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# Effect of Soil C, N and P Stoichiometry on Soil Organic C Fractions After Afforestation

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#### ABSTRACT

Afforestation is recognized as an important driving force for soil organic C (SOC) dynamics and soil element cycling. To evaluate the relationships between soil C:N:P stoichiometry and SOC fractions, soil C:N:P stoichiometry distributions at 0–200 cm soil depths were analyzed and the contents of SOC fractions were evaluated in 9 typical land-use systems on the Loess Plateau of China. The contents of light fraction organic C, particulate organic C (> 53, 53–2000, and > 2000  $\mu$ m), labile organic C, microbial biomass C, and dissolved organic C decreased with increasing soil depth and were higher in afforested soil than in slope cropland soil. Compared with the slope cropland, different vegetation types influenced soil C:N, C:P, and N:P ratios, especially when C:P and N:P ratios were significantly higher (P < 0.05). Moreover, SOC fractions at the 0–10 and 10–40 cm depths were particularly affected by soil C:P ratio, whereas those at the 40–100 and 100–200 cm soil depths were significantly affected (P < 0.05) by soil N:P ratio. These results indicate that changes in SOC fractions are largely driven by soil C:P and N:P ratios at different soil depths after afforestation.

Key Words: dissolved organic C, labile organic C, light fraction organic C, microbial biomass C, particulate organic C

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With increasing concerns about the climatic consequences of greenhouse-gas emissions, worldwide efforts are being made to increase C sequestration and reduce  $CO_2$  emissions (Deckmyn *et al.*, 2004). Afforestation of marginal agricultural lands offers opportunities to sequester soil organic C (SOC), improve the quality of degraded soils, and provide ecosystem services (Sauer et al., 2012). Although the studies investigating the considerable SOC sequestration potential of afforestation have reported inconsistent results (Cao et al., 2010), changes in SOC sequestration caused by afforestation are well recognized by researchers. Poeplau et al. (2011) found that in several midwestern USA sites SOC sequestration increased SOC to a greater level following tree planting than in cropland and in more nutrient-rich soils than in grasslands. Garcia-Franco et al. (2015) also reported that an increased SOC pool was linked to changes in microbial activity and fungal community structure after afforestation. Thus, a better understanding of the mechanisms and factors controlling the stabilization of SOC, especially SOC fractions following afforestation, is essential.

Following afforestation, changes inevitably occur in the physical and chemical elements of soil, especially in the three main elements: C, N, and P (Adams et al., 2001; Wei et al., 2009). In recent decades, great progress regarding the C:N:P stoichiometry has been made in terrestrial ecosystems, such as plant leaves and litter (Manzoni et al., 2010), forests (McGroddy et al., 2004), and microorganisms (Liu et al., 2010). Therefore, soil C:N:P stoichiometry has become a powerful tool that can be used to advance our understanding of biological processes and nutrient cycling in terrestrial ecosystems (Cleveland and Liptzin, 2007; Zhao et al., 2015). For example, Tian et al. (2010) documented that soil C:N, C:P, and N:P ratios in the organic C-rich topsoil could be good indicators of soil nutrient status during soil development. Jiao et al. (2013) also reported that soil nutrient stoichiometry played a substantial role in terrestrial C and nutrient cycling.

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However, the effect of afforestation on the C:N:P stoichiometry of soil is poorly understood, especially on the Loess Plateau of China.

The SOC is a complex and heterogeneous entity consisting of fractions varying in mean residence time (Campbell *et al.*, 1967), and is important for the physical, chemical, and biological properties of soil (Reeves, 1997). However, many previous studies only addressed general changes in SOC and offered limited understanding of the mechanisms contributing to the stabilization of SOC in soils. The SOC changes using labile C pools, such as microbial biomass C (Cambardella and Elliot, 1992; Freixo et al., 2002), light fraction organic C (Zhao et al., 2014), particulate organic C (De Moraes Sá and Lal, 2009), and dissolved organic C (Saha et al., 2011), is frequently used to assess the effects of management practices and land use change. Importantly, evidence suggests that, compared with total SOC, certain fractions of SOC are more sensitive indicators of the effects of management practices and land use change (Franzluebbers and Arshad, 1992; Strosser, 2010). Thus, using these fractions of SOC is an efficient way to assess the SOC storage after land use change. Unfortunately, the interactions between the stoichiometry and SOC fractions that affect SOC sequestration in soil are poorly understood. The Loess Plateau in China covers approximately  $62.4 \text{ km} \times 104$ km and is known for its long agricultural history and severe soil erosion (Chen et al., 2007). Historically, the native vegetation was destroyed to meet the food supply needs of an expanding population, which resulted in severe soil erosion and land degradation (Fu et al., 2005). To counteract soil erosion and other environmental problems, an environmental protection policy known as the Grain to Green Program (GTGP) was implemented by the Chinese central government. The purpose of GTGP is to convert low-yield slope croplands  $(> 25^{\circ})$  into forests, shrubs, or grasslands (An *et* al., 2013). In recent years, a lot of work has been conducted to investigate changes in soil C and N storage after large-scale afforestation (Han et al., 2010; Fang et al., 2012). However, studies on SOC fractions, soil C:N:P stoichiometry, and their relationships have been limited.

In the present study, it was hypothesized that SOC fractions were strongly influenced by soil C:N, C:P, and N:P ratios following large-scale afforestation. Therefore, the present study aimed to: 1) analyze soil C:N:P stoichiometry distributions in 0–200 cm soil depths; 2) determine the contents of SOC fractions including labile organic C (LOC), dissolved organic C (DOC), light fraction organic C (LFOC), microbial biomass C

(MBC), and particulate organic C (POC) at different soil depths under different land use types; and 3) evaluate the relationships between soil C:N:P stoichiometry and SOC fractions (LOC, DOC, LFOC, MBC, and POC).

# MATERIALS AND METHODS

### Study area

This study was conducted in the Wuliwan catchment in Ansai County, Shaanxi Province (36°46′42″- $36^{\circ}46'28''$  N,  $109^{\circ}13'46''-109^{\circ}16'03''$  E), which is located in the central region of the Loess Plateau, China. This area is characterized by a semi-arid climate and hilly loess landscape with an annual average temperature of 8.8 °C and an average annual precipitation of 505 mm (about 300 mm in dry years and > 700 mm in wet years). Most of the precipitation (60%) occurs between July and September. Arable farming mostly occurs on sloping lands without irrigation. The loess parent material at the site has an average thickness of 50–80 m and the soil in this region belongs to Calciustepts (Chen et al., 2007). Sand (2-0.05 mm) and silt (0.05-0.002 mm) account for approximately 29.2%and 63.5%, respectively, of the soil at soil depths of 0–20 cm. After 40 years of afforestation, the area proportion of forestland has significantly increased from 5% to 40% (Xue *et al.*, 2009). The major agricultural land use of the area is slope cropland. Agricultural management in this region, including the major crop types grown, has not changed significantly since the 1970s. After more than 40 years of comprehensive management, the ecological environment of the catchment area has significantly improved (Fu et al., 2010). Beginning in the late 1970s, slope cropland was replanted with forest, mainly Robinia pseudoacacia L. (RP) and *Caragana korshinskii* Kom. (CK), to control soil erosion. Cropland was also abandoned since the 1970s due to its extremely low productivity and long distance from farmers' residences (Li et al., 2004).

#### Experimental design and soil sampling

In June 2013, 27 study sites in this region were selected based on the history of land use, *i.e.*, major land use types (including RP, CK, orchard, abandoned land, and slope cropland) and restoration age (45, 40, and 25 years for RP, 40 years for mixed forest *Prunus davidiana* and CK, and 40 and 30 years for CK). Then, in each plantation, 3 plots of 30 m  $\times$  30 m were established. All sites were located in the same physiographical units with the same slope aspects, the same elevation (1 250 m), and a spatial distance of 1 200 m to ensure uniformity and homogeneity. A description of each land use type is shown in Table I.

Soil samples were obtained to a depth of 200 cm at a 10-cm interval in the upper 0–100 cm of the soil profile and at a 20-cm interval in the lower 100-200 cm of the soil profile. After removing the litter layer carefully by hand from the topsoil, soil samples were taken at several soil depths using a soil auger (5 cm diameter) from 10 points in an "S" shape at each plot. One portion was sieved through a 2-mm mesh for SOC, total N (TN), total P (TP), LOC, POC, and LFOC analyses. A second portion was sieved through a 2-mm mesh and stored at 4 °C for MBC and DOC analyses. The LF-OC and MBC analyses were conducted using the soil samples from 0-100 and 0-30 cm depths, respectively. Visible plant debris was removed immediately after sampling. Then, 10 soil samples from each depth of each plot were mixed to generate one sample. Samples were collected at least 80 cm away from any trees. All samples were sieved through a 2-mm screen. Additionally, 3 soil profiles were randomly dug in each plot and at each sampling interval 3 samples were obtained to determine soil bulk density.

# Laboratory analysis

The SOC, TN, and TP contents were determined using the  $K_2Cr_2O_7$  oxidation, Kjeldhal, and Mo-Sb antispectrophotography methods, respectively (Cheng *et al.*, 2015).

Soil LFOC fraction was extracted using NaI (Sheng et al., 2015). Briefly, 20 g air-dried soil sample (< 2 mm) was suspended in a 40-mL NaI solution (1.70 g cm<sup>-3</sup>), shaken for 1 h at 250 r min<sup>-1</sup>, and then centrifuged at 3 000 r min<sup>-1</sup> for 10 min. The supernatant was then filtered using a 0.45-mm glass-fiber micro-filtration membrane, and the remaining solution was collected for reuse. The separation method described above was repeated until no floating particles were visible on the membrane. The particles on the membrane were collected, washed with 75 mL of 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> solution followed by a wash with 200 mL deionized water to remove any residual NaI, dried at 60 °C for 48 h, weighed, and stored as the LFOC.

Soil POC fraction was separated as described by Cambardella and Elliott (1992) and Zhao *et al.* (2014). Briefly, 20 g air-dried soil sample was dispersed in 100 mL of 5 g L<sup>-1</sup> sodium hexametaphosphate and shaken for 18 h at 90 r min<sup>-1</sup>. The suspension was then filtered through 2000- and 53-µm sieves, and the filted suspension was collected. The material remaining on the sieves was thoroughly rinsed with deionized water, dried at 60 °C overnight, weighed, and stored as > 2000 and 53–2000 µm POC (POC3 and POC2, respectively). Next, the suspensions collected after filtration through 2000- and 53-µm sieves were dried at 60 °C using the water bath method, and stored as < 53 µm POC (POC1).

Soil LOC fraction was measured following the me-

## TABLE I

Detailed information for the study sites under different land use types, including 45-, 40-, and 25-year *Robinia pseudoacacia* (RP-45, RP40, and RP25, respectively), 40-year mixed forest *Prunus davidiana* and *Caragana korshinskii* (MF40), 40- and 30-year *C. korshinskii* (CK40 and CK30, respectively), abandoned land, orchard, and slope cropland

Land use type	Location	Elevation	Sand	Silt	Clay	Coverage	Main herbaceous types
		m		%			
RP45	36°50′47″ N, 109°21′12″ E	1206	$29.2 \pm 0.2^{\rm a}$	$63.1\pm0.8$	$7.7 \pm 1.1$	70	Artemisia gmelinii, Incarvillea sinensis
RP40	36°52′24″ N, 109°20′55″ E	1209	$30.1 \pm 1.0$	$64.5\pm2.2$	$5.4 \pm 1.2$	80	Artemisia gmelinii, Stipa bungeana
RP25	36°51′63″ N, 109°21′01″ E	1258	$28.3 \pm 1.3$	$60.2\pm2.1$	$11.5\pm0.9$	85	Heteropappus altaicus, Ixeris chinensis
MF40	36°50′15″ N, 109°21′10″ E	1158	$30.3 \pm 1.2$	$67.9 \pm 1.7$	$1.8\pm0.9$	75	Artemisia gmelinii, Cleistogenes chinensis Keng
CK40	36°51′16″ N, 109°21′01″ E	1259	$27.5\pm0.9$	$65.8 \pm 1.9$	$6.7 \pm 0.5$	75	Artemisia gmelinii, Potentilla tanacetifolia
CK30	36°53′65″ N, 109°21′29″ E	1279	$24.6\pm1.7$	$68.2\pm2.6$	$7.2\pm0.8$	85	Cleistogenes chinensis Keng, Incarvillea sinensis
Abandoned land	36°51′38″ N, 109°8′99″ E	1240	$32.8 \pm 1.8$	$60.3 \pm 1.9$	$6.9 \pm 1.0$		Heteropappus hispidus Less, Artemisia qmelinii
Orchard	36°51′97″ N, 109°20′55″ E	1209	$31.4 \pm 1.2$	$63.4\pm2.7$	$5.2 \pm 0.6$		U U
Slope cropland	36°51′98″ N, 109°20′51″ E	1214	$21.4 \pm 1.1$	$62.5 \pm 2.1$	$16.1 \pm 1.2$		

<sup>a)</sup>Means  $\pm$  standard deviations (n = 3).

thod described by Blair *et al.* (1995) and Zhao *et al.* (2014). An air-dried soil sample (2–6 g) was placed into a 50-mL centrifuge tube, 25 mL of 333 mmol  $L^{-1}$  KMnO<sub>4</sub> solution was added, and the mixture was shaken at 120 r min<sup>-1</sup> for 1 h and centrifuged for 5 min at 5000 × g. The upper clear solution was transferred, diluted 250-fold, and determined for absorbance at a wavelength of 565 nm. The absorbance of different KMnO<sub>4</sub> concentrations at 565 nm was also determined to generate a standard curve, which was subsequently used to determine KMnO<sub>4</sub> concentration remaining in the supernatant. The difference between the amount of KMnO<sub>4</sub> added and remaining was used to calculate the LOC in the soil sample.

Soil MBC fraction was measured according to the method of Sheng *et al.* (2015). Fresh soil sample (25 g) was fumigated with CHCl<sub>3</sub> vapor in a desiccator for 24 h. After removing any residual CHCl<sub>3</sub> by evacuation, the fumigated soil was extracted with 0.5 mol  $L^{-1}$  of K<sub>2</sub>SO<sub>4</sub> for 30 min. Non-fumigated soil was extracted following the same procedure. The MBC per sample was estimated as the difference between the amount of organic C extracted from the fumigated and non-fumigated soil multiplied by a factor of 2.22. Organic C extracted from the non-fumigated soil was also considered to be the DOC (Sheng *et al.*, 2015). The C:N, C:P, and N:P ratios were calculated as molar ratios (atomic ratios).

# Statistical analyses

All statistical analyses were carried out using SPSS 17.0. Analysis of variance and Duncan's multiple range test at a 5% level of significance were used to compare the differences in the contents of SOC, TN, TP, POC, LOC, DOC, LFOC, and MBC, and soil C:N, C:P, and N:P ratios under different land use types and at different soil depths. Spearman correlations between soil C:N, C:P, and N:P ratios and the contents of POC, LOC, DOC, LFOC, and MBC at different soil depths were performed in R v.3.1.3.

## RESULTS

## Soil stoichiometry under different land use types

The contents of SOC, TN, and TP and the C:N, C:P, and N:P ratios varied by soil depth and land use type (Fig. 1). The contents of SOC, TN, and TP at the 0–40 cm soil depth were higher than those at the 40–100 and 100–200 cm soil depths, being more than 11.13%–50.12% and 21.33%–38.19% higher, respectively. The contents of SOC, TN, and TP were higher under different vegetation types than in the slope cropland, especially for 45-year RP (RP45). For example, compared with the slope cropland, the SOC, TN, and TP contents were 439.91%, 443.94%, and 41.32% higher, respectively, under different vegetation types at the 0–10 cm soil depth, 546.88%, 396.86%, and 36.18% higher at the 10–40 cm soil depth, 340.82%, 177.63%, and 28.15%% higher at the 40–100 cm soil depth, and 392.69%, 237.91%, and 20.16% higher at the 100–200 cm soil depth. The C:N, C:P, and N:P ratios followed the same trends as the SOC, TN, and TP contents. However, the C:N ratio in the 40-year mixed forest *P*. *davidiana* and CK was higher than those under other land use types, and reached 1.34 times that found in the slope cropland.

## SOC fractions under different land use types

Differences in the contents of LOC, DOC, LFOC, MBC, and POC among different land use types and soil depths are shown in Figs. 2 and 3. Overall, the contents of LOC, DOC, and POC at the 0–10 cm soil depth were significantly higher (P < 0.05) than those at other soil depths. The contents of LOC, DOC, LFOC, MBC, and POC were significantly higher (P < 0.05) in RP45 than under other land use types. For example, the LOC, DOC, and POC contents in RP45 at the 0–10, 10–40, 40–100, and 100–200 cm soil depths were 41.78%–45.68%, 26.28%–37.19%, 13.99%–18.08%, and 5.04%–15.86% higher, respectively, than those in slope cropland.

# Effect of soil C, N and P stoichiometry on SOC fractions under different land use types

The SOC fractions (LOC, DOC, LFOC, MBC, and POC) at different soil depths were largely affected by soil C:N:P stoichiometry (Fig. 4). In general, the C:P ratio was the main factor influencing SOC fractions at the 0–10 and 10–40 cm depths, and the N:P ratio was the main influencing factor at the 40–100 and 100–200 cm soil depths (Fig. 4). The SOC and POC1 at the 0–10 cm soil depth were strongly affected by the C:P ratio, while LOC and POC1 were affected by soil N:P and C:P ratios (Fig. 4a). The N:P ratio had a significant influence on SOC, POC3, POC2, and POC1 at the 40–100 and 100–200 cm soil depths (Fig. 4c, d).

# DISCUSSION

#### SOC, TN, and TP under different land use types

Afforestation significantly affects the contents of SOC, TN, and TP (Grünzweig *et al.*, 2007). Our re-



Fig. 1 Variations of soil organic C (SOC), total N (TN), and total P (TP) contents and soil C, N, and P stoichiometry under different land use types, including 45-, 40-, and 25-year *Robinia pseudoacacia* (RP45, RP40, and RP25, respectively), 40- and 30-year *Caragana korshinskii* (CK40 and CK30, respectively), 40-year mixed forest *Prunus davidiana* and CK (MF40), orchard (OR), abandoned land (AL), and slope cropland (SC), at different soil depths. Horizontal bars indicate standard deviations of the means (n = 3).

sults showed that afforestation led to higher SOC, TN, and TP compared with the slope cropland (Fig. 1), which is consistent with the results of previous studies (Eaton *et al.*, 2008). A possible reason for this is that lower levels of residue in the soil of slope cropland lead to lower SOC, TN, and TP contents. Our results also showed that the SOC and TN contents in RP45 were higher than those of other land use types (Fig. 1). This suggests that the effects of RP45 on soil C and N play a significant role in land use and ecosystem management. These results are consistent with those reported by Qiu *et al.* (2010), who found that *R. pseudoacacia* forests had the potential to improve SOC content in the loessial gully region of the Loess Plateau and that SOC increased with increasing stand age, accounting for 83.2%–96.6% of the total C stored. Several studies have found substantial C sequestration rates under black locust trees, with the values ranging from 2.4 Mg ha<sup>-1</sup> year<sup>-1</sup> in Ohio, USA (Ussiri *et al.*, 2006) to 4.0–7.0 Mg ha<sup>-1</sup> year<sup>-1</sup> in Germany (Quinkenstein *et al.*, 2011; Matos *et al.*, 2012).

# SOC fractions under different land use types

Soil C accumulation may be largely driven by enhanced litter inputs during afforestation. Moreover, afforestation influences not only soil C stocks, but also



Fig. 2 Variations of labile organic C (LOC), dissolved organic C (DOC), light fraction organic C (LFOC), and microbial biomass C (MBC) under different land use types, including 45-, 40-, and 25-year *Robinia pseudoacacia* (RP45, RP40, and RP25, respectively), 40- and 30-year *Caragana korshinskii* (CK40 and CK30, respectively), 40-year mixed forest *Prunus davidiana* and CK (MF40), orchard (OR), abandoned land (AL), and slope cropland (SC), at different soil depths. Horizontal bars indicate standard deviations of the means (n = 3).



Fig. 3 Variations of particulate organic C in < 53, 53-2000, and  $> 2000 \mu m$  soil aggregates (POC1, POC2, and POC3, respectively) under different land use types, including 45-, 40-, and 25-year *Robinia pseudoacacia* (RP45, RP40, and RP25, respectively), 40- and 30-year *Caragana korshinskii* (CK40 and CK30, respectively), 40-year mixed forest *Prunus davidiana* and CK (MF40), orchard (OR), abandoned land (AL), and slope cropland (SC), at different soil depths. Horizontal bars indicate standard deviations of the means (n = 3).

the different SOC fractions within the soil (Zhao *et al.*, 2015). Our results indicated that the contents of LOC, DOC, LFOC, MBC, and POC differed by land use types, and were significantly higher in the soils with vegetation than with slope cropland (Figs. 2 and 3). These findings are consistent with those of Zhao

*et al.* (2014), who found that the conversion of slope cropland to vegetation improved the LOC, DOC, L-FOC, MBC, and POC contents. A possible reason for this is that the lower input of residue into the soil in slope cropland leads to lower SOC content.

The LOC, DOC, LFOC, MBC, and POC contents

### SOIL STOICHIOMETRY EFFECT ON C FRACTIONS



Fig. 4 Spearman correlations between soil C, N, and P stoichiomentry and soil organic C (SOC) fractions at soil depths of 0–10 (a), 10–40 (b), 40–100 (c), and 100–200 cm (d). The SOC fractions include dissolved organic C (DOC), light fraction organic C (LFOC), labile organic C (LOC), microbial biomass C (MBC), and particulate organic C in < 53, 53–2000, and  $> 2000 \mu m$  soil aggregates (POC1, POC2, and POC3, respectively).

under vegetation types were higher than those in slope cropland at both the 0–10 and 100–200 cm soil depths, especially in RP45. This indicated that afforestation not only affected SOC fractions in the surface soil, but also greatly influenced those in the deep soil. Similar results were found by Fu *et al.* (2010), who reported that the stability of SOC in deep soils was enhanced during afforestation on the hilly Loess Plateau, compared with that in the shallow soils. This is mainly because the most important factors protecting SOC fractions in subsoil include the spatial separation of soil organic matter, microorganisms, and extracellular enzyme activity related to the heterogeneity of C input (Guan *et al.*, 2015). As a result, stabilized SOC fractions in the subsoil are stratified horizontally.

# Effect of soil C, N and P stoichiometry on SOC fractions under different land use types

Following afforestation, the contents of C, N, and P inevitably change in the soil, and several studies have indicated that these elements are often closely related and influenced by soil C sequestration (Walker and Adams, 1958; Post *et al.*, 1982; Melillo *et al.*, 2003; Zhang *et al.*, 2013). The results of the present study showed that soil C:N:P stoichiometry influenced SOC fractions at different soil depths (Fig. 4), indica-

ting that SOC fractions were sensitive to soil C:N, C:P, and N:P ratios after afforestation. Furthermore, the mechanisms acting to increase soil C pools were largely affected by soil C:N:P stoichiometry after afforestation. Similar results were found by Clive *et al.* (2006), who reported that the more-stable fine fraction pool of soil organic matter (< 0.4 mm) contained more N, P, and S per unit of C. This is likely because afforestation affects the amount and quality of litter input, the litter decomposition rate, and the processes of organic matter stabilization in soils. In addition, the change in land use from agricultural to forestry means that the annual cycle of cultivating and harvesting crops is replaced by the much longer forest cycle. Consequently, this enables the development of high net primary productivity and reduces the degree of soil disturbance, leading to increased SOC fractions (Vesterdal et al., 2002). Moreover, the available P in soil controlled the C content by affecting microbial activity and biological N fixation (Tian et al., 2010), which largely affected SOC fractions. Conversely, plant roots change soil C components by absorbing or releasing C, N, and P, which strengthens the relationships between these elements. Thus, the effect of soil C:N:P stoichiometry on SOC fractions can provide significant information related to the mechanisms of C sequestration. However,

to better understand the mechanism of C sequestration through C:N:P stoichiometry in soil, further studies addressing the thresholds of soil C:N, C:P, and N:P ratios should be performed.

# CONCLUSIONS

The contents of LOC, DOC, LFOC, MBC, and POC differed by land use types and were significantly higher in afforested lands than in slope cropland. The SOC fractions at different soil depths were influenced by soil C:N:P stoichiometry under different land use types, indicating that SOC fractions were largely affected by soil C:N, C:P, and N:P ratios after afforestation. Thus, it could be concluded that changes in SOC fractions represented an important factor driven by changes in soil C:N:P stoichiometry.

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