



Responses of soil nutrients and enzyme activities to afforestation species and age on China's Loess Plateau: An investigation from soil aggregates aspect



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ABSTRACT

Farmland afforestation has long been considered an effective approach to improve soil structure and nutrients cycling. Whereas, how the tree species and afforestation ages affect soil enzymes associated with carbon (C), nitrogen (N), and phosphorus (P) cycling at aggregate scale has been less examined. In this study, we examined the effects of farmland afforestation with legume trees (Black locust, *Robinia pseudoacacia*) at three stand ages (10, 20, and 30 years) and with non-legume trees (Chinese pine, *Pinus tabulaeformis*) at 30 years on soil organic C (SOC), total N (TN), total P (TP) and the C-, N-, and P-acquiring enzyme activities (EEA_C, EEA_N, and EEA_P) in bulk soils and aggregates in the southern part of China's Loess Plateau. We found that afforestation with black locust significantly increased the proportion of > 2 mm aggregates and mean weight diameter (MWD) across the 10–30 years, whereas afforestation with Chinese pine reduced > 2 mm aggregates and MWD. Farmland afforestation increased SOC and TN, with greater effect occurring at greater ages for legume trees and in < 0.25 mm fraction, but uniformly decreased TP in soil aggregates. Afforestation increased soil enzyme activities, with greater effects on N enzymes for afforestation with non-legume trees at 0–10 cm and greater effects on C and P enzymes for afforestation with legume trees. In addition, EEA_C and EEA_N were highest in < 0.25 mm aggregate fraction, but EEA_P was independent of aggregate size. The enzyme activities in > 2 mm aggregates accounted for 54%–60% of the activities in bulk soils. Our results suggest that enzyme activity was determined mainly by the > 2 mm aggregates and was significantly influenced by afforestation species and ages through changing soil nutrients and aggregate size distribution.

1. Introduction

Afforestation of farmland, one of the most important agricultural land-use changes, is of utmost importance for ecological restoration and sustainable land management, particularly for ecologically vulnerable regions typified by the Loess Plateau (Kim et al., 2023; Prangel et al., 2024). The conversion of degraded farmland into forested ecosystem significantly protects soils from erosion while concurrently fostering the

accumulation of soil organic matter and accelerating the restoration of biodiversity. Moreover, afforestation sustains significant enhancements of ecosystem services, including carbon (C) sequestration and water conservation, which contribute meaningfully to the global efforts of climate change mitigation by reducing atmospheric carbon dioxide levels (Cheng et al., 2013). Therefore, afforestation on farmlands is a critical strategy for achieving ecological sustainability across degraded landscapes, addressing immediate challenges of amending soil

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degradation and ecosystem disruption, while also providing a more resilient and balanced ecological program (Morriën et al., 2017).

Soil aggregates are a crucial component of soil and the habitat of microbial communities, which support important functions from soils to ecosystems (Rabbi et al., 2016). The aggregation of soil particles facilitates C sequestration by restricting the accessibility of soil organic matter to soil microbiota and extracellular enzymes and thus increasing the physical protection of soil (Jastrow et al., 2007; Bai et al., 2021). Soil microbial communities and enzymes are intricately modulated by the quantity and chemical nature of organic matter, environmental conditions, and the decomposition potential of organic matter sequestered within soil aggregates of varying sizes (Davinic et al., 2012; Smith et al., 2014; Bach et al., 2018). Currently, there are some reports regarding the variations of soil microbiomes among aggregate size classes (Rabbi et al., 2016; Bach et al., 2018; Hu et al., 2023; Simon et al., 2024; Zhang et al., 2025), whereas how enzyme activities differ with various aggregate size classes is rarely reported. Given that soil enzymes are highly sensitive to resource availability and are critical for soil biogeochemical cycling, a deeper understanding of how land-use change influences enzyme activities in aggregates of varying sizes would offer a novel and underexplored perspective for elucidating the role of soil microbial activities in supporting ecological functionalities in a changing world.

It is well acknowledged that agricultural land-use change, particularly afforestation on degraded farmland, affects enzyme activities in bulk soils. For example, afforestation significantly increases soil enzyme activities, as evidenced by studies in China's Loess Plateau (Deng et al., 2019; Zhang et al., 2019) and abandoned croplands in southwestern Russia (Sun et al., 2022), which reported increased β -1,4-glucosidase, β -1,4-N-acetylglucosaminidase, and phosphatase. Furthermore, legume trees differ from non-legume trees in their organic inputs—such as leaf litter, fine roots, root nodules, and C rhizodeposition—which contain comparatively higher nitrogen contents, and thereby affect soil enzyme activities through root exudates or litter (Pirhofer-Walzl et al., 2012; Gou et al., 2023). For instance, comparative studies in the Loess Plateau between black locust (*Robinia pseudoacacia* Linn., a legume species) and Chinese pine (*Pinus tabuliformis* Carr., a non-legume species) showed that afforestation with black locust exhibited significantly higher β -1,4-glucosidase, β -D-cellobiosidase (Wu et al., 2025), and phosphatase (Xu et al., 2022) activities compared to Chinese pine, while showing reduced β -1,4-N-acetylglucosaminidase activity (Wu et al., 2025). Additionally, changes in soil carbon and nutrient availability with increasing afforestation ages further modulated enzyme dynamics (Zhong et al., 2020; Ren et al., 2021; Liu et al., 2023), highlighting the complex interplay between afforestation ages, substrate quality, and microbial metabolic adaptation during the process of converting farmland to forest.

Moreover, meta-analyses further confirm these trends globally, with enzyme responses influenced by restoration duration and tree species (Huang et al., 2022; Luo et al., 2023). Current understandings highlight the important role of soil enzymes in regulating the effects of land-use change on soil functions, whereas how enzymes respond to land-use change and support soil functionality at aggregates aspects is still not addressed. Additionally, afforestation has significant effects on soil nutrients in both bulk soils and aggregates (Zhang et al., 2020b; Yu et al., 2023; Su et al., 2025), but how such effects are linked to the effects on enzymes in soil aggregates is unknown. Such linkage is particularly important because of the close associations between enzymes and nutrients cycling (Jing et al., 2017; Yokoyama et al., 2017; Zhang et al., 2019, 2022a; Xu et al., 2020). These two knowledge gaps significantly limit our ability to explicitly explain the effects of agricultural land-use change on soil biogeochemical cycles and functionality.

To fill these knowledge gaps, we assessed how the species and ages of afforested trees affect soil nutrients and enzyme activities in soil aggregates in China's Loess Plateau. The Loess Plateau is one of the world's most fragile ecosystems, mainly due to extensive agricultural activities and induced soil erosion (Zhang et al., 2020b; Su et al., 2025). To protect soils from erosion and to restore the degraded environments in this

region, extensive farmland afforestation efforts have been launched since the 1980s (Deng et al., 2019; Rong et al., 2021), which have resulted in significant increases in vegetation coverage and ecological restoration (Zhang et al., 2020a; Liang et al., 2025). Such widely applied afforestation projects provide an ideal platform for us to examine the effects of afforestation on soil ecology from various aspects. In this study, we compared the effects between black locust and Chinese pine with the afforestation of 30 years to assess the effects of afforestation species, and compared the effects among 10, 20, and 30 years for the afforestation with black locust to assess the effects of afforestation ages. The effects of such agricultural land-use changes were evaluated by comparing the afforested land with adjacent farmland, where the afforested land was established. We aim to answer the following scientific questions: (1) how do enzyme activities vary with aggregate size? (2) how do afforestation species and ages influence enzyme activities in bulk soils and aggregate fractions? (3) what factors influence soil enzyme activities in afforested ecosystems?

2. Materials and methods

2.1. Study site, experimental design and soil sampling

Our study was conducted in Yeheshan Watershed (34°33'N, 107°56'E, 449–1662 m a.s.l.), Fufeng County, Shaanxi Province (Fig. 1). The watershed is located in the southern Loess Plateau, and has a semi-arid continental climate, with a mean annual temperature of 12.7°C, mean annual precipitation of 580 mm and mean annual evaporation of 1217 mm (Ma et al., 2019). The predominant soil type in the watershed is silt loam (Ma et al., 2019), with clay, silt, and sand contents of 23.64 %, 35.83 % and 40.53 %, respectively. The soil type is classified as Inceptisols according to the United States Department of Agriculture (USDA). The watershed was composed of farmland and forestland. The farmlands were rotated with winter wheat (*Triticum aestivum*, October to June) and summer maize (*Zea mays L.*, June to October). The forestlands were established in farmland following the afforestation projects with different trees at various times. The dominant trees in the forestland were black locust and Chinese pine. The forestlands were not fertilized or irrigated.

Our experiment was composed of two sub-experiments. The first sub-experiment was designed to compare the effects of tree species of afforestation (sub-experiment I), which was composed of black locust and Chinese pine after 30 years of afforestation (L₃₀ yr and P₃₀ yr), respectively. The second sub-experiment was designed to compare the effects of afforestation ages (sub-experiment II), which was composed of black locust forestlands with stand ages of 10, 20, and 30 years (L₁₀ yr, L₂₀ yr, and L₃₀ yr, respectively). Both sub-experiments shared the same adjacent farmlands as control (CK) for examining the effects of afforestation. The afforested forests in both sub-experiments were established on these adjacent farmlands following the implementation of "Grain for Green" project at various times. The selected farmlands and forestlands possessed identical soil types, comparable slopes, and similar slope positions, which guaranteed that observed differences between paired farmlands and forestlands could be attributed to afforestation effects.

In July 2019, three sampling plots (10 m × 10 m) were randomly established in each treatment (i.e., CK, L₁₀ yr, L₂₀ yr, L₃₀ yr, and P₃₀ yr), and the sampling plots were at least 50 m away from each other, and 100 m away from the boundary of the treatments. Soil samples were collected from the 0–10 cm and 10–30 cm depths in each plot with a 5-cm diameter soil auger using a five-point sampling method, and were combined into one composite sample for each sampling plot. The organic layer was removed before sampling in forestland. After removal of roots, litter, debris, and stones, the soil samples collected were air-dried for aggregate separation and laboratory analysis.

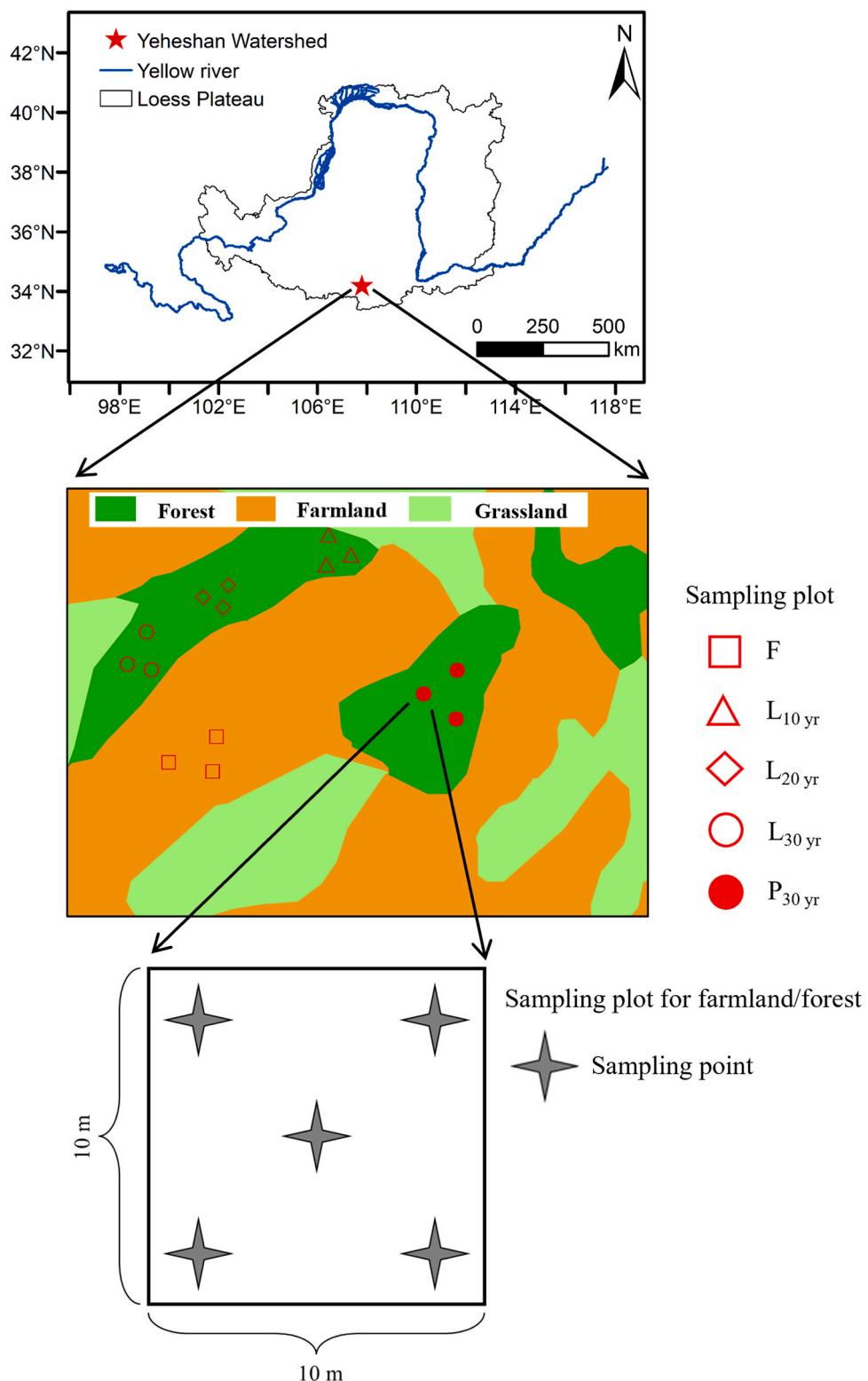


Fig. 1. Location of the study sites and sampling scheme. Note: F: farmland; L₁₀ yr: locust afforested 10 years; L₂₀ yr: locust afforested 20 years; L₃₀ yr: locust afforested 30 years; P₃₀ yr: pine afforested 30 years.

2.2. Laboratory analysis

We used a dry-sieving method to separate soil aggregates for the measurement of soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP) as well as enzyme activities in aggregates. We selected this method because it has been reported that air-dried soil samples did

not significantly impact microbial community composition and structure (Wang et al., 2021), and that the dry-sieving did not reduce measured aggregate extracellular enzyme activity compared to field-fresh whole-soil as predicted (Bach et al., 2014). In comparison, the immersion of soil into water when using wet-sieving method significantly affected these metrics.

Aggregate size distribution was determined by a dry-sieving method (Okolo et al., 2020). The 200 g air-dried soil was placed into a set of sieves with mesh diameters of 2, 1, and 0.25 mm and shaken for 10 min. All the aggregate fractions were weighed, and the proportion of each aggregate fraction was calculated.

The mean weight diameter (MWD, mm) of soil aggregates was calculated as (Kemper and Rosenau, 1986):

$$MWD = \sum_{i=1}^n x_i \times w_i$$

where w_i is the weight fraction (percent) in each aggregate class and x_i is the mean diameter of each class.

The SOC, TN, and TP in both bulk soils and aggregates were determined using the Walkley-Black method, the Kjeldahl method, and the sulfuric acid and perchloric acid digestion method, respectively (Page et al., 1982). Enzyme activities were measured fluorometrically using a solution of substrates labeled with 4-methylumbelliflone (MUB) or 7-amino-4-methylcoumarin (AMC). Briefly, 3.00 g soil was mixed and homogenized with 125 mL of a 100 mM Tris buffer using a magnetic stirrer for 5 min to prepare for a soil suspension. Five experimental groups were established: the sample wells (150 μ L sample suspension plus 50 μ L substrate solution), the blank wells (150 μ L soil suspension plus 50 μ L buffer), the negative control wells (150 μ L buffer plus 50 μ L substrate solution), the standard wells (150 μ L soil suspension plus 50 μ L standard solution (10 μ M 4-MUB, or 7-AMC for the LAP assays)), and the reference standard wells (150 μ L buffer plus 50 μ L standard solution). Eight technical replicates were prepared for each group. The microplate was horizontally shaken for 60 s for mixing, incubated at 25°C in the dark for 0.5 h (AP), 2 h (BG, LAP), or 4 h (CBH, BX, NAG), respectively. Fluorescence was measured using a microplate reader (λ 365 nm excitation and λ 450 nm emission). Enzyme activities were expressed as nanomoles of substrate released per hour per gram of dry soil ($\text{nmol g}^{-1} \text{h}^{-1}$). Throughout the experiment, the suspension was maintained under continuous magnetic stirring to ensure homogeneity.

The C-acquiring enzymes (EEA_C) were the sum of β -D-cellulosidase (CBH), β -1,4-glucosidase (BG) and β -1,4-xylanidase (BX), the nitrogen (N)-acquiring enzymes (EEA_N) were the sum of β -1,4-N-acetylglucosaminidase (NAG) and L-leucine aminopeptidase (LAP), and the phosphorus (P)-acquiring enzyme (EEA_P) was alkaline phosphatase (AP). The enzyme commission classifications of CBH, BG, BX, NAG, LAP, and AP were 3.2.1.91, 3.2.1.21, 3.2.1.37, 3.2.1.14, 3.4.11.1, and 3.1.3.1, respectively (Sinsabaugh et al., 2009).

2.3. Statistical analysis

We conducted a two-way analysis of variance (ANOVA) to examine the impacts of afforestation species or ages and their interactive effects with soil depth on: (1) the proportion of each aggregate fraction and MWD; (2) SOC, TN, and TP; and (3) EEA_C, EEA_N, and EEA_P. One-way ANOVA and Tukey's multiple comparisons were conducted to determine the differences in MWD, soil nutrients, and enzyme activities in different afforestation species and ages in the bulk soil and in each aggregate fraction under each soil depth (with a probability level of 0.05). If necessary, square root transformation was performed before analysis in order to conform to the assumptions of normality and homogeneity of variance. Multiple comparisons were assessed via Kruskal-Wallis test when the assumptions of normality and homogeneity of variance were not satisfied.

Contributions of different-sized aggregates to soil enzyme activities in bulk soils were calculated as $EEA_i \times w_i / EEA_{\text{bulk soils}} \times 100\%$, where EEA_i refers to enzyme activities in > 0.25 mm, 0.25–1 mm, 1–2 mm, and > 2 mm aggregate fractions, w_i is the weight fraction (percent) in each aggregate class, and $EEA_{\text{bulk soils}}$ refers to enzyme activity in bulk soils. We conducted Spearman's rank correlation analysis to assess the relationships among SOC, TN, and TP and enzyme activities. The

ANOVA and correlation analyses were conducted using Origin 2023 (OriginLab Corp.). Partial least squares-path modelling (PLS-PM) was used to evaluate the direct and indirect effects of afforestation species and ages, soil nutrients, MWD, and proportion of aggregate fraction on enzyme activities by using the R package 'plspm' (Tenenhaus et al., 2005). We constructed the conceptual framework for the direct and indirect effects of afforestation species or ages, MWD, proportion of each aggregate fraction, and soil nutrients on enzyme activities in bulk soils and aggregates (Fig. S1). This analysis was performed using the R statistical software (version 4.2.2, <http://www.r-project.org/>).

3. Result

3.1. Effects of afforestation species and age on soil aggregates

When averaged across two sub-experiments, the > 2 mm aggregate fraction dominated the soil mass in this study, with a proportion of 33 %–78 %, while the 1–2 mm, 0.25–1 mm, and < 0.25 mm aggregate fractions accounted for 10 %–20 %, 10 %–33 % and 2 %–14 % of the soil mass, respectively (Fig. 2). As to P₃₀ yr, afforestation decreased the proportion of the > 2 mm aggregate fraction (-25 % and -40 % for 0–10 and 10–30 cm; $P < 0.05$) and increased the proportion of the < 0.25 mm (+133 % and +160 % for 0–10 and 10–30 cm; $P < 0.05$), 0.25–1 mm (+3 % and +72 % for 0–10 and 10–30 cm; $P > 0.05$ and $P < 0.05$, respectively), and 1–2 mm (+18 % and +50 % for 0–10 and 10–30 cm; $P < 0.05$) aggregate fractions compared with farmland, and thus decreased the MWD ($P > 0.05$ and $P < 0.05$ for 0–10 and 10–30 cm). However, as to L₃₀ yr, afforestation increased the proportion of the > 2 mm aggregate fraction (+41 % and +6 % for 0–10 and 10–30 cm; $P < 0.05$ and $P > 0.05$, respectively) and decreased the proportion of the < 0.25 mm (-33 % and -40 % for 0–10 and 10–30 cm; $P > 0.05$), 0.25–1 mm (-41 % and -17 % for 0–10 and 10–30 cm; $P < 0.05$ and $P > 0.05$), and 1–2 mm (-12 % and +8 % for 0–10 and 10–30 cm; $P > 0.05$) aggregate fractions compared with farmland, and thus increased the MWD ($P > 0.05$ for 0–10 and 10–30 cm). As to different afforestation ages of black locust, the proportion of the > 2 mm aggregate fraction was increased by +43 %, +57 % and +41 % at 0–10 cm and +14 %, +20 % and +6 % at 10–30 cm in L₁₀ yr, L₂₀ yr, and L₃₀ yr treatments, respectively, while the proportion of other aggregate fractions decreased compared with farmland. The conversion of farmland to black locust increased the MWD value of soil aggregates, especially at 0–10 cm soil depth, but this effect was similar among different afforestation ages.

3.2. Effects of afforestation species and age on SOC and nutrients in bulk soils and aggregates

The SOC and TN were highest in the < 0.25 mm aggregate fraction and declined with increasing aggregate size, but there was no significant difference in TP among the aggregate fractions (Fig. S2a, b, c). The conversion of farmland to forests with two tree species with the same afforestation age (L₃₀ yr and P₃₀ yr) increased SOC and TN and decreased TP, and the effects of black locust on SOC and TN were greater than those of Chinese pine in bulk soils and aggregates at the 0–10 cm depth (Fig. 3a, b, c). Afforestation did not significantly change SOC and TN in bulk soils at the 10–30 cm depth. In addition, the percent increase in SOC and TN decreased with increasing aggregate size at the 0–10 cm depth, but the percent decrease in TP did not change with aggregate size. For example, compared with farmland, 30-year afforestation with black locust (L₃₀ yr) resulted in 482 %, 341 %, 279 % and 143 % increases for SOC ($P < 0.05$), 365 %, 231 %, 179 % and 133 % increases for TN ($P < 0.05$), and 50 %, 47 %, 50 % and 54 % decreases for TP ($P < 0.05$), for the < 0.25, 0.25–1, 1–2 and > 2 mm aggregate fractions, respectively.

For the conversion from farmland to black locust forests, afforestation increased SOC and TN with increasing age at the 0–10 cm depth

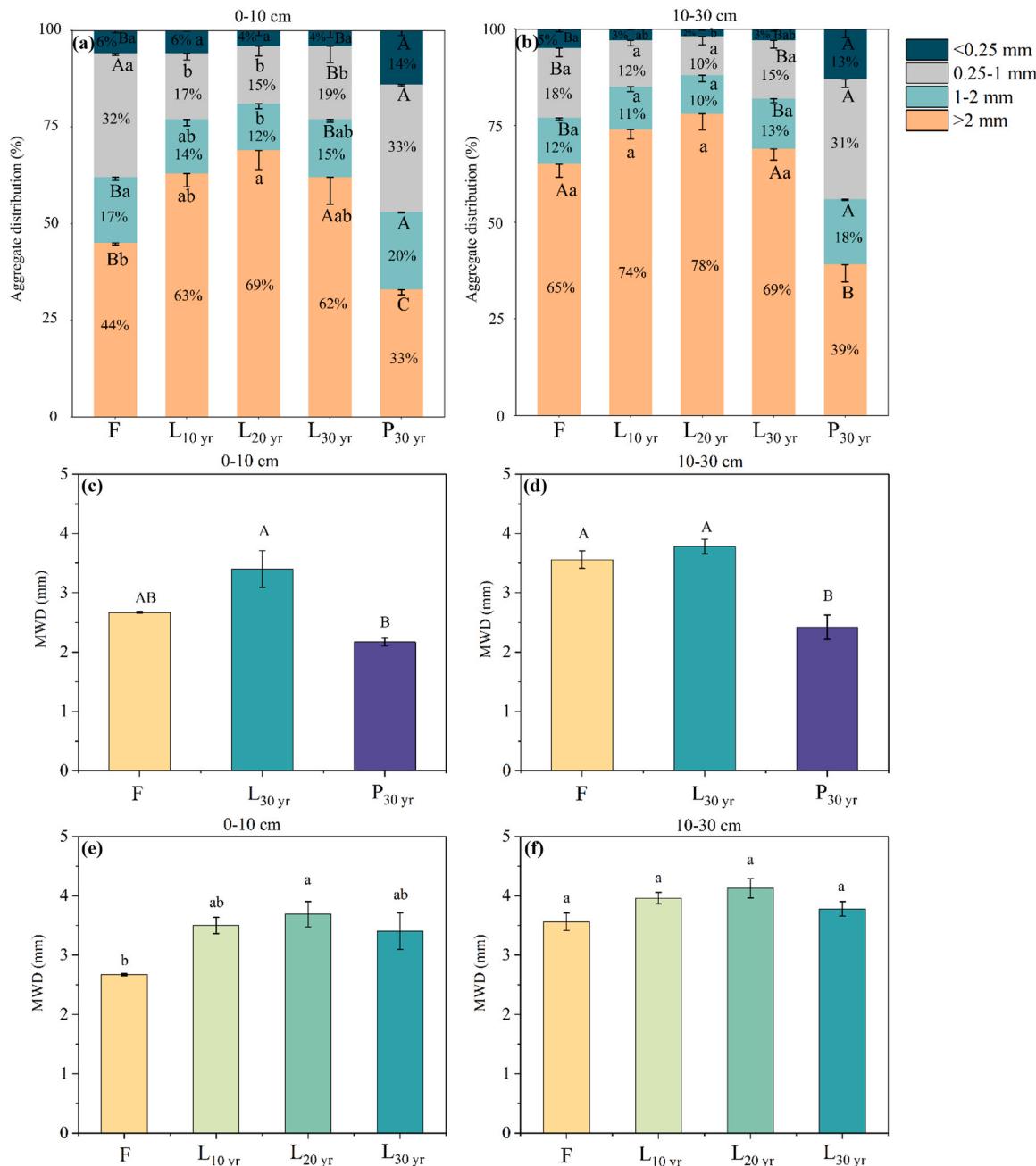


Fig. 2. Proportion of each aggregate fraction (a, b) and the mean weight diameter (MWD) (c, d, e, f) of soil aggregates at 0–10 (a, c, e) and 10–30 cm depth (b, d, f) for different afforestation species (c, d) and different afforestation ages (e, f). Note: The error bar represents standard error. F: farmland; L_{10 yr}: locust afforested 10 years; L_{20 yr}: locust afforested 20 years; L_{30 yr}: locust afforested 30 years; P_{30 yr}: pine afforested 30 years. Different capital letters indicate statistically significant differences among the different afforestation species (F, L_{30 yr}, P_{30 yr}) ($P < 0.05$). Different lowercase letters indicate statistically significant differences among the different afforestation ages (F, L_{10 yr}, L_{20 yr}, L_{30 yr}) ($P < 0.05$).

(Fig. 3d, e). Moreover, the increases were highest in the <0.25 mm aggregate fraction, and were more pronounced at the later ages of afforestation (L_{20 yr} and L_{30 yr}). For example, for the <0.25 mm aggregate, this land-use change increased SOC in the first 20 years, but it decreased in the later 10 years (20–30 years) (Fig. 3d), whereas the negative effect of afforestation ages on TP did not change with aggregate size (Fig. 3f). For example, compared with farmland, 10-year afforestation with black locust (L_{10 yr}) resulted in 25 %, 18 %, 22 % and 21 % decreases for TP at the 0–10 cm depth for the <0.25, 0.25–1, 1–2 and >2 mm aggregate fractions, respectively ($P > 0.05$).

3.3. Effects of afforestation species and age on enzyme activities in bulk soils and aggregates

The activities of EEA_C and EEA_N decreased with increasing size of aggregates, while the activity of EEA_P was similar among different aggregate sizes (Fig. S2d, e, f). Thirty-year afforestation with both black locust and Chinese pine increased enzyme activities at the 0–10 cm depth (Fig. 4a, b, c). The effects on EEA_C and EEA_P were greater for black locust (EEA_C, +62 % to +123 %; EEA_P, +211 % to +281 %; compared with farmland) than for Chinese pine (EEA_C, +15 % to +100 %; EEA_P, +117 % to +177 %; compared with farmland) in bulk soils and aggregates (except EEA_C in bulk soils and >2 mm aggregate fraction), while

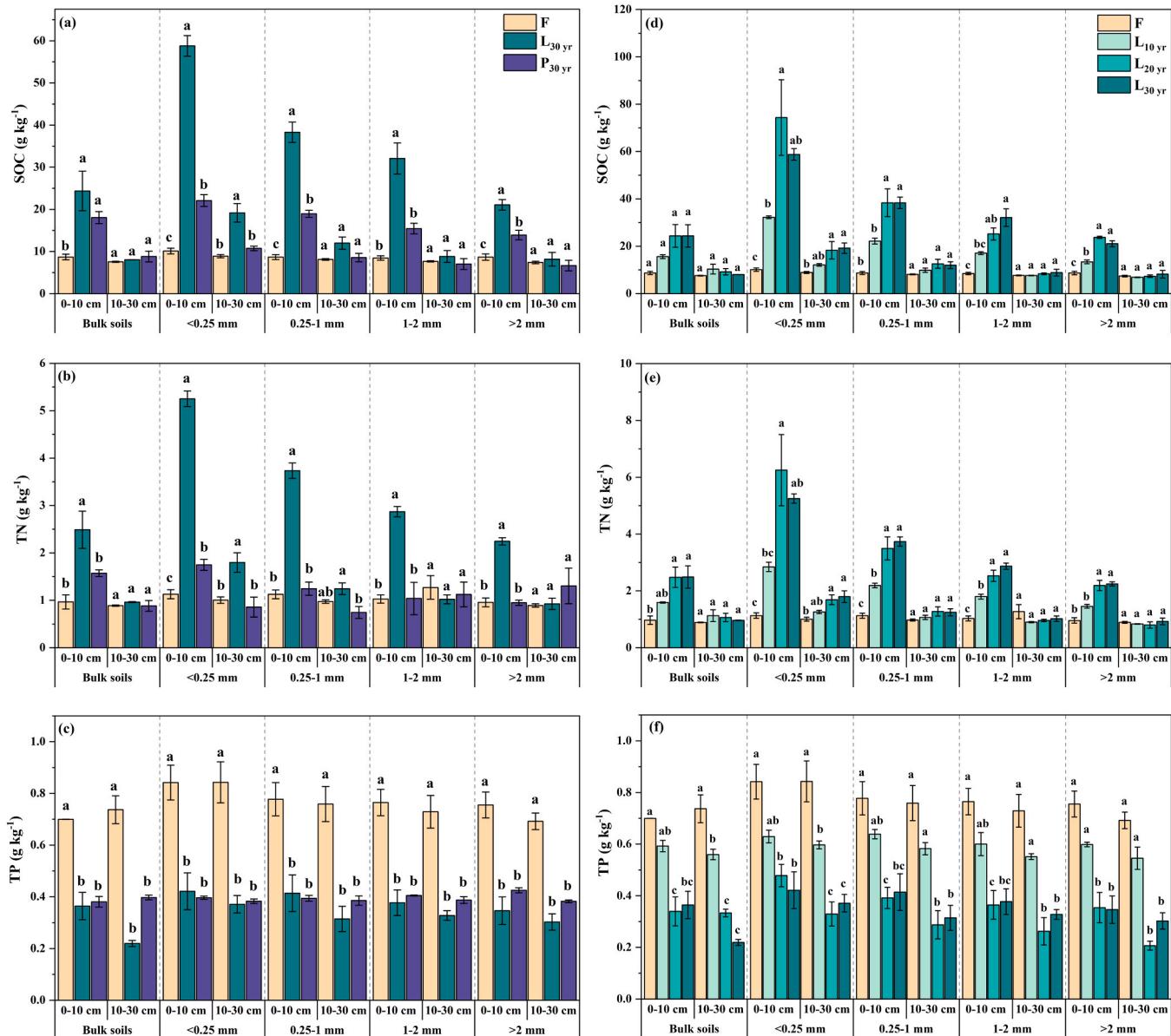


Fig. 3. Content of organic carbon (SOC) (a, d), total nitrogen (TN) (b, e) and total phosphorus (TP) (c, f) in bulk soils and aggregates at 0–10 cm and 10–30 cm depth for different afforestation species (a, b, c) and different afforestation ages (d, e, f). Note: The error bar represents standard error. F: farmland; L_{10 yr}: locust afforested 10 years; L_{20 yr}: locust afforested 20 years; L_{30 yr}: locust afforested 30 years; P_{30 yr}: pine afforested 30 years. Different lowercase letters indicate statistically significant differences among the different afforestation species (F, L_{10 yr}, L_{20 yr}, L_{30 yr}) ($P < 0.05$).

the effects on EEA_N were greater for Chinese pine (+30 % to +91 %; compared with farmland) than for black locust (-7 % to +66 %; compared with farmland) at the 0–10 cm depth. Additionally, the effects on EEA_C and EEA_N activities were greater in < 0.25 mm aggregate fraction than other aggregate fractions.

For conversion from farmland to black locust, the increases in enzyme activities were greater in 10- and 30-year afforestation than those in 20-year afforestation, and the increases of EEA_C and EEA_N were greater in < 0.25 mm aggregate fractions than other aggregate fractions (Fig. 4d, e, f). Therefore, the positive effect on EEA_C and EEA_N was greater in aggregates of small sizes, while the effect on EEA_P was independent of aggregate size.

3.4. Key drivers of enzyme activities

The enzyme activities in bulk soils were mainly affected by > 2 mm aggregate fractions (Fig. 5). > 2 mm aggregate fractions explained 54 %

of EEA_C variation in bulk soils, 58 % of EEA_N variation in bulk soils, and 60 % of EEA_P variation in bulk soils, respectively. Spearman's correlation indicated that the enzyme activities were mainly affected by the aggregate stability and soil nutrients (Fig. 6). For example, in bulk soils, EEA_C and EEA_N had the strongest correlation with MWD (-0.44 and -0.53), and EEA_P had the strongest correlation with TP (-0.67); in aggregates, EEA_C and EEA_N had the strongest correlation with SOC (0.70 and 0.55), and EEA_P had the strongest correlation with TP (-0.63).

PLS-PM showed that the effects of afforestation species and ages on enzyme activities varied with aggregate fractions (Fig. 7). For example, the effect of soil nutrients on EEA_C was greatest in 1–2 mm and > 2 mm aggregate fractions; for the effects of afforestation species on enzyme activities, the proportions of 0.25–1 mm and 1–2 mm aggregate fractions had significant effects on EEA_C, but the proportions of < 0.25 mm and > 2 mm aggregate fractions had no significant effects on EEA_C (Fig. 7a); afforestation species had significant direct effects on EEA_N in 0.25–1 mm and 1–2 mm aggregate fractions, but they had no significant

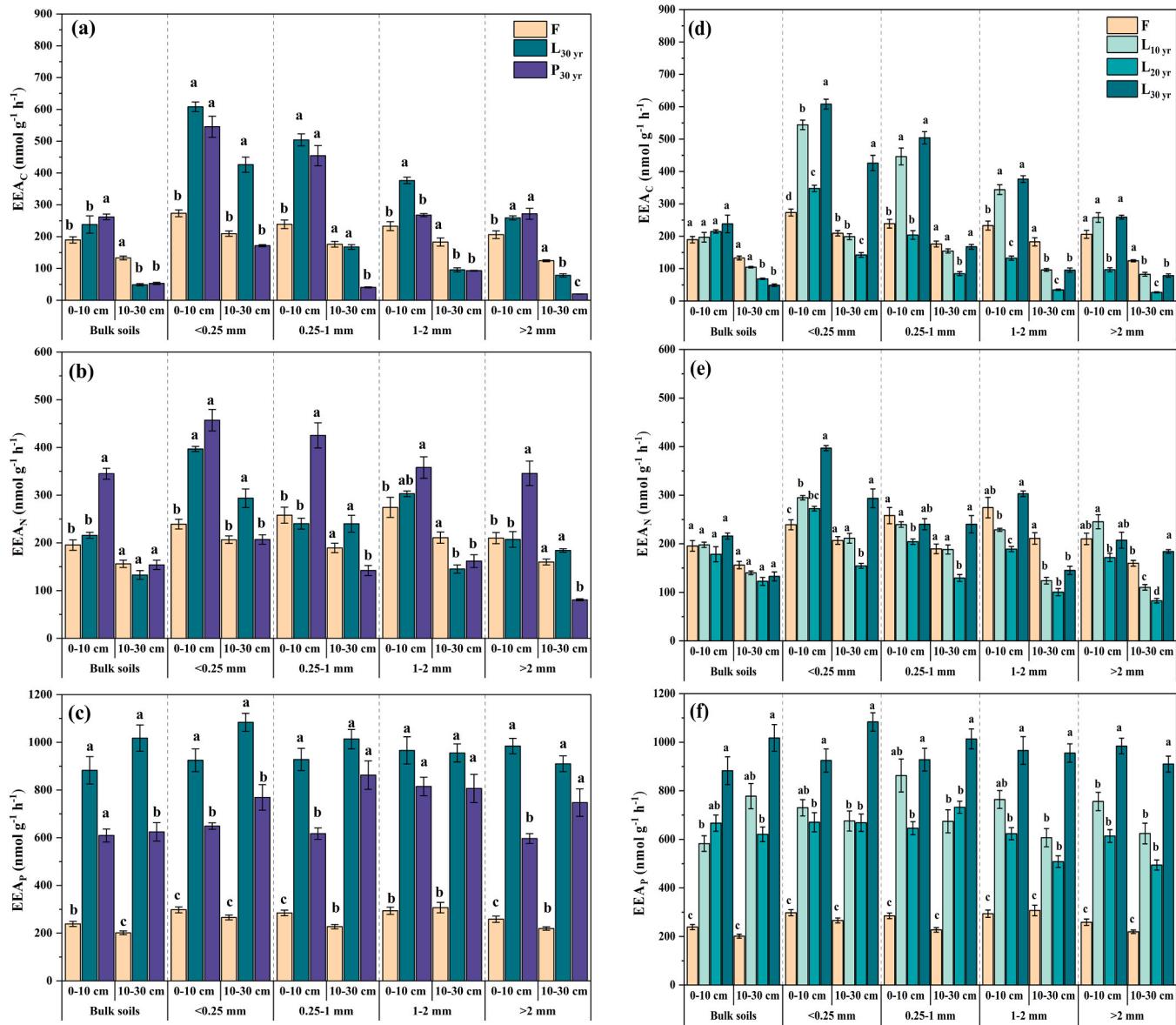


Fig. 4. Variations of EEA_C (a, d), EEA_N (b, e) and EEA_P (c, f) in bulk soils and aggregates at 0–10 cm (a, c, e) and 10–30 cm (b, d, f) depth for different afforestation species (a, b, c) and different afforestation ages (d, e, f). Note: The error bar represents standard error. EEA_C: C-acquiring enzymes activities; EEA_N: N-acquiring enzymes activities; EEA_P: P-acquiring enzyme activity; F: farmland; L_{10 yr}: locust afforested 10 years; L_{20 yr}: locust afforested 20 years; L_{30 yr}: locust afforested 30 years; P_{30 yr}: pine afforested 30 years. Different lowercase letters indicate statistically significant differences among the different afforestation species (F, L_{10 yr}, L_{20 yr}, L_{30 yr}) ($P < 0.05$).

direct effects on EEA_N in < 0.25 mm and > 2 mm aggregate fractions (Fig. 7a). Moreover, changes in EEA_P were mainly directly affected by afforestation species and ages, and changes in EEA_C and EEA_N were not only directly affected by afforestation species and ages, but also largely indirectly determined by afforestation-induced nutrients, aggregate stability and aggregate fraction proportion changes.

4. Discussion

4.1. The effects of afforestation on SOC, nutrients, and enzyme activity vary with afforestation species

Our study revealed distinct patterns in soil aggregate stability following farmland conversion to different forest types. Compared with farmland, black locust showed a relatively higher proportion of > 2 mm aggregate (Fig. 2). This observation can be attributed to the accumulation of organic matter from litter inputs in the forest soil and the fact that

the forest is not disturbed by tillage or fertilization (Burst et al., 2020; Zhang et al., 2020b). In the process of converting farmland to forestland, accumulated organic matter from litter inputs and metabolites generated by microbial activity are crucial binding agents in soil, ultimately increasing the proportion of larger aggregates (Zhang et al., 2018). As a result, the conversion of farmland to black locust was beneficial to the enhancement of soil aggregate stability, consistent with other studies in the Loess Plateau (Zhong et al., 2021; Zhang et al., 2022b). However, compared with farmland, pine showed relatively low aggregate stability, which might be due to the fact that a greater number of horizontal roots of pine (23 Ind Plant⁻¹), which was significantly higher than that of black locust (16 Ind Plant⁻¹), may have destroyed the unstable large aggregates (Denef et al., 2002; Zhang et al., 2024). Furthermore, compared with black locust litter (high N, low lignin), the lignin content in pine tree litter is higher and its decomposition rate is slower (Hattenschwiler et al., 2005; Rumeau et al., 2024), resulting in a weaker ability to promote aggregate formation through organic binding

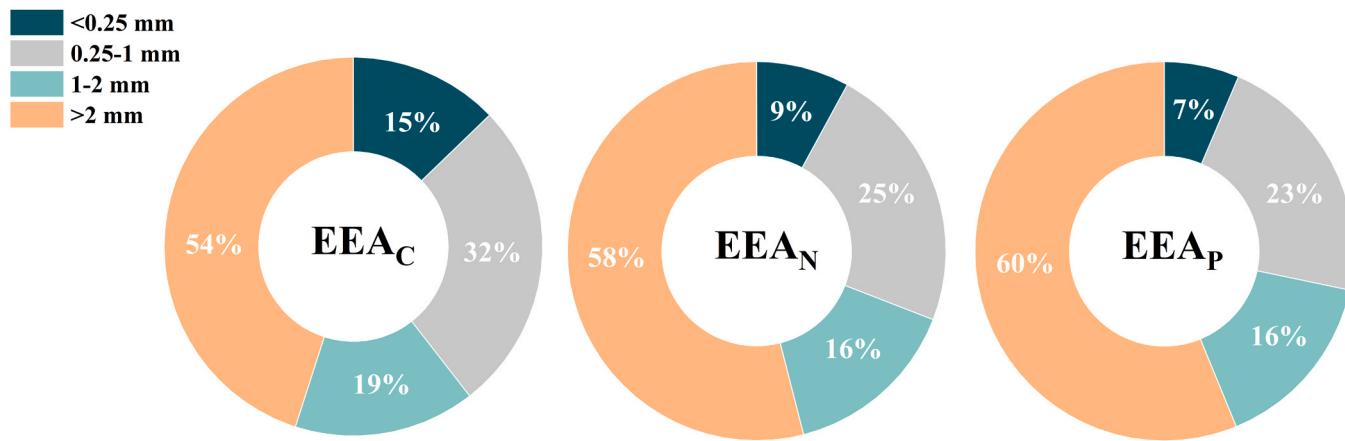
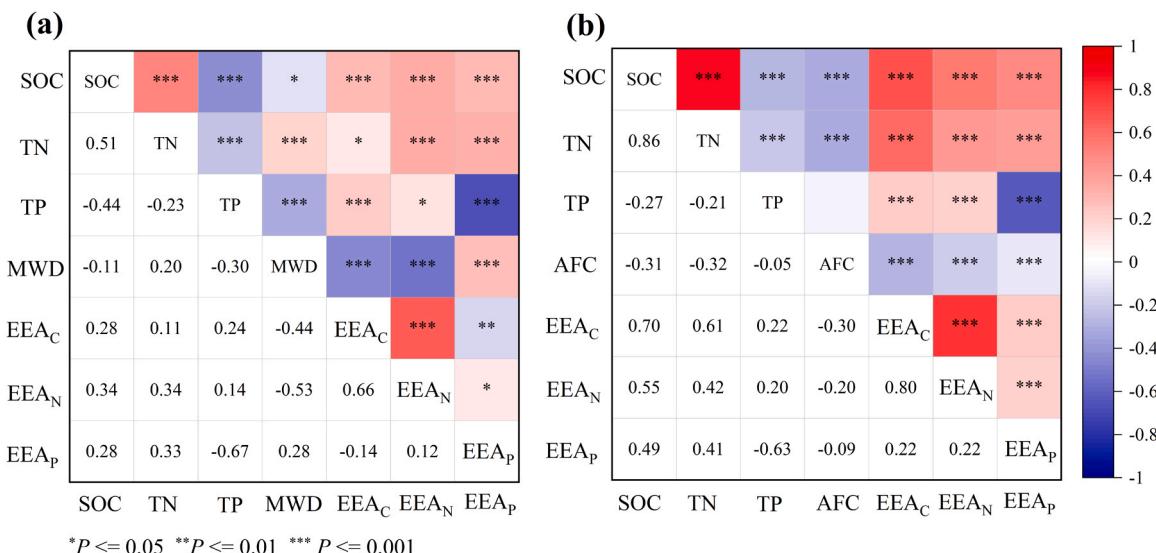


Fig. 5. Contributions of different-sized aggregates to soil enzymes activities (EEA_C, EEA_N, and EEA_P) in bulk soils. Note: EEA_C: C-acquiring enzymes activities; EEA_N: N-acquiring enzymes activities; EEA_P: P-acquiring enzyme activity.



*P <= 0.05 **P <= 0.01 ***P <= 0.001

Fig. 6. Heatmaps indicating potential relationships between enzyme activity (EEA_C, EEA_N and EEA_P), soil nutrients, MWD and proportion of each aggregate fraction in bulk soils (a) and aggregates (b). Note: EEA_C: C-acquiring enzymes activities; EEA_N: N-acquiring enzymes activities; EEA_P: P-acquiring enzyme activity; MWD: mean weight diameter; AFC, proportion of each aggregate fraction; SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus. *P < 0.05, **P < 0.01, and ***P < 0.001.

mechanisms. Therefore, for Chinese pine, the mechanical disturbance caused by penetrating horizontal roots may outweigh the binding effects of organic matter. These results indicated that converting farmland to black locust promoted soil aggregation stability, with consistent effects observed over 10–30 years, while afforestation with Chinese pine for 30 years may have negative effects on soil structure.

In this study, we showed that the conversion of farmland to forest resulted in the accumulation of SOC and TN at 0–10 cm, while the lack of significant changes was observed in deeper layer (10–30 cm) (Fig. 3a, b, d, e). These results were expected, as the accumulation of SOC and TN in the top layer was primarily driven by the large input of plant litter following afforestation, a process that is predominantly concentrated in the topsoil layer (Richter et al., 1999; Wei et al., 2012). Notably, the effect of afforestation on SOC and TN depended on the tree species (Table S1), and these differences could be primarily attributed to variations in litter quality. Specifically, the C/N ratio of pine litter was 35 while the C/N ratio of deciduous trees litter ranged from 14 to 21, which is the optimal C/N ratio for litter decomposition (Heal et al., 1997). The faster decomposition rate of black locust litter compared to pine litter likely explained why black locust afforestation exhibited greater

potential to enhance soil C and N accumulation than pine afforestation. By contrast, TP exhibited a decline after farmland afforestation likely due to plant absorption and lack of P fertilizer (Fig. 3c, f; Li et al., 2019; Yao et al., 2019), and the reduction in TP did not change with the variation of soil depth, which was consistent with the findings from other experiments (Xu et al., 2020; Zhao et al., 2024). These contrasting responses of aggregate stability and soil nutrients to afforestation together emphasize the critical role of tree species in mediating C and N dynamics, while P dynamics remain more uniformly affected by afforestation.

Our results showing an increase in soil enzyme activities (EEA_C, EEA_N, and EEA_P) at 0–10 cm by farmland afforestation (Fig. 4) could be ascribed to the accumulation of belowground biomass and litter from farmland to forest (Bowles et al., 2014), and this increasing effect exhibited surface layer dominance and diminishing impacts at greater depths, consistent with the effect of afforestation on SOC and TN (Fig. 3). The enzyme-nutrient coupling suggested preferential microbial investment in surface decomposition processes (Kong et al., 2022), where enzymes exhibited stronger dependence on labile organic inputs compared to the stabilized nutrient pools dominating deeper layer.

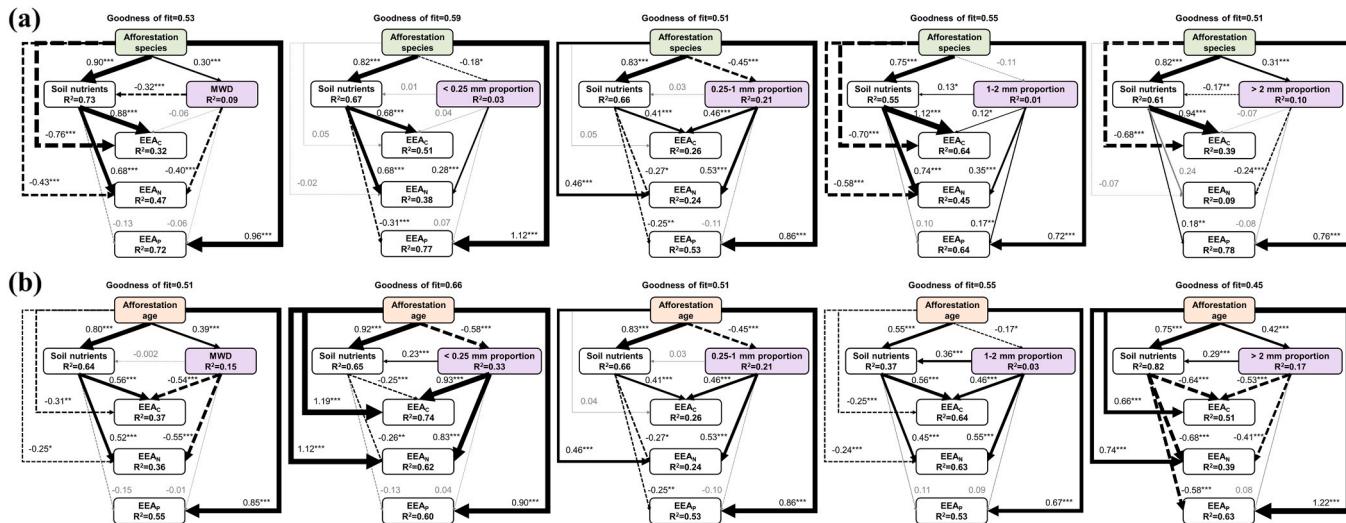


Fig. 7. Partial least squares path modelling (PLS-PM) assessing the direct and indirect effects of afforestation species (a) and ages (b), soil nutrients, mean weight diameter and proportion of each aggregate fraction on enzyme activity (EEAc, EEA_N and EEA_P). Numbers adjacent to arrows show standardized path coefficients. Solid and dashed lines indicate positive and negative relationships, respectively. Gray lines indicate nonsignificant pathways. The R² values indicate the proportion of variance explained. Soil nutrients is a latent variable by incorporating SOC, TN, and TP. Note: EEAc: C-acquiring enzymes activities; EEA_N: N-acquiring enzymes activities; EEA_P: P-acquiring enzyme activity; MWD: mean weight diameter; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus. *P < 0.05, **P < 0.01, and ***P < 0.001.

Furthermore, we found that the effects of farmland afforestation on enzyme activities varied significantly among different tree species (Table S1). This inconsistency might be related to differences in litter chemistry between two tree species, as evidenced by the superior capacity of high-nitrogen black locust litter (low lignin) to stimulate enzymes compared to pine litter with more lignin compounds that were difficult to decompose (Hattenschwiler et al., 2005; Prescott and Grayston, 2013). However, this great effect of black locust on enzyme activities may be changed by black locust as a leguminous species because black locust has higher N concentrations, and may alleviate microbial N limitation via root exudates or litter decomposition (Cui et al., 2020; Xu et al., 2022; Gou et al., 2023) and thus EEA_N of pine was significantly higher than that of black locust in our study (Fig. 4b). Hence, the conversion of farmland to forests (leguminous and non-leguminous species) may shape soil enzyme activity patterns through interactive mechanisms involving litter chemistry, root, and microbial resource allocation strategies, further influencing microbial mediation of nutrient cycling processes.

4.2. Effects of afforestation on SOC, nutrients, and enzyme activity vary with afforestation ages

The striking results of this study involved the dynamic relationship between afforestation ages of black locust and soil enzyme activities. Although afforestation generally enhanced enzyme activities through increased soil nutrient availability (Sinsabaugh et al., 2002; Xu et al., 2017), and black locust, which has more developed fine roots, could produce more enzymes through the release of exudates (Ren et al., 2016), we observed a paradoxical decline in enzyme activities at the 20-year black locust restoration (Fig. 4d, e, f). This nonlinear response might be related to the change of soil moisture during the conversion of farmland to black locust (Jia et al., 2017; Zhang et al., 2019). Previous studies in the Loess Plateau have reported soil moisture dynamics following afforestation, with the most severe moisture deficit occurring at the 20-year black locust restoration (Yang et al., 2022), suggesting that despite litter and root biomass contributing to SOC and nutrients, higher nutrients and lower moisture may limit microbial growth and inhibit soil enzyme activities (Wang et al., 2012; Liu et al., 2018). Future research integrating soil moisture monitoring is needed to validate this

hypothesis.

Additionally, although afforestation enhanced enzyme activity related to P acquisition, it failed to promote soil P accumulation (Fig. 3; Fig. 4). One possible explanation for this apparent decoupling might involve P demand outstripping supply—that is, P uptake by plantations or the growth needs of microorganisms may counteract or even exceed P input into the soil through litter and root exudates (Deng et al., 2017; Luo et al., 2023). The findings demonstrated that black locust was more effective than Chinese pine for farmland afforestation, significantly improving soil aggregate stability, carbon and nitrogen sequestration, and enzyme activity regulation in the 0–30 cm soil depth. However, the observed 20-year enzyme activity decrease of black locust—potentially linked to moisture deficit—underscores the need for optimized water management to ensure the sustainability of farmland-to-forest conversion in arid and semi-arid ecosystems.

4.3. SOC, nutrients and enzyme activity vary with soil aggregate sizes in afforested ecosystems

In this study, we showed that the SOC and TN were highest in < 0.25 mm aggregates and decreased with increasing aggregate size (Fig. S2a, b), which was inconsistent with previous studies reporting preferential SOC accumulation in large size aggregates (Liu et al., 2020; Wang et al., 2023). This inconsistency might result from variations in organic C components across aggregate fractions. For example, Ma et al. (2024) found that dissolved organic C was negatively correlated with aggregate size, while microbial biomass C increased with the increase in aggregate size; furthermore, Wang et al. (2023) observed that the readily oxidizable organic C in large aggregates was significantly higher than that in smaller fractions, suggesting inconsistent patterns in unstable C cycling in aggregates in current studies. While TP was similar among different aggregate sizes (Fig. S2c), this finding was consistent with previous results observed in the Loess Plateau (Wu et al., 2023). These findings collectively underscore the need for deeper investigation into organic C component dynamics and microbial regulation mechanisms following afforestation.

In our current study, the effect of afforestation on EEAc and EEA_N was greatest in < 0.25 mm aggregate, decreasing with the increase of aggregate size (Fig. 4), probably due to the larger specific surface area

and the abundance of C and N resources of < 0.25 mm aggregate fraction (Rabbi et al., 2016; Bach et al., 2018). By contrast, the EEA_P , similar to TP, exhibited no aggregate-size dependency (Fig. S2c, f). These findings collectively indicated the close association between nutrient availability and the variation of enzyme activities among different aggregate fractions (Chen et al., 2020). It should not be overlooked that although EEA_C and EEA_N activities in > 2 mm aggregate fractions were the lowest, they contributed the most to enzyme activities in bulk soils (Fig. 5), primarily because > 2 mm aggregate fraction dominated the soil mass (33 %–78 %), while < 0.25 mm aggregate fraction accounted for a relatively low proportion of the soil mass (2 %–14 %), resulting in limited contributions to the enzyme activities in bulk soils. Notably, the mechanisms of afforestation-driven enzyme activities were different at aggregate aspect, exhibiting different direct and indirect effects among four aggregate fractions (Fig. 7), which might be due to the distinct biogeochemical microenvironments (such as the distribution of organic matter, pore structure, moisture conditions, dominant phyla, microbial community structure) in different aggregate sizes (Tian et al., 2022; Zhang et al., 2023; Simon et al., 2024). The diverse responses of these aggregate-specific microenvironments to afforestation may drive the different pathways by which the enzyme activity was affected. Therefore, this study suggested that enzyme activities related to soil C, N, and P cycling in aggregates may be differentially influenced by multi-aspect mechanisms (e.g., specific surface area, nutrient availability, organic C composition, and microbial community), and thus future research should further focus on the dynamic coupling mechanisms between aggregate-scale multi-aspect mechanisms and microbial metabolic interactions.

4.4. Limitations and Future Recommendations

Given that our experimental design was based on the implementation of the “Grain for Green” project, it was impossible to select afforested lands with identical treatments (i.e., tree species and afforestation ages) under the same landscape conditions (e.g., slope position, gradient, and aspect) and initial soil conditions (e.g., soil texture). To ensure comparability, we established sampling plots within the same afforested land, thereby maintaining consistent landscape and initial soil conditions across sampling points. This approach was necessary because selecting widely separated plots could introduce variations in terrain and soil properties, potentially confounding the assessment of tree species and afforestation age effects. However, this experimental design may approximate pseudo-replication. To address this limitation, we recommend conducting long-term, controlled experiments across varying landscape and initial soil conditions. Such an approach would help explicitly disentangle the effects of these two factors on afforestation outcomes. Furthermore, our study was conducted exclusively in the semi-arid region of the southern Loess Plateau. This regional focus underscores the need for future cross-regional comparisons across diverse climatic zones and soil types to evaluate how these effects may vary depending on climate conditions and soil properties.

5. Conclusion

In this study, we examined the effects of afforestation on SOC, TN, TP and enzyme activities associated with C, N, and P cycling in both bulk soils and aggregates on the southern Loess Plateau. We found that afforestation significantly enhanced SOC and TN at 0–10 cm but reduced TP, with these effects varying by afforestation species and ages. Black locust afforestation improved aggregate stability, whereas pine afforestation decreased aggregate stability likely due to root-induced mechanical disruption. Furthermore, enzyme activities increased at 0–10 cm following afforestation, but the effect depended on tree species. Notably, the effects of afforestation ages on enzyme activities did not show a linear increasing trend, and these enzyme activities were lowest in 20 years for black locust. At the aggregate scale, SOC, TN, EEA_C and

EEA_N exhibited upward trends with decreasing aggregate size, while TP and EEA_P were unaffected by aggregate fractions, and the enzyme activities in bulk soils were mainly affected by > 2 mm aggregate fractions. PLS-PM demonstrated the significant influence of afforestation on soil enzyme activities, with soil nutrients, aggregate stability, and proportion of aggregate fraction playing a crucial role in different aggregate sizes. These findings advance mechanistic understanding at aggregate scale of ecosystem restoration in arid and semi-arid regions, and contribute to the formulation of appropriate forest management measures. Furthermore, our study was conducted exclusively in the semi-arid region of the southern Loess Plateau. This regional focus underscores the need for future cross-regional comparisons across diverse climatic zones and soil types to evaluate how these effects may vary depending on climate conditions and soil properties.

CRediT authorship contribution statement

Dixing Feng: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Yufei Yao:** Writing – review & editing, Visualization. **Jingyun Zhou:** Methodology, Investigation. **Weibo Kong:** Writing – review & editing, Methodology, Funding acquisition. **Jianlun Gao:** Resources, Investigation. **Qingyin Zhang:** Methodology, Investigation. **Xiaoxu Jia:** Methodology, Conceptualization. **Ming'an Shao:** Supervision. **Xiaorong Wei:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Liping Qiu:** Writing – review & editing, Supervision, Conceptualization.

Declaration of Competing Interest

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109804.

Data availability

Data will be made available on request.

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