C:N:P stoichiometry and nutrient storage at the ecosystem level in different secondary mixed forest types in the Qinling Mountains, China (#44136)

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C:N:P stoichiometry and nutrient storage at the ecosystem level in different secondary mixed forest types in the Qinling Mountains, China

Yue Pang [1] , **Jing Tian** [1] , **Xuan Zhao** [1] , **Zhi Chao** [1] , **Yuchao Wang** [2, 3] , **Xinping Zhang** [4] , **Dexiang Wang** Corresp. [1]

[1] Ecology, College of Forestry, Northwest A&F University, Yangling, China

[2] Institute of Botany of Shaanxi Province, Xi'an, China

[3] Shaanxi Engineering Research Centre for Conservation and Utilization of Botanical Resources, Xi'an, China

[4] School of art and design, Xi'an university of technology, Xi'an, China

Corresponding Author: Dexiang Wang
Email address: wangdx66@sohu.com

Background. Carbon (C), nitrogen (N), and phosphorus (P) are the main nutrient elements required for plant development, and their stoichiometric ratios and storage are important indicators of ecosystem function and productivity. However, few studies have assessed nutrient stoichiometry and storage characteristics at the ecosystem level, especially in mixed forest types.

Methods. We investigated the C, N, and P concentrations and stoichiometry ratios in trees, understory plants, litter, and soil layers in three different secondary mixed forest types, broadleaf mixed forests (BM), broadleaf-conifer mixed forests (BCM) and coniferous mixed forests (CM), in the Qinling Mountains.

Results. The results showed that significant differences in C:N:P stoichiometry were detected in multiple organs in the plant layers in the different forest types. Trees, shrubs and herbs all allocated more N and P in leaves and had a higher N:P ratio in leaves than in other organs. The C concentrations, C:N ratios and C:P ratios of all tree organs showed a decreasing order: BM < BCM < CM, while the N and P concentrations showed an increasing order: BM > BCM > CM. The leaf N:P ratios of all plants were less than 14. For litter and soil, BM had generally higher N and P concentrations than those of BCM and CM. The highest N and P storage was in tree branches-not in the stem, which had the highest biomass (except for P in CM). Compared with other forest types, CM shared more nutrients stored in the labile litter layer, while BM shared more nutrients stored in the stable soil layer. The net ecosystem nutrient element storage in BM was generally higher than that in BCM and CM.

Conclusion. Our findings demonstrate that BM has more advantages in C, N and P nutrient fertility and storage than BCM and CM in secondary succession communities, and this research imply the necessity of artificial intervention in secondary forests toward broadleaf mixed forests.

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[1]College of Forestry, Northwest A&F University, Yangling, 712100, Shaanxi, China
[2]Institute of Botany of Shaanxi Province, Xi’an, Shaanxi, China
[3]Shaanxi Engineering Research Centre for Conservation and Utilization of Botanical Resources, Xi’an, Shaanxi, China
[4]School of art and design, Xi'an university of technology

Corresponding Author:
Dexiang Wang[1]
College of Forestry, Northwest A&F University (No.) 3 Taicheng Road Yangling District, Shaanxi, 712100, China
Email address: wangdx66@sohu.com

Abstract

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Keywords C:N:P stoichiometry, nutrient storage, multiple organs, secondary mixed forest ecosystem

Introduction

A forest ecosystem is a collection of coordinated and unified development of material and energy, and forest nutrients are the cornerstone of a forest ecosystem community's survival, reproduction and development (Jordan 1985; Sharma & Sharma 2004; Waring & Schlesinger 1985). Carbon (C), nitrogen (N) and phosphorus (P) are key elements for ecosystem organism construction and play vital roles in ecosystem biogeochemical cycles and community succession (Jr et al. 2006; Song et al. 2014). Many studies have reported the C, N and P nutrient characteristics of vegetation, soil and litter in forest ecosystems (Cremer et al. 2016; Frédéric et al. 2010; Inagaki et al. 2004), directly promote the research process of the forest ecosystem nutrient cycle. However, these studies independently studied the nutrient characteristics of different components of the ecosystem, ignoring the overall attributes of the ecosystem. In nature, ecosystems are composed of biological communities and abiotic environments, and close correlations exist between different components of the ecosystem (Zhang et al. 2018a).

Therefore, investigations of the C, N and P nutrient characteristics, including diverse ecosystem components, might better reflect the nutrient cycling attributes in forest ecosystems and provide a better foundation for improving ecological models.

The global forest area is now approximately 4 billion ha (FAO 2015). Among this area, secondary forests (regenerating through natural processes after significant human or natural disturbance of the original forest vegetation) account for 59.5% of the forest cover and provide important ecosystem services, such as species conservation, carbon sink improvement, and water resources protection (Grimwood & Dobbs 2010; Mcdonald et al. 2002; Orihuela-Belmonte et al. 2013). Due to the differences in the time and intensity of disturbance factors, secondary forests are mainly mixed communities composed of multiple species (Peña-Claros 2003). This coexistence pattern of multiple species will lead to competition for resources and space between aboveground (canopies) and underground (roots) components (Cremer et al. 2016; Shanin et al. 2014), showing diversified niche partitioning and ecosystem combination (Büttner & Leuschner 1994; Prescott 2002), and may finally modify the allocation patterns of C, N and P among different levels in the ecosystem. Although most previous studies have analyzed soil carbon stocks, species composition and community structure, productivity and diverse ecological processes in secondary forests (Chai & Evj 2011; Fonseca et al. 2011; Kenzo et al. 2010), the C, N and P nutrient patterns at the ecosystem level in different secondary mixed forest communities remain unclear. This insufficient knowledge might lead to the inaccurate estimation of secondary forest nutrient reserves and underestimate the important role of secondary forests in the nutrient cycle (Attiwill & Adams 1993; Mcdonald & Healey 2000). Accordingly, exploring the C, N and P nutrient characteristics in different secondary mixed forests is urgently needed to meet the challenge of managing C and nutrient stocks worldwide.

A classical concept in the field of plant ecology indicates that organisms and their environments are closely connected by the exchange of chemical elements, and there is reciprocal control between different components in the ecosystem (Ladanai et al. 2010; Odum et al. 1972). Ecological stoichiometry, which focuses on the interaction of chemical resources (elements) in organisms and the environment, has been regarded as a scientific and effective approach for exploring the balance of energy and materials among the components in an ecosystem (Kennish 2016). Previous studies have analyzed the C:N:P stoichiometric characteristics of plant organs, litter and soil at regional and global scales to reveal nutrient limitations of plants, nutrient cycling and feedback relationships (Han et al. 2005; Yang et al. 2018). These studies have advanced our understanding of ecosystem stoichiometric characteristics to some extent; however, for plant stoichiometry, these studies have mainly focused on certain organs, such as leaves and roots. In fact, different plant organs play different functional roles, resulting in differences in nutrient concentrations among organs (Kerkhoff et al. 2006; Zhang et al. 2018c). Typically, plant leaves are responsible for photosynthesis and have higher nutrient concentrations (Wright et al. 2004). Therefore, it is important to quantify nutrient element stoichiometric variation in multiple plant organs, which will provide further insights into the functional diversity of vegetation and be useful in guiding forest management decisions ranging from forest health concerns to designing appropriate deforestation and fertilization programs.

The Qinling Mountains run across central China and are characterized by typical mountain forest. Forests in the Qinling Mountains suffered from extensive logging during the 1960s and 1970s, which promoted the regeneration of diverse secondary forests. To advance natural forest resource protection and improve the ecological environment, the Chinese government initiated the "Natural Forest Protection Program" (NFPP) (Xu et al. 2006). Through decades of hard work, the project has enhanced the plant community diversity and improved the primary productivity of forest ecosystems (Zhang et al. 2011), and this project has been particularly beneficial for the formation of secondary forest (Cao & Chen 2015). Now, secondary forests account for 80% of the Qinling forest area, which has become a typical secondary forest area in China (Chai et al. 2016). Previous studies have analyzed the structural characteristics of the community, soil nutrient characteristics, plant leaf C:N:P stoichiometry and microbial diversity among these secondary forests (Hou et al. 2018; Shi et al. 2019; Zhang et al. 2018b; Zheng et al. 2017). However, information about the effects of different mixed forest types on ecosystem C:N:P stoichiometry and nutrient storage characteristics has rarely been evaluated.

In this study, we determined the C, N, and P concentrations and stoichiometric ratios in trees, understory plants, litter, and soil collected from three different secondary mixed forest types, namely, broadleaf mixed forests (BM), broadleaf-conifer mixed forests (BCM) and coniferous mixed forests (CM), in the Qinling Mountains. We hypothesized that the C:N:P stoichiometries of different plant organs, litter and soil were diverse among different secondary mixed forest types. In addition, we predicted that the nutrient cycling rate and nutrient element storage were higher in BM than in BCM and CM. Therefore, the objectives of this research were to (1) examine the C, N and P concentrations and stoichiometric characteristic differences of multiple plant organs, litter and soil among different secondary mixed forest types; (2) quantify the nutrient storage capacity of the C, N and P elements in different secondary mixed ecosystems; and (3) provide suggestions for nutrient reserve management in secondary forest ecosystems.

Materials & Methods

Study site description

The field research was conducted at the Qinling National Forest Ecosystem Research Station (Huoditang Experimental Forest Farm of Northwest A&F University) in Ningshaan County (33°18′-33°28′N, 108°21′-108°39′E), Shaanxi Province, China. The landform of the station is characterized by an abrupt and broken landscape, with altitudes ranging from 800 to 2500 m and a mean slope of approximately 35°. The soil in this area is composed of Cambisols, Umbrisols and Podzols (FAO), and the mean soil depth is 50 cm. This region has a subtropical humid montane climate, with an average annual precipitation of 1000 mm. Over 50% of the precipitation falls from July to September, and the average annual humidity is approximately 77%. The average temperature is 10.5 °C, with an extreme minimum temperature of -9.5 °C and an extreme maximum temperature of 35 °C. The plant growth period is approximately 177 days, and the average frost-free period is approximately 199 days. The forests had been harvested or experienced man-made destruction during the 1960s and 1970s in the Huoditang Experimental Forest Farm, and much of the area is now covered by secondary growth. Currently, the main tree species in this area are *Ouercus aliena var. auteserrata*, *Quercus variabilis*, *Pinus armandii*, *Betula albosinensis*, *Picea asperata*, *Populus davidiana* and other broad leaf species.

Experimental design

The study was conducted from July to August 2017 at the Huoditang Experimental Forest Farm. Based on the vegetation deforestation history and restoration status, three secondary mixed forest types (BM, BCM and CM) were selected. Detailed information about each secondary mixed forest type is presented in Table 1. All selected sites were located on similar slopes, aspects, slope gradients, and elevations. Each secondary mixed forest type was represented by three independent replicate sites, and the space between any two sites was large enough to exclude spatial dependence for the soil variables. Three replicate plots (20 × 20 m) were randomly established at each site for the subsequent plant, litter and soil sampling (Fig.1). For each plant, litter and soil variable, the average value of the three replicated plots was taken as the observation for the whole site. Finally, in total, 9 observations were established (three different secondary mixed forest types × three replicate sites) for each variable.

Plant, litter and soil investigation and sampling

The diameter at breast height (DBH, 1.3 m) of all trees in each plot was measured, and the trees were also classified and counted by species. Additionally, different organ samples of trees were obtained. Newly mature and healthy leaves and branches (diameter <1 cm) were picked from the east, west, south and north directions in the upper, middle and lower parts of the canopies, and a machete and an increment bore we used to obtain the bark and stem samples, respectively, at the DBH location. Roots (diameter <1 cm) were dug up from the 0-60 cm soil layer, and root samples were collected by removing the surrounding soil along a main root of a specific plant species until the roots appeared. These organ samples from the trees were oven dried at 70°C to constant weight. Based on the DBH and tree height, the biomass of the components (leaves, branches, stems, bark and roots) of each tree species in the three secondary mixed forest plots was calculated using published species-specific allometric equations developed for trees within or near the study area (Table S1). To better reflect the relative contributions of multiple tree species at the community level, the different organ samples of the tree were mixed according to the ratios calculated from the allometric equations.

Shrub and herb biomass were determined using total harvesting destructive sampling techniques. Five shrub subplots (2 × 2 m) and five herb subplots (1 × 1 m) were established along the diagonals of each plot for sample collection. Shrub plants were separated into leaves, stems and roots, and herbs were separated into aboveground and belowground components. For litter sampling, all organic material within five 1 × 1 m subplots was collected from each plot. There were no corresponding allometric equations for shrubs and herbs in the study area, and the same components of shrubs, herbs and litter were mixed uniformly into one sample. Finally, the subsamples of shrub, herb and litter were transported to the laboratory and oven dried at 70 °C to a constant weight.

For soil sampling, nine replicate sampling points were established along an "S" shape in each plot. After removing the litter layer and biological crusts, nine soil samples at 0-20 cm, 20-40 cm and 40-60 cm were obtained separately from each point using a soil auger (400-mm inner diameter) and were fully homogenized to form one composite soil sample for each soil layer in each plot. The plant roots, fauna, and debris were removed by hand, and the gravel (rock fragments >2 mm) was reserved to measure the percentage of stones. The remaining soil samples were sieved (< 2 mm) and air dried at room temperature for chemical property analysis. Soil bulk density (BD) samples were obtained randomly from three points per plot by volumetric rings (100 cm3). The nutrient element storage of C, N and P in each soil layer was calculated using the following equation:

$$S_n = C_n \times BD_n \times L_n \times 10^{-1}$$

Where S_n is the C, N and P storage of soil in the n-th soil layer ($t \cdot ha^{-1}$); C_n, BD_n, and L_n are the C concentration ($mg \cdot g^{-1}$), soil bulk density ($g \cdot cm^{-3}$), and soil depth (cm) of the n-th soil layer, respectively; and 10^{-1} is the unit conversion factor.

Plant, litter and soil physicochemical measurements

The C, N, and P concentrations in the tree, shrub and herb organs and litter were analyzed after the samples were ground into a powder with a plant-sample mill (1093 Sample Mill, Hoganas, Sweden). The organic carbon (OC) contents of the plant, litter and soil samples were measured using the $K_2Cr_2O_7$ oxidation method. The total nitrogen (TN) and total phosphorus (TP) concentrations of the plant, litter and soil samples were determined with an automatic discontinuous elemental analyzer (Clever chem200+, Germany) after digestion with H_2SO_4 and H_2O_2. The volume of gravel (rock fragments >2 mm) was measured using the drainage method. The soil BD was determined using the soil core method and obtained by calculating the ratio of soil mass to total volume ($g \cdot cm^{-3}$) after oven dried at 105 °C to a constant weight.

Data analyses

The total ecosystem C, N and P storage values were based on the combination of trees, shrubs, herbs, litter and soil pool. The mean and standard error of the investigated variables (e.g., C, N and P concentrations, C, N and P stocks, C:N, C:P and N:P ratios) of plant organs, litter and soil mixtures were calculated for each organ, site and soil depth separately. Data were checked for normality and homogeneity of variance and, if necessary, were transformed. The effects of organ, soil layer and forest type on the concentration, stoichiometry and stocks of the nutrient elements (C, N and P) were tested using one-way ANOVA and least significant difference (LSD) multiple comparison ($p < 0.05$). All statistical analyses were performed using R version 3.5.0 (R Development Core Team 2018).

Results

Plant and litter biomass and soil bulk density

The biomass of plant organs was generally different among different organs and forest types (Fig. S1A, B). The highest biomass occurred in the stem for tree, root for shrub and aboveground portion for herb. Inconsistent biomass patterns of plant organs were observed in plant layers among different forest types; however, these patterns were ~~usually~~ nonsignificant. For total plant biomass (Table S2), the shrub total biomass in BCM was significantly higher than that in CM, and there were no significant differences between BCM and BM. The herb total biomass in CM was significantly higher than that in both BM and BCM. Although the tree total biomass was most often nonsignificant in the three forest types, it accounted for more than 96% of the ecosystem total plant biomass in all forest types. In addition, the litter biomass in CM was significantly higher than that in BM and BCM (Table S2).

Only in the BM was the soil BD of the 0-20 cm soil layer significantly lower than that of the 40-60 cm soil layer, although it was not statistically significant among the different soil layers in the BCM and CM (Fig. S1C). There was no notable difference in soil BD at the same soil layer between different forest types (Fig. S1C).

C:N:P stoichiometric characteristics in ecosystem components

The stoichiometry varied greatly in different plant organs, litter and soil layers under different forest types. In the tree layer, the C concentration was nonsignificant between different organs for all forest types (Fig. 2A). The C concentration of all organs in CM was notably higher than that in BM, while it was similar in BCM and the other forests (Fig. 2A). Among the tree organs in the three forest types, the leaves and stem had significantly higher and lower N and P concentrations, respectively, than the other organs (Fig. 2B, C). The N and P concentrations in all tree organs had the same pattern among the different forest types, showing the increasing order of BM > BCM > CM (Fig. 2B, C). In contrast, leaves and stems had the lowest and highest ratios of C:N and C:P for all forest types, respectively, showing a decreasing order of BM < BCM < CM (Fig. 2D, E). The N:P ratio in leaves was notably higher than that in other organs among all forest types (except leaves and branches in CM) (Fig. 2F).The N:P ratios of branches and bark in CM were significantly higher than those of the other two forest types, while the values were typically nonsignificant in other organs in all forest types (Fig. 2F).

In the shrub layer, the highest C concentration was observed in the branches for the three forest types, while the highest N and P concentrations were in the leaves (Fig. 3A-C). A significant difference was observed for only C and P in leaves and for N in branches among forest types. Shrub branches and leaves had the highest and lowest ratios of C:N and C:P for all forest types, respectively, while the highest N:P ratio was observed in leaves (Fig. 3D-F). Significant differences were observed for C:N in branches, C:P in leaves and N:P in leaves and branches among forest types. In the herb layer, the aboveground leaf C, N and P concentrations were significantly higher than those in the underground root, while the aboveground leaf C:N and C:P ratios were significantly lower than those in underground root (except for C:P in BCM)

(Fig. 3A-E). A significant difference was observed only for C in leaves, for P in roots and for C:P in both leaves and roots among forest types. Although the herb N:P ratio was nonsignificant among different organs, it was generally higher in leaves than in roots (Fig. 3F). The significant difference for N:P was observed only in leaves among forest types. In the litter layer, the C:N:P stoichiometric characteristics were similar to the results of the tree layer (except for N:P). The C concentration was generally higher in CM than in BM and BCM (Fig. 3A). The N and P concentrations in BM were significantly higher than those in BCM and CM, while the opposite trend was observed, i.e., the C:N and C:P ratios were significantly lower in BM than in BCM and CM (Fig. 3B-E). BM and BCM had relatively higher N:P ratios than CM (Fig. 3F).

For the soil level, the concentrations of C, N and P and the ratios of C:P and N:P in topsoil (0-20 cm) were significantly higher than those in undersoil (20-40 cm and 40-60 cm), while the C:N ratio was nonsignificant among different soil layers (Fig. 4A-F). The C and N concentrations in CM were significantly lower than those in BCM and BM only in topsoil, while the P concentration was significantly higher in the 0-20 cm and 20-40 cm soil layers in BM than in BCM and CM (Fig. 4A-C). The C:P and N:P ratios in the 0-20 cm and 20-40 cm soil layers in BCM were significantly higher than those in BM and CM, while the C:N ratio was nonsignificant among the different forest types (Fig. 4D-F).

C, N and P nutrient storage in ecosystem

The C, N and P nutrient storage varied greatly in the different plant organs, litter and soil layers in the different forest types (Figs. 5-7, Tables 2-4). For the plant layer, the highest C storage was observed in the stems of trees, roots of shrubs and leaves of herbs, while the highest N and P storage was generally observed in branches of trees (except for P in BCM and CM), roots of shrubs (except for N in BCM) and leaves of herbs (Figs. 5-7A, B). The C storage of tree stems in CM was significantly higher than that in BM, while the N and P storage values of tree branches in BM were significantly higher than those in BCM and CM (Figs. 5-7A). Additionally, the C, N and P storage values of shrub leaves and branches in BCM were generally higher than those in the other two forest types, while the C, N and P storage values of herb leaves in BM and BCM were considerably lower than those in CM (Figs. 5-7B). Regarding nutrient element storage of total plant biomass, the C, N and P storage values of shrub biomass in BCM were generally higher than those in BM and CM, while the C, N and P storage values of herb biomass in CM were significantly higher than those in BM and BCM (Tables 2-4). The nutrient element storage of total tree biomass had the highest percentage among the plant layer, and the P storage of total tree biomass in BM was significantly higher than that in CM (Tables 2-4). For the litter layer, the share of C, N and P stored in litter biomass in CM generally exceeded that in BM and BCM (Tables 2-4).

For the soil layer, the storage of C and N in the mineral topsoil (0–20 cm) was significantly higher than that in the undersoil (20-60 cm), while the P storage was nonsignificant among the different soil layers (Figs. 5-7C). Although there was no notable difference in nutrient element storage at the same soil layer among different forest types, the nutrient element storage in BM was generally higher than that in BCM and CM (Figs. 5-7C). Regarding the total soil nutrient element storage and net ecosystem nutrient element storage, the C, N and P storage values were all nonsignificant under the three forest types (Tables 2-4). However, the soil layer had the highest nutrient element storage among different ecosystem components, and both the total soil nutrient element storage and the net ecosystem nutrient element storage in BM were generally higher than those in BCM and CM (Tables 2-4).

Discussion

C:N:P stoichiometric characteristics in ecosystem components

In our study, significant differences in C:N:P stoichiometry were detected in multiple organs in the plant layer under the different forest types (Figs. 2-3). This difference is because the different taxonomic groups of plants have diverse genetic characteristics (Thiel-Egenter & Gugerli 2010), which determine the formation of multifunctional plant organs and control the complex physiological process of vegetation (Tian et al. 2015), ultimately leading to the differences in nutrient characteristics in multiple plant organs. Simultaneously, to adapt to the environment, plants gradually developed strong physiological and biochemical regulation abilities during the long course of evolution (Sterner & Elser 2002). Therefore, plants can actively adjust their demand for specific nutrient elements (Gong et al. 2017), which consequently results in C:N:P stoichiometric differences between plant organs (Sistla & Schimel 2012). Different plant functional groups (tree, shrub and herb) have a common set of rules that allocate more N and P in leaves and have a higher N:P ratio in leaves than in other organs (Figs. 2-3B, C, F). This finding aligns with previous studies ~~that~~ showed that plant leaves had higher nutrient concentrations than non-leaf organs (Hong et al. 2014; Zhang et al. 2018c). Leaves are responsible for many physiological functions (e.g., photosynthesis, transpiration and respiration) and require higher quantities of N and P to complete diverse biochemical processes (Minden & Kleyer 2014). Furthermore, leaves with higher metabolic activity (metabolic organs) can maintain a relatively constant higher N:P ratio to meet the physiological needs of metabolic processes, while structural organs, which have P concentrations that increase faster than N concentrations during growth, have a lower N:P ratio (Kerkhoff et al. 2006; Zhang et al. 2018a). These results imply that both the genetic characteristics and the environmental factors may have led to the diverse C:N:P stoichiometric characteristics among plant organs and that more metabolically active organs had higher N and P nutrient concentrations.

The C concentrations, C:N ratios and C:P ratios of all tree organs showed a decreasing order of BM < BCM < CM, while the N and P concentrations showed an increasing order of BM > BCM > CM. (Fig. 2A-E). These findings correspond with those of Cao and Chen (2017) and Han et al. (2005), who reported higher C concentrations, C:N ratios and C:P ratios in coniferous than in deciduous species and higher N and P concentrations in deciduous than evergreen species. On the one hand, conifers have many kinds of structural carbohydrates (C-rich), such as lignin, tannins and waxes, and lower N and P contents, resulting in higher C concentrations, C:N ratios and C:P ratios (Thomas & Martin 2012). On the other hand, the higher C:N and C:P ratios reflect higher plant N and P use efficiency (Ge & Xie 2017). Coniferous species are often confined to nutrient-limited habitats (Aerts & Iii 1999), but still maintain normal growth. The coniferous species have a higher N and P utilization efficiency, leading to higher C:N and C:P ratios. Moreover, a previous study proposed that the nutrient supply status can determine the nutrient concentrations in plant organs (He et al. 2008). In our study, the soil N and P concentrations were higher in BM than in the other forest types (Fig. 4B, C), which may have caused higher N and P contents and lower C:N and C:P ratios in organs in BM than in BCM and CM. In contrast, the C, N and P concentrations and stoichiometry of understory plants were also significantly different among the forest types, but the concentrations where different in different organs, with no consistent pattern among forest type (Fig. 3). A possible explanation for these results may be that different plant functional groups show some degree of below-ground niche partitioning and have different root depth distributions (Büttner & Leuschner 1994), leading to understory plants having different nutrient utilization strategies from trees, ultimately forming diverse nutrient characteristics patterns. A previous study suggested that a leaf N:P ratio <14 indicates N limitation, an N:P ratio between14 and 16 indicates both N and P limitation, and an N:P ratio >16 indicates P limitation (Güsewell 2004). In this study, the leaf N:P ratios of plant layers were all less than 14 (Figs. 2-3F), suggesting the occurrence of N limitation in the study area.

For litter, BM had higher N and P concentrations than BCM and CM, while BM had lower C concentrations, C:N ratios and C:P ratios; these results are consistent with the nutrient pattern of the tree layer (Fig. 3). The findings extend that of Megan et al. (2004), confirming that litter stoichiometric characteristics were generally aligned with those of plants (Megan et al. 2004). This relationship is because litter nutrients are derived from the nutrients in plant organs (Zhang et al. 2017). Additionally, trees can produce more litter biomass than understory species annually (Liu et al. 2018) and may have dominated the nutrient characteristics of litter. In the present study, topsoil (0-20 cm) had significantly higher C, N, and P concentrations and ratios of C:P and N:P than undersoil (40-60 cm) (Fig. 4A-C, E, F). This result is in general agreement with the results of previous studies conducted in forest and grassland systems (Prusty et al. 2009; Yang & Chen 2017). A possible explanation for the result is that topsoil nutrients are mainly affected by the return surface litter and soil microorganisms (Jobbagy & Jackson 2000). With increasing soil depth, the input of organic matter is limited by the permeability of the soil, and microbial decomposition activity gradually decreases (Berger et al. 2002), leading to the striking stratification characteristics of soil nutrients. Among the different forest types, the soil in CM had generally lower C, N and P concentrations and ratios of C:P and N:P than BM and CM (Fig. 4A-C, E, F). This result may be explained by the fact that litter in BM and BCM had relatively higher N and P concentrations (Fig. 3B-C), which can better stimulate microbial activity and invertebrate digestion (Kerkhoff et al. 2006), ultimately benefiting litter decomposition and promoting soil nutrient accumulation. Furthermore, the litter biomass in BM and BCM was notably lower than that in CM (Table S2), which also supported this explanation. In comparison, the C:N ratio was nonsignificant among the different soil layers and in different forest types (Fig.

4D), which may be due to the close temporal coupling of C and N contents in the litter decomposition process, which is consistent with the conclusion from a secondary forest study (Yang & Luo 2011). In general, these results, i.e., higher nutrient concentrations in litter and soil and lower litter biomass in BM, indicate that BM has a higher soil fertility and nutrient cycling rate, which is more conducive to healthy community development and succession.

C, N and P nutrient storage in ecosystem

The highest C storage was observed in the stems of trees, and the highest C, N and P storage values were observed in the roots of shrubs (except for N in BCM) and leaves of herbs (Figs. 5-7A, B). We can explain these findings by the higher levels of biomass in these plant organs (Fig. S1A, B) and the relatively higher nutrient concentration (Figs. 2-3A-C) (PEICHL et al. 2006; Yu et al. 2015). However, the highest N and P storage values in trees are not in the stem, which has the highest biomass (except for P in CM); rather, the highest values were generally in branches (Figs. 6-7A). This result corresponds with the results of Frédéric et al. (2010), who reported that the contribution of stem wood to total nutrient storage was generally lower than its contribution to total biomass. Among the different forest types, the nutrient storage of different organs and the total biomass nutrient storage were significantly different in the plant layers (Figs. 5-7A, B and Tables 2-4). This result is most likely associated with the diversity of species composition, biomass and nutrient concentration, which together determined the nutrient storage in the plant organs and different plant layers (Frédéric et al. 2010; Gong et al. 2017). Considering the management of nutrient element storage in ecological systems, these results suggested that under forest tending, removing only the tree stem from the forest and leaving the other organs in the forest can maintain fertility and promote community stability in the secondary mixed forest ecosystems.

Our study suggested that C, N and P storage in litter biomass in CM generally exceeded that in the BM and BCM (Tables 2-4). This finding agrees with previous studies ~~that~~ found that, compared with broadleaf tree species, conifers tend to store a relatively higher amounts of nutrient elements in a labile litter layer (Cremer et al. 2016). Because conifer litter had higher lignin and C/N ratios and lower Ca concentrations than broadleaf trees, litter decomposition and nutrient release was hampered in conifer forest (Hobbie et al. 2006). The storage of C and N in the topsoil was significantly higher than that in the undersoil because of the addition of litter fall from the more diverse canopy of trees and understory to the surface soil (Kassa et al. 2017). In contrast, the P storage was nonsignificant among the different soil layers (Fig. 7C). Soil P mainly comes from the weathering of soil rock parent material, which is a very slow process, thereby leading to relatively stable P storage under different soil layers (Tian et al. 2010). Nutrient element storage in different soil layers in BM was generally higher than that in BCM and CM (Figs. 5-7C). This result matches the previous conclusions that the annual litter biomass of aboveground and underground components in broadleaf forest is higher than that in coniferous forest (Finer et al. 2007; Li et al. 2005), and the broadleaf forest have more decomposable components and soil biological activity (Augusto et al. 2015), which enhances the soil C, N and

P storage. In total, these results indicate divergent forest nutrient conservation strategies, in which CM share more nutrients stored in the labile litter layer and BM share more nutrients stored in the stable soil layer.

Soil had the highest nutrient storage among ecosystem levels, while overstory trees, understory plants and litter had lower proportions (Tables 2-4). This finding corresponds with that of Wu et al. (2006), who reported that there were larger nutrient reserves in soil layers. This result is most likely because soil nutrient storage is more stable and normally continuously increasing due to the association with soil minerals and protection within aggregates (Lützow et al. 2006). Net ecosystem nutrient element storage in BM was generally higher than that in BCM and CM but with nonsignificant differences (Tables 2-4). This result agrees with the conclusion drawn from a previous study, in which the storage of the C, N and N elements in the coniferous forest was generally lower than that of deciduous species (Cao et al. 2016). However, for nonsignificant differences, this result may be because the community is in the initial stage of succession and has lower nutrient storage in aboveground organism components in our study area (Jiang et al. 2017). Therefore, these results validated our assumption that BM has more advantages in terms of C, N and P nutrient storage than do BCM and CM in secondary succession communities.

Conclusions

Our results demonstrate that stoichiometric and nutrient storage characteristics were significantly different for different components in three secondary mixed forest ecosystems. Significant differences in C:N:P stoichiometry were detected in multiple organs in the plant layer under the different forest types, indicating that genetic characteristics and environmental factors may together lead to diverse stoichiometric characteristics. All plants allocated the most N and P to leaves, suggesting that more metabolically active organs have higher N and P nutrient contents. The leaf N:P ratios of plant layers were all less than 14, suggesting that all plants in the study area are limited by N. Higher nutrient concentrations in litter and soil and lower litter biomass in BM indicate that BM has a higher soil fertility and nutrient cycling rate. The generally higher N and P storage in tree branches not in the stem, which has the highest biomass suggested that in forest tending, removing only the tree stem from the forest and leaving the other organs in the forest can maintain fertility and promote community stability in secondary mixed forest ecosystems. Compared with other forest types, CM share more nutrients stored in the labile litter layer, while BM share more nutrients stored in the stable soil layer. BM has more advantages in terms of C, N and P nutrient storage than do BCM and CM in the secondary succession community. Collectively, our findings imply the necessity of artificial intervention in secondary forests toward broadleaf mixed forests, this research provide valuable data for forest nutrient element storage management and establishing a nutrient cycle model.

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

Yue Pang designed the experiment; Yue Pang, Jing Tian carried out the field work and analyzed the data; Yue Pang wrote the manuscript; and Xuan Zhao, Zhi Chao, Yuchao Wang, Xinping Zhang and Dexiang Wang revising the draft manuscript.

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Figure 1

Geographic location of the Huoditang Experimental Forest Farm and the sampling plots

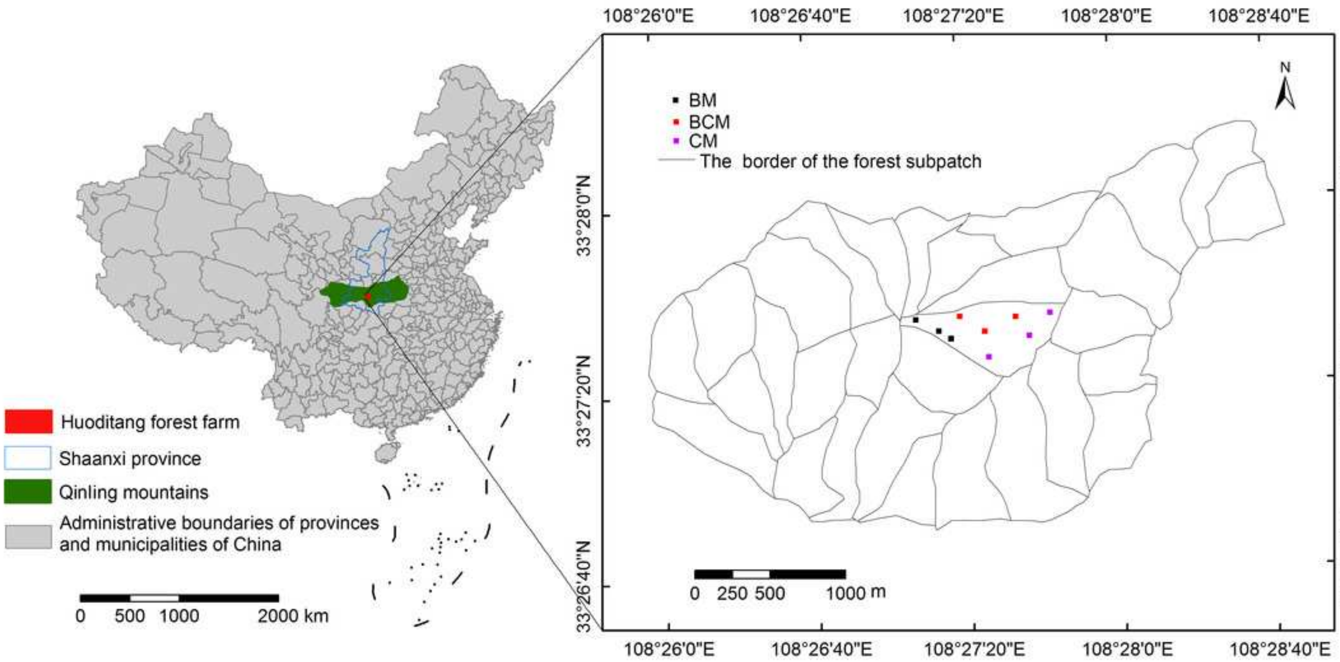

Figure 2

Stoichiometric characteristics of tree organ C, N, and P in three secondary mixed forests

Different lowercase letters above the bars indicate significant differences among different forest types for the same organ ($p < 0.05$), while different uppercase letters indicate significant differences among different organs for the same forest type ($p < 0.05$).

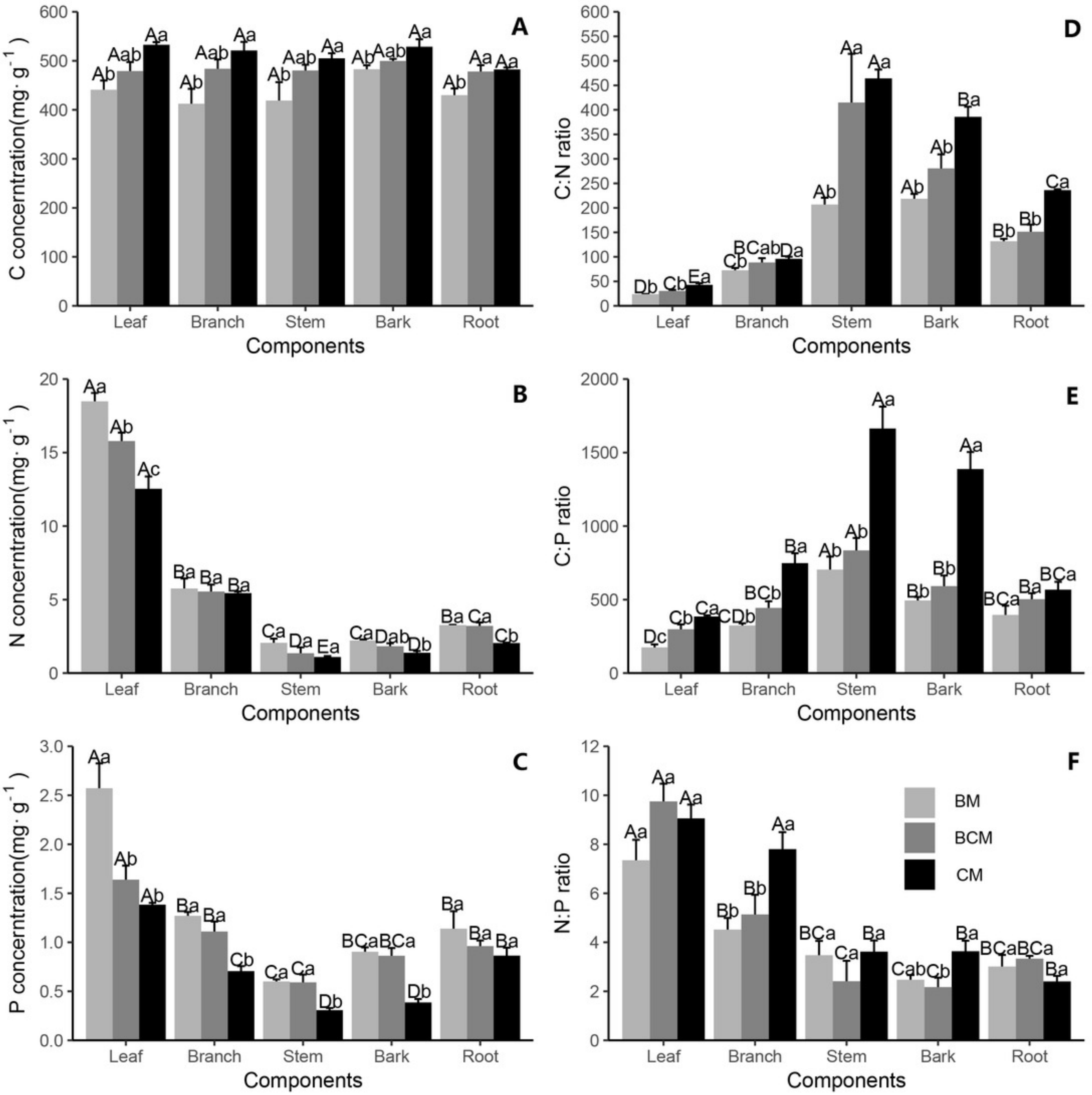
A
C concentration(mg·g^{-1})
0
100
200
300
400
500
600
Ab Aab Aa
Ab Aab Aa
Ab Aab Aa
Ab Aab Aa
Ab Aa Aa
Leaf
Branch
Stem
Bark
Root
Components
D
C:N ratio
0
50
100
150
200
250
300
350
400
450
500
550
600
Db Cb Ea
Cb BCab Da
Ab Aa Aa
Ab Ab Ba
Bb Bb Ca
Leaf
Branch
Stem
Bark
Root
Components
B
N concerntration(mg·g^{-1})
0
5
10
15
20
Aa Ab Ac
Ba Ba Ba
Ca Da Ea
Ca Dab Db
Ba Ca Cb
Leaf
Branch
Stem
Bark
Root
Components
E
C:P ratio
0
500
1000
1500
2000
Dc Cb Ca
CDb BCb Ba
Ab Ab Aa
Bb Bb Aa
BCa Ba BCa
Leaf
Branch
Stem
Bark
Root
Components
C
P concentration(mg·g^{-1})
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Aa Ab Ab
Ba Ba Cb
Ca Ca Db
BCa BCa Db
Ba Ba Ba
Leaf
Branch
Stem
Bark
Root
Components
F
N:P ratio
0
2
4
6
8
10
12
Aa Aa Aa
Bb Bb Aa
BCa Ca Ba
Cab Cb Ba
BCa BCa Ba
BM
BCM
CM
Leaf
Branch
Stem
Bark
Root
Components

Figure 3

Stoichiometric characteristics of shrub and herb organs and litter layer C, N, and P in three secondary mixed forests

Different lowercase letters above the bars indicate significant differences among different forest types for the same organ ($p < 0.05$), while different uppercase letters indicate significant differences among different organs for the same forest type ($p < 0.05$). SL: shrub leaf, SB: shrub branch, SR: shrub root, HA: herb aboveground, HU: herb underground, GL: ground litter.

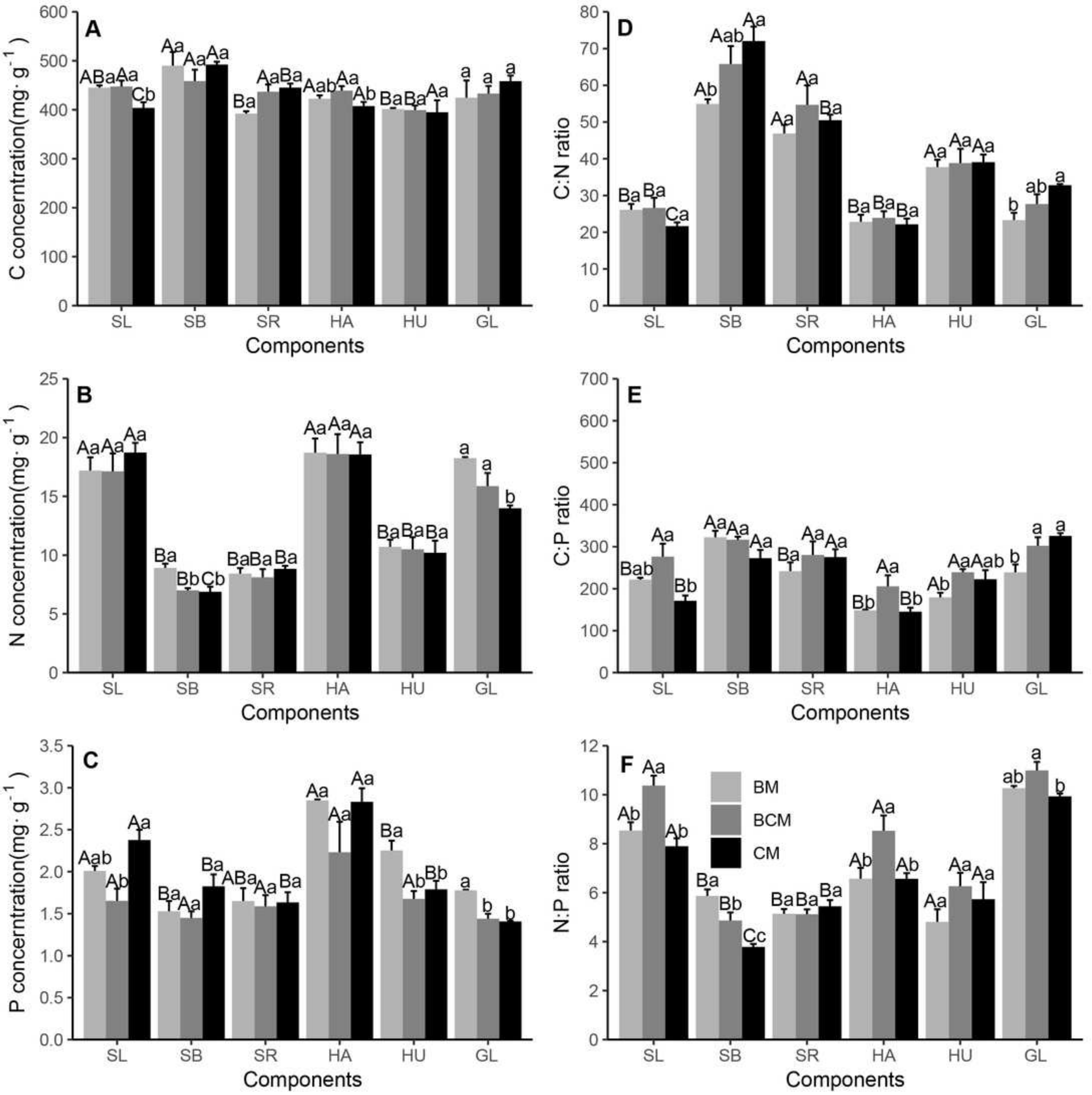
A
C concentration(mg·g^{-1})
600
500
400
300
200
100
0
ABa Aa Cb
Aa Aa Aa
Ba Aa Ba
Aab Aa Ab
Ba Ba Aa
a a a
SL SB SR HA HU GL
Components
B
N concentration(mg·g^{-1})
25
20
15
10
5
0
Aa Aa Aa
Ba Bb Cb
Ba Ba Ba
Aa Aa Aa
Ba Ba Ba
a a b
SL SB SR HA HU GL
Components
C
P concentration(mg·g^{-1})
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Aab Ab Aa
Ba Aa Ba
ABa Aa Ba
Aa Aa Aa
Ba Ab Bb
a b b
SL SB SR HA HU GL
Components
D
C:N ratio
80
70
60
50
40
30
20
10
0
Ba Ba Ca
Ab Aab Aa
Aa Aa Ba
Ba Ba Ba
Aa Aa Aa
b ab a
SL SB SR HA HU GL
Components
E
C:P ratio
700
600
500
400
300
200
100
0
Bab Aa Bb
Aa Aa Aa
Ba Aa Aa
Bb Aa Bb
Ab Aa Aab
b a a
SL SB SR HA HU GL
Components
F
N:P ratio
12
10
8
6
4
2
0
Ab Aa Ab
Ba Bb Cc
Ba Ba Ba
Ab Aa Ab
Aa Aa Aa
ab a b
BM
BCM
CM
SL SB SR HA HU GL
Components

Figure 4

Stoichiometric characteristics of soil layer C, N, and P in three secondary mixed forests

Different lowercase letters above the bars indicate significant differences among different forest types for the same soil layer ($p < 0.05$), while different uppercase letters indicate significant differences among different soil layers for the same forest type ($p < 0.05$).

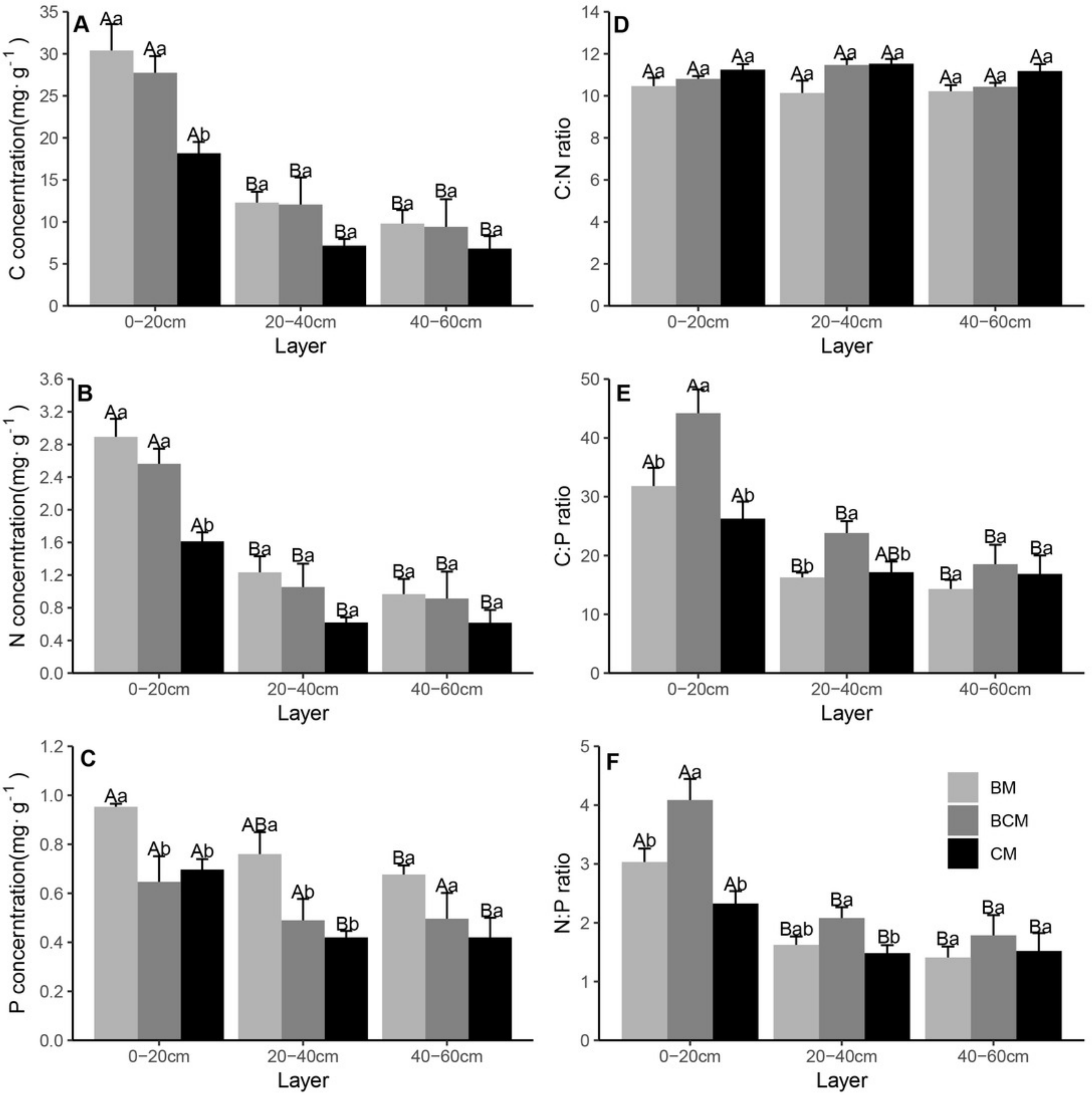
A
C concentration(mg·g⁻¹)
0
5
10
15
20
25
30
35
Aa
Aa
Ab
Ba
Ba
Ba
Ba
Ba
Ba
0−20cm
20−40cm
40−60cm
Layer
D
C:N ratio
0
2
4
6
8
10
12
14
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
B
N concentration(mg·g⁻¹)
0.0
0.4
0.8
1.2
1.6
2.0
2.4
2.8
3.2
3.6
Aa
Aa
Ab
Ba
Ba
Ba
Ba
Ba
Ba
E
C:P ratio
0
10
20
30
40
50
Ab
Aa
Ab
Bb
Ba
ABb
Ba
Ba
Ba
C
P concentration(mg·g⁻¹)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Aa
Ab
Ab
ABa
Ab
Bb
Ba
Aa
Ba
F
N:P ratio
0
1
2
3
4
5
Ab
Aa
Ab
Bab
Ba
Bb
Ba
Ba
Ba
BM
BCM
CM

Figure 5

Carbon storage of trees (A), understory plants (B) organs and soil layers (C) in three secondary mixed forests

Different lowercase letters above the bars indicate significant differences among different forest types for the same organ or soil layer ($p < 0.05$), while different uppercase letters indicate significant differences among different organs or soil layers for the same forest type ($p < 0.05$). SL: shrub leaf, SB: shrub branch, SR: shrub root, HA: herb aboveground, HU: herb underground.

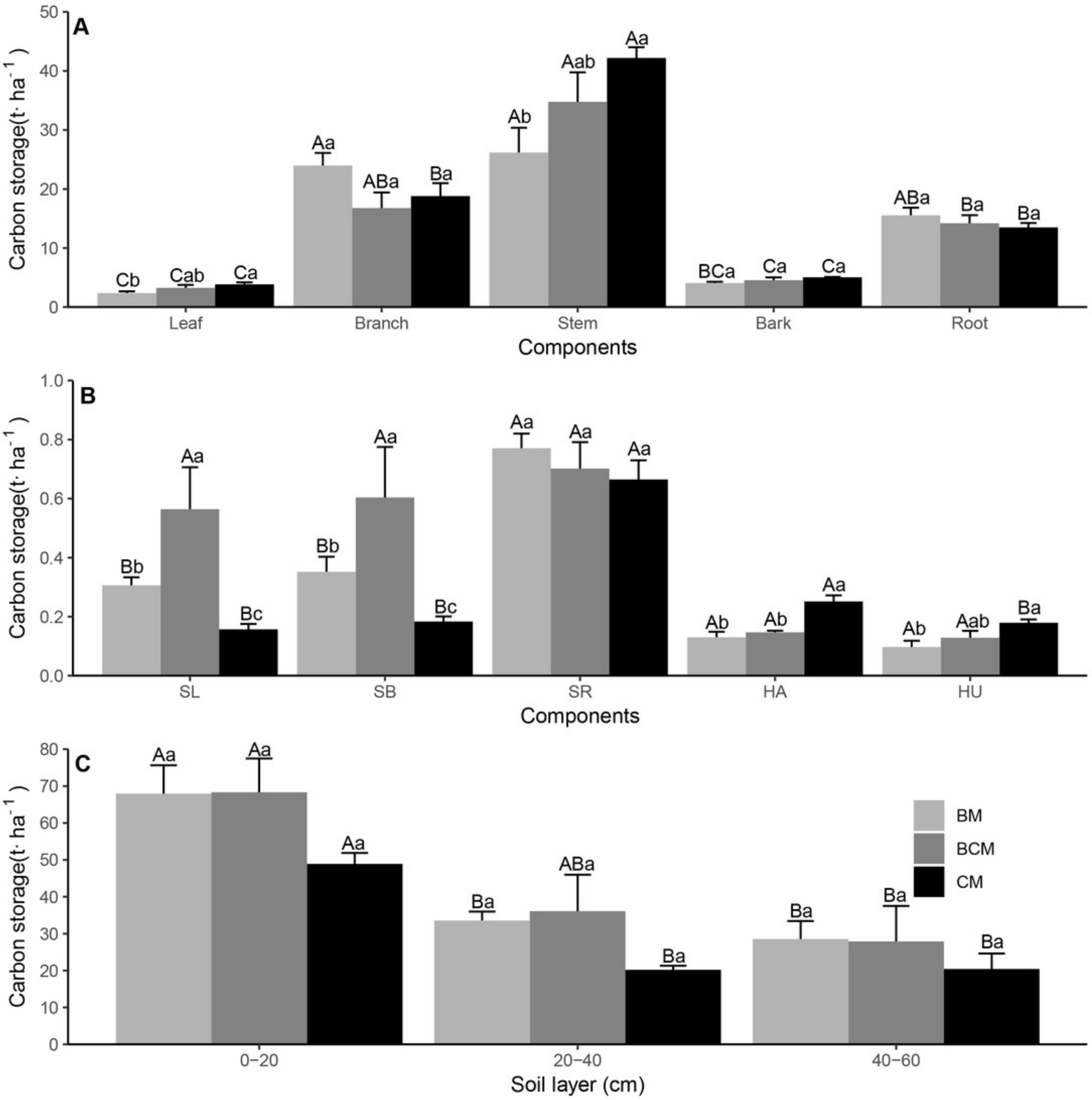
A
Carbon storage(t· ha^{-1})
0
10
20
30
40
50
Cb
Cab
Ca
Aa
ABa
Ba
Ab
Aab
Aa
BCa
Ca
Ca
ABa
Ba
Ba
Leaf
Branch
Stem
Bark
Root
Components
B
Carbon storage(t· ha^{-1})
0.0
0.2
0.4
0.6
0.8
1.0
Bb
Aa
Bc
Bb
Aa
Bc
Aa
Aa
Aa
Ab
Ab
Aa
Ab
Aab
Ba
SL
SB
SR
HA
HU
Components
C
Carbon storage(t· ha^{-1})
0
10
20
30
40
50
60
70
80
Aa
Aa
Aa
Ba
ABa
Ba
Ba
Ba
Ba
BM
BCM
CM
0–20
20–40
40–60
Soil layer (cm)

Figure 6

Nitrogen storage of trees (A), understory plants (B) organs and soil layers (C) in three secondary mixed forests

Different lowercase letters above the bars indicate significant differences among different forest types for the same organ or soil layer ($p < 0.05$), while different uppercase letters indicate significant differences among different organs or soil layers for the same forest type ($p < 0.05$). SL: shrub leaf, SB: shrub branch, SR: shrub root, HA: herb aboveground, HU: herb underground.

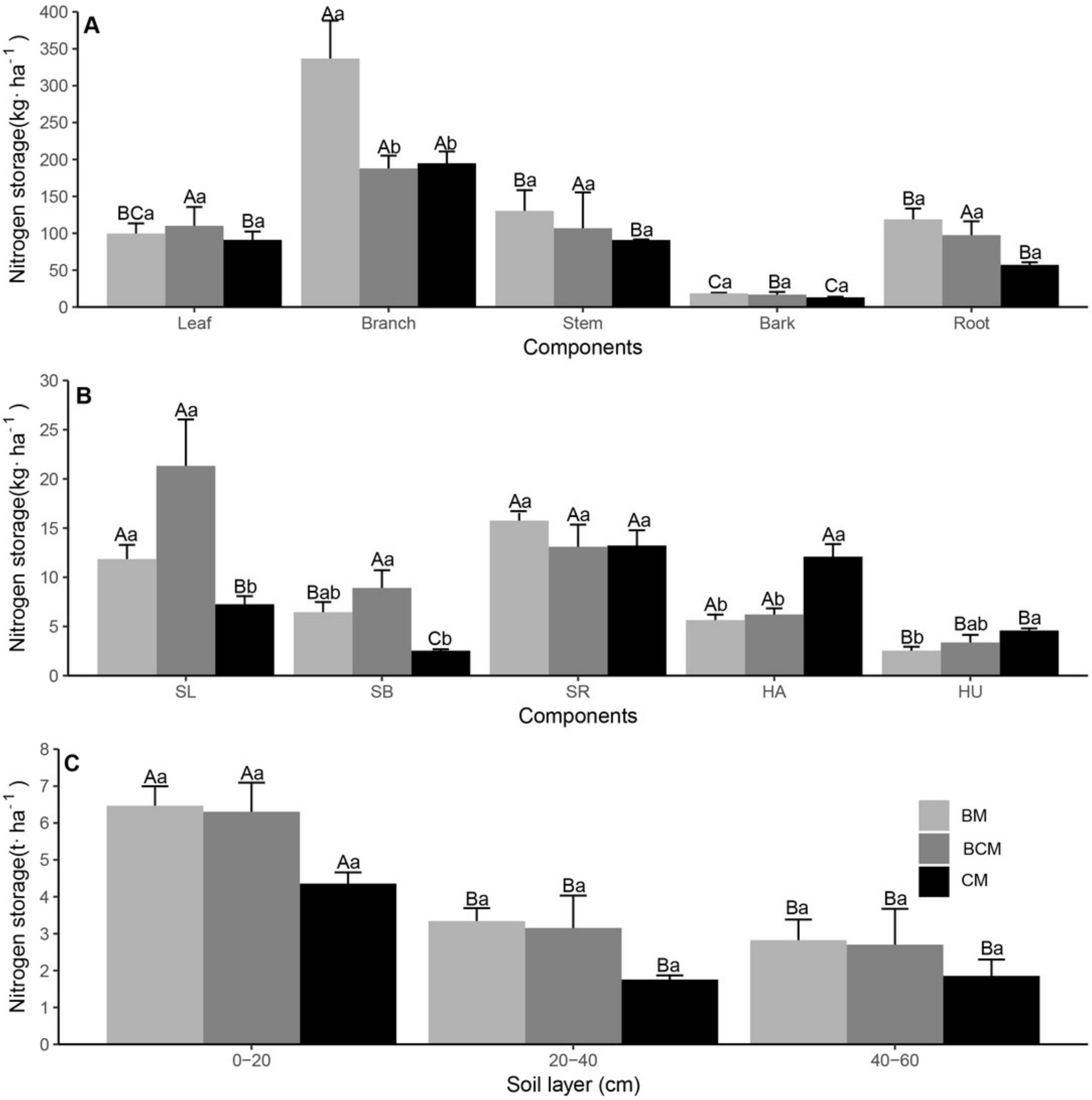
A
Nitrogen storage(kg· ha^{-1})
0
50
100
150
200
250
300
350
400
BCa
Aa
Ba
Aa
Ab
Ab
Ba
Aa
Ba
Ca
Ba
Ca
Ba
Aa
Ba
Leaf
Branch
Stem
Bark
Root
Components
B
Nitrogen storage(kg· ha^{-1})
0
5
10
15
20
25
30
Aa
Aa
Bb
Bab
Aa
Cb
Aa
Aa
Aa
Ab
Ab
Aa
Bb
Bab
Ba
SL
SB
SR
HA
HU
Components
C
Nitrogen storage(t· ha^{-1})
0
1
2
3
4
5
6
7
8
Aa
Aa
Aa
Ba
Ba
Ba
Ba
Ba
Ba
0–20
20–40
40–60
Soil layer (cm)
BM
BCM
CM

Figure 7

Phosphorus storage of trees (A), understory plants (B) organs and soil layers (C) in three secondary mixed forests

Different lowercase letters above the bars indicate significant differences among different forest types for the same organ or soil layer ($p < 0.05$), while different uppercase letters indicate significant differences among different organs or soil layers for the same forest type ($p < 0.05$). SL: shrub leaf, SB: shrub branch, SR: shrub root, HA: herb aboveground, HU: herb underground.

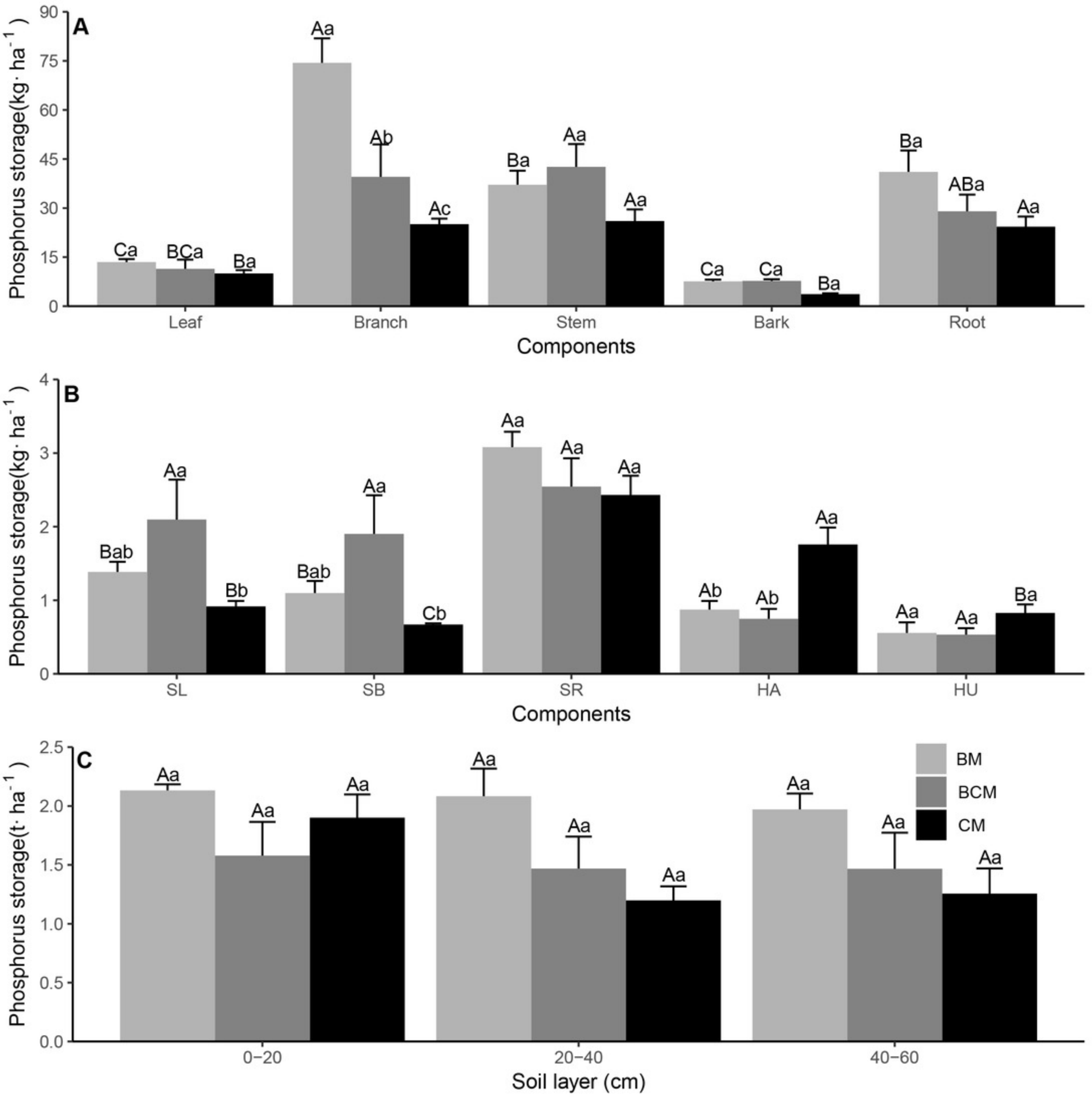
A
Phosphorus storage(kg· ha-1)
90
75
60
45
30
15
0
Ca
BCa
Ba
Aa
Ab
Ac
Ba
Aa
Aa
Ca
Ca
Ba
Ba
ABa
Aa
Leaf
Branch
Stem
Bark
Root
Components
B
Phosphorus storage(kg· ha-1)
4
3
2
1
0
Bab
Aa
Bb
Bab
Aa
Cb
Aa
Aa
Aa
Ab
Ab
Aa
Aa
Aa
Ba
SL
SB
SR
HA
HU
Components
C
Phosphorus storage(t· ha-1)
2.5
2.0
1.5
1.0
0.5
0.0
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
BM
BCM
CM
0−20
20−40
40−60
Soil layer (cm)

Table 1(on next page)

Characteristics of sample plots in three secondary mixed forests

Forest types	**BM**	**BCM**	**CM**
Altitude (m)	1900-2150	2000-2100	1800-2000
Slope aspect	Northwest	Northeast	Northwest
Slope position	Central	Central	Below
Slope gradient (°)	16-24	11-20	15-22
Fertigation	No	No	No
Shrubs	*Schisandra sphenanthera*	*Schisandra sphenanthera*	*Viburnum betulifolium*
	Viburnum betulifolium	*Smilax china*	*Lonicera fragrantissima*
	Rubus mesogaeus	*Viburnum betulifolium*	*Rubus mesogaeus*
Herbaceous	*Matteuccia intermedia*	*Tripterospermum chinense*	*Athyrium sinense*
	Lysimachia christinae	*Viola verecunda*	*Tripterospermum chinense*
	Carex duriuscula	*Carex duriuscula*	*Carex duriuscula*
DBH (cm)	17.24±1.76	13.98±0.74	19.06±0.52
Height (m)	10.85±0.22	11.84±0.56	19.79±0.34
Density (n ha^{-1})	933±246	1333±30	783±88

Table 2(on next page)

Carbon storage of plant total biomass, litter total biomass, soil and net ecosystem

Different letters indicate significant differences ($p < 0.05$) among forest types based on a one-way ANOVA followed by an LSD test.

Ecosystem pool	**BM**		**BCM**		**CM**	
	C storage	**percentage**	**C storage**	**percentage**	**C storage**	**percentage**
Tree (t ha^{-1})	72.09±4.82	35.09%	73.49±9.71	35.03 %	83.35±3.11	47.13%
Shrub (t ha^{-1})	1.43±0.06ab	0.70%	1.87±0.26a	0.89 %	1.00±0.07b	0.57%
Herb (t ha^{-1})	0.23±0.01b	0.11%	0.27±0.01b	0.13%	0.43±0.03a	0.24%
G-litter (t ha^{-1})	1.63±0.16b	0.80%	1.82±0.10b	0.87%	2.53±0.16a	1.43%
Soil (t ha^{-1})	130.05±13	63.30%	132.30±25	63.08%	89.54±4.01	50.63%
Net ecosystem (t ha^{-1})	205.43±10	100%	209.75±35	100%	176.86±7.14	100%

1

Table 3(on next page)

Nitrogen storage of plant total biomass, litter total biomass, soil and net ecosystem

Different letters indicate significant differences ($p < 0.05$) among forest types based on a one-way ANOVA followed by an LSD test.

Ecosystem pool	BM		BCM		CM	
	N storage	percentage	N storage	percentage	N storage	percentage
Tree (kg ha^{-1})	704.16±41	5.24%	518.96±112	4.05%	446.93±24	5.24%
Shrub (kg ha^{-1})	34.06±0.70a	0.25%	43.33±6.7a	0.34%	23.03±1.05b	0.27%
Herb (kg ha^{-1})	8.20±0.16b	0.06%	9.59±0.77b	0.07%	16.68±1.5a	0.20%
G-litter (kg ha^{-1})	70.35±5.07	0.52%	66.42±2.89	0.52%	77.27±4.51	0.91%
Soil (t ha^{-1})	12.63±1.33	93.93%	12.16±2.39	95.02%	7.97±0.29	93.43%
et ecosystem (t ha^{-1})	13.45±1.32	100%	12.80±2.5	100%	8.53±0.32	100%

Table 4(on next page)

Phosphorus storage of plant total biomass, litter total biomass, soil and net ecosystem

Different letters indicate significant differences ($p < 0.05$) among forest types based on a one-way ANOVA followed by an LSD test.

Ecosystem pool	BM		BCM		CM	
	P storage	percentage	P storage	percentage	P storage	percentage
Tree (kg ha^{-1})	173.67±13a	2.72%	130.27±23ab	2.8%	89.13±3.9b	2.00%
Shrub (kg ha^{-1})	5.56±0.19ab	0.09%	6.54±1.04a	0.14%	4.01±0.2b	0.09%
Herb (kg ha^{-1})	1.43±0.04b	0.02%	1.28±0.07b	0.03%	2.58±0.32a	0.06%
G-litter (kg ha^{-1})	6.86±0.53ab	0.11%	6.04±0.07b	0.13%	7.77±0.36a	0.17%
Soil (t ha^{-1})	6.19±0.39	97.06%	4.51±0.8	96.9%	4.36±0.35	97.76%
et ecosystem (t ha^{-1})	6.37±0.39	100%	4.66±0.84	100%	4.46±0.34	100%