Organic amendments combined with biochar for improving soil and plant quality in a *Torreya grandis* plantation

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Abstract

Purpose Biochar and organic amendments can improve soil quality, but their combined effects on plant growth as well as soil microbial functions deserve further investigation.

Materials and methods We established an 8-month field experiment to explore the effects of biochar (0, 10 t ha^{-1}), organic amendments (10 t ha^{-1} sheep manure and 2 t ha^{-1} rapeseed cake), and their combinations on plant growth and soil quality in a *Torreya grandis* plantation.

Results and discussion Organic amendments significantly ($P < 0.05$) increased soil available and total P contents as well as mineral nutrients in leaves. Biochar significantly increased soil available P and K and total K contents. Biochar combined with sheep manure rather than rapeseed cake displayed a significant ($P < 0.05$) increase in plant quality and soil nutrients in comparison with manure or rapeseed cake addition alone. Biochar combined with sheep manure significantly increased microbial activity (indicated by microbial substrate utilization rate) compared with manure alone treatment. In addition, organic amendments in combination with biochar generally had no significant effects on microbial diversity indices, but they had some interactive effects on some bacterial taxa, such as Proteobacteria and Actinobacteria, and predicted metabolism functions. Changes in soil nutrient contents were closely linked with soil bacterial community composition and improvements in plant quality.

Conclusions The results showed that 10 t ha^{-1} biochar co-applied with 10 t ha^{-1} sheep manure was suitable for the promotion of soil fertility and plant growth in a *Torreya grandis* plantation.

Keywords Biochar · Organic amendment · Soil quality · Microbial community

Abbreviations
SM   Sheep manure
RC   Rapeseed cake
BC   Biochar
BCSM Sheep manure co-applied with biochar
BCRC Rapeseed cake co-applied with biochar

Responsible editor: Shahla Hosseini Bai

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1 Introduction

Environmental degradation is one of the greatest challenges in the twenty-first century (Bai et al. 2010; Smith et al. 2019). The increase in the use of chemical fertilizer has caused environmental pollution and soil degradation through leaching and the loss of soil biodiversity, which subsequently affects plant growth (Chen et al. 2014; Cui et al. 2018; Bai et al. 2020). Chemical fertilizers incorporated in the soil can be

transformed into soluble compounds, which then leach into adjacent rivers, lakes, and waterways (Bahram et al. 2018; Zheng et al. 2020). This excess chemical fertilization, coupled with ongoing atmospheric N deposition, have caused soil acidification and affected soil community structure and activity in both agricultural and terrestrial ecosystems (Bai et al. 2020; Li et al. 2020). In light of the land degradation and ecological cost caused by chemical fertilizers, it is necessary to advance land management practices to improve soil quality to support sustainable ecosystem development.

Both in the ecologic and economic sense, the application of organic amendments in agriculture and forestry has received increasing attention (Han et al. 2016; Neto et al. 2016). Organic amendments, such as plant residues or livestock manures, are rich in organic carbon, available phosphates (AP), nitrogen (AN), and potassium (AK) sources for plant use (Meng et al. 2005) and can have substantial impacts on soil fertility, aggregation, and microbial activity (Ding and Han 2014; Wang et al. 2017a). Organic amendments have been added to increase soil cation-exchange capacity (Schulz and Glaser 2012), reduce carbon (C) and nitrogen (N) in runoff, and buffer soil acidification in tea plantations (Xie et al. 2020). Organic amendments also affect soil microbial C use profiles (Zhang et al. 2012), microbial abundance and diversity, and nutrient availability (Fu et al. 2021). In addition, several studies have shown that the long-term application of manure improves the growth and yield of maize (Meng et al. 2005; Han et al. 2016; Mahmood et al. 2017; Gu et al. 2019).

Biochar, a C-rich product obtained via the thermochemical conversion of biomass in an oxygen-limited environment (Kan et al. 2016), is capable of improving soil performance by increasing nutrient concentrations, retaining soil water content, and increasing soil porosity (Lehmann et al. 2011). Biochar-mediated increases in soil porosity can influence soil microbial biomass and community structure by providing a suitable habitat for microbes (Chen et al. 2013, 2018; Gomez et al. 2014; Xu et al. 2014; Palansooriya et al. 2019; Guo et al. 2020). Biochar application may increase soil C storage and reduce nitrate and ammonium leaching (Wang et al. 2017b), and help to increase the availability of nutrients to plants and improve plant growth and crop yields (Kavitha et al. 2018). The properties of biochar and the improvements in soil quality, however, are dependent on the type of biomass used, the pyrolysis conditions, and soil type (Xiao et al. 2018).

Studies have shown that fertilizer co-applied with biochar has a better performance than fertilizer application alone in terms of yield and nutrition concentrations in plants and soil fertility (Glaser et al. 2015; Zhang et al. 2017a). Biochar mixed with dairy manure improved the soil pH, organic carbon, C/N ratio, and enzyme activities (Bera et al. 2016). Biochar mixed with organic amendment changed the abundance, diversity, and

composition of nitrifying bacteria (Zhang et al. 2017a). A few studies have, however, reported that soil enzyme activities were unchanged with biochar addition mixed with manure (Elzobair et al. 2016), but they suspected the reason was the low amount of biochar added to soil. Schulz and Glaser (2012) found that biochar mixed with fresh leaf compost enhanced the N leaching. Another study indicated that the presence of biochar resulted in the decrease of N mineralization and soil microbial activity when co-applied with organic amendments (El-Naggar et al. 2019). Nevertheless, how biochar co-application with organic amendments affects microbial community structure is still not fully understood.

Torreya grandis (Taxaceae, subgenus Torreya) is an evergreen coniferous tree. China lists this species as a national second-grade key protected wild plant species (Yu 1999). Within this genus, *T. grandis* cv. Merrillii, a popular nut tree species, is endemic to South China (Li and Dai 2007). Over the years, long-term and improper chemical fertilization not only accelerated soil acidification in *T. grandis* plantations, but has also led to a lower nut yield due to imbalanced nutrient input, disease outbreaks, and insect infestations (Li 2016; Ye et al. 2018). An effective, sustainable, and nutritionally balanced fertilization method is required to stimulate the sustainable development and productivity of *T. grandis* plantations. In practice, manure, rapeseed cakes and biochar have been recently used as alternatives in *T. grandis* forest management. Our previous study demonstrated that biochar addition alone can mitigate the negative effects of soil acidification and land degradation on the nut quality of *T. grandis* plantations (Zhang et al. 2017b; Li et al. 2020). However, how biochar in combination with organic amendments affects plant growth and the composition and activity of the soil microbial communities remains unclear. We infer that (i) organic amendments, such as sheep manure (SM) and rapeseed cake (RC), can enhance the mineral nutrients concentrations in both soil and plant biomass in *T. grandis* plantations; and that (ii) SM and RC combined with biochar may perform better for soil fertility and plant quality than organic amendment alone, and they may have interactive effects on soil nutrients and the composition and functional activity of soil microbial communities. To test this, we established a field experiment and investigated the effects of SM and RC amended with and without biochar on the mineral nutrient concentrations in soil and plant biomass and the soil microbial community composition and activity in a *T. grandis* plantation.

2 Materials and methods

2.1 Site description

The experiment site of the *T. grandis* plantation was located in Lin'an district (30° 14′ N, 119° 42′ E), in the

northwestern region of Zhejiang Province, China. The site experiences a subtropical monsoon climate with distinct seasons. The mean annual precipitation is 1613.9 mm, and the mean annual temperature ranges between 4.5 and 28.9 °C. The minimum and maximum temperatures normally occur in January and July, respectively (Zhang et al. 2017b). The *T. grandis* orchard was established in 2016 with a density of 1000 trees per ha. The heights of the young trees were approximately 120 ± 7 cm height when the present study was conducted. Chemical fertilizer ($N:P_2O_5:K_2O$ 15:15:15, with a rate of 400 kg ha^{-1} in March before flowering and 350 kg ha^{-1} in September after the harvest of seeds) had been implemented in this plantation for two years (in 2016 and 2017) to improve the growth of young trees. Chemical fertilizers were incorporated into the 3–5 cm depth soil layer by tillage. The chemical fertilization practice ceased after March 2018 to reduce the legacy effect on soil. The experiment plantation had a loam soil, with a pH of 5.03, total potassium (K) content of 15.71 g kg^{-1}, total N content of 1.2 g kg^{-1}, total phosphorus (P) content of 1.1 g kg^{-1}, and soil organic C content of 9.52 g kg^{-1}.

2.2 Experimental design

The experimental design involved two main factors, namely biochar (application rates of 0 and 10 t ha^{-1}) and organic amendment (non-amended, control; sheep manure, applied at a rate of 10 t ha^{-1}; and rapeseed cake, applied at a rate of 2 t ha^{-1}). Thus, there were six treatment combinations: the control (CK) without organic amendments or biochar, sheep manure (SM) applied at a rate of 10 t ha^{-1}, rapeseed cake (RC) applied at a rate of 2 t ha^{-1}, biochar (BC) applied at a rate of 10 t ha^{-1}, sheep manure at 10 t ha^{-1} co-applied with biochar at 10 t ha^{-1} (BCSM), and rapeseed cake at 2 t ha^{-1} co-applied with 10 t ha^{-1} biochar (BCRC). The type and the amount of organic amendment were chosen in accordance with the amounts typically applied by local farmers that are optimal for plant growth. The biochar application rate was at a mid-range level, which was based on previous research studies that assessed both microbial and plant responses (Biederman and Harpole 2013; Gomez et al. 2014). The experiment was arranged in a completely randomized design with three replications of each treatment. Biochar was produced from rice straw through slow pyrolysis (500 °C) with a 5-h residence time in Liyang Desheng Activated Carbon Factory, Jiangsu Province, China. Organic amendment was obtained from Hangzhou Nianfeng Organic Fertilizer Co., Ltd. The chemical properties of the biochar and organic amendments are provided in Table S1. In November 2018, a 30-cm wide and 20-cm deep circular trench was excavated at 40 cm away from each tree body, and the biochar

and/or organic amendments were incorporated into the soil at a depth of 20 cm in each trench for each treatment. For the combined treatments of BCSM and BCRC, the biochar and organic amendments were first thoroughly mixed and then incorporated into the soil. For the CK treatment, the soil was excavated and processed in the same way with other treatments.

Samples of topsoil (0–20 cm) were collected on August 1, 2019, 1 year after biochar and organic amendment treatments. Five soil cores were randomly selected from each circular trench around each tree trunk. The five soil samples were mixed and homogenized into a composite sample, and about 10 g of fresh soil was immediately frozen in liquid nitrogen prior to DNA extraction. The rest of each composite sample was transferred immediately to a polyethylene bag and placed on ice before being transported to the laboratory. A portion of each sample was passed through a 2-mm sieve and stored at 4 °C prior to Biolog analysis. The remaining sample was air-dried at room temperature and held for physicochemical analysis. In August 2019, 20 current leaves and 20 fresh nuts of *T. grandis* were randomly collected from each tree. After cutting and homogenizing the leaves, 5 g of fresh leaves was used for chlorophyll measurement. The remaining leaves were dried to a constant mass at a temperature of 65 °C and ground prior to further analysis.

2.3 Analysis of soil chemical properties

The soil pH was determined using a pH meter (FE20 pH meter, Mettler Toledo GmbH, Greifensee, Switzerland) with a soil to water ratio of 1:2.5 (w/v). Soil organic carbon (SOC) was measured by the $K_2Cr_2O_7$ titration method. Total N (TN) was determined by the Kjeldahl method, while total phosphorus (TP) and potassium (TK) were analyzed using the HNO_3-H_2SO_4-$HClO_4$ method. The available N (AN) was determined using the hot alkaline hydrolysis method, the available phosphorus (AP) was analyzed colorimetrically through the molybdenum antimony blue method after being extracted from the soil with an NH_4F solution, and available K (AK) was determined using CH_3COONH_4 extraction-flame photometry. In addition, NO_3^--N was extracted using saturated calcium sulfate, NH_4^+-N was extracted using 2 M KCl, and extracts were measured using a UV–visible spectrophotometer (UV-2600, Kyoto, Japan). All the mentioned soil properties were determined based on the protocol described by Lu (1999).

2.4 Analysis of leaf nutrients and nut quality of *T. grandis*

The leaf samples were wet digested using the H_2SO_4-H_2O_2 method; the potassium (K), zinc (Zn), magnesium (Mg), and calcium (Ca) content of the *T. grandis* leaves were analyzed

using the flame photometric method, while leaf P content was analyzed by molybdenum antimony blue colorimetry. The leaf N content was measured by the Kjeldahl procedure. All of the above leaf properties were assayed following the protocol described by Lu (1999). Chlorophyll of chopped leaves was extracted with 95% alcohol, and the concentration of each sample was measured and analyzed in terms of absorbance at 470, 649, and 664 nm (Lichtenthaler 1987). Length, width, and weight of 20 fresh nuts were measured.

2.5 Soil DNA extraction, sequencing and bioinformatics analyses

Soil DNA for each sample was isolated from a 0.25-g soil sample using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). PCR primer sets (338 F and the 806 R PCR) were used to amplify the V3 and V4 regions of the bacterial 16S rRNA gene (Caporaso et al. 2010). The PCR mix was prepared in a 20-μl tube containing 4 μl of 5 × FastPfu Buffer, 2 μl of 2.5 mM dNTPs, 0.8 μl of each primer (5 μM), 0.4 μl of the Fast Pfu Polymerase (TransGen, China), 0.2 μl of BSA, 10 ng of template DNA, and DNA-free water. After initial denaturation (95 °C for 3 min), 27 cycles of denaturation (30 s, 95 °C), annealing (30 s, 55 °C), and elongation (45 s, 72 °C) were performed followed by a final extension for 10 min (72 °C). The PCR products were visualized in a 2% agarose gel using electrophoresis. Sequencing was done using a MiSeq 2000 sequencing system (Illumina Inc., San Diego, CA, USA) performed at Shanghai Majorbio Bio-pharm Technology Co., Ltd.

Raw sequence reads were de-multiplexed, quality-filtered using Fastp, and processed using FLASH (Tanja and Salzberg 2011). Operational taxonomic units (OTUs) were delineated at 97% sequence similarity using Usearch (vers. 7.0, http://drive5.com/uparse/) and an agglomerative clustering algorithm. Community alpha diversity indices were generated based on the obtained OTUs using QIIME. Function predictions were derived from the bacterial community data obtained based on the OTUs table using Phylogentic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (Langille et al. 2013). Prior to function prediction, OTUs were normalized by dividing each OTU against known 16S rRNA gene copy numbers. Function predictions were categorized into Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Chen et al. 2019).

2.6 Biolog analysis

The microbial community-level physiological profiles were measured using Biolog-ECO plates according to the procedure described by Girvan et al. (2003). Briefly, 10-g aliquots (dry-weight equivalent) were shaken with 100 ml sterile water, diluted by a factor of 1000, and inoculated (125-μl aliquot) into a microplate well. All plates were incubated at 25 °C for 168 h, and absorbance at 590 nm was recorded every 24 h for 7 days. The average well color development (AWCD) value was calculated for each sample at each time point. Utilization scores of the six substrate groups (including carbohydrates, amino acids, carboxylic acids, amines, polymers, and phenolic compounds) were analyzed at 168 h.

2.7 Statistical analysis

All statistical analyses were performed using SPSS 16.0 (IBM, Chicago, USA) and Origin Pro 8.6 (Origin Lab, Northampton, CA, USA). A two-way ANOVA was used to evaluate the effects of organic amendment, biochar, and their interaction on soil and plant properties. A multiple comparison of means was conducted using a Duncan test at $\alpha = 0.05$. Normality and variance homogeneity requirements were met, and no data transformation was necessary. The relationships between soil quality and leaf characteristics were tested with Pearson correlation. Redundancy analysis (RDA) was used to account for relationships among the microbial community and environmental factors.

3 Results

3.1 Soil chemical properties

Both organic amendment and biochar had notable effects on soil chemical properties (Table 1). AP and TP contents were significantly higher under the SM and RC treatments than in the CK. The soil AP, TP, and TK contents were significantly ($P < 0.05$) higher by 204.3%, 44.1%, and 62.5%, respectively, in the soils of biochar amendments compared to CK. SM displayed significantly higher soil NO_3^--N content than RC. In contrast, the NH_4^+-N content was higher in RC than in SM ($P < 0.05$). The interactive effects of organic amendment and biochar application were significant for AN, AP, TN, TP, TK, and SOC.

3.2 Plant properties

SM, RC, and biochar application significantly increased leaf P and Mg contents but had no effect on leaf N content (Table 2). The addition of biochar significantly increased the length, width, and fresh weight of nuts by 11.6%, 3.9%, and 17.6%, respectively ($P < 0.05$), in comparison with CK,

Table 1 Physicochemical properties of the soil of the *T. grandis* plantation under different fertilizer treatments

	CK	SM	RC	BC	BCSM	BCRC	OF	BC	OA×BC
pH	6.16±0.27b	6.50±0.14ab	6.90±0.30a	6.37±0.11ab	6.75±0.24ab	6.63±0.08ab	ns	ns	ns
AN (mg kg⁻¹)	82.68±4.22b	97.76±6.21b	130.17±16.91a	96.69±3.05b	95.39±9.06b	90.71±4.01b	ns	ns	0.023
AP (mg kg⁻¹)	75.59±2.16c	147.61±4.02b	242.16±43.40a	229.99±8.87a	208.56±10.43a	199.43±5.35ab	0.009	0.002	<0.001
AK (mg kg⁻¹)	714.10±45.51a	750.07±7.56a	741.86±24.38a	654.61±17.32a	650.87±24.06a	707.35±45.12a	ns	0.024	ns
NO_3^--N (mg kg⁻¹)	5.26±0.25b	7.59±0.42a	5.97±0.87b	5.93±0.27b	5.80±0.23b	5.59±0.59b	ns	ns	ns
NH_4^+-N (mg kg⁻¹)	18.82±1.67b	19.15±0.17b	25.03±3.62a	17.82±0.73b	18.50±1.32b	17.98±1.52b	ns	ns	ns
TN (g kg⁻¹)	1.13±0.10bc	1.32±0.07ab	1.42±0.06a	1.12±0.08bc	1.31±0.10ab	0.97±0.01c	ns	0.027	0.023
TP (g kg⁻¹)	0.68±0.02d	0.91±0.02bc	1.23±0.08a	0.98±0.02bc	1.01±0.06b	0.85±0.04c	0.002	ns	0.0001
TK (g kg⁻¹)	20.76±0.47c	24.16±0.79c	28.59±1.69b	33.73±1.12a	33.03±1.77a	31.72±0.09ab	ns	<0.001	0.004
SOC (g kg⁻¹)	11.3±0.58c	11.75±0.78bc	14.58±0.58a	12.00±0.34bc	13.42±0.65ab	10.26±0.46c	ns	ns	0.001

All values are mean±SE ($n=3$). Values with different lowercase letters differ significantly between treatments, based on Duncan's multiple range tests

CK the control without fertilizer or biochar, *SM* sheep manure, *RC* rapeseed cake, *BC* biochar, *BCSM* sheep manure plus biochar, *BCRC* rapeseed cake plus biochar, *AN* available nitrogen, *TN* total nitrogen, *AK* available potassium, *TK* total potassium, *AP* available phosphorus, *TP* total phosphorus, *SOC* soil organic carbon, NO_3^--N, nitrate nitrogen, NH_4^+-N ammonium nitrogen, *OA* organic amendment effect, *OA×BC* interaction effect of biochar and organic amendments

ns, $P>0.05$; *$P<0.05$; **$P<0.01$

whereas no significant changes in nut width were observed under SM and RC fertilizer application. Organic amendment and biochar applications displayed significant interaction for leaf and nut properties, except for leaf N, nut width, and fresh weight.

3.3 Soil bacterial community abundance and community composition

A total of 998,298 quality bacterial sequences ranging from 41,038 to 73,407 sequences per sample (55,461 on average), and 6919 OTUs were detected in the *T. grandis* plantation soil. The OTU numbers as well as the Shannon and Simpson diversity indices did not differ between the six treatments, except for the Chao1 diversity, which increased with biochar treatment (Table 3).

The most abundant phyla were Proteobacteria, followed by Acidobacteria, Chloroflexi, Actinobacteria, and Bacteroidetes, adding up to 85.8% relative abundance (Fig. 1). The SM treatment displayed a significantly higher relative abundance of Proteobacteria, but a lower abundance of Acidobacteria in comparison with RC. The abundance of Actinobacteria and Bacteroidetes showed, however, under biochar with the organic amendments, BCSM and BCRC, no significant differences. The relative abundance of Latescibacteria was significantly higher under the RC treatment. The combination of biochar and organic amendment treatment had interactive effects on the relative abundance of Proteobacteria, which were most abundant in the whole study. Some OTUs of Acidobacteria, Actinobacteria, and Bacteriodetes also had interactive effects (Table 4).

At the genus level, the bacterial communities were dominated by the following taxa: norank_c_*Acidobacteria* (average of 10.8%), norank_f_*Anaerolineaceae* (4.8%), norank_f_*Gemmatimonadaceae* (3.5%), norank_f_*Nitrosomonadaceae* (3.1%), and *Acidothermus* (1.8%) (Table 4). The norank_f_*BIrii41* was significantly more abundant in the SM treatment compared to RC. The abundance of *Acidothermus* (Actinobacteria) increased significantly with the addition of biochar. The relative abundances of genera Candidatus_*Solibacter* and *Bryobacter* increased significantly in the BCSM treatment, in comparison with SM alone (Table 4).

3.4 Prediction of metabolic function of bacterial communities

The PICRUSt analysis yielded a total of 46 level 2 KEGG Orthology groups (KOs). Twelve gene families of metabolic functions were detected with the algorithm, and half of them were affected by the application of organic amendment (Table 5). The predicted relative abundance of gene families related to the glycan biosynthesis and metabolism increased significantly under RC compared with SM. Biochar and organic amendment showed significant interactive effects on many predicted metabolic functions, except for carbohydrate metabolism, global and overview maps, energy metabolism, and metabolism of cofactors and vitamins (Table 5).

Table 2 Physicochemical properties of *T. grandis* leaves and nuts under different fertilizer treatments

	CK	SM	RC	BC	BCSM	BCRC	OA	BC	OA×BC
Leaf N (mg g^{-1})	24.21±0.93a	24.71±1.05a	27.15±0.69a	27.15±0.69a	21.97±0.69a	21.67±3.74a	ns	ns	ns
Leaf P (mg g^{-1})	2.65±0.07c	2.97±0.09ab	3.23±0.00a	3.08±0.13ab	3.06±0.07ab	2.91±0.05b	ns	ns	0.002
Leaf K (mg g^{-1})	22.63±0.63a	17.33±0.44bc	18.86±1.25b	16.37±0.30c	20.90±0.17a	18.46±0.31b	ns	ns	<0.001
Leaf Zn (mg kg^{-1})	56.75±0.42 cd	64.08±0.26ab	60.20±2.11bc	60.56±2.37bc	67.24±0.79ab	54.86±1.99d	<0.001	ns	0.023
Leaf Mg (mg g^{-1})	4.45±0.13b	4.98±0.10a	5.17±0.07a	4.95±0.01a	5.04±0.03a	5.02±0.09a	0.001	ns	0.006
Leaf Ca (mg g^{-1})	47.49±2.50b	49.23±1.61b	58.46±1.14a	51.23±2.01b	47.75±1.82b	51.94±1.47b	0.006	ns	0.046
Chlorophyll (mg g^{-1})	1.58±0.03bc	1.62±0.02b	1.87±0.03a	1.46±0.07c	1.71±0.02b	1.67±0.06b	<0.001	0.043	0.013
Length (mm)	28.82±0.15b	30.89±0.57a	31.23±0.09a	32.16±0.17a	32.47±0.37a	31.49±0.91a	ns	0.001	0.022
Width (mm)	21.49±0.16c	21.72±0.20bc	21.68±0.31bc	22.33±0.26ab	22.85±0.11a	22.78±0.29a	ns	<0.001	ns
Fresh weight (g)	8.52±0.14c	9.51±0.16ab	9.27±0.19bc	10.02±0.30ab	10.36±0.24a	10.14±0.49ab	ns	0.001	ns

All values are mean±SE (*n*=3). Values with different lowercase letters differ significantly between treatments, based on Duncan's multiple range tests

CK the control without fertilizer or biochar, *SM* sheep manure, *RC* rapeseed cake, *BC* biochar, *BCSM* sheep manure plus biochar, *BCRC* rapeseed cake plus biochar, *Leaf N* leaf nitrogen, *Leaf P* leaf phosphorus, *Leaf K* leaf potassium, *Leaf Zn* leaf zinc, *Leaf Mg* leaf magnesium, *Leaf Ca* leaf calcium, *Length* nut length, *Width* nut width, *Fresh weight* nut fresh weight, *OA* organic amendment effect, *OA×BC*, interaction effect of biochar and organic amendments

ns, $P > 0.05$; *$P < 0.05$; **$P < 0.01$

3.5 Microbial substrate utilization rate

Biolog analysis used for getting substrate utilization rates of the six treatments showed that the AWCD values increased rapidly from 24 to 96 h, and then gradually decreased after 96 h of incubation across all six treatments (Fig. 2). RC treatment showed significantly higher AWCD values than CK, but SM displayed significantly lower substrate use activity. The addition of biochar together with organic amendment treatments yielded contrasting results in substrate utilization activity; the BCSM displayed a significant increase in microbial metabolism compared with the SM treatment, but the BCRC showed a significant reduction in soil microbial

metabolic activity in comparison to RC alone. The microbial substrate utilization scores of amino acids and carboxylic acids increased significantly under the RC in comparison to SM (Fig. 3). The microbial substrate utilization scores of polymers, carbohydrates, phenolic compounds, and amine contents were not significantly different among the six treatments.

3.6 Linkages between soil chemical properties, plant properties and the soil microbial community

The RDA ordination plots (Fig. 4) showed that the composition of the soil bacterial community changed significantly with changes in AK (R^2=0.4689, P=0.008) and NO$_3^-$-N

**Table 3** The α-diversity indices of soil microbial communities in different treatments in *T. grandis* plantation

	CK	SM	RC	BC	BCSM	BCRC	OA	BC	OA×BC
OTUs	2697.67±74.88a	2633.33±83.96a	2679.33±13.20a	2822.67±34.14a	2824.67±104.68a	2643.00±119.18a	ns	ns	ns
Chao1	3758.78±158.54ab	3631.39±121.34b	3842.15±33.34ab	4001.49±72.69ab	4116.50±169.39a	3820.79±142.76ab	ns	0.041	ns
Shannon	6.86±0.06a	6.79±0.09a	6.82±0.02a	6.80±0.10a	6.81±0.12a	6.78±0.11a	ns	ns	ns
Simpson	0.0023±0.0002a	0.0026±0.0004a	0.0024±0.0001a	0.0029±0.0005a	0.0032±0.001a	0.0027±0.0005a	ns	ns	ns

All values are mean±SE (*n*=3). Values with different lowercase letters differ significantly between treatments, based on Duncan's multiple range tests

CK the control without fertilizer or biochar, *SM* sheep manure, *RC* rapeseed cake, *BC* biochar, *BCSM* sheep manure plus biochar, *BCRC* rapeseed cake plus biochar, *OA* organic amendment effect, *OA×BC* interaction effect of biochar and organic amendments

ns, $P > 0.05$; *$P < 0.05$; **$P < 0.01$
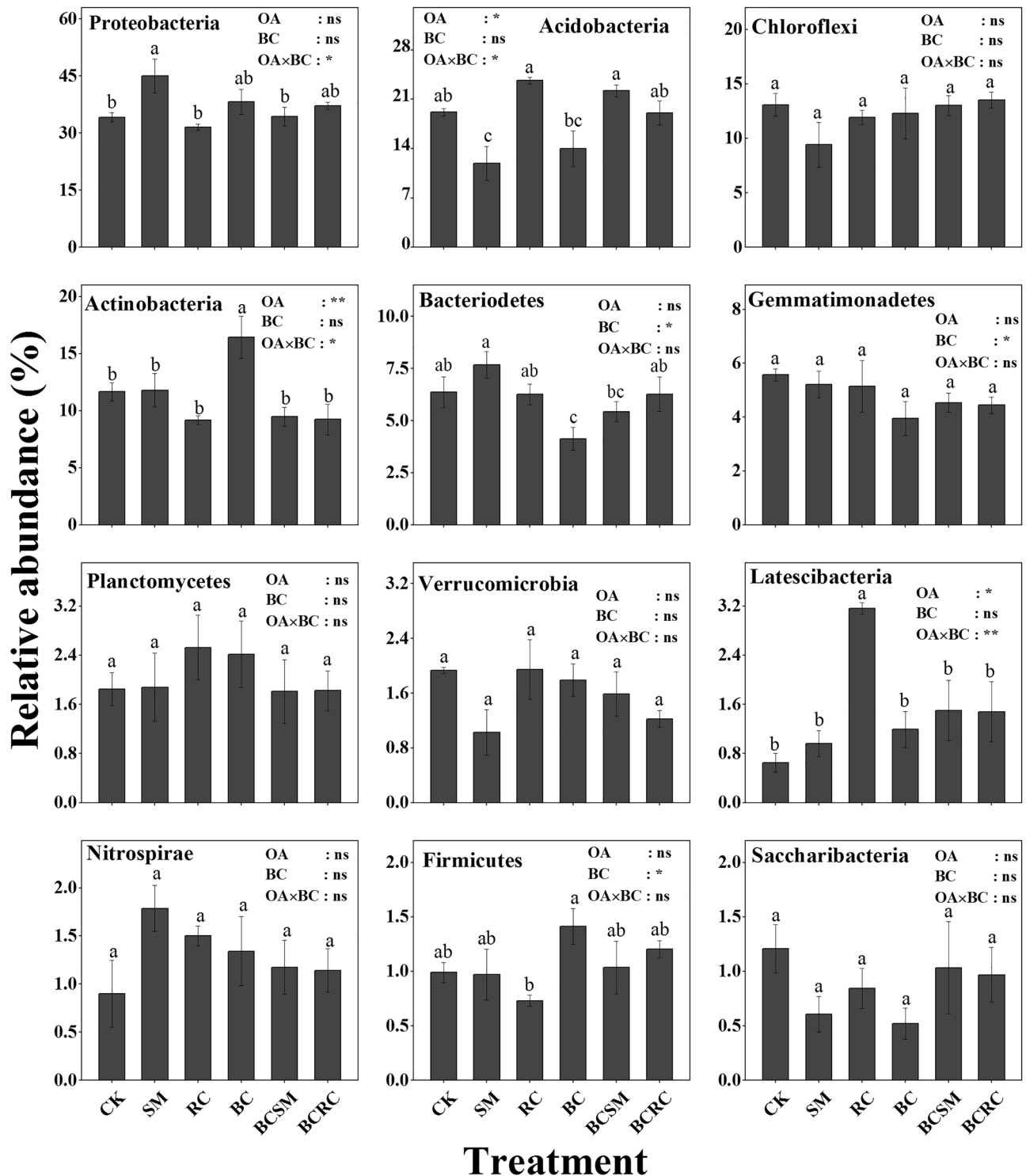
Fig. 1 Relative abundance of bacterial phyla based on amplicon 16S rRNA gene data from biochar and organic amendment treatments in a *T. grandis* plantation. Phyla representing < 1% of the bacterial population and unclassified bacteria are not shown. CK, the control without fertilizer or biochar; SM, sheep manure; RC, rapeseed cake; BC, biochar; BCSM, sheep manure plus biochar; BCRC, rapeseed cake plus biochar. All values are mean ± SE (*n* = 3). Different lowercase letters indicate values that differ significantly (*P* < 0.05). OA, organic amendment effect; OA × BC, the interaction effect of biochar and organic amendments; ns, *P* > 0.05; *P* < 0.05; **P* < 0.01

Table 4 Most abundant bacterial OTUs (%) under different treatments in *T. grandis* plantation

Phylum	Genus	CK	SM	RC	BC	BCSM	BCRC	OA	BC	OA×BC
Proteobacteria	*Acidibacter*	2.48±0.44a	1.77±0.21b	1.54±0.10b	1.63±0.04b	1.78±0.07b	1.67±0.19b	ns	ns	ns
	norank_f_*Blrii41*	1.31±0.05de	2.73±0.23a	1.53±0.04c	1.98±0.16b	1.12±0.14de	1.07±0.04f	0.002	0.001	<0.001
	Haliangium	1.60±0.00ab	1.80±0.10ab	1.60±0.10ab	2.00±0.30a	1.50±0.10b	1.30±0.10b	ns	ns	ns
	norank_o_*Xanthomonadales*	1.09±0.04a	1.57±0.19a	1.39±0.16a	1.40±0.14a	1.18±0.12a	1.36±0.17a	ns	ns	ns
	norank_f_*Nitrosomonadaceae*	2.96±0.33ab	3.70±0.31a	3.63±0.32ab	2.88±0.30ab	2.69±0.24b	2.90±0.24ab	ns	0.027	ns
Acidobacteria	norank_c_*Acidobacteria*	9.82±1.00a	9.70±1.00a	12.76±2.08a	9.72±0.91a	11.22±1.50a	11.78±1.78a	ns	ns	ns
	Acidobacteriaceae_Subgroup_1	2.26±0.90a	1.04±0.46a	1.42±0.32a	1.97±0.43a	2.42±0.15a	1.14±0.29a	ns	ns	ns
	Candidatus_Solibacter	1.51±0.15ab	1.04±0.22b	1.43±0.15ab	1.29±0.26ab	1.97±0.40a	0.97±0.11b	ns	ns	0.026
	Bryobacter	1.38±0.02b	1.31±0.14bc	1.28±0.16bc	0.85±0.10c	1.93±0.26a	1.28±0.09bc	0.014	0.014	ns
Chloroflexi	norank_f_*Anaerolineaceae*	4.62±0.49ab	4.66±0.46ab	5.41±0.55ab	3.58±0.35b	4.46±1.06ab	5.69±0.11a	ns	ns	ns
	norank_c_*KD4-96*	1.35±0.10b	1.53±0.32a	1.51±0.29a	2.23±0.21a	1.12±0.16b	1.39±0.32b	ns	ns	ns
Actinobacteria	*Acidothermus*	1.54±0.29b	1.27±0.07b	1.16±0.33b	3.47±0.15a	1.68±0.09b	1.39±0.32b	<0.001	0.001	0.006
Bacteroidetes	norank_f_*Cytophagaceae*	2.36±0.09a	1.62±0.08b	1.38±0.09b	1.22±0.15b	1.19±0.26b	1.15±0.17b	0.014	0.001	0.032
Gemmatimonadetes	norank_f_*Gemmatimonadaceae*	3.74±0.13a	3.58±0.13a	3.58±0.32a	2.82±0.20b	3.75±0.28a	3.31±0.14ab	<0.001	<0.001	ns
Latescibacteria	norank_p_*Latescibacteria*	0.44±0.07b	1.21±0.05ab	2.45±0.72a	1.52±0.30ab	1.46±0.44ab	1.48±0.49ab	ns	ns	ns

All values are mean±SE ($n=3$). Values with different lowercase letters differ significantly between treatments, based on Duncan's multiple range tests

CK the control without fertilizer or biochar, *SM* sheep manure, *RC* rapeseed cake, *BC* biochar, *BCSM* sheep manure plus biochar, *BCRC* rapeseed cake plus biochar, *OA* organic amendment effect, *OA×BC* interaction effect of biochar and organic amendments

ns, $P>0.05$; *$P<0.05$; **$P<0.01$

Table 5 Predicted metabolism functions of the bacterial communities by Phylogenetic investigation of Communities by Reconstruction of Unobserved States (PICRUSt) under different fertilizer treatments. Data given as metabolism abundance (%)

	CK	SM	RC	BC	BCSM	BCRC	OA	BC	OA×BC
Carbohydrate metabolism	19.16±0.04a	18.92±0.13b	19.08±0.02ab	19.11±0.07ab	19.05±0.07ab	19.00±0.02ab	ns	ns	ns
Global and overview maps	17.94±0.01a	17.97±0.08a	18.13±0.08a	17.93±0.09a	18.08±0.04a	18.05±0.04a	ns	ns	ns
Amino acid metabolism	16.64±0.03ab	16.73±0.03a	16.55±0.05b	1 6.67±0.06ab	16.59±0.01b	16.66±0.03ab	ns	ns	0.01
Energy metabolism	10.02±0.01a	10.10±0.00a	10.07±0.03a	10.03±0.03a	10.10±0.03a	10.08±0.02a	0.03	ns	ns
Metabolism of cofactors and vitamins	8.86±0.02ab	8.89±0.02ab	8.94±0.02a	8.78±0.05b	8.94±0.07a	8.92±0.03a	0.02	ns	ns
Nucleotide metabolism	6.70±0.01ab	6.67±0.02bc	6.83±0.04a	6.57±0.07c	6.78±0.03ab	6.77±0.05ab	0.04	ns	0.03
Lipid metabolism	5.47±0.01ab	5.54±0.04a	5.39±0.03b	5.56±0.04a	5.40±0.01b	5.41±0.03b	0.01	ns	0.07
Xenobiotics biodegradation and metabolism	3.91±0.04bc	4.14±0.07ab	3.66±0.08c	4.28±0.16a	3.82±0.06c	3.87±0.10bc	0.01	ns	0.08
Metabolism of other amino acids	3.41±0.01ab	3.48±0.03a	3.37±0.02b	3.43±0.04ab	3.40±0.01b	3.43±0.01ab	ns	ns	0.03
Biosynthesis of other secondary metabolites	2.75±0.02ab	2.65±0.02c	2.78±0.01a	2.68±0.04bc	2.74±0.01ab	2.7±0.00bc	ns	ns	0.004
Glycan biosynthesis and metabolism	2.60±0.02ab	2.50±0.03b	2.67±0.01a	2.39±0.04c	2.61±0.02ab	2.58±0.05ab	0.01	0.02	0.001
Metabolism of terpenoids and polyketides	2.55±0.01bc	2.57±0.01ab	2.52±0.00c	2.58±0.02a	2.50±0.03bc	2.52±0.02bc	ns	ns	0.03

All values are mean±SE ($n=3$). Values with different lowercase letters differ significantly between treatments, based on Duncan's multiple range tests

CK the control without fertilizer or biochar, *SM* sheep manure, *RC* rapeseed cake, *BC* biochar, *BCSM* sheep manure plus biochar, *BCRC* rapeseed cake plus biochar, *OA* organic amendment effect, *OA×BC* interaction effect of biochar and organic amendments

ns, $P>0.05$; *$P<0.05$; **$P<0.01$

($R^2=0.327$, $P=0.044$), while pH, AN, SOC, and the C/N ratio concentration had no significant effects on the community. Leaf P correlated positively with the AP and TP concentration ($P<0.05$, Table S2). Soil NO_3^--N was significantly and negatively correlated with carboxylic acid, while soil NH_4^+-N was positively correlated with phenolic compounds (Table S2).

4 Discussion

4.1 Effects of organic amendments and biochar addition on soil quality parameters

In partial support of our first hypothesis, both AP and TP contents were significantly higher in the sheep manure and rapeseed cake treatments than in control (Table 1), showing that organic amendments helped to increase soil P concentrations (Fu et al. 2021). The NO_3^--N content was higher in sheep manure than rapeseed cake treatment that most likely

is connected to the high ammonium N (NH_4^+-N) content of sheep manure, which is subjected to ammonium oxidation and nitrification. (Yang et al 2017). Organic fertilizer has been reported to reduce the leaching of inorganic nitrogen (Gu et al. 2019; Xie et al. 2020). In the current study, the addition of biochar resulted in clear improvements in soil AP, TP, and TK in the *T. grandis*, which was consistent with previous studies (Zhang et al. 2017b; Li et al. 2020). When biochar was added together with sheep manure and rapeseed cake, the positive effect of organic amendment on soil quality parameters was maintained but varied with organic amendment type. Biochar combined with sheep manure rather than rapeseed cake displayed a significant ($P<0.05$) increase in soil nutrients, such as TP, TN, and SOC contents. These findings may be related to the properties of organic amendments and their different interactions between each other, which is in line with a previous report showing that biochar mixed with dairy manure results in improved soil ecological functioning and higher soil C sequestration potential (Bera et al. 2016).
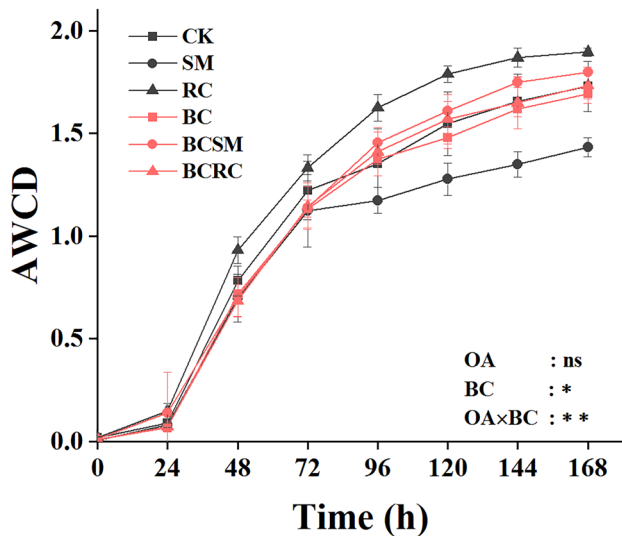
Fig. 2 Average well color development (AWCD) in Biolog EcoPlates for carbon source utilization under biochar and organic amendment treatments. CK, the control without fertilizer or biochar; SM, sheep manure; RC, rapeseed cake; BC, biochar; BCSM, sheep manure plus biochar; BCRC, rapeseed cake plus biochar. All values are mean ± SE (n = 3). OA, organic amendment effect; OA × BC, interaction effect of biochar and organic amendments; ns, $P > 0.05$; *$P < 0.05$; **$P < 0.01$. Two-way ANOVA for AWCD was an analysis covering 168 h

4.2 Effects of organic amendments and biochar addition on plant properties

Our study showed that the leaf P concentration was significantly higher under rapeseed cake in comparison to the control (Table 2), whereas it decreased significantly under rapeseed cake co-applied with biochar. This result was consistent with previous reports stating that organic amendments increased mineral nutrient contents in plants, but the addition of biochar led to an opposite result (Gent 2002; Glaser et al. 2015). This may be explained by the low efficiency of P adsorption and desorption caused by the addition of biochar (Morales et al. 2014). We found that leaf P content had significant and positive correlations with soil AP and TP (Table S2), which supports the concept that soil P concentration affects the P content in the leaves and plant biomass (Geesing et al. 2000). The chlorophyll and leaf Ca concentrations were tightly correlated with seed quality and yield (Santos et al. 2013; Chen 2018), and increased significantly under the rapeseed cake treatment compared to control (Table 2). The combination of rapeseed cake with biochar did not, however, promote nutrient accumulation in leaves in comparison with rapeseed cake alone. In contrast, sheep manure treatment significantly increased most of the plant and soil parameters compared with control, and biochar in combination with manure had some significant synergistic effects (Table 2). Thus, our study demonstrated

that biochar co-applied with sheep manure can improve soil and plant quality more than sheep manure addition alone, supporting our second hypothesis that biochar co-applied with organic amendment would have interactive effects on plant quality, depending on the characteristics of the organic amendment type (Bonanimi et al. 2017).

4.3 Effects of organic amendments and biochar addition on soil bacteria community diversity and abundance

Short-term fertilization with organic amendments and biochar did not have a clear impact on diversity of microbial communities. The fertilization impact was detected on relative bacterial abundance level showing differences among treatments. Differences in abundance was detected among the dominant phyla in treated soil and specific OTUs were observed that clearly correlated with treatments (Tables 3 and 4, Fig. 1). Studies have shown that the abundance and diversity of bacterial groups are correlated with specific soil parameters and the type of fertilizer used (Xu et al. 2014; Wang et al. 2015; Gu et al. 2019).

The most abundant phyla in our study, Proteobacteria and Acidobacteria, responded differently to organic amendments. The Proteobacteria lineages are known as copiotrophic, which increase their abundances when there are more easily available carbon substrates in soil (Fierer et al. 2012). In contrast, Acidobacteria phyla harbor several lineages that are oligotrophic in nature which use less accessible carbon compounds and they have clearly slower growth rates (Fierer et al. 2007, 2012). We observed a higher NO_3^--N content in the sheep manure treated plantation soil that introduced excess NH_4^+-N, which suggested active bacterial ammonia oxidation compared with rapeseed cake (Table 1). Bacterial ammonia oxidizers (AOB) conducting ammonium oxidation and nitrifiers belong to the Proteobacteria, which may partly explain the higher abundance of Proteobacteria in sheep manure-treated soil as they are favored in short term fertilization treatments (Tables 1 and 4) (Ying et al. 2017). Previous studies have also shown that NO_3^--N correlates positively with Proteobacteria (Rotaru et al. 2010; Trump et al. 2011). The relative abundance of norank_f_*BIrii41* (Proteobacteria) differed significantly between the sheep manure and rapeseed cake treatments and was clearly more abundant in the sheep manure treatment (Table 4). It has been demonstrated that Acidobacteria can use sugars and amino acids as carbon sources for growth (Challacombe et al. 2011; Kalam et al. 2020). Thus, the higher utilization scores of amino and carboxylic acids in rapeseed cake compared with that of sheep manure supports our results of higher Acidobacteria abundance (Fig. 3).

Latescibacteria represent a ubiquitous member of the Fibrobacteres–Chlorobi–Bacteroidetes super phylum

Fig. 3 Biolog carbon source utilization (carbon families) scores by soil microbial communities under different fertilizer treatments. CK, the control without fertilizer or biochar; SM, sheep manure; RC, rapeseed cake; BC, biochar; BCSM, sheep manure plus biochar; BCRC, rapeseed cake plus biochar. All values are mean ± SE (*n* = 3). Different lowercase letters indicate values that differ significantly (*P* < 0.05). OA, organic amendment effect; OA×BC, interaction effect of biochar and organic amendments; ns, *P* > 0.05; *P* < 0.05; **P* < 0.01
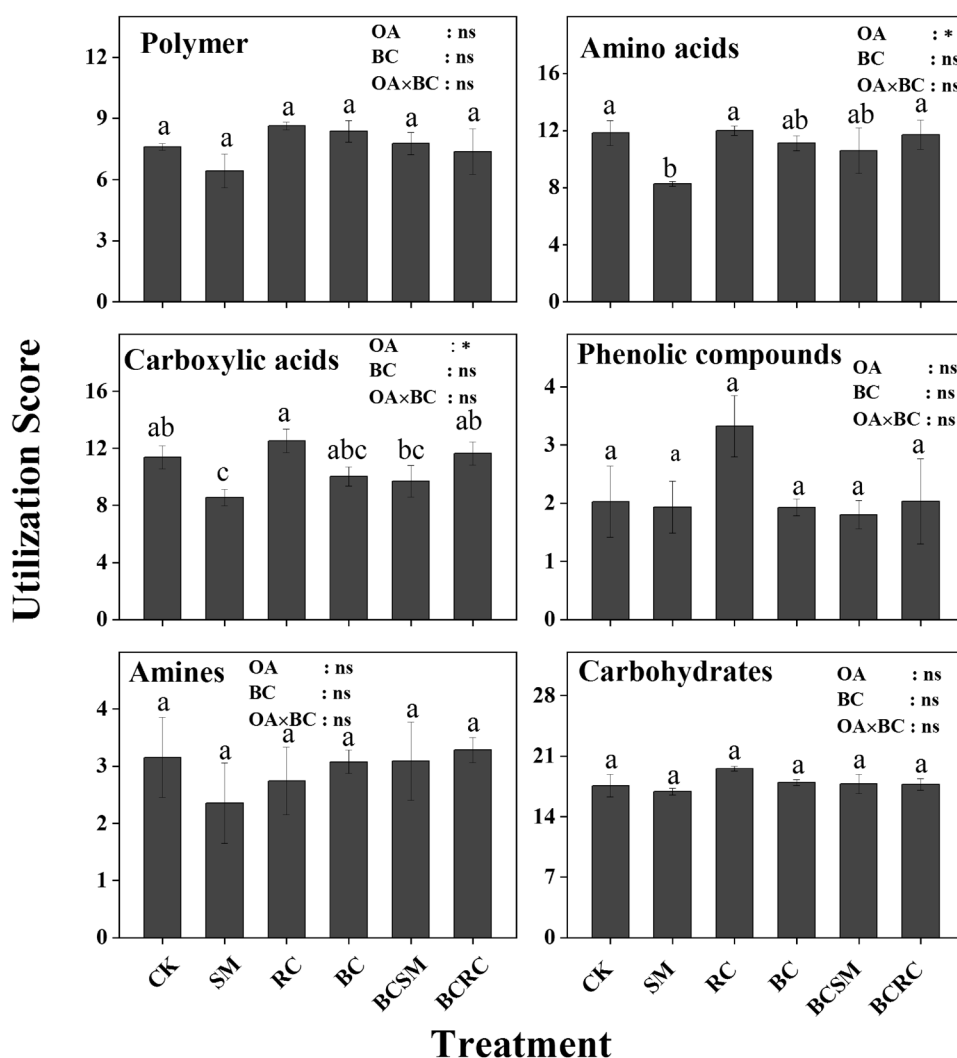
(Youssef et al. 2015; Farag et al. 2017). Our study showed that the relative abundance of Latescibacteria increased in all plots with organic amendments but was most significant in the rapeseed cake treatment, while rapeseed cake was also accompanied by higher metabolic activity towards amino and carboxylic acids (Figs. 1 and 3). The predicted metabolic function results showed a similar trend of glycan biosynthesis and metabolism (Table 5). This is well in line with other reports that indicate the members of Latescibacteria play an important role in the degradation of proteins, lipids, and polysaccharides present in plants and soil (Farag et al. 2017). Thus, our results suggested that rapeseed cake addition activated Latescibacteria involved in the utilization of amino and carboxylic acids. These results are in accordance with our hypothesis that the two organic amendments (sheep manure and rapeseed cake) may have interactive effects on the composition and functional activity of soil bacterial communities.

We observed a higher relative abundance of Actinobacteria with biochar addition compared to control, whereas their abundance decreased in mixed fertilization (Fig. 1). Actinobacteria are oligotrophic and have been found to be enhanced in soils amended with biochar (Khodadad et al. 2011; Palansooriya et al. 2019). Actinobacteria play an important role in the decomposition of organic matter and can preferentially use biochar-derived C instead of native soil organic matter (Dai et al. 2017). Members of the Actinobacteria genus *Acidothermus* detected in our study can produce thermostable cellulose-degrading enzymes (Challacombe et al. 2011). The relative abundance of Actinobacteria decreased in mixed fertilization, possibly because sheep manure and rapeseed cake provided large amounts of easily available organic C, favoring copiotrophic Proteobacteria (Table S1). Bacteroidetes is thought to be involved in the degradation of complex organic matter, especially in the degradation of polysaccharides and proteins (Thomas et al. 2011; Delgadobaquerizo et al. 2017). Previous studies have shown

Fig. 4 Redundancy analysis (RDA) of the soil microbial community constrained by soil chemical properties under fertilized *Torreya grandis* plantation. C/N ratio, soil organic carbon/ total nitrogen ratio; pH; SOC, soil organic carbon; AN, available nitrogen; AK, available potassium; NO_3^--N: nitrate nitrogen. CK, the control without fertilizer or biochar; SM, sheep manure; RC, rapeseed cake; BC, biochar; BCSM, sheep manure plus biochar; BCRC, rapeseed cake plus biochar. Asterisks (*) indicate soil parameters with a significant impact on bacterial communities ($P < 0.05$)
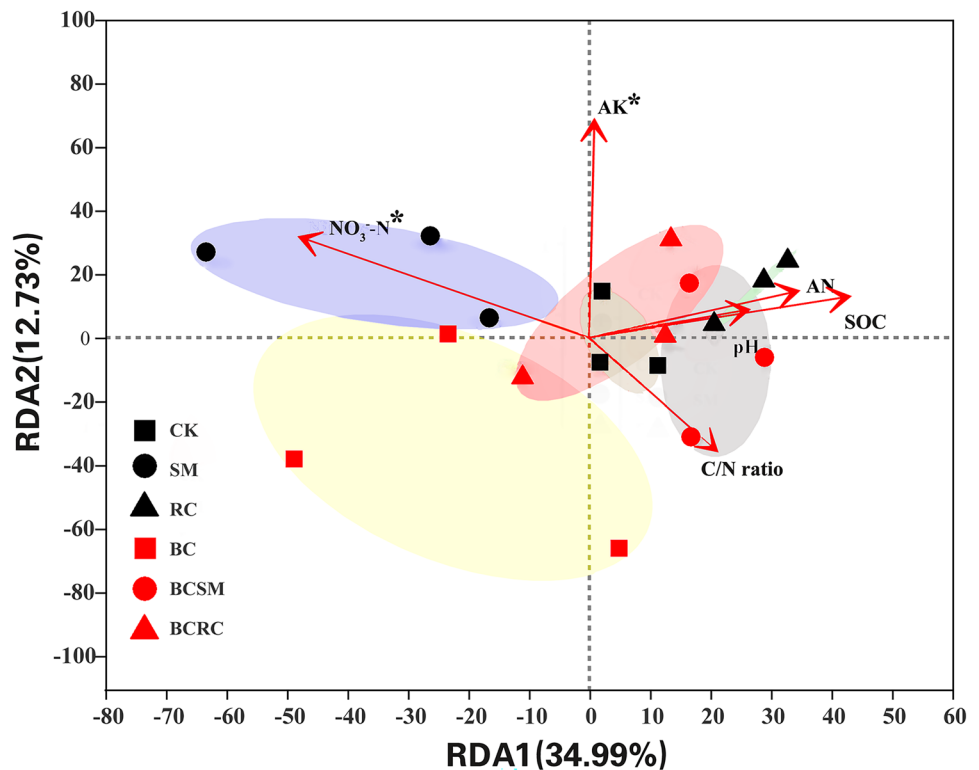
that Bacteroidetes prefer to use C substrates from organic amendment rather than from biochar (Delgadobaquerizo et al. 2017; Wolińska et al. 2017), which may explain the significant decrease in the abundance of Bacteroidetes under biochar addition compared to organic amendment. Indeed, a number of Bacteroidetes taxa act as key predictors of soil multi-functionality (Wolińska et al. 2017). At the genus level, the relative abundances of Candidatus_*Solibacter* and *Bryobacter* (Acidobacteria) were significantly increased with biochar combined with sheep manure (Table 4). *Solibacter* plays an important role in metabolic activity and disease resistance and provides a regulatory advantage in the soil environment (Challacombe et al. 2011). In a long-term chemical fertilization study, naturally grown walnut trees promoted some beneficial bacteria such as , *Nitrospira*, *Pseudomonas*, and Candidatus_*Solibacter* (Bai et al. 2020).

4.4 Effects of organic amendments and biochar on soil microbial metabolic activity

In our study, the microbial metabolic activity measured by Biolog Eco Plates was significantly higher under the rapeseed cake treatment than under sheep manure (Fig. 2). Similar results reported by Zhang et al. (2012) showed higher microbial activity in plant residues from farms than from manure. Liu et al. (2016) revealed that microbial activity increased significantly in soil with a decreased substrate C/N ratio. Rapeseed cake has a lower C/N ratio and can be decomposed

more easily than sheep manure (Table S1), since it can better satisfy the nutrient demand of microbes (Nguyen et al. 2016). Biochar amendment had no significant effect on soil microbial metabolic activity compared to control (Fig. 2), which was consistent with results of Steinbeiss et al. (2009), who also reported little effect of biochar amendment on the soil carbon balance and soil microbial activity. The microbial metabolic activity may decrease in soil with a decrease in soil nutrient content (Lagerström et al. 2009), which explains the changes in microbial activity under the rapeseed cake co-applied with biochar treatment where soil nutrients decreased with addition of biochar (Table 1 and Fig. 2). This is in accordance with our hypothesis that sheep manure and rapeseed cake combined with biochar have interactive effects on soil nutrients and the composition and functional activity of soil microbial communities.

The microbial substrate use rates for different carbon source was higher under rapeseed cake than under sheep manure, which may indicate a selection for specific microorganisms that can use more amino acids and carboxylic acids under rapeseed cake (Fig. 3) (Farag et al. 2017; Kalam et al. 2020). Rapeseed cake treatment had more nutrient and labile substrates than sheep manure, which may have stimulated microbial activity and nutrient transformation in the soil, as reported by Liang et al. (2007) who studied the effect of plant materials on the microbial transformation of amino sugars. One explanation to lower functional activity in sheep manure could also be that the excess ammonia

in sheep manure was already metabolized earlier under the previous higher functional activity (Table 1). A previous study indicated that the use of amino acids, carboxylic acids, and phenolic compounds by microorganisms was promoted under straw fertilizer (Shi et al. 2010), since the amino acids in straw residues provided readily available nutrient sources for soil microorganisms (Vinolas et al. 2001). We observed that carboxylic acids had significant negative correlations with soil NO_3^--N (Table S2), accompanied by low levels of NO_3^--N in rapeseed cake (Table 1). Our results indicated that alterations in soil nutrient availability under biochar and organic fertilization were important factors affecting soil microbial community activity in terms of the substrate metabolism rate. The soil microbial activity was significantly affected by biochar and organic amendments, but their interactive effects varied depending on the kind of organic amendment used. According to our findings combining organic agricultural waste for reuse with biochar is a plausible solution for combating the overall negative effects of traditional inorganic fertilization, causing acidification and a higher carbon footprint. Lower amounts of relatively expensive biochar can be applied, especially in combination with organic, environmentally friendly soil amendments.

5 Conclusion

In summary, our study demonstrated that organic amendments, such as rapeseed cake and sheep manure, improved plant quality and soil chemical properties in the *T. grandis* plantation. Their co-application with biochar had contrasting effects on the soil and plant quality, where sheep manure combined with biochar had a better performance. Biochar combined with sheep manure significantly increased the microbial metabolic activity compared with manure addition alone. In general, organic amendments in combination with biochar had no significant effects on microbial diversity indices and predicted metabolism functions. Application of biochar combined with sheep manure may be the better management strategy in the short term in *T. grandis* plantations, but long-term studies will show how the trends observed in this field study will prevail.

Author contribution Zhanhua Zhou: investigation, writing—original draft preparation. Kim Yrjälä: conceptualization, investigation, writing, and editing. Junhui Chen, Chenliang Yu, Wenhui Shi, Hua Qin: writing—review and editing. Yuanyuan Hu and Jiasheng Wu: conceptualization, writing—review and editing, supervisor. Weiwu Yu, Wensheng Dai, Jiasheng Wu: project administration.

Data sharing and accessibility All data generated or analyzed during this study are included in this article. The database or raw data generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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