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#### Original article

# Effects of phosphorus addition on soil microbial biomass and community composition in a subalpine spruce plantation



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#### ABSTRACT

Phosphorus (P) availability is expected to affect soil microbial community. However, our knowledge about the responses of soil microbial biomass and community composition to P availability is still limited. In this study, we established field plots with addition of 0, 5 (LP), 15 (MP) and 30 (HP) g P  $m^{-2}$  yr<sup>-1</sup> in a subalpine spruce plantation and investigated the responses of soil microbes. Chloroform fumigationextraction and phospholipid fatty acids (PLFA) analysis were used to determine soil microbial biomass and community composition, respectively. After two growing seasons of P addition, the HP treatment significantly increased soil microbial biomass carbon, nitrogen and P. The P addition exerted the specific influences on soil microbial community composition. The abundance of most groups of soil microbial community (bacteria, fungi, and arbuscular mycorrhizal fungi) increased in the HP treatment and the ratio of fungi to bacteria decreased in the LP and MP treatment, whereas the nonmetric multidimensional scaling ordination revealed no significant difference in the PLFA pattern between the P treatments and the control. Although soil P availability increased in all P treatments compared to the control, the dissolved organic carbon, indicative of soil carbon availability, was promoted only by the HP treatment. Besides, soil microbial biomass was positively correlated to soil carbon availability and pH. These results indicate that soil microbes are insensitive to the elevated P availability in this spruce plantation and P addition increases soil microbial biomass mainly through improving carbon availability and pH.

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#### 1. Introduction

Soil microbes play a critical role in regulating decomposition and thereby nutrient cycling in forest ecosystems [1]. In turn, soil nutrient availability, especially nitrogen (N) and phosphorus (P), can also affect the microbial biomass and community composition [2,3]. Treseder [2] reviewed the available studies addressing the effects of N on soil microbes and proposed that N addition reduced soil microbial biomass. Compared with microbial response to N availability, only a few studies focus on the effects

http://dx.doi.org/10.1016/j.ejsobi.2015.12.007 1164-5563/© 2015 Elsevier Masson SAS. All rights reserved. of P availability on soil microbes. Furthermore, the limited experimental data is rather controversial, and there is no general agreement on the effects of P availability on soil microbial biomass and community composition. DeForest et al. [4] found no significant changes in soil microbial biomass after P addition in either unglaciated or glaciated soil of temperate deciduous forests, while Li et al. [5] reported that P addition increased soil microbial biomass and fungi:bacteria (F:B) ratio and altered the community composition in P-deficient tropical forests. Other scholars found positive, neutral even negative effects of P on soil microbes in temperate forests [6–8]. Additionally, Liu et al. [3] found that responses of soil microbial biomass and community composition to P addition varied in three forest types in tropical China. These inconsistencies imply that the effects of P addition on soil microbial biomass and community composition may vary

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with the forest biome at global scale and the forest type at local scale, and that the exact relationship between P availability and soil microbial community is poorly understood [4,5]. Given the distinct discrepancy in climate, vegetation and soil types across forest biomes and among forest types, more studies from specific forest ecosystems are indispensable to conclude the effects of P availability on soil microbes.

To date, multiple mechanisms have been proposed to explicate the P effects on soil microbes [e.g., 3, 5, 7, 9] (Fig. 1). For instance, P addition can increase litter fall and fine root biomass, resulting in increased C availability to soil microbes, and eventually improved soil microbial biomass and altered the community composition [3,5]. Besides, the relieved P constraints and changes in soil pH and osmotic potential after P addition can also influence the microbial growth [5,7,10]. Nevertheless, the uncertainties of mechanisms for P effects on soil microbial community exist extensively. Therefore, it is necessary to explore how soil microbial biomass and community composition respond to P addition in a specific forest ecosystem.

As a unique group of cold-temperate coniferous forests, the subalpine coniferous forests of China, concentrating in areas of high mountains, deep valleys and plateaus in western China and dominated by *Picea* and *Abies* species, differ from the boreal forests of the cold-temperate zone and montane coniferous forests of the temperate zone mainly in the species composition and soil condition [11]. However, little is known about the relationship between soil microbes and P availability in this region, which hinders our accurate estimation of the decomposition and nutrient cycling because of the important role of soil microbes in regulating the two processes in forest ecosystems.

The objective of this study was to investigate the changes in soil microbial biomass and community composition after two growing seasons of P addition in a subalpine spruce plantation in the eastern Tibetan Plateau of China. We focused on two questions: (1) How soil microbial biomass and community composition respond to different doses of P addition? (2) Which soil parameters are correlated with the microbial community after P addition? We hypothesized that soil microbes were more sensitive to changes in soil pH and C availability after P addition than to the elevated P availability in this subalpine spruce plantation.

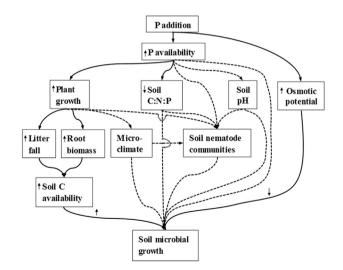


Fig. 1. Potential mechanisms for the P effects on soil microbial growth.

#### 2. Materials and methods

#### 2.1. Study site description

This study was conducted in a spruce (*Picea asperata*) plantation at Ma'erkang County in the northwest of Sichuan province, China (102°16'38.400" E, 31°43'11.142" N). This study site has an altitude of about 3724 m and a slope of 15° with a montane temperate climate with mean annual precipitation of 750 mm falling mainly from May to September. The mean annual temperature at the site is 4.9 °C with the mean maximum monthly temperature of 13.2 °C in July and the mean minimum monthly temperature of -4.7 °C in January [12].

The spruce plantation in our study site, one of the typical forest types in this region, was established on cutovers in 1992 by reforestation with 4 year-old spruce seedlings. The plantation did not receive any management after planting, but it has been slightly disturbed by the activity of yaks. In 2012, the tree density of the plantation was 2750 stems  $ha^{-1}$ . The tree canopy coverage was 89%. The trees had a mean height of 8.3 m and a mean diameter at breast height was 11.8 cm. The understory coverage was approximately 10%, dominated by some shade-tolerant species, and the rest was covered by litter. The litter layer was about 5.2 cm in depth and 3.5 kg  $m^{-2}$  in storage. The soil at the experimental site, which derived from metamorphic rocks including phyllite, slate and schist, was classified as Haplic Luvisol [12]. Before the P addition in November 2012, the mineral soil 0–10 cm had a pH of 5.18, organic C concentration of 82.9 g kg<sup>-1</sup>, total N concentration of 6.2 g kg<sup>-1</sup>, and total P concentration of 0.9 g kg $^{-1}$ .

#### 2.2. Experimental design

The fertilized experimental plots were established in October 2012 in a 5 ha spruce plantation, fenced by a wire netting against the disturbance of big animals' (e.g., yaks). Experimental plots were designed in a randomized complete block. The plot size was 5 m × 5 m, and a 2 m wide buffer strip was maintained between each plot. Four treatments included in this study were control (no P input, CK), low P input (5 g P m<sup>-2</sup> yr<sup>-1</sup>, LP), medium P input (15 g P m<sup>-2</sup> yr<sup>-1</sup>, MP) and high P input (30 g P m<sup>-2</sup> yr<sup>-1</sup>, HP). Each treatment was replicated three times.

The P was added as  $NaH_2PO_4 \cdot 2H_2O$ . The fertilizer was mixed with 3.75 L of water and uniformly sprayed on the forest floor in two monthly portions since December 2012 and continued through June 2014. Each control plot received the same water (3.75 L) with no fertilizer.

#### 2.3. Soil sampling and processing

The soil sampling was conducted in August 2014, after two growing seasons of P addition. Five soil cores with 5 cm in diameter were taken from each plot to a depth of 10 cm and mixed into one composite sample. After removing stones and coarse roots, the fresh soil sample was sieved to pass a 2 mm sieve. One subsample of the sieved fresh soil was kept in a refrigerator at 4 °C and analyzed within ten days after sampling for soil phospholipid fatty acids (PLFAs), microbial biomass C (MBC), microbial biomass N (MBN), microbial biomass P (MBP), soil dissolved organic C (DOC), and mineral N. Another subsample of the sieved soil was air-dried at the room temperature and homogenized for analysis of pH, soil organic carbon (SOC), total N, total P and available P (AP). All soil parameters values were presented in oven dried soil.

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