Hyphal-Mediated Nitrogen translocation is Optimized at Intermediate N Levels in Pinus massoniana from ^{15}N Tracing

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Abstract

Aims

Common mycorrhizal networks (CMNs) serve as essential pathways for belowground resource exchange, with a key function being hyphal-mediated nitrogen translocation across plant hosts. This study specifically investigates how CMNs, established by mixed mycorrhizal communities, facilitate interplant nitrogen translocation and regulate nitrogen allocation strategies. By elucidating this mechanism, our work provides critical insights for the restoration of degraded forest ecosystems.

Methods

This study developed a four-cell grid seedling apparatus (A-D chambers) to physically isolate root contact while preserving mycelial network connectivity. A sterile soil medium was used to maintain hyphal viability and prevent inter-chamber nutrient diffusion. A controlled experiment was conducted using *Pinus massoniana* seedlings inoculated with mixed mycorrhizal strains (Sm, comprised *Pisolithus orientalis* (Po), *Scleroderma citrinum* (Sc), and *Suillus luteus* (Sl), isolated and purified from *P.massoniana* rhizosphere soil samples.) versus non-inoculated controls (CK). Different nitrogen treatments were carried out through $(^{15}NH_4)_2SO_4$ solution isotope labeling (0 g/L (N0), 2 g/L (N2), 4 g/L (N4), and 6 g/L (N6)), we quantitatively assessed hyphal-mediated nitrogen translocation through CMNs across a 30-μm mesh barrier.

Results

CMNs established direct hyphal connections between donor and receiver seedlings, facilitating nitrogen translocation via hyphal pathways. The translocation pattern, which peaked at intermediate N levels, indicates a regulated interplant nitrogen redistribution mediated by the fungal network, rather than a mere enhancement of direct uptake by the receiver's mycorrhizae. Sm-inoculated seedlings exhibited a 2.62-fold increase in root biomass (N6) and 50.59% leaf nitrogen allocation (N4), significantly surpassing CK. The Sm-N4 treatment demonstrated optimal performance, enhancing photosynthetic capacity via prioritized nitrogen allocation to leaves. Notably, ^{15}N transfer ratios directly correlated with receiver seedling biomass and nutrient uptake, highlighting CMNs as a functional conduit for resource redistribution.

Conclusions

Our study shows that plants rely more on mycorrhizal fungi for nitrogen (N) when soil N levels drop. This dependence peaks at a critical threshold (N4), where fungal N translocation is most efficient. This supports the carbon-for-nitrogen trade model, where plants exchange carbon with fungi to acquire nitrogen, particularly for leaf development under N scarcity. Identifying N4 as the optimal level helps guide mycorrhizal use in reforesting N-poor areas.

Introduction

Plant roots and soil fungi combine to form the symbiotic association known as ectomycorrhiza (ECM), which exhibits distinct morphological characteristics and functional attributes (Strullu et al. 2018). Due to their broad host compatibility, the majority of ectomycorrhizal fungi form symbiotic relationships with multiple plant species through extraradical mycelium, facilitating the establishment of extensive mycelial networks termed common mycorrhizal

networks (CMNs) that interconnect plant root systems (Figueiredo et al. 2021; Oelmüller, R., 2019). The transfer of inorganic nutrients and organic photosynthetic products between plants is made possible by the common mycorrhizal network, which also serves as an essential conduit for information and material exchange between plants below ground (Song et al. 2019). Recent studies suggest that CMNs in conifers like *Pinus massoniana* exhibit species-specific nutrient transfer patterns, potentially driven by fungal carbon-nutrient trade-offs (Chen et al., 2021; Pena et al., 2023). We hypothesize that hyphal-mediated N translocation efficiency is maximized below a critical soil N availability threshold, where host carbon investment in fungal networks becomes optimally balanced with symbiotic nitrogen acquisition costs to sustain preferential allocation to photosynthetic tissues (Wipf et al. 2019; Wang, C. 2020).

Research on fungi that enhance plant nutrient uptake and utilization has advanced considerably in recent years (Musa et al. 2020; Cao et al. 2021; Wu et al. 2022; Lin et al. 2023); however, a predominant focus has been on arbuscular mycorrhizal fungi (AMF). (Liu et al. 2020; Chandrasekaran et al. 2020; Gao et al. 2021; Gui et al. 2023). Such as, AMF delivered 94% of the ^{15}N and ^{33}P through the mycelial network system between sorghum and flax, but only absorbed a little quantity of C from flax (Walder et al. 2012). Fewer studies have been conducted on ECM fungal nutrient acquisition and use (Formenti et al. 2023). As research has advanced, more and more emphasis has been drawn to the function of the common mycorrhizal network in the transmission of chemicals and information (Lian et al. 2019; Whiteside et al. 2019). It has been reported that ectomycorrhizal fungi (EMF) infiltrate mycelial networks made up of the roots of plants belonging to the same or different species, hence potentially translocating water, carbon, and nutrient across plants (Simard et al. 2012; Cahanovitc et al. 2022). Furthermore, evidence demonstrates that CMNs can assist host plants in transmitting chemical signals between communities (Gilbert et al., 2017).

Pinus massoniana, a widely distributed and common tree species in China, serves as a typical ectomycorrhizal host capable of participating in CMNs (Wang and Yang, 2022). One of the key elements preventing *P. massoniana* from growing and increasing forest production in southern China is the high acidity and deficiency in phosphate and nitrogen in soil (Dou et al., 2013; Guo et al., 2023). Thus, mycorrhizal symbiosis of *P. massoniana* is crucial for enhancing plant nitrogen absorption and preserving the nitrogen cycle in the environment. Prior research has revealed that *P. massoniana* seedlings can create a shared mycorrhizal network, and evidence suggests it allows for nutrient translocation and redistribution among seedlings (Tu et al. 2022). The exact mechanism underlying nutrient translocation within common mycorrhizal network remains unclear. A critical challenge in quantifying nutrient translocation via CMNs is distinguishing between true interplant movement (i.e., nutrient movement from one plant into the network and then to another plant) and the scenario where a shared mycelium directly absorbs nutrients from a localized source and delivers them to a receiver plant without prior incorporation into the donor plant (Teste et al., 2015). To address this, we employed a compartmented donor-receiver system with key design features: (1) a sterile growth medium to eliminate background soil hyphae and ensure that the only fungal pathway originated from the inoculated donor chamber; (2) a physical barrier (30-μm mesh) between the nitrogen labeling site (beneath donor chamber A) and the receiver chamber (D), preventing direct root or diffusive N access for the receiver plant. We hypothesize that under these constrained conditions, the ^{15}N detected in receiver seedlings predominantly results from hyphal-mediated translocation through the CMN established from the donor plant, and that this process is optimized at intermediate nitrogen levels. How do hyphal-mediated nitrogen translocation efficiency and allocation patterns vary across nitrogen gradients? Our study focused on the formation of a shared mycorrhizal network among *P. massoniana* seedlings, utilizing a four-cell grid seedling apparatus and building on the findings of previous research. Subsequently, we employed ^{15}N labeling to assess changes in growth, nutrient uptake, and transport in both donor and receiver seedlings at varying nitrogen doses.

Experimental material

Experimental strain

The mixed strains (SM) comprised *Pisolithus orientalis* (Po), *Scleroderma citrinum* (Sc), and *Suillus luteus* (Sl). These three ectomycorrhizal fungal species were originally isolated and purified directly from the fruiting bodies of *Pinus massoniana* forests. The fruiting bodies were collected from P. massoniana woodland soils, representing the most commonly encountered and widespread species found in these specific forest ecosystems.

These three ectomycorrhizal fungal species were kindly provided by Professor Ding of the Guizhou Forest Resources and Environment Research Center at Guizhou University (Guiyang, China). The three fungal species were all isolated and purified from their respective fruiting bodies. Following molecular biological identification, the obtained sequencing results were subjected to sequence alignment using the NCBI BLAST system and subsequently uploaded to GenBank, with accession numbers OM760642, MT994623, and MT994629 respectively. Three species of ectomycorrhizal fungi were first isolated and purified using solid culture medium, then mixed in a 1:1:1 concentration ratio for liquid medium amplification to form the final mixed inoculum solution. The growth status of three tested strains on flat solid medium in Fig.S1.

Experimental tree strain

Seeds of the Duyun-126 variety (thousand-grain weight: 11.3 g) were harvested from the *P. massoniana* improved seed base in Duyun, Guizhou Province, China (E107°31', N26°15'). The seedlings were disinfected as describe by Tu (2022). *P. massoniana* seeds were sterilized in a 0.5% potassium permanganate solution for 2 hours and then rinsed with sterile water 3 to 5 times. They were subsequently placed in sterile water at an initial temperature of 45 ℃, followed by immersion in an artificial climatic chamber at approximately 25 ℃ for 24 hours. Seeds were sown once they turned white, and afterward, they were placed in the artificial climatic chamber for seedling growth and emergence post-sowing.

Experimental setup

Using a plastic box, a four-cell grid seedling apparatus was constructed by hand (chamber A-D) as described by Tu (2022). The longitudinal section of the seedling equipment is shown in Fig. 1. Each seedling box included a donor chamber (A, 8.4cm), clay isolation layer (B, 3cm), mycelium chamber (C, 4cm), receiver chamber (D, 8.4cm), and labeling box. The compartments A and B, as well as C and D, were separated by 30-μm nylon mesh. The bottom of compartment A was perforated with the maximum feasible number of 3-mm diameter holes. A coarse nylon mesh with 1-mm apertures was installed between compartments B and C. *P. massoniana* seedlings were planted in A, and the mycelia in A could pass through the nylon net into B and C to reach D and form a mycelial network. A 30-μm nylon mesh separated compartments A-B and C-D. This pore size is well-established to preclude root penetration while permitting the passage of fungal hyphae. Crucially, a solid clay layer (compartment B) and an additional mesh barrier separated compartments B and C. This multi-layered physical isolation between the donor (A) and receiver (D) chambers was instrumental in preventing any direct diffusion of the ^{15}N label solution or root exudates from A to D through the soil matrix. The ^{15}N labeling box was positioned exclusively beneath compartment A. The design of this microporous supply box allowed partial penetration of donor plant roots from compartment A only. The receiver plant roots in compartment D were physically separated from the labeling box by the combined barriers of compartments B and C, and had no direct access to the ^{15}N source.

The chambers are filled with a lightweight medium, such as autoclaved soil, which serves to support the growth of microorganisms and fungi while maintaining the necessary humidity levels. This step eliminated any pre-existing native soil fungal networks, ensuring that the only source of extraradical mycelium in the system was the mixed

inoculum (Sm) introduced solely into compartment A during seedling transplantation. Therefore, any mycelium subsequently found connecting to the receiver seedlings in compartment D must have originated from and formed a network with the donor seedlings in compartment A. This design negates the possibility of receiver seedlings independently forming mycorrhizae with fungi that could directly access the ^{15}N label in compartment A. This design considerations align with findings from recent studies (Aleklett et al., 2021; Ghanbari et al., 2020).

In summary, this setup ensured that the only plausible pathway for ^{15}N to reach the receiver seedling in compartment D was via the extraradical mycelium of the fungi that had colonized the donor seedling in compartment A, traversed the barriers, and subsequently colonized the receiver seedling.

Medium

The soil was extracted from the *P. massoniana* forest, dried, and screened (1 cm × 1 cm). It was then combined with vermiculite and perlite in a 9:2:1 volume ratio. Every matrix was continually sterilized in an autoclave for 1 hour at 121°C and 0.14 MPa of pressure, and then packed into A, C, and D of the four-cell grid boxes at concentrations of 1.20, 0.60, and 1.20 kg/pot, respectively. In addition, clay sterilized under the same conditions was also loaded into B at 0.45 kg per basin (Tu et al. 2022). The physicochemical properties of the soil are shown in Table 1.

Experimental design

A two-factor experimental design was adopted, with Factor A being inoculation treatment, consisting of mixed inoculation (Sm) and non-inoculation (CK), where the mixed fungi were Po, Sc, and Sl mixed at a 1:1:1 ratio to simulate the actual ectomycorrhizal situation in *P. massoniana* forests; Factor B was different nitrogen application levels, with 4 nitrogen fertilizer levels: no nitrogen application 0 (N0), 2 g/L nitrogen (N2), 4 g/L (N4), and 6 g/L (N6), totaling 8 treatments, repeated 8 times, with 2 seedlings each in chamber A and D, 128 seedlings were used in total. After 40 days of growth (the seedlings are about 40 days old), uniformly grown seedlings were selected and transplanted into a four-cell grid seedling apparatus with mesh partitions. Two *P. massoniana* seedlings each were transplanted into Chamber A (donor chamber) and Chamber D (receiver chamber). Transplantation and inoculation were performed simultaneously, with only the seedlings in Chamber A being inoculated. Inoculation was performed concurrently with seedling transplantation in Chamber A. Briefly, 120 mL of the pre-cultured inoculum was thoroughly mixed into the soil substrate prior to transplanting *P. massoniana* seedlings. A 10 cm deep × 1 cm diameter hole was created adjacent to each seedling using a wooden stick, into which 5 mL of inoculum was introduced before backfilling. Additional 5 mL inoculum portions were deposited into two supplementary holes (5 cm depth) between neighboring seedlings to ensure uniform fungal distribution. This method aligns with protocols described by Tu et al. (2022) for establishing EMF symbioses under controlled conditions perform randomized sampling to quantify mycorrhizal colonization ratios and confirm successful establishment. Following a three-month growth establishment period, the ^{15}N labeling solution was added to the labelling box, such that the root systems of pre-colonized donor plants could make contact upon being aseptically transferred into Chamber A. To ensure isotopic specificity, the marker solution was replaced every 72 hours. This nitrogen supplementation, provided as ammonium sulfate ($(^{15}NH_4)_2SO_4$, isotopic abundance 98 atom%; Shanghai Zhenzhun Biotechnology Co., Ltd.), was dissolved in ultrapure water. All receiver plants were maintained in separation nutrient-deficient chambers connected via shared fungal networks to quantify the rhizosphere-to-rhizosphere transfer efficiency. Three months after the marking (the seedlings are about six months old), the *P. massoniana* seedlings were taken out for the index determination.

Index determination

Determination of seedling height, ground diameter, and biomass

After being cultivated at a room temperature of approximately 25°C, with 8 hours of natural light and a watering amount of 100-200 milliliters each day. Following three months of care, the *P. massoniana* seedlings were taken out and cleaned of dirt, perlite, vermiculite, and other impurities from the roots. The roots, stems, and leaves were then separation and placed in bags that could seal themselves. Every treatment was carried out three times. The gathered seedlings were weighed after 30 minutes at 105°C in the oven to determine their dry weights. Root biomass/above-ground biomass equaled the root-shoot ratio.

Mycorrhizal colonization ratio, mycorrhizal dependence, and mycelial contribution ratio

Mycorrhizal colonization ratio: the root samples were randomly selected from 30 trees per treatment and cut into 100 root segments of about 1 cm in length, and the mycorrhizal colonization ratio was observed under a stereo microscope. Mycorrhizal colonization ratio (%) = (the number of infested mycorrhizal root segments/the total number of root segments) × 100 (Qi et al., 2019). According to Zhang (2016), mycorrhizal dependency (%) was calculated as follows: dry weight with inoculation treatment * 100% / (dry weight with inoculation treatment - dry weight without inoculation treatment). According to Cai (2007), the mycelial contribution ratio (%) was (N uptake by mycorrhizal plants - N uptake by non-mycorrhizal plants) / N uptake by mycorrhizal plants *100%.

Nutrient content determination

Using a mixed ball mill (Retsch GmbH MM400, Germany) to grind the dry *P. massoniana* samples, the nutrients in the plant were identified. By using the sodium salicylate and sulfuric acid boiling methods, total nitrogen was ascertained. The ClerverChe automatic intermittent chemical analyzer (DeChem-Tech China Technical Service Center, Shenzhen Langcheng Technology Co., LTD. Chemical Analysis Technology Research and Development Center) was used to determine the total phosphorus using the sulfuric acid deboiling-molybdenum antimony method. Flame photometry using the FP series flame photometer FP6410 (Shanghai Jingke Industrial Co.Ltd.) was used to estimate the total potassium (Bao. 2015).

^{15}N abundance determination

The experiment used ^{15}N, and the abundance of ^{15}N was calculated as follows:

$$^{15}N\ atom\ \% = \frac{^{15}N}{^{14}N + ^{15}N} \times 100$$

The Elementar-Sercon Integra2 mass spectrometer (Element, Germam; Sercon Integra2, UK) was used to measure the ^{15}N abundance of plants.

Nitrogen utilization efficiency, allocation ratio and transfer ratio in *P. massoniana* seedlings

The calculations were performed following the method of Zhao (2006).

(1) N content by each organ = Dry weight of the organ × Total N content of the organ

(2) Total plant N content = N acquired by the shoot + N acquired by the root

(3) Proportion of ^{15}N in the N acquired of each organ (Ndff) = [(^{15}N atom% in plant sample - ^{15}N natural abundance [0.366%]) / (^{15}N atom% in labeled ammonium sulfate - ^{15}N natural abundance)] × 100%

(4) ^{15}N acquisition by each organ = N acquired by the organ × Ndff of the organ

(5) Total plant ^{15}N acquisition = ^{15}N acquired by the shoot + ^{15}N acquired by the root

(6) ^{15}N recovery (isotopic label recovery) = (Total plant ^{15}N acquisition / Total application ^{15}N amount) × 100%

(7) ^{15}N allocation [%] = (^{15}N acquired by each organ / Total plant ^{15}N acquisition) × 100%

(8) ^{15}N Transfer ratio [%] = [Amount of ^{15}N transferred via the mycelial bridge to the receiver plant] / [Total ^{15}N uptake by both donor and receiver plants] × 100%

Data analysis

Statistical analyses were performed using R 4.3.1 (lme4 and nlme packages) with mesocosm units (n=12) incorporatiod as a random effect to account for nested donor-receiver plant dependencies. Linear mixed-effects models (LMMs) were constructed to evaluate fixed effects of nitrogen administration levels while controlling for random mesocosm variation. Model residuals were verified for normality (Shapiro-Wilk) and homoscedasticity (Levene's test). Post-hoc comparisons between nitrogen treatments were conducted using Tukey's HSD through the emmeans package, with significance thresholds at α=0.05. Data visualization was implemented in OriginPro 2021, with results presented as mean ± SD (biological replicates: n=3 plants per mesocosm; experimental replicates: n=4 mesocosms per treatment).

Result

Root colonization ratio of donor/receiver of *P. massoniana* seedlings under different nitrogen levels

The results presented in Table 2 demonstrate the mycorrhizal colonization ratio of both donor and receiver plants without CK inoculation are 0%. The mycorrhizal colonization ratio of donor and receiver plants with nitrogen application are significantly higher than those without nitrogen (N0) ($P < 0.05$). The donor plants show the highest colonization ratio at the nitrogen application level of N6, which is 47.73% higher than N0, followed by N4. The receiver plants have the highest mycorrhizal colonization ratio at the nitrogen application level of N4, which is 65.91% higher than N0, followed by N6. Overall, the mycorrhizal colonization ratio of both donor and receiver plants at N6 and N4 are significantly greater than those at N0 and N2 ($P < 0.05$), and the colonization ratio of donor plants are higher than those of receiver plants.

Effect of common mycorrhizal network on biomass of *P. massoniana* seedlings under different nitrogen application levels

In non-inoculated (CK) donor seedlings, root, stem, and leaf biomass varied significantly with nitrogen levels, and the application level N4 produced the highest biomass, which was 1.42-, 3.02-, and 2.62-fold higher than that of N0, respectively (Table 3, $P < 0.05$). There was no significant difference between N2 and N0 ($P > 0.05$), but the root-shoot ratio progressively decreased across nitrogen levels and was considerably lower than N0 at N4 and N6 applications ($P < 0.05$). For inoculated (Sm) seedlings, root biomass showed an increase, the highest value at N6, which was 2.62 times greater than that of N0 and significantly different from other nitrogen levels ($P < 0.05$). Across nitrogen levels, leaf and stem biomass exhibited an initial increase followed by a decrease, peaking at N4 with values 2.44 and 2.60 times that of N0, respectively ($P < 0.05$). The root-to-shoot ratio of Sm-inoculated seedlings exhibited a fluctuating trend across nitrogen levels, initially increasing, then decreasing, and increasing again. The minimum value at N4 was significantly different from N0 ($P < 0.05$), while the maximum at N6 was the highest ($P < 0.05$) but not significantly different from N0 ($P > 0.05$). Similarly, mycorrhizal dependency across nitrogen levels displayed a rise–fall–rise pattern, reaching its maximum at N6; however, no statistically significant differences were observed among nitrogen

levels ($P > 0.05$). Except for root biomass at N0, the biomass of stems, leaves, and roots under Sm inoculation was higher than that of CK at the same nitrogen level.

For non-inoculated (CK) seedlings, root biomass gradually increased across nitrogen levels, while stem biomass showed an initial increase followed by a decrease, reaching its maximum at N4, which was 143.64% higher than that at N0 with a significant difference (Table 4, $P < 0.05$). Under Sm treatment, root and leaf biomass progressively decreased as nitrogen levels increased, peaking at N4 with values 111.94% and 26.81% higher than those at N0, respectively. Stem biomass exhibited an initial increase followed by a decrease across nitrogen levels, reaching its maximum at N4, which was 137.24% higher than that at N0 ($P < 0.05$). Sm inoculation increased the dry matter weight of roots, stems, and leaves of receiver seedlings compared to CK at the same nitrogen level, but no significant difference was observed in the root-to-shoot ratio ($P > 0.05$).

Effects of common mycorrhizal network on roots, stems, and leaves nutrients of *P. massoniana* seedlings under different nitrogen application levels

Total nitrogen in roots, stems, and leaves of donor/receiver of *P. massoniana* seedlings

The total nitrogen content of donor and receiver seedlings' roots under CK treatment peaked at N4 application (Fig. 2A, B), which was 32.29% ($P < 0.05$) and 11.10% ($P > 0.05$) greater than that of N0, respectively. The total nitrogen in the receiver seedlings' roots reached its maximum at N4 application, 11.26% higher than that of N0 ($P > 0.05$), whereas the total nitrogen in the donor seedlings' roots was lower than that of N0 ($P > 0.05$) under the Sm treatment. The total nitrogen of receiver seedlings was higher than that of N0 (Fig. 2D) only at N2 application, and the total nitrogen of donor/receiver seedlings reached the minimal value at N4 application. In stems, the total nitrogen of donor seedlings treated with CK and Sm was lower than that of N0 (Fig. 2C). The total nitrogen in the leaves of donor seedlings in CK and Sm increased, but as the nitrogen application ratio grew, it dropped (Fig. 2E). The maximal nitrogen application ratio in the CK treatment was 103.42% higher than in the N0 treatment ($P < 0.05$). Total nitrogen was at its greatest under the Sm treatment at N2 application, 5.97% greater than at N0 ($P > 0.05$). Leaf total nitrogen content of receiver plants in CK and Sm treatments showed a decrease-ascending-decreasing pattern with the increase of N application (Fig. 2F), and both of them reached the maximum value at N4 treatment, which was 17.05% and 3.54% higher than that of N0, respectively, but none of the differences was significant ($P > 0.05$). Although the total nitrogen content of stems did not show a significant difference compared to the CK group ($P > 0.05$), the total nitrogen content of leaves was significantly higher in the groups with N0 and N2 application ratios compared to the CK ($P < 0.05$). Additionally, the administration of Sm increased the total nitrogen content of seedlings that were both donors and receivers at the same nitrogen application ratio, with a significant difference observed at the N2 application ($P < 0.05$). Interaction effects between the factors mycorrhizal inoculation (Sm, CK) and nitrogen application levels (N0, N2, N4, N6) were extremely significant for both donor and receiver leaves (Table 5, $P<0.001$). The effect of mycorrhizal inoculation on nitrogen allocation to leaves is dependent on the nitrogen level, with the optimal benefit observed at the intermediate N4 level.

Total phosphorus in roots, stems, and leaves of donor/receiver of *P. massoniana* seedlings

Under CK treatment, donor/receiver seedlings' total phosphorus content changed in a fall-rise-fall manner as the ratio of nitrogen application increased (Fig. 3A, B). It peaked at the N4 application, when it was 60.27% and 131.45% higher than N0, respectively ($P < 0.05$). Total phosphorus in donor seedling stems grew initially, then dropped as nitrogen application increased. It reached its maximum value at N4 application, which was 171.72% greater than N0 application (Fig. 3C, D, $P < 0.05$). Total phosphorus in receiver seedling stems first declined, then grew as nitrogen treatment ratio increased (Fig. 3D). Total phosphorus reached its maximum value at N6 application, 9.10% more than that of N0 ($P <$

0.05). In the donor/receiver seedlings, overall total phosphorus increased initially, then decreased as the nitrogen application levels increased (Fig. 3E, F). Total phosphorus reached its maximum value under N4 application, which was 284.68% and 269.51% higher than that of N0, respectively ($P < 0.05$).

Under Sm treatment, the total phosphorus in the receiver's roots and stems and the donor seedling's roots and stems changed fall-rise-fall as the nitrogen application levels increased (Fig. 3), peaking at the N4 application. By 0.36% ($P > 0.05$), 41.42% ($P < 0.05$), 39.99% ($P < 0.05$), 32.35% ($P < 0.05$), and 49.31% ($P < 0.05$), it was more than N0, in that order. When the levels of nitrogen treatment was raised, the total phosphorus in the receiver seedlings' leaves declined initially, increased, and reached its maximum value at the N6 application (Fig. 3F), which was 29.91% higher than that of N0 ($P < 0.05$). Overall, at the N4 treatment, the seedlings inoculated with Sm exhibited the greatest effect on total phosphorus uptake and utilization. Highly significant interactions were observed for phosphorus content in the roots, stems, and leaves of receiver plants (Table 5, $P<0.001$), as well as in the stems and leaves of donor plants ($P<0.001$). This indicates that the CMN's role in enhancing phosphorus acquisition is also modulated by soil nitrogen availability.

Total potassium in roots, stems, and leaves of donor/receiver of *P. massoniana* seedlings

The total potassium of donor seedlings under CK treatment changed in a fall-rise-fall pattern as the levels of nitrogen application increased. It peaked at the application of N4, which was 11.55% greater than that of N0 (Fig. 4A, B, $P < 0.05$). Total potassium in the receiver seedlings' roots first rose, then fell as the amount of nitrogen applied increased. It reached its maximum value when N4 was applied, 18.53% more than N0 (Fig. 4C, D). With an increase in the nitrogen application levels, the total potassium of the donor and receiver seedlings changed overall in a fall-rise-fall pattern. It peaked at the N4 application, when it was 36.06% and 49.76% greater than the N0 application, respectively (Fig. 4E, F, $P < 0.05$).

Total potassium in the donor seedlings' roots, stems, and leaves increased initially under the Sm treatment, then decreased as the levels of nitrogen application increased (Fig. 4). The highest value was reached at the N4 application, which was 9.78% ($P < 0.05$), 9.02% ($P > 0.05$), and 28.46% ($P < 0.05$) higher than that of N0, among other values. When the levels of nitrogen application was increased, the total potassium in the receiver seedlings' roots and leaves changed in a fall-rise-fall pattern (Fig. 4B, F). In contrast, the total potassium in the stems first increased, then decreased (Fig. 4D), and finally reached its maximum at the N4 application. In comparison to N0, the total potassium content of the roots, stems, and leaves was 10.71% ($P < 0.05$), 64.29% ($P < 0.05$), and 21.04% ($P > 0.05$), respectively. Sm inoculation increased the total potassium in the roots, stems, and leaves of donor seedlings when compared to CK, and this difference was significant at the N4 application ($P < 0.05$). However, there was no significant difference in the total potassium in the roots, stems, and leaves of receiver seedlings inoculated with Sm when compared to CK ($P > 0.05$). A highly significant interaction was found for potassium in the stems of receiver plants (Table 5, $P<0.001$).

Nitrogen uptake and allocation in roots, stems, and leaves of donor/receiver of *P. massoniana* seedlings and mycelial contribution ratio

Table 6, $P < 0.05$, indicates that during the CK treatment, the nitrogen content of the donor's roots and leaves increased initially, then decreased as nitrogen application increased. Meanwhile, the stems exhibited a fall-rise-fall pattern, peaking at N4 application. The nitrogen content of the donor's roots, stems, and leaves was 1.90 times, 1.93 times, and 5.54 times that of N0. The receiver's roots and leaves under CK treatment exhibited a fall-rise-fall pattern as nitrogen application increased, whereas the stems increased initially before decreasing and reaching a maximum at N4 application. The nitrogen content of the roots, stems, and leaves was 1.55-, 1.94-, and 3.73-fold greater than that of N0, respectively ($P < 0.05$). Under the Sm treatment, the donor's stems and leaves absorbed more nitrogen as the amount of nitrogen applied rose. At the N6 application, the maximum amount of nitrogen absorbed was 2.59 times greater

than at the N0 application ($P < 0.05$). The uptake of nitrogen by stems and leaves had an initial increase, followed by a reduction as the nitrogen application levels was raised. The greatest value was attained at the N4 application, where the uptake of nitrogen was 2.23 and 1.93 times higher than that of N0 ($P < 0.05$). When the levels of nitrogen administration was increased, the receiver seedlings' roots and leaves absorbed less nitrogen, yet the stem initially expanded and later dropped. At the N4 treatment, the maximal nitrogen content by the roots, stems, and leaves was 1.41-fold ($P > 0.05$), 2.14-fold ($P < 0.05$), and 2.24-fold ($P < 0.05$), respectively. When donor/receiver seedlings were inoculated with Sm at the same nitrogen transfer ratio as CK, the roots, stems, and leaves of the former could absorb more nitrogen.

The mycelial contribution ratio was affected differently by varied nitrogen applications during Sm treatment (Table 7). With an increase in nitrogen treatment, donor seedlings' mycelial contribution ratio changed in a rise-fall-rise pattern. The mycelial contribution ratio peaked at the N2 application, when it was 27.47% greater than at N0, N6 followed suit, with an 18.38% higher ratio than at N0 ($P > 0.05$). Mycelial contribution ratio was the lowest with N4 application, and N0 was 1.29 times more than N4 ($P < 0.05$). The mycelial contribution ratio in the receiver seedlings gradually decreased as the nitrogen application increased; it peaked at N0 and decreased at N6, but there was no discernible difference in the mycelium contribution ratio between the various nitrogen application levels ($P > 0.05$).

Effects of common mycorrhizal network on ^{15}N acquisition, ^{15}N recovery, transfer ratio of donor/receiver of *P. massoniana* seedlings under different nitrogen application levels

Under CK treatment, donor seedlings' roots absorbed more ^{15}N initially, but as the levels of nitrogen application rose, it declined and finally peaked at N4 application. With an increase in nitrogen treatment levels, stem and leaf ^{15}N content increased and peaked at N6 application (Table 8). However, when the levels of nitrogen supply increased, the content reduced and peaked at the application of N4. With an increase of nitrogen application levels, stems acquisition more ^{15}N; this acquired peaked at N6 application. Under Sm treatment, the ratio at which donor seedlings' roots, stems, and leaves ^{15}N content increased as nitrogen was applied, peaking at N6 application. As the levels of nitrogen application increased, the ^{15}N acquisition of the receiver seedlings' stems changed in a rise-fall-rise pattern, peaking at the N6 application; in contrast, the ^{15}N acquisition of the leaves increased initially, then declined, peaking at the N4 application. With the exception of N2, Sm seedlings acquired more ^{15}N in their roots, stems, and leaves than CK seedlings did.

The donor/receiver seedlings under CK treatment have the highest ^{15}N recovery at the N2 application. With a significant difference from other conditions (Table 9, $P < 0.05$), the donor/receiver seedling's ^{15}N recovery is maximum with Sm treatment at the N4 application. However, there was no significant difference ($P < 0.05$) between the ^{15}N transfer ratios of Sm and CK when nitrogen levels were N4 and N6. N2 application under CK treatment had a greater ^{15}N transfer ratio than N4 and N6, whereas N4 application with Sm treatment had a higher ^{15}N transfer ratio than N2 and N6 ($P > 0.05$).

Effect of common mycorrhizal network on the ^{15}N allocation of donor/receiver of *P. massoniana* seedlings under different nitrogen application levels

Figure 5 shows that the ^{15}N allocation of the CK and Sm treatments were root > stem > leaf at the N2 and N6 application in the donor plant. The ^{15}N allocation was slightly higher in the root (72.15%) under the treatment of N2-CK.

The ^{15}N allocation of the CK and Sm treatments were root > stem > leaf at the N2 application in the receiver plant. Under CK treatment, the maximum of ^{15}N allocation at N4 application was 64.17% in roots, followed by 20.52% in

leaves; under Sm treatment, leaves (52.13%) were greater than roots (35.85%) and stems (12.02%). When N6 was applied, the stems had the highest ^{15}N allocation under the CK treatment (39.65%), followed by the roots (33.92%), and the roots had the highest ^{15}N allocation under the Sm treatment (73.59%), followed by the leaves (20.63%).

Principal Component and Correlation Analysis of Donor and Receiver Seedlings

Principal component analysis (PCA) was performed on 26 physiological and nutrient-related traits in donor and receiver seedlings of *P. massoniana* under different nitrogen (N) treatments (N0, N2, N4, N6) and inoculation status (Sm: inoculated; CK: non-inoculated).

For donor seedlings, the first three principal components (PCs) explained 80.09% of the total variance. PC1 (50.37%) was strongly associated with biomass, nitrogen uptake, and nitrogen allocation. PC2 (18.58%) correlated with potassium content and root ^{15}N uptake, while PC3 (11.15%) was linked to mycorrhizal colonization and phosphorus content（Table 10）.

PCA score plots revealed clear separation between Sm and CK treatments, with Sm samples clustering in positive regions of PC1 and PC2, indicating enhanced growth and nutrient uptake. Among N treatments, Sm-N4 showed the strongest association with stem biomass, nitrogen uptake, and mycorrhizal colonization, and achieved the highest comprehensive evaluation score (Table 11,13), identifying it as the optimal treatment.

For receiver seedlings, the first four PCs accounted for 78.59% of the variance. PC1 (46.00%) was positively correlated with biomass, nitrogen and ^{15}N uptake, and colonization ratio. PC2 (14.12%) was associated with root-shoot ratio and phosphorus content, while PC3 (10.56%) and PC4 (7.91%) related to ^{15}N transfer and allocation (Table 12).

Sm-treated seedlings clustered in positive regions of PC1 and PC2, confirming improved growth and nutrient assimilation. Sm-N4 and Sm-N6 showed the most favorable performance in biomass and nutrient uptake, with Sm-N4 also excelling in nutrient transfer efficiency.

Correlation analysis indicated strong positive relationships (Fig. S2, $P < 0.01$) between nutrient contents (K, N, P) and biomass traits in both donor and receiver plants. Mycorrhizal colonization was positively correlated with biomass and nitrogen uptake. Notably, ^{15}N transfer ratio was negatively correlated with ^{15}N uptake in donors but positively in receivers.

Discussion

Effects of ectomycorrhizal fungi and nitrogen application on the growth of donor/receiver of P. massoniana seedlings

Plant development and the distribution pattern of nutrients are significantly influenced by the ratio at which nitrogen is applied (Duan et al. 2022). In times of nitrogen scarcity, plants will allocate more photosynthates to the roots, improving the roots' ability to absorb nitrogen. Higher hyphal density under N4 treatment (44.48% infection ratio in donors) likely enhanced nitrogen transfer efficiency by expanding the contact area between fungal hyphae and root systems, as observed in *Pisolithus* associated networks (Simard et al., 2012). Reduced transfer ratios at N6 may reflect hyphal carbon allocation trade-offs under excessive nitrogen (Pena et al., 2023). Under the same inoculation treatment, nitrogen application promoted the growth of both donor and receiver *P. massoniana* seedlings in terms of height and diameter of the ground (Fig. S3), with the best performance at a nitrogen application ratio of N4 (4 g/L). In general, stem and leaf growth outpaced root growth, thus as nitrogen treatment increased, the root-shoot ratio decreased (Li et al. 2023). Additionally, the study discovered that when nitrogen delivery increased, the root-shoot ratio of donor seedlings receiving CK treatment progressively dropped. Furthermore, in line with the findings of Wang et al.

(2018), the biomass of donor/receiver seedlings grew initially before declining as nitrogen treatment increased. In line with other research (Guo et al. 2021; Zhou et al. 2020; Tu et al. 2022), inoculation may encourage the biomass accumulation of *P. massoniana* seedlings at the same nitrogen delivery ratio. The best performance was attained at the N4.

Effects of ectomycorrhizal fungi and nitrogen application on nutrient acquisition of donor/receiver of P. massoniana seedlings

Increased total nitrogen content results from fertilizing the seedlings, which aids in fostering their growth and bolstering their resilience (Zhu et al. 2011). According to Wang et al. (2023) and Fabiańska et al. (2020), ECM fungi have the ability to secrete phosphatase, increase soil phosphatase activity, improve soil phosphorus acquisition and transport capacity, and enhance phosphorus acquisition by host plants. These actions indirectly promote nutrient acquisition. Furthermore, ECM fungi's mycelium spreads throughout the soil, encouraging host plants to acquire nutrients and raising their levels of potassium, phosphate, and nitrogen (Xiao et al. 2023; Ortas et al. 2022). Our findings demonstrate that Sm inoculation significantly increased the concentrations of total nitrogen, potassium, and phosphorus in roots, leaves, and the stem. The nutrient demand of stems inoculated with Sm has likely reached saturation, as evidenced by the lower content of total potassium and total nitrogen in stems compared to CK. These results confirm that there are indeed significant interactive effects between the nitrogen application gradient and the mycorrhizal inoculation treatment. The impact of the mixed fungal consortium (Sm) on nutrient content (particularly N and P) is not consistent across all nitrogen levels; it is contingent upon the specific nitrogen availability. With the N4 application, there was an improvement in the acquisition of total phosphorus, total potassium, and total nitrogen.

This study suggests that inoculation may improve nitrogen content, as the roots, stems, and leaves of seedlings inoculated with Sm acquired more nitrogen than CK seedlings under identical nitrogen administration. Apart from the maximum uptake of nitrogen by the roots at N6, the maximum acquire of nitrogen by the donor/receiver stems and leaves happened at N4, and then at N6. While ECM fungi can supply nitrogen to their host plants, when nitrogen is scarce, they also store different amounts of nitrogen to suit their own requirements (Benavent et al. 2019; Gu et al. 2023). Additionally, the study discovered that donor/receiver seedlings acquired very little ^{15}N during N0 treatment, and that this may have been due to soil ^{15}N. When nitrogen application increased during the Sm treatment, the amount of ^{15}N acquired by the receiver's roots, donor roots, stems, and leaves grew progressively. The maximum amounts of ^{15}N acquired by the receiver's stems and leaves were N2 and N4, respectively.

Effects of ectomycorrhizal fungi and nitrogen application on ^{15}N recovery, transfer ratio and allocation ratios of donor/receiver of P. massoniana seedlings

One crucial indicator of a plant's capacity to acquire and use nitrogen is its nitrogen use efficiency. (Ghadirnezhad et al. 2024). The type of nitrogen present in the growth medium had an impact on plants' ability to utilize nitrogen (Martins et al. 2022). Higher plants predominantly utilize ammonium (NH_4^+) and nitratio (NO_3^-) as primary inorganic nitrogen sources, while also acquiring soluble organic nitrogen compounds (e.g., amino acids) as important supplementary forms. The bioavailability of nitrogen varies across plant species and environmental conditions, with nitrogen availability remaining a key factor influencing plant growth (Ma et al.2024; Deb et al. 2023). The fact that the study's donor/receiver ^{15}N consumption ratio under N0 was zero suggests that the meager amount of ^{15}N present in the soil was insufficient to meet the needs of the plants. The reason for the high transfer ratio could be that the donor and receiver seedlings have similar acquisition capacities of the natural ^{15}N content in the soil, meaning that there is little variation in their ^{15}N content.

The results demonstrated a distinct difference in ^{15}N allocation between donor and receiver seedlings across nitrogen treatments. The ^{15}N allocation [%] in the donor seedlings under Sm treatment was root > leaf > stem at N2 and N6, and leaf > root > stem at N0 and N4. In the receiver seedlings, at N0 and N6, root > stem > leaf; at N2 treatment, root > stem > leaf; and at N4 treatment, leaf > root > stem was the ^{15}N allocation [%] under Sm treatment. According to Qiang et al. (2023) increased N allocation [%] in leaves promotes the synthesis of photosynthetic pigments, which in turn increases seedlings' capability for photosynthetic processes. Additionally, it was discovered that the *P. massoniana* seedlings' mycorrhization reduced the roots' distribution ratios of N, P, and K (Sun et al. 2023). The superior performance of CMNs under N4 aligns with the 'carbon-for-nitrogen' trade-off model (Pena et al., 2023), where moderate N availability maximizes fungal carbon allocation to hyphal networks. Unlike arbuscular mycorrhizae (AMF), which prioritize phosphorus transfer, ECM fungi in *P. massoniana* likely optimize nitrogen redistribution to meet host carbon export demands, explaining the leaf-biased ^{15}N allocation at N4.

Effects of ectomycorrhizal and mycelial networks on plants

The role of ectomycorrhizal fungi (EMF) in enhancing plant nutrient acquisition is a major focus in plant-microbe interaction research. Recent studies by Pena et al. (2023) and Chen et al. (2021) highlight the impact of ectomycorrhizal (ECM) symbiosis on plant nutrient uptake and growth, with Chen's work further elucidating the role of various fungal species in enhancing nutrient enrichment through ECM. Our research indicates that the ^{15}N acquisition amount and distribution ratio of receptor plant roots show significant positive correlations ($P < 0.01$) with the biomass and N absorption of roots, stems, and leaves; Critically, the experimental evidence obtained through our compartmentalized ^{15}N tracing system directly reveals interplant, hyphal-mediated nitrogen translocation. This distinguishes it from the conventional role of mycorrhizal fungi in nutrient acquisition for a single host from the soil. Additionally, the mycorrhizal colonization ratio demonstratios significant positive correlations ($P < 0.01$) with the biomass of roots and stems, and N absorption of roots, stems, and leaves. This suggests that the biomass and N acquisition of donor and receptor plants increase with the rise in ectomycorrhizal colonization ratio. Related studies have also shown that ECM fungi are evidently essential to the nitrogen nutrition of seedlings, as they can boost the host plants' ability to acquire nutrients (Plett et al. 2023), and ectomycorrhiza can improve the efficiency of N use while encouraging the growth of seedlings (Sun et al. 2022).

In the field of plant-microbe interactions, the complex network of mycorrhizal associations has become a pivotal area of research. Some studies highlight the crucial function of mycorrhizal networks in mediating the dynamic exchange of resources within ecosystems, thereby enhancing the resilience and stability of plant communities (Tedersoo et al., 2020; Simard et al., 2012). Moreover, this research also revealed a significant negative correlation ($P < 0.01$) between transfer ratio and ^{15}N acquisition and distribution in roots, stems, and leaves of donor plants. In receiver plants, transfer ratio showed a significant positive correlation ($P < 0.01$) with ^{15}N acquisition in stems and leaves, ^{15}N distribution and biomass. This means that the ^{15}N content in the donor plant decreased with increasing transfer ratio, while the ^{15}N content in the receiver plant increased with increasing transfer ratio. And, this indicates that a portion of the ^{15}N from the roots, stems and leaves of the donor plant was transferred to the receiver plant through the mycelial network as the mycelial network was established.

Ecological implications for common mycorrhizal network-mediated nitrogen translocation

This study demonstrates that nutrient enrichment in receiver plants originates mainly from interplant nutrient transfer, providing evidence for the role of common mycorrhizal networks (CMNs) in this process. First, the use of a sterile growth medium and the unilateral inoculation solely in compartment A ensured that the extraradical mycelium

accessing the ^{15}N label in the donor chamber and subsequently colonizing the receiver chamber was physiologically and functionally integrated with the donor plant's root system. The carbon sustaining this mycelial network was primarily supplied by the donor plant, establishing a system where reciprocal resource exchange—fungal-delivered nitrogen for plant-derived carbon—is the fundamental driver, making the direct bypass scenario less likely. Second, the multi-layered physical isolation, comprising a clay layer and mesh barriers between the ^{15}N labeling site (beneath compartment A) and the receiver chamber (D), rendered it highly improbable for the receiver plant's mycorrhizae to directly and substantially access the ^{15}N source without the prior establishment of a hyphal network originating from the donor. The findings of this study demonstrate that at intermediate nitrogen levels, the common mycorrhizal network (CMN) established by mixed ectomycorrhizal fungal consortia most effectively facilitates nitrogen translocation among *P. massoniana* seedlings, with preferential allocation to photosynthetic organs such as leaves. This discovery provides important insights for understanding and utilizing mycorrhizal networks in ecological restoration. In degraded forest ecosystems where nitrogen is generally limited, establishing CMNs through inoculation with appropriate native ectomycorrhizal fungi can serve as an effective biological strategy to optimize internal nutrient resource allocation within plant communities, alleviate soil nitrogen constraints, and consequently promote the establishment and growth of pioneer species like *P. massoniana*, thereby accelerating the recovery of forest ecosystems.

Conclusions

(1) Hyphal network-mediated nitrogen translocation: The experimental design, featuring a sterile growth medium and physical barriers, effectively restricted the primary pathway of nitrogen delivery to the receiver seedling (compartment D) to the pre-established CMN originating from the donor (compartment A). The significant ^{15}N enrichment detected in receiver seedlings, coupled with the optimal allocation of 50.59% of absorbed nitrogen to photosynthetic organs at N4, demonstrates a efficient interplant nitrogen translocation facilitated by the fungal network, balancing metabolic costs and plant demand. (2) Threshold-driven efficiency: The N4 treatment maximizes biomass (2.62-fold root growth) and nutrient uptake (284% phosphorus increase), outperforming higher nitrogen levels (N6) where carbon allocation trade-offs reduce hyphal efficacy.

(3) Field relevance: Hyphal-mediated nitrogen translocation via CMNs mitigates soil nitrogen limitation in degraded *P. massoniana* ecosystems, proposing mixed fungal consortia as a sustainable reforestation tool.

(4) These findings redefine ECM-mediated nutrient dynamics, emphasizing fungal consortia and nitrogen thresholds for ecological restoration.

Declarations

Author Contributions: Y.W conceived and designed the experiment; T.W.T.L and M.Z analyzed the data and wrote the manuscript; Y.S performed the experiments and sorted out data; Z.Y.Z and W.Z revise the manuscript. All authors have revised the manuscript and approved the version submitted.

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Tables

Table 1 Soil physicochemical properties

pH	Total nitrogen (g/kg)	Alkaline nitrogen (mg/kg)	Total phosphorus (g/kg)	Available phosphorus (mg/kg)	Total potassium (g/kg)	Available potassium (mg/kg)	Organic matter (g/kg)
4.60	0.14	87.50	0.32	28.78	10.18	134.36	13.94

Table 2 Mycorrhizal colonization of donor and receiver *Pinus massoniana* seedlings under different nitrogen levels

	CK		Sm	
Treatment	Mycorrhizal colonization of donor plant (%)	Mycorrhizal colonization of receiver plant (%)	Mycorrhizal colonization of donor plant (%)	Mycorrhizal colonization of receiver plant (%)
N0	0	0	31.61±3.27 c	26.17±0.96 c
N2	0	0	38.42±0.81 b	31.10±1.83 b
N4	0	0	44.48±1.16 a	43.42±1.46 a
N6	0	0	46.69±1.23 a	41.33±2.61 a

N0、N2、N4、N6 were 0、2、4、6 g/L, respectively, nitrogen levels treatment; CK was not inoculated, Sm was inoculated; The data were mean ± standard deviation (n=3); Different lowercase letters indicate significant difference among different nitrogen treatment of the same strain ($P < 0.05$).

Table 3 The biomass of *Pinus massoniana* seedling's donor

Treatment	N level (g/L)	Biomass (g)			Root-shoot ratio	RMD (%)
		Root	Stem	Leaf		
CK	N0	0.15±0.01 Ab	0.06±0.02 Ab	0.14±0.05 Ab	0.80±0.26 Aa	-
	N2	0.15±0.02 Ab	0.07±0.01 Bb	0.14±0.01 Bb	0.72±0.12 Aa	-
	N4	0.21±0.03 Aa	0.19±0.04 Ba	0.37±0.02 Ba	0.38±0.03 Ab	-
	N6	0.13±0.00 Bb	0.11±0.05 Ab	0.29±0.08 Ba	0.37±0.13 Ab	-
Sm	N0	0.15±0.05 Ac	0.12±0.02 Ac	0.31±0.12 Ac	0.34±0.02 Ba	32.36±29.25 a
	N2	0.22±0.02 Ab	0.16±0.02 Ac	0.49±0.05 Ab	0.35±0.05 Aa	57.80±3.24 a
	N4	0.25±0.03 Ab	0.30±0.02 Aa	0.80±0.09 Aa	0.23±0.05 Bb	41.99±8.25 a
	N6	0.39±0.00 Aa	0.24±0.04 Ab	0.76±0.06 Aa	0.39±0.04 Aa	61.86±10.26 a

N0、N2、N4、N6 were 0、2、4、6 g/L, respectively, nitrogen levels treatment; CK was not inoculated, Sm was inoculated; Different lowercase letters indicate significant differences among nitrogen treatments within each inoculation status, whereas different capital letters indicate significant differences between inoculation statuses within each nitrogen treatment ($P < 0.05$).

Table 4 The biomass of *Pinus massoniana* seedling's receiver

Treatment	N level (g/L)	Biomass (g)			Root-shoot ratio	RMD (%)
		Root	Stem	Leaf		
CK	N0	0.09±0.01 Bb	0.07±0.01 Ac	0.13±0.02 Bc	0.49±0.03 Aab	-
	N2	0.11±0.02 Aab	0.07±0.02 Ac	0.10±0.03 Bc	0.65±0.23 Aa	-
	N4	0.13±0.00 Aa	0.16±0.01 Ba	0.40±0.02 Aa	0.24±0.01 Ac	-
	N6	0.13±0.01 Ba	0.11±0.02 Bb	0.22±0.02 Bb	0.41±0.02 Abc	-
Sm	N0	0.21±0.01 Aab	0.13±0.03 Ab	0.31±0.04 Ab	0.47±0.05 Aa	55.31±9.10 a
	N2	0.16±0.02 Ab	0.15±0.02 Ab	0.27±0.04 Ab	0.39±0.06 Aa	50.91±6.90 a
	N4	0.27±0.05 Aa	0.31±0.00 Aa	0.67±0.27 Aa	0.29±0.13 Aa	42.54±10.81 a
	N6	0.23±0.03 Aab	0.17±0.04 Ab	0.44±0.08 Aab	0.38±0.10 Aa	44.45±1.57 a

N0、N2、N4、N6 were 0、2、4、6 g/L, respectively, nitrogen levels treatment; CK was not inoculated, Sm was inoculated; Different lowercase letters indicate significant differences among nitrogen treatments within each inoculation status, whereas different capital letters indicate significant differences between inoculation statuses within each nitrogen treatment ($P < 0.05$).

Table 5 The interaction effects between the factors Mycorrhizal inoculation (Sm, CK) and Nitrogen application rate (N0, N2, N4, N6) on the nutrient content in root, stem and leaf of donor and receiver *Pinus massoniana* seedlings

Mycorrhizal * Nitrogen	Sum of Squares	df	MeanSquare	*F*	*P*
Donor-root-N	4.089	3	1.363	2.147	0.134
Receiver-root-N	2.808	3	0.936	4.197	*
Donor-stem-N	1.140	3	0.380	1.855	0.178
Receiver-stem-N	1.053	3	0.351	4.023	*
Donor-leaf-N	15.459	3	5.153	88.959	***
Receiver-leaf-N	2.533	3	0.844	8.749	***
Donor-root-P	0.435	3	0.145	3.175	0.053
Receiver-root-P	0.092	3	0.031	12.880	***
Donor-stem-P	0.414	3	0.138	115.208	***
Receiver-stem-P	0.328	3	0.109	39.880	***
Donor-leaf-P	1.202	3	0.401	257.649	***
Receiver-leaf-P	0.965	3	0.322	206.782	***
Donor-root-K	0.168	3	0.056	0.244	0.865
Receiver-root-K	1.490	3	0.497	2.331	0.113
Donor-stem-K	3.140	3	1.047	4.009	*
Receiver-stem-K	11.303	3	3.768	23.229	***
Donor-leaf-K	7.120	3	2.373	2.826	0.072
Receiver-leaf-K	8.465	3	2.822	1.745	0.198

Significance levels are indicated as follows: *P* < 0.05, ** P* < 0.01, *** P* < 0.001. Nitrogen (N), Phosphorus (P), Potassium (K).

Table 6 The nitrogen acquisition and allocation in the root, stem and leaf of *Pinus massoniana* seedling's donor/receiver

		N (mg)					
N level	Inoculation treatment	Donor			Receiver		
(g/L)		Root	Stem	Leaf	Root	Stem	Leaf
N0	CK	0.89±0.06 Ab	0.35±0.16 Ab	0.36±0.10Ac	0.63±0.12 Bb	0.21±0.06 Ab	0.51±0.04Bc
	Sm	1.33±0.44 Ac	0.61±0.07 Ab	1.85±0.61Ab	1.45±0.09 Aab	0.41±0.09 Ab	1.97±0.29Ab
N2	CK	0.95±0.22 Bb	0.29±0.04 Bb	0.50±0.02Bc	0.59±0.11 Ab	0.29±0.09 Aab	0.37±0.13Bc
	Sm	1.86±0.14 Abc	0.65±0.14 Ab	3.16±0.51 Aa	1.18±0.19 Ab	0.48±0.05 Ab	1.61±0.19Ab
N4	CK	1.69±0.39 Aa	0.68±0.11 Ba	1.98±0.31 Ba	0.98±0.07 Ba	0.40±0.04 Ba	1.90±0.14 Aa
	Sm	2.17±0.55 Ab	1.18±0.08 Aa	4.12±0.58 Aa	2.04±0.38 Aa	0.88±0.20 Aa	4.39±2.03 Aa
N6	CK	0.98±0.02 Bb	0.54±0.22 Aab	1.27±0.40 Ab	0.89±0.15 Aa	0.36±0.06 Aa	0.98±0.01 Bb
	Sm	3.43±0.08 Aa	1.05±0.33 Aa	3.57±0.87 Aa	1.62±0.58 Aab	0.51±0.11 Ab	2.32±0.54 Ab

N0、N2、N4、N6 were 0、2、4、6 g/L, respectively, nitrogen levels treatment; CK was not inoculated, Sm was inoculated; Different lowercase letters indicate significant differences among nitrogen treatments within each inoculation status, whereas different capital letters indicate significant differences between inoculation statuses within each nitrogen treatment ($P < 0.05$).

Table 7 Contribution ratio of ectomycorrhizal fungal hyphae under different nitrogen treatment

	Inoculation treatment	Donor	Receiver
N level (g/L)		Mycelial contribution ratio（%）	Mycelial contribution ratio（%）
N0	CK	-	-
	Sm	54.27±17.33 ab	64.48±5.85 a
N2	CK	-	-
	Sm	69.17±5.21 a	61.75±5.97 a
N4	CK	-	-
	Sm	41.94±5.90 c	53.2±12.29 a
N6	CK	-	-
	Sm	64.24±11.81 a	49.28±4.50 a

N0、N2、N4、N6 were 0、2、4、6 g/L, respectively, nitrogen levels treatment; CK was not inoculated, Sm was inoculated; Different lowercase letters indicate significant differences among nitrogen treatments within each

inoculation status, whereas different capital letters indicate significant differences between inoculation statuses within each nitrogen treatment ($P < 0.05$).

Table 8 The ^{15}N acquisition and allocation content in the root, stem and leaf of *Pinus massoniana* seedling's donor/receiver

		^{15}N(μg)					
N level	Inoculation treatment	Donor			Receiver		
(g/L)		Root	Stem	Leaf Total	Root	Stem	Leaf Total
N0	CK	0.003±0.00 Ab	0.001±0.00 Ab	0.000±0.00 Bb 0.00	0.002±0.00 Bd	0.000±0.00 Ac	0.001±0.00 Ac 0.00
	Sm	0.006±0.00 Ad	0.002±0.00Ab	0.001±0.00 Ab 0.00	0.001±0.00 Ab	0.001±0.00 Ac	0.002±0.00 Ab 0.00
N2	CK	175.27±40.91 Aa	23.90±3.12 Ab	43.77±1.33 Bb 242.94	2.50±0.46 Aa	0.40±0.10 Bb	0.30±0.10 Ab 3.20
	Sm	113.83±8.57 Ac	21.80±4.78 Ab	84.40±13.62 Ab 220.03	1.23±0.15 Ab	1.23±0.15 Aa	0.20±0.00 Ab 2.66
N4	CK	230.67±53.33 Aa	56.93±9.38 Ba	159.97±24.69 Ba 447.57	1.97±0.15 Bb	0.47±0.06 Bb	0.63±0.06 Aa 3.07
	Sm	260.60±65.52 Ab	85.63±5.78 Aa	283.63±39.86 Aa 629.86	3.37±0.60 Aa	1.13±0.23 Aa	4.90±2.25 Aa 9.40
N6	CK	221.57±4.60 Ba	71.40±29.75 Aa	161.50±50.64 Aa 454.47	0.77±0.15 Bc	0.90±0.17 Aa	0.60±0.00 Ba 2.27
	Sm	466.53±10.35 Aa	92.57±29.05 Aa	328.37±79.92 Aa 887.47	5.10±1.80 Aa	0.40±0.10 Bb	1.43±0.31 Ab 6.93

N0、N2、N4、N6 were 0、2、4、6 g/L, respectively, nitrogen levels treatment; CK was not inoculated, Sm was inoculated; Different lowercase letters indicate significant differences among nitrogen treatments within each inoculation status, whereas different capital letters indicate significant differences between inoculation statuses within each nitrogen treatment ($P < 0.05$).

Table 9 The ^{15}N recovery and transfer ratio of *P. massoniana* seedling's donor/receiver

N level (g/L)	Inoculation treatment	Donor	Receiver	
		^{15}N recovery (%)	^{15}N recovery (%)	Transfer ratio (%)
N0	CK	0.00±0.00 c	0.00±0.00 d	0.00±0.00 Aa
	Sm	0.00±0.00 c	0.00±0.00 c	0.00±0.00 Aa
N2	CK	2.02±0.36 Aa	0.03±0.00 Aa	1.31±0.18 Ab
	Sm	1.83±0.10 Ab	0.02±0.00 Ab	1.17±0.14 Ab
N4	CK	1.86±0.35 Ba	0.01±0.00 Bb	0.68±0.08 Ab
	Sm	2.62±0.23 Aa	0.04±0.01 Aa	1.47±0.42 Ab
N6	CK	1.26±0.22 Ab	0.01±0.00 Bc	0.50±0.13 Ab
	Sm	2.47±0.33 Aa	0.02±0.00 Ab	0.77±0.28 Ab

N0、N2、N4、N6 were 0、2、4、6 g/L, respectively, nitrogen levels treatment; CK was not inoculated, Sm was inoculated; Different lowercase letters indicate significant differences among nitrogen treatments within each inoculation status, whereas different capital letters indicate significant differences between inoculation statuses within each nitrogen treatment ($P < 0.05$).

Table 10 Principal component analysis of indexes of donor seedlings of P. massoniana treated with different nitrogen concentrations

Number	Initial feature root			Feature roots		
	Eigenvalue	Contribution ratio/%	Cumulative contribution ratio/%	Eigenvalue	Contribution ratio/%	Cumulative contribution ratio/%
1	13.095	50.365	50.365	13.095	50.365	50.365
2	4.830	18.576	68.941	4.830	18.576	68.941
3	2.898	11.147	80.088	2.898	11.147	80.088
4	1.381	5.310	85.398			
5	1.256	4.831	90.229			
6	0.831	3.197	93.425			
7	0.471	1.813	95.238			
8	0.314	1.207	96.445			
9	0.263	1.013	97.457			
10	0.227	0.874	98.331			
11	0.173	0.666	98.997			
12	0.081	0.31	99.307			
13	0.067	0.258	99.565			

Table 11 Scores of different nitrogen concentration treatment factors of donor seedlings of *P. massoniana*

	F1	F2	F3	F	Order
Sm-N4	4.9237	1.4157	-0.7426	5.5969	1
Sm-N6	5.0581	-2.2912	2.6259	5.3927	2
Sm-N2	0.9149	2.0572	0.6134	3.5855	3
Sm-N0	-1.7264	3.6711	0.3444	2.2892	4
CK-N4	1.1312	-0.0253	-3.0236	-1.9177	5
CK-N0	-5.6890	0.2430	1.8541	-3.5919	6
CK-N6	-1.0463	-2.6558	-0.8741	-4.5762	7
CK-N2	-3.5663	-2.4147	-0.7974	-6.7785	8

N0、N2、N4、N6 were 0、2、4、6 g/L, respectively, nitrogen levels treatment; CK was not inoculated, Sm was inoculated; F1: First principal component factor scores; F2: Second principal component factor scores; F3: Third principal component factor scores; F: Total principal component factor scores. Order: Factor score ordering.

Table 12 Principal component analysis of indexes of receiver seedlings of *P. massoniana* treated with different nitrogen concentrations

Number	Initial feature root			Feature roots		
	Eigenvalue	Contribution ratio/%	Cumulative contribution ratio/%	Eigenvalue	Contribution ratio/%	Cumulative contribution ratio/%
1	11.961	46.002	46.002	11.961	46.002	46.002
2	3.672	14.122	60.124	3.672	14.122	60.124
3	2.744	10.555	70.680	2.745	10.555	70.680
4	2.056	7.906	78.586	2.056	7.906	78.586
5	1.687	6.487	85.073			
6	1.049	4.034	89.107			
7	0.794	3.055	92.162			
8	0.684	2.631	94.793			
9	0.447	1.718	96.511			
10	0.328	1.262	97.773			
11	0.218	0.839	98.612			
12	0.145	0.556	99.168			
13	0.067	0.258	99.565			

Table 13 Scores of different nitrogen concentration treatment factors of receiver seedlings of *P. massoniana*

	F1	F2	F3	F4	F	Order
Sm-N4	7.088767	-0.147167	0.868833	-0.4049	7.405533	1
Sm-N6	2.051267	2.188	-0.154867	2.033167	6.117567	2
CK-N2	-4.167633	1.6615	2.377267	1.005767	0.8769	3
Sm-N2	0.4082	1.590633	0.167567	-2.5588	-0.3924	4
CK-N4	0.902067	-3.580067	0.047733	1.1041	-1.526167	5
CK-N6	-1.4253	-1.568933	1.128867	-1.013433	-2.8788	6
Sm-N0	-0.7482	1.1109	-3.389267	-0.0288	-3.055367	7
CK-N0	-4.109167	-1.2549	-1.046133	-0.137067	-6.547267	8

N0、N2、N4、N6 were 0、2、4、6 g/L, respectively, nitrogen levels treatment; CK was not inoculated, Sm was inoculated; F1: First principal component factor scores; F2: Second principal component factor scores; F3: Third principal component factor scores; F4: Fourth principal component factor scores; F: Total principal component factor scores. Order: Factor score ordering.

Figures

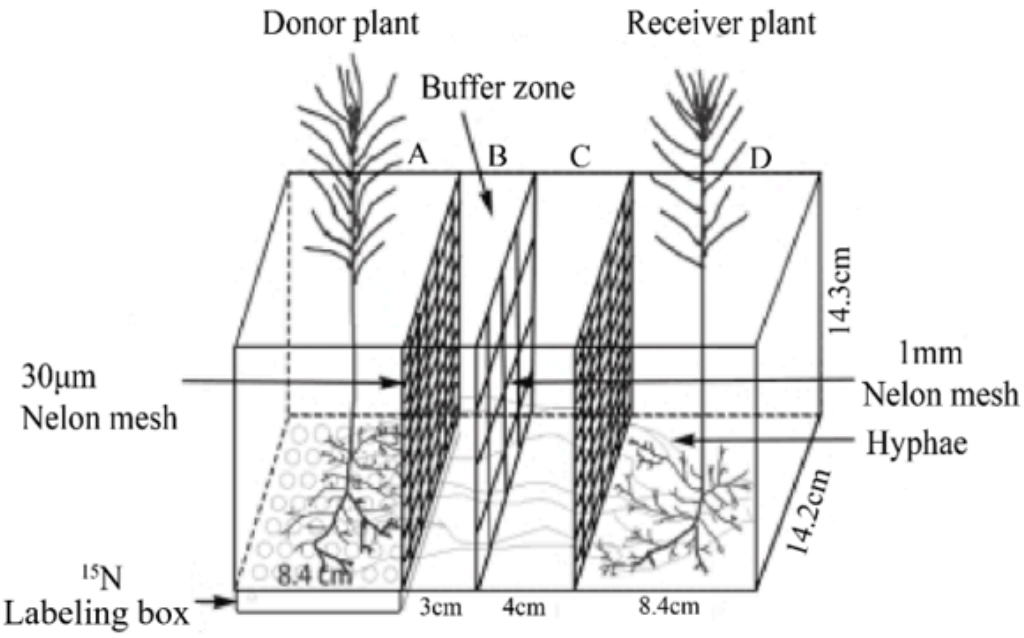

Figure 1

Four-cell grid seedling apparatus, four chambers (A, B, C, D) are arranged left to right, with A and D being 8.4cm×14.2cm×14.3cm, B being 3.0cm×14.2cm×14.3cm, and C being 4.0cm×14.2cm×14.3cm. Chambers A and B, as well as C and D, are divided by 30-μm nylon mesh, with 3-mm holes punched in A's bottom labeling box. A coarse nylon mesh with 1mm apertures separated B and C.

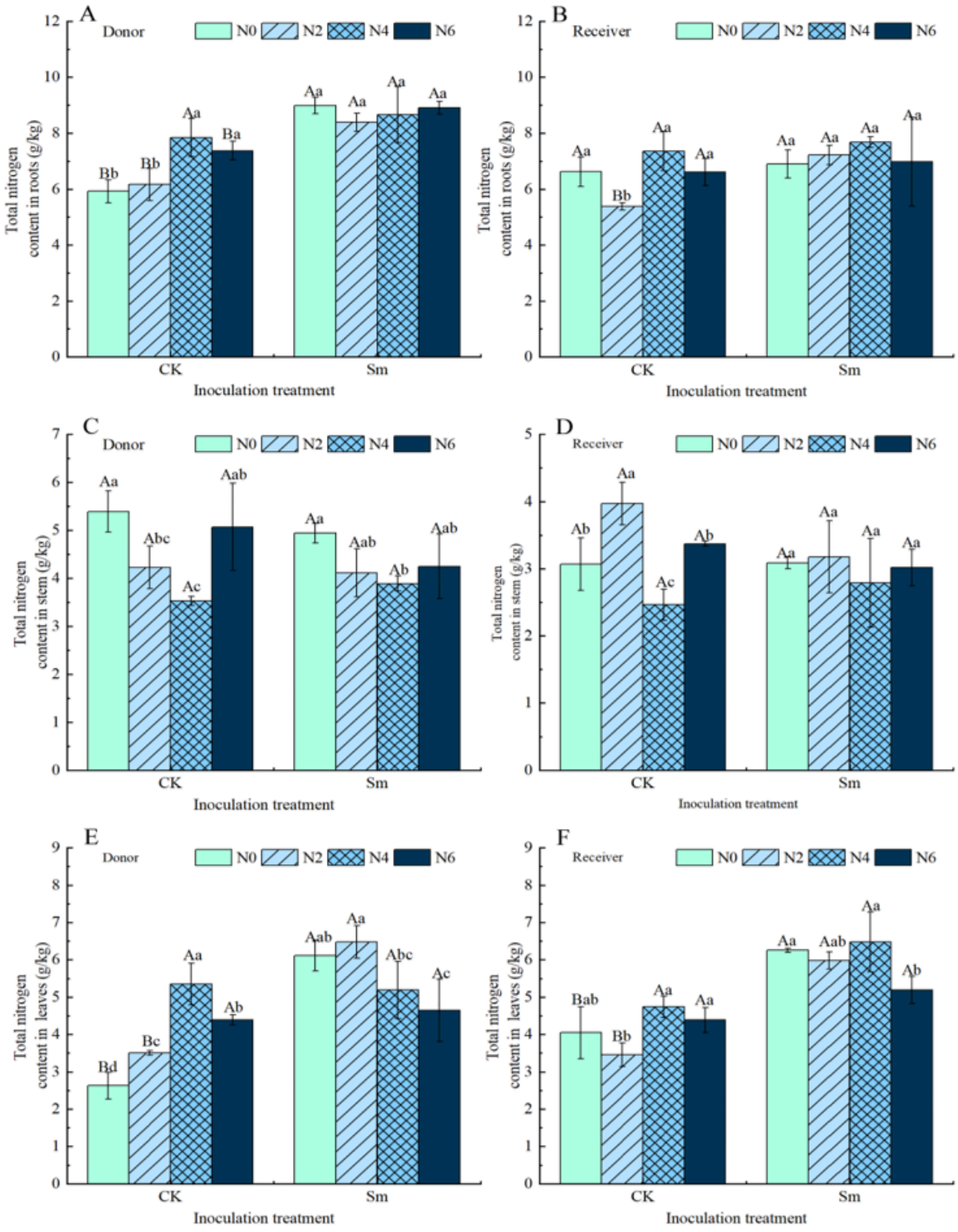

Figure 2

The nitrogen content difference in the roots (A, B), stems (C, D), and leaves (E, F) of *Pinus massoniana* donor/receiver seedlings. N0、N2、N4、N6 were 0、2、4、6 g/L, respectively, nitrogen treatment; CK was not inoculated, Sm was inoculated; The data were mean±standard deviation (n=3); Different lowercase letters indicate significant differences among nitrogen treatments within each inoculation status, whereas different capital letters indicate significant differences between inoculation statuses within each nitrogen treatment (P< 0.05).

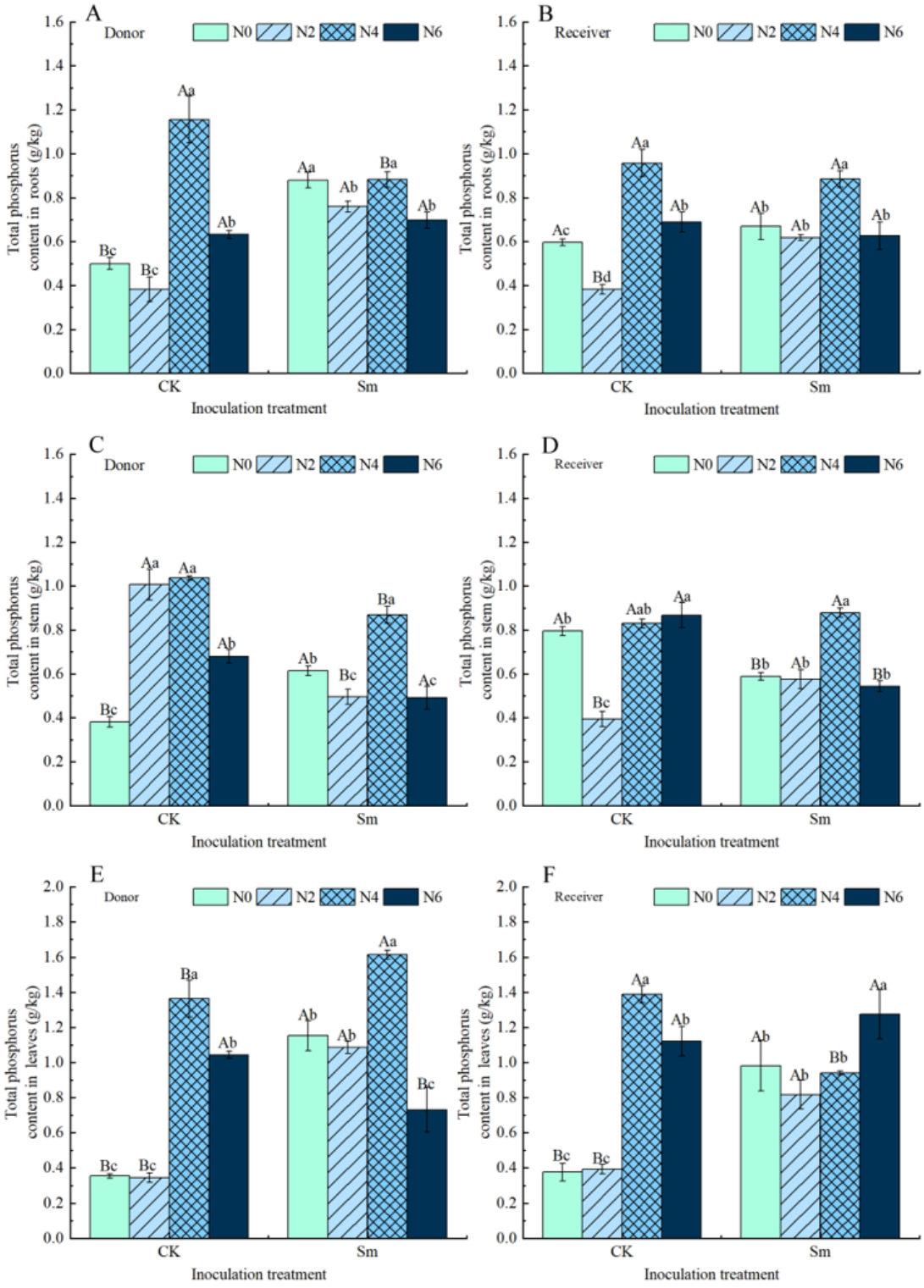

Figure 3

The phosphorus content difference in the roots (A, B), stems (C, D), and leaves (E, F) of *Pinus massoniana* donor/receiver seedlings. N0、N2、N4、N6 were 0、2、4、6 g/L, respectively, nitrogen treatment; CK was not inoculated, Sm was inoculated; The data were mean ± standard deviation (n=3); Different lowercase letters indicate significant differences among nitrogen treatments within each inoculation status, whereas different capital letters indicate significant differences between inoculation statuses within each nitrogen treatment ($P < 0.05$).

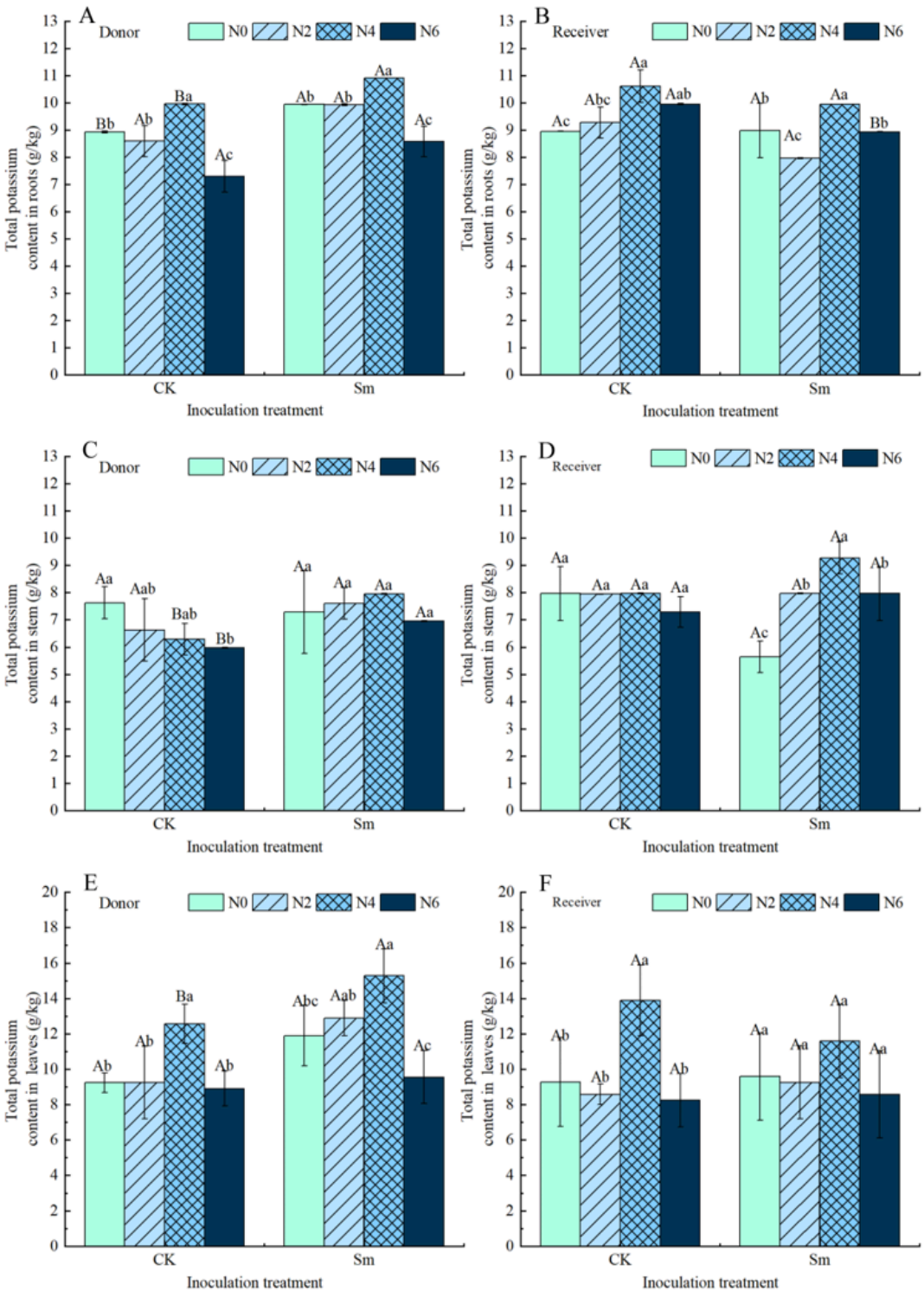

Figure 4

The potassium content difference in the roots (A, B), stems (C, D), and leaves (E, F) of *Pinus massoniana* donor/receiver seedlings. N0、N2、N4、N6 were 0、2、4、6 g/L, respectively, nitrogen treatment; CK was not inoculated, Sm was inoculated; The data were mean ± standard deviation (n=3); Different lowercase letters indicate significant differences among nitrogen treatments within each inoculation status, whereas different capital letters indicate significant differences between inoculation statuses within each nitrogen treatment ($P < 0.05$).

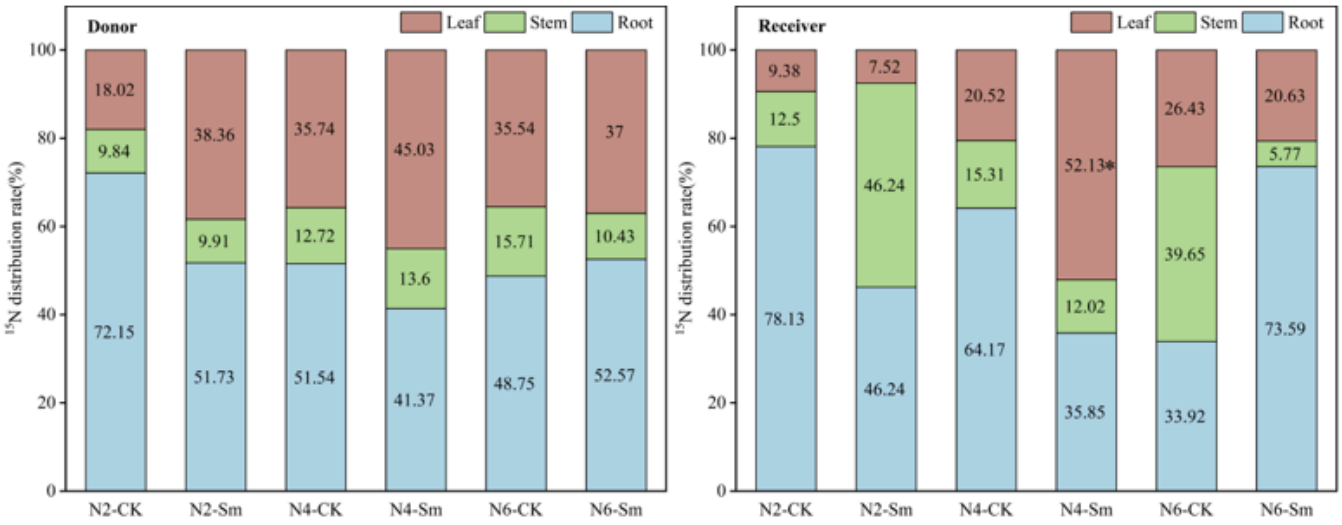

Figure 5

The ^{15}N allocation [%] of *P. massoniana* seedling's donor/receiver in roots, stems and leaves; N2、N4、N6 were 2、4、6 g/L, respectively, nitrogen treatment; CK was not inoculated, Sm was inoculated; *: ^{15}N allocation was significantly higher than other treatments, $P < 0.05$.

Supplementary Files

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