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Contrasting effects of biochar on N_2O emission and N uptake at different N fertilizer levels on a temperate sandy loam

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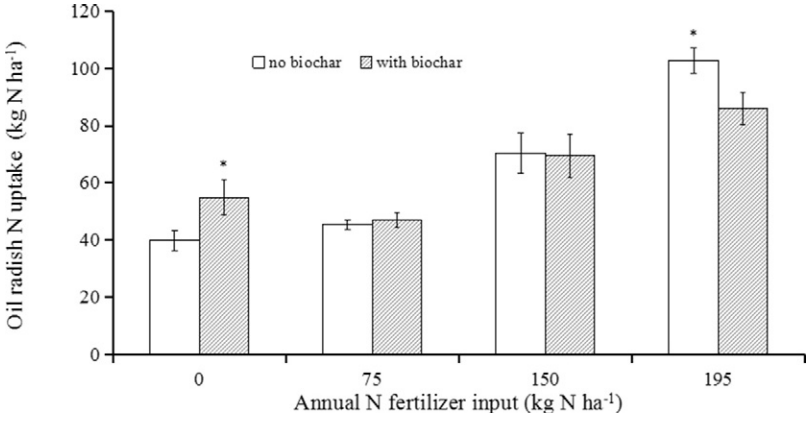
HIGHLIGHTS

- Biochar showed no effect on N_2O emissions and N uptake at the recommended N fertilizer rate.
- Without N fertilizer application, biochar induced higher N uptake of the catch crop.
- With increased N fertilizer application biochar decreased N_2O emissions.

GRAPHICAL ABSTRACT



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ABSTRACT

Biochar has been frequently suggested as an amendment to improve soil quality and mitigate climate change. To investigate the optimal management of nitrogen (N) fertilization, we examined the combined effect of biochar and N fertilizer on plant N uptake and N_2O emissions in a cereal rotation system in a randomized two-factorial field experiment on a sandy loam soil in Brandenburg, Germany. The biochar treatment received 10 Mg ha^{-1} wood-derived biochar in September 2012. Four levels of N fertilizer, corresponding to 0, 50%, 100%, 130% of the recommended fertilizer level, were applied in winter wheat (*Triticum aestivum* L.)) and winter rye (*Secale cereal* L.) in 2013 and 2014 followed by the catch crop oil radish (*Raphanus sativus* L. var. *oleiformis*). Biomass and N uptake of winter wheat and winter rye were significantly affected by the level of N fertilizer but not by biochar. For N uptake of oil radish an interaction effect was observed for biochar and N fertilizer. Without applied fertilizer, 39% higher N uptake was found in the presence of biochar, accompanied by higher soil NH_4^+ content and elevated cumulative CO_2 emissions. At 130% of the recommended fertilizer level, 16% lower N uptake and lower cumulative N_2O emissions were found in the biochar-mediated treatment. No significant change in abundance of microbial groups and nosZ gene were observed. Our results highlight that biochar can have a greenhouse gas mitigation effect at high levels of N supply and may stimulate nutrient uptake when no N is supplied.

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1. Introduction

While an increasing amount of N fertilizer is being synthesized to produce food for the growing world population (Erisman et al., 2011), the increased supply of N to agricultural soils is intensely challenging the agroecosystem in many regions and intensifying global climate change. Nitrous oxide (N_2O) emissions from agricultural production due to mineral or organic N fertilizer applications and the transformations of N in agricultural soils are one of the major contributors to global warming (Montzka et al., 2011). Furthermore, various transformations of N in the soil cause gas emissions, contamination of ground and surface water bodies with negative environmental effects (Galloway et al., 2003; Hashemi et al., 2016).

The integration of biochar in agricultural systems has been identified as an effective way to mitigate negative impacts of agricultural production by sequestering carbon in soils and conditioning reactive N in agricultural systems (Woolf et al., 2010; Clough et al., 2013; Benckiser et al., 2015). Lehmann and Joseph (2015) define biochar as a solid material produced by the thermochemical conversion of organic residuals in an environment that has limited or no oxygen. Biochar is a recalcitrant compound dominantly composed of aromatic carbon, and this inert feature of biochar was as a primary concern associated with incorporating biochar into soil to enhance soil carbon sequestration in the global warming context (Lehmann 2007). In addition, the direct and indirect effects of biochar on the N dynamics in soil and plants have been reported in numerous studies, but the interactions of biochar with N dynamics in plants and soil are far from being understood (Clough et al., 2013). The promising ability of biochar to suppress N_2O emissions from agricultural systems has received special attention because of its possible impact on climate change mitigation (Taghizadeh-Toosi et al., 2011; Cayuela et al., 2014). Several mechanisms or processes, such as elevation of the soil pH, enhanced soil air exchange due to changed soil structure, electro-chemical properties of the chars have been suggested, which may result in reduced N_2O emissions in presence of biochar (Ameloot et al., 2016). Soil microbial processes play a crucial role in the complex N transformations in presence of biochar, which still require substantial research before the biochar N interactions in soils are fully understood (Clough and Condron, 2010). Recently, special attention has been given to the electron donating capacity of biochars, which may be important for the promotion of the reduction of N_2O to N_2 in agricultural soils in presence of biochar (Cayuela et al., 2014; Prévoteau et al., 2016). Harter et al. (2013) found a mechanistic link between the abundance of functional marker genes of denitrifying bacteria (nosZ) and the emissions of N_2O from soil microcosms in the presence of biochar, which may help to understand the microbiological processes in the soil.

Besides microbiological processes, physical and chemical processes are also affected by biochar, which may impact the N dynamics in soils. Following biochar addition to soil, increased NH_4^+ sorption (Dempster et al., 2012; Jones et al., 2012; Yao et al., 2012) and reduced NH_4^+ and NO_3^- leaching (Dempster et al., 2012) have been numerously found. The enhancing NH_4^+ sorption were ascribed to the high cation exchange capacity (CEC) of biochar (Liang et al., 2006; Clough et al., 2013) and the elevated soil pH induced by alkaline biochar (Chan et al., 2007). In addition, increased water retention following biochar addition could also physically contribute to a reduction in NH_4^+ and NO_3^- leaching. On the other hand, increased immobilization of N associated with increasing microbial biomass was attributed to the higher C/N ratio of biochar compared with soil (Steiner et al., 2008) or to the labile carbon content of biochar (Ippolito et al., 2012). Thus, biochar might slow soil inorganic N release. Additionally, following biochar addition to soil, an increase in plant N uptake has been reported (Abbasi and Anwar, 2015). Together, these findings indicate that biochar may strongly interact with N transformation processes and then influence plant N uptake.

In agricultural production, the given N fertilizer level could be a crucial prerequisite to investigate the interactions between biochar and N dynamics. Dempster et al. (2012) found that the ability of biochar to adsorb NH_4^+ and NO_3^- varied depending on the initial concentration of NH_4^+ and NO_3^-, e.g., biochar can absorb more NO_3^- at a high NO_3^- concentration solution relative to the adsorption at a low NO_3^- concentration solution. However, there is limited knowledge on the combined effects of biochar and different N fertilizer levels on soil N and plant N uptake. This type of knowledge could help to devise a better strategy for fertilizer management in combination with biochar utilization in agricultural practices. Additionally, based on a review paper on the effects of biochar on soil N dynamics (Clough et al., 2013), long-term studies are highly recommended to obtain a holistic understanding of this interaction because some biochar effects are transient, such as the priming effect of soil organic matter (Kuzyakov et al., 2000) resulting from the labile carbon in biochar (Luo et al., 2012). Furthermore, the effects of biochar may also be plant species-specific.

Field trials are more realistic compared with pot or greenhouse experiments using artificial soil under controlled conditions. Results from a three-year field trial study conducted by Jones et al. (2012) showed an effect of biochar on N uptake of cultivated plants, however, no effect of biochar on dissolved organic N, NH_4^+ or NO_3^- pools size was observed, differing from the reduced N leaching or enhanced N adsorption obtained in a previous short-term laboratory incubation study (Clough et al., 2013). One important reason might be the absence of plants in some pot experiments, which could confound the understanding of biochar effects on soil N dynamics due to the missing role of plant N uptake. The study conducted by Xu et al. (2014) showed that the effects of biochar on the nitrification process differed considerably in the presence or absence of plants. Thus, a realistic and practical field study can provide a better understanding and an accurate evaluation of the interaction mechanisms between biochar and N dynamics with different N fertilizer levels.

The aim of our study was to investigate the combined effects of biochar and different N fertilizer levels on plant N uptake, N_2O emissions, and the potential mechanism underlying these effects. This study used data from a field experiment established in 2012 on an arable sandy loam soil in Germany. Biochar derived from wood chips was applied to the top soil at a rate of 10 Mg ha^{-1} (dry weight basis). Thereafter, following practical agricultural field management, N fertilizer was applied annually at four different levels. We hypothesized that the addition of biochar can facilitate plant N uptake and mitigate N_2O emissions, depending on the level of N fertilizer – from zero N fertilizer to excessive N fertilizer supply (130% of the recommended rate).

2. Materials and methods

2.1. Experimental field, biochar, and management

The experimental field site was located in Berge, Germany (52°63′N, 12°80′E) and operated by the research station of the Institute of Agricultural and Urban Ecological Projects, Berlin (IASP). Before this experiment, the field was cultivated by a farmer with a typical cereal-dominated crop rotation including wheat, rye, and maize. In 2012, the field was cultivated with oat (*Avena sativa* L.). The field had received regular mineral fertilizer and digestate from a biogas plant regularly as organic fertilizer, the last time solid digestate was applied in 2010. The soil is a haplic Cambisol with a texture of 71% sand, 22% silt, and 7% clay. Prior to the initiation of the experiment in August 2012, the soil had a pH of 6.0 (in $CaCl_2$), and the upper 20 cm soil contained 0.73% C, 0.07% N, 0.005% P (double lactate soluble P) and 0.011% K, respectively. The mean annual temperature at this field site is 8.7 °C and the mean annual precipitation is 503 mm.

The field experiment was initiated in September 2012 using a randomized block design including two factors, i.e., biochar and N fertilizer level. In total, eight treatments combinations were considered, i.e., four N fertilizer levels either with or without biochar ($n = 4$) (Table 1). The biochar was produced by Pyreg (Dörth, Germany) made from a mixture

Table 1
Experimental design with two factors, i.e., biochar and N fertilizer level (N0,N75,N150 and N195 mean 0, 75, 150 and 195 kg N ha^{-1} applied per year, respectively).

	Factors	
	Biochar (Mg ha^{-1})	Fertilizer (kg N ha^{-1} per year)
N0	0	0
N75	0	75
N150	0	150
N195	0	195
N0 + biochar	10	0
N75 + biochar	10	75
N150 + biochar	10	150
N195 + biochar	10	195

Table 2
The main crop and soil management events conducted throughout the experimental period.

Date	Management
12-Sep.-2012	Biochar application, tillage with cultivator and plow
01-Oct.-2012	Seeding of winter wheat (*Triticum aestivum* L.)
18-Nov.-2012	Second seeding of winter wheat
11-Apr.-2013	First N fertilizer application (0, 37.75, 75, 97.5 kg N ha^{-1})
06-May-2013	Second N fertilizer application (0, 37.75, 75, 97.5 kg N ha^{-1})
06-Jun.-2013	Fungicide application
15-Aug.-2013	Harvest of winter wheat (straw residues and grains removed from the field)
27-Aug.-2013	Tillage with disk harrow
17-Sep.-2013	Harrowing
24- Sep.-2013	Tillage
25-Sep.-2013	Seeding of winter rye (*Secale cereale* L.)
25-Oct.-2013	Herbicide application
06-Mar.-2014	First N fertilizer application (0, 37.75, 75, 97.5 kg N ha^{-1})
14-Mar.-2014	Fungicide application
14-Apr.-2014	Second N fertilizer application (0, 37.75, 75, 97.5 kg N ha^{-1})
07-Aug.-2014	Harvest of winter rye (straw residues and grains removed from the field)
12-Aug.-2014	Tillage with disk harrow
03-Sep.-2014	Sowing of oil radish (*Raphanus sativus* L.var. *oleiformis*)
28-Nov.-2014	Oil radish harvest and returned to the field
21-Apr.-2015	Plowing

of deciduous and coniferous wood chips by means of a screw pyrolyzer. The inlet gas temperature of the reactor's heating jacket was 850 ± 20 °C and lasted for 30 min. The hot biochar was then taken out and cooled down by adding water to about 40% dry matter. The biochar had 78% C (no carbonate contained), 0.72% total N (1.5 mg kg^{-1} mineral N), pH of 9.35, H/C 0.22, O/C 0.03, 0.58% K and 0.23% P, and 16.6% ash. As a mineral fertilizer, calcium ammonium nitrate (CAN) containing 27% N was used in this study. The four N fertilizer levels were 0, 75, 150 and 195 kg N ha^{-1}, which corresponded to 0, 50%, 100% and 130% of the recommended fertilizer level, respectively. The 100% recommended fertilizer level was assigned to the usual farmer practice based on the local field situation. Considering the limited yield potential of the field, crops received the same amount of N in each year during the three years of the experiment. Each plot measured 4.5 m × 10 m and contained two areas: one for soil sampling (1.5 m × 9 m) and another undisturbed one for evaluating crop yields and for determining N uptake (1.5 m × 9 m).

In September 2012, biochar (10 Mg ha^{-1}, dry weight basis) was incorporated into the top 30 cm of the soil by tillage with cultivator and plow to distribute the biochar homogeneously. Meanwhile, the treatment without biochar amendment was also plowed. At the beginning of October 2012, winter wheat (*Triticum aestivum* L.) was sown for use as fodder, and harvested in August 2013, followed by winter rye (*Secale cereale* L.) sown in September 2013 and harvested in August 2014. After harvest of rye, oil radish (*Raphanus sativus* L. var. *oleiformis*) was seeded as a catch crop in September 2014 for preventing nutrient leaching and improving soil structure over winter. The oil radish was mulched in April 2015 before seeding of the following maize. Fertilizer was applied in split applications (50%:50%) in 2013 and 2014 to the growing crops of winter wheat and winter rye; oil radish did not receive any mineral fertilizer. Further details on plant and soil management are summarized in Table 2.

2.2. Soil texture, pH, and soil N

Before biochar application, five points in each plot (crosswise sampling) were chosen from where soil samples were collected from 0 to 30 cm soil depth and pooled as a composite sample. The soil samples were air dried, crushed and sieved through a 2-mm mesh to determine soil texture, soil total N content (N_t), and soil pH. Soil texture was analyzed using the pipette method following DIN ISO 11277 (2002). Soil total N was measured by dry combustion using a Vario Max CNS elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Soil pH was determined using 0.01 M $CaCl_2$ (the ratio of soil to $CaCl_2$ is 1:2.5, m/v). All analyses were conducted in triplicate.

To investigate the effect of biochar and N fertilizer on the soil N pool, soil samples were collected from a 0–30 cm soil depth in 2013 after harvesting the winter wheat, and in 2014 after cultivation of the oil radish. The sampling procedure was the same as for soil texture detection mentioned previously. Additionally, soil mineral N (NH_4^+ and NO_3^-) was determined after the oil radish cultivation. To determine soil mineral N, 10 g of fresh soil was weighed into a 150-mL flask containing 50 mL of 0.0125 M $CaCl_2$, and was subsequently shaken for 60 min at 30 rpm. Then, the suspension was centrifuged at 2000 rpm for 2 min, and the supernatant was extracted and stored at 4 °C for further measurement based on flow-injection analysis (FIA System, MLE; Germany).

2.3. Gas emissions and water content

Soil carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions were measured weekly from 21 August 2014 (a few days prior to oil radish seeding) until 21 April 2015 (oil radish plowing) using closed round chambers (51 cm lower diameter, 39 cm upper diameter and 40 cm height). First, a plastic ring (base of the chambers) was installed in the upper 5 cm of the soil of each investigated plot. While measuring gas emissions, the chamber was placed on the ring and was hermetically sealed by filling the base ring with water. Gas samples were collected at 0, 20, 40 and 60 min after chamber closure using pre-evacuated glass vials. All vials were placed on a pressure-controlled autosampler and analyzed using a gas chromatograph (Shimadzu GC 14G injection system) equipped with an electron-capture detector to determine CO_2 and N_2O (Loftfield et al., 1997). Prior to gas sample analysis, standard gases were used to calibrate the system. The N_2O detection limit was approximately 5 ppb. The cumulative N_2O emissions were calculated by linearly interpolating the N_2O emitted over the measurement days (Suddick and Six, 2013); the same method was used to calculate the cumulative CO_2 emissions.

While collecting the gas sample, the in situ water content was recorded by time-domain reflectometry (TDR) using 10-cm long probes coupled to a cable tester (S/N: HSIAB332, Field Operated Meter, Poland). In addition, the rainfall data were recorded during the growth of oil radish (Fig. 1).

2.4. NH_4^+ and NO_3^- adsorption on biochar

The NH_4^+ and NO_3^- adsorption capacity of the biochar used in this study was determined by using 100 mg of biochar and 0.025 g mL^{-1} of NH_4NO_3 (pH adjusted to 6.0 to correspond to the pH of soil), equivalent to a total of 10 Mg ha^{-1} of biochar and 380 kg N ha^{-1} of N fertilizer applied at the field site within two years. Considering that the shaking instrument could break down the biochar particles, we developed an approach in which the solution could flow by gravity as follows: 100 mg of biochar was homogeneously mixed with 0.5 mL of N reagent

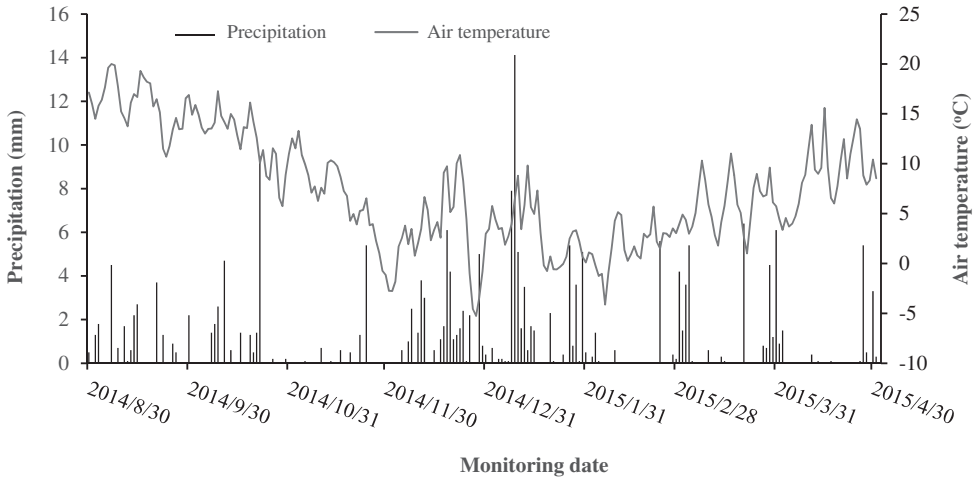

Fig. 1. Air temperature and precipitation during oil radish cultivation.

(NH_4NO_3) and 5 g of sand. Then, the sand-biochar mixture was transferred into a 10-mL syringe containing 5.5 g of wet sand (5 g of sand and 0.5 g of deionized water). The wet sand at the bottom of the syringe was used to prevent the biochar from leaching out. Thereafter, 2.2 g of wet sand (2 g of sand and 0.2 g of deionized water) was placed on the top of the biochar-sand mixture. A total of 15 mL of pH 6.0 water was used (the pH was adjusted using HNO_3; the amount of NO_3^- was negligible); each time, 5 mL was added to the top of the syringe and a 0.45-μg filter was placed at the bottom of the syringe to collect the leachate. The leachate was collected using a plastic bottle and was frozen at −20 °C until the NH_4^+ and NO_3^- concentrations were determined. Meanwhile, the control was prepared where only sand was used to replace the sand-biochar mixture. Additionally, the blank, which only contained the N reagent, was prepared. Four replicates were used for each treatment.

2.5. DNA extraction, nosZ abundance, and microbial community composition

We observed that the N_2O emissions differed between N195 and N195 + biochar and between N0 and N0 + biochar within the oil radish growing period. Therefore, soil samples from these treatments were collected in November 2014 to determine the composition of the microbial community and the abundance of the functional gene (nosZ) responsible for effecting the N_2O transformation.

DNA was extracted from soil sampled after the oil radish cultivation. Briefly, genomic DNA was extracted from 0.5 g of fresh soil using the Macherey-Nagel NucleoSpin® Soil Kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany), following the manufacturer's instructions. The resulting DNA samples were used to determine the total DNA amount and the nucleic acid purity using a NanoDrop 1000 μ-volume spectrophotometer (NanoDrop Products, Wilmington, DE, USA). The mean ratio of 260 nm/280 nm throughout all samples was 1.87 (± 0.6), suggesting that the DNA used in this study was pure, and proteins, phenols or other contaminants that absorb at approximately 280 nm were absent. For the quantitative PCR of the nosZ gene, the methodology described by Henry et al. (2006) was followed using the primers nosZ 2F (5′-CGGRACGGCAASAAGGTSMSSGT-3′) and nosZ 2R (5′-CAKRTGCAKSGCRTGGAGAA 3′).

To assess major groups of the microbial community, real-time quantitative PCR (qPCR) assays were performed using the QuantStudio™ 12 K Flex Real-Time PCR System containing 96-well polypropylene plates (Life Technologies, Grand Island, NY, USA). The reaction was performed by mixing the following reagents: 4 μL of 5× HOT FIREPol® EvaGreen® HRM Mix ROX (Solis Biodyne, Tartu, Estonia), 0.25 μL of the appropriate forward and backward primer (biomers.net, Ulm, Germany) diluted to 10 pM, 14.5 μL of Millipore H_2O and 1 μl of template DNA. The PCR was carried out for 15 min at 95 °C after activation of polymerase, followed by 40 cycles at 95 °C for 15 s, then 20 s at different annealing temperatures depending on the primer, and finally 30 s at 72 °C. The primers used for different target groups were documented in supplementary material (Table A.1). The DNA standards containing the target region for each primer set were based on the DNA extracted from the appropriate standard organisms: *Verticillium dahliae* EP806 (this laboratory) for fungi; *Acidobacterium capsulatum* (DSM11244) for Acidobacteria; *Streptomyces avermitis* (DSM46492) for Actinobacteria; *Agrobacterium tumefaciens* pGV2260 (this laboratory) for Alphaproteobacteria; *Burkholderia phymatum* (DSM17167) for Betaproteobacteria; *Escherichia coli DH5α* (this laboratory) for Gammaproteobacteria, and *Bacillus licheniformis* (this laboratory) for Firmicutes.

2.6. Soil microbial biomass carbon, basal soil respiration, and metabolic quotient

For better understanding the role of the soil microflora on the differences in N uptake in the N0 and N0 + biochar, and in the N195 and N195 + biochar treatments, we collected soil samples (0–30 cm soil depth) in June 2015. Briefly, soil samples were first equilibrated for two days at 20 °C following the protocol used by Anderson and Domsch (1978). Then, triplicate samples were mixed with glucose (3 mg g^{-1} soil) and incubated for up to 24 h at 20 °C. An automated infra-red analysis system (Heinemeyer et al., 1989) with a constant gas flow was used to measure CO_2 production of up to 24 samples. Soil microbial biomass carbon (C_{mic}) was determined at the initial maximum respiration response as C_{mic} (μg g^{-1} soil) = (μl CO_2 g^{-1} soil h^{-1}) × 40.04 + 0.37 (Anderson and Domsch, 1978). Soil basal CO_2 respiration was measured continuously in the absence of glucose for up to 24 h at 20 °C. The metabolic quotient (qCO_2) was calculated as the ratio of basal CO_2 respiration and the substrate-induced respiration, and was expressed as ng CO_2-C μg^{-1} C_{mic} h^{-1}.

2.7. Crop yield and plant N uptake

Both grains and straw were removed from the plots during harvest of winter wheat and winter rye. The above-ground oil radish products were harvested and mostly returned to the field after biomass evaluation, only a very small part was kept for determining N content. After harvest, fresh and dry matter of straw and grains of winter wheat and winter rye as well as the above-ground biomass of oil radish were determined. For each plot, the yield of winter wheat and winter rye was evaluated by the sum of dry matter of the straw and grains from a chosen small plot. Similarly, the above-ground biomass of oil radish of each

plot was evaluated by dry matter of the above-ground products. For N uptake determination, an aliquot of the straw and grain mixture was dried at 60 °C and then milled to ≤1 mm to measure the N content by dry combustion using a Vario Max CNS elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany), the same procedure was applied for determining N content of oil radish.

2.8. Statistical analysis

Statistical analysis was performed using SAS software (SAS version 9.4; SAS Institute Inc., Cary, NC; US). Two-way analysis of variance (ANOVA) was performed using biochar and N fertilizer level as the two main factors. Multiple comparisons were performed using the *Proc Mixed* function with the *LSMeans Simulate* test, and significance was considered at the three levels of $P < 0.001$, $P < 0.05$ and $P < 0.10$.

3. Results

3.1. Biomass, plant N uptake, soil N and water content

N fertilizer significantly affected biomass and N uptake of winter wheat in 2013 and winter rye in 2014 ($P < 0.001$) (Table 3) with a trend of higher values at higher N fertilizer rate. However, biochar and its interaction with N fertilizer had no significant effect on these parameters.

After winter rye in 2014, oil radish was grown as a catch crop for retaining nutrients before cultivating the next main crop. N fertilizer significantly affected the biomass of oil radish ($P < 0.001$) (Table 3), with a trend providing larger biomass at higher N fertilizer rate. Biochar and its interaction with N fertilizer had no significant effect on biomass of oil radish. With respect to N uptake of oil radish (Fig. 2), N fertilizer showed a significant effect ($P < 0.001$) but biochar had no significant effect (Table 3). However, the interaction between biochar and N fertilizer had a significant effect on N uptake ($P < 0.05$) with distinct effects of biochar on N uptake at different N fertilizer levels (Fig. 2). In the treatments, without N fertilizer application, the biochar-mediated treatment (N0 + biochar) had 39% higher N uptake ($P < 0.05$) compared with the treatment without biochar (N0). By contrast, at 130% of the recommended fertilizer level, the N uptake of the biochar-mediated treatment (N195 + biochar) was 16% lower ($P < 0.05$) than the treatment without biochar (N195).

The total soil N content (N_t) measured after harvesting the winter wheat in 2013 was not significantly affected by the biochar, N fertilizer level or their interaction (Table A.2). However, N_t measured after the cultivation of oil radish in 2014 was significantly affected by N fertilizer ($P < 0.001$) (Table A.2). After oil radish cultivation, without applied

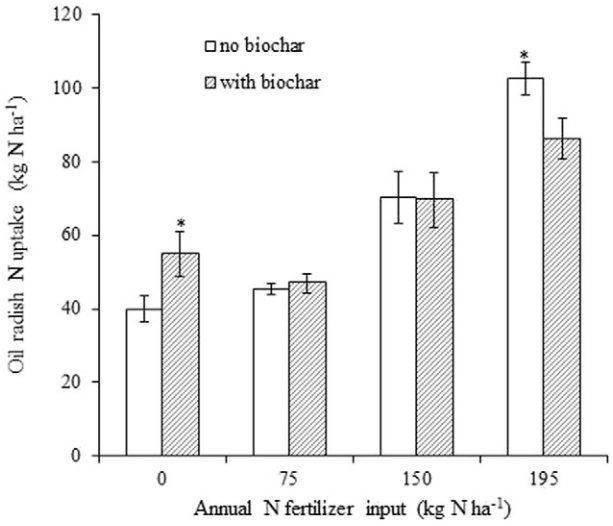

Fig. 2. N uptake by oil radish growing in soil with or without biochar under different N fertilizer levels. Data are shown as the means ± standard errors ($n = 4$). Asterisks indicate significant differences between the treatments with and without biochar for a given fertilizer input level (Simulate, $P < 0.05$).

fertilizer, NH_4^+ content was significantly higher in the biochar-mediated treatment compared with the treatment without biochar ($P < 0.10$) (Table 4). NO_3^- concentration was similar between the treatments with and without biochar (Table 4). At the applied fertilizer rates (50%, 100%, 130% of the recommended level), regardless of biochar or not, the content of NH_4^+ or NO_3^- in all the treatments was similar (Table 4) (data of 50% and 100% of the recommended fertilizer level is not shown).

The volumetric soil water contents of the four treatments (N0, N0 + biochar, N195, N195 + biochar) were similar throughout the monitoring period of growing oil radish (data not shown here).

3.2. NO_3^- and NH_4^+ adsorption

The biochar used (corresponding to the applied amount in the field) did not notably adsorb NO_3^- and NH_4^+. After adding the N reagent, the NO_3^- concentration in the leachate collected from the biochar-mediated treatment and the treatment without biochar was 0.77 ± 0.07 and 0.69 ± 0.03 mg/L ($n = 4$), respectively, similar to the added NO_3^- concentration (0.65 mg/L). The NH_4^+ concentrations in the leachate were 0.21 ± 0.02 and 0.19 ± 0.01 mg/L ($n = 4$) in the biochar-mediated

Table 3
Biomass and N uptake of winter wheat, winter rye and oil radish for the treatment factors biochar and fertilizer and their interaction (N0,N75,N150 and N195 mean 0, 75, 150 and 195 kg N ha^{-1} applied per year, respectively; biochar represents 10 Mg ha^{-1} biochar). Values of all parameters are shown as the means ± standard errors. The significance (*P* values) derived from the *F* test with multiple comparisons. Different letters indicate significant differences ($p < 0.05$).

	Plant biomass (Mg ha^{-1})				Plant N uptake (kg ha^{-1})		
Factor	Winter wheat		Winter rye	Oil radish	Winter wheat	Winter rye	Oil radish
Biochar							
Yes	11.4 ± 0.4		12.7 ± 0.4	2.2 ± 0.2	141.9 ± 8.0	141.2 ± 7.0	64.6 ± 6.7
No	10.9 ± 0.5		12.6 ± 0.5	2.2 ± 0.1	137.6 ± 9.9	142.4 ± 6.3	64.4 ± 4.6
Fertilizer							
N0	6.8 ± 0.5c		9.8 ± 0.4b	1.9 ± 0.1bc	73.3 ± 4.2c	95.5 ± 8.0c	47.4 ± 4.4c
N75	11.1 ± 0.6b		13.3 ± 0.3a	1.8 ± 0.2c	122.6 ± 10.2b	134.1 ± 5.3b	46.2 ± 1.5c
N150	13.0 ± 0.6a		13.9 ± 0.4a	2.5 ± 0.2ab	169.6 ± 9.8a	169.6 ± 7.4a	69.9 ± 4.8b
N195	13.9 ± 0.3a		13.6 ± 0.4a	2.6 ± 0.2a	193.6 ± 11.4a	167.9 ± 6.1a	94.4 ± 4.5a
Factor	*DF*[a]	*P value*					
Biochar	1	*0.202*	*0.537*	*0.975*	*0.758*	*0.821*	0.231
Fertilizer	3	***<0.001***	***<0.001***	*0.006*	***<0.001***	***<0.001***	***< 0.001***
Biochar Fertilizer*	3	*0.939*	*0.821*	*0.903*	*0.379*	*0.321*	**0.002**

a, degrees of freedom.

Table 4
The investigated soil parameters of four treatments (N0 and N195 indicate 0 and 130%, respectively, of the recommended fertilizer rate per year (150 kg N ha^{-1}); biochar refers to the biochar applied at 10 Mg ha^{-1}). Values of all parameters are shown as the means ± standard errors ($n = 4$). The italic bold values indicate significant difference derived from the *F* test with multiple comparisons; "ns" indicates no significant difference.

Treatment	NH_4^+ mg kg^{-1}	NO_3^-	Cum CO_2-C[a] g m^{-2}	Cum N_2O-N[b] mg m^{-2}	nosZ gene 10^7 copies g^{-1} soil	C_{mic}[c] μg g^{-1}	BR[d] μg CO_2-C g^{-1} soil h^{-1}	qCO_2[e] ng CO_2-C μg $^{-1}$ C_{mic} h^{-1}
N0	0.79 ± 0.20	2.08 ± 0.19	356.0 ± 35.5	34.7 ± 7.5	3.00 ± 0.25	51.0 ± 3.70	0.057 ± 0.009	0.97 ± 0.02
N0 + biochar	1.49 ± 0.19	1.67 ± 0.29	424.4 ± 21.1	54.2 ± 15.7	2.81 ± 0.22	49.6 ± 5.54	0.058 ± 0.002	1.21 ± 0.11
	0.050	*ns*	***0.013***	*ns*	*ns*	*ns*	*ns*	*ns*
N195	0.34 ± 0.20	3.78 ± 0.21	425.5 ± 55.0	113.7 ± 25.7	3.92 ± 0.20	53.6 ± 4.29	0.045 ± 0.004	0.85 ± 0.07
N195 + biochar	0.49 ± 0.26	3.58 ± 0.42	413.3 ± 23.3	73.85 ± 10.5	4.19 ± 0.11	41.9 ± 2.33	0.045 ± 0.003	1.09 ± 0.09
	ns	*ns*	*ns*	***0.074***	*ns*	*ns*	*ns*	*ns*

a, cumulative CO_2 emissions from oil radish seeding to plowing in the next year; b, cumulative N_2O emissions from oil radish seeding to plowing in the next year; c, soil microbial biomass carbon; d, basal respiration; e, metabolic quotient.

treatment and the treatment without biochar, respectively, similar to the added NH_4^+ concentration (0.20 mg/L).

3.3. Gas emissions and nosZ gene abundance

In the absence of N fertilizer, the emitted CO_2 did not differ significantly between the treatments with and without biochar at individual monitoring times (Fig. 3a). However, the cumulative CO_2 emissions from N0 + biochar were significantly higher than that from N0 ($P < 0.05$) (Table 4). On the other hand, at 130% of the recommended fertilizer level, the CO_2 emissions from the treatments with and without biochar were not significantly different (Fig. 3b), similar to the cumulative CO_2 emissions (Table 4).

For the emissions of N_2O (Fig. 3c, d), in the absence of fertilizer, at the individual monitoring times the N_2O emitted from the biochar-mediated treatment (N0 + biochar) was not significantly different from that emitted from the treatment without biochar (N0), and the cumulative N_2O emissions between the treatments with and without were also not statistically different. At 130% of the recommended fertilizer level, the N_2O emission measured in the biochar-mediated treatment (N195 + biochar) was not significantly different compared with the N195 treatment at the individual monitoring times. However, cumulative N_2O emissions of the N195 + biochar treatment were significantly lower than that of the N195 treatment ($P < 0.10$) (Table 4).

At the time of sampling the oil radish for biomass evaluation in November 2014, the mean nosZ gene in the N0 treatment was 2.7×10^7 copies/g soil; similar to that of the N0 + biochar treatment, i.e., 2.5×10^7 copies/g soil (Table 4). Also, the N195 and N195 + biochar treatments had similar nosZ gene abundance, i.e., 3.5×10^7 and 3.8×10^7 copies/g soil, respectively.

3.4. Microbial biomass, respiration and community composition

Without applied N fertilizer, similar microbial biomass, basal soil respiration, and qCO_2 values were measured in the treatments with and without biochar (Table 4). Also, for the abundance of main groups of microbial community, all the groups in the treatment N0 + biochar were not significantly different compared with those in the treatment

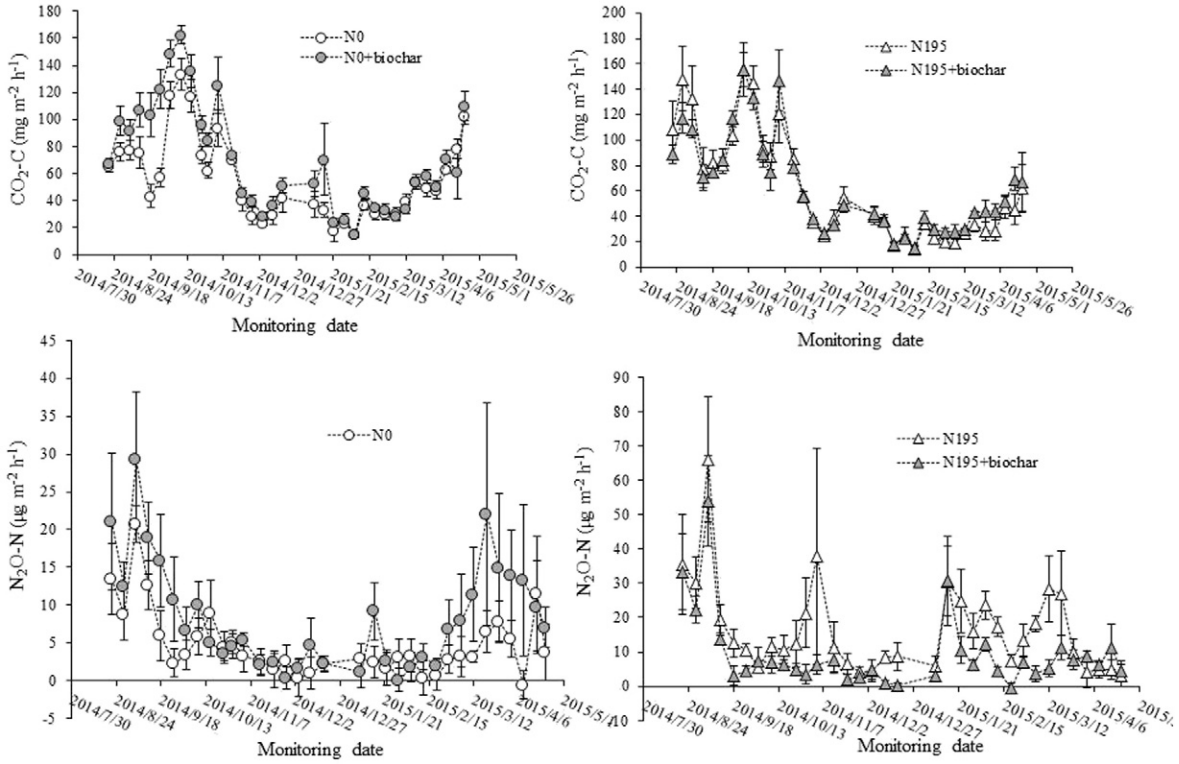

Fig. 3. Time series of CO_2 and N_2O from oil radish seeding until plowing in the next year. Red arrows indicate the sampling date for the nosZ gene measurement (N0 and N195 represent 0 and 130%, respectively, of the recommended fertilizer level per year (150 kg **N ha^{-1}**); biochar refers to the biochar applied at 10 **Mg ha^{-1}**). Data are shown as the means ± standard errors ($n = 4$).

N0 (Table A.3). Correspondingly, for the treatments applied with the highest fertilizer rate, similar basal soil respiration was measured in the N195 and N195 + biochar treatments, as well as microbial biomass, qCO_2, and abundance of main microbial groups (Table 4, Table A.3).

4. Discussion

4.1. Effect of biochar on N uptake

Biochar has been proposed as a soil amendment due to its beneficial function on N cycling processes, e.g., reduced N leaching or enhanced N mineralization (Clough et al., 2013), but studies which investigated how biochar interacts with N fertilizer in the presence of plants and how biochar affects plant N uptake on temperate arable soils are rather limited (Güereña et al., 2013; Karer et al., 2013), especially under conditions of agricultural field experiments. In our study, the addition of biochar did not alter N uptake of the cultivated crops at either 50% or 100% of the recommended fertilizer level. Similar findings were also identified by the study of Güereña et al. (2013) where N uptake of maize was not altered following the addition of biochar from 1 Mg ha^{-1} to 30 Mg ha^{-1} to a temperate American silt loam soil applied with 50%, 100% of the recommended fertilizer level. Additionally, the heterogeneous rainfall at field condition relative to the controlled moisture in the laboratory or other soil constrains would also impact N pool and then N uptake which needs further investigation. After the cultivation of winter wheat and winter rye, however, the N uptake of oil radish as a catch crop was notably changed, which was significantly increased in the biochar-mediated treatment without applied fertilizer and decreased in the biochar-mediated treatment applied with 130% of the recommended fertilizer level. To our knowledge, this is the first study identifying positive and negative effects of biochar on N uptake of a catch crop under increasing N fertilizer supply levels. This is in line with our initial hypothesis that the given N fertilizer rate is very important to be considered when using biochar as a soil amendment. The underlying potential mechanisms for varied N uptake are discussed in the following section 4.2 and 4.3.

4.2. Effect of biochar in the absence of N fertilizer supply

Without fertilizer application, higher N uptake of oil radish was observed in the treatment with biochar addition compared with the treatment without biochar, indicating a higher N availability in the presence of biochar. The direct input of mineral N from biochar was 15 g ha^{-1}, and it is an insignificant amount which somehow cannot contribute to the significant higher available N. Additionally, a significantly higher soil NH_4^+ concentration was observed in the biochar-mediated treatment at the time of sampling oil radish for N uptake determination, which was about twice of soil NH_4^+ concentration of the treatment without biochar. Possibly, in absence of N fertilizer biochar induced an enhanced mineralization of soil organic substance (Wang et al., 2016) (native organic matter or the enhanced mineralization of organic N (around 71 kg N ha^{-1}) from biochar itself), which is supported by elevated CO_2 emission, NH_4^+ concentrations and N in the oil radish biomass. In addition, following biochar addition to soil, an enhanced retention of soil inorganic N has been recorded due direct adsorption or indirectly by enhancing water retention which may also result in enhanced retention of inorganic N (Clough et al., 2013; Güereña et al., 2013), thus contributing to enhanced N availability for cultivated plants. Our results from the sorption experiment, however, could not prove higher retention of either NH_4^+ or NO_3^- with the wood-derived biochar used in our experiments. The study of Jones et al. (2012) noted that wood-based biochar at a rate of 50 Mg ha^{-1} could not absorb NO_3^-, and even absorbed less NH_4^+ than the sandy clay loam soil involved. However, this sorption result may not accurately reflect the field condition because of the potential biochar aging. On the other hand, the rainfall during the oil radish growth was rather low, which would not be enough to induce leaching. Furthermore, water contents among the treatments were similar throughout the period of oil radish growth, suggesting that the physically benefit on the retention of inorganic N via enhancing water retention could not be a reason.

Therefore, a plausible mechanism leading to the increased N uptake remains an elevated mineralization of organic matter or biochar, which is supported by a significantly higher cumulative CO_2 emissions in the biochar-mediated treatment compared with the treatment without biochar. This is in compliance with the meta-analysis of Wang et al. (2016) who reported that biochar addition to low fertility sandy soils induced promoted mineralization of organic carbon in soil. Following biochar addition, a positive priming effect of soil organic carbon has been reported in short term from a few days to less than one year (Luo et al., 2011; Zimmerman et al., 2011), while a negative priming effect of soil organic carbon was proposed after long term biochar addition (Zimmerman et al., 2011; Weng et al., 2015). For instance, Zimmerman et al. (2011) found that biochar produced at high temperature, similar to our biochar, resulted in high protection of organic matter and thus induced a negative priming of soil organic carbon after 250 days in a 500-day incubation experiment. From our study, more than two years after biochar application, the elevated cumulative CO_2 emissions contradict the proposed negative priming effect. The lack of N fertilizer input could be the reason for the enhanced mineralization of biochar or soil organic matter which needs further investigation, e.g., based on stable isotope techniques.

4.3. Effect of biochar with increased N fertilizer supply

In contrast to the treatments without N fertilizer supply, biochar showed no significant effects on measured soil N pool size and plant N uptake under 50% and 100% of the recommended fertilizer rates, also there were no changes in soil microbial diversity, microbial biomass, CO_2 and N_2O emissions. This indicates that soil N cycling runs independent of biochar under given mineral N input from fertilizer, or the effect of N fertilizer on soil N cycling overcame the effect of biochar if there were any. However, the treatments with increased N fertilizer supply (130% of the recommended fertilizer level) showed significantly lower N uptake by oil radish in the biochar-mediated treatment compared to the treatment without biochar. On the other hand, soil total N was identical between these two treatments, as well the investigated microbial parameters, i.e., microbial biomass, basal respiration and qCO_2, and abundance of main microbial groups. Thus, relative to the treatment without biochar, the lower N uptake together with the identical soil total N in the biochar-mediated treatment indicates that in the presence of biochar some part of the N must have been lost either as leachate or transformed and translocated as gas (N_2O, N_2). The increased loss of N in the form of leachate following the addition of biochar is not likely to be a reason due to the similar water content over the entire period of oil radish growth.

Nitrous oxide (N_2O) is an intermediate product of soil N transformation processes, which will either directly emit to the atmosphere or will finally be transformed into N_2 and then emit to the atmosphere. Throughout the entire monitoring period of growing oil radish, the notably lower cumulative N_2O emissions of the N195 + biochar treatment than the N195 treatment ($P < 0.10$) comply with the findings from previous studies (Suddick and Six, 2013; Cayuela et al., 2014) that biochar addition to soil can be an efficient way to reduce N_2O emissions from agricultural soils. However, at other fertilizer supply levels, a statistically proven reduction in N_2O emission was not seen following the addition of biochar. This was also true in the first year of the experiment during winter wheat cultivation (Dicke et al., 2015) where only the N_2O emissions at the 100% recommended fertilizer level were investigated. Regarding the lower flux rate of N_2O following biochar addition, the underlying mechanism for this process was reported due to microbial reduction of N_2O to N_2 via nosZ gene-containing microorganisms, as recently noted by Cayuela et al. (2014) and Harter et al. (2015). However,

in our study, the abundance of the nosZ gene in the biochar-mediated treatment was not significantly higher than that in the treatment without biochar. Furthermore, at the soil sampling time for nosZ gene analysis, the measured N_2O fluxes in these treatments with and without biochar were also similar. Due to the limited sampling times for nosZ gene analysis, we could not verify the nosZ gene abundance at the other N_2O monitoring times. In addition, the pathway for producing N_2O in this field site has been proposed mainly due to nitrification (Dicke et al., 2015). Further studies are needed to recognize such interrelations in more detail.

4.4. Implication of biochar-N interactions

From this study, biochar showed its potential to mitigate N_2O emissions from soils receiving an over-sufficient N fertilizer rate. Furthermore, this mitigation of N_2O emissions was evident under experimental field conditions which are rarely studied as compared to the numerous controlled incubation studies (Van Zwieten et al., 2015). In agricultural practices, we are faced with an N surplus due to the quest for higher food production which has created massive challenges by threatening soil quality, climate and human health. In Europe, the threats of N fertilizer overuse cost the European Union twice of the value of European farm income (Sutton et al., 2011). Also, this issue is rather serious in other regions such as China facing a daunting challenge to reduce emissions of reactive N (Liu et al., 2013). Hence, based on the observed lower N_2O emissions, the use of biochar in agriculture offers an effective way to mitigate the negative environmental problems caused by overuse of N fertilizers. On the other hand, in the case of no fertilizer, the beneficial function of the applied biochar on N uptake suggests an increased N availability, which could be valuable for some systems without mineral N fertilizer, or with reduced fertilizer application. In addition, biochar derived from different origins and processes vary with different N adsorption abilities (Clough et al., 2013), or with different stability (Wang et al., 2015). Thus, the type or amounts of biochar should be considered in further studies in order to better recognize combined effect of biochar and fertilizer management on soil N cycling.

5. Conclusion

Our study demonstrates that biochar can induce interaction effects with N fertilizer supply on a temperate sandy soil. In detail, the presence of biochar enhanced N uptake of the catch crop oil radish in a crop rotation without N fertilizer, showing a higher soil NH_4^+ concentration and higher cumulative soil CO_2 emissions, which indicates accelerated mineralization processes. However, this effect is not seen in the presence of N fertilizer. With over-sufficient N fertilizer application, after the addition of biochar the detected lower cumulative N_2O emissions suggest the potential of biochar to mitigate climate change caused by overuse of N in agriculture. Additionally, lower N uptake was also detected. The interrelation of lower cumulative N_2O emissions and lower N uptake following biochar addition needs further clarification.

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Appendix A. Supplementary data

Supplementary material.

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