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The nitrogen acquisition strategy of *Picea asperata* seedlings depended on their symbiotic ectomycorrhizal fungi community

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Introduction: Ectomycorrhizal symbiosis is pivotal for plant nutrient acquisition
in subalpine ecosystems, but the mechanism of ectomycorrhizal fungal (EMF)
communities regulating nitrogen (N) uptake in woody plants remains limited.

Methods: We investigated the effects of EMF communities sourced from a
N-rich natural forest and a N-poor plantation on N uptake and growth of *Picea*
asperata seedlings through a controlled inoculation experiment.

Results: Seedlings colonized by natural forest EMF exhibited 58% higher total
biomass than controls, owing to accelerated fungal colonization, optimized
root architecture, a 94% increase in root ammonium influx rates, and a
75% higher glutamine synthetase activity. In contrast, seedlings colonized by
plantation EMF exhibited a 49% increase in biomass, primarily via regulating
soil microbial enzymatic activities and community-level physiological profiles,
thereby sustaining soil N availability.

Discussion: These findings reveal that EMF communities originating from N-rich
soil tended to enhance the nutrient uptake capacity of host plants, while EMF
communities from N-poor soil preferred to improve soil nutrient availability,
regardless of the current soil nutrient status. This adaptive specialization
underscores the importance of tailoring EMF inoculants to native soil conditions
to enhance restoration efficacy in degraded subalpine forests.

KEYWORDS

ectomycorrhizal fungi, N uptake, *Picea asperata*, soil N availability, subalpine forest

Introduction

Mycorrhizal symbiosis is a key factor regulating plant nutrient uptake and growth
(Smith and Read, 2008). Generally, the formation of mycorrhizae on plant roots offers
multiple benefits, such as enhancing water and nutrient acquisition (Smith and Read, 2008;
van der Heijden et al., 2015), increasing resistance to environmental stress (Begum et al.,
2019), and improving pathogen defense (Cameron et al., 2013). Ectomycorrhizal fungi

(EMF), which are commonly associated with plants in boreal and temperate forests, play a significant role in enhancing plant growth and nutrient acquisition. EMF have been shown to facilitate N acquisition in N-limited ecosystems by increasing the surface area available for uptake (van der Heijden et al., 2015), promoting the N assimilation rate (Rineau et al., 2012), directly absorbing organic N (Chen et al., 2019), and indirectly decomposing litter to supplement N availability (Op De Beeck et al., 2018). However, the effect of the EMF community on plant growth is influenced by various factors, such as the composition and diversity of the EMF community, plant species, and soil nutrient availability (Nakashima et al., 2016; Hazard et al., 2017; Nash et al., 2021; Liu et al., 2020).

It has been reported that the effects of EMF on nutrition assimilation and plant growth varies among EMF species richness (Heijden et al., 1998; van der Heijden et al., 2015). Shoot biomass in *Pinus sylvestris* seedlings was enhanced by the presence of a single EMF species, which increased enzyme activity in the mycorrhizal root tips (Kipfer et al., 2012). Higher EMF species richness improves the host plant's nutrient acquisition potential by diversifying the exoenzyme repertoires (Heinonsalo et al., 2016; Karlsen-Ayala et al., 2022). However, despite the varying effects of different EMF species on plant growth, no complementary effects of EMF diversity were observed in another study (Shi et al., 2017). EMF colonization can significantly alter root morphology to facilitate greater N acquisition (Zhang et al., 2023), but the effects of EMF communities on root physiology and their preference for inorganic nitrogen forms (nitrate or ammonium) remain inconsistent (Kou et al., 2015; Corrales et al., 2017; De Quesada et al., 2024). Consequently, the N acquisition mechanisms of ectomycorrhizal (ECM) symbionts across different EMF communities remain unclear.

In addition to directly stimulating nutrient uptake in plants, EMF have also been reported to enhance plant nutrient uptake indirectly by regulating soil nutrient transformations (Ito and Reshi, 2013). It is reported that EMF regulate the activities of enzymes related to N transformation, promote the destabilization of mineral-associated organic matter by secreting ligands (e.g., oxalic acid), and release bound nutrients through the synergy of non-enzymatic desorption and a multi-enzyme system (proteases, oxidases, hydrolases) (Keiluweit et al., 2015; Li et al., 2021; Jilling et al., 2021). There are interspecific differences in their capacity to utilize soil organic matter among EMF species (Shah et al., 2016; Pellitier and Zak, 2017). Walker et al. (2015) found that ECM roots of seedlings grown in forests exhibit distinct exoenzyme activity profiles compared to those planted in clear-cut areas, but seedling biomass did not differ between these two environments. The high abundance of *T. terrestris* ectomycorrhizae in the clear-cut area may have facilitated seedling nutrient acquisition, even in soils with reduced EMF richness and low availability of nitrogen and phosphorus (Walker et al., 2015; Bennett et al., 2017). Additionally, higher soil N availability is generally expected to enhance plant N uptake and growth. However, previous studies (Liu et al., 2011; Umaña et al., 2020) have not consistently observed this effect, and the reasons for this discrepancy remain unclear. While EMF diversity effects are documented (Jonsson et al., 2001; Anthony, 2025), whether EMF functional traits vary with soil nutrient status remains poorly explored, particularly in degraded plantation ecosystems.

The subalpine coniferous forests located in the transition zone between the Qinghai-Tibet Plateau and the Sichuan Basin are characterized by low nutrient availability and a short growing season (Pang et al., 2011). Over the past century, more than one million hectares of natural forests dominated by *Abies faxoniana* and *Picea asperata* have been extensively logged and reforested with *P. asperata* seedlings. Soil N content differed significantly between the two forest types, with natural forests exhibiting a 3-fold higher value than *P. asperata* monoculture plantations (Xu et al., 2010). Previous studies have shown that ECM colonization rate and nitrogen content of *P. asperata* seedling roots were lower in plantations compared to nearby natural forests, however, no significant differences in biomass accumulation were observed between *P. asperata* seedlings grown in plantations and those in natural forests (Xu et al., 2012; Li et al., 2015). While higher soil N availability is generally considered beneficial for plant growth, this advantage was not evident in the previous studies. Given that the *P. asperata* seedlings in these studies originated from the same nursery, it raises the question of whether the initial ECM colonization on the roots plays a critical role in plant growth. Although soil microbial community composition differed significantly between the two forest types (Zhao et al., 2022), there was no significant difference in EMF biomass (Li et al., 2015). Nonetheless, the roles of EMF in nitrogen uptake and the growth of *P. asperata* seedlings remain poorly understood. Therefore, an inoculation experiment was conducted to evaluate the impact of soil EMF communities from two distinct forest types on the growth of *Picea asperata* seedlings. We hypothesized that: (1) EMF inoculation would significantly promote the growth of *P. asperata* seedlings, and (2) there would be no significant difference in growth promotion between EMF communities derived from plantation soils and those from natural forest soils.

Materials and methods

Experimental design and plant material

After removing surface litter, 0–20 cm topsoil samples were collected from natural forests and adjacent plantations with distinct fungal community compositions in the Miyaluo Experimental Forest (Supplementary Figure S1), eastern Tibetan Plateau (31°35'N; 102°35'E; 3,150 m a.s.l.). Soil from both forest types was divided into two portions: one portion was subjected to two cycles of autoclaving (121°C for 1 h per cycle) to eliminate soil biota, while the remaining fresh soil was immediately stored at 4°C for microbial inoculation within 5 days of collection. One subpart of the fresh soil was mixed with sterilized water at the ratio of 1 g soil: 1 mL water, shaken for 1 h on a shaker, and then filtered through a 38-μm mesh to remove mycorrhizal root fragments and spores, while allowing other soil organisms to pass through, thereby standardizing the bacterial actinomycetes and non-mycorrhizal fungi between mycorrhizal and non-mycorrhizal treatments (Koide and Li, 1989; Pérez and Urcelay, 2009). The microbial slurry from natural forest or plantation was added into sterilized soils at 12.5% (v/w) to establish non-mycorrhizal treatments. Parallel mycorrhizal treatments received an equivalent volume of sterile distilled water to maintain moisture consistency. Mycorrhizal treatments were

inoculated with fresh soil (from natural forest or plantation) containing intact mycorrhizal propagules and complete native microbial communities. An equal amount of sterilized soil as the inoculum was added to the non-mycorrhizal treatment to equalize nutrient discrepancies.

Picea asperata seeds were also collected from Miyaluo Experimental Forest. Seeds underwent surface-sterilized in 5% KMnO₄ solution for 2 h, followed by three rinses and soaked in with sterile water, and then germinated on moist filter paper at 25°C. Plastic boxes (62.5 × 52.5 × 15 cm) were used for planting *P. asperata* seedlings filled with inoculated soil (fresh natural forest soil or plantation soil) and autoclaved soil with microbial slurry or sterilized water (Table 1). Each box was filled with homogenized mixed soil, combining natural forest and plantation soils in a 1:1 mass ratio. The initial physicochemical properties of this mixed soil were as follows: total carbon 89 g kg⁻¹, total N 7.2 g kg⁻¹, total phosphorus 0.56 g kg⁻¹, total potassium 13.44 g kg⁻¹, and pH 6.67. 120 healthy 50-day old seedlings were transplanted into each box, and they were watered with sterilized water as needed. There were four treatments in this experiment: (1) PU, autoclaved soil with plantation microbial slurry; (2) PI, autoclaved soil with plantation inoculum; (3) NU, autoclaved soil with natural forest microbial slurry; (4) NI, autoclaved soil with natural forest inoculum. Each treatment included four plastic boxes as experimental replicates.

The determination of colonization rate

Ectomycorrhizal colonization rate was assessed monthly at 30, 60, 90, and 120 days post-inoculation. Three boxes were randomly selected for sampling, and six seedlings were chosen randomly from each selected container at every time point. The entire root systems of sampled seedlings were excised and thoroughly washed with deionized water. The roots were subsequently sectioned into 1-cm fragments using sterile scalpels. All root fragments were examined under a stereomicroscope (Stemi SV 11; Zeiss, Jena, Germany) and sorted into vital and non-vital tips. Vital root tips were distinguished as ectomycorrhizal or non-ectomycorrhizal by the presence or absence of fungal mantles. The colonization rate was calculated as follows: Colonization rate (100%) = Ectomycorrhizal root tips/total number of root tips × 100.

After 120 days of inoculation, all the remaining seedlings were harvested for the determination of plant biomass, the concentration of NH₄⁺-N and NO₃⁻-N, root architecture, ion influx rate and enzyme activities.

The determination of plant biomass and root architecture

Three seedlings in each box were randomly sampled and separated into shoots and roots. All plant tissues were oven-dried at 65°C until constant mass. The shoot / root ratio (S/R) was calculated based on the dry weight.

The root systems of three selected seedlings in each box were scanned by a scanner (HP 4Jc, Hewlett-Packard, D) for image acquisition. The length, surface area (SA), Average diameter, volume, and the number of root tips were analyzed using the

WinRHIZO image analysis software (Régent instruments, Quebec, QC, Canada). The dry weight of the scanned root was also determined, and the specific root length (root length/biomass) (SRL), Specific root area (surface area/ biomass) and Tissue density were calculated.

The determination of net fluxes of NH₄⁺ and NO₃⁻

Net fluxes of NH₄⁺ and NO₃⁻ were determined through non-invasive measurements at root surfaces using a scanning ion-selective electrode technique (SIET system, BIO-IM-XY, Younger United States Science and Technology Corp., MA, United States). Entire root systems of three seedlings in each box were randomly selected and immersed in a standardized measuring solution, containing 1 mM KCl, 0.1 mM CaCl₂ and 0.1 mM NH₄NO₃ (pH adjusted to 5.5). Ion-selective microelectrode with 2–4 μm aperture was manufactured and silanized by Xuyue Sci. & Tech. Co., Ltd. (Beijing, China), following established protocols for backfilling solution preparation and ion-selective liquid cocktail application as reported by previous studies (Luo et al., 2013). The ion-selective electrodes were calibrated before flux measurements according to Tang et al.'s (2019) study. Prior to flux measurements, electrodes were calibrated using standard solutions (1 mM KCl, 0.1 mM CaCl₂ and 0.1 mM NH₄NO₃) with two additional NH₄NO₃ concentrations of 0.05 and 0.5 mM. Electrodes were used when the Nernstian slopes were 58 ± 5 mV for NH₄⁺, and -58 ± 5 mV for NO₃⁻ per tenfold concentration difference. Based on the pre-experimental determinations, the net fluxes of NH₄⁺ and NO₃⁻ were measured on the 2–4 mm root segment proximal to the root apex, where maximal net ion uptake activity was observed for both two ions (Luo et al., 2013).

Determination of NH₄⁺-N and NO₃⁻-N concentrations and associated enzyme activities of the seedlings

NH₄⁺-N and NO₃⁻-N concentrations, along with nitrate reductase (NR) and glutamine synthetase (GS) activities, were determined using commercially available assay kits (Suzhou Comin Biotechnology Co., China) following manufacture procedures. The basic principles of the kits were shown as follows: NH₄⁺-N determination employed ninhydrin-mediated chromogenic reactions measured at 580 nm; NO₃⁻-N was determined using nitrosalicylic acid formation monitored through absorbance measurements at 410 nm. NR activity was determined based on the content of red azo-compound via 540 nm absorbance. GS activity was measured glutamyl-γ-hydroxamate formation at 550 nm absorbance.

Determination of soil parameters

Soil NH₄⁺-N and NO₃⁻-N were extracted with 2 M KCl solution and measured by colorimetry as described by Sattolo et al. (2016). Dissolved organic C (DOC) and total dissolved N (TDN)

TABLE 1 Experimental design and treatments.

Treatments	Autoclaved soil (Kg)		Inoculated soil (Kg)		microbial slurry (L)		Sterile water (L)
	Natural forest	Plantation	Natural forest	Plantation	Natural forest	Plantation	
PU	4	4	1	1		1	1
PI	4	3					
NU	4	4			1		1
NI	3	4					

were extracted by the method of Jones and Willett (2006). Briefly, 20 g fresh soil was mixed with 100 mL ultrapure water, shaken for 1 h, and then centrifuged. The supernatant was filtered through a 0.45 µm glass fiber filter. The C and N in the extracts were also measured using a C/N analyzer (Multi-N/C 2100, Analytik Jena AG, Germany). Dissolved organic N (DON) was calculated as $DON = TDN - (ammonium + nitrate)$.

Soil microbial biomass C (MBC) and N (MBN) were determined through chloroform fumigation-extraction (Vance et al., 1987). Both fumigated and non-fumigated soils were extracted with K_2SO_4 and analyzed for extractable organic C and N using a total C/N analyzer (Multi-N/C 2100, Analytik Jena AG, Germany). The MBC and MBN calculations employed conversion factors of 0.45 for MBC (Kec) and 0.54 for MBN (Ken), based on the differential between fumigated and non-fumigated extracts (Vance et al., 1987).

Activities of four extracellular enzymes were determined using modified protocols from Saiya-Cork et al. (2002) and German et al. (2011), including acid phosphatase (AP), β -1,4-Glucosidase (BG), peroxidase (PER) and polyphenol oxidase (PPO). Hydrolase activities (AP, BG) were measured fluorometrically using substrates linked to a fluorescent tag (4-methylumbelliferone), while oxidative enzymes (PER, PPO) were measured colorimetrically using L-3, 4-dihydroxyphenylalanine as substrate (German et al., 2011). All assays utilized 2 g soil suspensions in 100 mL 50 mM sodium acetate buffer (pH 5.0), with 200 µL aliquots incubated in 96-well microplates under controlled conditions: 25°C in darkness for 2 h (AP), 5 h (BG), and 4 h (PER, PPO).

Assessment of community-level physiological profiles

Microbial metabolic diversity was evaluated using Biolog EcoPlates™ (Biolog Inc., Hayward, CA) as described by Garland (1996). Each 96-well plate contains a triplicate set of 31 carbon substrates plus three control wells containing no carbon source. Briefly, 10 g dry weight of fresh soil was mixed with 100 mL of 0.85% NaCl, shaken for 10 min (300 rpm), and settled for 20 min; then, the suspensions were diluted to a 10^{-3} dilution. Subsequently, 125 µL of the supernatant was inoculated into each well of the Biolog plate, and incubated at 25° in darkness for 168 h. The optical density (OD) for each well was read at a wavelength of 590 nm every 24 h using a Biolog GEN III Microstation™ reader (United States). The data at the end of the exponential phase at 144 h were chosen for the principal component analysis (PCA) (Yu et al., 2015). The potential substrate utilization by the microbial community was measured by calculating the average well color

development (AWCD) using the following equation according to Garland (1996) (Equation 1):

$$AWCD = \sum_{i=1}^n (x_i - c) / 31 \quad (1)$$

Where x_i is the OD value in the substrate well, and c is the OD value measured in the control well. The 144 h OD value for each sample in triplicate, divided by their AWCD to normalize the values were used to calculate the functional diversities using Shannon index (Garland, 1996). The Shannon index is calculated as follows (Equation 2):

$$\text{Shannon index} = - \sum P_i (\ln P_i) \quad (2)$$

where p_i is the ratio of the activity on each substrate (OD_i) to the sum of activities on all substrates (ΣOD_i).

Data analysis

Duncan multiple comparison was used to identify the differences of the parameters among different treatments by the software statistical package for social science (SPSS) version 17. Net flux data were calculated using JCal V3.2.2 (or xuyue.net). The changes in patterns of 31 carbon sources utilization were analyzed using principal component analysis (PCA) and one-way permutational ANOVA (PERMANOVA) with Bray-Curtis similarity distance on Biolog data by using PAleontological STatistics (PAST, v3.18) software (Hammer et al., 2001).

Results

Colonization rate and biomass of the seedlings

During the experimental period, seedlings inoculated with natural forest inoculum (NI) demonstrated initial ectomycorrhizal fungal (EMF) colonization within 30 days post-inoculation, achieving a final colonization rate of 95.02% by experiment termination (Table 2). In contrast, seedlings inoculated with plantation inoculum (PI) exhibited delayed colonization onset at 60 days, with significantly lower maximum colonization rates compared to seedlings with NI ($p < 0.05$). Control treatments receiving autoclaved soil supplemented with microbial slurry from either plantation or NF sources showed minimal colonization (<2% in both cases) throughout the study period (Table 2).

EMF colonization significantly enhanced biomass accumulation by 49–58% ($p < 0.05$), with NI demonstrating

TABLE 2 The colonization rate of the seedlings during the experimental time (mean $\pm$ SE, $n = 3$).

Colonization rate (%)	PU	PI	NU	NI
30d	0.000 $\pm$ 0.000 b	0.000 $\pm$ 0.000 b	0.000 $\pm$ 0.000 b	10.99 $\pm$ 2.662 a
60d	0.305 $\pm$ 0.330 c	15.90 $\pm$ 2.890 b	0.820 $\pm$ 0.477 c	31.79 $\pm$ 3.673 a
90d	1.436 $\pm$ 0.500 c	84.57 $\pm$ 2.040 b	1.584 $\pm$ 0.243 c	93.45 $\pm$ 1.670 a
120d	1.96 $\pm$ 0.549 c	87.69 $\pm$ 0.870 b	2.025 $\pm$ 0.507 c	95.02 $\pm$ 0.692 a

Treatments labeled with different letters are significantly different at $p = 0.05$ for Duncan comparisons. PU, autoclaved soil with plantation microbial slurry; PI, autoclaved soil with plantation inoculum; NU autoclaved soil with natural forest microbial slurry; NI, autoclaved soil with natural forest inoculum.

superior promotion effects compared to plantation inoculum (PI), particularly in shoot biomass accumulation (Figure 1). This differential growth response resulted in significantly higher shoot-to-root ratios (S/R) in colonized seedlings. However, no significant difference of the biomass accumulation was observed between PU and NU treatments (Figure 1).

Root morphology

EMF colonization exerted inhibitory effects on most of the root morphological parameters (Figure 2). Root length, SRL, and root tip numbers decreased by about 10% under PI colonization and over 20% under NI colonization. Both inoculums increased mean root diameter, while divergent responses were observed for SA, SRA, and root volume. These parameters were increased by PI but decreased by NI (Figure 2).

N assimilation

NI significantly enhanced root net influxes of NH_4^+ -N and NO_3^- -N by 94 and 22% ($p < 0.05$), respectively, compared to NU controls. PI only significantly increased NH_4^+ -N influx by 40% relative to PU treatment ($p < 0.05$) (Figure 3a). There was no significant difference of NH_4^+ -N and NO_3^- -N influxes between NU and PU treatments. Notably, the net influx of NH_4^+ -N significantly exceeded NO_3^- -N influx across all treatments (Figure 3a).

Similar with root ion net influxes, the accumulation of NH_4^+ -N in seedlings were significantly increased by both inoculums ($p < 0.05$), especially for NI treatments, NH_4^+ -N contents increased by 149% in root and 160% in shoot. However, the significant increment of the NO_3^- -N accumulation by NI and PI inoculum was only observed in roots ($p < 0.05$) (Figure 3b). Both inoculums significantly elevated GS activities, with root GS showing 51% (PI) and 75% (NI) increases. However, the significant promotion of NR activity was only observed in root (Figure 3c).

Soil biochemical properties

NI inoculation decreased soil inorganic N content, particularly NH_4^+ -N content, while PI treatment maintained comparable NH_4^+ -N levels to controls with slight NO_3^- -N increases (Table 3). Additionally, MBC and MBN increased by 33 and 78% (PI), and 55 and 100% (NI), respectively compared to their controls. The activities of all soil enzymes determined were not significantly

affected by NI treatment, except BG (Table 3). On the contrary, PI treatment significantly enhanced AP, BG, PER and PPO activities by 46, 10, 68, and 35%, respectively (Table 3).

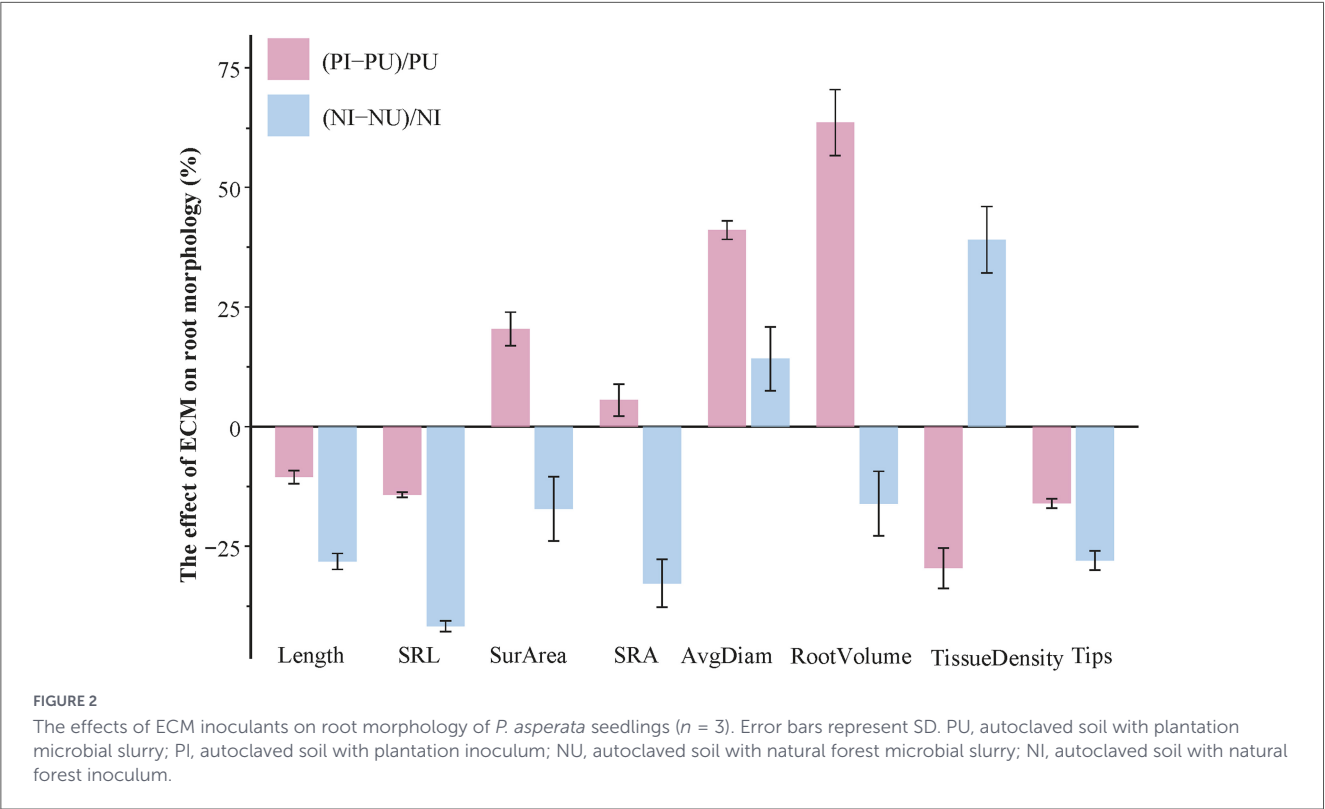
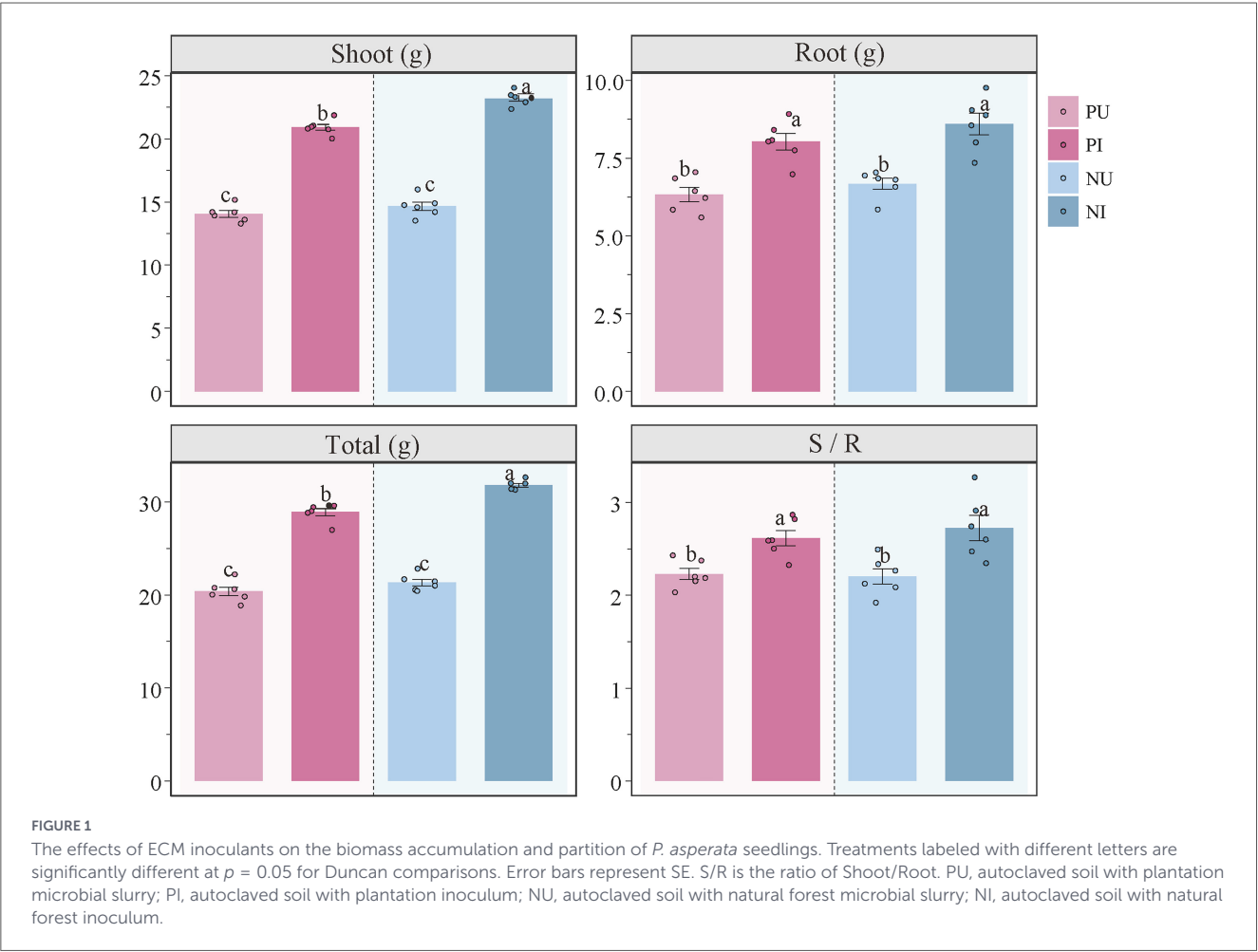
Community-level carbon utilization profiles

As the indicator of the potential substrate utilization of the soil microbial community, PI and PU treatments showed higher initial AWCD values (0–4 days), while PI and NI inoculation induced greater late-stage metabolic activity, especially for PI treatment (Figure 4a). PCA based on community-level physiological profiles (CLPPs) explained 58.7% variance (PC1:37.5%, PC2:21.2%) (Figure 4b). According to the loading plot, the carbon utilization patterns were more similar between NU and PU treatments, while PI and NI were different from other treatments indicating inoculum-specific metabolic signatures. PI had the highest utilization rate of carbohydrates, amino acids, amides, and acids, while NI do best in esters and alcohols (Figure 5a). Additionally, the diversity of the potential substrate utilization in PI was significantly higher than that in NI, PU and NU treatments (Figure 5b).

Discussion

Physiological and morphological strategies for N optimization by natural forest EMF inoculums

The significant growth enhancement in *P. asperata* seedlings induced by ectomycorrhizal fungal (EMF) colonization through both natural forest inoculum (NI) and plantation inoculum (PI), aligns with previous findings demonstrating EMF inoculation efficacy in enhancing plant growth (Pan et al., 2022; Rwizi et al., 2025; Wang et al., 2025). While both two inoculums positively influenced seedling biomass, NI exhibited significantly superior performance, particularly in shoot biomass accumulation (Figure 1). This likely stems from the relative N-rich environment of natural forests, where plant growth and survival hinges on competitive nutrient acquisition strategies (Liu W. et al., 2023; Wu et al., 2025). Seedlings inoculated with NI developed superior nitrogen acquisition capacity, leading to enhanced N assimilation and subsequent biomass gains. This phenomenon was further confirmed by the established correlations between tissue nitrogen content and plant growth in previous studies (Reich et al., 2006;



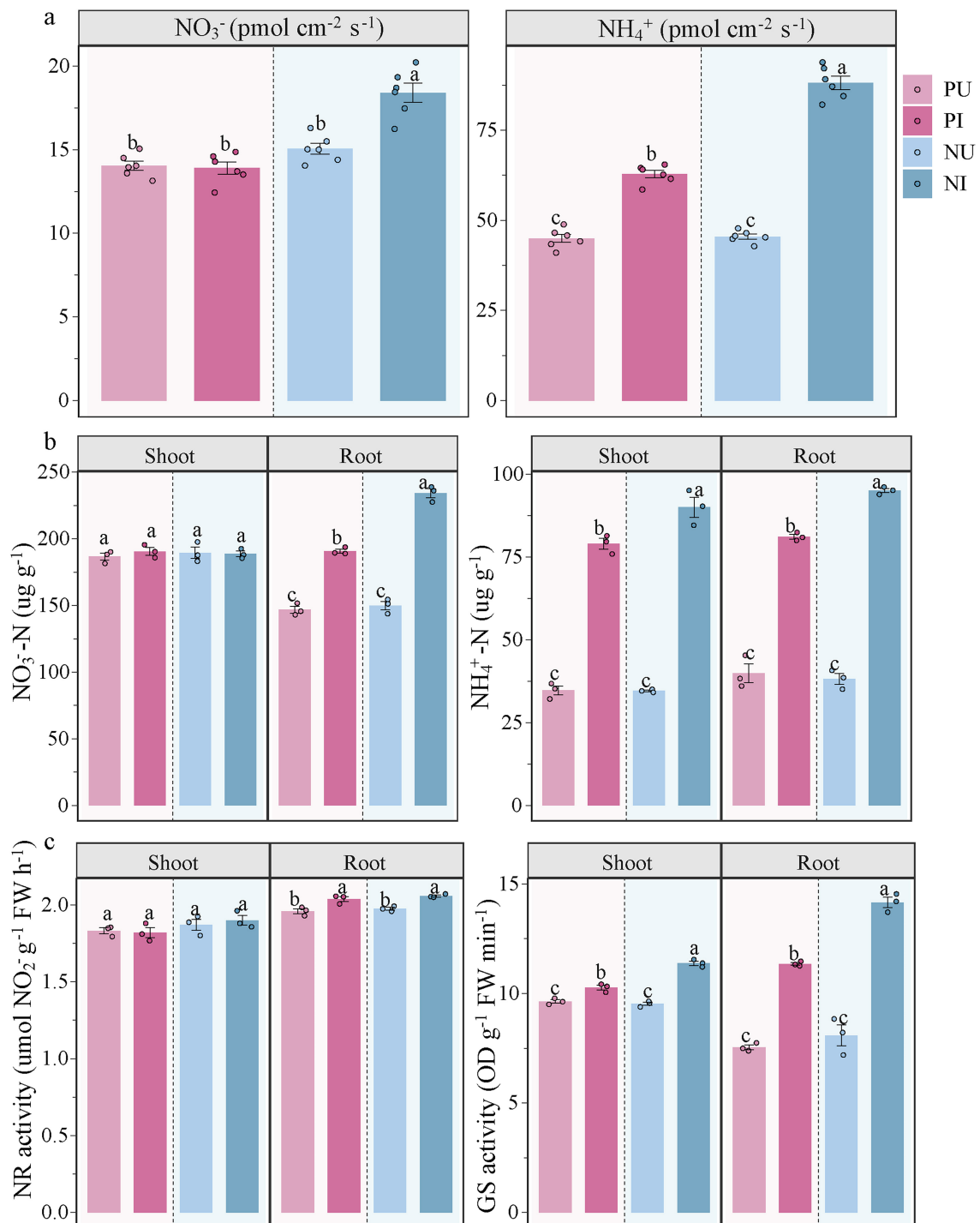


FIGURE 3

The effects of ECM inoculants on N-ion net influx (a), NO_3^- -N and NH_4^+ -N contents (b), and GS and NR activities (c) of the seedlings. Treatments labeled with different letters are significantly different at $p = 0.05$ for Duncan comparisons. Error bars represent SE. PU, autoclaved soil with plantation microbial slurry; PI, autoclaved soil with plantation inoculum; NU, autoclaved soil with natural forest microbial slurry; NI, autoclaved soil with natural forest inoculum.

Singh et al., 2022). Therefore, the enhanced N acquisition strategies of *P. asperata* seedlings by NI plays critical roles in promoting biomass accumulation.

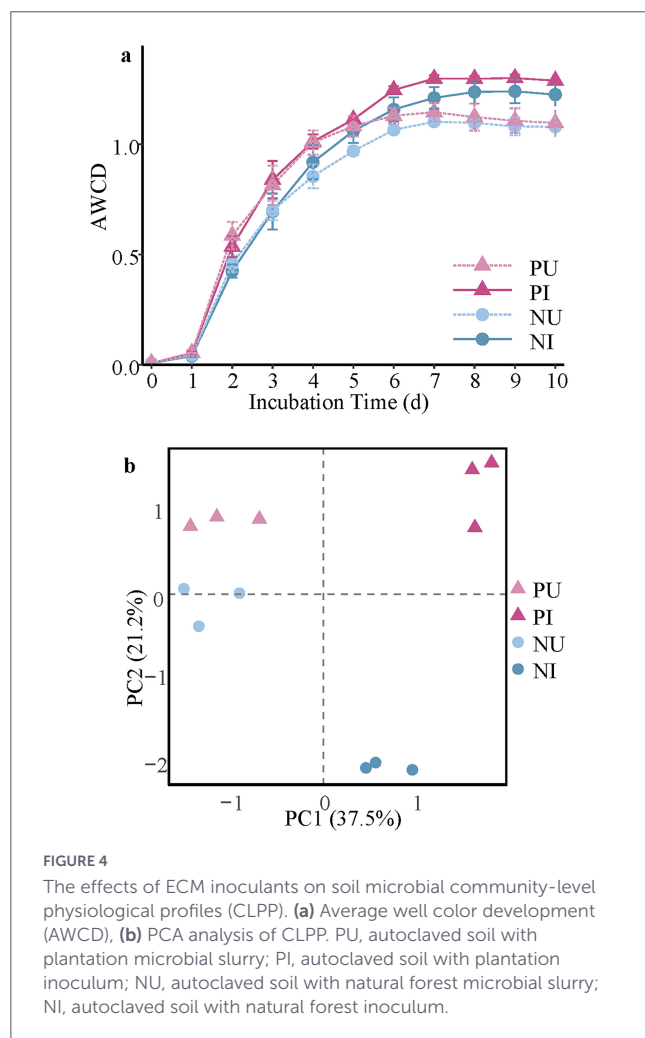
The pronounced N acquisition capacity of *P. asperata* seedlings colonized by natural forest EMF arises from a multi-layered adaptive strategy encompassing higher colonization rate, root

architectural modification, and physiological regulation. Compared to PI, the NI demonstrated the fastest and highest colonization rate (95%) in seedlings (Table 2), suggesting that soil ECM fungal community of natural forest was more efficient for forming ECM symbionts. The different species composition of the ECM fungal communities between NI and PI likely contributed most

TABLE 3 Effects of ECM on soil parameters (mean $\pm$ SE, $n = 3$).

Soil parameters	PU	PI	NU	NI
$\text{NH}_4^+ \text{-N}$ (mg Kg^{-1})	5.63 ± 0.18 a	5.53 ± 0.19 a	5.94 ± 0.07 a	4.98 ± 0.10 b
$\text{NO}_3^- \text{-N}$ (mg Kg^{-1})	24.61 ± 0.67 ab	25.25 ± 0.53 a	23.64 ± 0.40 ab	22.87 ± 0.56 b
DOC (mg Kg^{-1})	239.85 ± 6.52 a	251.82 ± 8.68 a	239.35 ± 13.47 a	262.59 ± 4.78 a
DON (mg Kg^{-1})	86.40 ± 4.40 a	87.52 ± 3.25 a	85.91 ± 3.38 a	94.85 ± 3.33 a
MBC (g Kg^{-1})	85.80 ± 5.49 b	113.92 ± 2.55 a	83.19 ± 7.69 b	128.96 ± 12.13 a
MBN (mg Kg^{-1})	22.15 ± 2.42 c	39.34 ± 3.55 ab	26.78 ± 1.47 bc	53.46 ± 8.10 a
AP ($\mu\text{mol h}^{-1} \text{g}^{-1} \text{MBC}$)	79.73 ± 2.60 b	116.46 ± 7.13 a	78.03 ± 11.07 b	80.92 ± 5.32 b
BG ($\mu\text{mol h}^{-1} \text{g}^{-1} \text{MBC}$)	50.62 ± 3.94 c	156.80 ± 7.56 a	42.48 ± 5.60 c	95.46 ± 8.78 b
PER ($\mu\text{mol h}^{-1} \text{mg}^{-1} \text{MBC}$)	2.71 ± 0.07 b	4.54 ± 0.51 a	2.97 ± 0.28 b	3.17 ± 0.35 b
PPO ($\mu\text{mol h}^{-1} \text{mg}^{-1} \text{MBC}$)	2.89 ± 0.15 b	3.90 ± 0.23 a	2.96 ± 0.37 b	2.82 ± 0.23 b

Treatments labeled with different letters are significantly different at $p = 0.05$ for Duncan comparisons. $\text{NH}_4^+ \text{-N}$, $\text{NO}_3^- \text{-N}$, DOC, DON, MBC, MBN, AP, BG, PER, and PPO represent ammonium nitrogen, nitrate nitrogen, dissolved organic carbon, dissolved organic nitrogen, microbial biomass carbon, microbial biomass nitrogen, acid phosphatase, β -1,4-glucosidase, peroxidase, and phenol oxidase in the soil, respectively. Treatments labeled with different letters are significantly different at $p = 0.05$ for Duncan comparisons. PU, autoclaved soil with plantation microbial slurry; PI, autoclaved soil with plantation inoculum; NU, autoclaved soil with natural forest microbial slurry; NI, autoclaved soil with natural forest inoculum.



to the higher and faster colonization (Supplementary Figure S1), since ECM species with different life history variations varied in their infecting rate during ECM formation (Molina and Horton, 2015). Therefore, the rapid symbiotic establishment and higher

colonization rate of natural forest EMF maybe enhance nitrogen acquisition in seedlings (Li et al., 2015).

Seedlings colonized by NI also exhibited distinct shifts in root morphology, characterized by increased average root diameter and tissue density, alongside decreased root length and root tip numbers (Figure 2). The increased diameter of ECM symbiotic roots is reported to enhance the capacity to transport nutrients from the soils with roots (Chen et al., 2018). This architectural optimization reduced carbon expenditure on exploratory growth, while increasing symbiotic interface development, as evidenced by the 95% colonization rate and reduced root length and root tip numbers (Figure 2 and Table 2). Such adaptations align with the strategy observed in late-successional forest trees, where prioritized mycelial network expansion enables efficient nitrogen capture from organic matter via proteolytic enzyme secretion (Bödeker et al., 2014; Kadowaki et al., 2018; Bönisch et al., 2024). However, a previous study showed that EMF inoculation can increase water uptake of *Pinus tabulaeformis* by enhancing the length, surface area and diameter of roots (Qi and Yin, 2023). This was primarily attributed to the antagonistic interaction between root growth and mycorrhizal associations, given that both depend on photosynthetic carbon allocated belowground (van der Heijden et al., 2015; Nash et al., 2021).

The 94 and 22% increases in $\text{NH}_4^+ \text{-N}$ and $\text{NO}_3^- \text{-N}$ influx rates (Figure 3a), reflect significantly enhanced ammonium and nitrate transporting ability in NI colonized roots. Previous studies also showed that mycorrhizal mediated N uptake via regulating mycorrhiza-specific ammonium/nitrate transporter abundance and transport activities in plant roots (Hui et al., 2022; Wang et al., 2020). GS is the primary mediator for converting inorganic nitrogen into organic nitrogen (Zhang et al., 2025), and NR also is one of the key enzymes regulating plant nitrogen assimilation (Fu et al., 2020). Concurrently, the significant rise in root GS, root NR, and shoot GS activities further confirmed that NI colonization could promote N assimilation by enhancing N metabolic enzyme activities (Figure 3c). Additionally, the 75 and 20% elevation in root and shoot GS activities of *P. asperata* also (Figure 3c) suggesting a critical adaptation to prevent ammonium toxicity

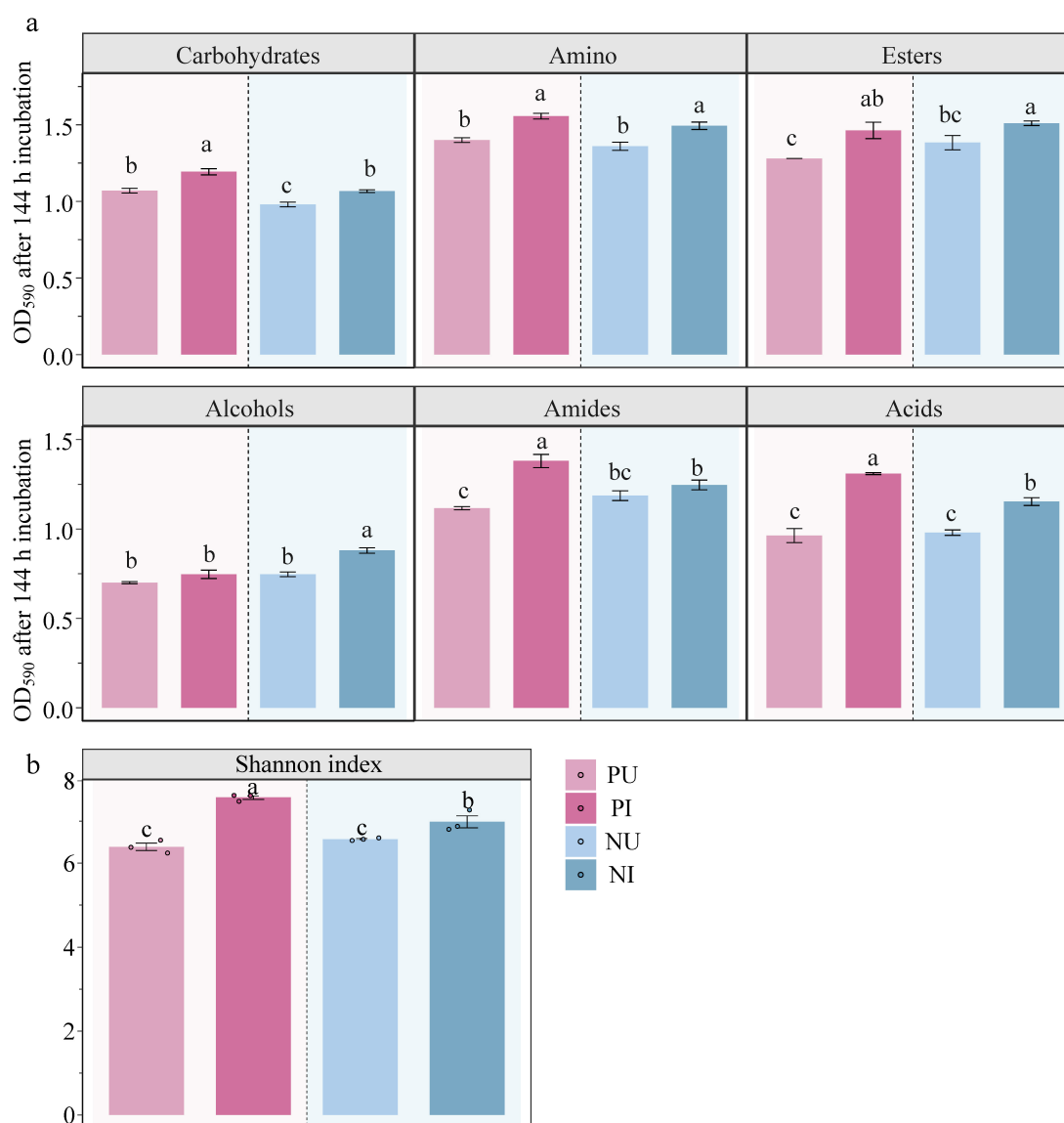


FIGURE 5

The effects of ECM inoculants on AWCD of different carbon sources (a) and soil microbial functional diversity (b). Treatments labeled with different letters are significantly different at $p = 0.05$ for Duncan comparisons. Error bars represent SE. PU, autoclaved soil with plantation microbial slurry; PI, autoclaved soil with plantation inoculum; NU, autoclaved soil with natural forest microbial slurry; NI, autoclaved soil with natural forest inoculum.

while maintaining amino acid biosynthesis in seedlings colonized by NI (Song et al., 2022).

Enhancing soil nutrient availability for host N acquisition by plantation EMF inoculums

Although the growth of *P. asperata* seedlings were enhanced, the shoot and total biomass accumulation of seedlings colonized by plantation inoculum (PI) were significantly lower than those with natural forest inoculum (NI) (Figure 1). This was mainly because the improvements in the colonization rate, NH_4^+ -N and NO_3^- -N influx rates, and GS activity induced by PI were lower than those under NI treatments (Figure 3 and Table 2). Unlike NI treatments, which prioritizes direct plant N assimilation, PI are

better to coordinating microbial activation and enzymatic priming (Table 3). Compared to the control, soil inorganic N availability is significantly decreased by NI application probably due to the enhanced uptake of the seedlings, but it is rarely affected in PI treatment even though seedlings also enhanced the N uptake (Table 3). This result indicated that maintaining and increasing soil N availability was likely the main strategy of PI to promote plant growth, regardless of soil nutrient condition. Some studies also showed that inoculums kept their functions and properties in new environments (Yadav et al., 2021; Sergio et al., 2021), and promoted plant growth by enhancing soil nutrient availabilities (e.g., N and P), improving soil structure and optimizing soil physiochemical properties (Mahanty et al., 2017; Jiang et al., 2021; Liu X. et al., 2023).

In the present study, the application of PI led to a notable enhancement in the activities of AP, BG, PER, PPO, NAG and

protease, whereas NI only increased BG and protease activities (Table 3). This result suggested that unlikely NI which only enhanced BG and protease activities to mobilize labile C and N, PI activated a broad enzymatic repertoire to degrade complex organic matter, enhanced soil multifunctionality and sustaining long-term soil fertility (Shah et al., 2021; Whalen et al., 2024). Previous studies also reported that the soil microbial community of poor soil will have the advantage of increase soil nutrient and enhanced seedlings survival (Li et al., 2024; van der Heijden et al., 2015). Therefore, higher microbial activities further supported that PI could maintain N availability. However, compared to the PU/NU treatments, the PI and NI treatments not only introduced EMF but also introduced an intact soil microbial community (including bacteria, other fungi, protozoa, etc.). Thus, treatment effects reflect not only EMF colonization but also combined effects of mycorrhizal symbiosis and other microbiomes.

Additionally, the higher initial average well color development (AWCD) in PI and PU treatments during the first 4 days likely reflects the rapid activation of microbial community to labile carbon sources (Figure 4a). This aligns with the “fast response” traits of microbiomes, which are often enriched with bacteria capable of exploiting simple carbohydrates and organic acids from root exudates (de Graaff et al., 2010). PI maintained higher AWCD throughout incubation and significantly greater substrate utilization diversity (Figure 5b), indicating enhanced capacity to decompose diverse substrates (Sousa et al., 2011; Mahmood et al., 2024). PCA showed that NU and PU samples clustered closely (similar labile carbon-focused utilization) mainly due to ECM fungi removal induced community simplification, while PI and NI were functionally distinct, reflecting exogenous inoculum effects (Figure 4b). PI had the highest utilization rate of carbohydrates, amino acids, amides, and acids, while NI do best in esters and alcohols (Figure 5a). Collectively, PI treatment with rapid labile carbon response and broad substrate utilization, carried important ecological implications. Such communities better adapt to fluctuating carbon inputs and improve soil carbon cycling efficiency (Wagg et al., 2019), making PI a promising candidate for ecological restoration or soil improvement. However, the EMF communities colonizing seedling root tips and microbial communities in all treatments were not characterized in the present study, which limits the further differentiation and in-depth functional interpretation of key EMF and other microbial taxa.

Conclusion

This study clarified two distinct adaptive strategies of EMF communities from nitrogen-rich natural forests (NI) and nitrogen-depleted plantations (PI) in regulating N acquisition and growth of *P. asperata* seedlings. NI enhanced total biomass via a plant-centric strategy, by accelerating EMF colonization, optimizing root morphology, elevating root N influx rates, boosting nitrogen metabolic enzyme activities, and collectively strengthening host N acquisition and assimilation. In contrast, PI promoted biomass accumulation through a soil-focused strategy, by activating a broad spectrum of soil enzymes, diversifying microbial substrate utilization, and sustaining soil N availability despite seedling N

uptake. These findings demonstrate that EMF communities from fertile soils prioritize direct host nutrient uptake, while those from poor soils specialize in improving soil multifunctionality, highlighting adaptive specialization shaped by native soil nutrient conditions. This study underscores the practical significance of matching EMF inoculants to target soil environments. Future applications should leverage these adaptive traits to tailor EMF based interventions, thereby improving the efficacy of forest restoration under varying soil nutrient conditions.

Data availability statement

The datasets analyzed in this study are available from the corresponding author upon reasonable request.

Author contributions

LHL: Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. XP: Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. XL: Data curation, Formal analysis, Writing – review & editing. YH: Investigation, Methodology, Writing – review & editing. LL: Investigation, Resources, Writing – review & editing. NT: Methodology, Validation, Writing – review & editing. YD: Investigation, Validation, Writing – review & editing. CZ: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ffgc.2026.1839862/full#supplementary-material>

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