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Biochar enhances nut quality of *Torreya grandis* and soil fertility under simulated nitrogen deposition

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A B S T R A C T

Torreya grandis, an economically important nut tree in Southeast China, is being subjected to increasing atmospheric nitrogen (N) deposition, and thus far its impact on nut quality remains unknown. Also, studies evaluating nut quality response to biochar application (commonly used as soil amendment) to soils under N deposition conditions are rare. Here, we investigated changes of nut physical characteristics (i.e., nut weight, length and width and kernel weight) and nut nutritional components (i.e., lipid, protein, starch and total soluble sugars) under a factorial combination of biochar application (0, 20 t ha^{-1}) and simulated additional nitrogen deposition (0, 30 and 60 kg N ha^{-1} yr^{-1}) treatments in a growing season (approximately 7 months). Results showed that N addition had a direct fertilizing effect, with a significant increase of nut weight, nut size, kernel weight, and nut nutritional components in terms of lipid, protein, starch and total soluble sugars. However, soil chemical properties were negatively affected by N addition with significant decreases in soil pH and available N, P and K. Biochar application showed a liming effect, with a significant increase of soil pH which was significantly correlated with nut weight and the main nut nutritional components. Additionally, biochar strongly interacted with additional N deposition by increasing the availability of N, P and K in soil. Our study suggested, for the first time, that the 'win-win' can be achieved, both nut quality of *T. grandis* and soil fertility can be improved by biochar application to soils suffering from N deposition. Results are relevant for the successful improvement of nut crop quality and development of sustainable agriculture under accelerated N deposition worldwide.

1. Introduction

Nitrogen (N) deposition, accelerated by increasing agricultural fertilization, fossil fuel consumption and excessive deforestation, has become a global issue (Dise and Wright, 1995; Holland et al., 2005; Liu et al., 2013b). It has been calculated that the global deposition rates of N increased from 34 Tg N yr^{-1} in the late 19th century to 100 Tg N yr^{-1} in the late 20th century, and the deposition rates are predicted to double from current values by the mid-21st century (Galloway et al., 2004). Increased N deposition has raised concerns about its negative effects on the earth system, such as soil and water acidification, increased susceptibility of plants to secondary stresses, and declines in biodiversity (Bobbink et al., 1998; Townsend et al., 2003; Liu et al., 2006; Phoenix et al., 2012). It is crucial for local and global economies to take actions to reduce N deposition and its negative impact (Liu et al., 2013b).

N fertilizer is one of the major sources of NH_3 emission, leading to increased NH_4-N in the atmosphere, which is the dominant form in N deposition (Ju et al., 2009). In China, the use of N fertilizer surpassed the United States and the European Union in 2000. Meanwhile, the deposition rates of N increased from 13.2 kg yr^{-1} ha^{-1} (1980s) to 21.1 kg yr^{-1} ha^{-1} (2000s), and the annual bulk deposition of N was up to 18 Tg, equaling to approximately 60% of the national N fertilizer consumption (Liu et al., 2013b). Despite deposited N is a natural nutritional source that can enhance plant productivity through fertilization (Phoenix et al., 2012; Binkley and Hogberg, 2016; Meunier et al., 2016), long legacy of N deposition can cause acidification of soil via the introduction of anions and H^+, and lead to enhanced availability of potentially toxic cations (e.g. Al^{3+}, Fe^{3+}), which can affect plant health (Gundersen and Rasmussen, 1990; Bowman et al., 2008; Phoenix et al., 2012). An understanding of impacts of N deposition on crop quantity and quality is needed for improving N-use efficiency and for planning of N fertilization strategies for reducing NH_3 emissions from agricultural processes.

Biochar is a solid organic carbon compound obtained from incomplete combustion of organic materials in an oxygen-limited environment (Sohi et al., 2010). Currently, it is widely advocated as a soil amendment, offering a number of benefits of soil health

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such as increased soil carbon content, decreased nutrient runoff, and improved soil fertility and soil tilth (Tang et al., 2013; Wang et al., 2016). Numerous controlled field and greenhouse experiments have demonstrated that biochar application to soils can increase crop yields (Biederman and Harpole, 2013; Cranedroesch et al., 2013). Furthermore, adding biochar to soil can reduce uptake of trace elements and their toxicities in plants, attributing to its distinct properties such as alkalinity and high cation exchange capacity (Rizwan et al., 2016). For this reason, biochar application should be considered as a way to alleviate acidification of soil by N deposition. However, thus far studies lack experimental designs to evaluate how biochar combined with N deposition affects crop production and quality and soil fertility. This information is needed for improving nut quality and for developing sustainable agriculture by proper soil amendment under the increasing atmosphere N deposition.

Chinese torreya (*Torreya grandis*), a species of Taxaceae family, is an economically important native nut tree species in Southeast China. *T. grandis* production in Zhejiang Province totaled 3 million kg in 2015, with a farm-gate value of over $13 million (Zeng et al., 2015). However, the main cultivation area of *T. grandis* is subject to increasing degrees of N deposition, and annual bulk N deposition averaged 30.9 kg N ha^{-1} yr^{-1} (Zhao et al., 2008). There are relatively few data on how plant health is influenced by N accumulation in the soil and how this consequently affects nut quality. In addition, whether biochar application alleviate or synergize the effect of N deposition on nut quality remains unknown.

In this study, we compared how nut physical characteristics and the nutritional components of nuts in *T. grandis* respond to a factorial combination of three simulated N deposition and two biochar levels. In addition, changes of soil chemical properties were measured for better understanding of the potential mechanisms of N and biochar effects. We hypothesized that: (1) N deposition will have positive effects on nut quality, but increasing N deposition will negatively affect soil fertility (available N, P and K) and decrease soil pH; (2) the addition of biochar will enhance nut quality, and positively influence soil fertility and increase soil pH; (3) the effects of biochar on nut quality and soil fertility will interact with N deposition, with positive effects on both nut quality and soil fertility and increase in soil pH. Testing these hypotheses will provide valuable insights into proper soil amendment, alternative soil fertilization strategies and improvement of nut quality under the increasing global N deposition.

2. Materials and methods

2.1. Experimental site

The experimental site is located in Lin'an, Zhejiang Province, in southeastern China (30°14′N, 119°42′E). Lin'an has a subtropical, monsoonal climate and clear-cut seasons with mean annual precipitation of 1613.9 mm and mean annual temperature of 15.6 °C, ranging from 4.5 °C in January to 28.9 °C in July.

The *T. grandis* plants have grown at the site since 2000s with standard maintenance. The seedlings of all trees were grafted with the cultivar 'Merrillii' with a density of 900–1000 trees per hectare. Trees were 15 year old, and each of them had on average of 18 kg nut production. The soil type is acidic. The soil belongs to the yellow-red soil class (Chinese system of soil classification), which is equivalent to Hapludult soil in soil taxonomy. The initial soil characteristics are shown in Table 1. Soils were plowed annually in later October after harvest and the trees were fertilized at the rate of 58.5–58.5–58.5 kg/ha of N-P-K, respectively.

2.2. Plot preparation and the experimental design

Experiments were arranged in a complete random design with 6 treatments by 3 replicates. According to the local deposition rates of N (30.9 kg N ha^{-1} yr^{-1}) (Zhao et al., 2008) and the widely used method to double and triple the local deposition rate in order to simulate additional N deposition (Mo et al., 2006; Fang et al., 2007; Song et al., 2016a), three levels of N addition treatments were included: 0 kg N ha^{-1} yr^{-1} (N0, as a control), 30 kg N ha^{-1} yr^{-1} (low N-addition, NL) and 60 kg N ha^{-1} yr^{-1} (high N-addition, NH). Within each N treatment, two levels of biochar were applied: 0 kg BC ha^{-1} yr^{-1} (No biochar application, BC0) and 20 t ha^{-1} (Biochar application, BC). Each treatment was conducted in a 4 × 4 m plot with each tree in the center. The source of N used in the treatments was NH_4NO_3 (ammonium nitrate), since ammonium nitrate is closest to the chemical composition of local wet N deposition (NH_4^+ and NO_3^- accounting for 56.1% and 43.9%, respectively) (Song et al., 2017). Starting from March 2015, N deposition was simulated at the beginning of each month. Quantified ammonium nitrate was weighed according to the N addition rate and dissolved in water. The ammonium nitrate solution was evenly sprayed from the top of the canopy of the *T. grandis* trees with an electric sprayer. Each control site received in a similar way equal amount of N-free water. Biochar was made from wheat straw through a pyrolysis process at 450 °C under anoxic conditions (Sanli New Energy Company, Henan, China). The original biochar mass was ground so that it could pass through a 2 mm sieve and then mixed thoroughly to obtain a fine granular consistency. Final biochar had a pH value of 9.8, and its basic properties are shown in Table 2. In March 2015, biochar was mixed thoroughly into the soil in 20 cm depth.

2.3. Nut quality characteristics measurement

In early October 2015, 50 fresh nuts were harvested from each tree, and weight, length, width and kernel weight of the nuts were measured. Then all kernels were stored at −80 °C for lipid, protein, starch and total soluble sugars analysis. The kernels were ground in an Osterizer blender (Galaxie Model Number 869-18R, Jarden Consumer Solutions, Boca Raton, Fla., U.S.A.) to a homogeneous flour (40-mesh). Lipid content was determined by the AOAC Official Method 948.22 (AOAC, 1995). Approximately 10 g per thimble of whole fat flour was defatted in a Soxhlet apparatus with petroleum ether solvent (boiling point range 38.7–54.8 °C) for 8 h. The defatted samples were dried overnight (10 to 12 h) in a fume hood to remove residual petroleum ether and weighed to calculate lipid content. Protein content was determined by the AOAC Official Method 950.48 (AOAC, 1995). Approximately 0.25 g of whole fat flour were used in the micro-Kjeldahl N analysis. Protein (%) was

Table 1
Soil physicochemical properties the experimental site in the present study.

pH	Bulk density (g cm^{-3})	Organic carbon (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Total K (g kg^{-1})	Available N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
5.30 ± 0.0	1.3 ± 0.1	26.1 ± 0.0	2.1 ± 0.0	0.8 ± 0.0	13.6 ± 0.0	80.4 ± 1.2	206.4 ± 1.8	173.8 ± 5.1

Means for 3 replicate measurements are given with standard deviations.

Table 2
Physicochemical properties of the biochar used in the present study.

PH	Bulk density ($g\,cm^{-3}$)	Specific surface area ($m^2\,g^{-1}$)	Cation exchange capacity ($cmol\,kg^{-1}$)	Organic carbon ($g\,kg^{-1}$)	Total N ($g\,kg^{-1}$)	Total P ($g\,kg^{-1}$)	Ash (%)
9.80	0.5	9.7	189.3	425.3	5.2	3.4	18.60

calculated using the formula N (%) × 5.32 (AOAC, 1995). Starch content was determined by Colorimetric Method ((Dubois et al., 2002). Total soluble sugar was determined by the phenol-sulphuric acid method (Dubois et al., 2002).

2.4. Soil sampling

In early October 2015, three soil core samples, each from 3.5 cm × 20 cm deep, were taken randomly from each plot and mixed. Soils were sieved (<2 mm) to remove plant residues, stones and roots, and the soil samples were air-dried before measuring chemical properties. 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. Total N was determined using a CN automatic analyzer (Sumigraph NC-80, Shimadzu, Japan). Soil organic matter (SOM) was determined by the $K_2Cr_2O_7$ titration method (Nelson and Sommers, 1974). Soil samples were digested in a mixture of HNO_3-H_2SO_4-$HClO_4$ in order to measure total phosphorus (P) and total potassium (K) (Jackson, 2005). Available N (N) was determined by alkaline-$KMnO_4$ method (Prasad, 1965). Available phosphorus (P) and available potassium (K) were extracted by shaking 1 g soil with 50 mL $NaHCO_3$ (0.5 mol L^{-1}, pH 8.5) for 1 h, and determining P and K concentration in the solution by a spectrophotometer (UV2550, Shimadzu, Japan).

2.5. Data analyses

All data were analyzed by SPSS (Version 16.0, SPSS Inc., Chicago, USA). To evaluate effects of biochar application and N additions on physical properties (weight, length, width and kernel weight) and nutritional compositions (lipid, protein, starch and total soluble sugar) of the nuts, the data were subjected to analysis of variance (ANOVA) and mean separation was done by Fisher's least significance difference (LSD) at $P \leq 0.05$. A two-way ANOVA method was used to evaluate the interactional effects of biochar application and N addition on nut physical and nutritional properties. Associations between nut properties and soil physicochemical properties were determined by Pearson correlation analysis.

3. Results

3.1. Physical properties of the nuts

With the exception of length, the physical properties of the nuts were significantly affected by the treatments with biochar and added nitrogen deposition (Table 3). However, the interaction of these two treatments was significant only in the case of nut length (Table 3). Without applying biochar, a low level of added nitrogen caused a significant, and a high level of added nitrogen an insignificant, increase of nut fresh weight (Fig. 1A), kernel weight (Fig. 1B), nut length (Fig. 1C) and nut width (Fig. 1D). Without added nitrogen, biochar application increased significantly all of these four physical parameters (Fig. 1A–D). Compared with the corresponding treatment with added nitrogen alone, biochar application generally increased the values of the physical parameters.

3.2. Biochemical properties of the nuts

The biochemical properties of the nuts were significantly affected by the treatments with biochar and added nitrogen deposition, and also by their interaction (Table 3). In general, the effects of the treatments were greater in the biochemical (Fig. 2) than in the physical properties (Fig. 1) of the nuts. Without applying biochar, both levels of added nitrogen significantly increased the lipid content of the nuts (Fig. 2A).

However, with biochar application the low level of added nitrogen significantly decreased, and the high level insignificantly increased, the lipid content (Fig. 2A). At each level of nitrogen deposition, the biochar application significantly increased the lipid content, the increase being 23.8% under ambient nitrogen, and 2.4% and 6.4% at low and high added nitrogen, respectively (Fig. 2A). Without biochar application, the nut protein content was significantly increased by both levels of added nitrogen (Fig. 2B). However, with biochar application both levels of added nitrogen decreased the protein content; the high level of addition resulting in a lower protein content than the low level of addition (Fig. 2B). At each level of nitrogen deposition, applying biochar increased the protein content, but at the high level of added nitrogen the increase was not significant (Fig. 2B).

Without biochar application, both levels of added nitrogen significantly increased contents of total starch (Fig. 2C) and soluble sugars (Fig. 2D) of the nuts. However, with biochar application low added nitrogen significantly increased and high added nitrogen significantly decreased both of the contents of starch (Fig. 2C) and total soluble sugars (Fig. 2D). At each level of nitrogen deposition, the application of biochar increased the starch content, even though the increase was not significant at the high level of nitrogen addition (Fig. 2C). For the contents of total soluble sugars the effect of biochar was more diverse, as it significantly increased the

Table 3
Two-way ANOVA analysis for the effects of Biochar application (BC) and N addition on physical and biochemical properties of *Torreya grandis*.

		Physical properties								Biochemical properties									
Source		Nut weight		Kernel weight		Nut length		Nut width		Lipid		Protein		Starch		Total soluble sugars		Total nutrition	
	df	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.
BC	1	24.41	<0.001	17.062	0.001	0.464	0.509	55.084	<0.001	329.639	<0.001	82.002	<0.001	173.419	<0.001	142.3	<0.001	1045.656	<0.001
N	2	8.66	0.005	5.599	0.019	2.959	0.090	13.103	0.001	35.052	<0.001	5.047	0.026	30.274	<0.001	90.87	<0.001	122.751	<0.001
BC * N	2	1.372	0.291	1.236	0.325	3.973	0.047	0.026	0.975	99.539	<0.001	23.289	<0.001	36.607	<0.001	16.516	<0.001	252.436	<0.001

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, NS: no significance.
Total nutrition means the main nutritional components including lipid, protein, starch and total soluble sugars.

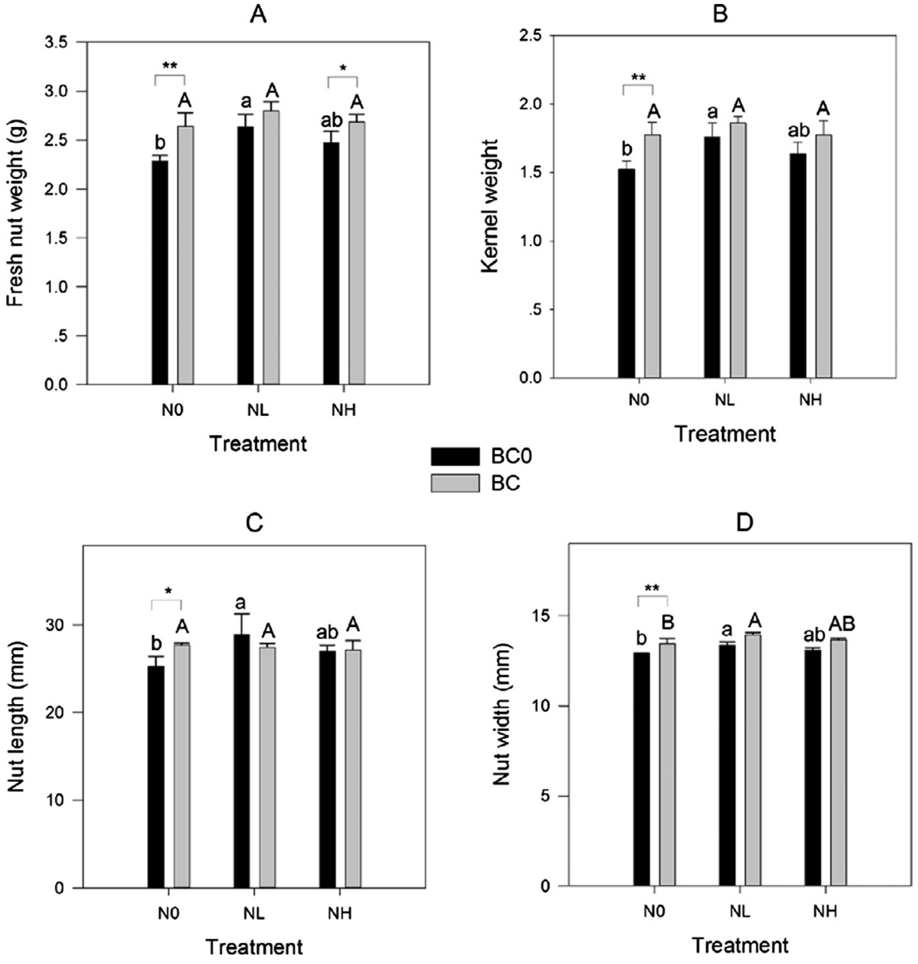

Fig. 1. Physical properties of nuts of *Torreya grandis* trees grown under nitrogen deposition and biochar treatments: fresh nut weight (A), kernel weight (B), nut length (C), and nut width (D). N0: ambient nitrogen deposition without added nitrogen. NL: treatment with low level of added nitrogen deposition (ambient plus 30 kg N ha^{-1} yr^{-1}). NH: treatment with high level of added nitrogen deposition (ambient plus 60 kg N ha^{-1} yr^{-1}). Bars with different letters indicate significant differences among nitrogen deposition treatments within none-biochar (BC0) treatments (lowercase letters), and correspondingly within biochar (BC) treatments (uppercase letters), both at $P \leq 0.05$. Asterisks indicate significant differences between BC0 and BC treatment within the same nitrogen treatment ($^{*}P < 0.05$, $^{**}P < 0.01$). All significances were calculated by Fisher's LSD.

content at ambient and at low added nitrogen levels, but significantly decreased the content at the high added nitrogen (Fig. 2D).

3.3. Soil chemical properties

The chemical properties of soil were significantly affected by the treatments with biochar and added nitrogen deposition, and also by their interaction (Table 4). Without biochar application, added nitrogen deposition significantly affected the soil properties (Fig. 3). Low level of added nitrogen increased the total content of phosphorus (Fig. 3C), but it decreased the total content of potassium (Fig. 3D) and the contents of available nutrients in the case of all three elements (Fig. 3E–G). High level of added nitrogen decreased SOM (Fig. 3A); and with the exception of available nitrogen (Fig. 3E), it decreased the total contents and the contents of available nutrients for the various elements (Fig. 3B, C, D, F, G). Both low and high level of added nitrogen resulted in a lower soil pH (Fig. 3H). Under ambient nitrogen deposition, biochar application significantly increased SOM (Fig. 3A) and total content of nitrogen (Fig. 3B), but it significantly decreased the total contents of the other two elements (Fig. 3C, D) and the contents of available nutrients for all three elements (Fig. 3E–G).

Under added nitrogen deposition the effects of biochar application changed dramatically, revealing a major interaction between the nitrogen deposition and application of biochar. Accordingly, with the exceptions of the content of total phosphorus under low level of nitrogen addition (Fig. 3C) and the content of available nitrogen under the high level of added nitrogen (Fig. 3E), the contents of the nutrients were increased by biochar application under conditions of added nitrogen deposition (Fig. 3B–G). Under all levels of nitrogen deposition, application of biochar increased the soil pH (Fig. 3H).

4. Discussion

4.1. Effects of N deposition on nut quality and soil properties

Our aim was to understand the individual and the interactive effects of N deposition and biochar application on nut quality, which is relevant for understanding of crop improvement, soil amendment and proper fertilization to reduce NH_3 emissions under the globalization of N deposition.

Consistent with our first hypothesis, that N deposition would positively affect nut quality, we found that nut weight, nut size and nutritional components in terms of lipid, protein, starch and sugars were all increased by N deposition (Figs. 1 and 2). These results show a direct fertilizing effect. Deposited N, given as a nutrient available to the trees, can be directly absorbed by the trees, resulting in the benefit of plant growth and production. This

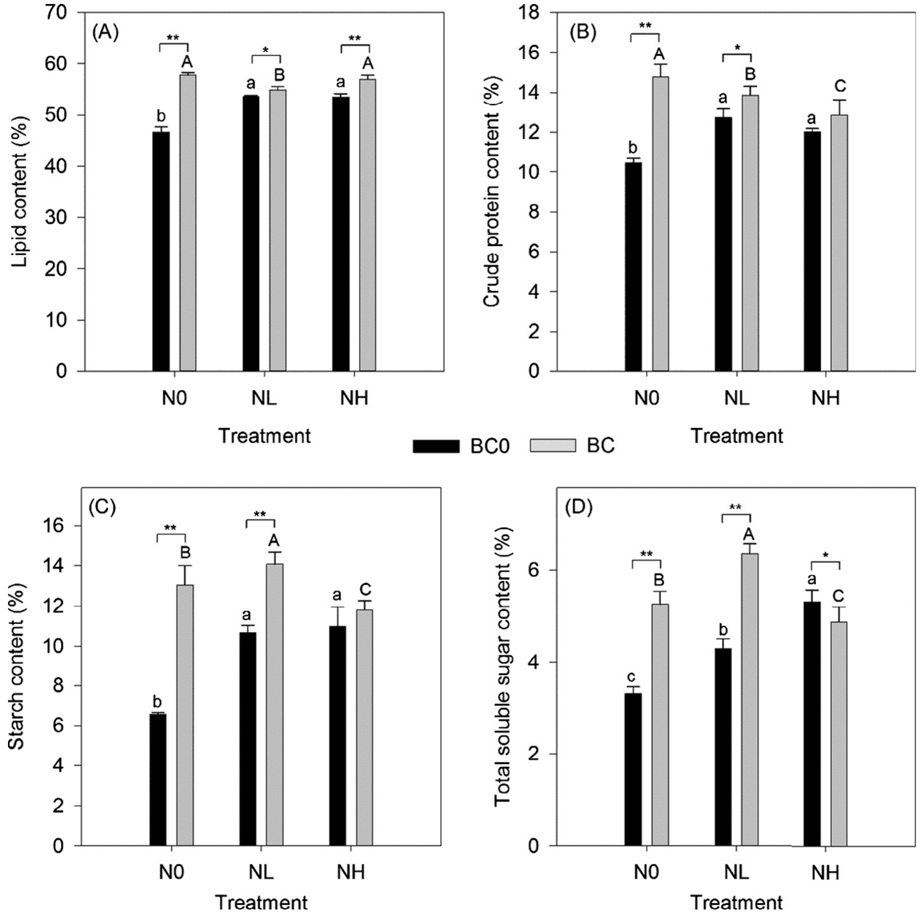

Fig. 2. Biochemical properties of nuts of *Torreya grandis* trees grown under nitrogen deposition and biochar treatments: nut lipid content (A), crude protein content (B), total soluble sugar content (C), and starch content (D). N0: ambient nitrogen deposition without added nitrogen. NL: treatment with low level of added nitrogen deposition (ambient plus 30 kg N ha^{-1} yr^{-1}). NH: treatment with high level of added nitrogen deposition (ambient plus 60 kg N ha^{-1} yr^{-1}). Bars with different letters indicate significant differences among nitrogen deposition treatments within none-biochar (BC0) treatments (lowercase letters), and correspondingly within biochar (BC) treatments (uppercase letters), both at $P \leq 0.05$. Asterisks indicate significant differences between BC0 and BC treatment within the same nitrogen treatment ($^{*}P < 0.05$, $^{**}P < 0.01$). All significances were calculated by Fisher's LSD.

Table 4

Two-way ANOVA analysis for the effects of Biochar application (BC) and Nitrogen (N) deposition on soil nutrition and pH.

Sourse	df	Soil organic matter		Total N		Total P		Total K		Available N		Available P		Available K		pH	
		F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.
N	2	20.485	<0.001	95.054	<0.001	901.872	<0.001	13.204	0.001	910.17	<0.001	1276.182	<0.001	321.801	<0.001	195.105	<0.001
BC	1	50.813	<0.001	502.368	<0.001	636.056	<0.001	59.054	<0.001	181.56	<0.001	18.402	0.001	3644.918	<0.001	6893.654	<0.001
BC * N	2	14.301	0.001	1.428	0.278	251.784	<0.001	89.059	<0.001	1705.66	<0.001	2465.611	<0.001	1236.327	<0.001	14.359	0.001

indicated that N was the limiting nutrient in our experimental field (Högberg et al., 2006; Plassmann et al., 2009). Another possible explanation of improved nut quality is that N deposition can increase the quantity of microorganisms in the soil and accelerate mineralization of organic compounds (Li et al., 2016). It is generally known that N addition can increase crop production. However, excessive N has showed negative effects (Taylor et al., 1991; Gray and Garrett, 1998; Ladha et al., 2005). In this study, we found that although both low and high N addition treatments positively influenced nut weight, high N addition led to lower nut weights compared to those under low simulated N deposition (Fig. 1). This indicates that N deposition exceeding 60 kg N ha^{-1} yr^{-1} reversed the positive effects.

N is necessary for life processes in plants, but N requirement varies greatly between species (Broadley and White, 2005). For example, in oat and corn, N addition increased protein content, but decreased oil content (Zhang et al., 1993; Humphreys et al., 1994). In walnut (lipid content was not influenced by the N addition Verardo et al., 2009). In this study, we demonstrated, for the first time, that both low and high N deposition increased all the main nutritional compositions (lipid, protein, starch and sugars) in nuts of *T. grandis* (Fig. 2), which suggested that *T. grandis* has high N demand for building these compounds. However, high N deposition did not increase the nutritional compounds as compared to low deposition (Fig. S1), suggesting that the optimal N supply for *T. grandis* was less than 60 kg N ha^{-1} yr^{-1} in this experimental field. These results provide valuable insight into how the global increasing N deposition may affect crop quality. Deposited N, as a natural nutritional source, showed positive effects on the nut quality in *T. grandis*. Thus, less N fertilization is recommended

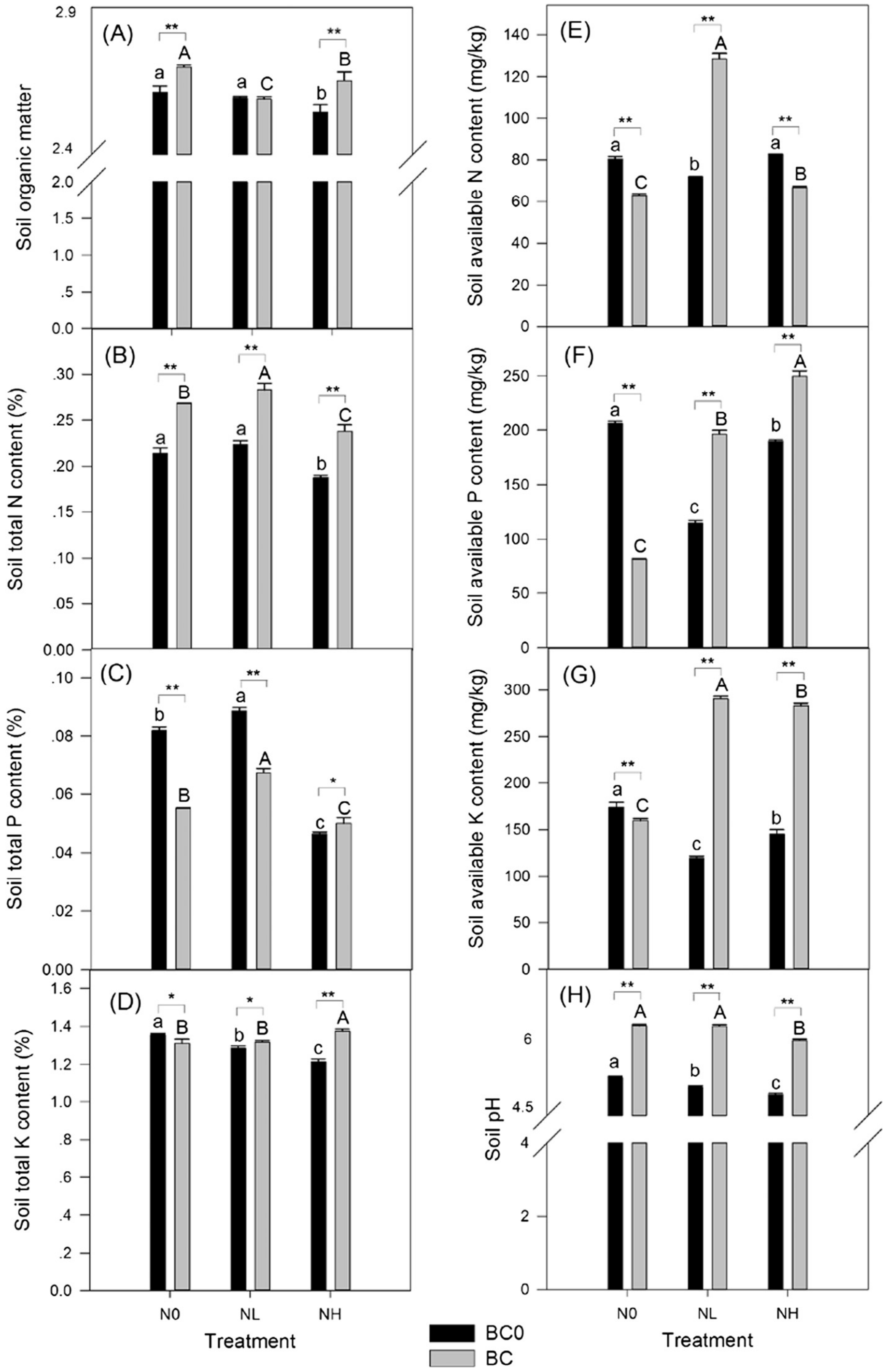

Fig. 3. Soil properties under nitrogen deposition and biochar treatments: organic matter (A), total nitrogen content (B), total phosphorus content (C), total potassium content (D), available nitrogen content (E), available phosphorus content (F), available potassium content (G), and pH (H). N0: ambient nitrogen deposition without added nitrogen. NL: treatment with low level of added nitrogen deposition (ambient plus 30 kg N ha^{-1} yr^{-1}). NH: treatment with high level of added nitrogen deposition (ambient plus 60 kg N ha^{-1} yr^{-1}). Bars with different letters indicate significant differences among nitrogen deposition treatments within none-biochar (BC0) treatments (lowercase letters), and correspondingly within biochar (BC) treatments (uppercase letters), both at $P \leq 0.05$. Asterisks indicate significant differences between BC0 and BC treatment within the same nitrogen treatment ($^{*}P < 0.05$, $^{**}P < 0.01$). All significances were calculated by Fisher's LSD.

in order to reduce NH_3 emissions by excessive N fertilization (Ju et al., 2009).

Our data also supported our first hypothesis that N deposition would negatively affect soil fertility and aggravate soil acidification. We found a small but significant decrease in soil pH after N deposition, where low and high N deposition decreased soil pH from 5.30 to 5.11 and 4.92, respectively (Fig. 3H). Meanwhile, soil available N, P and K were all significantly decreased by N deposition (Fig. 3E, F, G), except that high N deposition non-significantly increased soil available N. A plausible explanation for the effect of N deposition on soil available nutrition is that N deposition caused soil acidification, leading to enhanced availability of toxic metals and increased NO_3^- leaching, and causing depletion of base cations (Gundersen and Rasmussen, 1990; Bergkvist and Folkeson, 1992). In addition, high N deposition caused significantly reduced soil SOM and total N, P, and K (Fig. 3A–D), which was not beneficial to plant health from the long-term perspective (Broadley and White, 2005). Therefore, long-term effects of N deposition on nut production and quality is yet to be determined.

4.2. Effects of biochar application on nut quality and soil properties

Biochar, used as soil amendment, was commonly reported beneficial to crop production (Jeffery et al., 2011; Liu et al., 2013a). The biochar application level used in the present study (20 t/ha) has been generally used in the previous studies in order to examine the possible effects of biochar amendment on agroecosystems (Song et al., 2016b).

In this study, biochar application in the soil significantly increased fresh nut weight of *T. grandis* by 15.3%, which was higher than the average mean (approximately 10%) of 177 observations reported by Jeffery et al. (2011) using meta-analysis. In addition, biochar application to soils showed apparent improvement of nut quality and sharp increase of contents of lipid, protein, starch and total soluble sugars in nuts, which could contribute to better nut flavor and higher economic value (Li et al., 2005). Moreover, biochar application should also benefit *T. grandis* propagation, since seed germination and seedling survival would be facilitated by increased storage reserves in seeds (Bewley, 1997). It is known that biochar application increases crop production. However, the effect of biochar application on crop grain or nut quality remains inconsistent. In grape, neither of quality parameters (i.e., total sugar or total acidity) was influenced by wood biochar application in an alkaline soil (Schmidt et al., 2014; Genesio et al., 2015). In tomato, Petruccelli et al. (2015) found that sugar content was not affected by biochar, but secondary metabolite contents showed changes relating to biochar type. In this study, we proved, for the first time, that wheat-straw-biochar application to the acid soil positively influenced the *T. grandis* nut quality with significant increases of the content of nutritional components including lipid, protein, starch and total soluble sugars (Fig. 2). These results supported our second hypothesis that biochar would enhance nut quality in *T. grandis*.

Plant production is promoted by biochar application through several mechanisms, such as improved soil water-holding capacity (Karhu et al., 2011), enhanced soil fertility (Novak et al., 2009), and reduction in leaching losses of nutrients (Laird et al., 2010). In this study, the base soil was acidic (pH = 5.3, Table 1). Biochar application in the soil very effectively increased soil pH (Fig. 3H) in both control sites and added N deposition sites, out of which soil pH was slightly decreased by N deposition in the latter. Increased pH can reduce the concentration of toxic elements in the soil and it can be beneficial to plant health (Rizwan et al., 2016). Pearson correlation analysis indicated that nut weight and nutritional components (lipid, protein and starch) were significantly correlated with soil pH (Table S1). Thus, changes in *T. grandis* nut production and storage reserves were inferred to be consequences of an acid neutralization or a liming effect. Similar effects have been also found in other experiments, particularly in studies where soils were acidic like in the present study (Steiner et al., 2009; Hass et al., 2012). However, it is necessary to further establish an additional lime treatment to distinguish the biochar effects beyond pH effects. In addition, we found available N, P and K in soil were decreased by biochar application (Fig. 3E–G). This was probably due to the incomplete extraction of nutrients by the extraction methods used in this study. Nutrient may be retained in biochar pores, and repeated (10×) extraction and sequential washing of biochar particles has been suggested to release nutrients captured by biochar (Kammann et al., 2015; Haider et al., 2016).

Nevertheless, SOM and total N were both sharply increased by biochar application, which was presumably due to the N and organic compounds within the structure of the biochar material. These results partially supported our second hypothesis that biochar would enhance soil fertility with increased SOM and total N, and increase soil pH. However, according to the extraction method used in this study the availability of N, P, and K in the soil was reduced by biochar application. If the nutrients were retained in the biochar particles (Kammann et al., 2015; Haider et al., 2016), its capacity for retention and release of nutrients needs further investigation. Therefore, we cannot draw any conclusion on the long-term effects of biochar application alone on nut quality.

Additionally, when considering the high cost for application of 20 t ha^{-1} biochar (approximately $13,000 per ha), the biochar amendment maybe not the viable way to improve the quality of *T. grandis* nut in practical scale. However, biochar application has various benefits, such as carbon sequestration, mitigating soil greenhouse gas emission, and alleviation of toxicity of trace elements (Biederman and Harpole, 2013; Chan et al., 2007; Karhu et al., 2011; Rizwan et al., 2016). Thus, it is necessary to further examine the comprehensive benefits of biochar application.

4.3. Interactive effects of N deposition and biochar application on nut quality and soil properties

As discussed above, both N deposition and biochar application individually enhanced nut quality in *T. grandis* (Table 3, Fig. 2). However, soil availability of nutrients (N, P and K) was negatively affected by both of these two factors alone (Fig. 3E–G). In support of our third hypothesis, we found that biochar application showed strong interactive effects with N deposition on nut nutritional components (Table 3, Fig. 2) and soil chemical properties (Table 4, Fig. 3). First, biochar application combined with various N additions significantly increased nut weight and the main nutritional components compared to either low or high N addition treatment without using biochar (Figs. 1A and 3). These results were consistent with our third hypothesis that biochar combined with N addition would positively affect nut quality. Second, available N, P and K in the soil was significantly ($P < 0.01$) increased by biochar application in combination with N deposition relative to biochar application or N deposition addition separately, except that available N was decreased by biochar application and high N addition (Fig. 3E–G). This suggested complex interactions between biochar and N addition, where biochar's improvement on soil nutrition was associated with N addition. These results are in agreement with the results of the earlier studies where the effect of biochar applied to the soil together with inorganic fertilizer, on the crop quality and soil fertility, has been found to depend on soil N supply (Chan et al., 2007; Asai et al., 2009; Zwieten et al., 2010). One mechanism that could explain the increased availability of soil nutrients by biochar combined with N deposition is the porous structure and the high cation exchange capacity of biochar, which can absorb and retain nutrients to its surface (Lehmann et al., 2003; Jeffery et al., 2015). Although this potential feedback requires further investigation, our data suggested, for the first time, that biochar application improved nut quality in *T. grandis*, and enhanced soil fertility under stimulated N deposition conditions.

5. Conclusions

Our experiments provide a first-time evaluation of the interactive effects of biochar application and N deposition on nut quality in *T. grandis* and on soil fertility. These findings are relevant to the understanding how globally increasing N deposition affects agriculture, how to improve crop quality and develop sustainable agriculture through proper soil amendment, and how to reduce N deposition from agricultural practices by alternative fertilization strategies. In this short-term study, we found that both N deposition and biochar application had positive effects on nut quality in *T. grandis*. However, neither N deposition nor biochar application alone improved soil fertility in terms of available N, P and K. Low N deposition showed a direct fertilizing effect with increased nut weight and increased nutritional components in terms of lipid, protein, starch and sugars. However, high N deposition led to lower nut weights and did not cause further increase in the total nutritional compositions, as compared to low deposition, suggesting that the deposition of N of 60 kg N ha^{-1} yr^{-1} is excessive for *T. grandis* in our experimental field. Thus, deposited N should be considered as a natural nutrition source, and less fertilization in *T. grandis* fields is recommended for drawing up fertilization strategies. Biochar application showed acid neutralization or a liming effect with significantly increased soil pH. Moreover, the 'win–win' situation was achieved. Compared with the individual effects of the two treatments, when combined with N deposition, biochar affected positively nut quality and enhanced soil fertility with increased availability of N, P and K. Because our study covered a short response period, the long-term effects of biochar and N deposition on nut quality and soil fertility require further investigation.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2017.02.036.

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