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Effects of mixed plantations on soil microbial diversity and ecosystem multifunctionality across China: a meta-analysis

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Mixed plantations have emerged as a key afforestation strategy for enhancing the stability and sustainability of forest ecosystems. However, their effects on soil microbial diversity and ecosystem multifunctionality (EMF) remain inconsistent. Here, we compiled a dataset from 121 peer-reviewed publications and conducted a meta-analysis to evaluate the effects of different mixed plantation strategies on soil microbial diversity and EMF. The results showed that mixed plantations significantly increased bacterial and fungal Shannon indices by 1.43 and 7.4%, respectively, compared to monocultures. In addition, mixed plantations enhanced key ecosystem functions, including increased soil organic carbon, nutrient availability, and moisture retention, while reducing soil bulk density. Collectively, these improvements resulted in a 19.25% increase in the EMF index. Further analysis revealed significant positive correlations between both bacterial and fungal diversity and EMF, as well as significant associations between EMF and key soil physical properties. Moreover, the effects of mixed plantations on microbial diversity and ecosystem functions varied depending on the mixing strategy. Overall, our findings highlight the ecological benefits of mixed plantations for soil microbial diversity and EMF, supporting more effective and sustainable afforestation practices.

KEYWORDS
afforestation strategies, mixed plantations, soil microbial diversity, ecosystem multifunctionality, meta-analysis

1 Introduction

Biodiversity loss disrupts ecosystem structure and functioning, posing a major threat to long-term ecological stability and human wellbeing (Jaureguiberry et al., 2022). Forest ecosystems cover approximately 31% of the Earth's terrestrial surface and play a crucial role in maintaining global biodiversity and providing ecosystem services and functions, including carbon storage, nutrient cycling, water conservation, and climate regulation (FAO, 2024). In recent decades, with the growing demand for ecosystem services and the need to avoid the ecological crises caused by excessive logging of natural forests, the area of forest plantations now comprises approximately 7% of the global forest cover (Liu S. et al., 2018).

Notably, China has established the world's largest area of forest plantations through a series of major afforestation projects, representing more than 25% of the global planted forest area (Ma et al., 2025). China's forest plantations possess several distinctive characteristics that set them apart globally. In terms of dominant species, they are predominantly composed of fast-growing timber trees such as *Populus* species (Populus tomentosa) and *Cunninghamia lanceolata*, which are managed for high-yield timber production (Sui et al., 2022). Beyond their economic value, these plantations are strategically established to fulfill critical ecological functions, most notably soil and water conservation, which is vital for rehabilitating degraded land across the country. Among various afforestation models, monoculture plantations have become the prevailing afforestation model owing to their high productivity and management simplicity (Li T. et al., 2022). However, long-term, large-scale monoculture plantations are known to cause a range of ecological problems, such as soil erosion, declines in soil quality, reduced biodiversity, and diminished ecosystem service capacity (Li et al., 2021; Li P. et al., 2022; Zhou et al., 2024). In contrast, mixed plantations are receiving increasing attention due to their potential to enhance biodiversity, improve ecosystem resilience, and maintain multiple ecosystem functions (Gamfeldt et al., 2013; Xie et al., 2018; Zhou et al., 2024).

Soil microbial communities, as a vital component of global biodiversity, play key roles in ecosystem functioning (Delgado-Baquerizo et al., 2016; Bastida et al., 2021). Studies have shown that mixed plantations can influence soil microbial diversity by altering soil moisture content, pH, and nutrient availability (An et al., 2019; Ding et al., 2022; Xu et al., 2022). Despite increasing research on how mixed plantations affect soil microbial communities, results remain inconsistent and no clear consensus has emerged. Some studies report that mixed plantations significantly enhance soil microbial diversity (Xu et al., 2022; Liu L. et al., 2023; Li N. et al., 2024), while others report either negative effects or no significant differences compared to monocultures (Xu et al., 2021; Pan et al., 2023; Yang et al., 2024). Variations in tree species composition, stand age, and planting density may contribute to the variable effects on soil microbial diversity (Zhang et al., 2023; Zhang et al., 2024).

Biodiversity has been widely recognized as a key driver of ecosystem multifunctionality (EMF) in numerous studies (Lefcheck et al., 2015; Mensah et al., 2020; Yan et al., 2023). Earlier research tended to focus on the relationship between plant diversity and EMF (Maestre et al., 2012; van der Plas et al., 2016; Gross et al., 2017). The relationship between soil microbial diversity and EMF has received growing attention with the rapid advancement of molecular biology (Shu et al., 2023; Sun et al., 2023; Zhang et al., 2025). Previous studies have found a significant positive correlation between soil microbial diversity and EMF (Delgado-Baquerizo et al., 2020). As research has become more nuanced, some studies have revealed that fungal diversity positively influences EMF (Duan et al., 2021), whereas bacterial diversity appears to have a less pronounced effect (Chen et al., 2023). These findings suggest that trade-offs may exist between different types of soil microbial diversity and their contributions to EMF (Sun et al., 2023).

Although mixed plantations are increasingly adopted in afforestation practices, the specific relationships between mixed plantations, soil microbial diversity, and EMF remain unclear. Previous field-based empirical studies have been largely confined to local or small spatial scales, limiting their applicability to broader regions. Moreover, existing meta-analyses on mixed plantations have primarily focused on either soil microbial diversity or specific ecosystem functions (Gong et al., 2021; Yan et al., 2021; Xiang et al., 2022), with few attempts to quantitatively integrate both aspects. To address these gaps, this study conducts a quantitative meta-analysis across China, a representative region for large-scale plantation development, to simultaneously evaluate the effects of mixed plantations on soil microbial diversity and EMF. This integrated approach provides new insights for optimizing mixed plantation management and promoting ecosystem sustainability.

Here, we compiled a comprehensive database comprising 332 paired observations from monoculture and mixed plantations for meta-analysis to evaluate soil microbial diversity and EMF. Based on our meta-analysis, we aimed to (1) quantify the impact of mixed plantations on soil microbial diversity; (2) evaluate their effects on various ecosystem functions and EMF; and (3) explore the relationships between microbial diversity and EMF in mixed plantation systems. For robust assessment of microbial alpha diversity, we employed the Shannon index as our primary metric, following established methodologies (He et al., 2013). Furthermore, we hypothesized that factors such as the presence or absence of nitrogen-fixing tree species, the age structure of mixed plantations, and species mixture type significantly influence soil microbial diversity and ecosystem functioning.

2 Materials and methods

2.1 Data collection

We conducted a comprehensive literature search of peer-reviewed journal articles published before 31 December 2024, using the Web of Science and China National Knowledge Infrastructure (CNKI) databases. The literature screening was performed following the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Moher et al., 2009; Figure 1). Various keyword combinations were used for the search, such as: (mixed forest or mixed plantation or plantation or different forest stand types) AND (soil microbial diversity or soil fungal diversity or soil bacteria diversity or soil microbial biomass or soil microbial community) AND (ecosystem multifunctionality or soil physical and chemical properties). Additionally, relevant peer-reviewed literature was further supplemented by cross-checking the reference lists of the included articles.The studies were subsequently screened according to the following criteria: (1) only studies conducted within China were included; (2) only field studies conducted in plantation forests with at least three replicates were included, excluding those conducted in natural forests; (3) to ensure comparability, each study had to include both mixed plantations (treatment; composed of two tree species) and monoculture plantations (control; consisting of one of these two species) at the same experimental site; (4) only studies focusing on the entire community of soil bacteria and fungi were included, excluding those that focused on specific microbial taxa; (5) data were extracted only from the 0 to 20 cm soil layer; (6) the diversity index of soil microbial community was calculated based on the high-throughput sequencing method (Wang et al., 2023);

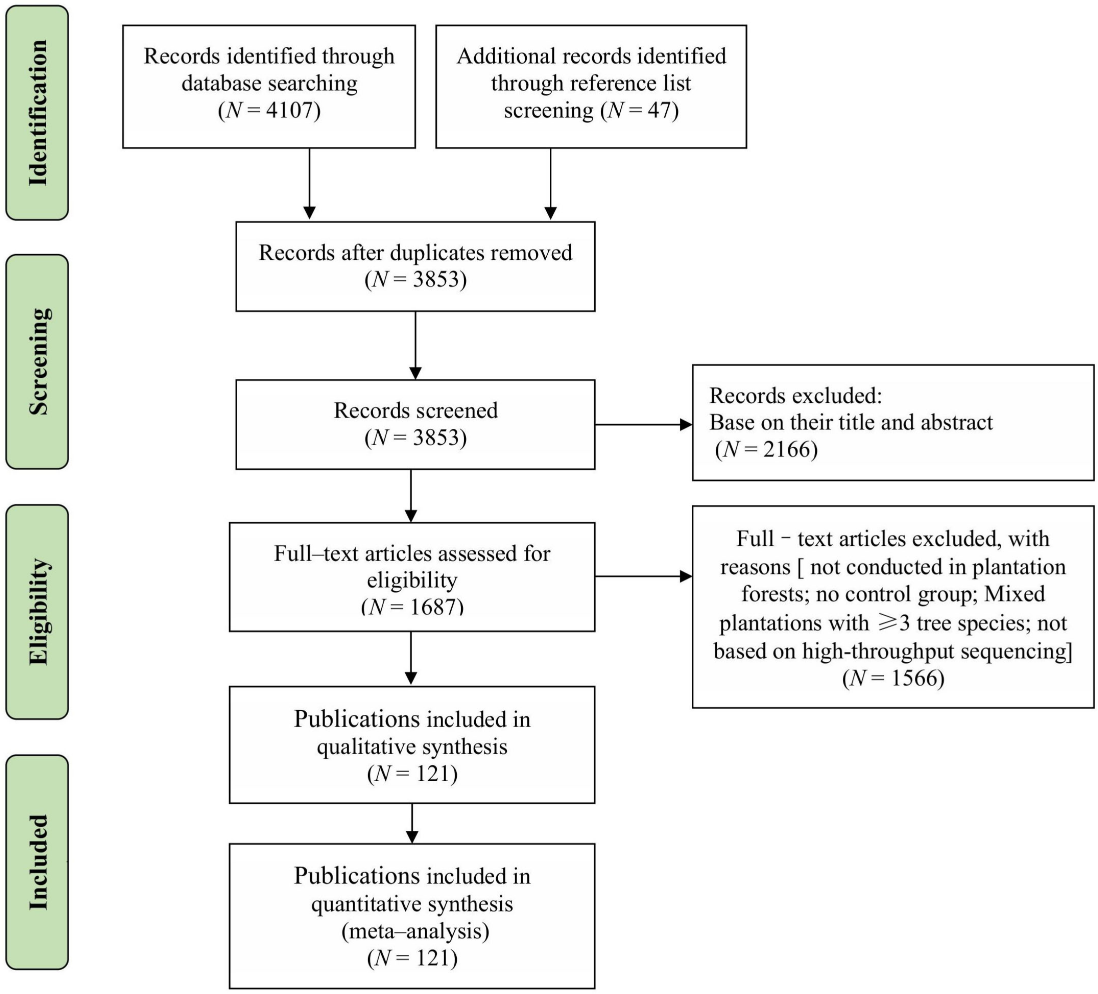

FIGURE 1
PRISMA diagram showing the process of locating publications included in this meta–analysis.

(7) if a publication included multiple experiments under different treatments, seasons or years, stand ages, or mixing proportions, each independent experiment was considered as one observation in the meta-analysis.

2.2 Data extraction

For each study included in this meta-analysis, we extracted key variables, including the mean, standard deviation (SD), and sample size for plant properties [litter biomass (LB), root biomass (RB)], soil physicochemical properties [bulk density (BD), soil moisture content (SM), soil pH (pH), soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), available phosphorus (AP), and available potassium (AK), ammonium nitrogen (NH_4^--N), nitrate nitrogen (NO_3^--N), SOC/TN ratio (C/N)], microbial attributes [microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), bacterial Shannon index, and fungal Shannon index, and enzyme activity urease activity (URE)].

All original data were extracted from the figures, tables, and supplementary material of the publications. Specifically, data presented in tables were directly extracted, whereas graphical data were digitized using the GetData Graph Digitizer (version 2.24). When only the median, range, or interquartile range was reported, we estimated the mean and standard deviation using the method proposed by Wan et al. (2014). If the original studies reported standard errors (SE) instead of SD, we converted SE to SD using the formula $SD = SE^* \sqrt{n}$. Additionally, we collected site characteristics, including geographic location (latitude and longitude), elevation, mean annual temperature, mean annual precipitation, mixed plantation type, mixing ratio, and stand age. Stand age was defined as the number of years between forest establishment (or species introduction) and sampling. Based on these criteria, the databases used for the Meta-analysis comprise 332 observations at 104 sites from 121 peer-reviewed publications. The study sites included in this study are shown in Figure 2.

We categorized the collected data based on three mixing strategy classifications:

(1) Nitrogen-fixing status (N-fixing status): classified based on the presence or absence of nitrogen-fixing tree species as

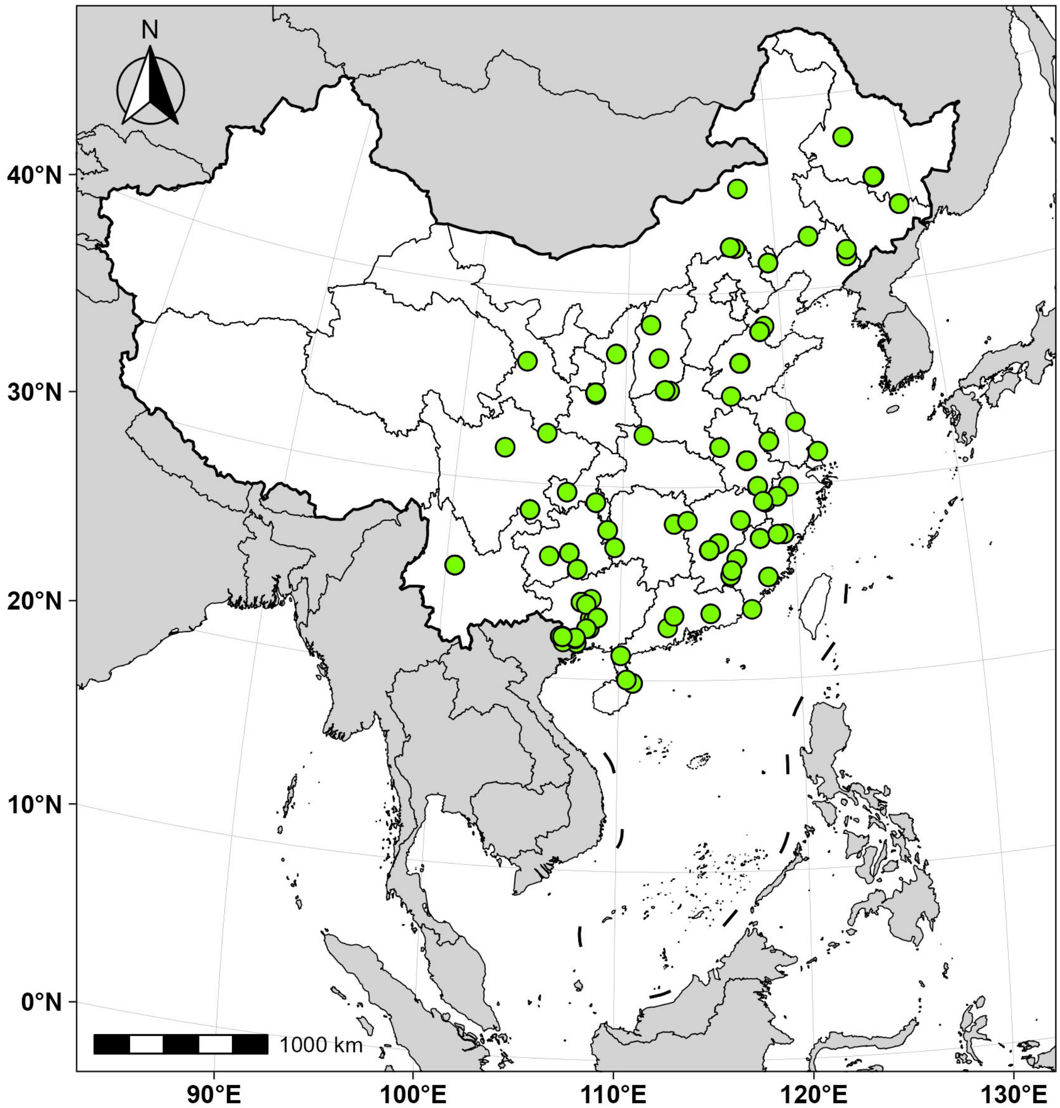

FIGURE 2
Distribution of all study sites included in this meta-analysis across China.

nitrogen-fixing mixed plantations (NF) and non-nitrogen-fixing mixed plantations (NNF).

(2) Age structure: classified as uneven-aged mixed plantations (UA) and even-aged mixed plantations (EA).

(3) Species mixture: classified as coniferous–broadleaf mixed plantations (CB) and broadleaf–broadleaf mixed plantations (BB). Coniferous–coniferous mixed plantations were excluded from the classification due to an insufficient number of available studies ($n < 3$).

2.3 Assessment of ecosystem multifunctionality index

We selected 13 indicators related to ecosystem functioning from the database to assess EMF, including LB, RB, SOC, TN, TP, AP, AK, NO_3^--N, NH_4^--N, MBC, MBN, C/N, and URE. These indicators were selected because they have been recognized in previous studies as good descriptors of ecosystem functions and services (Yuan et al., 2021; Liu et al., 2025). To ensure consistency in directional change, the values of the C/N ratio were multiplied by −1, since higher values represent a negative effect on ecosystem functioning (Ma et al., 2021). The EMF index was calculated as the mean of effect sizes across the 13 selected indicators, following the averaging approach widely used in multifunctionality studies (Shu et al., 2024). This approach was adopted because it provides an intuitive and easily interpretable metric that gives balanced consideration to the overall performance of multiple ecosystem functions.

2.4 Meta-analysis

We used the natural log-transformed effect size (RR) to quantify the impact of mixed plantations on soil microbial diversity and individual ecosystem functions (Hedges et al., 1999), as shown in Eq. (1):

$$RR = \ln(Xt/Xc) = \ln(Xt) - \ln(Xc) \quad (1)$$

where Xt and Xc are the average values of the selected variables in the mixed plantation (treatment group) and monoculture plantation (control group), respectively.

The variance (v) of RR was calculated using Eq. (2):

$$V = \frac{SD_t^2}{N_t X_t^2} + \frac{SD_C^2}{N_c X_c^2} \quad (2)$$

where Nt and Nc represent the sample size of mixed plantation and monoculture plantation, respectively; SDt and SDc denote the standard deviations (SD) of the mixed plantation and monoculture plantation, respectively. If the SD was unavailable and could not be estimated, it was imputed as 10% of the corresponding mean, following Li X. et al. (2020).

To facilitate interpretation, the weighted effect sizes (lnRR) were back-transformed and presented as percentage changes using Eq. (3) (Wang et al., 2023):

$$\text{Percentage change}(\%) = (\exp(\ln RR) - 1) \times 100\% \quad (3)$$

We used the "metafor" package (version 4.6.0) in R to perform a meta-analysis with a random-effects model (Viechtbauer, 2010). This model was chosen to account for heterogeneity arising from differences in site conditions and experimental designs among the included studies, which cannot be addressed by a fixed-effects model. The "rma" function was employed to calculate the lnRR and the corresponding 95% confidence interval (CI). If the 95% CI did not overlap with zero, the effect of mixing on the given variable was considered significant ($p < 0.05$).

We used between-group heterogeneity tests (Q_B) to compare effect sizes across different mixing strategies. A significant Q_B value ($P_{QB} < 0.05$) indicates significant differences in the weighted effect sizes between groups for a given variable.

To examine whether soil microbial diversity affected EMF, we used ordinary least squares regressions to analyze the relationships between soil bacterial and fungal diversity and EMF. In addition, we also analyzed the relationships between soil physical properties and EMF. For the regression model, we used the Shapiro-Wilk test to assess the normality of the residuals and the Breusch-Pagan test to examine homoscedasticity (Zhang et al., 2022). For both tests, $p > 0.05$ was considered to indicate that the assumptions of normality and homoscedasticity were not violated.

Given that some variables lacking reported SD were included in this meta-analysis, we conducted a sensitivity analysis to assess the possible bias introduced by this inclusion (Gao et al., 2021). Specifically, we calculated and compared the lnRR and 95% CIs using all available data and a subset excluding studies without SD. If the lnRR were similar and their 95% CIs overlapped, the inclusion of all data for that variable was deemed acceptable for our meta-analysis. Furthermore, we employed both Egger's regression test and Fail-Safe N analysis to comprehensively assess potential publication bias in this meta-analysis (Egger et al., 1997; Rosenberg, 2005). If the Fail-Safe N exceeded 5n + 10 (where n is the sample size), the results were considered robust and free from publication bias. Sensitivity analysis indicated that the inclusion of data without SD did not change the direction and statistical significance of our results (Table 1), and no potential publication bias was detected (Table 2).

All statistical analyses and visualizations were performed in R version 4.4.1 (R Core Team, 2024).

3 Results

3.1 Effects of mixed plantations on soil microbial diversity

The Shannon indices of bacterial and fungal communities were both significantly higher in mixed plantations than in monocultures, with increases of 1.43% ($p < 0.01$) and 7.4% ($p < 0.001$), respectively (Figure 3). Further analysis revealed that

TABLE 1 Sensitivity analysis for the effects of mixed plantations on soil microbial diversity and ecosystem multifunctionality.

Variables	Data class	Q_T	*n*	lnRR (95%CI)
LB	Full data	2253.4	49	0.2740 (0.1854, 0.3626)
	Reduced data	2175.3	46	0.2671 (0.1774, 0.3569)
RB	Full data	533.2	34	0.3415 (0.2529, 0.4301)
	Reduced data	473.7	28	0.3028 (0.2039, 0.4017)
SOC	Full data	7523.2	232	0.1822 (0.1495, 0.2150)
	Reduced data	7371.1	222	0.1899 (0.1570, 0.2228)
TN	Full data	4116.2	245	0.2120 (0.1802, 0.2438)
	Reduced data	3974.6	235	0.2101 (0.1779, 0.2422)
TP	Full data	12445.1	204	0.1351 (0.0913, 0.1788)
	Reduced data	11885.9	194	0.1379 (0.0967, 0.1792)
AP	Full data	13263.5	163	0.2059 (0.1450, 0.2669)
	Reduced data	12979.0	159	0.1896 (0.1302, 0.2491)
AK	Full data	19347.2	93	0.1323 (0.0658, 0.1988)
	Reduced data	19309.0	84	0.1305 (0.0578, 0.2031)
NH_4^--N	Full data	1619.9	115	0.1179 (0.0634, 0.1725)
	Reduced data	1619.4	114	0.1191 (0.0640, 0.1741)
NO_3^--N	Full data	14973.3	113	0.2235 (0.1112, 0.3358)
	Reduced data	14961.3	112	0.2259 (0.1126, 0.3392)
C/N	Full data	9964.6	90	−0.0318 (−0.0750, 0.0115)
	Reduced data	9964.3	89	−0.0309 (−0.0747, 0.0128)
$H_{bacteria}$	Full data	1441.1	94	0.0142 (0.0052, 0.0233)
	Reduced data	1439.0	91	0.0147 (0.0056, 0.0238)
H_{fungi}	Full data	155.8	56	0.0714 (0.0298, 0.1130)
	Reduced data	154.1	54	0.0764 (0.0334, 0.1194)

Full data includes all data from studies that directly provided standard deviation (SD) and those with SD obtained through calculation. Reduced data includes data only from studies that directly provided SD. Q_T, total heterogeneity; *n*, sample size; lnRR, weighted effect size; CI, confidence interval. The effect of mixed plantations is considered statistically significant if the 95% CI does not overlap with zero. LB, litter biomass; RB, root biomass; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; AP, available phosphorus; AK, available potassium; NH_4^--N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; C/N, SOC/TN ratio; $H_{bacteria}$, bacterial Shannon index; H_{fungi}, fungal Shannon index.

TABLE 2 Results of publication bias.

Variables	*n*	Egger's test		Exist of bias	Fail-safe number	Is the analysis robust
		z	*p*-value			
LB	49	0.7667	0.4433	No	45821	Yes
RB	34	0.6111	0.5412	No	7299	Yes
BD	71	−0.8986	0.3689	No	8799	Yes
SM	114	−0.0263	0.9791	No	24276	Yes
pH	199	−0.5416	0.5881	No	38103	Yes
SOC	232	0.3920	0.6950	No	260777	Yes
TN	245	0.6439	0.5197	No	239661	Yes
TP	204	−0.1269	0.8990	No	134160	Yes
AP	163	−0.6434	0.5200	No	195440	Yes
AK	93	−0.7276	0.4669	No	88751	Yes
NH_4^--N	115	1.1819	0.2372	No	9810	Yes
NO_3^--N	113	−2.5209	0.0117	Yes	62913	Yes
C/N	90	1.0708	0.2843	No	3633	Yes
MBC	83	−1.7033	0.0885	No	62229	Yes
MBN	57	−2.0445	0.0409	Yes	14740	Yes
URE	53	1.8059	0.0709	No	11672	Yes
$H_{bacteria}$	94	−0.5851	0.5585	No	5102	Yes
H_{fungi}	56	1.1110	0.2666	No	648	Yes

A p-value > 0.05 in Egger's regression test indicates no evidence of publication bias. If the fail-safe number is greater than $5n + 10$ (where n is the sample size), the results are considered robust regardless of any potential publication bias. LB, litter biomass; RB, root biomass; BD, bulk density; SM, soil moisture content; pH, soil pH; SOC, soil organic carbon; TN, soil total nitrogen; TP, total phosphorus; AP, available phosphorus; AK, available potassium; NH_4^--N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; C/N, SOC/TN ratio; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; URE, urease activity; $H_{bacteria}$, bacterial Shannon index; H_{fungi}, fungal Shannon index.

this increase was significant regardless of the age structure of the mixed plantations. Specifically, the Shannon indices of bacteria and fungi increased by 2.00 and 14.56%, respectively, in uneven-aged mixed plantations, and by 1.53 and 5.59% in even-aged mixed plantations (all $p < 0.05$) (Figure 3). While nitrogen-fixing mixed plantations did not significantly affect the bacterial Shannon index ($p > 0.05$) (Figure 3A), they significantly increased the fungal Shannon index by 7.14% ($p < 0.05$) (Figure 3B). In contrast, broadleaf–broadleaf mixed plantations significantly increased the bacterial Shannon index by 2.38% ($p < 0.05$) (Figure 3A), but had no significant effect on the fungal Shannon index ($p > 0.05$) (Figure 3B).

3.2 Effects of mixed plantations on ecosystem functions

Mixed plantations significantly increased plant biomass compared to monocultures, with LB and RB rising by 31.52 and 40.71%, respectively ($p < 0.001$) (Figure 4). Mixed plantations exhibited higher levels of SM, pH, SOC, TN, TP, AP, AK, NH_4^--N, and NO_3^--N concentrations, with varying magnitudes across indicators (Figure 4). Among all indicators, the NO_3^--N concentration showed the largest increase, rising by 25.04% overall ($p < 0.001$) (Figure 4), and by 61.91% specifically in nitrogen-fixing mixed plantations ($p < 0.001$) (Figure 5A). In contrast, the BD level in mixed plantations decreased by 5.66% ($p < 0.001$) (Figure 4). Notably, the C/N ratio decreased significantly by 13.39%, but only in uneven-aged mixed plantations ($p < 0.001$) (Figure 5B).

Microbial biomass was significantly greater in mixed plantations, with MBC and MBN increasing by 21.74 and 25.38%, respectively, compared to monocultures ($p < 0.001$) (Figure 4).

Soil urease activity also increased significantly in mixed plantations, showing a 22.98% rise compared to monocultures ($p < 0.001$) (Figure 4). This stimulatory effect on urease activity was consistent across all examined mixing strategies (Figures 5A–C).

3.3 Effects of mixed plantations on EMF and its drivers

Compared to monoculture plantations, mixed plantations significantly increased the EMF index by 19.25% ($p < 0.001$) (Figure 6). This positive effect was consistent across all mixing strategies, although the magnitude of the increase did not differ significantly among them ($p > 0.05$) (Table 3). Linear regression analysis showed that the EMF index was positively correlated with both bacterial and fungal Shannon indices ($p < 0.05$) (Figure 7 and Table 4). Additionally, among soil physical properties, EMF was positively associated with SM and negatively associated with BD (both $p < 0.001$) (Figure 8 and Table 4).

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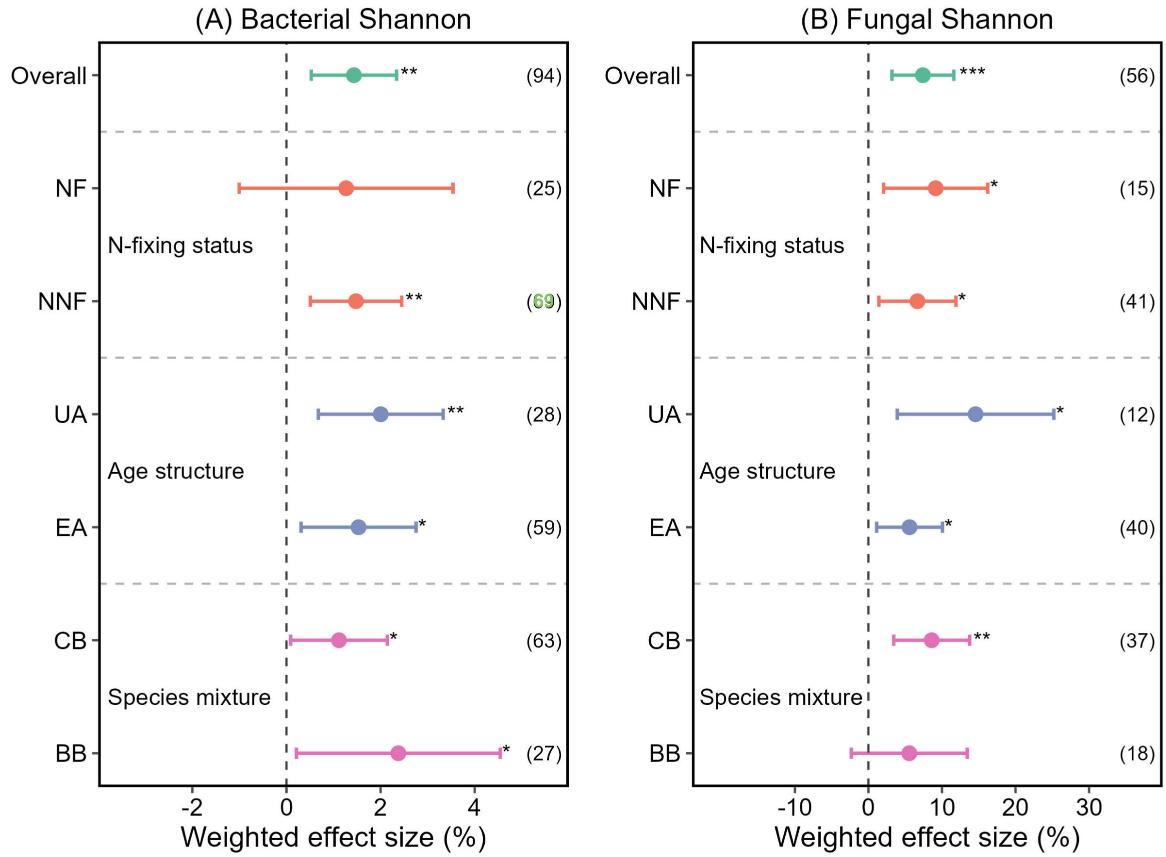

FIGURE 3
Weighted effect sizes **(A)** of soil bacterial Shannon index and **(B)** of soil fungal Shannon index in mixed plantations under different mixing strategies. Error bars represent 95% confidence intervals (CIs) of the weighted effect sizes. An effect is considered statistically significant if the 95% CI does not overlap with zero ($p < 0.05$). Sample sizes for each variable are shown in parentheses next to the corresponding labels. ***, **, and * denote $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively. NF, nitrogen-fixing mixed plantations; NNF, non-nitrogen-fixing mixed plantations; UA, uneven-aged mixed plantations; EA, even-aged mixed plantations; CB, coniferous-broadleaf mixed plantations; BB, broadleaf-broadleaf mixed plantations.

4 Discussion

4.1 Positive effects of mixed plantations on soil microbial diversity

Our meta-analysis showed that the effect sizes of mixed plantations on the Shannon index were positive (Figures 3a,b), suggesting an overall increase in soil microbial biodiversity. Previous studies have found that mixed plantations generally support higher microbial diversity than monocultures, mainly due to their more diverse tree species composition, which provides diverse organic inputs and fosters heterogeneous microhabitats (Li et al., 2015; Khlifa et al., 2017). Litter and root exudates from different tree species offer diverse nutritional substrates for microbes, while also improving the soil microenvironment and increasing the availability of soil nutrients, thereby supporting a more diverse microbial community (Li et al., 2015; Khlifa et al., 2017). Higher microbial diversity is widely recognized as an indicator of healthier soils, promoting more efficient nutrient cycling, organic matter decomposition, and resistance to pests and diseases (Zhu et al., 2021). Notably, microbial diversity is not determined solely by tree species composition; it is also shaped by multiple biotic and abiotic factors, such as pH, nutrient availability, and microbial interactions, which may influence the observed patterns (Ding et al., 2022; Wang Y. et al., 2022).

Moreover, our results revealed that different mixing strategies had divergent effects on bacterial and fungal diversity. Specifically, nitrogen-fixing mixed plantations significantly increased the fungal Shannon index, while bacterial diversity showed no significant response (Figure 3). This pattern suggests that fungal communities may be more responsive than bacterial communities to the presence of nitrogen-fixing species (Zhang et al., 2020; Liu et al., 2022). Introducing nitrogen-fixing species into monocultures may enhance plant nutrient-use efficiency, increasing the input of structurally complex plant residues and root exudates rich in macromolecular organic matter (Wu et al., 2025). Fungi, as primary decomposers of recalcitrant compounds such as lignin and cellulose, may benefit from these additional resources, potentially contributing to the observed increase in fungal diversity. While nitrogen-fixing species may contribute to higher fungal diversity, this effect likely operates together with other environmental and biological factors (Li N. et al., 2024). Moreover, bacterial diversity remained largely unaffected, possibly reflecting a lower sensitivity to tree species composition and a greater influence of abiotic factors such as soil depth and moisture (Xu Z. et al., 2023).

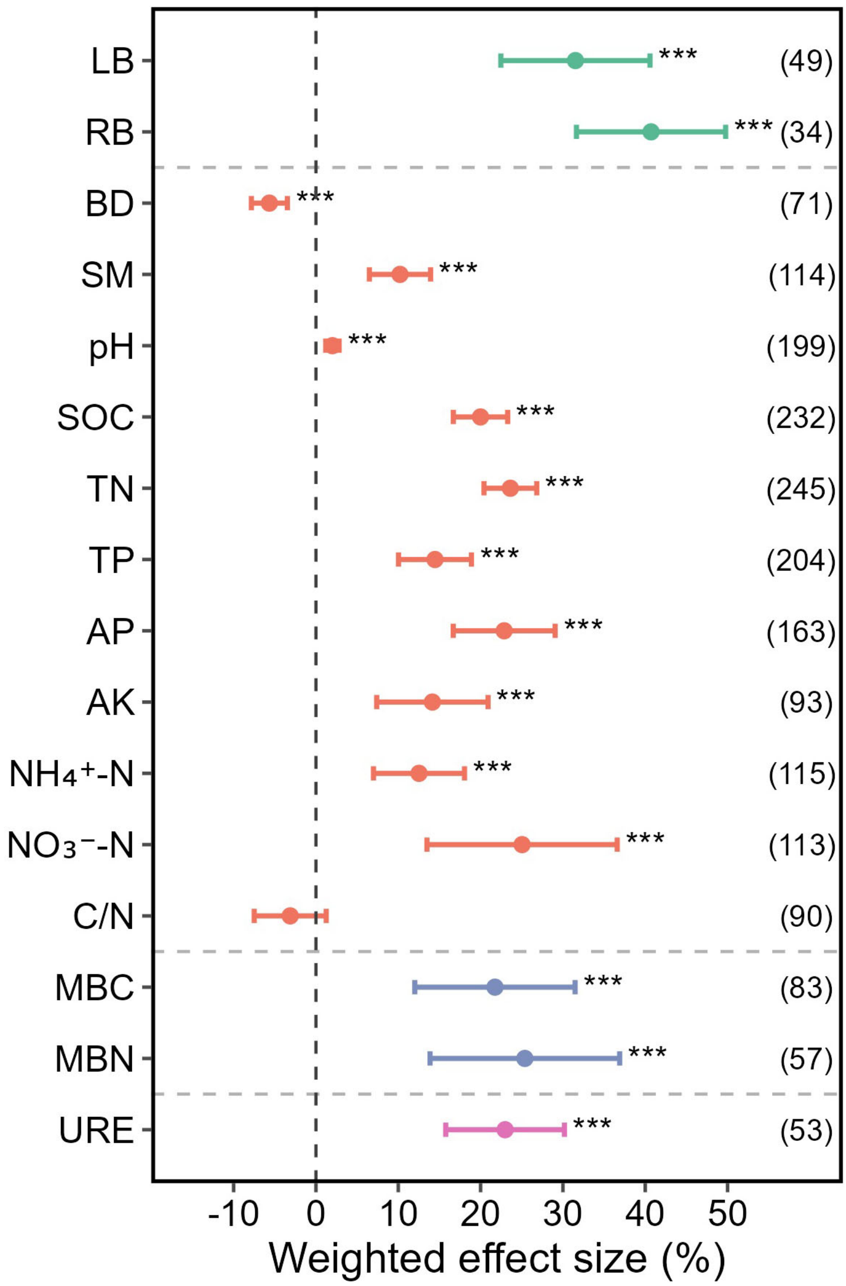

FIGURE 4
Weighted effect sizes of individual ecosystem functions (LB, RB, SOC, TN, TP, AP, AK, NH_4^--N, NO_3^--N, C/N, MBC, MBN, and URE) and soil properties, including physical (BD, SM) and chemical (pH) characteristics, in mixed plantations. Error bars represent 95% confidence intervals (CIs) of the weighted effect sizes. An effect is considered statistically significant if the 95% CI does not overlap with zero ($p < 0.05$). Sample sizes for each variable are shown in parentheses next to the corresponding labels. *** denote $p < 0.001$, respectively. LB, litter biomass; RB, root biomass; BD, bulk density; SM, soil moisture content; pH, soil pH; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; AP, soil available phosphorus; AK, soil available potassium; NH_4^--N, soil ammonium nitrogen; NO_3^--N, soil nitrate nitrogen; C/N, SOC/TN ratio; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; URE, urease activity.

In contrast, broadleaf–broadleaf mixed plantations increased the bacterial Shannon index only (Figure 3A), likely because faster decomposition of broadleaf litter accelerates nutrient turnover, providing more labile carbon and nutrients for bacterial communities (Wang et al., 2018; Zhuang et al., 2023). Collectively, these results suggest that bacterial and fungal communities exhibit distinct responses to different mixing strategies (Bai et al., 2025; Xiao et al., 2022).

4.2 Improved ecosystem functions in response to various mixing strategies

Our findings showed that mixed plantations significantly increased LB and RB compared to monocultures (Figure 4). This pattern may be driven by more efficient resource use and niche complementarity in mixed plantations. For instance, mixed plantations often develop stratified canopies that enable species to

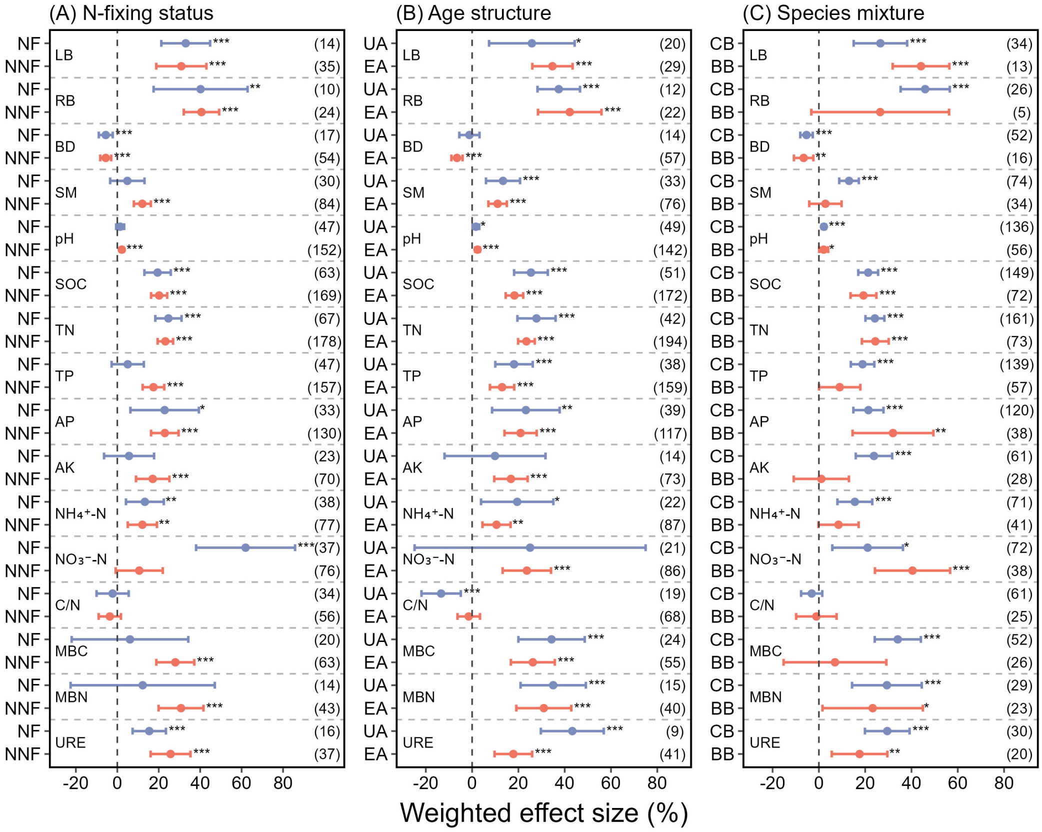

FIGURE 5
Effects of **(A)** N-fixing status (NF, nitrogen-fixing mixed plantations; NNF, non-nitrogen-fixing mixed plantations), **(B)** age structure (UA, uneven-aged mixed plantations; EA, even-aged mixed plantations), and **(C)** species mixture (CB, coniferous-broadleaf mixed plantations; BB, broadleaf-broadleaf mixed plantations) on multiple ecosystem functions and soil physical properties. Error bars represent 95% confidence intervals (CIs) of the weighted effect sizes. An effect is considered statistically significant if the 95% CI does not overlap with zero ($p < 0.05$). Sample sizes for each variable are shown in parentheses next to the corresponding labels. ***, **, and * denote $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively. See Figure 4 caption for abbreviation definitions.

optimize light capture through vertical niche differentiation. This structural advantage persists over time, resulting in higher litter production in mixed plantations than in monocultures, thereby increasing litter biomass (Wu et al., 2019). Moreover, root niche partitioning and complementary resource use in mixed plantations likely enhance fine root biomass by facilitating more efficient use of belowground resources (Ma and Chen, 2016).

Plants primarily absorb nitrogen in the forms of ammonium (NH_4^-) and nitrate (NO_3^-) from the soil (Wang et al., 2005). Mixed plantations have been shown to enhance the concentrations of both NH_4^--N and NO_3^--N in the soil (Yu et al., 2015; Zhou et al., 2023). In our study, the concentrations of NH_4^--N and NO_3^--N were both significantly increased in mixed plantations (Figure 4). Notably, NO_3^--N levels increased by 61.91% in nitrogen-fixing mixed plantations (Figure 5A), which was significantly higher than in non-nitrogen-fixing mixed plantations (Table 3). This finding aligns with previous studies, suggesting that nitrogen-fixing species enhance soil nitrogen availability (Hoogmoed et al., 2014). This increase is likely attributable to the ability of the associated tree species to fix nitrogen symbiotically, leading to greater nitrogen input into the soil (Mus et al., 2016). Furthermore, mixed plantations may promote nitrification by soil microbes, accelerating the conversion of NH_4^--N to NO_3^--N and further increasing NO_3^--N levels (Liu C.-A et al., 2018). While this shift improves nitrogen availability and promotes plant growth, it may also increase the risk of nitrogen loss, an aspect requiring further investigation (Liang et al., 2020).

In addition, we found that only uneven-aged mixed plantations significantly reduced the C/N ratio (Figure 5B). The C/N ratio is a proxy for nitrogen mineralization potential, with lower values typically indicating higher rates of nutrient release (Liu and Lu, 2024). The decrease in the C/N ratio in uneven-aged mixed plantations may be associated with the timing of species introduction and the resulting age structure (Ling et al., 2022). As new species are introduced, the quantity and quality of surface litter increase, providing more substrates for soil microbes. This stimulates microbial activity, leading to faster decomposition and increased nitrogen mineralization and release, significantly increasing soil nitrogen content and thereby reducing the C/N ratio (Ling et al., 2022; Zhang et al., 2023).

Our study also found that mixed plantations reduced BD (Figure 4). Lower bulk density improves soil aeration, which facilitates root respiration and enhances root growth and nutrient uptake, consistent with the findings of Guo et al. (2023). The reduction in soil bulk density often stems from the complementary distribution of deep and shallow roots in mixed plantations, which

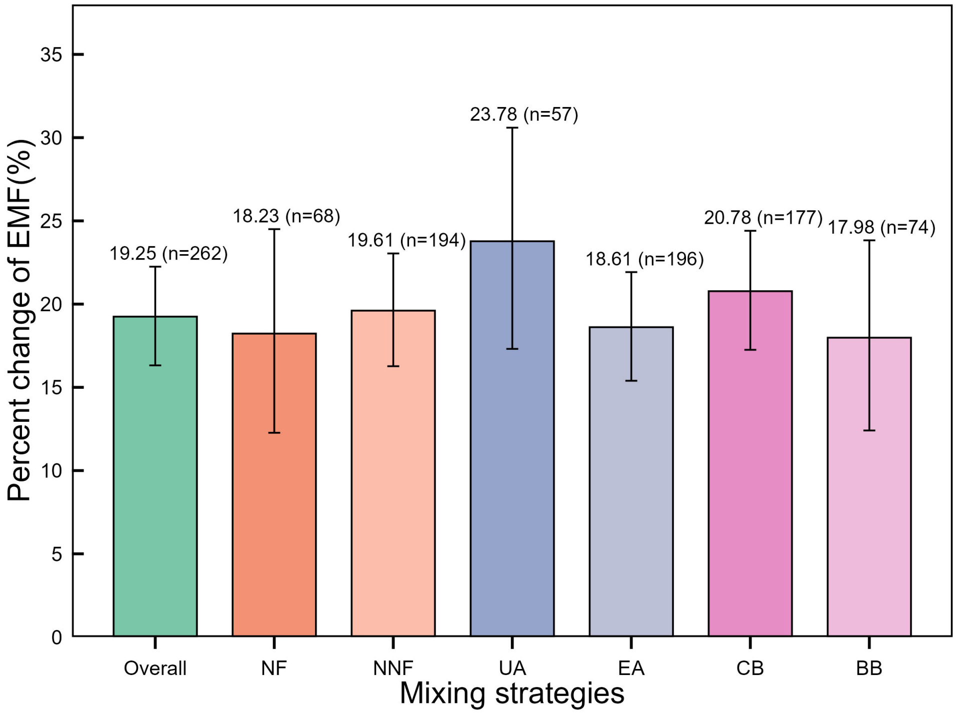

FIGURE 6
Percent change in ecosystem multifunctionality (EMF) across different mixing strategies in mixed plantations. NF, nitrogen-fixing mixed plantations; NNF, non-nitrogen-fixing mixed plantations; UA, uneven-aged mixed plantations; EA, even-aged mixed plantations; CB, coniferous-broadleaf mixed plantations; BB, broadleaf-broadleaf mixed plantations.

TABLE 3 Between-group heterogeneity (Q_b and *P*-values) for each variable under different mixing strategies.

Variables	N-fixing status		Age structure		Species mixture	
	Q_b	*P*	Q_b	*P*	Q_b	*P*
LB	0.04	0.843	0.61	0.433	1.76	0.185
RB	0.01	0.918	0.06	0.803	1.10	0.293
BD	0.02	0.883	3.43	0.064	0.24	0.623
SM	2.37	0.124	0.26	0.611	5.71	**< 0.05**
pH	0.58	0.448	0.54	0.464	0.01	0.932
SOC	0.03	0.865	2.11	0.146	0.22	0.641
TN	0.11	0.739	0.62	0.433	0.001	0.973
TP	4.52	**<0.05**	0.57	0.448	3.09	0.079
AP	0.0001	0.989	0.07	0.790	1.39	0.239
AK	1.72	0.189	0.38	0.538	8.27	**<0.01**
NH_4^+-N	0.04	0.851	0.98	0.322	1.13	0.288
NO_3^--N	11.33	**<0.001**	0.39	0.535	1.58	0.208
C/N	0.08	0.778	6.39	**<0.05**	0.18	0.672
MBC	2.74	0.098	0.43	0.512	4.35	**<0.05**
MBN	1.15	0.284	0.04	0.835	0.10	0.755
URE	0.84	0.361	4.81	**<0.05**	1.58	0.208
$H_{bacteria}$	0.02	0.890	0.2	0.651	1.49	0.222
H_{fungi}	0.30	0.581	2.33	0.127	0.48	0.490
EMF	0.16	0.690	2.02	0.156	0.68	0.409

Q_b values represent the between-group heterogeneity, with $p < 0.05$ indicating a statistically significant difference.

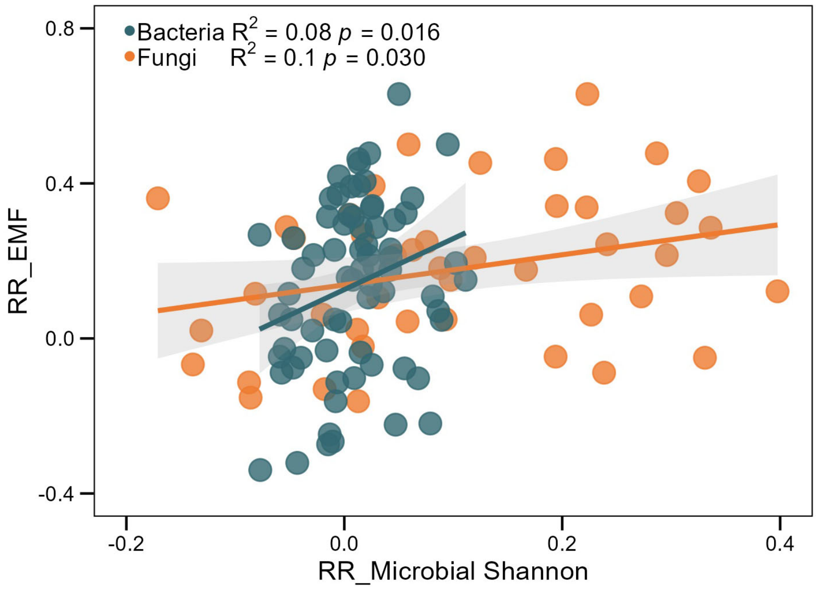

FIGURE 7
Relationships between the effect sizes (RR) of soil bacterial and fungal Shannon indices and the RR of ecosystem multifunctionality (EMF). Light gray areas indicate the 95% confidence intervals, with the central lines representing linear regressions.

TABLE 4 Linear relationships between the effect sizes (RR) of soil microbial diversity and physical properties, and the RR of ecosystem multifunctionality (EMF).

Variables	n	Intercept	Slope	R^2	P	Shapiro-Wilk		Breusch-Pagan	
						W	p	χ^2	p
$H_{bacteria}$	72	0.116	1.503	0.080	0.016	0.976	0.181	0.008	0.931
H_{fungi}	45	0.134	0.415	0.105	0.030	0.982	0.704	0.194	0.660
SM	114	0.104	0.413	0.156	< 0.001	0.995	0.940	0.103	0.748
BD	71	0.083	−0.867	0.211	< 0.001	0.975	0.163	0.316	0.574

"n" represents the sample size for each regression analysis. The Shapiro-Wilk and Breusch-Pagan tests were used to assess residual normality and homoscedasticity, respectively. For both tests, $p > 0.05$ indicates that model assumptions were met.

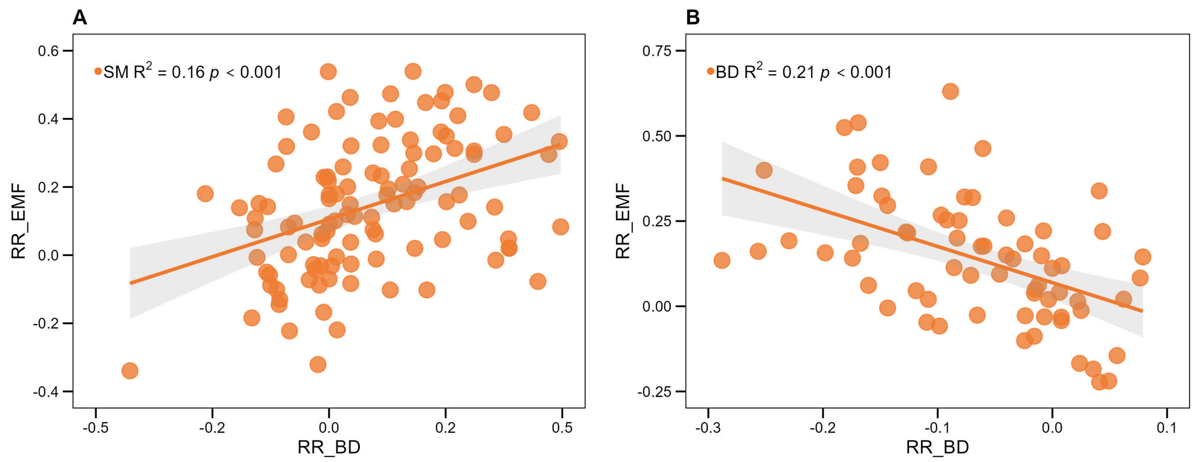

FIGURE 8
Relationships between the effect sizes (RR) of soil moisture content (SM, **A**) or soil bulk density (BD, **B**) and the RR of ecosystem multifunctionality (EMF). Light gray areas indicate the 95% confidence intervals, with the central lines representing linear regressions.

increases soil porosity (Liu et al., 2021). Additionally, the greater litter input and faster decomposition in mixed plantations promote the formation of soil aggregates via humus accumulation, thereby improving soil pore structure (Qin et al., 2013). In contrast, monocultures, with limited root distribution and lower, slower-decomposing litter input, tend to have higher soil bulk density (Qin et al., 2013; Liu et al., 2021). Furthermore, mixed plantations significantly increased SM (Figure 4), possibly due to the thick litter and debris layer retaining moisture and reducing water loss through evaporation (Liu B. et al., 2023). Mixed plantations have been confirmed to be more effective than monocultures in enhancing soil water retention (Gong et al., 2024). Therefore, from the perspective of soil and water conservation, afforestation with mixed species is preferable to monoculture.

Changes in soil microbial biomass reflect shifts in microbial community structure and serve as key indicators of soil fertility and microbial metabolic activity (Zhang et al., 2014). Urease activity, closely linked to soil nitrogen availability, is also a crucial indicator of soil quality (Dong et al., 2023). In this study, mixed plantations led to marked increases in MBC, MBN, and urease activity (Figure 4). Their higher species diversity results in a broader range of organic inputs, providing diverse substrates that stimulate microbial activity (Singh et al., 2012). Furthermore, differences in species composition in mixed plantations lead to higher nitrogen content and lower C/N ratios in litter, accelerating decomposition and enhancing microbial activity (Peng et al., 2025), thus increasing MBC and MBN relative to monocultures. However, it is worth noting that while litter can enhance microbial biomass, it may also trigger the priming effect, accelerating the decomposition of native soil organic carbon and potentially resulting in a net loss or reduced stability of soil carbon stocks (Feng et al., 2022). The increase in urease activity may be due to the elevated levels of inorganic nitrogen in mixed plantations, which provide more substrates for urease and stimulate its activity (Luo et al., 2022). Other studies have suggested that enhanced enzyme activity is often the result of multiple interacting factors, including increased soil pH, improved aeration, and higher availability of nutrients (Guo et al., 2023).

4.3 Correlations between the EMF and soil microbial diversity and physical properties

Numerous studies have demonstrated that mixed plantations generally outperform monocultures across a range of ecosystem functions (Pretzsch et al., 2015; Gong et al., 2021; Dai et al., 2023). Building on these findings, our study adopted an integrative perspective by examining EMF, and showed that mixed plantations significantly enhanced overall ecosystem performance (Figure 6), consistent with previous findings (Xu H. et al., 2023). This result highlights the ecological advantage of mixed plantations in simultaneously promoting multiple ecosystem functions. However, other studies have suggested that differences in EMF between mixed and monoculture plantations may depend on the specific tree species used in afforestation (Li X. et al., 2024). Species composition influences interspecific interactions—such as complementarity or competition—which in turn significantly affect EMF levels. These underlying mechanisms merit further investigation.

Regression analyses indicated a significant correlation between EMF and both soil microbial diversity and physical properties. Soil microbial communities were key predictors of EMF and important indicators for forest management (Wang J. et al., 2022). Consistent with previous findings (Shi et al., 2021; Li et al., 2023), the EMF index showed significant positive correlations with both bacterial and fungal Shannon indices (Figure 7). Such positive relationships may arise from multifunctional niche complementarity and interspecific functional dissimilarity among microbial taxa (Luo et al., 2018). Soil microbial communities contribute directly to essential processes such as organic matter decomposition and nutrient mineralization, which support multiple ecosystem functions (Delgado-Baquerizo et al., 2016). Moreover, EMF was positively correlated with SM but negatively correlated with BD (Figures 8A,B), indicating that improved soil structure and moisture conditions can enhance EMF. High bulk density may reduce soil porosity and aeration, constraining root growth and microbial activity and ultimately decreasing EMF (Niu et al., 2024), whereas optimal soil moisture facilitates nutrient transport and plant growth, thereby enhancing multiple ecosystem functions (Li P. et al., 2022; Wang et al., 2024). Although these factors were significantly correlated with EMF, they explained only a small proportion of its variation (Table 4), suggesting that other unmeasured factors such as aboveground biodiversity, litter quality, and climatic conditions likely also contribute to EMF regulation.

4.4 Limitations and future directions

Our meta-analysis demonstrates that mixed plantations generally exert positive effects on soil microbial diversity and a range of ecosystem functions. In addition, it provides insights into the drivers of EMF. Nonetheless, several limitations should be acknowledged. The main limitations of this study include: (1) a focus on widely reported factors, which precluded the inclusion of other key variables like mixing proportion and stand age due to data scarcity; (2) a restricted geographic scope and limited sample size, which reduced the representation of diverse mixing ratios; and (3) the lack of consideration for microbial communities in deeper soil horizons. Consequently, future studies should aim to broaden geographic coverage, investigate deeper soil profiles, and examine a wider range of species compositions to advance our understanding of how mixed plantations shape microbial diversity and ecosystem processes.

5 Conclusion

This meta-analysis revealed that mixed plantations increased soil microbial diversity and enhanced EMF. Importantly, the positive correlation between microbial diversity and EMF underscores a potential central role of soil microbial communities in sustaining multiple ecosystem functions. Moreover, the significant relationships between EMF and soil physical properties underscore the importance of a well-structured soil environment in supporting ecosystem functioning. Although the study confirms the general benefits of mixed plantations, it also reveals that their effectiveness varies with N-fixing status, age structure, and species mixture type. In summary, mixed plantations offer significant ecological advantages over monocultures by enhancing soil microbial diversity and improving multiple ecosystem functions. Consequently, mixed plantations are a more effective afforestation strategy than monocultures in maintaining biodiversity and enhancing ecosystem multifunctionality.

Data availability statement

The original contributions presented in this study are included in this article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

KX: Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft. YX: Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. XL: Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

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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.2025.1694630/full#supplementary-material

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