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LETTER

N use efficiencies and N_2O emissions in two contrasting, biochar amended soils under winter wheat—cover crop—sorghum rotation

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Abstract

Biochar, a carbon-rich, porous pyrolysis product of organic residues, is evaluated as an option to tackle major problems of the global food system. Applied to soil, biochar can sequester carbon and have beneficial effects on nitrogen (N) cycling, thereby enhancing crop yields and reducing nitrous oxide (N_2O) emissions. There is little understanding of the underlying mechanisms, but many experiments indicated increased yields and manifold changes in N transformation, suggesting an increase in N use efficiency. Biochar's effects can be positive in extensively managed tropical agriculture, however less is known about its use in temperate soils with intensive fertilisation. We tested the effect of slow pyrolysis wood chip biochar on N use efficiency, crop yields and N_2O emissions in a lysimeter system with two soil types (sandy loamy Cambisol and silty loamy Luvisol) in a winter wheat—cover crop—sorghum rotation. ^{15}N-labelled ammonium nitrate fertiliser (170 kg N ha^{-1} in 3 doses, 10% ^{15}N) was applied to the first crop to monitor its fate in three ecosystem components (plants, soil, leachate). Green rye was sown as cover crop to keep the first year's fertiliser N for the second year's sorghum crop (fertilised with 110 kg N ha^{-1} in two doses and natural abundance ^{15}N). We observed no effects of biochar on N fertiliser use efficiency, yield or N uptake for any crop. Biochar reduced leaching by 43 $\pm$ 19% but only towards the end of the experiment with leaching losses being generally low. For both soils N_2O emissions were reduced by 15 $\pm$ 4% with biochar compared to the control treatments. Our results indicate that application of the chosen biochar induces environmental benefits in terms of N_2O emission and N leaching but does not substantially affect the overall N cycle and hence crop performance in the analyzed temperate crop rotation.

1. Introduction

Global food production increases the demand for agricultural land, water and fossil energy and leads to high greenhouse gas (GHG) emissions and resource depletion (IAASTD 2009). These challenges for agriculture are reinforced by climate change (IPCC 2014) and an increasingly animal-based diet at a global scale (Stehfest *et al* 2009, Westhoek *et al* 2014). Improving nitrogen (N) use efficiency is an important factor for reducing inputs and lessening harmful impacts of agriculture on the environment (Decock *et al* 2015, Zhang *et al* 2015). The application of biochar to agricultural soils is discussed as an option to tackle several of these challenges agriculture faces today (Lehmann 2007). Biochar is produced by thermochemical transformation of organic residues and can be mixed with compost or be applied directly to soil (Schmidt *et al* 2014). Further, sequestration of carbon dioxide (CO_2) from the atmosphere via biochar application might help to mitigate climate change (Woolf *et al* 2010). Reduced fertiliser demand through

biochar application can reduce fossil energy consumption (Woolf *et al* 2014), and increasing yields (Crane-Droesch *et al* 2013) may reduce GHG emissions induced by land-use change for gaining additional cropland (Fearnside 2000). Biochar application is likely to impact soil N dynamics (Clough *et al* 2013) with a potential to reduce nitrous oxide (N_2O) emissions from agricultural fields as recently demonstrated (Cayuela *et al* 2015, Hüppi *et al* 2015) but also contradicted (Angst *et al* 2014, Verhoeven and Six 2014). Hence, an improved knowledge of biochar's influence on soil N processes in an agricultural context is still needed.

A positive response of crop yields after biochar application to soil is a major potential biochar benefit. Jeffery *et al* (2011) found a significantly higher mean crop productivity (+10%) in biochar-amended versus control soils in a meta-analysis (>60 studies). The yield response was highly variable and specific to soil and biochar properties. There are indications that positive yield effects are associated with the ability of biochar to reduce water stress in drought situations (Karer *et al* 2013). Increased maize yield after biochar application was also attributed to the enhanced availability of calcium (Ca) and magnesium (Mg) in soil (Major *et al* 2010). Furthermore, positive yield response with biochar was found in acidic and sandy textured soils (Liu *et al* 2013). These results show that yield response to biochar strongly depends on soil conditions. A meta-analysis by Crane-Droesch *et al* (2013) found both soil cation exchange capacity (CEC) and soil organic carbon (C) content to be strong predictors for positive yield responses with biochar.

Most relevant from an agronomic viewpoint is that biochar may also modulate plant N uptake and yield through its influence on N dynamics in the soil. Biochar can affect the agricultural N cycle by (i) reducing organic N transformation rates (Prommer *et al* 2014), (ii) accelerating gross nitrification (Anderson *et al* 2011, Nelissen *et al* 2012) and (iii) increasing soil N immobilisation (Bruun *et al* 2012, Zheng *et al* 2013, Nelissen *et al* 2015). Thus, yield effects after biochar application may be driven by changes in the soil's N cycle, e.g. via an increased N use efficiency. Further, many studies have shown that biochar may help to prevent N leaching (Laird *et al* 2010, Güereña *et al* 2013, Ventura *et al* 2013). However, there are currently no studies that tried to trace the fate of fertiliser N in the plant–soil system after biochar application.

Many of the above mechanisms how biochar might change N cycling in agricultural fields may also affect gaseous N emissions from soils, namely N_2O, nitrogen gas (N_2), nitric oxide (NO) and ammonia (NH_3). Biochar may increase N loss from NH_3 volatilisation (Taghizadeh-Toosi *et al* 2011) but may reduce N_2O emissions from soil (Cayuela *et al* 2015). Decreased N_2O emissions can be related to reduced inorganic N availability, caused by increased microbial N immobilisation or a decrease in nitrification rates (Cayuela *et al* 2013, Wang *et al* 2015). Moreover, elevated soil pH after alkaline biochar application could enhance N_2 formation by enhancing N_2O reductase activity (Harter *et al* 2013, Obia *et al* 2015). An increase in soil aeration, owing to the highly porous structure of biochar, may reduce anoxic sites for N_2O production in soil (Yanai *et al* 2007, Rogovska *et al* 2011). Finally, there is evidence that abiotic redox reactions on biochar play an important role for reduced N_2O emissions from soil (Quin *et al* 2015).

Together, there is still little direct evidence whether biochar changes the efficiency of N uptake by crops and how it influences the fate of fertiliser N in the soil–plant system. In this study, we traced fertiliser N in an open-air lysimeter system, filled with two different soils, over two years for three crops. In the first year, winter wheat was fertilised with ^{15}N-labelled ammonium nitrate. The label enabled us to assign the N to two different pools (labeled fertilizer versus soil N pool) in order to detect whether biochar alters the origin of N in plant N uptake, soil N content, N leaching and N_2O emissions. During consecutive planting of a cover crop during winter and sorghum in the subsequent year, the pathways and fate of the ^{15}N label were traced through the crop rotation to study mid-term effects of biochar on soil N cycling in an open-air environment. In particular, we wanted to test whether biochar application in these agricultural systems

- increases aboveground plant N uptake and plant yield,
- affects sources of N (fertiliser versus soil-derived),
- decreases N leaching and N_2O emissions, and
- increases retention of fertiliser N in soil.

2. Methods

2.1. Lysimeter system

The lysimeter system at the Agroscope research facility Reckenholz Zurich (47.43 °N, 8.52 °E) contains 16 pots of 0.6 m diameter (area of 0.28 m^2) and 0.6 m soil depth built in a concrete block in the open air. The lysimeter pots are arranged in a single line in this concrete block, each pot equipped with an outlet for the leachate at 70 cm depth. The 10 cm below the soils are filled with gravel and stones. The pots had been filled with two types of soil (eight pots each) in 1988; these soils differed in texture (sandy loam soil: 19% clay, 25% silt, 57% sand; and silt loam soil: 19% clay, 54% silt, 28% sand) and soil organic C content (0.7% and 1.7%, respectively). The initial soil pH was 6.9 and 5.9 for the sandy loam and the silt loam, respectively. The soil at the field site where the sandy loam was taken from is classified as a eutric Cambisol, and the loam soil was taken from a site with a haplic Luvisol (IUSS Working Group WRB 2014).

Since the initiation of the lysimeter system, pots have been cropped with various field crops. The climate at the study site is moist temperate, with a mean annual air temperature of 9.4 °C and mean annual precipitation of 1054 mm (climate data 1981–2010 from the 50 m nearby MeteoSwiss station Affoltern, Meteoswiss 2013).

2.2. Biochar

The biochar was produced at the PYREG reactor of Swiss Biochar GmbH in Lausanne, Switzerland, in early 2012. This is a commercial continuous slow pyrolysis production system that reaches highest treatment temperatures of 500 °C to 650 °C during 20 min of pyrolysis (Bucheli *et al* 2014, Bachmann *et al* 2016). The feedstock was partially composted wood chip residues after sieving from a compost production plant. We measured carbon (C), hydrogen (H) and nitrogen (N) by dry combustion of milled subsamples in an elemental analyzer equipped with GC-TCD (Hekatech, Germany). We measured oxygen (O) contents separately after pyrolysis at 1000 °C in the same analyzer. The organic elemental composition of the biochar was 0.7% N, 67.8% C, 1.1% H and 8.3% O, resulting in a C/N ratio of 99.5 by mass, and molar ratios of 0.09 for O/C and 0.20 for H/C. The specific surface area measured by N_2 adsorption was 226 $m^2\ g^{-1}$, the pH (1:5 biochar to 0.01 M calcium chloride [$CaCl_2$]) was 10.1, and the liming capacity corresponded to 15.4% calcium carbonate ($CaCO_3$). The biochar contained 19% ash. Differential scanning calorimetry at a heating rate of 10 °C min^{-1} under synthetic air revealed a 50% burnoff temperature of 468.1 °C and a biochar peak temperature of 486.6 °C (for method details see Leifeld 2007).

2.3. Experimental management

2.3.1. Preparation and biochar application

Soil in the 16 lysimeter pots was manually turned and mixed on the 10th of October 2012 down to 20 cm depth. The preceding crop, *L. Perenne,* was harvested, and soil was sampled and measured for total C and total N contents, pH and mineral N content. We analyzed yield and soil data and assigned biochar versus control treatments to the 2*8 pots in a way, that the starting conditions for the treatments were not different, i.e., to avoid pre-any experimental bias from different starting conditions. This still allowed for an alternating sequence of biochar and control treatments in the line of lysimeter pots.

Half of the lysimeters of each soil type were treated with 20 t ha^{-1} (0.566 kg biochar per 0.28 m^2 lysimeter pot) wood chip biochar on the 24th of October 2012. The first 10–15 cm of soil were taken out of the lysimeters and mixed with biochar by hand in multiple steps to evenly distribute biochar in the soil. The control pots were treated the same way but without biochar amendment. Each of the four replicates per treatment was equipped with a Decagon TE5 temperature and soil moisture probe at 6–9 cm depth, logging at a 30 min interval.

2.3.2. First year: winter wheat (Triticum aestivum)

One day after biochar application (24th of October 2012) and seedbed preparation, ammonium nitrate (LONZA-Ammonsalpeter 27.5% N, no ^{15}N enrichment), phosphorus (Landor, Tripelsuperphosphat 46% P), potassium (potash salt granulated 60% K) and Mg (Landor, Granumag 29% Mg + sulphur [S]) were applied to each pot at a rate of 43 kg N, 86 kg P, 114 kg K and 21 kg Mg ha^{-1}. One hundred and twenty seeds of winter wheat (breed: Siala) were sown in five lines approximately three cm deep.

^{15}N fertiliser was applied to all lysimeters in 3 applications with 10% ^{15}N double-labelled ammonium nitrate ($^{15}NO_3^{15}NH_4$). The first fertiliser application took place on the 23rd of April 2013 with 70 kg N ha^{-1} diluted in 1.5 l of water per lysimeter, with another 1.5 l water added after fertilisation (equal to 10.8 mm of rain for each pot). The second N application was performed on the 15th of May (50 kg N ha^{-1}) and a third N fertilisation on the 14th of June (50 kg N ha^{-1}; always with the same amount of water). Winter wheat was harvested on the 16th of July.

For the N balance calculations, we corrected the winter wheat grain yield for losses due to bird feeding. Details about the loss estimation and its uncertainty are given in the supplementary material.

2.3.3. Winter cover crop: green rye (Secale cereale)

On the 25th of September 2013, the soil in the lysimeters was turned and mixed by hand and green rye was sown as a winter cover crop. Plant material was sampled on the 23rd of December 2013 and 27th of March 2014 to determine ^{15}N uptake, and green rye was harvested on the 10th of April 2014. The amount of recovered N from the fertiliser applied in the previous year was calculated by the ^{15}N content (aboveground biomass only). On the 14th of April 2014, the cover crop harvest was fully returned to the soil and mixed via manual tillage.

2.3.4. Second year: sorghum (Panicum miliaceum)

After cover crop incorporation, sorghum (proso millet, breed: Quartet) was sown at a rate of 200 seeds per lysimeter on the 6th of May 2014. At the same time, unlabelled ground ammonium nitrate fertiliser was added to the seeding rows at a rate of 30 kg N ha^{-1}. On the 12th of June, 50 kg N ha^{-1} were spread with 1.5 l water, and another 30 kg N ha^{-1} were applied on the 21st of July. LONZA-Ammonsalpeter fertiliser was used without ^{15}N enrichment ($\delta^{15}N$ −6.14‰). Plant material was first sampled on the 2nd of July and again with the harvest on the 17th of September 2014. The sorghum yield was quantified as combined straw and grain yield.

2.4. Soil sampling and analysis

Soil (0–10 cm) and crops were sampled before each fertilisation event and at harvest. Soil pH was measured shortly after sampling, and an aliquot of 10 g was dried, ground and used for bulk ^{15}N measurement. The pH was measured in a 1:2.5 moist soil:water suspension, quickly shaken and equilibrated for at least 10 h and then measured using a PH100 ExStik pH meter (Extech Instruments Corp., Nashua, NH, USA). Soil CEC and base saturation were measured on 2.5 g (d.w.) aliquots after saturation with 0.1 M BaCl-solution buffered at pH 8.1 and determination of ions by ICP-AES (FAL 1998). For ammonium and nitrate measurements (soil mineral N content; $N_{(min)}$), N was extracted from 20 g field-moist soil (stored frozen) with a 2 M potassium chloride (KCl) solution and filtered. The filtrate was analysed by segmented flow injection analysis with a SKALAR SANplus analyser (Skalar Analytical B.V., Breda, The Netherlands). For the elemental analyses, soil samples were dried at 105 °C, sieved <2 mm and ground in a ball mill at a frequency of 25/s for 5 min

At the end of the experiment, on the 22nd of October 2014, all lysimeters were destructively sampled by taking two soil cores per lysimeter, each of 7.7 cm diameter and 60 cm length. Bulk density was calculated for each 10 cm segment from these soil cores. To quantify the soil's total ^{15}N content, an aliquot of each segment was taken, dried and ground for ^{15}N analysis.

2.5. ^{15}N measurement

The amount of ^{15}N in bulk samples was quantified by elemental analysis isotope ratio mass spectrometry (EA-IRMS) on an Integra2 instrument (Sercon, UK) at the University of Basel. Briefly, sample material was combusted in the presence of O_2 in an oxidation column at 1030 °C, combustion gases were passed through a reduction column (650 °C), and produced N_2 gas was purified (separated from CO_2) and transferred to the IRMS for online isotope measurements. The atom % ^{15}N of the samples was then calculated from $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$ peak heights according to Drury *et al* (1987).

2.6. Lysimeter leachate

The leachate from the lysimeters was sampled irregularly depending on the outflow (roughly after 20 l from each pot). The volume was measured and an aliquot was taken for further analysis. Ammonium and nitrate concentrations were measured on the same SKALAR SANplus analyser as for the soil samples. The ^{15}N content of the dissolved N residues was determined by EA-IRMS following freeze-drying of a leachate subsample.

2.7. Nitrogen fertiliser use efficiency calculation

The N use efficiency was defined as recovered ^{15}N in the yield of the ^{15}N-labelled amount of fertiliser applied to winter wheat. From each harvest (plant shoot and grain from winter wheat and total above-ground plant for sorghum), total dried matter was quantified. An aliquot was ground and measured for ^{15}N. Labelled fertiliser N was then calculated from the dry matter yield multiplied by the ^{15}N atom % (Drury *et al* 1987). Natural abundance $^{15}N/^{14}N$ ratios in soil and plant material prior to ^{15}N fertiliser application was subtracted from the measured ^{15}N. Residual ^{15}N stocks were then related to the total 17 kg ^{15}N ha^{-1} applied (10% ^{15}N in 170 kg N ha^{-1} applied as NH_4NO_3 fertiliser) during winter wheat cropping in 2013. In the second year during sorghum cropping, no additional ^{15}N label was applied. Hence, the 2 year rotation was designed to focus on N use efficiency from the fertiliser applied to winter wheat.

2.8. Greenhouse gas static chamber measurement

Greenhouse gas samples were collected from static opaque polyvinyl chloride chambers that were manually put over the entire lysimeter column. Chamber height was 25 or 65 cm depending on crop height. Chamber diameter was slightly larger than the lysimeter soil column (68 cm versus 60 cm) resulting in an effective chamber volume of 91.5 l and 238 l for the short and the tall chambers, respectively. For each measurement, chambers were manually placed in a ring with rubber sealing inside. Four 20 ml glass vials with rubber septa were filled with chamber air during the 30–45 min closure time. Automatic gas samplers were built to pump chamber air via injection needles through the sample vial. An electronic device controlled electromagnetic valves to open and close the chambers at predefined time steps to sample the chamber air regularly. Hence, the vials were not pre-evacuated but flushed with approximately 100 ml min^{-1} for at least 5 min.

Chamber gas samples were analysed within 4 weeks of collection on a gas chromatograph (7890A, Agilent Technologies, Santa Clara, CA, USA). As pre- and analytical column, a HayeSep Q 80/100 (Restek Corp., Bellefonte, PA, USA) was used at a length of 1.83 m and 2.44 m, respectively. The gas samples were loaded onto separate sample loops that were then carried to a flame ionisation detector via a methanizer with helium for CO_2 and to an electron capture detector by N_2 for N_2O detection. Oven temperature was set to 100 °C.

The N_2O flux for each chamber was calculated with the flux estimation procedure (R-script by R. Fuss on bitbucket.org, Fuss 2015) as used in Leiber-Sauheitl *et al* (2014). It is a modification of the HMR package (Pedersen *et al* 2010) that chooses between exponential curvature for nonlinear chamber behavior (Hutchinson-Mosier regression) and robust linear regression. However, the nonlinear model could never be fitted successfully, therefore 634 fluxes were calculated with the robust linear and 35 with simple linear regression.

The yearly N_2O emission budget was calculated using linear interpolation between days with flux measurement. Because the measurements did not cover the whole year regularly, we interpreted annual emissions with caution. We checked the reliability of this approach by comparing it with mean annual emissions. The latter showed the same order of magnitude of emissions and similar treatment effects from soil and biochar (see supplementary material).

2.9. $^{15}N_2O$ measurement

N_2O from two emission peaks was collected at the end of the chamber sampling in 180 ml glass bottles with rubber crimp caps. The total N_2O in each sample was purged with carrier helium directly into a gas bench modified according to McIlvin and Casciotti (2010) and analysed by continuous flow gas chromatography—IRMS (Thermo Finnigan DELTAplus XP). Even with strongly ^{15}N-enriched samples, atom % ^{15}N was calculated using the equations from Stevens and Laughlin (1994) based on mass 45/44 and 46/44 N_2O ratios.

From the ^{15}N content in the N_2O of the chamber air, the background atmospheric $^{15}N_2O$—with a concentration of 0.325 ppm and 0.3634% ^{15}N—was subtracted because it was already present at the beginning of the chamber measurement. This allowed us to determine soil-derived $^{15}N_2O$ emissions, which were then used to estimate the N source for N_2O production in soil:

$$\text{soil derived } N_2O\,[\text{at}\%\ ^{15}N] = \frac{^{15}N(\text{chamber air [at \%]}) * c\,(N_2O \text{ chamber air}) \text{ [ppm]} - {}^{15}N(\text{atm}) \text{ [at \%]} * c\,(N_2O \text{ atm}) \text{ [ppm]}}{c\,(N_2O \text{ increase in chamber air}) \text{ [ppm]}}.$$

2.10. Statistical analysis

Statistical analyses were performed with R software (version 3.0.1, R Core Team 2015). The significance level was chosen at $p < 0.05$ for all procedures, unless indicated otherwise. Significant treatment effects on the N pools were determined using a 2-way ANOVA from the rbase package (factor soil: sandy loam, silt loam; factor treatment: biochar, control).

3. Results

3.1. Meteorology and soil water content

Figures 1 and 2 show meteorological parameters from the winter wheat and cover crop–sorghum periods, respectively. The year 2013 started with relatively cold temperatures and two pronounced frost events that are reflected by below zero degree temperatures and low (liquid) volumetric water content (VWC) during soil frost (figure 1). In July 2013, there was a dry period coinciding with the last fertilisation. Total precipitation for 2013 was 1027 mm. The cropping period for green rye and sorghum (figure 2) began with relatively warm winter temperatures and a dry and warm period in April and June. Average temperatures in Switzerland during this period were 1.5 °C above the 1981–2010 norm. From July onwards, the summer was cold and wet compared with the climatic mean (Meteoswiss 2015). However, the precipitation sum for the whole year 2014 of 985 mm was lower than in 2013.

Soil VWC appeared to be higher in soils treated with biochar compared with the control, but only for 10 out of 735 days was this difference significant. Hence, there was no evidence that soils treated with biochar held significantly more water than non-treated soils.

3.2. Fertiliser balance from ^{15}N tracing

A large fraction (44% for sandy loam and 35% for silt loam) of the applied fertiliser from 2013 was still in soil at the end of 2014 (figure 3; table S1 supplement). There was neither a significant difference between the two soil types ($p = 0.07$) nor between biochar and control treatments ($p = 0.40$) for fertiliser-derived soil ^{15}N. Winter wheat grains took up 30% and straw 8% of the ^{15}N-label. The ^{15}N uptake was not affected by soil type or biochar application. The cover crop, green rye, took up 2.2% of the applied ^{15}N fertiliser (table S1 supplement); there were no differences between soil types ($p = 0.10$) or biochar treatments ($p = 0.57$). In the following year, after cover crop reincorporation, aboveground sorghum incorporated another 3% of the previous year's fertiliser. Note that the cover crop ^{15}N was available in soil again for sorghum growth. The ^{15}N uptake by sorghum was not affected by soil type or biochar application.

Leaching of ^{15}N was minimal and the leachate contained only around 0.4% of the labelled fertiliser after 1.5 years. Most of the ^{15}N label introduced by the fertiliser had not yet passed the soil column. Total leached ^{15}N over the experiment was not different between biochar and control treatments ($p = 0.18$), whereas there was a significant difference between soil types ($p = 0.03$); the sandy loam lost more N via leaching than the silt loam. Total N leaching in the second winter of the experiment was low, but biochar treatments reduced leaching significantly compared to the control ($p = 0.02$) during that period. Figure 4 shows the time series of NO_3^- and NH_4^+ N leachate measurements during the experiment with the major

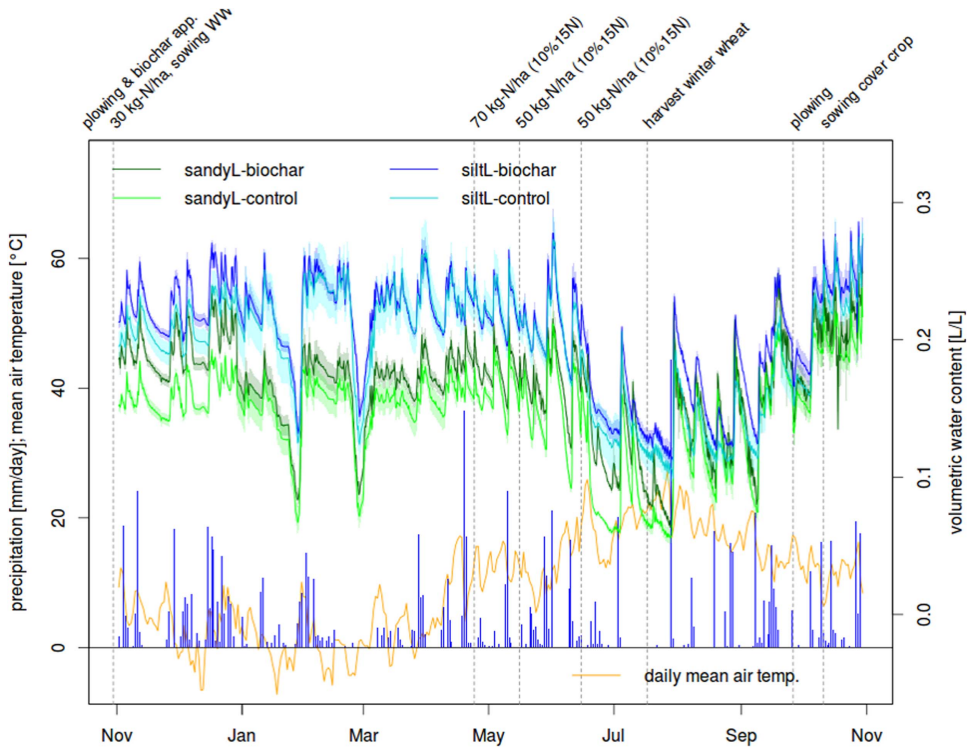

Figure 1. Volumetric water content of the four treatment combinations, temperature (orange line) and precipitation (blue bars) during winter wheat cropping from November 2012 to November 2013.

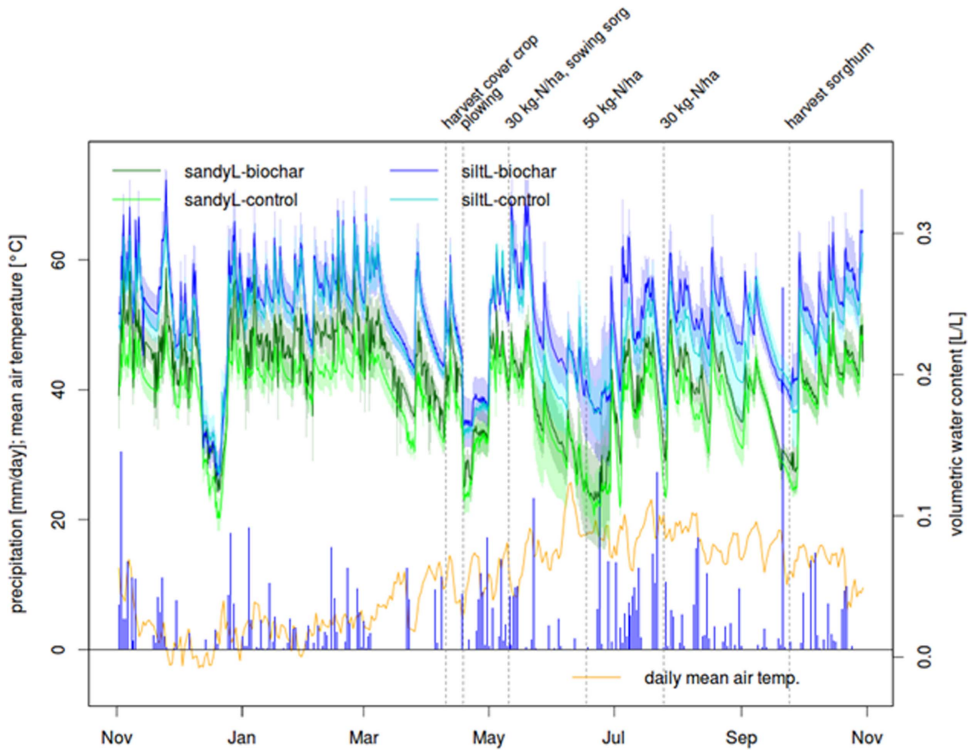

Figure 2. Volumetric water content of the four treatment combinations, temperature (orange line) and precipitation (blue bars) during cover crop and sorghum cropping from November 2013 to November 2014.

peaks in winter (2012–13 and 2013–14). Whereas leached amounts in the first winter were in the expected range of roughly 35 kg N ha^{-1}, the leached N in 2013 accounted for only 5 kg N ha^{-1}. Water amounts leached through the soil columns were about the same in both winters (80–100 l per lysimeter equal to

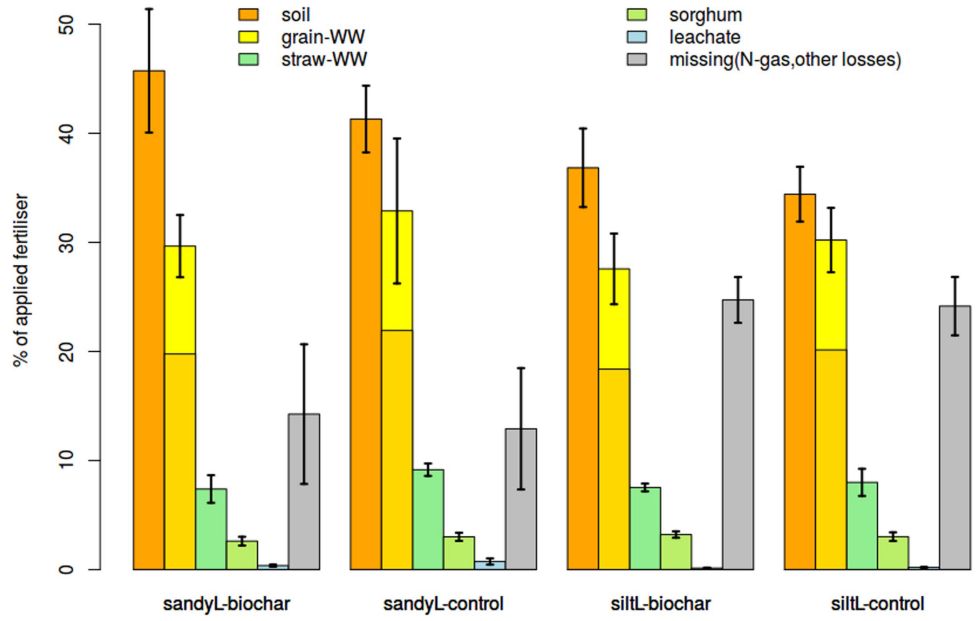

Figure 3. Two-year fertiliser balance of 170 kg N ha^{-1} with 10 % ^{15}N labelled (initial input: 17 kg ^{15}N). Error bars indicate the standard error from the four replicates. The lower part of the winter wheat (WW) grain column (gold) indicates the estimated two thirds of lost ^{15}N from yield depression and bird predation.

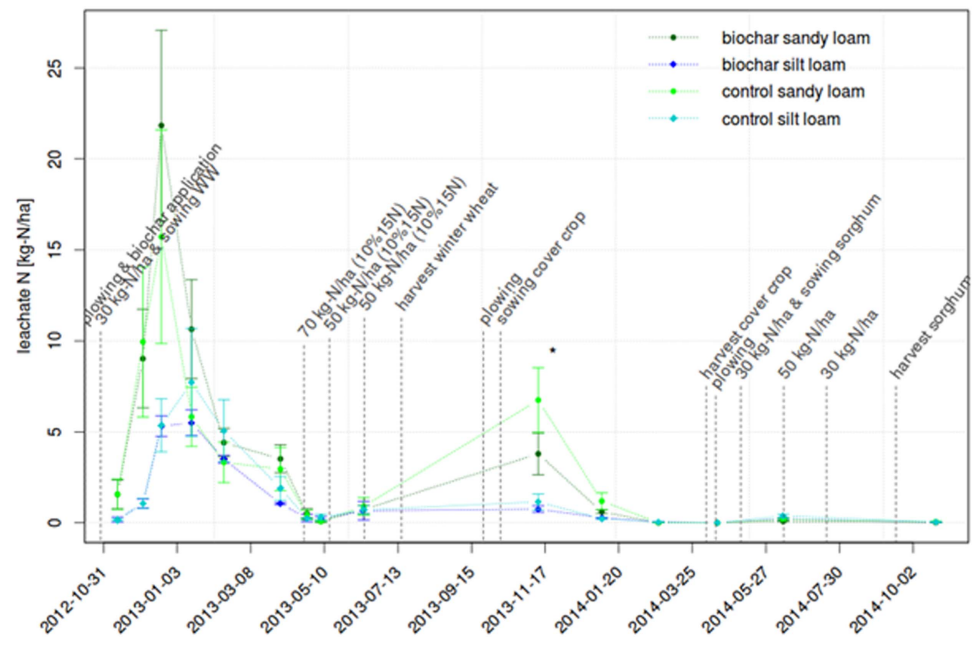

Figure 4. Mean N leaching from the lysimeters over time. Measurement with significant treatment effect is indicated with an asterisk. Error bars indicate the standard error from the four replicates.

285–360 mm, roughly one-third of the mean annual rainfall).

The missing amount of ^{15}N fertiliser in figure 3 refers to the difference between the applied amount of N fertiliser and the sum of ^{15}N recovered in soil, plants and leachate. The amount of missing fertiliser in the ^{15}N budget was 13.6% in the sandy loam and 24.5% in the silt loam. This missing fraction was not controlled by biochar application ($p = 0.84$) but differed between soil types ($p = 0.04$) (figure 3). The silt loam had a larger fraction of missing fertiliser N compared with the sandy loam, but with a smaller variability.

During the experiment there was mostly no difference in ^{15}N or total N uptake between the two soil types or between biochar versus control (figure 5). However, fertiliser uptake into winter wheat grain was higher in the silt loam than in the sandy loam ($p = 0.01$).

The two soil types had significantly different soil mineral N content (N_{min}), pH, CEC and base saturation (table 1). Biochar did not affect N_{min} or soil CEC

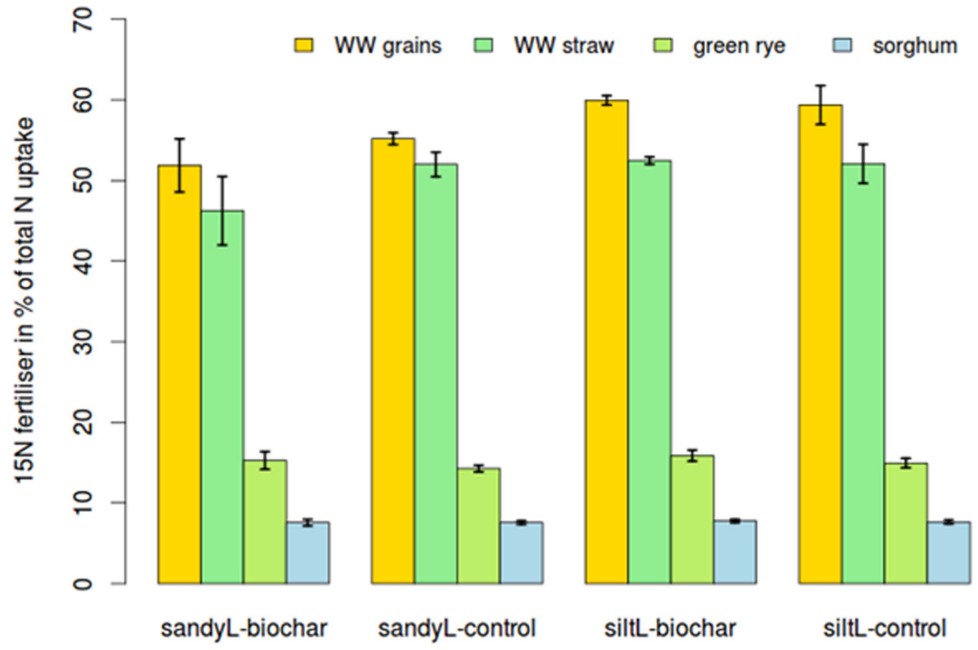

Figure 5. ^{15}N fertiliser uptake as a percentage of total N in plants for winter wheat (WW) grains and straw, the cover crop green rye and sorghum. Error bars indicate the standard error from the four replicates.

at any point in time. However, biochar application increased soil base saturation ($p < 0.001$) and pH ($p < 0.001$).

3.3. N_2O emissions

N_2O emissions were discontinuously measured. Still, both background emissions and emission peaks were captured (figure 6). Interpolated yearly N_2O emissions were around 1.5 kg N ha^{-1} yr^{-1} (figure 7). An ANOVA of the mean N_2O flux over the measured time span revealed a *p*-value of 0.026 for the biochar treatment and 0.039 for the soil types (table 2). This analysis indicates significantly higher emissions in the sandy loam than silt loam and a significant reduction of N_2O emissions by biochar compared to the control by 11% and 21%, respectively. Biochar pots tended to have lower emissions especially at peak events (figure 6). Yearly mean N_2O emission estimates resulted in N_2O emission factors of around 1%, being in the expected range from the IPCC (2014). Although N_2O emissions were different between treatments at the two campaigns when $^{15}N_2O$ was measured, we did not see any preferential N_2O release from labelled fertiliser (table 2).

4. Discussion

4.1. N balance

Our results for a temperate winter wheat—cover crop—sorghum rotation showed that application of 20 t of slow pyrolysis wood chip biochar neither led to a higher fertiliser N uptake by the crops nor did it increase or decrease yields. The N use efficiency from the first year's fertiliser application of approximately 40% throughout the whole rotation was not increased by biochar. Furthermore, N content in three ecosystem components, i.e. plant, soil and, in most cases, leachate, was not significantly altered in our system by biochar. Reported changes in N transformation with biochar (e.g. Prommer *et al* 2014, Nelissen *et al* 2014) may not immediately change gross N flows in a temperate agricultural system with high N inputs and already high soil fertility. For example, Prommer *et al* (2014) have shown that biochar significantly reduces gross rates of soil organic N transformation in the field but not gross mineralisation of organic N. The authors explained their findings by a decoupling of the soil organic and inorganic N cycles and concluded that the combined addition of biochar and fertiliser N would increase soil organic N and enhance soil C sequestration. Pereira *et al* (2015) observed increased N transformation rates with biochar but no change in plant productivity or leaf N content. Vaccari *et al* (2011) observed up to 30% increased biomass production without change in grain N content. In agreement with our results, this shows that small changes in N cycling with biochar (i.e. increased N transformation rates or increased biomass production) do not necessarily increase agricultural yields. Our results also indicate that plant growth was not limited by factors that were affected by biochar, i.e. soil pH and base saturation. Biochar also did not alter soil N content, plant available N, and CEC. Hence we cannot support the hypothesis that biochar can improve nutrient availability indirectly through changes in soil pH or CEC (Scott *et al* 2014).

In contrast to our results, many studies on biochar and N uptake found increasing yields (e.g., Jeffery *et al* 2011, Biederman and Harpole 2013). For example, van Zwieten *et al* (2010a) reported a 250% wheat biomass increase with biochar at 10 t ha^{-1} on a

Table 1. Soil parameters and ^{15}N content in soil (^{15}N at%) at several points in time (mean by treatment ± standard error); sandy loam and silt loam.

Parameter/unit	Date	sandyL-biochar	sandyL-control	siltL-biochar	siltL-control	*p*-value biochar	*p*-value soil
Base saturation (pre biochar) (%)	2012-10-15	78.0 ± 0.9	79.0 ± 2.3	56.3 ± 1.4	58.0 ± 1.9	0.43	<0.001
Base saturation (%)	2012-10-24	90.2 ± 1.1	80.0 ± 1.9	76.2 ± 1.7	59.5 ± 1.7	<0.001	<0.001
CEC (pre biochar) (cmol+/kg)	2012-10-15	13.6 ± 0.2	13.6 ± 0.2	11.9 ± 0.2	11.9 ± 0.2	0.95	<0.001
CEC (cmol+/kg)	2012-10-24	13.6 ± 0.3	13.6 ± 0.3	11.8 ± 0.1	12.2 ± 0.1	0.31	<0.001
N(min), (pre biochar) (mg N/kg soil)	2012-10-15	3.1 ± 1.1	3.3 ± 1.5	0.5 ± 0.2	0.2 ± 0.1	0.97	0.01
N(min) (mg N/kg soil)	2012-10-24	1.8 ± 0.3	1.5 ± 0.4	0.8 ± 0.3	0.8 ± 0.2	0.74	0.01
N(min) (mg N/kg soil)	2013-04-08	5.3 ± 0.4	6.3 ± 0.4	2.7 ± 0.5	3.0 ± 0.8	0.26	<0.001
N(min) (mg N/kg soil)	2013-05-08	6.4 ± 0.8	7.2 ± 0.5	2.4 ± 0.4	3.0 ± 0.8	0.33	<0.001
N(min) (mg N/kg soil)	2013-06-13	3.8 ± 0.2	3.8 ± 0.6	1.1 ± 0.3	1.3 ± 0.4	0.78	<0.001
N(min) (mg N/kg soil)	2014-07-04	1.3 ± 0.1	1.3 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.29	<0.001
N(min) (mg N/kg soil)	2014-04-14	1.1 ± 0.2	1.3 ± 0.1	0.8 ± 0.2	0.7 ± 0.2	0.83	0.02
pH (pre biochar)	2012-10-15	7.0 ± 0.0	6.9 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	0.74	<0.001
pH	2013-04-16	8.0 ± 0.1	7.3 ± 0.1	7.5 ± 0.2	6.4 ± 0.07	<0.001	<0.001
pH	2013-07-19	7.2 ± 0.2	6.8 ± 0.1	6.6 ± 0.1	5.9 ± 0.1	<0.001	<0.001
soil ^{15}N (^{15}N at%)	2013-07-16	0.68 ± 0.05	0.71 ± 0.03	0.80 ± 0.05	0.71 ± 0.02	0.40	0.15
soil ^{15}N (^{15}N at%)	2014-09-16	0.60 ± 0.03	0.57 ± 0.02	0.61 ± 0.03	0.59 ± 0.02	0.30	0.53

ferralsol and suggested an increase in N use efficiency. Petter *et al* (2016) showed an increased N use efficiency with biochar in an upland rice cropping system (soil pH 5.3, base saturation 41%). This discrepancy highlights the differential effects biochar application can have on highly weathered tropical soils compared to temperate fertile ones. Soils like those in our experiment with naturally high pH and base saturation may not benefit as much as less fertile and highly weathered acidic tropical soils (Crane-Droesch *et al* 2013). Jeffrey *et al* (2011) suggested that the main mechanisms for yield increase may be due to biochar's liming effect, improved water holding capacity and improved crop nutrient availability. In experiments by Karer *et al* (2013), positive yield effects were observed during drought situations but no significant effect was discernible in the following years and with other crops. In our case, we could not find increased yields with biochar although winter wheat plants may have slightly suffered from dry conditions during grain filling after the third fertilisation event.

Fertiliser N use efficiency of the first crop is typically around 30%–50% (Ladha *et al* 2005). Our results of fertiliser N uptake by winter wheat grains of roughly 30% was at the lower end of this estimate. Our yields had to be corrected (see supplement) because they were probably depressed due to the application of a growth regulator, made necessary owing to constrained chamber heights, and bird feeding (inhibited grain filling in addition to the estimated 67% yield loss, see supplement for details). Additionally, moderate drought in the sun-exposed concrete block of the lysimeter facility may have impaired plant growth and yield and may have had a negative influence on grain filling. These yield depressions were not treatment-specific and our 30% N use efficiency already consider these losses. In addition, birds partially also consumed the sorghum grain yield, which we not corrected for, because the overall ^{15}N uptake was already very low in the second year. Yet, there was no indication for a preferential predation by birds to certain treatments as they were mixed in the facility, all replicates were effected the same and there were no differences in plant growth for any crop. Our interpretation is also based on the ^{15}N label yield in the plants and grain, indicating no difference in fertiliser uptake.

There were two significant biochar effects observed for our system, namely reduced bulk N leaching in the second year and reduced N_2O emissions. Despite their environmental relevance, these N fluxes were very small compared with the overall N balance. Furthermore, the high natural variability in a field situation, i.e. soil heterogeneity, field management, weather conditions and extremes, reduce the sensitivity of our experiment for small effects that were frequently detected in laboratory incubations (Clough and Condron 2010).

Because not all N fluxes were measured, there was a quantifiable but unknown gap in the fertiliser N recovery of 13%–25% after 2 years. The extent of this gap may depend on experimental variability (larger for the sandy loam than the silt loam) and uncertainties in the balance calculation. The missing fraction was most likely related to the transformation of fertiliser N into gaseous forms, namely N_2, NH_3 and NO during denitrification, nitrification and ammonium volatilisation that can make up a significant proportion of the overall N budget (Martinez and Guiraud 1990, Clough *et al* 2001, Friedl *et al* 2016). The imbalance was significantly higher in the silt loam, which was less prone to leaching than the sandy loam. A proportionally higher leaching for both soil types could balance the gap only for the sandy loam, where greater losses due

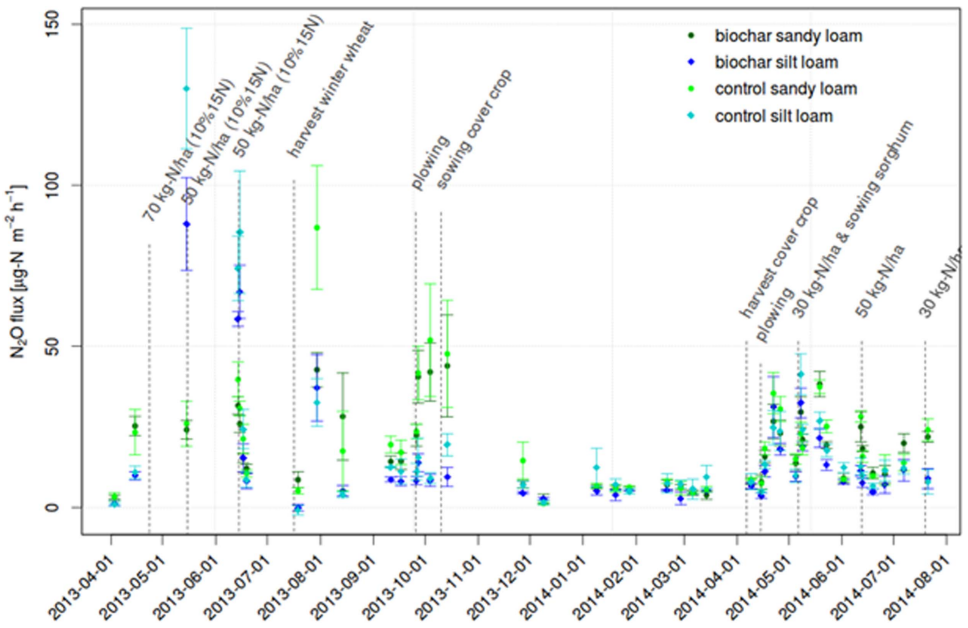

Figure 6. N_2O emissions during the 2 years of the experiment with winter wheat in 2013 and sorghum in 2014. Error bars indicate the standard error from the four replicates.

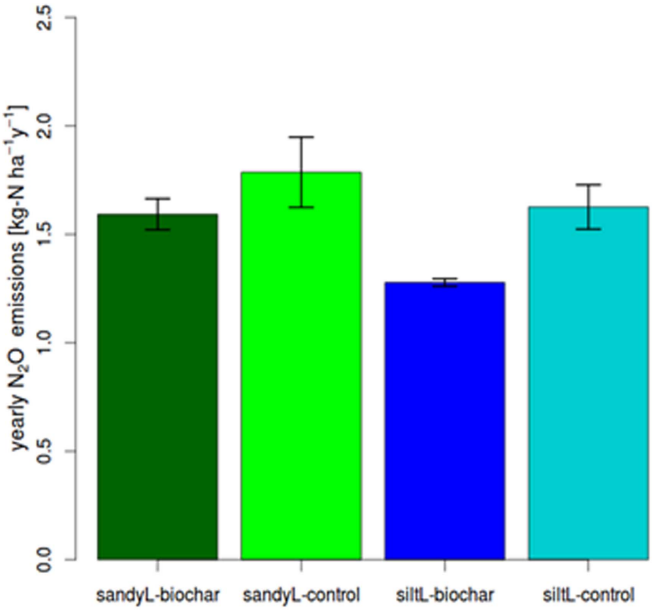

Figure 7. Total annual N_2O emissions as calculated from measured events; error bars = 1 SE; annual emissions estimated with fluxes interpolated between days with measurement.

to leaching were measured. Instead, the missing fertiliser ^{15}N can better be explained by the observed N_2O emission patterns: Although N_2O emissions are often one order of magnitude lower than N_2 emissions (Jambert *et al* 1997) and do not contribute significantly to the overall N balance, they may provide semi-quantitative information on the overall denitrification rate and hence N_2 loss. A higher denitrification rate (as suggested by higher N_2O accumulation; see below) may explain the larger gap in the ^{15}N balance of the silt loam compared with the sandy loam. Friedl *et al* (2016) demonstrated how cumulated N_2 emissions from an intensively managed subtropical pasture can account for up to 40% of the applied N. Considering this large potential for unmeasured gaseous losses, they may account for the gaps in N recovery.

To our knowledge, this is the first study that quantified N use efficiencies after biochar application on two different soils using ^{15}N fertiliser tracing. Previous work relied mostly on short-term laboratory experiments that do not allow to investigate the sustainability of biochar effects on the soil's N cycle at larger spatial or temporal scales (Scott *et al* 2014). For example, Nelissen *et al* (2015) showed how significant changes in soil N

Table 2. Yearly mean N_2O emissions and ^{15}N content of N_2O emissions at two sampling dates.

Parameter	Unit	Date	sandyL-biochar	sandyL-control	siltL-biochar	siltL-control	*p*-value biochar	*p*-value soil
Cumulative N_2O linear int.	kg N ha^{-1} yr^{-1}	2013–2014	2.00 ± 0.05	2.24 ± 0.29	1.74 ± 0.06	2.34 ± 0.11	0.02	0.60
Yearly mean N_2O	kg N ha^{-1} yr^{-1}	2013–2014	1.60 ± 0.08	1.79 ± 0.16	1.28 ± 0.02	1.63 ± 0.10	0.03	0.04
Soil-derived N_2O	^{15}N at%	2013-05-16	4.94 ± 0.55	5.91 ± 0.33	8.52 ± 0.17	8.65 ± 0.10	0.13	<0.001
Soil-derived N_2O	^{15}N at%	2014-05-08	1.16 ± 0.14	1.40 ± 0.13	1.11 ± 0.06	1.11 ± 0.06	0.27	0.14

transformation with fresh biochar completely vanished after one year. In order to better understand the underlying mechanisms of biochar-plant-soil-microorganism interactions, more longer-term field experiments (with aging biochar) are needed.

4.2. N_2O emissions

We found an average reduction in soil N_2O emissions of 15%, which is within the range of a recently published meta-analysis by Cayuela *et al* (2015) (mean of 28 ± 16% in field experiments). This concordance strengthens the evidence for the effectiveness of biochar to reduce N_2O emissions in the field. With the same biochar, Felber *et al* (2013) found a 21.5% reduction in N_2O emissions during one growing season on a grassland.

With a 0.20 H/C ratio, our biochar was in the range of low H/C ratio biochars that Cayuela *et al* (2015) identified as being most effective for reducing N_2O emissions from soil. These biochars have a condensed aromatic structure that allows electron transfer across conjugated pi-electron systems (Klüpfel *et al* 2014), which might be beneficial to the last step of denitrification (Cayuela *et al* 2013). Furthermore, our biochar had a high pH and especially a large liming capacity of 15.4% $CaCO_3$ equivalents. We observed an increase in soil pH after application of this alkaline biochar (table 1). The pH effect has been suggested previously as a possible mechanism for reduced N_2O emission from soil after biochar amendment (van Zwieten *et al* 2010b, Zheng *et al* 2012). With increasing soil pH, the denitrifying community tends to increase N_2O reduction activity, thereby reducing emissions as N_2O (Čuhel *et al* 2010), as also shown in biochar–soil slurries by Obia *et al* (2015). Although the pH hypothesis is plausible from our observations and data, it is still unclear if reductions in N_2O emissions can solely be assigned to the soil pH increase. Hüppi *et al* (2015) explicitly tested for the pH effect in a field trial but could not verify that the N_2O emission reduction was caused by an enhanced soil pH. Soil pH manipulations and their effects on N_2O emissions are driven by complex interactions (Baggs *et al* 2010) and are not yet finally understood.

The unmeasured gaps in the fertiliser N balance are mostly gaseous fluxes of N_2O, N_2, NO_x and NH_3 (Jambert *et al* 1997). From our flux measurements, we can roughly estimate the N_2O losses to be at the magnitude of 1% of applied fertiliser. Butterbach-Bahl *et al* (2013) estimated the mean N_2O share of denitrification from agricultural soils to be 15 ± 6%. If we estimate the N_2 emissions accordingly (i.e. N_2 being 6.7 ± 1.9 times the N_2O emissions), our system lost roughly 7% of fertiliser as N_2. This percentage accounts for half of the missing N in the sandy loam and about one-fourth in the silt loam. According to Jambert *et al* (1997), gaseous N losses from a mineral fertilised maize field can have the following shares: 1% as NH_3, 40% as NO, 14% as N_2O and 46% as N_2. Hence, NO emissions can be in the same order of magnitude as N_2 and explain another substantial fraction of the missing N. Nelissen *et al* (2014) tested various fertiliser types and found not only reduced cumulative N_2O (52%–84%) emissions with biochar but also reduction in NO (47%–67%). They explained the reduced emissions by increased NH_3 volatilisation, microbial N immobilisation and non-electrostatic sorption of NH_4^+ and NO_3^- as well as pH effects. However, our data do not suggest that there were large changes in N immobilisation (due to high fertiliser input) or sorption on biochar, because we did not observe changes in soil N content or plant N uptake.

With regard to fertiliser-derived N_2O our observations suggest that biochar application did not alter the N source for N_2O production in soil. This is the first study to show that the N source for N_2O in an experiment with reduced emissions by biochar in the field was not changed. This finding means that biochar neither reduced the availability of fertilizer nor that of soil-derived N for microbial N_2O production. Thus the (unknown) processes responsible for N_2O emission reduction may not be fertiliser specific. Further, this finding indicates that reduced N_2O emissions by biochar only depended on increased N_2O reduction (i.e. increased nosZ activity) but did not decrease the amount of N used for denitrification (Harter *et al* 2013, Obia *et al* 2015).

5. Conclusion

In our temperate lysimeter systems with sandy loam eutric Cambisol and silty loam haplic Luvisol, soil types that are among the most common agricultural soils in Central Europe, the applied slow pyrolysis woodchip biochar did not change N fertiliser use efficiency or N partitioning among the 3 ecosystem components (soil, plants or leachate) over the course of two years. Biochar treatment caused a decrease in N_2O emissions but no change in the source of N for

N_2O production. Although the observed effects due to biochar application (i.e. reduced N_2O emissions and leaching) apply to fluxes that are small within the overall N balance, they are environmentally significant and important for understanding biochar functioning in agricultural systems. Especially reduced N_2O emissions have a large relevance for climate mitigation and the overall biochar GHG balance. However, a comprehensive life cycle assessment is needed to verify if these improvements can counterbalance possible negative effects from biochar production (e.g. competition for biomass as resource) and other adverse effects (e.g. introduction of organic and inorganic pollutants to soil). We showed that application of the chosen biochar in the respective temperate agricultural soils has a small but significant potential to reduce environmental impacts of N fertilisation and does not impair crop yields.

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