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The fate of nitrogen in an Acrisol in Zambia under different management practices

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Acknowledgements

This thesis marks the end of my 5-year period as an NMBU student, within the “Environment and Natural Resources” program, and the end of my master´s degree, which focused “Soil and Environment”. This thesis is the end result of a yearlong study on the effects of management practice on the fate of nitrogen, with all the difficulties, wonderful learning experiences and accumulated knowledge that followed.

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Abstract

A 10-day ^{15}N tracer experiment, using ^{15}N-NO_3 and ^{15}N-NH_4, was conducted on an Acrisol in Mkushi, Zambia, in a rain-fed maize field. The addition of the ^{15}N label, as either ^{15}N-NO_3 or ^{15}N-NH_4, was equivalent to 0.1g/m^2 with an enrichment of 99.98%, giving no fertilizing effect of the soil. The main objective was to observe how the use of three different management practices; conservation farming (CFN), conservation farming with the addition of 4t biochar/ ha (CFB) and conventional farming (Conv) effected the pathway of nitrogen, especially focusing on the emission of N_2O.

The results from the 10-day ^{15}N tracer experiment showed that management practice affected the total N uptake in biomass, N_2O fluxes, ^{15}N recovery in N_2O and mean %loss of label in the soil pack. CFN was found to have the largest loss of label from the soil profile with a mean % loss of 62%, followed by Conv at 54%, while the smallest loss was found in CFB (36%). In coherence with this, the largest proportional uptake of the ^{15}N-NH_4 was in CFN>Conv>CFB, and Conv>CFN>CFB for the ^{15}N-NO_3. The differences in N uptake did however not have an effect on yield at harvest. Recovery of the added ^{15}N-label in N_2O varied from 0.45 -5.9%. No significant difference between management practice in %loss of the label within the measured system.

A notably large average flux of N_2O (221.28 μg N_2O-N/m^2/h) was measured from the Conv treated plots and was found to positively correlate to soil pH and KCl extractable N (8.14 mg NO_3/kg, 22mg NH_4/kg), possibly due to residual fertilizer left on the soil surface. N_2O fluxes from CFB (53.3 μg N_2O-N/m^2/h) and CFN (27.7 μg N_2O-N/m^2/h) were within range of what was found in similar studies. The source of N_2O was assessed by examining the relationship between gross nitrification rates with N_2O fluxes, expecting a significant positive relationship if the main N_2O emitting process was nitrification and by examining the relationship between AT% ^{15}N excess in the N_2O and AT% ^{15}N excess in the KCl extractable NO_3, expecting to find a significant positive relationship if the main N_2O emitting process was denitrification. Neither relationship was found to be significant, the results for main N_2O emitting process were therefore inconclusive.

Sammendrag

Norsk tittel: Tap, tilgjengelighet og opptak av nitrogen under ulike dyrkningssystemer i en Acrisol i Zambia.

Ett 10-dagers ^{15}N tracer eksperiment, tilført som $^{15}N\text{-}NO_3$ eller $^{15}N\text{-}NH_4$, ble gjennomført på en Acrisol i Mkushi, Zambia, på en nedbørsavhengig maisåker. Tilførselen av ^{15}N, som enten $^{15}N\text{-}NO_3$ eller $^{15}N\text{-}NH_4$, tilsvarte en tilførsel av 0.1g/m^2, med en anrikelse på 99.98%, med ingen gjødslingseffekt. Hoved objektivet var å observere hvordan bruken av tre ulike dyrkningssystemer; tradisjonelt jordbruk (Conv), presisjonsjordbruk (CFN) og presisjonsjordbruk med tilførsel av 4 tonn med biokull per hektar (CFB) påvirket tap, tilgjengelighet og opptak av nitrogen, med ett spesielt fokus på tap via N_2O utslipp.

Resultatet fra det 10 dagers lange studiet viste at dyrkningssystem påvirket det totale opptaket av nitrogen i biomassen, N_2O fluksene, den totale gjenværende mengden av ^{15}N i feltet og gjennomsnittlig prosentvis tap av ^{15}N i jordprofilet. Det største tapet av ^{15}N ble funnet i CFN, med ett gjennomsnittlig tap av 62%, etterfulgt av Conv med ett tap på 54%, mens det minste tapet ble funnet i CFB (36%). I samsvar med dette, ble det største proporsjonale opptaket av $^{15}N\text{-}NH_4$ funnet i rekkefølgen CFN>Conv>CFB, og i rekkefølgen Conv>CFN>CFB av $^{15}N\text{-}NO_3$. Forskjellen i opptak av N ble derimot ikke funnet å påvirke den totale avlingen. Funnet av ^{15}N isotopet i N_2O-utslippet varierte mellom 0.45-5.9%. Ingen signifikant forskjell mellom dyrkningssystemer ble funnet i forbindelse med prosentvis tap av ^{15}N i feltet.

En spesielt høy gjennomsnittlig flux av N_2O (221.28 µg N_2O-N/m^2/h) ble målt fra de Conv behandlede plottene. Disse viste seg å være positivt korrelert med jord pH og KCl utvinnbart nitrogen (8.14 mg NO_3/kg, 22mg NH_4/kg). De høye verdiene av KCl utvinnbart nitrogen funnet i Conv, i forhold til i CFN og CFB, kom muligens av gjødselrester på jordoverflaten. Gjennomsnittlig N_2O flukser fra CFB plottene (53.3 µg N_2O-N/m^2/h) og CFN plottene (27.7 µg N_2O-N/m^2/h) var innen rekkevidde av verdier funnet i lignende forsøk. Kilden av N_2O ble vurdert gjennom å se på forholdet mellom nitrifikasjonsrater og N_2O fluksene, der man hadde forventet å se ett signifikant positivt forhold mellom disse dersom nitrifikasjon var en viktig prosess for N_2O utslipp. Forholdet mellom AT% ^{15}N *excess* i N_2O og AT% ^{15}N *excess* i KCl utvinnbar NO_3, skulle ha vist ett signifikant positivt forhold dersom denitrifikasjon var en viktig prosess for N_2O utslipp. Da ingen av disse forholdene var signifikante, kan man ikke konkludere med hvilken prosess som står for N_2O utslippene fra åkeren.

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Introduction

With climate change, resulting in a number of droughts, seasonal floods, extreme temperatures and dry spells, affecting food security in the already vulnerable areas of the world, there is an increasing need for climate change adaption of agriculture (Zambia, 2011). In Sub-Saharan Africa (SSA) a continuously growing population, in an area highly at risk to climate changes, with relatively dry climates and largely nutrient depleted soils, finding an agricultural practice sustainable with the changing climate is a highly pressing matter (Rumley & Ong, 2007). In Zambia, small-scale farms (<2 ha) accounts for 70% of all farms, with maize accounting for 70% of the area planted and 50% of the caloric intake of the population (Hichaambwa et al., 2015; ZNFU, 2014). Maize being a drought prone, labor intensive crop in need of precise management, makes Zambia's agricultural practice exceedingly vulnerable (Aagaard, 2011). To counteract increasing food shortage and negative impacts of climate change the Conservation Farming Unit (CFU) has since 1995 been working to promote the climate smart agricultural practice in Zambia, a country with generally dry soils and a large population relying on agriculture as a source of income (Bank, 2003).

Conservation agriculture (CA) is an alternative to conventional farming practices that aims to create a more sustainable agriculture. The practice of CA is based on three principles: minimal soil disturbance, maintaining permanent soil cover (at least 30% mulch cover) and crop rotations with nitrogen-fixing leguminous species, as well as the inclusion of N fixating trees (faidherbia) between rows (Rumley & Ong, 2007). Based on experiences with CA from Zimbabwe, the Zambian National Farmers Union (ZNFU) set up the CFU in 1995. The aim of the CFU was to increase smallholder farm productivity while sustainably building soil fertility (Goeb, 2013). To achieve this in Zambia, where most farmers have under 2 ha of land and limited resources the CFU promoted a subcategory of CA not including, but still supporting, the use of N fixating trees called Conservation Farming (CF) (Haggblade & Tembo, 2003).

The practice of CA was developed and successfully adopted by large, commercial holdings in North America, Latin America and select areas of Asia, in sub-humid to humid regions (Rumley & Ong, 2007). In SSA however, crop responses to CF have been highly variable, resulting in low adoption rates among the small holders (Corbeels et al., 2015). In Zambia, through the work of the CFU involving trainings, demonstrations and farm days, adoption rates of (partial) CF have reached 10%, one of the highest in Africa (Haggblade & Tembo, 2003). However, short-term effects of CF in Zambia are also highly variable (Goeb, 2013).

The adoption of CF entails the making of rip lines or a precise grid of basins with a constant spacing. The basins are prepared in the dry season (June – October) leading up to the planting season starting in November (Haggblade & Tembo, 2003). (Haggblade & Tembo, 2003). Basins have a depth of 20 cm, whereas traditional plowing involves the upper 10 cm only. With their greater depth, the construction of basins breaks up any existing hard pan and facilitates percolation and root development (Mkomwa et al., 2015). All seeds, lime, are fertilizers are added in the basins before the onset of the rainy season. Each year the basins are re-opened during the dry-season. With early preparation of the fields, crops can benefit from the rain season in its entirety.

It is shown that, though a larger number of farmers are adopting parts of CF into their practice, implementing all three principles is difficult. Due to residues being used for other purposes, such as for animal feed, along with free-roaming animals and a common occurrence of wild-fires, retaining a permanent mulch coverage on the soil throughout the year is hard to achieve (Rumley & Ong, 2007). It is shown that only 25% of CF practicing farmers implement all three of the traditional principles, but higher maize yields are found also for these farmers (Rumley & Ong, 2007).

In Zambia, smallholder farmers traditionally burn crops after harvest and plow the soils, turning the surface entirely, after the first rains have arrived. The practice of tillage along with crop residue burning leaves the soil especially exposed to erosion (Farooq & Siddique, 2015).With continuous use of tillage in today's agricultural practices the global erosion rates on agricultural soils are shown to be one to two orders of magnitude greater than the erosion found under natural vegetation (Austin, 2015). The continued loss of agricultural soils, and thereby the loss of soil organic matter and nutrients, which are most abundant in the surface horizon, is expected to become critical for global food production. With the implementation of zero- or minimum tillage through the use of permanent planting basins, the disturbance of the soil is minimized.

The earths soil and vegetation stores three times as much carbon (C) as is present in the earth's atmosphere (Austin, 2015). The C is stored largely in soil organic matter (SOM), which is especially prevalent in the topsoil. The practice of tillage has shown to decrease

SOM and increase leaching of N (Lal et al., 2007). Through no tillage/minimum tillage Bai et al. (2009) and Wang et al. (2014) found a decrease in bulk density and increase in water stable aggregates associated with an increase in SOM. West and Post (2002) found that, for most crops, switching from conventional tillage to no tillage and including crop rotation also increased sequestration of SOM in soils. Permanent soil cover will reduce the risk of soil erosion, while increasing the soil's input of C & N, reducing the need for fertilizer and N loss through leaching (Pisante et al., 2015). Diversification of crops by the addition of leguminous (N fixing) plants will increase the soil's N supply, and most likely also increases the soil's C stock through atmospheric C sequestration (Pisante et al., 2015). The combination of zero/minimum tillage, permanent soil cover and crop diversification, as in CF is therefore related to a decrease in the large reductions of SOM from agricultural fields (Farooq & Siddique, 2015). SOM is found to contribute to increased soil fertility through increasing retention of water and nutrients, reducing bulk density and buffering soil pH (Srinivasarao et al., 2015).

By keeping crop residues on the soil surface, as a mulch, along with minimum tillage, including permanent planting basins, it is expected to see a better protection of the soil from water and wind erosion. In turn this leads to reduced water run-off (thus minimizing leaching) and water loss through evaporation, increased water retention and enhanced soil physical, chemical and biological properties for long-term sustainable productivity (Verhulst et al., 2010). Consequently, a build in SOM is expected over time, compared to fields under conventional practice, resulting in restored soil fertility (Srinivasarao et al., 2015).

Monocropping of maize is a common practice in rain-fed agriculture in Zambia, and generally in SSA, due to limited rain and short growing seasons. In Zambia the adoption of crop rotation is especially difficult as over 75% of Zambia's farmers are small holders, owning less than 5 ha, and 67% of the population depends on agriculture as their main source of income and thus food (Haggblade & Tembo, 2003). Thus, adopting crop rotation entails that a large proportion of farmers' holdings will be used for less-favored crops, like groundnuts. However, crop rotation, apart from improving soil quality, also helps moderate/mitigate weed pressure, plant diseases and pests, which may occur when not burning the fields after harvest. In addition, crop rotation may provide farmers with economically viable options of crops that are more resilient to weather irregularities than maize (Verhulst et al., 2010).

In recent years, biochar has been suggested as a soil enhancer with beneficial effects for crop production (Cornelissen et al., 2013; Glaser et al., 2002; Martinsen et al., 2014). Biochar is a charcoal product made through the pyrolysis of organic waste (Sparrevik et al., 2013). The idea of burning crop residue and incorporating the burnt material back into the soil stems from the Terra Preta soils found in central Amazonia. Terra Preta soils are ancient soils found to have large amounts of carbonized materials stored, likely originating from a high and continuous input of organic matter that has gone through incomplete combustion. These soils were found to have increased soil fertility and larger amounts of SOM, entailing large amounts of stored C (Glaser et al., 2002). Terra Preta soils have persisted for centuries, 500-2500 years, despite being located in tropical, humid regions characterized by relatively rapid mineralization rates of SOM. The fact that Terra Preta soils have significantly higher organic matter contents, and therefore high levels of stored C, shows the potential use of biochar to sequester C (Lehmann et al., 2003).

The process of producing biochar can be done in a multitude of ways, and the method will affect the quality of biochar. Using traditional kiln technologies without managing pyrolysis gases is most common in rural areas (Sparrevik et al., 2015). A newer technique for making biochar, which requires minimal investment from the farmers, is the Kon Tiki flame curtain method. The method is based on the fundamental principle of smokeless fire, making biochar production as accessible as possible with minimal GHG emissions (Schmidt & Taylor, 2014). The method involves digging a conically formed soil pit, where biomass is combusted layer-by-layer. Upon the appearance of grey ashes at the surface, a new layer of biomass is added until the pit is filled. A detailed explanation of the method is written by Pandit et al. (2017). The biochar needs to be mixed into the soil to decrease the risk of loss through erosion. Application of biochar in conventional agriculture can be done through mixing of biochar into the soil during tillage. Under CF, the implementation of biochar addition into the soil may be done in combination with basin preparation. This is done in the experimental field used in this thesis.

Both CF and biochar have been linked to a more sustainable agricultural practice, due to their potential for enhancement of soil fertility (chemical, physical and biological), their counteracting effect on agricultural GHG emissions and increased yields. However, studies on how these soil amendments function together are scarce. The few publications that exist have focused on yield (Martinsen et al., 2014). To some extent root architecture and some soil characteristics have also been studied in soils under both practices, but these studies did not include a comparison to the individual practices, nor conventional agriculture (Abiven et al., 2015; Obia et al., 2016). Thus, there is a clear knowledge gap with respect to the interactive effect of these two practices. This thesis aims to cover parts of this gap.

Biochar has shown to affect the soils chemical and physical characteristics, but how the soil is affected varies with the quality of the biomass used in production, the production process and the initial soil qualities. Biochar has shown to improve structure, water retention capacity, fertility and carbon sequestration in degraded soils. However, these effects are not found in more fertile soils Improvements to the soils structure and reduction of compaction/bulk density, affect the Water-Filled Pore Space, WFPS, which regulates soil aeration and hence the oxygen availability for microorganisms. These properties can come directly from the porous nature of the produced biochar, affecting bulk density, or indirectly due to soil aggregation induced by biochar addition (Mukherjee & Lal, 2013; Obia et al., 2015; Obia et al., 2016). The increased yields found in biochar-amended soils have also been linked to several chemical factors attributed to the addition of biochar, such as enhancement of pH, CEC and specific surface area (Cornelissen et al., 2013). Some effects of biochar have mostly been shown in greenhouse trials and incubation experiments (Obia et al., 2015; Pandit et al., 2017; Pandit et al., 2018), and findings may therefor vary when applied in field trials.

Several trials testing biochar have been related to its effect on GHG emissions. The main focus being the effect on C, due to biochar's C sequestration potential (Mukherjee & Lal, 2013). The application of biochar has also been found to affect the emission of nitrous oxide (N_2O, a potent greenhouse gas) of soils. The emission of N_2O from soils is a result of N turnover processes, where both denitrification and nitrification contribute, as illustrated by "the hole in the pipe model "Firestone and Davidson (1989). Denitrification is the dominant pathway returning reactive N to the atmosphere, with nitric oxide (NO) and N_2O being possible biproducts in the process. The availability of N is a known major driver for the emissions of N_2O from soil (Liu et al., 2017; Wang et al., 2018). The positive charge of NH_4^+ makes the ion less mobile in the soil through the soil's natural cation exchange capacity (CEC), while NO_3^- is more mobile and therefore more prone to loss through leaching. Biochar has shown to increase the soils CEC. In addition it may increase NO_3^- within its nanopores, when made at a temperature $\geq 600^{\circ}C$, thus minimizing N loss through leaching (Clough et al., 2013; Kammann et al., 2017). The sorption of NH_4^+ and NO_3^- to biochar also reduces the amount of available N for microbial nitrification and denitrification (Clough et al., 2013; Syakila & Kroeze, 2011).

The majority of studies on biochar's effect on the emissions of N_2O have shown that the addition suppresses emissions, though a few cases have found no effect or even increased N_2O emissions (Kammann et al., 2017; Spokas & Reicosky, 2009). The suppression has shown to partially be attributed to biochar's alkalizing effect on soils, creating an environment where N_2O reductase activity is enhanced, resulting in less of the N emissions escaping the soil as N_2O (Clough et al., 2013; Kammann et al., 2017; Obia et al., 2015). However, the increase in pH may increase soil N mineralization and nitrification and the increase in soil aeration may also increase nitrification, in turn resulting in more N_2O emissions (Kammann et al., 2017). The porous qualities of biochar have been suggested to also affect N_2O emissions. For example, due to increased soil aeration in denitrification dominated soils, the increased O_2 concentration will likely entail a decrease in N_2O emissions from the soil. However, this may increase nitrification, which in turn can result in larger N_2O emissions (Clough et al., 2013; Linn & Doran, 1984; Obia et al., 2015). Though most studies have found net decreases in N_2O emissions with biochar additions, this seems to be largely dependent on the importance of nitrification and denitrification as the main source-processes of N_2O emissions, which depends on soil characteristics and climate. The above-mentioned factors indicate that N_2O emissions may increase with biochar addition in highly nitrification dominated soils. This will be further investigated in this thesis.

Since the 1900's, anthropogenic influences have largely affected the N cycle, the largest contributor being the use of artificially made fertilizers in agriculture (VanLoon & Duffy, 2010). Through the Haber-Borsch method the non-reactive elementary form of N, that makes up 78 % of our atmosphere, is converted into reactive N (NH_3) that contributes to a larger amount of N in circulation (Galloway et al., 2008).

Increased amounts of available N (NO_3 & NH_4) in circulation is believed to be the main cause of increased N_2O in the atmosphere (Schlesinger, 2009).N_2O is an important greenhouse gas with a global warming potential 298 times that of CO_2 over a 100-year time period (Forster et al., 2007), and a retention time of 120 years (VanLoon & Duffy, 2010). About 60% of all anthropogenic emissions of N_2O can be sourced back to agriculture (Syakila & Kroeze, 2011). As found in several studies, the emissions of N_2O are mostly found to be higher in tilled fields than non-tilled fields and substantially higher with high applications of N-fertilizers (Baggs et al., 2003; Linn & Doran, 1984; MacKenzie et al., 1998; Malhi et al., 2006). Understanding which factors and processes affect the emission of N_2O from agricultural fields will be important to mitigate global N_2O emissions.

One of the three main principles of CF, to incorporate nitrogen-fixing leguminous species to induce biological N fixation, aims directly at increasing the soils N amount (Farooq & Siddique, 2015). Though the other principles of CF do not directly aim for this effect, several indirect effects on the N content are found. Both CF and the addition of biochar to soils are related to increases in SOM and therefore SOC, since SOC is estimated to be about 50% of SOM (Brady & Weil, 2010, p.373; Farooq & Siddique, 2015; Glaser et al., 2002). The same trend is therefore expected to be found for total N, as the N and C cycle are closely linked. With the implementation of zero or minimum tillage and permanent basins, a smaller amount of C & N is expected to be released from the soil's protected N pools, resulting in a significantly higher total N concentration compared to conventionally tilled soils (Govaerts et al., 2007). Another important factor affecting N_2O emissions through both nitrification and denitrification is the water content of the soil. Zhu et al. (2013) and Linn and Doran (1984) found a linear relationship between increased WFPS and N_2O emissions from WFPS values from 30% to 70%. Linn and Doran (1984) also found that maximum rates of nitrification were found at WFPS values around 60%, since microbial activity is found to be water limited under 60%, with the largest denitrification rates found at a WFPS>70%.

To further understand the behavior of N in the environment, ^{15}N tracer field experiments have been conducted (Boast et al., 1988; Hauck & Bouldin, 1961). The use of the stable isotope, ^{15}N, has increased the understanding of pathways and mechanisms in the N cycle. The main assumption in these studies is that the enriched ^{15}N-labelled substrate will mix fully with the native N pool, which implies that the area is uniformly labelled (Boast et al., 1988; Stevens et al., 1997). With the natural abundance of ^{15}N in the environment being known, (0.3663 at% (Barraclough, 1995)), and with the addition of a known amount of a ^{15}N tracer, the added ^{15}N label can be followed through the environment. The collected samples (gas, water, soil, plant) can then be analyzed for its ^{15}N abundance with the use of an Isotope Ratio Mass Spectrometry (IRMS).

The use of ^{15}N tracers have been extensively used in agricultural studies, with a large focus on ^{15}N recovery in different pools (Gardner & Drinkwater, 2009). Through the sampling of the different pools of N in the studied area, a ^{15}N mass balance can be constructed, giving important information on the movement of N in the system (Hauck & Bremner, 1976). The main pools sampled in previous agricultural N tracer studies have been total crop biomass N, total soil N, grain N, soil inorganic N and microbial biomass N in descending frequency (Gardner & Drinkwater, 2009). By adding the ^{15}N-label in NH_4 and NO_3, respectively, in replicated plots the transformations of N, including estimations of denitrification and nitrification rates, can be done (Morse & Bernhardt, 2013). Studies of these transformation have been included in a significantly smaller amount of studies (Gardner & Drinkwater, 2009). By adding the labels to plots under different agricultural management, e.g. with and without biochar, the obtained mass balance can be used to see how management affects pathways of N.

The method, as mentioned, is based on the assumption that the enriched ^{15}N-labelled substrate will mix fully with the native N pool (Boast et al., 1988; Stevens et al., 1997). Earlier studies such as by Recous et al. (1988), done with an application of 80 kg N ha^{-1} with an atom% excess of 3.63-5.68% to winter wheat crops, have shown a recovery varying from 71% to 122% 2 days after application, showing uncertainties in the method. However, a mass balance from the gas, water, soil and plant samplings will give a larger understanding of the endpoints and pathways of the added N. Especially important for both agriculture and the environment is to see if changes in management practice can increase nitrogen uptake in plants while decreasing loss of N through leaching and N_2O emissions.

In this thesis the main research question is: How does the use of different management practices (Conventional farming - Conv, CF - CFN and CF with biochar additions - CFB) and form of added N (NH_4-N or NO_3-N), affect the main N turnover processes (nitrification/denitrification), including N uptake in plants, storage in soil, and loss through water and gas emissions in a cultivated Acrisol in Zambia?

Objectives

1. Construct a ^{15}N mass balance for the soil-plant-atmosphere system to assess the fate of N including N uptake in plants, storage in soil and loss through gaseous emissions as affected by management practice and the form of added N (NH_4-N or NO_3-N).
2. Evaluate which soil chemical and physical properties vary with management practice, and how they affect N_2O emissions.
3. Assess if denitrification or nitrification is the dominant pathway for N_2O release in the area.

Hypotheses:

- In comparison with conventional management (Conv), both CF (CFN) and CF + biochar (CFB) will have increased SOM, increased water retention, reduced bulk densities and a higher pH. The effects will be greater in CFB plots compared to CFN plots.
- CFB plots will have a larger CEC, and CFB and CFN plots will have higher soil water content → Recovery of NH_4 in the soil pool will be greater than NO_3 and decrease in the order CFB>CFN>Conv for both forms of added N.
- All plots will show denitrification as the dominant N_2O emitting process.
- The pH, WFPS and concentration of available N (NO_3 & NH_4) will have a strong positive linear relationship with N_2O emissions.

Experimental site

All experiments were done on a large private farm (CENA Farms; Mount Isabel) with rain-fed maize in Mkushi, Central Province in Zambia (S13 45.684, E29 03.349). The soil was a sandy loam Acrisol (Obia et al., 2016). Until 2017, when the experimental site was set up, all plots had been under CFN practice for several years. The experimental site consists of 20 x 30 cm large basins with 90 x 80 cm spacing, resulting in 13890 basins per hectare. Within the experimental site there are plots with three different management practices including conventional farming (Conv.), CFN and CFB randomly distributed in triplicates within the area, resulting in 9 plots, as shown in Figure 1. In each plot, N in the form of 15-NO_3, 15-NH_4 and H_2O as a control was added to 3 of the 4 rows of maize. The last row acted as a border.

Within each CF plot there are 4 rows with 4 basins (20 x 30cm) in each basin 3 maize seeds are planted. Plots under CF practice were prepared on the 20th of November 2017 by opening the previously dug basins. All plots received fertilizer "Compound D" (N, P_2O_5, K_2O - 10:20:10) at a rate of 200 kg ha^{-1} yr^{-1} before planting. Planting occurred on the 22nd of November, before the forecasted first rains. In addition, urea was applied as top dressing at a rate of 100 kg ha^{-1} yr^{-1} four to five weeks and eight weeks after planting. This gives a total addition of 112 kg N ha^{-1} yr^{-1}. For the CFN and CFB treated plots the "Compound D" was mixed into the soil in the basins before planting. In the conventional treated plots, the "Compound D" was added at the same time as for the other management practices, but in the rows with planting stations. The emergence of the first maize plant was observed already on the 26th of November. The CFB plots had received 4t/ha of locally produced biochar from pigeon pea, prepared in a Kon Tiki kiln at 600 ˚C. The biochar had a pH of 10.4, TOC of 56.1 %, TON of 0.69% and CEC of 6.6 $cmol_{(+)}kg^{-1}$ (Munera-Echeverri et al., 2018).

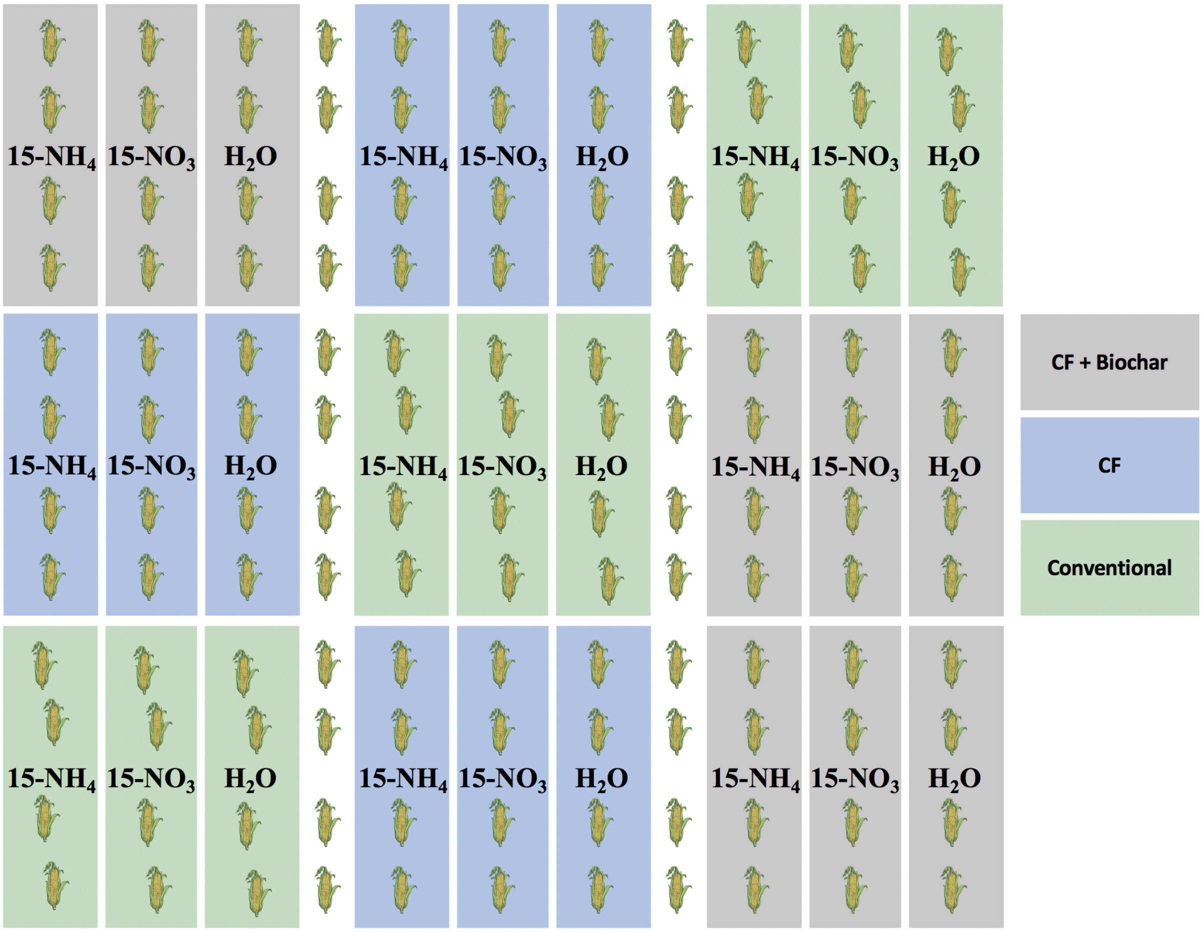

Figure 1. *Experimental site setup. Three blocks with one of each of the three management practices randomly distributed within the experimental site. Each block has four rows of four planting basins, each with three maize plants. The rows were labeled with* $^{15}N\text{-}NH_4$, $^{15}N\text{-}NO_3$, H_2O, *respectively, the final row of maize functions as a border.* [1]

Mkushi has an average temperature of 20.4°C and an annual average rainfall of about 1200 mm (Obia et al., 2016). Zambia's climate has a large seasonal variation resulting in only one growing season, from November – March, due to the distribution of rainfall, followed by a dry period from April – October (Obia et al., 2017). During the year of this field study, 2018, there was a large anomaly in the rainy season, where the first large rain events were not seen until February, at the time of field sampling, as told by the local farmers[2]. The abnormal rain distribution had a clear impact on crop growth all over Zambia.

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[1] The conventional plots do not have planting basins, the plants are there spread throughout the plot.

[2] Weather data from the field for the entire season was obtained, but the rain gauge was clogged when found, the only data collected is therefore from 10 days period of the experiment from the farmers own rain gauge.

Method:

^{15}N Label addition

The field experiment started with the addition of the two different ^{15}N labels, viz. ^{15}N-NH_4 and ^{15}N-NO_3. To get an even distribution within each plot a garden sprayer was utilized. The addition of the ^{15}N label was equivalent to 0.1g/m^2 (0.38-0.39 mg N/kg) with an enrichment of 99.98%, giving no fertilizing effect of the soil. For the CF plots this entailed an addition of 6 mg ^{15}N per basin (20 x 30 cm area) in three basins per plot, the firth basin was sprayed with 8 mg ^{15}N on a 20 x 40 cm large area, this basin was used for gas measurements. For the conventional plots 48 mg ^{15}N in total was added to rows with four planting stations (20 x 240 cm area).

Gas samples

Collecting the gas samples from the soil surface was done by first capturing the emissions in a chamber, with a known volume (2.95 dm^3), placed directly on the ground. The chamber used in this experiment was a bucket modified with an addition of a butyl rubber septum, to be able to extract the gas sample with a syringe and a needle. A rubber tube was used keep the pressure inside the bucket at 1 atmosphere. The bucket was pushed 4 cm into the ground to ensure that no air escapes during deployment. The gas samples from the bucket were transferred to a pre-evacuated crimp-sealed glass vial with a butyl rubber septum. When collecting the sample, the plunger was first pumped up and down 3 times to ensure complete mixing of gases in the bucket before the sample was collected and injected into the glass vial. Each ^{15}N labeled plot had a designated bucket and syringe, to prevent cross contamination between labels.

Image 1. *Gas chamber (for NO_3 labeled plots) used for collecting gas samples along with syringes and vials used to obtain samples.*

Samples were collected on 7 occasions (the day of ^{15}N application and 24, 48, 72, 168, 192, 216 and 240 hours after application) at 0, 15 and 30 minutes after deployment of the static chamber in the designated plot. Samples from six sampling events were collected in 10 ml glass vials. At one sampling event, 24 hours after application of ^{15}N, larger 120 ml glass vials were used to collect samples sufficiently large for ^{15}N analysis of N_2O. The larger samples were collected by using two three-way valves with one syringe inserted into the bucket and the other inserted into the pre-evacuated glass vial. The two valves were connected by a rubber tube.

Gas samples were collected both inside and outside basins. Inside the basins (CFN and CFB) and at the planting stations (Conv), this involved sampling as close to the plants as possible for each of the three labels in each plot. The samples collected outside basins was done between planting basins, with one sampling per plot. The temperature inside the chambers was recorded at beginning and end of chamber deployment to correct N_2O emission rates.

Gas samples – Analysis

The collected gas samples were analyzed for N_2O, CO_2 and CH_4 by an automated gas chromatograph (GC Model 7890A, Agilent, USA). N_2O fluxes were estimated by fitting 3 samplings; 0, 15 and 30 minutes after application of the chamber, along a linear regression to the increase of N_2O concentration over time and calculated as µg N_2O-N m^{-2} h^{-1} using measured chamber temperatures. As a quality control a similar procedure was followed with respect to increasing CO_2 concentrations, where CO_2 should increase consistently over time. In total 4 fluxes were excluded from further study due to a lack of CO_2 accumulation within the chamber, which likely can be explained by a leak in the chamber or the vial (3 from CFN plots, and 1 from a CFB plot). During transport 11 flasks broke, none of them were from the same sampling spot and time. The fluxes were then calculated from only 2 sampling times, as long as the CO_2 accumulation was as expected in the 2 remaining samples.

Soil samples

Soil samples were collected at five different sampling events after the application of the label. The first soil sample was taken directly after the application of the labeled N, further samplings were done at; 24, 72, 168 and 240 hours after the addition of the label. Six soil samples were collected using a soil auger (diameter of 0.5cm) at 0-5cm depth and bulked. Four soil samples were collected at 5-20 cm depth with a soil auger (diameter of 0.4 cm) and bulked. A subsample of the soil was used for the KCl extractions done in the field, while the rest of the soil sample was transferred directly into pre-labeled zip-lock bags which were sealed until they could be dried, sieved to collect the fine earth fraction (2mm) and pretreated for the specific experiments done in the lab at NMBU.

A subsample from the soil samples collected at 24 and 240 hours after application of the label were bulked together for further analysis for soil properties that may vary with management practice, but not time.

Soil samples – Weight estimates

All soil samples were weighed before and after drying, the following calculations were done based on these measurements:

Gravimetric moisture content: $\theta_g = \frac{m_{wet} - m_{dry}}{m_{dry}}$ (Bilskie, 2001)

Bulk density: $\rho_b = \frac{m_{dry}}{Volume^*} \left(\frac{g}{cm^3}\right)$ (Bilskie, 2001)

*Volume of auger

Volumetric moisture content: $\theta_v = \frac{\theta_g * \rho_b}{\rho_w{}^*}$ (Bilskie, 2001)

$^*\rho_w \sim 1$ and was therefore ignored

Water filled pore space: $WFPS = \frac{\theta_v}{\left(1 - \frac{\rho_b}{\rho_p{}^*}\right)} * 100\ (\%)$ (Zhu et al., 2018)

$^*\rho_p - Particle\ density = 2.65\ g/cm^3$ (Linn & Doran, 1984)

Bulk density was expected not to vary with time, however, when estimating the bulk density from all the soil sampling events significantly and consistently higher values were found at 168 hours after application of the label. The sampling at this time was done by another person, using a slightly different protocol, because of the inconsistency in bulk density from this time, compared to the other sampling days, the bulk density estimates from that sampling time were removed. All calculations involving bulk density values in the thesis, are therefore combined for all times and plots to get one estimate per management practice, label and depth, a total of 18 estimates. Label is included due to the setup of the experimental site (Figure 1), with the labels being systematically added in rows within each plot, meaning the ^{15}N-NH$_4$ is consistently located in the right side of the plots, ^{15}N-NO$_3$ in the middle of the plot and the control (H$_2$O) always on the left side of the plots, possibly making the location of the label affect the bulk density in the soil.

Bulked soil samples:

To understand which soil factors, that may vary according to management practice, contribute to the possible differences in distribution of ^{15}N, bulk samples from different time periods (24 and 240 hours after application) were made to have an adequate amount of sample for all the following analysis. This was done on the assumption that the soil properties analyzed would not vary in the small time-window of sampling (viz. 10 days).

Dry matter – Loss on ignition – Inorganic C:

This analysis was done in three steps. Dry matter was estimated by taking about 4 g of airdried fine earth samples and adding to individual crucibles with a prenoted weight. The crucibles, with the added sample, were then dried in a drying cabinet at 105°C for 5 hours and were then weighed again (Krogstad, 1992).

The following day, the samples were heated up to 550°C, to determine loss on ignition (LOI). LOI is an estimate of the soil organic matter content, but results may be overestimated for clay-rich material with significant amounts of crystal water. The samples were left in the oven, while it was turned on, for 6 hours before shutting it off, and the samples were left until the following day, to cool down. The samples were then weighed again (Krogstad, 1992).

The last step was heating the samples up to 900°C, to burn inorganic C sources. The samples were left for 6 hours, before they the oven was turned off. The samples were left until the following morning to cool down, before being weighed a final time (Ball, 1964).

The calculations are done by:

Dry matter (%):$\frac{m_{Crucible\ with\ sample\ weight\ after\ 105^\circ\mathrm{C}} - m_{Crucible}}{m_{crucible\ with\ original\ sample} - m_{crucible}} * 100$ (Krogstad, 2018)

Loss on ignition (%):$\frac{m_{Crucible\ with\ sample\ weight\ after\ 550^\circ\mathrm{C}} - m_{Crucible}}{m_{Crucible\ with\ sample\ weight\ after\ 105^\circ\mathrm{C}} - m_{crucible}} * 100$ (Krogstad, 2018)

LOI was corrected by subtracting a value of 1, due to a clay content of 6% as shown in (Krogstad, 2018)[3]

Inorganic C (%):$\frac{m_{Crucible\ with\ sample\ weight\ after\ 900^\circ\mathrm{C}} - m_{Crucible}}{m_{Crucible\ with\ sample\ weight\ after\ 550^\circ\mathrm{C}} - m_{crucible}} * 100$ (Ball, 1964)

All further analysis was corrected with respect to the dry matter content.

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[3] Table showing correction factors – Appendix 1.1, Table showing clay content in soil – Appendix 1.2

Tot C & N

Total C and N was analyzed after crushing soil samples in an agate mortar for 2 minutes, using a Leco TruSpec instrument. The result is given in weight % of the total sample.

P-Al

The Egners ammonium lactate (AL)-method has been used since 1960 to estimate the amount of plant available phosphorus in acidic soils. The method was done by weighing in 2 g of the fine earth into 100 ml acid washed glass bottles. 5 blanks and 2 samples with a known control soil was added. Next 40ml extraction AL-solution was added, before the samples were put on a shaker for 1.5 hours at 120 rpm (Egnér et al., 1960).

The sample was then filtered through "Whatman blue ribbon" filter paper and diluted 10 times with the addition of 9 ml of water to 1 ml of extract. After addition of 0,4 ml of ascorbic acid and 0,4 ml of molybdenum reagent to the diluted solution, the sample were analyzed after 10 minutes using a spectrophotometer at 700 nm (Egnér et al., 1960).

Cation exchange capacity (CEC)

Cation exchange capacity was determined, using 3 g of the fine earth.
The exchangeable base cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) were determined in 50 ml of 1M ammonium acetate extracts (NH_4OAc) buffered at pH 7, as explained by Krogstad (2018). Extractable acidity was determined by back-titration with 0.05 M sodium hydroxide to pH 7 (Krogstad, 2018). The sum of exchangeable base cations and exchangeable acidity was assumed to equal the effective CEC (Schollenberger & Simon, 1945). Six blanks and two samples with a standard soil were added to the series to check the solution, possible contaminations and measurement errors.

pH

Soil pH was determined by transferring 10 ml of the fine earth, to a graduated beaker, with an addition of 25 ml deionized water. The beaker was capped, and shaken well, before the sample was left to settle overnight. The beaker was then shaken again the following morning, left to settle again for 30 min, before the pH measurements were taken. The pH measurements were done with a glass electrode pH meter. The pH meter was first calibrated with two buffer solutions; at pH 4, then pH 7, to check the calibration a reference solution with pH 6.87 was used (Krogstad, 1992).

^{15}N soil-analysis (done at the University of California, UC Davis)

^{15}N, TOC and TON analysis of subsamples of all soil layers and vegetation were sent to the UC Davis Stable Isotope Facility. The amount of sample needed for the analyses was calculated according to instructions given on the webpage of the UC Davis Stable Isotope Facility (https://stableisotopefacility.ucdavis.edu/sample-weight-calculator.html). The samples were weighed in, using tin capsules (size: 8 x 5 mm), that were folded closed and pressed flat with a forming device.

Due to the small size, pretreatment of the soil's sub-samples was necessary to ensure a representative sample. A subsample of the fine earth was crushed in an automated agate mortar for 2 minutes.

NH_4 and NO_3 in KCl extracts

Following 4 of the soil sampling events ;1, 24, 72 and 240 hours after application of the label, a KCl extraction was done on all samples, to analyze for inorganic N (NO_3 & NH_4) as explained by (Maynard et al., 1993). The extractions were done on site to avoid changes of the NO_3 and NH_4 concentrations. The amount of possible contamination was checked using 12 blanks; three blanks for each series of extractions. Of each sub-sample, 11g of field-fresh soil was weighed into plastic tubes. 40 ml 1M KCl was then added to the tubes before they were capped and put on a table-shaker for about 60 minutes, to get equilibrium between the soil and the liquid. The samples were then passed through "Whatman blue ribbon" filter paper, that had been pre-washed with distilled water, in funnels, into new 50 ml plastic tubes until the tube was filled. The samples were the kept frozen until the analysis was done by a spectrophotometer.

Since the soil was not dried before weighing, the results, given in mg/l, were corrected for moisture content when converting samples to mg/kg. The obtained values were corrected for NH_4 and NO_3 concentration found in the blanks.

Determination of $d^{15}NO_3$ in KCl extracts

The modified denitrifier method described was developed by Zhu et al. (2018). The method is based on the ability of the bacterial culture (Pseudomonas Chlororaphis ss. Aureofaciens), which lacks N_2O reductase, to convert the NO_3 in the sample to N_2O.

The method was applied to the KCl extracts. The sample used should contain 100 nmol NO_3-N, and the required volume will therefore vary with each sample. The calculated sample volume is transferred to a sterile crimp-sealed gas vial with a butyl rubber septum, which has been acid-washed and autoclaved. The samples were kept in the freezer until the bacteria culture was ready for use.

The bacteria were grown on a pre-made NO_3^- free TSB medium, explained in detail by Zhu et al. (2018). On a laboratory clean bench 1ml of Pseudomonas chlororaphis ss. Aureofaciens was transferred to 120 ml flasks filled with 50ml TSB medium, this made the starting culture. The medium is then left in a water bath with stirring magnets in each flask until the optical density (OD) value of the medium was 0.1-0.3 (after about 9-11 hours). When the optimal level was reached 1 ml of the starting culture was transferred to new 120 ml flasks with 50 ml of the original medium, making the working culture. The number of working cultures is decided by the number of samples that have to be denitrified. In this experiment 3 working cultures were enough for 70 samples, 1 additional working culture was left as a blank. The working cultures were left in the water bath with stirring magnets until OD values reached between 0.3-0.5 (6-10 hours).

A few hours before the working culture was expected to be ready the previously frozen KCl extracts were thawed and 2 ml of working culture was added to each sample with a sterile syringe. Samples were then helium-washed with 5 cycles of evacuation and helium filling. The samples were put on a table shaker at moderate speed for 2 days. Finally, 0.2 ml 10M NaOH was injected into each sample to stop the denitrification process before analyzing them by the IRMS.

The AT% of each sample was calculated using the same equation as for ^{15}N in N_2O by *Stevens and Laughlin (1994),* to account for double substituted $^{15}N2O$:

Atom % ^{15}N in N_2O = 100 $({}^{45}R + 2\,{}^{46}R - {}^{17}R - 2\,{}^{18}R) / (2+2\,{}^{45}R+2\,{}^{46}R)$

The equation is used when the ^{15}N atoms are not expected to be randomly distributed throughout the N_2O, as is the case in this thesis, due to the high enrichment of the added label. ^{45}R being the ratio of ion currents (I) at m/z 45 to 44, $^{45}R={}^{45}I/I^{44}$, following this $^{46}R={}^{46}I/{}^{44}I$. ^{17}R & ^{18}R are set values when assuming the natural abundance of oxygen isotopes, values given by Stevens and Laughlin (1994).

At% excess is calculated by subtracting the at% values found in the KCl extracts of the H_2O-treated plots.

Vegetation samples

Vegetation samples were taken at 3 sampling events; 24, 168 and 240 hours after the application of the ^{15}N label. At each of these three sampling events one of the four basins was dug up in its entirety to collect most of the root along with the aboveground biomass. The roots of the maize plants were washed, to get rid of the soil trapped within the root system. Following the roots being washed the maize plants were cut at the bottom of the stem into roots and aboveground biomass. The parts were then weighed separately. First all three roots from the basin where measured together, the same for the aboveground biomass, then the largest plant was found, and the root and aboveground biomass of that plant was measured separately. The largest plant (aboveground biomass and root) from each basin was then collected and taken to a lab at the University of Zambia (UNZA) where roots and aboveground biomass were individually shredded into small pieces and homogenized in small plastic bags, before being shipped to NMBU. The plant samples were transferred to paper bags at NMBU and put in a drying cabinet over night before weighing the samples. Specific pretreatments for each lab experiments were done on the dried samples.

When estimating the total biomass per hectare, the weight of the single plant was multiplied by 3, to account for the 3 plants per basin. The number was then multiplied by number of basins per hectare.

^{15}N plant-analysis at UC Davis

As for the soil samples, a small subsample of the plant samples, pretreated to a sufficiently small particle size by being put through a ball mill for 3 minutes, was sent to UC Davis for ^{15}N analysis. Analysis on the C & N content of the plant samples were not done prior to sending the subsample to UC Davis Stable Isotope Facility, the estimated content of C & N in the plant samples was therefore taken from the above-mentioned web page, 0.8-1.3%N for roots and 0.4-0.8%N for stems, to send the desired sample size. The samples were then, like the soil samples, weighed into tin capsules (size: 8 x 5 mm).

Further calculations using ^{15}N data

Atom% of ^{15}N

The isotopic data obtained from the gas-, soil-, and vegetation samples were used to calculate AT% with the same equation by Stevens and Laughlin (1994), also used on the KCl-extractable NO_3^-:

Atom % ^{15}N in N_2O = 100 $({}^{45}R + 2\,{}^{46}R - {}^{17}R - 2\,{}^{18}R) / (2+2\,{}^{45}R+2\,{}^{46}R)$

^{15}N recovery (%)

The recovery (%) of ^{15}N in N_2O was found as the intercepting point between the AT% and $1/N_2O$(ppm).

The recovery (%) of ^{15}N in the soil-, KCl-extractable NO_3^-, and vegetation samples was found using the following method, as shown by Providoli et al. (2005):

Recovery (%): $\frac{\mathrm{g15N/cm^2}}{\mathrm{g\ 15N\ addition\ /cm^2}}$

$g^{15}N/cm^2 = (g\ N/cm^2) * X_{sample}$
(For the KCl-extractable $NO_{3\,-}$ gNO_3^-/cm^2)

X_{sample} includes the fractional abundance of ^{15}N in the sample, in the non-labelled plots and of the applied tracer (99.98 atom%). Further explanation of X_{sample} is offered by Providoli et al. (2005)

Proportional recovery was estimated assuming that the amount of ^{15}N recovered at each of the sampling events, by combining recovery from the soil and vegetation pools equals 100% recovery of the added label, even in cases where the total amount found is greater than amount added.

Estimation of nitrification rates

In-situ rates of gross nitrification were estimated by applying principals of ^{15}N pool dilution and NO_3^- mass balance (Kirkham & Bartholomew, 1954) from ^{15}N abundances in the $^{15}NO_3^-$ treated plots, assuming the mineralization rate = immobilization rate.

Statistical analysis

All statistical analysis was done using a 0.05% significance level. Statistical analysis was done using the Rcmdr-package Version 2.4-4.

ANOVA was applied to:

- All soil characteristic listed in Table 1 and Table 2 between management practices and depth or root/shoot.
- Weighted estimated averages for depth on KCl extractable NH_4 and NO_3 between management practices.
- KCl extractable NH_4 and NO_3 between sampling events for each individual management practice.
- WFPS (%) between management practices, for all times and for the mean values.
- For TON and KCl extractable N, between added label.
- % Loss of label within the entire field from 1 hour after application to 240 hours after application between management practice and form of added label.
- ^{15}N recovery (%) of ^{15}N-NH_4 and ^{15}N-NO_3 in N_2O between management practices and form of added label.

- % Loss of label in the soil profile from 1 hour after application to 240 hours after application between management practice and form of added label.
- ^{15}N proportional recovery of ^{15}N-NH_4 compared to ^{15}N-NO_3 in the KCl extractable NO_3 pool.
- ^{15}N proportional recovery in vegetation at the last sampling event (240 hours after application of the label) between management practices and form of added label.
- The mean N_2O fluxes within the timeframe of the experiment between placement of sample outside or inside of basins and management practice.
- AT% in N_2O and AT% in ^{15}N-NO_3 between management practice and form of added label.
- Nitrification rates between management practice, and depth.

If the F-test was significant then Tukey HSD was used as post hoc in all of the abovementioned ANOVA analysis.

All correlation analysis was done using Spearman's correlations test. Correlation was checked between:

- N_2O emissions (fluxes) and all sampled soil characteristics, using all data points from the field.
- N_2O emissions and the four characteristics that showed the best correlation when all data points were included were checked for each individual management practice.

A logarithmic regression was used, because of higher R^2 values than when linear regression was used in all cases, to illustrate the decrease in recovery of ^{15}N in soil for each management practice and form of added label.

Linear regressions were used to assess the relationship between:

- The AT% in NO_3 and in N_2O for each management practice and form of added label.
- Average N_2O emissions and the nitrification rate between 24-72 and 72-240 hours after application of the label for the entire field.

In Table 1 all values are from bulked soil samples, however, for TON (%) all samples were under the detection limit at NMBU (0.05%) and were therefore substituted with the TOC and TON (%) obtained from the soil samples sent to UC Davis. The same soil samples, and not bulked soil samples, were used to estimate the bulk density of the soil.

Results

Soil & biomass characteristics

Table 1 shows all results of the soil characteristics not expected to change with time, and therefore analyzed as bulked soil samples between multiple sampling events[4]. The pH, P-Al, TON and TOC in the Conv treated plots were all significantly different in the top 5 cm from the CFN and CFB treated plots. The pH values tended to be higher, albeit not significant, in the top 5 cm of the soil for all management practices. Between management practice however, there was a significantly lower pH found in the Conv treated plots, compared to the other two management practices, at both depths. Notably, the pH is slightly higher for the CFB plots than in the CFN plots, though not significant. P-Al showed a large and significant difference in the top 5 cm of the soil between Conv and the two CF management practices, while showing no significant difference between management practices at the lower depth (5-20 cm).

The soil's total carbon content (the sum of TOC and inorganic C) is about 1%. Both C pools contribute to around 50%. However, the inorganic C content does not differ between the management practices, and most of the variation in the total C pool, can therefore be attributed to changes in TOC. The presence of inorganic C is strange, seeing that pH <6.5 (Brady & Weil, 2010 p.274). TOC & TON varied significantly between all management practices in order CFB>CFN>Conv, TOC showed no difference between depths, while TON showed a significantly higher content in the top 5 cm for CFB and CFN. As expected, the C/N ratio was significantly greater under CFB than in CFN and conv, due to the addition of carbon-rich and nitrogen-poor biochar.

The CEC, potassium (K+), bulk density and estimated inorganic C showed no significant differences between management practices. Although not significant, the CEC showed, an increasing trend in the order Conv < CFN < CFB, i.e. the same order as found for TOC. Because no difference was found with depth for estimated inorganic C and SOM (LOI), the values were therefore combined to a weighted average per basin. A significantly higher SOM content was then found for the CFB treated plots compared to the Conv treated plots, as expected with the addition of biochar.

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[4] TOC and TON results from UC Davis replaced the C & N content found in the bulked samples. Both results for TOC are shown in appendix 2.

Table 1. *Soil characteristics by management practice and depth, showing mean values ± standard deviations (pH variation is shown by range). Letters indicate significant differences at p < 0.05 between depth and management practice for each soil characteristic.*

		CFB		**CFN**		**Conv**	
	n	**0 – 5 cm**	**5 – 20 cm**	**0 – 5 cm**	**5 – 20 cm**	**0 – 5 cm**	**5 – 20 cm**
pH (range)	3	6.47 (6.42-6.5)^{a}	6.16 (6.02-6.34)^{a}	6.23 (6.09-6.36)^{a}	6.12 (5.9-6.26)^{a}	5.62 (5.51-5.73)^{b}	5.22 (5.1-5.44)^{b}
P-Al mg/kg	3	12.04 ± 1.74^{c}	29.08 ± 5.31^{abc}	15.05 ± 4.6^{bc}	27.07 ± 10.86^{abc}	42.12 ± 8.67^{a}	30.74 ± 3.8^{ab}
CEC cmol$_c$/kg	3	8.4 ± 2.15^{a}	3.71 ± 0.37^{a}	7.42 ± 2.5^{a}	4.18 ± 0.64^{a}	6.16 ± 2.38^{a}	5.28 ± 1.68^{a}
K+ cmol$_c$/kg	3	0.14 ± 0.01^{a}	0.12 ± 0.01^{a}	0.11 ± 0.04^{a}	0.11 ± 0.04^{a}	0.10 ± 0.01^{a}	0.09 ± 0.01^{a}
BD g/cm^3	12	1.31 ± 0.14^{ab}	1.33 ± 0.16^{ab}	1.42 ± 0.13^{a}	1.29 ± 0.16^{b}	1.33 ± 0.23^{ab}	1.25 ± 0.16^{b}
TOC %	15	0.8 ± 0.18^{a}	0.89 ± 0.25^{a}	0.59 ± 0.13^{b}	0.53 ± 0.09^{bc}	0.46 ± 0.07^{c}	0.48 ± 0.06^{c}
TON %	15	0.06 ± 0.009^{a}	0.05 ± 0.007^{bc}	0.05 ± 0.01^{b}	0.04 ± 0.008^{cd}	0.04 ± 0.006^{d}	0.04 ± 0.005^{d}
C:N	15	14.24 ± 1.81^{b}	17.81 ± 3.81^{a}	11.68 ± 0.69^{c}	12.01 ± 0.85^{c}	11.14 ± 0.75^{c}	11.39 ± 0.87^{c}
In. C %	3	0.51 ± 0.03^{a}		0.54 ± 0.12^{a}		0.5 ± 0.03^{a}	
LOI (%)	3	1.33 ± 0.27^{a}		0.91 ± 0.24^{ab}		0.72 ± 0.08^{b}	

The biomass samples were taken about mid-way in the growing season. At the time of sampling there was a significantly higher TON content in both the root and shoot found in the biomass in the Conv treated plots than under CFB and CFN (Table 2). However, at the time of sampling there was also a significantly lower estimated total biomass in the Conv treated plots. Due to a lower estimated biomass in the Conv treated plots, there was also a lower estimated total N uptake from the field, and following, a smaller % uptake of the applied N by fertilizer application, consistent with the greater amounts of KCl extractable N left in the soil, as shown in Figure 2.

At the time of harvest no analysis was done on the plants. However, at the time of harvest both the total biomass collected from the plots and the cob yield showed no significant differences between management practices.

Table 2. *Biomass characteristics by management practice. Letters indicate significant differences at p < 0.05 between root/shoot and management practice for each biomass characteristic.*

		CFB		**CFN**		**Conv**	
	n	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**
TOC %	45	26.10 ± 5.94^{a}	42.23 ± 1.71^{b}	25.64 ± 7.13^{a}	42.52 ± 0.84^{b}	28.78 ± 7.04^{a}	41.40 ± 6.07^{b}
TON %	45	0.75 ± 0.25^{c}	1.52 ± 00.25^{b}	0.84 ± 0.27^{c}	1.66 ± 0.23^{b}	1.42 ± 0.51^{b}	1.92 ± 0.34^{a}
Biomass at time of sampling (ton/ha)[5]	45	3.99 ± 1.27^{a}		4.07 ± 1.1^{a}		2.84 ± 1.01^{b}	
Root/shoot	27	0.33 ± 0.39^{a}		0.24 ± 0.14^{a}		0.22 ± 0.12^{a}	
Tot N uptake (kg/ha)[6]	27	56.2 ± 21.87^{ab}		64.91 ± 21.45^{a}		48.41 ± 19.34^{b}	
Uptake of fertilizer N by biomass (%)	27	50.17 ± 19.53^{ab}		57.96 ± 19.15^{a}		43.22 ± 17.26^{b}	
Biomass at harvest (ton/ha)	3	9.04 ± 0.52^{a}		8.05 ± 1.95^{a}		7.66 ± 1.01^{a}	
Cob yield (ton/ha)	3	3.72 ± 0.19^{a}		3.64 ± 1.05^{a}		2.1 ± 0.39^{a}	

A large effect of management practice was found in both the KCl extractable NO_3 and NH_4, with significantly larger amounts found in the Conv treated plots compared to the CFB and CFN treated plots (Figure 2). The amount of NO_3 found in the Conv treated plots were, on average, 3.3- and 4.3-times higher than the amount found in the CFB and CFN respectively at the start of the experiment (Table 3). The same trend was found for the amount NH_4 in the soil, with 11.5- and 8.9-times higher values in the Conv treated plots compared to the CFB and CFN respectively. However, as seen in Figure 2, there was a decreasing trend in the amount of NH_4 with time. In fact, at the end of our sampling period, 240 hours after application of the label, no significant difference was found between management practices. For NO_3 the average amount also decreased with time, but not significantly, and the amount was still significantly larger at the end point of the sampling event compared to the other management practices.

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[5] Estimated by multiplying the measured weight of one plant by 3, to get weight per basin. The number was then multiplied by number of basins per hectare.

[6] Estimated by multiplying the total estimated biomass by TON (%).

The strikingly elevated amounts of KCl extractable NH_4 in the Conv treated plots showed a significant decrease with time within the 10-day period, for both depths, this significance was not found for CFN nor CFB. The amount of KCl extractable NO_3 was not found to decrease significantly within the time frame of our field experiment in any of the management practices. No significant differences were found for the depth for NH_4, whereas for the NO_3 the only significance found in depth was at the first KCl extraction (hour 1).

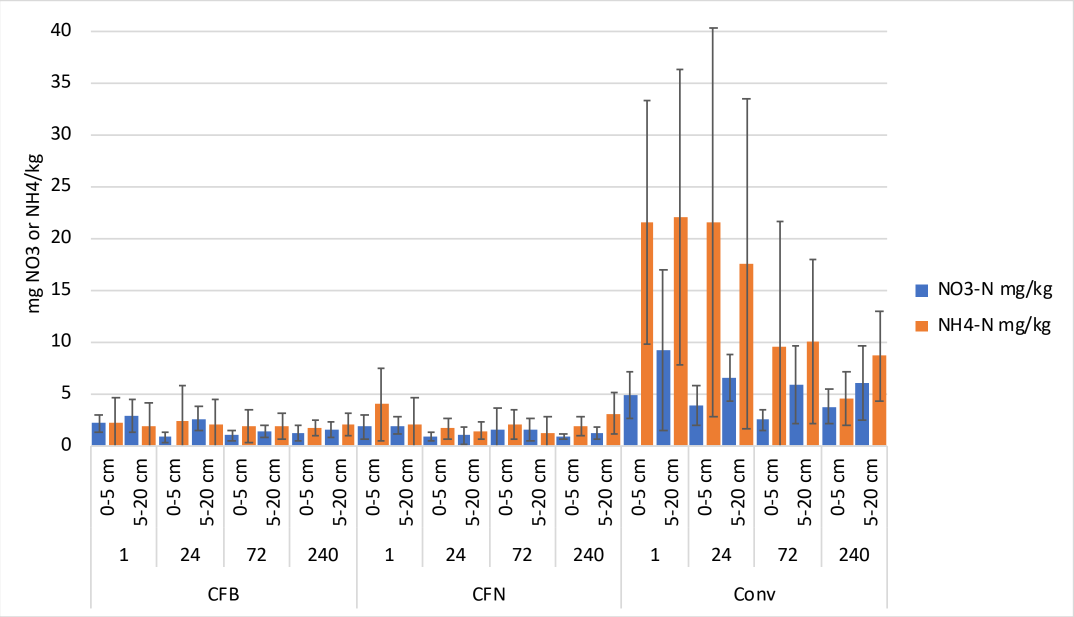

Figure 2. *Mean amounts of KCl extractable NO_3 and NH_4 in the soil for both sampled soil depths, 1, 24, 72 and 240 hours after start of the experiment, for all management practices. Error bars show standard deviation. n=9.*[7]

Table 3. *Mean estimated values for 0-20cm depth of KCl extractable NO_3 and NH_4 for each management practice, at each sampling event ± standard deviations. Letters indicate significant differences at $p < 0.05$ between management practices for NO_3 and NH_4 individually.* [8]

Management practice	**Hour**	**Depth (cm)**	**n**	**mg NO_3/kg**	**mg NH_4/kg**
CFB	1	0-20	9	2.65 ± 1.19^{bc}	1.91 ± 2.19^{b}
CFN	1	0-20	9	1.91 ± 0.79^{bc}	2.47 ± 2.53^{b}
Conv	1	0-20	9	8.14 ± 5.86^{a}	21.96 ± 12.14^{a}
CFB	240	0-20	9	1.44 ± 0.68^{c}	2.00 ± 0.94^{b}
CFN	240	0-20	9	1.16 ± 0.43^{c}	2.80 ± 1.55^{b}
Conv	240	0-20	9	5.51 ± 2.89^{ab}	7.63 ± 3.82^{b}

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[7] Values from all three differently labeled plots, which are in triplicates.

[8] Values are based on all sampling events and across the different forms of added N.

Due to clogging of the rain gauge, the weather data collected at the site from November to the time of the experiment, showed no precipitation, this is however consistent with observations from local farmers. The gauge was cleaned upon arrival (04. Feb 2018). During the experiment there was a substantial amount of precipitation in the area, a total amount of 270mm during the sampling period (04 -14 Feb. 2018), as measured by the local weather station after the gauge was cleaned. The WFPS, following the precipitation events increased from 38, 33 and 24% for CFB, CFN and Conv, respectively at the start of the study and increased steadily until 168 hours after the study started, to values of 66, 58 and 40% (Figure 3). As shown in Figure 3 there was a tendency in WFPS (%) to increase as CFB>CFN>Conv, though the significance is varying within the sampling events. When combining all sampling events, the trend CFB>CFN>Conv became significant.

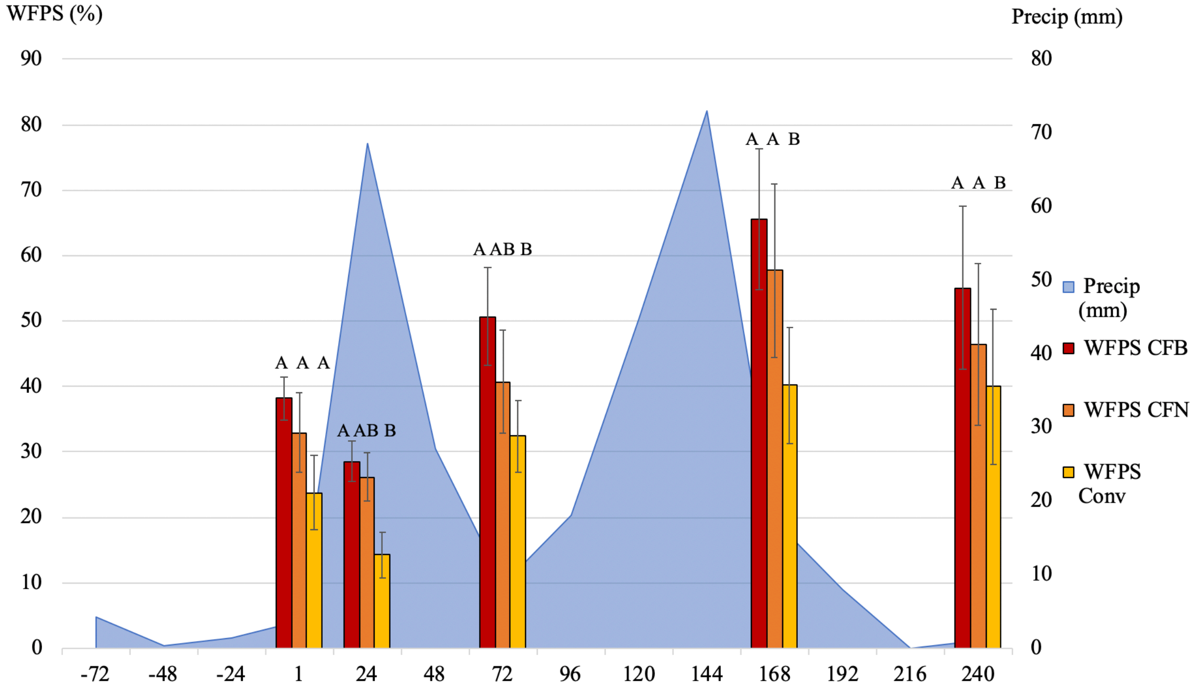

Figure 3.*Water-filled pore space (%) as a mean of each management practice, with standard deviation shown as error bars and precipitation (mm) in hours after application of the* ^{15}*N-label. Letters indicate significant differences at* $p < 0.05$ *between management practices.*

^{15}N mass balance for the soil-plant-gas system

The amount of N added through the addition of the ^{15}N are presented in Table 4. From the table we can calculate that the amount of added N accounts for 2 – 11% of the total amount of available N in the soil. The addition was also far lower than the standard deviation for both NH_4 and NO_3, showing that the addition of ^{15}N was likely too small to affect the N pool. The impact of the ^{15}N-label addition on the N pool, as TON and KCl extractable N, also showed no effect when testing for significant differences in levels against the control (water) plots.

Table 4. *Estimated mg ^{15}N added/kg soil per management practice as well as mean mg NH4/kg, mg NO3/kg and mg available N/kg ± standard deviation 1 hour after application of the label.*[9]

Management practice	Depth (cm)	mg added N/kg	n	mg NH_4/kg	mg NO_3/kg	Mg available N/kg[10]
CFB	0 – 20	0.38	35	1.98 ± 1.57	1.88 ± 1.01	3.86
CFN	0 - 20	0.38	36	2.06 ± 1.72	1.39 ± 0.82	3.45
Conv	0 - 20	0.38	36	14.51 ± 11.58	6.14 ± 3.73	20.65

Figure 4 is an overview of excess ^{15}N data, shown as ^{15}N recovery in all sampled pools. The dataset is not complete with samplings from all pools for all sampling events, and several figures using extractions from the dataset are therefore presented further down in the results to better show important findings. However, from the figure it is shown that the recovery in Conv, especially in the plots which received ^{15}N-NH_4, was unduly large, and about 100% greater than the amount of added ^{15}N to the area. Though the recovery was, on average, higher than 100% at the first sampling event, it is evident that the majority of the added ^{15}N label was recovered in the soil for all times, forms of added N and management practice, with a larger recovery being found from the ^{15}N-NH_4 compared to the ^{15}N-NO_3. The figure also shows that the ^{15}N recovery in the N_2O, measured only 24 hours after the start of the experiment, was a small fraction compared to the other pools, and the values are therefore shown in Table 5.

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[9] Calculation of mg added N/kg: mg added N per basin / (Volume of soil in each basin * mean bulk density for each management practice)

[10] Mg available N/kg = mg NH4/kg + mg NO3/kg

A clear decrease in recovery of the ^{15}N label with time was found for both forms of the added label, in all management practices. This decrease in recovery of the added ^{15}N-NH$_4$ in the CFB plots was notably lower than for the other management practices, though not significant. In addition, the CFB plots showed a lower recovery in the vegetation. There were however no significant differences in %loss between hour 1 and 240 between management practice nor label.

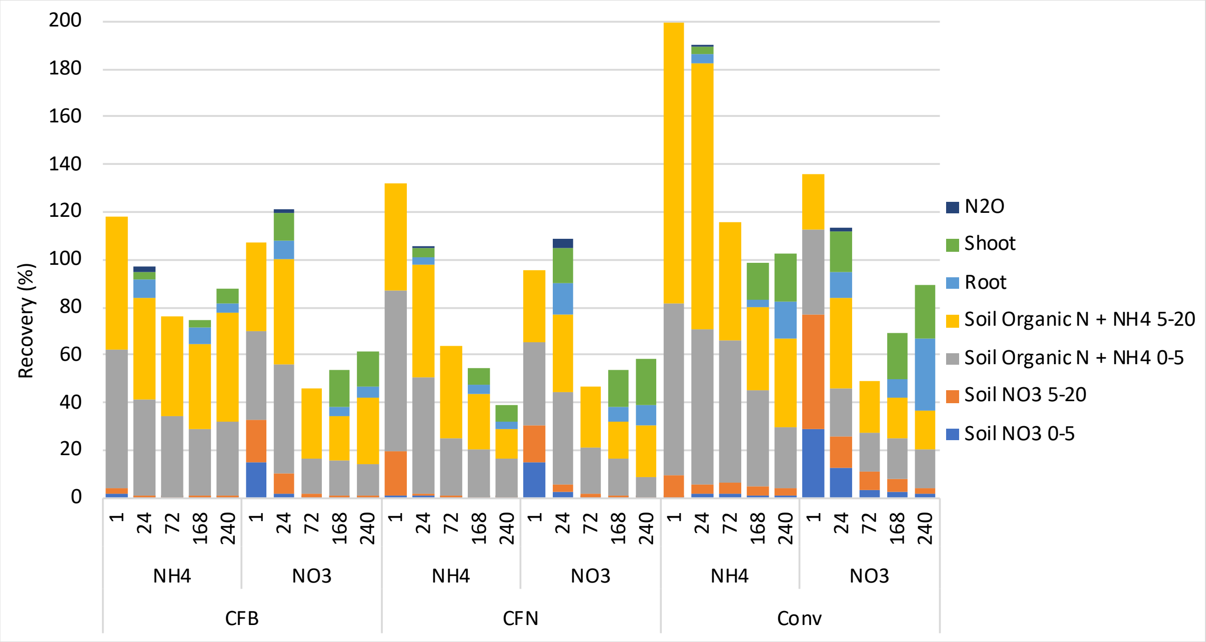

Figure 4. *Average recovery (%) in soil organic N and NH4 (sampled at all times) and soil* NO_3*-N (not sampled 168 hours after application) in both sampled depths, vegetation divided into roots and shoots (not sampled 1 & 72 hours after application) and* N_2O *(only 24 hours after application) in each of the management practices for each of the added forms of* ^{15}*N label (*^{15}*N -*NH_4 *or* ^{15}*N -*NO_3*).*

For the ^{15}N recovery in N_2O of the added ^{15}N-NH$_4$ there was a recovery between 0.78-1.95%, with no significant differences between management practice. For the ^{15}N recovery of the added ^{15}N-NO$_3$ in the N_2O the recovery was similar as for the ^{15}N-NH$_4$ but was significantly higher in the CFN, with an average recovery of over 4% (Table 5). No significant difference was found between added forms of label.

Table 5. *^{15}N recovery (%) of the ^{15}N-NH$_4$ and the ^{15}N-NO$_3$ label in the N$_2$O, 24 hours after application of the label. Letters indicate significant differences at p < 0.05 between management practices, for the ^{15}N-NH$_4$ and ^{15}N-NO$_3$ individually.*

Management practice	**Depth (cm)**	**n**	**^{15}N-NH$_4$**	**n**	**^{15}N-NO$_3$**
CFB	0-20	2	1.95 ± 1.11^a	3	1.48 ± 1.08^b
CFN	0-20	3	1.41 ± 0.48^a	3	4.21 ± 1.91^a
Conv	0-20	3	0.78 ± 0.53^a	3	1.55 ± 0.49^b

The major recovery of the ^{15}N was found to be in the soil pool throughout the experiment. Figure 5 shows ^{15}N recovery % in the soil pool (including all N pools), which showed a logarithmic decrease with time, 1, 24, 72, 168 and 240 hours after application of the label. The figure, with the estimated regression coefficients, showed the tendency of ^{15}N-NH$_4$ loss to be greater in the Conv treated plots, and lowest in the CFB, while the loss of ^{15}N-NO$_3$ showed little variation with management practice. When looking into the % loss of label from 1 hour after application to 240 hours after application in the soil profile, no significant differences were found between the added forms of the ^{15}N label. When not including form of added label, a significant difference was found between management practice at confidence level 0.90 (p=0.06), with the significant difference being found only between CFN (mean % loss of 62%) and CFB (mean % loss of 36%). Conv did not differ with a mean % loss of 54%.

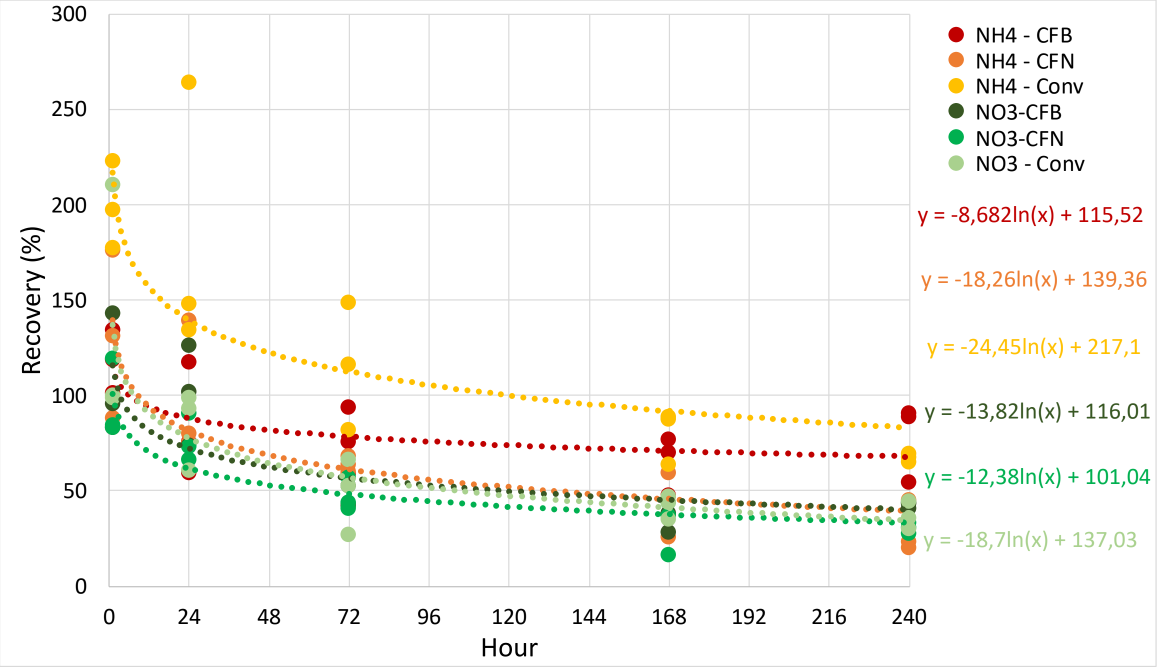

Figure 5. *Recovery (%) of soil N, in each of the management practices for each of the added forms of labeled* ^{15}N *(*^{15}N*-*NO_3 *or* ^{15}N*-*NH_4*), with equations for each logarithmic regression included.*

The proportional recovery (viz. the total recovery, normalized to 100%; Figure 6) showed that, an increase in proportional recovery in the vegetation is observed, for all management practices and forms of added N, with time. There was also a general trend of a larger proportional amount of the ^{15}N-NO_3 being found back in the maize, compared to the labeled ^{15}N-NH_4, significantly so in both CFB and Conv (Table 6). The uptake of ^{15}N-NO_3 increased CFB<CFN<Conv, with Conv having a significantly larger uptake of label compared to CFB.

Of the soil ^{15}N pool, the proportional recovery in the KCl extractable NO_3 varied highly with the form of ^{15}N added to the soil. A significantly smaller fraction of the added ^{15}N-NH_4 was found in the NO_3 pool if compared to the addition of ^{15}N-NO_3 (Figure 6). The exception being a notably high proportional recovery of 15% of the ^{15}N-NH_4 in NO_3, 1 hour after application in the CFN treated plots. However, within the triplicates used to estimate this value, only one plot has a notably high proportional recovery of 28.2%. Of the added ^{15}N-NO_3, only around 30% of the recovered signal was found back after 1 hour in the NO_3 pool for CFB and CFN. In the Conv treated plots this proportional recovery was almost twice as high, with 67% of the total recovery of the ^{15}N-NO_3 found back as NO_3, 1 hour after application. For all management practices, except the ^{15}N-NH_4 added label to the Conv treated plots, a decrease in proportional recovery as NO_3 was found with time.

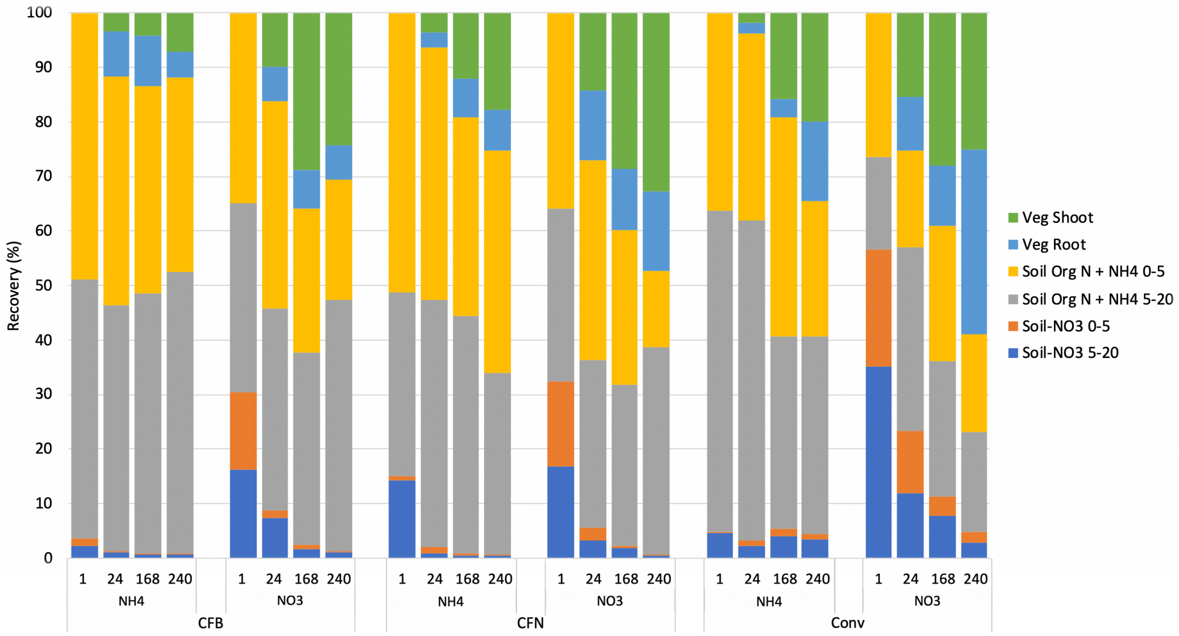

Figure 6. *Proportional recovery (%) in the different pools and depths/roots/shoots of the added* $^{15}N\text{-}NO_3$ *or* $^{15}N\text{-}NH_4$ *label to the three management practices at 1, 24, 168 and 240 hours after application.*[11,12]

Table 6. *Mean proportional recovery (%) of the* ^{15}N *label found in vegetation at 24, 168 and 240 hours after application of the label. Letters indicate significant differences at* $p < 0.05$ *between management practices and added form of label at the last sampling event.*

Management practice		**n**	**24**	**168**	**240**
CFB	**$^{15}N\text{-}NH_4$**	3	12.28	12.60	10.91^{a}
	$^{15}N\text{-}NO_3$	3	18.39	38.97	33.01^{bc}
CFN	**$^{15}N\text{-}NH_4$**	3	6.12	20.10	32.03^{bc}
	$^{15}N\text{-}NO_3$	3	22.96	41.72	45.42^{cd}
Conv	**$^{15}N\text{-}NH_4$**	3	4.18	14.05	24.25^{ab}
	$^{15}N\text{-}NO_3$	3	23.29	38.96	58.04^{d}

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[11] ^{15}N in vegetation was not sampled 1 hour after application but was assumed to be 0%.

[12] ^{15}N in KCl extractable NO_3 was not measured 168 hours after application, the value shown is an estimated average value between hours 72 and 240.

N_2O emissions:

The emissions of N_2O were clearly elevated in the Conv treated plots, compared the other management practices (Figure 7). As shown in Table 7, this elevation was only evident in these plots, with N_2O fluxes highly varying within each group. Note the tendency for smaller variations in N_2O flux outside basins, where no fertilizer was applied, compared to their respective management practices inside basins. Within the elevated flux found in the Conv treated plots, there was a significant decrease in N_2O fluxes found the first to the last sampling event.

Though not significant, the tendency of higher fluxes found in the CFB plots, were found to relate to one singe plot in the sampling pool (plot nr. 6; Figure 8). The deviating plot showed an increased N_2O flux of 593% compared to the two other NH_4 labeled plots, and an increase of 456 and 1066% compared the other NO_3 labeled plots. In the Conv treated plots, the large variations are present in all plots.

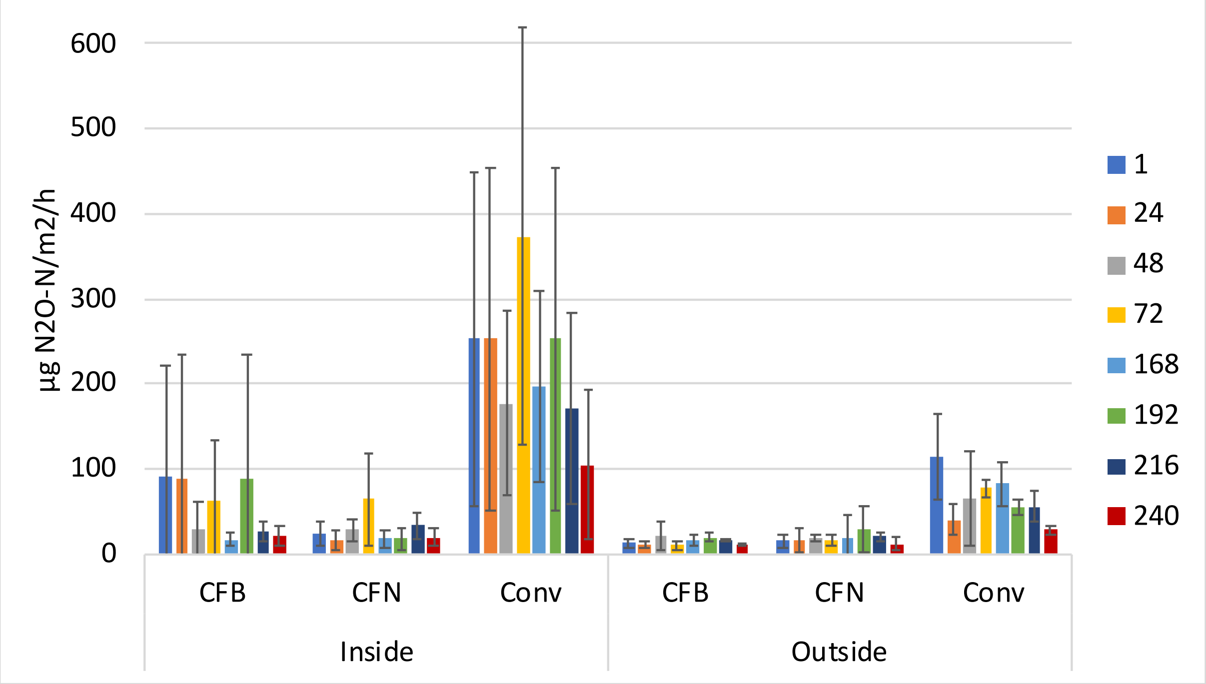

Figure 7. *Mean N_2O fluxes (µg N_2O-N/m^2/h) for each plot at 8 sampling events, shown as hours after application of the label, across forms of added N. Error bars show standard deviation for each bar. n=9 inside basins. n=3 outside basins.*[13]

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[13] CFN Hour 1 & 216 and Conv Hour 24: n=8

Table 7. *Mean N_2O flux ($\mu g\ N_2O\text{-}N/m^2/h$) calculated from the 8 different sampling events, across the different forms of added N ± standard deviations. Letters indicate significant differences between management practices at $p < 0.05$.*

In/Out	Management practice	n	$\mu g\ N_2O\text{-}N/m^2/h$
Inside basins	**CFB**	72	53.29 ± 92.29^b
	CFN	70	27.72 ± 26.25^b
	Conv	71	221.28 ± 177.13^a
Outside basins	**CFB**	24	14.71 ± 7.29^b
	CFN	24	17.96 ± 13.59^b
	Conv	24	64.93 ± 35.79^b

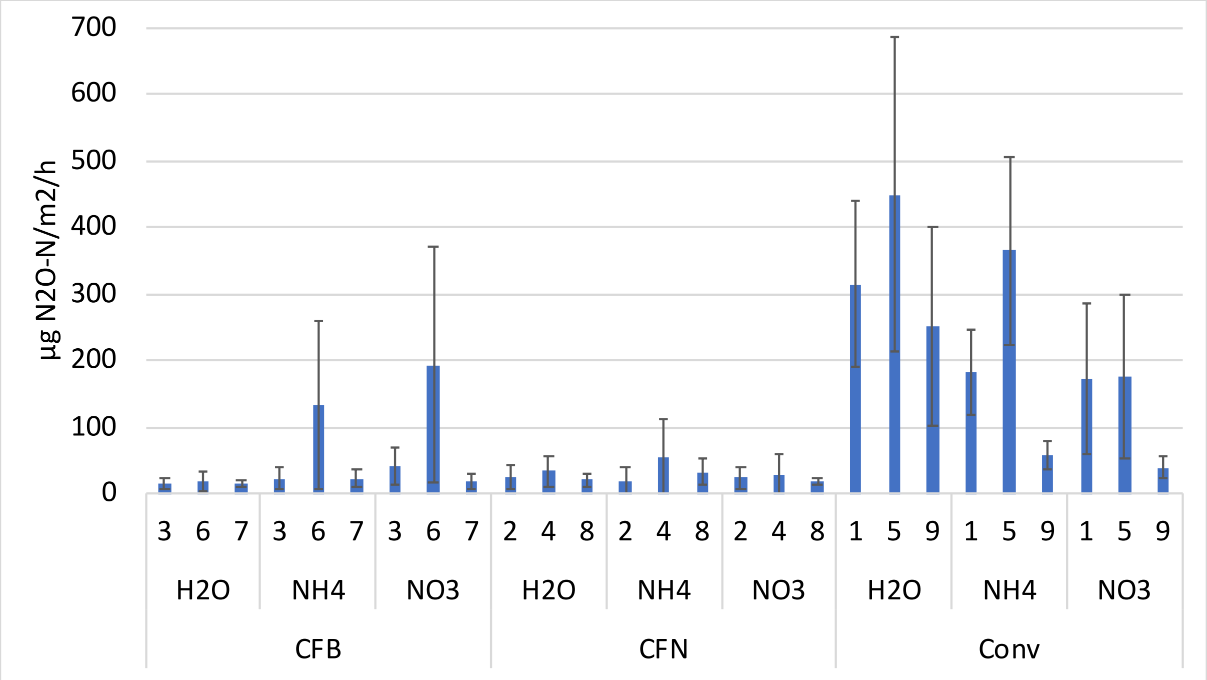

Figure 8. *Spatial variation in mean N_2O fluxes within each management practice. Error bars show standard deviation for each bar. n=9 inside basins. n=3 outside basins. n=8 sampling events.*

AT% ^{15}N of N_2O; indication of the source process of N_2O production

The N_2O emissions may result from denitrification or nitrification, the relationship between the AT% ^{15}N excess in the KCl extractable NO_3^- and the AT% ^{15}N excess of emitted N_2O, can indicate the main process. The AT% in N_2O was measured 24 hours after application of the label. Table 8 shows that the AT% in N_2O did not vary significantly between label nor management practice. The AT% in ^{15}N-NO_3 however, showed a significantly larger AT% ^{15}N-NO_3 when the label was added as ^{15}N-NO_3 in both CFB and Conv. A notably strange observation from Table 8 is a larger AT% ^{15}N excess in the N_2O than in the NO_3, for all plots labeled with ^{15}N-NH_4, and for the CFN treated plots labeled with ^{15}N-NO_3.

Table 8. *Mean AT%^{15}N excess in N_2O and NO_3 in each management practice for each added label.*

Label	Management practice	n	AT%^{15}N-N_2O	AT% ^{15}N-NO_3
^{15}N-NH_4	**CFB**	3	1.56 ± 1.11^{ab}	0.41 ± 0.13^{c}
	CFN	3	1.02 ± 0.48^{ab}	0.99 ± 0.47^{bc}
	Conv	3	0.38 ± 0.53^{b}	0.33 ± 0.24^{c}
^{15}N-NO_3	**CFB**	3	1.09 ± 1.08^{ab}	2.09 ± 0.48^{a}
	CFN	3	3.81 ± 1.91^{a}	1.87 ± 0.24^{ab}
	Conv	3	1.16 ± 0.49^{ab}	2.58 ± 0.30^{a}

The AT% in KCl extractable NO_3^- in the ^{15}N-NO_3 labeled plots were used to calculate the gross nitrification rates through pool dilution (Figure 9). Values averaged 1.1 mg N/kg soil/day. No significant difference in nitrification rate was found between management practice, nor depth. Though ^{15}N-NO_3 was measured 1, 24, 72 and 240 hours after application of the label, only the 24-72 hours after application measurements were used to estimate the nitrification rates. The gross nitrification rates from 1-24 were highly variable, the label likely not being homogenously distributed 1 hour after application, and therefore not included in further investigations. 240 hours after application the signal was very low, and therefore not included.[14]

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[14] Nitrification rates from all times are presented in Appendix 3.

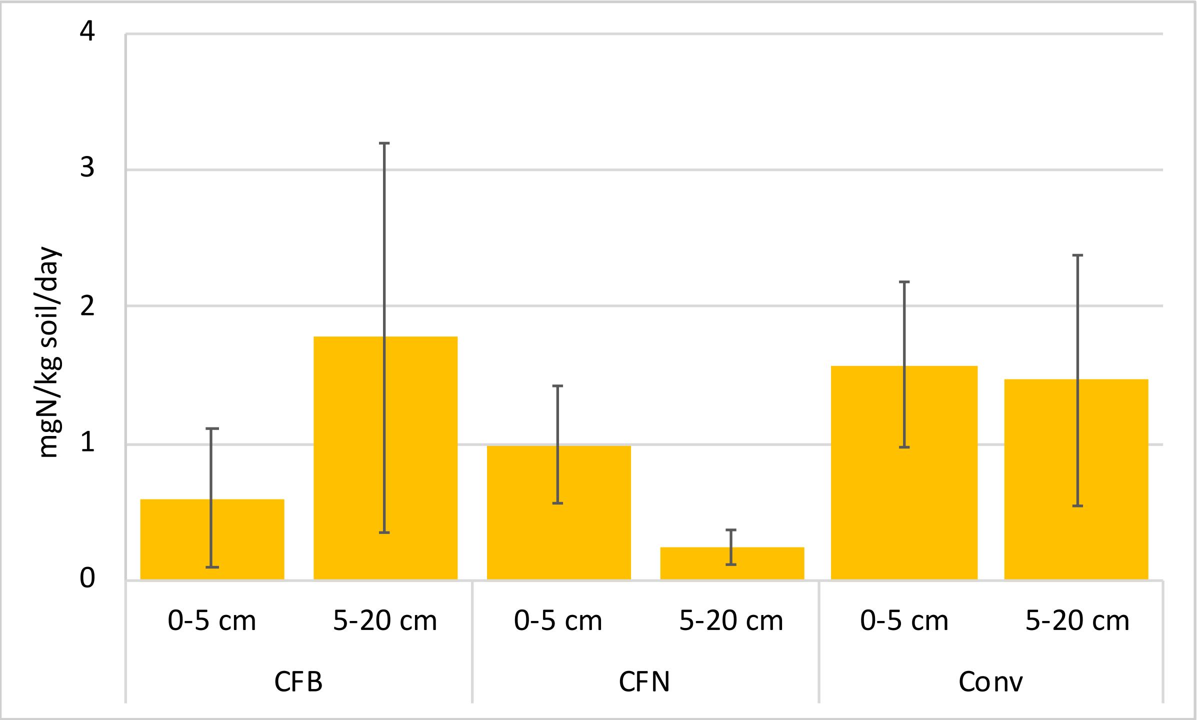

Figure 9. *Calculated gross nitrification rates (mg N/kg/day) at the two sampled depths 24 - 72 after application of the* ^{15}N *label. Error bars show standard deviation.*

Discussion:

Soil & biomass characteristics

Biochar addition in soil resulted in increases in CEC, K+, SOM, organic C, pH and water retention, when comparing CFB to CFN and Conv. However, though the tendency of an increase was seen, the degree of increase was smaller than expected, with only the TOC being significantly higher. These findings were coherent to earlier studies, where the studies indicated that the magnitude of the effect differs with the type of biochar, due to the effect of its production temperature and the type of biomass feedstock (Cornelissen et al., 2013; Mukherjee & Lal, 2013; Munera-Echeverri et al., 2018; Obia et al., 2015; Obia et al., 2016). The significance was also affected by the small sample size (n=3) for the bulked soil sample characteristics. Through visual analysis of the CFB plots, biochar was found not to be in close vicinity of the maize roots in all plots, which may have impacted the effect of the biochar in the soil. This is supported by the addition of 4t/ha of biochar, with a TOC of 56.1 % being equivalent to 161g C/basin. In the CFN plots there was an average TOC of 0.6%, equivalent to 94 g C/basin, with an additional addition of the 161 g C/basin, the amount of C should have increased 2.7 times, giving a TOC of 1.63%, an increase of 1% in TOC in the CFB plots compared to the CFN plots. The actual found increase was 0.21% in the top 5 cm and 0.36% in the lower 15 cm.

The most unexpected findings in the characteristics of the soil were the large elevated amounts of P-Al and KCl extractable NH_4 and NO_3, in the Conv treated plots compared to the CFB and CFN treated plots. Seeing that all these elements are present in the fertilizer applied to the field, a possible explanation is the different application method used on CF treated plots, compared to Conv treated plots. When adding fertilizer to CF plots, the fertilizer was mixed into the soil in the preexisting basins, however, in the Conv plots the fertilizer was spread within the entire planting row, possibly limiting the distribution within the soil profile and the availability of the fertilizer application to the plants. When looking into the difference in available N at the first sampling event (Table 3), the difference in available N between CFN/CFB and Conv was 25.5/25.7 mg/kg, respectively. This adds up to a total of 5.5 kg N/ha more in the Conv treated plots compared to both CFN and CFB treated plots. The total estimated N uptake (kg/ha) in CFB was 7.7kg N/ha larger in CFB compared to Conv, and 16.5 kg N/ha larger in CFN compared to Conv, which shows that the additional amount taken up by the biomass in the CF treated plots, accounts for the elevated amounts of N left in the soil in the Conv treated plots.

The distribution of these compounds in the soil is worth noting, with P-Al being elevated only in the top 5 cm of the soil (Table 1). By contrast, NH_4 and NO_3 were significantly elevated within the entire 20 cm, with NO_3 showing a tendency to be more elevated in the lower 15 cm. Cations, being adsorbed to the soil surface through CEC, are more stable in soils compared to anions. Phosphorus being a cation and having the specific adsorption to Al-, Fe- and Mn-oxides, therefore remains minimally mobile in the soil profile, though the specific adsorption was mostly prominent in lower pH. Ammonium is absorbed to the soil, but NO_3, on the other hand, is the most mobile element, being an anion, and thus little sorbed. Therefore NO_3 is at larger risk for loss through leaching and transport with soil water (Brady & Weil, 2010, p.404-405), coherent with the findings.

The small amounts of precipitation in the area, from the time of fertilizer application to the time of sampling, likely limited the vertical transport of nutrients within the soil profile. With the use of basins in CF practices the initial fertilizer application was thoroughly mixed into the soil, this was not done in the Conv treated plots, possibly explaining parts of the increased amounts of fertilizer compounds in the Conv plots. Another possible explanatory factor is the water harvesting quality of the basins used in CF (Goeb, 2013; Haggblade & Tembo, 2003).

The water harvesting qualities associated with basin use may therefore also contribute to a "flushing" effect, giving a larger water transport, and therefore nutrient transport. However, the "Compound D" fertilizer application also contains K, and an increased amount of K simultaneously with an increased amount of P and N would therefore be expected. However, this was not observed. Within the time frame of the experiment there were large amounts of precipitation, a total of 270mm, and a clear decrease in both NH_4 and NO_3 was recorded (Figure 2), suggesting that the increase in precipitation may have increased either the availability or loss of N. The decreasing trend observed within the time frame of the experiment for NH_4 and NO_3 suggests that the levels of NH_4 and NO_3 found in the soil under Conv were on their way to converge with the levels found in CFB and CFN. The possible physical nutrient limitation in the Conv treated plots is supported by the significantly smaller biomass found within these basins at the time of sampling. The significantly lower biomass found during the time of sampling was no longer prevalent at harvest, supporting the possibility of N being immobilized due to low precipitation, before being transported and therefore made available for plant uptake when the first major rains of the season occurred.

Besides the characteristics possibly affected by fertilizer application, some of the expected differences between CFN and Conv were not found. One of the expected impacts of practicing tillage, as done in Conv treated plots, was a decrease in SOM (Lal et al., 2007). The findings did show a significantly lower SOM content in the Conv treated plots compared to CFB treated plots, this significance was however lost when excluding the effect of biochar in the CFN plots. This may be due to the small sample size (n=3), but also due to low SOM contents across all management practices <1%, which was low even compared to highly degraded soils in arid areas (Brady & Weil, 2010, p.384). Similar levels were reported by Obia et al. (2016) and Martinsen et al. (2014), from the same study area. The entire field had also been under CF for many years before the field was converted into an experimental field, meaning that the Conv plots had only been tilled for one year at the time of this study, the SOC% in the Conv plots are therefore perhaps greater than one would expect if practiced for many years. The small increase in SOM was theorized to buffer pH, decrease BD and increase water content (Srinivasarao et al., 2015), and the amount of TOC, seeing that SOC accounted for about 58% of SOM (Pisante et al., 2015), which was also found in this study. The pH was significantly higher in CFN & CFB compared to Conv, as expected by literature, with CFB having a slightly higher mean than CFN (Srinivasarao et al., 2015). Bai et al. (2009), Wang et al. (2014) and Obia et al. (2016) also found a decrease in bulk density with CF, which was not found in this study.

Total organic nitrogen (TON) was generally low throughout the field, consistent with a low SOM, with N constituting between 1-6% of SOM (Brady & Weil, 2010, p.369). An increase in leaching of N has been found when tilling the soil, due to more of the compounds on stable aggregates being released (Lal et al., 2007). A significantly lower amount of TON, but significantly higher amount of NH_4 and NO_3 was found in the Conv treated compared to both CF treated plots, supporting the presence of possible fertilizer residue in the Conv plots. A decrease in disruption of soil aggregates, reducing the availability of the nutrients to microbes, can be a supportive factor in the found slight increases in TON in CFN and CFB (Farooq & Siddique, 2015; Govaerts et al., 2007). As expected, the effect on TON and TOC, and therefore C:N, was significantly different between all management practices, increasing as CFB > CFN > Conv.

^{15}N recovery

1 hour after application of the ^{15}N label it was expected to optimally find 100% recovery of the label in the soil. As seen in Figure 4, recovery % was consistently higher than 100%, for all management practices and for both added forms of N, 1 hour after application of the label. This was however also found in earlier studies, such as by Recous et al. (1988), where the highest found recovery was 122%, and was reasoned to be due to insecurities within several steps of the method, including uncertainties in pool estimates, %N etc., as well as possible a not perfectly homogenized distribution within the soil. In the conventional farming plots there was however an abnormally large recovery, up to 223% of the $^{15}N\text{-}NH_4$ label, and 210% of the $^{15}N\text{-}NO_3$. This indicates a possible fault with the application of the label. This may again be explained by the experimental setup of the Conv plots compared to the CFN and CFB plots. For both CF treated plots, four planting basins, with three maize plants per basin, were located on a line in the middle of each row, with the label being added to each basin. However, in the Conv plot, there were no planting basins, and the plants were therefore more spatially spread out within the line in the middle of each row, and the label was added to the entire line. Due to the spacing between basins, this means that the area where the label is added was twice as large for the Conv treated plots. To compensate for this, twice the amount of label was added, meaning that the recovery of 200% was consistent with the doubled amount of added label, but was not consistent with the intended spread of this on twice as large an area. Indicating a possible flaw in the addition of the label.

The unduly large recovery of the label in the Conv treated plots therefore makes the data hard to interpret as presented in Figure 4. However, the movement of label can be interpreted even if the addition of label was higher than intended. As shown in Table 4, the amount of added N in the Conv treated plots, even if doubled, was within the standard deviation of both NH_4 and NO_3 and should therefore still not influence the exchangeable NH_4 and NO_3 pool. Another odd observation was that for both CFB and CFN the recovery is higher 24 hours application when added as ^{15}N-NO_3, which was also found by Recous et al. (1988), likely due to the isotope not being homogenized in the soil 1 hour after application.

Most of the N in soils is organically bound, with a small amount of inorganic N (NO_3 and NH_4), which is available for direct uptake by plants. Organic N usually accounts for more than 90% of the total N pool (Harmsen & Kolenbrander, 1965). The percentage of available N of the total N pool within this study varied from 7.5% in the Conv treated plots at the start of the study, to 0.7-0.9% in the CF treated plots. The expectation was therefore that some of the added label, added as an inorganic form of N, would end up in the organic N pool, while some of the inorganic N would be taken up by the plants, some would be lost through volatile loss, as for example N_2O, and some would be lost through leaching (mostly NO_3). A loss of label with time, as seen in Figure 4, was therefore expected. As a general assumption, it is said that 1% of the N added as fertilizer is expected to escape as N_2O (De Klein et al., 2006). For all management practices, and forms of added N, except the Conv treated plots with additions of ^{15}N-NH_4, the escape was found to be higher than 1%. Especially high was the recovery in N_2O from the added ^{15}N-NO_3 in the CFN 24 hours after label addition. This may be explained by very small emissions from these plots, with a mean flux of 27.7 μg N_2O-$N/m^2/h$ (Table 7), compared the CFB and Conv treated plots, 24 hours into the experiment, meaning that a larger proportion of the N_2O had its origin in the label in the CFN plots. The sample of ^{15}N in N_2O was sampled at the time of lowest soil moisture, 24 hours after application, which may have given a smaller portion of ^{15}N in N_2O than would be found at other times.

As seen in Figure 4, the recovery of the added ^{15}N-NO_3 was generally lower than for the ^{15}N-NH_4 for both CFB and Conv, which was expected due to $NO3^-$ being a more mobile component as an anion, and therefore at larger risk of leaching, also found by Munera-Echeverri et al. (2019). In the CFN treated plots on the other hand, the recovery after 240 hours was in fact higher for the ^{15}N-NO_3, partially due to a greater fraction in the vegetation, though these differences are not significant. Figure 5 shows that the decrease in recovery was logarithmic, meaning that the loss was large immediately after application, and then started to stabilize. This was consistent with the loss being largest when a larger fraction was present as the mobile anion NO_3, before a larger proportion was incorporated into the soil organic pool or converted to NH_4 (Figure 6). The decreased amount of recovery of the ^{15}N-NO_3 with time did not vary much between management practices. However, the loss of ^{15}N-NH_4, being a cation, was expected to be affected by the soils CEC. With the addition of biochar, it was expected to see an increase in CEC (Martinsen et al., 2014), which was observed, but not significant. This increase in CEC was therefore expected to relate to a reduced loss of the NH_4 from the soil. As shown in Figure 5 the slope coefficient, and therefore loss, was largest for Conv, then CFN and lowest for CFB. Similarly, the CEC was lowest in CONV, and highest for the CFB, showing the expected abovementioned trend.

The form of N also effects the uptake in vegetation, shown in Figure 4. Though a larger recovery of the ^{15}N-NH_4 was found in vegetation in the CFN and Conv treated plots, there were lower recoveries in the vegetation than shown for the ^{15}N-NO_3. This was expected due to NO_3 being readily available for plant uptake, compared to NH_4 which needs to be further transformed before plant uptake (Mengel & Kirkby, 1978). Both the form of added N, and management practice has shown effects on the proportional distribution of the label between pools within the time frame. Especially prominent was the small proportional recovery of the ^{15}N-NH_4 in the vegetation in the CFB. This was coherent with the small loss observed in the soil for the same plots and label. This indicated that biochar reduces loss through leaching but may have increased immobilization due to the greater C:N ratio found in the soil (Table 1). However, approximately the same uptake of the ^{15}N-NO_3 was found for CFB and CFN, indicating no effect of biochar on NO_3, though NO_3 adsorption capacity has been seen to increase with the addition of biochar produced at temperatures over 600˚C (Clough et al., 2013). The similar proportional recovery in vegetation between Conv and CFN for NO_3, and to some degree NH_4, indicates no effect of CF on plant uptake.

The general expectation when adding an inorganic form of N to the soil is that some will be converted into the organic N pool, while the rest will be taken up by the vegetation or lost by leaching and gaseous loss, as seen in this study (Harmsen & Kolenbrander, 1965). However, earlier tracer studies, such as by Recous et al. (1988) done on winter wheat in France, found a much larger amount of the added inorganic forms of N, back as inorganic N, for example, up to 89% of the labeled ^{15}N-NO_3 was found back as inorganic N two days after application of the label, which was their first sampling event. A significant difference in the setup of this experiment, was that the total inorganic N pool is not measured, only the NO_3. The recovery of NO_3 in CFB and CFN are still very low compared to the findings in this study. After 192 hours Recous et al. (1988) reported a recovery of ^{15}N-NO_3 of 71%, which is significantly greater compared to this study, where already after 72 hours only a few % of the label was recovered in the NO_3 pool.

The expectation when adding the label as ^{15}N-NH_4 was that a very small fraction will be found as NO_3 1 hour after the application, since this implies that the nitrification process happened within one hour of the addition. For both CFB and Conv this was the case, but the recovery of 20% of the ^{15}N-NH_4 as NO_3 after 1 hour was unexpected. This could indicate a high nitrification in the soil, which was then taken up by the plants as NO_3, which explains the low recoveries at the following sampling events.

The large amounts of precipitation within the timeframe of the experiment, 270 mm, likely made loss through leaching, an especially important contributor to the decrease in recovery of the added label, found to be between 36-62%. A considerable amount of NO_3 was likely transported away with the precipitation, due to its mobility, but the heavy rains may also wash away large amounts of particles, and therefore the NH_4 sorbed to the soil surface. The loss of NH_4 may also be contributed to other gas emissions, due to NH_4 being a volatile compound, though likely not in considerable amounts due to a low pH of <6.4 (Brady & Weil, 2010, p.401-402). These pools were not sampled, the losses can therefore not be traced.

N_2O emissions:

The clearly elevated emissions found in the Conv treated plots were unexpected. However, as mentioned, a prominent elevated amount of NH_4, NO_3 and P-Al was observed in the same plots, due to likely residual fertilizer on the soil surface. A study conducted by Baggs et al. (2003) over two years looking into the effects of fertilizer application on N_2O emissions on tilled and non-tilled soils in England, found that the conventionally tilled soils had a delay in N_2O response to the addition of fertilizer, which they attributed to a quicker mineralization in non-tilled plots. The emissions found in the Conv treated plots in this study were very high for a not recently fertilized field (221 µg N_2O-N/m^2/h), however, if the assumption that fertilizer residue was left on the soil surface and was made available for biota during the time frame of the experiment, the emissions are similar to the emissions from recently fertilized conventionally tilled plots (peak of 234 µg N_2O-N/m^2/h), as found by Baggs et al. (2003).

A study, with a similar experimental setup on an Arenosol in Kaoma, Zambia, conducted by Munera-Echeverri et al. (2019) found significantly lower emissions from the Conv treated plots compared to CFN and CFB treated plots, with mean values of 8.4 µg N_2O-N/m^2/h in the Conv plots, 26.1 µg N_2O-N/m^2/h in the CFN treated plots and 34.2 µg N_2O-N/m^2/h in the CFB treated plots. Very similar fluxes in the CFN treated were found in this study, but both CFB and Conv are clearly higher, with the Conv treated plots being extremely elevated. With the opposite trend found for which management practice had the largest flux of N_2O, a coherent trend in the KCl was also found. Munera-Echeverri et al. (2019) found a generally higher KCl extractable N in the CF treated plots, possibly explaining the elevated emissions from these plots, as much higher KCl extractable N was found in this study along with clearly elevated N_2O fluxes in the Conv treated plots. Both soil moisture and pH were similar in to the values found in this study and did seemingly not have a large effect on N_2O emissions.

All variables tested in this study were checked for a correlation to the N_2O emissions (Table 9) when using the estimated values for the entire upper 20 cm of the soil profile. These characteristics were also checked when using the individual values from 0-5 and 5-20 cm, but only very small differences in the correlation coefficient were found, and therefore not included. All characteristics except for potassium (K+), bulk density (BD) and inorganic C correlated with the N_2O fluxes when all samplings from the field were included. NH_4, NO_3, P-AL and CEC being positively correlated to the N_2O emissions, while pH, TOC, TON, C:N, SOM and soil moisture being negatively correlated. A negative relationship TOC was unexpected, seeing that C in an important energy source for denitrifying bacteria (Burford & Bremner, 1975). The four variables, which were hypothesized to correlate with the N_2O emissions, NH_4, NO_3, pH and soil moisture, were further tested for correlation within each management practice, shown in Table 10.

Table 9. *Results from Spearman´s rank correlation test between the emissions of N_2O and all soil characteristics in Table 1, Gravimetric soil moisture and WFPS. * Indicates significant correlation.* [15]

Soil characteristics	Depth (cm)	n	p-value	ρ
NH_4 (mg/kg)	0-20	107	6.8E-04*	0.522
NO_3 (mg/kg)	0-20	106	1.2E-05*	0.578
pH	0-20	213	2.2E-16*	-0.636
P-Al mg/kg	0-20	213	6.2E-12*	0.448
CEC $cmol_c$/kg	0-20	213	5E-08*	0.363
K+ $cmol_c$/kg	0-20	213	0.1446	-0.1
BD g/cm^3	0-20	135	0.6519	-0.039
TOC %	0-20	135	6.6E-05*	-0.341
TON %	0-20	135	8E-04*	-0.151
C:N	0-20	135	5.1E-06*	-0.387
In. C %	0-20	213	0.4509	-0.052
SOM %	0-20	213	4.4E-08*	-0.338
Gravimetric soil moisture	0-20	133	9.3E-04*	-0.284
WFPS (%)	0-20	133	5.2E-04*	-0.297

Table 10 shows that the significant correlation was not found within each management practice, except for in the CFN treated plots, with pH, this is due to little variation in pH as illustrated in Figure 10. In Figure 10a) and b) the Conv plots show to be responsible for most of the high flux values, and all the highest values of NO_3 and NH_4. The elevated fluxes of N_2O found in plot 6 (Figure 8) were shown not to have comparatively high values of NH_4 nor NO_3. Figure 10c) shows that the spread in observations was generally very low in the CFN, a bit larger for CFB, but very large in Conv. In Figure 10a) the higher N2O fluxes were found at lower pH (Conv). The elevated fluxes of N_2O found in plot 6 had a lower pH compared to the other CFB plots. Figure 10d) shows that all elevated fluxes (>100 µg N_2O-N/m^2/h) had a WFPS under 50%, with the larger fluxes being found with decreasing WFPS%. Figure 10e) shows that the significant negative correlation found was highly impacted by the elevated

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[15] All values obtained as bulked samples were assumed to be constant throughout the addition of label and time frame of the experiment, and the 9 values (for each plot) were therefore used for all pairs.

Conv levels and affected by the elevated levels in some of the CFB plots, which could disrupt the trend due to the TOC in the added biochar mainly being non-labile organic C and cannot be used by organisms to get energy.

Table 10. *Results from Spearman's rank correlation test between the emissions of* N_2O *and the average estimated amount of* NH_4, NO_3, *pH and WFPS in the 0-20 cm depth of the soil, for all data points combined, and for each individual management practice. *indicates significant correlation.*

Management practice		NH4		NO3			pH			WFPS (%)	
	n	**p-value**	***ρ***	**p-value**	***ρ***	**n**	**p-value**	***ρ***	**n**	**p-value**	***ρ***
All	107	6.8E-4*	0.52	1.2E-5*	0.58	213	2.2E-16*	-0.64	133	5.2E-04*	-0.29
CFB	35	0.938	0.01	0.978	0.01	72	0.089	-0.20	45	0.884	-0.02
CFN	36	0.264	-0.19	0.088	0.29	70	0.022*	-0.27	44	0.140	0.23
Conv	36	0.152	0.24	0.212	0.21	70	0.149	0.17	44	0.126	-0.24

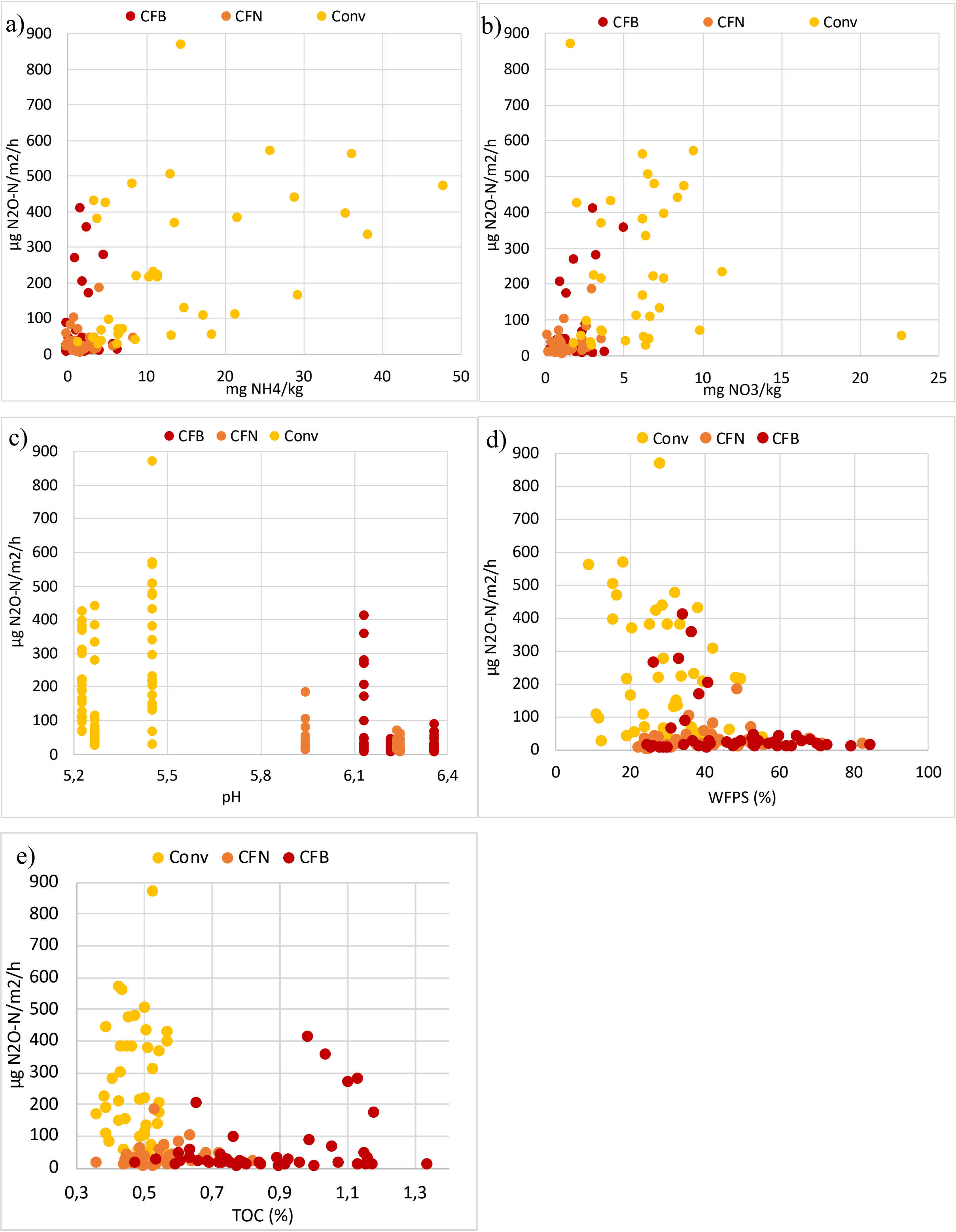

Figure 10. *Showing spread in data between the relationship in N_2O fluxes and a) NH_4, b) NO_3, c) pH, d) WFPS and e) TOC within and between management practices.*

Though the possible fertilizer residue in the Conv plots may have greatly affected the emissions of N_2O from these soils, significant relationships were found between the N_2O emissions and several soil characteristics not directly impacted by this residue (Table 9). Among these the amount TOC and TON, and the ratio between these C:N, was found to have a negative relationship with the N_2O emissions. Usually the expectation would be that there was a positive relationship, seeing that C is a needed substrate for the biota conducting both denitrification and nitrification. From Figure 10e the decreasing trend was clearly affected by the elevated emissions of N_2O found in the Conv treated plots. If the speculations about residual fertilizer is true, other factors, in this case likely the large amounts of NH_4/NO_3 in the Conv plots, may have affected the relationship by increasing N_2O emissions, despite of low TOC. The implementation of tillage has also shown to release more of the of C & N from the soil's protective pools (Govaerts et al., 2007), due to destruction of aggregates, which could have depleted the TOC in these soils. When not considering the Conv treated plots, all elevated N_2O emissions in the CFB plots were found to have higher amounts of C, possibly contributing to the reason why some plots have elevated N_2O emissions compared to the others. The same reasoning applies to the TON, the C:N ratio and the SOM content.

One of the best correlations in this study was found with both forms of available; NH_4 and NO_3. However, the correlation was not found within each management practice. From Figure 10 it's clear that it was the generally higher amount of NO_3, NH_4 and N_2O emissions from the Conv plots that dominated the scatter from all data points. This therefore indicates that a large increase in the amount of available forms of N as NO_3 and NH_4 will give large increases to the N_2O fluxes, but within the amount there may be large variation in N_2O responses. A similar trend was found by Baggs et al. (2003) where the N_2O emissions were higher in the fertilized plots with significantly higher amounts of available N compared to non-fertilized plots, but with no significant relationship between the emissions of N_2O and the amount of available N within the fertilized plots.

The other strong correlations with the N_2O emissions are the pH and WFPS (Table 9). The negative relationship between the N_2O emissions and WFPS indicates that the N_2O emissions were mainly dominated by nitrification, seeing that the denitrification happens under anaerobic conditions(Brady & Weil, 2010, p.403). This can also be indicated by the negative relationship with pH, seeing that nitrification is an acidifying process, possibly explain the lower pH in the plots with elevated N_2O emissions (Brady & Weil, 2010, p.402-403). This is also consistent with the findings of Bateman and Baggs (2005) who found that nitrification was the main N_2O emitting process at WFPS between 35-60%, while denitrification was the main contributor when the WFPS was above 70%, WFPS in this field was, with few exceptions far under 70%.

One of the expectations from literature was a decrease in N_2O emissions with the addition of biochar, due to the majority of studies on biochar's effect on the emissions of N_2O have shown that the addition suppresses some of the emission, though a few cases have found no effect or even increased N_2O emissions (Kammann et al., 2017; Spokas & Reicosky, 2009). In this study the latter was found, with no effect of biochar found for a majority of the plots. However, for two sampling spots, plot 6 in the NO_3 and NH_4 labeled spots, large increases of 456 – 1066% were found. One of the suggested explanations for the cases with highly elevated emissions with the addition of biochar is that the increase in pH may increase soil N mineralization and nitrification and that the increase in soil aeration may also increase nitrification, in turn resulting in more N_2O emissions (Kammann et al., 2017). The plots with elevated emissions had the lowest WFPS found in the CFB plots, supporting the explanation of increased soil aeration possibly stimulating nitrification and therefore N_2O emissions.

AT% ^{15}N of N_2O; indication of the source process of N_2O production

As mentioned, the emissions of N_2O can be a result of either nitrification or denitrification. Figure 11 shows that the AT% ^{15}N excess in the NO_3 when added as ^{15}N-NH_4 was lower than when added as ^{15}N-NO_3, as expected. However, the AT% ^{15}N excess in N_2O was not much lower when added as ^{15}N-NH_4 compared to when added as ^{15}N-NO_3. The use of the ^{15}N label to estimate which process was dominant is done in a multitude of ways (Stevens et al., 1997), one of which is by looking at the ratio between the AT% found in the N_2O and the AT% found in the KCl extractable NO_3. If the AT% in the N_2O is found to be as large as the AT% found in the KCl extractable NO_3, when the label is added as ^{15}N-NO_3, it implies that the all the N_2O originates from NO_3, then implying denitrification. However, the ratio found showed that the signal was higher in the AT% found in the N_2O compared to the AT% found in the KCl extractable NO_3, which should not be possible. There are several possible reasons for this, firstly the ^{15}N in the N_2O was only sampled at one time, 24 hours after application of the label, giving very few data points. Also, the AT% in the NO_3 was obtained from using the denitrifier method, increasing the amount of uncertainty of the values. The assumption made when interpreting Figure 11 is therefore that the possible errors are systematic and if there is a relationship between increases in the AT% found in the N_2O and the AT% found in the KCl extractable NO_3, the relationship would still be evident.

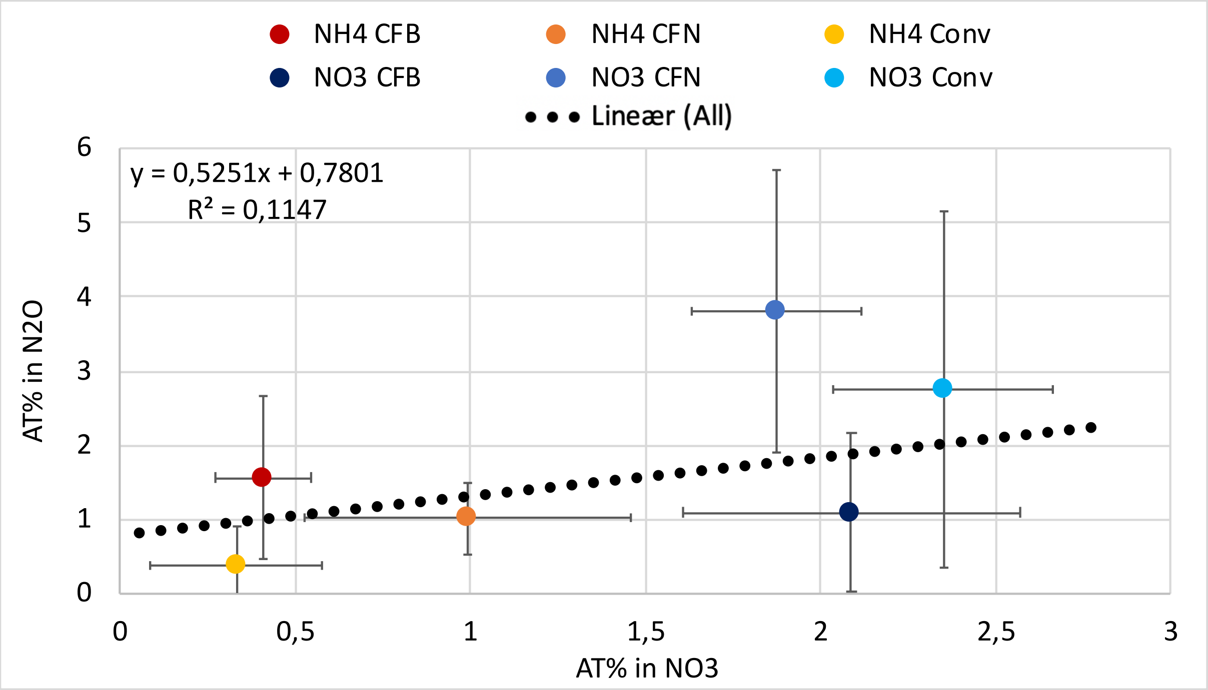

Figure 11. Relationship between AT% ^{15}N excess in the N_2O and AT% ^{15}N excess in the KCl extractable NO_3 for each management practice and both forms of added ^{15}N, shown as mean values with standard deviations (n=3). Regression line and equation is run for all data points (p=0.1263, n=18).

Figure 12 shows the lack of a significant relationship between the gross nitrification rates, between 24-72 after application of the ^{15}N label, and the averaged N_2O emissions within those time periods. When using the gross nitrification rates between 24-72 hours after application of the ^{15}N label, and the averaged N_2O emissions within those time periods no sign of a significant relationship was found, suggesting that nitrification was not a large contributor to N_2O emissions. However, due to the small sampling pool, and possible measuring errors, it is not possible to safely say which process is the main source of N_2O.

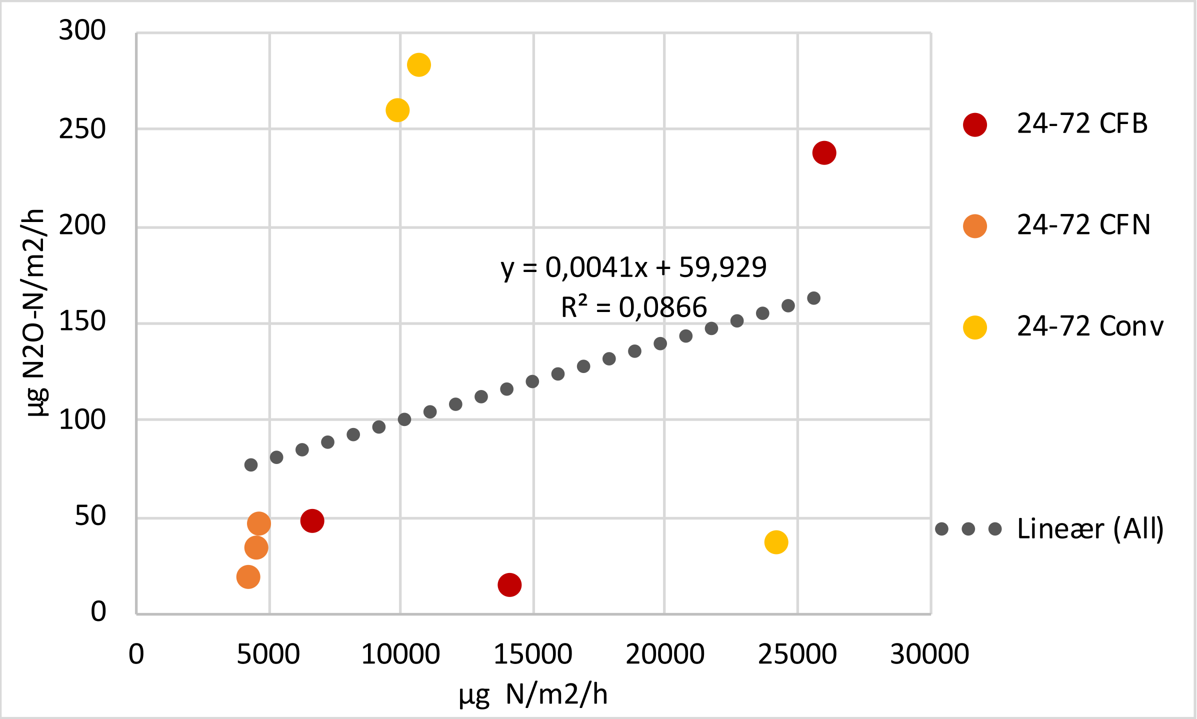

Figure 12. *Relationship between the N_2O flux (µg N_2O-N/m^2/h) and the gross nitrification rate (µg N/m^2/h) at 24-72 after application of the label for all management practices. Linear regression is run on all data (p=0.46, n=18).*

Further research

To be able to conclude with the processes behind the N_2O emissions a larger sample size and several sampling days throughout a longer period of time, should be done. To understand why the large amount of residual N in the Conv treated plots remained in the soil such a long period after application of the fertilizer a long-term drought experiment in a similar soil should be conducted, possibly showing another positive of CF in drought-prone areas.

Conclusions

The use of CF showed to increase both water retention and pH but did not show to have the intended effect on SOM nor bulk density. The ^{15}N tracer experiment showed that there was no significant effect of added form of ^{15}N on the loss within the soil profile. However, a significant effect of the label was found in the uptake of label in plants, with uptake of ^{15}N-NO_3 being significantly larger in CFB and Conv. There was also a significant difference between the ^{15}N uptake in biomass between management practices, with a significantly higher uptake of ^{15}N-NH_4 in CFN compared to in CFB. For ^{15}N-NO_3 a significantly higher uptake was found for Conv compared to CFB. In the N_2O no difference was found between added forms of label, but a significantly higher recovery was found in the CFN treated plots when the label was added as ^{15}N-NO_3.

The N_2O emissions showed to be within expected values in the CF treated plots, but the Conv treated plots showed a large deviation from literature. The extremely elevated emissions in N_2O in the Conv treated plots were found to correlate best to the amount of KCl extractable NH_4 and NO_3 present in the soil, as well as the pH in the plots. We are not able to quantify the relative contribution of nitrification or denitrification based on the data analyzed. However, the WFPS being >70%, the positive relationship between KCl-extractable NH_4 and the positive relationship between gross nitrification rates and N_2O are possible indicators of nitrification being an important N_2O emitting process. Albeit, the stronger positive relationship between AT% ^{15}N excess in the N_2O and AT% ^{15}N excess in the KCl extractable NO_3 and the very high N_2O fluxes found in the Conv treated plots, indicate that denitrification is an important N_2O emitting process. Further research with the use of several sampling days of ^{15}N in N_2O would need to be done to conclude.

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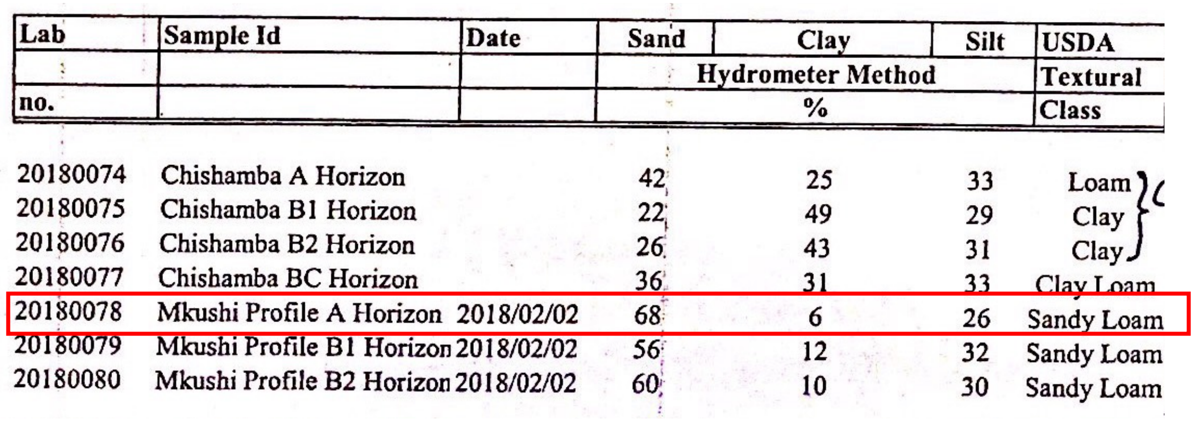
Supportive information:

Appendix 1.1 Correction factors for LOI with different clay contents. Source: (Krogstad, 1992).

Soil type	Clay content	Correction figure
Sand and silt	5-9%	1
Light clay	10-24%	2
Medium clay	25-39%	2.5
Stiff clay	40-59%	3.5
Very stiff clay	>59%	4.5

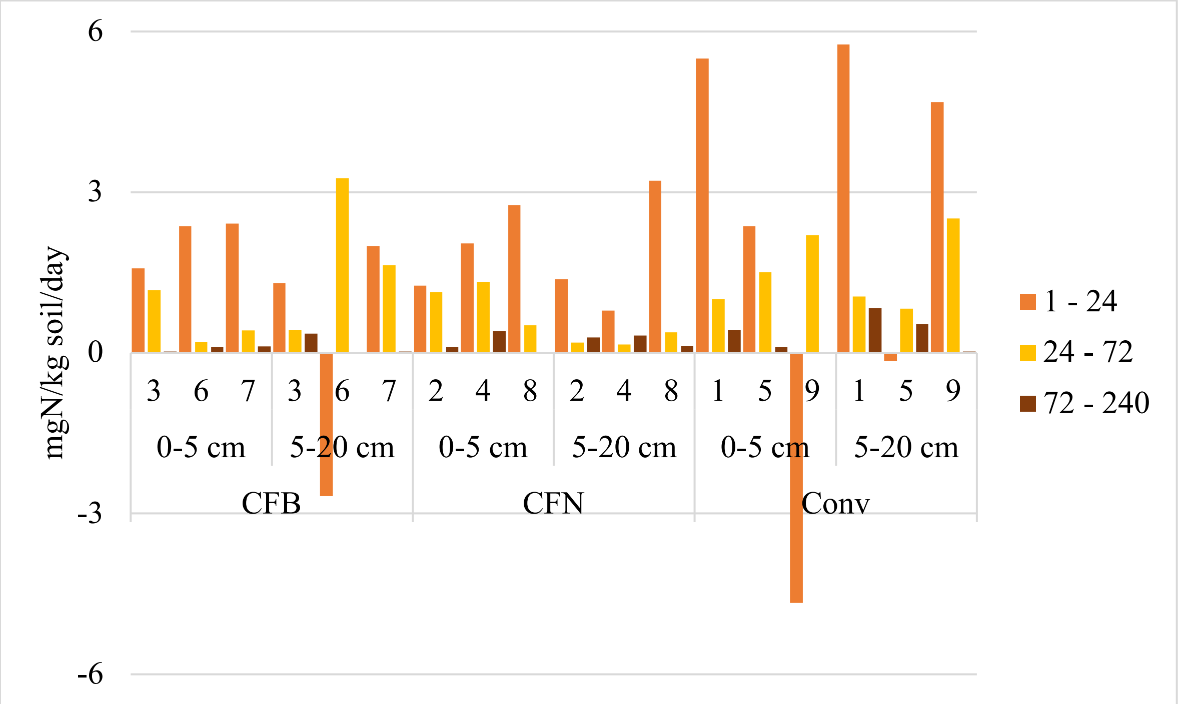
Appendix 1.2 Clay content found in previous sampling of the soil by UNZA. Red outline shows the relevant site, horizon and clay content.



Lab no.	Sample Id	Date	Sand	Clay	Silt	USDA Textural Class
			Hydrometer Method			
			%			
20180074	Chishamba A Horizon		42	25	33	Loam
20180075	Chishamba B1 Horizon		22	49	29	Clay
20180076	Chishamba B2 Horizon		26	43	31	Clay
20180077	Chishamba BC Horizon		36	31	33	Clay Loam
20180078	Mkushi Profile A Horizon	2018/02/02	68	6	26	Sandy Loam
20180079	Mkushi Profile B1 Horizon	2018/02/02	56	12	32	Sandy Loam
20180080	Mkushi Profile B2 Horizon	2018/02/02	60	10	30	Sandy Loam

Appendix 2. Comparison of TOC found through analysis of bulked samples, analyzed at NMBU and the TOC data obtained through the isotopic analysis of soil at UC Davis.

Management practice	**Depth**	**TOC (%) NMBU n=3**	**TOC % UC Davis n=15**
CFB	0 - 5 cm	0.85 ± 0.04	0.8 ± 0.18 ^{a}
	5 – 20 cm	0.92 ± 0.18	0.89 ± 0.25 ^{a}
CFN	0 - 5 cm	0.61 ± 0.09	0.59 ± 0.13 ^{b}
	5 – 20 cm	0.55 ± 0.05	0.53 ± 0.09 ^{bc}
Conv	0 - 5 cm	0.49 ± 0.02	0.46 ± 0.07 ^{c}
	5 – 20 cm	0.51 ± 0.03	0.48 ± 0.06 ^{c}



Appendix 3. Calculated gross nitrification rates (mg N/kg/day) at the two sampled depths 1-24, 24 - 72 and 72 - 240 hours after application of the ^{15}N label.

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