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Responses of soil phosphorus fractions after nitrogen addition in a subtropical forest ecosystem: Insights from decreased Fe and Al oxides and increased plant roots

Yuexin Fan[a,b], Xiaojian Zhong[a,b], Fang Lin[a,b], Chenchung Liu[c], Liuming Yang[a,b], Minhuang Wang[a,b], Guangshui Chen[a,b], Yuehmin Chen[a,b,d,*], Yusheng Yang[a,b,d,*]

[a] College of Geographical Science, Fujian Normal University, Fuzhou 350007, China
[b] State Key Laboratory of Subtropical Mountain Ecology (Funded by Ministry of Science and Technology and Fujian Province), Fujian Normal University, Fuzhou 350007, China
[c] Department of Environmental Engineering, National Ilan University, Ilan 260, Taiwan
[d] Institute of Geography, Fujian Normal University, Fuzhou 350007, China

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ABSTRACT

Elevated nitrogen (N) deposition may exacerbate soil phosphorus (P) limitations in P-deficient subtropical forest ecosystems. However, which abiotic factors predominantly contribute to soil P transformations and how plant roots affect soil P variations after N addition in these ecosystems are unclear. To address these issues, we studied a natural *Castanopsis carlesii* forest in Fujian, China subjected to 3 years of N addition. Soil P fractions, soil properties, iron (Fe) and aluminum (Al) oxides, root biomass (RB), and root length density (RLD) were investigated. The results showed that N application remarkably increased the concentrations of soil available P but significantly decreased content of soil moderately labile P. The quantities of free Fe and Al (Fe_d and Al_d) and organic-bound Fe and Al (Fe_p and Al_p) decreased after N addition. These changes indicated an important relationship between Fe and Al oxides and P fractions, especially Fe_p and moderately labile P, suggesting that Fe and Al oxides predominantly influenced soil P fractions in N-enriched plots. Meanwhile, RB and RLD were higher after N addition treatments than control treatment. Positive correlations between RB and RLD and P fractions (available P, labile P, and moderately labile P) indicated that plant roots exert an essential influence on changes in soil P fractions. In conclusion, N addition in subtropical forests significantly influenced soil P fractions, primarily by decreasing Fe and Al oxides and increasing plant root biomass and density.

1. Introduction

Nitrogen (N) and phosphorus (P) constrain plant productivity in the vast majority of terrestrial ecosystems (Elser et al., 2007; García-Oliva et al., 2018; Reich et al., 2004; Yang et al., 2015). Subtropical forest ecosystems are characterized by strongly weathered soil with high P-fixation capacities (Velásquez et al., 2016; Walker and Syers, 1976), making P as the limiting factor for structures, functions, and processes of these ecosystems (Hou et al., 2016; Penuelas et al., 2013; Turner, 2008; Vitousek et al., 2010). Furthermore, N deposition is especially serious in Southeast China due to human activity (Liu et al., 2011; Lu et al., 2014) and further stimulates plant P demands; thus; N deposition has been a research priority in recent decades (Deng et al., 2016; Fan et al., 2018; Sherman et al., 2006). However, N addition studies in this region have observed inconsistent soil P fraction responses (Fan et al., 2018; Huang et al., 2014; Mirabello et al., 2013), implying that N-mediated changes in soil P fractions and their mechanisms remain to be clarified. Consequently, N enrichment and associated P cycling require further evaluation in subtropical forest ecosystems.

Nitrogen deposition exerts a profound influence on soil abiotic properties, such as soil acidification (Bowman et al., 2008; Lu et al., 2014), iron (Fe) and aluminum (Al) oxide contents (Fernandez et al., 2003; Sherman et al., 2006), and soil exchangeable cations and their exchangeable capacity (Shi et al., 2016; Tian and Niu, 2015), which have been well documented as the main drivers of soil P availability in terrestrial ecosystems. For instance, Fe and Al oxides are the major minerals affecting P solubility in soils by sorption reactions (Reed et al., 2011; Vitousek et al., 2010). Changes in pH and soil cation levels induced by environment variations affected soil organic P forms and P saturation status (Hou et al., 2018b). A meta-analysis of 106 studies

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[*] Corresponding authors at: College of Geographical Science, Fujian Normal University, Fuzhou 350007, China.
E-mail addresses: ymchen@fjnu.edu.cn (Y. Chen), geoyys@fjnu.edu.cn (Y. Yang).

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revealed that global patterns of soil acidification in response to N addition had shifted into the Al buffering phase (Tian and Niu, 2015). Furthermore, soil buffering systems may shift from Al towards Fe at pH levels lower than 4 (Bowman et al., 2008). These results emphasize the pivotal role of Fe and Al oxides in slowing soil acidification related to soil P solubility, especially in acidic subtropical soil. In addition, P fractions bonded to Fe and Al account for approximately 18–33% of the total P in higher-weathered soils (Fan et al., 2018; Margenot et al., 2017) and ultimately determine available P content in subtropical forest soil. However, our understanding of changes in Fe and Al oxide levels and their influence on soil P fractions after N addition is incomplete.

N application alters forest soil nutrient status and further induces variations in plant root traits (Li et al., 2015). Numerous fertilization experiments have been conducted in tropical, temperate, and boreal forests to investigate plant root responses to N addition and have revealed inconsistent results (Clemensson-Lindell and Persson, 1995; Lee and Jose, 2003; Li et al., 2015; Li et al., 2018; Mo et al., 2007; Son and Hwang, 2003; Tian et al., 2016; Zogg et al., 1996). Even studies of similar tropical forest ecosystems have produced inconsistent observations (Adamek et al., 2011; Tu et al., 2011), suggesting unclear patterns of plant root responses to N application. Nevertheless, plant roots play a substantial role in the P cycling of forest ecosystems by uptake of P nutrients needed for plant production and by secretion of enzymes or organic acids that alter soil P fractions (Kitayama, 2013; Landeweert et al., 2001; Yokoyama et al., 2017). However, previous studies exploring the relationship between soil P and N addition have paid more attention to the effect of soil nutrient status (Hou et al., 2015; Velásquez et al., 2016; Yang et al., 2015), enzyme activity, and microbes on soil P fractions (Kitayama, 2013; Rosling et al., 2015; Turner et al., 2013) than on the function of plant roots in soil P dissolution and depletion. Lack of knowledge of the interactions between plant roots and soil P availability limits our ability to ameliorate plant P limitations in subtropical forest ecosystems.

In this study, we investigated soil P fractions, base cation levels, Fe and Al oxide levels, plant root biomass (RB), and plant root length density (RLD) in a natural *Castanopsis carlesii* forest in Southeast China after three consecutive years of N addition. We aimed to elucidate soil P fraction responses to N deposition, Fe and Al oxides (abiotic factors), and plant roots (biotic factors). We hypothesized that (i) Fe and Al oxides are the predominant abiotic factors affecting soil P availability, owing to a shift in the soil buffering system to Al and Fe at lower pH; and that (ii) plant roots significantly affect soil P fractions because N application enhances the P demands of plants in this area.

2. Materials and methods

2.1. Site description

This study was conducted at the *Castanopsis kawakamii* Nature Reserve in Fujian Province, Southeast China (117°28′E longitude and 26°11′N latitude), a region with a subtropical monsoon climate. The mean annual temperature is 19.4 °C, mean annual precipitation is 1700 mm, and relative humidity is 79%. The soil is an oxisol formed from sandstone and classified as red soil per Chinese soil classification, equivalent to an oxisol in USDA Soil Taxonomy. The soil depth is approximately 30–70 cm, and the soil texture is sandy clay. The natural *Castanopsis carlesii* forest has been undisturbed for > 200 years. We established a permanent plot in 2012 at an altitude of 300–315 m above sea level on a northeast-facing slope. *Castanopsis carlesii* is the predominant canopy species, and other species include *Schima superba* and *Pinus massoniana*. Canopy coverage is approximately 89%, and the mean tree height, mean tree diameter at breast height, and stand density were 11.9 m, 20.0 cm, and 1955 stem ha^{-1}, respectively (Guo et al., 2016).

2.2. Experimental design

In order to explore the response of soil P cycling to elevated anthropogenic N deposition in the future, we established three treatments in the natural forest, including a control treatment without N addition (CT), a low-N treatment of 40 kg ha^{-1} yr^{-1} (LN), and a high-N treatment of 80 kg ha^{-1} yr^{-1} (HN). Each treatment was replicated 4 times; 12 plots of 20 m × 20 m were established randomly, each surrounded by a 10 m wide buffer zone. Since November 2012, 20 L NH_4NO_3 solution (containing 381 g NH_4NO_3 in LN and 762 g NH_4NO_3 in HN) was applied by hand to the forest floor in the LN and HN treatment areas on a monthly basis. The same volume of distilled water was applied in CT.

2.3. Soil sample collection

Soil samples were taken from both the A (0–10 cm) and B layers (10–20 cm) in October 2015; five cores were collected randomly from each plot (after removing a litter layer), combined, and homogenized. In total, 24 samples (3 treatments × 4 replications × 2 layers) were placed in sealed plastic bags and immediately transported to the laboratory. The stones and roots were picked out, and each fresh soil sample was sieved into 2 mm particles, and then divided into two subsamples. One was refrigerated at 4 °C to measure NH_4^+-N and NO_3^--N, while the other was air-dried and used to analyze soil P fractions, Fe and Al oxides, and soil properties.

2.4. Plant root sampling and data collection

From each treatment area, 32 root cores were taken with a soil corer (diameter 50 mm) after removal of a litter layer. All soil cores were divided into 0–10 and 10–20 cm fractions according to sampling depth. Roots were separated from the soil by washing. The roots were sorted as living or dead based on shape, color, elasticity, and toughness; living roots with diameters < 5 mm were selected. These roots were scanned with an Epson Perfection V750 Pro scanner (Epson, Tokyo, Japan), and the images were analyzed with WinRHIZO Pro 2009b (Regent Instruments, Inc., Sainte-Foy, Canada) to determine RLD. The roots were then dried at 65 °C to a constant mass to estimate RB.

To compare the responses of plant roots to N addition in this study with those reported in other ecosystems around the world, data were collected from 22 journal articles (Supplementary Table S1) published since 1995 using the Web of Science resource and searching terms “nitrogen addition” and “root” or “fertilization” and “root”. We select observations based on the following criteria (i) field-simulated nitrogen deposition studies were selected, while laboratory incubation studies were not included; (ii) only control and simulated nitrogen deposition treatment data were selected in multifactorial studies; (iii) studies in which the fertilizers only contained nitrogen and no other nutrients (e.g. K, P, and Ca) were selected. Plant root biomass data were compiled from control and nitrogen deposition treatments in the experiments. Variations (v) in root biomass due to N enrichment in the different studies were calculated by:

$$v = \frac{R_t - R_c}{R_c} \times 100\%$$

where R_t and R_c are root biomass of the N-addition treatment and control groups, respectively. Patterns in root biomass variations after N application on a global scale were exhibited using ArcGIS 10.2 (ESRI Inc., Redlands, CA, USA).

2.5. Measurements of soil properties

The pH was determined using a glass electrode (Starter 300; Ohaus, Parsippany, NJ, USA) with a 1:2.5 soil:water solution (w/v). Soil total N (TN) and organic C (SOC) were measured using an elemental analyzer (Elementar Vario EL III; Elementar, Langenselbod, Germany). Soil

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