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Full Length Article

The Effect of Ectomycorrhizal Fungi on Litter Decomposition and Phosphorus Availability to *Pinus koraiensis*

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Abstract

Mycorrhizal fungi play an important role on litter decomposition and nutrient release, besides nutrient uptake and transfer to host plants. In this study, we evaluated the effects of an ectomycorrhizal (ECM) fungus *Suillus grevillei* on litter decomposition and the level of phosphorus (P) nutrition in *Pinus koraiensis* seedlings. Seedlings of annuals were grown under three external P (P0, P20 and P40) levels, with or without *S. grevillei* inoculation, and with or without leaf litter. We observed that the percentage of mycorrhizal colonization was the highest under P20 among all treatments. Biomass production of seedlings was significantly higher with mycorrhization than without mycorrhization, irrespective of P nutrition. Biomass production was positively correlated to the rate of root mycorrhizal colonization. The amount of P released to soil environment was significantly higher under mycorrhization than under non-mycorrhization irrespective of P nutrition, suggesting that ectomycorrhizal hyphae had transferred from litter-released P and released to host plant. The content of soil P was significantly higher in mycorrhizal rhizosphere than in non-mycorrhizal rhizosphere, and phosphatase activity of ECM-inoculated was higher than that of non-inoculated in the rhizosphere. Meanwhile, we found total Phosphorus concentrations in *P. koraiensis* seedlings had significant differences between ECM-inoculated and non-inoculated. Our results demonstrate that *S. grevillei* could promote litter decomposition, litter could promote the development of ectomycorrhizae between *S. grevillei* and *P. koraiensis,* ectomycorrhizal fungi could convert P stored in the matrix into an available form to plants and thus promote the growth of both fungi and host plants. Our results also provide implications for an enhanced P biogeochemical cycling in the plant-fungal-soil system.

Keywords: Litter decomposition; *Pinus koraiensis*; Plant biomass production; *Suillus grevillei*

Introduction

The *Pinus koraiensis* mixed broad-leaved forest is one of the typical forest vegetation types in Changbai Mountain and Xiaoxing'anling, China. *Pinus koraiensis* seeds have nutrition value for human consumption, and economic importance to the local economy (Yang *et al*., 2008; Su *et al*., 2009). It is not only a valuable tree species for economic use, but also has key roles in the organization and functioning of ecosystems. (Young *et al.,* 2014), so ecologists have been focusing on cultivating *P. koraiensis* to preserve the stability of its ecosystem recently.

Ectomycorrhizal (ECM) fungi colonize roots and supply nutrients to enhance growth of host plants (Anderson and Cairney, 2007; Courty *et al*., 2010). As important terrestrial mutualistic fungal groups, ECM fungi have been intensively studied regarding its effects on plant growth, plant communities, and ecosystem processes (Glen *et al.,* 2008). *P. koraiensis* similar to all tree species of nemoral and boreal forests, forms obligate symbiosis with ECM fungi, which was revealed both in the study of ground variety of fungi by collection of fruit bodies, and in morphological, anatomical and molecular analysis of mycorrhizal endings (Cairney, 2011; Jouni *et al.,* 2016). Lateral roots are well-developed compared with its tap root, and most nutrients are absorbed through mycorrhizae. *P. koraiensis* grows poorly or dies without ectomycorrhizal fungi (Johnson *et al*., 2012; Shalaka *et al*., 2014).

Litter decomposition releases nutrients, and is the main nutrition resources for trees in the process of material circulation (Jiang *et al*., 2014). Litter decomposition played a key role in ecosystem service provision by converting plant material into inorganic component that keep the forest soil fertility (Chigineva *et al*., 2009; Nina *et al*., 2016). Decomposing leaf litter was a major source of soil nutrients available to the mycorrhizae. These fungi and litter-derived

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nutrients are the main components of the nutrient cycle in *P. koraiensis* forests (Dai *et al.,* 2001). However major studies on litter decomposition had been limited to the effects of physical-chemical environment and the substrate quality, while only few studies had focused on contributions of soil community to litter decomposition (Guo *et al.,* 2006).

Currently, there are only few studies on *P. koraiensis* mycorrhizae, and these studies focus mainly on the formation of ectomycorrhizae and their effects on seedling growth (Wallander and Thelin, 2008). Although many researchers study on relationship of the litter nutrient, ectomycorrhizal fungi and host trees, most of them focus mainly on the release and uptake of nitrogen, only a few scholars conducted studies on phosphorus uptake, and alteration of nutrient composition of soils by leaf litter. It is likely that the nutrient composition of plant litters affect plant growth and mycorrhizal communities (Leifheit *et al.,* 2015). Therefore we evaluated litter decomposition, nutrient release and uptake, and transfer between ectomycorrhizal fungi and their host plant across an entire system. We study on relationship of the litter nutrient, mycorrhizal fungi and host trees under the different nutritional conditions, and hope the results of the study can make us fully understand ectomycorrhizal fungi play an important role in material cycle in Pinus koraiensis.

Materials and Methods

Plant Growth Box and Experimental Design

Experimental boxes (6.3 L plastic boxes, 70×30×30 cm) were divided by 37 μm nylon mesh into three chambers, including one middle chamber (40×30×30 cm) and two outside chambers (15×30×30 cm) (Fig. 1). The nylon mesh separation allowed mycorrhizal hyphal, but not root, penetration. The growth media in the middle chamber was inoculated with or without ECM, and supplied with or without external phosphorus (KH_2PO_4) at 0, 20 and 40 mg/kg media (designated as P0, P20 and P40, respectively). The outside chamber was filled with or without 1100 grams of oven-dried pine litter, segments which were originally collected from a local *P. koraiensis* forest in autumn (Table 1). The litter had 51.23±1.2% carbon, 0.79±0.02% nitrogen, 0.18±0.03% phosphorus, 0.55±0.02% potassium, 24.13±0.35% lignin and 31.43±0.67% cellulose. Each treatment had 5 replicates, and a total of 60 boxes were arranged in a randomized block design.

Preparation of Aseptic Ectomycorrhizal Seedlings

Sporocarps of *Suillus grevillei* were collected in *P. koraiensis* forest in the Fenglin Nature Reserve and cultured on potato dextrose agar plates. The *Suillus grevillei* culture was then cultivated on MMN medium in 300 mL flasks for 15 days (28 ± 2 °C, 140 rpm).

Seeds of *P. koraiensis* were surface-sterilized in 15% H_2O_2 for 5 min, rinsed with sterile water, and germinated on the autoclaved wet paper tissue. The germinated seeds were transplanted into the middle chamber containing 30 kg autoclaved soil in a glasshouse. The soil was taken from 0–20 cm depth in *P. koraiensis* forest in the Fenglin Nature Reserve. The soil had 2.45±0.12% organic matter, 0.14±0.04% total N, 0.045±0.006% P, 9.05±0.08 mg/g available N, 3.48±0.06 mg/g available P, and 11.06±0.12 mg/g K.

Roots of one year old non-mycorrhizal seedlings were soaked with *S. grevillei* slurry. Autoclaved mycorrhizal slurry was used as negative control to soak seedlings.. Seedlings were grown for 26 weeks in a glasshouse (28/23ºC, day/night), and were watered with 5.0 mL P-nutrient solution or P-free deionised water twice a week. To reduce water loss, the surface of the growth medium in each compartment was covered by a layer of high density polythene resin beads that were pre-sterilized by soaking into 75% ethanol and rinsed with water. The bases of the growth units were wrapped in aluminum foil to protect the roots from sunlight. The growth boxes were randomly repositioned twice a week to keep light exposure uniform.

Determination of Mycorrhizal Colonization and Biomass

The mycorrhizal colonization percentage of the seedlings was determined using the Phillip and Hayman staining method (KOH bleaching-acid fuchsin stain) with some modifications (Phillips and Hayman, 1970). Seedling height was measured by ruler, and then seedlings were oven-dried at 60□ until no weight change to compare biomass. Litter weight losses were determined by weight-loss method.

Determination of Phosphorus

Foliage, stems and roots in the growth chamber and the remaining needles in the two outside chambers were separately harvested and oven-dried at 105ºC for 30 min to cease metabolic activity and then at 65ºC for two days and finally ground to fine powder. P was determined according to Thomas technology (Thomas *et al.,* 1967). Litter P in the both side chambers was assayed using the molybdenum antimony method (Bao *et al.,* 2000).

The $HCLO_4$-H_2SO_4 method was used for determining total phosphorus in soil (Shi *et al*., 2004), the molybdenum antimony colorimetric method for available P in soil, and phenyl phosphate disodium colorimetric method for Phosphatase activity (Yao and Huang, 2006).

Data Analyses

Data (means ± SE, n = 5) were subjected to ANOVA procedures. Significant differences in means were compared with the Duncan's test at $P \leq 0.05$ using the SPSS Version 17 (Chicago, IL, USA).

Table 1: Experimental design for testing effects of ectomycorrhizal fungus *Suillus grevillei* and external chemical phosphorus (KH_2PO_4) on litter decomposition and phosphorus availability to host seedlings grown in three-chambered boxes

	Treatments		
	High P stress (0.0 mg/kg, P0)	Moderate P stress (20 mg/kg, P20)	No P stress (40 mg/kg, P40)
$Pine^{M+L+}$	150 mL EM inoculant to the middle chamber, litter to two outside chambers	150 mL EM inoculant to the middle chamber, litter to two outside chambers	150 mL EM inoculant to the middle chamber, litter to two outside chambers
$Pine^{M+L-}$	150 mL EM inoculant to the middle chamber, litter to two outside chambers	150 mL EM inoculant to the middle chamber, no litter to two outside chambers	Same 150 mL EM inoculant to the middle chamber, litter to two outside chambersP20
$Pine^{M-L+}$	150 mL EM inoculant to the middle chamber, litter to two outside chambers	150 mL MMN media to the middle chamber, litter to two outside chambers	Same 150 mL EM inoculant to the middle chamber, litter to two outside chambersP20
$Pine^{M-L-}$	150 mL EM inoculant to the middle chamber, litter to two outside chambers	150 mL MMN media to the middle chamber, no litter to two outside chambers	Same to 150 mL EM inoculant to the middle chamber, litter to two outside chambers

Abbreviations: L+ or L− represents with or without litter addition in the two outside chambers; M+ or M− represents with or without mycorrhizal inoculation in the middle chamber; P0, P20 and P40 represent 0, 20.0 and 40.0 mg external KH_2PO_4 added to per kg growth media

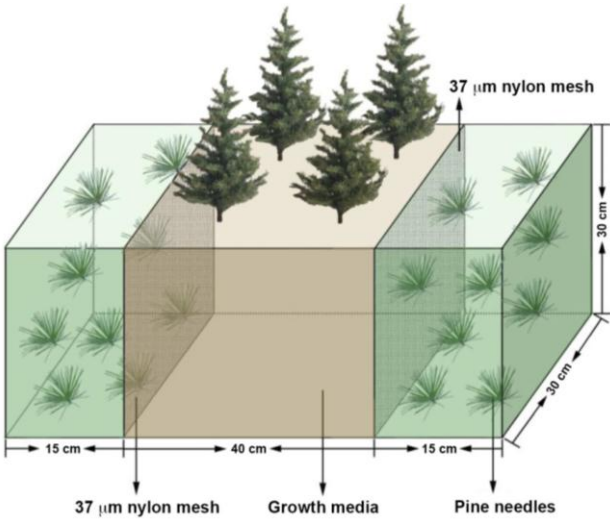

Fig. 1: Diagram (longitudinal section) of a three-chambered growth box

Results

Ectomycorrhizal Colonization

We did not observe ECM colonization to the roots and hyphae on the nylon mesh in non-mycorrhizal treatments. No roots crossed nylon mesh in any treatments. No matter what the external P addition level was, ECM colonization was significantly higher in $Pine^{M+L+}$ than in $Pine^{M+L-}$ treatment (Table 2). Meanwhile, significantly higher ECM colonization under P additions ranked as No P ≈ Low P>High P for the $Pine^{M+L+}$ treatment, while Low P > No P>High P under the $Pine^{M+L-}$ treatments.

Plant Height and Biomass Production

No matter what the external P addition level was, significantly higher both plant height and biomass production between treatments ranked as $Pine^{M+L+}$>$Pine^{M+L-}$ > $Pine^{M-L+}$ ≈ $Pine^{M-L-}$ (Table 3).

Table 2: Percentage of mycorrhizal colonization in 6-month-old *Pinus koraiensis* seedlings

Treatment	P0	P20	P40
$Pine^{M+L+}$	73.4±1.4a, x	76.5±1.2a, x	56.4±1.6a, y
$Pine^{M+L-}$	61.2±1.1b, y	70.2±1.5b, x	50.5±0.7b, z
$Pine^{M-L+}$	0c, x	0c, x	0c, x
$Pine^{M-L-}$	0c, x	0c, x	0c, x

Note: Data (means ± SE, n = 5) followed by different letters indicate significant difference (P≤0.05) between treatments for the same P fertilization (a, b) and P fertilizations for the same treatment (x, y, z), respectively

Abbreviations: L, litter addition status; M, mycorrhizal status; P, phosphorus

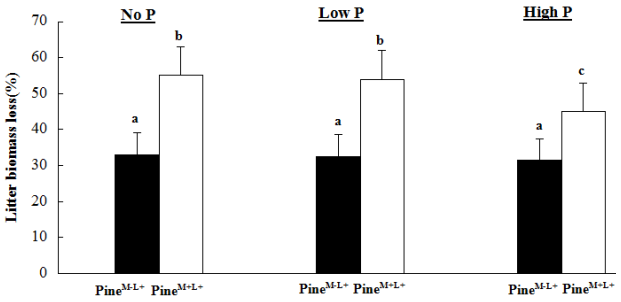

Fig. 2: Effects of phosphorus additions on pine litter biomass loss (%) after 6-month incubation in the two outside chambers of the growth box

Data (means ± SE, *n* = 5) followed by different letters indicate significant differences ($P < 0.05$) between treatments under the same P fertilization (a, b). Data were firstly averaged since the litter biomass loss was similar between the two outside chambers. Abbreviations: L+ or L−, with or without litter addition in the two outside chambers; M+ or M−, with or without mycorrhizal inoculation in the middle chamber; P0, P20 or P40 mg external P_2O_5 was added to per kg growth media

Meanwhile, significantly higher both plant height and biomass production under P additions ranked as High P ≈ Low P > No P for the $Pine^{M+L+}$ and $Pine^{M+L-}$ treatments, while Low P > No P > High P under the $Pine^{M-L+}$ and $Pine^{M-L-}$ treatments.

Changes in Litter Biomass

No matter what the external P addition level was, percentages of litter biomass loss were significantly higher in $Pine^{M+L+}$ than in $Pine^{M+L-}$ treatment. Meanwhile, significantly higher litter biomass loss under P additions ranked as No P ≈ Low P > High P for the $Pine^{M+L+}$ treatment, whilst were similar for the $Pine^{M+L-}$ treatment irrespective of external P addition levels (Fig. 2).

Phosphorus Concentrations in Plant Growth Media and Litter

Significantly higher phosphorus in growth media between treatments ranked as $Pine^{M+L+}$ > $Pine^{M+L-}$ > $Pine^{M-L+}$ ≈ $Pine^{M-L-}$ (Table 4). Meanwhile, significantly higher phosphorus in growth media under P additions ranked as High P ≈ Low P > No P for the $Pine^{M+L+}$ treatment, whilst as High P >Low P > No P for the $Pine^{M+L-}$, $Pine^{M-L+}$ and $Pine^{M-L-}$ treatments. On the other hand, significantly higher phosphorus in the remaining litter between treatments ranked as $Pine^{M-L+}$ > $Pine^{M+L+}$, irrespective of the external P addition level. In contrast, significantly higher phosphorus in the remaining litter under P additions ranked as High P ≈ Low P > No P for the $Pine^{M+L+}$ treatment, whilst were similar among different P additions for the $Pine^{M-L+}$ treatment.

Total Phosphorus Concentrations in Pinus Koraiensis Seedlings

Although the growth of ECM-inoculated seedlings was significantly better than that of non-inoculated plants, different processing (including under different phosphorus levels, whether to have ECM or litter) were no significant different in phosphorus concentrations of *Pinus koraiensis* seedlings. However, total Phosphorus concentrations in the seedlings were significantly different among treatments. The $Pine^{M+L+}$ treatment had fixed the most total phosphorus, reached 0.613 g/strain (Table 5).

Total Phosphorus in Soil and Phosphatase Activity

The total phosphorus concentrations in soil of ECM-inoculated seedlings was significantly lower than that of non-inoculated plants. This result showed the ECM could transform the total phosphorus into the phosphorus, which plants could absorb. Meanwhile, in the rhizosphere, phosphatase activity of ECM-inoculated was higher than that of non-inoculated. This result indicates the activity of phosphatase and mycorrhizal infection rate has certain correlation (Table 6).

Discussion

In natural forest ecosystems, ectomycorrhizal fungi have evolved for millions of years with plants, and play an important role in various ecological processes such as nutrient cycle, energy flow, biodiversity and productivity (Wang *et al.,* 2017). When soil nutrients are readily available, plants mainly absorb nutrients directly through their roots, but ectomycorrhizae usually plays increasingly important roles during the times of nutrient stress (Sousa *et al*., 2012; Liao *et al*., 2016). In this study, we investigated the formation of mycorrhizae between *S. grevillei* and *P. koraiensis* under three nutrient conditions (P0, P20, and P40 treatments). Our result showed that the mycorrhizal infection rates were lowest in seedlings growing in the nutrient-rich soil matrix (P40 treatments), and the highest infection rates was found under the moderately poor nutrient conditions (P20 treatments), while P0 treatments negatively impacted mycorrhiza, which might also require P for growth. Moreover, the biomass of seedlings with *S. grevillei* ectomycorrhizae was significantly higher than non-mycorrhizal seedlings under the same nutrient treatments, indicating that mycorrhizal fungi promoted seedling growth.

Previous studies suggested that mycorrhizal fungi could hardly use cellulose and other complex carbon sources (Mikryukov *et al*., 2015). Thus non-symbiotic mycorrhizal fungi in competition with saprophytic fungi in the soil are disadvantaged. Leaf-litter decomposition could affect the ectomycorrhizal community of *Pitch pine* seedlings (Purahong *et al*., 2014). This research showed that ectomycorrhizal fungi could promote litter decomposition, and this effect could be attributed to the following two aspects. One is the result of the direct involvement of mycorrhizal fungi in litter decomposition, and the other is ectomycorrhizae promoting the breakdown activity of saprophytic fungi. Our results indicates that litter nutrients were transferred to the plant for growth by ectomycorrhizal fungi. Meanwhile, litter decomposition promoted the propagation of ectomycorrhizal fungi and the formation of mycorrhizae. Ectomycorrhizae not only transmitted nutrients to plants, but also promoted the breakdown activity of saprophytic microorganisms, which played important roles in forest material cycles (Anna *et al*., 2014).

Ectomycorrhizal fungi are not only decomposers, which participate in ecosystem nutrient cycles and energy flow, but also fix nutrients and water in an ecosystem by their own growth and reproduction (Holste *et al*., 2016; Hirose *et al*., 2010). Mycorrhizal fungi play an important role in inorganic P cycling, accelerate the weathering of P-containing soil, and convert P stored in the matrix into a form available to plants (Kaiser *et al*., 2015).

Conclusion

The study showed the effects of an ectomycorrhizal (ECM) fungus *Suillus grevillei* on litter decomposition and phosphorus (P) nutrition of *Pinus koraiensis* seedlings, and found that the P content of mycorrhizal rhizosphere soil was significantly higher than that of non-mycorrhizal rhizosphere soil. Therefore, mycorrhizal fungi may participate directly in the ecosystem P cycle,

Table 3: Effect of ectomycorrhizal fungus on plant height and biomass production in 6-month-old *Pinus koraiensis* seedlings

Treatment	P0		P20		P40	
	Height (cm/plant)	Biomass (g/plant)	Height (cm/plant)	Biomass (g/plant)	Height (cm/plant)	Biomass (g/plant)
$Pine^{M+L+}$	7.32±0.25a, y	2.46±0.06a, y	9.55±0.12a, x	3.21±0.05a, x	9.32±0.14a, x	3.11±0.10a, x
$Pine^{M+L-}$	6.35±0.13b, y	2.31±0.06b, y	8.40±0.11b, x	2.75±0.03b, x	8.47±0.13b, x	2.88±0.03b, x
$Pine^{M-L+}$	5.88±0.41c, z	2.05±0.02c, z	6.62±0.12c, y	2.22±0.07c, y	7.60±0.21c, x	2.51±0.07c, x
$Pine^{M-L-}$	5.85±0.32c, z	2.08±0.05c, z	6.56±0.14c, y	2.18±0.03c, y	7.46±0.31c, x	2.45±0.04c, x

Data (means ± SE, n = 5) followed by different letters indicate significant differences (P≤0.05) between treatments under the same P fertilization (a, b, c) and between P fertilizations for the same treatment (x, y, z), respectively

Abbreviations: L+ or L−, with or without litter addition in the two outside chambers; M+ or M−, with or without mycorrhizal inoculation in the middle chamber; P0, P20 or P40, 0, 20.0 or 40.0 mg external P was added to per kg growth media

Table 4: Effect of ectomycorrhizal fungus on concentrations of available phosphorus in the plant growth media and remaining pine litter after 6-months of plant growth

Treatment	P0		P20		P40	
	Growth media (mg P/kg)	Remaining litter (mg P/kg)	Growth media (mg P/kg)	Remaining litter (mg P/kg)	Growth media (mg P/kg)	Remaining litter (mg P/kg)
$Pine^{M+L+}$	24.8±1.2a, y	0.105±0.007b, z	30.8±0.8a, x	0.118±0.005b, y	32.4±1.1a, x	0.131±0.004b, x
$Pine^{M+L-}$	16.5±0.6b, z	—	22.3±0.3b, y	—	29.1±0.6b, x	—
$Pine^{M-L+}$	13.1±1.1c, z	0.148±0.008a, x	17.9±0.5c, y	0.155±0.003a, x	27.1±0.7c, x	0.153±0.006a, x
$Pine^{M-L-}$	13.8±0.9c, z	—	18.4±0.9c, y	—	26.3±0.8c, x	—

Data (means ± SE, n = 5) followed by different letters indicate significant differences (P≤0.05) between treatments under the same P fertilization (a, b) and between P fertilizations for the same treatment (x, y, z), respectively

Abbreviations: L+ or L−, with or without litter addition in the two outside chambers; M+ or M−, with or without mycorrhizal inoculation in the middle chamber; P0, P20 or P40, 0, 20.0 or 40.0 mg external P_2O_5 was added to per kg growth media

Table 5: Effect of mycorrhization on percentage and total amount of phosphorus in 6-month-old *Pinus koraiensis* seedlings

Treatment	P0		P20		P40	
	Plant (%)	Total P (g/seedling)	Plant (%)	Total P (g/seedling)	Plant (%)	Total P (g/seedling)
$Pine^{M+L+}$	0.188±0.004a, x	0.462±0.008a, z	0.191±0.006a, x	0.613±0.015a, x	0.183±0.010a, x	0.569±0.017a, y
$Pine^{M+L-}$	0.183±0.002a, x	0.423±0.011b, z	0.180±0.011a, x	0.495±0.019b, y	0.182±0.003b, x	0.524±0.011b, x
$Pine^{M-L+}$	0.179±0.008a, x	0.367±0.009c, z	0.181±0.003a, x	0.402±0.014c, y	0.183±0.009a, x	0.459±0.007c, x
$Pine^{M-L-}$	0.181±0.007a, x	0.376±0.010c, z	0.179±0.008a, x	0.390±0.008c, y	0.184±0.005a, x	0.451±0.011c, x

Data (means ± SE, n = 5) followed by different letters indicate significant differences (P≤0.05) between treatments under the same P fertilization (a, b) and between P fertilizations for the same treatment (x, y, z), respectively

Abbreviations: L+ or L−, with or without litter addition in the two outside chambers; M+ or M−, with or without mycorrhizal inoculation in the middle chamber; P0, P20 or P40, 0, 20.0 or 40.0 mg external P_2O_5 was added to per kg growth media

Table 6: Effects of ectomycorrhizal fungus on concentrations of total phosphorus and phosphatase in the plant growth media

Treatment	P0		P20		P40	
	P (%)	Phosphatase (mg/g·h)	P (%)	Phosphatase (mg/g·h)	P (%)	Phosphatase (mg/g·h)
$Pine^{M+L+}$	0.031±0.003b, y	0.26±0.01a, x	0.028±0.001b, z	0.28±0.01a, x	0.037±0.002b, x	0.21±0.03a,y
$Pine^{M+L-}$	0.034±0.001b, y	0.22±0.01b,y	0.030±0.002b, z	0.25±0.01b, x	0.039±0.003b, x	0.19±0.02a,z
$Pine^{M-L+}$	0.041±0.003a, x	0.11±0.02c, x	0.042±0.004a, x	0.13±0.03c, x	0.043±0.001a, x	0.12±0.01b, x
$Pine^{M-L-}$	0.043±0.002a, x	0.13±0.01c, x	0.043±0.003a, x	0.12±0.02c, x	0.042±0.002a, x	0.14±0.02b, x

Data (means ± SE, n = 5) followed by different letters indicate significant differences (P≤0.05) between treatments for the same P fertilization (a, b) and between P fertilizations for the same treatment (x, y, z), respectively

Abbreviations: L, litter addition status; M, mycorrhizal status; P, phosphorus

increase the P content in the ecosystem P cycle, and absorb nutritive elements to generate more mycelia in the plant rhizosphere soil. Also demonstrate that ectomycorrhizal fungi could convert P stored in the matrix into an available form to plants and thus promote the growth of both fungi and host plants, which provide implications for an enhanced P biogeochemical cycling in the plant-fungal-soil system.

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