Forest Ecology and Management 432 (2019) 319–326

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Biochar application alleviates unbalanced nutrient uptake caused by N deposition in *Torreya grandis* trees and seedlings

Rui Zhang[a,b,1], Yuxin Zhao[a,b,1], Jianhong Lin[a], Yuanyuan Hu[a], Heikki Hänninen[a,b], Jiasheng Wu[a,b,*]

[a] State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Hangzhou 311300, China
[b] NFGA Engineering Research Center for Torreya grandis 'Merrillii', Zhejiang A&F University, Hangzhou 311300, China

ARTICLE INFO

Keywords:
N:P ratio
Foliar stoichiometry
Phosphorus limitation
Nutrient balance
Forest management

ABSTRACT

Foliar nitrogen: phosphorus (N:P) ratio is a valuable indicator of nutrient limitation in forest ecosystems. The ratio is currently dramatically affected by the increasing anthropogenic N deposition in China. Biochar, a soil amendment, has profound benefits for improving soil N and P availability. However, little is known about N and P uptake and nutrient balance of plants growing on biochar-amended soils under N deposition. Here, we conducted a two-year N addition and biochar application factorial experiment with *Torreya grandis* trees and seedlings, and the responses of concentrations of foliar N and P and the foliar N:P ratio were evaluated. N addition alone increased foliar N concentration, but did not change foliar P concentration, and thus increased foliar N:P ratio. Biochar application alone increased concentrations of both foliar N and P but did not cause any change in the N:P ratio. Most importantly, when applying N and biochar together, the concentration of foliar P increased and the foliar N:P ratio decreased, relative to each N treatment alone. This novel result suggests that biochar application can serve as an effective forest management tool, which can not only enhance the availability of N and P nutrients, but also alleviate the imbalanced uptake of nutrients indicated by the high foliar N:P ratios under increasing N deposition.

1. Introduction

Atmospheric nitrogen (N) deposition, mainly arising from agricultural N fertilization, fossil fuel consumption, and emissions from cowsheds and stables, is currently impacting forests, grasslands and heathlands (Liu et al., 2013; Phoenix et al., 2012). The N deposition rate in large regions of the world (i.e., United States, western Europe, and China) exceeds 10 kg N ha^{-1} yr^{-1}, which is more than twentyfold the N deposition rate in the less impacted boreal forests (Liu et al., 2013; Meunier et al., 2016). This increasing N input to ecosystems changes the global N cycle and has for this reason become a key driver of global change (Ferretti et al., 2014).

It is well-known that N deposition often has a direct fertilizing effect on trees in ecosystems where N is a limiting factor, resulting in this way in increased forest productivity (Binkley and Hogberg, 2016; Song et al., 2017a). Therefore, as N levels continue to rise, N limitation is subsequently alleviated (Tao and Hunter, 2012). Over long-time scales, N deposition can alter biogeochemical cycling in forest ecosystems by shifting nutrient dynamics, for instance by changing N and P contents of plants and soils (Du et al., 2016; Ferretti et al., 2014; Sardans et al., 2016). Indeed, major concerns continue to be raised regarding the long-term trends of unbalanced N:P ratios and declining P in foliar nutrition of trees across Europe (Binkley and Hogberg, 2016). Several studies in boreal, temperate, and subtropical forest ecosystems have demonstrated that the increasing N deposition can increase foliar N concentration, while other nutrients, such as P, may become limiting factors, leading to increased N:P ratios and a shift from N to P limitation (Huang et al., 2016; Jonard et al., 215; Sardans et al., 2016; Song et al., 2016; Talkner et al., 2015; Veresoglou wt al., 2014).

The foliar N:P ratio is an essential ecological trait indicating stoichiometric flexibility, which reflects plants' physiological capacity to adjust nutrient balance whilst maintaining physiological functions (Sardans et al., 2016; Sistla and Schimel, 2012). According to the growth-rate hypothesis, fast-growing organisms often need to invest relatively much resources to the phosphorus-rich RNA to support the rapid protein synthesis (Matzek and Vitousek, 2009). Therefore, a low N:P ratio is often found to be associated with a high growth rate (Elser et al., 2010; Matzek and Vitousek, 2009; Sardans et al., 2016).

* Corresponding author at: 666 Wusu Street, Hangzhou 311300, China.
E-mail address: wujs@zafu.edu.cn (J. Wu).
[1] These authors have contributed equally to this work.

https://doi.org/10.1016/j.foreco.2018.09.040
Received 13 June 2018; Received in revised form 19 September 2018; Accepted 22 September 2018
Available online 26 September 2018

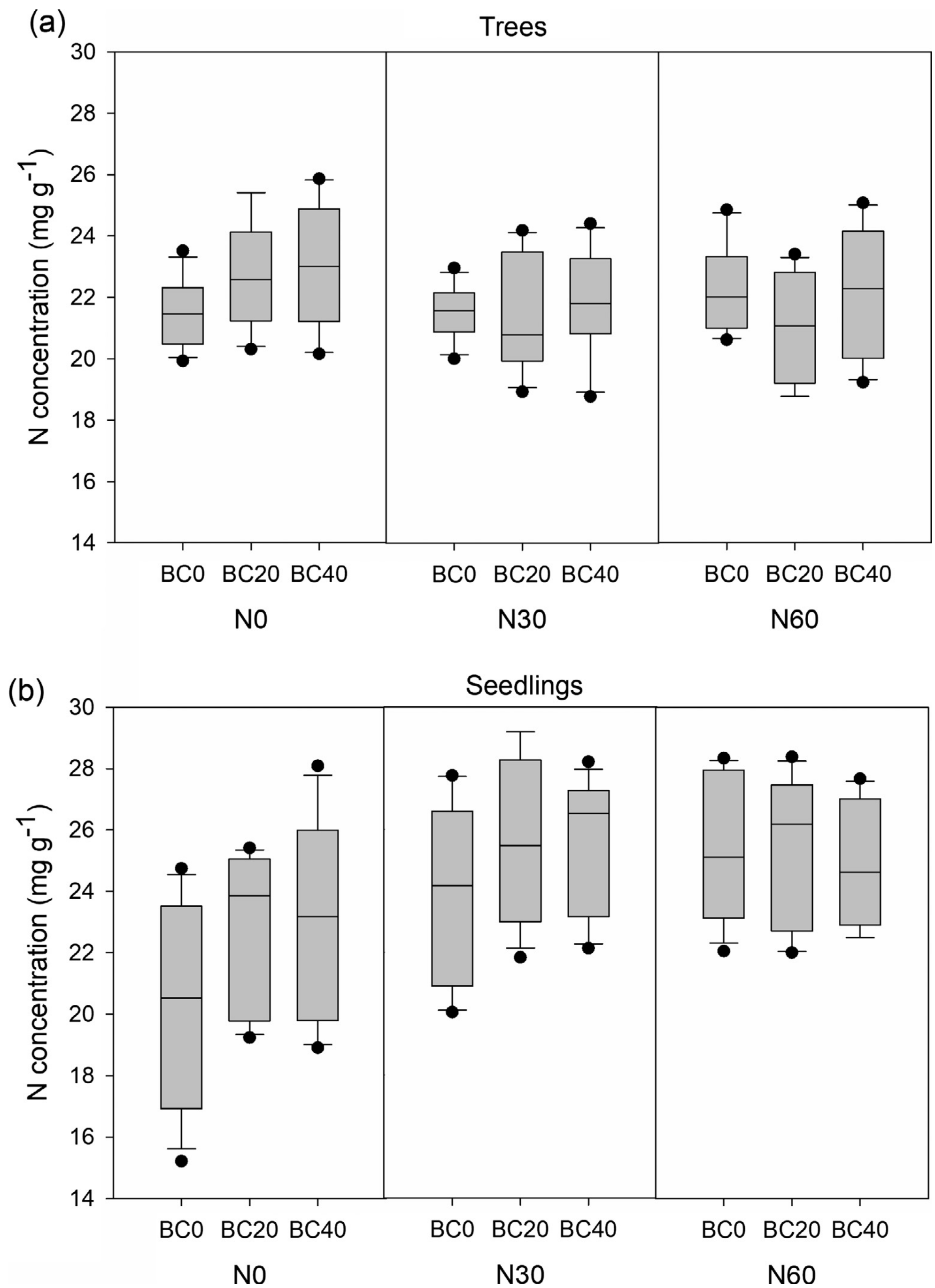

Fig. 1. Effects of additional N deposition and biochar treatments on the concentration of foliar nitrogen (N) in *Torreya grandis* trees (a) and seedlings (b). N0: ambient N deposition without added nitrogen (control). N30: treatment with a low level of added N deposition (ambient plus 30 kg N ha^{-1} yr^{-1}). N60: treatment with a high level of added N deposition (ambient plus 60 kg N ha^{-1} yr^{-1}). BC0: no biochar application (control). BC20: 20 t biochar ha^{-1}. BC40: 40 t biochar ha^{-1}. The box plots represent pooled data from four samplings carried out at the beginning of June and September both in 2016 and 2017.

Accordingly, an increasing N:P ratio caused by N deposition may limit plant growth. Furthermore, P limitation is an important mechanism underlying changes in plant community composition and ecosystem processes under globally increasing N deposition (Du et al., 2016; Sardans et al., 2016). Therefore, it is important to develop practical forest management strategies to avoid reductions of forest production due to P limitation caused by increasing N deposition.

Biochar is a solid carbon coproduct of incomplete combustion of organic materials in a high-temperature and oxygen-limited environment (Sohi et al., 2010). It has high surface area and high pH and contains various forms of N and P nutrients (i.e., NH_4^+ and ortho-P) (Gul and Whalen, 2016). Serving as an effective tool in soil amendment, biochar application has received increasing attention among researchers in the past decade (Jeffery et al., 2015). Today, the vast majority of biochar research has been conducted for determining its role in soil remediation (Wang et al., 2016). Biochar can improve soil water-holding capacity (Basso et al., 2013), increase microbial biomass (Li et al., 2018) and activity (Steinbeiss et al., 2009), reduce soil N_2O emissions (Cayuela et al., 2013), and immobilize contaminants (Ahmad et al., 2014). In addition, biochar has also been shown to increase long-term availability of nutrients in the soil by reducing nutrient leaching losses and accelerating N nitrification (Laird et al., 2010). Furthermore,

Table 1
Results from three-factor ANOVAs evaluating the effects of biochar application (BC; 0, 20 and 40 t ha^{-1}), nitrogen addition (NA; 0, 30 and 60 kg N ha^{-1}) and the time of measurement (time; June and September 2016, June and September 2017) on the foliar concentrations of nitrogen (N) and phosphorus (P) and the foliar N:P ratio in *Torreya grandis* trees and seedlings.

Source	N		P		N:P	
	F	*P*	*F*	*P*	*F*	*P*
Trees						
BC	20.142	< 0.001	40.117	< 0.001	47.681	< 0.001
NA	30.669	< 0.001	1.461	0.239	10.206	< 0.001
Time	2445.154	< 0.001	51.665	< 0.001	266.893	< 0.001
BC * NA	21.086	< 0.001	4.982	< 0.001	16.667	< 0.001
BC * Time	12.208	< 0.001	1.853	0.101	2.509	0.029
NA * Time	4.408	0.001	5.891	< 0.001	11.100	< 0.001
BC * NA * Time	2.389	0.012	2.179	0.022	3.134	0.001
Seedlings						
BC	45.194	< 0.001	156.046	< 0.001	98.850	< 0.001
NA	70.638	< 0.001	49.196	< 0.001	2.594	0.082
Time	1556.701	< 0.001	192.687	< 0.001	293.330	< 0.001
BC * NA	15.808	< 0.001	11.995	< 0.001	41.325	< 0.001
BC * Time	17.749	< 0.001	15.720	< 0.001	5.195	< 0.001
NA * Time	5.345	< 0.001	10.113	< 0.001	16.558	< 0.001
BC * NA * Time	4.319	< 0.001	3.731	< 0.001	4.461	< 0.001

biochar can interact with soil, enhancing soil fertility with increased soil N and P contents, thus contributing to increased plant growth (Biederman and Harpole, 2013; Gundale et al., 2016; Schulz et al., 2013; J. Zhang et al., 2017). However, there are relatively few studies addressing the potential effects of biochar application on the nutritional status, especially the N:P ratio, in plants (Jeffery et al., 2015). This is unfortunate, because understanding these mechanisms is needed to predict potential long-term changes in plant productivity in biochar-amended plantations.

Chinese torreya (*Torreya grandis* Fort. ex Lindl. cv. Merrillii.) is one of the economically most important evergreen forest tree species in Southeastern China. Zhejiang province, the main growing region of *T. grandis*, is subjected to a high N deposition with an average rate of 30.9 kg N ha^{-1} yr^{-1} (Song et al., 2017b; R. Zhang et al., 2017). However, studies addressing the potential effects of N deposition on foliar stoichiometry in *T. grandis* are still lacking. In a short-term study, R. Zhang et al. (2017) found that soil fertility can be improved by applying biochar to *T. grandis* plantation suffering from N deposition. However, little is known about how biochar and its interaction with N deposition may influence the foliar stoichiometry. Information of these potential effects is needed for designing forest management strategies under the changing environmental conditions in the future.

In order to study how N deposition and biochar application affect foliar stoichiometry in *T. grandis* trees and seedlings, we applied additional N deposition and biochar treatments in field plots in a subtropical *T. grandis* plantation in southeastern China. We hypothesized that: (1) Additional N deposition increases foliar N concentration, but foliar P concentration remains unchanged, thus resulting in an increase of foliar N:P ratio; (2) biochar application alone increases foliar concentrations of both N and P; and (3) biochar interacts with N addition, counteracting the increase of N:P ratio induced by N addition. Testing these hypotheses will provide novel insights into the potential function of biochar in alleviating tree nutrient limitation caused by increased N deposition in forest ecosystems.

2. Materials and methods

2.1. Study site and experimental design

The experiment was conducted in the Lin'an research area (30°14′N, 119°42′E) in southeastern China. Soil at this site is acidic and belongs to the Hapludult soil type in Soil Taxonomy (Gong, 2001). Details of the soil characteristics can be found in R. Zhang et al. (2017). The site has a subtropical, monsoonal climate and clear-cut seasons. The mean annual precipitation is 1614 mm. The mean annual air temperature is +15.6 °C, and the mean monthly air temperature in January and July is +4.5 °C and +28.9 °C, respectively.

The main part of the *T. grandis* plantation was established in 2000 with grafted seedlings of the 'Merrillii' cultivar at a density of 900 to 1000 trees per hectare (R. Zhang et al., 2017). Nuts of these trees are currently harvested annually and each of the trees produces on the average 18 kg nuts per year. A new *T. grandis* plantation was established in 2012, using the same procedures as when establishing the main plantation in 2000.

The experiments were conducted both with the mature trees established in 2000 (later "trees") and with the seedlings established in 2012 (later "seedlings"). For both trees and seedlings, we arranged a complete random design with nine treatments (3 × 3 nitrogen and biochar treatments) by three replicates (plots). According to the local deposition rate (30.9 kg N ha^{-1} yr^{-1}) and the widely used method to simulate N deposition (Song et al., 2017b; R. Zhang et al., 2017), we applied three levels of additional N deposition: Ambient N deposition without added nitrogen (nitrogen control, N0), a low level of added N deposition (ambient plus 30 kg N ha^{-1} yr^{-1}, N30), and a high level of added N deposition (ambient plus 60 kg N ha^{-1} yr^{-1}, N60). Based on our previous study (R. Zhang et al., 2017), we applied within each N treatment three levels of biochar: 0 (biochar control, BC0), 20 (BC20), and 40 (BC40) t biochar ha^{-1}. Each treatment was conducted in a 4 × 4 m plot with one experimental tree or seedling in the center. In this way, a total of 9 treatments × 3 plots/treatment × 1 tree (seedling)/plot = 27 trees (seedlings) were included in the study.

Ammonium nitrate (NH_4NO_3) was used to simulate additional N deposition, since it is closest to the chemical composition of local N deposition (NH_4^+:NO_3^- ≈ 56:44) (J. Zhang et al., 2017). From March 2015, additional N deposition was applied at the beginning of each month. Quantified NH_4NO_3 was weighted according to each N addition level, dissolved in water, and was then evenly sprayed to each plot with an electric sprayer.

Biochar was made from wheat straw through a pyrolysis process at 450 °C under anoxic conditions (Sanli New Energy Company, Henan, China), and was ground and sieved by 2 mm. The basic properties of the biochar applied can be found in R. Zhang et al. (2017). Briefly, it has high surface area (9.7 m^2 g^{-1}), is alkaline (pH = 9.8), and is rich in total N (5.2 g kg^{-1}) and P (3.4 g kg^{-1}). In March 2015, biochar was added to the uppermost approximately 20 cm of the soil in each plot assigned for biochar application. The biochar was mixed thoroughly into the soil by ploughing.

2.2. Foliar chemistry and soil physicochemical measurements

Leaves were sampled for chemical analyses on four occasions: at the beginning of June and September in both 2016 and 2017. On each occasion, we selected five mature and healthy sample leaves in the south facing side of the mid-canopy of each experimental tree and seedling. Leaves were stored in a box with ice and were immediately transferred to the laboratory. They were oven-dried at 65 °C for 48 h, and then ground before chemical analyses.

In order to understand the effects of the treatments on the foliar stoichiometry, the properties of the soil in each treatment were examined at the end of the experiment in September 2017. We collected randomly the top 20 cm soil from five spots in each experimental plot with a soil auger (3.5 cm diameter), and then mixed the samples originating from the same plot. The soil samples were air-dried and grounded before determining soil chemical properties.

Foliar N concentration and concentration of soil total N were measured by a CN analyzer (Sumigraph NC-80, Shimadzu, Japan). The P concentration of both the leaves and the soil was determined by

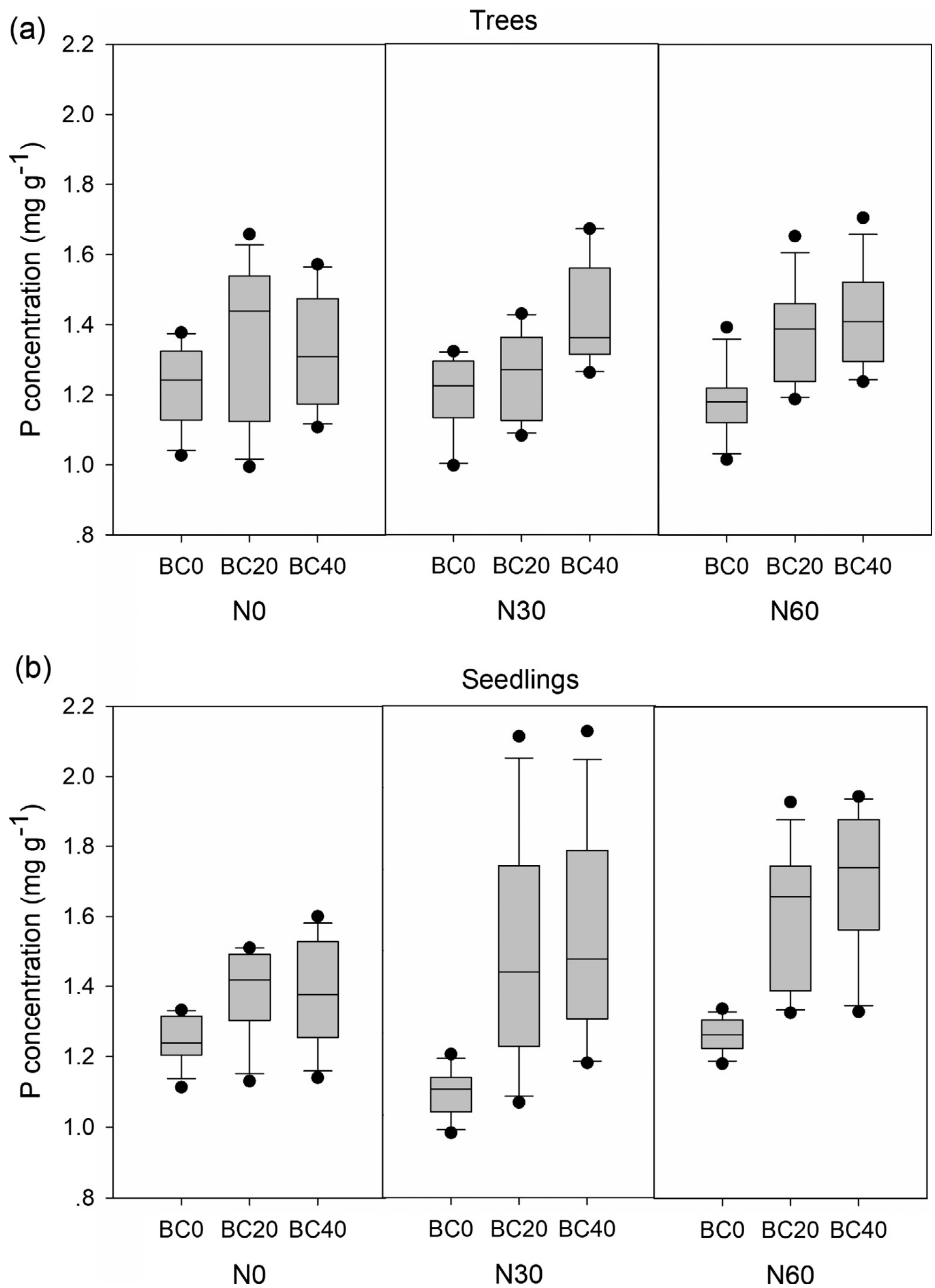

Fig. 2. Effects of additional N deposition and biochar treatments on the concentration of foliar phosphorus (P) in *Torreya grandis* trees (a) and seedlings (b). N0: ambient N deposition without added nitrogen (control). N30: treatment with a low level of added N deposition (ambient plus 30 kg N ha^{-1} yr^{-1}). N60: treatment with a high level of added N deposition (ambient plus 60 kg N ha^{-1} yr^{-1}). BC0: no biochar application (control). BC20: 20 t biochar ha^{-1}. BC40: 40 t biochar ha^{-1}. The box plots represent pooled data from four samplings carried out at the beginning of June and September both in 2016 and 2017.

colorimetric analysis with a modified Kjeldahl method (Song et al., 2016). Foliar N:P ratio was calculated as the mass ratio of these two elements. Soil pH was measured using a pH meter (FE20, Mettler Toledo, Switzerland) after shaking the soil water (1:2.5 w/v) suspension for 30 min. The concentration of soil available N was determined by the alkaline-$KMnO_4$ method (Prasad, 1965). The available P in the soil was first extracted by shaking a 1 g soil sample with 50 mL $NaHCO_3$ (0.5 mol L^{-1}, pH 8.5) for 1 h, and the concentration of the available P in the solution was subsequently determined by a spectrophotometer (UV2550, Shimadzu, Japan) (Song et al., 2016).

2.3. Statistical analysis

Data were analyzed with a three-factor ANOVA, with biochar treatment, N addition treatment, and sampling time as factors affecting foliar concentrations of N and P, the N:P ratio in the leaves, and the physicochemical properties of the soil. The data satisfied the assumption of homogeneity of variance. Following ANOVA, we compared means using Fisher's least significance difference (LSD) at $P \leq 0.05$. Pearson correlation analysis was performed to test the correlation between foliar N:P ratio and soil characteristics. All data were analyzed by SPSS software (version 16.0, SPSS Inc., Chicago, USA).

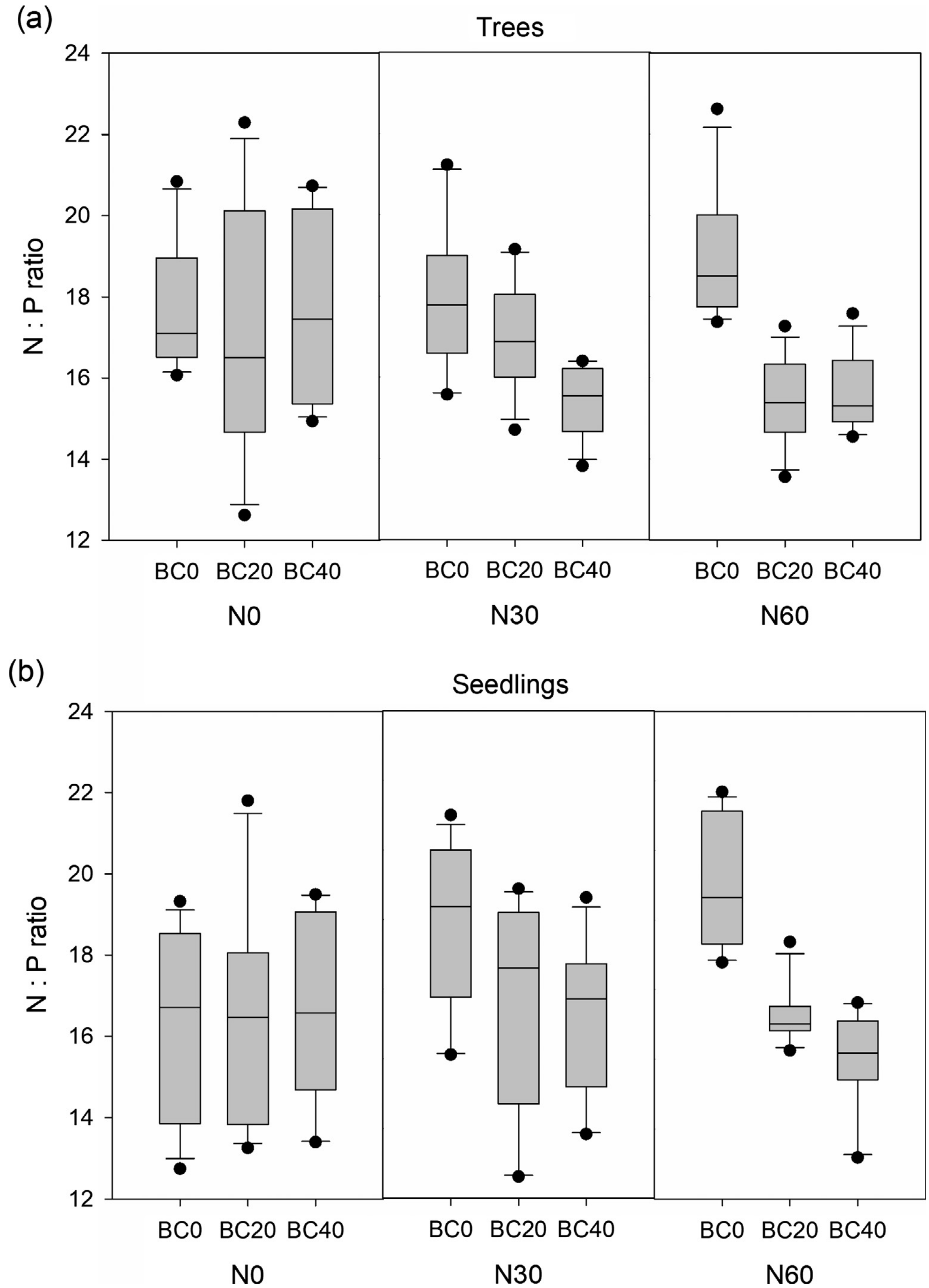

Fig. 3. Effects of additional N deposition and biochar treatments on the foliar nitrogen: phosphorus ratio (N:P) in *Torreya grandis* trees (a) and seedlings (b). N0: ambient N deposition without added nitrogen (control). N30: treatment with a low level of added N deposition (ambient plus 30 kg N ha^{-1} yr^{-1}). N60: treatment with a high level of added N deposition (ambient plus 60 kg N ha^{-1} yr^{-1}). BC0: no biochar application (control). BC20: 20 t biochar ha^{-1}. BC40: 40 t biochar ha^{-1} The box plots represent pooled data from four samplings carried out at the beginning of June and September both in 2016 and 2017.

Table 2
Pearson correlation coefficients of the foliar N:P ratio with soil characteristics at the end of the experiment in September 2017. The asterisks ** and * indicate statistical significances at α = 0.01 and 0.05, respectively.

	Total N	Total P	Available N	Available P	pH	Total N: Total P
Trees	−0.519**	−0.560**	0.006	0.096	−0.483*	0.083
Seedlings	−0.225	−0.572**	0.138	0.490**	−0.545**	0.562**

3. Results

3.1. Foliar N concentration

The average foliar N concentration over the four sampling times was in the double control (N0 + BC0) 21.23 mg g^{-1} in the trees and 20.35 mg g^{-1} in the seedlings (Fig. 1a, b). Compared with the double control, N addition alone generally increased foliar N concentration in the trees in all of the four sampling times (Fig. 1a; Fig. S1), and in a few cases, the increases were significant. For instance, N60 significantly increased foliar N concentration in the measurements in September 2016 (Fig. S1b) and September 2017 (Fig. S1d). Meanwhile, more significant increases of foliar N concentration in response to N addition occurred in the seedlings in all four sampling times (Fig. 1b; Fig. S1e, f, g and h). Compared with the double control, biochar application alone significantly increased foliar N concentration in both trees and seedlings in all four sampling times, with the exception of the measurement carried out with the seedlings in June 2017 (Fig. S1).

Biochar application interacted with N addition significantly in determining the foliar N concentration (Table 1). Compared with the controls (N0, BC0), many significant increases of the concentration occurred in response to biochar application and N addition in both trees and seedlings (Fig. 1; Fig. S1), the trees investigated in June 2017 being an exception (Fig. S1c). However, compared with the treatments with N addition alone, the patterns of foliar N concentration in response to biochar application combined with N addition were diverse (Fig. 1). For instance, as compared with the corresponding N addition alone, as a result of using the combination of biochar application and N addition foliar N concentration either increased (Fig. S1e, 1h), decreased (Fig. S1b, 1c, 1f), or remained unchanged (Fig. S1a).

3.2. Foliar P concentration

The average foliar P concentration over the four sampling times was in the double control (N0 + BC0) 1.22 mg g^{-1} in the trees and 1.24 mg g^{-1} in the seedlings (Fig. 2a, b). Compared to the double control, N addition alone did not cause any significant changes in the foliar P concentration in either the trees or the seedlings in any of the four sampling times (Fig. 2; Fig. S2). When only biochar was applied, compared with the double control, several significant increases of P concentration occurred (Fig. 2; Fig. S2a, c, d, e, f, and g).

The interactive effects of biochar application and N addition on foliar P concentration were significant (Table 1). Compared with the double control (N0 + BC0), many significant increases in foliar P concentration occurred in response to interactions of biochar application and N addition (Fig. 2; Fig. S2a, b, d, e, f, g, and h). Biochar application interacted with N addition as the foliar P concentration increased when N addition was combined with biochar application, but not when N addition was carried out alone (Fig. 2; Fig. S2a, b, d, e, f, g, and h).

3.3. Foliar N:P ratio

The average N:P ratio over the four sampling times was in the double control (N0 + BC0) 17.4 mg g^{-1} in the trees and 16.4 mg g^{-1} in the seedlings (Fig. 3a, b). Compared with the double control, N addition alone significantly increased the N:P ratio in the trees in the measurements in June and September 2017 (Fig. S3c and d). In the seedlings, N addition alone increased the N:P ratio in all four sampling times (Fig. 3b; Fig. S3e, f, g and h).

As compared with the double control, biochar application alone did not generally affect the N:P ratio (Fig. 3a, b). However, the ratio was decreased by the BC20 treatment in the trees in June 2017 (Fig. S3c) and increased by the BC40 treatment in seedlings in June 2017 (Fig. S3g).

The interaction of biochar application and N addition on the foliar N:P ratio was significant (Table 1). Compared with the double control (N0 + BC0), the N:P ratio decreased significantly in response to the combination of biochar application and N addition, both in trees and in seedlings in all four sampling times, the measurements with seedlings in June 2017 being an exception (Fig. S3g). Furthermore, compared with each N addition alone treatment, biochar application significantly decreased the N:P ratio in almost all cases (Fig. 3; Fig. S3).

3.4. Relationships between foliar stoichiometry and soil physicochemical properties

The results for the soil physicochemical properties are reported in the Supplementary material (Figs. S4 and S5). We found that in most cases the combination of biochar application and N addition increased significantly soil total P concentration and pH in both the tree and the seedling plantation (Fig. S4 and S5). Pearson correlation analysis showed that foliar N:P ratio correlated negatively with concentration of soil total P concentration and soil pH in both the tree and the seedling plantation (Table 2). Furthermore, foliar N:P ratio correlated negatively with concentration of soil total N in the tree plantation, and positively with the soil N:P ratio in the seedling plantation (Table 2).

4. Discussion

4.1. Foliar stoichiometric responses to N addition

We found for the *T. grandis* trees and seedlings growing in current environmental conditions (double control) higher foliar N concentrations, lower foliar P concentrations, and higher foliar N:P ratios than the corresponding average values found by Han et al. (2005) for 753 Chinese terrestrial plant species. *T. grandis* is classified as a slow-growing species (Shen et al., 2014). Our results supported, then, the growth-rate hypothesis that a relatively high N:P ratio is often found to be associated with a low growth rate (Matzek and Vitousek, 2009).

In support of our first hypothesis, we found that increased N deposition generally leads to increased foliar N concentrations in both *T. grandis* trees and seedlings. This is consistent with the general recognition that N deposition often has direct fertilizing effects on trees in forest ecosystems (Huang et al., 2016; Sardans et al., 2016; Song et al., 2016). These results also suggested that the concentration of available N in our study area was limiting the growth of the *T. grandis* trees and seedlings. Furthermore, seedlings seemed to be more sensitive to N addition than trees. This is probably due to the generally higher requirement of N nutrient for vegetative growth in seedlings than in trees (Ishida et al., 2005).

The concentration of available P in the soil is generally rather low in subtropical areas in China, so that growth of the plants growing in these areas is generally strongly limited by the availability of P (Han et al., 2005). In the present study, no changes in foliar P concentration were found in respond to N addition in either trees or seedlings in any of the four sampling times carried out during the study period lasting for 16 months. This indicates a limited capacity of the *T. grandis* trees and seedlings to acquire P when its concentration in the soil is low. This restricted response is consistent with the weak relationship between foliar P concentration and N deposition found in coniferous species growing on infertile soils with low availability of P (Crowley et al., 2012). On the contrary, plants growing on fertile soils with high availability of P can maintain the nutrition balance by increasing P uptake when N loading increases (Phoenix et al., 2012; Sardans et al., 2016).

Although N addition increased the concentration of foliar N in the present study, it failed to promote P uptake in the *T. grandis* trees and seedlings, resulting in an increased foliar N:P ratio. According to a generalization proposed by Gusewell (2004), N:P ratio often negatively correlates with rates of plant growth and biomass production, and P limitation often occurs when foliar N:P ratio exceeds 20. In our study, N addition increased the N:P ratio, and in a few cases, its value was near or above 20 (Fig. S3), indicating a N-addition-induced P limitation. These results not only support our first hypothesis stating that N addition increases the N:P ratio but were also in agreement with previous studies with forest trees demonstrating that increasing N deposition may aggravate P limitation (Huang et al., 2016; Sardans et al., 2016; Song et al., 2016).

4.2. Foliar stoichiometric responses to biochar and its interactions with N addition

Consistent with our second hypothesis, we found that biochar application generally increased the concentrations of both foliar N and P. A plausible explanation for these strong effects of biochar on concentrations of foliar N and P is that biochar itself contains nutrients (i.e., NH_4^+, ortho-P) on its surface, contributing to the increased uptake of both N and P (Gul and Whalen, 2016). However, availabilities of N and P in the soil were not consistent with the increasing pattern of concentrations of foliar N and P after biochar application, and in some cases, the concentrations of available N and P in the soil were even decreased by biochar (Fig. S4, S5). This is probably due to its high surface area with strong adsorption capacity and slow release of nutrients from it to the soil (Biederman and Harpole, 2013; R. Zhang et al., 2017). Another possible explanation is that ammonium was converted into oxidized forms of nitrogen during the drying of the soil (Patrick and Wyatt, 1964). Interestingly, biochar application alone generally did not affect the foliar N:P ratios, suggesting that biochar can provide a balanced availability of nutrients in the soil, so that the *T. grandis* trees and seedlings can maintain their stoichiometric homoeostasis with similar changes in N and P uptake.

In the present study, both N addition and biochar application alone increased the concentration of foliar N. However, their interaction was not always positive, and in some cases, it was even negative compared with the separate effects of each factor alone. A possible explanation for these negative interactions is that the uptake rate of N may become saturated by one of the factors alone (Crowley et al., 2012), so that biochar application combined with N addition did not increase the uptake further, or it even lead to toxicity due to excessive availability of N in the soil (Song et al., 2016). In contrast, compared with each N treatment alone, the concentration of foliar P was generally increased by the combination of biochar and N addition. These results suggest that due to the increased N deposition, N limitation would be gradually alleviated. However, plant growth would still be limited by the availability of P if biochar is not applied. This is in line with the general conclusion of Han et al. (2005) who stated that plant growth in the subtropical areas of China is in general limited more by the availability of P than that of N.

According to the above reasoning, due to the increased N deposition there is no longer any N limitation in the subtropical areas of China, but the growth limitation caused by low availability of P has been aggravated due to increased foliar N:P ratio (Gusewell, 2004). Though biochar application alone did not affect foliar N:P ratio, it interacted with N addition by effectively decreasing N:P ratios in the treatment groups with nitrogen addition. This is consistent with our third hypothesis. The decrease of the N:P ratio was a result of the sharply increased foliar P concentration in response to the simultaneous biochar application and N addition (Fig. 2). These results indicated that rather than biochar application or N addition alone, a combination of them is efficient in promoting availability of P to the *T. grandis* trees and seedlings. Our Pearson correlation analysis also showed that the decrease of foliar N:P ratio correlated with the increase of concentration of total P in the soil for both *T. grandis* trees and seedlings (Table 2). Indeed, our data showed that, in most cases, the combination of biochar application and N addition increased the concentration of total P in the soil (Fig. S4, S5). This result is consistent with several earlier experimental studies suggesting that biochar strongly affects soil P availability and that soil interactions and the effects of biochar depend on soil N availability (Asai et al., 2009; Gul and Whalen, 2016; R. Zhang et al., 2017). The combination of biochar application and N addition may enhance plant acquisition of P in several ways. Biochar may favor organic P hydrolysis due to a high microbial carbon concentration in biochar-amended soil (Gul and Whalen, 2016). Furthermore, the increase of soil pH caused by biochar can promote the mineralization of soil P (Gundale et al., 2016). However, these potential explanations remain largely hypothetical, so that further studies addressing these mechanisms are needed.

As discussed above, biochar application was related with enhanced uptake of P and decreased foliar N:P ratios. Notably, the N:P ratios which in a few cases exceeded 20 by N addition alone were decreased below 20 after biochar application (Fig. S3), suggesting alleviation of P limitation induced by N addition (Gusewell, 2004). In China, increased N deposition may shift large regions of forests to P limitation, leading to limited plant performance and decreased forest productivity (Gusewell, 2004; Du et al., 2016). Our study suggested, for the first time, that by effectively decreasing the foliar N:P ratios biochar application can provide for forest management a valuable tool to alleviate the P limitation induced by N deposition.

5. Conclusions

Our experiments provide a first-time evaluation of the interactive effects of biochar application and N addition on foliar stoichiometry of *Torreya grandis* trees and seedlings in a forest ecosystem exposed to increasing N deposition. These interactive effects facilitate maintaining nutrient balance of forests under global change. Our results suggested that N addition alone increased concentrations of foliar N but not those of foliar P, resulting in increased N:P ratios, and in some cases, in P limitation of growth. Biochar application under the current deposition climate increased concentrations of both foliar N and P, but did not affect the N:P ratio, suggesting that biochar application can provide the *Torreya* trees and seedlings a well-balanced nutrient uptake and stoichiometric homoeostasis. Furthermore, significant interactive effects of biochar application and N addition on the concentrations of foliar N and P were found. Notably, biochar application interacting with N addition sharply increased concentrations of foliar P. Finally, our results show, for the first time, that biochar application combined with N addition significantly declined foliar N:P ratio. This suggests that biochar application would be an effective way to alleviate P limitation induced by N deposition. The underlying mechanisms about the interactive effects of biochar and N deposition on foliar stoichiometry remain to be determined in future studies.

Acknowledgements

The authors thank the Chinese National Natural Science Foundation (Grant Nos. 31570616, 31800579), Zhejiang Provincial Natural Science Foundation of China (Grant No. LQ18C160001), the Key Research Program of Zhejiang Province (2018C02004), and the Selective Breeding of New Cultivars in Torreya grandis (Grant 2016C02052-12) for financial support.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2018.09.040.

References

Ahmad, M., Rajapaksha, A.U., Lim, J.E., Zhang, M., Bolan, N., Mohan, D., Vithanage, M., Lee, S.S., Ok, Y.S., 2014. Biochar as a sorbent for contaminant management in soil and water: a review. Chemosphere 99, 19–33.

Asai, H., Samson, B.K., Stephan, H.M., Songyikhangsuthor, K., Homma, K., Kiyono, Y., Inoue, Y., Shiraiwa, T., Horie, T., 2009. Biochar amendment techniques for upland rice production in Northern Laos : 1. Soil physical properties, leaf SPAD and grain

yield. Field Crops Res. 111, 81–84.
Basso, A.S., Miguez, F.E., Laird, D.A., Horton, R., Westgate, M., 2013. Assessing potential of biochar for increasing water-holding capacity of sandy soils. Global Change Biol. Bioenergy 5, 132–143.
Biederman, L.A., Harpole, W.S., 2013. Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. Global Change Biol. Bioenergy 5, 202–214.
Binkley, D., Hogberg, P., 2016. Tamm review: revisiting the influence of nitrogen deposition on Swedish forests. For. Ecol. Manage. 368, 222–239.
Cayuela, M.L., Sánchez-Monedero, M.A., Roig, A., Hanley, K., Enders, A., Lehmann, J., 2013. Biochar and denitrification in soils: when, how much and why does biochar reduce N2O emissions? Sci. Rep. 3, 1732.
Crowley, K.F., McNeil, B.E., Lovett, G.M., Canham, C.D., Driscoll, C.T., Rustad, L.E., Denny, E., Hallett, R.A., Arthur, M.A., Boggs, J.L., Goodale, C.L., Kahl, J.S., McNulty, S.G., Ollinger, S.V., Pardo, L.H., Schaberg, P.G., Stoddard, J.L., Weand, M.P., Weathers, K.C., 2012. Do nutrient limitation patterns shift from nitrogen toward phosphorus with increasing nitrogen deposition across the Northeastern United States? Ecosystems 15, 940–957.
Du, E., de Vries, W., Han, W., Liu, X., Yan, Z., Jiang, Y., 2016. Imbalanced phosphorus and nitrogen deposition in China's forests. Atmos. Chem. Phys. 16, 8571–8579.
Elser, J.J., Sterner, R.W., Gorokhova, E., Fagan, W.F., Markow, T.A., Cotner, J.B., Harrison, J.F., Hobbie, S.E., Odell, G.M., Weider, L.J., 2010. Biological stoichiometry from genes to ecosystems. Ecol. Lett. 3, 540–550.
Ferretti, M., Marchetto, A., Arisci, S., Bussotti, F., Calderisi, M., Carnicelli, S., Cecchini, G., Fabbio, G., Bertini, G., Matteucci, G., De Cinti, B., Salvati, L., Pompei, E., 2014. On the tracks of Nitrogen deposition effects on temperate forests at their southern European range – an observational study from Italy. Glob. Change Biol. 20, 3423–3438.
Gong, Z., 2001. Chinese Soil Taxonomy. Science Press, Beijing, China.
Gul, S., Whalen, J.K., 2016. Biochemical cycling of nitrogen and phosphorus in biochar-amended soils. Soil Biol. Biochem. 103, 1–15.
Gundale, M.J., Nilsson, M.C., Pluchon, N., Wardle, D.A., 2016. The effect of biochar management on soil and plant community properties in a boreal forest. Global Change Biol. Bioenergy 8, 777–789.
Gusewell, S., 2004. N:P ratios in terrestrial plants: variation and functional significance. New Phytol. 164, 243–266.
Han, W., Fang, J., Guo, D., Zhang, Y., 2005. Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. New Phytol. 168 (2), 377–385.
Huang, Z., Liu, B., Davis, M., Sardans, J., Penuelas, J., Billings, S., 2016. Long-term nitrogen deposition linked to reduced water use efficiency in forests with low phosphorus availability. New Phytol. 210, 431–442.
Ishida, A., Yazaki, K., Hoe, A., 2005. Ontogenetic transition of leaf physiology and anatomy from seedlings to mature trees of a rain forest pioneer tree, Macaranga gigantea. Tree Physiol. 25, 513.
Jeffery, S., Bezemer, T.M., Cornelissen, G., Kuyper, T.W., Lehmann, J., Mommer, L., Sohi, S.P., van de Voorde, T.F.J., Wardle, D.A., van Groenigen, J.W., 2015. The way forward in biochar research: targeting trade-offs between the potential wins. Global Change Biol. Bioenergy 7, 1–13.
Jonard, M., Fürst, A., Verstraeten, A., Thimonier, A., Timmermann, V., Potočić, N., Waldner, P., Benham, S., Hansen, K., Merilä, P., Ponette, Q., Cruz, A.C., Roskams, P., Nicolas, M., Croisé, L., Ingerslev, M., Matteucci, G., Decinti, B., Bascietto, M., Rautio, P., 2015. Tree mineral nutrition is deteriorating in Europe. Glob. Change Biol. 21, 418–430.
Laird, D., Fleming, P., Wang, B., Horton, R., Karlen, D., 2010. Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma 158, 436–442.
Li, Q., Lei, Z., Song, X., Zhang, Z., Ying, Y., Peng, C., 2018. Biochar amendment decreases soil microbial biomass and increases bacterial diversity in Moso bamboo (*Phyllostachys edulis*) plantations under simulated nitrogen deposition. Environ. Res. Lett. 13, 044029.
Liu, X., Zhang, Y., Han, W., Tang, A., Shen, J., Cui, Z., Vitousek, P., Erisman, J.W., Goulding, K., Christie, P., 2013. Enhanced nitrogen deposition over China. Nature 494, 459–462.
Matzek, V., Vitousek, P.M., 2009. N:P stoichiometry and protein:RNA ratios in vascular plants: an evaluation of the growth-rate hypothesis. Ecol. Lett. 12, 765–771.
Meunier, C.L., Gundale, M.J., Sanchez, I.S., Liess, A., 2016. Impact of nitrogen deposition on forest and lake food webs in nitrogen-limited environments. Glob. Change Biol. 22, 164–179.
Patrick, W.H., Wyatt, R., 1964. Soil nitrogen loss as a result of alternate submergence and drying 1. Soil Sci. Soc. Am. J. 28 (5), 647–653.
Phoenix, G.K., Emmett, B.A., Britton, A.J., Caporn, S.J.M., Dise, N.B., Helliwell, R., Jones, L., Leake, J.R., Leith, I.D., Sheppard, L.J., Sowerby, A., Pilkington, M.G., Rowe, E.C., Ashmorek, M.R., Power, S.A., 2012. Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments. Glob. Change Biol. 18, 1197–1215.
Prasad, R., 1965. Determination of potentially available nitrogen in soils — a rapid procedure. Plant Soil 23, 261–264.
Sardans, J., Alonso, R., Janssens, I.A., Carnicer, J., Vereseglou, S., Rillig, M.C., Fernandez-Martinez, M., Sanders, T.G.M., Penuelas, J., 2016. Foliar and soil concentrations and stoichiometry of nitrogen and phosphorous across European Pinus sylvestris forests: relationships with climate, N deposition and tree growth. Funct. Ecol. 30, 676–689.
Schulz, H., Dunst, G., Glaser, B., 2013. Positive effects of composted biochar on plant growth and soil fertility. Agron. Sustain. Dev. 33, 817–827.
Shen, C., Hu, Y., Du, X., Li, T., Tang, H., Wu, J., 2014. Salicylic acid induces physiological and biochemical changes in *Torreya grandis* cv. Merrillii seedlings under drought stress. Trees 28, 961–970.
Sistla, S., Schimel, J., 2012. Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change. New Phytol. 196, 68–78.
Sohi, S.P., Krull, E., Lopezcapel, E., Bol, R., 2010. A review of biochar and its use and function in soil. Adv. Agron. 105, 47–82.
Song, X., Gu, H., Wang, M., Zhou, G., Li, Q., 2016. Management practices regulate the response of Moso bamboo foliar stoichiometry to nitrogen deposition. Sci. Rep. 6, 24107.
Song, X., Chen, X., Zhou, G., Jiang, H., Peng, C., 2017a. Observed high and persistent carbon uptake by Moso bamboo forests and its response to environmental drivers. Agric. For. Meteorol. 247, 467–475.
Song, X., Li, Q., Gu, H., 2017b. Effect of nitrogen deposition and management practices on fine root decomposition in Moso bamboo plantations. Plant Soil 410, 207–215.
Steinbeiss, S., Gleixner, G., Antonietti, M., 2009. Effect of biochar amendment on soil carbon balance and soil microbial activity. Soil Biol. Biochem. 41, 1301–1310.
Talkner, U., Meiwes, K.J., Potočić, N., Seletković, I., Cools, N., Vos, B.D., Rautio, P., 2015. Phosphorus nutrition of beech (*Fagus sylvatica* L.) is decreasing in europe. Ann. Forest Sci. 72 (7), 919–928.
Tao, L., Hunter, M.D., 2012. Does anthropogenic nitrogen deposition induce phosphorus limitation in herbivorous insects? Glob. Change Biol. 18, 1843–1853.
Veresoglou, S.D., Peñuelas, J., Fischer, R., Rautio, P., Sardans, J., Merilä, P., Tabakovic-Tosic, M., Rillig, M.C., 2014. Exploring continental-scale stand health – N:P ratio relationships for European forests. New Phytol. 202, 422–430.
Wang, J.Y., Xiong, Z.Q., Kuzyakov, Y., 2016. Biochar stability in soil: meta-analysis of decomposition and priming effects. Global Change Biol. Bioenergy 8, 512–523.
Zhang, J., Lv, J., Li, Q., Ying, Y., Peng, C., Song, X., 2017. Effects of nitrogen deposition and management practices on leaf litterfall and N and P return in a Moso bamboo forest. Biogeochemistry 134, 115–124.
Zhang, R., Zhang, Y., Song, L., Song, X., Hänninen, H., Wu, J., 2017. Biochar enhances nut quality of *Torreya grandis* and soil fertility under simulated nitrogen deposition. For. Ecol. Manage. 391, 321–329.