

Article

Mixed-Species Stands Improve the Coordination between Leaf and Fine Root Traits in a Common Garden Experiment

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Abstract: The coordination between leaf and root traits is conducive to an integrated understanding of whole-plant ecological strategies and reveals how community composition and diversity contribute to defining the functions and services of ecosystems. However, there is limited understanding regarding the impact of species richness and trait categories on the coordination between leaf and root traits. Based on a 9-year common garden experiment, we investigated the leaf and fine root traits of 56 plots (25.8 m × 25.8 m) encompassing various trait categories (trait categories were defined according to the root depth, leaf habit, and mycorrhizal type) and different levels of species richness (1, 2, 4, 8) in the context of a forest biodiversity and ecosystem functioning experiment conducted in subtropical China (BEF-China). We found the following: (1) Our findings indicate that there was generally a significant difference in leaf traits, occasionally in absorptive root traits, and no difference in transport root traits between different trait categories. (2) Conversely, species richness significantly influenced all transport root traits except root nitrogen and most leaf and absorptive root traits. (3) The results demonstrated that trait categories played a crucial role in the coordination between leaf and fine root traits. Additionally, the coordination between leaf and fine root traits increased with higher species richness, particularly in deep-rooted, evergreen, and ectomycorrhizal fungi species. Furthermore, the coordination between leaf and fine root traits was significantly lower in monocultures compared to four- and eight-species mixtures. These results suggest that a significant mixture effect exists in the coordination between leaf and fine root traits due to the comprehensive and divergent capture of above- and belowground resources and reduced intraspecific competition. Therefore, compared to monocultures, mixed-species stands can enhance the coordination of leaf and fine root traits, and it is advisable to establish forests with mixtures of more than four species, dominated by deep-rooted, evergreen, and ectomycorrhizal fungi species, to maintain ecosystem stability and functional integrity.

Keywords: functional traits; species richness; aboveground–belowground linkages; root depth; leaf habit; mycorrhizal types



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1. Introduction

Plant functional traits can reflect trade-offs between different physiological and ecological functions by responding to ecological strategies and environmental adaptations [1]. Functional traits are defined as key dimensions of ecological strategies directly related to growth, survival, and reproduction [2], forming a spectrum of phenotypic traits along the gradients of ecological and life-history strategies. A major achievement in trait-based ecology is the determination of the plant economics spectrum (PES), which helps to conceptually organize the trade-offs between conservative and acquisitive resources [3]. Functional traits refer to the morphological, chemical, physiological, and phenological features that

directly impact plant survival, growth and reproduction. They are crucial markers of how effectively a community acquires and makes use of plant resources. By identifying relationships between forest structures, processes, and ecosystem services, plant functional traits can explain trade-offs and synergies between these services [4]. Plant traits can be divided into morphological traits related to variations in the morphology and structure of plant organs and chemical traits associated with the allocation of nutrient elements [5].

Leaf traits can mirror plant adaptations to survival environments and play an important role in affecting ecosystem functioning, including productivity and carbon and water cycles [6]. The leaf economics spectrum (LES) is defined as the coordinated change in leaf structure and chemical traits along the resource availability gradient [7], and the worldwide leaf economic traits are roughly divided into structural, chemical, and photosynthetic traits [8]. Reich indicated that coordinated changes in leaf morphology (e.g., specific leaf area (SLA)), chemistry [e.g., leaf nitrogen and phosphorus (LN and LP)], and metabolism (e.g., photosynthetic capacity) help explain species strategy, community assembly, and ecosystem structure and function. Leaf dry matter content (LDMC), SLA, leaf area (LA), and LN were considered to be important traits in the LES [9]. Chlorophyll (CHL) is the key pigment for photosynthesis in plants, which is closely related to the growth and development of plants [10]; the LA of plant species controls surface availability and determines the photosynthetic capacity and water evaporation of plants [11]; the SLA reflects the absorption and utilization efficiency of light energy by plants [12]; the LDMC reflects the growth rate and biomass of the plant [13]; and LN and LP play crucial roles in plant metabolism and function, serving as reliable indicators of plant photosynthetic capacity and strategies for resource utilization, which play an important role in plant growth and reproduction [14]. The LES represents a combination of direct and indirect causal relationships among leaf traits [7]. It reveals consistent correlations across a range of leaf traits that reflect a continuum from conservative to acquisitive plant strategies [15]. The fast acquisition of resources is usually associated with a high SLA, LN, and LP, while a high LDMC reflects resource-conservative strategies [16]. Acquisitive species are characterized by rapid rates of growth and leaf turnover and being resource-acquisitive, whereas conservative species exhibit slower rates of growth and turnover [13].

Fine roots, which are traditionally defined as roots with a diameter of ≤ 2 mm, play a critical role in the acquisition of water and mineral nutrients from the soil [17]. Additionally, fine root traits can serve as indicators of a plant's adaptability to the soil environment [18]. Fine roots can be categorized into two types: absorptive roots and transport roots. Absorptive roots, primarily responsible for acquiring and absorbing soil resources, constitute the most distal root system. In contrast, transport roots are more abundant in the branch hierarchy and serve mainly structural and transport functions, while also possessing some storage capacity [19]. In the largest root database to date, the global root traits (GRooT) database [20], the results suggest that the most commonly measured root morphological and chemical parameters are specific root length (SRL; 31.7% of all species in the database), root nitrogen concentrations (RN; 27.6%), root diameter (DIA; 26.0%), and root tissue density (RTD; 23.6%) [21]. The DIA reflects the thickness of plant roots, which is closely related to the water and nutrient uptake capacity of plants; the SRL and specific root area (SRA) reflect the length and surface area of plant roots, respectively, and are related to plant growth and adaptability; the RTD reflects the density and mechanical strength of plant roots [22]; and RN and root phosphorus (RP) are essential nutrients for plants, playing a critical role in plant growth and reproduction [23]. Fine-root morphological traits are indicative of plants' feedback mechanisms to ecosystem processes [22]. The presence of a root economics spectrum (RES) in plants (analogous to LES) embodies trade-off strategies in root economics between growth productivity and structural persistence [24]. Root traits have diverged along a continuum defined by two antithetical life strategies: an ancestral 'conservative' strategy, where plants with thicker roots rely on symbiotic relationships with mycorrhizal fungi for nutrient acquisition, and a derived 'acquisitive' strategy, wherein thinner roots facilitate a more efficient utilization of photosynthetic carbon for soil explo-

ration [25]. Unique root morphology and chemical traits (i.e., high SRL, SRA, RTD, and root nitrogen concentrations) indicate resource-acquisitive plants, while a large DIA and root C:N ratio indicate resource-conservative plants [26].

Leaves and roots are the aboveground and belowground structures of plants, respectively, serving the primary function of acquiring resources for growth and defense [27]. The linkages between leaf and root traits are currently a prominent area of research in understanding the covariation in traits in entire plants, playing a crucial role in comprehending the ecological strategies adopted by whole plants [28]. In resource-limited environments, plants actively allocate resources among various functional traits, thereby establishing internal connections and trade-off relationships [29]. Fundamental trade-offs exist between plant function and functional traits related to resource-acquisitive or resource-conservative strategies [24]. The plant economics spectrum hypothesis suggests that under conditions of strong biophysical limitation and environmental selection, there is coordination between aboveground and belowground features, tissue biomass construction costs, and resource uptake [30]. The coordination between leaf and root traits can jointly determine the performance of species [8]. It helps predict the properties of underground organs that cannot be seen [28]. Leaf and root traits can adapt to environmental changes through trade-offs or coordinated effects, enabling plants to adopt corresponding ecological strategies to maintain growth [31]. Multidimensional coordination between root and leaf traits may result in certain root traits being coordinated with leaf traits, while others vary independently in terms of resource acquisition techniques [32]. Strong correlations between traits with different functions reveal trade-offs or a coordination that limits and harmonizes plant function. For example, low-cost morphological phenotypes of leaves and absorbing roots (high SLA and SRL) are often associated with other traits that promote rapid growth and resource acquisition (high nutrient concentrations) [33]. However, there is still a lack of studies on the trade-offs or coordinated effects between leaves and roots across different species richness levels.

There are differences in leaf economic traits between evergreen and deciduous species. Deciduous species, characterized by frequent defoliation and new leaf growth, have a high demand for soil nutrients and adopt a rapid resource acquisition strategy [13]. In contrast, evergreen species have a long leaf lifespan, a low demand for nutrients, and adopt a conservative resource utilization strategy [34]. Different types of mycorrhizal fungi, such as ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM), can result in variations in root morphology [35]. AM species typically adopt a “quick investment-return” strategy [36], while ECM species prefer a “slow investment-return” strategy [37]. Based on their root depth characteristics, species can be classified as either deep-rooted or shallow-rooted. Deep-rooted tree species primarily rely on groundwater absorption from deep soil to enhance their survival ability [38], whereas shallow-rooted species are typically found in shallow soil and mainly rely on precipitation to access shallow soil water [39].

Tree species richness is expected to induce physiological responses in trees, which are reflected in changes in functional leaf traits, especially those related to light interception, photosynthesis, and drought stress response [40]. High species richness may increase resource–use complementarity, leading to niche-partitioning effects and reducing competition for space and nutrients. This leads to increased resource uptake by hybrid species, more vigorous growth, and increased investment in chemical and physical defenses [41]. Plant functional traits vary widely across habitats and species globally [42]. Plant functional traits can predict how species respond to interspecific interactions at the individual level [43], as well as how a community utilizes limited resources at the community level [44]. However, there is limited understanding regarding how plant traits of different trait categories respond to changes in species richness. The approach of using common garden experiments has a long-standing tradition in the field of plant evolutionary ecology, where multiple genotypes are grown in a shared environment, allowing for the attribution of observed phenotypic variation in the field to genetic causes or environmental factors [45]. The study of the variation in and correlation of plant functional traits can objectively reflect

the differences in physiological processes and adaptation strategies of plants to the external environment, thus providing a significant breakthrough in understanding ecological topics such as plant community assembly and biodiversity maintenance mechanisms [46]. In our 9-year common garden experiment, we examined the variations in leaf and root traits among different trait categories at varying levels of species richness, i.e., the number of tree species. Our study aimed to address the following questions: (i) How do leaf and root traits respond to tree diversity among different trait categories? (ii) How does the coordination between leaf and root traits change with different species richness?

2. Materials and Methods

2.1. Study Site

The study was conducted in the forest biodiversity and ecosystem functioning experiment in subtropical China (BEF-China) experiment near Xingangshan township, Dexing, in southeast China's Jiangxi Province (latitude 29°08'–29°11' N, longitude 117°90'–117°93' E). The region has a typical subtropical climate, characterized by a mean annual temperature of 16.7 °C and an annual precipitation of 1821 mm. The natural vegetation in the region consists of subtropical forests that include a mixture of evergreen and deciduous species [47]. The area is dominated by Cambisols and Cambisol derivatives as the main soil types. Regosols are found on the ridges and crests, while Anthrosols result from colluvial deposits on the foot slopes and valley floors [44]. This study focused on site A of the BEF-China experiment, which was established by 400 one-year-old seedlings with a 1.29 m spacing distance for each plot in the spring of 2009. Site A covers a hilly area of 26.6 ha, with an altitude ranging from 105 to 275 m and slopes varying from 0 to 45 degrees. It consists of a total of 271 plots. Each plot has a projected ground area of 666.7 m² (25.8 × 25.8 m). Six levels of tree species richness were established, namely 1, 2, 4, 8, 16, and 24, using a broken-stick design that ensures equal representation of all tree species across the different diversity levels [47].

2.2. Study Plots

In July 2018, field sampling was conducted in 56 plots of a random extinction series with two replicates of each species' composition. These compositions followed a broken-stick design and included a pool of 16 species [47]. Two species, *Rhus chinensis* and *Castanopsis eyrei*, were excluded from this study due to their small sample size and low survival rate (Table 1). Based on different classification standards of root depth, leaf habit, and mycorrhizal type, all tree species were divided into three categories. These categories included 9 deep-rooted species, 5 shallow-rooted species, 4 evergreen species, 10 deciduous species, 6 ECM species, and 8 AM species. The classification of mycorrhizal types was referred to in a previous study in BEF-China [48]. In total, we sampled 56 plots, which consisted of twenty-eight monocultures, sixteen 2-species mixtures, eight 4-species mixtures, and four 8-species mixtures.

Table 1. List of tree species in plots.

| Species Name | Abbreviation | Family Name | Root Depth | Leaf Habit | Mycorrhizal Type |
|---|--------------|----------------|----------------|------------|------------------|
| <i>Castanea henryi</i> (Skan) Rehd. et Wils. | CaHe | Fagaceae | deep-rooted | deciduous | ECM |
| <i>Castanopsis sclerophylla</i> (Lindley & Paxton) Schottky | CaSc | Fagaceae | deep-rooted | evergreen | ECM |
| <i>Choerospondias axillaris</i> (Roxb.) Burt et Hill | ChAx | Anacardiaceae | shallow-rooted | deciduous | AM |
| <i>Cyclobalanopsis glauca</i> (Thunberg) Oersted | CyGl | Fagaceae | deep-rooted | evergreen | ECM |
| <i>Cyclobalanopsis myrsinifolia</i> (Blume) Oersted | CyMy | Fagaceae | deep-rooted | evergreen | ECM |
| <i>Koelreuteria bipinnata</i> Franch. | KoBi | Sapindaceae | shallow-rooted | deciduous | AM |
| <i>Liquidambar formosana</i> Hance | LiFo | Hamamelidaceae | deep-rooted | deciduous | AM |

Table 1. Cont.

| Species Name | Abbreviation | Family Name | Root Depth | Leaf Habit | Mycorrhizal Type |
|---|--------------|---------------|----------------|------------|------------------|
| <i>Lithocarpus glaber</i> (Thunb.) Nakai | LiGl | Fagaceae | deep-rooted | evergreen | ECM |
| <i>Nyssa sinensis</i> Oliver | NySi | Nyssaceae | deep-rooted | deciduous | AM |
| <i>Quercus fabri</i> Hance | QuFa | Fagaceae | deep-rooted | deciduous | ECM |
| <i>Quercus serrata</i> Murray | QuSe | Fagaceae | deep-rooted | deciduous | ECM |
| <i>Sapindus saponaria</i> L. | SaSa | Sapindaceae | shallow-rooted | deciduous | AM |
| <i>Schima superba</i> Gardn. et Champ. | ScSu | Theaceae | shallow-rooted | evergreen | AM |
| <i>Triadica sebifera</i> (Linnaeus) Small | TrSe | Euphorbiaceae | deep-rooted | deciduous | AM |

Note: ectomycorrhiza (ECM); arbuscular mycorrhizal (AM).

2.3. Data Collection

In the 9-year experiment, a total of 672 individuals were sampled, including 6 individuals per species in each plot. This common garden experiment had the same stand age (9 years), stand density (6000 individuals/ha), row spacing (1.29 m), and similar tree heights, basal diameters, and basal areas (Table 2). In July 2018, ten intact green and mature leaves exposed to sunlight were collected from the upper part of the canopy for each individual [18]. The leaf samples were submerged in water overnight (approximately 12 h), then blotted with clean paper and scanned using a photo scanner (Epson Perfection V39; Epson, Suwa, Japan). Leaf area (cm²) was determined from these images using ImageJ [28]. All leaf samples were oven-dried at 65 °C for more than 48 h until a constant mass was achieved and then retained for subsequent chemical analysis. The specific leaf area (SLA; mm² mg⁻¹) of each sample was calculated as the ratio of total leaf area to oven-dry weight, and the leaf dry matter content (LDMC; mg g⁻¹) was measured as the ratio of leaf oven-dry weight to water-saturated weight. The chlorophyll content in fresh leaves was determined using the alcohol extraction–colorimetric method [49].

Table 2. List of basic information about forest stands of different species richness.

| Species Richness | Tree Height (m) | Basal Diameter (cm) | Basal Area (cm ²) |
|------------------|-----------------|---------------------|-------------------------------|
| 1 | 5.36 ± 2.36 a | 7.84 ± 4.17 a | 61.84 ± 64.44 a |
| 2 | 4.69 ± 2.85 a | 7.24 ± 4.43 a | 56.48 ± 65.38 a |
| 4 | 5.49 ± 2.50 a | 8.51 ± 5.28 a | 78.52 ± 84.79 a |
| 8 | 4.98 ± 2.21 a | 7.16 ± 4.33 a | 54.74 ± 58.85 a |

Note: the lowercase letters mean the differences among different species richness ($p < 0.05$).

For all individuals sampled from leaves, at least two intact parts of the root systems (with a maximum diameter of 2~5 mm) were randomly selected by tracing lateral roots through the surface soil (depth 0~15 cm). The sampled parts of the root systems were placed in a plastic bag and stored in a portable cooler for transportation from the field to the laboratory. Fine roots (≤ 2 mm) were functionally classified into two types: shorter-lived absorptive roots and longer-lived transport fine roots, as defined by the method of McCormack [19]. Roots were scanned with a high-resolution scanner (Epson Perfection LA324000; Epson, Suwa, Japan) and analyzed with WinRHIZO Pro software (v2009, Regent Instrument, Quebec, Canada) to measure root diameter and lengths. After trait measurements, the root samples were oven-dried at 65 °C for a minimum of 48 h, and the weights were recorded after confirming a constant weight. Specific root area (SRA; m² kg⁻¹) was calculated as the ratio of root surface area to dry mass. Specific root length (SRL; m g⁻¹) was calculated as the ratio of root length to dry mass. Root tissue density (RTD; g cm⁻³) was calculated as the ratio of root dry mass to fresh volume.

All the leaf and root samples were dried in an oven at 65 °C for 48 h and then crushed. The total nitrogen concentrations of the leaf and root samples were determined using an Elemental Analyzer (Elementar Vario EL III; Elementar, Langensfeld, Germany). The total phosphorus concentrations of the leaf and root samples were examined using the

molybdenum–antimony anti-colorimetric method [50]. The abbreviations of all parameters are given in Table 3.

Table 3. Abbreviations and meanings of all parameters.

| Parameters | Abbreviation | Parameters | Abbreviation |
|-------------------------|--------------|----------------------------|--------------|
| Species richness | SR | Leaf habit | LH |
| Root depth | RD | Mycorrhizal type | MT |
| Chlorophyll | CHL | Root diameter | DIA |
| Specific leaf area | SLA | Specific root length | SRL |
| Leaf area | LA | Specific root surface area | SRA |
| Leaf dry matter content | LDMC | Root tissue density | RTD |
| Leaf nitrogen | LN | Root nitrogen | RN |
| Leaf phosphorus | LP | Root phosphorus | RP |

2.4. Statistical Analysis

To examine the effects of different trait categories (root depth, leaf habit, mycorrhizal type) and species richness on the variations in leaf, absorptive root, and transport root traits, a two-way ANOVA, followed by Tukey’s multiple comparisons test ($p < 0.05$), was conducted. A redundancy analysis (RDA) was carried out for each trait category on the relationships between leaf, absorptive root, and transport root trait matrixes along different species richness levels. In the RDA, the leaf trait matrixes were used as explanatory variables, and the absorptive root and transport root trait matrixes were used as response variables to analyze the data [51]. The degree to which leaf traits are correlated with fine root traits (the explained variation in the RDA, R^2) was determined to reveal the coordination between leaf and root traits across different trait categories and species richness levels in this study. All statistical analyses and plots were created using the statistical software R4.3.1 [52].

3. Results

3.1. Variations in Leaf and Root Traits among Different Trait Categories at Different Species Richness Levels

3.1.1. Variations in Leaf Traits across Different Trait Categories at Different Species Richness Levels

The root depth, leaf habit, and mycorrhizal type of tree species shown in Table 1 exhibited significant effects on the amount of CHL and the SLA, LA, LDMC, LN, and LP (Figure 1 and Table S1). Species richness had a significant effect on the SLA, LA, LDMC, LN, and LP, regardless of trait categories, but did not significantly affect CHL content. The interaction between root depth and tree species richness significantly influenced the CHL, LN, and LP contents, while the interaction between leaf habit and tree species richness significantly affected the SLA and LN. The interaction between mycorrhizal type and tree species richness significantly impacted the CHL and LN contents. The deep-rooted species generally had a lower SLA and LP but a higher LA and LDMC compared to the shallow-rooted species. The deciduous species exhibited a lower CHL and LDMC but a higher SLA, LA, LN, and LP compared to the evergreen species. The ECM species demonstrated a higher CHL and LDMC but a lower SLA, LA, and LP compared to the AM species.

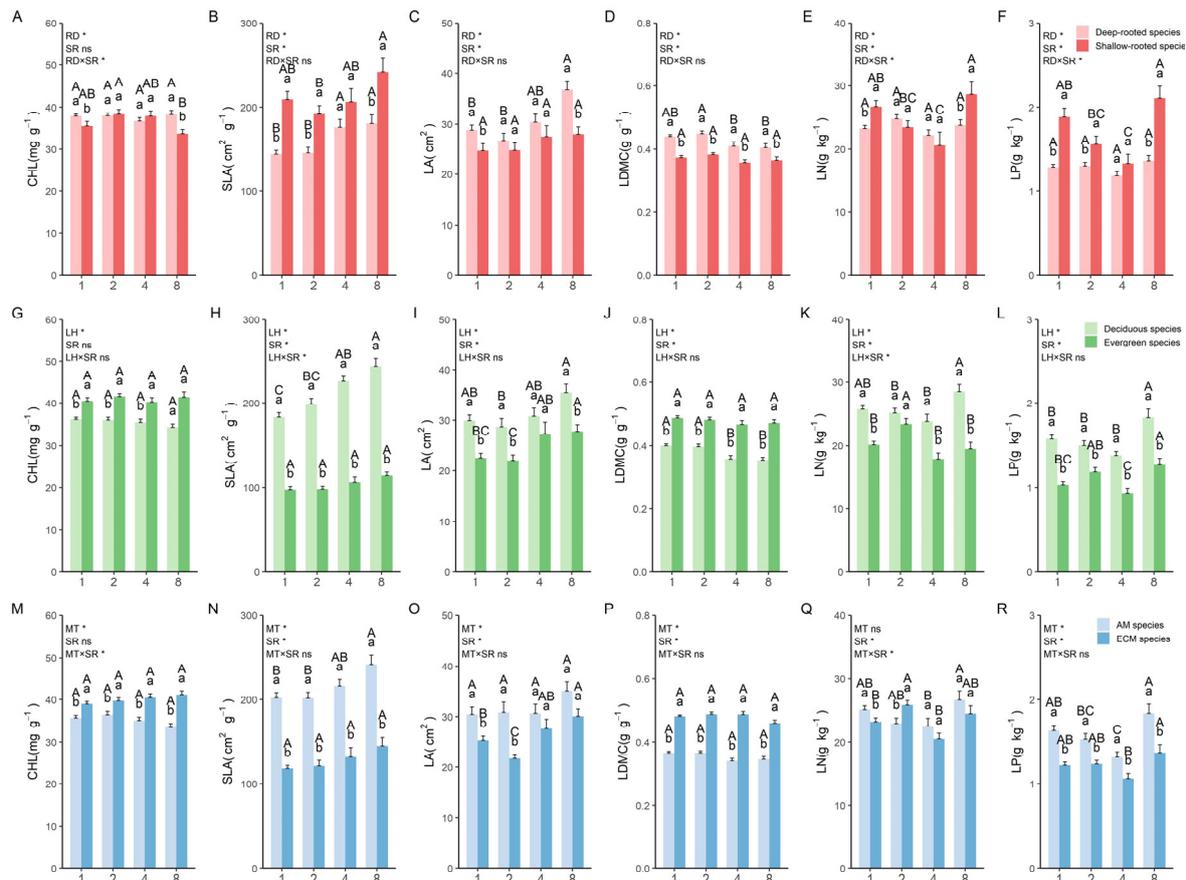


Figure 1. Variations in the CHL (A), SLA (B), LA (C), LDMC (D), LN (E) and LP (F) for root deep-rooted and shallow-rooted species; Variations in the CHL (G), SLA (H), LA (I), LDMC (J), LN (K) and LP (L) for deciduous and evergreen species; Variations in the CHL (M), SLA (N), LA (O), LDMC (P), LN (Q) and LP (R) for AM and ECM species. Note: The lowercase letters mean the differences among different trait categories ($p < 0.05$). The uppercase letters mean the differences among different species richness levels ($p < 0.05$). The “ns” indicates no significant difference ($p > 0.05$), whereas the “*” indicates a significant difference ($p < 0.05$) among different trait categories, species richness, and the interactions between trait categories and species richness (Tukey’s HSD). Root depth (RD), species richness (SR), leaf habit (LH), mycorrhizal type (MT), chlorophyll (CHL), specific leaf area (SLA), leaf area (LA), leaf dry matter content (LDMC), leaf nitrogen (LN), and leaf phosphorus (LP).

3.1.2. Variations in Absorptive Root Traits across Different Trait Categories at Different Species Richness Levels

In the absorptive root, root depth significantly affected SRL, SRA, and RP, while leaf habit significantly influenced the DIA, RTD, and RN. However, the mycorrhizal type only showed significant effects on the RP (Figure 2 and Table S2). Regarding root depth types, species richness had a significant effect on the SRL, SRA, RTD, and RP. In terms of leaf habit types and mycorrhizal types, species richness significantly influenced the SRL, SRA, RTD, and RP. The interaction between leaf habit and tree species richness significantly influenced the SRL, SRA, RTD, and RP, while the interaction between mycorrhizal type and tree species richness only had a significant effect on SRL. The deep-rooted species generally exhibited a higher SLA, SRA, and RP compared to the shallow-rooted species, while there was no significant effect on the DIA, RTD, and RN between the deep-rooted and shallow-rooted species. The deciduous species generally showed a higher DIA and RN compared to the evergreen species, while there was no significant effect on the SRL, SRA, RTD, and RP between the deciduous and evergreen species. Similarly, there was

no significant effect on the DIA, SRL, SRA, RTD, RN, and RP between the AM and ECM species.

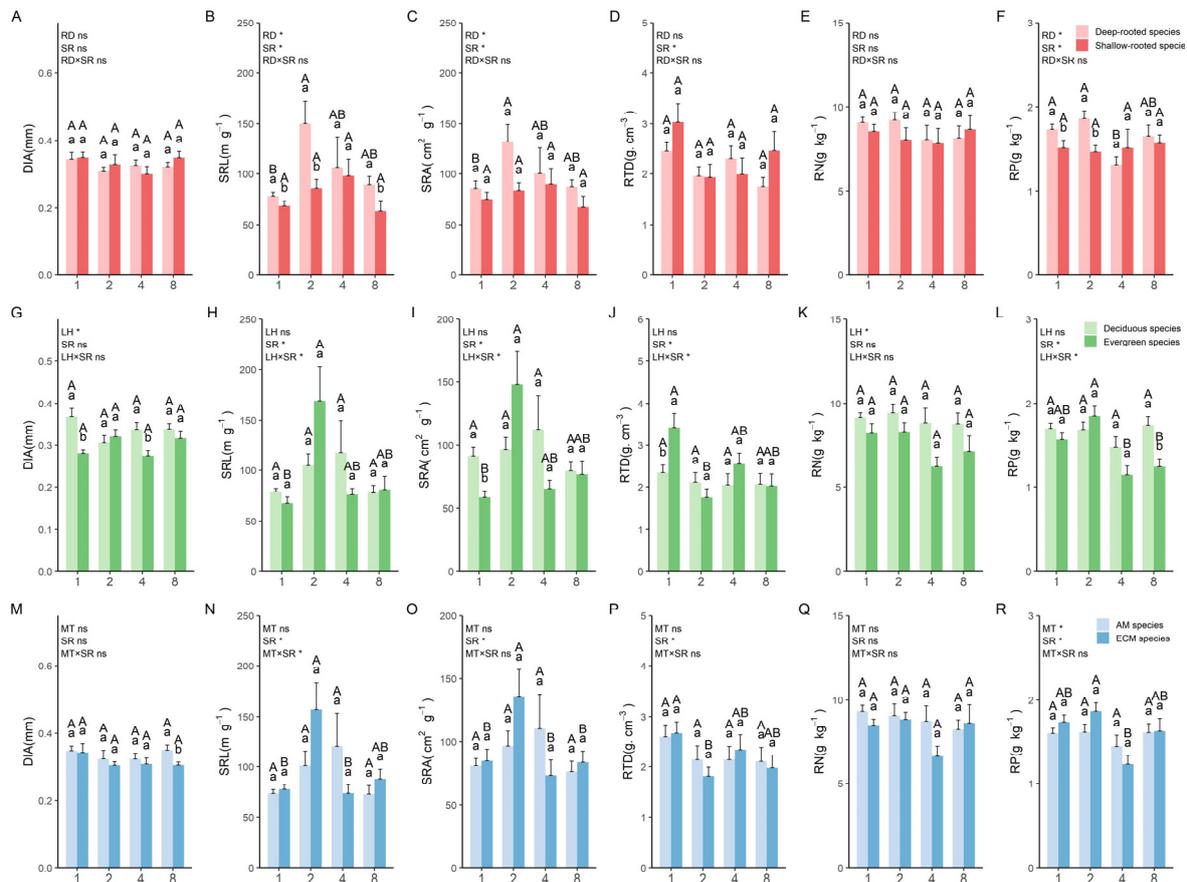


Figure 2. Variations in the DIA (A), SRL (B), SRA (C), RTD (D), RN (E) and RP (F) of absorptive roots for root deep-rooted and shallow-rooted species; Variations in the DIA (G), SRL (H), SRA (I), RTD (J), RN (K) and RP (L) of absorptive roots for deciduous and evergreen species; Variations in the DIA (M), SRL (N), SRA (O), RTD (P), RN (Q) and RP (R) of absorptive roots for AM and ECM species. Note: The lowercase letters mean the differences among different trait categories ($p < 0.05$). The uppercase letters mean the differences among different species richness levels ($p < 0.05$). The “ns” indicates no significant difference ($p > 0.05$), whereas the “*” indicates a significant difference ($p < 0.05$) among different trait categories, species richness, and the interactions between trait categories and species richness (Tukey’s HSD). Root depth (RD), species richness (SR), leaf habit (LH), mycorrhizal type (MT), root diameter (DIA), specific root length (SRL), specific root surface area (SRA), root tissue density (RTD), root nitrogen (RN), and root phosphorus (RP).

3.1.3. Variations in Transport Root Traits across Different Trait Categories at Different Species Richness Levels

There were no significant differences in the DIA, SRL, SRA, RTD, RN, and RP for the transport roots between the different trait categories (root depth, leaf habit, and mycorrhizal type), while species richness showed significant effects on all the transport root traits except RP (Figure 3 and Table S3). Among the interactions, only the interaction between leaf habit and tree species richness significantly affected the RTD. In the deep-rooted species, the DIA and SRA increased with increasing species richness, while the RTD decreased. In the evergreen, deciduous, and AM species, the DIA increased with increasing species richness, while the RTD decreased.

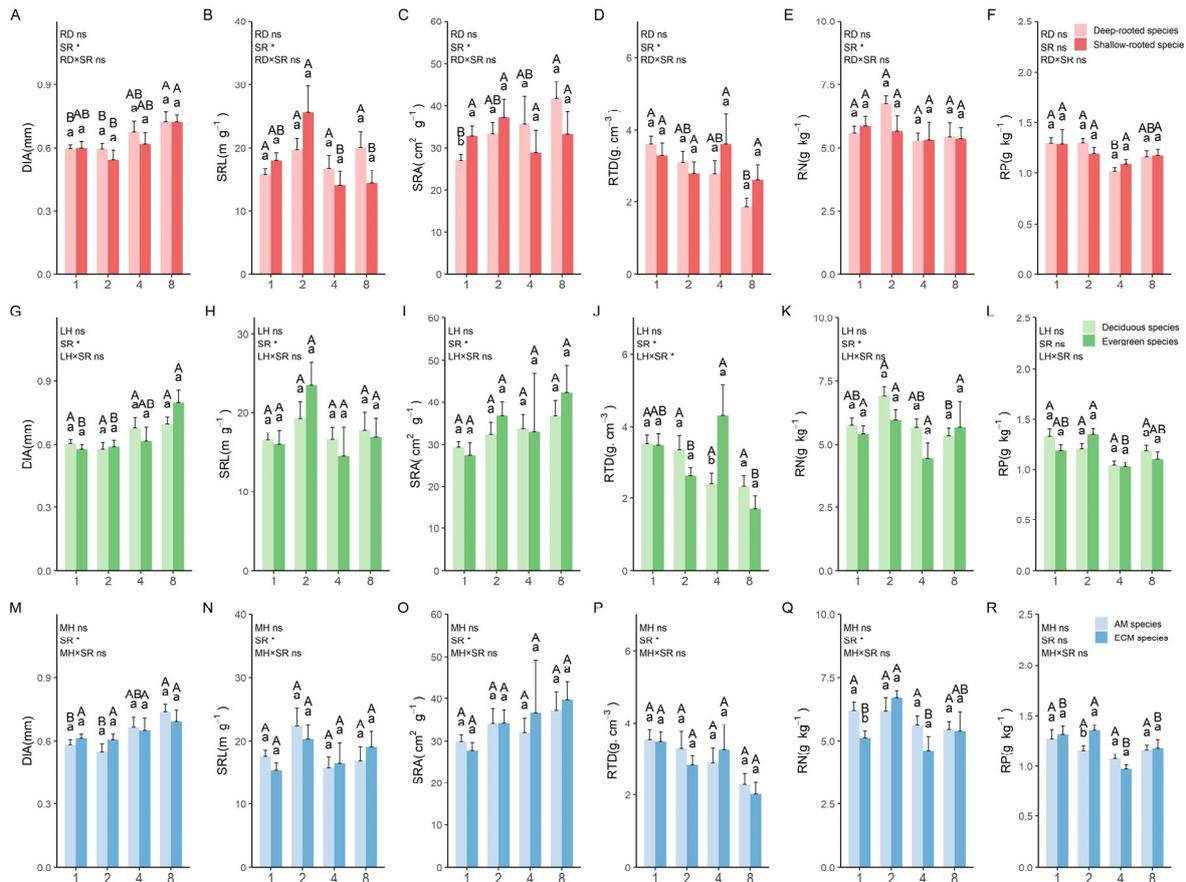


Figure 3. Variations in the DIA (A), SRL (B), SRA (C), RTD (D), RN (E) and RP (F) of transport roots for root deep-rooted and shallow-rooted species; Variations in the DIA (G), SRL (H), SRA (I), RTD (J), RN (K) and RP (L) of transport roots for deciduous and evergreen species; Variations in the DIA (M), SRL (N), SRA (O), RTD (P), RN (Q) and RP (R) of transport roots for AM and ECM species. Note: The lowercase letters mean the differences among different trait categories ($p < 0.05$). The uppercase letters mean the differences among different species richness ($p < 0.05$). The “ns” indicates no significant difference ($p > 0.05$), whereas the “*” indicates a significant difference ($p < 0.05$) among different trait categories, species richness, and the interactions between trait categories and species richness (Tukey’s HSD). Root depth (RD), species richness (SR), leaf habit (LH), mycorrhizal type (MT), root diameter (DIA), specific root length (SRL), specific root surface area (SRA), root tissue density (RTD), root nitrogen (RN), and root phosphorus (RP).

In summary, there was generally a significant difference in leaf traits, occasionally in absorptive root traits, and no difference in transport root traits between the different trait categories. Conversely, species richness significantly influenced all the transport root traits except RN and most leaf and absorptive root traits. Overall, the interaction between trait categories and species richness did not significantly affect the leaf and fine root traits.

3.2. The Coordination between Leaf and Fine Root Traits

The redundancy analysis revealed that the explained variation between the leaf and fine root traits in the deep-rooted, evergreen, and ECM species increased with increasing species richness (Figure 4A). In the shallow-rooted species, the explained variation between the leaf and fine root traits increased from the one- to the four-species mixture but decreased from the four- to the eight-species mixture. In the deep-rooted species and ECM species, the explained variation between the leaf and fine root traits gradually increased from the one- to the two-species mixture and then sharply increased to approximately 50% from the two- to the eight-species mixture. In the evergreen species, the explained variation

between the leaf and fine root traits increased linearly to half of the total variation (52.8%) from the one- to the eight-species mixture. In the deciduous species and AM species, the explained variation between the leaf and fine root traits fluctuated between 8.6% and 20.6% from the one- to the eight-species mixture. On average, the explained variation between the leaf and fine root traits in the monocultures (11%) was significantly lower than that in the four- and eight-species mixtures (over 35% in Figure 4B). The explained variation between the leaf and fine root traits was higher in the shallow-rooted species from the one- to the four-species mixture but lower in the eight-species mixture compared to the deep-rooted species.

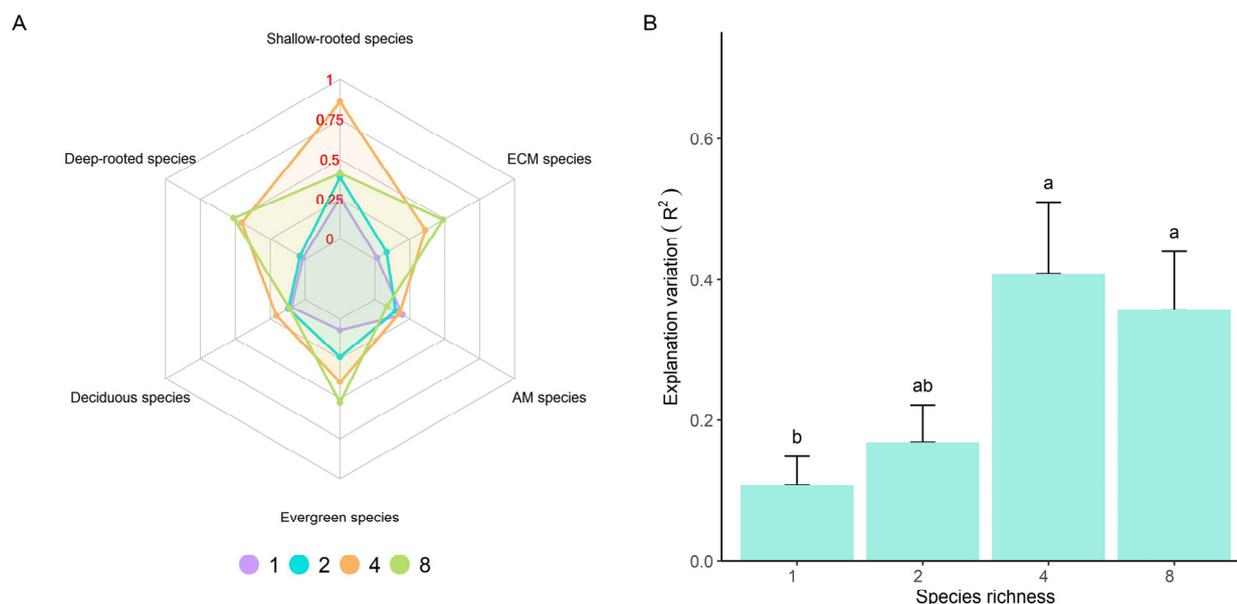


Figure 4. Radar plot of the coordination between leaf and fine root traits across different trait categories at different species richness levels (A) and the average explained variation across different tree species richness levels (B). Note: the lowercase letters mean the differences among different species richness levels ($p < 0.05$).

The explained variation between the leaf and fine root traits in the monocultures was similar (about 10%) in both the deciduous and evergreen species, but the gap between the deciduous and evergreen species quickly widened from the two- to the eight-species mixture. The explained variation between the leaf and fine root traits was higher in the AM species than in the ECM species from the one- to the two-species mixture, but it sharply reversed from the four- to the eight-species mixture.

In conclusion, trait categories played a crucial role in the coordination between the leaf and fine root traits, while species richness had a significant impact on this coordination in the shallow-rooted, deep-rooted, evergreen, and ECM species.

4. Discussion

4.1. Effects of Trait Categories and Species Richness on Leaf and Root Traits

4.1.1. Effects of Trait Categories and Species Richness on Leaf Traits

We found there were significant differences in the leaf and fine root traits across different species in the monocultures (Figures S1 and S2). These tree species were divided into different trait categories, and each trait category represents a unique ecological strategy and way of using resources [8]. Our results showed that species richness had a significant effect on SLA, LA, LDMC, LN, and LP, regardless of trait categories, implying that an increase in species richness would have an important impact on the morphological and physiological characteristics of plants [53]. Due to the complementarity of resources among different species, higher species richness will reduce the level of competition between

species and reduce environmental pressure [53]. Our results showed that there were significant differences in the leaf and root traits between the monocultures and species mixtures. Similarly, a meta-analysis of 43 studies related to eight physical and chemical leaf traits showed that all traits except leaf thickness were highly plastic and exhibited significant differences between plant monocultures and species mixtures [41].

Usually, there were lower SLA and LP and higher LA and LDMC in the deep-rooted species than in the shallow-rooted species (Figure 1). Water is more abundant in deep soil, so a longer (deeper) root system is required to reach it [38]. More water provides the opportunity to grow LA and more evaporation, which cools the plant in high-temperature conditions [6]. A larger LA allows photosynthetic chloroplasts to arrange themselves over a larger surface, so they do not have to rely on a larger SLA because they can evaporate more. Deep-rooted species grown in nitrogen-restricted soils have higher leaf thickness and dry mass content values, typically associated with a “resource conservation” strategy and low relative growth rates [54]. The root depth of shallow-rooted tree species is relatively shallow, mainly dependent on the nutrient supply in the topsoil [39]. When the root system is concentrated close to the surface, the transpiration associated with hydraulic lift increases more strongly [55]. Intense water stress may inhibit plant growth to some extent, leading to a higher SLA and a lower LDMC [56].

Our study found significant differences in the leaf traits between deciduous and evergreen species (Figure 1). Because deciduous species grow faster and adopt “resource-acquisitive” strategies, they have higher SLA, LA, LN, LP and lower LDMC [13]. However, in order to withstand physical damage by constructing tissues with thick layers or high tissue densities that are expensive, evergreen species exhibit a “resource conservation” strategy with a low SLA and a high LDMC [34]. Evergreen species have longer leaf lifespans and require more biomass investment to control their structural integrity and defend against disturbances [57]. In order to improve resource use efficiency and enhance the adaptability of poor habitats, the leaf traits of evergreen species maintain low photosynthesis and transpiration rates [58]. On the contrary, deciduous species have high nutrient requirements for nitrogen and phosphorus in order to achieve stronger photosynthetic capacity and faster nutrient cycling, as well as to maximize the capture of resources during the limited growing season [58].

There were higher CHL and LDMC and lower SLA, LA, and LP in the ECM species than in the AM species. ECM species tend to have a “resource conservation” strategy, and ECM fungi promote the enhancement of their defense ability, i.e., a higher LDMC [59]. ECM fungi have evolved a unique ability to obtain nutrients directly from organic matter, reducing their carbon investment in nutrient uptake, resulting in lower nitrogen and phosphorus uptake [48]. AM species may suffer more pathogenic stress in the same soil [60]. As a result, AM species employ a “resource acquisition” strategy, where AM fungi are highly effective at absorbing inorganic nutrients and can absorb large amounts of nutrients to resist environmental stresses, reduce the accumulation of specific pathogens, and enhance interspecific (e.g., synergistic or competitive) relationships [60]. The SLA of the AM species was higher, indicating that their resource utilization efficiency was higher, which was conducive to the acquisition of photosynthesis and improved the nutrient competitiveness of the AM tree species [61].

4.1.2. Effects of Trait Categories and Species Richness on Root Traits

In order to maximize the uptake and utilization of water and nutrients in the soil, there are significant differences in root functional traits among different tree species (Figure S2), where the absorptive roots focus on extracting resources from the soil, while the transport roots are responsible for transporting these resources to various parts of the tree [62]. Our study demonstrated that there was generally a significant difference in leaf traits, occasionally in absorptive root traits, and no difference in transport root traits between the different trait categories [63]. These results may be due to the different functions of different organs of plants. The leaves are exposed to rapid changes in climatic factors. Leaf

functional traits are very sensitive to climate change, reflecting the high adaptability of plants to the environment and their ability to self-regulate in complex habitats [64]. The degree of plasticity of the absorptive roots varies from species to species in response to changing soil environmental conditions, including nutrient and water availability and soil chemistry and structure. In contrast, transport roots are less susceptible to changes in the soil environment [65]. Transport roots, as higher-order structures within the plant, are responsible for the internal transport of nutrients and carbon compounds and do not exchange carbon and nutrients directly with the soil [65].

Higher water and nutrient availability in the topsoil can promote the growth of absorptive roots compared to deeper soils, and absorptive roots located in the topsoil layer can produce a higher benefit-to-cost ratio (resource access versus carbon investment) [66]. Therefore, the SLA, SRA, and RP of the deep-rooted species were higher than those of the shallow-rooted species in the absorptive roots. Previous studies have also confirmed that the carbon uptake efficiency of deep-rooted species is generally higher than that of shallow-rooted species [67], suggesting that deep-rooted species are less expensive to maintain compared to higher construction investments. When species richness is high, deep-rooted species may be subject to intense competition for water and nutrient resources in deep soils. To better adapt to this competitive environment, deep-rooted species can reduce the number of roots and improve their quality, resulting in more efficient access to water and nutrients [66]. The results showed that the transport root DIA and SRA increased with increasing species richness, while the RTD decreased with increasing deep-rooted species richness. The appearance of new leaves in deciduous species is relatively frequent, and in order to meet the nutrient requirements of plants, there is a large demand for soil nutrients [58]. Therefore, in order to form a competitive advantage in coexistence with evergreen species, deciduous tree species must obtain large amounts of nutrients such as nitrogen and phosphorus and need to penetrate the soil through thicker roots, making water and nutrient transport more efficient [68].

4.2. The Coordination between Leaf and Fine Root Traits

The redundancy analysis showed that the explained variation between the leaf and fine root traits in the deep-rooted, evergreen, and ECM species increased with an increase in species richness (Figure 4). Moreover, the explained variation between the leaf and fine root traits in the monocultures was significantly two times lower than that in the four- and eight-species mixtures. These results indicate that there is a significant mixture effect in the coordination between leaf and fine root traits. Higher explained variation provides a large synchronization between above- and belowground systems, indicating greater coordination between leaves and roots. The coordination between leaves and fine roots means that the aboveground and under-ground parts must be coordinated in terms of access to a distribution of limited resources and adaptation to environmental factors [29]. However, there are also “management constraints” that may lead to functional convergence between aboveground and belowground traits, i.e., leaf and fine root traits exhibit coordinated changes at the community level [69]. Different plants may exhibit a convergence of functional traits under the same habitat conditions, as plants with similar functional traits may be more likely to gain an advantage in competition and thus achieve population continuity [70,71]. In an ecosystem with a high level of diversity, different species may develop different ecological niches using the resources they are good at and maximizing their own growth and reproduction through resource differentiation [72]. Furthermore, when species richness increases, intraspecific competition and negative density dependency are alleviated, and different species are better able to use and allocate different resources, making leaves and fine roots more effective in resource acquisition and utilization [4]. By influencing the functional diversity, ecological adaptation strategies, and environmental responsiveness of vegetation, species richness can promote the efficient use of resources by plant communities and maintain the stability and functional integrity of ecosystems [72]. The typical vegetation of subtropical evergreen broad-leaved forests in China has the characteristics

of evergreen, leathery leaves and a bright surface, and Fagaceae is one of the main tree species in this type of vegetation [73], with a high ectomycorrhizal fungal infection rate and rich fungal diversity [74]. This is consistent with our findings that evergreen, ECM, and deep-rooted species have advantages in the coordination between leaf and fine root traits.

The root system of shallow-rooted species is densely distributed in the surface soil, and its contact area with nutrient resources is larger, meaning it can obtain more water and nutrients in the soil and promote the root growth of shallow-rooted species [39]. In order to gain more aboveground biomass, shallow-rooted species may improve the nutrient uptake and utilization efficiency of the root system by increasing the coordination between leaf and fine root traits [75]. However, the roots of deep-rooted species grow in deeper soils and need to allocate more biomass for root growth [76], which may lead to low coordination of nutrient supply between the leaves and roots, resulting in relatively weak aboveground growth. This different biomass allocation mechanism may result in less coordination between the leaf and fine root traits in deep-rooted species than in shallow-rooted species (Figures 4 and S3).

Studies have shown that evergreen species generally have higher coordination between leaf and fine root traits than deciduous species (Figures 4 and S4) [77]. Changes in the integration of leaf and root traits are a key feature of the evolution of deciduous habits, allowing leaves to escape environmental stresses and altering the relationship between leaves and roots. Specifically, deciduous tree species lose their leaves during the winter or dry season, resulting in a break in the root–leaf relationship [78]. The leaves of evergreen species are often photosynthetic, providing a continuous supply of energy while also transporting organic matter produced by photosynthesis to the roots, allowing the roots to continuously absorb water and nutrients [79]. Thus, evergreen tree species have the characteristic of maintaining green leaves all year round, and their relationship between the root system and the leaves is more close and continuous [80]. Evergreen and deciduous species coexist through different ecological strategies to reduce the intensity of competition at the local scale. Evergreen species improve their resource use efficiency by sustaining photosynthesis and maintaining a continuous leaf–root connection, while deciduous species adapt to seasonal changes and avoid direct competition with evergreen species by shedding leaves and regrowing new leaves at the right time [58].

Our results suggest that the coordination between leaf and fine root traits in ECM species is more sensitive in response to changes in species richness than that in AM species (Figures 4 and S5). AM fungi invade the inside of plant roots after making contact with them and form enlarged structures on the root surface [81]. The symbiotic relationship between AM fungi and plant roots is stable and is not easily affected by changes in the soil environment [82]. Species richness enhances the complexity of the fungal symbiosis network associated with AM species, allowing fungal species to interact more extensively, and this widespread distribution and symbiosis may lead to a relatively stable explanatory variation in the leaf and fine root traits in AM species [60]. In contrast, ECM fungi predominantly form massive Hartig nets in intercellular spaces and hyphal mantles around the root tip, facilitating higher branching intensity to promote the colonization of root tips by ECM fungi [81]. ECM fungi exhibit high host specificity and can establish a fungal network that transmits nutrients and signals between trees of the same species [83]. The ECM tree can obtain resources and nutrients from a wide network of mycorrhizal fungi, alleviate the conspecific negative density dependence of ECM, and improve its adaptability to various environments [60]. Different ECM species have different types of mycelial reticulations that help plants access and utilize resources, and they have different adaptations to the nitrogen and phosphorus needs of plants [84]. When species diversity is abundant and multiple species coexist together, the mycelial reticulations of different ECM species can overlap, and this overlap reduces competition for nutrients and water [85], improving the coordination between leaf and fine root traits.

5. Conclusions

The coordination between leaf and root functional traits maximizes resource acquisition and utilization efficiency, improves our understanding of the ecological strategies above and below ground in plants, and is helpful in elucidating how community composition and species richness shape ecosystem functions and processes. Our findings provide insights into the differences in leaf–root trait coordination across different species richness levels and trait categories. Specifically, leaf and absorptive root traits often differed between trait categories, while species richness significantly influenced most of the leaf traits and absorptive and transport root traits. Additionally, trait categories played a crucial role in the coordination between leaf and fine root traits, which increased with species richness in the deep-rooted, evergreen, and ECM species. Therefore, our results imply that mixed forests dominated by deep-rooted, evergreen, and ectomycorrhizal fungi species show a greater advantage than monocultures in the coordination of leaf and fine root traits. This may be due to the presence of a significant mixture effect in the coordination between leaf and fine root traits through the comprehensive and divergent utilization of above-ground and belowground resources, as well as the alleviation of intraspecific competition. Therefore, when implementing afforestation practices such as establishing new forests, reforestation, or forest transformation, we recommend the creation of mixed forests with over four species, with a dominance of deep-rooted, evergreen, and ectomycorrhizal fungi species, to maintain ecosystem stability and functional integrity.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f15050744/s1>, Table S1: Results of statistical analyses for leaf traits; Table S2: Results of statistical analyses for absorptive root traits; Table S3: Results of statistical analyses for transport root traits; Figure S1: Variations of leaf traits across different species in monocultures; Figure S2: Variations of fine root traits across different species in monocultures; Figure S3: Redundancy analysis (RDA) about the relationships between leaf traits and root traits in deep-rooted and shallow-rooted species at different species richness; Figure S4: Redundancy analysis (RDA) about the relationships between leaf traits and root traits in deciduous and evergreen species at different species richness; Figure S5: Redundancy analysis (RDA) about the relationships between leaf traits and root traits in AM and ECM species at different species richness.

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