Applied Soil Ecology 124 (2018) 218–228

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Changes in nitrogen-cycling microbial communities with depth in temperate and subtropical forest soils

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ARTICLE INFO

Keywords:
Temperate forest
Subtropical forest
Soil depth profile
Nitrogen functional gene abundance
N-cycling microbial community structure

ABSTRACT

The soil nitrogen (N) cycle, as a network of interlinked processes, is mainly driven by microbial communities. However, the patterns and factors that influence the abundance of the different N-cycling microbial communities in temperate and subtropical forest soils are still poorly understood. Moreover, recent studies have tended to focus on surface soils, and have neglected the fact that there may be steep gradients in nutrient concentrations in forest soil profiles. The objective of our study was to determine whether the distributions and compositions of N-cycling microbial communities changed with depth in subtropical and temperate forest soils. We collected soil samples from the surface to 80 cm deep from 2 forests, a native conifer mixed broadleaf temperate forest in northeast China and a native evergreen broadleaf subtropical forest in south eastern China. We used high-throughput sequencing to fingerprint the soil microbial community structures and real-time quantitative PCR to measure the abundances of N-cycling functional genes (NFGs), specifically those involved in organic N decomposition (*chiA*), N fixation (*nifH*), archaeal and bacterial ammonia oxidation (*amoA*; AOA and AOB, respectively), and nitrite reduction (*nirK* and *nirS*). The composition and diversity of N-cycling microbes shifted between the upper and lower soils. Because of changes in soil nutrient concentrations and soil conditions with soil depth, N-cycling microbial taxa searched for different ecological niches. The absolute abundances of NFGs decreased with depth in both forest soils, indicating that N transformations mainly occurred in surface soil. The relative abundances of NFGs differed between the two forest soils. Because the soil C/N ratios declined with depth in the soil profile, N-cycling microbes invested less energy in N-supplying mineralization and fixation processes that were C-intensive and invested more energy in ammonia oxidization to balance the soil stoichiometry in the subtropical forest subsoils. The *nifH*, which dominated the soil profiles of the temperate forest were mainly controlled by the soil N concentrations, indicating the high microbial demand for N. We also found that the microbial N storage potentials were lower, and the NO_3^--N leaching potentials were higher, in the N-rich subtropical forest surface soils and subsoils than in the temperate forest subsoils and surface soils, and that these potentials were significantly correlated with the soil N/P ratios. This suggests that N-cycling microbes change their energy investments at the molecular scale depending on the level of N saturation in the soil.

1. Introduction

There is considerable variation in the numerous microbial communities that drive biogeochemical cycling throughout soil profiles (Wu et al., 2016). However, most recent studies of microbial communities have mainly focused on surface soils, and have ignored the fact that there are large stores of soil organic matter and vast numbers of microorganisms in some subsoils (Fierer et al., 2003; Agnelli et al., 2004; Stone et al., 2015).

Environmental conditions, such as climate change, plant diversity, soil pH, and nutrient concentrations (Nelson et al., 2016), tend to change dramatically with depth in the soil profile. Microbial communities, tend to shift in response to these variations by changing the absolute or relative abundance of taxa in the community (Allison et al., 2013). Therefore, it is reasonable to assume that spatial heterogeneity in soil properties causes vertical diversity in microbial communities. It has been shown that the microbial mass declines with soil depth and that microorganisms tend to inhabit unique vertical environment niches

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https://doi.org/10.1016/j.apsoil.2017.10.029
Received 14 June 2017; Received in revised form 13 October 2017; Accepted 26 October 2017

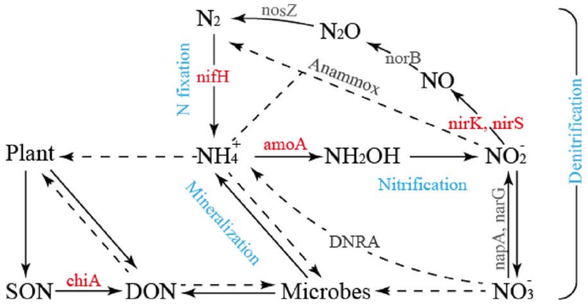

Fig. 1. Main nitrogen cycle processes in forest soils, of which the ones considered in this study and their common corresponding functional genes are shown in blue and red, respectively (adapted from Levy-Booth et al. (2014)). DNRA = dissimilatory nitrate reduction to ammonium. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Agnelli et al., 2004; Hansel et al., 2008; Eilers et al., 2012); we cannot therefore assume that the metabolic activities and functions of microorganisms in subsoils reflect those in surface soils.

While nitrogen (N) is an essential driver of plant production and microbial functions in terrestrial ecosystems, it is frequently limiting (Marklein and Houlton, 2012). The N cycle in soil is a network of interlinked processes that is catalyzed by a series of microbes (Fig. 1). For example, diazotrophic microorganisms can fix atmospheric N_2 into bioavailable N. As a proxy for mineralization, chitinase catalyzes the decomposition of chitin, one of the most abundant N-containing natural macromolecules (Brankatschk et al., 2011). Both N fixation and mineralization are responsible for the acquisition of bioavailable N at the expense of soil organic matter and energy (Geisseler et al., 2010; Dos Santos and Dean, 2011). Ammonia oxidization is the first and rate-limiting step of autotrophic nitrification (Frijlink et al., 1992), in which ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) acquire energy by oxidizing ammonia to hydroxylamine and fixing inorganic carbon (He et al., 2012; Stone et al., 2015). Denitrification involves consecutive dissimilatory sequential reduction of nitrate to dinitrogen gas; nitrite reduction is an important component in this process that results in the loss of gaseous N (Levy-Booth et al., 2014). Studies have shown that the abundances of ammonia oxidizing and denitrifying microbes decline markedly with soil depth (Fischer et al., 2013; Stone et al., 2015). Information about the energy investment strategies and function of N-cycling microbes is urgently needed, as the mechanisms that control the distribution and structures of different functional N-cycling groups along the soil profile are poorly understood.

Fig. 1 There are vast differences between the climatic conditions, plantation diversities, and soil properties of subtropical and temperate forests in southeastern and northeastern China, respectively. For example, precipitation and temperature are higher in the former than the latter; subtropical soils are generally N-rich and P deficient, and are exposed to N deposition, while temperate forest soils have relatively high P and organic C concentrations (Xu et al., 2017; Zhang et al., 2017). These environmental differences can influence soil microbial communities. Studies have shown that soil P concentrations, pH, and redox potential were important influences on NFGs involved in N-cycling in subtropical forest soils (Zhang et al., 2014a; Tang et al., 2016), and that NFGs were positively correlated with soil C and N concentrations (Levy-Booth and Winder, 2010; Szukics et al., 2012; Norman and Barrett, 2014) in temperate forest soils. We are still not sure if the same factors control microbes in surface soils and subsoils, nor do we have a comprehensive understanding of how microbial N-cycling strategies, such as potential N storage, NO_3^--N leaching, and gaseous N loss, differ between temperate and subtropical forest soils with very different N saturation conditions. Answers to these questions would help us to predict and understand the controls on N turnover in forest ecosystems in the context of global warming and increasing N deposition.

In this study, we measured the abundances of NFGs and used *nifH* to represent N transformations during N fixation, *chiA* to represent organic N decomposition, *amoA* (including AOA and AOB) to represent ammonia oxidation, and *nirK* and *nirS* to represent nitrite reduction. We also examined how the community structures of N-cycling microbes such as *nifH*, *chiA*, AOA, and *nirS* varied at different depths in the temperate forest soil profiles where there were more sources of C and the vertical nutrient variation was more distinct than in the subtropical forest. We predicted that the N-cycling microbial community structures would differ between surface soils and subsoils because of decreases in soil C and N resources with soil depth. Reflecting the different plantations and edaphic properties, we hypothesized that the N-cycling microorganism communities would differ in size but would both decrease with soil depth in these two forests. We predicted that, because of the high N deposition in the subtropical forest soils, ammonia oxidizers would be more abundant in subtropical soils than in the temperate forest soils, and that *nifH*, *chiA*, *nirK*, and *nirS* would be more abundant in the temperate forest soils, because of the higher soil C resources. We also hypothesized that N-cycling microbes would occupy different vertical niches; that is, soil C and nutrient resources would become increasingly scarce with depth in the soil, so the relative abundance of energy-consuming microorganisms with *nifH*, *chiA*, *nirK* and *nirS* would decrease, and the relative abundance of energy-acquiring ammonia oxidizers would increase. We also wanted to examine how the N turnover potential varied under different conditions. We used the (*nifH* + *chiA*)/(AOA + AOB) *amoA* and (AOA + AOB) *amoA*/(*nirK* + *nirS*) gene abundance ratios and the abundance of the *nirK* + *nirS* genes as indicators of potential microbial N storage, NO_3^--N leaching, and gaseous N emissions, respectively. We acknowledge that, as we did not measure all possible N-cycling processes, such as dissimilatory nitrate reduction to ammonium and mineralization by fungi, our approach does not provide a complete understanding of microbial N turnover. We believe however that it provides information about the capacity of microbes to both store and release N under different conditions. We predicted that, to balance the soil stoichiometry, potential N loss would be high, and potential N storage would be low, under relatively high N saturation. We also wanted to confirm the main controls on the abundance, distribution, and changes in the community structure of N-cycling microbes, as we predicted that changes in N-cycling communities were mainly driven by soil C and N concentrations.

2. Materials and methods

2.1. Site description

The study sites were in the Changbai Mountain (CBM) temperate native forest (42°24′02″N, 128°05′42″E) and Dinghu Mountain (DHM) subtropical native forest (23°10′13″N, 112°32′26″E) (Table 1). Changbai Mountain is a native conifer, mixed broad-leaf forest with Korean pine (*Pinus koraiensis*) and mixed Mongolian oak (*Quercus mongolica*) as the main species. The DHM forest, a native evergreen broad-leaf forest, is dominated by Chinese cherry (*Schim asuperba*), chinquapin (*Castanopsis chinensis*), and Chinese cryptocarya (*Cryptocarya chinensis*). The CBM site has a continental temperate monsoon, a mean annual temperature of 2.8 °C, and a mean annual precipitation of 731 mm. The DHM site has a subtropical monsoon climate, a mean annual temperature of 22.0 °C, and a mean annual precipitation of 1733 mm. The soils in CBM and DHM are classified as Argosols and Latosols, respectively (Soil Survey Staff, 2010).

2.2. Soil collection and physico-chemical analysis

Soil samples were collected in July and August of 2016 in the CBM and DHM forests, respectively. For each site, we excavated 5 random soil subsamples of mineral soil from 0 to 80 cm deep using a soil auger