Impacts of nitrogen and phosphorus additions on the abundance and community structure of ammonia oxidizers and denitrifying bacteria in Chinese fir plantations

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ABSTRACT

Nitrogen (N) cycling in forest ecosystems has been extensively studied over the past decade. However, even with this concentrated research effort, we still do not have a comprehensive understanding of how N-cycling microbial communities in subtropical plantations respond to N and phosphorus (P) additions. We designed an on-going fertilization experiment in which varying amounts of N or/and P were added to forest soil so that the effects of N and P applications on the abundances and community structures of nitrifying and denitrifying microbes in Chinese fir plantations in subtropical China could be explored using quantitative real time PCR (*q*PCR) and high-throughput sequencing methods. The results showed that most N-cycle functional genes responded differently to N or/and P additions depending on the type and amount of nutrients added. Ammonia oxidizing archaea (AOA) were more abundant and active than ammonia oxidizing bacteria (AOB) in all treatments. While the abundances of *nirK* and *nirS* decreased under the high N applications, the abundances of *narG* increased. With the exception of AOB *amoA* and *nirS*, the abundances of all the functional genes were strongly and positively correlated with the concentrations of soil available P (AP) and negatively correlated with the ratio of available N to AP, which suggested that P had an important influence on the abundances of N-cycling microbes in these forest ecosystems. Shifts in communities of both AOA and *nirK*-type bacteria were driven by combined application of N and P, with group I.1a-associated and Rhizobiales as the most relatively abundant groups, respectively.

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1. Introduction

As the most limiting biological nutrients, nitrogen (N) and phosphorus (P) play important roles in controlling plant productivity and microbial functions in terrestrial ecosystems (Marklein and Houlton, 2012). The atmospheric N deposition observed in forest ecosystem is expected to increase further in the future which will significantly modify nutrient availability (Janssens et al., 2015). Nitrogen is generally considered as the main element that controls plant productivity, species composition, and diversity (Weand et al., 2010). Excessive N inputs into forest ecosystems can also result in negative consequences, e.g. increasing soil acidification (Lu et al., 2014), nitrate leaching (Dise et al., 2009), and greenhouse gas emissions (IPCC, 2007). Phosphorus is also a limiting element for primary productivity in terrestrial ecosystems, both individually and in combination with N (Vitousek et al., 2010). The natural P supply is poor in China's southern subtropical regions (Chen et al., 2014a). Unlike N, which can accumulate either rapidly by biological N fixation or atmospheric deposition, P does not increase at the same rate because of losses from land via runoff and transformations of soil mineral P (Huang et al., 2016; Turner et al., 2012). Further, N deposition can lead to an element imbalance and may enhance P limitations in forest ecosystems (Chen et al., 2015).

Soil microorganisms are key drivers in ecosystem N cycling

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(Huang et al., 2013; Wang et al., 2006). Although nitrification can be performed completely by *Nitrospira* bacteria (Daims et al., 2015), aerobic chemoautotrophic nitrification is traditionally considered to involve two steps, namely ammonia oxidation followed by conversion from nitrite to nitrate (Prosser, 1989). Ammonia oxidation, the first and rate-limiting step of nitrification that involves the conversion of ammonia to nitrite, is considered central to the global nitrogen cycle (Kowalchuk and Stephen, 2001) (Fig. 1). Because of the significance of ammonia oxidation in the global N cycle, the *amoA* gene, the alpha subunit of the ammonia monooxygenase has been widely studied as a molecular marker in various ecosystems (Hu et al., 2015). However, for over a century, ammonia oxidizing bacteria (AOB) were considered as the main driver for ammonia oxidization until ammonia oxidizing archaea (AOA) were discovered (Venter et al., 2004).

Denitrification involves consecutive dissimilatory reduction during which NO_3^--N is transformed into N_2 ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) by different groups of bacteria (Geets et al., 2007) (Fig. 1). Firstly, nitrate is reduced to nitrite ($NO_3^- \rightarrow NO_2^-$) with *narG* and *napA* genes encoding the nitrate reductase. The second step ($NO_2^- \rightarrow NO$) is catalyzed by two different nitrite reductases (NIR) encoded as *nirK* (copper-containing) or *nirS* (cytochrome cd1-containing) that normally do not appear in the same organism (Zumft, 1997). NIR is thought to be the most important enzyme in the denitrification process because of its role in gas formation (Levy-Booth et al., 2014). The next step leads to N_2O formation (NO $\rightarrow N_2O$), which is mediated by nitric oxide reductase (NOR) encoded by *cnorB* or *qnorB* genes, after which N_2O reduction to N_2 ($N_2O \rightarrow N_2$) is catalyzed by nitrous oxide reductase (NOS) with *nosZ* as the gene marker.

As far as we know, nitrogen cycle is a network of interlinked processes (Shen et al., 2014) which is responsible for N losses by NO_3^--N leaching and NO, N_2O or N_2 emissions caused by both nitrifiers and denitrifiers. To date, most current studies have focused on individual steps of the N cycle, such as ammonia oxidation (Chen et al., 2014b; Long et al., 2012), NO_2^--N reduction (Chen et al., 2010; Yoshida et al., 2009), and the production or reduction of N_2O (Morales et al., 2010). To obtain a comprehensive understanding of N transformations, studies that consider all the key steps in N cycling are needed.

As one of the major plantation tree species in China, the Chinese fir (*Cunninghamia lanceolata*) has been extensively planted because of its yield and excellent timber quality. It occupies 9.11 million hectares and accounts for about 18% and 5% of all plantations in China and worldwide, respectively (Ma et al., 2007). A variety of management approaches have been employed to improve the soil fertility and forest productivity, among which fertilization is the most effective and feasible. An increasing number of studies have

reported the influence of nutrient additions on C and N sequestration in Chinese fir plantations (Fan et al., 2014; Janssems et al., 2015; Wei et al., 2012). However, relatively few studies have focused on soil microbial properties and the microbes involved in N cycling. Moreover, in the context of increasing atmospheric N deposition and N fertilization, we need an improved understanding of the influence of P on N cycling in forest ecosystems that receive exogenous N additions.

We can use molecular measurements on N-cycle functional genes to assess the relationships between soil amendments, soil characteristics, and microbial community functions. Numerous studies have examined the effects of N additions on N cycling using the real-time quantitative PCR (qPCR) approach. Carey et al. (2016), from their meta-analysis of effect of integrated applications of various fertilizer types on different ecosystems, showed that AOB were more dynamic and had greater potential for nitrification than AOA after additions of N. Out of the denitrifying genes, fertilizer had the greatest influence on the population of *narG*, and least on the population of *qnorB* (Chen et al., 2012). There is also a study indicating that long-term fertilizer applications had a significant impact on the size of the *nirK* community, but had little impact on the *nirS* community (Chen et al., 2010). Previous studies have shown that the abundance and community compositions of N-cycle functional genes can be affected by N forms (Szukics et al., 2009) and N deposition levels (Bárta et al., 2010). However, few studies have addressed the influence of P additions, and, in particular, the interactions between N and P, on forest soil microbial communities involved in N cycling. In fact, reports of the interactions between P and N inputs in subtropical forests are rare (Chen et al., 2015). There is therefore a need for an improved understanding of how nutrient additions influence the N cycling process in subtropical forest ecosystems.

In this study, we reported the effects of N, P, and combined N and P additions on soil properties and N-cycling microorganisms in a Chinese fir plantation. We measured the abundances of nitrifying (AOA, AOB *amoA*) and denitrifying (*narG*, *nirK*, *nirS*, *qnorB* and *nosZ*) genes and examined the community structures of AOA *amoA* and *nirK* to investigate their response to N and P additions to the Chinese fir soils. We hypothesized that low NH_4NO_3 application rates would increase the N-cycle functional gene abundances because of the nutrient-poor conditions in subtropical forests in southern China (Chen et al., 2015), while high NH_4NO_3 application rates would decrease the abundance of N-cycling microorganisms because of the decrease in soil pH, as was reported in a similar study carried out in a grassland system (Ning et al., 2015). We also hypothesized that P applications would increase the abundance of N-cycle genes and consequently increase the potential for N losses from the forest, as P additions would mitigate the P limitations to microorganisms. We evaluated the responses of N-cycling microorganisms as part of an integrated process rather than on an individual basis in a forest and compared them with those previously reported for other ecosystems so as to obtain an improved understanding of the mechanisms of N-cycling in forest ecosystems.

2. Materials and methods

2.1. Site description and experimental design

The experimental site is part of the Chinese Academy of Sciences Ecological Research Station at Qianyanzhou, Jiangxi Province, Southern China (115°03′29.2″E, 26°44′29.1″N). A subtropical monsoon climate dominates in this region. The mean annual temperature and precipitation at the site were 17.9 °C and 1600 mm, respectively (Chen et al., 2015). The mean soil temperature and soil gravimetric water content were 17.4 °C and 22.3% in
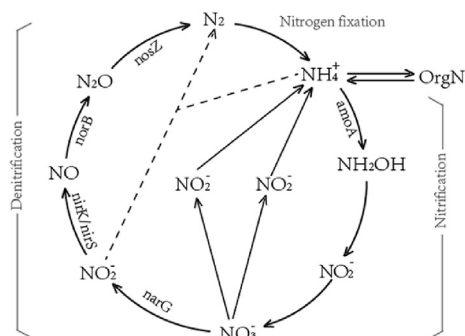

Fig. 1. Functional genes for nitrification and denitrification (adapted from (Yang et al., 2013)).