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Parent material and vegetation influence bacterial community structure and nitrogen functional genes along deep tropical soil profiles at the Luquillo Critical Zone Observatory

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ABSTRACT

Microbial communities mediate every step of the soil nitrogen cycle, yet the structure and associated nitrogen cycle functions of soil microbial communities remain poorly studied in tropical forests. Moreover, tropical forest soils are often many meters deep, but most studies of microbial nitrogen cycling have focused exclusively on surface soils. The objective of our study was to evaluate changes in bacterial community structure and nitrogen functional genes with depth in soils developed on two contrasting geological parent materials and two forest types that occur at different elevations at the Luquillo Critical Zone Observatory in northeast Puerto Rico. We excavated three soil pits to 140 cm at four different sites representing the four soil × forest combinations ($n = 12$), and collected samples at ten-centimeter increments from the surface to 140 cm. We used bacterial 16S rRNA gene-DGGE (denaturant gradient gel electrophoresis) to fingerprint microbial community structures, and quantitative PCR to measure the abundance of five functional genes involved in various soil nitrogen transformations: *nif*H (nitrogen fixation), *chi*A (organic nitrogen decomposition), *amo*A (ammonia oxidation), *nir*S (nitrite reduction) and *nos*Z (nitrous oxide reduction). Multivariate analyses of DGGE fingerprinting patterns revealed differences in bacterial community structure across the four soil × forest types that were strongly correlated with soil pH ($r = 0.69$, $P < 0.01$) and nutrient stoichiometry ($r^2 \geq 0.36$, $P < 0.05$). Across all soil and forest types, nitrogen functional genes declined significantly with soil depth ($P < 0.001$). Denitrification genes (*nir*S and *nos*Z) accounted for the largest proportion of measured nitrogen functional genes. Measured nitrogen functional genes were positively correlated with soil carbon, nitrogen and phosphorus concentrations ($P < 0.001$) and all genes except *amoA* were significantly more abundant in the Inceptisol soil type compared with the Oxisol soil type ($P < 0.03$). Greater abundances and a stronger vertical zonation of nitrogen functional genes in Inceptisols suggest more dynamic nitrogen transformation processes in this soil type. As the first study to examine bacterial nitrogen functional gene abundances below the surface 20 cm in tropical forest soils, our work provides insight into how pedogenically-driven vertical gradients control the nitrogen-cycling capacity of soil microbial communities. While previous studies have shown evidence for redox-driven hotspots in tropical nitrogen cycling on a watershed scale, our study corroborates this finding on a molecular scale.

1. Introduction

Microbial communities influence biogeochemical cycles throughout soil profiles, yet information on how community structure and functional characteristics change with soil depth is scarce (Fierer et al., 2003b). Tropical forest soils are often many meters deep and subsoils store substantial quantities of soil organic carbon (C) in low concentrations, contributing approximately 50% of the estimated C pool below 1 m (Jobbágy and Jackson, 2000). A large proportion of Bacteria and Archaea are able to live beneath the upper few meters of the earth's surface in the so-called "deep biosphere" (Whitman et al., 1998; Hoehler and Jorgensen, 2013), in environments that provide only marginal energy for cell growth and division. It is thus reasonable to predict that metabolically active microbial communities exist throughout the first several

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meters of tropical soil profiles. A more detailed characterization of these communities and their relationship to environmental variations is warranted to understand their roles in mediating biogeochemical cycles.

In surface soils, litter inputs principally define the energy resources and nutrients available to microbial communities (Hobbie, 1992; Waldrop et al., 2006), and as such the amount and chemical quality of litter can have large impacts on microbial community structure and functions (Chapman and Newman, 2010; Nemergut et al., 2010). Abiotic environmental properties such as redox potential (DeAngelis et al., 2010), nutrient concentrations (Cusack et al., 2011), soil texture (Sessitsch et al., 2001; Rousk et al., 2010), pH (Rousk et al., 2010) and mineralogy (Heckman et al., 2009) can also affect community structure. All of these properties can change dramatically with soil depth (Holden and Fierer, 2005; Hansel et al., 2008). Microbial biomass generally declines exponentially with soil depth, following declines in soil C availability (Blume et al., 2002; Fierer et al., 2003b; Stone et al., 2014). Molecular studies using both fingerprinting and metagenomic techniques have indicated that community composition also changes with depth (Agnelli et al., 2004; Hansel et al., 2008; Eilers et al., 2012). Changes in both microbial community composition and the physiochemical environment with soil depth suggests that subsoil microbial communities are specialized for their environmental niches such that their metabolic functions cannot be easily inferred from their surface counterparts (Ghiorse and Wilson, 1988; Zvyagintsev, 1994).

A key ecological function of microbial communities is their participation in all steps of the soil nitrogen (N) cycle. For instance, nitrogen fixation, organic N decomposition, nitrification and denitrification are mediated largely by soil microbes (Robertson and Groffman, 2007). Tropical forests are often characterized by a dynamic microbial N cycle, with relatively high levels of N loss via denitrification (Vitousek and Sanford, 1986; Livingston et al., 1988; Silver et al., 2000) balanced by high levels of N fixation (Reed et al., 2007; Cusack et al., 2009). While tropical forests are often considered N-rich relative to temperate forests, N deposition is increasing rapidly in the tropics (Galloway et al., 2004) and the response of soil microbial communities remains uncertain. Future changes to the N cycle of tropical forests will depend on the response of microbial N transformations to global change drivers. The environmental gradients represented by soil depth profiles may improve our understanding of the major environmental drivers of microbial N cycling. Several studies have found that abundances of N-fixing, nitrifying and denitrifying bacteria decline with depth in temperate forest soils (Mergel et al., 2001), temperate grassland soils (Marhan et al., 2011; Regan et al., 2011), agricultural soils (Forbes et al., 2009) and artificial Technosols (Hafeez et al., 2012), but the depth distribution of N-cycling bacteria has not, to our knowledge, been investigated in tropical soils. Measurements of nitrogen functional genes (NFGs) using quantitative PCR (qPCR) provide a powerful tool for evaluating microbial contributions to different stages of the N cycle. The abundance of NFGs provides insight into the biological capacity for N-cycle processes to occur, including process rates, substrate availability and population density of functional groups of microorganisms (Wallenstein and Vilgalys, 2005).

The goal of this study was to evaluate how bacterial community structure and NFGs change with soil depth in the context of landscape-scale gradients. Our study site, the Luquillo Critical Zone Observatory (LCZO) in northeast Puerto Rico, offers a unique natural experiment setting for examining the influence of landscape-scale drivers (contrasting geologic parent materials and climactically-driven forest types) on microbial communities along deep soil profiles. Previous studies have found rapid rates of N cycling in this ecosystem (Silver et al., 2005; Templer et al., 2008; Cusack et al., 2009) but research has been restricted to surface soils and above-ground vegetation. Here we coupled a molecular fingerprinting approach (DGGE) with measurements of NFGs using qPCR to simultaneously assess changes in bacterial community structure and functions. The five NFGs we measured encode enzymes responsible for major N transformations in soils, including N fixation (*nif*H), nitrite reduction (*nir*S), nitrous oxide reduction (*nos*Z), ammonia oxidation (*amo*A) and organic N decomposition (*chi*A) (Wallenstein and Vilgalys, 2005; Lindsay et al., 2010). We hypothesized that forest type would be an important control on community structure and NFGs in surface soils, as different vegetation assemblages lead to organic matter inputs of varying chemical quality. In subsoils, we predicted that community structure and NFG abundances would be relatively decoupled from forest type but that soil parent material will be more important. Finally, we predicted that the distribution of NFGs would change with depth to reflect variations in the dominant microbial processes along environmental gradients. We predicted that organic N decomposition and N fixation would be more important processes in surface soils, where organic matter is more abundant and N may be more limiting due to high plant demand. In subsoils, we predicted the importance of anaerobic metabolism and non-C energy sources would increase, which would be reflected by increased microbial denitrification activity.

2. Methods

2.1. Study site, sample collection and soil characteristics

This study was conducted using soils collected from the Luquillo Critical Zone Observatory (LCZO) in northeastern Puerto Rico

Table 1
General site and soil characteristics for the four soil × forest types. MAT = mean annual temperature, MAP = mean annual precipitation, ANPP = annual net primary productivity.

	Oxisol		Inceptisol	
	Tabonuco	Colorado	Tabonuco	Colorado
Location	18°18′50.0″N 65°44′25.0″W	18°16′39.1″N 65°50′54.0″W	18°15′46.3″N 65°47′35.5″W	18°17′28.7″N 65°47′44.2″W
Elevation (m)	360	790	360	780
MAT (°C)[a]	24	21	24	21
MAP (mm yr^{-1})[a]	3500	4200	3500	4200
Aboveground biomass (t ha^{-1})[b]	190	130	190	130
ANPP(t ha^{-1} yr^{-1})[b]	10.5	7.6	10.8	4.05
Texture class	Silty clay loam	Silty clay	Sandy loam	Sandy loam
Soil taxonomy[c]	Humaquox, Aquic & Inceptic Hapludox	Humaquox, Aquic & Inceptic Hapludox	Histic Humaquepts, Aquic & Histic Dystrudepts	Histic Humaquepts, Aquic & Histic Dystrudepts

[a] McDowell et al. (2012).
[b] Weaver and Murphy (1990).
[c] Classification of soils according to US Taxonomy Soil Survey Staff (2013).