Effects of nitrogen deposition and phosphorus addition on arbuscular mycorrhizal fungi of Chinese fir (Cunninghamia lanceolata)

Chuyu Lin
Zhejiang A&F University   https://orcid.org/0000-0002-0151-2927

Yaoxiong Wang
Zhejiang A&F University

Meihua Liu
Zhejiang A&F University

Quan Li
Zhejiang A&F University

Wenfa Xiao
Chinese Academy of Forestry

Xinzhang Song (✉ songxinzhang@gmail.com)
https://orcid.org/0000-0003-2434-7466

Research article

Abstract

Background Nitrogen (N) deposition is a key factor that affects terrestrial biogeochemical cycles with a growing trend, especially in the southeast region of China, where shortage of available phosphorus (P) is particularly acute and P has become a major factor limiting plant growth and productivity. Arbuscular mycorrhizal fungi (AMF) establish a mutualistic symbiosis with plants, and play an important role in enhancing plant stress resistance. However, the response of AMF to the combined effects of N deposition and P additions is poorly understood. Results Our results showed that N deposition significantly increased AMF root colonization rates and spore density, but inhibited both symbiotic relationship and spore propagation. In N-free plots, P addition significantly increased AMF root colonization rates, but did not significantly alter spore density. In low-N plots, AMF root colonization rates significantly decreased under low P addition, but significantly increased under high P addition, and spore density exhibited a significant decline under both low and high P additions. In high-N plots, AMF root colonization rates and spore density significantly increased under P additions. In general, AMF were closely related to the relative content of N and P in the soil. Interactive effects of simulated N deposition and P addition on both AMF root colonization rates and spore density were significant. AMF root colonization rates were significantly negatively correlated with soil moisture. Conclusions Moderate N deposition or P addition can weaken the symbiotic relationship between plants and AMF, significantly reducing AMF colonization rates and inhibiting spore propagation. However, a moderate addition of P greatly enhances spore yield. Soil moisture content is the main factor regulating AMF colonization rates. In the case of interactive effects, the AMF colonization rates and spore density are affected by the relative content of N and P in the soil.

Background

Arbuscular mycorrhizal fungi (AMF), a heterogeneous group of diverse fungal taxa and the most widespread fungal symbionts of plants, can establish mutualistic associations with the roots of over 80% of all terrestrial plant families, and plays a critical role in plant nutrient acquisition, growth, and ecosystem sustainability [1–3]. The network of AMF extraradical mycelium in the soil supports water and mineral nutrition of the host plant, especially for enhancing the supply of phosphates, by effectively absorbing and translocating mineral nutrients (e.g., N and P) beyond the depletion zones of the plant rhizosphere [4, 5]. In return, plants provide carbon to AMF (as a carbon (C) source) by transferring hexose produced by photosynthesis via roots [6, 7]. Typically, 5–10% of photosynthetically fixed C is allocated to the fungal partner [8]. Owing to their filamentous organization, fungi exploit diverse substrates on the basis of their nutritional strategy [9] and significantly contribute to the uptake of soil nutrients, increase plant biomass, and improve the plant resistance to stress and pathogens. Therefore, fungi have received a great deal of attention from researchers [10]. The symbionts formed through AMF colonization of plants plays an essential role in the N cycle by affecting processes such as organic N mineralization, biological N fixation, and eluviation, thereby improving the nitrogenase activity of plants and increasing the N transport pathway of AMF to different forms [11]. Moreover, AMF colonization can alter the pH of the rhizosphere soil, which can activate insoluble phosphate to improve its availability, as well as

promote the growth and development of the host plants by inducing the expression of the P transporter gene in plants, altering the kinetic parameters of plant nutrient absorption, and promoting photosynthetic phosphorylation.

Nitrogen deposition is a crucial issue with regard to global climate change. The increasing consumption of fossil fuels and application of agricultural fertilizers have largely enhanced the input of anthropogenic available N into ecosystems worldwide in the last few decades [12, 13]. It is predicted that by the middle of the 21st century, global N deposition levels will appear to be twice what they were at the end of the 20th century [14]. China is currently one of the three areas worldwide with the greatest concentration of N deposition. Particularly in the southeast regions, the most severe N deposition has reached 63.53 kg·ha^{-1}·yr^{-1} [15, 16]. Nitrogen deposition has resulted in increases in soil available N and changes in soil pH and N:P ratios. The activities of N metabolism enzymes and the accumulation of N assimilates in plants may also be altered, thereby affecting plant growth [17]. In addition, the change in soil properties and physiological function of plants will directly affect colonization, growth, N metabolism, and P uptake of AMF [18]. Studies have shown that high N levels (10 mmol·L^{-1} NH_4C1-NH_4NO_3 3:1) contributed to excessive N inputs into the soil, which weakens the symbiotic synergy between AMF and host plants [19]. Furthermore, N deposition also affects plant P uptake. He et al. [20] reported that the P content in the aboveground part of the plant inoculated with AMF increased with increasing N content, while the P content in the root decreased continuously.

Phosphorus is one of the most vital nutrients affecting plant growth and metabolism. However, in south China, the shortage of available P in subtropical acidic soil is particularly severe because large quantities of P remain insoluble and easily fixed. Therefore, low P stress has become one of the key factors restricting plant growth and productivity. Arbuscular mycorrhizal (AM) symbiosis plays an essential role in the adaptation and tolerance of plants to low P stress, and is the most important mechanism for improving the efficient utilization of plant P [21]. After forming the AM symbiosis, the absorption range of the symbiont expands through the symbiont mycelium, thereby greatly improving the symbionts absorption capacity for P [22, 23]. Mycorrhiza can also activate insoluble P to improve the absorption efficiency of P in plants by secreting organic acid and acid phosphatase [24]. Studies have demonstrated that the ectomycorrhiza of many coniferous species can replace the absorptive roots and become the main organ of the root system for obtaining soil resources [25]. However, the availability of P in soil is affected the colonization of AMF on plants, and is not conducive to the formation of mycorrhiza if out of range [26]. It has been reported by Zhang et al. [27] that AMF mycorrhizal colonization rates, mycelium density, and spore numbers of corn (*Zea mays)* were significantly higher at the P50 level (50 mg·kg^{-1}) than those without P addition. As the P treatment increased to 200 mg·kg^{-1} and 500 mg·kg^{-1}, mycorrhizal colonization rates declined.

Chinese fir (*Cunninghamia lanceolata (*Lamb.) Hook.) is a unique tree species for afforestation and timber use in China, with a widespread natural distribution and artificial cultivation. It plays an important role in China's artificial forests and has become a common tree species in Chinese subtropical forest

ecosystems [28]. With regard to global climate change, N deposition and low P stress pose a threat to the yield of Chinese fir forests, while the requirements of a well-established AM symbiosis for induced resistance to stress is generally accepted [29]. However, the effects of simulated N deposition and P addition on AM symbiosis remains unclear, and using commercial timber species as host plants under natural field conditions remain poorly investigated. Therefore, the aim of this study was to examine the influence of N deposition and P addition on AMF by imposing simulated N deposition and P additions to the soil in a Chinese fir forest. The objectives of the study were to identify the main factors affecting the AMF mycorrhizal effect of Chinese fir trees and to demonstrate their importance for regulating and improving the utilization efficiency of soil N and P nutrients of Chinese fir trees. This will provide a theoretical basis and reference for the application of AMF for improving the development and management of the timber forest industry under the background of global environmental change.

Results

In the P-free plots, N addition reduced colonization rates, and maximum mycorrhizal colonization rates (77.1%) were evident in the N0+P0 treatment ($P < 0.05$) (Fig. 1). Moreover, mycorrhizal colonization rates under the N60+P0 treatment were significantly lower than that under the N0+P0 and N30+P0 treatment($P < 0.05$). With the addition of P alone, colonization rates decreased with increasing additions of P. In the N30 plots, mycorrhizal colonization initially decreased and then increased with increasing additions of P, with a significant decline in colonization rates under the N30+P20 treatment ($P < 0.05$) (Fig. 1). However, in the N60 plots, colonization increased under N60+P20 treatment and decreased under N60+P40 treatment. Compared with the N60+P0 and N60+P40 treatments, colonization rates of the N60+P20 treatment increased significantly ($P < 0.05$) (Fig. 1).

In P-free plots, AMF spore density decreased with increases in the N level. Compared with CK (control check), spore density under the N60+P0 treatment decreased by 45.2% ($P < 0.05$). In the N0 plots, there was no significant variation in the spore density of AMF with increases in P addition (Fig. 2). In the N30+P20 and N60+P40 treatments, P addition significantly reduced spore density ($P < 0.05$). Compared with the N30+P0 treatment, spore density decreased by 27.3% under the N30+P40 treatment ($P < 0.05$). In the N60 plots, AMF spore density significantly increased with P additions ($P < 0.05$), and the AMF spore density in the rhizosphere under the N60+P40 treatment was the highest (4.83 /20g dry soil), which was 2.26 times higher than that of N60+P0 (Fig. 2).

We determined physicochemical characteristics in a Chinese fir forest and made correlation analysis (Table 1). AMF colonization rates were significantly negatively correlated with soil moisture ($P < 0.05$), but there was no significant correlation with the other soil physicochemical factors ($P > 0.05$) (Table 2). There was also no significant correlation between AMF spore density and soil moisture content, soil pH, available N, available P, total N, and total P ($P > 0.05$). Consequently, soil moisture content may be the main soil physicochemical factor affecting AMF colonization rates (Table 2).

The two-way ANOVA analysis revealed that N deposition and P addition significantly affected root colonization rates of AMF ($P < 0.05$). In addition, the contribution of N deposition was greater than the independent effects of P additions and their interaction (Table 3). Except for P additions, both N deposition and interaction significantly affected AMF spore density in rhizosphere soil. Moreover, the contribution of the interaction was greater than that of N deposition.

Discussion

Effects of N deposition on AMF

The present study showed that the effect of simulated N deposition on AMF colonization and spore growth was dependent on the different additions of P to the soil. In the P-free plots, atmospheric N deposition increased soil N availability whereas ectomycorrhizal colonization rates decreased, which is consistent with the findings in previous studies [30]. With excessive N input into the soil, the increasing NH_4^+ concentration will cause mitochondrial swelling and cytoplasmic cracking in the plant's root system, influencing the growth and development of the host plant since ammonium nitrogen must be assimilated in the root immediately after being absorbed by the mycorrhiza, which consumes a large amount of carbohydrates [31]. Moreover, the extraradical fungal hyphae will be poisoned and the function of AMF altered, thereby affecting the synergistic relationship of the symbionts [32]. Under the conditions of P additions, colonization rates will be reduced once soil N content has improved. According to the cost-benefit modelling approach [33], the demand relationship between plants and AMF will be weakened when N and P obtained by plants is sufficient. As the mechanisms of underground nutrients change, the amount of photosynthetic products allocated to the mycorrhiza may be also reduced [34]. In addition, the decline in colonization rates leads to a corresponding reduction in spore density. The functional prediction model indicates that in soils with a high P content, an increase in N would reduce the number of arbuscules, vesicles, to name a few. Colonization rates affect the capacity of AMF to confer its associated host plant with soil nutrients to obtain the C it needs for growth, which directly affects spore germination and the growth of fungal hyphae [35]. This reveals that the quantity of carbohydrates provided by the root system of the Chinese fir host was an important determinant factor in AMF spore yield [36]. Numerous field studies have shown that AMF colonization rates and spore density are closely related to soil factors [37]. Nonetheless, excessive soil nutrient content was generally thought to inhibit mycorrhizal symbiosis [38]. In our study, the correlation analysis showed that colonization rates and moisture content were negatively correlated at the 0.05 level. We surmise that the long precipitation period prior to sampling reduced the soil oxygen content, which is not conducive to AM symbiosis [39, 40]. An alternate possibility is that high soil moisture content affected the absorption of soil nutrients (especially P) by plant roots, which alters the synergistic relationship between AMF and plants [41].

Effects of P addition on AMF

P is also an important factor affecting AMF colonization intensity and spore growth. In the N-free plots, P additions improved soil nutrients to a certain extent. As plant dependence on AMF decreased, colonization rate decreased, which is consistent with the research conducted by Zhang et al [42]. It has been reported that when soil P is abundant, plants can obtain more P nutrients through their roots, which contributes to the inhibition of the growth, extension rate, and fungal activity of AMF extraradical fungal hyphae. Thereafter, with a reduction in colonization rates, the effect of mycorrhiza on promoting P uptake and growth of plants would be reduced [43, 44]. Under N30+P0 treatment, P deficiency is relatively exacerbated by N addition to P-deficient soils, which contributes to strengthening the AM symbiosis. AMF obtain a large amount of carbohydrates from host plants, thereby enhancing the growth of symbionts and the absorption of soil P. At the same time, sporulation activity of AMF is also promoted, with the number of spores increasing to a certain extent. However, in our study, colonization rates significantly increased under the N30+P40 treatment, which indicates that a high P gradient caused the relative deficiency in soil N. The incongruous relative content of N and P enables plants to initiate physiological mechanisms to enhance the absorption of N, thus strengthening the symbiotic relationship between AMF and plants. This provides a possible explanation for the reduction in the colonization rates under the N30+P20 treatment, mainly owing to the relative harmonious proportion of N and P content. Under high N (N60) treatments, a reduction in available P caused N deposition and colonization rates greatly increased with low P additions. Nevertheless, when an excessive amount of P is added and the demand for P by host plants is adequate, the increasing P content in the plant will generate a decrease in secretion quantity or a change in secretion composition in the root system. This will have an inhibitory effect on AMF reproduction and mycorrhizal colonization [45], thereby leading to a reduction in colonization rates under the N60+P40 treatment. Spores are an important P pool [46], and a moderate amount of P addition to the soil enhances spore numbers, which probably caused the significant increase in spore density under the N60+P40 treatment. Previous studies have shown that P content in the soil is an important factor affecting the growth of AMF [47], and the effect of AMF on improving P nutrition in plants depends strongly on the nutrient status of the soil, especially concerning the available P level in the soil [48].

Combined Influence of N deposition and P addition on AMF

We inferred from our results that N deposition could alter the relative P content in the soil, exacerbating the effects of high P sensitivity on symbionts and P uptake, revealing simultaneously the importance of P in alleviating the N deposition stress on AMF by affecting the synergistic relationship between AMF and host. Many studies indicate that soil properties have a direct effect on the formation and development of arbuscular mycorrhiza and number of propagates, and that AMF colonization rates are closely related to soil N and P [49]. In our study, two combination treatments N60+P20 and N30+P40 caused a soil structural disequilibrium. The increasing N content in the soil will stimulated more P demand for the host plants, which are relatively deficient. Hence, the root system of the plant will invest in AMF to resist adversity, which will significantly increase colonization rates. Furthermore, colonization rates of the N60+P20 treatment were higher than that of the N30+P40 treatment, whereas spore density was lower. This suggests that P has a more robust impact on mycorrhizal formation than N, and P deficiency is more likely to increase colonization rates and decrease spore-producing activity. Research has already

demonstrated that decreases in the relative availability of soil P due to the large supply of N will increase the absorption and utilization of soil P through mycorrhiza, to improve plant P nutrition and balance the N:P ratio in vivo [50]. Well-structured soil nutrients can weaken the symbiotic relationship between plant and AMF, which may provide an explanation for AMF colonization rates of N60+P20 and N30+P40 treatments being higher than that of N30+P20 and N60+P40 treatments. However, AMF spore density increased significantly under the N60+P40 treatment, indicating that under adequate soil nutrients conditions, spores are used to store nutrients and promote their continuous growth and reproduction in order to cope with an unpredictable future environment [51, 52].

Conclusions

Under both simulated N deposition and P addition treatments, AMF colonization rates were significantly reduced, the symbiotic relationship was weakened due to a lack of C resources needed by AMF from the roots of the fir trees, and spore propagation was inhibited. N deposition and P addition improved soil nutrients, thereby weakening the demand and dependence between the trees and AMF, thus threatening the symbiotic relationship. However, the relationship strengthened under low-N high-P treatments, as this caused the relative deficiency of soil N, which enhanced N absorption by Chinese firs. Under high-N treatments, a large supply of N reduced the relative availability of soil P, causing significant increases in colonization rates, while high P treatments increased spore yield. A moderate N deposition or P addition can weaken the symbiotic relationship. However, in the case of interactive P and N effects, AMF colonization rates and spore density were affected by the relative content of N and P in the soil. There are other soil physicochemical characteristics affect AMF. Soil moisture content was the main environmental factor affecting AMF colonization rates. This study provides a new approach to improving the management and productivity of Chinese fir forests under the background of global environmental change.

Methods
Study site

This study was conducted in Gaokan village (119°67'E, 30°21'N), Lin'an District, Zhejiang Province, China. It is located on the northern margin of the subtropical monsoon climatic zone, and characterized by four distinct seasons, mild climate, and abundant precipitation. The average annual temperature of the site was 15.6 °C with an average annual precipitation of 1420 mm, and an average of 230 frost-free days per year. The soil is classified as Ferrisols derived from granite [16], and the terrain is comprised of low hills. Local N deposition rate is $30-37$ kg·hm^{-2}·a^{-1} [16].

Experimental design

We selected 27 ten-year old Chinese fir trees with a similar growth form and average height of approximately 3 m in the sample forest. A 3 m × 3 m independent plot was established at the center of each tree, with an interval distance of at least 2 m. Based on actual N deposition and the increasing trend in subtropical areas of China [53–55], we set up two treatments: a low N (30 kg·hm^{-2}·a^{-1}) treatment (N30) and high N (60 kg·hm^{-2}·a^{-1}) treatment (N60). Two P addition treatments were also established after referring to relevant foreign and domestic research [56,57]: a low P (20 mg·kg^{-1}) treatment (P20) and a high P (40 mg·kg^{-1}) treatment (P40). Another four N and P compound treatments (N30+P20, N30+P40, N60+P20, and N60+P40) and a control group were established, with a total of nine treatments and three replicates in completely randomized blocks, with one replicate plot per treatment per block. Calcium magnesium phosphate fertilizer (main ingredients including $Ca_3(PO_4)_2$, $CaSiO_3$, $MgSiO_3$, and 12–18% P_2O_5) was sprayed evenly and then thoroughly mixed into the soil with a deep plough to a 0.3 m depth on April 2017, so that the available P content in the upper layer of the soil reached to P20 and P40 levels. Following April 2017, quantitative NH_4NO_3 was weighed, mixed with 0.225 L of water, and sprayed evenly from above the tree crown of each plot using an electric sprayer at the beginning of every month for 12 equal applications (over an entire year) throughout the experimental period. Each control treatment plot (N- and P-free) received 0.225 L of N-free water to control for the effects of the added water.

Sampling

The distribution of the root systems of the selected Chinese fir trees was observed by the trench method [58]. Soil samples of 20–30 cm deep in the rhizosphere of the fir trees were dug in a circle (with a radius of 30 cm) with a spade at the base of each treatment plot (this was to remove topsoil to reduce experimental interference). Soil cores were sampled in four directions from each plant in each treatment plot, and mixed and sieved (2 mm mesh size) to remove stones, coarse roots and other plant residues. The soils loosely and tightly bound to the surface of roots were removed by clean tweezers and brush, and defined as rhizosphere soil samples [59]. Then, they were placed in sterile sealed bags and immediately transferred to the laboratory. Sifted and dried soil samples were stored at 4 °C in the refrigerator for determining physical and chemical properties and AMF spore density. Root samples were preserved with formaldehyde-acetic acid alcohol (FAA) fixative (38% formalin, glacial acetic acid, 70% alcohol in a ratio of 1:1:9 (v:v:v)) prepared in advance in glass tubes with screw caps.

Measurements

The samples of fine roots were stained with Acid Fuchsin according to a modified staining protocol [60], and quantified by dividing the cross points with infected mycorrhizal by the total cross points, according the magnified gridline intersection method developed by McGonigle et al. [61]. The AMF spores were isolated from 20 g dried rhizosphere soil samples using wet sieving, decanting, and the sucrose gradient centrifugation method [62]. Spore density (total number of spores in 20 g of dry soil) was determined by

counting the number of spores with a normal appearance (based on color, shape, surface condition and examination of spore contents) were counted under a compound microscope (40X) [63]. Soil moisture content was determined by the drying method (105°C, 8h), and soil pH was determined by a portable pH meter (FE20, Mettler Toledo, Switzerland) after the mixture (soil: water (w/v) ratio is 1:2.5) was shaken for 30 minutes. Soil effective N was determined by the alkali-hydrolysis diffusion method [64], and soil available P was extracted by the diacid method and determined by molybdenum-antimony colorimetry [65]. The content of soil N was determined by H_2SO_4-H_2O_2 decoction and semi-micro Kjeldahl method [66], and the content of soil P was determined by H_2SO_4-H_2O_2 decoction and molybdenum-antimony colorimetry [67].

Statistical analysis

Statistical analysis was performed using SPSS 19.0 for Windows software (SPSS Inc., Chicago, IL) with a one-way analysis of variance (ANOVA) followed by least significant difference (LSD) to establish quantitative differences between treatments. The relationship between soil characteristics, AMF root colonization rates, and spore density were tested using the Spearman's correlation. Two-way ANOVA was performed to evaluate the combined influence of N deposition level and P addition on AMF root colonization rates and spore density. All data were tested for homogeneity of variance and normality of distribution prior to conducting the ANOVA. The data satisfied the assumption of homogeneity of variance. The images were all produced by SigmaPlot12.5 (Systat Software Inc., San Jose, CA, USA).

Declarations

Ethics approval and consent to participate

Not applicable. We did not handle animals, thus no ethics protocols were required to be followed.

Consent for publication

Not applicable.

Availability of data and material

The datasets in the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

Authors' contributions

XS designed the study and was responsible for funding acquisition and resources. CL and YW performed experiments and analyzed data. ML, QL, XS and WX discussed and wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

References

1. Smith SE, Read DJ. Mycorrhizal symbiosis. New York: Academic press; 2012. p. 321-322.

2. Campos-Soriano L, Segundo BS. New insights into the signaling pathways controlling defense gene expression in rice roots during the arbuscular mycorrhizal symbiosis. Plant Signaling Behav. 2011; 6 (4):553-557.

3. He XH, Duan YH, Chen YL, Xu MG. A 60-year journey of mycorrhizal research in China: Past, present and future directions. Science China Life Science. 2010; 53 (12):1374-1398.

4. Mao L, Liu YJ. The eco-physiological functions of arbuscular mycorrhizal fungi: a review. Sciencepap Online. 2018; 11 (3):236-245.

5. Karasawa T, Hodge A, Fitter AH. Growth, respiration and nutrient acquisition by the arbuscular mycorrhizal fungus *Glomus mosseae* and its host plant *Plantago lanceolata* in cooled soil. Plant Cell Environ. 2012; 35:819-828.

6. Pozo MJ, Azcón-Aguilar C. Unraveling mycorrhiza-induced resistence. Curr Opin Plant Biol. 2007; 10 (4): 393-398.

7. Parniske M. Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol. 2008; 6 (10): 763-775.

8. Bryla DR, Eissenstat DM. Respiratory costs of mycorrhizal associations. In: Lambers H, Ribas Carbo M Editors. Plant Respir. Springer, The Netherlands; 2005. p. 207-224.

9. Bonfante P, Genre A. Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. Nat Commun. 2010; 48 (1):1038-1046.

10. Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M, Pascale SD, Bonini P, Colla G. Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Sci Hortic. 2015; 196: 91-108.

11. Veresoglou SD, Chen BD, Rillig MC. Arbuscular mycorrhiza and soil nitrogen cycling. Soil Biology and Biochemistry. 2012; 46:53-62.

12. Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science. 2008; 320: 889-892.

13. Song XZ, Gu HH, Wang M, Zhou GM, Li Q. Management practices regulate the response of Moso bamboo foliar stoichiometry to nitrogen deposition. Sci Rep. 2016; 6:24107.

14. Galloway JN, Cowling EB. Reactive nitrogen and the world: 200 years of change. Ambio. 2002; 31 (2):64-71.

15. Liu J, Zhang Y, Han WX, Tang AH, Shen JL, Cui ZL, Vitousek P, Erisman JW, Goulding K, Christie P, Fangmeier A, Zhang F. Enhanced nitrogen deposition over China. Nature. 2013; 494 (7438): 459-462.

16. Li Q, Song XZ, Scott X. Chang, Peng CH, Xiao WF, Zhang JB, Xiang WH, Li Y, Wang WF. Nitrogen depositions increase soil respiration and decrease temperature sensitivity in a Moso bamboo forest. Agric For Meteorol. 2019; 268: 48-54.

17. Wang JF, Liu NK. Research progress on mechanisms of atmospheric nitrogen deposition and its ecological impact. Pollut Control Technol. 2018; 31 (6):17-21+39.

18. Ling JX, Yang YH, Wang YN, Yan XL, Shao S, Yan XF. Research progress on effects of nitrogen deposition on symbiont of plant-Arbuscular mycorrhizal. Grassland and Turf. 2015; 35 (3): 88-94.

19. Wang YN. Physiological responses of *Leymus chinens*-arbuscular mycorrhizal symbiont to the interaction of nitrogen deposition and salt-alkali stress. *PhD thesis*. Northeast Forestry University; 2016.

20. He XL, Liu T, Zhao LL. Effects of inoculating AM fungi on physiological characters and nutritional components of *Astragalus membranaceus* under different N application levels. Chin J of Appl Ecol. 2009; 20 (9): 2118-2122.

21. Hodge A. The plastic plant, root responses to heterogeneous supplies of nutrients. New Phytol. 2004; 162: 9-24.

22. Lin SS, Sun XW, Wang XJ, Dou CY, Li YY, Luo QY, Sun L, Jin L. Mycorrhizal studies and their application prospects in China. Acta Prataculturae Sinica. 2013; 22 (5):310-325.

23. Liu RJ, Chen YL. Mycorrhizology. Beijing: Science Press. 2007; 1-447.

24. Su YB, Lin C, Zhang FS, Yang XL. Effect of arbuscular mycorrhizal fungi (*Glomus Mosseae, Glomus Versiformea, Gigaspora Margarita* and *Gigaspora Rosea*) on phosphate activities and soil organic phosphate content in clover rhizosphere. Soils. 2003; 35 (4):334-338.

25. Bauhus J, Messier C. Soil exploitation strategies of fine roots in different tree species of the southern boreal forest of eastern Canada. Canadian Journal of Forest Resources. 1999; 29: 260-273.

26. Feng HY, Feng G, Wang JG, Li XL. Regulation of P status host plant on alkaline phosphatase (ALP) activity in intraradical hyphae and development of extraradical hyphae of AM fungi. Mycosystema. 2003; 22 (4):589-598.

27. Zhang SB, Wang YS, Yin XF, Liu JB, Wu FX. Development of arbuscular mycorrhizal (AM) fungi and their influences on the absorption of N and P of maize at different soil phosphorus application levels. Journal of Plant Nutrition and Fertilizer. 2017; 23 (3): 649-657.

28. Shi SZ, Xiong DC, Feng JX, Xu CS, Zhong BY, Deng F, Chen YY, Chen GS, Yang YS. Ecophysiological effects of simulated nitrogen deposition on fine roots of Chinese fir (*Cunninghamia lanceolata*) seedlings. Acta Ecol Sin.

2017; 37 (1):74-83。

29. Slezack S, Dumas-Gaudot E, Paynot M, Gianinazzi. S: Is a fully established arbuscular mycorrhizal symbiosis required for bioprotection of *Pisum sativum* roots against *Aphanomyces euteiches*? Mol Plant-Microbe Interact. 2000; 13:238-241.

30. Yang JY, Wang YH, Wen GS, Yi LT. Effects of AM fungi and simulated nitrogen deposition on the growth and biomass accumulation of *Solidago canadensis* seedlings. Chin J of Ecol. 2013; 32 (11): 2953-2958.

31. Marschner H. Marschner's mineral nutrition of higher plants. New York: Academic press; 2012. p. 122-123.

32. García IV, Mendoza RE. Relationships among soil properties, plant nutrition and arbuscular mycorrhizal fungi-plant symbioses in a temperate grassland along hydrologic, saline and sodic gradients. FEMS Microbiol Ecol 2008; 63 (3):359-371.

33. Eissenstat DM, Yanai RD. The ecology of root lifespan. Adv Ecol Res. 1997; 27: 1-60.

34. Pang L, Zhou ZC, Zhang Y, Feng ZP, Feng ZP. Effects of atmospheric N sedimentation on growth and P efficiency of *Pinus Massoniana* mycorrhizal seedlings under low P stress. Journal of Plant Nutrition and Fertilizer. 2016; 22 (1):225-235.

35. Cai XZ. Effects of nitrogen addition on arbuscular mycorrhizal fungi and nitrogen uptake of Chinese fir seedlings. *PhD thesis*. Fujian Normal University; 2017.

36. Bago B, Cano C, Azcón-Aguilar C, Samson J, Coughlam A, Piché Y. Differential morphogenesis of the extraradical mycelium of an arbuscular mycorrhizal fungus grown monoxenically on spatially heterogeneous culture media. Mycologia. 2004; 96 (3):452-462.

37. Zhang X, Pei ZP, Sun G, Wang Y, Su XP. Correlation between physicochemical properties of rhizosphere soil and arbuscular mycorrhizal fungi in *Medicago sativa* grassland. Northern Horticulture. 2016; 13:172-177.

38. Wang JF, Liu NK. Research progress on mechanisms of atmospheric nitrogen deposition and its ecological impact. Pollut Control Technol. 2018; 31 (6):17-21+39.

39. Ipsilantis I, Sylvia DM. Interactions of assemblages of mycorrhizal fungi with two Florida wetland plants. Appl Soil Ecol. 2007; 35(2): 261-271.

40. Ma LM , Wang PT , Wang SG. Effect of flooding time length on mycorrhizal colonization of three AM fungi in two wetland plants. Environ Sci. 2014; 35(1):263-270.

41. Sharma D, David K. Moisture-a regulator of arbuscular mycorrhizal fungal community assembly and symbiotic phosphorus uptake. Mycorrhiza. 2015; 25:67-75.

42. Zhang XH. The adaptability of arbuscular mycorrhizal fungi to different soil environmental factors. *PhD thesis*. Agricultural University of Hebei; 2003.

43. Li YF, Ju LH, Zhang LY, Zhang LY, Xu GH. Effects of AM fungi on plant growth and nitrogen and phosphorus utilization in rice/mungbean intercropping under different phosphorus application levels. Jiangsu Agricultural Sciences. 2013; 41 (3):51-55.

44. Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Bücking H. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science. 2011; 333: 880-882.

45. Sun XW, Wang XJ, Chen M, Chen M, Dou CY, Gao FX, Jin L. Effects of eco-enviromental factors on the production and distribution of arbuscular mycorrhizal fungal spores. Acta Prataculturae Sinica. 2011; 20 (1): 214-221.

46. Sun JH, Bi YL, Qiu L, Jiang B. A review about the Effect of AMF on Uptaking Phosphorus by Host Plants in Soil. Chin J of Soil Sci. 2016; 47 (2): 499-504.

47. Subhashini DV. Effect of NPK fertilizers and co-inoculation with phosphate solubilising arbuscular mycorrhizal fungus and potassium mobilizing bacteria on growth, yield, nutrient acquisition and quality of tobacco (*Nicotiana tabacum*) . Commun Soil Sci Plant Anal. 2016; 47 (3): 328-337.

48. Liu L, Liao H, Wang XR, Yan XL. Regulation effect of soil P availability on mycorrhizal infection in relation to root architecture and P efficiency of Glycine max. Chin J of Appl Ecol. 2008; 19 (3):564-568.

49. Tessa C, Stefan H, Jürgen H, Horn S, Velescu A, Wilcke W, Rillig M. Nitrogen and phosphorus additions impact arbuscular mycorrhizal abundance and molecular diversity in a tropical montane forest. Glob Change Biol. 2014; 20:3646-3659.

50. Gusewell S. N:P ratios in terrestrial plants: variation and functional significance. New Phytol. 2004; 164 (2): 243-266.

51. Xia TT, Wang ZP, Zhang LC, Jing HR. Effects of phosphorus concentration on growth of arbuscular mycorrhizal sweet corn. Ind Microbiol. 2017; 47 (3):49-53.

52. Qiu JJ. Molecular mechanism of phosphorus uptake by the interaction of arbuscular mycorrhizal fungi and maize. *PhD thesis*. Shandong Agricultural University; 2017.

53. Reay DS, Dentener F, Smith P, Grace J, Feely RA. Global nitrogen deposition and carbon sinks. Nat Geosci. 2008; 1 (7): 430-437.

54. Fang H, Mo J, Peng S, Li Z, Wang H. Cumulative effects of nitrogen additions on litter decomposition in three tropical forests in southern China. Plant Soil. 2007; 297:233-242.

55. Mo J, Brown S, Xue JH, Li ZA, Li DJ, Dong SF. Response of nutrient dynamics of decomposing pine (*Pinus massoniana*) needles to simulated N deposition in a disturbed and a rehabilitated forest in tropical China. Ecol Res. 2007. 22:649-658.

56. Chen ZY, Wu PF, Zou XH, Wang P, Ma Jing, Ma QX. Relationship between growth and endogenous hormones of Chinese fir seedlings under low phosphorus stress. Scientia Silvae Sinicae. 2016; 52 (2):57-66.

57. Leng HN, Chen YT, Duan HP, Rao LB, Wang YJ, Hu YX. Effects of phosphorous stress on the growth and nitrogen and phosphorus absorption of different formosan sweet gum provenances. Chin J of Appl Ecol. 2009; 20 (4): 754-760.

58. Wu JJ, Yang ZJ, Liu XF, Xiong DC, Lin WS, Chen ZQ, Wang XH. Analysis of soil respiration and components in *Castanopsis carlesii* and *Cunninghamia lanceolata* plantations. Chin J of Plant Ecol. 2014; 38 (1): 45-53.

59. Yang YR, Song YY, Scheller HV, Ghosh A, Ban YM Chen H, Tang M. Community structure of arbuscular mycorrhizal fungi associated with *Robinia pseudoacacia* in uncontaminated and heavy metal contaminated soils. Soil Biol Biochem. 2015; 86:146-158.

60. Berch SM, Kendrick B. Vesicular-arbuscular mycorrhizae of southern Ontario ferns and fern-allies. Mycologia. 1982; 74 (5): 769.

61. McGonigle TP, Miller MH, Evans DG,. Fairchild GL, Swan JA. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 1990; 115: 495-501.

62. Gerdemann JW. Nicolson TH. Spores of Mycorrhizal Endogone Species Extracted from Soil by Wet Sieving and Decanting. Transactions of British Mycological Society. 1963; 46 (2): 235-244.

63. Eom AH, Wilson GW, Hartnett DC. Effects of ungulate grazers on arbuscular mycorrhizal symbiosis and fungal community structure in tallgrass prairie. Mycologia. 2001; 93(2):233-242.

64. Jackson ML. Soil chemical analysis. New Jersey: Prentice-Hall Inc; 1973. p. 205-226.

65. Olsen SR. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Miscellaneous Paper Institute for Agricultural Research Samaru; 1954.

66. Hess TM. Tropical Soil Biology and Fertility: A Handbook of Methods. Anderson JM and Ingram JSI editors. Wallingford: CAB International; 1990. p. 245-245.

67. Zhang WR, Yang GJ, Tu XN, Zhang P. Forestry Industry Standard of the People's Republic of China: Forest Soil Analysis Method. Beijing: China Standards Press; 1999.

Tables

Table 1. Soil physicochemical characteristics in a Chinese fir forest under N deposition and P addition treatments

	Soil moisture (%)	pH	AN (mg/kg)	TN (g/kg)	AP (mg/kg)	TP (g/kg)
CK	27.6±2.2Aa	4.96±0.09Ab	132.53±26.28Aa	1.2±0.1Aa	0.73±0.07Bb	0.05±0.01Ab
LP	26.2±3.0a	5.02±0.15b	136.27±1.62a	1.4±0.3a	0.91±0.08b	0.12±0.02a
HP	28.3±4.7a	5.23±0.06a	144.20±4.20a	0.9±0.3a	1.51±0.37a	0.08±0.04ab
LN	28.2±2.4Ab	5.15±0.21Aa	88.67±3.52aB	1.1±0.1Aa	0.90±0.03Ab	0.05±0.01Aa
LN-LP	30.9±1.7ab	5.42±0.42a	135.80±7.41b	1.2±0.3a	1.10±0.21b	0.06±0.01a
LN-HP	32.4±1.9a	5.27±0.22a	158.67±11.91ab	1.0±0.1a	8.87±0.79a	0.11±0.09a
HN	31.0±1.8Aa	5.07±0.04Ab	139.07±5.83Aa	1.2±0.3Aa	0.79±0.02aB	0.08±0.02Ab
HN-LP	27.7±1.3b	5.14±0.04b	136.27±21.39a	1.2±0.2a	2.33±0.03b	0.12±0.01a
HN-HP	30.1±1.7ab	5.27±0.03a	134.87±4.50a	1.4±0.2a	2.78±0.66a	0.14±0.01a

AN: available nitrogen; TN: total nitrogen; AP: available phosphorus; TP: total phosphorus. Different lowercase letters indicate significant differences under the same simulated N deposition gradient, while different uppercase letters indicate significant differences under the same P addition gradient, $P < 0.05$.

Table 2 Correlations between colonization and spore density of arbuscular mycorrhizal fungi (AMF) and soil physicochemical characteristics

	Soil moisture	pH	AN	TN	AP	TP
Root colonization rates	-0.402*	-0.118	-0.140	-0.185	-0.076	-0.154
Spore density	0.000	0.217	-0.061	0.042	0.164	0.024

AN: available nitrogen; TN: total nitrogen; AP: available phosphorus; TP: total phosphorus.

*$P < 0.05$.

Table 3 Two-way ANOVA showing the effects of N and P treatment on arbuscular mycorrhizal fungi (AMF)

Factors	Source of Variation	SS	df	MS	F
Root colonization rates	N Deposition	19287.87	2	9643.94	712.25**
	P Addition	451.99	2	226.00	16.69**
	N×P Interaction	1015.09	4	253.77	18.74**
Spore density	N Deposition	6.82	2	3.41	4.64*
	P Addition	1.34	2	0.67	0.91
	N×P Interaction	17.92	4	4.48	6.11**

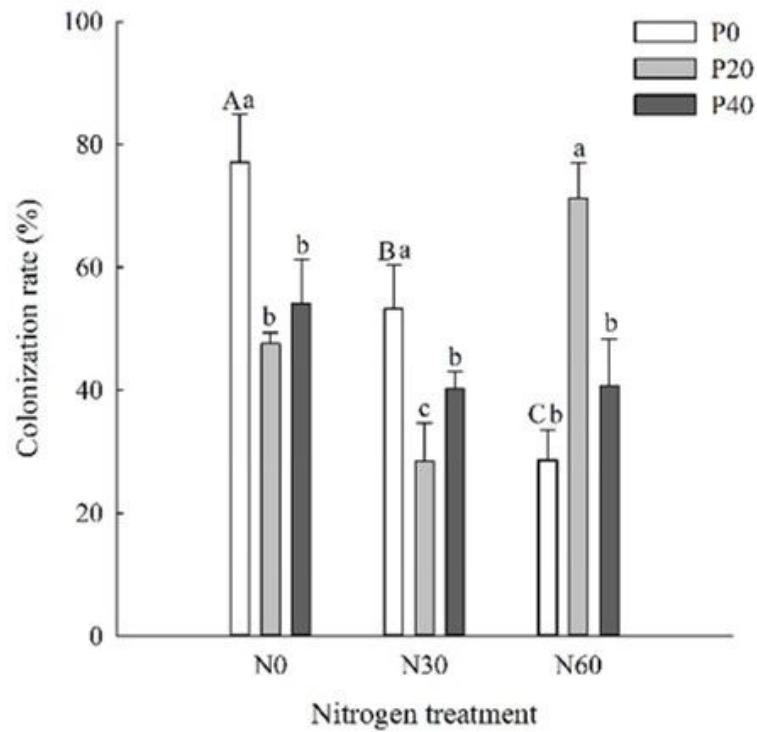* $P < 0.05$ and ** $P < 0.01$, respectively.

Figures

Fig. 1

Figure 1

Arbuscular mycorrhizal fungi (AMF) root colonization of Cunninghamia lanceolata under nitrogen and phosphorus treatments. Different lowercase letters indicate significant differences under the same simulated N deposition gradient, while different uppercase letters indicate significant differences under the same P addition gradient, $P < 0.05$. Bars denote the standard error ($n = 3$).
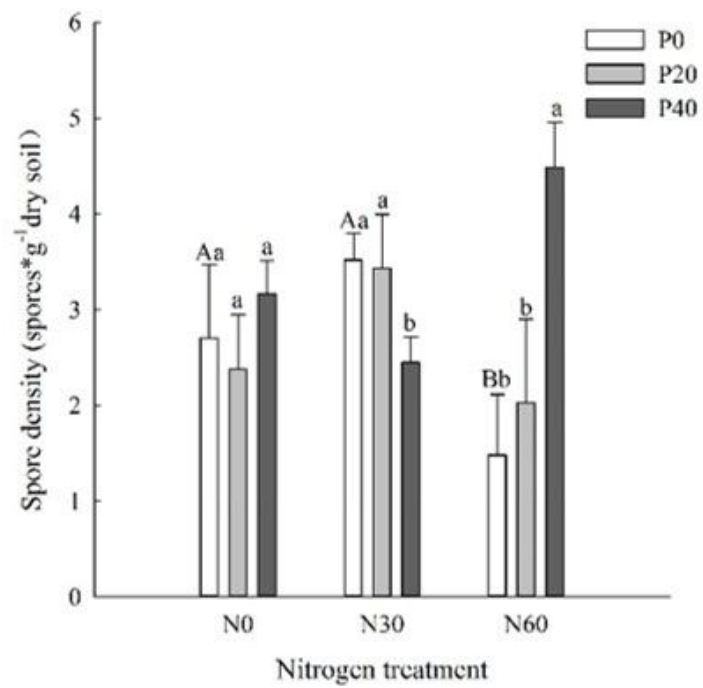
Fig. 2

Figure 2

Arbuscular mycorrhizal fungi (AMF) spore density of Cunninghamia lanceolata under nitrogen and phosphorus treatments. Bars denote the standard error (n = 3)