Norwegian University of Life Sciences
Faculty of Environmental Sciences
and Natural Resource Management (MINA)

Philosophiae Doctor (PhD)
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Effects of conservation farming and biochar on carbon and nitrogen cycling in light textured soils in the sub-humid tropics

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“The most incomprehensible thing about the universe is that it is comprehensible”

Albert Einstein

“An expert is a person who has found out by his own painful experience all the mistakes that one can make in a very narrow field”

Niels Bohr

“Study hard what interests you the most in the most undisciplined, irreverent and original manner possible.”

Richard Feynman

Contents

Summary

Biochar has received attention in the research community for being one of the most promising technologies for carbon (C) sequestration in soils, improvement of soil fertility and reduction of N_2O emissions. Biochar is used as amendment in agricultural soils due to its liming potential, its capacity to supply nutrients and its positive effect on cation exchange capacity (CEC). Often, it is not clear if the reported positive effects of biochar addition are due to the effect of the ash component or the effects of the porous C-rich material (charcoal). Even the characterization of parameters such as CEC in biochar is challenging for the same reason, the content of ash and its porous structure. Current methods for CEC analysis of soils have been applied to biochar without proper validation and literature values for CEC of biochar are highly variable and are often poorly reproducible. Thus, in paper I, the objective was to modify and critically assess different steps in the existing ammonium acetate (NH_4OAc) method for CEC analysis of biochar where ammonium (NH_4^+) is displaced by potassium chloride (KCl) following removal of excess NH_4OAc with isopropanol, in batch mode. We introduced a pre-treatment that allowed us to remove the cations associated with ashes and to reduce pH to 7, which is critical for comparison between biochars since CEC is pH-dependent. Subsequently, we compared CEC estimates by sum of base cations in NH_4OAc with those obtained by displacement of NH_4^+ by KCl after washing with isopropanol. We found better CEC values based on sum of exchangeable cations, likely because isopropanol fails to effectively remove the excess NH_4^+ trapped inside the nano-pores of biochar. The CEC normalized to C content of biochar varied from 10.8 $\text{cmol}_{(+)}$ kg C^{-1} to 119.6 $\text{cmol}_{(+)}$ kg C^{-1} which is much lower than the values reported for native soil organic carbon (221 $\text{cmol}_{(+)}$ kg C^{-1} to 810 $\text{cmol}_{(+)}$ kg C^{-1}).

Conservation farming (CF) involves different forms of minimum tillage, crop residue retention and crop rotation. Conservation farming may increase yields in Sub-Saharan Africa (SSA) and reduce losses of soil organic carbon (SOC) as compared to conventional tillage. Most studies on the effect of CF on SOC have focused on total SOC, whereas the impact of SOC fractions such as particulate organic matter (POM, mainly formed by partly decomposed plant material) and hot-water extractable C (HWEC; labile SOC fraction) has received little attention. Fractionation of SOC may be used to understand stabilization mechanisms of soil organic matter (SOM), which are relevant for C sequestration and N mineralization. In paper II, we assessed the effects of seven years of CF in the form of permanent planting basins under hand-hoe tillage on build-up of SOC,

in an Acrisol in Mkushi, Zambia. Moreover, we investigated N mineralization and HWEC in soil samples collected inside and outside permanent planting basins. The results showed higher SOC concentration, C stock, HWEC and N mineralization rate inside basins than in between basins. The significant increase of SOM inside basins was attributed to greater root biomass, since the basins were carefully maintained in the same place during the seven years of CF practice. The relative increase of SOC was 2.95‰ per year in the upper 20 cm of the soil.

In paper III, we used the same CF field as in paper II and started a new experiment in which we analyzed the effect of a range of soil management practices involving tillage, residue retention and biochar addition on the amount and quality of SOM. The soil management practices were: i) conventional tillage without residue retention, ii) CF with residue removal, iii) CF with the addition of biochar inside basins and iv) the continuation of the already established CF practice. The experiment lasted three growing seasons (2.5) years and involved a maize-soya-maize rotation with the addition of fertilizer to maize only (117 kg N ha^{-1}). In this experiment, we measured crop yields and stover production in each treatment. We investigated the amount and quality of SOM in response to residue removal under CF, and in addition, CF was compared with the conventional treatment. We studied the effect of biochar addition inside basins on the relationship between SOC, total N, HWEC, N mineralization rate. Results showed that soya yield under CF treatments was higher than in the conventional treatment, whereas none of the treatments affected maize yield. We found that residue removal did not affect SOC, HWEC, POM and N mineralization. Particulate organic matter in CF was higher inside basins than outside, likely due to roots biomass inputs. Soil organic C, HWEC and N mineralization rate inside basins decreased upon conventional tillage. We found that biochar significantly increased SOC but it did not affect amounts of HWEC or N mineralization rates. The results suggest that conservation farming has a positive effect on yield compared to conventional farming when little or no fertilizer is added (when ample fertilizer is supplied this effect on yield disappears). Biochar was effective sequestering C in soil but it did not have agronomic benefits.

To our knowledge, the combined effect of conservation farming and biochar addition on N_2O emissions has not been investigated in SSA. In paper IV, the objective was to study the effect of CF with and without biochar, as well as conventional tillage with residue removal on N_2O emissions in an Arenosol in Kaoma, Zambia. We aimed at elucidating the factors controlling N_2O

emissions that are affected by soil management practices and at assessing the partitioning of ammonium nitrate (NH_4NO_3) and urea (CH_4N_2O) between soil and maize. We added and traced a small dose of ^{15}N in the plant-soil system and in the extractable nitrate (NO_3^-) pool. Results showed that N_2O emissions were explained best by the NO_3^- concentration in soil. Nitrous oxide flux and NO_3^- were lower in conventional than in CF treatments, probably due to different methods of fertilizer application rather than to differences in soil moisture. The amount of fertilizer applied was the same for the CF and conventional plots, but the fertilizer was more concentrated inside CF basins compared to the conventional plots where the fertilizer was applied to a greater area resulting in a dilution

Although biochar increased amounts of extractable NO_3^- and gross nitrification rates, it did not have any impact on N_2O emissions and did not affect the recovery of ^{15}N in the plant-soil system. Literature suggests that N_2O emissions may be modified by soil pH, however, none of the treatments (including biochar addition) had an effect on pH. Our results show a correlation between gross nitrification rates and N_2O fluxes, which suggests that nitrification is an important source of N_2O in this soil. The water-filled pore space values during the sampling campaign were below 60% which is a moisture content favorable for nitrification rather than denitrification. This could explain why biochar did not increase N_2O emissions, despite increasing extractable NO_3^- and soil moisture. There was a clear effect of the form of ^{15}N added on the total recovery of ^{15}N in the plant-soil system, being higher when $^{15}NH_4^+$ (86%) was applied as compared to $^{15}NO_3^-$ (38%). The total recovery was dominated by ^{15}N in soil and maize uptake of ^{15}N was higher when applied as NO_3NH_4 than as urea.

Sammendrag

Biokull har fått mye oppmerksomhet i forskningsmiljøer for å være en av de mest lovende teknologiene for karbonlagring i jord. I tillegg kan bruk av biokull forbedre jordas fruktbarhet og redusere utslipp av lystgass (N_2O). Biokull kan brukes til jordforbedring på grunn av sitt kalkingspotensial, evne til å forsyne næringsstoffer og øke kationbyttekapasiteten (CEC). Det er uklart om den positive effekten av biokull er på grunn av tilføring av aske eller som følge av biokullets høye porøsitet og karboninnhold. Bestemmelse av CEC i biokull er utfordrende på grunn av kullets porøse struktur og innhold av alkalisk aske. Nåværende metoder for CEC-analyse av jord har blitt anvendt på biokull uten tilstrekkelig validering. I tillegg er rapporterte litteraturverdier for CEC av biokull svært variable og ofte dårlig reproduserbare. I artikkel I var derfor målet å utføre et risteforsøk for å modifisere og kritisk vurdere forskjellige trinn i den eksisterende ammoniumacetat (NH_4OAc) metoden for CEC-analyse av biokull, hvor ammonium (NH_4^+) byttes ut av kaliumklorid (KCl) etter fjerning av overskudds NH4OAc med isopropanol. Metoden inkluderte forbehandling av biokullet med fortynnet saltsyre (HCl) for å redusere pH til 7 og for å fjerne lettoppløselige kationer som stammer fra asken og ikke fra negativt ladede ionebytteseter. Behandling med HCl tillater også vurdering av CEC ved pH 7, noe som er viktig for sammenligning av CEC mellom ulike biokulltyper siden CEC er pH-avhening. CEC-estimatene basert på sum av basekationer i NH4OAc ble sammenlignet med de som ble oppnådd ved utbytting av NH_4^+ med KCl etter vasking med isopropanol. Vi fant at utbytting av NH_4^+ med KCl kan føre til overestimering av CEC, antagelig fordi isopropanolmolekylene er større enn et stort antall av biokullets nanoporer, som medfører at isopropanol ikke er effektivt nok til å fjerne overskudds NH_4^+ inne i porene. CEC-normalisert til karbon(C)-innholdet av biokull varierte fra 10.8 $cmol_{(+)}$ kg C^{-1} til 119.6 $cmol_{(+)}$ kg C^{-1}, som er mye lavere enn verdiene som er rapportert for naturlig jordorganisk karbon (221 $cmol_{(+)}$ kg C^{-1} to 810 $cmol_{(+)}$ kg C^{-1}).

Conservation farming (CF; “bevaringsjordbruk” eller “klimasmart jordbruk”) involverer ulike former for redusert jordarbeiding, at planterester fra forrige avling blir liggende på jorden, samt vekselbruk. Det er vanligvis rapportert at CF kan øke avlinger og redusere tap av jordorganisk karbon (SOC) i forhold til konvensjonell jordarbeiding i Afrika sør for Sahara (SSA). De fleste studier som omhandler effekter av CF på SOC har fokusert på total SOC, mens effekten på ulike SOC-fraksjoner slik som partikulært organisk materiale (POM, som hovedsakelig er dannet av delvis nedbrutt plantemateriale) og varmtvannsekstraherbart C (HWEC, en labil SOC-fraksjon)

har fått liten oppmerksomhet. Fraksjonering av SOC kan brukes til å forstå stabiliseringsmekanismer av jordorganisk materiale (SOM), som er relevante for karbonlagring og nitrogen (N)-mineralisering. I artikkel II vurderte vi effekten av syv år med CF i form av permanente plantingsbassenger under jordarbeiding-med-hakke på oppbygging av SOC, i en Acrisol i Mkushi, Zambia. Videre undersøkte vi N mineralisering og HWEC i jordprøver tatt i og utenfor de permanente plantingsbassengene. Resultatene viste høyere SOC-konsentrasjon, C-lager, HWEC og N-mineraliseringshastighet inne i bassenger enn mellom bassenger. Den betydelige økningen av SOM i bassengene ble tilskrevet større rotbiomasse, siden bassengene var nøye opprettholdt på samme sted i løpet av de syv årene med CF-praksis. Den relative økningen av SOC var 2,95 ‰ per år i de øvre 20 cm av jorda.

I artikkel III brukte vi det samme CF-feltet som i artikkel II og startet et nytt eksperiment der vi analyserte effekten av ulike jordarbeidingsmetoder slik som full jordarbeiding, fjerning eller ikke fjerning av planterester fra forrige avling og biokull på mengde og kvalitet av SOM. Jordarbeidingsmetodene inkluderte: i) konvensjonell jordbearbeiding uten planterester på jorda, ii) CF med fjerning av planterester, iii) CF med tilsetning av biokull i plantebassenger, og iv) videreføring av den allerede etablerte CF-praksisen. Forsøket pågikk i tre vekstsesonger (2.5 år) og inkluderte et mais-soya-maisvekselbruk med tilsetning av gjødsel kun til mais (117 kg N ha^{-1}). Avling og total biomasseproduksjon i hver behandling ble målt. I tillegg så vi på mengde og kvalitet av SOM som følge av fjerning av biomasserester under CF, i tillegg til at CF ble sammenlignet med konvensjonell jordbearbeiding. Vi studerte effekten av biokulltilsetning i bassenger på forholdet mellom SOC, total N, HWEC, og N mineraliseringshastighet. Resultatene viste at soyaavlingene under CF-behandlingene var høyere enn ved konvensjonell jordbearbeiding, mens ingen av behandlingene påvirket maisavlingene. Vi fant at fjerning av biomasserester ikke påvirket SOC, HWEC, POM og N mineralisering. Partikulært organisk materiale i CF var høyere i plantebassengene enn utenfor, sannsynligvis på grunn av biomassetilførsel fra røtter. Jordorganisk C, HWEC og N mineraliseringshastighet i bassengene ble redusert ved konvensjonell jordbearbeiding. Vi fant at biokull økte SOC betydelig, men det påvirket ikke mengden av HWEC eller N mineraliseringshastighetene. Resultatene tyder på at conservation farming har en positiv effekt på avling sammenlignet med konvensjonell jordbearbeiding når det tilføres lite eller ingen gjødsel. Når det tilføres rikelige mengder med gjødsel forsvinner denne effekten. Biokull var effektiv for karbonlagring i jord, men hadde utover dette ingen agronomiske fordeler.

Etter vår kunnskap er den kombinerte effekten av conservation farming og biokulltilsetning på N_2O-utslipp ikke undersøkt i SSA. I artikkel IV var målet å studere effekten av CF med og uten biokull, samt konvensjonell jordarbeiding med fjerning av biomasserester på N_2O-utslipp i en Arenosol i Kaoma, Zambia. Målet var å belyse faktorer som styrer N_2O-utslipp som påvirkes av jordarbeidingspraksis samt å se på fordelingen av ammoniumnitrat (NH_4NO_3) og urea (CH_4N_2O) mellom jord og mais. Vi tilførte og sporet en liten dose av ^{15}N i plante-jordsystemet og i lageret av ekstraherbar nitrat (NO_3^-). Resultatene viste at N_2O-utslippene var best forklart av NO_3-konsentrasjonen i jorda. Flukser av lystgass og NO_3^- var lavere i konvensjonelle enn i CF-behandlinger, sannsynligvis på grunn av ulike metoder for gjødseltilførsel snarere enn forskjeller i jordfuktighet. Selv om samme mengde gjødsel ble tilført i CF og konvensjonell behandling, ble gjødselen mer konsentrert inne i bassengene under CF, mens den ble spredt utover et større areal i den konvensjonelle behandlingen, noe som forårsaket en fortynning av gjødselen. Selv om biokull økte mengden av ekstraherbar NO_3^- og brutto nitrifiseringshastigheter, hadde det ingen innvirkning på N_2O-utslipp og påvirket ikke mengden av ^{15}N som ble funnet tilbake i plante-jordsystemet. Tidligere studier antyder at N_2O-utslipp kan modifiseres med endringer i jordas pH, men ingen av behandlingene (inkludert biokulltilførsel) hadde en effekt på pH. Våre resultater viser en korrelasjon mellom brutto nitrifiseringshastigheter og N_2O-fluks, noe som tyder på at nitrifikasjon er en viktig kilde til N_2O i denne jorda. Andelen av porevolumet som var fylt med vann under prøvetakingskampanjen var under 60%, som er et fuktighetsinnhold som er gunstig for nitrifikasjon i stedet for denitrifikasjon. Dette kan forklare hvorfor biokull ikke økte utslippene av N_2O, til tross for økt mengde ekstraherbar NO_3^- og jordfuktighet. Det var en klar effekt av form av ^{15}N tilsatt på total mengde av ^{15}N funnet tilbake i plante-jordsystemet, som var høyere når $^{15}NH_4^+$ (86%) ble tilsatt sammenlignet med $^{15}NO_3^-$ (38%). Total mengde av ^{15}N som ble funnet tilbake var dominert av ^{15}N i jord, og opptak av ^{15}N i mais var høyere når det ble tilført som NO_3NH_4 enn som urea.

List of papers

I) **Cation exchange capacity of biochar: An urgent method modification**

Munera-Echeverri J.L.[a], Martinsen V.[a*], Strand L.T.[a], Zivanovic, V[a]., Cornelissen, G[a,b]., Mulder J.[a]. Sci Total Environ, 2018. **642**: p. 190-197.

II) **Significant build-up of soil organic carbon under climate-smart conservation farming in Sub-Saharan Acrisols**

Vegard Martinsen, Jose Luis Munera-Echeverri, Alfred Obia, Gerard Cornelissen Jan Mulder. Science of The Total Environment, 2019. **660**: p. 97-104.

III) **Effect of conservation farming and biochar addition on fractions of SOC, N mineralization and crop productivity in a light-textured Acrisol in the sub-humid tropics**

Munera-Echeverri J.L.[a], Martinsen V.[a*], Strand L.T.[a], Cornelissen, G[a,b]., Mulder J.[a]

Manuscript.

IV) **N partitioning, N_2O fluxes and *in situ* gross nitrification in a rain-fed tropical biochar-amended Arenosol planted to maize – effect of soil management**

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

Manuscript.

Introduction

Climate-smart agriculture in Sub-Saharan Africa

Sub-Saharan Africa (SSA) has the fastest population growth in the world and this trend is expected to continue in the decades to come (OECD/FAO, 2016). Likewise, crop production in SSA will have to increase to meet the population's needs for food (OECD/FAO, 2016). However, climate change in rain-fed agriculture is already affecting crop production negatively and this is a serious threat to food security (Knox et al., 2012). Therefore, there is a need for improved agricultural practices that increase climate resilience of food production systems. These type of practices are known as climate-smart, and in theory, they should i) improve the adaptation to the negative effects of climate change (erratic rainfall in the case of SSA), ii) mitigate climate change by sequestering C and decreasing the emissions of greenhouse gases and iii) sustain crop productivity (FAO, 2013; Paustian et al., 2016).

Conservation farming (CF), including different forms of minimum tillage, crop residue retention and crop rotation, has been shown to offer adaptation to drought by increasing soil moisture, water infiltration in soils and increasing yields in SSA (Thierfelder et al., 2017; Thierfelder et al., 2015; Thierfelder and Wall, 2009). In countries like Zambia, minimum tillage is practiced in the form of ripping lines or planting basins (Ngoma et al., 2015). Planting basins is a system for smallholder farmers without access to mechanization and consist of holes dug using hand-hoe in grids of about 15.850 basins ha^{-1} (Haggblade and Tembo, 2003), which has been found to perform well in arid conditions (Thierfelder et al., 2015). In addition, CF may offset anthropogenic CO_2 emissions by restoring C in soils due to the addition of residues and the reduced tillage (Lal, 2015). Yet, less is known about its potential of sequestering C in soils and reducing greenhouse gases such as nitrous oxide (Thierfelder et al., 2017).

Biochar application to soils has been suggested as a multiple-win strategy for a number of sustainability-related issues, including i) C sequestration, ii) improvement of soil fertility, and iii) agricultural waste management (Jeffery et al., 2015). It has been suggested that biochar has the greatest potential for increasing crop yields in sandy acidic soil, which constitute an important proportion of the soils in SSA (Tully et al., 2015), due to enhanced soil water retention (Obia et al., 2016) and amelioration of soil acidity (Cornelissen et al., 2018; Martinsen et al., 2015). Studies have shown that biochar can reduce nitrous oxide emissions and prevent losses of mineral N in soil due to its liming effect and ion exchange capacity (Borchard et al., 2019; Cayuela et al., 2014; Obia et al., 2015).

1.2 Zambia

The Republic of Zambia is a landlocked country located in Southern Africa, with an extension that covers 752 610 km^2 and a cultivable area of 16.35 million ha (FAO, 2005; Sinyenga, 2018). The population of Zambia was estimated at 16.6 million in 2017 with a growth rate of 2.9% per year (Sinyenga, 2018). The agricultural sector in Zambia represents about 20% of the GDP (FAO, 2015) and it employs 48% of the working population (WorldBank, 2017). Maize is the dominant crop in Zambia with a production estimated at 3.6 million tons in 2016-17, followed by wheat with 0.2 million tons. Maize production in Zambia has increased in the last decade due to an expansion of the area planted, whereas maize yields have grown modestly (WorldBank, 2017).

The precipitation pattern in Zambia is unimodal, with rainfalls starting on November and finishing on April/May (Libanda et al., 2015). The average precipitation in the country is 1020 mm and the mean temperature varies from 16 ˚C to 21 ˚C in the dry season, while it is around 21 ˚C in the wet season (FAO, 2005). The country has been divided into three main agro-ecological regions (I, II and III; Figure 1.1) representing the southern, central and northern parts of Zambia respectively (FAO, 2015). A gradient of precipitation that increases from south (less than 800 mm year^{-1}) to north (1200 mm year^{-1}) determines the different agro-ecological zones, being the northern areas of the country more rainy and with poorer soils (FAO, 2015; Libanda et al., 2015). Region II has been dived in two (IIa and IIb; Figure 1.1) due to differences is soil types. Region IIa consists of Acrisols, Luvisols and Vertisols, whereas in the western parts Arenosols are predominant (JAICAF, 2008).

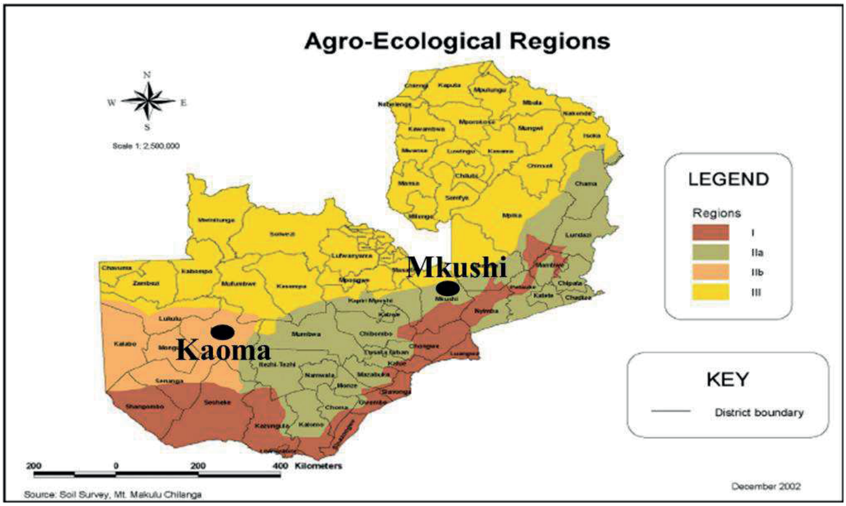


Figure 1.1. Map of agro-ecological regions of Zambia with the two experimental sites of the present study in Mkushi and Kaoma. Source: FAO (CFA, Zambia Brach Homepage).

1.3 Conservation farming

1.3.1 Yields

Conservation farming has been promoted since the 1990s in Zambia as an alternative to conventional soil management practices, involving tillage and residue removal (Farooq and Siddique, 2015; Thierfelder and Wall, 2010). Conservation farming must include complementary practices (on-time fertilization, pest control and weed management, among others), which determine largely the success of farmers following this technology (Thierfelder et al., 2018). Research has shown that CF systems in SSA often yield more than adjacent conventionally tilled plots and the reasons include higher soil water retention and higher water infiltration (Thierfelder et al., 2015; Thierfelder et al., 2013b). Research showed that yield benefits of CF increased with increasing number of years practicing CF, since farmers need first to gain experience to master important practices such as timely planting, weeding, fertilizer application, among others (Thierfelder et al., 2015). Therefore, adoption of CF among smallholder farmers is limited (Giller et al., 2015; Giller et al., 2009; Thierfelder et al., 2015). Nevertheless, conservation farming has been shown to work well under dry climates due to improved soil water retention (Thierfelder et al., 2015). Research has suggested that yields under basins tillage are higher than in conventional tillage if combined with early land preparation (Ngoma et al., 2015). However, basins were found to increase labor burden with no yield benefits (Rusinamhodzi, 2015).

1.3.2 Effect of conservation farming on C sequestration

Soil organic C is one of the main components of soil organic matter (SOM), which plays an important role in ecosystem services and crop production (Manlay et al., 2007). SOM contributes to the mitigation and adaptation to climate change as it acts as a sink for CO_2, a major greenhouse gas, it helps to store plant nutrients (Paustian et al., 2016) and it makes crop production more resilient to drought conditions by promoting soil aggregation (Oades, 1984) and water infiltration (Franzluebbers, 2002). There are general claims that conservation farming may increase C sequestration in soils due to minimum tillage and residue retention (Farooq and Siddique, 2015; Lal, 2015). However, the recent evidence seems to point out in the direction that the effect of conservation farming on soil C in SSA is overestimated (Powlson et al., 2016; Sommer et al., 2018). There are significant number of cases in the Sub-Saharan countries with no measurable increases in C and many studies have overestimated the increase in C because of errors introduced by inappropriate soil sampling (Powlson et al., 2016). Some studies have suggested that the positive effects of conservation farming on C sequestration in soil are site-specific depending largely on soil texture (Swanepoel et al., 2018), while others report only minor increases (Martinsen et al., 2017). More recently, research by Sommer et al. (2018 in Kenya has suggested that conservation farming reduces C losses compared to conventional tillage but it fails to sequester C. Most of these studies have been conducted under farmers' fields, comparing conservation farming with adjacent conventional plots (Martinsen et al., 2017; Thierfelder et al., 2013b). This introduces uncertainties since not all the farmers follow the recommended guidelines to the same extent. Thus, studies under controlled conditions are needed to assess the potential of conservation farming on C sequestration under SSA conditions.

Limited residue retention has been suggested as the reason for the low SOC increase in conservation farming in SSA (Cheesman et al., 2016). Crop biomass is low in smallholder farms in SSA (Dzanku et al., 2015) and there are competing uses of residues such as feed for livestock (Giller et al., 2015). In the case of maize, aboveground biomass was found to contribute little to C stabilization in soil, whereas root biomass is found to be more stable in soils (Kong and Six, 2010). Little research has focused on the effect of root-derived C on SOC under conservation farming systems in SSA. Manually dug basins offer the possibility to keep the same planting position season after season, that otherwise is difficult to achieve under other forms of no-till such as mechanized ripping lines. In addition, most of the studies on the effect of conservation farming on SOC in SSA have focused on total amounts of C, whereas there are few studies on SOC fractions, which are important to understand stabilization and decomposition mechanisms of C and N in agricultural soils (von Lützow et al., 2007). There is little research on the possible effects of improved SOC under conservation farming on N mineralization in soil, which is important for crop productivity, N leaching and emissions of nitrous oxide.

1.3.3 Nitrous oxide under conservation farming practices

Nitrous oxide (N_2O) is a non-CO_2 greenhouse gas produced in agricultural soils, which has a high warming potential despite its low concentration in the atmosphere (Montzka et al., 2011). The concentration of N_2O in the atmosphere has been rising due to the extensive use of fertilizer nitrogen (N) worldwide (Davidson and Kanter, 2014; Montzka et al., 2011). Nitrous oxide is produced during nitrification, i.e. the microbial oxidation of ammonia (NH_3) to nitrite (NO_2^-) and NO_3^- under aerobic conditions (Butterbach-Bahl et al., 2013; Linn and Doran, 1984). In addition, N_2O is produced by denitrification, i.e. the microbial reduction of NO_3^- to N_2 via the gaseous intermediates NO and N_2O under anaerobic conditions (Zumft, 1997). Both processes are driven by soil micro-organisms viz. bacteria, archaea and fungi. The N_2O product ratio of denitrification, $N_2O/(N_2+N_2O)$, increases with decreasing soil pH (Bakken et al., 2012; Wang et al., 2018), whereas the N_2O yield of nitrification, $N_2O/(NO_2^- + NO_3^-)$ is believed to be unaffected by pH within a pH range common in agriculture (Mørkved et al., 2007).

Measured N_2O emissions from soils in Sub-Saharan Africa (SSA) are scarce (Wang et al., 2018) and flux values are generally estimated based on the Intergovernmental Panel on Climate Change's (IPCC) Tier 1 approach (1% of added N is emitted as N_2O-N; (Hickman et al., 2011)). The few reported values are highly variable. Brummer et al. (2008) reported an average flux of 19 μg N m^{-2} h^{-1} with the addition of 52 Kg N ha^{-1} and a mean of 80 μg N m^{-2} h^{-1} 24 days after addition of 140 kg N ha^{-1} in croplands in Burkina Faso. Rosenstock et al. (2016) reported an average N_2O flux of 12 μg N m^{-2} h^{-1} in agricultural soils in Kenya and Tanzania and a wide range of values (from -2 to 300 μg N m^{-2} h^{-1}), under different farming systems, weather conditions and soil texture. Scaling up N_2O fluxes to annual emissions over certain area may introduce uncertainty in the estimates due to the nature of N_2O emissions, which is characterized by hot-spots and hot-moments and a large spatio-temporal variability (Butterbach-Bahl et al., 2013). Nevertheless, Kim et al. (2016 reviewed annual emission estimates from different types of agricultural systems, which varied from 4.0±1.5 kg N_2O ha^{-1} yr^{-1} (64% of N) in crop land to 120.1±26.1 kg N_2O ha^{-1} yr^{-1} in vegetable gardens, which corresponded to a N_2O emission factor of 0.5±0.2 to 3.5±0.5 % of the N input, respectively.

Little is known about the effect of the principles of conservation farming on N_2O emissions worldwide (Thierfelder et al., 2017). There are concerns about higher N_2O emissions under conservation farming systems, since soil moisture retention may increase (Grandy et al., 2006; Thierfelder et al., 2017) and conditions for denitrification may be favored, or enhanced N mineralization which leads to higher substrate availability for N_2O production (Grandy et al., 2006). Studies aiming at understanding the mechanisms responsible for N_2O emissions in agro-ecosystems in SSA are lacking (Rosenstock et al., 2016). Research has suggested that a better understanding of plant-microbe interactions in the rhizosphere may improve our knowledge on the intrinsic variability of N_2O fluxes at the soil-atmosphere interface (Butterbach-Bahl et al., 2013). Factors affecting N_2O production include availability of mineral N in soil (which is affected by crop uptake), soil pH and soil moisture (Butterbach-Bahl et al., 2013; Wang et al., 2018). Regarding mineral N, studies focusing on its short-term dynamics in crop systems under field conditions are scarce, probably because from an agronomical perspective it is not so important. However, short-term dynamics of mineral N is important to understand the variability of N_2O emissions in crop systems.

1.4 Biochar

Biochar, the byproduct of the pyrolysis process of agricultural wastes, has been suggested as a way of sequestering C in soil while improving soil fertility (Lehmann et al., 2006). Interest in biochar started after finding large contents of black C in a type of human-made soils in the Amazon, called Terra Preta or Amazonian Dark Earths, which developed in areas where ancient indigenous communities dumped different types of wastes such as charcoal, ash, bones, excrements and potsherds (Glaser et al., 2000). Over millennia, these soils developed a higher fertility than the surrounding soils and sequestered large amounts of C (Alho et al., 2019; Glaser et al., 2000; Liang et al., 2006). Biochar is rich in black C, which is a condensed aromatic structure present in char, soot and graphite (Hammes et al., 2007; Liang et al., 2008; Pignatello et al., 2006). Black C is highly persistent in nature, with a residence time that may vary between hundreds to thousands of years (Liang et al., 2008).

Biochar has been found to increase soil water-holding capacity (Obia et al., 2016), ameliorate soil acidity (Cornelissen et al., 2018; Martinsen et al., 2015) and supply nutrients such as potassium (Pandit et al., 2018). All these factors may contribute to higher yields. It has been shown that biochar has its maximum potential for enhancing yields under sandy acidic soils (Jeffery et al., 2017), which represent about 13% of the land in SSA (Hartemink and Huting, 2008). Previous research has reported positive effects of biochar on maize yield in Zambia under conservation farming (Cornelissen et al., 2013; Martinsen et al., 2014). The mechanisms behind the positive effects include i) liming effect and nutrient content of the alkaline ashes of biochar, ii) the ion exchange capacity of the black C-rich material and iii) the porous structure of the carbonaceous material that increases water-holding capacity in the soil (Jeffery et al., 2017; Obia et al., 2016; Pandit et al., 2018). In addition, biochar has been suggested as a way to reduce N_2O emissions due to its liming potential (Cayuela et al., 2014; Obia et al., 2015) but also, it has been shown to increase nitrification and denitrification upon ageing (Duan et al., 2018). Biochar may increase the amounts of extractable NO_3^- and NH_4^+ but it is not clear how this may affect N_2O emissions (Borchard et al., 2019; Clough et al., 2013). A mechanistic understanding of the reductions of N_2O, including the role of biochar-N-soil interactions (Clough et al., 2013) and the presence of plants, is needed.

The alkalizing effect of biochar, which is associated with the ashes, has been shown to be short to medium lived in acidic soils, varying with feedstock (Cornelissen et al., 2018; Jeffery et al., 2015). By contrast, the carbonaceous material of biochar is responsible for the long-term effects, such as retention of nutrients by ion exchange reactions, retention of water due to its porous structure and C sequestration in condensed aromatic rings. It has been found that black C increases cation exchange capacity (CEC) of soils (Liang et al., 2006) and thus, biochar, which also contains black C, may have the potential to increase CEC (Jeffery et al., 2015). Surfaces in biochar particles gradually oxidize after addition to soils and thus, cation exchange capacity is believed to develop over time (Jeffery et al., 2015). However, biochar is a difficult material for analysis of CEC due to its porous structure and the presence of alkaline ashes. Literature values for the CEC of biochar are surprisingly variable [from 5 to 204 $cmol_{(+)}$ kg^{-1}; (Berek and Hue, 2016; Budai et al., 2014;

Mukherjee et al., 2011; Pandit et al., 2018)] and are often poorly reproducible, suggesting methodological problems.

Analysis of CEC in biochar has been done following methods developed for soils, such as the displacement after washing method, using ammonium acetate (NH_4OAc) at pH 7, isopropanol and potassium chloride (Chapman, 1965; Schollenberger, 1945). By this method, there are two approaches for estimating CEC: i) sum of base cations in NH_4OAc extract and ii) displacement of NH_4^+ by K^+. To our knowledge, there is no study comparing both approaches in biochar samples. However, the methods to analyze CEC in soils have potential sources of error when applied to biochar. As example, the release of base cations coming from the dissolution of carbonates and silicates present in ashes, which can interfere with the sum of exchangeable base cations approach leading to an overestimation of CEC (Graber et al., 2017). Therefore, proper validation of a methodology for CEC analysis is lacking.

1.5 Objectives of the study

In paper I, the objective was to validate a method for cation exchange capacity analysis of biochar that uses ammonium acetate, isopropanol and potassium chloride (KCl) in batch mode. A pre-treatment, washing the biochar with dilute hydro-chloric acid (HCl), was introduced to reduce the pH of the biochar to 7, while removing readily soluble cations, derived from ash and not from the negative exchange sites of biochar. The HCl treatment also allows the assessment of the CEC at pH 7, which is important for comparison between biochars. The specific objectives of the study were: i) to find the optimal conditions for every step of the displacement after washing method using NH_4OAc, isopropanol and KCl. ii) To assess the relative contribution of readily soluble salts derived from ashes vs. exchangeable cations. iii) To compare CEC estimates of biochar based on the displacement of NH_4^+ by K^+ and CEC based on the sum of base cations.

In paper II, we investigated the effects of seven years of strict conservation farming husbandry in the form of planting basins on the build-up of soil organic C and N mineralization in an Acrisol at Mkushi, Zambia. Attention was given to keep the planting basins in the same position every growing season. Crop residue was placed in between rows of basins after harvest in each season, in a two-year maize-groundnuts rotation. We compared the quality and quantity of SOC inside vs. outside planting basins. *Planting basins are hypothesized to increase SOC content, due to the high density of biomass derived from roots inside as compared to outside basins.*

From the seventh year onwards, we investigated the effect of a range of soil management practices involving tillage, residue retention and biochar addition on yields, amount and quality of SOC and N mineralization in the same Acrisol in Mkushi, Zambia in a maize-soya-maize rotation (3 seasons; paper III). The soil management practices were: i) conventional tillage without residue retention, ii) CF with residue removal, iii) CF with the addition of biochar inside basins and iv) the continuation of the normal CF practice. In this experiment, the specific objectives were: ii) to measure crop yields and stover production in each treatment. ii) To investigate the amount and quality of SOM and N mineralization in response to residue removal under CF, and in addition, to compare CF with the conventional treatment. iii) To study the effect of biochar addition inside basins on the relationship between SOC, total N, HWEC, N mineralization rate. *It is hypothesized that biochar addition has a positive effect on crop yields by a positive priming effect on N mineralization. Also that CF gives higher crop yield than conventional tillage due to higher N mineralization rate inside basins. Lastly, that residue retention is effective increasing the amount and improving the quality of SOM.*

In paper IV, we aimed at investigating the effect of conservation farming with and without biochar, as well as conventional tillage with residue removal on N_2O emissions in an Arenosol in Kaoma, Zambia. Simultaneously, we studied factors that are known to affect N_2O production such as mineral N content, soil moisture and soil pH, and traced a small dose of ^{15}N in the extractable nitrate pool and in the plant-soil system. ^{15}N pool dilution was used to estimate *in-situ* nitrification rates. In addition, we assessed the effect of biochar addition and tillage form on maize yield. *It was hypothesized that biochar reduces N_2O emissions by increasing soil pH. Moreover, we hypothesize that CF leads to higher N_2O fluxes due to increased soil moisture retention, which favors anoxic conditions for denitrification. Lastly, biochar increases the short-term recovery of ^{15}N in the plant-soil system as either ammonium nitrate or urea.*

2. Materials and methods

2.1 Biochars

For the validation of the method for CEC analysis (paper I), we used four types of biochar. i) Pigeon pea biochar (made at 600 ˚C in flame-curtain kiln at Mkushi, Zambia), ii) cacao shell biochar [made in a locally constructed kiln at Bogor, Indonesia at 350 ˚C (Hale et al., 2013)], iii)corncob biochar [made in a traditional earth-mound kiln in Zambia at 400 ˚C during 7 days (Cornelissen et al., 2013)] and iv) rice husk biochar [made at 400-500˚C in a drum retort kiln at Chisamba, Zambia at 350 ˚C (Obia et al., 2016)]. The biochar used in the field experiments was prepared from pigeon pea (*Cajanus cajan*) stems in a flame curtain kiln at 600 ˚C in October 2015. The same batch of biochar was used in the experiments in paper III and IV.

2.1.1 Cation exchange capacity

The "displacement after washing method" for CEC analysis using ammonium acetate (NH_4OAc), isopropanol and potassium chloride (KCl) consists of a number of consecutive steps that include: saturation with NH_4OAc, washing of excess NH_4^+ with isopropanol and displacement of NH_4^+ by K^+ (Figure 2.1). The modification of the method for CEC analysis was done in batch, which is a novelty because normally CEC is analyzed in column experiments. In our modification of the method, we introduced a pre-treatment in which pH was adjusted and readily soluble cations were removed. Cation exchange capacity of biochar was estimated by the sum of base cations in ammonium acetate extract and by displaced NH_4^+ by K^+. Base cations extracted in the pre-treatment and in the ammonium acetate solution were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES), whereas Displaced NH_4^+ was analyzed in 2M KCl extracts by spectrophotometry.

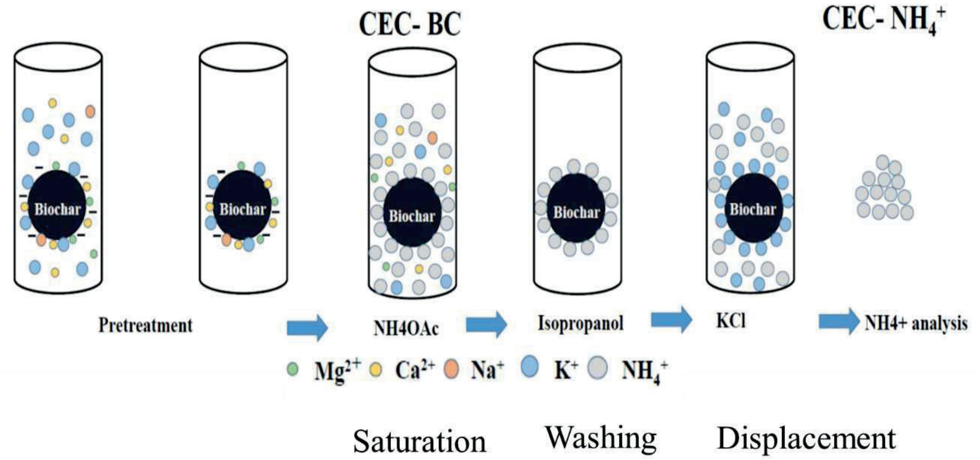


Figure 2.1 Summary of the “displacement after washing method” for CEC analysis of biochar using ammonium acetate (NH_4OAc), isopropanol and potassium chloride (KCl). Figure from the graphical abstract paper I.

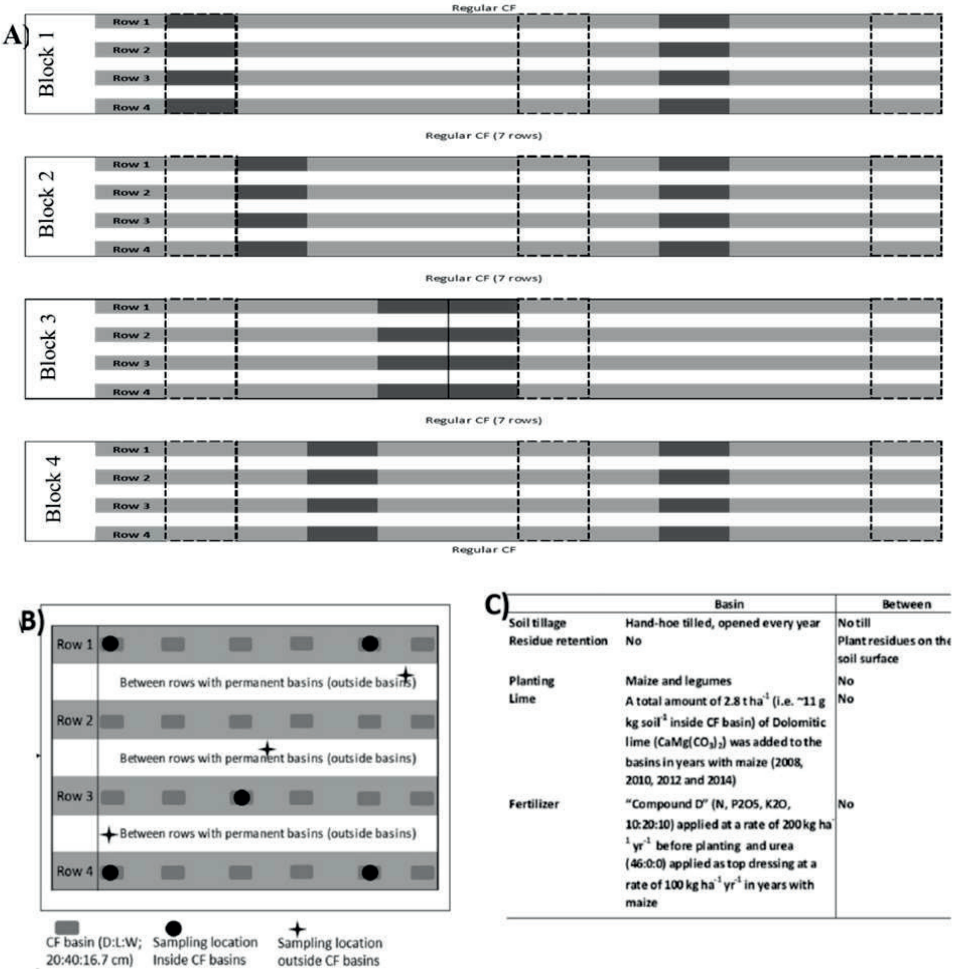
2.2 Field experiments

Field experiments were established on two private farms in Zambia: one in Mkushi (S13°45′25.7″ E29°03′55.5″) and other in Kaoma (S14 51.004 E24 49.148; Figure 1.1), with an average annual precipitation of 1220 and 930 mm and a mean temperature of 20.4 ˚C and 20.8 ˚C, respectively. The experimental sites are located in the agro-ecological regions IIa and IIb, respectively (Figure 1.1). In Kaoma, the soil is an Arenosol (WRB, 2015) containing 89% sand, 3.5% clay and 7.5% silt (sandy). In Mkushi, an Acrisol having 68% sand, 22% silt and 10% clay (sandy loam).

2.2.1 Mkushi

In Mkushi, we conducted two experiments. In the first, we evaluated the effects of seven years of conservation farming practices on quantity and quality of soil organic matter in a number of plots of 24 m^2, distributed in four blocks (Figure 2.2; paper II). The practice of conservation farming involved maintaining the same position of the basins each growing season, residue retention in the area outside basins and a two-year maize-groundnuts rotation. Soil samples were taken from inside and outsides basins with a cylindrical soil auger. The samples were dried, sieved (< 2mm) and soil organic C, total N and hot-water extractable C determined. A laboratory incubation at 20˚C during 60 days was carried out to determine potential N mineralization rates (Martinsen et al., 2017).

Other important soil parameters such as CEC, plant-available phosphorus, bulk density, and pH were analyzed.



	Basin	Between
Soil tillage	Hand-hoe tilled, opened every year	No till
Residue retention	No	Plant residues on the soil surface
Planting	Maize and legumes	No
Lime	A total amount of 2.8 t ha^{-1} (i.e. ~11 g kg $soil^{-1}$ inside CF basin) of Dolomitic lime ($CaMg(CO_3)_2$) was added to the basins in years with maize (2008, 2010, 2012 and 2014)	No
Fertilizer	"Compound D" (N, P2O5, K2O, 10:20:10) applied at a rate of 200 kg ha^{-1} yr^{-1} before planting and urea (46:0:0) applied as top dressing at a rate of 100 kg ha^{-1} yr^{-1} in years with maize	No

Figure 2.2. a) Experimental setup in Mkushi, Zambia (paper II) consisting of four blocks that were divided in plots for sampling purposes. b) Plot containing four rows of six basins each and showing how the sample was conducted inside and outside basins. c) Description of the management practice. Figure 1 from paper II.

From the 7th year onwards, four soil management practices were introduced in the same field and the effects on crop biomass, soil organic C and N mineralization were investigated in a maize-soya-maize rotation. The management practices were: i) conventional tillage without residue retention (CONV) , ii) CF with residue removal (CF-NO-RES) , iii) CF with the addition of biochar inside basins (CF-BC) and iv) the continuation of the normal CF practice (CF-NORM; Figure 2.3). Each management practice was evaluated in plots of about 24 m^2 randomly distributed in four blocks (Figure 2.4; paper III). We analyzed crop biomass (grain and stover) at the end of each growing season, studied the effects of tillage and residue removal on SOC, total N, hot-water extractable C and N mineralization. In addition, we determined particulate organic matter for the normal practice of conservation farming and conservation farming without residues.

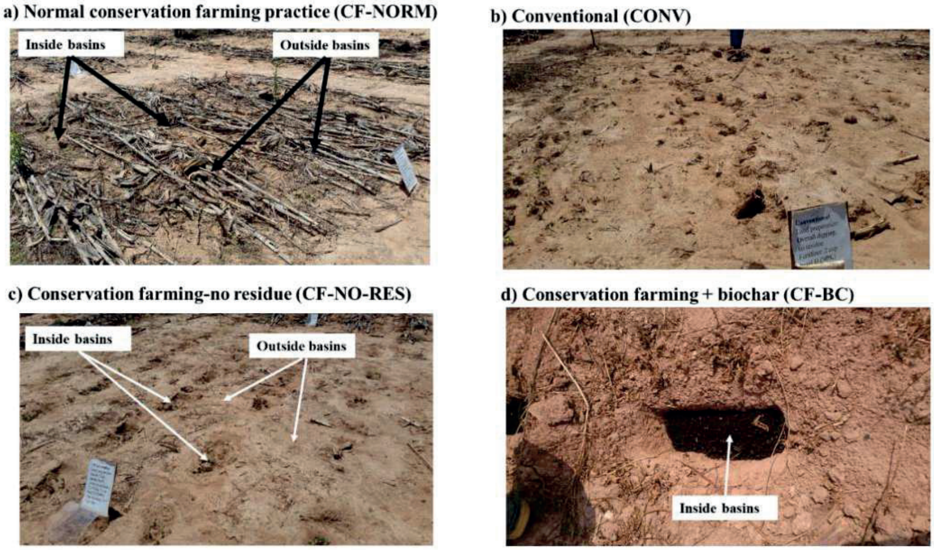


Figure 2.3. Soil management practices evaluated in Mkushi. Figure S1 from paper III.

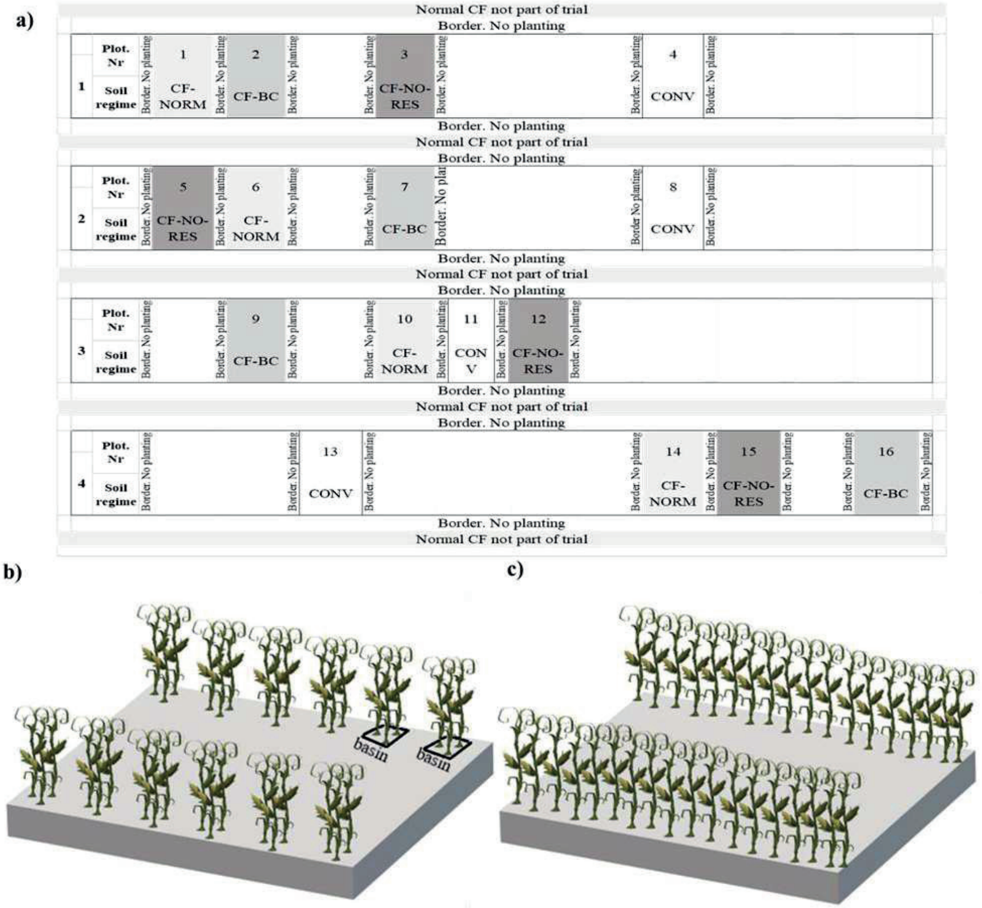


Figure 2.4. a) Experimental setup of the second experiment in Mkushi (paper III). The four soil regimes were randomly distributed in 4 blocks. Each plot was about 24 m^2 and consisted of 4 rows of six basins in all the conservation farming treatments and 4 rows of plants in conventional (CONV) plots. b) Conservation farming plots. Each row consisted of 6 planting basins with 3 plants of maize. c) Conventional plots. Each row had 18 plants. Figure S2 from paper III.

2.2.2 Kaoma

In Kaoma, we conducted an experiment involving three soil management practices: i) normal conservation farming practice, ii) conservation farming with the addition of pigeon pea biochar inside basins and iii) conventional tillage with residue removal (Figure 2.5; paper IV). Maize was planted under the three management practices and the experiment lasted one growing season. Each treatment was evaluated in plots of about 20m^2, containing seven rows of plants with four basins each, and distributed randomly in three blocks (Figure 2.6a). This experiment involved the addition of a single minor dose of ^{15}N that was traced in plants, soil and extractable NO_3^- during 10-days.

Figure 2.5. Soil management regimes in ^{15}N labeling experiment in Kaoma. a) Conservation farming with biochar addition inside planting basins and crop residue retention between rows. b) Conservation farming normal: planting basins with crop residue retention between rows. c) Conventional: overall plough without crop residue retention. Figure S1 from paper IV.

^{15}N labeling experiment

The ^{15}N labeling experiment started 7 weeks after planting, on 16/01/17 and the sampling campaign lasted for 10 days. The experimental setup was a split-plot design, with each plot divided into 4 split-plots that corresponded to the 3 forms of ^{15}N application and the unlabeled (water addition only) reference (Figure 2.6). Three different ^{15}N forms were tested, $NH_4{}^{15}NO_3$, $^{15}NH_4NO_3$, ^{15}N-Urea. In all nine plots, each of the three ^{15}N tracers (99.98 atom%) and the reference (H_2O) were assigned to one row, while one buffer row of maize was kept in between labelled rows to avoid cross contamination (Figure 2.6). Soil samples were taken 1.5, 24, 72 and 240 hours after ^{15}N addition from 0-5 cm and 5-20 cm for ^{15}N analysis. Potassium chloride extracts were prepared *in-situ* within 3 hours and analyzed for NO_3^- and NH_4^+ and the isotopic composition of the extractable NO_3^- was determined. Plant samples were taken 10 days after addition of ^{15}N (Figure 2.7). A mass balance of ^{15} N was calculated.

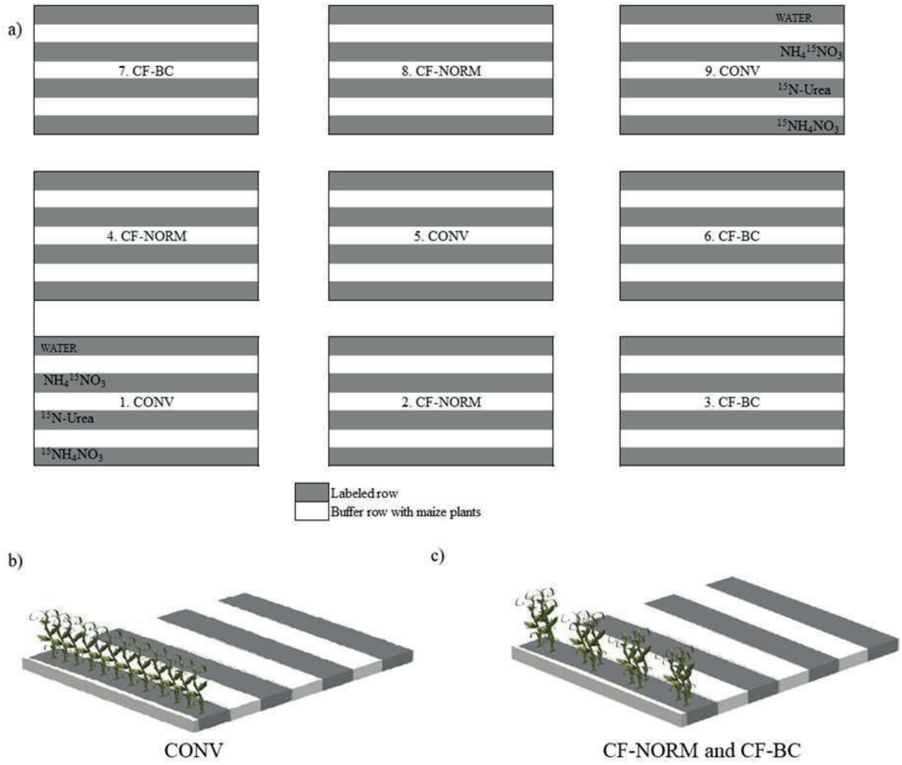
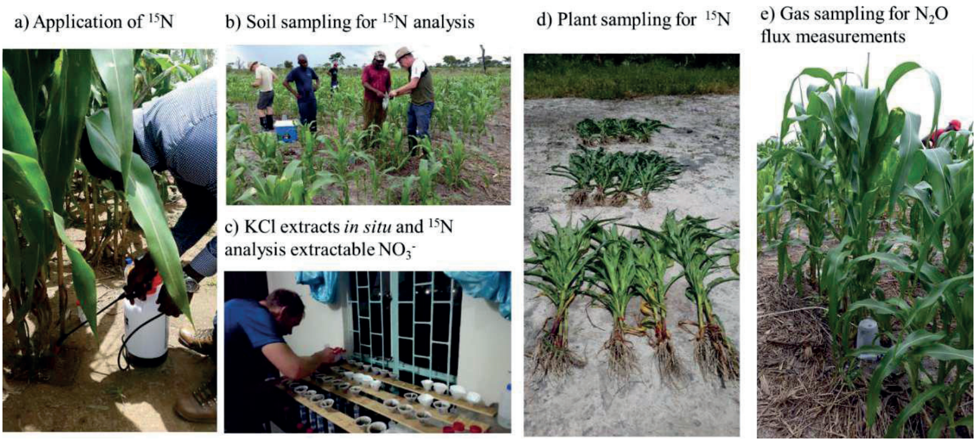


Figure 2.6. a) Overall experimental set-up and detailed planting schemes for b) conventional (CONV) and c) conservation farming with (CF-BC) or without biochar (CF-NORM). CONV had 12 equidistant (21 cm) maize plants per row while CF-BC and CF-NORM had four planting basins per row with 3 maize plants per basin. Each plot had 7 rows.

Nitrous oxide emissions

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 under CF-NORM and CF-BC; for the CONV treatment the chamber was placed in the proximity of the brace roots, which is the zone where fertilizer and ^{15}N were applied (Figure 2.7). Nitrous oxide 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 butyl septa (Chromacol).

Samples were taken 1, 15, 30 and 45 minutes after 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}.


a) Application of ^{15}N

b) Soil sampling for ^{15}N analysis

c) KCl extracts *in situ* and ^{15}N analysis extractable NO_3^-

d) Plant sampling for ^{15}N

e) Gas sampling for N_2O flux measurements

Figure 2.7. a) Application of ^{15}N with a hand sprayer in a planting basin. b) Soil sampling after the ^{15}N application. c) KCl extraction in a makeshift field lab. d) Plant sampling for ^{15}N analysis at the end of the 10-day experiment. e) Static chamber deployment for N_2O flux measurements.

2.3 Data analysis

All the data analysis in the four papers was conducted using package RcmdrPlugin.NMBU of R software (R-Core-Team, 2017). One-way ANOVA was used to assess the effect of shaking time with ammonium acetate and potassium chloride on CEC estimates of biochar (paper I). Two-sided paired *t*-test was used for the comparison of soil parameters inside and outside basins (paper II).We used one-way ANOVA to determine differences in crop biomass and C and N stocks between treatments (paper III). Two way ANOVA (treatment 3 levels and in vs. out 2 levels) was used to assess differences in SOC, total N, N mineralization rate and HWEC between treatments and between inside and outside basins (CF) or rows (CONV; paper III). Linear mixed effect model (Fixed factors: treatment 2 levels, year 2 levels) was used to assess the effects on conservation farming with and without biochar on SOC, total N, N mineralization rate and HWEC over time at 0 to 8 cm and at 8 to 20 cm separately, where block was included as a random factor (paper III).

The spatial autocorrelation between repeated measurements in 2016 and 2018 was assumed constant between the different treatment combinations. Two way ANOVA (treatment 2 levels and in vs. out 2 levels) was used to assess the difference in the particulate organic matter between CF-NORM and CF-NO-RES inside and outside basins in each depth independently (paper III). Linear regression was used to assess the relationship between HWEC and potential N mineralization rates (paper III). Linear mixed effect models were used to test differences between soil management practices, forms of ^{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, and atom% $^{15}N_{soil}$ and atom% $^{15}N_{NO3-}$ at 0 to 5 cm and 5 to 20 cm, respectively (Fig. 5; paper IV). N form, nested in soil management practice, nested in block was used as random factor. The spatial autocorrelation between repeated measurements was assumed constant between the different treatment combinations. The effect of soil management on soil properties, biomass, grain yield, N_2O flux and nitrification rates and effect of soil management and N form on ^{15}N recovery (paper IV) was tested by using linear mixed effect models with block as a random factor. A multiple regression model was set up to link N_2O fluxes with extractable NO_3^- logarithmic conversion of the data (paper IV).

3. Main results and discussion

3.1 Paper I: Cation exchange capacity of biochar

A pre-treatment using diluted hydro-chloric acid was introduced to assess CEC at pH 7, which is important for comparison between biochars, and to remove readily soluble cations, which are derived from salts and not from the negative exchange sites of biochar. Subsequently, we found the ideal conditions for extraction of exchangeable cations, removal of excess ammonium and saturation with potassium. The results showed that about 83% of all exchangeable base cations of the biochar samples were extracted in the first extraction with 20 ml of ammonium acetate. We found that the readily soluble and the exchangeable cations were two different pools, which differed in the relative contribution of the main cations (Table 3.1). The results showed that biochar has more readily soluble than exchangeable cations (Table 3.1). The results showed that at least 4 washings with isopropanol were needed to obtain small amounts of excess NH_4^+. The first extraction with 20 ml 2M KCl following isopropanol washing removed 93% of the adsorbed NH_4^+ for all the biochars.

The sum of base cations in ammonium acetate extract provided a smaller and better estimate of the CEC than KCl-extractable NH_4^+ (Figure 3.1). We hypothesized that the overestimation of the CEC based on KCl-extractable NH_4^+ is due to the ineffectiveness of the relatively large isopropanol molecules to remove the excess of ammonium acetate in biochars rich in nano-pores, due to size exclusion. In this paper, we also suggested using ethanol or even water instead of isopropanol. In a further experiment (non-published data), we found that our hypothesis was correct and that ethanol was better than isopropanol removing excess NH_4^+ due to smaller size of molecules. The CEC normalized to C content of biochar varied from 10.8 to 119.6 $cmol_{(+)}\ kg^{-1}$ which is much lower than the values reported for native SOC [221 $cmol_{(+)}\ kg\ C^{-1}$ to 810 $cmol_{(+)}\ kg\ C^{-1}$; (Martinsen et al., 2017; Parfitt et al., 2008)].

Table 3.1. Base cations removed in the pretreatment and exchangeable base cations for 4 types of biochar. Table S2 in Munera-Echeverri et al. (2018.

	Cation	**Cacao Shell cmol(+)/kg**	**Corncob cmol(+)/kg**	**Rice husk cmol(+)/kg**	**Pigeon Pea cmol(+)/kg**
Base cations removed in the pretreatment	**Ca**	16.1	1.1	2.0	7.6
	s.d	*0.8*	*0.1*	*0.04*	*0.5*
	Mg	33.5	0.8	1.1	10.3
	s.d	*2.8*	*0.2*	*0.06*	*0.5*
	K	200	18.3	10.5	38.6
	s.d	*6.5*	*0.4*	*0.4*	*0.8*
	Na	1.0	0.2	0.2	0.1
	s.d	*0.01*	*0.2*	*0.04*	*0.002*
	Sum of cations	**250.6**	**20.4**	**13.8**	**56.6**
Exchangeable base cations	**Ca**	37.5	2.5	1.7	3.5
	s.d	*2.5*	*1.5*	*0.03*	*0.8*
	Mg	12.3	0.6	0.6	2.0
	s.d	*1.4*	*0.2*	*0.0*	*0.6*
	K	9.2	2.9	3.9	1.1
	s.d	*0.3*	*0.24*	*0.03*	*0.29*
	Na	0.1	0.001	0.1	0.008
	s.d	*0.006*	*0.001*	*0.004*	*0.001*
	CEC-BC	**59.1**	**6.0**	**6.2**	**6.6**
	s.d	*4.2*	*1.8*	*0.03*	*1.6*

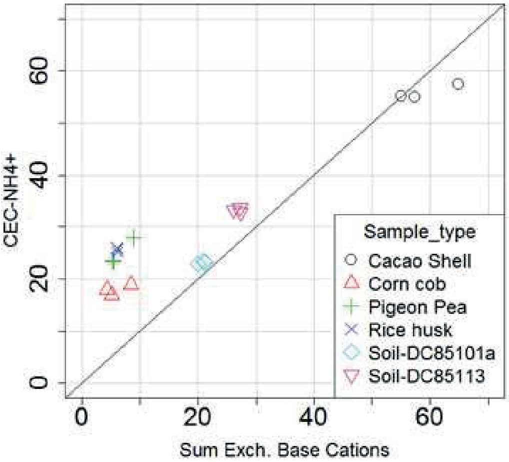


Figure 3.1. CEC-NH_4^+ ($cmol_{(+)}$/Kg) as function of CEC-BC ($cmol_{(+)}$/Kg;). The line is 1:1 line. Figure 2 in Munera-Echeverri et al. (2018).

3.2 Paper II: Build-up of soil organic C and N mineralization inside basins

The results showed that 7 years of conservation farming practices led to higher SOC concentration, C stock and CEC inside basins than outside (Figure 3.2). The relative increase of SOC was 2.95‰ per year, which is lower than the aimed by the "4 per 1000" initiative (www.4p1000.org). The significant increase of SOC inside basins was attributed to root biomass, since the basins were carefully maintained in the same place during the seven years of CF practice. Therefore, we confirmed the hypothesis that CF leads to higher SOC inside basins, but we still did not have enough evidence to attribute it to root biomass. Hot-water extractable C was higher inside basins than outside and NO_3^- production rate in an incubation experiment was higher inside than outside basins at 8 to 20 cm (Figure 3.3). Results suggested higher N mineralization inside basins than outside, which may be attributed to the higher amounts of labile C (HWEC) inside basins (Figure 3.3). The N mineralization essay showed that nitrate accumulates over time while ammonium is immobilized during incubation at 20°C for 60 days. This is in agreement with results by Martinsen et al. (2017, who found net nitrate mobilization and net ammonium immobilization in agricultural soils in Eastern and Central Providence in Zambia.

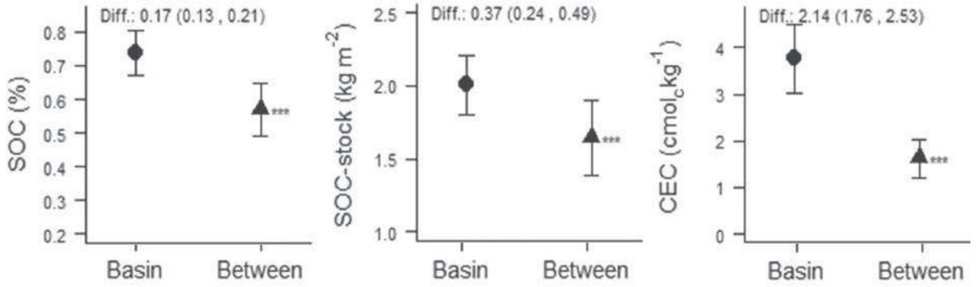


Figure 3.2. Mean (±sd) of concentration soil organic carbon (SOC; %), C stock (kg C m^{-2}) and cation exchange capacity (CEC) at 0 to 20 cm under CF inside and in between basins. *** p<0.001, based on two-sided paired *t*-test. Modified from figure 2 in Martinsen et al. (2019.

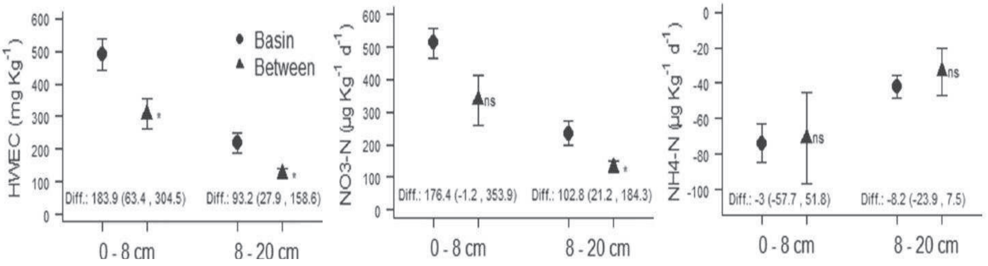


Figure 3.3. Mean (±sd) hot water extractable carbon (HWEC,mg kg soil^{-1}) and net rates of NO_3-N and NH_4-N production (µg N kg soil^{-1} day^{-1}) during 60 days of incubation of soils (0–8 cm and 8–20 cm) from inside CF basins and in rows between CF basins (outside), Mkushi, Zambia. Modified from figure 3 in Martinsen et al. (2019.

3.3 Paper III: Effect of conservation farming and biochar on crop biomass, SOC and N mineralization

Soya yield and stover were lower in CONV (2.1 ton ha^{-1}) than in CF treatments (3.0 ton ha^{-1}; Table 3.2), when no fertilizer was applied. The negative effect of CONV on yield was not observed in the case of maize yield, likely due to the ample application rate of urea and NPK fertilizer. We found no significant effect of biochar on biomass production. In average, the maize stover production corresponded to 4.9 ton ha^{-1} season^{-1} and the soya stover to 2.8 to ton ha^{-1} season^{-1} in CF treatments and 1.9 ton ha^{-1} season^{-1} under conventional tillage (Table 3.2).

Table 3.2 Grain yield and stover production in the growing seasons 2015-16, 2016-17, 2017-18 in Conservation farming (CF) with biochar (CF-BC), normal CF (CF-NORM), CF with no residue (CF-NO-RES) and conventional (CONV). Standard error of the mean (s.e), n = 4.

Growing season		**CF-BC**	**CF-NORM**	**CF-NO-RES**	**CONV**
2015-16 Maize	Grain (ton ha^{-1})	5.0	5.2	6.1	4.7
	s.e	*0.4*	*0.6*	*0.4*	*0.4*
	Stover (ton ha^{-1})	4.9	4.4	5.3	3.7
	s.e	*0.3*	*0.1*	*0.7*	*0.4*
2016-17 Soya	Grain (ton ha^{-1})	3.0^{a}	3.4^{a}	3.6^{a}	2.1^{b}
	s.e	*0.2*	*0.3*	*0.2*	*0.3*
	Stover (ton ha^{-1})	2.5^{a}	2.9^{a}	3.1^{a}	1.9^{b}
	s.e	*0.3*	*0.3*	*0.2*	*0.2*
2017-18 Maize	Grain (ton ha^{-1})	3.1	3.5	3.6	2.2
	s.e	*0.2*	*0.3*	*0.3*	*0.5*
	Stover (ton ha^{-1})	5.7	4.9	5.7	4.7
	s.e	*0.4*	*0.1*	*0.5*	*0.5*

The results showed that residue removal did not have any effect on particulate organic matter, hot-water extractable C, N mineralization and SOC after 3 growing seasons (Figure 3.4; Table 3.3). This may be explained by high decomposition rates of crop residues (Six et al., 2002). However, residue losses due to termite activity, which increases with increasing residues (Mutsamba et al., 2016), or transport by wind and water may also have contributed to the lack of effect of residues on SOC and its particulate and soluble fractions. Therefore, we reject the hypothesis that residue retention has a positive effect on the amount and quality of SOC and N mineralization rate. However, the fact that residues do not have an effect on either soil organic matter does not imply that they do not provide other benefits such as soil moisture retention and weed control if they are kept as mulch (Turmel et al., 2015).

Table 3.3. Particulate organic matter (POM) in the bulk soil in CF-NORM and CF-NO-RES inside and outside basins at 0 to 8 cm and 8 to 20 cm in 2018. Lowercase letters denote differences in POM between inside and outside basins independent of the soil management treatment. Modified version of Table 2 in paper III.

	POM in bulk soil (%)	
	CF-NO-RES	**CF-NORM**
Inside	**a**	
0-8 cm	0.82±0.02	0.81±0.13
8-20 cm	0.43±0.03	0.44±0.06
Outside	**b**	
0-8 cm	0.40±0.03	0.44±0.03
8-20 cm	0.20±0.02	0.26±0.02

Under conservation farming, particulate organic matter increased inside basins (Table 3.3), which indirectly confirms the hypothesis in paper II that the increase of SOC inside basins is due to root biomass inputs. Our data show that root biomass is more effective than aboveground biomass in restoring particulate organic matter, which is in agreement with previous research (Kong and Six, 2010). The data also showed that the positive effects of conservation farming on SOC and N mineralization, that were accumulated over seven years inside basins, disappeared upon conventional tillage, since SOC and N mineralization decreased to the levels observed outside basins. This could tentatively explain the fact that conservation farming had positive effects on crop yield as compared to conventional farming only when fertilizer was not applied. When ample amount of fertilizer was used, the difference in yield was not significant. Therefore, we confirmed the hypothesis that CF gives higher crop yields attributed to some extent to the higher N mineralization rate inside basins.

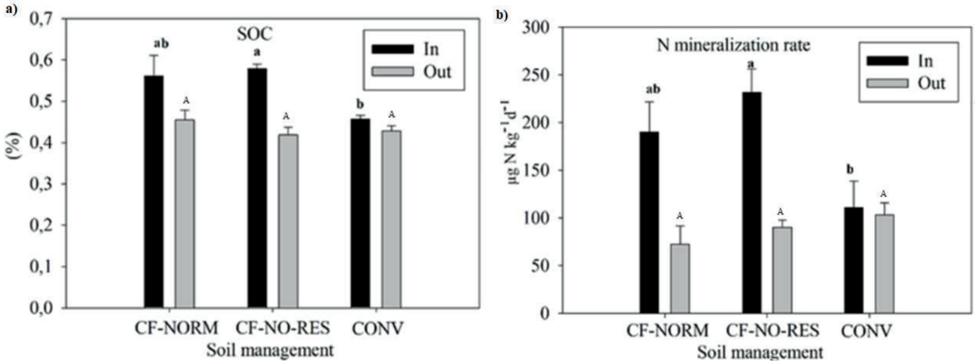


Figure 3.4. (a) Soil organic C and(b) N mineralization rate inside and outside basins under CF-NORM and CF-NO-RES and inside and outside planting rows in CONV in 2018 at 0 to 20 cm depth. Error bars represent standard error (n=4). Lower case letters indicate significant differences ($p<0.05$) between soil management practices inside and outside basins (or inside and outside rows of plants under CONV). Modified version of Figure 1 in paper III.

The data shows that biochar increased SOC but it did not affect either hot-water extractable C or had any priming effect on N mineralization (Figure 3.5 a and b). Nitrogen mineralization rates were highly correlated with HWEC independently from soil management practice or total amount of SOC (Figure 3.5b). Previous studies that suggest that biochar is a stable material in soil that persists for hundreds of years (Budai et al., 2016). The lack of priming effect on N mineralization contrasts with other studies that find either positive or negative priming effects on SOC (Budai et al., 2016; Lu et al., 2014; Zimmerman et al., 2011). Therefore, we found evidence for rejecting the hypothesis than biochar increases crop yields by positive priming on N mineralization.

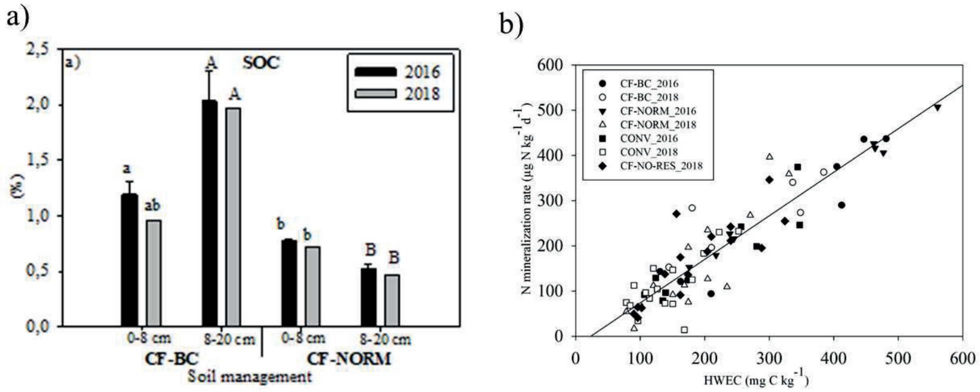


Figure 3.5**.** a) Soil organic C (%) inside planting basins in CF-NORM and CF-BC at 0 to 8 cm and 8 to 20 cm depth in 2016 and 2018. Lower case letters denote differences between treatments at 0 to 8 cm. Upper case letters show differences between treatments at 8 to 20 cm. b) Relationship between HWEC and potential N mineralization rates in CF-BC, CF-NORM, CF-NO-RES and CONV in the sampling campaigns of 2016 and 2018 [$R^2 = 0.82$; N min rate (µg-N $kg^{-1}d^{-1}$) ~ 0.9622 x HWEC (mg C Kg^{-1}) – 22.359]. Modified version of figure 2a and 3 from paper III.

3.4 Paper IV: Nitrous oxide emissions, partitioning of mineral N in the plant-soil system and gross nitrification under conservation farming with biochar amendment

Nitrous oxide emissions

A positive relation with extractable NO_3^- in soil explained N_2O fluxes best (Figure 3.6). The average N_2O flux in CONV (8.4 ±0.7 µg N_2O-N m^{-2} hr^{-1}) was significantly lower than in conservation farming with (34.1±4.1 µg N_2O-N m^{-2} hr^{-1}) and without (26.1±3.8 µg N_2O-N m^{-2} hr^{-1}) biochar addition, whereas biochar did not affect N_2O emissions (Table 3.4). Biochar did not affect soil pH (details in paper IV), thus, we reject the hypothesis that biochar addition decreases N_2O emissions by the liming effect. Although biochar increased extractable NO_3^- (Figure 3.7), it did not affect N_2O emissions (Table 3.4). This could suggest that denitrification was not the source of N_2O in this soil. The water-filled pore space values below 60 % during sampling (details in paper IV) and the significant relationship between gross nitrification rates and N_2O fluxes (Figure 3.8) support nitrification as the main source of N_2O. Conservation farming had higher N_2O emissions than CONV and this was due to larger amounts of mineral N (Figure 3.7) rather than to higher soil moisture retention under CF (details in paper IV). Lower mineral N in CONV could be explained by the way the fertilizer is applied in CF vs in CONV. The amount of fertilizer per hectare was the same, but the amount for 3 plants was concentrated inside basins in CF, whereas every plant along the rows received a dash of fertilizer in CONV.

The data suggest that biochar increased gross nitrification rate at 5 to 20 cm, the depth at which the biochar was applied, whereas in CF-NORM and in CONV gross nitrification rates were the same at both depths (Table 3.4). Biochar increased extractable NO_3^- likely by increasing gross nitrification rate (Figure 3.8; Table 3.4). The data suggest that biochar increased gross nitrification as compared to conventional tillage ($p=0.024$) and normal conservation farming ($p=0.061$), however, the variability of rates was large in the treatment with biochar addition (Figure 3.8).

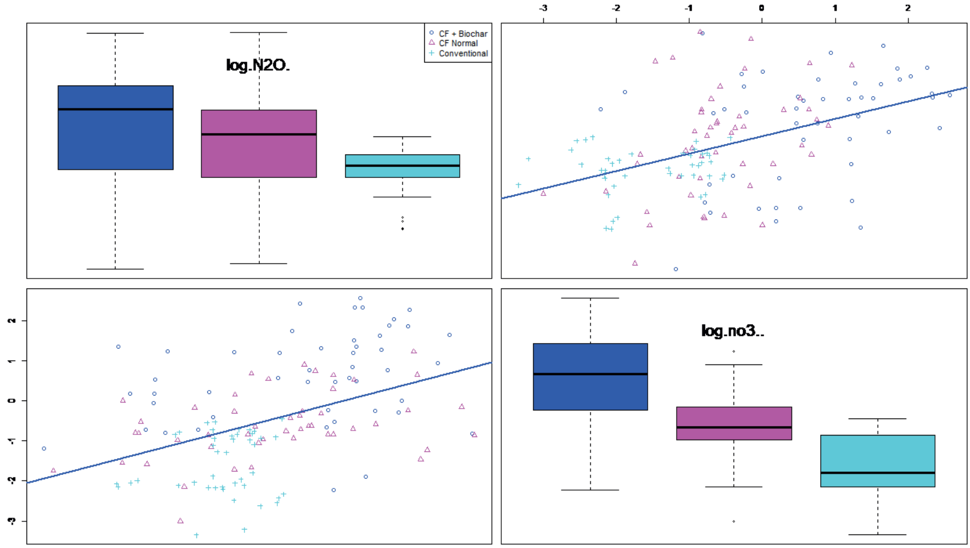


Figure 3.6. Natural logarithm of N_2O fluxes as a function of natural logarithm of KCl-extractable NO_3^- [log(N_2O) ~ 2.79662 + 0.38392*log (KCl-NO_3^-); $p<0.001$, $R^2 = 0.20$]. Figure S3 from paper IV.

Tab. 3.4. Average N_2O flux and estimated gross nitrification rates in CF-BC, CF-NORM and CONV at 0 to 5 cm and 5 to 20 cm. Values are means with standard errors. For N_2O fluxes n=21 and for gross nitrification n=3. The average temperature during the gas sampling campaign was 26˚C. Table 2 from paper IV.

	Units	**CF-BC**	**CF-NORM**	**CONV**
Mean N_2O flux	µg N m^{-2} hr^{-1}	34.1 (4.1)^a	26.1 (3.8)^a	8.4 (0.7)^b
Gross nitrification				
0-5 cm	mg N kg^{-1} d^{-1}	0.6 (0.60)^b	0.2 (0.17)	0.2 (0.11)
5-20 cm	mg N kg^{-1} d^{-1}	4.5 (1.91)^a	0.9 (0.78)	0.1 (0.03)

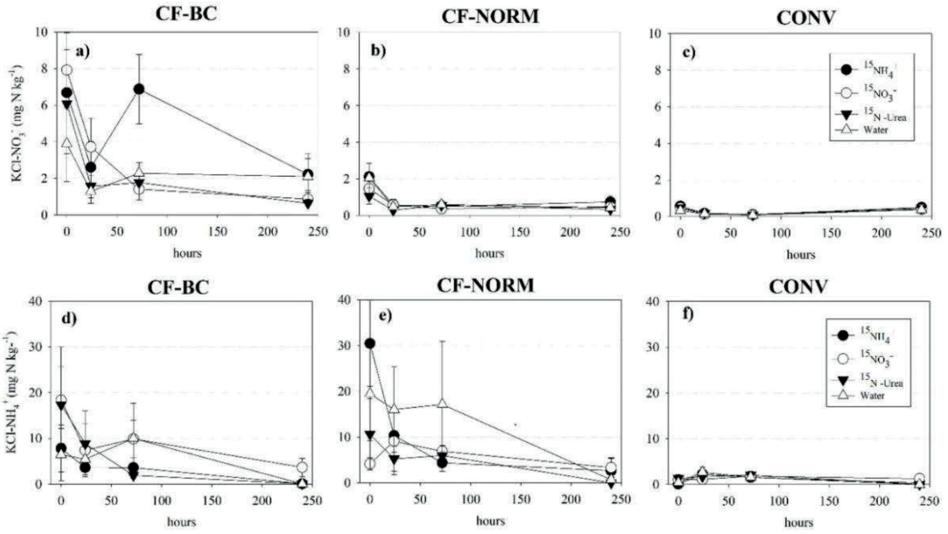


Figure 3.7. Weighted average (0-20 cm) of KCl-NO$_3^-$ (mg N kg^{-1}; a, b and c), NH$_4^+$ (mg N kg^{-1}; d, e and f) and N_2O fluxes (µg N m^{-2} h^{-1}; g, h and i) in CF-BC, CF-NORM and CONV in response to the addition of ^{15}NH$_4^+$, ^{15}NO$_3^-$, ^{15}N-Urea or water during the 240-hour labeling experiment. Values are means and standard errors (n=3). Figure 3 from paper IV.

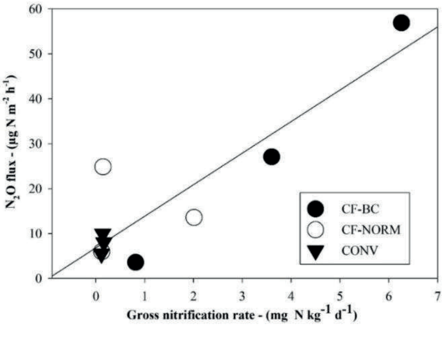


Figure 3.8. Relationship between N_2O fluxes in the ^{15}NO$_3^-$ treatments and gross nitrification rates based on ^{15}N pool dilution (depth-weighted 0 – 20 cm). Nitrification estimates are based on mass balance and change in atom $^{15}N_{NO3}$ from 24 hours to 72 hours (R^2=0.78; N_2O flux ~ 6.855 + 7.021* gross nitrification rate). Figure 5 from paper IV.

Mass balance of ^{15}N

The ^{15}N mass balance showed no effect of soil management practice, including the addition of biochar, on the total recovery of ^{15}N after 10 days (Figure 3.9). Biochar did not have an effect on the ^{15}N recovery in soil and maize plants. Biochar only increased the amount of ^{15}N recovered as NO_3^- when ^{15}N was applied as $^{15}NH_4^+$ but this contributed few to the total ^{15}N mass balance. Therefore, we reject the hypothesis that biochar increases the short-term retention of N in the plant-soil system. There was a clear effect of the form of ^{15}N added. Total recovery of ^{15}N (plants, soil and nitrate) was 38%, 64% and 86% of added ^{15}N, if applied as $^{15}NO_3^-$, ^{15}N-Urea and $^{15}NH_4^+$, respectively. This indicates large losses of NO_3^- under field conditions, tentatively due to leaching, as it has been reported in Arenosols in Zimbabwe (Nyamangara et al., 2003).The total ^{15}N recovery was dominated by recovery in soil and this was lower if added as $^{15}NO_3^-$ than as $^{15}NH_4^+$ and ^{15}N-Urea (Figure 3.9). Uptake of ^{15}N in maize was lower if added as ^{15}N-urea than as $^{15}NH_4^+$ and $^{15}NO_3^-$.

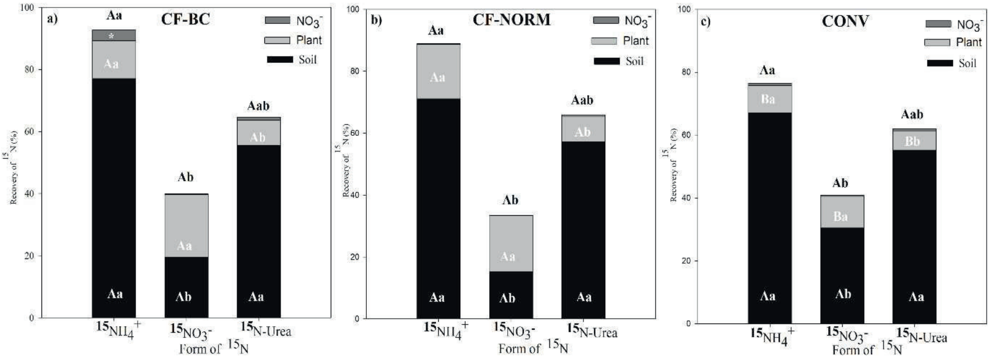


Figure 3.9. ^{15}N recovery (%) in KCl-extractable NO_3^-, in residual soil from 0 to 20 cm and in maize plants in a) CF-BC, b) CF-NORM and c) CONV, labelled with $^{15}NH_4^+$, $^{15}NO_3^-$ and ^{15}N-Urea, respectively. Uppercase letters indicate the difference between soil management, whereas the lowercase letters indicate the difference between forms of ^{15}N for the same management. The black letters above the bars indicate the difference in total recovery, the white letters the recovery in soil. Figure 6 in paper IV.

4. Conclusions and implications

In this study, we contributed to the understanding of the CEC values of the carbonaceous material of biochar. We investigated the base cations associated with the ash component of biochar and the cations associated with the exchange sites of the C-rich material. While the effect of the ashes is short-lived, the effects of the carbonaceous material last longer, and in the case of CEC, is it thought to increase overtime upon biochar ageing under field conditions. We showed that some biochars, such as cacao shell biochar, may have a large amount of cations and alkalinity associated with soluble salts of the ash component, while others, such as rice husk biochar, have few cations and low alkalinity. The results suggest that when readily soluble cations are high, there is a high probability of overestimating CEC by sum of base cation in NH_4OAc. We compared two ways of analyzing CEC (sum of base cations after pre-treatment and displaced NH_4^+ by K^+ after washing with isopropanol). We found better estimates by sum of base cations in NH_4OAc extract, since isopropanol is not effective removing all the excess of NH_4^+ from the nano-pores of biochar. In general, CEC values normalized to C content of biochar are low as compared to the CEC values of native soil organic C. Further research is needed to understand the effect of biochar ageing in the field on CEC.

The results show that conservation farming in the form of permanent basins with residue retention increased SOC while having positive agronomic effects in Zambian Acrisols. The increase of SOC occurred in the area inside basins rather than outside basins. Since the area inside basins occupies about 10 % of the land, the increase of SOC inside basins vs outside basins was low (2.9‰ SOC yr^{-1} 20 cm deep). Yet, this low increase in SOC led to N mineralization rates about 2.5 times higher inside basins than outside. Higher particulate organic matter and hot-water extractable C inside basins vs outside indicate that root biomass was more effective than residue biomass in increasing the quality and amount of soil organic matter. The results show that residues did not play an important role in increasing either yield or SOC. Conventional tillage led to a significant decrease in soya yield in a maize-soya-maize rotation that involved the addition of fertilizer only to maize and not to soya. Soil organic C, hot-water extractable C and N mineralization rate decreased upon tillage to the same level observed in the area outside basins. This could tentatively explain the decrease in crop yield upon conventional tillage only when fertilizer was not added. Biochar addition inside basins increased SOC and C stock but it did not have any effect on crop yields and N mineralization.

This study shows that soil extractable NO_3^- was the factor explaining best N_2O emissions rather than soil pH and soil moisture. The results show a high correlation between gross nitrification rates and N_2O fluxes and water-filled pore space values lower than 60%, which suggest that nitrification was a dominant source of N_2O during sampling. This explains why biochar did not have an effect on N_2O despite increasing extractable NO_3^-. Biochar did not have any effect on soil pH, therefore we could not assess the effect of liming on N_2O fluxes. However, the effect of soil pH on N_2O fluxes in a system dominated by nitrification is perhaps not so relevant at a pH suitable for crops. We found that biochar increased extractable NO_3^- in soil likely due to an increase in gross nitrification rate. Biochar did not have an effect on the partitioning of ^{15}N in the plant-soil system. We found higher mineral N in conservation farming systems vs conventional and we attributed this to the differences in the fertilizer application method. However, from our data, it is not possible to determine if this is always the case. Therefore, further research on the dynamics of mineral N under conservation farming systems vs conventional systems is needed since it has implications for understanding the spatio-temporal variability of N_2O fluxes. The N_2O fluxes in this study were low in the case of conventional tillage to medium in CF systems as compared to values reported in the literature. The plant uptake of ^{15}N under conventional tillage was lower than in CF systems likely due to the lower amounts of mineral N in the soil. The form of ^{15}N added affected the total recovery of ^{15}N after 10 days, with a high proportion of the ^{15}N recovered (86%) if added as $^{15}NH_4^+$ while low recovery if added as $^{15}NO_3^-$. Our results suggest large losses of NO_3^- most likely due to leaching rather than gaseous losses.

5. Outlook

In this study, I validated a methodology for analysis of cation exchange in biochar. I gained an understanding of how CEC estimates of biochar compare with the values of soil organic matter. The method still needs further development in the washing step after saturation with NH_4OAc, namely, to assess the effect of other solvents such as ethanol or water on CEC estimates based on displaced NH_4^+. However, this method is a good starting point for studying the effect of biochar ageing in the field on CEC. It would be of interest to study the change in CEC overtime in already existing long-term experiments involving amendment with biochar. Also, I found that some biochars can have a notably high CEC and alkalinity like cacao shell biochar, whereas others such as corncob, rice husk or pigeon pea biochars have low CEC and low alkalinity. I think it is needed a larger screen of the properties of biochars made from other types of accessible feedstock in rural communities of developing countries. This may help to predict the agronomical performance of a pool accessible biochars, their effects on N_2O emissions and to explore their effect on remediation of cationic pollutants in drinking water, such as methylmercury and other types of heavy metals.

I found interesting the effect of permanent planting basins on N mineralization rates because it shows how plant roots can improve the rhizosphere soil by inputs of particulate and labile organic matter; and make it more active biologically than the surrounding soil. This also has implications for crop productivity. I would like to know more about how farmers can make use of root biomass inputs for their own interest. Further research is needed on root-derived SOM under other forms of minimum tillage or no-till technologies. Also, the effect of different crop rotations on root-derived SOC. The dynamics of soil mineral N in response to conservation farming vs conventional systems need further research since it has implications for N_2O emissions.

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Paper I

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Cation exchange capacity of biochar: An urgent method modification

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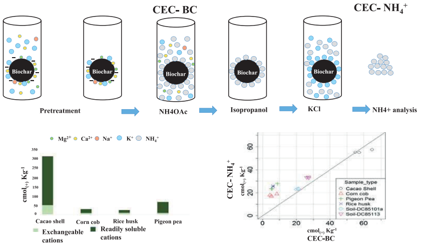
HIGHLIGHTS

- The ammonium acetate (NH_4OAc) method was validated in batch for different biochars.
- CEC estimates were based on sum of base cations and displaced NH_4^+ by K^+.
- Optimal equilibration time and amount of NH_4OAc and KCl were established.
- The role of readily soluble and exchangeable cations was compared.
- Isopropanol did not penetrate all the micro-pores of some biochars.

GRAPHICAL ABSTRACT



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ABSTRACT

Biochar, produced through pyrolysis of organic matter, is negatively charged, thus contributing to electrostatic adsorption of cations. However, due to its porous structure and contents of alkaline ashes, the determination of the cation exchange capacity (CEC) is challenging. Literature values for the CEC of biochar are surprisingly variable and are often poorly reproducible, suggesting methodological problems. Here, we modify and critically assess different steps in the existing ammonium acetate (NH_4OAc) method (pH 7), where ammonium (NH_4^+) is displaced by potassium chloride (KCl), following removal of excess NH_4OAc with isopropanol, in batch mode. We used pigeon pea biochar to develop the method and conducted a test on three additional biochars with different acid neutralizing capacity. A pretreatment step of biochar was introduced, using diluted hydrochloric acid, to decrease biochar pH to near neutral, so that 1 M NH_4OAc effectively buffers the biochar suspension pH at 7. This allows the CEC of all biochars to be determined at pH 7, which is crucial for biochar comparison. The dissolution of ashes may cause relatively large weight losses (e.g. for cacao shell biochar), which need to be accounted for when computing the CEC of raw biochar. The sum of NH_4OAC-extractable base cations provided a smaller and better estimate of the CEC than KCl-extractable NH_4^+. We hypothesize that the overestimation of the CEC based on KCl-extractable NH_4^+ is due to the ineffectiveness of the relatively large isopropanol molecules to remove excess NH_4OAc in biochars rich in micro-pores, due to size exclusion. The amount of base cations removed in the pretreatment was about three (rice husk biochar) to ten times (pigeon pea biochar) greater than the amount of exchangeable cations. The CEC values of biochar increased from 10.8 cmol/Kg carbon to 119.6 cmol/Kg carbon. These values are smaller than reported CEC values of soil organic carbon.

1. Introduction

Biochar is a carbon-rich product made by pyrolysis of organic waste, which may be used as a soil enhancer. Particularly, in tropical soils biochar has been shown to have a positive impact on soil fertility, including

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increased potassium (K^+) content, pH, water retention capacity, and cation exchange capacity (Jeffery et al., 2011; Liang et al., 2006; Martinsen et al., 2015), which all contribute to increased crop yield (Jeffery et al., 2017). Moreover, biochar is emerging as an alternative for heavy metal remediation in soil and water (Ahmad et al., 2014). For the assessment of the effect of biochar on soil properties and its potential as remediation agent, it is important to have reliable and accurate determination of its contribution to the soil's cation exchange capacity (CEC). However, in the literature CEC estimates for biochar are highly variable, commonly ranging from 5 to 50 $cmol_{(+)}$ Kg^{-1} (Agegnehu et al., 2016; Berek and Hue, 2016; Budai et al., 2014; Gamage et al., 2016; Nelissen et al., 2015; Singh et al., 2010; Song and Guo, 2012) and even reaching values as high as 69 to 204 cmol $_{(+)}$ Kg^{-1} (Lou et al., 2016; Mukherjee et al., 2011; Pandit et al., 2018; Yuan et al., 2011). The large variability in CEC may be due to the number of factors affecting the surface properties of biochar, such as charring temperature and feedstock (Budai et al., 2014; Suliman et al., 2016). However, it also may be attributed to errors in the analytical method, which have not yet been properly addressed. Previous research (Graber et al., 2017) summarized the potential sources of error in CEC determination of biochar, which may relate to the presence of ashes, porosity and the intrinsic hydrophobicity of biochar:

1. Incomplete saturation of exchange sites, due to hydrophobicity of biochar, which may cause poor wetting of the sample and thus underestimation of the CEC.
2. Slow diffusion of replacing cations, due to the micro-porous structure of biochar, which can prolong the equilibration time, potentially causing underestimation of the CEC.
3. Floating and non-settling particles can increase substantially the mass losses during the decantation process may cause underestimation of the CEC.
4. Release of base cations coming from the dissolution of carbonates and silicates present in ashes can interfere with the sum of exchangeable base cations (overestimation of CEC).
5. Soluble carbonates in biochar can change the pH of the replacing solution, with increasing pH causing an increase in CEC.

Methodologies, where displacement after washing is applied are commonly used for CEC analysis in soil (Bache, 1976; Rhoades, 1982) and more recently in biochar (Graber et al., 2017). Often, base cations extracted by NH_4OAc at pH 7 (Chapman, 1965; Hendershot et al., 2008; Schollenberger, 1945) are used to estimate sum of exchangeable base cations, which, in near-neutral soils, often is assumed to be equal to CEC. Alternatively, the CEC is based on displaced NH_4^+ (CEC-NH_4^+) by KCl in a subsequent step, after washing with an organic solvent, such as isopropanol or ethanol. Advantages of NH_4OAc include the avoidance of hydrolysis problems of non-neutral salts, the high wetting and penetration capacity (e.g. compared with barium chloride), and the inexpensive reagents (Schollenberger, 1945). Moreover, NH_4OAc (pH 7) allows comparison of different biochars at neutral pH, which is important, due to the pH dependency of CEC and the high variability in surface properties among different biochars (Mukherjee et al., 2011). However, biochar's CEC may differ considerably between its natural pH and pH 7. In addition, the alkalinity of biochar is highly variable and for some chars it may be needed to adjust pH with acid before using NH_4OAc (pH 7). This may cause problems such as removal of some cations, electrostatically bound to weak acid functional groups at the biochar surface, due to protonation of these groups in response to acid addition, and thus, affecting CEC based on sum of base cations (CEC-BC).

Graber et al. (2017) modified a two-step procedure for determination of CEC of biochar using 1 M NH_4OAc (pH 7), isopropanol and 2 M KCl. In brief, biochar was shaken with 1 M NH_4OAc at pH 7 in batch mode and then transferred into a mechanical extractor, where more NH_4OAc was added, before washing with alcohol and subsequently with KCl. According to these authors the method has a number of aspects which need further development, including: 1) The biochar: solution (B:S) ratio required for the saturation with NH_4^+ and displacement with K^+. 2) The equilibration time of the saturation of exchange sites with NH_4^+ and its subsequent displacement with K^+. 3) Amount of extraction steps required to get full saturation and subsequent removal of NH_4^+. In the present study we explore most of these aspects and suggest ways forward. The batch approach ensures contact between the biochar and the solution, which is important when assessing the reaction time with NH_4OAc, KCl and isopropanol and it avoids problems such as preferential flow in the column.

In our study we will critically assess (A) the B:S ratio required for the saturation with NH_4^+ and displacement with K^+, the equilibrium time with 1 M NH_4OAc and 2 M KCl, the volume of isopropanol needed to wash the excess NH_4^+, the optimal volume of 1 M NH_4OAc to sufficiently displace cations on biochar surface and the volume of KCl to replace NH_4^+ from the exchange sites, (B) to assess the relative contribution of readily soluble salts vs. exchangeable cations and (C) to compare CEC estimates based on displacement of NH_4^+ by K^+ and CEC based on sum of base cations.

2. Materials and methods

2.1. Biochar samples

Four types of biochar were used in this study: pigeon pea biochar (made at 600 °C in an earth-mound kiln at Mkushi, Zambia), cacao shell biochar (made in a locally constructed kiln at Bogor, Indonesia at 350 °C (Hale et al., 2013)), corncob biochar (made in a traditional earth-mound kiln in Zambia at 400 °C during 7 days (Cornelissen et al., 2013)) and rice husk biochar (made at 400–500 °C in a drum retort kiln at Chisamba, Zambia at 350 °C (Obia et al., 2016)). The biochar production methods are discussed in the references. In addition, two certified soils materials (NCS DC85101a and NCSDC85113 (NCS, 2017)) were included in the batch experiment. Biochar samples of about 5 L were taken to the Norwegian University of Life Sciences (NMBU), where they were crushed and sieved to 0.5 mm to 2 mm.

2.2. Biochar pretreatment

For some of the biochars, the buffered NH_4OAc (pH 7) solution may not adjust the pH to 7 (this is especially important for biochars with high alkalinity). Therefore, CEC estimates with and without pretreatment may diverge, since CEC is pH-dependent. To circumvent this problem, the biochar samples were washed with deionized water and the pH values adjusted to pH 7, using 0.05 M hydrochloric acid (HCl), thus removing readily soluble cations associated with salts, ashes and weak acid functional groups. First, 1 g of biochar (1.0 ± 0.005) and 20 ml of deionized water were added to 50 ml polypropylene tubes. The tubes were shaken at 200 rpm in a horizontal shaker overnight to ensure a proper wetting of the sample. Next, 0.05 M HCl was gradually added to the tubes until reaching pH 7.0 ± 0.3. Subsequently, the tubes were shaken during 24 additional hours. The amount of acid added was recorded and the amount of H^+ to reach pH 7 was calculated to determine the acid neutralizing capacity (ANC_{pH7}). Next, the tubes were centrifuged at 1700 *g* during 15 min, the supernatants were removed using a 10 ml pipette and the biochar slurry in the tubes was washed twice more with 20 ml deionized water and shaken for 24 h each time. The supernatant in the third washing should have EC values < 0.2 mS cm^{-1} to avoid significant amounts of base cations in the slurry prior to NH_4OAc addition, which may cause overestimation of exchangeable base cations in the NH_4OAc extracts. The total amount of water and dilute HCl used for each biochar were recorded and the supernatants were stored for analysis of base cations by inductively coupled plasma optical emission spectrometry (ICP-OES).

2.3. Release of exchangeable cations in NH_4OAc (pH 7)

To know the adequate amount of NH_4OAc needed to extract all exchangeable cations, the four types of biochar and two certified soil materials (1 g dry weight of each) were extracted four times consecutively with 20 ml 1 M NH_4OAc each time. In the first extraction, the tubes were shaken horizontally during 24 h at 200prm, then centrifuged at 1700*g* during 15 min and the supernatants collected and stored until analysis. In the three subsequent extractions, the supernatants were collected after 2 h shaking. The extracts were analyzed for Ca^{2+}, Mg^{2+}, K^+, Na^+, Fe, Al and Mn by ICP-OES and the CEC calculated based on sum of exchangeable base cations.

2.4. Washing of excess NH_4OAc with isopropanol and subsequent NH_4^+ extraction with KCl

The adequate amount of isopropanol needed to remove excess NH_4^+ after shaking with NH_4OAc, was determined, using pigeon pea biochar. The test involved four washings steps with 20 ml 99% isopropanol in triplicate. The biochar was pre-treated as described above; subsequently, 20 ml of 1 M NH_4OAc was added and the tubes were shaken at 200 rpm during 24 h. After extraction with NH_4OAc, the biochar slurry was washed with 20 ml 99% isopropanol, once, twice, three and four times, respectively. In each case, 20 ml of 2 M KCl were added subsequently and the tubes were shaken during 24 h at 200 rpm. The tubes were centrifuged at 1700 *g*, and the supernatants were analyzed for NH_4^+ (for details see next paragraph). In addition, we determined NH_4^+ in isopropanol extracts after three and four washings in the other biochar types and certified soils.

2.4.1. Biochar to solution ratios and equilibration time for KCl extraction

A factorial 3 × 3 × 2 experiment was set up to assess the importance of diffusion of replacing cations (NH_4^+ and K^+) in micro-pores over prolonged time by equilibrating pigeon pea biochar with 1 M NH_4OAc (pH 7) for 1 and 7 days, respectively, and for 1, 3 and 7 days with 2 M KCl. In addition, the effect of three different biochar to solution (B:S) ratios (1:15, 1:30 and 1:45) on the final CEC estimates, based on displaced NH_4^+ in the KCl extract was assessed. One gram of biochar and 15 ml of NH_4OAc and KCl were used for the treatment 1:15, while 0.5 g and 15 ml and 22.5 ml were used in the 1:30 and 1:45 treatments, respectively. Only one loading with 1 M NH_4OAc and one with 2 M KCl was done. The pH adjustment and the washing with water was done in the same way as described above. Every treatment was assessed in triplicates. All the CEC estimates were based on displaced NH_4^+ by K^+.

2.4.2. Extractions of NH_4^+ by K^+

The amount of 2 M KCl needed to displace NH_4^+ by K^+ was determined through three subsequent extractions with 20 ml 2 M KCl each time. One gram of the four types of biochar and the two certified soils was weighed in 50 ml plastic tubes. For biochars, the pH and EC adjustment was done as described above. The certified soils were not pretreated. Subsequently, the samples were saturated with NH_4OAc, washed four times with 20 ml of 99% isopropanol and then extracted three consecutive times with 2 M KCl in triplicates. The CEC was estimated based on displaced NH_4^+ in the KCl extracts.

2.5. Chemical analysis

The NH_4OAc extracts were analyzed for base cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) by ICP-OES. Displaced NH_4^+ was analyzed in 2 M KCl extracts spectrophotometrically (see Supplementary data). All results were corrected for dry matter content of biochar and background concentration in the sequential extractions. Moreover, the biochars were analyzed for total amount of Ca, Mg, K, Na and after decomposition with nitric acid (HNO_3) and hydrofluoric acid (HF).

2.6. Reproducibility

We evaluated the reproducibility of the CEC-NH_4^+ estimates by using coefficients of variation (CV), i.e. the relative standard deviation, of those experiments in which we carried out 3 and 4 washings with isopropanol. For CEC-BC we used CV of experiment 2 (Table 1), in which four consecutive extractions with 1 M NH_4OAc (pH 7) were done.

2.7. Mass loss

After the extraction with 2 M KCl, the biochar slurries were washed 3 times with 35 ml deionized water to remove the excess of salt, until EC ≈ 0.4 mS cm^{-1} and dried during a week at 70 °C. The mass loss during all the procedure was determined and the CEC values referred to the initial raw biochar, corrected for dry matter content.

2.8. Statistical analysis

Statistical analysis of the experiment described in Section 2.6 was done using the R software (R-Core-Team, 2017). The CEC estimates of the samples shaken for 1 and 7 days with NH_4OAc, 1, 3 and 7 days with 2 M KCl and the three different B:S were subjected to one-way ANOVA.

Table 1
Overview of the consecutive experiments.

Experiment	Type of biochar	Objective	Description/Treatments
1. Biochar pretreatment	Pigeon pea, rice husk, cacao shell and corncob	To remove acid-soluble salts and adjust pH	pH adjustment to 7 and washing of samples until EC < 0.2 mS·cm^{-1}
2. Release of exchangeable cations in NH_4OAc (pH 7)	Pigeon pea, rice husk, cacao shell and corncob	To know the amount of NH_4OAc needed to exchange the base cations	Four consecutive extractions with 20 ml NH_4OAc in each extraction.
3. Washing of excess NH_4OAc with isopropanol and subsequent NH_4^+ extraction with KCl	1st part: Pigeon pea	To know the right amount of isopropanol needed to remove the excess NH_4^+	1st part: 1, 2 and 3 washings with 20 ml isopropanol each time. Analysis of NH_4^+ in KCl extracts of pigeon pea biochar.
	2nd part: Pigeon pea, rice husk, cacao shell and corncob		2nd part: analysis of NH_4^+ in isopropanol extracts in the 3rd and 4th washing for all the biochars.
3.1 Biochar to solution ratios and equilibration time for KCl extraction	Pigeon pea	To assess the diffusion of NH_4^+ and K^+ in micro-pores and the effect of three different B:S on the CEC estimates, based on NH_4^+ in KCl extract	B:S: 1:15/1:30/1:45 NH_4OAc: 1 and 2 days KCl: 1, 3 and 7 days
3.2 Extractions of NH_4^+ by K^+	Pigeon pea, rice husk, cacao shell and corncob	To know the amount of KCl needed to fully displace NH_4^+	Three consecutive extractions with 20 ml KCl in each extraction

Table 2
pH, electrical conductivity (EC), acid neutralizing capacity (ANC$_{pH7}$) and the accumulated sum of base cations (Ca, Mg, K, Na) removed during the three washings of the pretreatment of biochar with water and acid. The first washing was done with water and acid followed by two consecutive washings with 20 ml deionized water each time. The values are referred to raw biochar.

	[a]pH	s.d	[b]EC mS cm^{-1}	s.d	ANC$_{pH7}$ cmol$_{(+)}$ Kg^{-1}	1st cmol$_{(+)}$ Kg^{-1}	2nd cmol$_{(+)}$ Kg^{-1}	3rd cmol$_{(+)}$ Kg^{-1}	[c]Δ cmol$_{(+)}$ Kg^{-1}
Pigeon Pea	10.4	*0.03*	1.4	*0.02*	49	47.2 ± 0.3	7.4 ± 0.2	2.0 ± 0.07	7.6
Cacao Shell	9.6	*0.03*	8.9	*0.18*	134	219 ± 5.0	24.5 ± 0.4	7.5 ± 0.4	117
Corncob	8.5	*0.04*	0.8	*0.01*	7	15.7 ± 0.4	3.4 ± 0.2	1.3 ± 0.09	13.4
Rice husk	8.7	*0.05*	0.4	*0.01*	4	10.1 ± 0.2	2.7 ± 0.3	1.0 ± 0.05	9.8

[a] Measured in 1:5 deionized water and raw biochar.
[b] Measured in 1:20 deionized water using raw biochar.
[c] Difference between accumulated sum of cations removed in the pretreatment and ANC$_{pH7}$.

3. Results and discussion

3.1. Pretreatment

The ANC$_{pH7}$ was largest in cacao shell biochar followed by that of pigeon pea biochar despite the higher pH of the latter (Table 2). Rice husk and corncob biochar had a relatively small ANC$_{pH7}$, although also these biochars had pH > 8.5. In accordance with this, the cumulative amount of base cations (expressed in cmol$_{(+)}$/Kg) removed in three consecutive washings and the initial EC decreased in the same order with cacao shell biochar > pigeon pea biochar > corncob biochar > rice husk biochar (Table 2). In the third washing of the pretreatment the removal of base cations declined to only 7.5 cmol$_{(+)}$ Kg^{-1} (cacao shell biochar), 2 cmol$_{(+)}$ Kg^{-1} (pigeon pea biochar), 1.3 cmol$_{(+)}$ Kg^{-1} (corncob biochar), 1 cmol$_{(+)}$ Kg^{-1} (rice husk biochar), thus contributing little to ANCpH7. The cumulative amount of removed cations in the three washings exceeded ANC$_{pH7}$ (Table 2) indicating that a considerable proportion of the extracted cations are due to dissolution of salts (47 to 71%, except pigeon pea biochar for which this was 14%).

3.1.1. Release of exchangeable cations in the NH$_4$OAc (pH 7) extract

On average, 83% of all exchangeable base cations of the biochar samples were extracted in the first extraction with 20 ml NH$_4$OAc (Table 3). Both certified soils had very similar patterns as pigeon pea and rice husk BC, with 88% of the base cations removed in the first extraction. After four extractions, the sum of exchangeable base cations decreased in the order cacao shell biochar > pigeon pea biochar > rice husk biochar > corncob biochar (Table 3). The average sum of exchangeable base cations of the certified soils was 20.7 and 26.9 cmol$_{(+)}$ Kg^{-1} for DC85101a and DC85113, respectively. The certified values of sum of exchangeable cations for these soils are 23.4 ± 2.4 and 29 ± 1.4 cmol$_{(+)}$/Kg, respectively (NCS, 2017).

Among the exchangeable base cations, Ca^{2+} is the most abundant in cacao shell and pigeon pea biochars (63 and 53% of the total respectively), followed by Mg^{2+} (20.8 and 30%) K^{+} (15.5 and 16.6%) and Na^{+} (0.15 and 0.14%) (Fig. 1b). In corncob and rice husk biochars, K^{+} was the most abundant (48 and 63% respectively), followed by Ca^{2+} (41 and 27%) Mg^{2+} (10 and 9.6%) and Na^{+} (0.2 and 0.9%). This difference in Ca^{2+} and K^{+} saturation is related to the type of feedstock, with grass-type feedstocks being richer in K^{+} than the other biochars used in this study. In both soil samples Ca^{2+} represented about 70% of the total exchangeable cations, whereas this was about 24%, 3% and 1% for Mg^{2+}, K^{+}, and Na^{+}, respectively. As expected at high pH, exchangeable Fe, Al and Mn in the biochars were small (Table S1).

3.1.2. Cations removed in the pretreatment vs exchangeable cations

The amount of cations removed during the pretreatment was higher than the amount of exchangeable cations for all the biochars (Table S2). When combining the exchangeable cations and the cations removed in the pretreatment, the exchangeable fraction accounts for <31% (Fig. 1c.) with the highest values observed for rice husk biochar (31%), followed by corncob (22.7%), cacao shell (19%) and pigeon pea (10.4%) biochar. The proportion of individual base cations relative to total exchangeable cations differed from that observed in the pretreatment (Fig. 1a. and b.). In general, K^{+} is the most abundant base cation removed in the pretreatment (> 68%) for all the biochars (Fig. 1a), while Ca^{2+} was more important in the exchangeable fraction, particularly for pigeon pea and cacao shell biochars as indicated above (Fig. 1b). The proportion of Mg^{2+} to the total readily soluble cations was 3.9% for corncob, 7.9% for rice husk, 13.3% for cacao shell and 18.2% for pigeon pea biochar. In general, Mg^{2+}is more abundant in the exchangeable fraction of cations than in the readily soluble ones. The contribution of Na^{+} to the exchangeable fractions and the readily soluble cations was minor, being the highest for rice husk biochar with 1.6% of the exchangeable cations and 1.4% of the cations removed in the pretreatment.

By pretreating biochar, we washed away the readily soluble cations, associated with ashes and salts, which otherwise would have dissolved in NH$_4$OAc (pH 7). The amount of base cations removed in the third washing of the pretreatment (Table 2) was considerably lower than CEC-BC (Table 3). This was the reason for setting 0.2 mS cm^{-1} as EC threshold in the pretreatment. Previous research (Martinsen et al., 2015) using a sub sample of the same cacao shell biochar showed that CEC-BC without any pretreatment was 197 cmol$_{(+)}$/Kg, while in our experiment this was 59.1 cmol$_{(+)}$ Kg^{-1} with pretreatment. Furthermore, Graber et al. (2017) found significant differences between CEC-BC and CEC- NH$_4^+$ for some of the biochars they analyzed. Without pretreatment, they found that for wheat straw biochar made at 550 °C and at 700 °C, the sum of base cations in NH$_4$OAc was 4 and 5 times greater than CEC-NH$_4^+$. Therefore, when the sum of exchangeable cations is used to estimate CEC, a pretreatment removing readily soluble salts is compulsory, to prevent the contribution of soluble components of ashes, especially for biochars with high EC.

Table 3
Percentage of exchangeable base cations extracted in four consecutive extractions with 20 ml 1 M NH$_4$OAc (pH 7) for four types of biochars and two certified soils. CEC values are referred to raw biochar.

	CEC-BC cmol$_{(+)}$ Kg^{-1}	s.d	First (%)	s.d	Second (%)	s.d	Third (%)	s.d	Fourth (%)	s.d
Pigeon Pea	6.6	*1.6*	77.5	*0.3*	13.6	*0.3*	5.9	*0.2*	3.0	*0.1*
Cacao Shell	59.1	*4.2*	75.6	*2.5*	12.2	*1.0*	8.4	*0.7*	3.8	*0.8*
Corncob	6.0	*1.8*	90.1	*1.1*	5.2	*1.2*	3.0	*0.2*	1.7	*0.1*
Rice husk	6.2	*0.03*	87.7	*1.4*	7.8	*1.2*	2.9	*0.3*	1.5	*0.1*
DC85101a	20.7	*0.5*	88.5	*0.4*	7.6	*0.2*	2.8	*0.1*	1.2	*0.2*
DC85113	26.9	*0.5*	87.6	*0.7*	8.8	*0.7*	2.6	*0.1*	1.1	*0.0*

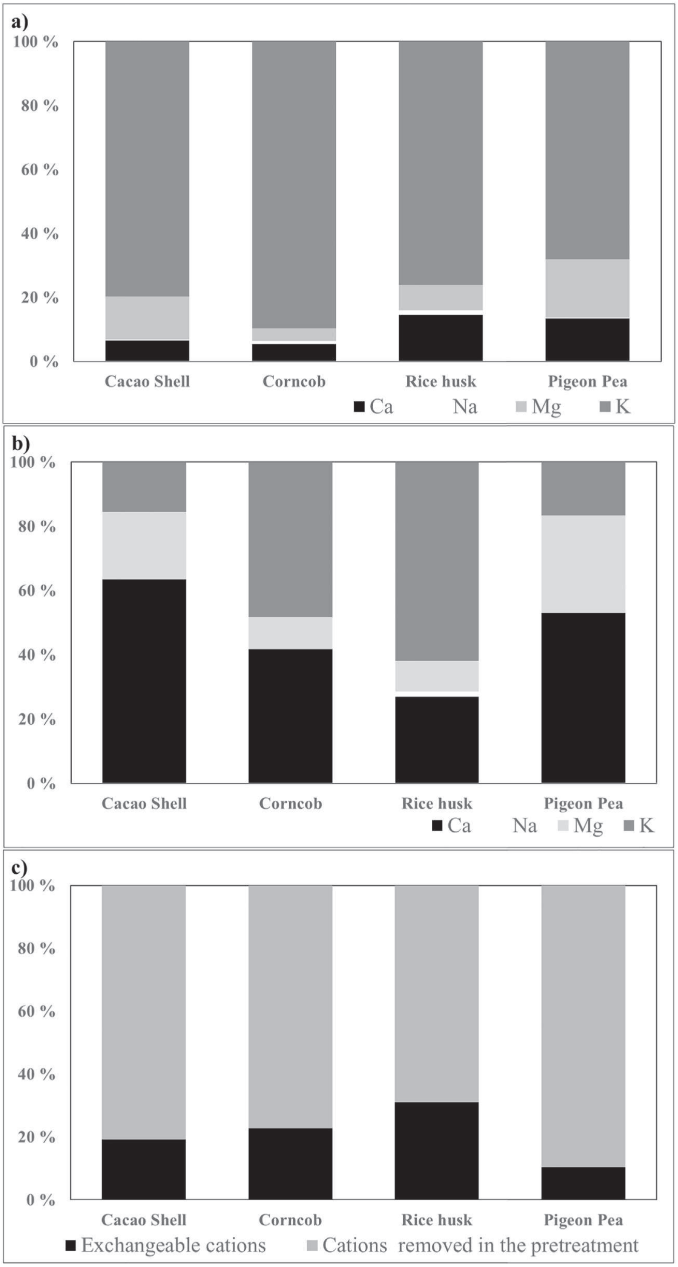

Fig. 1. a) Contribution of individual cations to the total amount readily soluble cations removed in the pretreatment, b) Contribution of individual cations to the total amount of exchangeable cations. c) Relative contribution of cations removed during the pretreatment and the exchangeable cations.

3.2. Washing of excess NH_4OAc with isopropanol and subsequent NH_4^+ extraction with KCl

The 2 M KCl extractable NH_4^+ significantly decreased upon washing with isopropanol with a reduction of 66% from the first to the third washing (Fig. S1). The analysis of NH_4^+ in isopropanol extracts after washing three and four times revealed small amounts of excess NH_4^+ for all biochars and soils (Table S3).

3.2.1. Biochar to solution ratios and equilibration time for KCl extraction

There were no significant ($p = 0.64$, Table S4) differences in the CEC estimates of pigeon pea biochar based on KCl extractable NH_4^+, if the biochar had been shaken with NH_4OAc for 1 or 7 days (27.3 (±2.3) cmol $_{(+)}$ Kg^{-1} and 27.0 (±2.4) $cmol_{(+)}$/Kg, respectively). This indicates that in there was no diffusion limitation to saturate biochar with NH_4^+ despite the presence of micro-pores that could restrict the entrance of NH_4^+ ions. In addition, there was no significant ($p = 0.88$, Table S4) difference in CEC estimates of pigeon pea biochar if shaken with 2 M KCl, for 1, 3 or 7 days. This indicates that within one day there is full displacement of NH_4^+. Moreover, there were no significant differences between the three B:S ratios evaluated ($p = 0.54$, Table S4). The average CEC values were 26.6 (±2.2) $cmol_{(+)}$/Kg, 27.4 (±1.6) $cmol_{(+)}$ Kg^{-1} and 27.4 (±3.0) $cmol_{(+)}$ Kg^{-1} for 1:15, 1:30 and 1:45 treatments, respectively. In all cases, the B:S ratio was enough to fully saturate the exchange sites. To have ample solution volume for chemical analysis of the extracts, we used 20 ml of 1 M NH_4OAc and 2 M KCl.

3.2.2. Extractions of NH_4^+ by K^+

CEC-NH_4^+ decreased in the order cacao shell biochar > rice husk biochar > pigeon pea biochar > corncob biochar (Table 4). The first extraction with 20 ml 2 M KCl following isopropanol washing removed 93% of the adsorbed NH_4^+ for all the biochars (Table 4) and 98.7% for the certified soils. The certified CEC values for soils are 21.6 ± 1.6 and 31 ± 1 $cmol_{(+)}$ Kg^{-1}; ((NCS, 2017); note that the certified CEC values are different than sum of exchangeable base cations) and our results summing the three consecutive extractions were 23.8 and 34 cmol $_{(+)}$ Kg^{-1} for DC85101a and DC85113. However if we only consider the value from the first extraction with 2 M KCl, the CEC values fit in the certified intervals. This indicates that at least one extraction with 20 ml 2 M KCl is enough to have a reasonable estimate of CEC-NH_4^+ values of biochar and soil samples.

3.3. Mass loss

On average, the mass loss of the raw biochars during the batch procedure was 26%, 1.7%, 1.6% and 10% of the raw biochar for cacao shell biochar, corncob biochar, rice husk biochar and pigeon pea biochar, respectively. The pretreatment data indicate that a significant amount of Ca, Mg and K was lost as salts and also through dissolution of carbonates and silicates (included in ANC_{pH7}). Based on the removal of Ca, Mg, K and Na in the pretreatment we estimated a weight loss of 8.6%, 0.7%, 0.5% and 1.8% for cacao shell, corncob, rice husk and pigeon pea biochars, respectively. Presumably the loss is at least twice as much, because the dissolution of cations is coupled with the dissolution of anions like chlorides, sulfates, carbonates and silicates. Based on this it is likely that nearly all mass loss is due to the removal of salts, carbonates and silicates in the pretreatment, whereas the loss of pure biochar was limited.

During the pretreatment, the supernatant of corncob biochar was the only with dark color (Fig. S2), however, the mass loss of this biochar was very low. Probably the loss of pure biochar was small, as the extracts were carefully pipetted instead of decanted and because the ionic strength of the extracting solutions caused flocculation and settling of the brown colored colloids of the biochar suspensions. In addition, three additional washings with water were done after the extraction with 2 M KCl in order to remove the excess of salt and to estimate the total mass loss. Thus, the total mass loss during the batch procedure is an overestimation.

Table 4
CEC based on displaced NH_4^+ (CEC-NH_4+) and percentage of NH_4^+ extracted in 3 consecutive extractions with 2 M KCl. CEC values are referred to raw biochar.

Sample type	CEC-NH_4^+ cmol(+)/Kg	C·V (%)	First KCl (%)	Second KCl (%)	Third KCl (%)
Pigeon Pea	26,7	8.4	93.5	4.5	2.0
Cacao Shell	59.7	2	93.4	4.2	2.4
Corncob	19.1	4.6	96.3	1.3	2.5
Rice husk	27,3	1.2	89.1	7.7	3.2
Soil-DC85101a	23.8	0.25	99.3	0.4	0.3
Soil-DC85113	34.0	1.3	98.1	1.6	0.3

3.4. Comparison of CEC-BC and CEC-NH_4^+ for biochar

For cacao shell biochar and the two certified soils, CEC-NH_4^+ to CEC-BC ratios were 1.0, 1.1 and 1.2 respectively, indicating reasonable correspondence between both methods (Fig. 2). However, for the other biochars, CEC-NH_4^+ was 3.9, 4.1 and 3.2 times greater than CEC-BC (Fig. 2). A likely explanation is that these biochars are rich in micro-pores (not analyzed) and isopropanol does not effectively remove excess NH_4^+ trapped in the micro-pores, presumably because of size exclusion of isopropanol molecules. Exclusion of organic molecules from small pores due to steric effects was reported earlier for wood charcoals (Zhu et al., 2005). Previous research (Pignatello et al., 2006) described a molecular sieving effect in which large natural organic compounds were more restricted to the external sites of environmental black carbon and thus, blocking the entrance of N_2 to the interior of the narrowest pores when analyzing surface properties. Moreover, previous research indicated that biomass-based activated carbon was dominated by pores in the size range of 3.5–15 Å (Amstaetter et al., 2012). Also torrefied maize and cotton stalks made at 290 °C had a significant amount of pores between 4 and 5 Å (Chen et al., 2014). Considering that the molecular diameter of isopropanol is 5.2 Å (Van der Bruggen et al., 1999), it is likely that it cannot penetrate the smallest pores, thus reducing its effectiveness to remove excess NH_4OAc. Therefore, we recommend to wash excess NH_4OAc in biochar with solvents having smaller molecular size than isopropanol, e.g. ethanol or even water. To our knowledge there is no study of the effectiveness of different solvents to remove excess index cations in CEC methodologies for biochar. Due to the limitations of using isopropanol as a washing agent, we believe that CEC-BC after pretreatment of biochar provides a better estimate of its CEC than CEC-NH_4^+.

The CEC- BC was 119.6 $cmol_{(+)}$ Kg C^{-1}, 11.6 $cmol_{(+)}$ Kg C^{-1}, 10.8 $cmol_{(+)}$ Kg C^{-1}, and 14.1 $cmol_{(+)}$ Kg C^{-1} for cacao shell biochar, pigeon pea biochar, corncob biochar and rice husk biochar, respectively. These values are smaller compared to those reported for soil organic C, (221–330 $cmol_{(+)}$ Kg C^{-1} (Parfitt et al., 2008); 370–500 cmol $_{(+)}$ Kg C^{-1} (Gruba and Mulder, 2015); 540–810 $cmol_{(+)}$ Kg C^{-1} (Martinsen et al., 2017)) and closer to what other studies have

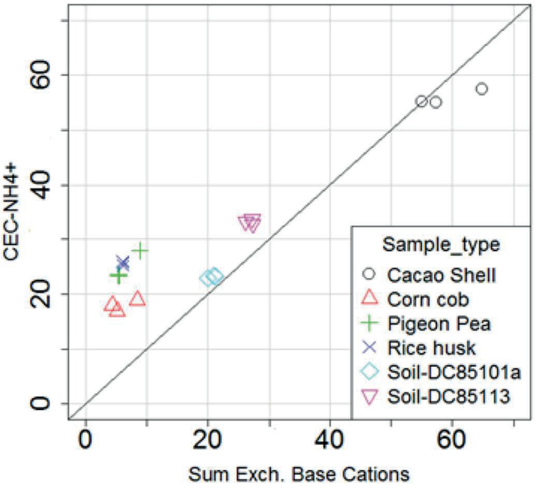

Fig. 2. CEC-NH_4^+ ($cmol_{(+)}$/Kg) as function of CEC-BC ($cmol_{(+)}$/Kg;). The line is the 1:1 line.

Table 5
CV of CEC-NH_4^+ combining all the experiments in which 3 and 4 washing with isopropanol were done and CV of CEC-BC of the experiment with four consecutive extractions with 1 M NH_4OAc.

Feedstock	CEC-NH_4^+				CEC-BC	
	Replicates	CEC-NH_4^+ ($cmol_{(+)}$/kg)	*Sd*	CV (%)	Replicates	CV (%)
Pigeon Pea	60	27.1	*2.3*	8.4	3	24.4
Cacao Shell	11	58.1	*4.4*	7.5	3	7.1
Corncob	11	22.4	*3.3*	14.5	3	29.5
Rice husk	11	26.7	*0.8*	3.0	3	0.4

published for biochar (75 $cmol_{(+)}$ Kg C^{-1}, (Silber et al., 2010)). Total organic carbon of the biochars are given in Table S5.

3.5. Reproducibility

The reproducibility of CEC-BC was good for the biochars of rice husk and cacao shell, and rice husk biochar, but less so for corncob and pigeon pea biochars (Table 5). Probably, the CV values for CEC-BC were relatively high, because they are a summation of four cations, each adding to the uncertainty of the sum. Although, representing an overestimation, due to ineffective washing of excess NH_4OAc, the reproducibility of CEC-NH_4^+ was superior and if a more appropriate solvent is found it is to be preferred over CEC-BC. Even if CEC-NH_4^+ estimates with 3 and 4 washings with isopropanol were bulked (Table 5), CV was lower than 10% for cacao shell, pigeon pea biochar and rice husk biochar, while this was slightly higher for corncob biochar (14.5%, Table 5). CV values of CEC-NH_4^+ may be decreased further for all the biochars if only the values from the experiment with four isopropanol washings are included (Table 4). The experiments with 3 and 4 washings with isopropanol were combined because there were only three replicates using 4 washings with isopropanol.

4. Conclusions

The NH_4OAc (pH 7) method for CEC determination based on displacement of NH_4^+ by KCl was tested for biochar in batch mode and compared with CEC values obtained from the sum of extractable cations in NH_4OAc.

A compulsory pretreatment was shown to be necessary to remove readily soluble cations from salts, carbonates and silicates and adjust biochar pH to near neutral. The amount of readily soluble cations removed in the pretreatment was several times greater than the exchangeable fraction for all biochars. Skipping the pretreatment causes a major overestimation of the CEC of biochar. Pretreatment of biochar also causes major mass losses, due to the removal of salts, carbonates and silicates. These mass losses have to be quantified in order to be able to compute the CEC of the initial raw biochar. The most abundant cation in the readily soluble fraction of the pretreatment of all biochars was K^+. The exchangeable cations (CEC-BC) were dominated by K+ for rice husk and corncob biochars, whereas this was Ca^{2+} for pigeon pea and cacao shell biochars.

This study shows that shaking 1 g of biochar, following pretreatment, with 1 M NH_4OAc during 24 h was enough to saturate the exchange sites with NH_4^+. Additional 1 M NH_4OAc was needed to fully extract the exchangeable cations. There was no additional NH_4OAc removed after four consecutive washings with 20 ml isopropanol. However our results suggest that isopropanol may not penetrate the smallest pores of some biochars and we conclude that other liquid compounds are required to fully remove excess NH_4OAc. Shaking with 2M KCl during 24 h fully displaced NH_4^+ from the exchange sites. The biochar to solution ratios did not affect the CEC estimates.

CEC-NH_4^+, although representing an overestimation due to the inefficient removal of excess NH_4OAC by isopropanol, was more reproducible than CEC-BC.

A detailed description of the protocol for the modified method in batch is presented in the Supplementary data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.06.017.

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Supplementary material

Cation Exchange Capacity of biochar: An urgent method modification

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Table S1. Exchangeable Fe, Mn and Al in biochar

	Fe	Mn	Al
Biochar type	**cmol+/kg**	**cmol+/kg**	**cmol+/kg**
Pigeon Pea	1.7E-03	5.2E-02	2.2E-02
Cacao Shell	5.6E-03	2.2E-01	1.4E-02
Corn cob	1.5E-02	1.1E-01	5.1E-02
Rice husk	4.5E-02	2.3E-01	1.2E-02

Table S2. Base cations removed in the pretreatment and exchangeable base cations for 4 biochars and 2 certified soils.

	Cation	**Cacao Shell cmol(+)/kg**	**Corncob cmol(+)/kg**	**Rice husk cmol(+)/kg**	**Pigeon Pea cmol(+)/kg**	**Soil-DC85101a cmol(+)/kg**	**Soil-DC85113 cmol(+)/kg**
Base cations removed in the pretreatment	**Ca**	16.1	1.1	2.0	7.6	-	-
	s.d	*0.8*	*0.1*	*0.04*	*0.5*	-	-
	Mg	33.5	0.8	1.1	10.3	-	-
	s.d	*2.8*	*0.2*	*0.06*	*0.5*	-	-
	K	200	18.3	10.5	38.6	-	-
	s.d	*6.5*	*0.4*	*0.4*	*0.8*	-	-
	Na	1.0	0.2	0.2	0.1	-	-
	s.d	*0.01*	*0.2*	*0.04*	*0.002*	-	-
	Sum of cations	**250.6**	**20.4**	**13.8**	**56.6**		
Exchangeable base cations	**Ca**	37.5	2.5	1.7	3.5	14.7	19.5
	s.d	*2.5*	*1.5*	*0.03*	*0.8*	*0.5*	*0.4*
	Mg	12.3	0.6	0.6	2.0	5.0	6.5
	s.d	*1.4*	*0.2*	*0.0*	*0.6*	*0.1*	*0.1*
	K	9.2	2.9	3.9	1.1	0.8	0.8
	s.d	*0.3*	*0.24*	*0.03*	*0.29*	*0.03*	*0.02*
	Na	0.1	0.001	0.1	0.008	0.2	0.2
	s.d	*0.006*	*0.001*	*0.004*	*0.001*	*0.008*	*0.005*
	CEC-BC	**59.1**	**6.0**	**6.2**	**6.6**	**20.7**	**26.9**
	s.d	*4.2*	*1.8*	*0.03*	*1.6*	*0.5*	*0.5*

Table S3. NH_4^+ ($cmol_{(+)}$/Kg) in isopropanol extracts after the third and fourth washing.

	[1] NH_4^+ 3rd washing *cmol(+)/Kg*	[2] NH_4^+ 4th washing *cmol(+)/Kg*
Pigeon Pea	6.53	0.73
Cacao Shell	2.20	0.53
Corncob	1.99	0.50
Rice husk	3.69	0.49
DC85101a	1.02	0.43
DC85113	1.88	0.74

[1] Cumulative volume of isopropanol: 60ml

[2] Cumulative volume of isopropanol: 80ml

Table S4. ANOVA table, biochar to solution ratios (Solid.Solution; **B:S :** 1:15/1:30/1:45**) and equilibration time with 1M NH_4OAc (1 or 7 days) and 2M KCl (**1, 3 and 7 days**).**

```
LinearModel.1 <- lm(CEC_cmolc.Kg ~ KCl + NH4OAc + Solid.Solution,
+   data=Dataset.tmp)
Anova Table (Type II tests)
Response: CEC_cmolc.Kg
```

	Sum Sq	Df	F value	Pr(>F)
KCl	1.389	2	0.1225	0.8850
NH4OAc	1.182	1	0.2086	0.6499
Solid.Solution	6.990	2	0.6167	0.5439
Residuals	271.994	48		

Table S5. Organic carbon content and total amount of Ca, K, Na, Mg

Biochar	OC %	Ca g/kg	K g/kg	Na g/kg	Mg g/kg
Pigeon Pea	56.1	12	15	0.30	8.3
Cacao shell	52.1	20	80	0.3	12
Corn cob	55.5	2.4	12	1.2	1.5
Rice husk	45.5	1.7	10	0.21	1.1

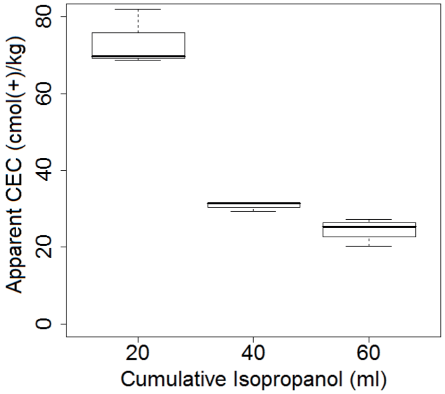

Figure S1. Effect of three consecutive washings with 99% propanol on the amount of NH_4^+ in the 2M KCl extracts (apparent CEC) of pigeon pea biochar. The figure emphasizes the importance of washing excess NH_4^+ with propanol in the modified batch procedure for CEC determination.

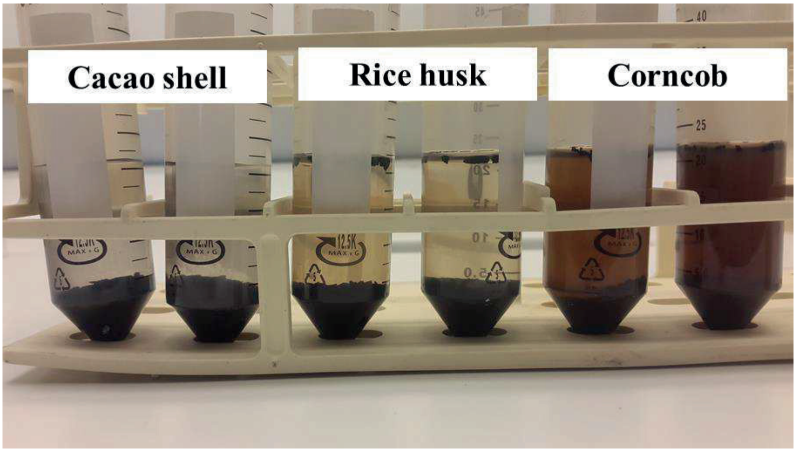

Figure S2: Cacao shell, rice husk and corncob extracted with water in the third washing of the pretreatment. The color of the supernatants differ between biochars. There was no relation between the color of the supernatant and the mass losses. The highest mass losses occurred in cacao shell biochar (26%), followed by pigeon pea (10%), corncob (1.7%) and rice husk (1.6%). Corncob's supernatant was the darkest in the third washing with water. When 1M NH_4OAc, isopropanol and 2M KCl were added, the supernatants were clear.

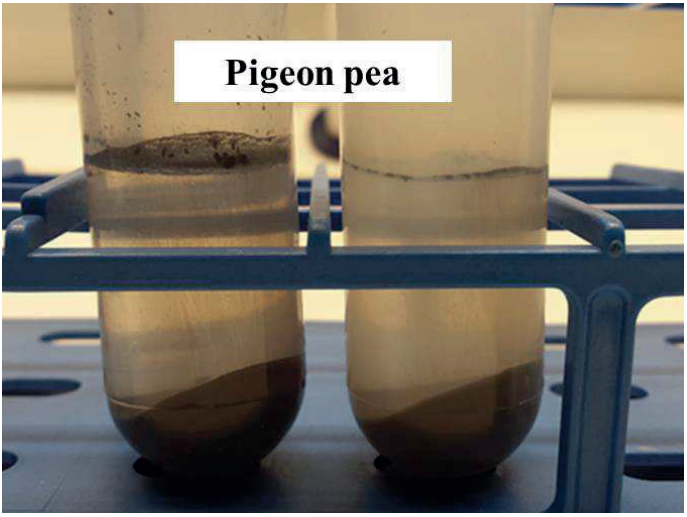

Figure S3. Pigeon pea biochar extracted with water in the third washing of the pretreatment.

Analytical procedure

Based on the results of our experiments, we recommend the next steps for CEC determination based on displaced NH4+ and for the extraction of water-soluble and exchangeable base cations of biochar in batch.

1. Pretreatment

a. Weigh 1 gram of biochar in 50ml plastic tubes. Record the weight of the tubes with the lids and record the dry matter content of biochar by taking a subsample and drying it at 105 ˚C during 24 hours.

b. Add 20 ml of deionized water and shake horizontally at 200 rpm during 4 hours to ensure proper wetting of the sample. Once the tubes have been shaken for 4 hours, add 0.05M HCl gradually until pH 7. Record the volume of 0.05M HCl. Check pH at different intervals and if needed add more 0.05M HCl.

c. After 48 hours, centrifuge the tubes at 1700 g Pipette out the supernatant avoiding the floating biochar particles, add 20ml of deionized water again and shake horizontally during 1 hour.

d. Centrifuge the tubes at 1700 g and discard the supernatants. Repeat the washings with water until EC < 200µS/cm.

2. Saturation with 1M NH_4OAc
   a. Add 20 ml of 1M NH_4OAc (pH7) and shake the tubes horizontally at 200 rpm during 24 hours. If you are interested in the exchangeable base cations, extract twice more with 20 ml 1M NH_4OAc (pH7) and shaking during 2 hours each time. Centrifuge at 1700 g and collect the supernatants for analysis of base cations. Correct for the background levels of cations in the sequential extractions.
3. Washing with 99% isopropanol
   a. Add 20 ml of 99% isopropanol to the tubes and shake horizontally at 200 rpm during 2 hours.
   b. Centrifuge the tubes at 1700 g and pipette out the supernatant. Repeat three times more. In total the samples are washed 4 times with alcohol. As quality control, NH_4^+ should be analyzed in the supernatant after the last addition of isopropanol.
4. Displacement of NH_4^+ with K^+

a. Add 20 ml of 2M KCl, shake the tubes horizontally at 200 rpm during 24 hours and centrifuge the tubes at 1700 g.

b. Collect the supernatants, store them in the fridge and analyze NH_4^+ as soon as possible.

5. Mass loss:

   After the displacement with KCl is finished and the supernatants have been collected, wash 2-3 times with 35 ml deionized water to remove excess KCl until EC < 200µS/cm. Centrifuge the tubes at 1700 g, pipette out the excess water and dry the tubes at 65 ˚C during 5 days. Record the weight of the tubes with biochar, calculate the total mass loss using the initial weight of the tubes and biochar corrected for dry matter content. The mass loss at a certain stage of the CEC analytical process is weighed according to the number if extractions at that specific point and the values are referred to the initial biochar.

Ammonium analysis

Ammonium was analyzed spectrophotometrically, following the salicylate method [1] at 655 nm. Briefly, a reagent A was prepared by dissolving 1.0 g of salicylic acid and 0.1g of sodium nitroprusside in 100ml of citrate buffer (0.27M trisodium citrate dehydrate, 0.054M NaOH). Reagent B was prepared by dilution of 2 ml of 6% sodium hypochlorite (NaOCl) in 100 ml of water. A calibration curve was made, using standard solutions of 0, 50, 75, 100, 500 and 1200 NH_4^+-N µg/l. Next, 0.5 ml of reagent A and B were added to 10ml plastic tubes containing 3 ml of the diluted 2M KCl extracts of every sample. The samples were shaken using a vortex shaker and after three hours the absorbance values were read at 655nm.

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Paper II

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Short Communication

Significant build-up of soil organic carbon under climate-smart conservation farming in Sub-Saharan Acrisols

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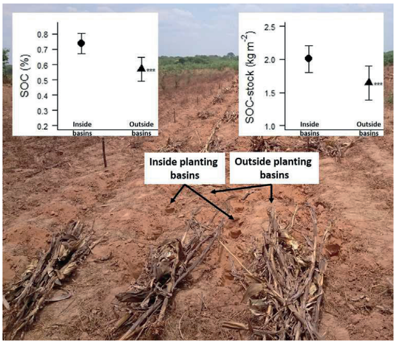
HIGHLIGHTS

- Soil fertility build-up inside vs. outside planting basins under conservation farming in SSA
- Absolute increase of 0.05 t C ha^{-1} yr^{-1} inside vs. outside 20 cm deep planting basins
- Relative increase of 2.95 ‰ SOC yr^{-1} inside vs. outside 20 cm deep planting basins
- Increase in labile C, pH, CEC and potential nitrification inside planting basins

GRAPHICAL ABSTRACT



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ABSTRACT

Conservation farming (CF) involving minimum tillage, mulching and crop rotation may offer climate change adaptation and mitigation benefits. However, reported effects of CF, as applied by smallholders, on storage of soil organic carbon (SOC) and soil fertility in Sub-Saharan Africa differ considerably between studies. This is partly due to differences in management practice, soil type and adoption level between individual farmers. Where CF involves planting basins, year-to-year changes in position of basins make SOC stock estimates more uncertain. Here we assess the difference in SOC build-up and soil quality between inside planting basins (receiving inputs of lime and fertilizer; basins opened each year) and outside planting basins (no soil disturbance or inputs other than residues) under hand-hoe tilled CF in an Acrisol at Mkushi, Zambia. Seven years of strict CF husbandry significantly improved soil quality inside planting basins as compared with outside basins. Significant effects were found for SOC concentration (0.74 ± 0.06% vs. 0.57 ± 0.08%), SOC stock (20.1 ± 2.0 vs. 16.4 ± 2.6 t ha^{-1}, 0–20 cm), soil pH (6.3 ± 0.2 vs. 4.95± 0.4) and cation exchange capacity (3.8 ± 0.7 vs. 1.6 ± 0.4 $cmol_c$ kg^{-1}). As planting basins only occupy 9.3% of the field, the absolute rate of increase in SOC, compared with outside basins, was 0.05 t C ha^{-1} yr^{-1}. This corresponds to an overall relative increase of 2.95‰ SOC yr^{-1} in the upper 20 cm of the soil. Also, hot water extractable carbon (HWEC), a proxy for labile organic matter, and

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Abbreviations: CF, conservation farming; CA, conservation agriculture; SOC, soil organic carbon; CEC, cation exchange capacity; HWEC, hot water extractable carbon; P, phosphorus; K, potassium; N, total nitrogen; SOM, soil organic matter; SOC, soil organic carbon; BD, bulk density; NH_4-N, ammonium; NO_3-N, nitrate.

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potential nitrification rates were consistently greater inside than outside basins. The significant increase in quantity and quality of SOC may be due to increased inputs of roots, due to favorable conditions for plant growth through input of fertilizer and lime, along with increased rainwater infiltration in the basins.

1. Introduction

Soil organic matter (SOM) is important for soil structure, water holding capacity and release and retention of plant nutrients that are crucial for agricultural productivity (FAO, 2017a). Soil organic carbon (SOC) is a major constituent of SOM and SOC sequestration mitigates climate change (Lal, 2004a, 2004b), while a decline in SOC may lead to soil degradation that poses a threat to global climate and food security (Lal, 2013). Recently, the "4 per mille" initiative (4p1000.org/) was launched at the COP 21 with an aspiration to increase global SOC stocks by 4 per thousand per year (0.4%) as a compensation for the global emissions of greenhouse gases from anthropogenic sources (Minasny et al., 2017). In a survey of SOC stock and sequestration potential for 20 regions in the world, Minasny et al. (2017) found that a sequestration rate of 4‰ can be accomplished under best management practices. Also, Paustian et al. (2016) reported that improved soil management could make "climate smart soils" in terms of increased C sequestration and reduced greenhouse gas emissions. However, others such as Poulton et al. (2018) have highlighted limitations to achieving the 4‰ goal in practical agriculture due to e.g. lack of resources or because practices are uneconomic or undesirable for food production.

Adoption of sustainable production systems and practices has been suggested to increase resilience and help mitigate climate change (FAO, 2017b). Conservation agriculture (CA) comprising the principles of minimum tillage, residue retention and crop rotation (Mafongoya et al., 2016; Powlson et al., 2016) may offer climate change adaptation and mitigation benefits, due to increased SOC storage, soil fertility, water conservation and productivity (Corbeels et al., 2018; Lal, 2015; Pisante et al., 2015). However, reported effects of CA on SOC sequestration and soil quality in Sub-Saharan Africa differ considerably between studies (Cheesman et al., 2016; Corbeels et al., 2018; Corbeels et al., 2015; Sommer et al., 2018; Thierfelder and Wall, 2012) and the mitigation potential of CA systems remains unclear (Thierfelder et al., 2017). A recent review by Corbeels et al. (2018) showed that annual SOC accumulation rates in response to treatments with all three principles of CA varied enormously, from −96 to 176‰ yr^{-1}, with half of the observations reporting relative SOC build-up rates exceeding 34‰ yr^{-1}. It is believed that climatic and edaphic conditions, combined with management practices such as seeding system, degree of residue retention, fertilizer addition, weeding and crop rotation, determine whether CA has positive, negative or no effect on yields and soil fertility (Gatere et al., 2013; Giller et al., 2009; Nyamangara et al., 2014; Steward et al., 2018; Thierfelder et al., 2016).

Due to mulching, using crop residue, CF increases the input of organic carbon in soil, thus enhancing soil structure, water infiltration and biological activity (Lal et al., 2007; Powlson et al., 2014). In addition, no-till, due to its minimum soil disturbance, is effective in controlling soil evaporation, sequestering SOC and minimizing erosion losses (Lal et al., 2007). According to Powlson et al. (2014) potential disadvantages of no-till include relatively small additions of SOC to the whole profile (i.e. increases occur largely near the soil surface), more challenges in weed control (extra hand weeding or reliance on herbicides), increased BD and in some cases increased nitrous oxide emission. No-till in combination with residue retention and crop rotation increases crop yields under rain fed agriculture in dry climates (Pittelkow et al., 2015). However, no-till alone may reduce yields (Pittelkow et al., 2015) and inappropriate management, such as insufficient weeding and lack of early planting, constrains yields on CA farms (Gatere et al., 2013). Farmers may struggle to follow all principles of CA such as maintaining sufficient crop residues, due to e.g. burning, removal and grazing that will reduce carbon inputs to the soil (Cheesman et al., 2016; Chivenge et al., 2007; Thierfelder et al., 2013; Umar et al., 2011). In addition, CA technologies (e.g. direct seeding/dibble stick, hand hoe-basin systems and ripping) and fertilizer application rates vary among individual farmers (Johansen et al., 2012; Mafongoya et al., 2016; Thierfelder et al., 2015) resulting in variations in soil disturbance and input of organic carbon and nutrients to the soil. Together with inherent site/farm heterogenity (intrinsic soil properties, micro-climate) affecting crop production, these factors may partly explain the large variation found in the literature with respect to yield and soil quality effects of CA.

In Zambia, the CA system, with hand-hoe prepared planting basins and animal-drafted or mechanized rip lines (Johansen et al., 2012), has been promoted by the Conservation Farming Unit (CFU). CFU uses the term conservation farming (CF) for conservation tillage (i.e. minimum tillage (MT), using planting basins or ripping), retention of crop residues and the incorporation of legumes in crop rotation (CFU, 2011; Gatere et al., 2013). Recently, Sommer et al. (2018) reported reduced losses of SOC (0–15 cm) but no net carbon sequestration under CA in two long term (12 years) trials in Western Kenya. A large number of on-farm sites in Zambia (Martinsen et al., 2017) and Zimbabwe (Nyamangara et al., 2013) indicate small effects of CF on soil C stocks. Comparisons of soils under CA (up to 9 years) and adjacent conventional fields from 450 farms in 15 districts in Zimbabwe revealed generally low SOC contents (<1%) without clear difference between the two management practices (Nyamangara et al., 2013). Results from 40 on-farm sites in Zambia showed small differences in soil quality parameters between CF and conventional practices at smallholder farms after maximum 12 years since CF adoption (Martinsen et al., 2017). In both studies, there were only small differences in amount of SOC, total phosphorus and pH when comparing inside and outside CF planting basins. Martinsen et al. (2017) attributed this to a gradual year-to-year shift in position of the basins and large variability between study sites.

Here, we assess the effect of seven years of hand-hoe tilled CF on soil quality and build-up of SOC by comparing soil from inside vs. outside planting basins under controlled conditions in Acrisols, Mkushi, Zambia. Considerable attention was given to keep basins in the same position and fertilizer and lime were added to basins only. Planting basins are hypothesized to increase SOC content, due to the high root density and increased biomass production, associated with elevated soil moisture, as basins favor the accumulation of runoff from the surrounding outside basin areas.

2. Material and methods

2.1. Study design and sampling

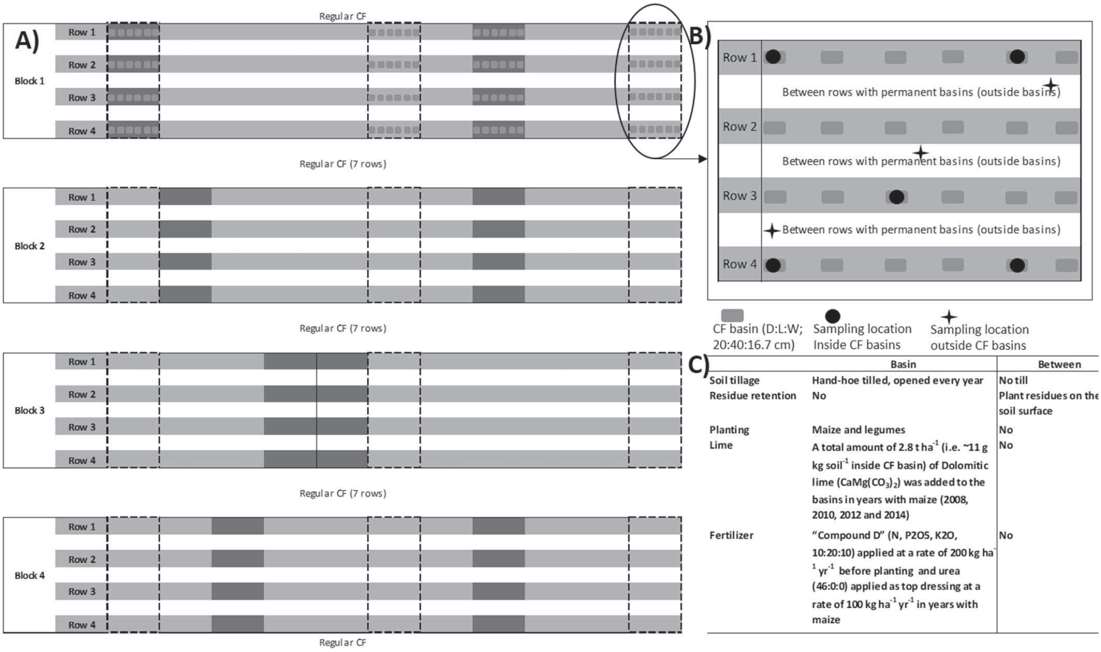
The study was conducted on a private farm (Mt Isabel) in Mkushi (S13°45′25.7″ E29°03′55.5″), central Zambia. The average annual rainfall and temperature were 1220 mm and 20.8 °C, respectively. The soil type was sandy loam Acrisol (Obia et al., 2016). Land use prior to soil sampling in 2015 included seven years of strict conservation farming (CF) husbandry. Before application of CF, land use was conventional, including continuous maize cropping with minimal inputs of fertilizer and lime and poor weed control. Conversion to CF in 2008 included dry season (May–August) preparation of permanent planting basins using hoes (min tillage), two year crop rotation (maize-ground nuts) and residue retention (mulching without chopping, i.e. leaving plant residues on the soil surface in between planting rows). The CF practice included preparation of rows of permanent basins (Fig. 1, Fig. S1) with a spacing of 90 cm between rows and 80 cm between basins within rows (~13,890 basins ha^{-1}). Each basin has an area of ~0.07 m^2 and a volume of ~13.4 L (20 cm depth, 40 cm length, 16.7 cm width). Herbicides (Glyphosate, Atrazine/Cyanazine mix and Gramoxone) and hand weeding were used for weed control. For maize, fertilizer "Compound D" (N, P_2O_5, K_2O, 10:20:10) was applied at a rate of 200 kg ha^{-1} yr^{-1} before planting and urea (46:0:0) applied as top dressing at a rate of 100 kg ha^{-1} yr^{-1} about four to five weeks as well as eight weeks after planting (i.e. a total of 200 kg ha^{-1} yr^{-1}). Legumes used in rotation received no fertilizer; so, the site was fertilized every second year, i.e. four times in the period 2008–2015. The total amount of NPK on elemental basis corresponded to an application of 112 kg N ha^{-1} yr^{-1}, about 17.5 kg P ha^{-1} yr^{-1} and about 16.5 kg K ha^{-1} yr^{-1} during the four years when fertilizer was applied. A total amount of 2.8 t ha^{-1} (i.e. ~11 g kg $soil^{-1}$ inside CF basin) of Dolomitic lime ($CaMg(CO_3)_2$) was added to the basins in years with maize (2008, 2010, 2012 and 2014).

Soil sampling was conducted in October 2015 and in May 2016. After sampling, the soil was air dried prior to transport and analyzed. In October 2015 soil sampling was conducted at three selected plots (each ~24 m^2) in each of four blocks (each block ~250 m^2, Fig. 1a). Each plot consisted of four rows of six permanent planting basins (i.e. 24 basins per plot, Fig. 1b). At each of the 12 plots, five soil samples (0–20 cm) were collected in planting basins and three soil samples were collected between basin rows (i.e. outside basins) using a hand hoe. The five and three samples, respectively, were bulked prior to chemical analysis ($n = 12$ for both CF basins and outside CF basins, i.e. a total of 24 samples) to make a composite sample per plot. When sampling, we focused on the top 20 cm of the soil, which was the basin depth. In an earlier study we showed that maize roots tended not to go deeper than the basin, with 95% of maize roots occurring in the top 25 cm of the soil (Abiven et al., 2015). Undisturbed soil samples were collected at 2–7 cm soil depth using 100 cm^3 steel rings in two plots per block in basins and between rows to determine plant available water and bulk density (BD). One sample from between rows in block 1 was lost in transport, so the average of CF basins in block 1 was used to allow for a paired comparison per block ($n = 7$ for both CF basins and between basin rows).

In May 2016 at harvest, four soil samples at two plots per block were collected inside basins and between rows at 0–3, 3–8, 8–13 and 13–20 cm soil depth, using a cylindrical soil auger. Hot water extractable carbon (HWEC) was determined on all samples and potential N-mineralization rate was determined on samples bulked at 0–8 cm and 8–20 cm soil depth. In order to report the results for the same depth intervals, values for HWEC were bulked at the same depth intervals based on depth-weighted average for 0–3 and 3–8 cm and 8–13 and 13–20 cm, respectively. Thus, the total amount of samples at both of the depths 0–8 and 8–20 was $n = 4$ for both CF basins and for between rows of CF basins (i.e. a total number of 16 samples).

2.2. Soil analysis and statistics

A detailed description of the methods can be found in Appendix A (Supplementary data). Briefly, bulk density (BD), volume percentage water at field capacity (pF 2) and at wilting point (pF 4.2), as well as total porosity were determined using undisturbed soil cores of 100 cm^3 according to Obia et al. (2016). The amount of plant available water was calculated as the difference between volume percentage water at field capacity and at wilting point. Prior to all other analyses, soil samples were air-dried and sieved at 2 mm. Soil texture was determined using the Pipette method (Elonen, 1971). In the pipette method,



	Basin	Between
Soil tillage	Hand-hoe tilled, opened every year	No till
Residue retention	No	Plant residues on the soil surface
Planting	Maize and legumes	No
Lime	A total amount of 2.8 t ha^{-1} (i.e. ~11 g kg $soil^{-1}$ inside CF basin) of Dolomitic lime ($CaMg(CO_3)_2$) was added to the basins in years with maize (2008, 2010, 2012 and 2014)	No
Fertilizer	"Compound D" (N, P2O5, K2O, 10:20:10) applied at a rate of 200 kg ha^{-1} yr^{-1} before planting and urea (46:0:0) applied as top dressing at a rate of 100 kg ha^{-1} yr^{-1} in years with maize	No

Fig. 1. A) Experimental setup, Mkushi, Zambia. The sampling was conducted at 4–5 plots (each ~24 m^2) in four blocks (each ~4 m width and 61 m length i.e. ~250 m^2). Plots used for chemical analysis and BD are highlighted with a dashed border (three plots in each block sampled October 2015) and plots used for determining hot water extractable C and potential N mineralization are highlighted dark gray (two plots per block sampled May 2016). **B**) Plot (24 m^2, 24 planting basins) used for soil sampling. **C**) Management.

soil texture classes (clay, silt, sand) are estimated in terms of size and distribution of primary particles by sieve and sedimentation analysis. Soil pH was determined in 0.01 M $CaCl_2$ using a soil to solution ratio of 1:2.5 with a digital pH meter. Subsamples of the air-dried and sieved samples were dried at 60 °C to determine dry matter content and then milled prior to determination of organic carbon (C) and total nitrogen (N). Total soil carbon was determined by dry combustion (EC12, C determinator, Leco Corporation) (Nelson and Sommers, 1982). Since soil pH was below 6.5, total C was used as a measure of soil organic carbon (SOC). Total N was determined on untreated soil samples by the Dumas method (TruSpec, CHN analyzer, Leco Corporation; Bremmer and Mulvaney, 1982). Carbon and N stocks were calculated based on volume of soil by multiplying depth of sampling, BD and elemental concentration. Mean values of BD for basins and between rows, respectively, were used per block to calculate C stocks. In addition, C stocks were calculated based on an equivalent mass of soil since equal depth sampling may overestimate C stocks in treatments with greater BD (Ellert and Bettany, 1995; Wendt and Hauser, 2013).

Exchangeable base cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) and exchangeable Al^{3+} were determined in 1 M ammonium nitrate extracts (NH_4NO_3, unbuffered). Exchangeable acidity was determined by back-titration with 0.05 M sodium hydroxide to pH 7. The sum of exchangeable base cations and exchangeable acidity was assumed to equal the effective cation exchange capacity (CEC) according to Schollenberger and Simon (1945). Total, inorganic and organic P was determined according to Møberg et al. (1990). Acid oxalate extractable Fe, Al and P were determined according to van Reeuwijk (1995).

Potential nitrogen mineralization rates were determined in an incubation experiment (60 days, 20 °C) using air dried and sieved soil samples with an adjusted water content of ~31 vol% (modified after Martinsen et al. (2017); see Appendix). Rates of net ammonification and net nitrification were determined by subtracting initial (day 0) KCl-extractable soil NH_4-N and NO_3-N from final amounts (after 60 days) of extracted NH_4-N and NO_3-N, respectively. Extractable NH_4-N and NO_3-N were analyzed using a flow injection analyzer (FIA Star 5010). Hot water extraction of organic C was done to determine the labile fraction of SOM according to Ghani et al. (2003). Briefly, 30 mL of distilled water was added to polypropylene tubes with 5 g of soil. The suspensions were gently shaken in a vortex shaker prior to extraction of dissolved organic carbon in a temperature-controlled hot water bath (80 °C during 16 h). Dissolved organic carbon was measured after filtration of the extract (0.45 μm), using a total organic carbon analyzer (TOC-V CPN, Shimadzu).

Comparison of mean soil parameters for basins and between rows was done using two-sided paired *t*-tests. Difference between means and 95% confidence intervals of the estimated differences are shown in Figs. 2 and 3 and in Table 1. To assess propagation of error (Ku, 1966),

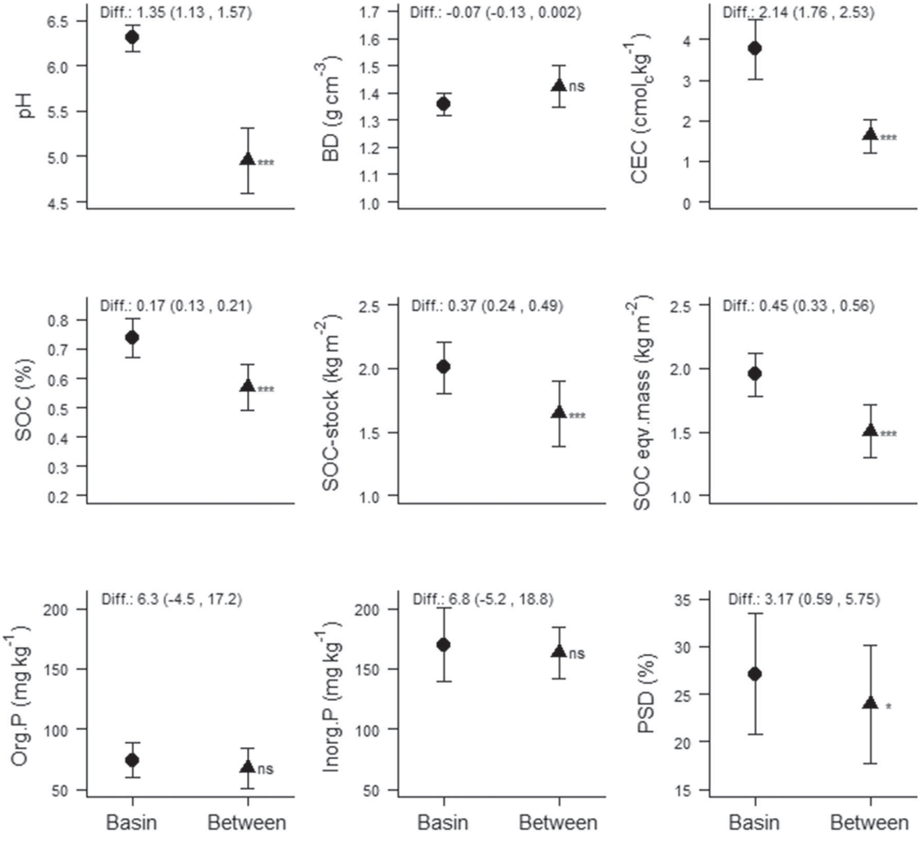


Fig. 2. Mean (± sd) soil properties (0–20 cm) of CF basins (inside) and in between rows of CF basins (outside), Mkushi, Zambia. Difference between means and 95% confidence intervals of the estimated differences are shown. N = 12 for both CF basins and between rows of CF basins except for bulk density (BD, n = 7). "ns"; p > 0.05, "*";p < 0.05, "**";p < 0.01 and "***";p < 0.001 based on two-sided paired *t*-tests. BD is bulk density, CEC is cation exchange capacity, SOC is soil organic carbon, Org. P and Inorg. P are organic and inorganic P, respectively. PSD is P saturation degree (see Appendix for calculation). The equivalent mass of soil is 265 kg soil (BD 1.33 g cm^{-3}, depth 20 cm at CF basin in block four).

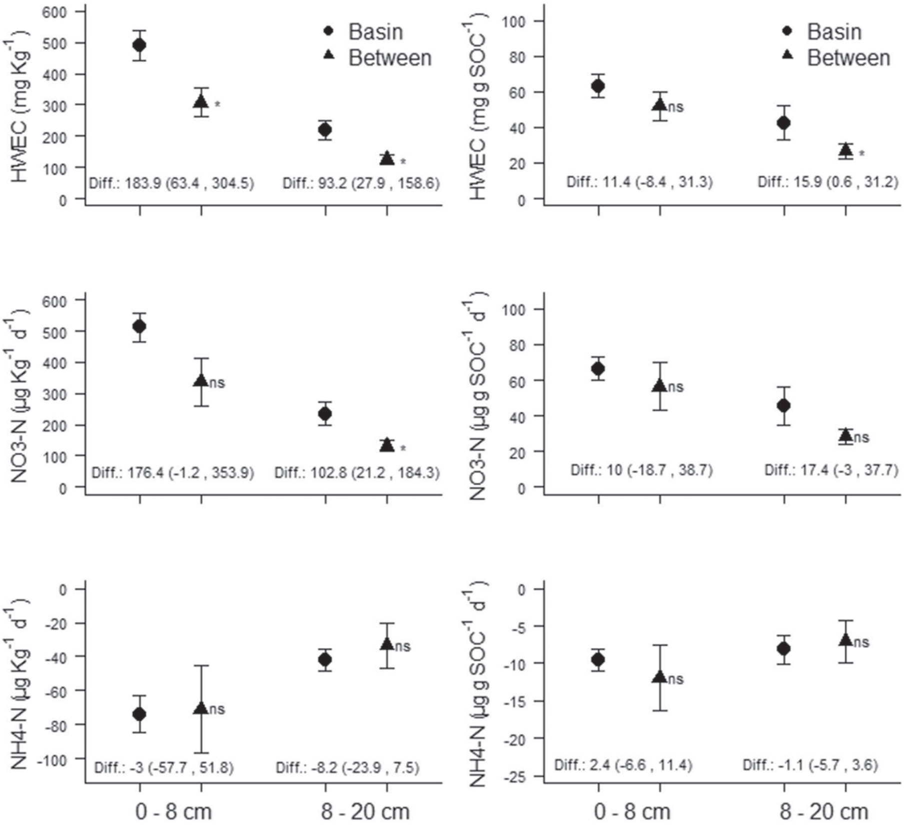


Fig. 3. Mean (± sd) hot water extractable carbon (HWEC, mg kg soil^{-1} and mg g SOC^{-1}) and net rates of NO_3-N and NH_4-N production (μg kg soil^{-1} day^{-1} and μg g SOC^{-1} day^{-1}) during 60 days of incubation of soils (0–8 cm and 8–20 cm) from inside CF basins and in rows between CF basins (outside), Mkushi, Zambia. Difference between means and 95% confidence intervals of the estimated differences are shown. N = 4 for both CF basins and between rows of CF basins at each of the depths (total n = 16). Positive and negative rates of NO_3-N and NH_4-N indicate a net mobilization and net immobilization of N, respectively. "ns"; $p > 0.05$, "*"; $p < 0.05$, "**"; $p < 0.01$ and "***"; $p < 0.001$ based on two-sided paired *t*-tests between CF basins and between rows of CF basins for each soil depth separately.

for normalized values (i.e. CEC per g of SOC, HWEC in mg g SOC^{-1} and net rates of NO_3-N and NH_4-N in μg g SOC^{-1} day^{-1}), standard deviation (sd) was calculated as $\mathrm{sd} = |R| * \sqrt{(sdX/X)^2 + (sdY/Y)^2}$. Here R is the normalized mean, sdX is standard deviation of SOC, X is mean of SOC, and sdY and Y and is standard deviation and mean of HWEC or net rates of NO_3-N and NH_4-N, respectively. Linear regression was used to assess the relationship between CEC, SOC and pH, and between net nitrification rate and HWEC. The statistical software package "R" version 3.4.4 (R-Core-Team, 2018) was used for all statistical analysis.

3. Results and discussion

3.1. Soil organic carbon

Seven years of strict CF husbandry including all three elements of CF and basins in fixed positions resulted in significant differences in properties of soil inside and outside planting basins (Table 1, Fig. 2). Concentrations of SOC were significantly greater inside than outside basins (0.74 ± 0.06% vs. 0.57 ± 0.08%, respectively). Although bulk density did not differ significantly (1.36 ± 0.04 g cm^{-3} vs. 1.42 ± 0.08 g cm^{-3} inside and outside basins, respectively), SOC stocks were significantly greater inside basins (2.01 ± 0.20 vs. 1.64 ± 0.26 kg m^{-2}). Also, if based on the equivalent mass of soil, the SOC stock was significantly greater inside than outside basin (Table 1).

After seven years of CF the SOC stock was 0.365 kg m^{-2} greater inside than outside basins (Fig. 2). This corresponds to an increase of 0.522 t C ha^{-1} yr^{-1}, assuming unchanged SOC stocks outside basins since adoption of CF. Based on the pool of SOC outside basins (16.4 t C ha^{-1}, Fig. 2) the relative increase in SOC in basins (viz. the upper 20 cm of the soil) was 31.8‰ per year. Since the fraction of the field occupied by basins per ha at the experimental farm was ~9.3%, the corrected absolute change in organic C-stock of the field was $3.65 * 0.093 = 0.34$ t C ha^{-1} after seven years. This corresponds to an absolute annual increase of 0.05 t C ha^{-1} yr^{-1} or a relative increase of 2.95‰ per year, which is smaller than aimed for in the four per mille initiative 4p1000.org/;(Minasny et al., 2017). Note that this increase is limited to the upper 20 cm of the soil and that the total increase in SOC stocks may have been a bit higher. Previously, smaller differences in SOC between inside and outside planting basins were reported (Martinsen et al., 2017; Nyamangara et al., 2013). As discussed by Martinsen et al. (2017) the small differences between inside and outside basins may in part be due to large variability between study sites and year to year movement of basins. Yet, in the present study, with fixed basins and controlled conditions, the differences were significant. Clearly, SOC inside planting basins is affected by maize plants, in particular maize roots,

Table 1
Mean (± sd) soil properties (0–20 cm) of CF basins (inside) and in between rows of CF basins (outside basins), Mkushi, Zambia after 7 years of CF. Difference between means and 95% confidence intervals of the estimated differences are shown.

	Basin	Between	Diff.	95% CI
	Mean sd	Mean sd		
Clay/%	9.23 (±0.85)	9.48 (±1.04) ns	−0.25	(−1.04, 0.54)
Silt/%	21.45 (±3.11)	22.00 (±3.39) ns	−0.55	(−3.33, 2.23)
Sand/%	69.34 (±2.73)	68.53 (±2.93) ns	0.81	(−1.40, 3.02)
Total porosity[a]/%	51.42 (±1.32)	51.30 (±0.75) ns	0.11	(−0.98, 1.21)
FC (pF 2)[a]/Vol%	21.91 (±1.53)	22.18 (±1.68) ns	−0.27	(−1.81, 1.27)
WP (pF 4.2)[a]/Vol%	5.40 (±0.54)	5.10 (±0.58) ns	0.30	(−0.16, 0.76)
PAW[a]/Vol%	16.51 (±1.36)	17.08 (±1.55) ns	−0.57	(−1.93, 0.79)
Tot.N/%	0.03 (±0.01)	0.02 (±0.01) ns	0.01	(−0.002, 0.02)
Tot.N-stock (0–20 cm)/kg m^{-2}	0.08 (±0.04)	0.05 (±0.03) ns	0.02	(−0.01, 0.06)
Ca/cmol$_c$ kg^{-1}	2.49 (±0.56)	0.86 (±0.35) ***	1.63	(1.26, 2.01)
Mg/cmol$_c$ kg^{-1}	1.03 (±0.18)	0.34 (±0.12) ***	0.69	(0.54, 0.83)
K/cmol$_c$ kg^{-1}	0.19 (±0.03)	0.19 (±0.05) ns	−0.01	(−0.04, 0.02)
Na/cmol$_c$ kg^{-1}	0.05 (±0.07)	0.07 (±0.09) ns	−0.02	(−0.06, 0.03)
H/cmol$_c$ kg^{-1}	0.00 (±0.00)	0.16 (±0.34) ns	−0.16	(−0.37, 0.06)
Base saturation/%	100.00 (±0.00)	90.75 (±18.14) ns	9.25	(−2.28, 20.78)
Total P/mg kg^{-1}	243.98 (±31.67)	230.86 (±29.25) ns	13.13	(−4.12, 30.37)
Al (Ox)/mmol kg^{-1}	15.72 (±3.51)	17.95 (±4.83) ***	−2.23	(−3.31, −1.14)
Fe (Ox)/mmol kg^{-1}	6.94 (±0.96)	7.19 (±1.17) ns	−0.25	(−0.58, 0.07)
P (Ox)/mmol kg^{-1}	3.06 (±0.81)	2.94 (±0.71) ns	0.11	(−0.16, 0.39)
PSC/mmol kg^{-1}	11.33 (±2.14)	12.57 (±2.95) **	−1.24	(−1.92, −0.56)

[a] N = 12 for both CF basins and between CF basins except for total porosity, volume % water at field capacity (FC) and wilting point (WP) and amount of plant available water (PAW) where n = 7 for each. Fe (Ox), Al (Ox) and P (Ox) is oxalate extractable Fe, Al and P, respectively. PSC is phosphorus sorption capacity calculated according to Breeuwsma and Silva (1992), see Appendix. CEC is cation exchange capacity (unbuffered, sum of base cations and acidity). CN ratio was not calculated because Tot. N < 0.05%. "ns"; p > 0.05, "*"; p < 0.05, "**"; p < 0.01 and "***"; p < 0.001 based on two-sided paired *t*-tests.

and the micro-topography that favors the accumulation of runoff water (Obia et al., unpublished). The significant net increase of SOC inside basins compared with outside basins thus indicates that these factors are more important than the input of crop residues, which were primarily added in between rows.

3.2. Hot water extractable carbon and potential N mineralization

The amount of hot water extractable carbon (HWEC), a measure of labile SOC that correlates with microbial biomass in soils with low content of SOC (Sparling et al., 1998; Wang and Wang, 2011), was consistently greater inside than outside basins (Fig. 3). The use of HWEC is a sensitive method to determine effects of changes in soil management on soil carbon (Ghani et al., 2003). Greater amounts of HWEC inside than outside basins indicate a greater pool of labile SOC inside basins that may be lost easily, due to decomposition and reduced C inputs (Chivenge et al., 2007; Six et al., 2002). The fraction of HWEC of SOC was 6.4 ± 0.7% and 5.2 ± 0.82% inside and outside basins, respectively, at 0–8 cm soil depth and 4.2 ± 0.9% and 2.7 ± 0.4% inside and outside basins, respectively, at 8–20 cm soil depth (Fig. 3). The differences of HWEC as a fraction of SOC between inside and outside basins were only statistically significant at 8–20 cm soil depth ($p = 0.045$). The HWEC fraction of SOC in the upper 8 cm of the Mkushi soil was at the high end of values found in a review by von Lützow et al. (2007), who reported the fraction of HWEC to vary between 1% and 5% of total SOC.

Rates of nitrification followed the same pattern as that of HWEC, with greater nitrification rates inside than outside basins (Fig. 3). However, the difference was only significant at 8–20 cm soil depth (235 ± 38 vs 133 ± 18 μg NO_3-N kg soil^{-1} day^{-1}). Ammonium was immobilized throughout the incubation period at both soil depths (Fig. 3). Net potential nitrification rates were significantly correlated with HWEC (1.04 μg increase in NO_3-N d^{-1} per mg of HWEC, $R^2 = 96$, $p < 0.001$). Previously, Ghani et al. (2003) found a significant correlation between mineralizable N and HWEC in allophanic soils from New Zealand. Curtin et al. (2017) also reported a significant correlation between HWEC and mineralizable N in soil samples from 130 sites representing major agricultural regions of New Zealand.

3.3. Soil acidity

Soil pH was significantly higher inside basins than outside basins (6.30 ± 0.15 vs. 4.95± 0.37; $p < 0.05$). Marginally higher soil pH inside planting basins compared with outside basins was reported previously by Nyamangara et al. (2013) for smallholder farms in Zimbabwe. In the present study a total amount of 2.8 t ha^{-1} of dolomitic lime was added to the basins in years with maize, corresponding to ~11 g kg soil^{-1} (i.e. an alkalinity of ~240 mmol$_c$ kg soil^{-1}). This is well in excess of the amount of exchangeable acidity outside the basins (1.6 mmol$_c$ kg^{-1}, Table 1) and shows that the rate of liming was enough to eliminate soil acidity inside the planting basins in addition to neutralizing the annual acid input, due to carbonic acid and cation uptake by plants (van Breemen et al., 1984).

3.4. Cation exchange capacity

The cation exchange capacity (CEC) was significantly greater inside (3.76 ± 0.73 cmol$_c$ kg^{-1}) than outside (1.62 ± 0.41 cmol$_c$ kg^{-1}) basins. Normalizing CEC per g of SOC also revealed significantly greater CEC inside (0.51 ± 0.11 cmol$_c$ g SOC^{-1}) than outside (0.29 ± 0.08 cmol$_c$ g SOC^{-1}) basins. The larger CEC inside basins is due to greater amounts of SOC, in addition to a higher pH (6.30 ± 0.15 vs. 4.95 ± 0.37), which causes an increase in the number of binding sites per g SOC (Gruba and Mulder, 2015). There was a significant linear relationship between CEC and amount of SOC (0.87 ± 0.20 and 0.26 ± 0.17 cmol$_c$ increase in CEC per g of SOC for inside and outside basins, respectively, $R^2 = 0.89$, $p < 0.001$) with a significantly ($p = 0.03$) greater slope (i.e. stronger increase in CEC per unit increase in SOC) inside than outside basins. The importance of soil organic matter controlling CEC in this sandy loam is similar to previously reported values (Martinsen et al. (2017) from different sites in the eastern and central provinces of Zambia (0.54–0.81 cmol$_c$ increase in CEC per g of SOC).

3.5. Phosphorus, potassium and nitrogen

The amount of total (Table 1), organic and inorganic P (Fig. 2) did not differ significantly between inside and outside basins. However, the P saturation degree (%PSD, Fig. 2) was significantly greater inside than outside basins mainly because of a greater P sorption capacity (due to more Al oxides and lower pH) outside basins (Table 1). This indicates a slightly higher availability of P inside as compared to outside basins. The amount of total N was low and often below detection limit (0.05% N). Low N status of agricultural soils is common in Sub-Saharan Africa (Martinsen et al., 2017; Mloza-Banda et al., 2016). Exchangeable potassium (K^+) was ~0.19 cmol_c kg^{-1} both inside and outside the basins (Table 1). The non-significant differences in N, P and K between outside and inside the planting basins suggest that the amount of fertilizer added in years with maize (i.e. 8.1 g basin^{-1}, 1.3 g basin^{-1} and 1.2 g basin^{-1} of N, P and K, respectively) is about the same as the sum of the amount lost, including leaching (mostly N and K) and removal in biomass at harvest. Yields under CF for the season 2015–2016 (sampled May 2016) were 5.2 (±0.84 SD) t ha^{-1} and 4.6 (±0.44 SD) t ha^{-1} for maize grain and maize stover (stems and leaves), respectively. Assuming average grain yields of 5.2 t ha^{-1} and assuming that all stover (4.6 t ha^{-1}) is used as residue (i.e. returned to the soil between basins), the amount of NPK removed from basins in years with maize corresponded to ~5.7 g basin^{-1}, 1.1 g basin^{-1} and 1.2 g basin^{-1} of N, P and K, respectively (Table S1.). These numbers are close to those for annual inputs, when also taking into account some loss of N through leaching and gaseous emissions (McNeill and Unkovich, 2007).

3.6. Soil physical properties

The fraction of clay, silt and sand were similar inside and outside planting basins (Table 1). In addition, soil physical properties including texture, BD, soil porosity, percentage water at field capacity and wilting point as well as the amount of plant available water were similar to values reported in previous studies from the same area (Obia et al., 2017; Obia et al., 2016) and did not differ between inside and outside basins (Table 1, Fig. 2). The lack of significant differences in BD may have been due to i) soil heterogeneity such that the difference was rendered insignificant, ii) smaller differences in BD than expected, because increased termite activity under mulch in between the rows (Mutsamba et al., 2016) or iii) limited compaction outside basins in the oxide-rich Acrisols. The observed lack of effect on water retention characteristics is contrary to what would be expected of soil with increased soil organic matter (Obia et al., 2016). Despite being significant, the increase in SOC (from 0.57 to 0.74%) apparently has been too small to cause an increase in porosity, water content at field capacity and wilting point.

4. Conclusion

Seven years of CF, following recommended guidelines, using the same basin location each year, significantly increased storage of SOC inside planting basins. The increase in SOC was most likely caused by increased inputs of roots, due to favorable conditions for plant growth through increased water availability and input of fertilizer and lime. In addition, biogeochemical properties such as pH, CEC, HWEC and potential nitrification rates were significantly enhanced inside planting basins after seven years of strict CF husbandry. Our study highlights the important role of basins in build-up of SOC and underscores the importance of appropriate soil sampling schemes to account for the spatial variability between inside and outside basins when studying effects of CF vs. other management practices.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.12.452.

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Appendix A. Supplementary data

Significant build-up of soil organic carbon under climate-smart conservation farming in Sub-Saharan Acrisols

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Soil analysis (detailed description)

Bulk density (BD), volume percentage water at field capacity (pF 2) and at the wilting point (pF 4.2) as well as total porosity was determined on undisturbed soil cores of 100 cm^3. Water retention at the different matrix potentials was determined using ceramic pressure plates and BD determined on the dry matter mass of soil (Obia et al., 2016). The amount of plant available water was calculated as the difference between volume percentage water at field capacity and at the wilting point. All soil samples were air-dried and sieved at 2 mm prior to further analysis. Soil texture was determined using the Pipette method.

Soil pH was determined in 0.01M $CaCl_2$ using a soil to solution ratio of 1:2.5 with a digital pH meter. Subsamples of the air-dried and sieved samples were dried at 60 °C to determine dry matter content and then milled prior to determination of organic carbon (C) and total nitrogen (N). Total soil carbon was determined by dry combustion (EC12, C determinator, Leco Corporation) (Nelson and Sommers, 1982). Since soil pH was below 6.5, total C was used as a measure of soil organic carbon (SOC). Total N was determined on untreated soil samples by the Dumas method (TruSpec, CHN analyzer, Leco Corporation) (Bremmer and Mulvaney, 1982). Carbon and N stocks were calculated based on volume of soil by multiplying depth of sampling, BD and elemental concentration. Mean values of BD per block for CF basins and between rows of basins, respectively, was used to calculate C-stocks. In addition, C-stocks were calculated based on an equivalent mass of soil since equal depth sampling may overestimate C stocks in treatments with greater BD (Ellert and Bettany, 1995; Wendt and Hauser, 2013). Since all sampling was done to a depth of 20 cm, the sub-plot with the lowest BD, and thus the smallest soil mass was designated as the “equivalent” soil mass. This was done in order to avoid going deeper than 20 cm, since our %SOC measurements were based on 0-20 cm only. The plot with the lowest soil mass was in block 4 inside CF basins with BD of 1.33 g cm^{-3} and an equivalent mass of soil corresponding to 265 kg m^{-2}.

Exchangeable base cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) and exchangeable Al^{3+} were determined in extracts after extraction with 1M ammonium nitrate (NH_4NO_3, unbuffered). Extractable acidity was determined by back-titration with 0.05 M sodium hydroxide to pH 7. The sum of exchangeable base cations and exchangeable acidity was assumed to equal the effective cation exchange capacity (CEC) according to Schollenberger and Simon (1945). Total (based on crushed and ignited (550 °C) soil samples) and inorganic P (based on crushed but not ignited soil samples) was determined according to Møberg et al. (1990) in soil extracts after addition of 6M H_2SO_4 and heating in a water bath (~70 °C). Phosphate in the extracts was determined using the Molybdenum-blue method according to Murphy & Riley (1962). Organic P was calculated as the difference between total P and inorganic P. Acid oxalate extractable Fe, Al and P were determined according to van Reeuwijk (1995). The sample was shaken in an acid ammonium oxalate solution (pH 3) dissolving the “active” or short-range order (amorphous) compounds of Fe and Al. Phosphorus sorption capacity (PSC) and phosphorus saturation degree (PSD) was calculated according to Breeuwsma and Silva (1992):

$$\text{PSC (mmol kg}^{-1}\text{)} = 0.5*[\text{Al}_{ox}\text{ (mmol kg}^{-1}\text{)} + \text{Fe}_{ox}\text{ (mmol kg}^{-1}\text{)}] \qquad (1)$$

$$\text{PSD (\%)} = [\text{P}_{ox}\text{ (mmol kg}^{-1}\text{)/PSC}] * 100 \qquad (2)$$

where Al_{ox}, Fe_{ox} and P_{ox} are oxalate extractable Al, Fe and P.

Potential N mineralization rates were determined in an incubation experiment using air dried and sieved soil samples (modified after Martinsen et al. (2017)). At the start of the experiment (day 0), 8 g of soil was added to each of six PP tubes from each of the eight samples. To each PP tube 1.8 mL of distilled water corresponding to ~31 vol% water was added. One set of triplicate sub-samples were immediately frozen (background level), while the remaining triplicate sub-samples were incubated (dark) in an incubation cabinet at 20 °C. After 60 days of incubation, the remaining triplicate sub-samples were removed and frozen. After thawing, the soils were extracted in 20 mL 2M KCl (Øien and Selmer-Olsen, 1980) and filtered prior to analysis of NH_4-N and NO_3-N using a flow injection analyzer (FIA Star 5010). Rates of net ammonification and net nitrification were determined by subtracting initial extractable soil NH_4-N and NO_3-N from final amounts (after 60 days) of extracted NH_4-N and NO_3-N, respectively. The sum of produced NH_4-N and NO_3-N represents net mineralization (Vestgarden and Kjönaas, 2003). The average of each of the triplicate sub-samples was used in the statistical analysis. Hot water extraction (80 °C during 16 hours) of carbon was done to determine the labile fraction of soil organic matter according to Ghani et al. (2003). Briefly, 30 ml of distilled water was added to PP tubes with 5 g of soil. The suspensions were gently shaken in a vortex shaker prior to extraction (0.45 µm) of dissolved organic carbon (DOC). The DOC was measured using a total organic carbon analyzer (TOC-V CPN, Shimadzu).

Comparison of mean soil parameters for basins and between rows was done using two-sided paired t-tests. Difference between means and 95% confidence intervals of the estimated differences are shown in Figures 2 and 3 and in Table 1. To assess propagation of error (Ku, 1966), for normalized values (i.e. CEC per g of SOC, HWEC in mg g SOC^{-1} and net rates of NO_3-N and NH_4-N in µg g SOC $^{-1}$ day^{-1}), standard deviation (sd) was calculated as sd= $|R| * \sqrt{(sdX/X)^2 + (sdY/Y)^2}$. Here R is the normalized mean, sdX is standard deviation of SOC, X is mean of SOC, and sdY and Y and is standard deviation and mean of HWEC or net rates of NO_3-N and NH_4-N, respectively. Linear regression was used to assess the relationship between CEC, SOC and pH, and between net nitrification rate and HWEC. The statistical software package "R" version 3.4.4 (R-Core-Team, 2018) was used for all statistical analysis.

Fig. S1. Plot (24 m^2, 24 planting basins) used for soil sampling, Mkushi, Zambia. The picture shows rows of permanent basins with a spacing of 90 cm between rows and 80 cm between basins within rows. Residues (maize stover) are placed between basins at the end of the dry season (October).

Table S1. Theoretical calcualtion of net input and removel of N, P and K at Mkushi, Zambia.

Budget; input and output of N, P, K: Mkushi				
Volume plant basin (cm3)		13360.00		
Bd (g cm3)		1.36		
Amount of soil per basin (kg)		18.17		
Additions (total per year)		**N**	**P**	**K**
Fertilizer "2 cup 2 cup" [200 kg N,P2O5,K2O (10:20:10) + 200 (46:0:0)] in **kg/ha**		112.00	17.46	16.60
Amount added per basin (g) per year for the years with fertiilzer addition (4 years before sampling)		8.06	1.26	1.20
Addition of N, P and K in mg/kg soil per basin for the years with fertiilzer addition (4 years before sampling)		443.78	69.16	65.79
Addition of N, P and K in mmol/kg soil per basin for the years with fertiilzer addition (4 years before sampling)		31.70	2.30	1.68
Removal in biomass (elemental concentration based on data from Mongu (from the report "Biochar in conservation farming in Zambia, Phase 2, report 2, 18 February 2013 AND Martinsen et al. 2014))				
Elemental concentration in maize stover (N in % and P+K in mg/kg): Ca in mg/kg		1.01	2262.00	7107.00
Elemental concentration in maize grain (N in % and P+K in mg/kg): Ca in mg/kg		1.51	2972.00	3310.00
Assumption: yield stover and grain at CF sites in Mkushi 2015-16: 4.6 t/ha + 5.2 t/ha	4.6			
	5.2			
Amount removed in stover (kg/ha) in years with maize		46.46	10.41	32.69
Amount removed in stover per basin (g) in years with maize		3.34	0.75	2.35
Amount removed in grain (kg/ha) in years with maize		78.52	15.45	17.21
Amount removed in grain per basin (g) in years with maize		5.65	1.11	1.24
Amount removed total (kg/ha) in years with maize		124.98	25.86	49.90
Amount removed total per basin (g) in years with maize		9.00	1.86	3.59
Net added-removed based on a harvest of 5.2 t/ha grains only per year: In years with maize (assuming roots remain in basin) and residues are put between rows with basins				
g per basin		***2.41***	***0.14***	***-0.04***
Net added-removed based on a harvest of 5.2 t/ha grains and 2.3 t/ha stover (i.e. half of nutirents in residues returned to the basin) per year: In years with maize (assuming roots remain in basin) and residues are put between rows with basins				
g per basin		***0.74***	***-0.23***	***-1.22***
Net added-removed based on a harvest of 4.6 t/ha + 5.2 t/ha per year (stover and grains): In years with maize (assuming roots remain in basin) and residues are put between rows with basins				
g per basin		***-0.93***	***-0.61***	***-2.40***

*Values for elemental concentrations: (Martinsen et al., 2014).

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Paper III

Effect of conservation farming and biochar addition on SOC quality, N mineralization and crop productivity in a light-textured Acrisol in the sub-humid tropics

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Highlights

- Conservation farming had a positive effect on yield compared to conventional farming when little or no fertilizer was added.
- 9.5 year of conservation farming led to higher amounts of particulate organic matter and labile C inside planting basins compared to the surrounding soil.
- The removal of residue during three growing seasons did not have any significant effect on the quality of the soil organic matter.
- Tillage (in combination with residue removal) reduced the amount of labile SOC and N mineralization.
- Biochar addition had no influence on yield and N mineralization but is highly stable in soil.

Abstract

Agricultural practices, involving tillage and the no addition of organic amendments, lead to soil organic matter (SOM) decline and this is a problem that affects crop production and environmental quality. Conservation farming (CF) including minimum tillage, using permanent planting basins, and crop residue retention with and without biochar addition may help to mitigate negative impacts of conventional practices. In this study, the effects of CF on the amount and quality of SOM and potential nitrogen (N) mineralization were investigated in a maize – soya rotation in an Acrisol in Zambia. A large number of CF plots, were run for 9.5 years, the last three growing seasons (2015-2018) four alternative management practices including biochar addition were introduced to study effects on soil characteristics. Here, we tested i) CF without residue retention (CF-NO-RES); ii) CF normal (CF-NORM), which is a continuation of regular CF; iii) Conventional tillage (CONV), with full tillage and removal of crop residues; and iv) CF with 4 ton ha^{-1} pigeon pea biochar inside basins and residue retention (CF-BC). The maize – soya rotation also implies an alternation in fertilizer regime, maize receives fertilizer (237 kg NPK and 200 kg Urea ha^{-1}) while soya receives none. Soya yield, when fertilizer was not applied, was significantly higher in CF systems than in CONV. Maize yields were not affected by the different management practices probably due to the high rate of fertilizer addition. Conventional tillage reduced both quality and quantity of SOM compared to conservation farming. Removal of crop residues did not affect any of the tested soil quality parameters. Particulate organic matter (POM) under conservation farming was larger inside basins than in the surrounding soil (outside basins) due to a greater amount of roots inside basins. CF-NORM had higher stock of soil organic carbon (SOC), higher N mineralization rates and more hot-water extractable carbon (HWEC; labile SOC). CF-BC increased the amount of SOC as compared with CF-NORM, whereas N mineralization rate and HWEC remained unaffected. The biochar appears to be relative stable as, 84 to 90% of the initial biochar was retrieved 2.5 years after addition. Results suggest long-term benefits on grain yield of minimum tillage in the form of permanent planting basins, no short-term effect of crop residues on build-up of SOC, and high stability of biochar in soil.

Introduction

Soil organic matter (SOM) is important for agricultural and ecosystem services (Manlay et al., 2007). SOM contributes to the mitigation and adaptation to climate change as it acts as a sink for CO_2, a major greenhouse gas, it helps storing plant nutrients (Paustian et al., 2016) and it makes crop production more resilient to drought conditions by promoting soil aggregation (Oades, 1984) and water infiltration (Franzluebbers, 2002). In Sub-Saharan Africa (SSA) SOM depletion is one of the major causes of soil degradation (Tully et al., 2015). Thus, there is a need for improved soil management alternatives that may contribute to increases in SOM. Conservation farming (CF), a set of practices including i) minimum or no tillage ii) retention of crop residues and iii) crop rotation, has been suggested as a way to increase soil organic C (SOC) if its three principles are strictly applied (FAO, 2013; Powlson et al., 2016). CF has been promoted among small-scale farmers in countries like Zambia for more than two decades (Corbeels et al., 2015) with yield benefits for farmers if combined with complementary practices such as the use of improved crop varieties, adequate weed and pest control, among others (Thierfelder et al., 2018; Thierfelder et al., 2013). Most of the studies assessing the effect of CF on SOC have focused on the total amount of organic C, whereas SOC fractions have been overlooked. Fractionation of SOC can be used to assess the quality of SOC, thus understanding the effect of soil management practices on decomposition and stabilization processes of SOM (Poeplau et al., 2018).

Among the multiple fractionation methods, particulate organic matter (POM; density lower than 1.6-2.0 g cm^{-3}; not strongly bound to minerals) and hot-water extractable C (HWEC at 80 ˚C; dissolved organic C with a diameter < 0.45µm) have been shown to be sensitive to changes in soil management and can be used to isolate labile to intermediate fractions, which are expected to be affected most by CF practices (Ghani et al., 2003; Powlson et al., 2016; Tivet et al., 2013). It has been shown that cultivation reduces the amount of POM in tropical and subtropical regions (Tivet et al., 2013) due to increased decomposition and/or reduced input of biomass. Biomass inputs may help restoring the initial amount of POM (Leifeld and Kögel-Knabner, 2005), being root biomass more effective than aboveground biomass (Kong and Six, 2010). In addition, HWEC has been found to be a good predictor of potential mineralizable N, which can be used to assess the capacity of soils to supply N (Ros et al., 2011) under different soil management practices (Ghani et al., 2003).

The present research builds on a previous study of the effects of seven years of CF using permanent planting basins (a form of minimum tillage that is practiced by smallholder farmers in arid environments) in Zambian Acrisols and a two-year maize-groundnuts rotation. This study found an increase of SOC of about 2.9‰ yr^{-1} and an increase of N mineralization rates inside basins as compared to the adjacent soil (Martinsen et al., 2019). Root biomass and not residue retention was suggested to be the reason for the changes in the amount and quality of SOC. Further study of the effect of tillage practice and residue retention is needed though.

Biochar, the C-rich pyrolysis product of agricultural waste, has been suggested as a way of sequestering C while improving soil fertility (Lehmann et al., 2009; Lehmann et al., 2006). Soil amendment with biochar may alleviate soil acidity, improve soil water-holding capacity and prevent leaching of plant nutrients (Jeffery et al., 2017; Major et al., 2012), which all contribute to increased crop yields. Previous research in SSA showed that biochar addition inside planting basins in CF husbandry increased maize yield after one season (Cornelissen et al., 2013; Martinsen et al., 2014), nevertheless, there are no studies of biochar in CF conducted over a greater number of growing seasons including a crop rotation. Moreover, biochar has been shown to impact SOC decomposition rates both positive and negatively, a process known as priming effect that may last from days to years (Weng et al., 2015; Zimmerman et al., 2011). Priming effect in biochar research has been measured mainly as the changes of CO_2 efflux in incubation experiments, while measurements of N mineralization rate are scant in spite of being another known way of determining this phenomenon (Kuzyakov, 2010).

In the present study, the objective was to investigate the effects of i) normal CF, ii) CF without residue retention, iii) conventional tillage without retention of crop residue and iv) CF with biochar addition inside basins on crop biomass, SOC, HWEC, soil N content, N mineralization rates in a rain-fed maize-soya-maize rotation (2.5 years). In addition, we aimed at assessing the build-up of POM inside permanent planting basins and compare it with the surrounding soil with and without retention of crop residues.

Materials and methods

Land use history and experimental setup

The experiment, done on private farm (CENA farms; Mount Isabel), Mkushi, Zambia (S13°45′25.7″ E29°03′55.5″), is conducted on a sandy loam (Acrisol) with an average annual precipitation of 1220 mm and a mean annual temperature of 20.4 ˚C (Martinsen et al., 2019; Obia et al., 2017). Prior to the experiment, the site had been under CF (planting basins and maize – ground nut rotation) for 7 years. For details about soil characteristics and soil management reference is made to Martinsen et al. (2019). Different treatments were established in 24 m^2 plots, randomly distributed in 4 blocks. All CF plots had four rows of six planting basins (distance 80 cm, 90 cm between rows, basins were about 40 cm x 20 cm and 20 cm deep), that were planted with either three maize plants or 8 seeds of soya beans. The variety of maize was MRI 634. The conventionally tilled plots (CONV) had four rows with 18 (equally spaced) maize plants each. In October 2015 four soil management practices were assigned to one plot in each of the four blocks (viz. four replications per treatment). Treatments included i) Conventional (CONV): full tillage to a depth of 10 cm and no residue retention. ii) CF Normal (CF-NORM): continuation of the practice implemented during 7 years prior to 2015, with basins fully opened before planting and crop residue added after harvest (4.4 ton ha^{-1} and 2.9 ton ha^{-1} of maize and soya residues, respectively). iii) CF no residue (CF-NO-RES): residue removal after harvest - retention of residue under CF-NORM corresponded to 4.4 ton ha^{-1} maize stover in 2015-16 and 2.9 ton ha^{-1} soya stover in 2016-17. iv) CF + Biochar (CF-BC): addition of 4 ton ha^{-1} of pigeon pea biochar inside basins before planting in October 2015 only, while crop residues were applied as in CF-NORM. Biochar was added at a depth of 8 to 20 cm. Seeds and fertilizer were mixed into the upper 8 cm of the basins.

The experiment was done for 3 growing seasons (2015 – 2018). Maize was planted in 2015/2016, soya in 2016/2017 and again maize in 2017/2018. Fertilizer was applied only to maize while soya did not receive any. All the soil management practices received the same amount of NPK fertilizer and urea per hectare. The fertilizer was applied differently in CF treatments and CONV. In all CF treatments, 17.1±0.8 g of NPK (N, P_2O_5, K_2O; 10-20-10) was applied per basin at pre-planting, corresponding to 237 kg ha^{-1}. Five and eight weeks after planting, topdressing with urea (46:0:0) was applied, corresponding to 100 kg ha^{-1} each time (200 kg ha^{-1} Urea). In the CONV treatment, the NPK fertilizer was applied at emergence along the rows of maize plants. Five and eight weeks after planting a topdressing with urea was applied (200 kg ha^{-1}). The application of N to maize crop corresponded to 116 kg N ha^{-1} yr^{-1}.

Biochar

Biochar feedstock was pigeon pea (*Cajanus cajan*) stems, produced in a flame curtain kiln (Cornelissen et al., 2016) at 600 ˚C. The chemical characteristics of the biochar were as described by Munera-Echeverri et al. (2018) and include pH (10.4), Electrical Conductivity (1.4 mS cm^{-1}), acid neutralizing capacity (ANC_{pH7}; 49 $cmol_{(+)}kg^{-1}$), total organic carbon (56.1%), Total N (0.69%), total H (1.1%) and cation exchange capacity (6.6 $cmol_{(+)}kg^{-1}$).

Biomass production

Stover biomass and grain yield were measured immediately after harvest. The values measured in the field were corrected for dry matter content. Root to shoot ratios of maize plants were measured in CF-BC, CF-NORM and CONV before harvest (Feb.2018; 12 weeks after planting) by digging the whole root system of 1 basin (3 plants) per plot in CF treatments and 3 consecutive plants in CONV. Root biomass was calculated by multiplying total aboveground biomass (grain plus stover) measured at the end of the growing season and root to shoot ratios. Root depth and width determined was measured.

Soil sampling

Soil was sampled in CF-NORM and CF-NO-RES outside and inside basins. In CONV, we sampled inside rows of maize plants and in between rows. CF-BC was sampled inside basins only. The first sampling campaign was carried out in May 2016 (end the growing season 2015-16, after harvest; CF-NORM and CF-BC) and the second in February 2018 (in the middle of the season 2017-18; all the treatments). Samples inside basins were taken by mixing one soil core of 2 cm diameter from five different basins (i.e. one bulked sample per treatment plot). Samples outside basins were taken from the area in between rows of basins in the same way. Samples were collected from 0 to 8 cm and from 8 to 20 cm depth. The samples were dried at 40 ˚C over a 7 days period before being sieved (2mm) at the Norwegian University of life Sciences (NMBU). The sample of one of the plots in CF-BC in 2018 was lost in the travel from Zambia to Norway.

C and N analyses and stocks

Soil organic carbon (SOC) was analyzed using a TruSpec CHN analyzer (Leco Corporation). Since soil pH was below 6.5 (Table S1) total C was used as a measure of SOC. Total N was analyzed by the Dumas method (Bremner and Mulvaney, 1982) using a TruSpec CHN analyzer (Leco Corporation). Stocks of organic C and N (ton ha^{-1}) were calculated by multiplying elemental concentration (inside and outside basins), bulk density (BD) and depth of sampling. C and N stocks in CF-BC outside basins was assumed to be the same as in CF-NORM. Bulk density was determined using 100 cm^3-steel rings at 5 to 10 cm in the season 2016-17 inside and outside basins in each treatment. The samples were transferred to plastic bags and dried at 105 ˚C at NMBU

N mineralization

Nitrogen mineralization rates were determined in a 60-day incubation experiment at 20 ˚C. A total of 8 g of dried soil was added to 50 ml polypropylene tubes and the moisture content was adjusted to 30% (v/v) by adding 1.8 ml distilled water. The lids were placed loosely on the tubes, allowing gas exchange. Water was replenished every 12 -14 days after weighing. The tubes containing the samples at time 0 were frozen at -18 ˚C and stored until KCl extraction. Mineral N (NH_4^+ and NO_3^-) was determined before and after incubation, using 20 ml 2M KCl. Tubes were shaken horizontally during 1 hour at 200 strokes per minute and filtered using Whatman filter (589/3). Extracts were analyzed for NH_4^+ and NO_3^- by Flow Injection Analysis (FIA tar 5010). Potential N mineralization rates were calculated by subtracting the initial amount of NH_4^+ and NO_3^- at time 0, from the amount determined after 60 days. Net production of NH_4^+ and NO_3^- (both in g kg^{-1} day^{-1}) after 60 days were summed and potential N mineralization rate per day was calculated.

Hot-water extractable C (HWEC)

The HWEC was determined as described in (Ghani et al., 2003). In brief, 5 grams of dried soil and 30ml of deionized water were added to 50ml polypropylene tubes. The tubes were closed, shaken in vortex-shaker during one minute and placed in a laboratory water bath during 16 hours at 80 ˚C. Subsequently, the tubes were centrifuged at 1700 g and the supernatant was filtered using a 0.45 µm polyethersulfone filter. The samples were analyzed for dissolved organic carbon (DOC) using a total organic analyzer (TOC-V CPN, Shimadzu).

Density fractionation

Density fractionation is a laboratory procedure used to isolate light SOC with density lower than 1.6-2.0 g cm^{-3}, which is not strongly bound to minerals, better known as particulate organic matter (POM), composed mainly of partially decomposed fragments of roots and aboveground biomass

(Boone, 1994). POM can be either free (fPOM) or occluded (oPOM) in soil aggregates. Density fractionation was carried out following a modified method based on Leifeld & Kögel-Knabner (2005) to determine POM, which was a combination of fPOM (wet sieved and density < 1.8 g cm^{-3}) and oPOM (ultrasonic dispersion with 22 J ml^{-1}, < 1.8 g cm^{-3}) fractions. In a pre-experiment, we found it difficult to differentiate between fPOM and oPOM as the amount of fPOM was very small. A sodium polytungstate ($Na_6(H_2W_{12}O_{40}) \times H_2O$) solution was prepared and its density adjusted to 1.8 g cm^{-3}. A total of 20 g dried and sieved soil (< 2mm) was weighted into 50ml polypropylene tubes and 30 ml of sodium polytungstate was added. The tubes were shaken gently 10 times end-over-end. Subsequently, the samples were dispersed ultrasonically with 22 J ml^{-1} and centrifuged at 1700 g for 12 minutes. The supernatant with floating particles (POM) was transferred onto a 20 µm sieve and rinsed with distilled water until the electrical conductivity dropped to < 100 µS cm^{-1}. The POM was dried at 105 ˚C for 24 hours and weighed. Next, the samples were milled and analyzed for total C and total N. The amount of POM relative to the bulk soil, the contribution of POC to SOC, as well as contribution of POM-N (PON) to total N was calculated.

Statistics

Statistical analyzes were conducted using R software (R-Core-Team, 2017). We used one-way ANOVA to determine differences in crop biomass (Tab. 1) and C and N stocks between treatments (Tab. 3). Two way ANOVA (treatment 3 levels and in vs. out 2 levels) was used to assess differences in SOC, total N, N mineralization rate and HWEC between treatments and between inside and outside basins (CF) or rows (CONV; Fig. 1). Linear mixed effect model (Fixed factors: treatment 2 levels, year 2 levels) was used to assess the effects on conservation farming with and without biochar on SOC, total N, N mineralization rate and HWEC (Fig 2) over time at 0 to 8 cm and at 8 to 20 cm separately, where block was included as random factor. The spatial autocorrelation between repeated measurements in 2016 and 2018 was assumed constant between the different treatment combinations. Two way ANOVA (treatment 2 levels and in vs. out 2 levels) was used to assess the difference in POM, POC, PON and C:N ratio of POM between CF-NORM and CF-NO-RES inside and outside basins in each depth independently. Differences between variables retained in the parsimonious models were analyzed by Tukey test at 0.05 significance. Linear regression was used to assess the relationship between HWEC and potential N mineralization rates.

Results

Biomass production and grain yield

Soya yield and stover (season 2016-17), were significantly smaller under CONV (2.1 tons of grain ha^{-1}) than under CF-NORM, CF-NO-RES and CF-BC (3.4 tons, 3.6 tons and 3.0 tons of grain ha^{-1}, respectively; Table 1). Maize yield and maize stover production did not show any significant differences between treatments (i.e. seasons 2015-16 and 2017-18). Maize yields were higher in 2015-16 (5.2 ton grain ha^{-1}) than in the season 2017-18 (3.1 ton grain ha^{-1}).Retention of residue under CF-NORM corresponded to 4.4 ton ha^{-1} maize stover in 2015-16 and 2.9 ton ha^{-1} soya stover in 2016-17. Maize root to shoot ratios were not significantly different between treatments. The calculated root biomass was 3.2 ton ha^{-1} in average (Tab. 1). The maximum depth and width of maize root system was 23 cm and 18.8 cm respectively (Tab. 1).

Effect of conservation farming on SOC, SOC fractions, total N and N mineralization

There was no effect of residue removal on SOC, HWEC, total N and mineralizable N after 3 growing seasons (Fig. 1, Tab 2). Under CF-NORM and CF-NO-RES, SOC, HWEC and N mineralization rate were significantly greater inside basins as compared to outside ($p<0.01$ in both cases). Yet, total N did not significantly differ between inside and outside basins (Fig 1). Likewise, POM was not affected by residue removal ($p=0.51$; Tab. 2). The results show larger amounts of POM inside basins than outside basins both at 0 to 8 cm and at 8 to 20cm ($p<0.01$ in all cases; Tab. 2). The quality of the POM was not affected by residue removal either, as indicated by similar contribution of POC to SOC, PON to total N and CN ratio in POM in both treatments. Instead, the results show differences in the above-mentioned parameters between inside and outside basins (Tab. 2). In CONV, the four parameters were the same inside and outside rows of plants. Total N did not show significant differences between the soil regimes. Soil Organic C, HWEC and N mineralization rate were higher in CF systems inside basins than in CONV inside rows (Fig. 1).

Effect of biochar, time and depth application inside basins

Biochar addition inside basins in CF significantly increased the amount of SOC as compared to CF-NORM at 8 to 20 cm depth where most of the biochar was added (Fig. 2a). The amount of C added as biochar was 2.2 ton C ha^{-1}, while the difference in C stock inside basins in 2018 between CF-BC and either CF-NORM or CF-NO-RES corresponded to 2 ton C ha^{-1} (Tab. 3), which is a recovery of 90% of the biochar added. Total C stock per hectare in 2018 showed significant differences between CF-BC and CONV only ($p<0.01$; Tab. 3). However, CF-BC had significantly higher C stock inside basins than both CF-NORM and CF-NO-RES ($p < 0.01$ in both). In 2018, concentration of total N (%) was increased upon biochar addition as compared to CF-NORM at 8 to 20 cm only (Fig. 2b). However, results showed no differences in N stock per hectare when CF-NO-RES, CF-NORM and CF-BC are included in the analysis (Tab.3). Nitrogen mineralization rates and HWEC in CF-BC were not different as compared to CF-NORM in 2016 nor 2018 (Fig. 2). Biochar increased amount of SOC but not its more labile fraction, as estimated by hot water extraction (HWEC). The correlation of N mineralization rate and HWEC was clear and it was not affected by any of the soil regimes including CF-BC (R^2=0.81; Fig. 3).

Discussion

Crop biomass-effect of soil management

Soya yield and stover were significantly lower in CONV than in CF systems in 2016-17 (Tab. 1), when no fertilizer was applied. We found no significant effect of treatment (including biochar) on maize yield. Maize yields in our experiment were greater (Tab.1) than those generally obtained by small-scale farmers in SSA, which were estimated to be 1.4 ± 1.0 ton ha^{-1} for maize (Dzanku et al., 2015). The reason is likely to be the high N application rate (116 kg N ha^{-1}) in our experiment. By contrast, in small-scale farmers in SSA use on average 17 kg NPK ha^{-1} (Thierfelder et al., 2018). Consequently, amounts of maize and soya residues were high (4.4 and 2.9 ton ha^{-1} y^{-1}, respectively). Research conducted in SSA has suggested 3 ton ha^{-1} of crop residues as threshold value to reach an initial soil cover of 30% of the land at the beginning of each growing season (Vanlauwe et al., 2014). Often, small-scale farmers do not manage to produce that amount of residues. The reason for higher maize yield in 2015-16 than in 2017-18 as likely a dry-spell that affected Zambia mainly during January 2018.

Results showed no effect of soil treatment (including biochar addition) on root to shoots ratio. Previously, Abiven et al. (2015) found more developed maize roots systems and greater yields in biochar amended plots compared to normal CF plots on the same site in Zambia. However, the application of N in the Abiven et al. (2015) study was not higher than 30 kg N ha^{-1}. Maize root to shoot ratio reported by Abiven et al. (2015) was about 0.037 to 0.045 which is much smaller than those found in our study (0.26 to 0.49, Tab. 1). Our values are in the range of what was reported for maize in North America (Anderson, 1988; Bolinder et al., 1999). Results showed estimates of maize root biomass that varied from 1.9 ton ha^{-1} in CONV to 3.5 ton ha^{-1} in CF-BC (Tab. 1).

Effect of permanent planting basins, tillage and residue retention on SOC, C fractions, total N and N mineralization

The experimental field at Mkushi had been managed with permanent planting basins and residue retention between rows of basins for 7 years prior the establishment of the treatments. At the onset of the present study SOC, HWEC and N mineralization rate were therefore higher inside permanent planting basins as compared to outside (Martinsen et al., 2019). Upon full tillage in CONV management, SOC, N mineralization rate and HWEC decreased to the same levels observed for the area outside basins in CF (Fig. 1). This could be explained by the redistribution of soil upon conventional tillage that diluted the HWEC that was gained inside basins. Other studies have shown sharp long-term decrease of HWEC and other labile SOC fractions upon full tillage has been reported in other tropical, subtropical as well as temperate soils mainly in the upper 5 cm (Tebrügge and Düring, 1999; Tivet et al., 2013). HWEC and N mineralization were highly correlated (Fig. 3), therefore, N mineralization in CONV was also lower than in CF inside basins (Fig. 1).

Results suggests no effect of residue removal on amount and quality of SOM (Fig. 1, Tab. 2). Previous research has suggested limited effect of crop residues on SOC increments in soils in South Africa and Kenya over longer periods of time than used in the experiments at Mkushi (Sommer et al., 2018; Swanepoel et al., 2018). This may be due to high decomposition rates of crop residues (Six et al., 2002). However, residue losses due to termite activity, which increases with increasing residues (Mutsamba et al., 2016), or transport by wind and water may also have contributed to the lack of effect of residues on SOC and its particulate and soluble fractions. The fact that residues do not have an effect on either SOM or POM (< 2 mm) does not imply that they do not provide other benefits such as soil moisture retention and weed control if they are kept as mulch (Turmel et al., 2015).

Amount of SOC, HWEC and N mineralization inside basins were greater than outside (Fig. 1; Tab. 2). The accumulation of root-derived biomass inside basins may explain this pattern as suggested by Martinsen et al. (2019). Inside basins (10% of the area), about 3.5 ton ha^{-1} of root biomass in CF-NORM (Tab. 1) was concentrated and incorporated into the soil, while outside basins about

4.5 ton ha^{-1} (in the case of maize) of crop residues was distributed over a 10 times larger area and placed on soil surface. Previous research has shown that root biomass is retained as SOM more efficiently than shoot biomass (Rasse et al., 2005). Often this has been attributed to the differences in decomposability of roots vs. shoots (Bolinder et al., 1999; Rasse et al., 2005). The observation that POM was higher inside basin than outside at both 0 to 8 am and 8 to 20 cm also favors the root carbon retention hypothesis, since residue biomass is applied at the surface while root biomass can penetrate deeper (Tab. 2).

Effect of biochar on amount and quality of SOC over time and depth

CF-BC significantly increased SOC and C stocks inside basins as compared to CF-NORM and CF-NO-RES (Fig.2; Tab. 3). In addition, C stock per hectare in CF-BC was significantly higher than in CONV (Tab. 3). The reason for not finding significant differences between in C stocks per hectare between CF-BC and CF-NORM may be attributed to the fact that biochar was added only to about 10% of the land and most of the C in the field was found outside basins (Tab. 3). C stocks in the present study were low as compared to the values reported by Martinsen et al. (2017) for other soils in the Eastern and Central provinces in Zambia in conventional and CF fields (28 to 45 ton C ha^{-1} vs. about 12.7 ton C ha^{-1}). The high recovery of biochar (90%) 2.5 years after addition suggests high stability of biochar in agreement with Kuzyakov et al. (2014), as well as limited lateral BC transport, i.e. floating up followed by erosion. Lateral BC transport was observed to be up to 30-40% in a similar soil in Zambia (Obia et al., 2017). Vertical BC transport was observed to be limited to 1-2 cm per year, and thus vertical transport to below 20 cm depth would not be expected. Despite larger SOC in CF-BC, HWEC was unaffected by biochar addition (Fig 2). HWEC decreased with depth with and without biochar. HWEC has been found to decrease with depth in uncultivated, agricultural and forest soils (Fan et al., 2013; Zhao et al., 2013). Likewise, CF-BC did not affect N mineralization rate and consequently, we did not find evidence for positive or negative priming effect of biochar on SOM decomposition. The net N mineralization rate increased with about 96 µg-N $kg^{-1}d^{-1}$ when HWEC increased by 100 mg kg^{-1} (Fig 3). HWEC was about 2.3% of the total SOC in CF-BC, 4.0 % in CF-NORM, and 3.4% in CONV. The decrease of HWEC from 2016 to 2018 in CF-NORM (Fig. 2) may be partially explained by the differences in maize root C inputs throughout the growing season, since sampling in 2016 was done at the end of the season (maximum amount of C inputs) whereas in 2018 it was done in the middle. In addition, maize biomass in the season 2017-18 decreased as compared to 2015-16, due water-stress. Fluctuations in HWEC across seasons has been reported in pine forest in Korea and this was linked to changes in the amount of extractable carbohydrates (Zhao et al., 2013). Soluble carbohydrates have been found to constitute about 79% of the root exudates of maize plants (Hütsch et al., 2002). In addition, soil pH inside basins decreased during the experiment from 6.3±0.2 (Martinsen et al., 2019) to 4.5 (with and without biochar). This could also explain the decrease in HWEC. In fact, solubility of SOM increases at increasing pH (Curtin et al., 2016). The decrease in HWEC from 2016 to 2018 can explain the decrease in N mineralization rate since DOM is an important substrate for microbial activity (von Lützow et al., 2007).

Conclusions and recommendations

Results showed that conservation farming had a positive effect on yield compared to conventional farming when no fertilizer is added (when ample fertilizer was supplied the effect on yield disappeared). In this experiment, special attention was given to keeping the planting basins in the same position in each growing season for 9.5 years. In conservation farming SOC, particulate organic matter and hot-water extractable C were larger inside basins than outside basins due to the continuous allocation of root biomass inside basins. Nitrogen mineralization was enhanced inside basins due to the increase of labile C. Upon tillage, the build-up of SOC, hot-water extractable C and mineralizable N decreased as compared to the amounts measured inside basins in the normal conservation farming practice. This suggests that it takes few seasons of "mismanagement" to undo the long-term benefits of conservation faming on soil C and N mineralization.

Research has suggested that planting basins increase labor burden with no benefits for farmers in the short-term (Rusinamhodzi, 2015). By contrast, other studies have suggested positive effect of basins on crop yields in Zambia (Ngoma et al., 2015). However, the fact is that the adoption of planting basins among small-scale farmers in sub-Saharan Africa is limited and mechanized ripping lines, by tractor or animal traction, seem more preferred in the future. Keeping the planting stations (basins in this case) in the same position may be challenging other types of conditions or even other forms minimum tillage. Therefore, further research is needed on root-derived soil organic matter under other types of minimum tillage in Sub-Saharan Africa. Our results do not suggests further increase of SOC in conservation farming inside basins from the 7th to the 9th year and a half, if the study by Martinsen et al. (2019). This suggests that more time is needed to detect significant increases in SOC overtime and that the rotation maize-groundnuts (before the 7th year) is more effective increasing SOC than maize-soya. Therefore, more studies like this one are needed but including different crop rotations.

Residue removal is the common practice in Sub-Saharan Africa since there are competing uses such as animal feed or fuel (Giller et al., 2015). The results did not show any significant effect in the short-term of residue removal on soil organic C, hot-water extractable C, total N, N mineralization rates and particulate organic matter, which is in agreement with previous studies. Therefore, the use of other types of organic inputs under conservation farming should be investigated. In the case of pigeon pea biochar, we showed that its incorporation inside permanent basins under conservation was effective in increasing amounts of SOC as compared to CF-NORM and conventional tillage. However, biochar addition did not affect crop biomass, labile soil C and N mineralization rate.

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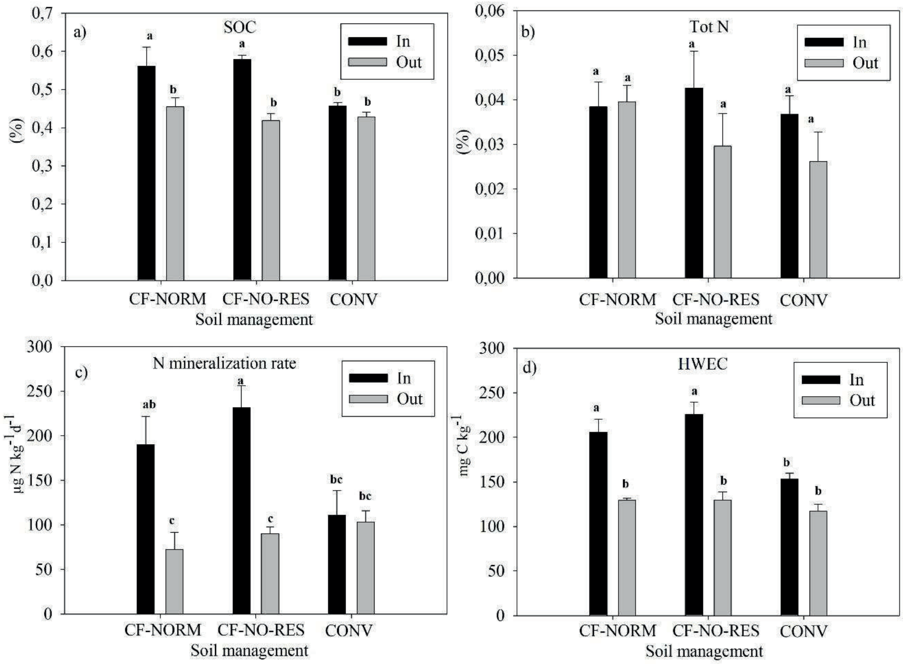

Fig. 1 (a) Soil organic C, (b) total N, (c) N mineralization rate and (d) HWEC inside and outside basins under CF-NORM and CF-NO-RES and inside and outside planting rows in CONV in 2018 at 0 to 20 cm depth. The vales are depth weighted averages of the values from 0 to 8 cm and 8 to 20 cm. Error bars represent standard error (n=4). Lower case letters indicate significant differences ($p<0.05$) between soil management practices inside and outside basins (or inside and outside rows of plants under CONV).

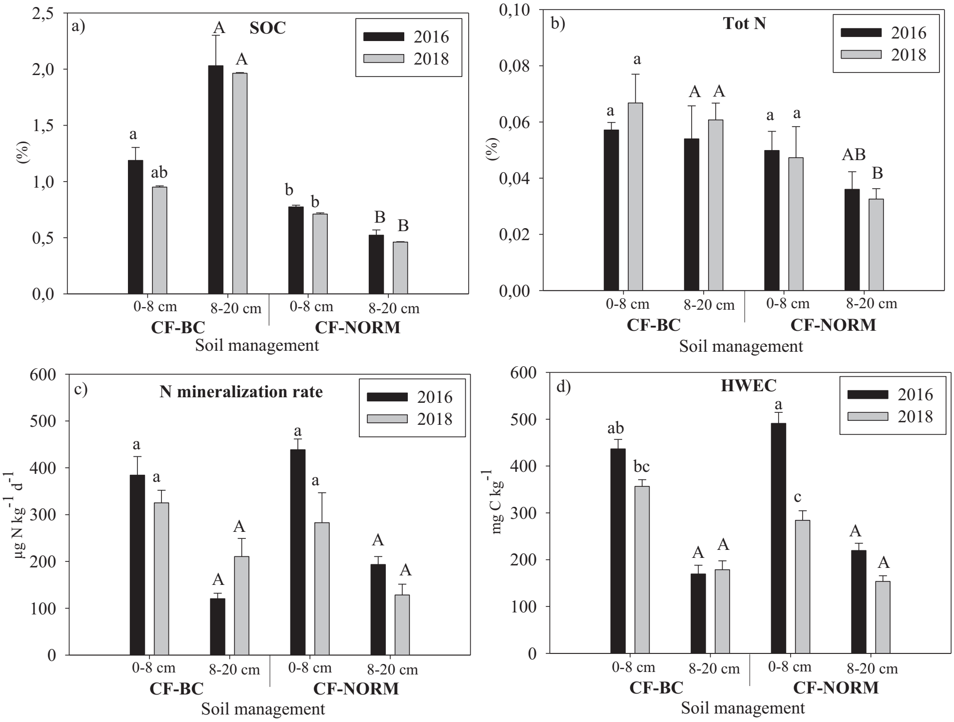

Fig 2. (a) SOC, (b) total N, (c) N mineralization rate and (d) HWEC inside planting basins in CF-NORM and CF-BC at 0 to 8 cm and 8 to 20 cm depth in 2016 and 2018. Lower case letters denote differences between treatments in 2016 and 2018 at 0 to 8 cm. Upper case letters show differences between treatments in 2016 and 2018 at 8 to 20 cm.

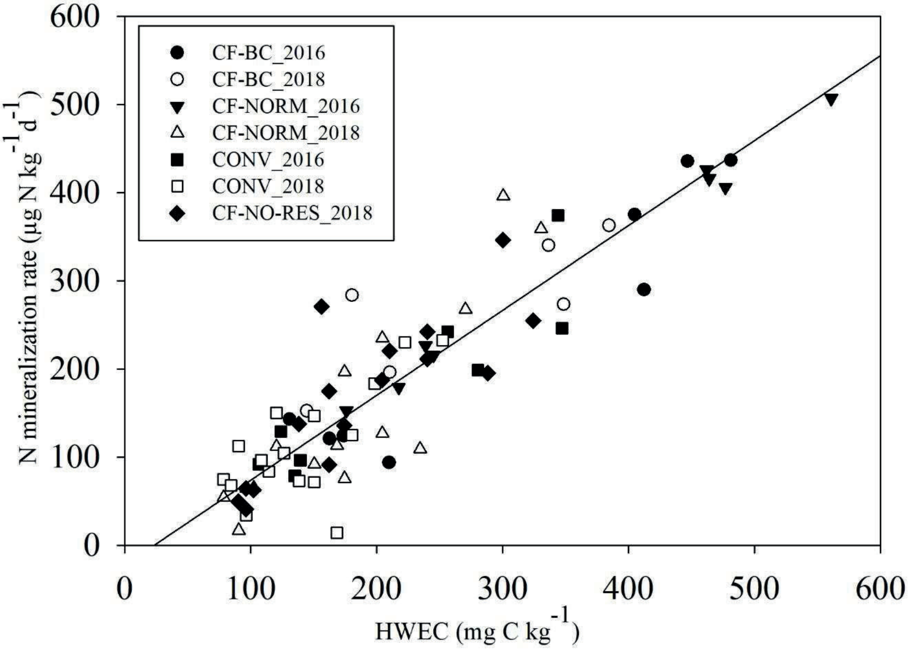

Fig. 3. Relationship between HWEC and potential N mineralization rates in CF-BC, CF-NORM, CF-NO-RES and CONV in the sampling campaigns of 2016 and 2018 [$R^2 = 0.82$; N min rate (μg-N kg^{-1}d^{-1}) ~ 0.9622 x HWEC (mg C Kg^{-1}) – 22.359].

Tab. 1. Grain yield and stover production in the growing seasons 2015-16, 2016-17, 2017-18 in Conservation farming (CF) with biochar (CF-BC), normal CF (CF-NORM), CF with no residue (CF-NO-RES) and conventional (CONV). Standard error of the mean (s.e), n = 4. For root dimension n=12.

Growing season		**CF-BC**	**CF-NORM**	**CF-NO-RES**	**CONV**
2015-16 Maize	Grain (ton ha^{-1})	5.0^{a}	5.2^{a}	6.1^{a}	4.7^{a}
	s.e	*0.4*	*0.6*	*0.4*	*0.4*
	Stover (ton ha^{-1})	4.9^{a}	4.4^{a}	5.3^{a}	3.7^{a}
	s.e	*0.3*	*0.1*	*0.7*	*0.4*
2016-17 Soya	Grain (ton ha^{-1})	3.0^{a}	3.4^{a}	3.6^{a}	2.1^{b}
	s.e	*0.2*	*0.3*	*0.2*	*0.3*
	Stover (ton ha^{-1})	2.5^{a}	2.9^{a}	3.1^{a}	1.9^{b}
	s.e	*0.3*	*0.3*	*0.2*	*0.2*
2017-18 Maize	Grain (ton ha^{-1})	3.1^{a}	3.5^{a}	3.6^{a}	2.2^{a}
	s.e	*0.2*	*0.3*	*0.3*	*0.5*
	Stover (ton ha^{-1})	5.7^{a}	4.9^{a}	5.7^{a}	4.7^{a}
	s.e	*0.4*	*0.1*	*0.5*	*0.5*
	Root:shoot	0.49^{a}	0.38^{a}	-	0.26^{a}
	s.e	*0.21*	*0.10*	-	*0.04*
	*Root biomass (ton ha^{-1}; calculated)	4.3^{a}	3.5^{a}	-	1.9^{a}
	s.e	*1.9*	*(1.0)*	-	*(0.5)*
Root dimension	Max depth (cm)	22.9	23.1	-	-
	s.e	*0.6*	*0.4*	-	-
	Max width (cm)	19.1	18.4	-	-
	s.e	*1.1*	*0.8*	-	-

*Root biomass was estimated only in 2017-18 in maize by multiplying root to shoot ratios and total biomass (grain + stover).

Table 2. Particulate organic matter in the bulk soil, contribution of particulate organic C (POC) to SOC, contribution of particulate organic N (PON) to total N and CN ratio in POM in CF-NORM and CF-NO-RES inside and outside basins at 0 to 8 cm and 8 to 20 cm in 2018. Lowercase letters denote differences in POM and quality of POM between inside and outside basins independent of the soil management treatment.

	POM in bulk soil (%)		**POC to SOC (%)**		**PON to Tot N (%)**		**C:N in POM**		**C:N in bulk soil**	
	CF-NO-RES	CF-NORM	CF-NO-RES	CF-NORM	CF-NO-RES	CF-NORM	CF-NO-RES	CF-NORM	CF-NO-RES	CF-NORM
Inside	a		a		a		b		a	
0-8 cm	0.82±0.02	0.81±0.13	30.8±0.8	33.2±3.9	31.8±11.2	33.9±10.4	16±0.6	17.3±0.5	15.8±4.6	17.0±3.4
8-20 cm	0.43±0.03	0.44±0.06	21.5±1.2	21.9±2.3	17.6±5.2	15.0±2.2	20.5±0.4	21.4±1.7	17.2±5.6	14.5±1.4
Outside	b		b		b		a		a	
0-8 cm	0.40±0.03	0.44±0.03	23.7±0.7	22.8±2.7	19.6±3.7	13.2±2.7	20.7±0.8	19.7±0.7	17.3±3.7	11.3±1.4
8-20 cm	0.20±0.02	0.26±0.02	13.9±0.5	18.3±1.6	10.2±5.1	6.0±2.0	30.8±6.4	45.8±5.7	18.9±7.0	13.5±2.6

Tab. 3. Carbon and Nitrogen stocks per hectare in the upper 20 cm in CF-NORM, CF-NO-RES, CONV and CF-BC in 2018. C and N stocks inside basins were compared between Conservation Farming (CF) systems only. In the three CF treatments, area dedicated to planting basins was 9.7% of the field whereas outside basins was 90.3%. The comparison of total C and N stock included CF systems as well as CONV. C and N stocks outside basins in CF-BC were assumed the same as in CF-NORM. Lower case letters indicate significant differences in C and N stocks between treatments, comparing either inside, outside basins or the total C stock per hectare.

		Soil management practices			
		CF-BC	**CF-NORM**	**CF-NO-RES**	**CONV**
C stock	*Inside*	3.5 (0.32)^{a}	1.5 (0.13)^{b}	1.5 (0.03)^{b}	-
(ton C ha^{-1})	*Outside*		11.2 (0.60)^{a}	11.0 (0.48)^{a}	-
	Total	14.1 (0.3)^{a}	12.7 (0.6)^{ab}	12.5 (0.5)^{ab}	11.4 (0.3)^{b}
N stock	*Inside*	143 (17)^{a}	100 (14)^{a}	111 (21)^{a}	-
(kg N ha^{-1})	*Outside*		976 (92)^{a}	779 (192)^{a}	-
	Total	1029(13.3)^{a}	1076 (93)^{a}	890 (205)^{a}	739 (159)^{a}

Effect of conservation farming and biochar addition on SOC quality, N mineralization and crop productivity in a light-textured Acrisol in the sub-humid tropics

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Supplementary Material

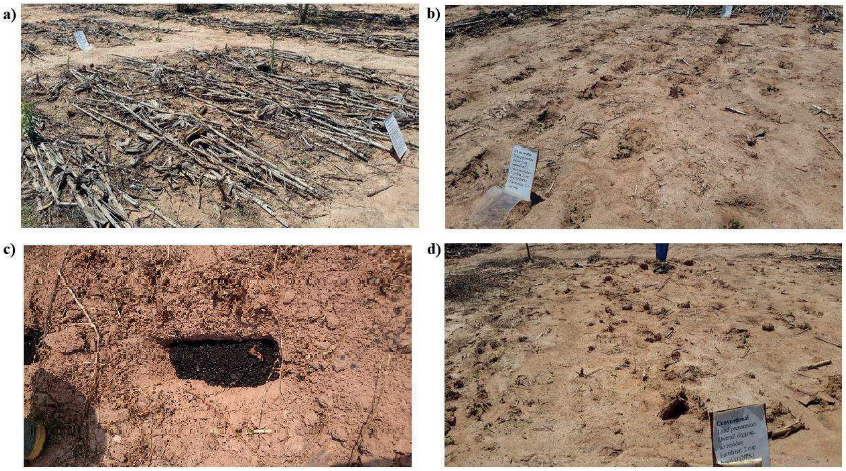

Fig. S1. Soil regimes: a) CF-NORM: residue retention, permanent basins, b) CF-NO-RES: no crop residues without residue retention, c) CF-BC: addition of pigeon pea biochar inside basins and d) CONV: full tillage at a depth of 20 cm and no residue retention.

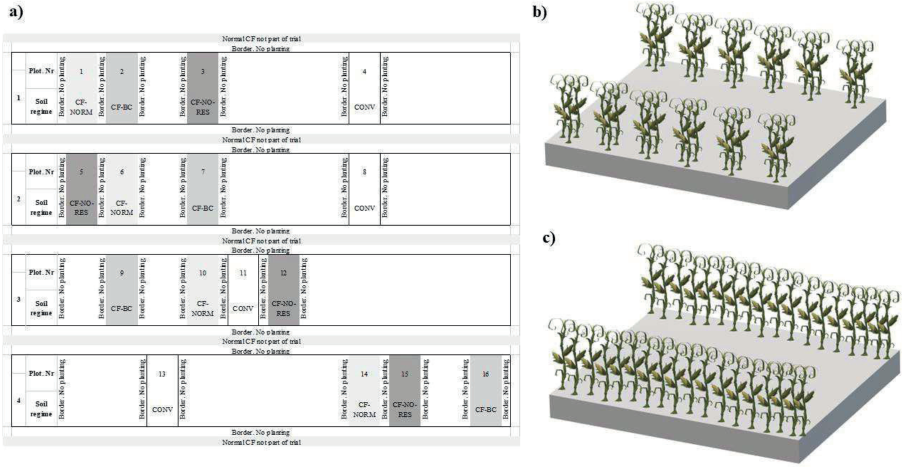

Fig. S2. a) Experimental setup. The four soil regimes were randomly distributed in 4 blocks. Each plot was about 20 m^2 and consisted of 4 rows of six basins in all the Conservation Farming (CF) treatments and 4 rows of plants in conventional (CONV) plots. b) CF plots. Each row consisted of 6 planting basins with 3 plants of maize. c) CONV plots. Each row had 18 plants.

Tab. S1. Soil pH inside and outside basins in 2016 and 2018.

Soil pH	**2016**	**2018**
Inside basins		
CF-BC	6.5 (0.10)	4.5 (0.04)
CF-NO-RES	-	4.8 (0.13)
CF-NORM	6.4 (0.10)	4.5 (0.12)
CONV	5.5 (0.12)	4.6 (0.04)
Outside basins		
CF-NO-RES	-	4.9 (0.32)
CF-NORM	-	4.5 (0.14)
CONV	-	4.9 (0.12)

Paper IV

N partitioning, N_2O fluxes and *in situ* gross nitrification in a rain-fed tropical biochar-amended Arenosol planted to maize – effect of soil management

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Key words: ^{15}N, pool dilution, biochar, maize, smallholder farm, conservation agriculture, N_2O, Sub-Saharan Africa; Arenosol

Abstract

Studies aiming at understanding the mechanisms behind N_2O emissions under conservation farming (CF) with biochar amendment are lacking. The objectives of this research are to elucidate the factors controlling N_2O emissions that are affected by CF and biochar addition and to assess the partitioning of ammonium nitrate (NH_4NO_3) and urea between maize plants and soil. We measured N_2O fluxes, mineral N, soil pH and soil moisture and we added and traced a small dose of ^{15}N in the plant-soil system and in the extractable nitrate (NO_3^-) in an Arensol in western Zambia. We compared three different soil management regimes: CF, using planting basins (CF-NORM), CF, where biochar had been added to planting basins at a rate of 4 ton ha^{-1} (CF-BC) and conventional tillage (CONV). Seven weeks after maize planting, plots were labelled with either nitrate ($^{15}NO_3^-$), ammonium ($^{15}NH_4^+$) or urea (^{15}N-Urea) and traced during ten days. Results showed that N_2O emissions were explained best by the NO_3^- concentration in soil. Literature suggests that N_2O emissions may be modified by soil pH, however, none of the treatments (including biochar) had an effect on it. Biochar did not have any impact on N_2O emissions, although biochar increased amounts of extractable NO_3^- and gross nitrification rates. Our results show correlation between gross nitrification rates and N_2O fluxes, which suggests that nitrification is an important source of N_2O in this soil. Nitrous oxide flux and mineral N were lower in CONV than in CF treatments. The reason for lower mineral N is likely the different methods of fertilizer application since in CF the amount of fertilizer for 3 plants was concentrated inside basins, while in CONV every plant along the rows received a dash of fertilizer, yet the amount per hectare was the same. There was a clear effect of the form of ^{15}N added on the total recovery of ^{15}N in the plant-soil system, being higher when $^{15}NH_4^+$ (86%) was applied as compared to $^{15}NO_3^-$ (38%).This indicates large losses of NO_3^- under field conditions, likely due to leaching. The total recovery was dominated by ^{15}N in soil and maize uptake of ^{15}N was higher when applied as NO_3NH_4 than as Urea. Biochar did not affect the recovery of ^{15}N in the plant-soil system.

Introduction

Nitrous oxide (N_2O) is a non-CO_2 greenhouse gas produced in agricultural soils, which has a high warming potential despite its low concentration in the atmosphere (Montzka et al., 2011). The concentration of N_2O in the atmosphere has been raising due to the extensive use of fertilizer Nitrogen (N) worldwide (Davidson and Kanter, 2014; Montzka et al., 2011). Measured N_2O emissions from soils in sub-Saharan Africa (SSA) are scarce (Wang et al., 2018) and N_2O flux estimates are generally based on the Intergovernmental Panel on Climate Change's (IPCC) Tier 1 approach (Hickman et al., 2011), assuming that 1% of added N is emitted as N_2O-N. Nitrous oxide emissions in SSA are expected to increase due to the increasing N inputs needed to meet the growing food demand. For this reason, it is necessary to evaluate management options for N_2O mitigation in crop systems in SSA.

Nitrous oxide is produced during nitrification, i.e. the microbial oxidation of ammonia (NH_3) to nitrite (NO_2^-) and NO_3^- under aerobic conditions (Butterbach-Bahl et al., 2013; Linn and Doran, 1984). In addition, N_2O is produced by denitrification, i.e. the microbial reduction of NO_3^- to N_2 via the gaseous intermediates NO and N_2O under anaerobic conditions (Zumft, 1997). Both processes are driven by soil micro-organisms viz. bacteria, archaea and fungi. The N_2O product ratio of denitrification, $N_2O/(N_2+N_2O)$, increases with decreasing soil pH (Bakken et al., 2012; Wang et al., 2018), whereas the N_2O yield of nitrification, $N_2O/(NO_2^- + NO_3^-)$ is believed to be unaffected by pH within an agronomically relevant pH range (Mørkved et al., 2007). This notion was recently challenged by Hink et al. (2017), who reported inherently lower N_2O yields of ammonia oxidizing archaea (AOA), which outnumber ammonia oxidizing bacteria (AOB) at low pH (Nicol et al., 2008). Hence, increasing the soil pH by liming or biochar addition may favor AOB over AOA, resulting in higher N_2O yields of nitrification.

Conservation farming (CF) has been suggested as one promising technology for agriculture in SSA (Farooq and Siddique, 2015; Thierfelder et al., 2017; Thierfelder et al., 2015). CF involves three principles: i) minimum tillage, ii) crop residue retention and iii) crop rotation. CF may increase crop resilience to climate change by improving water infiltration, water-holding capacity of soils and yields. In some cases, CF may mitigate climate change by restoring soil organic carbon stocks and thus, offsetting CO_2 production from other sectors (Powlson et al., 2016; Swanepoel et al., 2018; Thierfelder et al., 2017; Thierfelder et al., 2015). However, less is known about the effect of the principles of conservation farming on N_2O emissions worldwide and particularly in SSA (Thierfelder et al., 2017). There are concerns about higher N_2O emissions in CF systems in SSA, since CF may increase soil moisture retention (Grandy et al., 2006; Thierfelder et al., 2017) and conditions for denitrification may be favored.

The use of biochar as soil amendment has been suggested as an alternative to mitigate N_2O emissions in agriculture (Borchard et al., 2019; Cayuela et al., 2014). Biochar is charred organic material derived from agricultural residues in a process known as pyrolysis. Biochar is a complex material that comprises a porous carbonaceous matrix mixed with ashes, which are responsible for biochar's alkalinity. Biochar may increase soil pH due to its alkalinity and might therefore reduce N_2O emissions by decreasing the product ratio of denitrification (Cayuela et al., 2014; Obia et al., 2015). Biochar has been shown to increase nitrification and denitrification upon ageing (Duan et al., 2018) and to increase the amounts of extractable NO_3^- and NH_4^+ (Borchard et al., 2019; Clough et al., 2013) but it is not clear how this may affect N_2O emissions. A mechanistic understanding of the reductions of N_2O, including the role of biochar-N-soil interactions (Clough et al., 2013) and the presence of plants, is needed. To our knowledge, there is no study on effect of biochar on N_2O emissions in conservation farming systems in SSA.

In-situ application of ^{15}N tracers is a classical tool to study the fate of N fertilizers, and the partitioning of N between plants and soils in agroecosystems (Gardner and Drinkwater, 2009; Recous et al., 1988; Recous et al., 1992). Furthermore, gross rates of nitrification can be estimated (Rutting et al., 2011; Rütting et al., 2015) based on ^{15}N pool dilution (Kirkham and Bartholomew, 1954). So far, the majority of *in-situ* ^{15}N studies has been conducted in the temperate zone while only few studies have been reported for tropical regions (Gardner and Drinkwater, 2009; Nyamangara et al., 2003; van der Kruijs et al., 1988).

By combining ^{15}N tracer with measurements of N_2O fluxes, soil extractable mineral N, soil pH and soil moisture, we aimed at i) elucidating the factors controlling N_2O emissions such as mineral N content, soil moisture, soil pH and at ii) studying the partitioning of N, applied as NO_3^-, NH_4^+ or urea, between soil and maize plants as affected by soil tillage and biochar addition. For this, we applied small doses of highly (99 atom%) ^{15}N-enriched NO_3, NH_4 and urea to different management systems seven weeks after fertilization and traced this N during 10 days through various pools. ^{15}N pool dilution was used to estimate *in-situ* nitrification rates. In addition, we assessed the effect of biochar addition and tillage form on maize yield.

Materials and Methods

Experimental site and design

The ^{15}N labeling experiment was conducted on a private farm in western Zambia (coordinates S14 51.004 E24 49.148) in January 2017. Average annual precipitation and temperature are 930 mm and 20.8 ˚C, respectively (Obia et al., 2016). The soil is an Arenosol (WRB, 2015) containing 89% sand, 3.5% clay and 7.5% silt.

Land use prior to the experiment was four years of conservation farming, comprising planting basins, crop residue retention between rows and crop rotation (maize-groundnuts). Maize (*Zea mays*, PAN 53, Pannar) was planted on December 1, 2016 under three different soil management practices (Fig. S1) distributed in three blocks with three plots each (Fig. 1). The three experimental blocks were surrounded by maize of the same variety planted in basins. Each plot of the experimental blocks was about 20 m^2, accommodating seven planting rows. Each row had twelve planting stations (one maize plant per station in CONV) or four planting basins with three maize plants per basin, either with biochar mixed into the basin (CF-BC) or without biochar (CF-NORM). In CONV, rows were 90 cm apart and the distance between plants was 21 cm. The distance between basins (app. 20 x 30 x 20 cm) was 70 cm and between rows 90 cm in CF-NORM and CF-BC.

The CF-NORM plots received 16±1.5 g of NPK (10-20-10) per basin at planting (250 kg NPK fertilizer ha^{-1}). Top dressing with urea, common N fertilizer in SSA (Masso et al. (2017), was applied on January 27, 2017 (92 kg urea-N ha^{-1}). All CF-BC plots received 250 g pigeon pea biochar per basin (4 ton t/ha), placed at a depth between 5 and 20 cm before planting and filling the basin with soil. Fertilizer rates were the same as for CF-NORM. Conventional farming

(CONV) had overall digging and no residue retention. NPK (64±6 g per row, which is 250 kg/ha) was applied at the surface after germination. Top dressing with urea was applied 8 weeks after planting (92 kg urea-N ha^{-1}). Further details can be found in Fig. 1 b and c.

Biochar

Biochar was prepared from pigeon pea (*Cajanus cajan*) stems in a Kon-Tiki kiln (Cornelissen et al., 2016) at 600 ˚C. The chemical characteristics of the biochar, analyzed according to (Munera-Echeverri et al., 2018), include pH (10.4), Electrical Conductivity (1.4 mS cm^{-1}), acid neutralizing capacity (ANC_{pH7}; 49 $cmol_{(+)}kg^{-1}$), total organic carbon (56.1%), Total N (0.69%), total H (1.1%), and CEC (6.6 $cmol_{(+)}$ kg^{-1}).

^{15}N application

The ^{15}N labeling experiment started 7 weeks after planting, on 16/01/17 and the sampling campaign lasted for 10 days. The experimental setup was a split-plot design, with each plot divided into 4 split-plots that corresponded to the 3 forms of ^{15}N application and the unlabeled (water addition only) reference (Fig. 1 a). Three different ^{15}N forms were tested, $NH_4{}^{15}NO_3$, $^{15}NH_4NO_3$, ^{15}N-Urea. In all nine whole plots, each of the three ^{15}N tracers (99.98 atom%) and the reference (H_2O) were assigned to one row (Fig. 1 a), while one buffer row of maize was kept in between labelled rows to avoid cross contamination (Fig. 1). At all CF-NORM and CF-BC plots, two basins were selected for ^{15}N application (i.e. six plants in total) in each of the rows treated with $NH_4{}^{15}NO_3$, $^{15}NH_4NO_3$, ^{15}N-Urea and water, respectively. In the CONV plots (Fig. 1), two groups of three neighboring plants (at 21 cm distance between plants) were treated, each in a 10 cm x 60 cm planting row (viz. having a similar surface area as one basin; 30cm x 20cm). The total amount of ^{15}N added per basin (CF-NORM and CF-BC) and per 10 cm x 60 cm planting row (CONV) was 6.0 mg ^{15}N. This is equivalent to 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). Addition of ^{15}N was done in solution, after its dissolution in distilled water (concentration of 24.0 mg ^{15}N L^{-1}). Hand sprayers were used to add 250 ml of the labelled solutions (water at the reference) evenly to the soil surface (Fig. S2 a). This volume was equivalent to about 4 mm per basin (or planting row in case of CONV). Subsequently, 15 mm of clean water was added to wash the label into the soil. The reference received and equivalent volume (19 mm water).

Soil, gas and plant sampling and analyses

Soil samples were taken 1.5, 24, 72 and 240 hours after ^{15}N addition from 0-5 cm (n=144) and 5-20 cm (n=144). At each sampling, a bulked sample from each of the two labeled basins (CF-NORM and CF-BC) and planting rows (CONV) was taken. Bulked soil samples consisted of seven (0-5 cm) or three (5-20 cm) cores taken with an 8 mm diameter auger. 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. Dry soil samples were sieved (2 mm), milled and packed into 8 x 5mm tin capsules and shipped to the Stable Isotope Facility, University of California, Davis for ^{15}N, total N and total C analysis using isotope ratio mass spectrometry (IRMS). The amount of dry soil in each bulked soil sample and the volume of the auger were used to estimate an average bulk density (BD) for each split-plot at 0 to 5 cm and 5 to 20 cm depth.

The KCl extracts were prepared by adding 11 g of field moist soil and 40 ml of 1 M KCl to 50 ml centrifuge tubes, shaken horizontally at 200 strokes per minute for one hour and filtered using Whatman filters grade 589/3. 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 with 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]).

Plant samples were taken after 10 days by collecting the aboveground biomass and digging out the entire root system of the three plants of one of the labeled basins under CF-NORM and CF-BC, respectively, and one of the labelled groups of plants (i.e. the three plants) under CONV (n=9). The roots were washed in the field (Fig. S2 d), the plants cut at brace roots height and split into roots, stems and leaves and the fresh biomass recorded. The 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 and weighed in tin capsules for ^{15}N analysis at University of California, Davis. At the end of the growing season, maize yield, as well as total aboveground biomass were measured for each of the plots.

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 under CF-NORM and CF-BC; for the CONV treatment the chamber was placed in the proximity of the brace roots, which is the zone where fertilizer and ^{15}N were applied. 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 butyl septa (Chromacol). Samples were taken 1, 15, 30 and 45 minutes after chamber deployment. Temperature inside the chambers was recorded at beginning and 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

The WFPS was calculated by converting gravimetric (ω) to volumetric (θ) moisture using soil bulk density (BD, g cm^{-3}) and soil particle density (PD; 2.65 g cm^{-3}):

$$\text{WFPS (\%)} = \theta / (1-BD/PD) \times 100\%$$

where $\theta = \omega * BD$ (L/L)

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

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.:

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

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 ($^{17}O/^{16}O$) = 3.8861×10^{-4}; ^{18}R ($^{18}O/^{17}O$) = 2.0947×10^{-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:

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

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

$$X_{sample} = (F_{sample} - F_{reference}) / (F_{tracer} - F_{reference})$$

Here, F_{sample} is the fractional abundance of ^{15}N in the samples ($^{15}N/(^{15}N + ^{14}N)$). $F_{reference}$ is the fractional abundance of ^{15}N in the reference treatments. F_{tracer} is the fractional abundance of applied tracer (0.9998, i.e. 99.98 atom%). $N_{content}$ 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 and KCl-extractable NO_3^- per unit area of basin (CF-NORM, CF-BC) or labelled planting row (CONV) (g m^{-2}). The ^{15}N recovered (%) was relative to the amount of ^{15}N applied (0.1g ^{15}N m^{-2} basin or planting row). The recovery of ^{15}N in the soil residual N was defined as the recovery of ^{15}N in the bulk soil minus the ^{15}N recovery in the KCl-NO_3^- pool. Note that soil residual ^{15}N comprises the ^{15}N in the NH_4^+ pool.

Gross nitrification

Rates of gross nitrification were estimated applying principals of ^{15}N pool dilution and NO_3^- mass balance (Kirkham and Bartholomew, 1954) from ^{15}N abundances in the $^{15}NO_3^-$ treatments under all three types of management.

Statistical analysis

Statistical analyses were performed using the package RcmdrPlugin.NMBU of R software (R-Core-Team, 2017). The effect of soil management on soil properties, biomass, grain yield (Tab. 1), N_2O flux and nitrification rates (Tab. 2) and effect of soil management and N form on ^{15}N recovery (Fig. 6) was tested by using linear mixed effect models with block as a random factor. Further linear mixed effect models were used to test differences between soil management practices, forms of ^{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 (Fig. 4), and atom% $^{15}N_{soil}$ and atom% $^{15}N_{NO3-}$ at 0 to 5 cm and 5 to 20 cm, respectively (Fig. 5). N form, nested in soil management practice, nested in block was used as random factor. The spatial autocorrelation between repeated measurements were assumed constant between the different treatment combinations. The parsimonious models were chosen using using Akaike information criterion (AIC) and R^2 values. The parameterization chosen for the linear mixed models was sum to zero. Differences between treatments were assessed by Tukey test at a level of significance of 0.05. A multiple regression model was set up to link N_2O fluxes with extractable NO_3^- logarithmic conversion of the data. Linear relationships between gross involving gross nitrification rate were assessed by ordinary linear regression at the plot level (i.e. across the different forms of added N) using 10-day averages.

Results

The total precipitation during the 10 days of the experiment was 116 mm, occurring mostly as nocturnal rainfall except for January 17 (24 hr after ^{15}N addition), when it rained during sampling. Gravimetric soil moisture was consistently and significantly higher in CF-BC (11.5 ± 0.2 %) than in CF-NORM (9.8 ± 0.2 %; $p<0.01$) and CONV (9.6 ± 0.2 %; $p<0.01$) (Fig. 2 a). Despite the higher gravimetric soil moisture in CF-BC, average WFPS was significantly lower (28.4%) than in CONV (30.3%; $p=0.013$) and CF-NORM (31.2%; $p<0.01$; Fig.2 b).

Soil properties and maize biomass

Between 5 and 20 cm soil depth, total organic C was significantly higher in CF-BC as compared to CF-NORM and CONV ($p< 0.05$; Tab. 1), due to the addition of biochar. By contrast, at 0 to 5cm depth total organic C was not significantly different between CF-NORM, CF-BC and CONV (Tab. S1). Soil pH did not change significantly upon biochar addition at CF-BC, as compared to CF-NORM and CONV. Similarly, the addition of biochar did not result in a significant increase in plant available phosphorus (P-AL) and exchangeable base cations between the three soil management practices from 0 to 5cm and from 5 to 20cm depth (Tab. S1).

At the end of the ^{15}N experiment, i.e. 10 days after its initiation and 8 weeks after planting, the maize plants were in the phenological stage 35-37 of the BBCH scale (Lancashire et al., 1991). At this point, there were no significant differences in root and aboveground biomass between the three soil management regimes when scaled per hectare (Tab. 1). Nitrogen content in maize plants eight weeks after planting was significantly lower in CONV as compared to CF-NORM and CF-BC. N content in maize compartments increased in the order roots > stems >leaves (Fig. S2). There was no significant differences in grain yield between the three soil management practices ($p>0.05$).

KCl-extractable mineral N in soil

The CF-BC and CF-NORM treatments had greater values of KCl-extractable NO_3^--N and NH_4^+-N in soil than CONV (Fig.3). In general, KCl-NH_4^+-N prevailed over NO_3^--N, irrespective of soil management. Immediately following N addition, total KCl-extractable NO_3^--N and NH_4^+-N in mg per basin (CF-BC and CF-NORM) or in mg per three maize plants (CONV) was up to 90 times larger than the added amount of labeled N (Fig. 3e). This indicates that the added amount of labeled N was minor compared to the already available KCl-extractable NO_3^--N and NH_4^+-N in soil. On average during the four sampling events, soil NO_3^- concentrations were significantly larger in CF-BC (3.2 mg kg^{-1}) than in CF-NORM (0.8 mg kg^{-1}; $p<0.05$) and CONV (0.3 mg kg^{-1}; $p<0.01$) with no significant difference between the latter two ($p = 0.58$). Highest NO_3^- concentrations were found in the $^{15}NH_4^+$ treatments of CF-BC mainly due to a peak after 72 h. Amounts of KCl-NH_4^+ were significantly larger for CF-NORM (9.2 mg kg^{-1}) as compared to CONV (1.2 mg kg^{-1}; $p = 0.02$), whereas there was no significant difference between the two latter and CF-BC (6.5 mg kg^{-1}; $p = 0.36$ and $p = 0.07$, respectively). There was a significant decrease in KCl-extractable NO_3^--N and NH_4^+-N with time ($p<0.01$).

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

Atom% $^{15}N_{soil}$ decreased significantly over time in 0 to 5 cm ($p<0.01$; Fig. 4, triangles), 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 the former. Atom% excess $^{15}N_{soil}$ was not by soil management ($p=0.466$). As expected, atom% $^{15}N_{NO3-}$ 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, circles). Thereafter it converged at both depths 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 irrespective of soil management practices, mainly at 0 to 5 cm, and it decrease to close to 0 atom% excess at 240 hours.

Gross nitrification

The abundance of ^{15}N in KCl-extractable NO_3^-, sampled at 24 and 72 hours after label application, were used to estimate gross nitrification rates. These time points were chosen as they best fulfilled the assumptions of the ^{15}N pool dilution technique (homogenous ^{15}N distribution, no or little re-mineralization of assimilated ^{15}N and uniform distribution within the soil profile; Davidson et al., 1991) . Gross nitrification rates were greater in CF-BC than in CONV (p=0.024) and CF-NORM (p=0.061; Tab. 2). Nitrification in CF-BC was significantly greater at 5 to 20 cm than at 0 to 5 cm (p = 0.037), whereas in CF-NORM and CONV there were no significant differences between both depths (p = 0.98 and p = 0.98, respectively). The results were more variable for the CF-BC treatment as compared with CF-NORM and CONV (Tab.3).

N_2O emissions

Average N_2O flux in CONV (8.4 µg N_2O-N m^{-2} hr^{-1}) was significantly lower than in CF-NORM (26.1 µg N_2O-N m^{-2} hr^{-1}; $p < 0.01$) and CF-BC (34.1 µg N_2O-N m^{-2} hr^{-1}; $p < 0.01$), whereas there was no differences between the latter two (p=0.20; Tab. 2). The fluxes were highly variable, ranging from 1.3 to 23 µg N_2O-N m^{-2} hr^{-1} in CONV, 1.0 to 160.6 µg N_2O-N m^{-2} hr^{-1} in CF-NORM and 0.3 to 156.6 µg N_2O-N m^{-2} hr^{-1} in CF-BC. There was no effect of the form of N added (NH_4^+-N, NO_3^--N or urea-N) on N_2O fluxes (p=0.235). We found that, after logarithmic transformations, N_2O fluxes were significantly explained by a positive relation with the logarithm of KCl-extractable NO_3^- ($p<0.001$, $R^2 = 0.20$; Fig. S3).

Average N_2O flux from per plot was positively correlated with gross nitrification rate ($p<0.01$, $R^2=0.78$; Fig. 5). The mean apparent N_2O yield (N_2O-N/gross nitrification) for all plots was 0.6%. Gross nitrification rates were positively correlated with i) the average KCl-extractable NH_4^+ (R=0.90; $p<0.01$), KCl-extractable NO_3^- ($R^2_{adj}=0.92$; $p<0.01$) in the $^{15}NO_3^-$ plots during the ten-day experiment. Multiple regression analysis showed that nitrification rates were best explained by KCl-NH_4^+ ($p<0.01$) and bulk density [$p<0.01$; $R^2_{adj}=0.97$; nitrification rate ~ 12.06949 + 0.21907(KCl-NH_4^+) – 8.65651(BD)].

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

The total ^{15}N recovery (soil, plants and NO_3^-) was dominated by residual soil ^{15}N (Fig. 6). The form of the added ^{15}N significantly affected ($p < 0.05$) both the recovery of ^{15}N in soil and the total recovery (Fig. 6). In contrast, there was no significant effect of soil management practice, neither on ^{15}N recovery in residual soil nor on total ^{15}N recovery. Total ^{15}N recovery and ^{15}N recovery in soil were significantly smaller if added as $^{15}NO_3^-$ than as $^{15}NH_4^+$ ($p<0.01$). The ^{15}N recovery in soil was lower if added as $^{15}NO_3^-$ than as either $^{15}NH_4^+$ or ^{15}N-Urea ($p < 0.05$; Fig 6). The low total ^{15}N recovery in the $^{15}NO_3^-$ treatments can be explained by high leaching rather than losses as N_2O. Even if 100% of the N_2O would be assumed to be ^{15}N (which is unlikely), the losses during the 10-day experiment would vary between 3.6 to 14% of the added ^{15}N.

^{15}N recovery in KCl-extractable NO_3^- was neither affected by soil management practice ($p=0.058$) nor by N application form ($p=0.077$), except for CF-BC, where KCl-extractable $^{15}NO_3^-$ was relatively large in response to the addition of $^{15}NH_4^+$ (Fig. 6a). The ^{15}N recovery in maize plants was significantly smaller in CONV than in CF-NORM ($p<0.01$) and CF-BC ($p=0.03$), whereas the difference between CF-NORM and CF-BC was not significant ($p = 0.83$). Significantly less ^{15}N was recovered in maize in response to ^{15}N-Urea than to $^{15}NO_3^-$ ($p<0.01$) and $^{15}NH_4^+$ ($p=0.02$). No significant difference in ^{15}N uptake was found in response to $^{15}NO_3^-$ and $^{15}NH_4^+$ addition ($p=0.22$). In maize plants, most ^{15}N was recovered in stems, whereas smaller fractions were recovered in roots and leaves (Tab. S2).

Discussion

We found no significant effect of management practice on maize yield (Tab. 1), which were substantially greater than the average yield of small-scale farmers in SSA, probably because the 2016-2017 season was relatively wet and the N fertilizer inputs were relatively high (117 kg N ha^{-1}). The commonly observed positive effect of biochar in combination with CF (viz. CF-BC) 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. Addition of biochar only increased soil organic C (Tab 1) and soil moisture (Fig. 2) during the ten-day experiment as compared to CF-NORM and CONV. The lower amount of mineral N in CONV may be explained by the method of application of NPK fertilizer in CF vs CONV. The amount of NPK per hectare was the same but the amount of fertilizer for 3 plants was concentrated inside basins in CF, whereas every plant along the rows received a dash of fertilizer in CONV. The lower availability of N in CONV may explain the lower N in maize plants in CONV 8 weeks after planting. Still, CONV did not have any effect on grain yield.

Nitrification upon biochar addition

Gross nitrification rates were in the range of what has been reported for agricultural soils in temperate regions (0.2 to 13.9 mg N kg^{-1} d^{-1}; Norton (2008). The nitrification rates in CONV and CF-NORM were low whereas in CF-BC were moderately high as compared to the values by Norton (2008). Nitrification rates were larger in CF-BC at 5 to 20 cm depth where most of the added biochar was. Unfortunately, we did not find values for agricultural soils in SSA for comparison. In a montane forest soil in Congo, gross nitrification was found to be 1.54±0.22 mg N kg^{-1} d^{-1} (Rütting et al. 2015).

The high correlation of gross nitrification rates with extractable NH_4^+ and the negative relation with BD, suggests that nitrification was substrate controlled and that lower BD enhanced nitrification most likely due to improved soil aeration. Nitrification rates explained the amounts of KCl-extractable NO_3^- in the experimental field. Increased nitrification upon biochar addition was the main reason for higher NO_3^- in CF-BC. In addition, the biochar we used is likely to have NO_3^- retention capacity since it has been suggested that biochars pyrolized at $\geq$ 600˚C (as the one we used) have the highest adsorption capacity (Clough et al., 2013; Yao et al., 2012). In this study, nitrification is likely to be the main source of N_2O as indicated by the correlation of N_2O fluxes and gross nitrification (Fig. 5). Furthermore, WFPS below 60% is favorable for nitrification (Linn and Doran, 1984) and in our experiment WFPS never exceeded 60%. The quick conversion of $^{15}NH_4^+$ and ^{15}N-Urea in to $^{15}NO_3^-$ just after 1.5 hr of tracer addition (Fig. 4) also indicates favorable nitrification.

N_2O fluxes

In general, the N_2O fluxes in our study are in the same range as compared with what has been reported in Africa in agricultural soils (Brummer et al., 2008; Kim et al., 2016; Rosenstock et al., 2016) and in other tropical regions (Bayer et al., 2015). The high variability in N_2O fluxes in our experiment is in agreement with what others have reported in SSA (Kim et al., 2016; Rosenstock et al., 2016). For example, Brummer et al. (2008) reported an average flux of 19 µg N m^{-2} h^{-1} with the addition of 52 Kg N ha^{-1} and a mean of 80 µg N m^{-2} h^{-1} 24 days after addition of 140 kg N ha^{-1} in croplands in Burkina Faso. Rosenstock et al. (2016) reported an average N_2O flux of 12 µg N m^{-2} h^{-1} in agricultural soils in Kenia and Tanzania and a wide range of values (from -2 to 300 µg N m^{-2} h^{-1}), depending on soil texture, farming systems and weather conditions. The latter study reported the highest N_2O flux in pasture land in the wet season (~60 µg N m^{-2} h^{-1}) and lower emissions in land dedicated to crops (~10 µg N m^{-2} h^{-1}).

When our N_2O fluxes are scaled-up (0.74 kg N ha^{-1} yr^{-1} in CONV and 3.0 kg N ha^{-1} yr^{-1} in CF-BC), they are ranked as low in CONV to medium in CF-NORM and CF-BC as compared to the values by Kim et al. (2016) in SSA (-0.06 kg N ha^{-1} yr^{-1} to 8.64 kg N ha^{-1} yr^{-1}). However, direct scaling up per hectare of the N_2O fluxes in CF systems most likely overestimates the emissions since the fertilizer was applied only inside basins and the area they occupy is roughly 10% (Martinsen et al., 2014). Therefore, flux outside basins is likely to be lower. In addition, as the precipitation pattern in Zambia is unimodal, emissions are likely to be negligible in the dry season when there are no rain-fed crops. If we assume the emissions outside basins in CF to be similar to the fluxes in CONV and a 6-month long rainy season (Libanda et al., 2015), N_2O emissions amount 0.46±0.22 kg N_2O-N ha^{-1} yr^{-1} in CF-BC, 0.45±0.22 mg N_2O-N ha^{-1} yr^{-1} in CF-NORM and 0.38±0.18 kg N_2O-N ha^{-1} h^{-1} in CONV. These N_2O emissions correspond to 0.32% to 0.39 % of the annual N input as fertilizer. Yield-scaled N_2O emissions correspond to 0.08 to 0.09 g N_2O-N kg^{-1} yield, which is low as compared to the values reviewed by Kim et al. (2016). Although N_2O fluxes were higher inside basins in CF systems as compared to CONV, our results do not suggest differences emissions (scaled-up per ha per year) between CONV and CF treatments. This is supported by Kimaro et al. (2016), who did not find differences in N_2O emissions (kg N_2O-N ha^{-1} yr^{-1}; measured at different intervals during 21 months) between reduced tillage with residue retention and conventional tillage planted to maize in a sandy loam in Tanzania. Likewise, Bayer et al. (2015) reported no effect of full and no tillage in N_2O emissions in maize crop in an Acrisol in Brazil.

Biochar is commonly advocated for N_2O mitigation (Borchard et al., 2019; Cayuela et al., 2014; Obia et al., 2015). However, in our experiment biochar addition did not decrease N_2O fluxes. The liming effect of pigeon pea biochar was null and therefore, we could not asses the importance of this mechanism for reduction of N_2O emissions. Biochar increased extractable NO_3^- and gross nitrification, without increasing N_2O. This suggests that N_2O is not produced from NO_3^- (denitrification) but from nitrification, in agreement with what was shown in Fig 5. The N_2O produced during nitrification is a byproduct of a chemical process, i.e. spontaneous oxidation of hydroxylamine (van Groenigen et al., 2015) and it has been shown that N_2O during nitrification is derived from NO_2^- rather than directly from NH_4^+(Yoshida, 1988). Therefore, the higher gross nitrification rates and the lack of effect on N_2O emissions upon biochar amendment make sense. Although, the logarithm of extractable NO_3^- was the best predictor of the N_2O fluxes, it does not necessarily implies that the source of N_2O is denitrification.

^{15}N recovery and partitioning between soil and maize

Different management practices (CF-BC, CF-NORM and CONV) had no impact on the total and soil ^{15}N recovery. However, the total and the soil ^{15}N recoveries were significantly affected by the form of ^{15}N added (Fig.6). Recovery of ^{15}N in the residual soil pool decreased in the order $^{15}NH_4^+$ and ^{15}N-Urea > $^{15}NO_3^-$ and total recovery in the order $^{15}NH_4^+$> $^{15}NO_3^-$, with no differences between the two latter and ^{15}N-Urea (Fig. 6). $^{15}NO_3^-$addition shows a large deficit in ^{15}N recovery possibly attributed to leaching of $^{15}NO_3^-$ from the soil (Fig. 4). N_2O emissions were small and are not likely to account for the ^{15}N losses. Most of the added NH_4^+ remained in soil after 10 days, either adsorbed in soil or taken up by microbes. Eventually, 3.4% of the added $^{15}NH_4^+$ was converted into NO_3^- after 10 days in the CF-BC but in CF-NORM and CONV this was less than 0.6% of the added $^{15}NH_4^+$ (Tab. S2). The results show that some urea can be converted into NO_3^- (Fig 4). However, we did not assess other mechanisms of urea losses, which may be more important, such as hydrolysis and ammonia volatilization. Our results show that urea was less available for maize than NO_3^- and NH_4^+ (Fig 6; Tab S2).There was no difference in ^{15}N recovery in maize plants between the addition of $^{15}NO_3^-$ and $^{15}NH_4^+$. It is worth stressing the large differences in ^{15}N losses between the addition of $^{15}NO_3^-$ and $^{15}NH_4^+$ and the similarity in ^{15}N uptake by maize.

Conclusions

This study showed that the N_2O fluxes were explained best by soil extractable NO_3^-. Nitrous oxide flux and NO_3^- were lower in conventional than in CF treatments, probably due to the different methods of fertilizer application rather than to differences in soil moisture. The amount of fertilizer for 3 plants was concentrated inside basins in CF, whereas every plant along the rows received a dash of fertilizer in conventional. Although biochar increased amounts of extractable NO_3^- and gross nitrification rates, it did not have any impact on N_2O emissions. The effect of the alkalinity of biochar on N_2O emissions could not be assessed since biochar did not affect soil pH. The results show correlation between gross nitrification rates and N_2O fluxes, which suggests that nitrification is an important source of N_2O in this soil. The water-filled pore space values during the sampling campaign were below 60% which is a moisture content favorable for nitrification rather than denitrification. This could explain the why biochar did not increase N_2O emissions, despite increasing gross nitrification, extractable NO_3^- and soil moisture. The dynamics of mineral N in response to CF and conventional practices needs further investigation, since mineral N is an important factor explaining N_2O emissions.

There was a clear effect of the form of ^{15}N added on the total recovery of ^{15}N in the plant-soil system, being higher when $^{15}NH_4^+$ (86%) was applied as compared to $^{15}NO_3^-$ (38%). The total recovery was dominated by ^{15}N in soil and maize uptake of ^{15}N was higher when applied as NO_3NH_4 than as Urea. Biochar did not affect the recovery of ^{15}N in the plant-soil system, although it increased nitrification. ^{15}N recovery in plants in CONV was lower than in CF-BC and CF-NORM most likely due to lower availability of mineral N.

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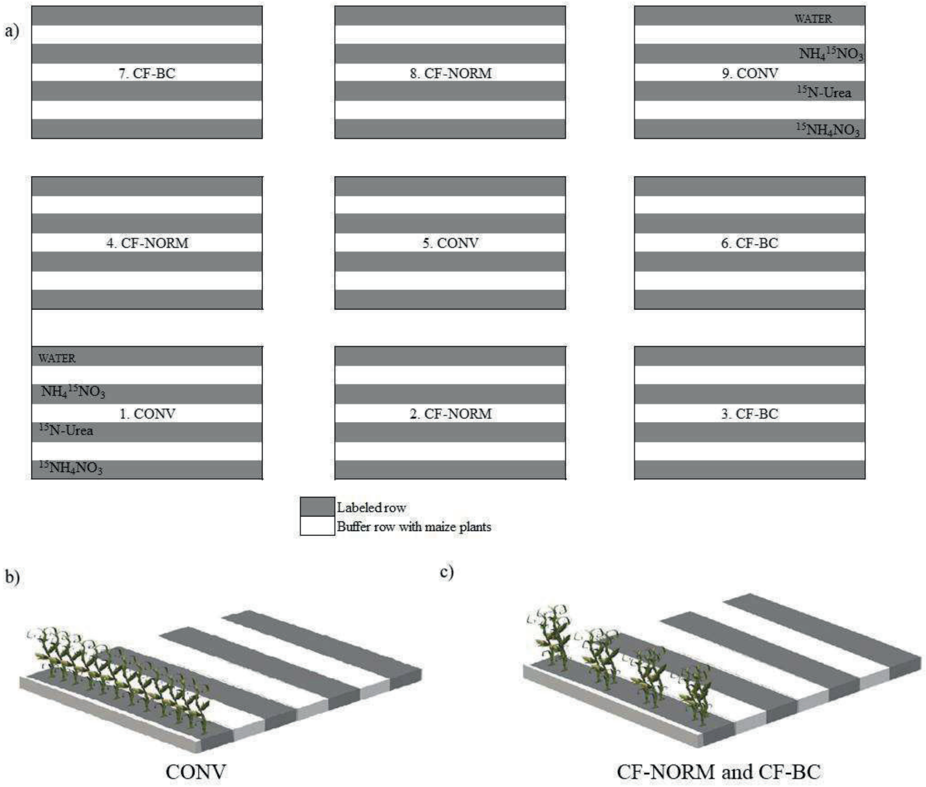

Fig. 1. a) Overall experimental set-up and detailed planting schemes for b) conventional (CONV) and c) conservation farming with (CF-BC) or without biochar (CF-NORM). CONV had 12 equidistant (21 cm) maize plants per row while CF-BC and CF-NORM had four planting basins per row with 3 maize plants per basin. Each plot had 7 rows.

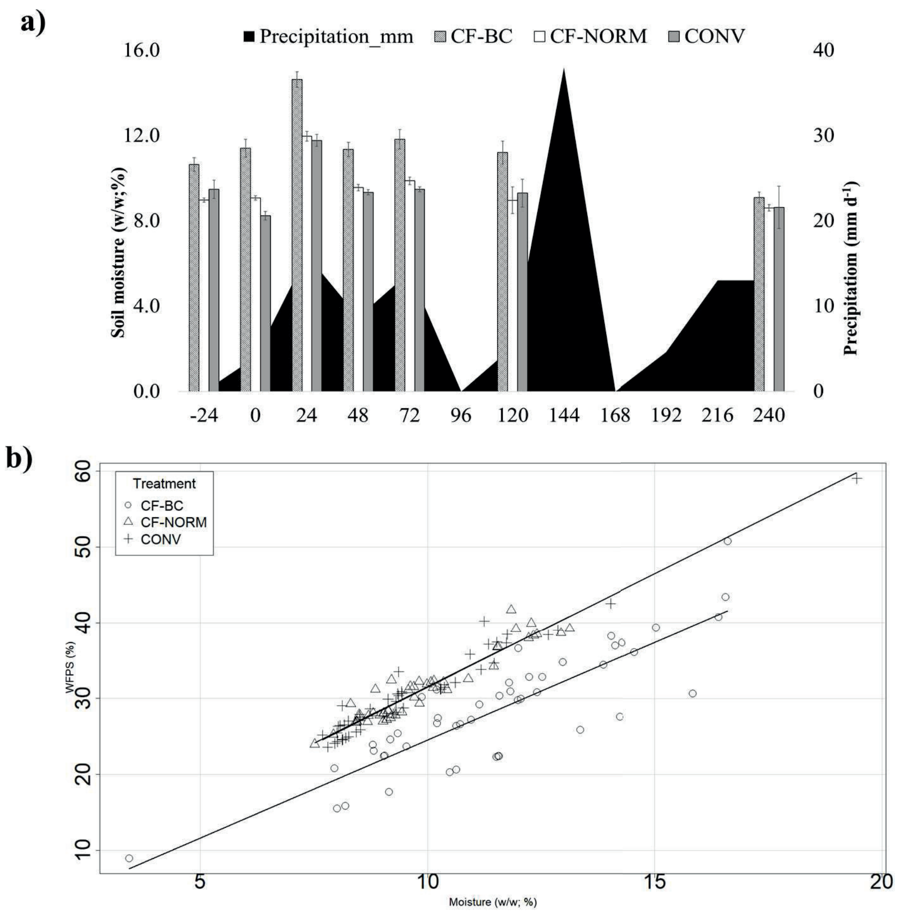

Fig. 2. a) Precipitation and gravimetric soil moisture content (w/w) during the ^{15}N labelling experiment as affected by soil management practice. Time is in hours before (negative) or after (positive) ^{15}N addition. Error bars represent standard error (n=12). b) Relationship between WFPS and soil moisture content. Regression lines for CF-NORM and CONV overlap [(CONV) $y = 1.4212x + 0.18888$; (CF-NORM) $y = 1.44x + 0.0027$; (CF-BC) $y = 1.3142x-0.4782$].

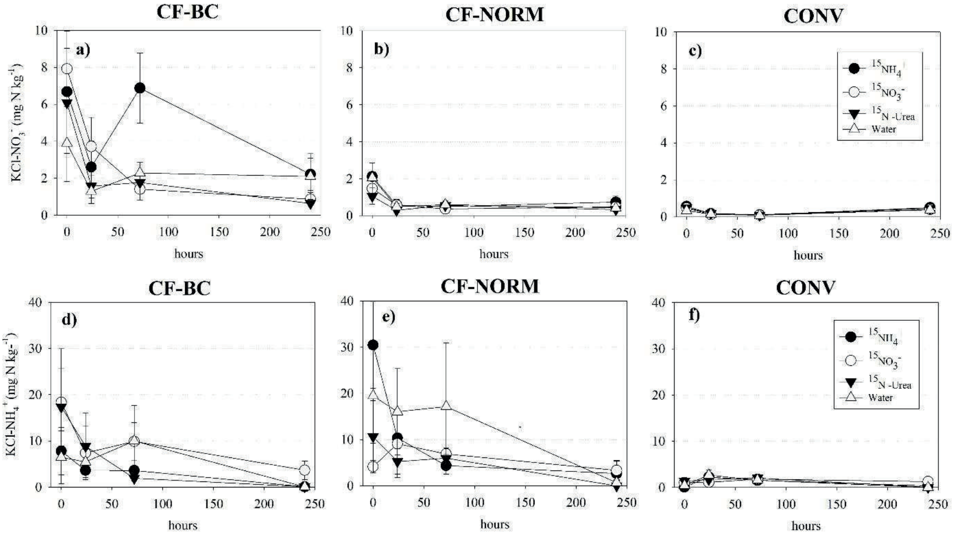

Fig. 3. Weighted average (0-20 cm) of KCl-NO_3^- (mg N kg^{-1}; a, b and c), NH_4^+ (mg N kg^{-1}; d, e and f) and N_2O fluxes (µg N m^{-2} h^{-1}; g, h and i) in CF-BC, CF-NORM and CONV in response to the addition of $^{15}NH_4^+$, $^{15}NO_3^-$, ^{15}N-Urea or water during the 240-hour labeling experiment. The initial observation of KCl-extractable N was done 1.5 hours after ^{15}N application. Values are means and standard errors (n=3).

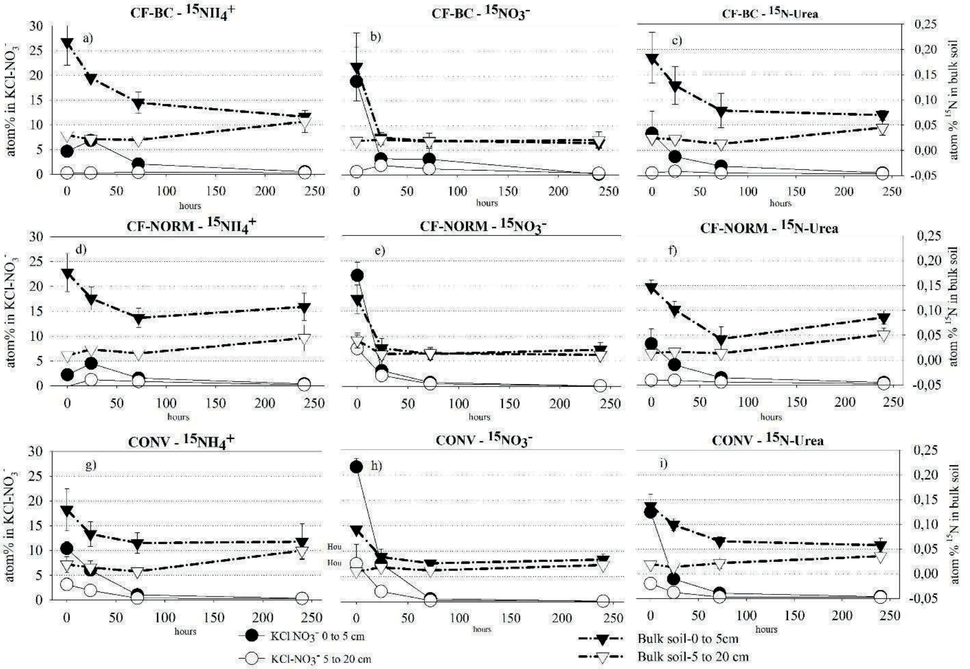

Fig. 4. Atom% excess $^{15}NO_3^-$ (circles) and atom%$^{15}N_{soil}$ (triangles) in CF-BC (a to c), CF-NORM (d to f) and CONV (g to i) at 0 to 5 cm (black) and 5 to 20 cm (white) depth in response to the addition of $^{15}NH_4^+$, $^{15}NO_3^-$ and ^{15}N-Urea. Values are means and standard errors (n = 3).

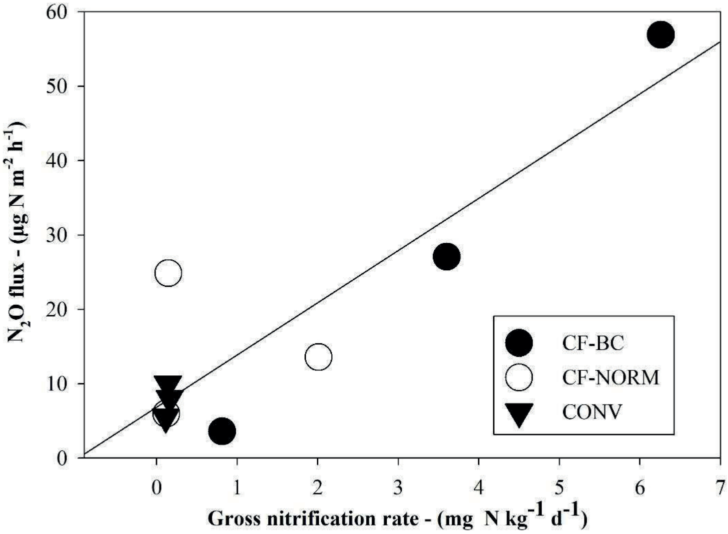

Fig. 5. Relationship between N_2O fluxes in the $^{15}NO_3^-$ treatments and gross nitrification rates based on ^{15}N pool dilution (depth-weighted 0 – 20 cm). Nitrification estimates are based on mass balance and change in atom $^{15}N_{NO3}$ from 24 hours to 72 hours (R^2=0.78; N_2O flux ~ 6.855 + 7.021* gross nitrification rate).

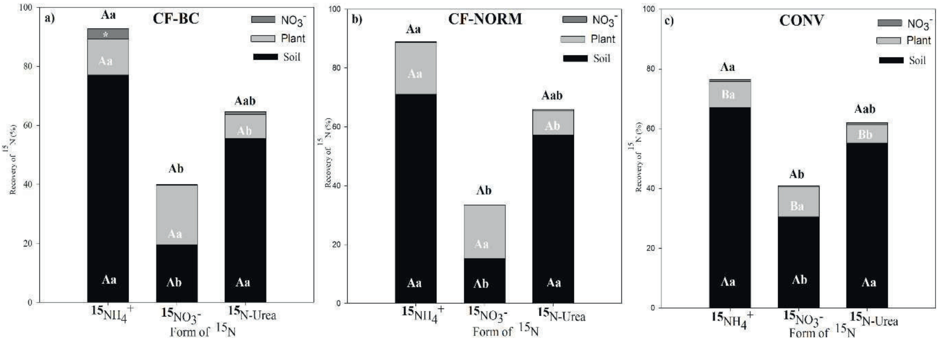

Fig. 6. ^{15}N recovery (%) in KCl-extractable NO_3^-, in residual soil from 0 to 20 cm and in maize plants in a) CF-BC, b) CF-NORM and c) CONV, labelled with $^{15}NH_4^+$, $^{15}NO_3^-$ and ^{15}N-Urea, respectively. Uppercase letters indicate difference between soil management, whereas the lowercase letters indicate the difference between forms of ^{15}N for the same management. The black

letters above the bars indicate the difference in total recovery, the white letters the recovery in soil and plants. The ^{15}N recovery in NO_3^- in CF-BC in response to $^{15}NH_4^+$ application was larger than the average of the other treatments (indicated by the star). Note that in most cases the recovery of ^{15}N in NO_3^- is barely visible.

Tab. 1. Soil properties (0-20 cm*) measured 7 weeks after maize planting, 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. Values are mean with standard errors (n=3). The suffix letters correspond to the grouping of the Tukey test (0.05).

		Units	**CF-BC**	**CF-NORM**	**CONV**
Soil	pH		6.25(0.05)^{a}	5.94(0.15)^{a}	6.22(0.02)^{a}
	Tot organic C	%	1.1(0.16)^{a}	0.49(0.04)^{b}	0.56(0.02)^{b}
	Tot N	%	0.046(0.002)^{a}	0.036(0.003)^{b}	0.040(0.002)^{ab}
	BD	g/cm3	1.27(0.05)^{a}	1.44(0.01)^{a}	1.44(0.02)^{a}
Maize	**Root biomass	ton/ha	0.50(0.05)^{a}	0.38(0.05)^{a}	0.43(0.07)^{a}
	**Root N	%	0.98 (0.11)^{a}	1.25 (0.12)^{a}	0.67 (0.04)^{b}
	**Aboveground biomass	ton/ha	1.96(0.15)^{a}	1.54(0.2)^{a}	1.52(0.17)^{a}
	**Aboveground N	%	1.46 (0.09)$_{a}$	1.55 (0.10)^{a}	1.08 (0.17)^{b}
	Grain Yield	ton/ha	4.9(0.6)^{a}	4.7(0.8)^{a}	4.8(0.3)^{a}

[*] Based on depth-weighted average of 0-5 and 5-20 cm, Table S1.

[**] Measured at the end of the 10-day experiment i.e. 8 weeks after planting.

Tab. 2. Average N_2O flux and estimated gross nitrification rates in CF-BC, CF-NORM and CONV at 0 to 5 cm and 5 to 20 cm depth. Values are means with standard errors. For N_2O fluxes n=21 and for gross nitrification n=3. The average temperature during the gas sampling campaign was 26˚C.

	Units	**CF-BC**	**CF-NORM**	**CONV**
Mean N_2O flux	$\mu g\ N\ m^{-2}\ hr^{-1}$	34.1 (4.1)^a	26.1 (3.8)^a	8.4 (0.7)^b
Gross nitrification				
0-5 cm	$mg\ N\ kg^{-1}\ d^{-1}$	0.6 (0.60)	0.2 (0.17)	0.2 (0.11)
5-20 cm	$mg\ N\ kg^{-1}\ d^{-1}$	4.5 (1.91)^a	0.9 (0.78)	0.1 (0.03)

N partitioning, N_2O fluxes and *in situ* gross nitrification in a tropical biochar-amended Arenosol planted to maize – effect of soil management

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Fig. S1. Soil management regimes. a) Conservation farming with biochar addition inside planting basins and crop residue retention between rows. b) Conservation farming normal: planting basins with crop residue retention between rows. c) Conventional: overall plough without crop residue retention.

Fig. S2. a) Application of ^{15}N with a hand sprayer in a planting basin. b) KCl extraction in a make-shift field lab. c) Soil sampling after the ^{15}N application. d) Plant harvest and root washing at the end of the 10-day experiment.

Tab. S1. Soil properties measured 7 weeks after maize planting in two depth layers (0 to 5 cm and 5 to 20 cm). Different letters indicate differences between soil practices at a level of significance $p<0.05$. n=3. Plant available phosphorus was extracted using ammonium lactate (Krogstad et al., 2008) and base cations (Ca, Mg, K and Na) using ammonium acetate (Schollenberger, 1945).

		CF-BC		**CF-NORM**		**CONV**	
		0-5cm	**5-20cm**	**0-5cm**	**5-20cm**	**0-5cm**	**5-20cm**
	Units						
pH		6.4(0.02)^{a}	6.2(0.06)^{ab}	6.3(0.19)^{a}	5.8 (0.14)^{b}	6.3(0.05)^{ab}	6.2(0.02)^{ab}
Tot C	%	0.6(0.05)^{b}	1.3(0.22)^{a}	0.6(0.06)^{b}	0.5(0.04)^{b}	0.6(0.05)^{b}	0.5(0.01)^{b}
Tot N	%	0.043(0.003)	0.046(0.002)	0.039(0.003)	0.035(0.002)	0.044(0.002)	0.038(0.003)
BD	g cm^{-3}	1.5(0.02)^{a}	1.2(0.07)^{b}	1.5(0.02)^{a}	1.5(0.01)^{a}	1.5(0.01)^{a}	1.5(0.02)^{a}
P-AL	mg Kg-1	15.3(1.3)^{a}	30.3(7.2)^{a}	22.9(13.1)^{a}	21.3(8.4)^{a}	9.6(0.3)^{a}	7.4(0.3)^{a}
Ca	cmol$_{(+)}$ Kg^{-1}	1.6(0.3)^{a}	1.6(0.2)^{a}	1.6(0.2)^{a}	1.2(0.2)^{a}	1.8(0.3)^{a}	1.7(0.2)^{a}
Mg	cmol$_{(+)}$ Kg^{-1}	0.34(0.05)^{a}	0.35(0.04)^{a}	0.34(0.04)^{a}	0.24(0.02)^{a}	0.40(0.05)^{a}	0.35(0.03)^{a}
K	cmol$_{(+)}$ Kg^{-1}	0.15(0.04)^{a}	0.08(0.02)^{a}	0.13(0.02)^{a}	0.09(0.004)^{a}	0.11(0.01)^{a}	0.08(0.02)^{a}
Na	cmol$_{(+)}$ Kg^{-1}	0.02(0.004)^{a}	0.01(0.01)^{a}	0.04(0.03)^{a}	0.01(0.01)^{a}	0.07(0.03)^{a}	0.01(0.004)^{a}

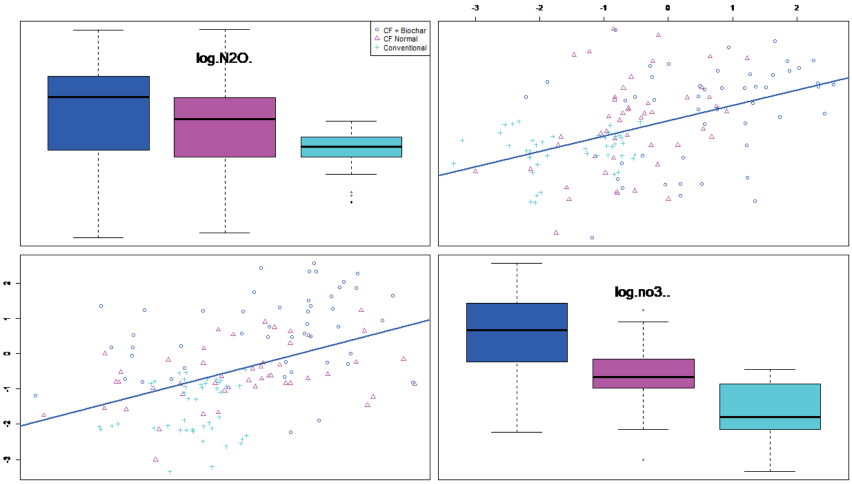

Fig. S3. Natural logarithm of N_2O fluxes as a function of natural logarithm of KCl-extractable NO_3^- [$\log(N_2O) \sim 2.79662 + 0.38392*\log(KCl\text{-}NO_3^-)$; $p<0.001$, $R^2 = 0.20$].

Tab. S2. ^{15}N recovery (%) in KCl-extractable NO_3^- from 0 to 5 cm and 5 to 20 cm, residual soil from 0 to 5 cm and 5 to 20 cm and in maize plants in CF-BC, CF-NORM and CONV with applications of $^{15}NH_4^+$, $^{15}NO_3^-$ and $^{15}N_2$-Urea.

	CF-BC			**CF-NORM**			**CONV**		
Recovery (%)	**$^{15}NH_4^+$**	**$^{15}NO_3^-$**	**^{15}N-Urea**	**$^{15}NH_4^+$**	**$^{15}NO_3^-$**	**^{15}N-Urea**	**$^{15}NH_4^+$**	**$^{15}NO_3^-$**	**^{15}N-Urea**
Soil									
-0 to 5 cm	21.9±*3.7*	5.0±*3.5*	21±*4.2*	34.6±*7.3*	6.2±*3.5*	20.5±3.6	23.4±*11.3*	9.9±*3.1*	18±*4.2*
-5 to 20cm	55.2±*23.2*	14.6±*11.1*	34.4±*12.3*	36.5±*17.1*	9.1±*4.4*	36.8±8.5	43.7±*13.1*	20.5±*3.2*	37.2±*5.7*
Total soil	77	19.6	55.4	71.1	15.3	57.3	67.1	30.5	55.2
	Average $^{15}NH_4^+$: 71.7^a			*Average $^{15}NO_3^-$: 21.8^b*			*Average ^{15}N-Urea:56^a*		
	Average CF-BC: 50.7^a			*Average CF-NORM: 47.9^a*			*Average CONV: 50.9^a*		
KCl-NO_3^-									
-0 to 5 cm	2.1±*1.8*	0.1±*0.1*	0.5±*0.03*	0.3±*0.1*	0.04±*0.02*	0.3±*0.1*	0.3±*0.04*	0.1±*0.02*	0.4±*0.1*
-5 to 20 cm	1.3±*0.7*	0.3±*0.2*	0.4±*0.1*	0.03±*0.2*	0.1±*0.03*	0.2±*0.1*	0.3±*0.1*	0.2±*0.02*	0.2±*0.02*
Total KCl-NO_3^-	3.4*	0.4	0.9	0.3	0.1	0.5	0.6	0.3	0.6
	Average $^{15}NH_4^+$:1.5^a			*Average $^{15}NO_3^-$: 0.2^a*			*Average ^{15}N-Urea: 0.7^a*		
	Average CF-BC: 1.5^a			*Average CF-NORM: 0.3^a*			*Average CONV: 0.5^a*		
Plants									
Stems	7.8±*1.1*	14.3±*3.3*	6.2±*1.9*	11±*1.3*	11.2±*1.2*	6.2±2.3	5.9±*1.3*	6.9±*1.7*	4±*0.6*
Roots	2.1±*0.3*	3.3±*0.6*	1.2±*0.01*	3.3±*0.3*	3.4±*1.0*	1.2±0.5	1.3±*0.2*	1.7±*0.2*	0.9±*0.1*
Leaves	2.4±*0.6*	2.5±*0.5*	0.8±*0.2*	3.3±*0.3*	3.5±*1.1*	0.7±0.2	1.6±*0.2*	2.2±*0.2*	1.3±*0.4*
Total in plants	12.2±*1.8*	20.1±*3.5*	8.3±*2.0*	17.6±*1.7*	18.1±*3.0*	8.2±2.9	8.8±*1.6*	10.1±*1.7*	6.2±*0.5*
	Average $^{15}NH_4^+$: 12.8^a			*Average $^{15}NO_3^-$:16.1^a*			*Average ^{15}N-Urea: 7.5^b*		
	Average CF-BC: 13.5^a			*Average CF-NORM: 14.6^a*			*Average CONV: 8.4^b*		
Total	**92.7±*27***	**40.0±*15***	**64.6±*15***	**89.0±*16***	**33.5±*6***	**65.9±*14***	**76.4±*26***	**40.8±*7***	**62.0±*9***
	Average $^{15}NH_4^+$: 86^a			***Average $^{15}NO_3^-$: 38.1^b***			***Average ^{15}N-Urea: 64.2^{ab}***		
	Average CF-BC: 65.7^a			***Average CF-NORM: 62.8^a***			***Average CONV: 59.8^a***		

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Errata list

PhD candidate:

Thesis: Effects of conservation farming and biochar on carbon and nitrogen cycling in light textured soils in the sub-humid tropics

Date: 07/05/2019

Side	**Line**	**Original text**	**Corrected text**
ii		Mumbia	Mumbi
3	72	addition	The addition
4	104	correlation	A correlation
12	309	storing	To store
12	327	Low	The low
15	398	All this	All these
16	435	A proper	Proper
16	446	Sum of	The sum of
17	463	hypothesize	hypothesized
17	475	lead	leads

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