Article

Soil Carbon, Nitrogen, and Phosphorus Storages and Their Stoichiometry Due to Mixed Afforestation with *Hippophae rhamnoides* in the Loess Hilly Region, China

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Abstract: Mixed-species tree plantations have additional ecological benefits over single-species tree plantations, such as habitat restoration and increasing biodiversity. However, changes in the soil carbon, nitrogen, and phosphorus storages and stoichiometry after mixed afforestation with the N-fixing tree species under the “Grain for Green Project” in the Loess Plateau of China are not well understood. Typical restoration types, including the mixed plantations of *Pinus tabulaeformis* with *Hippophae rhamnoides* (H_tP_t) and *Robinia pseudoacacia* with *H. rhamnoides* (H_tR_p), as well as the pure forests of *P. tabulaeformis* (P_t) and *R. pseudoacacia* (R_p), were chosen to examine changes in the storages and stoichiometry of soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) in 0–100 cm soil layers. The results showed that compared with the corresponding pure forest, H_tR_p significantly increased the SOC content in the 0–20 cm soil layer and the SOC storage in the 0–100 cm layer, while H_tP_t significantly increased the SOC content in the 0–10 cm layer, but there was no significant difference for SOC storage in the 0–100 cm layer between P_t and H_tP_t . Similarly, H_tR_p significantly increased the TN content in the 0–10 cm layer and the TN storage in the 0–100 cm layer, but there was no significant difference in TN storage between P_t and H_tP_t . Furthermore, H_tR_p significantly increased the TP content in the 0–100 cm layer and TP storage was higher than that of R_p , while there were no significant differences in TP content and storage between P_t and H_tP_t . In the 0–10 cm soil layer, H_tR_p significantly reduced C:N and increased N:P, but H_tP_t significantly increased C:P. In addition, compared with the pure forest, the soil physical and chemical properties had a stronger control effect on the soil storages and stoichiometric ratios in the mixed forests. In summary, compared with *P. tabulaeformis*, the introduction of N-fixing tree species into the *R. pseudoacacia* forest was more conducive to the accumulation of SOC, TN, and TP reserves and the improvement of the N and P utilization efficiency. These results have important implications for the restoration of degraded vegetation and scientific management of mixed plantations on the Loess Plateau and can provide basic data for the assessment of soil quality at the regional scale.

Keywords: mixed afforestation; pure plantation; carbon storage; nitrogen storage; phosphorus storage; stoichiometry; N-fixing tree species

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1. Introduction

Nitrogen (N) and phosphorus (P) are important nutrient elements for tree growth, where these nutritive element storages in soil may represent the crucial ecological factors that affect the primary productivity of a forest ecosystem [1]. Meanwhile, as the largest carbon (C) pool in the terrestrial biosphere, any minor change in soil C content and storage will have a significant impact on global warming [2]. Afforestation was acknowledged as

an atmospheric CO₂ sequestration measure through the storage of C in soil and improving the nutrient status [3,4]. However, large-scale and high-density planting of pure forests, which may easily cause problems, such as soil fertility decline and slow nutrient turnover, eventually affecting the accumulation of soil C, N, and P, and even the ecosystem functions [5]. Ecological stoichiometry addresses the equilibrium or interactions of the main elements, as well as the correlations between elements and ecosystem functioning, which can help to determine the responses of soil quality to afforestation [6].

Mixed-species tree planting has received intensive interest and is increasingly established throughout the world due to its potential influence on soil quality improvement [7]. Conversely, changes in soil nutrient storage and stoichiometry will affect ecosystem functions and processes. A clear evaluation of soil nutrients in ecological restoration areas is essential for understanding the ecological consequences of mixed afforestation and may have meaningful implications for long-term nutrient availability and the sustainable management of plantations [8].

Different tree species have different effects on soil stoichiometric characteristics in a mixed forest [9]. The soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) contents of a *Eucalyptus grandis* plantation could be changed significantly by the introduction of *Acacia mearnsii* [10]. Mixing in a *Prunus davidiana* forest had a significant effect on soil SOC, TN, C:P, and N:P in the south Taihang Mountains, but had a minor effect on TP [11]. The mixing of *Fraxinus chinensis* and *Robinia pseudoacacia* led to a decrease in soil SOC [12], while the mixing of *Ailanthus altissima* and *R. pseudoacacia* significantly increased the soil SOC content [13]. It can be seen that a reasonable mixing mode can effectively improve the soil nutrient status. However, the current research on the effect of mixed afforestation mainly focuses on the changes in nutrient content [8]. In terms of soil nutrient availability, nutrient cycling, and balance mechanisms, understanding of the impacts on SOC, TN, and TP storages and their ecological stoichiometric characteristics also need to be strengthened.

Indigenous Chinese pine (*Pinus tabulaeformis*) and N-fixing black locust (*R. pseudoacacia*) are the dominant tree species for afforestation in the Loess Plateau, which play an important role in regional soil erosion control and landscape maintenance. However, due to the lack of scientific guidance in the initial stage of ecological restoration, large areas of pure artificial forests have been built, resulting in poor growth and low carbon sink function [14]. After long-term afforestation practices, *Hippophae rhamnoides* (N-fixing tree species) are often included in these pure plantations to improve the stability of an ecosystem through a process of facilitation [15]. The introduction of N-fixing tree species may help to increase the soil SOC and TN contents [16], while the mixing of dual N-fixing tree species was found to be beneficial to the increase of soil TP content [17]. Therefore, it is speculated that the introduction of *H. rhamnoides* has different effects on these two typical forests, but the specific response characteristics are unclear. Furthermore, little is currently known about whether soil under dual N-fixing trees contained more nutrient storage than the N-fixing and non-N-fixing trees in the mixed planting [18].

In this study, we investigated the response of SOC, TN, and TP storages and stoichiometry in *P. tabulaeformis* and *R. pseudoacacia* to mixed afforestation with N-fixing tree species by measuring SOC, TN, and TP content in 0–100 cm soil profiles. Our aims were to (1) analyze the differences in SOC, TN, and TP storages and their stoichiometric ratios between pure and mixed forests, and (2) determine the main influencing factors of storage and stoichiometry to identify the differential response. This study contributes to the ecological restoration of degraded vegetation and the sustainable management of mixed forests on the Loess Plateau.

2. Materials and Methods

2.1. Site Description

The study was conducted at the Ansai National Ecological Experimental Station (36°51' N, 109°19' E), which is located in Shaanxi province, China. The region belongs to a

typical temperate semi-arid climate with a mean annual temperature of 10.4 °C. The mean annual rainfall is approximately 500 mm, where about 60% falls from July to September. The main soil type is Huangmian soil, which was developed from wind-deposited loessial parent material. The dominant woody plants are *P. tabulaeformis*, *Platycladus orientalis*, *R. pseudoacacia*, *H. rhamnoides*, and *Caragana korshinskii*.

2.2. Experimental Material

Based on the long-term fixed monitoring forest land in the field of the mountain test site, the pure plantations of *P. tabulaeformis* (P_t) and *R. pseudoacacia* (R_p) and the mixed plantations of *P. tabulaeformis* with *H. rhamnoides* (H_rP_t) and *R. pseudoacacia* with *H. rhamnoides* (H_rR_p) were selected as the research objects (Figure 1). For each forest type, three 20 m × 20 m study plots were selected as the sample. The original plantation density was widely used in the central part of the Loess Plateau: stem densities of P_t and R_p in pure plantations were 1667 and 1000 stems ha⁻¹, respectively. The stem densities in the corresponding mixed plantation were 1111 and 833 stems ha⁻¹. In addition, the initial afforestation density in the pure *H. rhamnoides* plantation was 3300 stems ha⁻¹, and the stem density in the corresponding mixed plantations was 1900 stems ha⁻¹.

Figure 1. Four typical restoration types: (a) the pure forest of *Pinus tabulaeformis* (P_t), (b) the mixed forest of *P. tabulaeformis* and *Hippophae rhamnoides* (H_rP_t), (c) the pure forest of *Robinia pseudoacacia* (R_p), and (d) the mixed forest of *R. pseudoacacia* and *H. rhamnoides* (H_rR_p).

According to a survey conducted in 2017, a similar stem density was observed among three replicate plots in the same plantation type. The mean densities of *P. tabulaeformis* and *R. pseudoacacia* in pure plantations were 1175.8 and 897.3 stems ha⁻¹, respectively. The stem densities of H_rP_t were 850 and 1775 stems ha⁻¹, respectively, and for H_rR_p , the stem densities were 800 and 1617 stems ha⁻¹, respectively. The basic characteristics of the sample plots are shown in Table 1.

Table 1. The general information of the study plots.

Sample Plot	Tree Species	Altitude (m)	Stand Age (a)	Tree Height (m)	DBH/DGH (cm)	Crown Diameter of (EW × SN) (m × m)
P_t	<i>P. tabuliformis</i>	1189	17	3.69 ± 0.09	7.43 ± 0.27	(2.96 ± 0.12) × (3.36 ± 0.11)
R_p	<i>R. pseudoacacia</i>	1287	17	7.59 ± 0.65	9.40 ± 1.50	(4.36 ± 0.39) × (4.24 ± 0.32)
H_tP_t	<i>P. tabuliformis</i>	1270	17	4.01 ± 0.17	7.11 ± 0.48	(2.01 ± 0.09) × (2.04 ± 0.11)
	<i>H. rhamnoides</i>			3.08 ± 0.14	5.43 ± 0.33	(2.62 ± 0.12) × (2.60 ± 0.14)
H_tR_p	<i>R. pseudoacacia</i>	1302	17	5.92 ± 0.52	7.80 ± 1.02	(1.79 ± 0.10) × (1.80 ± 0.09)
	<i>H. rhamnoides</i>			2.69 ± 0.10	4.63 ± 0.32	(3.36 ± 0.35) × (3.25 ± 0.34)

Note: P_t and R_p represent the pure plantations of *P. tabuliformis* and *R. pseudoacacia*, respectively. H_tP_t and H_tR_p represent the mixed plantations of *P. tabuliformis* with *H. rhamnoides* and *R. pseudoacacia* with *H. rhamnoides*, respectively. DBH and DGH represent the diameters at the breast and ground heights, respectively. The abbreviations mean the same below.

2.3. Soil Sampling and Laboratory Analyses

With the aim to examine changes in the storages and stoichiometry of SOC, TN, and TP in *P. tabuliformis* and *R. pseudoacacia* after mixed afforestation with *H. rhamnoides*, field soil sampling was conducted in August 2017. A total of 36 soil profiles were collected from 12 plots (three soil profiles per plot along the diagonal). Soil samples were taken from each profile at depth ranges of 0–10, 10–20, 20–50, and 50–100 cm, which were brought back to the laboratory and divided into two parts. One part was naturally air-dried; the plant residues and rocks were removed; and the soil was sieved before measuring the SOC, TN, TP contents and available P. The other part was stored in a refrigerator at 4 °C to be analyzed for other indicators.

The soil bulk density (BD), total porosity, and field capacity of each depth were measured using the cutting ring method. Soil water content (SWC) was obtained using oven-dried at 105 °C for 48 h until a constant weight was achieved. SOC was determined using the wet oxidation method, TN was measured using the Kjeldahl method, and TP and the available P were measured using the $\text{HClO}_4\text{-H}_2\text{SO}_4$ colorimetric and $\text{NaHCO}_3\text{-H}_2\text{SO}_4$ colorimetric methods, respectively. Nitrate N and ammonium N were determined using continuous flow injection analysis [19]. The basic soil physicochemical properties of the test soil are shown in Table 2.

Table 2. Physicochemical properties in the soil profile under pure and mixed forests.

Soil Depth (cm)	Sample Plot	SWC ($\text{m}^3 \text{m}^{-3}$)	Field Capacity (%)	BD (g cm^{-3})	Soil Total Porosity (%)	Nitrate N (g kg^{-1})	Ammonium N (g kg^{-1})	Available P (g kg^{-1})
0–10	P_t	0.14 ± 0.002	17.94 ± 0.24	1.23 ± 0.01	51.49 ± 0.59	0.21 ± 0.01 b	1.86 ± 0.11	0.31 ± 0.02 b
	H_tP_t	0.13 ± 0.004	17.14 ± 0.20	1.22 ± 0.03	51.86 ± 0.60	0.39 ± 0.02 a	1.49 ± 0.09	0.41 ± 0.02 a
	R_p	0.11 ± 0.001	13.72 ± 0.16 b	1.35 ± 0.02	46.94 ± 0.54 b	0.86 ± 0.05	1.13 ± 0.06 b	0.31 ± 0.02 b
	H_tR_p	0.11 ± 0.003	15.47 ± 0.18 a	1.29 ± 0.01	50.49 ± 0.58 a	0.85 ± 0.05	1.93 ± 0.11 a	0.50 ± 0.02 a
10–20	P_t	0.12 ± 0.002	15.51 ± 0.31 b	1.34 ± 0.02 a	47.11 ± 0.82 b	0.26 ± 0.04	1.86 ± 0.32	0.36 ± 0.06
	H_tP_t	0.13 ± 0.005	18.21 ± 0.32 a	1.17 ± 0.03 b	51.77 ± 0.90 a	0.33 ± 0.06	1.41 ± 0.24	0.37 ± 0.06
	R_p	0.09 ± 0.002	15.72 ± 0.27 b	1.25 ± 0.02 a	50.99 ± 0.88 b	0.56 ± 0.10	1.14 ± 0.20	0.19 ± 0.03 b
	H_tR_p	0.12 ± 0.005	19.01 ± 0.33 a	1.11 ± 0.03 b	55.41 ± 0.96 a	0.46 ± 0.08	1.42 ± 0.25	0.37 ± 0.06 a
20–50	P_t	0.13 ± 0.002	15.45 ± 0.22	1.35 ± 0.02	47.66 ± 0.69	0.15 ± 0.02	1.73 ± 0.20	0.30 ± 0.04
	H_tP_t	0.11 ± 0.004	16.56 ± 0.24	1.28 ± 0.03	50.27 ± 0.73	0.15 ± 0.02	1.27 ± 0.15	0.37 ± 0.04
	R_p	0.09 ± 0.001	14.39 ± 0.21	1.35 ± 0.02	47.38 ± 0.68	0.45 ± 0.05	1.34 ± 0.16	0.18 ± 0.02 b
	H_tR_p	0.11 ± 0.006	15.93 ± 0.23	1.31 ± 0.03	47.83 ± 0.73	0.37 ± 0.04	1.32 ± 0.15	0.33 ± 0.04 a
50–100	P_t	0.11 ± 0.003	15.51 ± 0.31	1.37 ± 0.03	48.38 ± 0.98	0.12 ± 0.03	1.72 ± 0.40	0.35 ± 0.08
	H_tP_t	0.08 ± 0.005	16.08 ± 0.37	1.29 ± 0.04	50.91 ± 1.07	0.17 ± 0.04	1.36 ± 0.32	0.36 ± 0.08
	R_p	0.10 ± 0.002	16.06 ± 0.32	1.39 ± 0.03	45.39 ± 0.92	0.33 ± 0.07	1.16 ± 0.23	0.21 ± 0.05
	H_tR_p	0.08 ± 0.009	16.29 ± 0.29	1.38 ± 0.04	46.79 ± 1.01	0.27 ± 0.06	1.28 ± 0.33	0.29 ± 0.07

Note: Different lowercase letters indicate that there are significant differences between pure and mixed forests in the same soil layer at 0.05 levels. These lowercase letters mean the same below.

2.4. Data Processing

The SOC, TN, and TP storages of each soil layer were calculated as follows [20]:

$$\text{SOC}_{i\text{Storage}} = \text{SOC}_i \times \text{BD}_i \times D_i / 10 \quad (1)$$

$$\text{TN}_{i\text{Storage}} = \text{TN}_i \times \text{BD}_i \times D_i / 10 \quad (2)$$

$$\text{TP}_{i\text{Storage}} = \text{TP}_i \times \text{BD}_i \times D_i / 10 \quad (3)$$

where SOC_i , TN_i , and TP_i are the SOC, TN, and TP contents of the i th layer of soil (g kg^{-1}), respectively. BD_i is the soil bulk density of the i th layer (g cm^{-3}) and D_i is the soil depth of the i th layer (cm). The soil storages of the 0–100 cm layer were equal to the sum of the four layers.

One-way analysis of variance (ANOVA) was used to compare significant differences in the SOC, TN, and TP contents, storages, and stoichiometry between the pure and mixed forests. Two-way analysis of variance was used to determine the impact of tree species, the mixing, and their interaction on SOC, TN, TP content and storages and the C:N, C:P, and N:P ratios. Stepwise regression was employed to study the relationships between the SOC, TN, and TP storages and stoichiometric ratios and their influencing factors, and a P-P plot was used to test the normality of the regression residuals (Figure S1). All statistical analyses were performed with SPSS 19.0. Figures were plotted using SigmaPlot 12.5.

3. Results

3.1. Soil Physical and Chemical Properties and SOC, TN, and TP Contents

There were different significance variations in the physical and chemical indexes among pure and mixed forests (Table 2). SWC ranged from 0.08 to 0.14 $\text{m}^3 \text{m}^{-3}$, and there was no significant difference in the same soil layer between pure and mixed forests. BD ranged from 1.11 to 1.39 g cm^{-3} . BD in the mixed forest was lower than that of the pure forest, and the significant differences were concentrated in the 10–20 cm layer. The field capacity and soil total porosity ranged from 13.72 to 19.01% and 45.39 to 55.41%, respectively. These two indexes of the mixed forest were higher than that of the pure forest, and significant differences were observed in the 0–10 and 10–20 cm layers. The content (g kg^{-1}) of soil nutrient elements in pure and mixed forests ranged from 0.12 to 0.86 for nitrate N, 1.14 to 1.98 for ammonium N, and 0.16 to 0.50 for available P. Compared with P_t , H_tP_t significantly increased the nitrate N and available P contents in the 0–10 cm layer, with increases of 83.06 and 31.01%. Compared with R_p , H_tR_p significantly increased the ammonium N content of the 0–10 cm layer and available P of the 0–10, 10–20, and 20–50 cm layers, with increases of 71.70, 58.62, 93.75, and 86.75%, respectively.

The contents (g kg^{-1}) of soil elements in the pure and mixed plantations ranged from 3.87 to 13.92 for SOC, 0.18 to 0.79 for TN, and 0.46 to 0.58 for TP. Compared with P_t , H_tP_t significantly increased the SOC and TN contents of the 0–10 cm soil layer, with increases of 35.84 and 27.50%, and in the same soil layer, there was no significant difference in TP between P_t and H_tP_t . By contrast, compared with R_p , H_tR_p significantly increased the SOC content of the 0–10, 10–20 cm soil layers, with increases of 17.07 and 27.16%, as well as the TN content of the 0–10 cm soil layer, with an increase of 68.09%. Furthermore, the TP content in the 0–10, 10–20, 20–50, and 50–100 cm layers of H_tR_p was increased by 20.83, 14.58%, 17.39, and 6.12%, respectively ($p < 0.05$) (Figure 2). The mixing and tree species had significant effects on SOC in the 0–10 cm layer, and the 10–20 cm layer was mainly affected by their interaction ($p < 0.05$). TN in the 0–10 cm layer was influenced by the mixing, tree species, and their interaction ($p < 0.01$). Except for the mixing, which had a significant influence on TP in the 50–100 cm layer ($p < 0.05$), other soil layers were mainly affected by the mixing and their interaction ($p < 0.01$) (Table 3).

Figure 2. Soil organic carbon, total nitrogen, and total phosphorus contents in the 0–100 cm layers of the pure and mixed forests. Different lowercase letters indicate that there are significant differences between pure and mixed forests in the same soil layer at 0.05 levels.

Table 3. Variance analysis of the mixed effect and tree species type on the SOC, TN, and TP concentrations in the soil profile.

Soil Depth (cm)	SOC (g kg ⁻¹)			TN (g kg ⁻¹)			TP (g kg ⁻¹)		
	ME	TS	ME × TS	ME	TS	ME × TS	ME	TS	ME × TS
0–10	5.13 *	19.52 ***	3.22	57.60 ***	40.19 ***	13.84 ***	35.74 ***	1.41	27.67 ***
10–20	0.56	2.82	8.75 **	0.29	1.98	3.50	9.12 **	0.75	27.68 ***
20–50	0.67	3.42	2.64	1.40	3.04	3.73	13.38 ***	3.89	23.34 ***
50–100	0.99	2.75	3.80	2.17	1.99	3.12	5.10 *	1.45	3.61

Note: ME represents the mixing effect, TS represents the tree species, and ME × TS represents the interaction between the mixing effect and tree species, respectively. * Indicates the significance level of $p < 0.05$, ** indicates the significance level of $p < 0.01$, and *** indicates the significance level of $p < 0.001$. The abbreviations and significance levels mean the same below.

3.2. SOC, TN, and TP Storages

The mean storage (t hm⁻²) of 10 cm thickness in soil layers of pure and mixed plantations ranged from 5.01 to 18.03 for SOC, 0.24 to 1.02 for TN, and 0.60 to 0.73 for TP. Compared with P_t , $H_t P_t$ significantly increased the SOC and TN storages of the 0–10 cm soil layer, with increases of 46.14 and 37.78%, respectively, but in the other soil layers, there was a decreasing trend, and a significant difference in SOC storage in the 10–20 cm soil layer was observed. In contrast, compared with R_p , $H_t R_p$ significantly increased the TN

storage of the 0–10 cm soil layer, with an increase of 59.38%, but significantly decreased the SOC and TN storages of the 50–100 cm soil layer ($p < 0.05$). Moreover, there was no significant difference due to mixed afforestation on TP storage in each soil layer (Figure 3). SOC storage in the 0–10 cm layer was affected by the mixing and tree species ($p < 0.01$); in the 20–50 cm layer, SOC was mainly influenced by their interaction ($p < 0.01$); and in the 50–100 cm layer, SOC was affected by the mixing, tree species, and their interaction ($p < 0.05$). The mixing, tree species, and their interaction had significant effects on the TN storage in the 0–10 ($p < 0.05$) and 50–100 cm layers ($p < 0.01$) (Table 4).

Figure 3. The storage of soil organic, total nitrogen and total phosphorus in the 0–100 cm layers of the pure and mixed forests. Different lowercase letters indicate that there are significant differences between pure and mixed forests in the same soil layer at 0.05 levels.

Table 4. Variance analysis of the mixing effect and tree species type on the storages of SOC, TN, and TP at varying depths in the soil profile.

Soil Depth (cm)	SOC Storage (t hm ⁻²)			TN Storage (t hm ⁻²)			TP Storage (t hm ⁻²)		
	ME	TS	ME × TS	ME	TS	ME × TS	ME	TS	ME × TS
0–10	14.62 **	30.41 **	2.28	45.26 ***	50.27 ***	7.06 *	4.54	2.37	0.51
10–20	2.06	0.44	12.70 **	3.35	2.04	0.88	2.04	2.04	2.83
20–50	2.92	4.59	4.82	1.91	1.20	2.92	0.18	0.26	3.16
50–100	13.95 **	37.10 ***	6.53 *	23.73 **	56.27 ***	21.75 **	0.02	0.18	0.96

Note: * Indicates the significance level of $p < 0.05$, ** indicates the significance level of $p < 0.01$, and *** indicates the significance level of $p < 0.001$.

To further elucidate the impact of the mixed afforestation on the soil storages, data sets of SOC, TN, and TP storages in the 0–100 cm layers were analyzed. The results indicated that the SOC and TN storages in the 0–100 cm layers in H_tR_p were significantly higher than that of R_p ($p < 0.05$); there were no significant differences between P_t and H_tP_t ; and the SOC, TN, and TP storages in 0–100 cm layers of *R. pseudoacacia* were greater than that of *P. tabulaeformis* (Table 5).

Table 5. SOC, TN, and TP storages of the 0–100 cm layers in pure and mixed forests.

Sample Plot	SOC Storage ($t\text{ hm}^{-2}$)	TN Storage ($t\text{ hm}^{-2}$)	TP Storage ($t\text{ hm}^{-2}$)
P_t	86.46 ± 5.00	3.92 ± 0.23	6.87 ± 0.40
H_tP_t	85.19 ± 4.92	3.88 ± 0.22	6.50 ± 0.38
R_p	$104.47 \pm 6.03\text{ b}$	$5.39 \pm 0.31\text{ b}$	6.83 ± 0.37
H_tR_p	$108.34 \pm 6.26\text{ a}$	$5.86 \pm 0.34\text{ a}$	7.01 ± 0.41
ME	0.05	0.59	0.12
TS	13.60 **	37.84 ***	0.00
ME $\times$ TS	6.21 *	0.83	1.69

Note: Different lowercase letters indicate that there are significant differences between pure and mixed forests in the same soil layer at 0.05 levels. * Indicates the significance level of $p < 0.05$, ** indicates the significance level of $p < 0.01$, and *** indicates the significance level of $p < 0.001$.

3.3. SOC, TN, and TP Stoichiometry

The ratios of the soil stoichiometry in the pure and mixed plantations ranged from 15.64 to 25.05 for C:N, 7.54 to 24.82 for C:P, and 0.35 to 1.36 for N:P. In the same soil layer, there was no significant difference in soil C:N between P_t and H_tP_t . Compared with P_t , H_tP_t significantly increased the C:P and N:P of the 0–10 cm soil layer, with increases of 31.58 and 25.15%. By contrast, compared with R_p , H_tR_p significantly decreased C:N of the 0–10 cm soil layer, with a decrease of 29.42%, but significantly increased N:P of the 0–10 cm soil layer, with an increase of 40.07%. In addition, in the same soil layer, there was no significant difference in soil C:P between R_p and H_tR_p (Figure 4). In the 0–10 cm layer, the mixing and their interaction had significant effects on C:N ($p < 0.01$), C:P was influenced by the tree species and their interaction ($p < 0.05$), and the mixing and tree species had significant effects on N:P ($p < 0.001$) (Table 6).

Table 6. Variance analysis of the mixing effect and tree species type on the stoichiometric ratios in the soil profile.

Soil Depth (cm)	C:N			C:P			N:P		
	ME	TS	ME $\times$ TS	ME	TS	ME $\times$ TS	ME	TS	ME $\times$ TS
0–10	8.76 **	0.27	16.59 ***	3.27	18.64 ***	6.68 *	28.43 ***	35.95 ***	3.45
10–20	0.20	1.86	2.11	0.00	1.88	2.16	2.96	1.03	0.74
20–50	0.09	1.53	0.01	2.36	2.84	0.14	1.91	2.06	0.01
50–100	0.07	0.01	0.31	2.30	1.11	3.89	3.35	3.62	3.85

Note: * Indicates the significance level of $p < 0.05$, ** indicates the significance level of $p < 0.01$, and *** indicates the significance level of $p < 0.001$.

Figure 4. Soil carbon, nitrogen, and phosphorus stoichiometric ratios in the soil profile of pure and mixed forests. Different lowercase letters indicate that there are significant differences between pure and mixed forests in the same soil layer at 0.05 levels.

3.4. Correlations between the Soil Storages, Stoichiometry, and Physical and Chemical Properties

For the pure plantation, the SOC storage was positively correlated with the SOC, TN, and nitrate N contents ($p < 0.05$), but significantly negatively correlated with BD ($p < 0.01$). The TN storage was positively correlated with the SOC, TN, and nitrate N contents ($p < 0.01$), but significantly negatively correlated with BD, SWC, ammonium N, and available P ($p < 0.05$). The TP storage was positively correlated with total porosity, field capacity, and the TP content ($p < 0.05$). Soil C:N was negatively correlated with BD ($p < 0.05$), but significantly positively correlated with total porosity ($p < 0.01$). Soil C:P and N:P were negatively correlated with SWC, ammonium N, and available P ($p < 0.05$), but significantly positively correlated with the SOC, TN, and nitrate N contents ($p < 0.01$) (Table 7). For the mixed plantation, the SOC and TN storages were positively correlated with the SOC, TN, TP, nitrate N, and ammonium N contents ($p < 0.05$). The TP storage was positively correlated with the TP, ammonium N, and available P contents ($p < 0.05$), but significantly negatively correlated with BD ($p < 0.01$). Soil C:N was negatively correlated with the SWC and TN and nitrate N contents ($p < 0.05$), but significantly positively correlated with total porosity and field capacity ($p < 0.05$). Soil C:P and N:P were negatively correlated with the TP content ($p < 0.05$), but significantly positively correlated with the SOC, TN, nitrate N, and ammonium N contents ($p < 0.05$) (Table 7).

Table 7. Correlation between the different soil physicochemical properties; SOC, TN, and TP storages; and stoichiometric ratios in the study area.

Forest Type	Soil Property	BD	SWC	Total Porosity	Field Capacity	SOC	TN	TP	Nitrate N	Ammonium N	Available P
Pure forest	Cs	−0.937 **				0.999 **	0.939 **		0.816 *		
	Ns	−0.831 *	−0.804 *			0.981 **	0.993 **		0.928 **	−0.757 *	−0.746 *
	Ps			0.887 **	0.846 *			0.867 *			
	C:N	−0.791 *		0.979 **							
	C:P		−0.850 *			0.963 **	0.999 **		0.955 **	−0.807 *	−0.796 *
	N:P		−0.915 **			0.917 **	0.995 **		0.987 **	−0.882 *	−0.873 *
Mixed forest	Cs	−0.820 *				0.970 **	0.919 **	0.946 **	0.904 **	0.979 **	
	Ns					0.999 **	0.981 **	0.854 *	0.974 **	0.913 **	
	Ps	−0.995 **						0.979 **		0.945 **	0.873 *
	C:N		−0.871 *	0.822 *	0.997 **		−0.804 *		−0.825 *		
	C:P	−0.761 *				0.990 **	0.954 **	−0.908 **	0.943 **	0.954 **	
	N:P				−0.731 *	0.992 **	0.999 **	−0.767 *	0.997 **	0.842 *	

Note: Cs, Ns, and Ps represent the SOC, TN, and TP storages, respectively. * Indicates the significance level of $p < 0.05$, ** indicates the significance level of $p < 0.01$.

To further reveal the influence of the mixed afforestation on soil storage and stoichiometry, stepwise regression equations were established (Table 8). The results indicated that for the pure plantation, the main factors that affected the SOC storage was the SOC content, TN storage was affected by the SWC and TN content, TP storage was affected by the total porosity and available P, C:N was affected by the total porosity, C:P was affected by the field capacity and TP content, and N:P was affected by the TN content and total porosity. In comparison, for the mixed plantation, the main factors that affected the SOC storage were BD, SWC, and the SOC content; TN storage was affected by the BD and TN, TP, and nitrate N contents; TP storage was affected by BD, field capacity, and the TP and nitrate N contents; C:N was affected by BD, total porosity, field capacity, and the TN content; C:P was affected by the SOC, TP, and nitrate N contents; and N:P was affected by the TN and nitrate N contents.

Table 8. Stepwise regression analysis of the soil physicochemical properties; SOC, TN, and TP storages; and stoichiometric ratios in the study area.

Forest Type	Soil Property	Equation	R ²
Pure forest	C _s	$C_s = 22.79SOC - 85.46$	0.996
	N _s	$N_s = 11.53SWC + 22.61TN - 5.33$	0.998
	P _s	$P_s = 0.43X_1 + 4.87X_4 - 15.69$	0.987
	C:N	$C:N = 0.80X_1 - 17.31$	0.948
	C:P	$C:P = 0.12X_2 - 39.42TP - 0.88$	0.998
	N:P	$N:P = 2.91TN - 0.01X_1 + 0.27$	0.986
Mixed forest	C _s	$C_s = 142.89BD - 165.37SWC + 9.07SOC - 145.37$	0.989
	N _s	$N_s = 4.20BD + 6.01TN + 4.08TP + 2.50X_3 - 6.23$	0.996
	P _s	$P_s = 2.48BD + 24.38TP + 0.30X_2 - 10.51X_3 - 10.72$	0.999
	C:N	$C:N = 14.26BD + 0.90X_1 - 21.29TN - 0.87X_2 - 19.24$	0.993
	C:P	$C:P = 0.69SOC - 30.25TP + 12.45X_3 + 1.25$	0.999
	N:P	$N:P = 1.08TN + 0.36X_3 + 0.21$	0.995

Note: X₁, X₂, X₃, and X₄ represent the soil total porosity, field capacity, and nitrate N and ammonium N contents, respectively.

4. Discussions

4.1. Effects of Mixed Afforestation on the SOC, TN, and TP Storages

SOC, TN, and TP storages are important soil quality evaluation indicators in the process of vegetation restoration and reconstruction in forest ecosystems. Mixed afforestation restoration modes are increasingly being built and were acknowledged as contributing to the improvement of soil nutrient conditions, especially in ecologically fragile regions [21]. Our research found that the mixing species to provide N-fixing significantly changed the SOC content and storage in surface soil (Figures 2 and 3). However, due to the differences in tree species properties, the responses of the selected tree species to mixing were different. Compared with the pure forest, H_rP_t significantly increased the SOC content and storage of the 0–10 cm soil layer. In contrast, H_rR_p significantly increased the SOC content of the 0–20 cm soil layers, while in the same soil layer, no significant difference for the SOC storage was observed (Figures 2 and 3). This might have been related to the transfer of C to the soil through the roots of ground vegetation and the decomposition of soil organic matter [22]. The decomposition rate of conifer litter is lower than that of the deciduous tree litter. Therefore, there is more accumulation of conifer litter and the SOC is also greater [23]. Previous studies showed that the mixing of N-fixing and non-N-fixing tree species contributed to accelerating the decomposition rate of litter in non-N-fixing tree species [24], indicating that more organic matter was returned to the soil per unit mass from coniferous tree species. In addition, the amount of litter may also be an important reason for the sensitive response differences to mixed afforestation among tree species. The amount of litter in H_rR_p was significantly higher than that of H_rP_t in the Loess Plateau region [25]. Therefore, we speculated that although the mixed N-fixer was beneficial regarding an increase in the return of organic matter in H_rP_t , no significant increase in SOC storage of

the 0–100 cm soil layer in H_tP_t was found, and its storage was significantly lower than that of H_tR_p (Table 5).

Similarly, the mixing with N-fixing tree species significantly affected the soil TN content and storage, but there was no significant difference in TP storage between these two pure forests and their mixed forests (Figures 2 and 3). This was mainly due to the differences in the source of N and P in the soil. N-fixing trees could directly fix N from the atmosphere to support their own growth, giving them more of an advantage over non-N-fixing tree species, especially in ecosystems with limited N [15]. The non-N-fixers may benefit from lowered competition for the available soil N through root exudates or the establishment of interconnected mycorrhizal networks between the N-fixing tree species [26,27]. As a result, the introduction of N-fixers in mixed woody plants may have had an important impact on the N dynamics of these two plantations (Table 5). In contrast, the source of P was relatively singular, which was mainly affected by the soil parent material. However, the mixing of N-fixing and N-fixing tree species is beneficial to the release of P in soil [17]. In addition, due to the increase in the amount of litter after mixing, the available P produced by the decomposition of litter was quickly absorbed by the plants [28]. As the soil parent material and environment condition were similar for all plantations, the variability and mobility of soil TP were not as obvious as those of SOC and TN, and mixed afforestation had little effect on soil TP.

4.2. Effect of Mixed Afforestation on Stoichiometric Ratios

Since the stoichiometric rates can be used as the indexes of soil nutrient status [6], clarifying its differential response to mixed afforestation is essential for revealing ecosystem functions. In this study, the mixed species of N-fixing significantly changed the stoichiometric ratios of the 0–10 cm soil layer (Figure 4), which may have been due to the susceptibility of topsoil layer environmental factors and litter decomposition [29]. Generally, soil C:N is not only a sensitive index of the C and N storage but also the quality of the soil in the forest ecosystem [30,31]. In our experiment, the average C:N ratio was 20.79 in the selected plantations, which was higher than the global average C:N of 14.3 [32]. In addition, there was no significant difference in C:N between P_t and H_tP_t , but H_tR_p significantly reduced the soil C:N ratio, which indicated that H_tR_p was helpful for accelerating the mineralization rate of organic matter. This could be due to the mixing of N-fixing and N-fixing tree species, which can provide a sufficient N source for soil microorganisms [33]. Soil C:P represents the ability of soil P mineralization, and a lower ratio indicates that it was more favorable for microorganisms to decompose litter, thereby increasing the available P content in the soil [34]. In this study, the average ratios of C:P for these four plantations were lower than China's average of 61.0 [34]. Additionally, H_tP_t significantly increased the value of soil C:P, but H_tR_p reduced the soil C:P, which indicated that H_tR_p was helpful for alleviating the limitation of P on plant growth to some extent. This may have been because the roots of N-fixing tree species were beneficial in terms of secreting organic acids and other substances, activating the insoluble P in soil, and promoting the accumulation of the soil TP content [35]. The N:P ratio in soil reflects the saturation state of N and is used to judge the threshold of soil nutrient limitation [30,36]. In this research, the averages of soil N:P in the examined plantations were lower than that of the global and Chinese average (9.3 and 13.1, respectively) [32]. Insufficient or lower contents of N and P lead to an increase in the ratios of C:N and C:P, while their excessive content might lead to insufficient C content [37]. Furthermore, N:P in H_tP_t and H_tR_p were significantly higher than that of P_t and R_p , which may have been because of the relatively higher soil TN content in mixed plantations.

4.3. Influencing Factors of the Storage of SOC, TN, and TP, and the Stoichiometric Ratios

The soil's physical and chemical properties significantly affected the SOC, TN, and TP storages in the pure and mixed forests (Table 7). The SOC and TN storages were significantly positively correlated with the nitrate N but negatively correlated with BD.

This was consistent with the research results of [38,39]. In addition, both soil C:P and N:P were negatively correlated with SWC. This may have been because the higher SWC could affect soil organic matter decomposition via its negative impact on the biological activity of microorganisms [40]. It is generally believed that the lack of P in the soil in semi-arid regions, which seriously affects the growth of plants and the development of forest ecosystems [41]. There is no unified conclusion on the phenomenon that soil TP decreased synchronously with SOC and TN after afforestation [41]. In the present study, the SOC and TN storages were significantly positively correlated with the SOC and TN contents, while the TP storage was significantly positively correlated with the TP content. This may have been due to the variations of SOC and TN, which were affected by the litter decomposition and biological N-fixation, but TP was mainly affected by the soil parent materials. The soil C:N ratio was positively correlated with the total porosity, and the soil C:P and N:P ratios were significantly positively correlated with the SOC, TN, and nitrate N contents. Furthermore, compared with the pure forests, soil physical and chemical properties had a stronger control effect on the soil nutrient storages and stoichiometric ratios in the mixed forests (Table 8). Mixed afforestation can significantly change the environmental conditions, such as light and water in the forest, and changes the microbial activity, which may ultimately lead to differences in the stoichiometric characteristics of the forest soil [42,43].

5. Conclusions

Artificial afforestation is an important measure for achieving vegetation construction in the Loess Plateau of China. This paper discussed the effects of mixed afforestation on soil storage and stoichiometry in two dominant tree species in this region. The results revealed that H_tR_p and H_tP_t significantly increased the SOC content in the 0–20 cm and 0–10 cm soil layers, respectively. Meanwhile, H_tR_p and H_tP_t significantly increased the TN content in 0–10 cm. The TP contents in the 0–10, 10–20, 20–50, and 50–100 cm layers of H_tR_p were significantly higher than that of R_p , but there were no significant differences between P_t and H_tP_t . Compared with R_p , the SOC and TN storages in the 0–100 cm layer of H_tR_p significantly increased and the TP storage was higher than that of R_p ; in contrast, no significant differences between P_t and H_tP_t were observed. In the 0–10 cm layer, H_tR_p significantly reduced C:N and increased N:P, while H_tP_t significantly increased C:P. There were no significant differences in the stoichiometric ratios between pure and mixed forests in other soil layers. Compared with the pure forest, soil physical and chemical properties had stronger control effects on the soil nutrient storages and stoichiometric ratios in the mixed forests. This study provides a theoretical basis for the scientific and sustainable management of regional artificial mixed-species restoration plantings. However, due to the complexity and variability of the internal ecological environment of mixed forest ecosystems, sampling data only during the period of vigorous growth may have limited our understanding of the nutrient mechanisms. Therefore, in further study, the frequency of the inter-annual or intra-annual observations, combined with isotope technology, should be improved to explore the influence mechanism of the introduction of nitrogen-fixing tree species on soil reserves and stoichiometric characteristics.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/f12121718/s1>, **Figure S1:** Normal P-P plots of the standardized regression residuals.

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