Soil stoichiometry influence C, N, and P distribution in soil aggregates after afforestation

Após a restauração de vegetação, a razão estequiométrica do solo afeta a distribuição de carbono, nitrogênio e fósforo no solo agregado

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Abstract

Soil carbon stabilization depends on its distribution in structural aggregates, and nutrient ratios in soils (mainly carbon (C), nitrogen (N), and phosphorus (P)). However, The relationship between the soil C:N:P stoichiometry and soil C, N, and P content in soil aggregates after afforestation are poorly understood. We investigated changes in soil C:N:P stoichiometry and soil C, N, and P content in soil aggregates at 0-20 cm and 20-40 cm depths on lands that were converted from slope croplands (SC) to forests on the Loess Plateau in China. Our results showed that soil organic carbon (SOC), soil total nitrogen (STN), soil total phosphorous (STP), and soil C:N, C:P, and N:P increased after afforestation. Compared with SC, the SOC, STN, and STP content in soil aggregates greatly increased in small macro-aggregates (0.25–5 mm). Furthermore, Beerkan Estimation of Soil Transfer (BEST) model results indicated that SOC, STN, and STP contents in soil aggregates were significantly affected by soil C:N, C:P, and N:P ratios. Likewise, redundancy analysis (RDA) showed that soil C:P and N:P ratios were the main factors to influence SOC, STN, and STP contents in small macro-aggregates in surface soil (0–20 cm). These results suggested that SOC accumulation after afforestation was due to its accumulation in small macro-aggregates and this increase was largely affected by soil C:N:P stoichiometry in surface soil.

Keywords: Afforestation; Soil C:N:P; Soil aggregates; BEST model; Loess Plateau.

Resumo

A estabilização do carbono no solo depende de sua distribuição em agregados estruturais e das proporções de nutrientes nos solos (principalmente carbono (C), nitrogênio (N) e fósforo (P)). No entanto, a relação entre a estequiometria do solo, nitrogênio e fósforo do solo após a restauração de vegetação e o conteúdo de carbono, nitrogênio e fósforo do solo agregado ainda não está muito clara. Este estudo enfoca as mudanças na estequiometria de carbono, nitrogênio e fósforo do solo e as mudanças no conteúdo de carbono, nitrogênio e fósforo em agregados do solo nas camadas de solo de 0-20 cm e 20-40 cm das terras agrícolas após a arborização no Planalto de Loess, na China. Nosso resultados mostraram que o carbono orgânico do solo (SOC), nitrogênio total do solo (STN), fósforo total do solo (STP) e C: N, C: P e N: P do solo aumentaram após o florestamento. Comparado com SC, o conteúdo de SOC, STN e STP aumentaram muito no solo macroagregado (0,25–5 mm). Além disso, os resultados do modelo de BEST (Estimativa de Transferência de Solo de Beerkan) indicaram que os conteúdos de SOC, STN e STP no solo agregado
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foram significativamente afetados pelas relações C: N, C: P e N: P do solo. Ao mesmo tempo, a RDA (análise de redundância) mostrou que as relações C: P e N: P do solo foram os principais fatores a influenciar os conteúdos de SOC, STN e STP no pequeno solo agregado do solo superficial (0–20 cm). Esses resultados sugerem que o acúmulo de SOC após a arborização foi devido ao seu acúmulo em pequeno solo agregado e esse aumento foi amplamente afetado pela estequiometria de carbono, nitrogênio e fósforo no solo superficial.

Palavras-chave: Arborização; Solo C:N:P; Solo agregado; Modelo; Loess Plateau.

INTRODUCTION

Afforestation usually affects soil aggregate size distribution and stability, which are important for improving soil quality and protecting soil organic carbon (SOC) (Barthès & Roose, 2002). Vegetation restoration has been found to increase aggregates, enhancing the uniformity of the distribution of soil aggregate size and inducing greater SOC sequestration (Six & Paustian, 2014; Cheng et al., 2015; Li et al., 2015). Soil aggregates are often grouped by size: macro-aggregates (>0.25 mm) and micro-aggregates (<0.25 mm) (Yamashita et al., 2006). Size group properties differ, such as in binding agents, carbon (C), and total nitrogen (STN) distribution (Gelaw et al., 2015). Therefore, the location of SOC in the hierarchical structure of the soil aggregate system has been observed to be crucial in the sequestration and transformation of SOC (Golchin et al., 1994; Yamashita et al., 2006; Guan et al., 2015). Previous studies indicated that most SOC is occluded within macro-aggregates in forest soil (Caravaca et al., 2004; Bronick & Lal, 2005), and conversion from forest to farmland can decrease the amount of macro-aggregates, resulting in a loss of total soil SOC and macro-aggregate-associated SOC (Grandy & Robertson, 2007; Wei et al., 2012b; An et al., 2013). However, factors affecting these changes are not well understood, especially using indicators such as soil C:N:P stoichiometry (Zhao et al., 2015).

Soil C:N:P stoichiometry can be a powerful tool for advancing our understanding of biological processes and nutrient cycling in terrestrial ecosystems (Cleveland & Liptzin, 2007; Zhao et al., 2015). In recent decades, great progress in C:N:P stoichiometry research has been made in leaves and litter (Manzoni et al., 2010), forests (McGroddy et al., 2004), and microorganisms (Liu et al., 2010). Well-balanced C:N:P ratios of 186:13:1 and 60:7:1 for soil and soil organisms, respectively, have been determined on a global scale (Cleveland & Liptzin, 2007). More importantly, changes in C, N, and P content in soil aggregates can be explained in terms of alterations in the stoichiometric ratios of the soil's components themselves. For example, O'Brien & Jastrow (2013) documented that the duration of linear soil C and N accumulation differed among aggregate-occluded pools in relation to the combined influences of increases in C and N concentrations. An et al. (2013) also reported that soil nutrient stoichiometry plays a substantial role in terrestrial carbon and nutrient cycling, especially in aggregate formation. A variety of mechanisms in C and N cycling have direct and indirect effects (Falkowski et al., 2000; Mooshammer et al., 2012). Unfortunately, the interactions between the stoichiometry (especially for C, N and P) and C, N, and P content in soil aggregates were poorly understood.

Robinia pseudoacacia L. (Black locust) is a tree species suitable for afforestation in arid regions of the world because of its rapid growth and ability to fix atmospheric nitrogen in disturbed soil ecosystems (Bolat et al., 2016). It is well-adapted to grow on different types of soil and in various
environmental conditions, though it does not grow well in compacted or wet soils. It can significantly improve soil N content and availability (Tateno et al., 2007), accelerate soil organic C sequestration (Ussiri et al., 2006), promote soil biological properties (Xue et al., 2007), and improve biodiversity (Evans et al., 2013; Zhao et al., 2015). Recently, the use of black locust in bioenergy has been researched. Straker et al. (2015) reviewed its propagation practices, biomass and energy yield estimates, environmental risks and benefits, and economic considerations for this promising feedstock. Therefore, planting of black locust in the Loess Plateau is particularly important in improving the region’s ecological environment. Knowledge about soil C, N, and P content in soil water-stable aggregates over time under black locust plantations on degraded soil would be helpful for understanding the mechanism of soil carbon sequestration and cycling.

The objectives of this study were to assess changes in soil stoichiometry due to vegetation cover, and the relationship between soil stoichiometry and soil C, N, and P content in soil water-stable aggregates after afforestation on the Loess Plateau of China. The following hypotheses were tested in this study: (1) afforestation would increase SOC, STN, and STP content in small macro-aggregates (0.25–2 mm); and (2) soil C:N, and especially C:P, and N:P ratios, would influence soil C, N, and P content in soil water-stable aggregates.

METHODS AND MATERIALS

Research area.

The study was conducted in the Wuliwan catchment of central Loess Plateau (Li et al., 2016). The Wuliwan catchment is one of the experimental sites of the Institute of Soil and Water Conservation, Chinese Academy of Science (CAS). This area is characterized by a semi-arid climate and hilly loess landscape in the Loess Plateau with an annual average temperature of 8.8°C, and an average annual precipitation of 505 mm. Sixty percent of the precipitation occurs between July and September (~ 300 mm in dry while > 700 mm in wet years) (Zhao et al., 2014b; Li et al., 2016). Arable farming mostly occurs on sloping lands without irrigation (mainly millet). Agricultural management in this region has not changed significantly since the 1970s. The soil in this region is Calciustepts (Gong et al., 1999), with approximately 29.2% sand (2–0.05 mm) and 63.6% silt (0.05–0.002 mm) at 0-20 cm soil depth, and 27.8% sand (2–0.05 mm) and 62.7% silt (0.05–0.002 mm) at 20-40 cm depth (Table 1) (Zhao et al., 2014b; Li et al., 2016). After 40 years of afforestation, the forest area significantly increased from 5% to 40% (Xue et al., 2009). 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, which was approximately 29.22% sand (2–0.05 mm) and 63.6% silt (0.05–0.002 mm). Abandoned cropland was also afforested during this period due to its extremely low productivity and long distance from farmers' residences (Li et al., 2004).

Experimental design.

In June 2013, three replicates for each vegetation type were selected, including 40 year-old RP and CK on abandoned land (AB) and slope cropland (SC). We then established three random 30 × 30 m subplots within each replicate. To avoid possible error introduced by different physiographical conditions, we selected cropland with a similar slope and gradient.
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All sites were located on the same physiographical units with the same slope aspects, the same elevation of 1250 m and a spatial distance of 1200 m to ensure uniformity and homogeneity. The characterization of each vegetation type is shown in Table 1.

Table 1. Detailed information for land use types.

| Vegetation type | Location | Elevation (m) | Sand (%) | Silt (%) | Clay (%) | Coverage | Mainly Vegetation Types of Herb |
|-----------------|----------|---------------|----------|----------|----------|----------|---------------------------------|
| R. pseudoacacia (RP40a) | 36°52′24″N; 109°20′55″E | 1209 | 30.1±1.0 | 64.5±2.2 | 5.4±1.2 | 0.8 | Artemisia gmelinii |
| | 36°51′16″N; 109°21′01″E | 1259 | 27.5±0.9 | 65.8±1.9 | 6.7±0.5 | 0.75 | Artemisia gmelinii |
| C. korshinskii (CK40a) | 36°51′38″N; 109°18′99″E | 1240 | 32.8±1.8 | 60.3±1.9 | 6.9±1.0 | | Stipa bungeana |
| | 36°51′88″N; 109°20′51″E | 1214 | 21.4±1.1 | 62.5±2.1 | 16.1±1.2 | | Potentilla tanacetifolia |
| Abandon land (AB) | 36°51′38″N; 109°20′51″E | 1214 | 21.4±1.1 | 62.5±2.1 | 16.1±1.2 | | Heteropappus |
| slope cropland (SC) | 36°51′88″N; 109°20′51″E | 1214 | 21.4±1.1 | 62.5±2.1 | 16.1±1.2 | | Artemisia gmelinii |

Values are represented as mean ±SD (n=3).

Soil sampling.

Soil samples were obtained from a depth of 40 cm at a sampling interval of 20 cm. After removing the litter layer (carefully removed by hand from topsoil), soil samples were taken from 0–20 and 20–40 cm soil depths using a soil auger (diameter 5 cm) from ten points within an “S” shape in each subplot. Visible plant debris and stones larger than 2 mm were removed immediately after sampling. Then, ten soil samples at each depth of each plot were mixed to make one sample. Samples were collected at least 80 cm away from the trees. All samples were sieved through a 2 mm screen. In addition, from each plot, three undisturbed soil samples were collected in aluminum containers from soil depths of 0–20 cm and 20–40 cm. The samples were air dried at room temperature for laboratory analysis.

Laboratory analysis.

The aggregate separation was performed using a modified Yoder method (Zhu, 1982; Cheng et al., 2015). Briefly, 100 g of air-dried (8-mm-sieved) soil was placed on top of a 5-mm sieve and submerged for 5 min in deionized water at room temperature. The sieving was performed manually by moving the sieve up and down 3 cm, 50 times, for 2 min-to achieve aggregate separation. A series of four sieves (2, 1, 0.5 and 0.25 mm) was used to obtain six aggregate fractions. The aggregate-size classes were oven dried (70 °C), weighed, and stored in glass jars at room temperature. Collected samples were ground to pass through a 0.25 mm sieve and SOC, TN, and TP were measured.

SOC (g.kg⁻¹), TN (g.kg⁻¹), and total soil phosphorus contents (g.kg⁻¹) were determined using K₂Cr₂O₇ oxidation method, Kjeldhal method, and the Mo-Sb anti-spectro-photography method, respectively (Bao, 2000).
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Statistical analyses.

We used the Beerkan Estimation of Soil Transfer (BEST) model building procedure in PRIMER v.7 to identify all possible combinations of factors that contributed to the C:N, C:P, and N:P ratios (Clarke & Warwick, 1994; Freedman & Zak, 2015), which account for the greatest proportion of SOC, TN, and TP content in different soil aggregates (<0.25 mm, 0.25–0.5 mm, 0.5–1 mm, 1–2 mm, and 2–5 mm, >5 mm). The factors were evaluated stepwise and were based on sufficient improvement in the model's R value (Table 2). All statistical analyses were carried out with SPSS 17.0 and CANOCO 4.5 software package (Braak & Smilauer, 2002).

Table 2. Results from ‘Best’ model selection procedure presented for different vegetation types and soil depth

| Soil depth | Number variables | R       | Predictor variables | Land use type | Number variables | R       | Predictor variables |
|------------|------------------|---------|---------------------|---------------|------------------|---------|---------------------|
| SOC        | 1                | 0.897   | Soil C:P            | 1             | 0.752            | Soil N:P |
|            | 1                | 0.861   | Soil N:P            | 1             | 0.625            | Soil C:N |
|            | 1                | 0.412   | Soil C:N            | 1             | 0.468            | Soil C:P |
|            | 2                | 0.910   | Soil C:P, Soil N:P  | 2             | 0.691            | Soil C:P, Soil N:P |
|            | 2                | 0.741   | Soil C:N, Soil C:P  | 2             | 0.651            | Soil C:N, Soil N:P |
|            | 2                | 0.677   | Soil C:N, Soil N:P  | 2             | 0.591            | Soil C:N, Soil C:P |
|            | 3                | 0.797   | Soil C:N, Soil C:P, Soil N:P | 3 | 0.616 | Soil C:N, Soil C:P, Soil N:P |
| TN         | 1                | 0.916   | Soil C:P            | 1             | 0.727            | Soil N:P |
|            | 1                | 0.898   | Soil N:P            | 1             | 0.689            | Soil C:N |
|            | 1                | 0.340   | Soil C:N            | 1             | 0.652            | Soil C:P |
|            | 2                | 0.931   | Soil C:P, Soil N:P  | 2             | 0.757            | Soil C:N, Soil N:P |
|            | 2                | 0.727   | Soil C:N, Soil C:P  | 2             | 0.734            | Soil C:P, Soil N:P |
|            | 2                | 0.663   | Soil C:N, Soil N:P  | 2             | 0.710            | Soil C:N, Soil C:P |
|            | 3                | 0.777   | Soil C:N, Soil C:P, Soil N:P | 3 | 0.760 | Soil C:N, Soil C:P, Soil N:P |
| TP         | 1                | 0.404   | Soil N:P            | 1             | 0.747            | Soil C:N |
|            | 1                | 0.396   | Soil C:P            | 1             | 0.727            | Soil N:P |
|            | 1                | 0.282   | Soil C:N            | 1             | 0.501            | Soil C:P |
|            | 2                | 0.424   | Soil C:P, Soil N:P  | 2             | 0.760            | Soil C:N, Soil N:P |
|            | 2                | 0.357   | Soil C:N, Soil C:P  | 2             | 0.718            | Soil C:N, Soil C:P |
|            | 2                | 0.320   | Soil C:N, Soil N:P  | 2             | 0.678            | Soil C:P, Soil N:P |
|            | 3                | 0.376   | Soil C:N, Soil C:P, Soil N:P | 3 | 0.743 | Soil C:N, Soil C:P, Soil N:P |

Note: Significance level of sample statistic: 0.1%; Number of permutations: 999 (Random sample)

RESULTS AND DISCUSSION

Afforestation effects on SOC, TN, and TP content and soil C:N:P stoichiometry.

The contents of SOC, TN, and TP and soil C:N, C:P, and N:P ratios varied with the soil profile and vegetation types (Figure 1a-f). SOC, STN, and STP contents were 125.4% %, 16.9 higher in RP40a than AB and CK40a among vegetation types in 0–20 cm soil depth. The SOC, TN, and TP
content in RP40a was higher than in SC by 22.8%, 134.5%, and 253.1% at 0–20 cm soil depth and 7.8%, 254.6%, 326.6% at 20–40 cm soil depth. Moreover, the SOC content, TN, and TP at 0–20 cm were higher than at 20–40 cm soil depth in RP40a by 23.2%, 28.3% and 33.0%.

**Figure 1.** Characteristics of SOC, TN and TP content and soil C:N:P stoichiometry after afforestation. Different capital letters indicate a significant difference among different vegetation types at P<0.05 level in the same soil depth, different small letters indicate significant difference among same vegetation types at P<0.05 level.

Soil C:P and N:P ratios increased among different vegetation types (Figure 1d-f) relative to SC. The soil C:P ratio in AB, CK40a, and RP40a were 187.5%, 58.8% and 31.0% higher than SC at 0-20 cm soil depth, and 331.7%, 204.3% and 9.4% higher at 20-40 cm soil depth, respectively. Likewise, the N:P ratio in AB, CK40a, and RP40a were also 498.1%, 131.8%, 20.2%,
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Soil C, N, and P stoichiometry differs with vegetation type and is highly complex (Zhang et al., 2013). In our study, soil C:P and N:P ratios were significantly higher in RP40a and CK40a than in SC (Figure 1e, 1f; p<0.05), which suggested that the conversion of farmland to forest significantly changed the nutrient cycle and that a large amount of phosphorus was consumed to balance C and N rehabilitation. Thus, afforestation increased the ratios of C:P and N:P, which may result in the occurrence of a relative soil phosphorus deficiency. However, the ratios of C:N were not the same as the other two ratios (C:P, N:P), which indicated that afforestation did not significantly influence C:N (p<0.05). Thus, the balance between carbon and nitrogen did not change during the rehabilitation process. The results were consistent with other studies, which found that the different types of land use exhibited different soil C:N:P ratios due to differences in vegetation type and land management practices (Zhao et al., 2015; Li et al., 2012; Aponte et al., 2010).

SOC, TN, and TP contents in soil aggregates after afforestation.

The differences in SOC, TN, and TP content in soil aggregates between the three forest/shrub types (RP40a, CK40a, and AB) and SC are shown in Figure 2. The forest/shrub SOC content in soil aggregates was 203.9%, 160.0% higher than SC at 20–40 cm and 0–20 cm soil depth, respectively (Figure 2a, 2b). Moreover, the content of SOC in small macro-aggregates (0.25–0.5 mm, 0.5–1 mm, 1–2 mm, and 2–5 mm) was higher than the others. For example, the content of SOC in 0.5–1 mm aggregate in RP40a was 19.5% higher than >5 mm aggregate, which in turn was 91.2% higher than <0.25 mm aggregate at 0–20 cm soil depth.

Similar trends were found in the TN content of different sized aggregates (Figure 2c, 2d). Relative to SC, on average TN content was 226.9%, 185.6% higher in forested soils at 20–40 cm and 0–20 cm soil depth, respectively. In addition, the content of TN in small macro-aggregates (0.25–0.5 mm, 0.5–1 mm, 1–2 mm, and 2–5 mm) was also higher than others. The content of TP in soil aggregates was higher in the forested plots than in SC, however, the difference was not significant (Figure 2e, 2f). Meanwhile, the TP content in soil aggregates was higher in large macro-aggregates (>5 mm) than in micro-aggregates (<0.25 mm) after afforestation.

Soil aggregates >0.25 mm were strongly related to soil erodibility (Gao, 1991). Revegetation was observed to be an efficient means of increasing soil aggregate stability on the Loess Plateau (An et al., 2013), and it could have enhanced carbon stocks in small macro-aggregates (0.25–2 mm) (Li et al., 2015). We found that SOC content in small macro-aggregates (0.25–5 mm) was higher than large macro-aggregates (>5 mm) and micro-aggregates (<0.25 mm), which suggested that the small macro-aggregates were favorable for the improvement of soil quality in our study area. Our results are in line with the findings of Wei et al. (2012a), Haile et al. (2008), and Cheng et al. (2015) but contrary to An et al. (2013). Although vegetation and land use are key factors affecting soil stability (Cerdà, 1998, 2000), the difference in these results could be due to the various ages of the rehabilitated plots (Cheng et al., 2015), physical control (Arjmand Sajjadi & Mahmoodabadi, 2015), and amendments such as humic acid applications on aggregate stability (Pulido Moncada et al., 2015). In addition, micro-aggregates (<0.25 mm) are formed within macro-aggregates (Oades, 1984) after the binding agents in macro-aggregates have degraded. Thus, micro-aggregates have lower SOC content than small macro-aggregates. The main links to large macro-aggregates
 (>5 mm) are plant roots, whereas small macro-aggregates (0.25–5 mm) are largely associated with clay particles and are probably linked to each other by roots, hyphae, and calcium carbonate (Cheng et al., 2015). Thus, small macro-aggregates have a more robust network than micro and larger macro-aggregates. Therefore, we concluded that small macro-aggregates (0.25–5 mm) facilitated carbon sequestration in soil during vegetation restoration.

![Figure 2](image)

**Figure 2.** Distribution of SOC, TN and TP contents in soil water-stable aggregates after afforestation. Different small letters indicate significant differences among different size aggregates under the same vegetation types at P<0.05 level, different capital letters indicate significant differences among vegetation types in the same size aggregates at P<0.05 level.

**Response of soil C, N, and P stoichiometry to SOC, TN, and TP contents in soil aggregates.**

SOC, TN, and TP contents in soil aggregates were significantly affected by soil C:N, C:P, and N:P ratios after afforestation (Table 2, Figure 3). The BEST model procedure indicated that
Soil C:P and N:P ratios significantly affected the SOC, TN, and TP contents in soil aggregates (Table 2). However, soil N:P ratio had influenced only the SOC and TP in soil aggregates, and C:N, C:P, and N:P ratios had affected the TN content in soil aggregates under different vegetation types.

The RDA analysis indicated that soil C:P and N:P ratios were the most significant factors influencing SOC$_{0.25-1mm}$, SOC$_{1.2mm}$, TN$_{>5mm}$, TN$_{0.5-1mm}$, TP$_{0.25-1mm}$, and TP$_{0.5-1mm}$ at 0–20 cm soil depth (Figure 3). Furthermore, the SOC$_{<0.25mm}$, TN$_{<0.25mm}$, TN$_{>5mm}$, and TP$_{<0.25mm}$ at 20–40 cm soil depth was affected by the soil C:P and N:P ratios. As a whole, soil the C:P and N:P ratios affected the content of TP content in small macro-aggregates (0.25–0.5 mm, 0.5–1 mm, 1–2 mm, and 2–5 mm) in surface soil (0–20 cm) as well as in large macro-aggregates and micro-aggregates (>5 mm and <0.25 mm) in subsoil (20–40 cm).

The interplay between the soil nutrient cycling processes involved in aggregate formation is highly complex and affected by a range of soil properties. Various factors have been reported in the literature to affect aggregation, especially SOC, TN, and TP content in soil aggregates (Wei et al., 2012b), organic binding agents (Qiu et al., 2012), and soil texture (Wei et al., 2012a). However, our results indicated that soil C:P and N:P ratios were the main factors that influenced SOC, TN, and TP content in small macro-aggregates (0.25–0.5 mm, 0.5–1 mm, 1–2 mm, and 2–5 mm) in surface soil (0–20 cm) after afforestation (Figure 2). Adding different types of plant biomass, such as litter and fine roots, to the soil has
different effects that result in the variation of soil C, N, and P concentration/ratios under different vegetation types. These effects can lead to significant variations in SOC, TN, and TP contents in soil aggregates (Lehmann et al., 2001; Shepherd et al., 2001; Ren et al., 2016). On the other hand, evidence indicates that the SOC, TN, and TP contents in soil aggregates can respond to afforestation through changes in the soil enzyme activities (Roldán et al., 2005; Udawatta et al., 2008), which closely reflects the degree of changes in soil C, N, and P concentration/ratios (Ren et al., 2016). These results agree with García-Gil et al. (2000). Wu et al. (2012) reported that the increase in β-glucosidase from poorly to better structured soils was likely related to changes in soil nutrients (mainly C and N). Nie et al. (2014) also found that increased enzyme activities related to C decomposition with decreasing aggregate size may be due to a higher SOC and TN concentration in soils.

More importantly, SOC, TN, and TP content in soil aggregates might be altered due to changes in the soil microbial community (Deng et al., 2014), which is tightly related to the soil C:N:P ratio (Ren et al., 2016; García-Franco et al., 2015). Differences in the C nutrient ratios closely reflect those of fungal hyphae vs. bacteria and macro-aggregates vs. micro-aggregates (Singh & Singh, 1995; García-Franco et al., 2015). It was also reported that macro-aggregates were believed to have more fungal-dominated microbial communities while bacteria were assumed to be dominant in micro-aggregates (Väisänen et al., 2005). Makino et al. (2003) indicated that heterotrophic bacteria could regulate their elemental composition homeostatically, which results in relatively narrow ranges of C:P and N:P ratios. The reasons for this variability might be that microorganisms, which are able to take up resources in excess and to store them in the form of glycogen or polyphosphates, leading to changes in the soil C:N:P ratio and affecting SOC, TN, and TP content in soil aggregates (Achbergerová & Nahálka, 2011). Considering the number of factors that affect nutrient cycling in aggregate formation in a wide range of soil types, our results indicated that soil the C:N:P ratio contributed significantly to variances in soil aggregate nutrients. To better understand this complex phenomenon, additional studies are needed to quantify the relationships between microbe abundance and enzyme activities under various soil management and land use practices.

**CONCLUSION**

Our study found that 40 years of afforestation on abandoned land and cropland significantly improved soil structure and resulted in the accumulation of SOC in bulk soils and macro-aggregates. SOC content in small macro-aggregates (0.25–5 mm) was higher than in large macro-aggregates (>5 mm) and micro-aggregates (<0.25 mm), which indicated that small macro-aggregates (0.25–5 mm) were the major contributor to SOC accumulation in afforested soil. Moreover, soil C:P and N:P ratios were the main factor that influenced SOC, TN, and TP content in small macro-aggregates (0.25–0.5 mm, 0.5–1 mm, 1–2 mm, and 2–5 mm) in surface soil. The results suggested that SOC accumulation in afforested soils was due to its accumulation in small macro-aggregates and this increase was largely affected by soil C:N:P stoichiometry in surface soil after afforestation.
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