



Mixed-species plantations enhance soil carbon stocks on the loess plateau of China

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Abstract

Background and aims Afforestation is considered one important means of mitigating climate change. However, it is still controversial whether mixed-species plantations (MP) were more conducive to the soil organic carbon (SOC) stocks than monoculture plantations (PP). **Methods** We conducted a meta-analysis based on 21 publications to assess the effects of different afforestation modes as well as controlling factors of SOC stocks on the Loess Plateau of China.

Results Compared with monoculture plantations, mixed-species plantations could significantly increase

the response size of SOC stocks by 28%. For different species combinations, tree-shrub mixtures were more conducive to increasing the SOC stocks. In climate-limited regions (MAT <8 °C, MAP <500 mm), the response size of SOC stocks of mixed-species plantations was 34% higher than that of monoculture plantations. Furthermore, significant differences were found in the response size of SOC stocks at young (< 10 yr) and mature stages (> 20 yr). Compared with abandoned land, afforestation on cropland, especially mixed-species plantations, could increase the SOC stocks by 10%. Additionally, compared with monoculture plantations, a stronger coupling relationship was observed between the soil total nitrogen (STN) and SOC stocks in mixed-species plantations.

Conclusion Our results suggest that for the Loess Plateau of China, planting mixed-species plantations containing nitrogen-fixing plants is a more effective approach to enhancing the SOC stocks than monoculture plantations.

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Introduction

The carbon (C) in the soil is mainly stored as soil organic matter (SOM), which is about 2.3 times greater than the C in atmospheric CO₂ and 3.5 times greater than the C in all terrestrial plants (Lal 2004; Schleuss et al.

2014). As an important terrestrial carbon sink, forest ecosystems cover an area of approximately 4 billion hectares worldwide and store approximately 70% of soil organic carbon (SOC) (Batjes 1996; Six et al. 2002; Carvalhais et al. 2014). Afforestation, forest planting in areas that previously were not classified as forests, is seen as an effective method of controlling climate change and increasing soil carbon sinks, and it is also an important means of reducing the pressure on existing forests (Wolfe et al. 2015; Pugh et al. 2019). However, due to the limitations of planting costs and survival rates and the demands of human beings, artificial afforestation is mostly conducted as monocultures (Richards et al. 2010; Chomel et al. 2014). Although monoculture plantations have been well-recorded in forest studies, in the face of increasing climate change and ecological degradation, there is an increasing interest in mixed-species plantations (Hulvey et al. 2013; Metz et al. 2016). Previous studies have shown that in addition to improving the productivity and stability of forest ecosystem, mixed species are more resilient and more resistant to biotic stresses (Zhang et al. 2012; Pretzsch and Schütze 2016). However, mixed-species plantations can potentially affect the chemical, physical, and biological processes of soil ecosystems as a consequence of changes in diverse stands and tree species composition (Coll et al. 2018; Liu et al. 2018). Especially for the SOC stocks, diverse stands will alter the balance between the input and loss of C by affecting the litterfall and rhizodeposition of plants and the structure of soil microbial communities (Wang et al. 2013; Prommer et al. 2019). As a result, it is still controversial that whether mixed-species plantations will more conducive to increasing the SOC stocks than monoculture plantations. Therefore, improving the understanding of how mixed-species plantations affect the SOC stocks will have an important impact on the development of forest-based carbon offsets and policies.

In general, mixed-species plantation is the system that composed of woody vegetation of multiple genotypes, species, structures and functions, and it can be arranged with variations in species composition, spatial arrangement and age structure (Griess and Knoke 2011; Manson et al. 2013; Liu et al. 2018). Ecological theories suggest that multi-species assemblages often show large differences in ecological strategies to cope with environmental changes (Richards et al. 2010; Mina et al. 2018). Some previous studies have also reported

conflicting findings about the relationship between the SOC stocks and plant diversity (Diaz et al. 2009; McKinley et al. 2011; Yang et al. 2019). One view showed that mixed-species plantations were more conducive to increasing the SOC stocks than monoculture plantations. In particular, mixed-species plantations can reduce competition for limited resources due to niche separation, thereby strengthening the carbon sequestration capacity of the region (Chomel et al. 2014; Liu et al. 2018). For example, mixed-species plantations can be used to stratify resources uptake (light, water, and nutrients) by adjusting crown width and tree height, thereby promoting the growth of vegetation and understory herbs and increasing the source of SOC (Forrester et al. 2006; le Maire et al. 2013). In addition, mixed-species plantations can provide a variety of habitats, thereby increasing the diversity of animals and microorganisms in the forest and the SOC cycle (Kooch et al. 2017). A contrasting ecological hypothesis showed that multi-species forests tend to occupy overlapping niches, leading to increased competition, which directly leads to more consumption of SOC than monoculture plantations (Manson et al. 2013). In addition, as many studies have demonstrated, the SOC stocks is also affected by many biotic and abiotic factors, such as vegetation type, planting age, climate, soil qualities and prior land use/land cover (Davidson and Janssens 2006; Berthrong et al. 2009; Doetterl et al. 2015). However, it is unclear that the potential differences of those factors between mixed-species plantations and monoculture plantations. Overall, due to the diversity of driving factors and complex plant-soil feedback mechanisms, it is still unknown whether mixed-species plantations will more conducive to increasing the SOC stocks than monoculture plantations.

Due to steep topography, frequent heavy rainfall in summer months, sparse vegetation cover, and improper land use, the Loess Plateau of China, with an area of 6.4×10^5 km², is considered one of the most severe soil and water losses areas in the world (Liu et al. 2007; Gao et al., 2016). To control soil erosion and increase vegetation coverage, a series of nationwide conservation projects focusing on soil and water conservation have been launched since the 1980s, such as the “Grain for Green” Program, which aims to restore degradation

lands (e.g., cropland and bare land) to forest, shrub land and grassland (Chang et al. 2014; Yuan et al. 2014). Over the past two decades, the vegetation coverage of the Loess Plateau has increased from 31.6% in 1999 to 59.6% in 2013, and the amount of sediment entering the sea from the Loess Plateau in 2015 was only 8.9% of that in the 1950s (Chen et al., 2015; Li et al., 2019). However, most of the plantations are established with fast-growing tree species (e.g., *Pinus tabulaeformis*) and exotic species (e.g., *Robinia pseudoacacia*), which will usually have a negative impact on the ecosystem stability (Cao et al. 2011; Brockerhoff et al. 2013). Especially for the Loess Plateau, due to limited rainfall and high evaporation from soil, large-scale afforestation operations will directly affect the balance between regional water supply and demand, which results in long-term soil water deficit and forest degradation (Cao 2011; Jia et al. 2017). Due to better stand structure and community characteristics of mixed-species plantations lead to more advantages in controlling soil erosion and alleviating water stress compared with that of monoculture plantations, mixed-species plantations are also widely cultivated in the Loess Plateau (Liang et al. 2012; Gong et al. 2020). Although previous researches have shown that afforestation programs on the Loess Plateau may indeed increase the SOC stocks (Chen et al. 2007; Deng et al. 2014), whether mixed-species plantations are more conducive to increasing the SOC stocks and stability than monoculture plantations on the Loess Plateau remains controversial (Wang et al. 2012; Gao et al. 2018). Furthermore, for the Loess Plateau, although factors affecting the SOC stocks after afforestation have been studied (Liu et al. 2011; Deng et al. 2014), how these factors regulate differences in the SOC stocks between different afforestation modes remains unknown.

Therefore, we extracted from the published papers for a meta-analysis to quantify the effects of different afforestation modes (mixed-species plantations and monoculture plantations) on the SOC stocks in the Loess Plateau of China. Based on complementary effects in species mixtures, we hypothesized that the SOC stocks of mixed-species plantations is higher than that of monoculture plantations, and answered the following questions: (1) how do SOC stocks change following

different afforestation modes compared with the replaced ecosystems; (2) are there any differences in the SOC stocks between different species combinations; and (3) how do prior land use/land cover, climate and planting age affect the SOC stocks following different afforestation modes.

Materials and methods

Data compilation

In this study, the literature on changes in SOC in different afforestation modes was compiled, using the Web of Science (United States) and CNKI (China Knowledge Resources Comprehensive Database, China) up to 1 July, 2019. Different combinations of search terms were used: “soil organic carbon” or “soil organic matter” and “Loess Plateau” and “mixed plantation” or “mixed forest” or “mixed species” or “species diversity” or “tree diversity” or “tree species” or “shrub species” or “afforestation”. To avoid publication bias, the following criteria were chosen to select the relevant research:

- (1). the SOC stocks was provided or could be calculated based on the SOC or SOM content, bulk density (BD) and soil depths;
- (2). data on afforested sites (mixed-species and monoculture plantations) and control sites (cropland or abandoned land) were included;
- (3). the experiments used paired-site, chronosequence, or retrospective design, with similar soil conditions for both the afforested sites and the control sites;
- (4). only data from field monitoring studies, excluding laboratory control experiments, were included; and
- (5). the plants studied were non-native species, plants consisting of natural forests were excluded.

In addition, all available data were extracted from the publications, including the location, latitude (N), longitude (E), mean annual temperature (MAT), mean annual precipitation (MAP), slope angle, slope aspect, planting age, sample size, etc.

In total, the final dataset comprised 92 observations in 17 sites in the top 30 cm of the soil from 21 peer-reviewed articles across the Loess Plateau of China (Fig. 1). All the raw data were extracted from the text,

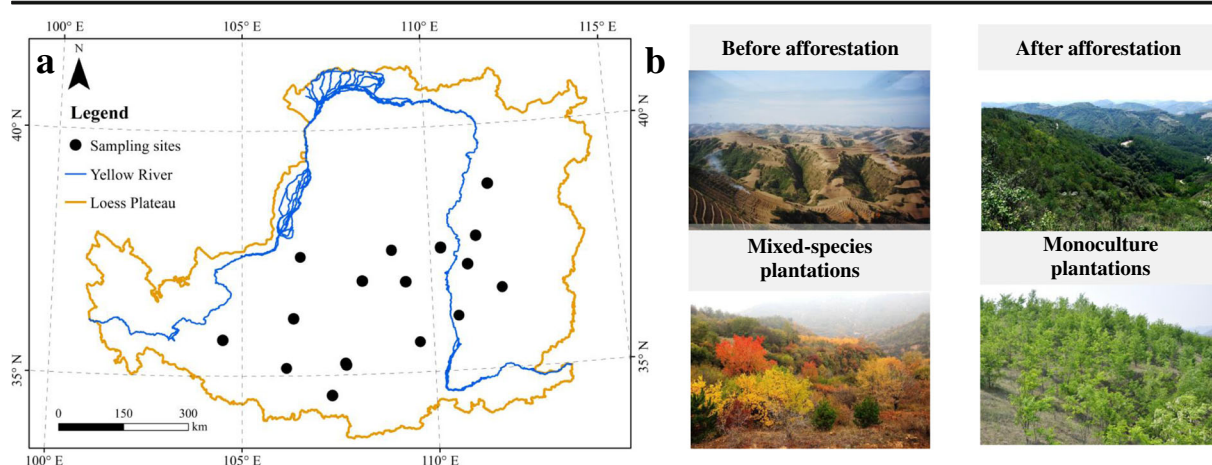


Fig. 1 Location of this study on the Loess Plateau. Note: (a) distribution of the sampling sites on the Loess Plateau. (b) landscape change and main vegetation before and after afforestation on the Loess Plateau

tables, charts and appendices in the publications. When data were presented graphically, the numerical data were obtained using GetData Graph Digitizer ver. 2.24 (Russian Federation). All raw data for SOC were standardized in $\text{g}\cdot\text{kg}^{-1}$. If a specific temporal or retrospective study involved observations of multiple plantation ages, the observations for each age were considered an independent study and included in the analysis. To depict a clearer trend in the SOC pools, the forest restoration time was divided into three time periods: young stage (<10 yr), middle stage (10–20 yr) and mature stage (>20 yr).

Data calculation

If there were only SOM values in the study, the SOC of the sample was calculated using the relationship between SOM and SOC as follows:

$$\text{SOC} = \text{SOM} \times 0.58 \quad (1)$$

where *SOC* represents soil organic carbon ($\text{g}\cdot\text{kg}^{-1}$) and *SOM* represents soil organic matter ($\text{g}\cdot\text{kg}^{-1}$).

The SOC stocks was calculated using the following equation:

$$C_s = \frac{\text{SOC} \times \text{BD} \times D \times (1-P)}{10} \quad (2)$$

in which, C_s is the SOC stocks ($\text{Mg}\cdot\text{ha}^{-1}$), *SOC* is the SOC concentration ($\text{g}\cdot\text{kg}^{-1}$), *BD* is the soil bulk density ($\text{g}\cdot\text{cm}^{-3}$), and *D* is the soil thickness (cm), and *P* is the fraction (%) of gravels with size of >2 mm in the soils.

Because the soil gravel size of Loess Plateau of China is mostly below 2 mm, this fraction (*P*) was assumed to be 0 (Wang et al. 2010).

BD values of soil are critical for calculating the SOC stocks, but many studies did not measure this parameter. We extrapolated the missing BD values based on the empirical relationship between the SOC concentration and BD values in the literature (Wu et al. 2003). BD values were calculated as follows:

$$\text{BD} = -0.1229 (\text{SOC}) + 1.2901 (\text{SOC} < 6\%) \quad (3)$$

$$\text{BD} = 1.3774 e^{-0.0413 \text{SOC}} (\text{SOC} > 6\%) \quad (4)$$

To increase the comparability of data obtained from the different studies, the methodology by Jobbagy and Jackson (2000) was adopted to convert the original SOC stocks into the SOC stocks in the top 30 cm:

$$Y = 1 - \beta^d \quad (5)$$

$$X_{30} = \frac{1 - \beta^{30}}{1 - \beta^{d_0}} \times X_{d_0} \quad (6)$$

where *Y* is the cumulative ratio of the SOC stocks from the soil surface to depth *d* (cm), β is the relative reduction rate of the soil carbon pool and soil depth (0.9786), X_{30} is the SOC stocks in the upper 30 cm, d_0 is the original soil depth available in individual studies (cm), and X_{d_0} is the original SOC stocks.

Meta-analysis

The response ratio is typically used as a measure of the experimental effect, and the proportional change produced by the experimental operation can be quantified (Hedges et al. 1999). In this study, the response ratio (r) is defined as the ratio of the SOC stocks of the current land use type (X_e) to the SOC stocks of the control plot (X_c). The formula is as follows:

$$r = \frac{X_e}{X_c} \quad (7)$$

As with classic meta-analyses, most studies only report an average of the data, and standard deviations or standard errors are not reported. To incorporate more research and to better analyze the laws and characteristics of the data, we used an unweighted meta-analysis (Powers et al. 2011; Su and Shangguan 2019). We use the negative (or positive) change of the mean response size (R) which indicates a decrease (or increase) in the value under the current land use (X_e) relative to control plots (X_c). The formula is defined as follows:

$$R = r - 1 \quad (8)$$

To better reflect the dynamic changes in the SOC stocks, the 95% confidence interval (CI) of means for the SOC stocks was calculated using a method described in previous studies (Luo et al. 2006; Deng et al. 2016) as shown in eqs. (9) and (10):

$$SE_R = \sqrt{\frac{V_R}{N}} \quad (9)$$

$$95\%CI = 1.96 \times SE_R \quad (10)$$

where SE_R represents the standard error of the relative change in the SOC stocks, and V_R and N are the variance and sample size of the relative SOC stocks change, respectively. In this study, the 95% CI for each category was calculated. If the 95% CI does not include zero, then the observed effect size is considered to be significantly different from zero.

Data analysis

All statistical analyses were performed using the SPSS statistical package version 24.0 (SPSS Inc., Chicago, Illinois, USA), and the figures were generated using Origin 9.0 (Originlab Corporation, Hampton, USA).

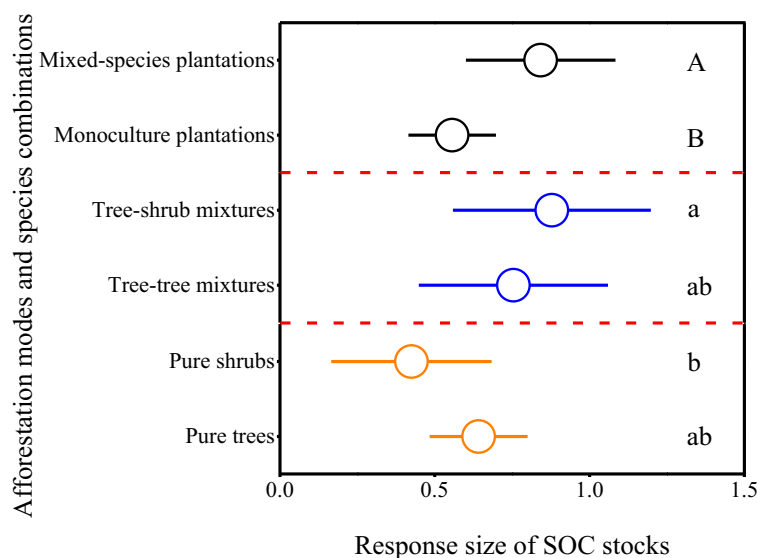
The Shapiro-Wilk test and the Levene's test were used to check the distribution and homogeneity of all data, respectively. One-way analysis of variance (ANOVA) was performed to assess whether there were significant differences in the response size of SOC stocks under different afforestation modes, planting age, climate and prior land use/land cover, and a least significant difference (LSD) test was used for multiple comparisons. Meanwhile, t-tests were conducted to evaluate whether planting age, climate and prior land use/land cover significantly affected the response size of SOC stocks under different afforestation modes. A regression analysis was conducted to analyze the relationships between the response size of SOC stocks and the soil total nitrogen (STN) stocks under different afforestation modes. Differences were evaluated at the 0.05 significance level ($P < 0.05$).

Results

Overall, compared with the control system (i.e. cropland and barred land), the response size of SOC stocks was significantly increased by 84% in mixed-species plantations and 65% in monoculture plantations (Fig. 2). Specifically, for different afforestation modes, the response size of SOC stocks of mixed-species plantations (0.84) was 28% higher than that of monoculture plantations (0.56) (Fig. 2). For different species combinations, the response size of SOC stocks of tree-shrub mixtures (0.88) was 46% higher than that of pure shrubs (0.42), but there was no significant difference from that of tree-tree mixtures (0.75) and pure trees (0.64) ($P > 0.05$).

The response size of SOC stocks significantly increased with planting ages (Fig. 3a). Specifically, for different afforestation modes, the response size of SOC stocks in mixed-species plantations at young stage (< 10 yr, 0.49) and mature stage (> 20 yr, 1.25) was significantly higher than that of monoculture plantations (< 10 yr, -0.13 ; > 20 yr, 0.78), although significant differences under different afforestation modes were not observed at middle stage (10–20 yr, MP = 0.56, PP = 0.54) (Fig. 3b). In addition, we observed that the response size of the SOC stocks of monoculture plantations had a negative value at young stage (< 10 yr, -0.13), indicating that the initial stage of afforestation would have a negative impact on the SOC stocks (Fig. 3b).

Fig. 2 The response size of SOC stocks in different afforestation modes and species combinations. Note: Different upper-case letters indicate significant difference among the different afforestation modes at 0.05 level ($P < 0.05$), and different lower-case letters indicate significant difference among the different species combinations at 0.05 level ($P < 0.05$)



After afforestation, the response size of SOC stocks generally showed a significantly increasing trends with the increase of MAT and MAP (Fig. 4a, b). Specifically, for different afforestation modes, significant differences were found in the response size of SOC stocks when the MAT < 8 °C (MP = 0.42, PP = 0.09) and MAP < 500 mm (MP = 0.42, PP = 0.06) (Fig. 4c, d). For different temperature zones, the response size of SOC stocks under different afforestation modes was the highest when MAT > 10 °C (MP = 1.16, PP = 0.92), and significant differences in the response size of SOC stocks under different afforestation modes were observed when MAT < 8 °C (MP = 0.42, PP = 0.09) and MAT > 10 °C (MP = 1.16, PP = 0.92) (Fig. 4c). Similarly, for different precipitation zones, the response size of SOC stocks under

different afforestation modes was the highest when MAP > 600 mm (MP = 1.22, PP = 0.89), and significant differences in the response size of SOC stocks under different afforestation modes were observed when MAP < 500 mm (MP = 0.42, PP = 0.06) and MAP > 600 mm (MP = 1.22, PP = 0.89) (Fig. 4d).

Overall, whether afforestation occurred on cropland or on abandoned land, the response size of SOC stocks was positive, but the prior land use/land cover had no significant effect on the response size of SOC stocks (Fig. 5a). Specifically, for different prior land use/land cover, the response size of SOC stocks following afforestation on cropland was slightly higher than that on abandoned land, and this difference was nonsignificant (Fig. 5a). For different afforestation modes, the response

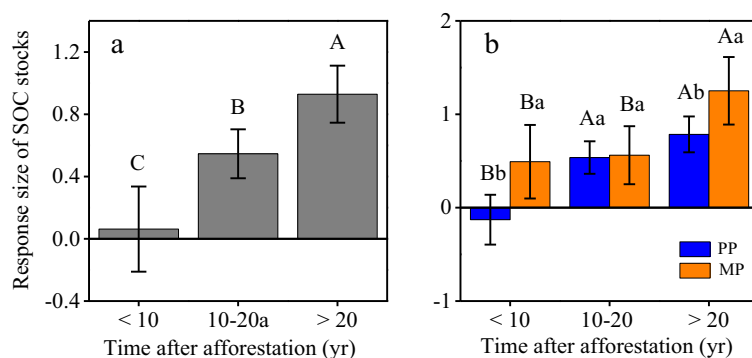
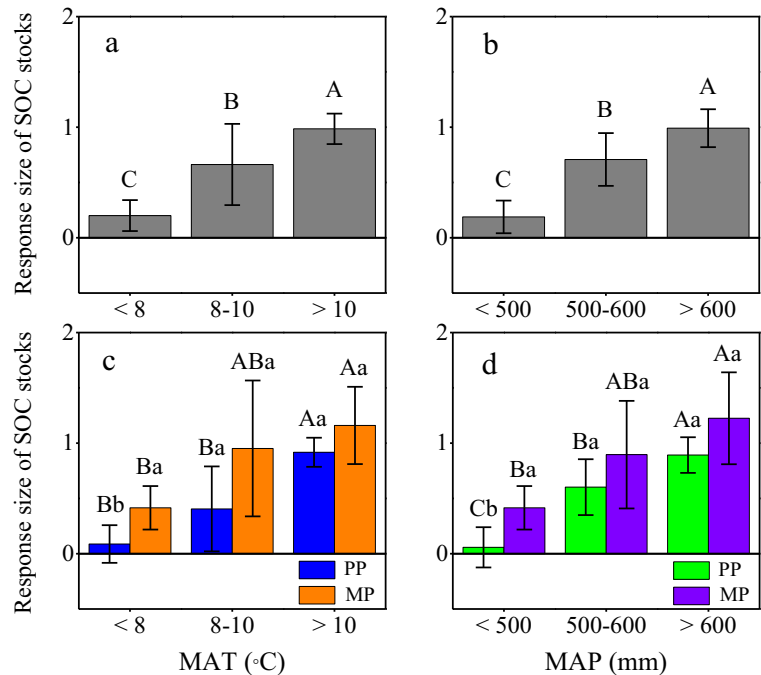


Fig. 3 The response size of SOC stocks at different planting age. Note: PP: monoculture plantations; MP: mixed-species plantations. The values representing response size are the mean \pm 95% CI. Different upper-case letters indicate significant difference

among the different planting age at 0.05 level ($P < 0.05$), and different lower-case letters indicate significant difference among the different afforestation modes at 0.05 level ($P < 0.05$)

Fig. 4 The response size of SOC stocks in different climatic zones, Note: PP: monoculture plantations; MP: mixed-species plantations. The values representing response size are the mean \pm 95% CI. Different upper-case letters indicate significant difference among the different climate zones at 0.05 level ($P < 0.05$), and different lower-case letters indicate significant difference among the different afforestation modes at 0.05 level ($P < 0.05$)



size of SOC stocks of planting mixed-species plantations on cropland (0.87) was 34% higher than that of monoculture plantations (0.53) (Fig. 5b).

There was a significant, positive, linear correlation between the response size of SOC stocks and STN stocks, indicating that SOC change was strongly coupled to STN change ($P < 0.01$, Fig. 6a, b). In addition, the synergistic relationship between the response size of STN stocks and SOC stocks in mixed-species plantations was stronger than that in monoculture plantations ($R^2 = 0.67 > 0.58$).

Discussion

Ecological and earth science studies over the past few decades have demonstrated that it is still controversial whether planting mixed-species plantations is an effective means of increasing the SOC stocks and contributing to the mitigation of global warming (McKinley et al. 2011; Yang et al. 2019). Therefore, we analyzed the effect of different afforestation modes on the SOC stocks on the Loess Plateau of China by integrating related studies, with a view to providing reference for

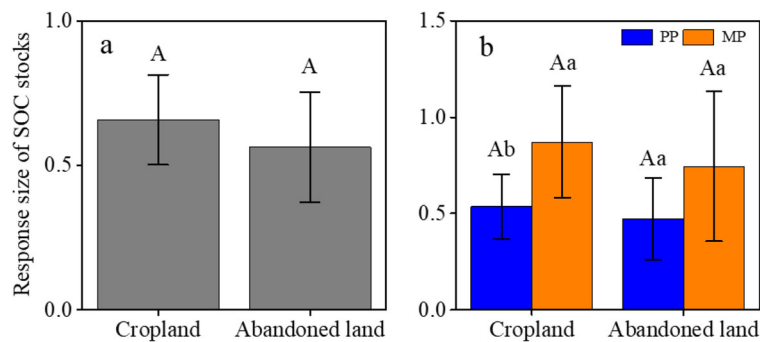


Fig. 5 The response size of SOC stocks in different prior land use/land cover, Note: PP: monoculture plantations; MP: mixed-species plantations. The values representing response size are the mean \pm 95% CI. Different upper-case letters indicate significant difference

among the different prior land use/land cover at 0.05 level ($P < 0.05$), and different lower-case letters indicate significant difference among the different afforestation modes at 0.05 level ($P < 0.05$)

the scientific management of future regional forest ecosystem.

Changes in the SOC stocks in different afforestation modes and species combinations.

By comparing the changes in the SOC stocks between monoculture plantations and mixed-species plantations, we found that the response size of SOC stocks of mixed-species plantations was significantly higher than that of monoculture plantations (Fig. 2). This result supports our hypothesis. Several possible mechanisms may underlie this result. First, aboveground leaf litter and belowground roots are the main source of organic matter input into the soil (Laganiere et al. 2010; Zhao et al. 2015). Mixed-species plantations with high species diversity and stand structure diversity can increase the amount and variety of litter (Hooper et al. 2012; Pretzsch and Schütze 2016), the root turnover, and the root exudates (Lei et al. 2012; Brassard et al. 2013), thereby resulting in higher accumulation of SOC stocks than monoculture plantations. Second, properties of microbes in soil may also alter SOC cycling and storage (Waring et al. 2013; Cheng et al. 2018). Mixed-species plantations usually have higher plant productivity and amounts of plant residue inputs than monoculture plantations (Pretzsch and Schütze 2016), which enhance the SOC stocks by increasing amounts of microbial biomass and necro-mass (Liang et al. 2011). Furthermore, fungal hyphae, microbes and plant roots can promote the development and stability of soil aggregates through the production of exudates, secondary metabolites and organic inputs, thus provided physical protection for SOC (Deng et al. 2018; Zhu et al. 2018). Some studies have found that the number of macroaggregates (0.25–2 mm) of soil in mixed-species plantations was higher than that in monoculture plantations (Shrestha et al. 2007; Kooch and Bayranvand 2017). Especially for the Loess Plateau, positive changes in macroaggregates could reduce

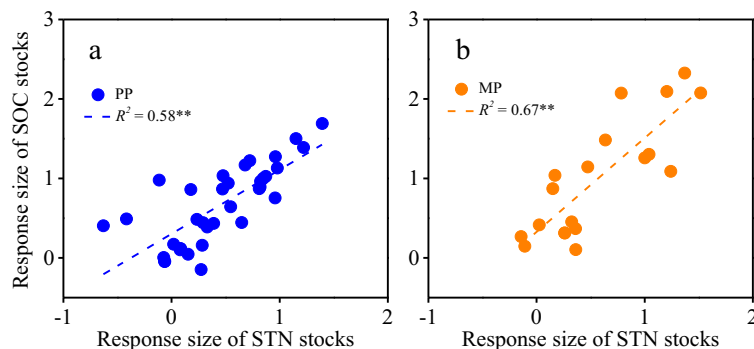
the loss of SOM caused by soil erosion, thereby promoting the accumulation of SOC (Gao et al. 2013; Wang et al. 2019).

Various combinations of different vegetation species have large differences in the SOC stocks. In this study, we found that the response size of SOC stocks was highest in the case of tree-shrub mixtures (Fig. 2), which is consistent with the results of previous studies (Zhang and Chen 2007; England et al. 2016). This phenomenon probably because of the differences in rotation periods and niche of trees and shrubs and thus a larger input of organic matter and the positive below-ground interaction (Kahmen et al. 2006; Gao et al. 2018). For the Loess Plateau, soil moisture often has constraint effects on the soil carbon sequestration (Lu et al. 2014; Wang et al. 2017). Gong et al. (2020) showed that mixed-species plantations, especially the tree-shrub mixtures are more able to maintain soil moisture than monoculture plantations, which will improve the growth of vegetation to a certain extent and increase the SOC stocks. In addition, this phenomenon may be due to the shrubs on the Loess Plateau are mostly nitrogen-fixing vegetation (e.g., *Hippophae rhamnoides*), the increase of STN content will increase the SOC stocks to some extent (Fig. 6) (Pereira et al. 2011; Luo et al. 2016).

Factors affecting the SOC stocks

Over time, different afforestation modes will have different effects on the SOC stock. Specifically, the SOC stocks will increase, remain the same, or decrease with planting ages (Smal and Olszewska 2008; Kurganova et al. 2015; Fataei and Varamesh 2016). Our findings showed that the response size of SOC stocks in monoculture and mixed-species plantations increased with planting ages on the Loess Plateau (Fig. 3a). Especially at young stage (<10 yr) and mature stage (>20 yr), the

Fig. 6 Effects of different afforestation modes on the carbon-nitrogen coupling relationship. Note: PP: monoculture plantations; MP: mixed-species plantations. STN: soil total nitrogen. The dotted line is the linear regression line. ** represents a significant linear regression at $P < 0.01$



response size of SOC stocks in mixed-species plantations was significantly higher than that in monoculture plantations (Fig. 3b). However, we found that at the young stage of afforestation (<10 yr), the response size of SOC stocks in monoculture plantations has a negative value (Fig. 3b), which may be attributed to the following factors. 1) In the early stage of afforestation, vegetation grows slowly, and the amount of litter is small, resulting in less carbon input into the soil, and the competition between sunlight, water and nutrients between individual plants further limits the ability of forest carbon sequestration (Barcena et al. 2014); 2) Disturbances in afforestation may damage the physical structure of the soil (e.g., soil aggregates, soil bulk density) and change the microclimate, thereby stimulating the decomposition of microorganisms and resulting in larger amount of soil carbon loss (La Scala et al. 2005; Mataix-Solera et al. 2011). 3) Serious soil erosion can cause great soil C losses in the Loess Plateau (Deng et al. 2019). Due to plantations have low vegetation cover (Feng et al., 2018) and sparse undergrowth vegetation (Chen et al., 2007) in the early stage, these conditions are difficult to effectively control soil erosion, which in turn cause the loss of SOC (Mongil-Manso et al. 2019). However, forest carbon sequestration is a slow process (Guo et al. 2018; Poorter et al. 2016). Therefore, it is necessary to further study the changes of SOC stocks as planting age.

Climate affects soil carbon accumulation by affecting the biological processes associated with both vegetation productivity and the rate of organic matter decomposition (Iglesias et al. 2012; Campo and Merino 2016). Specifically, precipitation is a critical variable that affects forest production, especially in arid and semiarid regions (Callesen et al. 2003; Luyssaert et al. 2007). Increased rainfall can significantly increase the biological productivity and thus promote SOC accumulation (Wei et al. 2009; Berthrong et al. 2012). Our data also supported this relationship (Fig. 4b). In addition, the large number of experiments showed that the climatic conditions in high temperature regions would stimulate the activity of soil microbes, and then accelerate the decomposition of SOC (Campo and Merino 2016; Canarini et al. 2016). However, we indicated that the response size of SOC stocks significantly increased with MAT (Fig. 4a), probably due to the Loess Plateau being characterized by a low MAT, which

could slow down the rate of litter decomposition, and was beneficial to the accumulation of SOC (Liu et al. 2011). We also found that the response size of SOC stocks of mixed-species plantations was significantly higher than that of monoculture plantations when the MAT <8 °C and MAP <500 mm (Fig. 4c, d), which may be due to the stand structural heterogeneity of mixed-species plantations (Zeller et al. 2017). The structure could improve the microclimate and light transmittance of mixed-species plantations (Cavanaugh et al. 2011; Edwards et al. 2014). In addition, our research found that when MAT >8 °C and MAP >500 mm, there was no significant difference in the response size of SOC stocks between mixed-species plantations and monoculture plantations (Fig. 4c, d). This further illustrated that mixed-species plantations may increase more the SOC stocks than monoculture plantations in climate-limited areas (MAT <8 °C, MAP <500 mm).

Prior land use/land cover has also a great impact on the SOC stocks. We found that the response size of SOC stocks of afforestation on cropland was higher than that on abandoned land (Fig. 5a), this probably due to tillage in croplands resulting in the breakup of aggregates, thereby facilitating the decomposition of SOM (Balesdent et al. 2000). In contrast, because of its high vegetation coverage and abundant root system, abandoned land also increases the amount of herbaceous litter while controlling soil erosion, resulting in higher SOC reserves (Kuzyakov and Domanski 2000). However, the accumulation of SOC in abandoned land often depends on how long the land had been abandoned. Due to past cultivation practices and current plant community (mostly annual species) following abandonment, the SOC stocks of abandoned land will be significantly higher than that of cropland needs at least 10 years grass succession in the Loess Plateau of China (He et al. 2016). In general, the equilibrium state of a cropland system usually results in a lower SOC value, and afforestation on cropland are likely to increase more the response size of SOC stocks than that on abandoned land. Moreover, planting mixed-species plantations on cropland could significantly increase the response size of SOC stocks by 34% than monoculture plantations (Fig. 5b). In addition to these above factors, it may be related

to the fact that mixed-species plantations can increase the source of SOC and reduce the decomposition of SOC.

This meta-analysis found that the SOC stocks are strongly coupled to STN stocks (Fig. 6), which is consistent with previous studies (Nwaogu et al. 2018; Shi et al. 2019). In this study, the soil carbon-nitrogen coupling relationship mixed-species plantations is better than that of monoculture plantations (Fig. 6a, b). This may be related to the species composition of mixed-species plantations. According to our dataset (Appendix S1), 67% of the mixed-species plantations on the Loess Plateau contain at least one nitrogen-fixing species (e.g., *Robinia pseudoacacia* - *Hippophae rhamnoides*, *Robinia pseudoacacia* - *Platycladus orientalis*). In general, the presence of nitrogen-fixing species could improve soil nitrogen pools and benefit adjacent non-nitrogen-fixing species (Rice et al. 2004; Tateno et al. 2007). Previous studies have shown that nitrogen-fixing vegetation may increase the root N concentrations and soil NO₃⁻, thereby improving the soil N and SOC pools (Forrester et al. 2005; Hu et al. 2017). In particular, the plantations in the Loess Plateau are mostly nitrogen-fixing species (such as *Robinia pseudoacacia*, *Hippophae rhamnoides*). Since these species usually have rich root nodules, they will have significant positive effects on community structure, soil physical and nutrient conditions in mixed-species plantations (Zhang and Chen 2007; Du et al. 2019). Additionally, nitrogen-fixing vegetation can provide positive feedback to plant communities by controlling the growth of mycorrhizal fungi. For example, Chen et al. (2018) showed that *Robinia pseudoacacia* in the Loess Plateau can improve the availability of soil moisture, nitrogen, and potassium by suppressing the development of arbuscular mycorrhizal (AM) symbiosis in *Platycladus orientalis* roots, thereby promoting the growth of *Platycladus orientalis*. Therefore, compared with monoculture plantations, mixed-species plantations containing nitrogen-fixing species are a better choice for accumulating the SOC stocks and the STN stocks in the Loess Plateau of China.

Implications for forest management

The carbon sink of forests does not come without a cost, and it may cause the changes of other ecological processes (Cao et al. 2011). Most notably, forests need to use more water while absorbing carbon dioxide (Liu and

Yang 2012). Especially for arid and semiarid regions, water is one of the key factors needed for the sustainable management of forest ecosystems (Cao et al. 2009). The ecological functions of vegetation can only be realized under the premise of ensuring normal vegetation growth. Therefore, afforestation is an inappropriate choice for areas where the MAP is close to or less than the potential evapotranspiration (Jian et al. 2015; Deng et al. 2016). Previous studies have shown that reducing tree density and increasing shrub planting can reduce soil water consumption and improve vegetation survival (Guo and Shao 2013; She et al. 2014). However, these modified approaches may still be too aggressive for areas where the natural plant is predominantly grassland or other non-woody plants (Koulouri and Giourga 2007; Cao et al. 2011). Compared with monoculture plantations, mixed-species plantations not only increase the SOC stocks, but also have advantages in increasing the productivity of plants (Pretzsch and Schütze 2016), maintaining soil moisture content (Gong et al. 2020), and supporting aesthetic and recreational values (Felton et al. 2016). Therefore, based on our findings, we suggest that before any afforestation plan is initiated, various factors such as regional climate, planting age and prior land use/land cover, should be taken into account, and afforestation should be transformed from a simple species-based measure to the best combination of plant species, especially tree-shrub mixtures that containing nitrogen-fixing plants (Zhang and Chen 2007; Hu et al. 2017).

This study showed that the response size of SOC stocks of mixed-species plantations was 28% higher than that of monoculture plantations. However, due to regional restrictions and the SOC stocks were affected by many factors, whether mixed-species plantations were more conducive to increase the SOC stocks remains controversial. In addition, although our research can provide references for related research around the world, still requires additional supplemental evidence.

Limitations of the study and future research directions

This study used a synthesis of 92 observations from 21 studies to describe differences in the SOC stocks between mixed-species plantations and monoculture plantations across the Loess Plateau of China. Although we can generate testable hypotheses based on new research and draw important conclusions, they also have

limitations. In general, meta-analysis does not permit adequate direct testing of hypotheses regarding mechanisms. Moreover, variation in describing methods and the level of detail in results among the selected studies, which may bias our results. In particular, because many studies did not long-term observations, thereby increasing the uncertainty of the study. In addition, for better comparison, we performed depth correction of the dataset, although previous studies have shown that depth correction does not change the overall pattern of soil carbon pool dynamics during vegetation development (Yang et al. 2011; Li et al. 2012). However, potential uncertainties may be introduced due to differences in the carbon distribution in soil profiles at different growth stages of vegetation. In addition, due to other soil parameters data (such as soil pH, total P, and soil water) could not be found in some collected papers, so we could not conclude the effects of other soil parameters on the SOC stocks. Therefore, future research should report the effect of corresponding soil characteristics on the SOC stocks, and at the same time, increase the understanding of the relationship between the surface and deep SOC stocks.

Conclusions

The response size of SOC stocks of mixed-species plantations was 28% higher than that of monoculture plantations on the Loess Plateau. In addition, species combinations, climate, planting age and prior land use/land cover were important factors affecting the SOC stocks. In particular, compared with monoculture plantations, mixed-species plantations had a greater buffering potential on the SOC stocks in response to climate-limited regions (MAT <8 °C, MAP <500 mm). Moreover, the positive relationship between STN stocks and SOC stocks of mixed-species plantations was stronger than that of monoculture plantations. Therefore, in the future, to improve the capacity of soil carbon sequestration, it would be better to choose mixed-species plantation containing nitrogen-fixing plants.

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Author contributions C.G. and Q.T conceived the study. C.G., M.X. and G.L. designed the study. C.G. and M.X. analyzed the data, all authors wrote and edited the manuscript.

Compliance with ethical standards

Conflict of interests The authors declare no competing interests.

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