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Environmental variables better explain changes in potential nitrification and denitrification activities than microbial properties in fertilized forest soils

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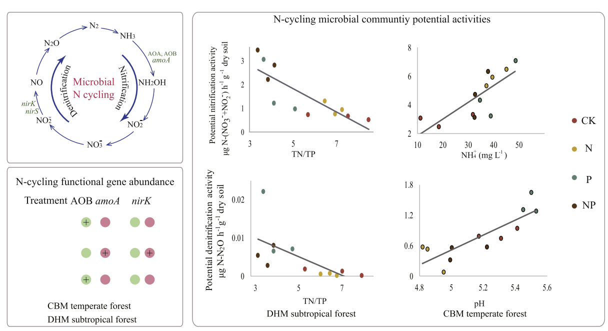
HIGHLIGHTS

- Both PNA and PDA increased when P was added to subtropical forest soils.
- PNA increased and PDA decreased when N was added to temperate forest soils.
- The composition of functional groups had minimal effects on PNA or PDA.
- PNA and PDA in subtropical forest soils were mainly controlled by the soil TN/TP.
- Overall, NH_4^+ and pH were the main drivers of PNA and PDA, respectively.

GRAPHICAL ABSTRACT



AOA: ammonia-oxidizing archaea; AOB: ammonia-oxidizing bacteria; DHM: Dinghu mountain; CBM: Changbai mountain; TN/TP: total N to total P ratio; CK: control; N: nitrogen fertilizer addition; P: phosphorus fertilizer addition; NP: combined nitrogen and phosphorus fertilizer addition; +: Increasing effect.

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ABSTRACT

Because of increases in atmospheric nitrogen (N) deposition worldwide, nutrient imbalances and phosphorus (P) limitations in soil are aggravated, with the result that P fertilizer applications to terrestrial ecosystems worldwide may increase. Nitrification and denitrification in soil are major sources of nitrous oxide emissions, especially in soils treated with fertilizers. However, few researchers have studied how forest soils respond to nutrient additions, so we are not sure how the potential nitrification and denitrification activities (PNA and PDA, respectively) and microbial communities involved in these processes might respond when N and P are added to temperate and subtropical forest soils. We investigated how the PNA, PDA, the abundances and community compositions of nitrifiers and denitrifiers, and environmental properties, including soil pH, soil total and dissolved organic carbon, total and available N and phosphorus P, changed when N and/or P were added to subtropical and temperate forest soils. We quantified the abundance, and analyzed the composition, of functional marker genes of nitrifiers (ammonia-oxidizing bacteria and archaea *amoA*) and denitrifiers (*nirK* and *nirS*) using quantitative PCR and sequencing, respectively. We found that the PNA and PDA in the subtropical soil increased when P was added and PNA in the temperate forest soil increased when either N or P was added. The PNA and PDA were positively correlated with the abundance of ammonia-oxidizing bacteria and *nirK*-denitrifiers, respectively, in the subtropical forest soil but were not correlated with changes in corresponding community compositions in either of the forest soils. The soil total N to total P ratio explained most of the variabilities in the PNA and PDA in the subtropical forest soils, and the soil exchangeable ammonium concentrations and pH were the main controls on the PNA and PDA,

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respectively, in the temperate forest soils. Our results indicate that soil environmental conditions have more influence on variations in the PNA and PDA in forest soils fertilized with N and P than the corresponding microbial properties.

1. Introduction

In recent years, nitrogen (N) deposition has increased worldwide, especially in forest ecosystems (Galloway et al., 2008). This increase in N inputs to terrestrial ecosystems may aggravate nutrient imbalances and phosphorus (P) limitations in soils, such that it may be necessary to apply P fertilizer to maintain net primary production (Marklein and Houlton, 2012; Lebauer and Treseder, 2008). The interactions between nutrient additions to soils, either via atmospheric deposition or anthropogenic fertilization, and nitrous oxide (N_2O) emissions from soils have attracted considerable attention because of the contribution of ongoing increases in greenhouse gas emissions to climate change (Hu et al., 2015). Soil ecosystems constitute the largest source of N_2O emissions, mainly from nitrification and denitrification (Baggs and Philippot, 2009; Hu et al., 2015). However, despite their potential to contribute to N_2O emissions, the effects of N and P additions on the abundances, compositions, and activities of nitrifying and denitrifying communities in forest soils are poorly understood.

Except some *Nitrospira* bacteria are known to perform comammox (Daims et al., 2015), nitrification is traditionally considered a two-step oxidation process, of which ammonia oxidation is the first and rate-limiting step (Frijlink et al., 1992) and is catalyzed by two functional microbial guilds, namely ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB). The availability of ammonia is considered the primary determinant of the growth of AOA and AOB (Verhamme et al., 2011). Ammonia-oxidizing archaea are smaller, have a wider surface-to-volume ratio (Martens-Habbena and Stahl, 2011), a stronger affinity for ammonia (Martens-Habbena et al., 2009), and are more energy efficient than AOB (Konneke et al., 2014), all of which help to define their different niches in the environment. During denitrification, nitrate is converted to dinitrogen gas in a series of consecutive dissimilatory processes by particular groups of heterotrophic bacteria. The reduction of nitrite to nitric oxide, the first gas-producing step in denitrification, is catalyzed by two different nitrite reductases (NIR), encoded by *nirK* (copper-containing NIR) or *nirS* (cytochrome *cd*1-containing NIR) genes. These two structurally different but functionally equivalent genes are generally used to indicate the overall biological potential for denitrification (Attard et al., 2011; Morales et al., 2010; Yin et al., 2015).

Potential nitrification and denitrification activities can be assumed as proxies of the corresponding enzyme concentrations (Tiedje, 1982). Changes in PNA or PDA may result from changes in the physiological activity of individual cells, and changes in the abundance of corresponding microorganisms and/or changes in their community compositions (Attard et al., 2011). So studies have reported that genetic characteristics and environmental factors were the main influences on PNA and PDA. From their meta-analysis, Carey et al. (2016) reported that AOB responded more dynamic than AOA when inorganic N and P were added, and that the increases observed in the PNA when N was added were significantly correlated with only AOB abundance. Shen et al. (2008) suggested that the soil pH was an important driver of the abundance of AOB, and that the AOB abundance was significantly related to the PNA in soils that had been treated with chemical and/or organic fertilizers over the long-term. While Wertz et al. (2012) found that there were significant relationships between the soil NO_3^- concentration, AOB abundance, and PNA, they suggested that there needed to be a shift in the AOB community composition before the PNA would increase in soils to which N was applied. Horz et al. (2004) found that the positive correlation between soil NH_4^+ concentrations and AOB community composition was associated with changes in the PNA caused by increases in N deposition. Yao et al. (2011), however, suggested that the community compositions of both AOA and AOB were related to variations in the soil pH, but a significant relationship between community abundance and PNA was observed only for AOA in soils with different pH and N inputs. Song et al. (2016) reported that the roles of AOA and AOB in nitrification depended on the pH in soils that were gradually acidifying, and that soil dissolved organic carbon (DOC) was an important influence on the community compositions of AOA and AOB, and consequently was related to PNA. Studies have also reported contradictory results about PDA. For example, Cuhel et al. (2010) suggested that the soil pH was a fundamental control on denitrification, and that the PDA was correlated with the abundance of the *nirS*-denitrifier. Petersen et al. (2012) suggested that the *nirK/S* gene abundance and soil NO_3^- concentration had indirect effects on the PDA. Yin et al. (2014) reported that the decrease in the PDA was mirrored by the abundance of *nirS*-denitrifers when inorganic fertilizer N, P and potassium were added to a black soil. However, Shrewsbury et al. (2016) suggested that environmental variables, like the soil water content and total soluble N, were more closely related to the PDA than to the denitrifier abundance. Similarly, Attard et al. (2011) found that the soil C, oxygen, and NO_3^- were more useful for predicting changes in the PDA than the denitrifier abundance and community structure. These related literature shows that we still do not know the relative importance of environmental and microbiological factors on explaining PNA and PDA in forest soils to which N and/or P have been added.

The environmental conditions vary considerably between subtropical and temperate forests in eastern China. Subtropical soils are highly weathered and organic matter mineralizes rapidly. Soils in the subtropical forests are subject to heavy N deposition, but receive very little biological or atmospheric P inputs, which means that soils are relatively N-rich and P-deficient. Temperate forest soils, on the other hand, are generally rich in P and organic C, but are relatively deficient in N (Xu et al., 2017; Zhang et al., 2018). The abundance of N-cycling microbes was reported limited by P (Tang et al., 2016). While the abundance and composition of N-cycling microbial communities in temperate soils may be influenced by soil C and N concentrations (Levy-Booth et al., 2014; Norman and Barrett, 2014). The increases in atmospheric N deposition observed in both subtropical and temperate forests in eastern china (Zhu et al., 2015) exacerbated the existing stoichiometric nutrient imbalance and caused P limitations (Deng et al., 2017). There are very few N and P fertilizer trials in these types of forest, so we have limited information about how additions of N and P might affect the abundances, compositions, and activities of nitrifying and denitrifying communities in these forest soils.

In this study, we applied N and/or P to the forest soils to mimic N deposition and P fertilization in forest soils with contrasting properties. We studied the effects of N and P fertilizer additions, both individually and combined, on soil properties and nitrifying and denitrifying communities in subtropical and temperate forest soils in eastern China. Our specific objectives were to determine the influence of N and P additions on the abundances, compositions, and potential activities of nitrifying and denitrifying communities; and to evaluate to what extent changes in the soil PNA and PDA could be explained by the microbial characteristics of the corresponding communities and soil conditions in different forest soils. We hypothesized that 1) the nitrifying and denitrifying communities and potential activities in the subtropical forest soils would be stimulated by P additions, and that 2) soil organic C, as an energy source, and N availability, as a substrate, would be the main controls on the abundances and compositions of these communities