. Soil and plant factors driving the
400 community of soil-borne microorganisms across chronosequences of secondary succession of
401 chalk grasslands with a neutral pH. *FEMS Microbiology Ecology* 77, 285-294.

402 Lebauer, D.S., Treseder, K.K., 2008. Nitrogen limitation of net primary productivity in terrestrial
403 ecosystems is globally distributed. *Ecology* 89, 371-379.

404 Li, M., Cao, H., Hong, Y., Gu, J., 2011. Spatial distribution and abundances of ammonia-
405 oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) in mangrove
406 sediments. *Applied Microbiology and Biotechnology* 89, 1243-1254.

407 Lindsay, E.A., Wakelin, S.A., Gibb, N.L., Colloff, M.J., 2010. The abundance of microbial
408 functional genes in grassy woodlands is influenced more by soil nutrient enrichment than by
409 recent weed invasion or livestock exclusion. *Applied and Environmental Microbiology* 76,
410 5547-5555.

411 Lu, L., Han, W., Zhang, J., Wu, Y., Wang, B., Lin, X., et al., 2012. Nitrification of archaeal
412 ammonia oxidizers in acid soils is supported by hydrolysis of urea. *ISME Journal* 6, 1978-
413 1984.

414 National Soil Survey Center. 2014. Separating roots from the soil by hand sieving.

415 Nemergut, D. R., Cleveland, C. C., Wieder, W. R., Washenberger, C. L., Townsend, A. R., 2010.
416 Plot-scale manipulations of organic matter inputs to soils correlate with shifts in microbial
417 community composition in a lowland tropical rain forest. *Soil Biology & Biochemistry* 42,
418 2153-2160.

419 Perveen, N., Barot, S., Alvarez, G., Klumpp, K., Martin, R., Rapaport, A., et al., 2014. Priming
420 effect and microbial diversity in ecosystem functioning and response to global change: A
421 modeling approach using the SYMPHONY model. *Global Change Biology* 20, 1174-1190.

422 Philippot, L., Spor, A., Henault, C., Bru, D., Bizouard, F., Jones, C. M., et al., 2013. Loss in
423 microbial diversity affects nitrogen cycling in soil. *ISME Journal* 7, 1609-1619.

424 Ragab, M., 1993. Distribution pattern of soil microbial population in salt-affected soils. In: H.
425 Lieth, & A. Al Masoom (Eds.), Springer, Netherlands, p. 467-472.

426 Rath, K. M., Rousk, J., 2015. Salt effects on the soil microbial decomposer community and their
427 role in organic carbon cycling: A review. *Soil Biology and Biochemistry* 81, 108-123.

428 Rhoades, J.D., Manteghi, N.A., Shouse, P.J., Alves, W.J., 1989. Estimating soil salinity from
429 saturated soil-paste electrical conductivity. *Soil Sci. Soc. Am. J.* 53, 428-433.

430 Rosier, C. L., Van Stan, J. T., Moore, L. D., Schrom, J. O. S., Wu, T., Reichard, J. S., et al.,
431 2015. Forest canopy structural controls over throughfall affect soil microbial community
432 structure in an epiphyte-laden maritime oak stand. *Ecohydrology* 8, 1459-1470.

433 Rothauwe, J. H., Witzel, K. P., Liesack, W., 1997. The ammonia monooxygenase structural
434 gene amoA as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing
435 populations. *Applied and Environmental Microbiology* 63, 4704-4712.

436 Saetre, P., Bååth, E., 2000. Spatial variation and patterns of soil microbial community structure
437 in a mixed spruce–birch stand. *Soil Biology and Biochemistry* 32, 909-917.

438 Sayer, E.J., 2006. Using experimental manipulation to assess the roles of leaf litter in the
439 functioning of forest ecosystems. *Biological Reviews* 81, 1-31.

440 Schimel, J., Balser, T. C., Wallenstein, M., 2007. Microbial stress-response physiology and its
441 implications for ecosystem function. *Ecology* 88, 1386-1394.

442 Stark, J. M., Firestone, M. K., 1995. Mechanisms for soil moisture effects on activity of
443 nitrifying bacteria. *Applied and Environmental Microbiology* 61, 218-221.

444 Su, J., Ding, L., Xue, K., Yao, H., Quensen, J., Bai, S., et al., 2015. Long-term balanced
445 fertilization increases the soil microbial functional diversity in a phosphorus-limited paddy
446 soil. *Molecular Ecology* 24, 136-150.

447 Terahara, T., Ikeda, S., Noritake, C., Minamisawa, K., Ando, K., Tsuneda, S., et al., 2009.
448 Molecular diversity of bacterial chitinases in arable soils and the effects of environmental
449 factors on the chitinolytic bacterial community. *Soil Biology and Biochemistry* 41, 473-480.

450 Van Stan, J. T., Pypker, T. G., 2015. A review and evaluation of forest canopy epiphyte roles in
451 the partitioning and chemical alteration of precipitation. *Science of the Total Environment* 536,
452 813-824.

453 van der Heijden, M.G., Bardgett, R. D., Van Straalen, N. M., 2008. The unseen majority: Soil
454 microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology*
455 *Letters* 11, 296-310.

456 Vance, C., Uhde-Stone, C., Allan, D., 2003. Phosphorus acquisition and use: Critical adaptations
457 by plants for securing a nonrenewable resource. *New Phytologist* 157, 423-447.

458 Wankel, S. D., Mosier, A. C., Hansel, C. M., Paytan, A., Francis, C. A., 2011. Spatial variability
459 in nitrification rates and ammonia-oxidizing microbial communities in the agriculturally
460 impacted elkhorn slough estuary, California. *Applied and Environmental Microbiology* 77,
461 269-280.

462 Wilkinson, S. C., Anderson, J. M., 2001. Spatial patterns of soil microbial communities in a
463 Norway spruce (*Picea abies*) plantation. *Microbial Ecology* 42, 248-255.

464 Williamson, N., Brian, P., Wellington, E., 2000. Molecular detection of bacterial and
465 streptomycete chitinases in the environment. *Antonie Van Leeuwenhoek International Journal*
466 *of General and Molecular Microbiology* 78, 315-321.

467 Zhang, W., Liu, C., Wang, Z., Zhang, L., Luo, X., 2014. Speciation and isotopic composition of
468 sulfur in limestone soil and yellow soil in karst areas of southwest china: Implications of
469 different responses to acid deposition. *Journal of Environmental Quality* 43, 809-819.

470

Tables

Table 1. Means and standard deviations of soil edaphic conditions measured during the study period beneath each cover type. Sample size differs for root biomass ($n = 45$), physicochemical variables ($n = 60$). Tukey-Kramer tests were run to determine significance, results are displayed as F -value (p -value). Superscripts indicate whether differences are significant..

Soil parameter	Cover type			F (p)
	Gap	-B	+B	
Root Biomass (g m^{-2})	265 ± 130	370 ± 180	275 ± 180	1.69(0.20)
Texture (%)				
Sand	88.3	87	88.4	0.3 (0.68)
Silt	4.7	4.7	3.3	0.31 (0.70)
Clay	7	8.3	8.3	0.32 (0.71)
Chemistry				
OM (%)	3.5 ± 0.1	3.9 ± 0.2	6.0 ± 2.0	1.4 (0.25)
P (ppm)	102.3 ± 5.3	82.9 ± 7.7	92.1 ± 7.0	2.1 (0.13)
K^+ (ppm)	61.9 ± 4.3	72.3 ± 3.3	67.0 ± 3.8	1.8 (0.17)
Mg^{2+} (ppm)	59.2 ± 9.3	45.9 ± 5.0	62.7 ± 5.0	1.7 (0.18)
Ca^{2+} (ppm)	395.5 ± 82.3	237.9 ± 35.3	430.1 ± 79.3	2.2 (0.12)
Na^+ (ppm)	40.5 ± 0.7^a	43.2 ± 1.0^a	47.8 ± 1.4^b	11.5 (<0.0001)
NO_3^- (ppm)	5.2 ± 0.05	4.0 ± 0.2	$5.4 \pm .07$	2.4 (0.10)
CEC (meq 100 g^{-1})	$5.8 \pm .5$	5.9 ± 0.5	7.3 ± 0.9	1.5 (0.22)
pH	4.6 ± 0.1^a	$4.1 \pm .1^b$	4.3 ± 0.2^{ab}	3.2 (0.04)

477 Table 2. Analysis of similarity (ANO-SIM) results from DGGE banding pattern
 478 presence/absence data from each sample showing, despite occasional significant differences in
 479 banding patterns between sites, no consistent significant patterns across cover type.

Sampling		Pairwise tests			Total
		<i>F</i> (<i>p</i>)			<i>F</i> (<i>p</i>)
AOA <i>amoA</i>	Gap vs -B	Gap vs +B	-B vs +B		
March	0.19(0.03)	0.11(0.16)	0.15(0.89)	0.11(0.16)	
May	0.04(0.34)	0.09(0.22)	0.21(0.11)	0.09(0.22)	
July	0.04(0.48)	0.14(0.18)	0.47(0.06)	0.14(0.18)	
September	0.16(0.23)	0.19(0.03)	0.31(0.09)	0.19(0.03)	
<i>chiA</i>					
March	0.02(0.49)	0.03(0.36)	0.02(0.51)	0.03(0.36)	
May	0.03(0.37)	0.14(0.15)	0.11(0.25)	0.14(0.15)	
July	0.12(0.80)	0.01(0.45)	0.19(0.11)	0.01(0.45)	
September	0.04(0.56)	-0.12(0.86)	0.08(0.74)	0.12(0.86)	

480

481 **Figure captions**

- 482 Figure 1. Site location and visual representation of soil sampling design. The five groups of
483 the blocked design are shown within the same hectare that also contained the
484 throughfall gauges. All grouping areas were no larger than 25 m² and were at least
485 10 m from the nearest trunk. Grouping areas contained a 1 m² sampling area for
486 each of the coverage types.
- 487 Figure 2. Throughfall rate and chemistry beneath a canopy gap (Gap), bare canopy (-B),
488 and *T. usneoides* covered canopy (+B). (a) Medians of spatially averaged
489 throughfall rate of each coverage type for the 29 storms measured throughout the
490 sampling period. Differences in the median and 25-75% observation range of (b)
491 all significantly different ions and (c) N containing ions for each coverage type.
492 Test statistics are provided from the Kolmogorov-Smirnov tests as $D(n)$, p -level
493 and superscripts indicate whether differences are significant. No superscripts are
494 provided in panel (b) since all ions are significantly different across all cover
495 types.
- 496 Figure 3. Mean and standard error of (a) Shannon's diversity index, (b) Margalef's richness
497 index, and (c) Pielou's evenness derived from DGGE banding patterns of *amoA*-
498 AOA *amoA* and *chiA*. One way ANOVA results are represented as $F(df)$, p -level.
499 Tukey HSD tests were run to compare between groups and superscripts indicate
500 whether differences are significant.
- 501 Figure 4. Average richness per sampling of (a) AOA *amoA* plotted against mean
502 throughfall ion concentration averages, (b) *chiA* versus throughfall SO₄²⁻ and Cl⁻
503 averages, as well as soil Na⁺ concentration, and (c) the relationship between soil
504 salinity (total dissolved solids) and both *chiA* and AOA *amoA* mean species
505 richness (Margalef's d). All show significant negative correlations. Throughfall
506 concentration averages were from storms occurring within the 2 month period
507 before soil sampling.

Figure 1

Figure 2

Figure 3

Figure 4

Na^+ , Cl^- , PO_4^{3-} , SO_4^{2-} deposition

Gap

Bare
cover

Epiphyte
cover

Species richness
(Margalef's d)

Microbial N-functional genes:

[Throughfall]