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RESEARCH ARTICLE

Biochar suppresses N_2O emissions and alters microbial communities in an acidic tea soil

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Abstract

Biochar has been considered as a promising soil amendment for improving fertility and mitigating N_2O emission from the arable land. However, biochar's effectiveness in acidic tea soil and underlying mechanisms are largely unknown. We conducted a short-term microcosm experiment using two biochars (1% w/w, LB, generated from legume and NLB, non-legume biomass, respectively) to investigate the effects of biochar amendments on soil chemical properties, N_2O emission, and microbial community in an acidic soil. Soil and headspace gas samples were taken on 1, 10, and 30 day's incubation. Biochar amendment increased soil pH and DOC, however, significantly reduced soil inorganic N. Both biochars at ~ 1% addition had little effect on microbial CO_2 respiration but suppressed soil N_2O emission by ~ 40% during the incubation. The divergence in N_2O efflux rates between soils with and without biochar addition aligned to some degree with changes in soil pH, inorganic N, and dissolved organic C (DOC). We also found that biochar addition significantly modified the fungal community structure, in particular the relative abundance of members of Ascomycota, but not the bacterial community. Furthermore, the copy number of *nosZ*, the gene encoding N_2O reductase, was significantly greater in biochar-amended soils than the soil alone. Our findings contribute to better understanding of the impact of biochar on the soil chemical properties, soil N_2O emission, and microbial community and the consequences of soil biochar amendment for improving the health of acidic tea soil.

Keywords Biochar · N_2O emission · Tea plantation · Microbial community · Acidic soil · Denitrification

Introduction

Tea (*Camellia sinensis*) an important cash crop often requires a high rate of N fertilization (i.e., > 300 kg N ha^{-1}) and slightly acidic soil conditions (pH 4.5–6.0) to grow well (USEPA 2008; Xue et al. 2010). However, the long-term and high rate of N fertilization may accelerate the acidification of originally acidic soil, resulting in a further decline of soil available nutrients, such as P, Ca, and Mg and yet an increase of Al toxicity (Han et al. 2008; Li et al. 2016a; Alekseeva et al. 2010). The long-term and high rate of N fertilization also enhances soil nitrous oxide (N_2O) emissions compared to arable lands cultivated with other grain and cash crops, such as maize, wheat, and rice (Oertel et al. 2016). It is estimated that ~ 61 kg N_2O-N ha^{-1} has been emitted annually from the soil of tea plantations in East Asia (Li et al. 2016c). Substantial N_2O emission generates not only an economic loss but also great environmental concerns given that N_2O contributes to global warming and damages the stratospheric ozone (Ravishankara et al. 2009). Hence, there is an urgent need for developing management practices that can help maintain soil pH and yet reduce soil N_2O emissions from tea plantations. Land application of biochar has been considered as a promising management strategy to ameliorate soil physical, chemical, and biological properties as well as mitigate soil N_2O emissions.

The impacts of biochar on soil physical properties have been largely investigated (Blanco-Canqu 2017). In general, biochar amendment can reduce soil bulk density by 3–31% and increase soil porosity by 14–64%, aggregate stability by 3–226%, and water availability by 4–130%. Biochar also offers many opportunities to manipulate soil chemical

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properties. Given its alkalinity, liming potential represents one of the significant impacts of biochar, specifically for a highly weathered and acidic soil. Soil pH may be increased by up to one unit, although this requires a large amount of biochar application (e.g., 40 tons ha^{-1}) (Laird et al. 2010; Chintala et al. 2014). The pH improvement often leads to enhanced soil fertility and a reduction in exchangeable Al, and thus Al toxicity (Syuhada et al. 2016; Domingues et al. 2017). Further, biochar can help enhance soil nutrient retention through its capacity for cation and/or anion exchanges, and this trait is particularly beneficial for soil having extra N of plant needs.

The concept that land application of biochar can effectively reduce N_2O emissions has been fairly well recognized. Underlying mechanisms have also been elucidated to some extent. Under anoxic conditions, for example, biochar may limit the total rate of denitrification and also stimulate the complete reduction from NO_3^- to N_2 by regulating soil pH, buffering capacity, moisture, aeration, and substrate availability (i.e., dissolved organic C (DOC) and NO_3^-) (Cayuela et al. 2013; Clough et al. 2013). Molecular-level investigations further demonstrated that biochar's mitigation on soil N_2O emission could be at the population and pertinent gene expression levels (Ducey et al. 2013; Harter et al. 2014;Wang et al. 2013; Li et al. 2016b; Xu et al. 2014). In general, biochar has suppressive or no effects on the abundances of *nirS* and *nirK*, genes encoding NO reductase for catalyzing the reduction from NO to N_2O, but often stimulates the abundance of *nosZ*, the gene encoding N_2O reductase for catalyzing the reduction from N_2O to N_2. Accordingly, biochar does not affect or even abate N_2O production and yet enhances N_2O consumption.

Besides denitrification, other microbial processes, such as nitrification and fungal NO reduction, can also contribute to soil N_2O emissions (Chen et al. 2014; Huang et al. 2017; Li et al. 2018). However, biochar's influences on the ammonia oxidizing microorganisms and fungal community are largely unknown. In this work, we examined the impacts of biochar on chemical and biological properties of a soil collected from a tea plantation. Specifically, we aimed to compare biochar's influences on population sizes of ammonia oxidizing archaea, ammonia oxidizing bacteria, and bacterial denitrifying organisms and their associations with the rates of soil N_2O emissions. We also evaluated the fungal community compositional changes in response to biochar addition. Here, two biochars generated from legume and non-legume biomass, respectively, were used for making better inferences of mechanisms by which biochar may help improve acidic tea soil health. Our hypotheses were that (1) both biochars were able to improve soil pH and reduce soil N_2O emissions, and (2) effects could occur at microbial population size and/or community compositional levels.

Materials and methods

Soil and biochar samples

The soil sample (0–20 cm depth) was collected in March 2017 from a garden of famous Chinese green tea (West lake Longjing) in Hangzhou (30° 11' N, 120° 5' E), eastern China, where the annual mean temperature was ~ 17 °C, and annual precipitation ranged from 1720 to 2100 mm. This soil was developed from Anshan quartz-free porphyry and classified as ultisols (Han et al. 2013). Fertilizer N was applied at ~ 900 kg N ha^{-1} $year^{-1}$ to this 36-year-old tea plantation, leading to a higher N_2O emissions compared with that in adjacent vegetable and forest systems (Han et al. 2013). The soil had pH 3.7, 58 g organic C kg^{-1} soil, and 4.6 g organic N kg^{-1} soil.

Legume and non-legume biomass biochars (i.e., LB and NLB) were made from the pyrolysis of soybean and rice straws, respectively, under 500 °C and N_2 for 6 h (Khan et al. 2015). LB and NLB were similar in pH 10.1 and NH_4^+-N ~ 11 mg kg^{-1}, but differed significantly in electrical conductivity (EC) and NO_3^--N. The EC and NO_3^--N in NLB were 10.4 mS cm^{-1} and 6.6 mg kg^{-1}, respectively, which were ~ 2 times greater than the respective values in LB.

Experimental setup

An incubation experiment was set up to examine the impacts of biochar amendment on acidic tea plantation soil N_2O emissions and also soil chemical and biological properties. Three treatments were (1) soil alone (CK), (2) soil with the addition of 1% LB, equivalent to a biochar application rate of 20 Mg ha^{-1}, and (3) soil with the addition of 1% NLB. For improving homogenous mixing with 2-mm-sieved soil sample, biochar was ground to fine powder (< 0.9 mm). Respective treatments (10 g soil of dry weight equivalent) were packed into 120-mL serum bottles (5.5 cm dia.) in three replicates at a bulk density of 1.1 g cm^{-3}, adjusted soil moisture to 60% water holding capacity, and incubated at 25 ± 0.5 °C in an incubator for 30 days. During the incubation, serum bottles remained capped but air flushed for 30 min after soil and headspace gas samples were taken on day 1, 10, and 30 (Yu et al. 2019). At each time point, gas samples were withdrawn from the headspace of each bottle using an auto-determination system a gas chromatography, and soil of each bottle was sampled for determined basic properties and stored at 4 °C.

Measurements of soil pH, inorganic N, and DOC

Soil pH was measured using a pH meter at a soil-to-water ratio of 1:2.5. Soil NH_4^+- and NO_3^--N were analyzed using a continuous flow colorimeter (SEAL AutoAnalyzer 3, Southampton, UK) after soil samples were extracted with 2 M KCl solution (1:5 w/v), shaken for 1 h, and filtered.

DOC was measured using a total C analyzer (vario TOC, Elementar, Germany) after soil samples were extracted with 0.05 M K_2SO_4 (1:10 w/v) and filtered following the method of Jones and Willett (2006).

N_2O and CO_2 effluxes and biological contribution

Gas samples (3 ml) were withdrawn from the headspace of serum bottles using an auto-sampler (CTC analytics AG), and pumped by a Gilson Minipuls® 3 peristaltic pump into a gas chromatography (Agilent 7890A, Agilent, Palo Alto, CA, USA) for measuring N_2O with an electron capture detector and CO_2 with a thermal conductivity dector. Soil N_2O (or CO_2) efflux (μg N kg^{-1} soil day^{-1}) was calculated by the equation: $((C_{sample} - C_{air}) \times M \times V)/(r \times m \times t)$, where C_{sample} and C_{air} are N_2O (or CO_2) concentrations in the headspace gas sample and atmosphere (ppbv), respectively; M is molar mass of N_2O (or CO_2, kg mol^{-1}); V is the volume of serum bottle headspace (L); r is the molar volume at 25 °C and 1 atm. (24.45 L mol^{-1}); m is the dry weight of soil (kg); and *t* is the measuring time (day).

For determining the contribution of nitrification and denitrification to soil N_2O emissions, another 3-day incubation experiment was conducted to compare soil N_2O emissions with or without the addition of a nitrification inhibitor. Concerned over < 100% inhibition efficiency, we used two nitrification inhibitors, acetylene (0.1% v/v) and dicyandiamide (DCD, 20 μg g^{-1} soil) to examine nitrification-mediated N_2O emissions. Headspace N_2O was sampled and measured on day 3 according to the methods described previously.

qPCR and MiSeq sequencing

At the end of incubation, soil genomic DNA was extracted from 0.5 g soil with the PowerSoil® DNA Isolation Kit (MoBio, Carlsbad, CA) according to the manufacturer's protocols. DNA concentration was measured using a NanoDrop ND 2000 spectrometer (DE, United States) and stored at – 20 °C prior to qPCR and high-throughput sequencing.

qPCR was performed to determine copy numbers of functional genes involved in nitrification and denitrification. The genes *amoA*, *nirS*/*nirK*, and *nosZ* that encode ammonia monooxygenase in both ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA), nitrite reductase, and nitrous oxide reductase, respectively, were quantitatively amplified on a 96-well plate with a real-time PCR detection system (Light Cycle 480, Roche, United States) using respective target primers (Table A1) (Throback et al. 2004; Tourna et al. 2008; Yu et al. 2019). Each 20 μL qPCR reaction solution contained 0.4 μL primer pair at respective concentration, 1 μL sample DNA (~ 10 ng), 10 μL 2 × SYBER Green qPCR Master Mix (Thermo Scientific, NY, United States), and 8.6 μL milli-Q water.qPCR cycling conditions included initial denaturation at 95 °C for 3 min, followed by 40 cycles of 30 s at 95 °C for denaturation, 30 s at respective annealing temperature required for individual primer pairs, and 30 s at 72 °C for extension, and then 5 min final extension at 72 °C. The specificity of the qPCR reaction was estimated by melting curve analysis (60–95 °C). Negative controls were also included in the qPCR analysis. The standard curve for determining the respective functional gene copy number was made using a series of 10-fold dilution of plasmid DNA that contained the target functional gene with known copy number (Yu et al. 2019).

MiSeq sequencing was run on an Illumina MiSeq platform (300 × 2 paired end, Illumina, San Diego, CA, USA) after the preparation of 16S rRNA gene and ITS region libraries. First, PCR amplifications were made for bacteria 16S rRNA gene and fungal ITS region using the primer pairs 515F/907R and ITS1F/ITS2R, both with barcodes attached to 5' end, respectively (Wang et al. 2018). A total of 50 μL of PCR reaction comprised 25 μL of 2 × GoTaq Green master mix (Promega, Madison, WI, USA), 2 μL (~ 10 ng/μL) of template DNA, 1 μL of 10 mM of forward and reverse primers containing barcodes, and 21 μL of milli-Q water. PCR conditions included 5 min initial denaturation at 95 °C; 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 55 °C for bacterial 16S rRNA gene, and 60 °C for fungal ITS region, and 45 s extension at 72 °C, followed by 7 min final extension at 72 °C. Then, PCR products were purified using a TIANgel Midi Purification Kit (Tiangen Biotech, Beijing, China) and quantified using a NanoDrop™ 2000 spectrophotometer (Thermo Scientific, NY, USA). Purified products were pooled in equimolar amounts for sequencing. Sequence reads have been deposited into the NCBI Sequence Read Archive (SRA) under the BioProject PRJNA517215.

Data analysis

Two-way analysis of variance (ANOVA) was used to evaluate if differences in chemical and biological properties were significant among three treatments (CK, LB, and NLB) over the incubation using SPSS 20.0 (IBM Corp, Armonk, N.Y., USA). Raw reads were demultiplexed, trimmed to the expected size (300–385 bp for 16S rRNA gene and 190–380 bp for ITS region), and then chimeras removed using USEARCH v6.1.544. After quality trimming, sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity and taxonomy was assigned using sequences available in the SILVA databases.

Operational taxonomic units (OTUs) with 97% similarity were picked up using QIIME 1.9.1 and taxonomy was assigned using sequences available in the Greengenes database (13.8) for 16S rRNA gene sequencing data and UNITE database (12.11) for fungal ITS region sequencing data. OTUs

were analyzed for alpha and beta diversity at sequencing depth 35,000 for bacteria and 27,000 for fungi. Distance-based redundancy analysis (dbRDA) was made from Bray-Curtis distance and soil chemical properties using the PRIMER (Plymouth Routines in Multivariate Ecological Research Statistical Software, v7.0.13, PRIMER-E Ltd, UK).

Results

Temporal changes in soil pH, inorganic N, and DOC following biochar addition

Biochar addition did not alter dynamic patterns of soil pH, inorganic N, and dissolved organic C during the incubation process, but led to increases or decreases in respective soil properties, compared to the soil alone treatment (Fig. 1). Soil pH was increased immediately by ~ 0.3 and 0.5 units after NLB and LB addition, respectively, and thereafter remained relatively stable over the incubation. Soil pH in the CK treatment also increased during first 10-day incubation perhaps due to rapid degradation of organic acids. Nonetheless, soil pH in the CK treatment was statistically lower than the pH of soil with biochar addition, specifically with LB. Regardless of soil treatments, soil NH_4^+-N increased rapidly and peaked 10 days after the incubation and thereafter declined; the differences between start and end of the incubation were ~ 2-fold on average. In contrast, soil NO_3^--N increased with the incubation, specifically for the 10 to 30-day period. Nevertheless, biochar addition reduced both soil NH_4^+ and NO_3^--N over the incubation, and the largest reduction, ~ 22% in soil inorganic N occurred in the LB treatment. Like inorganic N, DOC was similar on day 1, but diverged between soils with or without biochar addition over time. At the end of incubation, DOC was ~ 7% greater in biochar-amended soils than in the soil alone treatment, although DOC declined over the incubation irrespective of soil treatments.

N_2O and CO_2 effluxes following biochar addition

Soil N_2O efflux rates were similar for soil with or without the addition of nitrification inhibitors (acetylene and DCD) (Fig. A4), indicating that denitrification was the dominant process for soil N_2O emissions. Compared to the soil alone, biochar-treated soil consistently emitted less N_2O over the incubation (Fig. 2), and biochar mitigation effects appeared to be stronger during the earlier period. Consequently, soil cumulative N_2O emissions were reduced by ~ 40% on average by LB or NLB amendment. However, biochar addition had little influence on CO_2 effluxes; there were ~ 525 mg CO_2-C kg^{-1} soil emitted over the incubation.

Microbial community compositional and functional alterations following biochar addition

In total, 482,530 fungal ITS gene sequences passed quality control with sequences ranging from 27,419 to 179,470 for ITS genes. In addition, 484,953 bacterial 16S gene sequences passed quality control with sequences ranging from 35,831 to 82,689 for 16S genes. Biochar addition modified bacterial and fungal Shannon index but not observed OTUs (Table 1). Compared to the soil alone, LB promoted bacterial Shannon diversity, but reduced fungal Shannon diversity. NLB also reduced fungal Shannon diversity. The dbRDA analysis showed that only fungal community compositions diverged significantly between soils with and without biochar addition (Fig. 3). Together, the four soil properties, i.e., pH, NH_4^+, NO_3^-, and DOC, explained ~ 55% of variations in the fungal community; NH_4^+ contributed most (22%), followed by DOC (~ 18%), and least for NO_3^- and pH (~ 15%). However, no individual soil properties contributed > 10% of variations of bacterial community.

Of 35 bacterial phyla detected, a few were dominant, including Actinobacteria (~ 43% on average across three soil treatments, CK, LB, and NLB), Firmicutes (~ 20%), Proteobacteria (~ 18%), Bacteroidetes (~ 13%), Acidobacteria (~ 2%), Chloroflexi (~ 1%), and Gemmatimonadetes (~ 1%) (Supplementary Fig. A1). However, biochar addition had little influence on the relative abundances of these dominant phyla and neither did for classes, orders, families, genera, and species (Supplementary Fig. A2). Only Enterobacteriaceae, a class of Gammaproteobacteria, and its order Enterobacteriales were greater in LB-amended soil (7%) than the soil alone (2%).

Of 15 fungal phyla detected, Ascomycota dominated, accounting for ~ 72% on average across the three soil treatments, CK, LB, and NLB, followed by Basidiomycota (~ 13%) (Supplementary Fig. A1). Significant differences in the relative abundance were not detected at the phylum level, but at the level of class, order, family, genus, and species (Supplementary Fig. A3). For example, Eurotiomycetes, a class of Ascomycota, was less abundant in biochar-amended soils (~ 7%) than the soil without biochar addition (~ 22%), but Sordariomycetes was more abundant in biochar-amended soils (~ 38%) than the soil alone (~ 16%). The relative abundance of Hypocreales, an order of Sordariomycetes, was significantly ($P < 0.1$) greater in biochar-amended soils (~ 13%) than the soil alone (~ 4%). In contrast, Helotiales, an order of Leotiomycetes, was less abundant in biochar-amended soils (~ 2%) than the soil alone (~ 11%). At the family level, Nectriaceae was more abundant in biochar-amended soils (~ 9%) than the soil alone (~ 2%). Biochar addition also stimulated the species *Trichoderma hamatum* and *Phialophora mustea*, but reduced *Pseudophialophora eragrostis* compared to the soil alone.

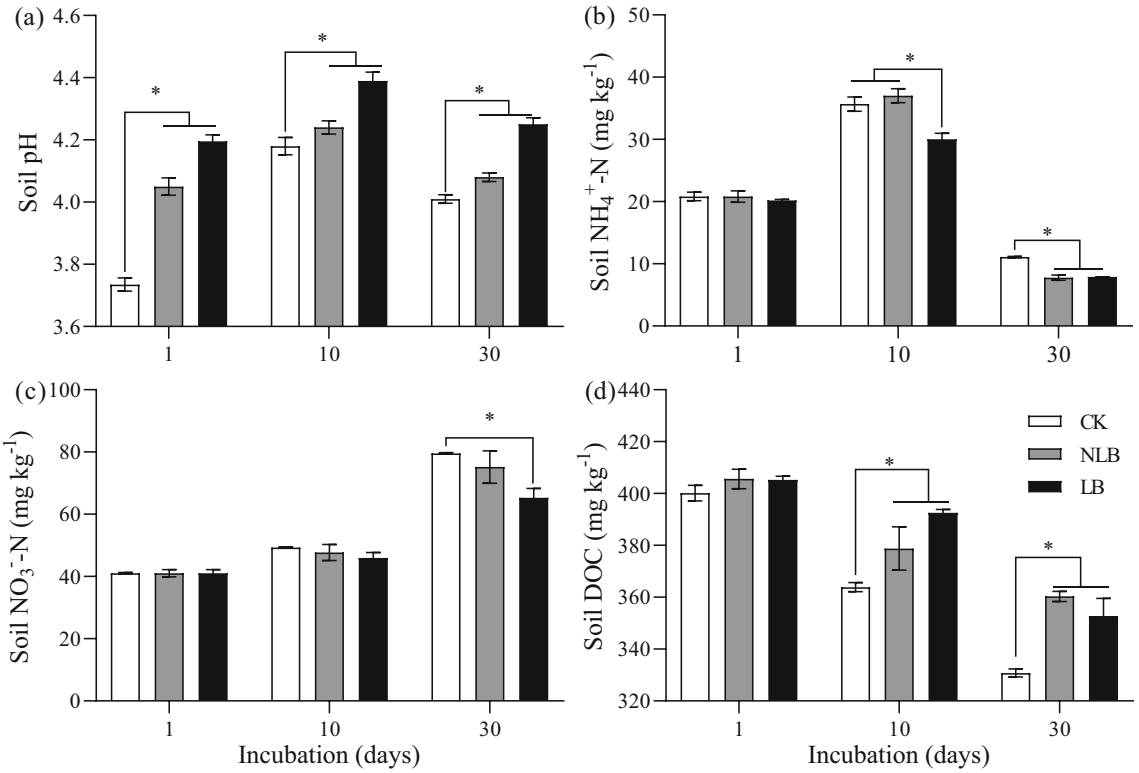

Fig. 1 Temporal changes of soil pH (a), inorganic N (b, c), and dissolved organic C (DOC) (d) over the 30-day incubation for soil alone (CK), soil with 1% addition of non-legume biochar (NLB), and soil with 1% addition of legume biochar (LB). Error bars represent standard errors of means (n = 3). "*" represents significant at the 0.05 level

Neither did biochar addition affect the *amoA* abundances of bacterial and archaeal ammonia oxidizers nor the *nirS* and *nirK* abundances of bacterial nitrite reducers (Fig. 4). However, it significantly promoted the *nosZ* abundance of bacterial nitrous oxide reducers. As such, the ratio of *nirK*/*nosZ* gene copy number was significantly lower in biochar-amended soils than the soil alone treatment.

Discussion

First, biochar moderately ameliorated the soil acidity and reduced the presence of extra inorganic N of tea plant need. Second, biochar substantially mitigated soil N_2O emission. Third, biochar shaped the fungal community despite little influence on the bacterial community. Because fungi play an important role in soil organic matter turnover and even contribute to soil N_2O production (Chen et al. 2014; Huang et al. 2017), biochar-induced changes in the fungal community structure were assumed responsible in part for changes in soil properties, nutrient (e.g., N) cycling, and thus the fate of nutrients (e.g., N) in the environment. Our study provided several lines of evidence to support that biochar would help improve the tea soil health.

Biochar application often mitigates soil N_2O emissions, specifically under O_2-limited conditions (Sánchez-García et al. 2014). Two independent meta-analyses using research articles published in different periods (2007-2013, 2011-2016) both documented that biochar application could suppress soil N_2O emissions by 33–54% (Cayuela et al. 2014; Schirrmann et al. 2017). This general statement was also confirmed by our data that biochar application substantially reduced soil N_2O effluxes (~ 40%) from the acidic tea plantation.

It is well known that nitrification and denitrification both can contribute to soil N_2O emission; however, in this study, denitrification was likely the main culprit due to the experimental setup. We measured CO_2 and N_2O emissions using a closed-container approach with varying measurement periods, i.e., 1, 9, and 18 days. Accumulation of emitted gasover time (e.g., > 30 min.) in the container headspace might reduce the gas diffusion rate from soil to air, resulting in an underestimation of gas efflux rate (Freijer and Bouten 1991). However, this approach could help create O_2-limited conditions due to long-lasting microbial respiration and yet no external O_2 supply. Given that the tea plantation contained a great amount of organic C (~ 5.8%), it was reasonably assumed that O_2 in soil pores was consumed rapidly, thereby generating O_2-limited conditions and favoring denitrification. Based on soil bulk density (1.1 g cm^{-3}), gravimetric water content (~ 27%, equivalent to 60% water holding capacity), and CO_2 respiration rate at ~ 50 mg C kg^{-1} soil on day 1 of the incubation, we estimated

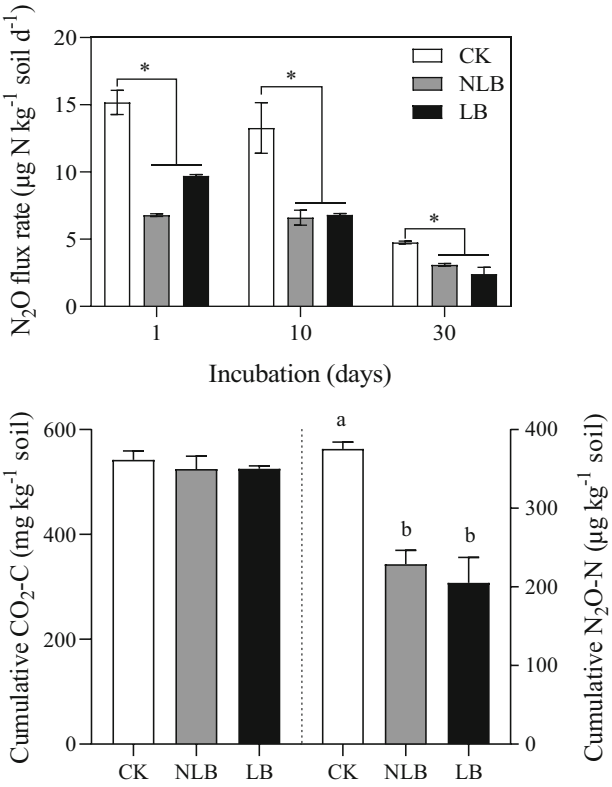

Fig. 2 Soil N_2O efflux rates (a) and total CO_2 and N_2O emissions (b) over the 30-day incubation for CK-, NLB-, and LB-treated soils. Error bars represent standard errors of means ($n = 3$). "*" represents significant at the 0.05 level. Different letters indicate significant difference $\alpha = 0.05$. See Fig. 1 for the abbreviation of treatments

that O_2 concentration in the soil profile could drop to ~ 2% at the absence of diffusion. The phenomenon that respiration can substantially lower O_2 concentrations and therefore create sub-oxic conditions for denitrification has been well described (Morley and Baggs 2010). Little effects of nitrification inhibitors on soil N_2O efflux rates also indicated the dominance of denitrification and thus O_2-limited conditions over the incubation.

Biochar's mitigation on soil N_2O emissions has been attributed to influences on the rate and/or the completeness of denitrification (Cayuela et al. 2013; Clough et al. 2013). In the acidic tea soil, however, biochar's mitigation effects were unlikely through influences on the rate of denitrification because CO_2 efflux rates imparted by microbial respiration were similar between soils with and without biochar addition. Also, biochar-induced direct reduction in soil NO_3^--N, the substrate of denitrification, was much less than the biochar's other N_2O mitigation effect. Further, the relative abundances of *nirK* and *nirS* genes encoding the NO reductase for catalyzing NO reduction to N_2O were similar between soils with and without biochar addition. All together, these suggested that biochar's N_2O mitigation effects were mainly through influences on the completeness of denitrification. This supposition was substantiated by the observation that the relative abundance of *nosZ*, the gene encoding N_2O reductase for improving the completeness of denitrification, was significantly greater in biochar-amended soils than the soil alone. This finding was in agreement with results from other similar studies (Ducey et al. 2013; Harter et al. 2016; Xu et al. 2014).

Table 1 Soil alpha diversity of bacterial and fungal communities structure determined on day 30

	Observed OTUs		Shannon diversity index	
	Bacteria	Fungi	Bacteria	Fungi
CK[#]	1958 a	731 a	5.61 b	5.55 a
NLB	1956 a	730 a	5.59 b	5.31 b
LB	2142 a	735 a	6.62 a	5.30 b

[#] CK, soil alone; NLB, soil with 1% addition of non-legume biochar; and LB, soil with 1% addition of legume biochar. Different letters indicate significant difference $\alpha = 0.05$

It is reasonable to assume that biochar addition could significantly modify soil properties, critical to dictate whether the end product of denitrification is N_2O versus N_2, such as pH, porosity and aeration, and availability of C versus N. Despite high porosity, biochar's notable influence on total soil porosity is moderate even at the high rate application (Hardie et al. 2014), suggesting that porosity alone cannot explain biochar's mitigation effects. Indeed, biochar-induced changes in porosity have been considered to have little influence on N_2O emission mitigation (Case et al. 2012; Cayuela et al. 2013). It was also unlikely that biochar's N_2O mitigating effect was through a control on the relative availability/limitation of organic C versus NO_3^-. Soil NO_3^- was ~ 40 mg N kg^{-1} at the beginning of the incubation and increased over time, suggesting that C might be limiting factor for respiratory denitrification and thus favoring a shortcut of NO_3^- reduction with N_2O as the end product. Biochar's addition did improve DOC content, but this improvement was much stronger towards the end of incubation, which did not align with the biochar's stronger effects on N_2O emission mitigation at the beginning of incubation. Instead, the liming effects of both NLB and LB were more potent at the beginning of incubation. As such, we considered the liming effects might be the key for biochar to mitigate soil N_2O emission in the tea soil. However, the observation that significantly larger pH effect of LB than NLB generating no difference in N_2O emission mitigation between the two implied that factors other than pH might also be important. Cayuela et al. (2013) proposed that biochar could serve as a reducing agent due to containing redox-reactive elements, such as Mn (IV) and Fe (III) for facilitating electron transfers to soil bacterial denitrifiers, resulting in enhanced reduction of N_2O to N_2. Given 2 times greater EC than LB, NLB might be more robust as a reducing agent than LB to promote the completeness of denitrification. Thus a combined

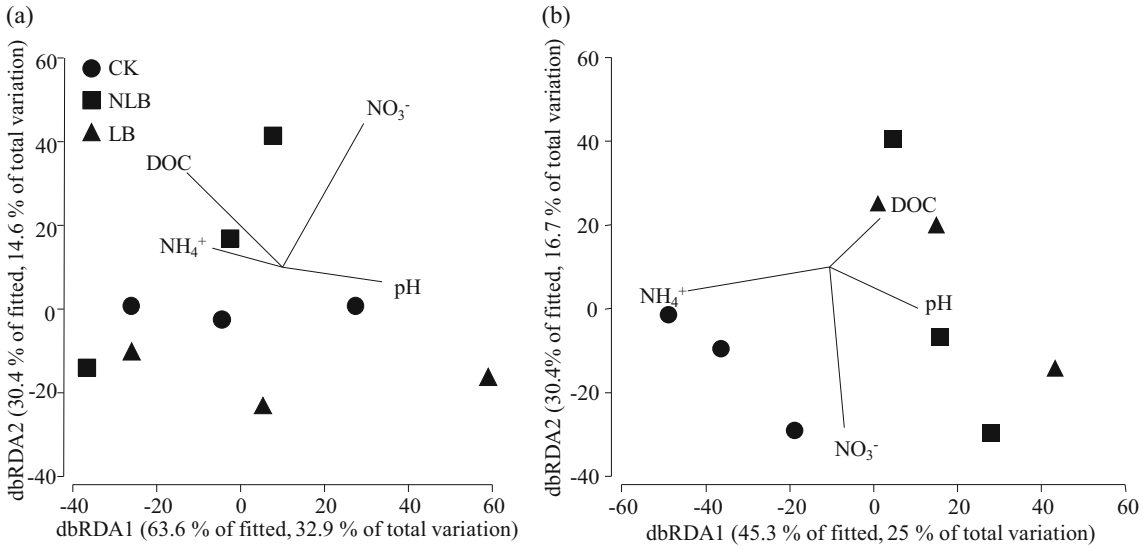

Fig. 3 Distance-based redundancy analysis (dbRDA) of bacterial (a) and fungal (b) communities with soil properties as explanatory variables in CK-, NLB-, and LB-treated soils. See Fig. 1 for the abbreviation of treatments

effect of liming and reducing agent made NLB and LB has comparable N_2O mitigation effects in the tea soil.

Biochar's effects on the soil bacterial community are often inconsistent across published data, perhaps due to large variations in the types, application rates, and influencing periods (long- versus short-term) of biochar as well as study sites (Chen et al. 2013; Jenkins et al. 2017). Thus, it was not surprising to observe that the bacterial community composition was unaffected by the addition of biochar, except for alpha diversity enhanced by LB. However, fungal community composition changed dramatically, suggesting that fungal community was more sensitive than the counterpart, bacterial community to the biochar amendment. This phenomenon was also documented in the work of Jenkins et al. (2017), showing that different responses of bacterial and fungal communities to biochar addition were time issues, being short-term for the

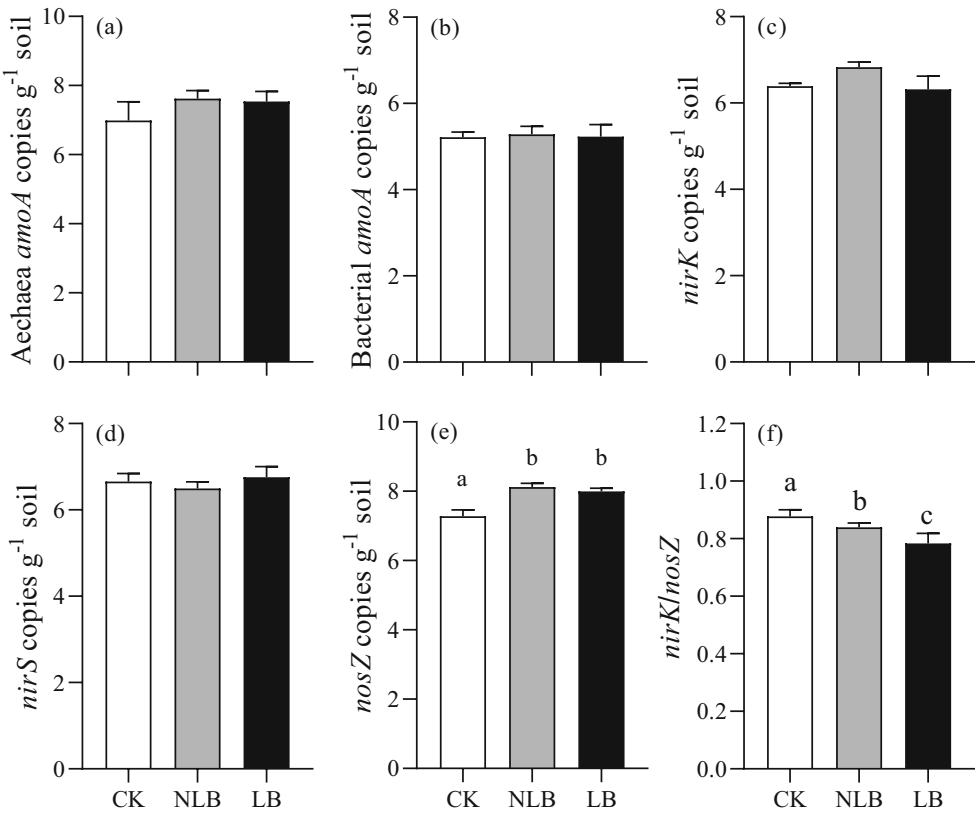

Fig. 4 Nitrogen-cycling gene abundances determined by qPCR; (a,b) *amoA* of archaea and bacteria, (c) *nirK*, (d) *nirS*, (e) *nosZ* and (f) *nirK*/*nosZ* for CK-, NLB-, and LB-treated soils. Error bars represent standard errors of means ($n = 3$). See Fig. 1 for the abbreviation of treatments

fungal community, i.e., 1 month after biochar addition and long-term for the bacterial community, i.e., 1 year after biochar addition. Nonetheless, our findings add to the argument that the direction and magnitude of biochar's effects are dependent on the soil environment and also indigenous microbial community.

Tea soil used in this work was very acidic (pH ~ 3.7), and dominant bacterial phyla were Actinobacteria, followed by Proteobacteria, Bacteroidetes, and Firmicutes. Although pH is a key regulator of the soil bacterial community (Lauber et al. 2009; Rousk et al. 2010), moderate changes in soil pH 1 month after biochar amendment might not be sufficient to shape the community. Rapid responses of the soil fungal community to the biochar addition have been associated with biochar's properties, e.g., mineralizable C and inorganic nutrients (Dai et al. 2018). Authors considered that biochar-associated available C and nutrients might promote proliferation of some saprophytes, resulting in a reduction in fungal diversity as well as a change in community compositions. Indeed, dbRDA showed that DOC and N availability were significant explanatory variables for differences in the fungal community between soil with and without biochar addition. Now the question is whether this shift in the soil fungal community affected the soil processes, such as N_2O emission.

A recent work using an isotopomer technique for the source tracking of soil N_2O emission showed that the site preference (~ 26‰), i.e., the difference between ^{15}N-N_2O in the center position ($\delta^{15}N^{\alpha}$) and the end position ($\delta^{15}N^{\beta}$), was much greater in biochar-amended soil-manure system than the system without biochar (~ 17‰) (Yuan et al. 2017). Although biochar stimulation on N_2O reduction could increase the site preference (Winther et al. 2018), fungal NO reduction should not be ruled out for soil N_2O emission given that the site preference of ^{15}N-N_2O from fungal NO reduction is often around 30‰ (Chen et al. 2016). In this study, most significant changes of the fungal community were for the members of Ascomycota, the phylum including diverse N_2O-producing fungi (Bollag and Tung 1972; Chen et al. 2014; Maeda et al. 2015; Mothapo et al. 2015; Huang et al. 2017). Specifically, the class Sordariomycetes and its order Hyprocreale, which contain numerous members of N_2O-producing capability, were significantly increased by LB and NLB additions. Further, biochar also stimulated the proliferation of *Trichoderma harzianum*, a known N_2O-producing fungus. Altogether, these observations seem to suggest that biochar amendment might affect the source of N_2O production, bacteria versus fungi.

Conclusions

Biochar was effective to suppress N_2O emission from the tea plantation. The underlying mechanisms were likely complex. On one hand, biochar's liming and/or reducing effects promoted N_2O consumption, leading to soil N_2O emissions mitigation. On the other hand, biochar might regulate the relative contribution of bacteria versus fungi to N_2O emission given that biochar promoted the fungal taxa capable of N_2O production. However, caution should be taken regarding biochar's long-term effectiveness in that the microcosm experiment only lasted for a month under the absence of growing plants. It should be also noted soil disturbance might greatly mask the in situ effects of biochar. The observation that soil pH in the soil alone treatment increased rapidly during the first 10 days of incubation indicated that organic acids were decomposed quickly due to soil mixing and homogenization. This would unlikely happen under the field conditions, thereby making biochar's liming effects much more significant compared to soil without biochar addition. Nonetheless, this work is significant since it provides direct evidence that biochar amendment could mitigate N_2O emission, improve soil pH, and reduce soil extra N in the acidic tea soil.

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