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ORIGINAL PAPER

Biochar alters nitrogen transformations but has minimal effects on nitrous oxide emissions in an organically managed lettuce mesocosm

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Abstract We investigated the effect of biochar type on plant performance and soil nitrogen (N) transformations in mesocosms representing an organic lettuce (*Lactuca sativa*) production system. Five biochar materials were added to a silt loam soil: Douglas fir wood pyrolyzed at 410 °C (W410), Douglas fir wood pyrolyzed at 510 °C (W510), pine chip pyrolyzed at 550 °C (PC), hogwaste wood pyrolyzed between 600 and 700 °C (SWC), and walnut shell gasified at 900 °C (WS). Soil pH and cation exchange capacity were significantly increased by WS biochar only. Gross mineralization increased in response to biochar materials with high H/C ratio (i.e., W410, W510, and SWC), which can be favorable for organic farming systems challenged by insufficient N mineralization during plant growth. Net nitrification was increased by W510, PC, and WS without correlating with the abundance of ammonia oxidizing gene (*amoA*). Increases in N transformation rates did not translate into increases in plant productivity or leaf N content. WS biochar increased the abundance of *amoA* and nitrite reductase gene (*nirK*), while SWC biochar decreased the abundance of *amoA* and nitrous oxide gene (*nosZ*). Decreases in N_2O emissions were only observed in soil amended with W510 for 3 days out of the 42-day growing season without affecting total cumulative N_2O fluxes. This suggests that effects of biochar on decreasing N_2O emissions may be transient, which compromise biochar's potential to be used as a N_2O mitigation strategy in organic systems. Overall, our results confirm that different biochar materials can distinctively affect soil properties and N turnover.

Keywords Mineralization · Nitrification · Functional gene abundance · Lettuce · Organic farming · Greenhouse gas emissions

Introduction

Managing nitrogen (N) dynamics represents a challenge for organic cropping systems. In particular, sufficient N mineralization is not always achieved; timing of N release and plant uptake is often not synchronized (Pang and Letey 2000), and subsequent N transformations of mineralized N may be released to the atmosphere as nitrous oxide (N_2O), a potent greenhouse gas. Emissions of N_2O are responsible for 61 % of agricultural greenhouse gas (GHG) emissions and are mainly related to N fertilization in field crop agriculture (Montzka et al. 2011). A meta-analysis by Tuomisto et al. (2012) reported that, compared to conventional systems, N_2O emissions were 31 % lower in organic systems when the impact was allocated per unit of field area but 8 % higher when the impact was allocated per unit of end product. The increasing market demand for organic food calls for an intensification and expansion of this production system (Li et al. 2007; Niggli et al. 2008; Willer and Kilcher 2012). Hence, organic farmers are challenged to increase yields without causing additional environmental harm.

One alternative proposed to increase N use efficiency and reduce N_2O emissions from soils is the amendment with biochar. The term "biochar" defines the charcoal obtained from

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the intended thermal conversion of waste biomass into energy in processes such as pyrolysis and gasification (Spokas et al. 2012). When amended to soils, biochar may alter many soil properties such as soil pH, cation exchange capacity (CEC), moisture content, and microbial activity that intrinsically regulate N transformations (Clough and Condron 2010; Clough et al. 2013). Studies have often linked increases in plant productivity and N uptake with an increased N availability in biochar-amended soils due to high N retention by cation exchange capacity (Chan et al. 2008a; Chan et al. 2008b; Steiner et al. 2007). A practice that improves plant N uptake would be very advantageous to organic farming systems challenged by the restrictions of using organically sourced N.

A growing number of studies demonstrate decreases in N_2O emissions associated with the effects of biochar on N availability, increases in soil pH, or aeration soil structure. Studies that relate decrease in N_2O emissions with lower inorganic N availability report either increased microbial N immobilization or decreased nitrification rates (Wang et al. 2011; Cayuela et al. 2013). Other reports suggest that the elevated soil pH resulting from biochar amendment enhances N_2O reductase activity and thus promotes N_2 formation and higher N_2/N_2O ratios (Clough and Condron 2010; Singh et al. 2010; Zheng et al. 2012). Finally, the highly porous structure of biochar can increase soil aeration, thereby reducing anaerobic sites for N_2O production (Yanai et al. 2007; Rogovska et al. 2011). However, studies also have documented that amendments with biochar lead to N_2O increases (Clough et al. 2010; Bruun et al. 2011; Saarnio et al. 2013, Singla et al. 2014, Verhoeven and Six 2014) and null effects (Castaldi et al. 2011; Karhu et al. 2011; Scheer et al. 2011; Suddick and Six 2013). Given such a variety of N_2O responses to biochar amendment, it is likely that effects vary with biochar materials (Guerrero et al. 2005), and soil and fertilization management (Spokas and Reicosky 2009).

To detect whether biochar can effectively increase N availability and plant productivity, as well as reduce N_2O emissions, we monitored these parameters in microcosms amended with five different biochar materials during the production of organic lettuce. We also assessed the abundance of N-cycling microorganisms by targeting functional bacterial genes that encode for enzymes involved in ammonia oxidation (*amoA*) and nitrite (*nirK* and *nirS*) and N_2O reduction (*nosZ*).

Materials and methods

Greenhouse bioassay description

The experiments were carried out at the Environmental Horticulture Greenhouse Facility, University of California, Davis. Lettuce (*Lactuca sativa*) was grown for 42 days under a constant temperature of 25 °C in the greenhouse. Plants were grown under mesocosm conditions that explicitly simulated field conditions in terms of irrigation and organic fertilization management. Six different biochar treatments were used in this study: control (no amendment), Douglas fir wood pyrolyzed at 410 °C (W410), Douglas fir wood pyrolyzed at 510 °C (W510), pine chip pyrolyzed at 550 °C (PC), hogwaste wood pyrolyzed between 600 and 700 °C (SWC), and walnut shell gasified at 900 °C (WS) (Table 1). Mesocosms were arranged in a completely randomized design that consisted of 19-L pots as experimental units with three replicates per treatment. The pots were filled with the A horizon of a Yolo silt loam soil (0–15 cm) to a depth of 20 cm. Soil was collected from the riparian zone of the Century Experiment at Russell Ranch Sustainable Agriculture Facility, Davis, CA. This soil is approximately 46 % sand, 32 % silt, and 22 % clay with a pH of 6.7, 7.30 g C kg^{-1} soil, and 0.82 g N kg^{-1} soil. The soil was sieved (<8 mm) and amended with fertilizer and biochar. The biochar was applied at a rate equivalent to 10 metric t ha^{-1}. The fertilization rate was split into two applications of 95 kg N ha^{-1} spaced by 30 days from each other using a compost mix of animal and plant manure. The plants received daily irrigation and soil moisture was maintained at about 20 % (*w/w*). Dry biomass (60 °C, 7 days) was recorded. Plant tissues were ball milled before N concentrations were analyzed by combustion (Costech Instruments ECS 4010). Nitrogen use efficiency (NUE) was calculated as the amount of N uptake by plants per kilogram of N supplied through fertilization.

Biochar and soil characterization

Table 1 shows some physicochemical properties of the biochar materials, and a complete characterization can be found in Mukome et al. (2013). Biochar and soil pH were measured in deionized water using a 1:2 and 1:1 (*w/w*) ratio, respectively, with an equilibration time of 1 h. The pH was measured on an Orion 4 Star, Thermo Fisher Scientific pH meter. Ash content of biochar materials was determined according to ASTME 1755-95 (1995).

Cation exchange capacity (CEC) measurements of biochar and soil samples were adapted from Mulvaney et al. (2004) and Gaskin et al. (2008). Following Mukome et al. (2013), for each sample, 0.5 g of biochar or soil was weighed into a solid phase column and 30 mL of 1 M NaOAc was added to the column, which was then vacuum-filtered. The samples were washed with 30 mL of isopropanol to remove excess sodium (Na). Sodium ions were displaced with NH_4OAc (pH 7) three times and measured by atomic adsorption spectroscopy at the UC Davis Analytical Laboratory. Biochar C, H, and N concentrations were determined by combustion at Galbraith Laboratories, Inc. (Knoxville, TN, USA). C input to respective treatment is presented in Table 1. Samples for measurement of pH, CEC, N transformation processes, and functional

Table 1 Feedstock, production temperature, and selected physicochemical properties of the biochar materials

Biochar material	Feedstock	Production temperature (°C)	pH	Ash (%)	N (%)	C (%)	H/C ratio	C input (metric ton ha^{-1})	CEC (meq 100 g^{-1})
Douglas fir (W410)	Softwood	410	7.1	2.6	0.21	83.9	0.8	8.39	10.0
Douglas fir (W510)	Softwood	510	7.3	3.0	0.36	65.7	0.27	6.57	12.0
Pine chip (PC)	Wood chips	550	7.9	17	0.91	71.2	0.47	7.12	3.2
Soft wood chips (SWC)	Softwood	600–700	7.5	2.4	0.51	68.2	0.64	6.82	26.2
Walnut shell biochar (WS)	Walnut shell	900	9.7	40.4	0.47	55.3	0.19	5.53	33.4

CEC cation exchange capacity

gene assessments were collected from mesocosms (0–10-cm depth) during the harvest of lettuce.

DNA extraction

Soil samples for DNA extractions were stored (−80 °C) for 3 weeks until DNA extractions. DNA was extracted from 0.5-g aliquots of soil using the FastDNA Spin Kit for Soil (MP Biomedicals, Illkirch, France). To improve DNA quality, an additional cleaning step was performed with the addition of 5.5 M guanidine isothiocyanate (Porteous et al. 1994). Final DNA extracts were stored at −20 °C before analysis using real-time (RT) PCR. The concentration of DNA in the extracts was determined using Qubit with Quant-iT dsDNA HS Assay Kits (Invitrogen, Carlsbad, CA, USA). DNA purity was determined using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE) by measuring the A_{260nm}/A_{280nm} absorbance ratio.

Real-time quantitative PCR assay

Abundances of total bacteria, nitrifier, and denitrifiers were estimated by real-time qPCR targeting of universal bacterial gene (16S rDNA), ammonia monooxygenase gene (*amoA*), nitrite reductase genes (*nirK* and *nirS*), and nitrous oxide reductase gene (*nosZ*), respectively. All genes were amplified using an Applied Biosystems 7300 Real-Time PCR system (Foster City, CA, USA), with three analytical replicates per sample, and references are shown in Table 2.

The quantification of the 16S rDNA gene used to estimate the total bacterial abundance was performed using 4 μL of template DNA, 10 μL of TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 4 μL of H_2O, 800 nM each of forward and reverse primers, and 0.4 μL of the fluorogenic probe (TM1389: 50-CTT GTA CAC ACC GCC CGTC-30; 200 nM) (Suzuki et al. 2000). Real-time quantitative PCR of the *amoA* gene was performed using 5 μL of template DNA, 10 μL of ABI Power SYBR Green PCR (Applied Biosystems, Foster City, CA, USA), and 0.3 μM of the A189 forward and 0.9 μM of the amoA-2R′ reverse primers (Okano et al. 2004). Real-time quantitative PCR of the *nirK* gene was performed using 5 μL of template DNA, 12.5 μL of ABI Power SYBR Green PCR Master Mix, and 0.5 μM each of forward and reverse primers (Henry et al. 2004). *NirS* gene was also amplified by using 5 μL of template DNA, 12.5 μL of ABI Power SYBR Green PCR Master Mix, and 0.5 μM each of forward and reverse primers (Throbäck et al. 2004; Kandeler et al. 2006). For the *nosZ* gene, qPCR was performed using 5 μL of template DNA, 10 μL of ABI Power SYBR Green PCR Master Mix, and 0.3 μM each of forward and reverse primers (Henry et al. 2006).

Standard curves were generated for each gene by using serial dilutions of a standard containing a known number of the target sequences. To prepare the 16S standard, we used the pT7Blue-3 Perfectly Blunt® Cloning Kit and pT7Blue-3 vector (EMD Millipore, Bedford, MA, USA). The 16S rDNA fragment was amplified from *Escherichia coli* (strain K-12). To prepare standards for the N cycling genes, we used TOPO TA cloning kit with pCR2.1-TOPO TA cloning vector and OneShot TOP10 chemically competent *E. coli* (Invitrogen, Carlsbad, CA, USA). DNA was extracted with a Plasmid Mini Kit (Qiagen) from four transformants containing *amoA* (GenBank: Z97833), *nirK* (GenBank: NC003037.1), *nirS* (GenBank: AF197466.1), and *nosZ* (GenBank: AF197468); gene fragments were amplified from *Nitrosomonas europaea* (ATCC 19718), *Sinorhizobia meliloti*, *Pseudomonas fluorescens*, and *Bradyrhizobium japonicum* (strain USDA 110), respectively. The concentration of plasmid DNA was quantified spectrofluorometrically using the Quant-iT fluorescent dye method (Molecular Probes, Invitrogen, Paisley, UK). Standard curves were linear over 5 orders of magnitude, and the detection limit was approximately 100 copies for the *amoA*, *nirK*, *nirS*, and *nosZ* real-time qPCRs and 1000 copies for the 16S rDNA real-time qPCR (r^2>0.98). The number of copies of 16S rDNA, *amoA*, *nirS*, *nirK*, and *nosZ* in soil extracts was calculated from the respective concentrations of extracted plasmid DNA.

We also calculated the ratios between the abundances of nitrite reductase genes (*nirK* and *nirS*) and nitrous oxide gene (*nosZ*) to detect any changes in the compostion of producers and reducer of N_2O, respectively. We report ratios of "*nirK*/*nosZ*," "*nirS*/*nosZ*," and "*nirK*+*nirS*/*nosZ*" abundances

Table 2 Primer sets and thermal profiles used for quantification of total bacterial gene (16S rDNA) and functional genes involved in the N cycle (*amoA*, *nirK*, *nirS*, and *nosZ*)

	Primer/probe	Primer sequence (5′-3′)	Thermal profile	Reference
16S rDNA	BACT1369F PROK1492R TM1389[a]	CGGTGAATACGTTCYCGG AAGGAGGTGATCCRGCCGCA CTTGTACACACCGCCCGTC	50 °C, 2 min, 95 °C, 10 min 40 cycles: 95 °C, 15 s, 56 °C, 60 s	Suzuki et al. (2000)
Bact. *amoA*	amoA189F amoA-2R'	GNG ACT GGG ACT TCT GG CCC CTC KGS AAA GCC TTC TTC	15 s at 95 °C, 40 cycles: 95 °C, 15 s 55 °C, 15 s, 72 °C, 31 s 95 °C,15 s, 60 °C, 30s, 95 °C, 15 s	Okano et al. (2004)
nirK	nirK876 nirK1040	ATYGGCGGVCAYGGCGA GCCTCGATAGRTTRTGGTT	95 °C, 30 s, 5 cycles: 95 °C, 15 s, 63–59 °C (-1 °C per cycle), 30 s, 72 °C, 30 s, 30 cycles: 95 °C, 15 s, 63 °C, 30 s, 72 °C, 30 s	Henry et al. (2004)
nirS	nirSCd3aF nirSR3cd	AACGYSAAGGARACSGG GASTTCGGRTGSGTCTTSAYGAA	95 °C, 10 min, 35 cycles: 95 °C, 15 s, 63 °C, 30 s, 72 °C, 30 s, 80 °C, 30 s	Throbäck et al. (2004) Kandeler et al. (2006)
nosZ	nosZ2F nosZ2R	CGC RAC GGC AAS AAG GTS MSS GT CAK RTG CAK SGC RTG GCA GAA	95 °C, 10 min 6 cycles: 95 °C, 15 s, 65 °C, 30 s, 72 °C, 30 s, 40 cycles: 95 °C, 15 s, 60 °C, 15 s, 72 °C, 30 s, 83 °C, 30 s	Henry et al. (2006)

[a] Fluorogenic probe, labeled with FAM and BHQ1 at the 5′- and 3′-ends, respectively

because no one bacterium possesses both the copper-based (*nirK*) and the heme-based (*nirS*) nitrite reductases (Throbäck 2006). Additionally, to our knowledge, it is difficult to predict the representative nitrite reductase gene for a specific environment and the factors that induce changes in the abundance of *nirK* or *nirS*.

Soil N transformations

We quantified gross mineralization and nitrification rates using ^{15}N pool dilution techniques (Barraclough 1991). In brief, for each mesocosm replicate, we re-wetted four 10-g soil subsamples with solutions of $(^{15}NH_4)_2SO_4$ (99 at.%) for potential gross mineralization incubations and $K^{15}NO_3$ (99 at.%) for potential gross nitrification. The addition of the ^{15}N solutions resulted in an approximate soil moisture level of 60 % water-holding capacity (WHC). The amount of ^{15}N added to each replicate was adjusted to achieve approximately 10 at.% ^{15}N enrichment. For the potential gross mineralization assay, two subsamples were incubated for 3 h (t_0) and 1 day (t_1). For the gross nitrification measurement, the two remaining replicates were incubated for 3 h (t_0) and 3 days (t_3). After the incubation period, samples were extracted with 2 M K_2SO_4 and pool sizes of NH_4^+-N and NO_3^--N were estimated colorimetrically by using the Berthelot reaction for NH_4^+-N (Forster 1995) and the vanadium (III) chloride reduction method for NO_3^--N (Doane and Horwáth 2003). The samples were diffused onto acidified disks (Stark and Hart 1996), and the ^{15}N isotopic signatures of NH_4^+-N and NO_3^--N were analyzed with a PDZ Europa 20-20 isotope ratio mass spectrometer (Crewe, UK; Stable Isotope Facility of the University of California, Davis). Rates for gross mineralization and nitrification rates were calculated from the rate at which the relative abundance of ^{15}N and the size of the NH_4^+-N and NO_3^--N pool changed over time (Barraclough 1991):

$$m = \theta \times \left[\frac{\log\left(A_0^*/A_t^*\right)}{\log[(1+\theta t)/C_0]} \right] \quad (1)$$

where m=potential gross mineralization or nitrification rate, θ=the rate of change in the size of the NH_4^+ or NO_3^- pool, A_0^* = the ^{15}N at.% excess of the NH_4^+ or NO_3^- pool at t_0, A_t^* = the ^{15}N at.% excess of the NH_4^+ or NO_3^- pool at t_1 or t_3, respectively, and C_0=the size of the NH_4^+ or NO_3^- pool at t_0.

Net nitrification rates were estimated by incubating 50 g air-dried soil for 7 days. The moisture of the soil samples was brought to 60 % WHC on the first day of incubation. NH_4^+-N and NO_3^--N of two 10-g subsamples were extracted at the first and seventh day of incubation using the same extraction and quantification methods described above for the gross mineralization and nitrification rate measurements.

Nitrous oxide fluxes

Nitrous oxide evolved from soil was sampled by an adapted vented-closed-flux chamber made of polypropylene material. These chambers (0.33-m height by 0.18-m diameter) securely fit on to the pots to create an airtight seal. At sampling time, gas samples (20 mL) were drawn from the headspace of the chambers with 25-mL polypropylene syringes and transferred into 12-mL pre-evacuated exetainers 0, 20, 40, and 60 min after the chambers were closed. Samples were taken two times a week or for 7 days after each fertilization event over the 42-day growth period. N_2O fluxes were analyzed by electron capture gas chromatography (GC-2014 Shimadzu Gas

Chromatograph, Kyoto, Japan). Gas concentrations were tested for linearity (Hutchinson and Mosier 1981) to determine the best flux and finally converted to g N_2O-N ha^{-1} day^{-1}. The cumulative N_2O emissions across the biochar materials were calculated by interpolating the emissions between each sampling day.

Data analyses

Gross mineralization, gross nitrification, net nitrification, and copy number of 16S rDNA, *amoA*, *nirK*, *nirS*, and *nosZ* genes were analyzed by one-way analysis of variance (ANOVA). For cumulative N_2O fluxes, we performed a mixed model (PROC MIXED, SAS) between treatments and sampling date with repeated-measures ANOVA to determine differences between biochar amendment and control across time. Dunnett post hoc analysis was used to compare the treatment against the control. When data were not normally distributed or showed heterogeneity of variances, they were log-transformed before analysis. Pearson correlation analysis (PROC CORR Pearson, SAS) was used to examine the relationships among functional gene abundance, soil, and biochar properties. All analyses were performed using SAS statistical package.

Results

Soil properties and plant performance

Soil pH was significantly increased only by WS biochar (Table 4) whose pH is the highest among the biochar materials used in this study. Correlation analyses indicate that soil pH correlated well with biochar pH and ash content (Table 5). WS biochar also increased significantly soil CEC by 64 % relative to the control (Table 4), likely due to increases in soil pH. Although changes in soil properties were observed, none of the biochar amendments altered plant performance indicators, such as plant biomass, leaf N concentration, and N use efficiency (Table 3).

N transformations and nitrous oxide emissions

Gross mineralization rates were increased by W410, W510, and SWC (Table 4). The highest gross mineralization rate was observed by the W410 biochar treatment, which was more than two times that of control. Gross mineralization rates were positively correlated with biochar H/C ratio (Table 5). Gross nitrification rates were not affected by any of the biochar materials and did not correlate with any of the variables measured. Soils amended with WS, PC, and W510 doubled net nitrification, while W410 and SWC did not alter net nitrification. Net nitrification was negatively correlated with biochar H/C ratio.

Table 3 Dry matter, leaf N concentration, and nitrogen use efficiency (NUE) in lettuce plants grown in soils amended with different biochar amendments

	Dry matter (g $plant^{-1}$)	Leaf N (mg g^{-1})	NUE (%)
Control	12.6±0.6	26.5±1.3	29.4±2.9
W410	14.7±0.9	31.9±3.2	41.3±5.7
W510	13.3±1.7	31.6±3.4	36.3±3.9
PC	12.4±2.6	32.3±8.2	31.6±0.8
SWC	10.9±1.9	29.9±5.0	27.3±2.1
WS	12.5±1.0	28.1±0.8	30.6±1.7

Values are means±standard errors (n=3) with no statistical differences between the means (p<0.05).

None of the biochar materials affected the total cumulative N_2O emissions (Table 4), which averaged 0.48 kg N_2O-N ha^{-1} over the 42-day growing season. However, biochar material W510 significantly decreased N_2O emissions for a short time of the growth period (Fig. 1b). W510 had lower cumulative N_2O fluxes for three consecutive days after the second application of fertilizer compared to the control (p-values=0.048, 0.026, and 0.042). We did not observe any changes in N_2O emissions in the remaining biochar treatments across the growing period compared to the control. Total cumulative emissions of N_2O did not correlate with any of the studied parameters.

Total bacterial, nitrifier, and denitrifier community abundances

The abundance of 16S rDNA and N cycling genes in biochar-amended soil is presented in Table 6. The abundance of total bacteria estimated by 16S rDNA gene was not affected by the biochar treatments. Results for the nitrifying and denitrifying gene abundance, however, revealed a general trend across the biochar materials; the abundance of *amoA*, *nirK*, and *nosZ* were the highest in WS biochar-amended soil and lowest in soils amended with SWC biochar. WS biochar significantly increased the abundance of *amoA* gene by 45 % whereas SWC decreased it by 65 %, compared to control. Additionally, biochar W410 decreased *amoA* abundance by 38 % compared to control. For the abundance of the denitrifying gene *nirK*, WS biochar-amended soil significantly increased (40 %) and SWC biochar decreased (26 %) numbers compared to the control. The abundance of *nosZ* gene was increased by 32 % in WS treatment and significantly decreased by 48 % in SWC treatment relative to the control. None of the biochar treatments altered the abundance of denitrifier gene *nirS*. To better understand how biochar materials influenced the composition of denitrifier community, we also

Table 4 Soil pH, cation exchange capacity (CEC), gross mineralization, gross nitrification, net nitrification, and cumulative N_2O emissions in biochar-amended soil after 42 days of lettuce growing period

	Soil pH	Soil CEC (meq 100 g^{-1})	Gross mineralization (μg N g^{-1} soil day^{-1})	Gross nitrification (μg N g^{-1} soil day^{-1})	Net nitrification (μg N g^{-1} soil day^{-1})	Cumulative N_2O (kg N_2O-N ha^{-1})
Control	6.7±0.1	33.5±2.3	2.1±0.1	1.3±0.5	0.2±0.0	0.6±0.1
W410	6.8±0.0	37.7±7.1	5.5±0.9*	0.8±0.3	0.2±0.0	0.5±0.1
W510	6.9±0.1	35.9±6.5	3.8±0.3*	1.3±0.1	0.4±0.0*	0.3±0.0
PC	6.8±0.1	27.6±0.8	3.2±0.1	0.7±0.3	0.4±0.0*	0.7±0.3
SWC	6.8±0.1	27.2±4.1	4.0±0.1*	1.1±0.2	0.2±0.0	0.4±0.1
WS	7.5±0.2*	55.0±0.6*	1.8±0.1	1.0±0.2	0.4±0.0*	0.4±0.0

Values are means±standard errors (n=3)

*Mean values significantly different from control (Dunnett's test, $p<0.05$)

calculated combinations of the ratios of N_2O reducer abundance to N_2O producer abundance (Fig. 2): *nosZ*/(*nirK*+*nirS*), *nosZ*/(*nirK*), and *nosZ*/(*nirS*). The highest *nosZ*/(*nirK*+*nirS*) and *nosZ/nirS* ratios were found in the WS biochar treatment, indicating a higher relative abundance of N_2O reducers to N_2O producers. Dunnett means separation test also revealed that SWC decreased the ratio of *nosZ*/(*nirS*+*nirK*) relative to the non-amended control soil, indicating a lower relative abundance of N_2O reducers to N_2O producers. The *nosZ/nirK* ratio was not significantly affected by any of the biochar treatments.

Correlation analyses for functional genes and biochar-related properties are presented in Table 5. The abundance of bacterial 16S rDNA gene positively correlated with biochar pH, biochar ash content, and soil pH. Abundances of *nirK* gene also correlated with biochar pH, ash content, soil pH, and soil CEC. The abundance of *amoA* gene positively correlated with biochar ash content and soil pH, but negatively correlated with biochar H/C ratio. *NosZ* correlated well with soil CEC. The abundance of *nirS* gene did not correlate with any of the biochar and soil properties.

Discussion

Among the measured biochar and soil parameters, gross mineralization rates positively correlated with H/C ratio, itself inversely proportional to biochar's aromaticity and recalcitrance (Hammes et al. 2008; Krull et al. 2009). We suggest that less recalcitrant chars presenting high H/C ratios (i.e., W410, W510, and SWC) increase mineralization rates, as they are more likely to be decomposed and thereby free up

Table 5 Pearson correlation coefficient for the relationships among bacterial gene abundance, biochar, and biochar-amended soil properties

	Biochar pH	Biochar ash	Biochar H/C	Soil pH	Soil CEC	16S rDNA	*amoA*	*nirK*	*nirS*	*nosZ*	Cum. N_2O	G. mineraliz.	Net nitrif.
Biochar pH	–	–	–	–	–	–	–	–	–	–	–	–	–
Biochar ash	0.99**	–	–	–	–	–	–	–	–	–	–	–	–
B. H/C	NS	NS	–	–	–	–	–	–	–	–	–	–	–
Soil pH	0.93*	0.93*	−0.64**	–	–	–	–	–	–	–	–	–	–
Soil CEC	NS	NS	NS	0.92*	–	–	–	–	–	–	–	–	–
16S rDNA	0.98**	0.99**	NS	0.92*	NS	–	–	–	–	–	–	–	–
amoA	ns	0.92*	−0.70**	0.93*	NS	0.93*	–	–	–	–	–	–	–
nirK	0.97**	0.98**	NS	0.97**	0.89*	0.95*	0.91*	–	–	–	–	–	–
nirS	NS	NS	NS	NS	0.91*	NS	0.91*	NS	–	–	–	–	–
nosZ	NS	NS	NS	NS	NS	NS	0.91*	NS	NS	–	–	–	–
Cum. N_2O	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	–	–	–
G. mineraliz	NS	NS	0.76**	0.84**	NS	NS	NS	NS	NS	NS	NS	–	–
Net nitrif.	NS	NS	−0.82**	NS	NS	NS	NS	NS	NS	NS	NS	NS	–

*$p<0.05$; **$p<0.01$

NS non-significant, *B. H/C* biochar H/C ratio, *Cum. N_2O* cumulative N_2O emissions, *G. mineralization* gross mineralization, *Net nitrif.* net nitrification

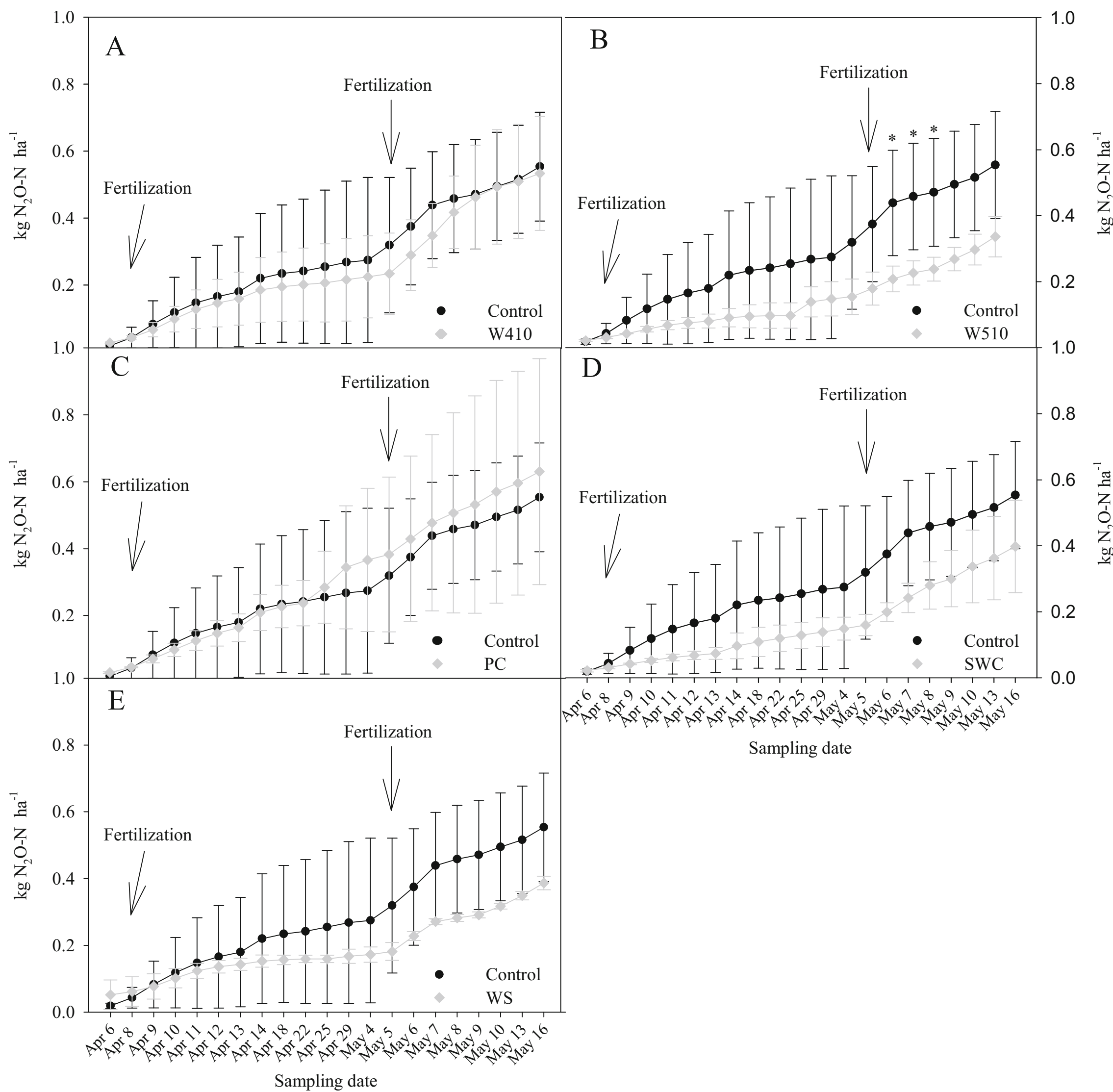

Fig. 1 Cumulative N_2O fluxes over 42 days in biochar-amended soil under lettuce cultivation. Mean values with *asterisks* are significantly different from control (Dunnett's test, $p<0.05$). *Error bars* indicate standard deviation of the mean ($n=3$)

Table 6 Abundance of total bacterial (16S rDNA), nitrifying (*amoA*), and denitrifying (*nirK*, *nirS*, and *nosZ*) genes in soil amended with different biochar materials expressed as number of gene copies per gram of soil

	16S rDNA (10^6 copies g^{-1} soil)	*amoA*	*nirK*	*nirS*	*nosZ*
Control	862±73	21±2.7	3.4±0.1	11±1.8	6±0.4
W410	621±2.4	13±0.8*	2.7±0.6	10±1.5	6±0.6
W510	669±87	17±1.1	2.6±0.6	8±0.9	5±0.9
PC	759±70	17±2.8	2.9±0.3	9±1.0	5±0.2
SWC	631±256	7±0.7*	2.5±0.5	7±1.3	3±1.0*
WS	954±158	31±0.7*	4.9±0.1*	11±2.3	8±1.9

Values are means±standard errors ($n=3$)

*Significantly different from control (Dunnett's test, $p<0.05$)

N trapped in the char into the mineral pool (Mukherjee and Zimmerman 2013). Whether the amount of N mineralized in this method represents the majority of mineralization, or represents a priming function for the bulk soil as reported in other studies (Wardle et al. 2008, Zimmerman et al. 2011) may require a full accounting of the N pools.

Net nitrification increased twofold in soils amended with W510, PC, and WS biochars. A higher net nitrification rate indicates higher N availability for plant uptake or for losses via denitrification or leaching (Piccolo et al. 1994; Singla et al. 2014). Song et al. (2014) performed a thorough study of the nitrification process and revealed that potential ammonia oxidation was sustained for 2 weeks longer in biochar-amended soils than in control soils (alkaline coastal soil). They associate this effect to an increased abundance of nitrifying bacteria and archaea in biochar-amended soils. In our study, out of the

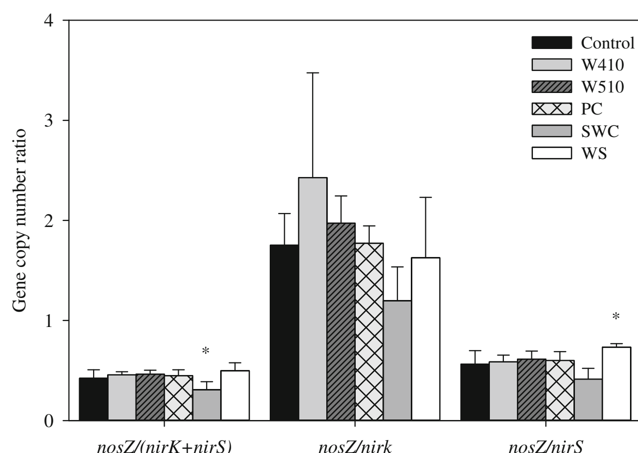

Fig. 2 Ratios of *nosZ*/(*nirK*+*nirS*), *nosZ*/*nirK*, and *nosZ*/*nirS* in biochar-amended soil under lettuce cultivation. Mean values with *asterisks* are significantly different from control within ratio group (Dunnett's test, $p<0.05$). *Error bars* indicate standard deviation of the mean ($n=3$)

biochar materials that increased net nitrification, only WS significantly increased bacterial *amoA* abundance. Although bacterial *amoA* is more abundant than archaeal *amoA* in soils, Song et al. (2014) suggested that there is a stronger correlation to nitrification with the latter, and this may be good cause for investigation of archaea nitrification with respect to biochar.

Increases in gross mineralization and net nitrification rates were not translated into increments in plant biomass. Sun et al. (2014) suggests that biochar effects on plant production may be more pronounced in deprived agroecosystems than in non-acidic and fertile soils. Additionally, Sun et al. (2014) suggests that biochar effects may be compromised by sufficient fertilization, which was the case for these mesocosms that received 190 kg ha^{-1} to reproduce the rates used by lettuce producers in the state of California (Rosenstock et al. 2013).

By altering chemical, physical, and hydrological properties of soils, several studies suggest that biochar can decrease N_2O fluxes (Yanai et al. 2007; Spokas et al. 2009; van Zwieten et al. 2010; Wang et al. 2011; Zheng et al. 2012), but the effects are site and biochar type specific (Spokas and Reicosky 2009). In this study, none of the biochar materials affected the total cumulative emissions of N_2O (Table 4). Significant effects of biochar on N_2O fluxes were only detected for a short period of the growing season and in soils amended with W510 biochar. With the techniques used in this study, it was not possible to determine the reason for this temporal decrease in N_2O emissions as W510 did not seem to limit N availability or restrain the presence of denitrifying microorganisms. Transient effects of biochar on N_2O emissions observed here are in accordance with previous studies carried out in fertilized and cultivated trails for mid to long term. Castaldi et al. (2011) monitored N_2O emissions during the growing period of wheat (approximately 60 days) at the field scale. In their study, although emissions in control plots were higher compared with biochar-treated plots, only in two occasions was the difference statistically significant. Similarly, Scheer et al. (2011) found that the effect of biochar on decreasing N_2O emissions is highly episodic with no effects on net N_2O flux over the entire sampling period (55 days). They monitored N_2O fluxes throughout the growing season of ryegrass in an Australian ferrosol and observed that only when fluxes were generally low (<50 μg N_2O-N m^{-2} h^{-1}) and during specific short periods, N_2O emissions were significantly lower in the biochar-amended plots. The occasional decreases in N_2O emissions observed in this study highlight the importance of long-term observations to comprehensively capture the effect of biochar on N_2O emissions.

Using the ^{15}N gas flux methods, Cayuela et al. (2013) observed that N_2O/N_2 ratio decreased in 15 agricultural soils after biochar amendment, pointing to denitrification as the main pathway for decreases in N_2O emissions in biochar-amended soils, by enhancing the reduction of N_2O to N_2. Given the form of fertilizer applied (organic) and the moderately low soil moisture of 20 % (*w*/*w*), it is possible that denitrification was not the predominant source of N_2O in our study; instead, nitrification may have played an important role in producing N_2O. Thus, decreases in N_2O fluxes from soils amended with W510 could be related to a decline in N_2O production from nitrification. Future studies should consider investigating the contribution of nitrification and denitrification to N_2O emission in biochar-amended soils to predict N_2O mitigation opportunities of biochar.

We assessed the abundance of nitrifying bacterial gene *amoA* and denitrifying *nirK*, *nirS*, and *nosZ* genes to verify whether differences in nitrification rates and N_2O emissions could be related to changes in the size of microbial community possessing the different N cycling genes. Neither nitrification nor N2O emissions correlated with gene abundance. Similarly, Kong et al. (2010) found that although soil management influenced nitrifying and denitrifying community abundance, changes in community sizes did not relate to changes in mineral N concentrations, mineralization, and nitrification rates. Gene abundances showed relationships with biochar properties or biochar-induced soil properties. Biochar ash content correlated well with *amoA* and *nirK* abundances. A higher abundance of *nirK* and *nosZ* was measured in the WS treatment and correlated well with increases in soil CEC. WS biochar, which significantly increased soil pH, *amoA*, and *nosZ*, shifted the *nosZ*/*nirS* ratio in favor of N_2O reducers without decreases in N_2O emissions. Therefore, our data suggest that biochar can alter abundances of N cycling genes; however, in order to link changes in process rates and functional microorganisms, an assessment of gene expression would be necessary.

Conclusions

We investigated the effects of five biochar materials on N transformations from mesocosm soils representing an organic lettuce production system. Our results indicate that biochar effects are specific to individual soil-biochar combinations. Biochars with high H/C ratios showed potential to increase plant N availability through increases in gross mineralization. This would be beneficial for organic farming systems challenged by insufficient N mineralization during plant growth. Our study also found minimal effects of biochar on N_2O emissions from soils. Significant decreases were only observed occasionally during the growing period and for only one biochar material used in this study. Our results confirm that changes in soil properties, microbial community composition, and N cycling are biochar type specific and biochar effect on N_2O mitigation is occasional and may not necessarily persist in the long term. It must be noted that these findings were obtained under specific mesocosm conditions representing an organic lettuce production system, and although they are valid for the studied scenario, they should be extrapolated with care to other agroecosystems.

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