Pigeon pea biochar addition in tropical Arenosol under maize increases gross nitrification rate without an effect on nitrous oxide emission

J. L. Munera-Echeverri, V. Martinsen, P. Dörsch, A. Obia, J. Mulder

Pigeon pea biochar addition in tropical Arenosol under maize increases gross nitrification rate without an effect on nitrous oxide emission

Munera-Echeverri J.L.[ab*], Martinsen V.[a], Dörsch P.[a], Obia, A.[c], Mulder, J.[a]

[a] Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, P.O. Box 5003, 1432 Ås, Norway.

[b] INRAE, InfoSol, 45075, Orléans, France

[c] Department of Agronomy, Faculty of Agriculture and Environment, Gulu University, P.O Box 166, Gulu, Uganda

*Corresponding author: jose-luis.munera-echeverri@inrae.fr

Abstract

Aims: To assess how biochar addition in rainfed conservation agriculture affects short-term transformation, plant uptake, retention of nitrogen (N) in soil, and nitrous oxide (N_2O) fluxes in a tropical Arenosol planted to maize. Methods: A ten-day *in situ* ^{15}N pool dilution and N cycling experiment, using tracer amounts (0.1 g m^{-2}) of ^{15}N labeled ammonium ($^{15}NH_4^+$), nitrate ($^{15}NO_3^-$) or ^{15}N-urea, was carried out seven weeks after planting of maize (*Zea mays* L.) under conservation agriculture in Zambia, using planting basins without (CA) and with pigeon-pea biochar (BC) addition (4 t ha^{-1}). Results: Pigeon-pea biochar increased soil NO_3^- concentration, gross nitrification rate, ^{15}N recovery in extractable soil NO_3^-, and soil moisture. However, effects of biochar on soil N retention and plant N uptake were not significant. Likewise, biochar did not affect N_2O fluxes. Conclusions: At low dosage, pigeon pea biochar has a positive effect on gross nitrification rate but does not affect short-term N retention in soil, N_2O fluxes, nor does it help increasing the uptake of N by maize.

Key words: ^{15}N, N_2O, pool dilution, biochar, maize, conservation agriculture

Introduction

Minimum tillage, mulching and crop rotation with legumes are the main pillars of conservation agriculture, which has been suggested to be more productive and sustainable than conventional practices in sub-humid regions of Sub-Saharan Africa (Thierfelder et al. 2017; Thierfelder et al. 2015). Among smallholders in Zambia, conservation agriculture using planting basins or rip lines are advocated as an alternative for conventional tillage. Basins occupy only ~10% of the land area, and appear to be effective with respect to rainwater harvesting (Obia et al. 2020; Thierfelder and Wall 2009). In planting basins, soil amendments and fertilizers can be placed in the direct vicinity of plant roots, making soil amelioration more effective. In general, soils in SSA are acidic and poor in nutrients and often smallholder farmers do not have the resources to purchase mineral fertilizer (Edmonds et al. 2009). Therefore, sustainable management practices should have a particular focus on limiting nutrient losses, especially nitrogen (N).

Biochar is a pyrolyzed organic material derived from organic feedstock. Biochar consists of a porous carbonaceous matrix and ash (Budai et al. 2014; Munera-Echeverri et al. 2018) and has the potential to ameliorate degraded soils and avoid nutrient losses (Angst et al. 2013; Clough et al. 2013; Major et al. 2012). In previous studies in Sub-Saharan Africa (SSA), biochar addition to soil was shown to increase plant available water, soil organic carbon (SOC), and potassium (K^+), while increasing plant biomass and crop yield (Abiven et al. 2015; Cornelissen et al. 2013; Kätterer et al. 2019; Munera-Echeverri et al. 2020; Obia et al. 2020). Biochar addition typically increases water retention in (micro) pores (Obia et al. 2020; Obia et al. 2016) and was shown to enhance soil aggregation (Obia et al., 2016). The ash contained in biochar neutralizes soil acidity and adds important plant nutrients to the soil, particularly K (Cornelissen et al. 2018; Jeffery et al. 2017; Martinsen et al. 2015; Martinsen et al. 2014). The carbonaceous matrix of biochar contributes to the retention of cations such as ammonium (NH_4^+), due to its negative charge (Munera-Echeverri et al. 2018). Retention of anions such as nitrate (NO_3^-) has been reported in some cases and was attributed to base functional groups (Clough et al. 2013; Nguyen et al. 2017), but this was not confirmed by other studies (Hale et al. 2013). Also, biochar can decrease the availability of NH_4^+ and NO_3^- due to immobilization by soil microbes (Liu et al. 2018; Nguyen et al. 2017). Potentially this could impact losses of N from the plant-soil system as well as N_2O emissions.

Multiple studies have reported smaller N_2O emissions from biochar amended soils, suggesting that biochar has the potential to mitigate N_2O emissions (Borchard et al. 2019; Cayuela et al. 2014; Obia et al. 2015). Apart from affecting substrate availability for N_2O, biochar-mediated changes in soil pH impact the two main pathways for N_2O emissions, i.e. nitrification and denitrification. Increases in soil pH due to biochar addition (Martinsen et al. 2015), result in a decrease in the $N_2O/(N_2+N_2O)$ ratio during denitrification (Obia et al. 2015; Weldon et al. 2019), whereas the N_2O yield of nitrification, $N_2O/(NO_2^-+NO_3^-)$ increases (Hink et al. 2017; Tzanakakis et al. 2019). In addition, biochar has been suggested to reduce N_2O emissions by acting as a redox mediator for N_2O reduction to N_2 in soil (Cayuela et al. 2013). Overall, it is worth noting that the above listed benefits of biochar are not universal because the effects are controlled by a multitude of factors including biochar feedstock, production method, as well as site edaphic and climatic conditions. This explains why biochar has been reported to have positive as well as negative effects on N_2O emission (Biederman and Harpole 2013; Cayuela et al. 2014).

Few studies have focused on effects of biochar on soil N losses, N transformations and N_2O emissions in in maize under conservation agriculture in SSA. Crops, combining high yields and significant biomass such as pigeon peas (*Cajanus cajan*) with its woody biomass, were found to provide abundant feedstock for biochar (Obia, 2019). Also, few studies have focused on the effects of biochar on short-term N transformations (days to weeks) in the soil-plant (maize) system in the initial phase of the growing season. This is a critical phase for N losses (either via leaching or gaseous loss) due to the high demand of N

required for crop development. In addition, the study of short-term N transformations upon biochar addition in the presence of crops can improve the understanding of the interaction between biochar and NO_3^- and NH_4^+ in the plant-soil system. The effect of biochar on N transformations after the application of urea is also of interest since urea is common as a top-dressing in maize in SSA.

Here, we study the effects of biochar addition in basin tillage on the rate of gross nitrification, retention of ^{15}N in soil and ^{15}N uptake in maize, using ^{15}N tracing. In addition, we studied the effect of biochar on N_2O fluxes.

The following hypotheses were tested: 1) Biochar accelerates N transformations, particularly nitrification that constitutes a "bottleneck" in N cycling by controlling the conversion of NH_4^+ to more mobile NO_3^-. 2) Biochar addition in basins reduces N losses from the plant-soil system either by increasing soil nutrient retention (e.g. sorption) ability or by enhancing plant N uptake. 3) Biochar decreases N_2O emissions.

Materials and Methods

Biochar

Biochar was prepared from pigeon pea stems in a Kon-Tiki kiln (Cornelissen et al. 2016). The temperature in the kiln at 3-5 cm below the flame curtain, measured using a Fluke 51 II Digital thermometer, equipped with an 0.8 m external sensor probe, (max temp 1372 °C) varied between 600-750˚C. The biochar had a cation exchange capacity (CEC) of 6.6 $cmol_c\ kg^{-1}$, pH 10.4 and total C and N of 56.1% and 0.69, respectively. Other chemical characteristics of the biochar, analyzed following Munera-Echeverri et al. (2018), can be found in Table S1.

Research site and experimental design

The ^{15}N labeling experiment was conducted on a smallholder farm in Kaoma, western Zambia in January 2017. The average annual precipitation and temperature for the area are 930 mm and 20.8 ˚C, respectively (Obia et al. 2016). The soil, an Arenosol (WRB 2015), is slightly acidic (pH 6.1), contains 89% sand, 3.5% clay and 7.5% silt, is low in soil organic matter (0.5% total organic C, 0.04% total N) and exchangeable cations (1.9 cmol (+) kg^{-1}) and has a bulk density (BD) of 1.6 g cm^{-3}.

Land use prior to the experiment was four years of conservation agriculture, with planting basins, crop residue retention and crop rotation (maize-*Arachis hypogaea*). Land preparation for the experiment was done in October 2016. This included planting basins, constructed by hand with a Chaka hoe, and where crop residues were left at the soil surface (in the study this is referred to as CA). In selected plots, pigeon pea biochar was added to the planting basins (referred to as BC; Fig. S1). Each of the two management types (CA and BC) were assigned to one plot per block, with three blocks in total (Fig. 1). The third plot of each block was conventionally tilled, and is not included in the present study (Fig 1). The three blocks planted with maize (*Zea mays*, PAN 53, Pannar) on December 1, 2016, were surrounded by maize of the same variety. Each plot was about 20 m^2 and accommodated seven rows. Each row had four planting basins

with three maize plants in each basin. The total plant population was the same for both management types. The basins were 0.2 m wide, 0.3 m long and 0.2 m deep in accordance with local recommendations of CA (CFU 2011). The distance between basins was 0.7 m and between rows 0.9 m (15873 basins ha^{-1}, covering ~10% of the land area). In the BC plots, biochar was applied manually to the basins, placed at a depth between 5 and 20 cm, and mixed with soil and fertilizer using a hoe. The biochar-soil mixture in the basin was covered with some soil to reduce erosion, seeds were added and covered with additional soil to fill up the basin. All BC plots received 250 g biochar per basin, equivalent to 1.6% w w^{-1} or 4.0 t ha^{-1}. The 4.0 t ha^{-1} biochar applied in the basins would amount to 40 t/ha if the same biochar concentration was applied to entire land surface. Therefore, basin tillage reduces the amount of biochar required for field application. The CA and BC basins received 16±1.5 g NPK (10% N, 20% P_2O_5, 10% K_2O) at planting (this amounts to 250 kg NPK fertilizer ha^{-1}) applied on December 1, 2016. Topdressing with urea (92 kg N ha^{-1}, as urea) was applied to all treatments inside basins on January 27, 2017, after completion of the 10-day ^{15}N labeling experiment.

^{15}N application

The 10-day ^{15}N labeling experiment started 7 weeks after planting, on January 16, 2017. The experimental setup was a split-plot design, with each plot divided into 4 split-plots, corresponding to the three forms of applied ^{15}N and the unlabeled control (water addition only) (Fig. 1). The three forms of applied ^{15}N were $NH_4{}^{15}NO_3$ (referred to as $^{15}NO_3^-$), $^{15}NH_4NO_3$ (referred to as $^{15}NH_4^+$) and ^{15}N-Urea (Fig. 1). In all six plots, each of the three ^{15}N tracers (99.98 atom%) and the reference (H_2O) were assigned to one row (Fig. 1), while one buffer row of maize plants was kept in between labeled rows to avoid cross-contamination. In all CA and BC plots, of the four basins per row only two were selected for treatment with $NH_4{}^{15}NO_3$, $^{15}NH_4NO_3$, ^{15}N-Urea and water, respectively. We added 0.1g ^{15}N m^{-2} (0.2 g N m^{-2} and 0.1 g N m^{-2} when added as NH_4NO_3 and urea, respectively), amounting to a total amount of 6.0 mg ^{15}N added per basin (0.3 mg ^{15}N kg^{-1}). Labeled NH_4NO_3 and urea were added dissolved in distilled water (24.0 mg ^{15}N L^{-1}) by spraying 250 ml evenly on the soil surface (Fig. S2 a). This volume was equivalent to about 4 mm. Subsequently, 15 mm of N-free groundwater was added to wash the label into the soil. The reference received an equivalent volume (19 mm, which is similar to the commonly received rainfall events in the region) of water.

Sampling and analyses of soil, N_2O and biomass

Soil samples were taken 1.5, 24, 72 and 240 hours after ^{15}N addition from 0-5 cm (n=96) and 5-20 cm (n=96). For each sampling, a bulked sample from each of the two labeled basins was taken. Bulked soil samples consisted of seven (0-5 cm) or three (5-20 cm) cores taken with an 8 mm diameter auger, which

we used to minimize the disturbance of the soil caused by repeated sampling. Different number of individual cores to obtain the composite samples were needed to reach the same amount of soil required for the chemical analyses. The samples were stored in a cooling box on ice and sub-samples were extracted on-site, within 4 hours with 1M KCl (Yu et al. 2017). The remaining soil was dried at 40˚C for one week to determine the gravimetric moisture content. Also, we measured volumetric moisture content at 0-10 cm depth in all split-plots, using a hand-held TDR (Hydraprobe; Stevens Water Monitoring Systems, USA), from 24 hours onwards. Dried soil samples were sieved (2 mm), milled in a mechanical mortar and packed into 8 x 5mm tin capsules and shipped to the Stable Isotope Facility, of the University of California, Davis for ^{15}N analysis. Total N and total C (all C is considered as organic carbon due to the absence of carbonates) were determined using an Elementar Vario EL Cube elemental analyzer (Elemental Analysensysteme GmbH, Hanau, Germany) interfaced to an isotope ratio mass spectrometry (IRMS) to analyze ^{15}N. The large combustion columns of the Elementar Vario EL Cube systems allows using big bulk samples. The weight of the sample was optimized according to the N content, which was about 75±0.818 mg g^{-1} corresponding to about 30.00±6.51 µg N. The amount of dry soil in each bulked soil sample and the volume of the auger were used to estimate an average BD of the four soil sampling events, for each split-plot at 0 to 5 cm and 5 to 20 cm depth. Although a small auger was used, the seven and three samples for 0 to 5 cm and 5 to 20 cm depth, respectively, repeated four times, allowed good estimation of BD. For validation, the BD estimates were compared with the values obtained by Obia et al. (2017), who studied the effect of biochar on soil physical parameters in a nearby Arenosol in Kaoma, Zambia. The dried and sieved soil samples were analyzed for exchangeable base cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) in ammonium acetate at pH 7 (Schollenberger 1945). Exchangeable acidity (H^+) was determined by back titration with sodium hydroxide to pH 7. Plant available phosphorus was determined by the ammonium lactate (P-AL) method described by Krogstad et al. (2008). Soil pH was determined in 0.01 M $CaCl_2$ using a solid to solution ratio of 1:2.5.

The KCl extraction of NO_3^- (KCl-NO_3^-) and NH_4^+ (KCl-NH_4^+) were done in a make-shift laboratory on site by adding 11 g of field moist soil and 40 ml of 1 M KCl to 50 ml centrifuge tubes. The tubes were shaken horizontally at 200 strokes per minute for one hour and filtered using Whatman filters grade 589/3 (Fig. S2 b). The supernatants were frozen immediately and transported to the Norwegian University of Life Sciences (NMBU), where NO_3^- and NH_4^+ contents were analyzed by flow injection analysis (FIA star 5020, Tecator, Sweden). ^{15}N abundance in NO_3^- was determined following the denitrifier method of Zhu et al. (2018), which converts NO_3^- quantitatively to N_2O before analyzing ^{15}N by PreCon- GC-IRMS (Thermo Finnigan MAT, Germany) at NMBU.

Plant samples were taken 10 days after applying the label by collecting the aboveground biomass and digging out the entire root system of the three plants of one of the labeled basins. The roots were washed

in the field (Fig. S2). Maize plants were cut at brace root height and split into roots, stems, and leaves and the fresh biomass recorded. Plant samples were taken to the University of Zambia (UNZA), where they were oven-dried at 70˚C and ground. The dry biomass was weighed, and the samples were transported to NMBU to be milled in a horizontal ball mill and weighed in tin capsules for ^{15}N analysis at University of California, Davis using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 IRMS. The sample weight was 4.474±0.419 mg, corresponding to 66.79±24.03 µg N. At the end of the growing season, maize yield, as well as total aboveground biomass were measured for each of the plots and corrected for plant removal during the experiment.

Fluxes of N_2O were measured 24 hours before and 1.5, 24, 48, 72, 120, and 240 hours after ^{15}N addition (n=252). A closed static chamber of 143 cm^2 (13.5 cm diameter) and 1.9 L headspace was gently pressed inside the planting basins. N_2O fluxes were measured in the four split-plots of each plot. Gas samples were collected using a 20 ml syringe coupled to a 3-way valve; gas samples were transferred to pre-evacuated 10 ml glass vials crimp-sealed with a butyl septum (Chromacol). Samples were taken 1, 15, 30 and 45 minutes after chamber deployment. The temperature inside the chambers was recorded at the beginning and the end of chamber deployment. The glass vials were shipped to Norway and analyzed for N_2O by automated gas chromatography (GC Model 7890A, Agilent, USA). The N_2O fluxes were estimated by linear regression of N_2O concentration change over time and calculated as µg N_2O-N m^{-2} h^{-1}. N_2O fluxes during the ten-day experiment were cumulated split- plot-wise using linear interpolation (Buchen et al. 2017).

Calculations

Analysis of ^{15}N in KCl-extractable NO_3^- and bulk soil

The atom% ^{15}N of KCl-NO_3^- was calculated according to Stevens and Laughlin (1994), using the mass to charge ratios (m/z) 45 and 46 of the N_2O with a non-random distribution to account for double substituted $^{15}N_2O$ produced in the denitrifier method:

$$\text{Atom\% } {}^{15}NO_3 = 100\frac{{}^{45}R+2\,{}^{46}R-\,{}^{17}R-2\,{}^{18}R}{2+2\,{}^{45}R+2\,{}^{46}R} \quad (1)$$

where ^{45}R is the ratio of the ion currents (I) at m/z 45 and 44 ($^{45}I/^{44}I$); $^{46}R = {}^{46}I/^{44}I$; ^{17}R ($^{17}O/^{16}O$) = 3.8861 x 10^{-4}; ^{18}R ($^{18}O/^{17}O$) = 2.0947 x 10^{-3}. Oxygen isotopes were assumed to be at natural abundance.

Atom% ^{15}N excess values of NO_3^- (atom% $^{15}N_{NO3-}$) and bulk soil (atom% $^{15}N_{soil}$) were calculated by subtracting the atom% ^{15}N of the non-labeled reference treatments.

^{15}N mass balance after 240 hours

The mass of ^{15}N recovered (g m^{-2}) in each N pool 240 hours after application was calculated as:

$$Mass\ {}^{15}N = X_{sample} * N_{content} * Mass \quad (2)$$

where X_{sample} is the ^{15}N fraction in the sample calculated as suggested by Providoli et al. (2005):

$$X_{sample} = \frac{F_{sample} - F_{reference}}{F_{tracer} - F_{reference}} \quad (3)$$

Here, F_{sample} is the fractional abundance of ^{15}N in the samples ($^{15}N/(^{15}N + ^{14}N)$), while $F_{reference}$ is the fractional abundance of ^{15}N in the reference treatments (~0.4 atom %) and F_{tracer} is the fractional abundance of applied tracer (0.9998, i.e. 99.98 atom%). The $N_{content}$ is the concentration of N in plant material, bulk soil and KCl-extractable NO_3^- (g g^{-1}), respectively. Mass is the total plant biomass, soil mass and KCl-extractable NO_3^- per unit area of basin. The ^{15}N recovery (%) is given relative to the amount of ^{15}N applied (0.1g ^{15}N m^{-2} basin). The residual ^{15}N remaining in the soil was defined as the ^{15}N content in bulk soil minus the amount of ^{15}N recovered in the KCl-NO_3^- pool. Note that soil residual ^{15}N includes ^{15}N in the NH_4^+ pool.

Gross nitrification

Rates of gross nitrification and gross NO_3^- consumption were estimated based on ^{15}N pool dilution and the NO_3^- mass balance (Kirkham and Bartholomew 1954) in the $^{15}NO_3^-$treatments. The abundance of ^{15}N in KCl-extractable NO_3, sampled at 24 and 72 hours after label application, was used to estimate gross nitrification rates. These time points were chosen as they best fulfilled the assumptions of the ^{15}N pool dilution technique (viz. homogenous ^{15}N distribution, no or little re-mineralization of assimilated ^{15}N and uniform distribution within the soil profile (Davidson et al., 1991). The equation for estimating gross nitrification rates assumes that gross production of NO_3^- (m) equals immobilization (i), i.e. no change in concentration of NO_3^- over this time lapse (Kirkham and Bartholomew 1954; Yu et al. 2017). The equation is:

$$m = i = (M_0/t)\log(H_0/H) \quad (4)$$

where M_0 represents the size of the $^{14+15}NO_3^-$ pool, t time in days, H_0 mass of $^{15}NO_3^-$ at the start and H mass of $^{15}NO_3^-$at the end of the period.

Statistical analysis

Statistical analyses were performed using the packages lme4 and lmerTest of R software (R-Core-Team 2020). The effects of biochar on soil properties, biomass, grain yield, the average N_2O flux, soil KCl-NH_4^+, soil KCl-NO_3^-, and effect of biochar and N form on ^{15}N recovery were tested by using linear mixed effect models with block as a random factor. Soil parameters at 0 to 5 cm and 5 to 20 cm were analyzed separately. The model for gross nitrification included biochar application and depth as fixed factors and block as random factor. Further linear mixed effect models were used to test differences between biochar application, forms of added ^{15}N and the change over time of the weighted average of KCl-NO_3^- and KCl-NH_4^+ at 0 to 20 cm soil depth, atom% $^{15}N_{soil}$ and atom% $^{15}N_{NO3-}$ at 0 to 5 cm and 5 to 20 cm, respectively, ^{15}N recovery in KCl-NO_3^-, and N_2O fluxes. N form, nested in biochar application, nested in block was used as random factor. The most parsimonious models were chosen based on the Akaike information criterion

(AIC) and R^2 values. Model checking was based on plotting (histograms and QQ plots) and visual inspection of residuals, fitted values and predicted random effects to assess normality and potential outliers. Also, Shapiro-Wilk test of the residuals was performed (α=0.05). N_2O fluxes, KCl-NO_3^- and KCl-NH_4^+ were ln transformed to fulfill model assumptions. The spatial autocorrelation between repeated measurements was assumed constant between the different treatment combinations. Differences between treatments were assessed by least-squares means using the function difflsmeans of the package lmerTest (Kuznetsova et al. 2017).

Results

The total precipitation during the 10 days of the experiment was 116 mm (Fig. 2.), occurring mostly as nocturnal rainfall except for January 17 (24 h after ^{15}N addition), when it rained during sampling. Gravimetric soil moisture was consistently and significantly higher in BC (7.7% to 15.3%) than in CA (7.6% to 11.5%; *p<0.01*), as well as the volumetric moisture content measured independently by TDR from 24 hours onwards (9.4% to 23.6% in BC and 8.5% to 21.4% in CA; Fig. S3).

Biochar characteristics, soil properties and maize biomass

Between 5 and 20 cm soil depth (where biochar was applied; Table 1), SOC concentrations were significantly greater in BC as compared to CA (*p = 0.02*; Table 1). Pigeon pea biochar is depleted in N, having a C/N ratio as high as 81 (Table S1), and its addition to the soil resulted in a no significant increase of N stock (Table 2), despite the low but significant increase in total N concentration at 5 to 20 cm (Table 1). The difference in C stock between BC and CA (~ 1.0 t C ha^{-1}; Table 2) suggest that about 42% of the added biochar (2.2 t ha^{-1}) was recovered. BD of BC plots was significantly lower than BD of CA (*p = 0.04*; Table 1). Biochar did not significantly increase soil pH, exchangeable cations, and P-AL (Table 1).

At the end of the ^{15}N experiment, i.e. 10 days after label addition and 8 weeks after planting, the maize plants were at the phenological stage of stem elongation [BBCH 35-37; Lancashire et al. (1991)]. At this growth stage, root and aboveground biomass were greater in BC (0.50 and 1.96 t ha^{-1}, respectively) than in CA (0.38 and 1.54 t ha^{-1}, respectively; Table 3), but the differences were not significant. Biochar did not have a significant effect on maize yield at the end of the growing season (*p=0.372*, Table 3).

KCl-extractable soil mineral N

The sum of KCl-extractable NO_3^--N and NH_4^+-N was up to 90 times greater than the added amount of labeled N (~0.3 mg ^{15}N kg^{-1} in 20 cm; Fig. 3). In general, KCl-NH_4^+ prevailed over NO_3^-, seven weeks after fertilization, irrespective of biochar addition (Fig. 3). On average during the four sampling events, the weighted average concentration of soil NO_3^--N was significantly greater in BC (3.2 mg N kg^{-1}, weighted average of 1.1 mg N kg^{-1} at 0 to 5 cm and 4.0 mg N kg^{-1} at 5 to 20 cm, Table 4) than in CA (0.8 mg N kg^{-1}; Table 4; *p<0.05*). Weighted average concentrations of KCl-NH_4^+ at 0 to 20 cm were not significantly

affected by biochar (9.2 mg kg^{-1} in CA vs 6.5 mg kg^{-1} in BC; *p=0.11*). Biochar did not significantly increase the total amount of mineral N (sum of KCl-extractable NO_3^--N and NH_4^+-N; Table 4). There was one conspicuous difference in NO_3^- concentration between NH_4NO_3 labeling treatments with a peak in NO_3^- concentration in the $^{15}NH_4^+$ treatments of BC at 72 hours (Fig. 3). This peak was absent in the $^{15}NO_3^-$ treatment, suggesting considerable variability between replicates. There was a significant decrease in KCl-extractable NO_3^--N and NH_4^+-N with time (*p<0.01*) in both BC and CA treatments (Fig. 3). The amounts of KCl-NO_3^- decreased significantly from 0 to 24 hours but remained stable after that until the end of the experiment, whereas the amounts NH_4^+-N were stable from 0 to 72 hours and decreased significantly at 240 hours (Fig. 3). The latter may be due to the large precipitation event on 22 January 2017, about 144 hours after the| start of the experiment.

Atom% ^{15}N excess in KCl-extractable NO_3^- and bulk soil

In both BC and CA plots, atom% $^{15}N_{soil}$ decreased significantly over time at 0 to 5 cm soil depth (*p<0.01*; Fig. 4), while the atom% excess $^{15}N_{soil}$ of the 5 – 20 cm soil layer slightly increased with time. Eventually, the atom% $^{15}N_{soil}$ in the two layers converged. This convergence occurred earlier (24 hours) and at a lower level of $^{15}N_{soil}$ (atom% excess ~ 0.025) in the $^{15}NO_3^-$ treatment than in the ^{15}N-Urea and $^{15}NH_4^+$ treatments (240 hours; atom% excess 0.05 – 0.1; Fig. 4), suggesting a more rapid downward transport of NO_3^-. Atom% excess $^{15}N_{soil}$ was not affected by biochar (*p=0.466*). As expected, atom% $^{15}NO_3^-$ in the upper 5 cm of the soil was highest in the $^{15}NO_3^-$ treatments 1.5 hours after ^{15}N addition (Fig. 4). Thereafter it converged at the two depths in both BC and CA treatments within 24 hours and it reached values close to 0 atom% excess after 240 hours (Fig. 4). Notably, the NO_3^- pool became enriched 1.5 hours after $^{15}NH_4^+$ and ^{15}N-Urea addition either in the presence or absence of biochar, mainly at 0 to 5 cm, and it decreased to close to 0 atom% excess at 240 hours.

Gross nitrification rates

The distribution of the $^{15}NO_3^-$ label in soil and KCl-NO_3^- became homogeneous after 24 hours as the atom% excess ^{15}N in both depths converged (Fig. 4 b and e). Therefore, gross nitrification rates were calculated for the interval 24 – 72 h. In this interval, there was no significant change in KCl-NO_3^- in the split-plots receiving $^{15}NO_3^-$ neither in BC nor in CA and consequently, the equation where m (nitrification) is equal to i (NO_3^- immobilization) was chosen. BC had significantly larger gross nitrification rates than CA at 5 to 20 cm (*p=0.01*, Table 4). Gross nitrification rates were significantly greater at 5 to 20 cm in BC (where the biochar was placed) than in 0 to 5 cm topsoil, which did not receive BC. Also, we obtained greater gross nitrification in BC at 5 to 20 cm by following the other set of equations proposed by Kirkham and Bartholomew (1954) where i is larger than m, and that could apply for the current experiment due to the decrease of KCl-NO_3^- from 1.5 hours to 240 hours.

^{15}N recovery in different pools after 240 hours

The form of the added ^{15}N significantly affected the total ^{15}N recovery at the end of the 10-day experiment in KCl-extractable NO_3^-, in residual soil from 0 to 20 cm (viz. ^{15}N content in bulk soil minus the amount of ^{15}N recovered in the KCl-NO_3^- pool) and in maize plants (Fig. 5). The recovery of ^{15}N was significantly smaller if added as $^{15}NO_3^-$ than as $^{15}NH_4^+$ ($p<0.007$), whereas ^{15}N recovery did not differ significantly between the ^{15}N-urea treatment and former two ($p=0.11$ and $p=0.15$, respectively). Figure 5 indicates that a major fraction of the added $^{15}NO_3^-$ and a lower fraction of ^{15}N-urea was lost from the soil during the 10 days of the experiment (63% and 34.7%, respectively), whereas ^{15}N added as $^{15}NH_4^+$ was largely recovered (90.8%; Fig. 5, Table S2). If added as $^{15}NH_4^+$ or ^{15}N-urea, ^{15}N was predominantly recovered in the residual soil ^{15}N pool (Fig. 5). By contrast, the limited amount of ^{15}N recovered after $^{15}NO_3^-$ addition was found in plant biomass and residual soil in nearly equal amounts after 10 days (Fig. 5).

There was no significant effect of biochar on the recovery of ^{15}N in soil nor in maize plants (Fig. 5). Recovery of ^{15}N in soil extractable NO_3^-, if ^{15}N was added as $^{15}NH_4^+$ or ^{15}N-Urea, was larger in BC than in CA ($p < 0.001$ and $p=0.02$, respectively; Fig. S4.). By contrast, biochar did not affect the recovery of ^{15}N in KCl-NO_3^- if ^{15}N was added as $^{15}NO_3^-$ ($p= 0.17$). The recovery of ^{15}N in KCl-NO_3^- decreased with time in plots with all three forms of added ^{15}N. After 240 hours, only the recovery of ^{15}N in KCl-NO_3^-, if added as $^{15}NH_4^+$, was positively affected by biochar, while this was not the case if ^{15}N had been added as $^{15}NO_3^-$ or ^{15}N-Urea (Fig. 5 and Fig. S4). A significant amount of the added $^{15}NH_4^+$ and ^{15}N-Urea (7.4% and 10.4%, respectively) was found in KCl-NO_3^- after just 1.5 hours, and the amount decreased with time (Fig. S4). The different N forms did affect ^{15}N recovery in maize; significantly less ^{15}N was recovered in maize in response to ^{15}N-Urea than to $^{15}NO_3^-$ ($p<0.01$) and $^{15}NH_4^+$ ($p=0.02$). In maize plants, most ^{15}N was recovered in stems, whereas smaller fractions were recovered in roots and leaves (Table S2).

N_2O emissions

The fluxes were highly variable, ranging from 1.0 to 160.6 µg N_2O-N m^{-2} h^{-1} in CA and from 0.3 to 156.6 µg N_2O-N m^{-2} h^{-1} in BC (Fig. S5). We found no significant effect ($p >0.05$) of biochar on N_2O fluxes (Fig. 6). The average flux in CA was 26.1 µg N_2O-N m^{-2} h^{-1}, while in BC this was 34.1 µg N_2O-N m^{-2} h^{-1} (Table 4). In CA, N_2O fluxes significantly dropped at 24 and 120 hours, whereas in BC, only the decrease at 120 hours was significant (Fig. 6). In CA, N_2O fluxes were not affected by the different ^{15}N treatments (Fig S5). In BC, fluxes of N_2O were larger in the $^{15}NH_4^+$ subplots than in the $^{15}NO_3$, ^{15}N-Urea and water subplots, while there were no significant differences between the three latter treatments (Fig. S5).

Discussion

In Zambia, the core of the rainy season is from December to February, a period accounting for 60% of the annual precipitation (Libanda et al. 2019). The average rainfall in January from 1960 to 2016 was 220 mm. In wet years such as 2007, the precipitation may reach 300 mm in January (Libanda et al. 2019). Therefore, our data, with 116 mm of precipitation for 10 days, indicate that the study period in January 2017 was relatively wet. We did not observe a correlation between precipitation and soil moisture since most of the precipitation occurred at night, and at the time of sampling great part of the water had already drained. The greater soil moisture content (volumetric and gravimetric) in the biochar-amended plots is in line with a recent study by Obia et al. (2020) indicating significantly greater volumetric soil moisture contents in a light-textured Acrisol amended with pigeon pea biochar in conservation agriculture. By contrast, Jeffery et al. (2015) found that biochar did not improve soil water retention in a sandy soil, which they attributed to the hydrophobicity of biochar blocking access of water into the micropores. The increased soil moisture content of BC plots in our study was likely caused by increased porosity due to reduced BD as reported by Obia et al. (2016). BD values reported by Obia et al. (2017), a in an adjacent Arenosol amended with biochar (1.27 g cm^{-3} with biochar and 1.40 g cm^{-3} without biochar), were similar to our estimated values obtained by cores at 5 to 20 cm (Table 1).

Maize biomass and soil properties

In the experiment, the N fertilizer inputs were relatively high (117 kg N ha^{-1}) compared to what is usually added by smallholder farmers in SSA, and the maize yield at the end of the season (4.7-4.9 t ha^{-1}) was substantially greater than the average yield in the region. Abiven et al. (2015) and Martinsen et al. (2014) reported yields of 1.7-3.4 t ha^{-1} and 3.8-4.2 t ha^{-1} at CA plots without and with added biochar, respectively in the sandy soils of Kaoma. Despite a tendency for greater aboveground and root biomass under biochar we found no significant effect of biochar on maize yield nor on aboveground and root biomass (Table 3). Previously, Abiven et al. (2015) reported an average grain yield increase of ~45% upon biochar addition relative to fertilized control plots at four farms in Zambia with root biomass about twice as large for plots that received biochar. The lack of significant effects of biochar in our study was probably because the 2016-2017 season was relatively wet and drought was not a limiting factor (Musonda et al. 2020). Thus, the commonly observed positive effect of biochar in combination with conservation agriculture on maize yield in these soils (Cornelissen et al. 2013; Martinsen et al. 2014), which has been attributed to increased water retention and thus increased water availability, may have been less important in this relatively wet year. We found no differences in the amount of N taken up by maize in root and aboveground biomass between CA and BC. This suggests that biochar did not immobilize added N in soil, nor did it increase N availability.

The lack of effect of pigeon pea biochar on soil pH and other soil nutrients such as Ca, Mg, K and P, may also account for the lack of a clear effect on the productivity of maize (Table 1). It is worth noting, however,

that the amount of biochar retrieved was low (42%) compared to the amount added prior to the 10-day experiment (Table 2) and thus, a significant proportion of the alkalinity and nutrients derived from biochar was not sampled. Possibly, the auger (diameter 8 mm only) used missed the larger biochar chunks while sampling. In a recent paper, Munera-Echeverri et al. (2020) found back about 90% of the added pigeon biochar in an Acrisol in Zambia 2.5 years after its application, using a soil auger with a larger diameter (50 mm). Previously, Obia et al. (2017) reported only limited downward migration of biochar in Arenosols (adjacent site to the current study) and Acrisols in Zambia upon biochar addition in the upper 0-5 cm surface layer, and the major loss of biochar was associated with lateral transfer through erosion. In the current study erosion of biochar was not likely since the biochar was applied at 5-20 depth and buried with a 5 cm-layer of soil reducing the risk of erosion and lateral losses.

Mineral N and gross nitrification

The concentration of mineral N (sum of NH_4^+ and NO_3^-) was not affected by biochar, suggesting that pigeon pea biochar did not increase N mineralization. Similar results have been reported by Munera-Echeverri et al. (2020) in an Acrisol in Zambia. However, biochar did improve the conditions for nitrification, as higher concentrations of NO_3^- and greater rates of gross nitrification were found in BC than in CA plots (Fig. 3, Table 4), which agrees with previous research (Berglund et al. 2004; Prommer et al. 2014; Sánchez-García et al. 2014). The increase in gross nitrification and NO_3^- concentration was observed at 5 to 20 cm (where most of the biochar was found, Table3). Also, the stimulation of nitrification by biochar was confirmed by the greater recovery of ^{15}N in KCl-NO_3^- in BC split-plots receiving $^{15}NH_4^+$ and ^{15}N-Urea (Fig. S4). A higher soil pH upon biochar addition may promote NH_3 over NH_4^+, thus increasing the availability of ammonia (NH_3) substrate for nitrification (Liu et al. 2018; Nelissen et al. 2012). However, in the current experiment biochar did not affect soil pH. The reason for greater gross nitrification is likely related to the effect of biochar on soil physical properties rather than its effect on soil pH or the availability of soil mineral N. Biochar may stimulate the populations of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) by increasing soil porosity and soil moisture in well-aerated soils (Nguyen et al. 2017; Prommer et al. 2014). Also, biochar may increase nitrification by adsorbing nitrifier inhibitors (Berglund et al. 2004) .

^{15}N recovery in different pools after 240 hours

Our results show that the form of added N had the greatest impact on the recovery of ^{15}N in soils and plants, while biochar did not have a significant effect (Fig. 5, Table S2). Most of the added NH_4^+ remained in the soil after 10 days, either adsorbed in soil or taken up by microbes, while a larger portion of the added $^{15}NO_3^-$ was lost likely due to leaching, considering the high rainfall during the 10-day experiment (116mm), the low water retention capacity of sandy soils (Obia et al. 2016), and that N_2O emissions were small and are not likely to account for the ^{15}N losses. In line with this, Nyamangara et al. (2003) reported high leaching

406 of NO_3^- (about 50% of the N input) in Arenosols planted to maize in Zimbabwe. Although NO_3^- has been
407 shown to have an advantage over NH_4^+ as fertilizer of cereals in semiarid climates where rainfall is low and
408 leaching is limited (E.Engel et al. 2019), our results show no difference in N recovery in maize biomass
409 between both forms of N. This may be explained by the high NO_3^- leaching and the quick conversion of a
410 significant part of the added $^{15}NH_4^+$ into $^{15}NO_3^-$.
411 ^{15}N-Urea was lost from the system likely due to hydrolysis instead of volatilization, considering the slightly
412 acidic soil (pH 6) and the moderate soil pH effect that a low ^{15}N-urea dose (0.1 g N m^{-2} applied to ~10% of
413 the land) may have. Volatilization of NH_3 at < pH 7 is minor (Rochette et al. 2013). However, losses of ^{15}N
414 due to nitrification and the subsequent NO_3^- leaching cannot be discarded. For example, van der Kruijs et
415 al. (1988) reported that 30% of the added ^{15}N-urea fertilizer was found as $^{15}NO_3^-$ in the soil leachate in
416 maize crop in Nigeria. Our results show that about 10% of the ^{15}N added as urea and NH_4^+ can be quickly
417 converted into NO_3^- (Fig. S4) within hours after addition. In SSA, this quick N transformation (within
418 hours) of added ^{15}N into $^{15}NO_3^-$ had been reported in forest soils in Congo (Rütting et al. 2015). To our
419 knowledge, the current study is the first showing high initial conversion of added ^{15}N-Urea and $^{15}NH_4^+$ in
420 agricultural soils in SSA. Hence, our results are in line with previous research showing significant NO_3^-
421 leaching and high nitrification potential in SSA soils, including Arenosols. However, not all the non-
422 recovered ^{15}N can be considered as a loss from the system because maize roots can take up N at a depth
423 greater than 20 cm, and we did not study beyond that.
424 The main effect of biochar was observed in the recovery of ^{15}N (added as $^{15}NH_4^+$ and ^{15}N-Urea,) in KCl-
425 NO_3^-, which had little contribution to the total ^{15}N mass balance (Fig. 5). Biochar did not affect ^{15}N loss
426 from the plant-soil system (Fig. 5). Previous studies have reported lower NH_4^+ and urea loss in soil columns
427 amended with biochar (Ding et al. 2010; Shi et al. 2020), as well as lower NO_3^- leaching in field and column
428 studies (Angst et al. 2013; Major et al. 2012). However, our study showed that pigeon pea biochar added at
429 a rate of 1.6% (by mass) did not avoid losses of any of the forms of added ^{15}N in the short-term. One
430 explanation is the low CEC of pigeon pea biochar (6.6 $cmol_{(+)}kg^{-1}$; close to the actual soil CEC) in the case
431 of NH_4^+ retention, and the absence of NO_3^- retention capacity of biochar (Hale et al. 2013). In addition,
432 biochar did not affect uptake of ^{15}N in maize biomass, that was higher if added as NH_4NO_3 than as urea, in
433 agreement with previous research (Mérigout et al. 2008).
434
435 *Nitrous oxide*
436 The N_2O fluxes of the current field experiment were highly variable, and the mean values agree with earlier
437 field studies in SSA (Kim et al. 2016; Raji and Dörsch 2020; Rosenstock et al. 2016). Although biochar is
438 believed to contribute to the mitigation of N_2O emissions (Case et al. 2015; Cayuela et al. 2015; Cayuela et
439 al. 2014; Obia et al. 2015), our results show that pigeon pea biochar did not affect N_2O fluxes (Tab 3, Fig.

6, Fig. S5). Biochar's $H:C_{org}$ and C/N ratios are two parameters that help predict whether a biochar can decrease N_2O emissions (Cayuela et al. 2015; Cayuela et al. 2014). Pigeon pea biochar has a $H:C_{org}$ ratio of 0.24 and a high C/N ratio (81; Table S1). Biochars with C/N ratios > 30 have been suggested to decrease N_2O emissions by temporarily immobilizing soil N (Cayuela et al. 2014). Also, biochars with $H:C_{org}$ ratios < 0.3 have been suggested to have the greatest potential for N_2O mitigation, due to a larger aromaticity and polymerization that facilitates electron transfer to denitrifiers in the last step of denitrification, promoting the reduction of N_2O to N_2 (Cayuela et al. 2015). However, a well-drained Arenosol might not be most favorable environment for denitrification. In addition, loss of denitrification substrate (nitrate) due to leaching may have affected the N_2O flux. Thus, we conclude that pigeon pea biochar did not lower N_2O flux and reject our third hypothesis.

Our results show that despite the increased gross nitrification rate, due to biochar addition, the N_2O fluxes are not affected. This is surprising and not in accordance with other studies (Sánchez-García et al. 2014). Ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) are responsible for nitrification (Hink et al. 2017). Generally, AOA dominate over AOB in acidic conditions (Prosser and Nicol 2012). The N_2O production rate of AOA have been shown to be close to 0 at pH > 5.5 , and increases dramatically at pH < 4.5 (Tzanakakis et al. 2019). Thus, it is likely that AOA were dominant due to the slightly acidic soil (pH 6.1), and due to the lack of response of N_2O flux upon increased gross nitrification rates in BC plots.

Conclusions

This study confirmed the hypothesis that biochar increases gross nitrification in Arenosols in Southern Africa. Addition of pigeon pea biochar significantly increased soil extractable NO_3^- and *in situ* gross nitrification which appeared to be linked to physical effects of biochar on soil structure and water retention rather than pH increase or increased or availability of nitrification substrate. The added biochar did not increase N retention nor N uptake by the maize crop possibly because its CEC was too similar to that of the soil. Future studies into nutrient retention by biochar should therefore choose biochars with relatively high CEC compared to that of the soil. Overall, we did not find any biochar effect on N_2O emissions and thus add to the body of evidence that biochar does not invariably reduce N_2O emissions. However, neither did we find increased N_2O emissions under conditions supporting nitrification and therefore conclude that that biochar can be applied in SSA crop production for other co-benefits without increasing N_2O emissions.

Acknowledgements

We are thankful to Dr. Victor Shitumbanuma and Edward Bwalya of the Soil Science Department of University of Zambia and to Mwenya Silombe and Phillip Maskbo for their support during the field experiment. We are thankful to Martin Muti Mafomafo, the farmer who made his farm available for the

experiment, and to Matthews Chewe from the Conservation Farming Unit (CFU) in Zambia for his assistance in the field.

Declarations

Funding

The study was funded by NMBU PhD (15/02524-1750092003) internal financing to the first author and by the Faculty of Environmental Sciences and Nature Resource Management at NMBU as part of the stipend to Vegard Martinsen granted for young talented scientists. Peter Dörsch received funding from the FACCE-ERA-GAS project MAGGE-pH under the Grant Agreement No. 696356.

Conflicts of interest/Competing interests

The authors declare no conflicts of interest

Availability of data and material

The data has been included as electronic supplementary material.

Code availability

Not applicable

Table 1. Soil properties measured 7 weeks after maize planting at two depths (0 to 5 cm and 5 to 20 cm) in conservation agriculture plots with biochar (BC) and without biochar (CA). Values are mean and standard error in parentheses (n=3).

		0-5cm			5-20cm		
Soil properties	**Units**	**BC**	**CA**	***p***	**BC**	**CA**	***p***
Organic C	%	0.6(0.05)	0.6(0.06)	*0.629*	1.3(0.22)	0.5(0.04)	***0.02***
Total N	%	0.043(0.003)	0.039(0.003)	*0.096*	0.046(0.002)	0.035(0.002)	***0.03***
C:N		13.8(0.9)	14.1(0.5)	*0.717*	27.4(5.4)	13.3(0.6)	*0.11*
pH		6.4(0.02)	6.3(0.19)	*0.591*	6.2(0.06)	5.8 (0.14)	*0.11*
[1]P-AL	mg Kg^{-1}	15.3(1.3)	22.9(13.1)	*0.597*	30.3(7.2)	21.3(8.4)	*0.12*
Ca	$cmol_{(+)}$ Kg^{-1}	1.6(0.3)	1.6(0.2)	*0.741*	1.6(0.2)	1.2(0.2)	*0.19*
Mg	$cmol_{(+)}$ Kg^{-1}	0.34(0.05)	0.34(0.04)	*0.089*	0.35(0.04)	0.24(0.02)	*0.12*
K	$cmol_{(+)}$ Kg^{-1}	0.15(0.04)	0.13(0.02)	*0.368*	0.08(0.02)	0.09(0.004)	*0.59*
Na	$cmol_{(+)}$ Kg^{-1}	0.02(0.004)	0.04(0.03)	*0.438*	0.01(0.01)	0.01(0.01)	*0.85*
[2]H^+	$cmol_{(+)}$ Kg^{-1}	2.47(0.59)	2.50(0.59)	*0.422*	3.30(0.15)	3.13(0.23)	*0.58*
Sum cat.	$cmol_{(+)}$ Kg^{-1}	2.13(0.30)	2.15(0.25)	0.837	2.06(0.19)	1.55(0.19)	*0.17*
CEC	$cmol_{(+)}$ Kg^{-1}	4.59(0.31)	4.65(0.38)	*0.517*	5.36(0.29)	4.69(0.05)	*0.08*
BD	g cm^{-3}	1.45(0.02)	1.46(0.02)	*0.483*	1.21(0.07)	1.43(0.02)	***0.04***

[1] Plant available phosphorus determined by the ammonium lactate method.

[2] Determined by back titration to pH 7 with sodium hydroxide in ammonium acetate.

Table 2. Carbon and nitrogen stocks inside planting basins (~10% of the total area) in conservation agriculture with biochar (BC) and without biochar (CA). Values are mean and standard error in parentheses (n=3).

		0-20cm (weighted average)		
		BC	**CA**	***p-value***
C stock	C ton ha^{-1}	1.76(0.35)	0.82(0.06)	0.058
N stock	N kg ha^{-1}	68.27(5.13)	61.08(3.65)	0.184

Table 3. Maize root and aboveground biomass and N content at the end of the ^{15}N labeling experiment (8 weeks after planting) and maize grain yield at the end of the growing season. Differences between conservation agriculture with biochar (BC) and without (CA) are based on least-squares means at a level of significance $p < 0.05$. Values are mean and standard error in parentheses (n = 3).

[1]Eight weeks after planting		**BC**	**CA**	***p-value***
Root biomass	ton ha^{-1}	0.50(0.05)	0.38(0.05)	*0.106*
Root N	%	0.98 (0.11)	1.25 (0.12)	***0.042***
N roots	kg N ha^{-1}	4.68(0.78)	4.75(1.03)	*0.909*
Abovegr. biomass	ton ha^{-1}	1.96(0.15)	1.54(0.2)	*0.137*
Aboveground N	%	1.46 (0.09)	1.55 (0.10)	*0.088*
Aboveground N	kg N ha^{-1}	29.17(6.25)	25.06(5.41)	*0.272*
[2]End of growing season				
Grain Yield	ton ha^{-1}	4.9(0.6)	4.7(0.8)	*0.372*

[1] Measured on 26.01.2017

[2] Measured on 24.05.2017

505 **Table 4**. Average N_2O flux, concentration of nitrate and ammonium in soil, and estimated gross nitrification rates in
506 conservation agriculture with biochar (BC) and without biochar (CA) at 0 to 5 cm and 5 to 20 cm depth. Values are
507 means with standard errors. For N_2O fluxes n=21, NO_3^- n=12, NH_4^+ n= 12, and for gross nitrification n=3. The average
508 temperature during the gas sampling campaign was 26˚C. There were four sampling events for mineral N and seven
509 for N_2O fluxes. Differences between CA and BC are based on least-squares means at a level of significance $p < 0.05$.

	Units	**BC**	**CA**	***p-values***
Mean N_2O flux	µg N_2O-N m^{-2} hr^{-1}	34.1 (4.1)	26.1 (3.8)	*0.27*
Nitrate (NO_3^-) concentration				
0-5 cm	mg N kg^{-1}	1.1 (0.3)	0.8 (0.2)	***0.03***
5-20 cm	mg N kg^{-1}	4.0 (0.8)	0.8 (0.2)	*p<0.001*
Ammonium (NH_4^+) concentration				
0-5 cm	mg N kg^{-1}	4.3 (1.1)	6.8 (1.7)	***0.03***
5-20 cm	mg N kg^{-1}	7.4 (2.2)	10 (2.3)	*0.16*
Sum Mineral N (NO_3^- + NH_4^+)				
0-5 cm	mg N kg^{-1}	5.5(0.9)	7.6 (1.3)	*0.08*
5-20 cm	mg N kg^{-1}	11.2(1.9)	10.7(2.1)	*0.81*
Gross nitrification rate				
0-5 cm	mg N kg^{-1} d^{-1}	0.6 (0.60)	0.2 (0.17)	*0.75*
5-20 cm	mg N kg^{-1} d^{-1}	4.5 (1.91)	0.9 (0.78)	***0.01***

510
511

Figures

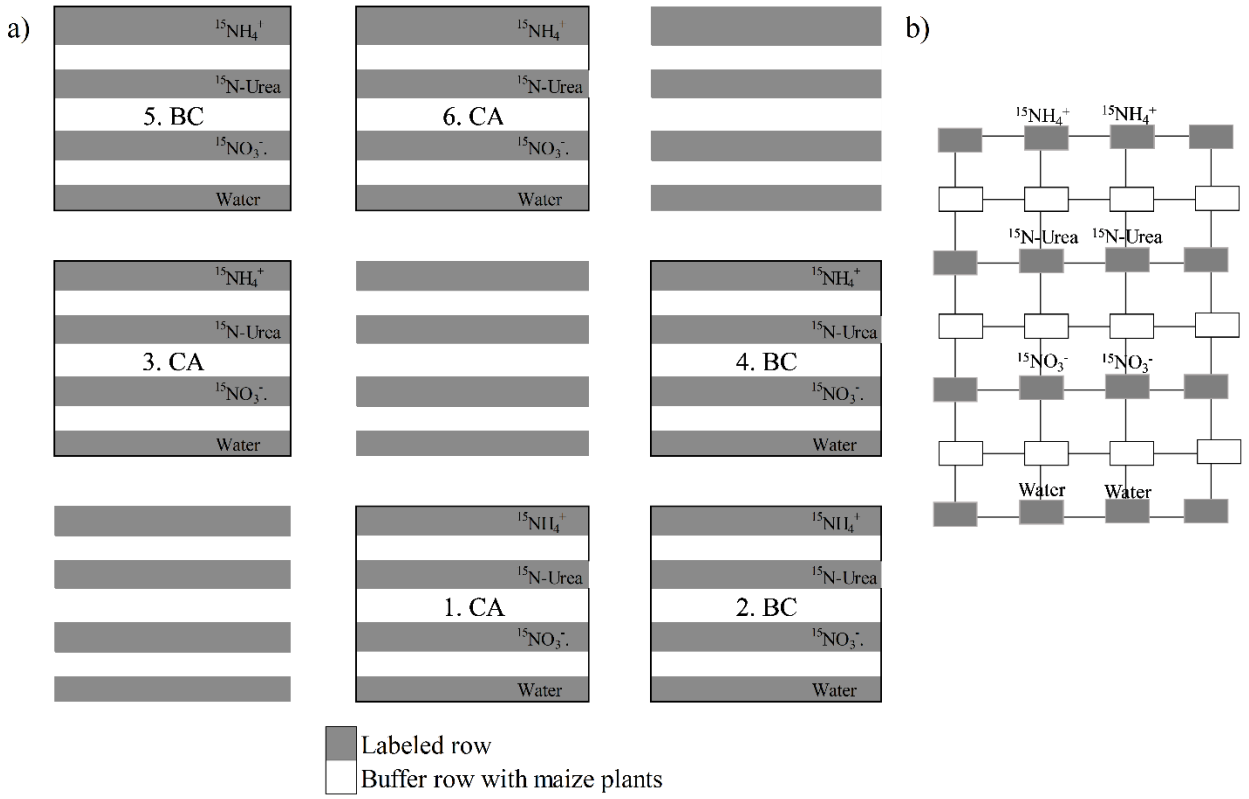


Fig. 1 a) Overall experimental set-up and detailed planting schemes used for conservation agriculture with biochar (BC) or without biochar (CA) in planting basins. CA and BC had four planting basins per row and 3 maize plants per basin. Each plot had seven rows of maize plants, represented by the grey and white colors. ^{15}N and the water control were added in the gray rows, while the white rows represent a buffer zone with maize plants present. The plots in diagonal represent the adjacent conventionally tilled plots that were not included in the present study. b) Representation of a plot with seven rows of maize plants at 90 cm distance. Each row had four planting basins at 70 cm distance. The dimensions of each basins were 0.2 m wide, 0.3 m long and 0.2 m deep, each one with three maize plants. ^{15}N addition was carried out in two basins of each labeled row

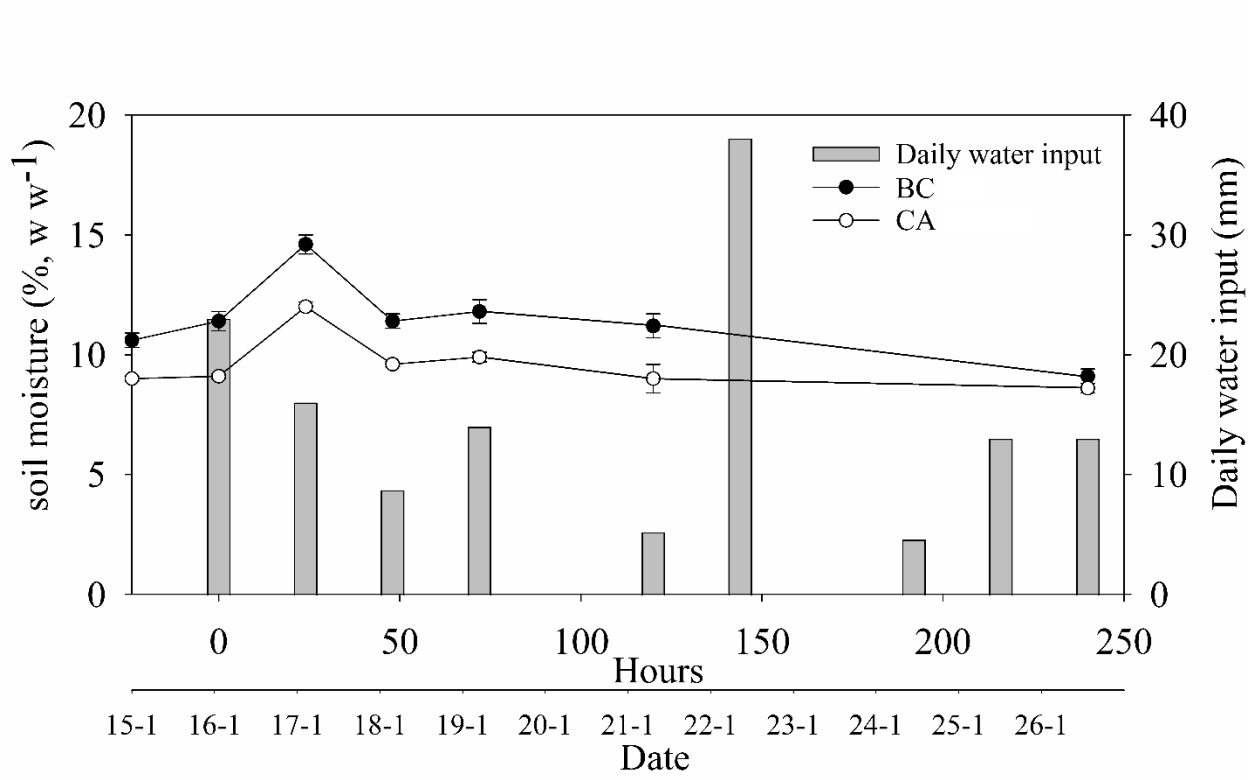
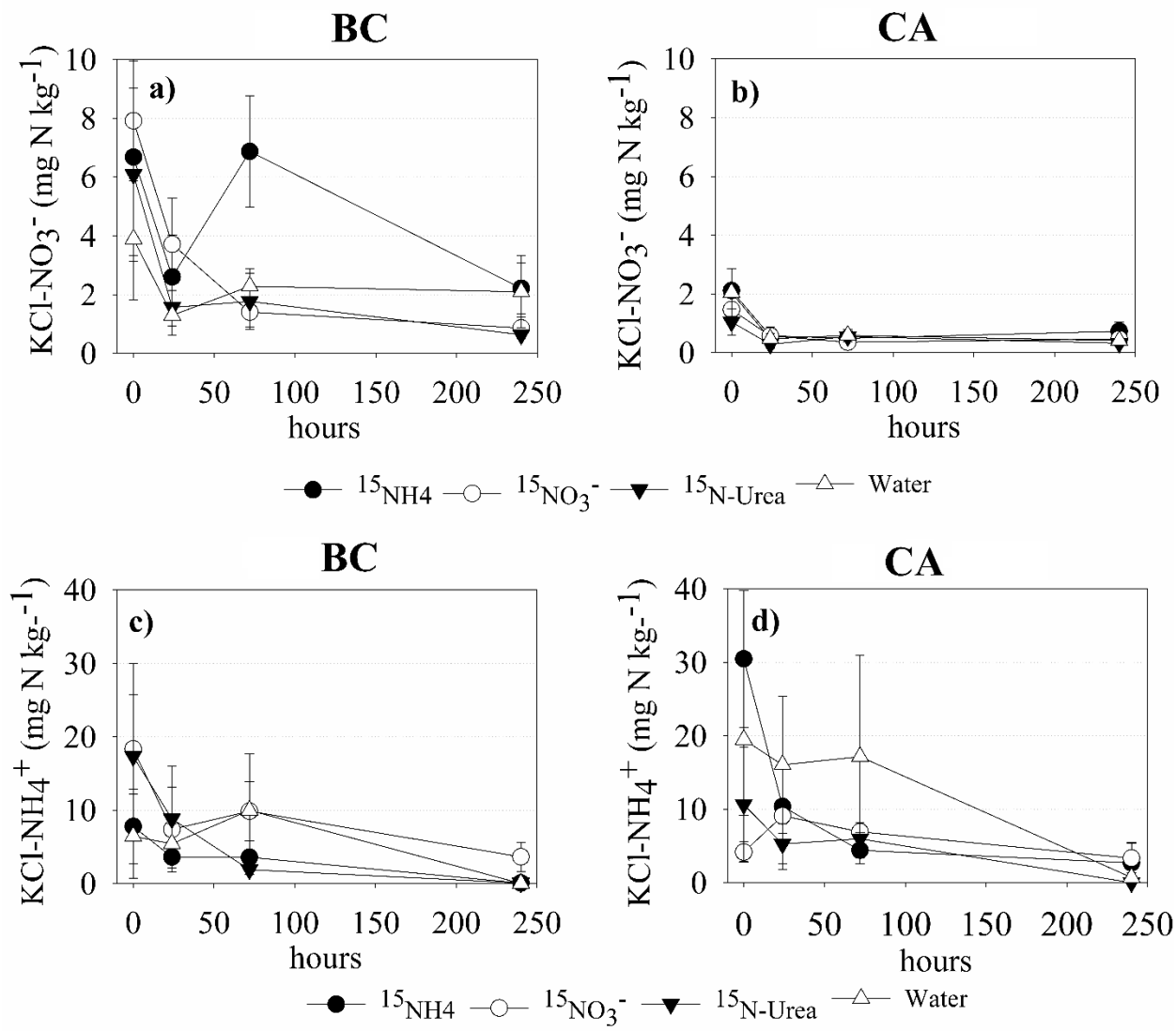


Fig. 2 Daily water input (sum precipitation and added water) and mean (with standard error bars) gravimetric (w w^{-1}) soil moisture content during the 10-days experiment. The x axis shows the hours after addition of ^{15}N label, and the date from 15.01.2017 (24 hours before ^{15}N application) until 26.01.2017 (~240 hours after ^{15}N application). On 16.1.2017 (addition of ^{15}N, 0 hour), the precipitation was 4 mm and the water used to add the ^{15}N was 19 mm. For the following nine days the daily water input was equal to the precipitation. The figure shows gravimetric soil moisture in planting basins with biochar (BC) and without biochar (CA). Soil depth average 0-20, n=12 for each day



535

536 **Fig. 3** Weighted average (0-20 cm) of KCl-NO_3^- (mg N kg^{-1}; a, b), NH_4^+ (mg N kg^{-1}; c, d) in
537 planting basins with (BC) and without (CA) biochar following the addition of $^{15}NH_4^+$, $^{15}NO_3^-$, ^{15}N-
538 Urea or water during the 240-hour labeling experiment. The initial observation of KCl-extractable
539 N was done 1.5 hours after ^{15}N application. Values are means and standard errors (n=3)

540

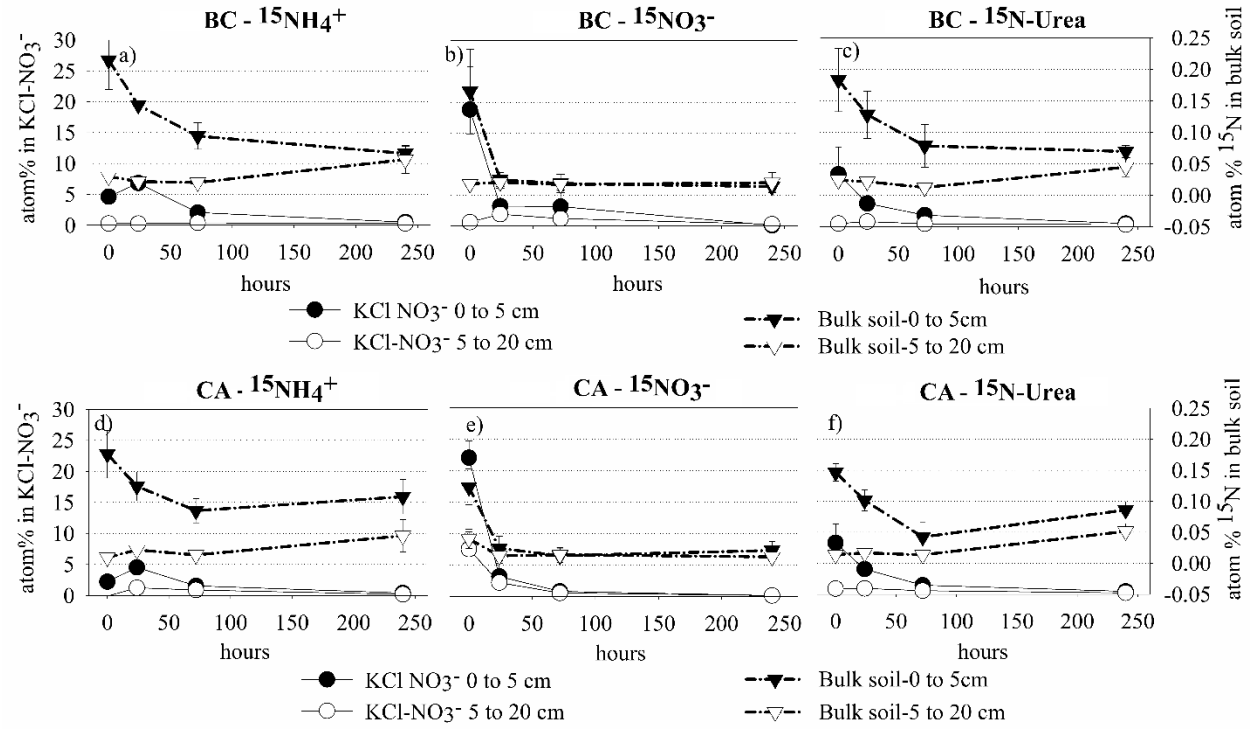


Fig. 4 Atom% excess $^{15}NO_3^-$ (circles) and atom%$^{15}N_{soil}$ (triangles) in planting basins with biochar (BC; a to c), and without biochar (CA; d to f) at 0 to 5 cm (black) and 5 to 20 cm (white) depth in response to the addition of $^{15}NH_4^+$, $^{15}NO_3^-$ and ^{15}N-Urea. Values are means and standard errors (n = 3). The initial observation of KCl-extractable N was done 1.5 hours after ^{15}N application

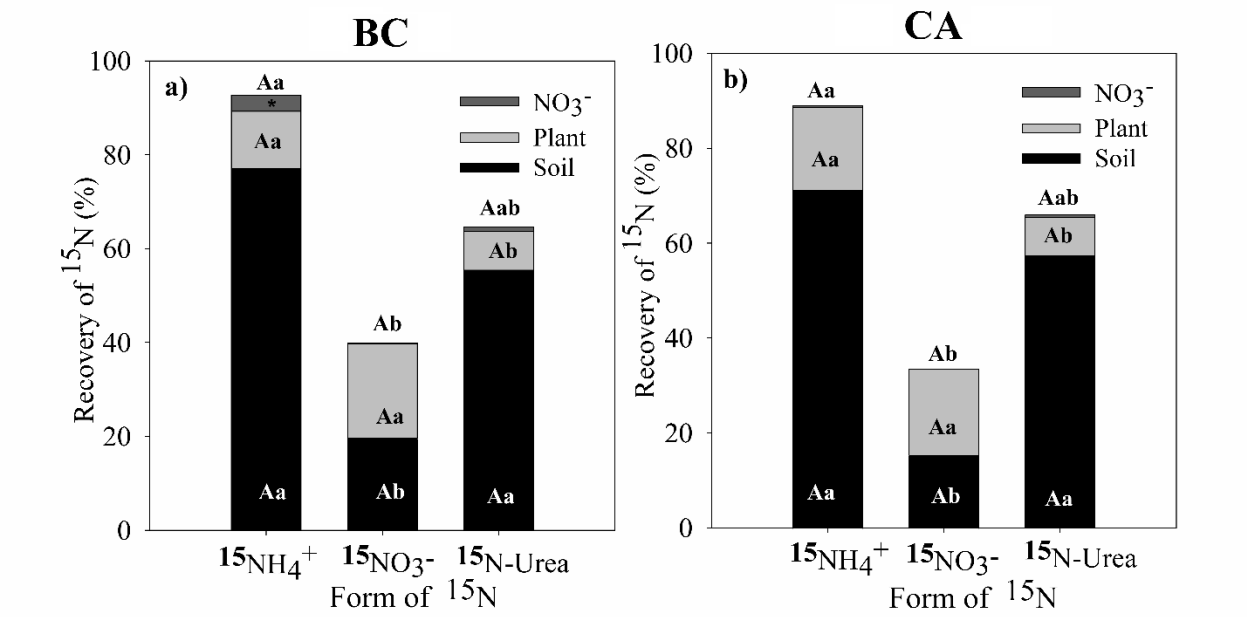


Fig. 5 Total ^{15}N recovery (%) in KCl-extractable NO_3^-, in residual soil from 0 to 20 cm and in maize plants in a) basins with biochar (BC) and b) without biochar (CA), labelled with $^{15}NH_4^+$, $^{15}NO_3^-$ and ^{15}N-Urea, respectively 240 hours after label addition. Uppercase letters indicate difference between CA and BC for each N form separately, whereas the lowercase letters indicate the difference between forms of added ^{15}N for within either CA or BC. Letters above the bars indicate the difference in total recovery (sum of soil, plant and NO_3^-). The letters inside each section of the stacked columns represent the differences between the recovery of ^{15}N either in soil or plants ($p<0.05$). Note that in most cases the recovery of ^{15}N in NO_3^- is barely visible. * Denotes significantly higher ^{15}N recovery in extractable NO_3^- for BC as compared to CA with $^{15}NH_4^+$ addition (based on least-squares means at $p < 0.05$)

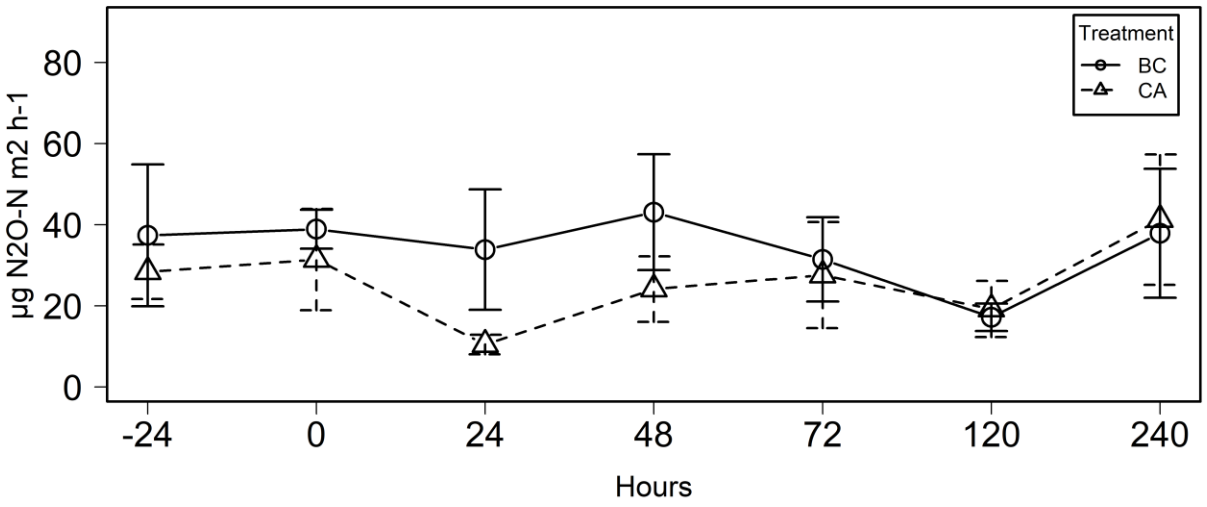


Fig. 6 Mean (+/- SE) nitrous oxide fluxes in conservation agriculture with biochar (BC) and without (CA) addition of pigeon pea biochar, from 24 hours before addition of ^{15}N to 240 hours after label addition (n=12 in each day)

References

Abiven S, Hund A, Martinsen V, Cornelissen G (2015) Biochar amendment increases maize root surface areas and branching: a shovelomics study in Zambia. Plant and Soil 395: 45-55. doi: 10.1007/s11104-015-2533-2.

Angst TE, Patterson CJ, Reay DS, Anderson P, Peshkur TA, Sohi SP (2013) Biochar diminishes nitrous oxide and nitrate leaching from diverse nutrient sources. J Environ Qual 42: 672-682. doi: 10.2134/jeq2012.0341.

Berglund LM, DeLuca TH, Zackrisson O (2004) Activated carbon amendments to soil alters nitrification rates in Scots pine forests. Soil Biology and Biochemistry 36: 2067-2073. doi: https://doi.org/10.1016/j.soilbio.2004.06.005.

Biederman LA, Harpole WS (2013) Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. GCB Bioenergy 5: 202-214. doi: https://doi.org/10.1111/gcbb.12037.

Borchard N, Schirrmann M, Cayuela ML, Kammann C, Wrage-Monnig N, Estavillo JM, Fuertes-Mendizabal T, Sigua G, Spokas K, Ippolito JA, Novak J (2019) Biochar, soil and land-use interactions that reduce nitrate leaching and N2O emissions: A meta-analysis. Sci Total Environ 651: 2354-2364. doi: 10.1016/j.scitotenv.2018.10.060.

Buchen C, Well R, Helfrich M, Fuß R, Kayser M, Gensior A, Benke M, Flessa H (2017) Soil mineral N dynamics and N 2 O emissions following grassland renewal. Agriculture, Ecosystems & Environment 246: 325-342. doi: 10.1016/j.agee.2017.06.013.

Budai A, Wang L, Gronli M, Strand LT, Antal MJ, Jr., Abiven S, Dieguez-Alonso A, Anca-Couce A, Rasse DP (2014) Surface properties and chemical composition of corncob and miscanthus biochars: effects

of production temperature and method. J Agric Food Chem 62: 3791-3799. doi: 10.1021/jf501139f.
Case SDC, McNamara NP, Reay DS, Stott AW, Grant HK, Whitaker J (2015) Biochar suppresses N2O emissions while maintaining N availability in a sandy loam soil. Soil Biology and Biochemistry 81: 178-185. doi: 10.1016/j.soilbio.2014.11.012.
Cayuela ML, Jeffery S, van Zwieten L (2015) The molar H:Corg ratio of biochar is a key factor in mitigating N2O emissions from soil. Agriculture, Ecosystems & Environment 202: 135-138. doi: 10.1016/j.agee.2014.12.015.
Cayuela ML, Sánchez-Monedero MA, Roig A, Hanley K, Enders A, Lehmann J (2013) Biochar and denitrification in soils: when, how much and why does biochar reduce N2O emissions? Scientific Reports 3: 1732. doi: 10.1038/srep01732.
Cayuela ML, van Zwieten L, Singh BP, Jeffery S, Roig A, Sánchez-Monedero MA (2014) Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis. Agriculture, Ecosystems & Environment 191: 5-16. doi: 10.1016/j.agee.2013.10.009.
CFU (2011) The Practice of Conventional and Conservation Agriculture in East and Southern Africa.
Clough T, Condron L, Kammann C, Müller C (2013) A Review of Biochar and Soil Nitrogen Dynamics. Agronomy 3: 275-293. doi: 10.3390/agronomy3020275.
Cornelissen G, Jubaedah, Nurida NL, Hale SE, Martinsen V, Silvani L, Mulder J (2018) Fading positive effect of biochar on crop yield and soil acidity during five growth seasons in an Indonesian Ultisol. Sci Total Environ 634: 561-568. doi: 10.1016/j.scitotenv.2018.03.380.
Cornelissen G, Martinsen V, Shitumbanuma V, Alling V, Breedveld G, Rutherford D, Sparrevik M, Hale S, Obia A, Mulder J (2013) Biochar Effect on Maize Yield and Soil Characteristics in Five Conservation Farming Sites in Zambia. Agronomy 3: 256-274. doi: 10.3390/agronomy3020256.
Cornelissen G, Pandit NR, Taylor P, Pandit BH, Sparrevik M, Schmidt HP (2016) Emissions and Char Quality of Flame-Curtain "Kon Tiki" Kilns for Farmer-Scale Charcoal/Biochar Production. PLoS One 11: e0154617. doi: 10.1371/journal.pone.0154617.
Davidson EA, Hart SC, Shanks CA, Firestone MK (1991) Measuring gross nitrogen mineralization, immobilization, and nitrification by 15N isotopic pool dilution in intact soil cores. J Soil Sci 42: 335-349.
Ding Y, Liu Y-X, Wu W-X, Shi D-Z, Yang M, Zhong Z-K (2010) Evaluation of Biochar Effects on Nitrogen Retention and Leaching in Multi-Layered Soil Columns. Water, Air, & Soil Pollution 213: 47-55. doi: 10.1007/s11270-010-0366-4.
E.Engel R, M.Romero C, PatrickCarr, A.Torrion J, AnneNaeth M (2019) Performance of nitrate compared with urea fertilizer in a semiarid climate of the northern Great Plains. 99: 345-355. doi: 10.1139/cjss-2019-0047.
Edmonds DE, Abreu SL, West A, Caasi DR, Conley TO, Daft MC, Desta B, England BB, Farris CD, Nobles TJ, Patel NK, Rounds EW, Sanders BH, Shawaqfeh SS, Lakmini, Lokuralalage, Manandhar R, Raun WR (2009) Cereal Nitrogen Use Efficiency in Sub Saharan Africa. Journal of Plant Nutrition 32: 2107-2122. doi: 10.1080/01904160903308184.
Hale SE, Alling V, Martinsen V, Mulder J, Breedveld GD, Cornelissen G (2013) The sorption and desorption of phosphate-P, ammonium-N and nitrate-N in cacao shell and corn cob biochars. Chemosphere 91: 1612-1619. doi: 10.1016/j.chemosphere.2012.12.057.
Hink L, Lycus P, Gubry-Rangin C, Frostegard A, Nicol GW, Prosser JI, Bakken LR (2017) Kinetics of NH3 - oxidation, NO-turnover, N2 O-production and electron flow during oxygen depletion in model bacterial and archaeal ammonia oxidisers. Environ Microbiol 19: 4882-4896. doi: 10.1111/1462-2920.13914.

632 Jeffery S, Abalos D, Prodana M, Bastos AC, van Groenigen JW, Hungate BA, Verheijen F (2017) Biochar
633 boosts tropical but not temperate crop yields. Environmental Research Letters 12: 053001. doi:
634 10.1088/1748-9326/aa67bd.
635 Jeffery S, Meinders MBJ, Stoof CR, Bezemer TM, van de Voorde TFJ, Mommer L, van Groenigen JW (2015)
636 Biochar application does not improve the soil hydrological function of a sandy soil. Geoderma
637 251-252: 47-54. doi: 10.1016/j.geoderma.2015.03.022.
638 Kätterer T, Roobroeck D, Andrén O, Kimutai G, Karltun E, Kirchmann H, Nyberg G, Vanlauwe B, Röing de
639 Nowina K (2019) Biochar addition persistently increased soil fertility and yields in maize-soybean
640 rotations over 10 years in sub-humid regions of Kenya. Field Crops Research 235: 18-26. doi:
641 10.1016/j.fcr.2019.02.015.
642 Kim DG, Thomas AD, Pelster D, Rosenstock TS, Sanz-Cobena A (2016) Greenhouse gas emissions from
643 natural ecosystems and agricultural lands in sub-Saharan Africa: synthesis of available data and
644 suggestions for further research. Biogeosciences 13: 4789-4809. doi: 10.5194/bg-13-4789-2016.
645 Kirkham D, Bartholomew WV (1954) Equations for Following Nutrient Transformations in Soil, Utilizing
646 Tracer Data Soil Sci Soc Am Proc 18: 33-34.
647 Krogstad T, Øgaard AF, Kristoffersen AØ (2008) New P recommendations for grass and cereals in
648 Norwegian agriculture. NJF Seminar: 42-46.
649 Kuznetsova A, Brockhoff PB, Christensen RHB (2017) lmerTest Package: Tests in Linear Mixed Effects
650 Models. Journal of Statistical Software 82. doi: 10.18637/jss.v082.i13.
651 Lancashire PD, Bleiholder H, van den Boom T, Langelüddecke P, Stauss T, Weber E, Witzenberger A (1991)
652 An uniform decimal code for growth stages of crops and weeds. Ann appl Biol 119: 561-601.
653 Libanda B, Zheng M, Ngonga C (2019) Spatial and temporal patterns of drought in Zambia. Journal of Arid
654 Land 11: 180-191. doi: 10.1007/s40333-019-0053-2.
655 Liu Q, Zhang Y, Liu B, Amonette JE, Lin Z, Liu G, Ambus P, Xie Z (2018) How does biochar influence soil N
656 cycle? A meta-analysis. Plant and Soil 426: 211-225. doi: 10.1007/s11104-018-3619-4.
657 Major J, Rondon M, Molina D, Riha SJ, Lehmann J (2012) Nutrient leaching in a Colombian savanna Oxisol
658 amended with biochar. J Environ Qual 41: 1076-1086. doi: 10.2134/jeq2011.0128.
659 Martinsen V, Alling V, Nurida NL, Mulder J, Hale SE, Ritz C, Rutherford DW, Heikens A, Breedveld GD,
660 Cornelissen G (2015) pH effects of the addition of three biochars to acidic Indonesian mineral
661 soils. Soil Science and Plant Nutrition 61: 821-834. doi: 10.1080/00380768.2015.1052985.
662 Martinsen V, Mulder J, Shitumbanuma V, Sparrevik M, Børresen T, Cornelissen G (2014) Farmer-led maize
663 biochar trials: Effect on crop yield and soil nutrients under conservation farming. Journal of Plant
664 Nutrition and Soil Science 177: 681-695. doi: 10.1002/jpln.201300590.
665 Mérigout P, Gaudon V, Quilleré I, Briand X, Daniel-Vedele F (2008) Urea Use Efficiency of Hydroponically
666 Grown Maize and Wheat. Journal of Plant Nutrition 31: 427-443. doi:
667 10.1080/01904160801894970.
668 Munera-Echeverri JL, Martinsen V, Strand LT, Cornelissen G, Mulder J (2020) Effect of conservation
669 farming and biochar addition on soil organic carbon quality, nitrogen mineralization, and crop
670 productivity in a light textured Acrisol in the sub-humid tropics. PLoS One 15: e0228717. doi:
671 10.1371/journal.pone.0228717.
672 Munera-Echeverri JL, Martinsen V, Strand LT, Zivanovic V, Cornelissen G, Mulder J (2018) Cation exchange
673 capacity of biochar: An urgent method modification. Sci Total Environ 642: 190-197. doi:
674 10.1016/j.scitotenv.2018.06.017.
675 Musonda B, Jing Y, Iyakaremye V, Ojara M (2020) Analysis of Long-Term Variations of Drought
676 Characteristics Using Standardized Precipitation Index over Zambia. Atmosphere 11. doi:
677 10.3390/atmos11121268.

678 Nelissen V, Rütting T, Huygens D, Staelens J, Ruysschaert G, Boeckx P (2012) Maize biochars accelerate
679 short-term soil nitrogen dynamics in a loamy sand soil. Soil Biology and Biochemistry 55: 20-27.
680 doi: 10.1016/j.soilbio.2012.05.019.
681 Nguyen TTN, Xu C-Y, Tahmasbian I, Che R, Xu Z, Zhou X, Wallace HM, Bai SH (2017) Effects of biochar on
682 soil available inorganic nitrogen: A review and meta-analysis. Geoderma 288: 79-96. doi:
683 10.1016/j.geoderma.2016.11.004.
684 Nyamangara J, Bergström LF, Piha MI, Giller KE (2003) Fertilizer Use Efficiency and Nitrate Leaching in a
685 Tropical Sandy Soil. Journal of Environment Quality 32. doi: 10.2134/jeq2003.5990.
686 Obia A, Børresen T, Martinsen V, Cornelissen G, Mulder J (2017) Vertical and lateral transport of biochar
687 in light-textured tropical soils. Soil and Tillage Research 165: 34-40. doi:
688 https://doi.org/10.1016/j.still.2016.07.016.
689 Obia A, Cornelissen G, Martinsen V, Smebye AB, Mulder J (2020) Conservation tillage and biochar improve
690 soil water content and moderate soil temperature in a tropical Acrisol. Soil and Tillage Research
691 197. doi: 10.1016/j.still.2019.104521.
692 Obia A, Cornelissen G, Mulder J, Dorsch P (2015) Effect of Soil pH Increase by Biochar on NO, N2O and N2
693 Production during Denitrification in Acid Soils. PLoS One 10: e0138781. doi:
694 10.1371/journal.pone.0138781.
695 Obia A, Mulder J, Martinsen V, Cornelissen G, Børresen T (2016) In situ effects of biochar on aggregation,
696 water retention and porosity in light-textured tropical soils. Soil and Tillage Research 155: 35-44.
697 doi: 10.1016/j.still.2015.08.002.
698 Prommer J, Wanek W, Hofhansl F, Trojan D, Offre P, Urich T, Schleper C, Sassmann S, Kitzler B, Soja G,
699 Hood-Nowotny RC (2014) Biochar Decelerates Soil Organic Nitrogen Cycling but Stimulates Soil
700 Nitrification in a Temperate Arable Field Trial. PLOS ONE 9: e86388. doi:
701 10.1371/journal.pone.0086388.
702 Prosser JI, Nicol GW (2012) Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche
703 specialisation and differentiation. Trends in Microbiology 20: 523-531. doi:
704 https://doi.org/10.1016/j.tim.2012.08.001.
705 Providoli I, Bugmann H, Siegwolf R, Buchmann N, Schleppi P (2005) Flow of Deposited Inorganic N in Two
706 Gleysol-dominated Mountain Catchments Traced with 15NO3− and 15NH4+. Biogeochemistry 76:
707 453-475. doi: 10.1007/s10533-005-8124-1.
708 R-Core-Team (2020) R: A Language and Environment for Statistical Computing Version 3.4.3. R Foundation
709 for Statistical Computing, Vienna, Austria.
710 Raji SG, Dörsch P (2020) Effect of legume intercropping on N₂O emissions and
711 CH₄ uptake during maize production in the Great Rift Valley, Ethiopia.
712 Biogeosciences 17: 345-359. doi: 10.5194/bg-17-345-2020.
713 Rochette P, Angers DA, Chantigny MH, Gasser M-O, MacDonald JD, Pelster DE, Bertrand N (2013) NH3
714 volatilization, soil concentration and soil pH following subsurface banding of urea at increasing
715 rates. Canadian Journal of Soil Science 93: 261-268. doi: 10.4141/cjss2012-095.
716 Rosenstock TS, Mpanda M, Pelster DE, Butterbach-Bahl K, Rufino MC, Thiong'o M, Mutuo PK, Abwanda S,
717 Rioux J, Kimaro AA, Neufeldt H (2016) Greenhouse gas fluxes from agricultural soils of Kenya and
718 Tanzania. J Geophys Res Biogeosci 121: 1568-1580. doi: 10.1002/2016JG003341.
719 Rütting T, Cizungu Ntaboba L, Roobroeck D, Bauters M, Huygens D, Boeckx P (2015) Leaky nitrogen cycle
720 in pristine African montane rainforest soil. Global Biogeochemical Cycles 29: 1754-1762. doi:
721 10.1002/2015gb005144.
722 Sánchez-García M, Roig A, Sánchez-Monedero MA, Cayuela ML (2014) Biochar increases soil N2O
723 emissions produced by nitrification-mediated pathways. 2. doi: 10.3389/fenvs.2014.00025.
724 Schollenberger CJ (1945) Determination of exchange capacity and exchangeable bases in soil-Ammonium
725 acetate method. Soil Sci 59: 13-24.

Shi W, Ju Y, Bian R, Li L, Joseph S, Mitchell DRG, Munroe P, Taherymoosavi S, Pan G (2020) Biochar bound urea boosts plant growth and reduces nitrogen leaching. Sci Total Environ 701: 134424. doi: 10.1016/j.scitotenv.2019.134424.

Stevens RJ, Laughlin RJ (1994) Determining Nitrogen-15 in Nitrite or Nitrate by Producing Nitrous Oxide. Soil Sci Soc Am J 58: 1108-1116.

Thierfelder C, Chivenge P, Mupangwa W, Rosenstock TS, Lamanna C, Eyre JX (2017) How climate-smart is conservation agriculture (CA)? – its potential to deliver on adaptation, mitigation and productivity on smallholder farms in southern Africa. Food Security 9: 537-560. doi: 10.1007/s12571-017-0665-3.

Thierfelder C, Matemba-Mutasa R, Rusinamhodzi L (2015) Yield response of maize (Zea mays L.) to conservation agriculture cropping system in Southern Africa. Soil and Tillage Research 146: 230-242. doi: 10.1016/j.still.2014.10.015.

Thierfelder C, Wall PC (2009) Effects of conservation agriculture techniques on infiltration and soil water content in Zambia and Zimbabwe. Soil and Tillage Research 105: 217-227. doi: 10.1016/j.still.2009.07.007.

Tzanakakis VA, Taylor AE, Bakken LR, Bottomley PJ, Myrold DD, Dörsch P (2019) Relative activity of ammonia oxidizing archaea and bacteria determine nitrification-dependent N2O emissions in Oregon forest soils. Soil Biology and Biochemistry 139. doi: 10.1016/j.soilbio.2019.107612.

van der Kruijs ACBM, Wong MTF, Juo ASR, Wild A (1988) Recovery of 15N-labelled fertilizer in crops, drainage water and soil using monolith lysimeters in south-east Nigeria. Journal of Soil Science 39: 483-492.

Weldon S, Rasse DP, Budai A, Tomic O, Dörsch P (2019) The effect of a biochar temperature series on denitrification: which biochar properties matter? Soil Biology and Biochemistry 135: 173-183. doi: 10.1016/j.soilbio.2019.04.018.

WRB IWG (2015) World reference base for soil resources 2014, update 2015. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No 106. FAO, Rome.

Yu L, Kang R, Mulder J, Zhu J, Dörsch P (2017) Distinct fates of atmogenic NH4+ and NO3− in subtropical, N-saturated forest soils. Biogeochemistry 133: 279-294. doi: 10.1007/s10533-017-0332-y.

Zhu J, Yu L, Bakken LR, Morkved PT, Mulder J, Dorsch P (2018) Controlled induction of denitrification in Pseudomonas aureofaciens: A simplified denitrifier method for dual isotope analysis in NO3(). Sci Total Environ 633: 1370-1378. doi: 10.1016/j.scitotenv.2018.03.236.