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Initial soil condition, stand age, and aridity alter the pathways for modifying the soil carbon under afforestation

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Abstract

Afforestation is a crucial pathway for ecological restoration and has the potential to modify soil microbial community, thereby impacting the cycling and accumulation of carbon in soil across diverse patterns. However, the overall patterns of how

afforestation impacts below-ground carbon cycling processes remain uncertain. In this comprehensive meta-analysis, we systematically evaluated 7045 observations from 210 studies worldwide to evaluate the influence of afforestation on microbial communities, enzyme activities, microbial functions, and associated physicochemical properties of soils. Afforestation increases microbial biomass, carbon and nitrogen hydrolase activities, and microbial respiration, but not carbon oxidase activity and nitrogen decomposition rate. Conversely, afforestation leads to a reduction in the metabolic quotient, with significant alteration of bacterial and fungal community structures and positive effects on the fungi: bacteria ratio rather than alpha and beta diversity metrics. We found a total 77% increase in soil organic carbon (SOC) content after afforestation, which varied depending on initial SOC content before afforestation, afforestation stand age, and aridity index of afforestation sites. The modified SOC is associated with bacterial community composition along with intracellular metabolic quotient and extracellular carbon degrading enzyme activity playing a role. These findings provide insights into the pathways through which afforestation affects carbon cycling via microorganisms, thus improving ourknowledge of soil carbon reservoir's responses to afforestation under global climate change.

Keywords

Afforestation; Initial soil organic carbon; Stand age; Aridity index; Microorganisms; Carbon accumulation

Introduction

Afforestation is becoming one increasingly popular type of land use change project primarily designated for ecosystem restoration (Bastin et al., 2019), soil and water conservation (Zhang et al., 2016a), and climate change mitigation (Bonan et al.,2008). Up to 2015, the global planted forest area amounted to approximately 173 million hectares, about half of which were in China (45.1 million hectares), the UnitedStates (25.1 million hectares) and France (15.9 million hectares), with a substantial proportion attributing to afforestation (Harris et al., 2019). The deliberate establishment of trees or shrubs on previously non-forested land is commonly known as afforestation (Watson et al., 2000; Liu et al., 2018; Chen et al., 2021). Afforestationleads to changes in the litter quantity and quality as well as root exudates, whichexerts substantial impacts on the equilibrium between the vegetation and soil carbon, affecting the soil organic carbon (SOC) dynamic (Li et al., 2012; Panchal et al., 2022). These changes can profoundly affect microbial communities and their activities (Brant et al., 2006). The varied microbial compositions, enzyme activities, and carbonuse efficiency are tightly coupled with soil carbon (C) accumulation (Graham et al., 2016; Luo et al., 2022). Therefore, a better understanding of the underlying mechanisms by which afforestation regulates microorganisms and soil C is essentialto predict the dynamics of the soil C reservoir. This knowledge is vital for identifying whether terrestrial ecosystems act as a sink or source of C in the context of afforestation.

Differences in substrate availability affect the reassembly and metabolism of microbial communities (Machado et al., 2021). The control of microbial processes through the interaction between substrate availability and potential decomposers is contingent upon the initial soil status (Hicks et al., 2019). For instance, C-rich soils were more vulnerable to warming than C-poor soils, attributed to sensitive fungal communities that enhance the C metabolism and high utilization ability of soil C (Crowther et al., 2016; Huang et al., 2021). However, it remains unexplored whether the effect of land use change on soil microorganisms differs from the initial SOC status. Moreover, afforestation age regulates the accumulation of dead plant materials and root exudates, thereby modifying the microenvironment and impacting the response patterns of fungal and bacterial communities (Singh et al., 2012; Wang et al.,2022; Zhang et al., 2023). It has been reported that microbial respiration, metabolic quotient, and enzyme activities respond differently during the period of afforestation (Veres et al., 2015; Ren et al., 2016; Zhao et al., 2019; Huang et al., 2022). To further elucidate the relationship between microbial communities and C accumulation following afforestation, it is essential to understand how microbial activity evolves as the afforestation advances.

The effects of afforestation on soil microorganisms may depend on prior land use type, tree types, and climate conditions (Zhang et al., 2016b; Zhang et al., 2017; Zhao et al., 2019). For example, afforestation on abandoned cropland significantly affected the composition of microbial communities compared to afforestation on grassland (Zhang et al., 2016b). The introduction of broad-leaved tree species led to greater

changes in microbial diversity than coniferous species (Deng et al., 2019). A previous meta-analysis showed that the increase in microbial biomass after afforestation is more pronounced in arid regions than in humid regions (Zhao et al., 2019). Nevertheless, it remains unexplored whether the effects of afforestation on microbial metabolic activity differ among these factors. Identifying consistent effects of afforestation on soil microbiology and functions is challenging because afforestation and its impacts are site-specific. While myriad results of field studies have been documented, the broader patterns and mechanisms remain poorly understood, leading to substantial uncertainty when predicting the consequences of afforestation. Revealing general patterns in the responses of soil microbial communities and functions to afforestation requires a comprehensive synthesis of the studies.

Large uncertainties remain about belowground C cycles since the dynamics of soil C are mediated by soil microbial activities under afforestation. The microorganisms influence SOC through two major pathways: extracellular modification and *in vivo* turnover, which include anabolism and catabolism processes (Liang et al., 2017). The catabolism of soil organic matter is predominantly influencedby microbial community composition and biomass (Bailey et al., 2002; Fierer et al., 2007; Deng et al., 2016; Chen et al., 2021), which could further affect soil extracellular activities and microbial C use efficiency. Microbial C use efficiency is defined as the ratio of the amount of C used in new biomass, excluding the C excretedin the form of metabolites and enzymes (Manzoni et al., 2012; Sinsabaugh et al.,

2013). It is commonly utilized to quantify how the C is allocated for microbial growth

and respiration. Previous studies have indicated that high microbial C use efficiency promotes soil C accrual since more C was retained as microbial biomass (Mao et al., 1992; Malik et al., 2018; Zhu et al., 2021). Xu et al. (2017) demonstrated that the metabolic quotient can function as an indicator of microbial C use efficiency. Several modelling efforts have confirmed that incorporating microorganisms into soil C models can substantially improve the accuracy of depicting both the direction and magnitude of C-climate feedback (Perveen et al., 2014; Delgado-Baquerizo et al., 2016; Delgado-Baquerizo et al., 2017). A better understanding of how microorganisms respond to land-use changes and provide feedback to soil C cycling beyond the microhabitat scale is crucial in both microbial ecology and ecosystem modelling communities (Pointing et al., 2016). However, C-cycle models do not account for the variation in microbial community structure and function due to land use change, as the mechanisms by which land use alters microbial contributions to soil C-cycling remains uncertain. It's essential to clarify how C accumulation responds to afforestation in terms of microbial community composition and physiological acclimation and/or adaptation.

Previous meta-analyses have investigated the impacts of afforestation on soil physicochemical properties (Berthrong et al., 2009; Li et al., 2012; Liu et al., 2018; Li et al., 2019; Wang et al., 2020a), microorganisms (Zhang et al., 2017; Zhao et al., 2019; Huang et al., 2022), enzyme activities (Huang et al., 2022), and so on. However, a comprehensive understanding of the mechanisms by which soil microorganisms influence C accumulation remains elusive. This knowledge gap

hinders the accurate prediction of long-term SOC dynamics following afforestation. To address these questions, it is essential to conduct large-scale comparative studies at afforested and non-afforested control sites. Therefore, this study performed a global synthesis of 225 publications and aimed to elucidate: (1) the general patterns of afforestation effects on soil microorganisms and activities, and (2) how these microbial attributes influence the cycling and accumulation of soil C under afforestation.

Methods

Data collection

We systematically searched the peer-reviewed journal articles that investigated the effects of afforestation on soil microbial attributes. A survey of articles published until 16th October 2023 was conducted through the ISI Web of Science Core Collection and Google Scholar databases using the combinations of keywords "soil* AND (OTU OR microb* OR bacteri* OR fung* OR extracellular enzyme OR N mineralization OR metabolic quotient OR qCO_2 OR microbial respiration OR basal respiration) AND (reforest* OR afforest* OR tree plantation)". The datasets of two meta-analyses that studied microbial attributes were also included (Zhao et al., 2019; Huang et al., 2022). Each article was reviewed to determine its eligibility based on four selection criteria:

1. at least one microbial metric, either microbial community or activities, was

reported;

2. focusing on the 0-30 cm topsoil and observations for deep soil layers were discarded;
3. the experiments were conducted under natural field conditions, and had at least one pair of data points comparing tree plantations (shrubland or woodland) versus adjacent unforested ecosystems (cropland and uncultivated land);
4. only the first rotation of afforestation was included.

When multiple publications included the same data from one study, we recorded the data only once. If a study included afforestation in various locations, with different stand ages, tree species, and soil layers, we considered them as distinct observations. The literature selection followed the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (Figure S1; Moher et al., 2009). Ultimately, we obtained a meta-dataset of 7045 observations and 210 studies from 225 publications. The dataset consisted of ten microbial community metrics, six microbial activities, and eight soil physicochemical properties. Microbial community metrics included microbial biomass, the biomass of fungi: bacteria ratio, fungal and bacterial Shannon diversity, richness, beta diversity, and community structure. The six microbial activities were C hydrolase (β-glucosidase, α-glucosidase, Cellobiohydrolase, Dehydrogenase, Xylosidase, Saccharase, Invertase, Cellulase, and Sucrase), C oxidase (phenol oxidase, peroxidase, and catalase), nitrogen (N) hydrolase (β-1,4-N-acetylglucosaminidase, urease, protease, and leucine aminopeptidase), microbial respiration, N decomposition rate, and metabolic quotient.

Additionally, soil physicochemical properties included SOC, dissolved organic C, total N, inorganic N, C: N ratio, soil water content, pH, and sand content. More details are provided in Table S1.

All the original data of the mean ($\overline{X}$), standard deviations (SD), and sample sizes (n) were extracted from the text, tables, figures, and appendices of the publications. When the results were graphically presented, data were extracted digitally using the Digitizer tool GetData Graph Digitizer (version 2.24). If standard errors (SE) were reported, we calculated SD using the following equation:

$$SD = SE \times \sqrt{n} \quad (1)$$

When the studies had no SD or SE values, we assigned an SD value of 1/10 of the mean, following the approach used by Luo et al. (2006). Afforestation characteristics (prior land use type, tree types, and stand age) and geographical locations (latitude and longitude) were obtained from the original publications. The study locations are shown in Figure 1. The aridity index (AI, mean annual precipitation divided by potential evaporation) of each location was extracted from the database (http://www.worldclim.org/). The AI values were accordingly higher in humid regions with more precipitation, and lower in drylands with strong atmospheric evaporation demand (Trabucco and Zomer, 2018).

Data analysis

The effect of afforestation on individual variables, except for the response ratio of microbial beta diversity (RR_{Beta}) and community structure ($RR_{Structure}$) was

estimated for each case study as the response ratio (*RR*):

$$RR = \ln\left(\frac{\overline{Xt}}{\overline{X}c}\right) = \ln\left(\overline{Xt}\right) - \ln\left(\overline{X}c\right) \quad (2)$$

where $\overline{X}_t$ and $\overline{X}_c$ are the mean values for the variable of the treatment and control, respectively. The variances (*v*) were calculated as:

$$v = \frac{S_t^2}{n_t \overline{X}_t^2} + \frac{S_c^2}{n_c \overline{X}_c^2} \quad (3)$$

where n_t and n_c are the sample sizes for the variable of the treatment and control, respectively; s_t and s_c are the standard deviations for the variable of the treatment and control, respectively. The microbial composition was analyzed using ordination techniques to demonstrate beta diversity within each treatment and variations in community structure among treatments (Zhou et al., 2020). We extracted the first two ordination axes of microbial composition using various ordination methods to calculate the Euclidean distances among different samples. This included distances within the control (D_c), within the treatment (D_t), and between the control and treatment (D_b) (Table S1, Zhou et al., 2020). RR_{Beta} and $RR_{Structu}$ were given by:

$$RR_{Beta} = \ln\left(\frac{\overline{D_t}}{\overline{D_c}}\right) \quad (4)$$

$$RR_{Structure} = \ln\left(\frac{\overline{D_b}}{\overline{D_c + D_t}}\right) \quad (5)$$

where $\overline{D_c}$, $\overline{D_t}$, $\overline{D_b}$, and $\overline{D_c + D_t}$ are the means of the D_c, D_t, D_b, and D_c+D_t, respectively. Their corresponding variances were calculated using equation (2). A

negative RR_{Beta} indicates a decrease in beta diversity in response to afforestation, while a positive value suggests an increase. A higher positive value of $RR_{structure}$

indicates a greater magnitude of community structure change; if $RR_{structure} < 0$, it signifies no effect. In the meta-analyses, the effect size estimations depend on how the individual observations are weighted and were given as follows (Chen et al., 2019; Chen et al., 2022; Wang et al., 2023):

$$W_r = \left(N_c \times N_t\right) / \left(N_c + N_t\right) \quad (6)$$

where W_r is the weight for each observation, and N_t and N_c are the numbers of replications for the afforestation treatment and the control, respectively.

The variance of effect sizes on microbial attributes and physiochemical properties was not only derived from the sampling error of the studies but alsodepended on differences in environmental factors and management practices among the studies. We tested the effect sizes of all attributes and investigated whether the effect sizes were influenced by five types of candidate predictors: (1) initial soil organic C content before afforestation (ISOC); (2) afforestation standage (SA); (3) AI in afforestation sites; (4) type of non-afforested control sites (CO, including cropland and uncultivated land); and (5) afforestation leaf types (LT, including conifer, broadleaf, and mixed plantation), using linear mixed-effect models. Models were implemented using restricted maximum likelihood (REML), with *Wr* being the weightfor each corresponding *RR* observation, using the R package *lme4* (Bates et al., 2015).We scaled the continuous predictors (ISOC, SA, and AI) and selected the mostparsimonious model with the lowest second-order Akaike information criterion (AIC) values to prevent overfitting (Table S3). The effects of the ISOC, SA, and AI, as well

as their interactions, were estimated by:

$$\ln RR = \beta_0 + \beta_1 \cdot ISOC + \beta_2 \cdot SA + \beta_3 \cdot AI + \beta_4 \cdot ISOC \times SA + \beta_5 \cdot ISOC \times AI + \beta_6 \cdot SA \times AI + \beta_7 \cdot ISOC \times SA \times AI + \pi_{study} + \varepsilon \quad (7)$$

where β_i are coefficients, π_{study} is random effects accounting for the autocorrelation among observations in each study, and ε is the sampling error. The CO and LT were individually modelled since simultaneous modelling would result in high inherent multicollinearity (Zuur et al., 2010). Model selection was implemented using the "dredge" function from the "MuMln" package (Bartoń, 2009). For the continuous predictors, we compared the linear and logarithmic-linear responses using alternative models and selected the most parsimonious model that retained the aforementioned predictors under CO and LT (Table S3). The selected models showed no multicollinearity (VIF < 3) (Graham et al., 2003). Pearson correlation analysis was used to explore the association between the C cycle-related variables. The SA was classified as 0-20 years and >20 years and the AI was classified as dryland (AI less than 0.65) and humid regions (AI more than 0.65) (Deng et al., 2017; Zhao et al., 2019; Qi et al., 2022). Within each variable, t-tests were used to assess the significance of the differences between the two groups. The response of the variables

to afforestation was also estimated as follows:

$$Effect\ size\ (\%) = (e^{\ln RR} - 1) \times 100\% \quad (8)$$

The publication bias was evaluated using Begg's test, Egger's regression (Koricheva et al., 2013), and file drawer analysis (Feng et al., 2022). All of the analyses were performed using the R program (v.4.0.2, R Core Team. 2021).

Results

The effects of afforestation on belowground processes

Afforestation generally significantly increased microbial biomass, the fungi: bacteria ratio, and the magnitude of change in bacterial and fungal community structures. However, the fungal and bacterial alpha diversity (Shannon and richness) and beta diversity remained consistent (Figure 2). Notable increases were observed in soil C and N hydrolase enzyme activities, microbial respiration, soil C and N concentrations (SOC, dissolved organic C, total N, and inorganic N), C: N ratio, and soil water content (Figure 2). There was a distinct decrease in the metabolic quotient and pH values (Figure 2). No significant changes were observed in the C oxidase, N decomposition rate, and sand content (Figure 2). Regarding specific enzymes, afforestation stimulated both hydrolytic and oxidase activities, and the increase in hydrolase activities surpassed that of oxidase (Figure S2).

The most parsimonious model identified that the ISOC, SA, and AI collectively affected the changes in SOC, total N, and C and N hydrolase activities (Figure 3; Table S4). The changes in microbial biomass, metabolic quotient, inorganic N, and soil water content demonstrated joint influences from ISOC and SA (Figure 3a and b),whereas fungal beta diversity and pH were concomitantly influenced by ISOC and AI (Figure 3a and c). There are also some soil properties affected by single factors. For instance, ISOC significantly affects bacterial beta diversity and fungi: bacteria ratio (Figure 3a), SA significantly affects fungal community structure (Figure 3b), and AI significantly affects bacterial community structure and beta diversity (Figure 3c).

In afforested land, SOC, dissolved organic C, microbial respiration, total N, inorganic N, microbial biomass, C and N hydrolase activities, and soil water content decreased significantly with increasing ISOC (Figure 3a). Conversely, higher ISOC leads to increased bacterial beta diversity, fungi: bacteria ratio, and metabolic quotient after afforestation (Figure 3a). As afforestation progressed, fungal beta diversity and community structure, microbial biomass, C and N hydrolase activities, organic C, total N, inorganic N, and soil water content increased, while the metabolic quotient decreased significantly (Figure 3b). Higher AI equated to more humidity in the afforested region and more afforestation increased bacterial community structure, fungal beta diversity, C and N hydrolase activities, N decomposition rate, soil organic C, and total N (Figure 3c). However, the *RR*s of fungal Shannon diversity andrichness and pH decreased with the increasing AI (Figure 3c).

Except for bacterial alpha diversity, C oxidase activity, and N decomposition rate, the response of other soil properties to afforestation did not change among non-afforested control sites and afforestation leaf types (Table S4). Afforestation on uncultivated land was more conducive to bacterial alpha diversity, including a 7.3% increase in Shannon diversity and a 29.7% increase in richness than cropland (Table S4). Compared to broadleaf forests, C oxidase activity after afforestation with conifer and mixed plantation forests increased by 103.4% and 44.8%, respectively, and the N decomposition rate of mixed plantation increased by 301.5% (Table S4). In addition, the most parsimonious model showed that *RR*s of sand content and C: N ratio showed no variation among the ISOC, SA, AI, CO, and LT (Table S4).

The relationship between soil microbial communities, soil enzyme activities, and C cycling

Afforestation-induced changes in bacterial and fungal community composition significantly affect soil enzyme activities and SOC (Figure 4). Specifically, the *RR*s of C hydrolase activity and SOC increased with the *RR*s of bacterial Shannon diversity and richness (Figure 4a and d). The increase in bacterial beta diversity and changes in community structure associated with afforestation contribute to the increase in SOC (Figure 4d). For fungi, the changes in community composition (including Shannon and beta diversity, richness, and community structure) had a significantly positive effect on the N hydrolase activity (Figure 4h). In addition, fungi: bacteria ratios also significantly affected the enzyme activities, and the afforestation-induced changes in C and N hydrolase activities decreased (Figure 4i and k) and C oxidase activity increased (Figure 4j) with the increasing fungi: bacteria ratios.

The *RR*s of SOC, dissolved organic C, and microbial biomass induced by afforestation had a significantly positive correlation with C hydrolase and oxidase activities (Figure 5a-c). Microbial biomass was more affected by C hydrolase activity ($R^2 = 0.447$, $P < 0.001$) rather than C oxidase activity ($R^2 = 0.039$, $P < 0.05$). In contrast, afforestation-induced changes in microbial respiration and metabolic quotient were only significantly affected by C hydrolase activities, with *RR* of microbial respiration increased ($P < 0.05$) and *RR* of the metabolic quotient ($P < 0.001$) decreased with the increasing C hydrolase activity (Figure 5d and e).

Factors affecting soil organic C across interannual variations and aridity index

Under afforestation exceeding 20 years, we observed a significantly higher increase in SOC, dissolved organic C, microbial biomass, and microbial respiration compared to afforestation less than 20 years (Figure 6a-d). However, the response of metabolic quotient for afforestation more than 20 years was lower than that of 0–20 years ($P < 0.1$, Figure 6e). The increase in SOC was mainly associated with the decrease in microbial quotient and the increase in microbial biomass after 20 years of afforestation (Figure 8a).

The positive effect of dryland afforestation on C hydrolase and oxidase activities decreased with the increasing ISOC (Figure S5b and c). In contrast, the effect of afforestation on C oxidase activity varied from negative to positive with the increasing ISOC, while the positive effect on C hydrolase activity did not vary with the ISOC in humid afforestation regions (Figure S5b and c). Afforestation in humid regions had a stronger positive effect on C hydrolase activity than in dryland afforestation regions (Figure 7c and e), whereas there were no significant differences in *RR*s of SOC and C oxidase activity (Figure 7a and d). When separately considering afforestation in C-poor ($SOC < 5 g\ kg^{-1}$) and C-rich ($SOC > 5 g\ kg^{-1}$) soils, still no difference in SOC was found for C-poor soils afforestation in different climate zones (Figure S6a). However, afforestation in C-rich soils of humid regions resulted in a significantly higher increase in SOC compared to dryland ($P < 0.001$) (Figure S6b). Inhumid afforestation regions, significantly lower soil pH had positive and negative

effects on C hydrolysis and oxidase activities, respectively (Figures 7b and 8a). An increase in soil water content due to afforestation was conducive to an increase in C hydrolase activity and the accumulation of SOC (Figure 8a). The potential alteration in the microorganisms and enzyme activities on C accumulation by afforestation are shown in Figure 8b.

Discussion

Variation in the response of soil microorganisms to afforestation

Afforestation caused an increase in the fungi: bacteria ratio (Figure 2) may conceivably arise from the high C: N ratio of litter and rhizosphere-to-bulk soil ratio after afforestation, which favours oligotrophic fungi (Fierer et al., 2012; Delgado-Baquerizo et al., 2016). This result is consistent with the findings of Luo et al. (2023) that afforestation increased fungal biomass more than bacterial biomass. Our study further found that the *RR*s of fungi: bacteria ratio increased logarithmically with ISOC (Figure S3j). The result was contrary to the uniformly decreasing pattern in the *RR* of bacterial alpha diversity change (Figure S3f and g). Slow-growing oligotrophic fungi have a competitive advantage in C-poor environments, to the detriment of bacterial growth (De Vries et al., 2013; Huang et al., 2021). The C-rich soils tend to support the growth and reproduction of bacteria with low substrate affinity and high growth rate (Leff et al., 2015). Bacteria suffering from C starvation in C-poor soils can exhibit

greater activity to the available C added by afforestation litter (Kong et al., 2018; Zhang et al., 2019a). After afforestation, the enhanced bacterial alpha diversity in C- poor soils can make bacteria more competitive in capturing C than fungi, resulting ina relatively lower value of fungi: bacteria ratio (Kaiser et al. 2010; Razanamalala et al., 2018). This result was supported by the significantly negative correlation between *RR*s of dissolved organic C and fungi: bacteria ratio (Figure 8a). In addition, the increased bacterial alpha diversity was significantly correlated with SOC (Figure 4d), suggesting that the bacterial diversity was beyond the threshold of functional redundancy under C starvation conditions (Nielsen et al., 2011; Delgado-Baquerizo et al., 2016; Li et al., 2024). Due to the greater tolerance to aridity (Bapiri et al., 2010; De Vries et al., 2012; Barnard et al., 2013), fungi can maintain higher metabolic readiness than bacteria in arid environments. A relatively higher positive response of fungal alpha diversity to afforestation occurred in dryland than in humid regions (Figure S4a and b), owing to that afforestation consequently mitigated the negative effects of aridity on fungal alpha diversity (Wang et al., 2021).

The rise in the alpha and beta diversity of both bacteria and fungi showed a smaller magnitude of change than the alteration in bacterial and fungal community structure (Figure 2). Univariate representation of microbial diversity masks the differences between groups and individual populations (Hartmann and Widmer 2006; Luo et al., 2022). Moreover, the changed fungal community structure increased with the afforestation stand age (Figure 3b) due to the increased presence of ectomycorrhizas and arbuscular mycorrhizal fungi (Qi et al., 2022). These changes

occur when the mycorrhizal fungi increase and plant litter decomposition is enhanced during afforestation (Zumsteg et al., 2012; Zhang et al., 2018). The AI of afforestation sites is the dominant factor affecting the bacterial community structure, and theminimal changes in the response of bacterial community structure to afforestation in arid regions may keep in line with the limited reliance of arid-adapted bacterial populations on external litter sources (Fuchslueger et al., 2016). Simultaneously, the changes in bacterial community structure across the aridity gradients affected SOC (Figure 4d), and attention should be paid to the variations of the community structure of specific taxa or functional groups of bacteria for large-scale afforestation under climate change.

Distinct response of carbon hydrolase and oxidase activities to afforestation

Afforestation increased C hydrolase rather than C oxidase activity (Figure 2). C hydrolases and oxidases play distinct roles in the degradation of cellulose and lignin present in apoplastic materials, respectively (Chen et al 2022a). Soil microorganisms tend to prioritize the metabolism of easily decomposable cellulose over complex structured lignin (Kaiser et al., 2010). The introduction of litter with abundant organic matter by afforestation may lead microorganisms to reduce the production of Coxidase, affecting the decomposition of recalcitrant compounds (Craine et al., 2007).

In C-poor soils, the increased enzyme activities would enhance the hydrolysis of organic matter to relieve C limitation for microbes caused by low dissolved organic C (Allison and Vitousek, 2005). On the contrary, the relatively smaller changes in

enzyme activities observed in C-rich soils likely reflect that soil microbes do not require additional C delivery from afforestation, which is consistent with microbial biomass (Xu et al., 2022). The relatively higher enzyme activity observed in humid regions may be attributed to the positive interaction between afforestation and soil moisture (Xiao et al., 2018). Unexpectedly, the negative response of C oxidase activity in C-poor soils and humid regions after afforestation (Figure S5c) may be derived from a reduction in the fungi: bacteria ratio and pH. This observation supportsthe theoretical framework that fungi are the primary producers of oxidase (Piton et al.,2020; Wu et al., 2022), thus decreased fungi: bacteria ratios in C-poor soils (Figure S3j) lead to a decrease in oxidase activity. In addition, the larger negative effect of reduced pH on C oxidase activity than on C hydrolase activity in humid regions (Figure 7b) coincided with the finding of Sinsabaugh et al. (2008) reinforces this conclusion.

Within a certain SA range, afforestation results in more water consumption by increased canopy transpiration, but increasing canopy coverage reduces surface soil evaporation (Tanaka and Hashimoto, 2006; Schwärzel et al., 2020). Larger SA implies an increasing water-holding capacity of litter materials, which improves precipitation interception and reduces soil evaporation, leading to increased soil infiltration and surface soil water content (Figure 2a, Xia et al., 2019; Bai et al., 2021). The increasing positive effect of SA on the response of C hydrolase activity is consistent with that on soil water content (Figure 8a), which favours the hydrolase activity (Zuccarini et al., 2023). The C oxidase activity does not change with the SA due to its

weak dependence on soil moisture (Zuccarini et al., 2023). However, the response of C oxidase activity was significantly altered by the LT. Compared with afforestation with broadleaf, the C oxidase activity showed greater changes under afforestation with conifer-leaved species and mixed plantation (Table S4). This difference can be attributed to the higher content of hard-to-degrade lignin and thus a higher C: N ratio of conifer litter (Brunel et al., 2017). The lignin-rich conifer litter is more prone to decomposition by C oxidase, leading to more pronounced changes in C oxidase activity. Moreover, microorganisms produce specialized extracellular enzymes to meet their ample soil nutrient requirement for triggering an upsurge in C-catabolic enzyme production (Sinsabaugh and Follstad Shah, 2011; Mooshammer et al., 2014; Luo et al., 2019). The observed increase in N hydrolase activity ensures the availability of adequate nutrients (Figure 8b). The activities of C hydrolase andoxidase activities showed distinct responses when afforestation occurs due to the variations in microbial needs, fungal-to-bacterial ratios, soil moisture, and pH.

Afforestation alters soil carbon accumulation pathways

The SOC after afforestation increased by 77% to that of non-forested sites, reflecting the accelerated turnover between the input and decomposition of plant residues from afforestation (Fujii et al., 2020). Our results also indicated that the SOC increased with SA (Qi et al., 2022) and AI (Jia et al., 2019; Chen et al., 2020b). However, the positive response of SOC decreased with ISOC (Hong et al., 2020), regardless of LT and CO (Table S4).

A lowered metabolic quotient and increased microbial biomass contributed to the increased SOC (Figure 8a) when afforestation occurs in low ISOC soils or has been afforested for more than 20 years. The pivotal role of initial soil conditions on the response of heterotrophic metabolism capacity to exogenous C addition from afforestation keeps in line with the finding of Wang et al. (2020b). Temporal variations in microbial respiration, microbial biomass, and metabolic quotient (Figure 6c-e) underscore the dynamic nature of microbial responses to afforestation, suggesting potential shifts in microbial metabolic strategies over time (Su et al., 2023). The progression of afforestation encourages a substantial allocation of photosynthates to belowground components, thereby escalating the input of labile root-derived carbon and the content of dissolved organic C (Hong et al., 2020). The transformation of degradable organic matter into microbial biomass substantially contributes to SOC accumulation (Sokol and Bradford, 2019; Zhang et al., 2023).

The lower metabolic quotient indicates a higher C use efficiency andconsequential microbial necromass contribution to C accumulation (Xu et al., 2017;Su et al., 2023). Despite both C hydrolase and oxidase activities were positively related to microbial biomass (Figure 5c), the dominance of a more energy-efficient C hydrolase pathway became evident. More energy was consumed by microorganismsto stimulate the C oxidase activity for recalcitrant C pool degradation than that used for stimulating the C hydrolase activity to decompose the easily degradable C pool (Zhang et al., 2019 b). Therefore, energy partitioning with C hydrolase is more

conducive to improving microbial C use efficiency. Soil C hydrolase activity has

direct effects on microbial respiration and C availability for microbial growth under afforestation (Chen and Chen 2018; Zhang et al., 2021; Xu et al., 2022). The materials produced by C-catabolic enzymes serve as substrates for both microbial catabolism and anabolism (Zhang et al., 2021). Though soil respiration increased with the increasing C hydrolase activity, the simultaneous increases of C use efficiency (decreased metabolic quotient) and SOC (Figure 5a, d, and e) suggest that the C hydrolase activity stimulates more anabolism than catabolism (Chen and Chen, 2019; Zhang et al., 2021). Overall, the shifts of microbial intracellular anabolism shaped the changes in SOC with the SA and ISOC.

The higher C hydrolase and oxidase activities in C-rich soils of humid regions favoured the increase of SOC after afforestation. The C hydrolase and oxidase activities directly decompose cellulose and lignin to partly decomposed plant materials and labile C (Liang et al., 2017; Chen et al., 2018). The unobvious response of SOC to afforestation in C-poor regions may be attributed to the reduced C oxidase activity. The reduced C oxidase activity after afforestation in C-poor humid regions offsets the accumulation of SOC from increased C hydrolase activity (Figure S5b and c). These findings explore the potential pathways of SOC accumulation under afforestation and improve the knowledge of long-term and large-scale soil C dynamics.

Limitations and Future Study

Using the meta-analysis of the database from peer-reviewed literature, this study

exhibited the global-scale patterns and regulations of afforestation on microorganisms, enzyme activities, and SOC. Compared to the worldwide maps of planted tree distribution (Harris et al., 2019), our study lacks the afforestation sites in North America and has relatively limited data in Europe (Figure 1). To ensure the usability of the data obtained, strict screening criteria were developed which excluded the studies in these regions (Table S5). However, the potential publication bias analysis showed that Rosenthal's fail-safe number was much larger than 5n+10 (n, number of observations, Table S2), the absence of these data would not impact the effect sizes (Feng et al., 2022).

The results showed that afforestation increased C hydrolase activity more than oxidase activity, the ratio of C hydrolase to oxidase activities would increase in a certain period. Xu et al. (2023) showed that the increase of soil C hydrolase to oxidaseactivity was closely related to the increase of SOC stability. Therefore, the effect of soil C hydrolase to C oxidase activity on SOC stability under afforestation should be followed up in future studies. Moreover, the lack of a soil C saturation capacity monitor poses a challenge in determining the most suitable sites for afforestation to maximize soil C sink. This challenge is complicated in drylands, where conflicts arise between afforestation and the availability of water resources (Teng et al., 2022). Inthis study, the contribution of afforestation to C accumulation was significantly lower in C-rich drylands than in humid regions (Figure S6b). This result highlights the importance of considering climatic conditions when assessing the potential for C sequestration under ecological restoration programs, especially in fragile ecosystems

in arid regions. Recent studies have employed machine learning algorithms to predict the soil C saturation capacity based on soil properties, land-use history, and climatic conditions (Szatmári et al., 2023). Efforts incorporating long-term monitoring, machine learning modelling techniques, and interdisciplinary approaches are crucial for addressing these challenges and identifying appropriate sites for ecological restoration in future.

Conclusion

This meta-analysis expands our understanding of the effect of afforestation on soil microbial, physical, and chemical properties, and provides evidence that afforestation has a substantial impact on C accumulation processes. Overall, afforestation is particularly conducive to enhancing SOC accumulation, especially in C-poor or humid regions, as well as during the post-afforestation period (>20 years). The impact of afforestation on SOC may be predominantly associated with changes in bacterial community composition. Microbial activities contributed to the changes in SOC under afforestation by both intracellular pathways (metabolic quotient) and extracellular metabolic pathways (enzyme activities). Moreover, the altered microbial biomass, metabolic quotient, and C hydrolase activity were highly correlated with water content and played a significant role in C accumulation processes under afforestation. These results highlight the importance of considering water availability when evaluating the effectiveness of microbial factors on C sequestration. Explicit consideration of these factors can contribute to the development of personalized

optimization strategies tailored to specific afforestation scenarios.

Author contributions

D. Li, Y. Zheng and J. Pei conceived the study. J. Ye, Y. Zheng, J. Pei, W. Ke, and C. Fang jointly analyzed data and drafted the paper. J. Peñuelas., J. Sardans, J. Ye, D. Li, C. Fang, W. Ke and X. Song wrote and edited the final version of the manuscript.

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References

Allison, S. D., Vitousek, P. M., 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biology and Biochemistry 37(5), 937–944.

Bai, Y., Zhou, Y., Du, J., Zhang, X., Di, N., 2021. Effects of a broadleaf-oriented transformation of coniferous plantations on the hydrological characteristics of litter layers in subtropical China. Global Ecology and Conservation 25, e01400.

Bailey, V. L., Smith, J. L., Bolton Jr, H., 2002. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. Soil Biology and Biochemistry 34(7), 997–1007.

Bastin, J. F., Finegold, Y., Garcia, C., Mollicone, D., Rezende, M., Routh, D., Zohner, C. M., Crowther, T. W., 2019. The global tree restoration potential. Science 365(6448), 76–79.

Bapiri, A., Bååth, E., Rousk, J., 2010. Drying–rewetting cycles affect fungal and bacterial growth differently in an arable soil. Microbial Ecology 60, 419–428.

Barnard, R. L., Osborne, C. A., Firestone, M. K., 2013. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. The ISME journal7(11), 2229–2241.

Bartoń, K., 2009. MuMIn: multi-model inference. http://r-forge. r-project. org/projects/mumin/.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67(1): 1-48.

Berthrong, S. T., Jobbágy, E. G., Jackson, R. B., 2009. A global meta-analysis of soil exchangeable cations, pH, carbon, and nitrogen with afforestation. Ecological Applications 19(8), 2228–2241.

Bonan, G. B., 2008. Forests and climate change: forcings, feedbacks, and the climate benefits of forests. Science 320(5882), 1444–1449.

Brant, J. B., Sulzman, E. W., Myrold, D. D., 2006. Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. Soil

Biology and Biochemistry 38(8), 2219–2232.

Brunel, C., Gros, R., Ziarelli, F., Da Silva, A. M. F., 2017. Additive or non-additive effect of mixing oak in pine stands on soil properties depends on the tree species in Mediterranean forests. Science of the Total Environment, 590, 676–685.

Chen, X., Chen, H. Y., 2018. Global effects of plant litter alterations on soil CO_2 to the atmosphere. Global Change Biology 24(8), 3462–3471.

Chen, J., Luo, Y. Q., García-Palacios, P., Cao, J. J., Dacal, M., Zhou, X. H., Li, J. W., Xia, J. Y., Niu, S. L., Yang, H. Y., Shelton, S., Guo, W., van Groenigen, K. J., 2018. Differential responses of carbon-degrading enzyme activities to warming: Implications for soil respiration. Global Change Biology 24(10), 4816–4826.

Chen, X., Chen, H. Y., 2019. Plant diversity loss reduces soil respiration across terrestrial ecosystems. Global Change Biology 25(4), 1482–1492.

Chen, C., Chen, H. Y., Chen, X., Huang, Z., 2019. Meta-analysis shows positive effects of plant diversity on microbial biomass and respiration. Nature Communications 10(1), 1332.

Chen, J. I., Elsgaard, L., van Groenigen, K. J., Olesen, J. E., Liang, Z., Jiang, Y., Lærke, P. E., Zhang, Y. F., Luo, Y. Q., Hungate, B. A., Sinsabaugh, R. L., Jørgensen, U. 2020a. Soil carbon loss with warming: New evidence from carbon-degrading enzymes. Global Change Biology 26(4), 1944–1952.

Chen, Z., Yu, G., Wang, Q., 2020b. Effects of climate and forest age on the ecosystem carbon exchange of afforestation. Journal of Forestry Research 31(2), 365–374.

Chen, L. F., He, Z. B., Wu, X. R., Du, J., Zhu, X., Lin, P. F., Tian, Q. Y., Kong, J. Q.,

2021. Linkages between soil respiration and microbial communities following afforestation of alpine grasslands in the northeastern Tibetan Plateau. Applied Soil Ecology 161, 103882.

Chen, Q., Long, C., Chen, J., Cheng, X., 2021. Differential response of soil CO2, CH4, and N2O emissions to edaphic properties and microbial attributes following afforestation in central China. Global Change Biology, 27(21), 5657−5669.

Chen, X., Chen, H. Y., Chang, S. X., 2022. Meta-analysis shows that plant mixtures increase soil phosphorus availability and plant productivity in diverse ecosystems. Nature Ecology & Evolution 6(8), 1112–1121.

Craine, J. M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88(8), 2105–2113.

Allison, S. D., Blair, J. M., Bridgham, S. D., Burton, A. J., Carrillo, Y., Reich, P. B., Clark, J. S., Classen, A. T., Dijkstra, F. A., Elberling, B., Emmett, B. A., Estiarte, M., Frey, S. D., Guo, J., Harte, J., Jiang, L., Johnson, B. R., Kröel-Dulay, G., Larsen, K. S., Laudon, H., Lavallee, J. M., Luo, Y., Lupascu, M., Ma, L. N., Marhan, S., Michelsen, A., Mohan, J., Niu, S., Pendall, E., Peñuelas, J., Pfeifer-Meister, L., Poll, C., Reinsch, S., Reynolds, L. L., Schmidt, I. K., Sistla, S., Sokol, N. W., Templer, P. H., Treseder, K. K., Welker, J. M., Bradford, M. A., 2016. Quantifying global soil carbon losses in response to warming. Nature 540(7631), 104–108.

De Vries, F. T., Liiri, M. E., Bjørnlund, L., Bowker, M. A., Christensen, S., Setälä, H.

M., Bardgett, R. D., 2012. Land use alters the resistance and resilience of soil food webs to drought. Nature Climate Change 2(4), 276–280.

De Vries, F. T., Shade, A., 2013. Controls on soil microbial community stability under climate change. Frontiers in Microbiology 4, 265.

Delgado-Baquerizo, M., Giaramida, L., Reich, P. B., Khachane, A. N., Hamonts, K., Edwards, C., Lawron, L. A., Singh, B. K., 2016. Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning. Journal of Ecology 104(4), 936–946.

Delgado-Baquerizo, M., Eldridge, D. J., Ochoa, V., Gozalo, B., Singh, B. K., Maestre, F. T., 2017. Soil microbial communities drive the resistance of ecosystem multifunctionality to global change in drylands across the globe. Ecology Letters 20(10), 1295–1305.

Deng, Q., Cheng, X., Hui, D., Zhang, Q., Li, M., Zhang, Q., 2016. Soil microbial community and its interaction with soil carbon and nitrogen dynamics following afforestation in central China. Science of the Total Environment 541, 230–237.

Deng, J., Zhang, Y., Yin, Y., Zhu, X., Zhu, W., Zhou, Y., 2019. Comparison of soil bacterial community and functional characteristics following afforestation in the semi-arid areas. PeerJ 7, e7141.

Feng, J., He, K., Zhang, Q., Han, M., Zhu, B., 2022. Changes in plant inputs alter soil carbon and microbial communities in forest ecosystems. Global Change Biology 28(10), 3426–3440.

Fierer, N., Bradford, M. A., Jackson, R. B., 2007. Toward an ecological classification

of soil bacteria. Ecology 88(6), 1354–1364.

Fierer, N., Lauber, C. L., Ramirez, K. S., Zaneveld, J., Bradford, M. A., Knight, R. 2012., Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. The ISME Journal 6(5), 1007–1017.

Fuchslueger, L., Bahn, M., Hasibeder, R., Kienzl, S., Fritz, K., Schmitt, M., Watzka, M., Richter, A., 2016. Drought history affects grassland plant and microbial carbon turnover during and after a subsequent drought event. Journal of Ecology 104(5), 1453–1465.

Fujii, K., Morioka, K., Hayakawa, C., Inagaki, Y., Hangs, R. D., Anderson, D. W., McConkey, B. G., 2020. Litter decomposition and soil organic carbonstabilization in a Kastanozem of Saskatchewan, Canada. Geoderma Regional 23, e00348.

Graham, M. H., 2003. Confronting multicollinearity in ecological multiple regression. Ecology 84(11), 2809–2815.

Knelman, J.E., Schindlbacher, A., Siciliano, S., Breulmann, M., Yannarell, A., Beman, J.M., Abell, G., Philippot, L., Prosser, J., Foulquier, A., Yuste, J.C., Glanville, H.C., Jones, D.L., Angel, R., Salminen, J., Newton, R.J., Bürgmann, H., Ingram, L.J., Hamer, U., Siljanen, H.M.P., Peltoniemi, K., Potthast, K., Bañeras, L., Hartmann, M., Banerjee, S., Yu, R.-Q., Nogaro, G., Richter, A., Koranda, M., Castle, S.C., Goberna, M., Song, B., Chatterjee, A., Nunes, O.C., Lopes, A.R., Cao, Y., Kaisermann, A., Hallin, S., Strickland, M.S., Garcia-Pausas, J., Barba,

J., Kang, H., Isobe, K., Papaspyrou, S., Pastorelli, R., Lagomarsino, A., Lindström, E.S., Basiliko, N., Nemergut, D.R., 2016. Microbes as engines of ecosystem function: when does community structure enhance predictions of ecosystem processes? Frontiers in Microbiology 7, 214.

Harris, N., Goldman, E. D., Gibbes, S., 2019. Spatial database of planted trees (SDPT) version 1.0. Technical Note, World Resources Institute.

Hartmann, M., Widmer, F., 2006. Community structure analyses are more sensitive to differences in soil bacterial communities than anonymous diversity indices. Applied and Environmental Microbiology 72(12), 7804–7812.

Hicks, L. C., Meir, P., Nottingham, A. T., Reay, D. S., Stott, A. W., Salinas, N., Whitaker, J., 2019. Carbon and nitrogen inputs differentially affect priming of soil organic matter in tropical lowland and montane soils. Soil Biology and Biochemistry 129, 212–222.

Hong, S., Yin, G., Piao, S., Dybzinski, R., Cong, N., Li, X., Wang, K., Peñuelas, J., Zeng, H., Chen, A., 2020. Divergent responses of soil organic carbon to afforestation. Nature Sustainability 3(9), 694–700.

Huang, R., Crowther, T. W., Sui, Y., Sun, B., Liang, Y., 2021. High stability and metabolic capacity of bacterial community promote the rapid reduction of easily decomposing carbon in soil. Communications Biology 4(1), 1376.

Huang, H., Tian, D., Zhou, L., Su, H., Ma, S., Feng, Y., Tang, Z., Zhu, J., Ji, C. Fang, J., 2022. Effects of afforestation on soil microbial diversity and enzyme activity: A meta-analysis. Geoderma 423, 115961.

Jia, X., Wang, X., Hou, L., Wei, X., Zhang, Y., Shao, M. A., Zhao, X., 2019. Variable response of inorganic carbon and consistent increase of organic carbon as a consequence of afforestation in areas with semiarid soils. Land Degradation & Development 30(11), 1345–1356.

Kaiser, C., Koranda, M., Kitzler, B., Fuchslueger, L., Schnecker, J., Schweiger, P., Rasche, F., Zechmeister-Boltenstern, S. Sessitsch, A. Richter, A., 2010. Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. New Phytologist 187(3), 843–858.

Kong, Y., Zhu, C., Ruan, Y., Luo, G., Wang, M., Ling, N., Shen, Q. Guo, S., 2018. Are the microbial communities involved in glucose assimilation in paddy soils treated with different fertilization regimes for three years similar? Journal of Soils and Sediments 18, 2476–2490.

Koricheva, J., Gurevitch, J., Mengersen, K., 2013. Handbook of meta-analysis in Ecology and Evolution. Princeton University Press, Princeton

Leff, J. W., Jones, S. E., Prober, S. M., Barberán, A., Borer, E. T., Firn, J. L., Harpole, W. S., Hobbie, S. E., Hofmockel, K. S., Knops, J. M. H., McCulley, R. L., La Pierre, K., Risch, A. C., Seabloom, E. W., Schütz, M., Steenbock, C., Stevens, C. J., Fierer, N., 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proceedings of the National Academy of Sciences 112(35), 10967–10972.

Liang, C., Schimel, J. P., Jastrow, J. D., 2017. The importance of anabolism in

microbial control over soil carbon storage. Nature Microbiology 2(8), 1–6.

Li, D., Niu, S., Luo, Y., (2012). Global patterns of the dynamics of soil carbon and nitrogen stocks following afforestation: a meta-analysis. New Phytologist 195(1), 172–181.

Li, X., Li, Y., Peng, S., Chen, Y., Cao, Y., 2019. Changes in soil phosphorus and its influencing factors following afforestation in Northern China. Land Degradation & Development 30(14), 1655–1666.

Li, Y., Chen, Z., Wagg, C., Castellano, M. J., Zhang, N., Ding, W., 2024. Soil organic carbon loss decreases biodiversity but stimulates multitrophic interactions that promote belowground metabolism. Global Change Biology 30(1), e17101.

Liu, X., Yang, T., Wang, Q., Huang, F., Li, L., 2018. Dynamics of soil carbon and nitrogen stocks after afforestation in arid and semi-arid regions: A meta-analysis. Science of the Total Environment 618, 1658–1664.

Luo, Y., Hui, D., Zhang, D., 2006. Elevated CO_2 stimulates net accumulations of carbon and nitrogen in land ecosystems: A meta-analysis. Ecology 87(1), 53-63.

Luo, X., Hou, E., Zhang, L., Zang, X., Yi, Y., Zhang, G., Wen, D., 2019. Effects of forest conversion on carbon-degrading enzyme activities in subtropical China. Science of the Total Environment 696, 133968.

Luo, R., Kuzyakov, Y., Zhu, B., Qiang, W., Zhang, Y., Pang, X., 2022. Phosphorus addition decreases plant lignin but increases microbial necromass contribution to soil organic carbon in a subalpine forest. Global Change Biology 28(13), 4194–4210.

Luo, X., Hou, E., Zhang, L., Kuang, Y., Wen, D., 2023. Altered soil microbialproperties and functions after afforestation increase soil carbon and nitrogen but not phosphorus accumulation. Biology and Fertility of Soils 1–14.

Machado, D., Maistrenko, O. M., Andrejev, S., Kim, Y., Bork, P., Patil, K. R., Patil, K. R., 2021. Polarization of microbial communities between competitive and cooperative metabolism. Nature Ecology & Evolution 5(2), 195–203.

Malik, A. A., Puissant, J., Buckeridge, K. M., Goodall, T., Jehmlich, N., Chowdhury, S., Gweon, H. S., Peyton, J. M., Mason, K. E., Agtmaal, M., Blaud, A. Clark, I. M., Whitaker, J., Pywell, R. F., Ostle, N., Gleixner, G., Griffiths, R. I., 2018. Land use driven change in soil pH affects microbial carbon cycling processes. Nature Communications 9(1), 3591.

Manzoni, S., Taylor, P., Richter, A., Porporato, A., Ågren, G. I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytologist 196(1), 79–91.

Mao, D. M., Min, Y. W., Yu, L. L., Martens, R., Insam, H., 1992. Effect of afforestation on microbial biomass and activity in soils of tropical China. Soil Biology and Biochemistry 24(9), 865–872.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., PRISMA Group*, T., 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Annals of Internal Medicine 151(4), 264–269.

Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J., Takriti, M., Watzka, M., Wild, B., Keiblinger, K. M.,

Zechmeister-Boltenstern S., Richter, A., 2014. Adjustment of microbial nitrogen use efficiency to carbon: nitrogen imbalances regulates soil nitrogen cycling. Nature Communications 5(1), 3694.

Nielsen, U. N., Ayres, E., Wall, D. H., Bardgett, R. D., 2011. Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity–function relationships. European Journal of Soil Science 62(1), 105–116.

Panchal, P., Preece, C., Peñuelas, J., Giri, J., 2022. Soil carbon sequestration by root exudates. Trends in Plant Science 27(8), 1360–185.

Perveen, N., Barot, S., Alvarez, G., Klumpp, K., Martin, R., Rapaport, A., Herfurth, D., Louault, F., Fontaine, S., 2014. Priming effect and microbial diversity in ecosystem functioning and response to global change: a modeling approachusing the SYMPHONY model. Global Change Biology 20(4), 1174–1190.

Piton, G., Foulquier, A., Martínez-García, L. B., Legay, N., Hedlund, K., da Silva, P. M., Nascimento, E., Reis, F., Sousa, J. P., De Deyn, G. B., Clement, J. C., 2020. Disentangling drivers of soil microbial potential enzyme activity across rain regimes: An approach based on the functional trait framework. Soil Biology and Biochemistry 148, 107881.

Pointing, S. B., Fierer, N., Smith, G. J., Steinberg, P. D., Wiedmann, M., 2016. Quantifying human impact on Earth's microbiome. Nature Microbiology 1(9), 1–2.

Qi, W., Zhang, Q., Chen, Y., Li, X., Cheng, X., Ye, C., Lu, F., Dang, H., Zhang, K., 2022. Afforestation alters functions of soil and microbiome but does not drive

soil carbon accumulation in two decades. Authorea Preprints.

Razanamalala, K., Fanomezana, R. A., Razafimbelo, T., Chevallier, T., Trap, J., Blanchart, E., Bernard, L., 2018. The priming effect generated by stoichiometric decomposition and nutrient mining in cultivated tropical soils: Actors and drivers. Applied Soil Ecology 126, 21–33.

Ren, C., Kang, D., Wu, J., Zhao, F., Yang, G., Han, X., Feng, Y., Ren, G., 2016. Temporal variation in soil enzyme activities after afforestation in the Loess Plateau, China. Geoderma 282, 103–111.

Schwärzel, K., Zhang, L., Montanarella, L., Wang, Y., Sun, G., 2020. How afforestation affects the water cycle in drylands: A process-based comparative analysis. Global Change Biology 26(2), 944–959.

Singh, K., Singh, B., Singh, R. R., 2012. Changes in physico-chemical, microbial and enzymatic activities during restoration of degraded sodic land: Ecological suitability of mixed forest over monoculture plantation. Catena 96, 57–67.

Sinsabaugh, R. L., Lauber, C. L., Weintraub, M. N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A. R., Cusack, D., Frey, S., Gallo, M. E., Gartner, T. B., Hobbie, S. E., Holland, K., Keeler, B. L., Powers, J. S., Stursova, M., Takacs-Vesbach, C., Waldrop, M. P., Wallenstein, M. D., Zeglin, L. H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11, 1252–1264.

Sinsabaugh, R. L., Follstad Shah, J. J., 2011. Ecoenzymatic stoichiometry of recalcitrant organic matter decomposition: the growth rate hypothesis in reverse.

Biogeochemistry 102, 31–43.

Sinsabaugh, R. L., Manzoni, S., Moorhead, D. L., Richter, A., 2013. Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. Ecology Letters 16(7), 930–939.

Sokol, N. W., Sanderman, J., Bradford, M. A., 2019. Pathways of mineral-associated soil organic matter formation: Integrating the role of plant carbon source, chemistry, and point of entry. Global Change Biology 25(1), 12–24.

Su, Z., Zhong, Y., Zhu, X., Wu, Y., Shen, Z., Shangguan, Z., 2023. Vegetation restoration altered the soil organic carbon composition and favoured its stability in a Robinia pseudoacacia plantation. Science of The Total Environment 899, 165665.

Szatmári, G., Pásztor, L., Laborczi, A., Illés, G., Bakacsi, Z., Zacháry, D., Filep, T., Szalai, Z., Jakab, G., 2023., Countrywide mapping and assessment of organic carbon saturation in the topsoil using machine learning-based pedotransfer function with uncertainty propagation. Catena 227, 107086.

Tanaka, K., Hashimoto, S., 2006. Plant canopy effects on soil thermal and hydrological properties and soil respiration. Ecological Modelling 196(1–2), 32– 44.

Trabucco, A., Zomer, R. J., 2018. Global aridity index and potential evapotranspiration (ET0) climate database v2. CGIAR Consortium for Spatial Information 10, m9.

Teng, Y., Zhan, J., Su, M., Wu, Y., Zhang, Y., Yu, C., Xu, C., 2022. Identifying

prioritized afforestation types in ecologically vulnerable zones of Northern China considering reducing water consumption and increasing carbon sequestration. Ecological Indicators 145, 109734.

Veres, Z., Kotroczó, Z., Fekete, I., Tóth, J. A., Lajtha, K., Townsend, K., Tóthmérész, B., 2015. Soil extracellular enzyme activities are sensitive indicators of detrital inputs and carbon availability. Applied Soil Ecology 92, 18–23.

Wang, S., Huang, Y., 2020a. Determinants of soil organic carbon sequestration and its contribution to ecosystem carbon sinks of planted forests. Global Change Biology 26(5), 3163–3173.

Wang, X., Zhang, W., Zhou, F., Liu, Y., He, H., Zhang, X., 2020b. Distinct regulation of microbial processes in the immobilization of labile carbon in different soils. Soil Biology and Biochemistry 142, 107723.

Wang, S., Zuo, X., Awada, T., Medima-Roldan, E., Feng, K., Yue, P., Lian, J., Zhao, S., Cheng, H., 2021. Changes of soil bacterial and fungal community structure along a natural aridity gradient in desert grassland ecosystems, Inner Mongolia. Catena 205, 105470.

Wang, M., Masoudi, A., Wang, C., Yang, J., Zhai, Y., Wu, C., Yu, Z., Liu, J., 2022. Plantation type and afforestation age disclose variable influences on soil microbial compositions in man-made forests in the Xiong'an New Area, China. Land Degradation & Development 33(16), 3058–3073.

Wang, Y., Luo, G., Li, C., Ye, H., Shi, H., Fan, B., Zhang, W., Zhang, C., Xie, M., Zhang, Y., 2023. Effects of land clearing for agriculture on soil organic carbon

stocks in drylands: A meta-analysis. Global Change Biology 29(2), 547–562.

Watson, R. T., Noble, I. R., Bolin, B., Ravindranath, N. H., Verardo, D. J., Dokken, D. J., 2000. Land use, land-use change and forestry: a special report of the Intergovernmental Panel on Climate Change. Cambridge University Press.

Wu, J., Zhang, Q., Zhang, D., Jia, W., Chen, J., Liu, G., Cheng X., 2022. The ratio of ligninase to cellulase increased with the reduction of plant detritus input in a coniferous forest in subtropical China. Appl Soil Ecology 170, 104269.

Xia, L., Song, X., Fu, N., Cui, S., Li, L., Li, H., Li, Y., 2019. Effects of forest litter cover on hydrological response of hillslopes in the Loess Plateau of China. Catena 181, 104076.

Xiao, W., Chen, X., Jing, X., Zhu, B., 2018. A meta-analysis of soil extracellular enzyme activities in response to global change. Soil Biology and Biochemistry 123, 21–32.

Xu, X., Schimel, J. P., Janssens, I. A., Song, X., Song, C., Yu, G., Sinsabaugh, R. L., Tang, D., Zhang, X., Thornton, P. E., 2017. Global pattern and controls of soil microbial metabolic quotient. Ecological Monographs 87(3), 429–441.

Xu, Q., Chen, J., van Groenigen, K. J., Li, D., Moorhead, D., Osborne, B., Ma, Z., Olesen, J., Hungate, B., Smith, P., Sun, X., Chu, C., Chen, H., 2022. Divergent responses of carbon-degrading enzyme activities to litter alterations: Implications for soil respiration. Authorea Preprints.

Xu, M. P., Zhi, R. C., Jian, J. N., Feng, Y. Z., Han, X. H., Zhang, W., 2023. Changes in soil organic C fractions and C pool stability are mediated by C-degrading

enzymes in litter decomposition of Robinia pseudoacacia plantations. Microbial Ecology 86(2), 1189–1199.

Zhang, W., Hu, G., Dang, Y., Weindorf, D. C., Sheng, J., 2016a. Afforestation and the impacts on soil and water conservation at decadal and regional scales in Northwest China. Journal of Arid Environments 130, 98–104.

Zhang, Q., Wu, J., Yang, F., Lei, Y., Zhang, Q., Cheng, X., 2016b. Alterations in soil microbial community composition and biomass following agricultural land use change. Scientific Reports 6(1), 36587.

Zhang, Q., Yang, J., Koide, R. T., Li, T., Yang, H., Chu, J., 2017. A meta-analysis of soil microbial biomass levels from established tree plantations over various land uses, climates and plant communities. Catena 150, 256–260.

Zhang, K., Cheng, X., Shu, X., Liu, Y., Zhang, Q., 2018. Linking soil bacterial and fungal communities to vegetation succession following agricultural abandonment. Plant and Soil 431, 19–36.

Zhang, X., Xin, X., Yang, W., Zhu, A., Ding, S., 2019a. Short-term decomposition, turnover and retention of residue-derived carbon are influenced by the fertility level in a sandy loam soil. Geoderma 349, 68–78.

Zhang, Q., Feng, J., Wu, J., Zhang, D., Chen, Q., Li, Q., Long, C., Feyissa, A., Cheng, X., 2019b. Variations in carbon-decomposition enzyme activities respond differently to land use change in central China. Land Degradation & Development 30(4), 459–469.

Zhang, B., Cai, Y., Hu, S., Chang, S. X., 2021. Plant mixture effects on carbon-

degrading enzymes promote soil organic carbon accumulation. Soil Biology and Biochemistry 163, 108457.

Zhang, Y., Zeng, D. H., Lei, Z., Li, X., Lin, G., 2023. Microbial properties determine dynamics of topsoil organic carbon stocks and fractions along an age-sequence of Mongolian pine plantations. Plant and Soil 483(1), 441–457.

Zhao, F. Z., Ren, C. J., Han, X. H., Yang, G. H., Wang, J., Doughty, R., Wang, Z. T., 2019. Trends in soil microbial communities in afforestation ecosystem modulated by aggradation phase. Forest Ecology and Management 441, 167– 175.

Zhou, Z., Wang, C., Luo, Y., 2020. Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. Nature Communications 11(1), 3072.

Zhu, E., Cao, Z., Jia, J., Liu, C., Zhang, Z., Wang, H., Dai, G., He, J., Feng, X., 2021. Inactive and inefficient: Warming and drought effect on microbial carbon processing in alpine grassland at depth. Global Change Biology 27(10), 2241–2253.

Zumsteg, A., Luster, J., G¨oransson, H., Smittenberg, R., Brunner, I., Bernasconi, S., Zeyer, J., Frey, B., 2012. Bacterial, archaeal and fungal succession in the forefield of a receding glacier. Microbial Ecology 63, 552–564.

Zuccarini, P., Sardans, J., Asensio, L., Peñuelas, J., 2023. Altered activities of extracellular soil enzymes by the interacting global environmental changes. Global Change Biology 29(8), 2067–2091.

Zumsteg, A., Luster, J., Göransson H., Smittenberg, R. H., Brunner, I., Bernasconi, S. M., Zeyer, J., Frey, B., 2012. Bacterial, archaeal and fungal succession in the forefield of a receding glacier. Microbial Ecology 63, 552–564.

Zuur, A. F., Ieno, E. N., Elphick, C. S., 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1(1), 3–14.

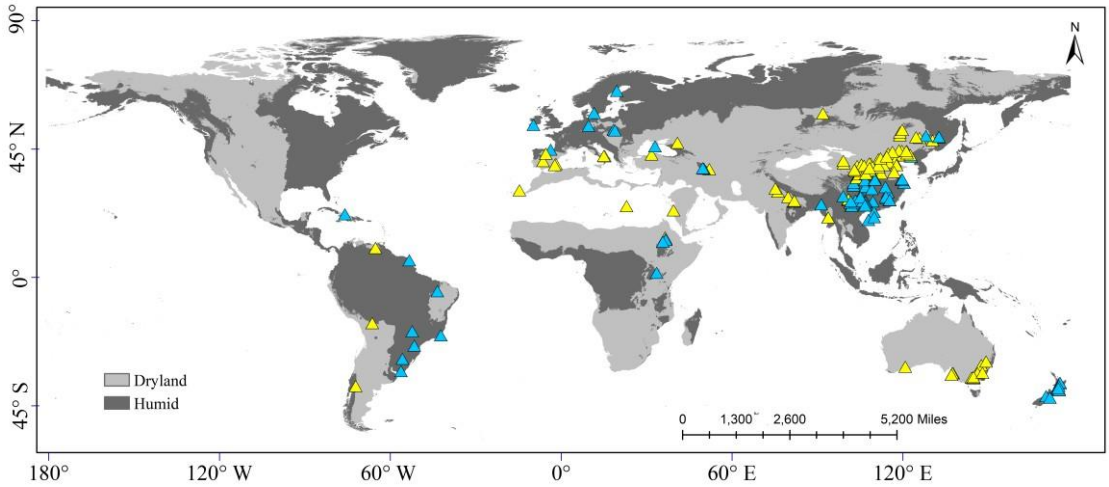


Figure 1. Locations of study sites used in this meta-analysis. Afforestation in dryland (aridity index < 0.65) and humid (aridity index > 0.65) regions are shown as coloured triangles. The yellow triangles indicate dryland region sites and the blue triangles indicate humid region sites.

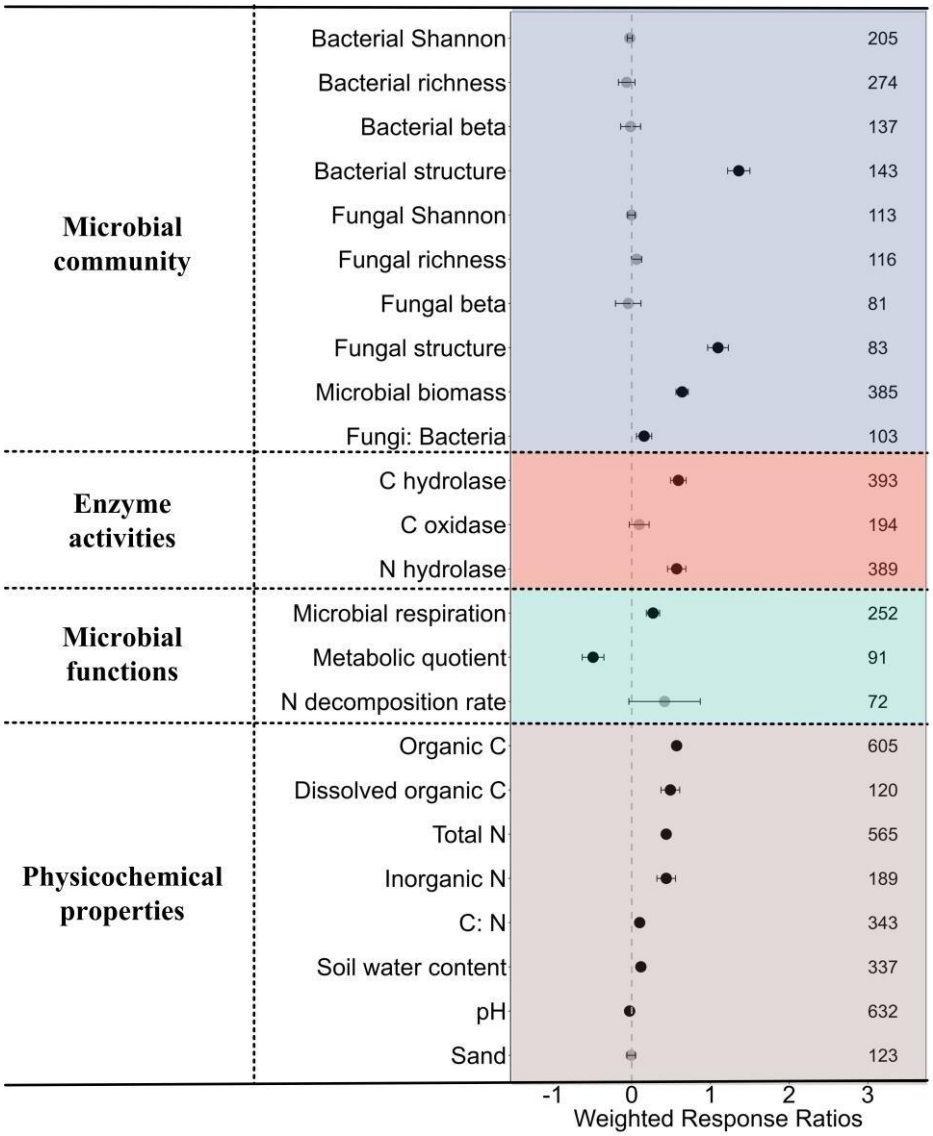


Figure 2. Meta-analysis of the weighted response ratios of afforestation on belowground processes. The error bars represent 95% confidence intervals (CIs), and they are not shown when the error bars are smaller than the symbol. The black dot indicates a significant effect of plant detritus manipulation at $P < 0.05$, whereas the grey dot indicates $P > 0.05$. The numbers are sample numbers.

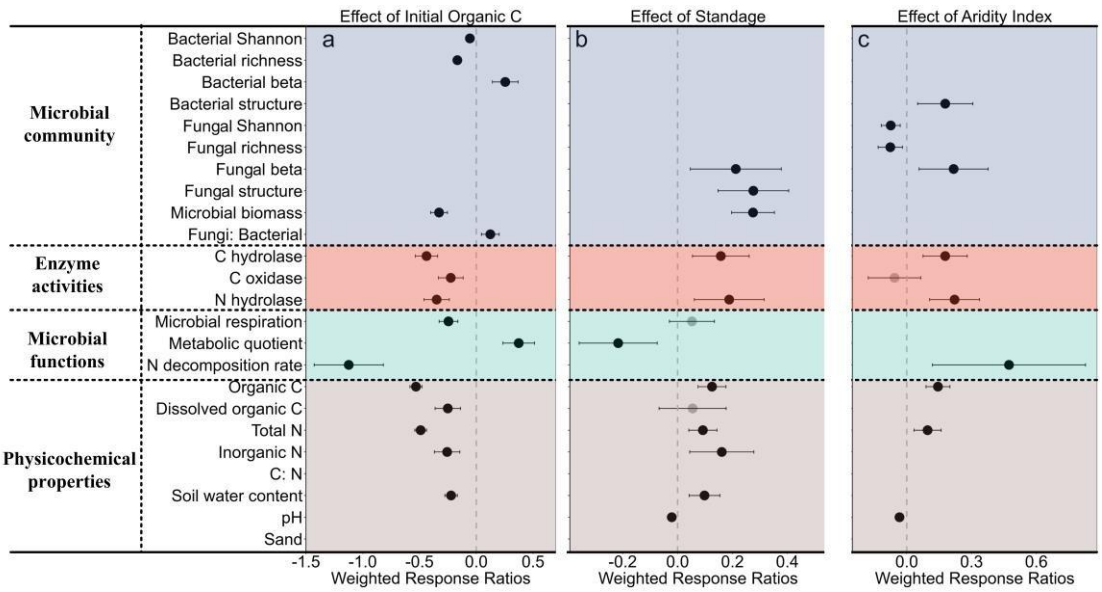


Figure 3. Changes in belowground processes driven by (a) initial soil organic carbon content before afforestation (ISOC), (b) afforestation standage (SA), and (c) aridity index in afforestation sites (AI). A high AI means a humid climate while a low AI means an arid climate. Circles with error bars represent overall mean ± 95% confidence intervals (CIs) when the continuous drivers (i.e., ISOC, SA, and AI) increase one scale unit. The black dot indicates a significant effect of plant detritus manipulation at $P < 0.05$, whereas the grey dot indicates $P > 0.05$. The vertical dashedlines are zero lines.

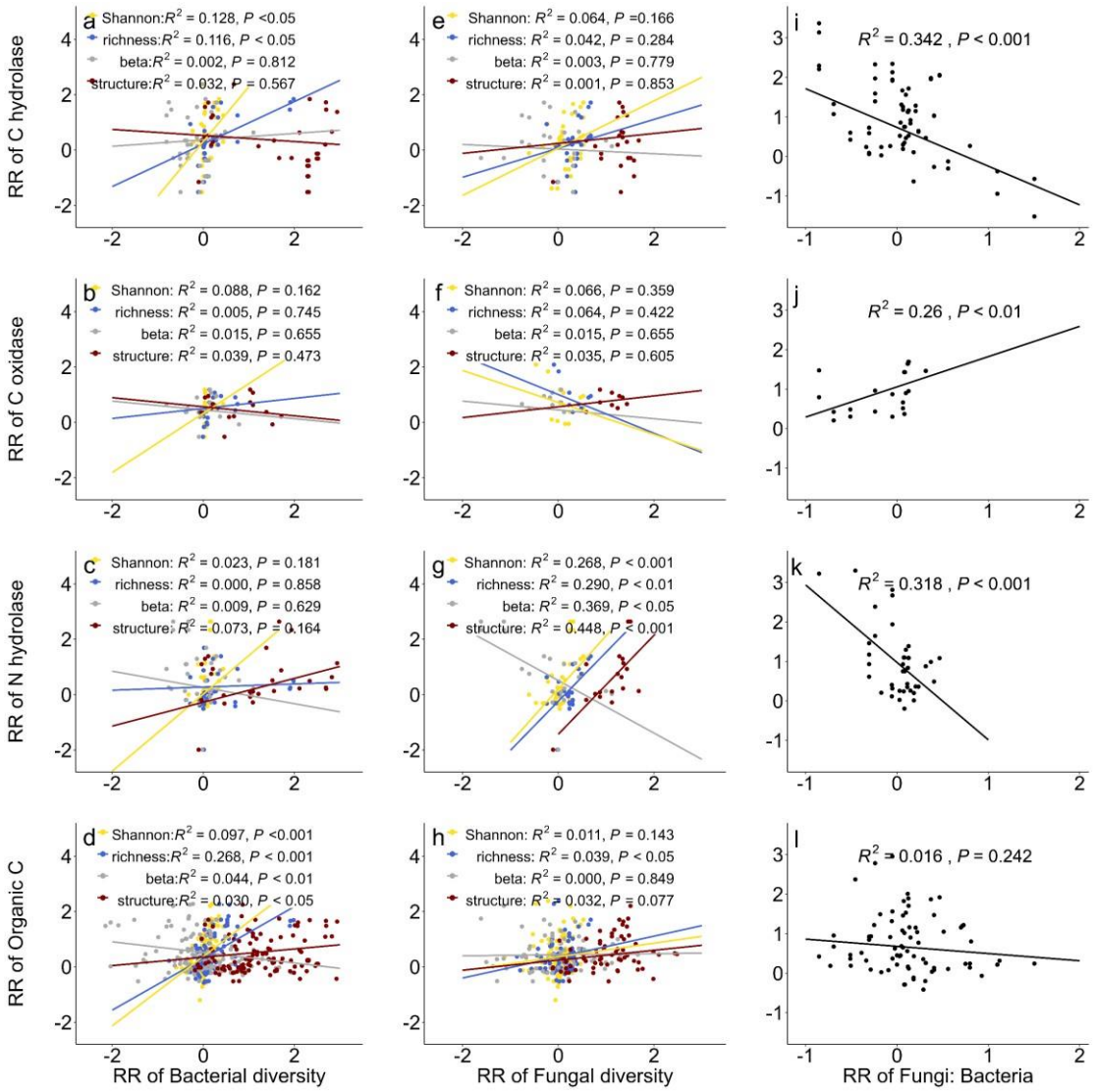


Figure 4. Linear relationships between the response ratios (*RR*s) of (a, e, and i) carbon (C) hydrolase activity, (b, f, and j) C oxidase activity, (c, g, and k) nitrogen (N) hydrolase activity, and (d, h, and l) soil organic C and the response ratios (*RR*s) of (a-d) bacterial and (e-h) fungal Shannon diversity, richness, beta diversity, and community structure, and (i-l) fungi: bacteria ratio.

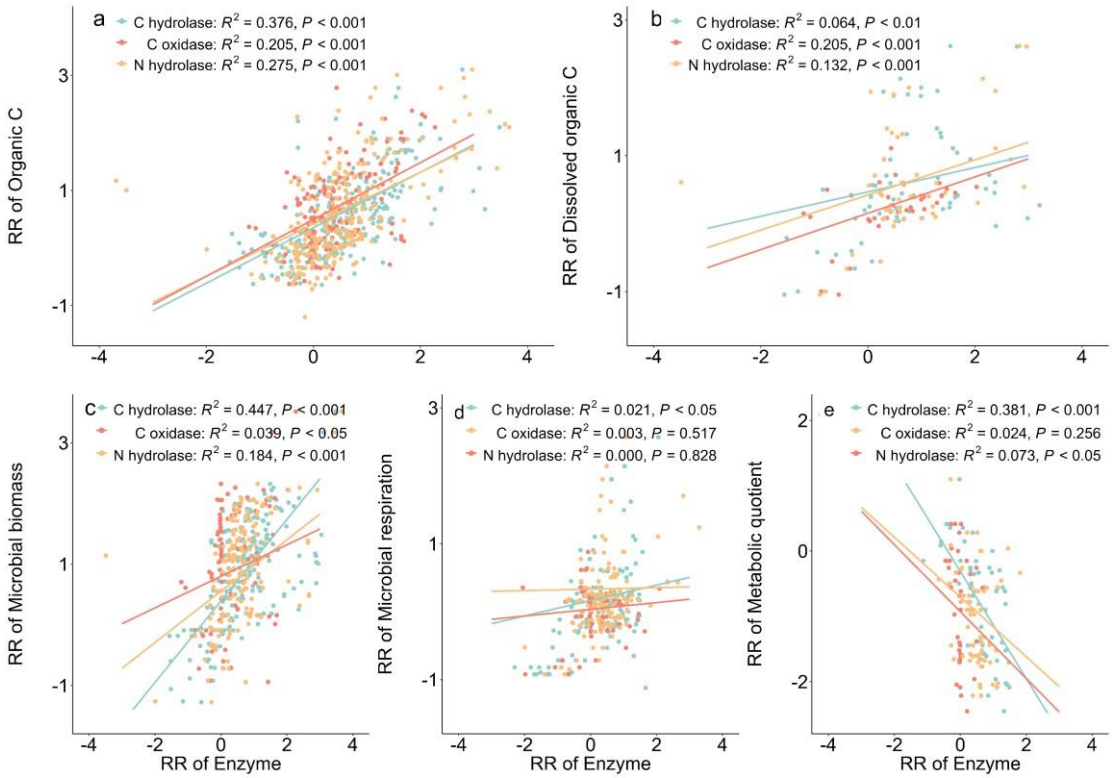


Figure 5. Linear relationships between the response ratios (*RR*s) of (a) soil organic carbon (C), (b) dissolved organic C, (c) microbial biomass, (d) microbial respiration, and (e) metabolic quotient and the response ratios (*RR*s) of C and nitrogen (N)hydrolase and C oxidase activities.

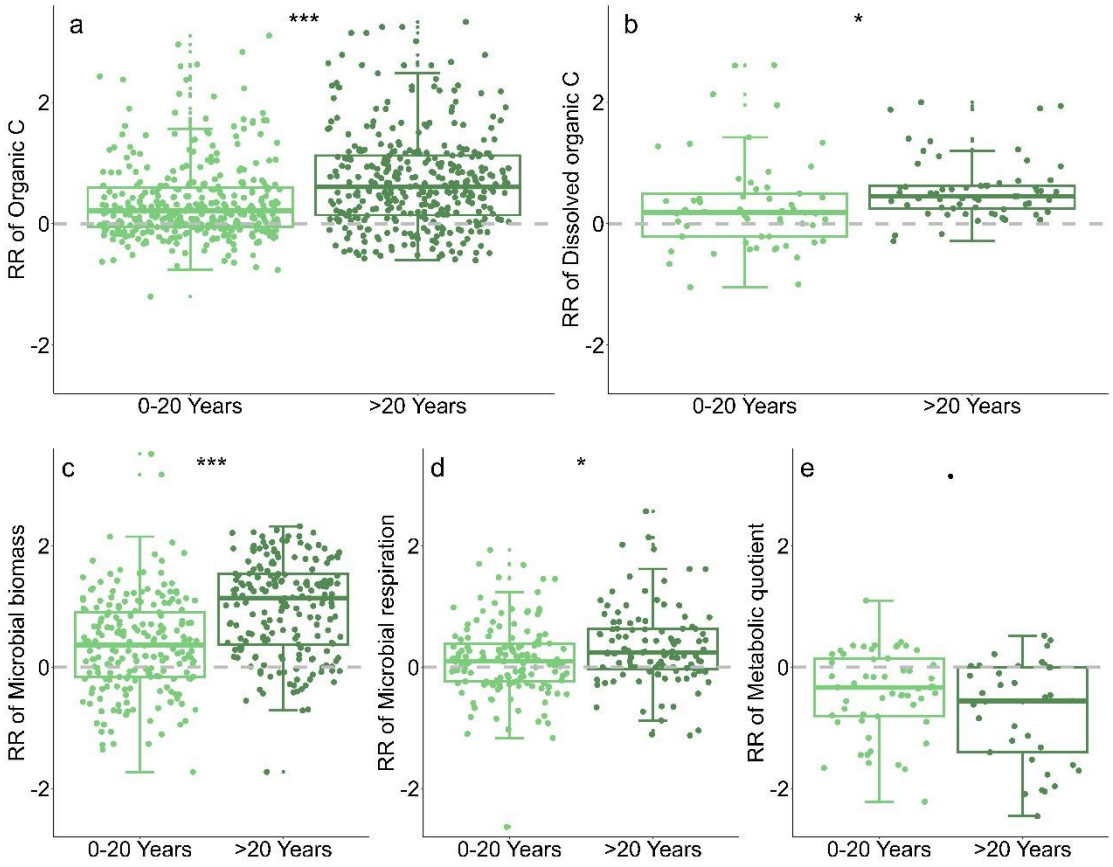


Figure 6. Changes of afforestation on (a) soil organic carbon (C), (b) dissolvedorganic C, (c) microbial biomass, (d) microbial respiration, and (e) metabolic quotientunder different standage (0–20 years and >20 years). The changes are quantified as the response ratios (*RR*s) after afforestation compared to the corresponding unplanted sites. Significant levels: *** P < 0.001; * P < 0.05; . P < 0.1; ns, non-significant (P > 0.1).

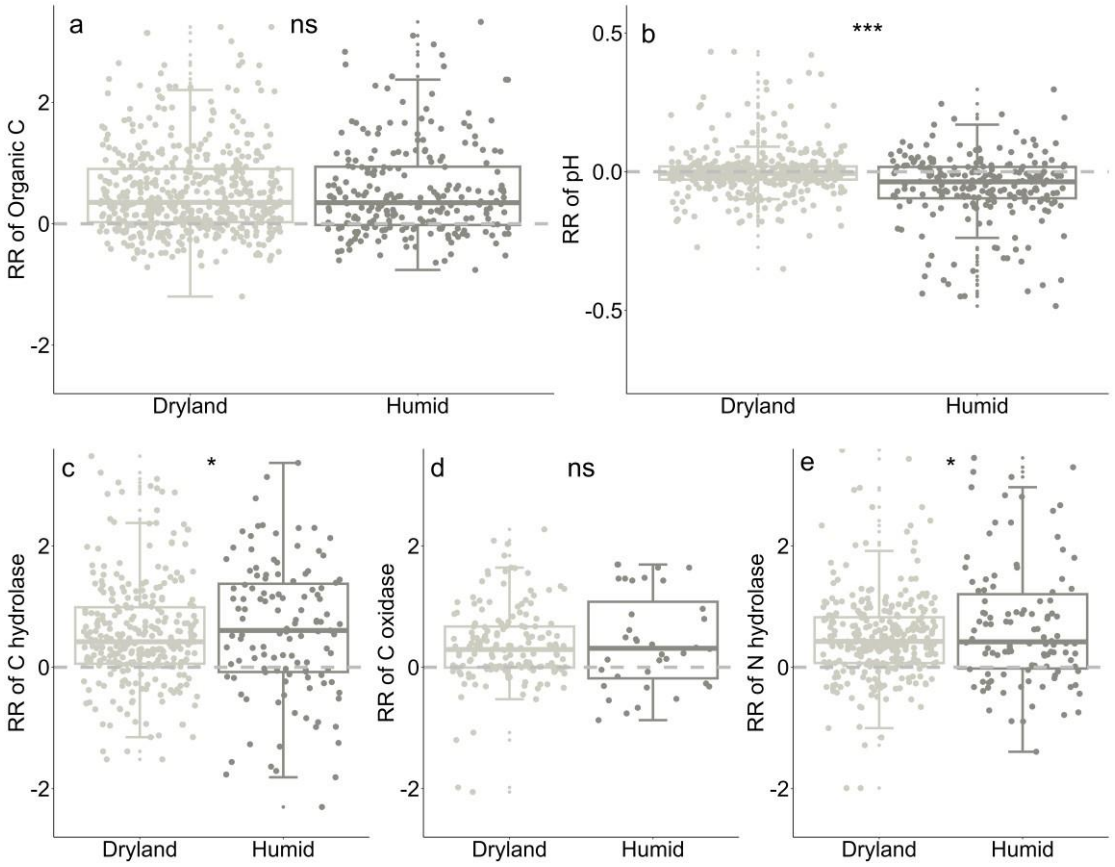


Figure 7. Changes of afforestation on (a) soil organic carbon (C), (b) pH, (c) C hydrolase, (d) C oxidase, and (e) nitrogen (N) hydrolase activities in dryland (aridity index < 0.65) and humid (aridity index > 0.65) regions. The changes are quantified as the response ratios (*RR*s) after afforestation compared to the corresponding unplanted sites. Significant levels: ***P < 0.001; * P < 0.05; . P < 0.1; ns, non-significant P > 0.1.

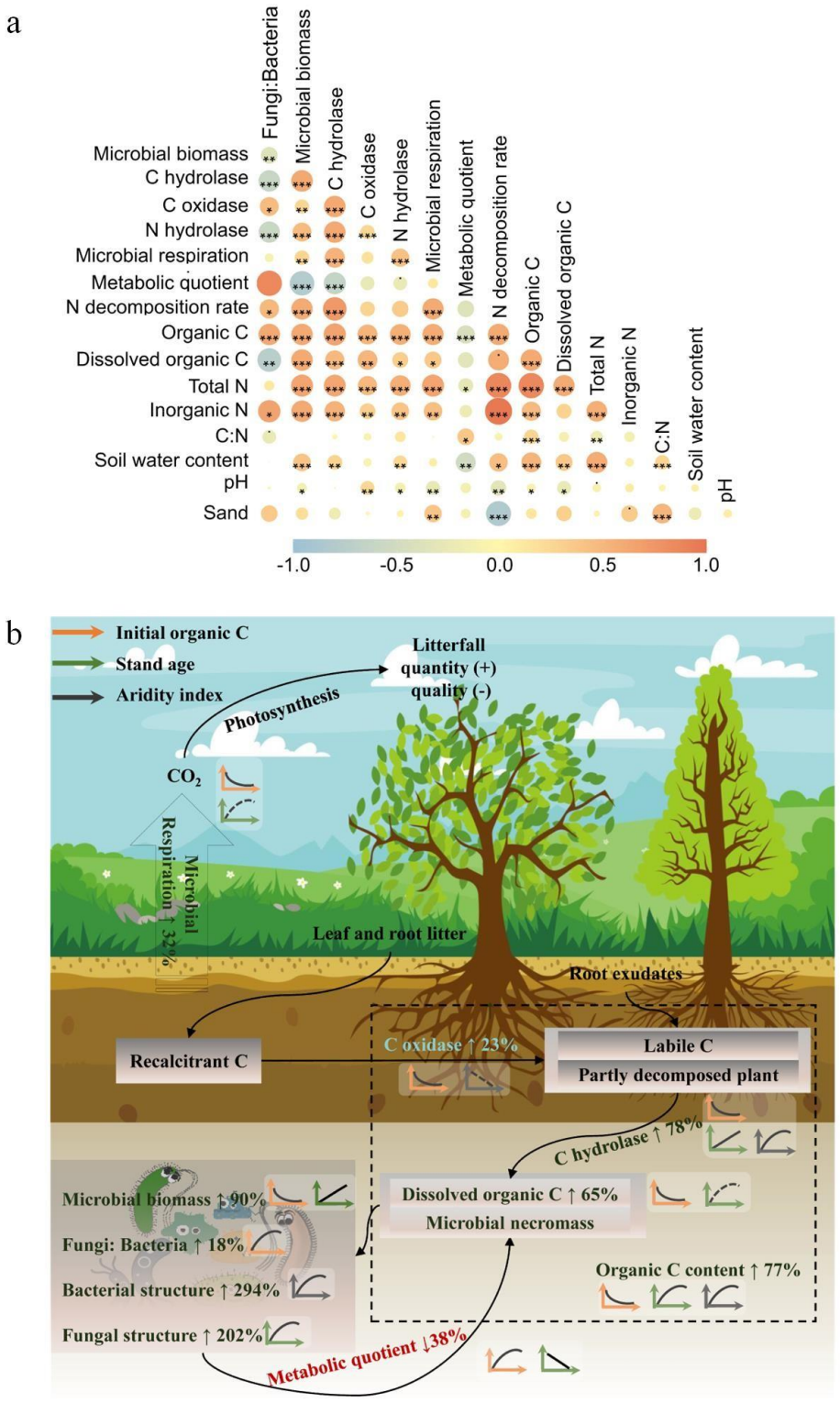


Figure 8. (a) Pearson correlation coefficients among the fungi: bacteria, microbial biomass, carbon (C) hydrolase, C oxidase, and nitrogen (N) hydrolase activities, microbial respiration, metabolic quotient, soil organic C, dissolved organic C, total N, inorganic N, C: N, soil water content, pH, and sand content. Positive correlations are labeled in red and negative correlations are labeled in blue. (b) Conceptual illustration of how afforestation affects soil microorganisms involved in the C cycle. Recalcitrant C represents lignin and other large aromatic C compounds while labile C represents

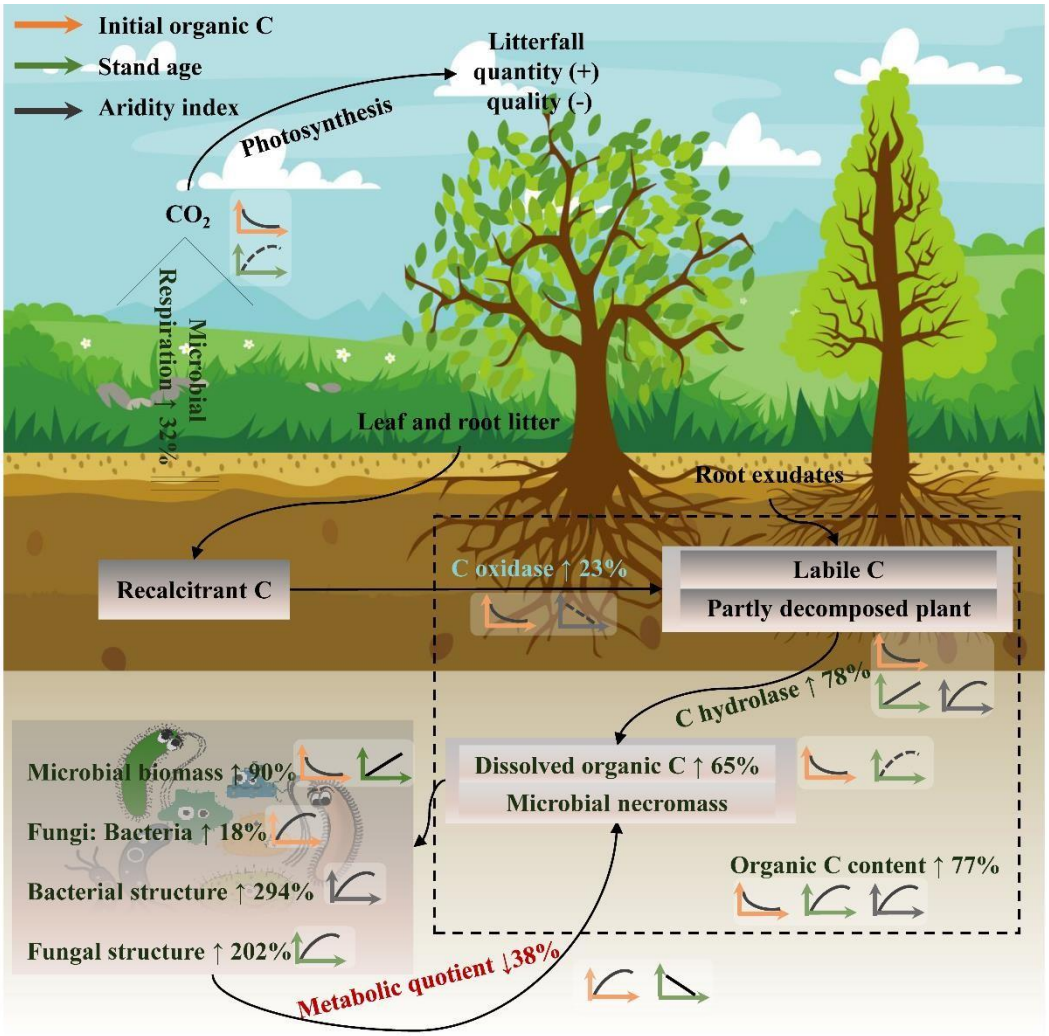
cellulose, hemicellulose, etc. compounds. All the effects (except C oxidase) are significant at $P < 0.05$. The values show the percentage change in the variables due to afforestation. The up (green) and down (red) arrows indicate increasing and decreasing trends, respectively. The horizontal coordinates of the axes in different colours are initial organic C, stand age and aridity index respectively, and the vertical coordinates are the corresponding effect sizes. Dashed lines indicate that the trend is not significant. The significant level is set at *** $P < 0.001$; ** $P<0.01$; * $P < 0.05$; . $P < 0.1$; ns, non-significant $P > 0.1$.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical abstract



Note: A higher positive value of bacterial or fungal structure indicates a greater magnitude of community structure change. The horizontal coordinates of the axes in different colors are initial organic C, stand age and aridity index respectively, and the vertical coordinates are the corresponding effect sizes.

Highlights

- SOC increases more in older stands, more humid regions, and initial soil C-poor afforestation scenarios.
- Afforestation changes the structure rather than the alpha and beta diversity of fungal and bacterial communities.
- Differences of C hydrolase and oxidase activities in response to afforestation.
- The impact of afforestation standage on SOC response via intracellular anabolism pathway.