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N use efficiencies and N$_2$O 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$_2$O) 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$_2$O 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 ± 19% but only towards the end of the experiment with leaching losses being generally low. For both soils N$_2$O emissions were reduced by 15 ± 4% with biochar compared to the control treatments. Our results indicate that application of the chosen biochar induces environmental benefits in terms of N$_2$O 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 (Schmitd 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 (N₂O) emissions from agricultural fields as recently demonstrated (Cayuela et al 2015, Hüppi et al 2015) but also contradicted (Angst et al 2014, Verhoveen 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, Guereña 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 by which biochar might change N cycling in agricultural fields may also affect gaseous N emissions from soils, namely N₂O, nitrogen gas (N₂), nitric oxide (NO) and ammonia (NH₃). Biochar may increase N loss from NH₃ volatilisation (Taghizadeh-Toosi et al 2011) but may reduce N₂O emissions from soil (Cayuela et al 2015). Decreased N₂O 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 N₂ formation by enhancing N₂O 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 N₂O 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 N₂O 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 ¹⁵N-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 N₂O emissions. During consecutive planting of a cover crop during winter and sorghum in the subsequent year, the pathways and fate of the ¹⁵N label were traced through the crop rotation to study midterm 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 N₂O 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 m²) 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).

2
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 N2 adsorption was 226 m2 g−1, the pH (1:5 biochar to 0.01 M calcium chloride [CaCl2]) was 10.1, and the liming capacity corresponded to 15.4% calcium carbonate (CaCO3). 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 m3 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 TES5 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 15N 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. 15N fertiliser was applied to all lysimeters in 3 applications with 10% 15N double-labelled ammonium nitrate (15NO3:15NH4). 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 15N 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 15N 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 15N enrichment (δ15N = −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$_2$-solution buffered to pH 8.1 and determination of ions by ICP-AES (FAL 1998). For ammonium and nitrate measurements (soil mineral N content; $N_{(\text{min})}$), 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 aboveground 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$_4$NO$_3$ 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$_2$O detection. Oven temperature was set to 100 °C.

The N$_2$O 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\textsubscript{2}O 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. \textsuperscript{15}N\textsubscript{2}O measurement

\textsuperscript{15}N\textsubscript{2}O 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\textsubscript{2}O in each sample was purged with carrier helium directly into a gas bench—IRMS (Thermo Finnigan DELTAplus XP). Even with strongly \textsuperscript{15}N-enriched samples, atom % \textsuperscript{15}N was calculated using the equations from Stevens and Laughlin (1994) based on mass 45/44 and 46/44 N\textsubscript{2}O ratios.

From the \textsuperscript{15}N content in the N\textsubscript{2}O of the chamber air, the background atmospheric \textsuperscript{15}N\textsubscript{2}O—with a concentration of 0.325 ppm and 0.363\% \textsuperscript{15}N—was subtracted because it was already present at the beginning of the chamber measurement. This allowed us to determine soil-derived \textsuperscript{15}N\textsubscript{2}O emissions, which were then used to estimate the N source for N\textsubscript{2}O production in soil:

\[
s\text{soil derived } N_2O \text{ [at\% } ^{15}\text{N}] = \frac{^{15}N(\text{chamber air [at \%]})c(\text{N}_2O \text{ chamber air [ppm]}) - ^{15}N(\text{atm}) [\text{at \%}]c(\text{N}_2O \text{ atm [ppm]})}{c(\text{N}_2O \text{ increase in chamber air [ppm]})}
\]

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 \textsuperscript{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 \textsuperscript{15}N. Winter wheat grains took up 30\% and straw 8\% of the \textsuperscript{15}N-label. The \textsuperscript{15}N uptake was not affected by soil type or biochar application. The cover crop, green rye, took up 2.2\% of the applied \textsuperscript{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 \textsuperscript{15}N was available in soil again for sorghum growth. The \textsuperscript{15}N uptake by sorghum was not affected by soil type or biochar application.

Leaching of \textsuperscript{15}N was minimal and the leachate contained only around 0.4\% of the labelled fertiliser after 1.5 years. Most of the \textsuperscript{15}N label introduced by the fertiliser had not yet passed the soil column. Total leached \textsuperscript{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\textsubscript{3}– and NH\textsubscript{4}+ N leachate measurements during the experiment with the major
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

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

The missing amount of 15N fertiliser in figure 3 refers to the difference between the applied amount of N fertiliser and the sum of 15N recovered in soil, plants and leachate. The amount of missing fertiliser in the 15N 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 15N 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 (Nmin), pH, CEC and base saturation (table 1). Biochar did not affect Nmin or soil CEC...
at any point in time. However, biochar application increased soil base saturation \((p < 0.001)\) and pH \((p < 0.001)\).

3.3. N\(_2\)O emissions

\(\text{N}_2\O\) emissions were discontinuously measured. Still, both background emissions and emission peaks were captured (figure 6). Interpolated yearly \(\text{N}_2\O\) emissions were around 1.5 kg N ha\(^{-1}\) yr\(^{-1}\) (figure 7). An ANOVA of the mean \(\text{N}_2\O\) 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 \(\text{N}_2\O\) 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 \(\text{N}_2\O\) emission estimates resulted in \(\text{N}_2\O\) emission factors of around 1%, being in the expected range from the IPCC (2014). Although \(\text{N}_2\O\) emissions were different between treatments at the two campaigns when \(^{15}\text{N}\O\) was measured, we did not see any preferential \(\text{N}_2\O\) 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 \textit{et al} 2014, Nelissen \textit{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 \textit{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 \textit{et al} (2015) observed increased N transformation rates with biochar but no change in plant productivity or leaf N content. Vaccari \textit{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 \textit{et al} 2014).

In contrast to our results, many studies on biochar and N uptake found increasing yields (e.g., Jeffery \textit{et al} 2011, Biederman and Harpole 2013). For example, van Zwieten \textit{et al} (2010a) reported a 250% wheat biomass increase with biochar at 10 t ha\(^{-1}\) on a
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-Drosch 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 15N 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 15N 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 N2O 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 N2, NH3 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

| 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 | 7.3 ± 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 15N (15N at%) | 2012-10-15 | 78.0 ± 2.3 | 56.3 ± 1.4 | 58.0 ± 1.9 | <0.001 | <0.001 |
| soil 15N (15N at%) | 2013-04-16 | 0.68 ± 0.05 | 0.71 ± 0.03 | 0.80 ± 0.05 | 0.71 ± 0.02 | 0.40 | 0.15 |
| soil 15N (15N at%) | 2014-09-16 | 0.60 ± 0.03 | 0.57 ± 0.02 | 0.61 ± 0.03 | 0.59 ± 0.02 | 0.30 | 0.53 |
to leaching were measured. Instead, the missing fertiliser-15N can better be explained by the observed N2O emission patterns: Although N2O emissions are often one order of magnitude lower than N2 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 N2 loss. A higher denitrification rate (as suggested by higher N2O accumulation; see below) may explain the larger gap in the 15N balance of the silt loam compared with the sandy loam. Friedl et al (2016) demonstrated how cumulated N2 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 15N 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

**Figure 6.** N2O 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 N2O emissions as calculated from measured events; error bars = 1 SE; annual emissions estimated with fluxes interpolated between days with measurement.
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. \textit{N}_2\textit{O} emissions

We found an average reduction in soil \textit{N}_2\textit{O} 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 \textit{N}_2\textit{O} emissions in the field. With the same biochar, Felber et al. (2013) found a 21.5\% reduction in \textit{N}_2\textit{O} 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 \textit{N}_2\textit{O} 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\textsubscript{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 \textit{N}_2\textit{O} 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 \textit{N}_2\textit{O} reduction activity, thereby reducing emissions as \textit{N}_2\textit{O} (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 \textit{N}_2\textit{O} 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 \textit{N}_2\textit{O} emission reduction was caused by an enhanced soil pH. Soil pH manipulations and their effects on \textit{N}_2\textit{O} 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 \textit{N}_2\textit{O}, \textit{N}_2, \textit{NO}_3 and NH\textsubscript{3} (Jambert et al. 1997). From our flux measurements, we can roughly estimate the \textit{N}_2\textit{O} losses to be at the magnitude of 1\% of applied fertiliser. Butterbach-Bahl et al. (2013) estimated the mean \textit{N}_2\textit{O} share of denitrification from agricultural soils to be 15 ± 6\%. If we estimate the \textit{N}_2 emissions accordingly (i.e. \textit{N}_2 being 6.7 ± 1.9 times the \textit{N}_2\textit{O} emissions), our system lost roughly 7\% of fertiliser as \textit{N}_2. This percentage accounts for half of the missing \textit{N} in the sandy loam and about one-fourth in the silt loam. According to Jambert et al. (1997), gaseous \textit{N} losses from a mineral fertilised maize field can have the following shares: 1\% as NH\textsubscript{3}, 40\% as NO\textsubscript{3} and 46\% as \textit{N}_2. Hence, NO emissions can be in the same order of magnitude as \textit{N}_2 and explain another substantial fraction of the missing \textit{N}. Nelissen et al. (2014) tested several fertiliser types and found not only reduced cumulative \textit{N}_2\textit{O} (52\%–84\%) emissions with biochar but also reduction in NO (47\%–67\%). They explained the reduced emissions by increased NH\textsubscript{3} volatilisation, microbial \textit{N} immobilisation and non-electrostatic sorption of NH\textsubscript{4} and NO\textsubscript{3} as well as pH effects. However, our data do not suggest that there were large changes in \textit{N} immobilisation (due to high fertiliser input) or sorption on biochar, because we did not observe changes in soil \textit{N} content or plant N uptake.

With regard to fertiliser-derived \textit{N}_2\textit{O} our observations suggest that biochar application did not alter the \textit{N} source for \textit{N}_2\textit{O} production in soil. This is the first study to show that the \textit{N} source for \textit{N}_2\textit{O} 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 \textit{N} for microbial \textit{N}_2\textit{O} production. Thus the (unknown) processes responsible for \textit{N}_2\textit{O} emission reduction may not be fertiliser specific. Further, this finding indicates that reduced \textit{N}_2\textit{O} emissions by biochar only depended on increased \textit{N}_2\textit{O} reduction (i.e. increased nosZ activity) but did not decrease the amount of \textit{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 silt 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 \textit{N} fertiliser use efficiency or \textit{N} partitioning among the 3 ecosystem components (soil, plants or leachate) over the course of two years. Biochar treatment caused a decrease in \textit{N}_2\textit{O} emissions but no change in the source of \textit{N} for
N$_2$O production. Although the observed effects due to biochar application (i.e. reduced N$_2$O 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$_2$O 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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