The Introduction of Broad-Leaved Tree Species Drives The Process of Nutrient Cycling in Forest Soil

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Research

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Abstract

Background

Pure cypress forests experience problems such as reductions in biodiversity, lowered capabilities for water and soil conservation, decreased underground productivity and land degradation. To improve the conditions of pure forests, we studied the effects of mixed gaps on the cypress (*Cupressus funebris*) pure forest, selecting the Chinese toon (*Toona sinensis*), which is a deciduous broad-leaved tree, as the mixed tree species. We examined the variations in the concentrations of C, N, and P and their ratios in plant tissues, litter and soils in different seasons in pure cypress forests with 4 different sizes of mixed gaps (50, 100, 150, 200 m²).

Results

The leaf N:P ratios of cypress and Chinese toon were 10.77 and 12.74, respectively, and N was the main limiting factor for tree growth in the study area. The N and P resorption rates of the cypress pure forest were 57.4% and 60.7%, respectively, and mixed gaps with Chinese toon increased the resorption rates. An analysis of correlations among leaf-litter-soil stoichiometry indicated that the correlations between the soil nutrient elements and the corresponding plant leaves and litter increased when the broadleaf tree species was introduced into the cypress pure forest.

Conclusions

These results indicate that the introduction of broad-leaved species was favorable for triggering the forest soil nutrient recycling process.

Background

The basic elements (C, N, and P) in forest ecosystems play an important role in plant development and physiological functions(Elser et al. 2008). The C storage in plants is controlled by the availability of N and P, and the coupling of C, N and P is stronger than that of other elements(LeBauer and Treseder 2008; Reich et al. 2009; Yu et al. 2010). Current theories hold that the growth rates and nutrient use efficiency of plants in habitats can be obtained by comparing the C:N and C:P ratios(Ågren et al. 2013), while the N:P ratio is most often used to determine the extent to which a plant population or community is restricted by N and P(Aerts and Chapin 1999; Güsewell and Koerselman 2002; Güsewell et al. 2005). Roots absorb nutrients in the soil, store C through leaf photosynthesis, and return nutrient elements such as N and P to the soil in the form of litter, which forms potential nutrient cycles in the leaf-litter-soil system(Zhang et al. 2017). Litter is the basic carrier of nutrients from plants to soil, and the heterogeneity of the nutrient return process of different plant types makes the chemical measurement of C, N and P coupling
relationships complex in the forest ecosystem, which has become the focus of research (Herbert et al. 2003; Hessen et al. 2004).

Previous studies have reported that pure forests experience problems such as decreases in stand stability and declines in soil fertility, that need to be solved urgently (Grodzińska and Szarek-Łukaszewska 1997). In a degraded stand, species allocation can be used to build a compound forest ecosystem, which can effectively promote ecosystem circulation. In particular, of the use of gap disturbances to introduce mixed tree species can change the secondary vegetation species in the gaps, as well as the nutrient distribution with the succession, and the ecological stoichiometry characteristics of C, N and P before and after the formation of forest gaps may differ. It is important to understand the distribution of the regulation mechanism of the nutrient ratio in the leaf-litter-soil system and the effect of the nutrient return ratio of litter on soil nutrient cycling.

There is a large area of inefficient pure cypress forest in the hilly area of the central Sichuan basin. In this study, gap disturbance was applied to a cypress forest, with the introduction of a native broad-leaved tree species, the Chinese toon. The gaps were 50 m$^2$, 100 m$^2$, 150 m$^2$, 200 m$^2$ and mixed the broad-leaved tree species with the cypress forest. We studied the significance of the plant-leaf-soil relationships during plant growth with seasonal changes and to improve the management of low-efficiency forests in this area. We hypothesized that (1) the transformation that occurs by replanting Chinese toon has an effect on the C:N:P ratios of the plants, litter, soil, as well as the relationship between the three (2) and that the Chinese toon, a native broad-leaved tree species, can change the elemental balance of the regional environment of the forest community.

**Results**

**C, N and P concentrations and C:N:P ratios in leaf and litter layers**

The green leaf nutrient concentrations and their stoichiometric ratios differed among seasons. A seasonal research method was used to study the nutrient contents of the leaves and litter of cypress and Chinese toon, and four gap sizes were investigated in the same year. Due to the characteristics of deciduous trees, green leaves were not collected in winter, and litter was not collected in April and July for the four different gap sizes with Chinese toon. In the same season, the leaf C and litter C of cypress were significantly higher than those of the mixed gaps of Chinese toon ($P < 0.05$, Fig. 1a, b). There were no significant differences in leaf C among the different sizes of Chinese toon mixed gaps, and the only significant difference in litter C occurred in December, with a significantly lower value in the 50 m$^2$ gap than in the 200 m$^2$ gap ($P < 0.05$). The leaf C and litter C of cypress showed the same seasonal changes: spring > autumn > winter > summer. The leaf C of Chinese toon did not show a significant change with the seasons, and the winter litter C was lower than the autumn litter C. The seasonal variation in N and P in the leaf and litter of cypress and Chinese toon was as follows: spring < summer < autumn < winter.
The C:N and C:P of cypress leaves were significantly higher than those of Chinese toon leaves (Fig. 1g,i). There was no significant difference among the different Chinese toon mixed gap areas. The leaf C:N was higher for the 50 m$^2$ gap than for the 200 m$^2$ gap in spring and summer, and the leaf C:P was higher for the 50 m$^2$ gap than for the 200 m$^2$ gap in spring. The N:P of the cypress leaves was significantly lower than that of the Chinese toon mixed gap leaves in spring and summer, but the N:P of cypress leaves was significantly higher than that of Chinese toon leaves in autumn and winter ($P < 0.05$, Fig. 1k). The C:N:P stoichiometric ratio of leaves and litter varied with the seasons as follows: spring < summer < autumn < winter.

The leaf C: P in different seasons was ordered from high to low as follows: winter, autumn, summer, spring (Table 4). The N:P in autumn and winter increased from 10:1 to 12:1 in spring and summer, respectively. C:P decreased with increasing gap size in the mixed gaps with Chinese toon, and the C:P was the lowest in the 200 m$^2$ gap in the three seasons. The N:P ratio was the highest in summer (14:1), the N:P in the 150 m$^2$ and 200 m$^2$ gaps was higher than that in the 50 m$^2$ and 100 m$^2$ in autumn, and the N:P of the 200 m$^2$ gap in spring (11:1) was lower than that in other seasons.

The CRE in the pure cypress forest was the lowest, and the CRE of the four sizes of gaps with Chinese toon was significantly higher than that of the pure cypress forest, and the CRE of the 50 m$^2$ gap was the largest (Table 2). The NRE and PRE of the cypress forest were also significantly lower than those of the mixed gaps with Chinese toon, and there were no significant differences in these values among different gap sizes (Table 2).

**Patterns of C, N, and P concentrations and ratios in the soil**

The 0–5 cm soil C of the cypress forest was significantly lower than that of the mixed gaps with Chinese toon, and the 0–5 cm soil C of the 200 m$^2$ gap was significantly higher than that of smaller gap sizes (Fig. 2a). The above rules were consistent in all four seasons. The 5–20 cm soil C values of the cypress forest and the 50 cm$^2$ gap were the same, and the 5–20 cm soil C of 150 cm$^2$ and 200 cm$^2$ gaps were still significantly higher than those of the smaller gap sizes (Fig. 2b). With the deepening of the soil layer, the differences among the soil C values of different gap sizes gradually disappeared, and the difference in soil C between the cypress forest and the mixed gaps of different sizes also disappeared (Fig. 2c). Furthermore, with increasing soil layer depth, the soil C showed a decreasing trend.

The 0–5 cm soil N of the cypress forest was significantly higher than that of the mixed gaps with Chinese toon, and the 0–5 cm soil C of 200 m$^2$ gap was significantly higher than that of the smaller gap sizes. In the 5–20 cm soil layer, the soil N decreased in the following order: 150 m$^2$ gap, cypress forest, 100 m$^2$ gap, 200 m$^2$ gap, and 50 m$^2$ gap (Fig. 2d, e, f). The 0–5 cm soil P of the cypress forest was lower than that of the mixed gap with Chinese toon, and the soil P of the larger gap sizes (150 m$^2$, 200 m$^2$) was higher than that of the smaller gap sizes (50 m$^2$, 100 m$^2$) (Fig. 2g).
The soil C:N of the cypress forest was less than that of the 4 gap sizes in the three soil layers 0–5 cm, 5–20 cm, and 20–40 cm. The soil C:P of the cypress forest was higher than that of the 4 gap sizes in 5–20 cm and 20–40 cm soil layers. The soil N:P of the cypress forest was higher than that of the 4 gap sizes in the three soil layers, i.e., 0–5, 5–20, and 20–40 cm (Fig. 2p, q, r).

In the cypress forest, the soil C:P ratio was higher in the 20–40 cm layer than in the 0–5 cm layer and was lowest in the 5–20 cm layer. This ratio was the highest in the 0–5 cm layer in the mixed gap with Chinese toon (Table 4). The N:P of the cypress forest was higher in the 0–5 cm layer than in the 5–20 layer and was the lowest in the 20–40 cm layer, and trend also occurred in the mixed gaps with Chinese toon, except for in the 150 m² gap. The mean value of C:P in the soil (0–40 cm) of different gap sizes decreased as follows: 0 > 100 m² > 200 m² > 50 m² > 150 m². In addition, the soil N:P of the cypress forest was higher than that of the mixed gaps with Chinese toon.

**Relationships between leaf and litter stoichiometry**

There is a correlation between the element contents and stoichiometry ratios in leaves and litter. In the cypress forest, there was a significant positive correlation between litter C and leaf C, litter N, litter P, leaf N and leaf P were significantly positively correlated with each other, and there was a significant negative correlation between litter N and P and leaf C:N:P (Table 3). The leaf N and leaf P showed a significant negative correlation with litter C:N and litter C:P, while the leaf C only showed a significant negative correlation with litter N/P ($P < 0.05$). There was a significant or very significant positive correlation between the stoichiometric characteristics of leaves and litter. The leaf N and leaf P showed a significant negative correlation with litter C:N and litter C:P, while the leaf C only showed a significant negative correlation with litter N/P ($P < 0.05$). There was a significant or very significant positive correlation between the stoichiometric characteristics of leaves and litter. This correlation changed under the mixed gaps with Chinese toon. The positive correlation between leaf N and P and litter nutrients became a significant negative correlation, and the only positive correlation remaining was between leaf C and litter nutrients. At the same time, the significant negative correlation between leaf N and P and the litter stoichiometry ratios also changed to a positive correlation.

As for the two-way ANOVAs conducted for the leaves, seasonal factors significantly affected the N and P and C:N:P ratios, while the interaction effects for seasons and gap size were only significant for the C, N, and P contents and did were not significant for the C:N:P ratio (Table 5). Two-way ANOVAs indicated that season significantly affected the leaf N and P and leaf C:N:P ratios. In the two-way ANOVAs of litter, the gap showed an insignificant effect on N:P that was inconsistent with the fresh leaves, and the significant interaction between the season and the gap disappeared. The soil layer was introduced in the analysis of variance for soil, and the soil nutrients and nutrient ratios showed significant responses for the soil layer, season, and gap size (Table 5). The interaction between the soil layer and gap also responded significantly to soil nutrients and nutrient ratios, and this significant response disappeared during the interaction between the soil layer and season, and in the interaction of the soil layer, season and gap only responded significantly to soil N and P. A two-way ANOVA indicated that both season and soil depth
affected the soil stoichiometric characteristics. The interaction between season and soil depth was not significant.

**Discussion**

**Leaf C, N and P concentrations and stoichiometry**

In this study, the annual average C concentration in cypress and Chinese toon leaves was higher than that in global terrestrial plants (464 g kg\(^{-1}\)), and the leaf C concentration of cypress was higher than that of coniferous forests with cypress as the main species in the Pearl River Delta (517.85 g kg\(^{-1}\)). The Chinese toon is a deciduous broad-leaved tree species with a higher leaf C concentration (469.12 g kg\(^{-1}\)) than evergreen broad-leaved forests such as *Mytilaria laosensis* forests (Elser et al. 2000). The average green leaf N and P concentrations of cypress in our study were 6.05 and 0.56 g/kg, respectively, and the average green leaf N and P concentrations of Chinese toon were 15.03 and 1.18 g/kg, respectively, which were lower than the critical nutrient concentrations reported in a global study (18.6 g kg\(^{-1}\) for N and 1.21 g kg\(^{-1}\) for P) by Han et al. (Han et al. 2005). The leaf C:N and C:P ratios indicate the productivity generated per unit nutrient supply or, to some extent, the nutrient use efficiency of plants (Ågren 2008). The storage of high C and low N and P levels made forest growth of the study area show high C:N and C:P ratios, meaning that the plants had a high C assimilation ability and high nutrient utilization capacity (Huang et al. 2007). Due to the different growth and development requirements of tree species, the demand for proportions of elements also differs, and the leaf C:N and C:P ratios of cypress were significantly higher than those of Chinese toon. In this study, the C:N of cypress was 1560:12 in December, which was substantially higher than the value of 84.91 in the spring of the same year; this large decline was due to the contrast between the high growth period in April and the low growth dormancy period in December (Table 4).

Conditions restricting plant growth can also be determined by stoichiometric ratios. The theory of ecological chemometrics suggests that the nutrient composition of normal organisms is basically stable, and beyond this range, organisms are limited by elements (Elser et al. 2000; He et al. 2008). The leaf N:P ratio is usually used to reflect the limitation of plants by N or P; when leaf N:P < 14, plant growth is mainly restricted by N, when N:P > 16, plant growth is mainly restricted by P, and when 14 < N:P < 16, plant growth is limited by N and P (Güsewell et al. 2003; Zhang et al. 2015). Notably, the leaf N:P ratios of cypress and Chinese toon were 10.77 and 12.74, respectively, which were obviously below the N-limitation threshold. The plant growth in the study area was mainly restricted by N, and N is the main limiting factor for tree growth in the central Sichuan hilly area (Merino et al. 2004). The growth rate hypothesis suggests that individual growth rate is inversely related to N:P (Elser et al. 1996). However, in this study, the significant difference between the growth rate of cypress and Chinese toon was not reflected in the N:P stoichiometric ratio, which cannot be explained by the above hypothesis and is consistent with the study of different stand ages of cypress.

**Leaf nutrient resorption efficiency**
The nutrients of litter are derived from leaves, and changes in vegetation types can significantly change the stoichiometric characteristics of litter. Leaves transfer nutrients to other tissues before they fall, and this ability to reabsorb and redistribute nutrients can effectively improve the utilization efficiency of nutrients and increase the time that nutrients remain in the plant body (Wardle et al. 2004; Chen et al. 2012). The reabsorption of nutrients by different plant leaves is not consistent. The nutrient recirculation rates in the litter of the cypress forest and in the litter of the mixed gaps with Chinese toon showed significant differences (Table 2). The recirculation rates of N and P of Chinese toon were significantly higher than those of cypress. In this study, the N and P reabsorption rates of the cypress forest were significantly lower than the global N and P reabsorption rates (57.4%, 60.7%). However, the N reabsorption rates of the four sizes of mixed gaps with Chinese toon were higher than the global average, and the P reabsorption rates of the smaller gaps (50 m$^2$ and 100 m$^2$) were also higher than the global average. These results suggest that plants can adapt to the environment primarily in ways that increase nutrient absorption rather than nutrient reabsorption, given a relatively abundant supply of soil nutrients (Han et al. 2013; Fan et al. 2015). The leaf C:N, C:P and N:P ratios showed significant increases seasonally in the litter of cypress and Chinese toon (Fig. 1). The leaf and litter C:N of Chinese toon were lower than those of cypress, and it is generally suggested that plant residues with the C:N ratio of the Chinese toon leaf and litter are more susceptible to decomposition and have a faster rate of decomposition. At the same time, an increase in litter N or a decrease in the C:N ratio would increase the rate of litter decomposition (Mooshammer et al. 2012; Ågren et al. 2013). The seasonal increase in N:P indicated that the reabsorption rate of N in plants was greater than that of P in plants, and the decomposition process was strongly restricted by P. The mixing of Chinese toon can effectively reduce the N:P of litter, which indicates that the introduction of broadleaf species is beneficial for increasing the relative rate of decomposition and facilitating the circulation of nutrients.

**Soil C, N, and P concentrations and stoichiometry**

The soil C:N of the cypress forest was 12.4, which was slightly higher than the average C:N value of soil (10–12) in China. When the Chinese toon was mixed into the forest, the soil C:N increased significantly and reached the global average (Cleveland and Liptzin 2007). The ecological stoichiometry of soil changes with the nutrient changes of aboveground plants and litter, but this relationship is very complicated and does not have a single, linear correlation (Table 2). The stoichiometric index of soil is an important indicator of the effectiveness of soil resources. The soil C:N is inversely proportional to the decomposition rate of organic matter, and the soil C:P can indicate the validity of P. In the cypress forest, the soil C:N increased with the depth of the soil layer, but the soil C:P did not change regularly with soil layer depth, and the effectiveness of P in different soil layers was more stable. The soil C:N in cypress was lower than that in Chinese toon, but the soil C:P of the mixed gaps with Chinese toon was not significantly different from that of the cypress forest (Fig. 2). The nutrient decomposition rate of Chinese toon was higher than that of cypress, and the increase in the coefficient of variation in the soil layer also indicated an increase in the soil dissimilation process.
Correlation analysis of stoichiometric ratios of plant-litter-leaf C, N and P in a cypress plantation and mixed gaps with Chinese toon

There are many influencing factors in the process of soil nutrient change. Research on the ecological stoichiometry of C, N and P is helpful to understand the cyclical process of basic elements in the soil-litter-vegetation interface of forest ecosystems. In this study, the C and N concentrations of two tree species decreased from leaf to litter to soil, and the P concentrations broadly decreased from leaf to soil to litter, which indicated that the nutrient storage in the leaves was the highest, followed by that in the litter and soil. This regular distribution showed consistency between the tree species. Changes in the leaf C:N:P can affect soil nutrient relationships, which in turn can provide feedback on the availability of plant nutrients (McGroddy et al. 2004). The concentrations of C, N and P in litter were significantly lower than those in leaves, which indicated that the reabsorption of nutrients before and after fading is a mechanism that plants have evolved in environments with a limited nutrient supply (Drenovsky and Richards 2004).

Conclusions

(1) The growth rate of Chinese toon was higher than that of cypress, but the growth rate hypothesis did not effectively indicate the difference between the production rate of cypress and Chinese toon.

(2) The leaf N:P ratios of cypress and Chinese toon were 10.77 and 10.64, respectively, which were obviously below the N-limit threshold. Furthermore, the growth process of cypress and Chinese toon was mainly restricted by N, and N is the main limiting factor for tree growth in the hilly area of central Sichuan.

(3) The litter N concentrations of mixed gaps with Chinese toon were higher than those of cypress, the litter C:N and N:P of mixed gaps with Chinese toon were lower than those of cypress, and the correlation between soil and "plant-litter" increased. Therefore, the introduction of broad-leaved tree species was beneficial to increasing the relative rate of decomposition and triggering the nutrient cycling process in forest soils.

Materials And Methods

Study site and experimental design

The study was conducted at Yongxin Village, Sichuan Province (31°5′-31°20′N, 104°15′-104°33′E), near the northern edge of the central Sichuan hilly area. The landforms of this area are dominated by low hills, and the soil type is alkaline purple soil. The area is characterized by a subtropical monsoon wet climate, with high temperatures. The area is rainy in summer but warm and humid in winter and has an average annual temperature of 16.0℃. The annual average rainfall is 905.9 mm, and the annual total precipitation of 87%-89% and is mostly concentrated from May to October. The average annual sunshine is 1215.4 h, with a frost-free period of 271 days.
The monoculture cypress plantation in this area was established in the mid-1980s at the middle and upper reaches of the Yangtze River. During this time, there were neither forestry management practices nor tending management. The stand density is 2300–2570 trees/hm$^2$, and the average tree height measured in 2015 was 6.5 m. The average diameter at breast height (DBH) was 6 cm, and the clear bole height was 3 m. The canopy density of stands is mostly 0.7–0.8, with densities reaching 0.9. In this study, four different gap sizes (50, 100, 150, 200 m$^2$ for artificial regeneration in forest gaps) were used, and pure cypress forests were used as controls (Table 1). Each treatment was repeated 3 times for a total of 15 test areas.

**Leaf, litter, and soil sampling**

In April 2016, after three years of harvesting the gaps, the Chinese toon grew as the main forest layer. In each treatment plot, we marked three plants of Chinese toon with a generally similar growth status and similar growth position evenly and set litterfall traps. There were 36 litterfall traps in the treatment plot. For the controls, in the center of each plot, we randomly marked a cypress plant and set litterfall traps; these control plants all had a generally similar growth status and growth position. There were 9 litterfall traps in each control plot. These 36 litterfall traps were nylon net with 100 mesh and an area of 1 m*1 m and were placed 5 cm from the ground.

In April, June, October, and December 2016, green leaves of Chinese toon were collected from 3 randomly selected trees in each plot, and the cypress’s green leaves were collected from the trees in the center of the control plots. All leaf samples were taken to the laboratory immediately and fixed in a drying oven at 105°C for 15 minutes. Subsequently, the samples were dried at 65°C for 48 h, ground with a grinder, and sieved to 2 mm mesh size for C, N, and P analysis.

The litter set in this study was separated by the quartering method and taken to the laboratory. Impurities were removed, and the litter was put into the nylon net. Then, the litter was dried at 65°C for 48 h, ground with a grinder, and sieved to 2 mm mesh size for C, N, and P analysis.

Soil samples were collected from 0–5 cm, 5–20 cm, and 20–40 cm depths in each plot. A soil sampler with a diameter of 6 cm was used to collect approximately 0.5 kg of soil from each sample in a sealed pocket. These soil samples were taken to the laboratory and air-dried; subsequently, living roots and stones were removed, and the samples were passed through 2-mm and 0.15-mm sieves for soil C, N, and P analysis.

**Sample determination**

The concentrations of C, N and P were measured by the potassium dichromate oxidation–ferrous sulfate titrime try method (Nelson and Sommers 1996), the Kjeldahl method (Bremner and Mulvaney 1996), and the colorimetric method (Parkinson and Allen 1975), respectively.

**N and P nutrient return rate**
The capability of litter nutrient return can be expressed as the nutrient return rate (R), which is defined as the difference between the nutrient content of the normal growth organs of a plant and the nutrient content in the ground litter in autumn, namely, the amount of nutrients that the litter diverts before it is littered. This return rate is calculated as follows:

$$R(\%) = \frac{(T_{\text{leaf}} - T_{\text{litter}})}{T_{\text{leaf}}} \times 100$$

where R is the nutrient return rate, $T_{\text{leaf}}$ is the nutrient content of the normal growth organs of the plant (g/kg), and $T_{\text{litter}}$ is the nutrient content in the ground litter (g/kg).

**DATA ANALYSIS**

Data were processed and plotted using Microsoft Excel 2016, and SPSS 22 was used to test the significance of differences (LSD method, $\alpha = 0.05$). One-way ANOVA and Tukey’s HSD tests were used to test the significant differences in C, N, and P and the stoichiometric ratios in the leaves, litter and soil layers of different tree species among different seasons in cypress stands. The seasonal dynamic maps of the C, N, and P contents were plotted with Origin8.5. Pearson’s correlation analysis was used to test the correlation between the soil basic properties and soil C, N, and P contents and their stoichiometric ratios.

** Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Availability of data and material**

Not applicable.

**Competing interests**

There are no competing interests.

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**Authors’ contributions**

Y.S. led the writing of the manuscript, and executed the technical assays and statistical analysis. X.W.L.
designed the experiment and edited the manuscript. H.F.Y, S.Z.L,C.F.,and R.H. contributed to data collection and the interpretation of the data. All authors read and approved the final manuscript.

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References

1. Aerts R, Chapin FS (1999) The Mineral Nutrition of Wild Plants Revisited: A Re-evaluation of Processes and Patterns. Adv Ecol Res 30:1–67. https://doi.org/10.1016/S0065-2504(08)60016-1
2. Ågren GI (2008) Stoichiometry and nutrition of plant growth in natural communities. Annu Rev Ecol Evol Syst 39:153–170
3. Ågren GI, Hyvönen R, Berglund SL, Hobbie SE (2013) Estimating the critical N: C from litter decomposition data and its relation to soil organic matter stoichiometry. Soil Biol Biochem 67:312–318. https://doi.org/10.1016/j.soilbio.2013.09.010
4. Bremner JM, Mulvaney CS (2015) Nitrogen-Total. John Wiley & Sons, Ltd, pp 595–624
5. Chen FS, Niklas KJ, Chen GS, Guo D (2012) Leaf traits and relationships differ with season as well as among species groupings in a managed Southeastern China forest landscape. Plant Ecol 213:1489–1502. https://doi.org/10.1007/s11258-012-0106-5
6. Cleveland CC, Liptzin D (2007) C:N:P stoichiometry in soil: Is there a “Redfield ratio” for the microbial biomass? Biogeochemistry 85:235–252. https://doi.org/10.1007/s10533-007-9132-0
7. Drenovsky RE, Richards JH (2004) Critical N:P values: Predicting nutrient deficiencies in desert shrublands. Plant Soil 259:59–69. https://doi.org/10.1023/B:PLSO.0000020945.09809.3d
8. Elser JJ, Dobberfuhl DR, MacKay NA, Schampel JH (1996) Organism size, life history and N:P stoichiometry. Bioscience 46:674–685. https://doi.org/10.2307/1312897
9. Elser JJ, Sterner RW, Gorokhova E et al (2008) Biological stoichiometry from genes to ecosystems. Ecol Lett 3:540–550. https://doi.org/10.1111/j.1461-0248.2000.00185.x
10. Elser O'Brien, Dobberfuhl D (2000) The evolution of ecosystem processes: Growth rate and elemental stoichiometry of a key herbivore in temperate and arctic habitats. J Evol Biol 13:845–853. https://doi.org/10.1046/j.1420-9101.2000.00215.x
11. Fan H, Wu J, Liu W et al (2015) Linkages of plant and soil C:N:P stoichiometry and their relationships to forest growth in subtropical plantations. Plant Soil 392:127–138. https://doi.org/10.1007/s11104-015-2444-2
12. Grodzińska K, Szarek-Łukaszewska G (1997) Polish mountain forests: Past, present and future. Environ Pollut 98:369–374. https://doi.org/10.1016/S0269-7491(97)00145-0
13. Güsewell S, Jewell PL, Edwards PJ (2005) Effects of heterogeneous habitat use by cattle on nutrient availability and litter decomposition in soils of an Alpine pasture. Plant Soil 268:135–149. https://doi.org/10.1007/s11104-004-0304-6
14. Güsewell S, Koerselman W (2002) Variation in nitrogen and phosphorus concentrations of wetland plants. Perspect Plant Ecol Evol Syst 5:37–61. https://doi.org/10.1078/1433-8319-0000022

15. Güsewell S, Koerselman W, Verhoeven JTA (2003) Biomass N:P ratios as indicators of nutrient limitation for plant populations in wetlands. Ecol Appl 13:372–384. https://doi.org/10.1890/1051-0761(2003)013[0372:BNRAIO]2.0.CO;2

16. Han W, Fang J, Guo D, Zhang Y (2005) Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. New Phytol 168:377–385. https://doi.org/10.1111/j.1469-8137.2005.01530.x

17. Han W, Tang L, Chen Y, Fang J (2013) Relationship between the relative limitation and resorption efficiency of nitrogen vs phosphorus in woody plants. PLoS One 8: https://doi.org/10.1371/journal.pone.0083366

18. He JS, Wang L, Flynn DFB et al (2008) Leaf nitrogen:phosphorus stoichiometry across Chinese grassland biomes. Oecologia 155:301–310. https://doi.org/10.1007/s00442-007-0912-y

19. Herbert DA, Williams M, Rastetter EB (2003) A model analysis of N and P limitation on carbon accumulation in Amazonian secondary forest after alternate land-use abandonment. Biogeochemistry 65:121–150. https://doi.org/10.1023/A:1026020210887

20. Hessen DO, Ågren GI, Anderson TR et al (2004) Carbon sequestration in ecosystems: The role of stoichiometry. Ecology 85:1179–1192. https://doi.org/10.1890/02-0251

21. Huang J, Wang X, Yan E (2007) Leaf nutrient concentration, nutrient resorption and litter decomposition in an evergreen broad-leaved forest in eastern China. For Ecol Manage 239:150–158. https://doi.org/10.1016/j.foreco.2006.11.019

22. LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89:371–379. https://doi.org/10.1890/06-2057.1

23. McGroddy ME, Daufresne T, Hedin LO (2004) Scaling of C:N:P stoichiometry in forests worldwide: Implications of terrestrial redfield-type ratios. Ecology 85:2390–2401

24. Merino A, Fernández-López A, Solla-Gullón F, Edeso JM (2004) Soil changes and tree growth in intensively managed Pinus radiata in northern Spain. For Ecol Manage 196:393–404. https://doi.org/10.1016/j.foreco.2004.04.002

25. Mooshammer M, Wanek W, Schnecker J et al (2012) Stoichiometric controls of nitrogen and phosphorus cycling in decomposing beech leaf litter. Ecology 93:770–782. https://doi.org/10.1890/11-0721.1

26. Nelson DW, Sommers LE (2018) Total carbon, organic carbon, and organic matter. In: Methods of Soil Analysis, Part 3: Chemical Methods. wiley, pp 961–1010

27. Parkinson JA, Allen SE (1975) A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. Commun Soil Sci Plant Anal 6:1–11. https://doi.org/10.1080/00103627509366539

28. Reich PB, Oleksyn J, Wright IJ (2009) Leaf phosphorus influences the photosynthesis-nitrogen relation: A cross-biome analysis of 314 species. Oecologia 160:207–212.
29. Wardle DA, Walker LR, Bardgett RD (2004) Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305:509–513. https://doi.org/10.1126/science.1098778
30. Yu Q, Chen Q, Elser JJ et al (2010) Linking stoichiometric homoeostasis with ecosystem structure, functioning and stability. Ecol Lett 13:1390–1399. https://doi.org/10.1111/j.1461-0248.2010.01532.x
31. Zhang G, Zhang P, Peng S et al (2017) The coupling of leaf, litter, and soil nutrients in warm temperate forests in northwestern China. Sci Rep 7:. https://doi.org/10.1038/s41598-017-12199-5
32. Zhang W, Zhao J, Pan F et al (2015) Changes in nitrogen and phosphorus limitation during secondary succession in a karst region in southwest China. Plant Soil 391:77–91. https://doi.org/10.1007/s11104-015-2406-8

Tables

Table 1
The basic characteristic of forest gap

| Gap size(A/m²) | Arbor height(m) | Arbor BDH(cm) | Main plants                                                      | Number of species | litter layer thickness |
|---------------|-----------------|---------------|-----------------------------------------------------------------|-------------------|-----------------------|
| 0(pure cypress forests) | 6.5             | 12            | *Cupressus funebris, Ligustrum lucidum, Vernicia fordii(Hemsl.), Moraceae broussonetia, Alnus cremastogyne, Quercus acutissima* | 12                | 0.4                   |
| 50            | 4.5             | 7             | Toona sinensis                                                  | 2.1               | 28                    |
| 100           | 4.7             | 7             | Toona sinensis                                                  | 2.5               | 38                    |
| 150           | 5.1             | 7             | Toona sinensis                                                  | 2.8               | 40                    |
| 200           | 4.9             | 7             | Toona sinensis                                                  | 3.2               | 35                    |
Table 2
Mean ± SE of C, N and P resorption efficiency (CRE, NRE and PRE) in different forest gap sizes

| Gap size (A/m²) | CRE%       | NRE%       | PRE%       |
|----------------|------------|------------|------------|
| 0              | 2.72 ± 0.54a | 18.34 ± 6.65a | 50 ± 3.58a |
| 50             | 17.19 ± 1.73b | 59.54 ± 1.59b | 61.32 ± 2.41b |
| 100            | 16.28 ± 0.73b | 59.98 ± 2.18b | 60.86 ± 0.88b |
| 150            | 15.42 ± 0.42b | 59.68 ± 3.61b | 58.33 ± 3.08b |
| 200            | 14.85 ± 2.61b | 58.24 ± 2.44b | 58.51 ± 2.31b |

Different lowercase letters indicate significant differences (P < 0.05) of mean values among the five different gap sizes.
Table 3
Spearman correlations for leaf and litter

| Gap sizes (A/m²) | Leaf C | Leaf N | Leaf P | Leaf C/N | Leaf C/P | Leaf N/P |
|------------------|--------|--------|--------|----------|----------|----------|
| 0                | Litter C  | 0.97** | 0.75   | 0.72     | -0.62    | -0.66    | -0.66    |
|                  | Litter N  | 0.53   | 0.89*  | 0.91*    | -0.87*   | -0.93**  | -0.93**  |
|                  | Litter P  | 0.73   | 0.96** | 0.99**   | -0.90*   | -0.96**  | -0.96**  |
|                  | Litter C/N| -0.52  | -0.87* | -0.91*   | 0.85*    | 0.92**   | 0.95**   |
|                  | Litter C/P| -0.72  | -0.94**| -0.97**  | 0.88*    | 0.95**   | 0.96**   |
|                  | Litter N/P| -0.84* | -0.91* | -0.91*   | 0.83*    | 0.86*    | 0.85*    |
| 50               | Litter C  | 0.46   | -0.96**| -0.99**  | 0.92*    | 0.98**   | -0.13    |
|                  | Litter N  | 0.34   | -0.89* | -0.94**  | 0.83*    | 0.92*    | -0.03    |
|                  | Litter P  | 0.35   | -0.94**| -0.98**  | 0.89*    | 0.96**   | -0.09    |
|                  | Litter C/N| 0.14   | 0.33   | 0.38     | -0.26    | -0.34    | -0.14    |
|                  | Litter C/P| -0.19  | 0.86*  | 0.90*    | -0.80    | -0.88*   | 0.06     |
|                  | Litter N/P| -0.40  | 0.84*  | 0.86*    | -0.82*   | -0.86*   | 0.18     |
| 100              | Litter C  | 0.71   | -0.90* | -0.96**  | 0.89*    | 0.97**   | 0.63     |
|                  | Litter N  | 0.47   | -0.94**| -0.95**  | 0.91*    | 0.92**   | 0.43     |
|                  | Litter P  | 0.45   | -0.98**| -0.94**  | 0.96**   | 0.92*    | 0.27     |
|                  | Litter C/N| 0.25   | 0.48   | 0.42     | -0.46    | -0.33    | 0.14     |
|                  | Litter C/P| -0.07  | 0.75   | 0.67     | -0.71    | -0.62    | 0.01     |
|                  | Litter N/P| -0.20  | 0.70   | 0.63     | -0.67    | -0.60    | -0.02    |
| 150              | Litter C  | 0.30   | -0.97**| -0.96**  | 0.96**   | 0.95**   | 0.34     |
|                  | Litter N  | 0.13   | -0.85* | -0.87*   | 0.83*    | 0.85*    | 0.56     |
|                  | Litter P  | 0.40   | -0.99**| -0.98**  | 0.98**   | 0.97**   | 0.41     |

* Significance is indicated by < 0.05

** Significance is indicated by < 0.01
| Gap sizes (A/m²) | Leaf C | Leaf N | Leaf P | Leaf C/N | Leaf C/P | Leaf N/P |
|-----------------|--------|--------|--------|----------|----------|----------|
| Litter C/N      | 0.05   | 0.42   | 0.46   | -0.38    | -0.42    | -0.58    |
| Litter C/P      | -0.44  | 0.90*  | 0.91*  | -0.89*   | -0.90*   | -0.47    |
| Litter N/P      | -0.63  | 0.84*  | 0.81   | -0.86*   | -0.82*   | -0.05    |
| 200             | Litter C | 0.64   | -0.97**| -0.95**  | 0.94**   | 0.95**   | 0.71     |
|                 | Litter N | 0.71   | -0.95**| -0.92*   | 0.94**   | 0.93**   | 0.65     |
|                 | Litter P | 0.76   | -0.98**| -0.93**  | 0.98**   | 0.95**   | 0.60     |
|                 | Litter C/N | -0.60  | 0.69   | 0.67    | -0.71    | -0.69    | -0.46    |
|                 | Litter C/P | -0.85* | 0.92** | 0.83*   | -0.94**  | -0.87*   | -0.43    |
|                 | Litter N/P | -0.34  | 0.31   | 0.19    | -0.32    | -0.24    | 0.08     |

* Significance is indicated by < 0.05
** Significance is indicated by < 0.01

Table 4
stoichiometric characteristics of leaf and soil C:N:P

| variable         | C:N:P as a function of plantation gap |
|------------------|----------------------------------------|
|                  | 0          | 50         | 100        | 150        | 200        |
| season           | Spring     | 858:10:1   | 326:12:1   | 305:12:1   | 298:12:1   | 251:11:1   |
|                  | Summer     | 899:10:1   | 489:14:1   | 440:14:1   | 427:14:1   | 393.8:14:1 |
|                  | Autumn     | 1220:12:1  | 550:12:1   | 540:12:1   | 525:13:1   | 519:13:1   |
|                  | Winter     | 1560:12:1  |           |           |            |            |
|                  | average    | 1134:11:1  | 455:13:1   | 429:13:1   | 416:13:1   | 388:13:1   |
| soil depth(cm)   | 0–5        | 91:9:1     | 76:3:1     | 84:5:1     | 53:2:1     | 62:3:1     |
|                  | 5–20       | 76:6:1     | 36:1:1     | 59:3:1     | 45:3:1     | 59:2:1     |
|                  | 20–40      | 95:5:1     | 33:1:1     | 38:1:1     | 46:1:1     | 44:1:1     |
|                  | average    | 87:7:1     | 48:2:1     | 60:3:1     | 45:2:1     | 55:2:1     |
Table 5 F and P values for the effects of season, gap, and soil depth on leaf, litter and soil C:N:P stoichiometric characteristics.

Two-way ANOVA for effects of forest gap size and seasonality on fresh leaf C, N, and P concentrations, and fresh leaf C:N:P stoichiometric ratios in the forest plantation

| Component | Factor                        | F(P)value | C    | N    | P    | C:N  | C:P  | N:P  |
|-----------|-------------------------------|-----------|------|------|------|------|------|------|
| leaf      | season                        | 1.051     | 219.189* | 330.148* | 169.752* | 173.583* | 12.538* |
|           | gap                           | 233.881*  | 315.333* | 230.226* | 509.698* | 246.543* | 10.763* |
|           | season * gap                  | 9.614*    | 13.619* | 18.334* | 0.290 | 3.397 | 2.900 |
| litter    | season                        | 97.357*   | 65.509* | 124.130* | 90.489* | 204.929* | 18.635* |
|           | gap                           | 268.051*  | 9.468* | 19.716* | 10.923* | 9.740* | 2.239 |
|           | season * gap                  | 4.085     | 0.857 | 1.281 | 0.090 | 0.282 | 0.199 |
| soil      | soil depth                    | 1242.095* | 2367.139* | 17.700* | 54.007* | 48.399* | 122.690* |
|           | season                        | 45.205*   | 15.234* | 9.032* | 2.106 | 9.994* | 4.448 |
|           | gap                           | 108.940*  | 267.590* | 77.279* | 21.132* | 53.977* | 166.574* |
|           | soil depth * season           | 1.260     | 3.812 | 2.091 | 0.869 | 2.326 | 2.615 |
|           | soil depth * gap              | 41.428*   | 109.459* | 16.246* | 2.461 | 10.501* | 7.905* |
|           | season * gap                  | 0.976     | 3.463* | 0.984* | 0.342 | 2.729 | 1.923 |
|           | soil depth * season * gap     | 0.870     | 1.228 | 1.029 | 0.565 | 1.414 | 1.616 |

NS not significant

*P < .001

Figures
Figure 1

The C, N, and P concentrations and C:N:P stoichiometry in the leaves, and litter in different gap sizes. The vertical bars represent the standard deviations, different capital letters indicate significant difference at the P<0.05 level between the different gap sizes.
Figure 2

The C, N, and P concentrations and C:N:P stoichiometry in the soil in different gap sizes. The vertical bars represent the standard deviations, different capital letters indicate significant difference at the P<0.05 level between the different gap sizes.