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Philosophiae Doctor (PhD), Thesis 2015:100

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Faculty of Environmental Science and Technology
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Impact of biochar on soil physical characteristics and greenhouse gas emissions

Effekt av biokull på jordfysiske egenskaper og klimagassutslipp

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Summary

Climate change and production of adequate amounts of food to feed the growing human population are key challenges facing the modern world. These two challenges often involve a trade-off between solving the one, while exacerbating the other. Application of biochar (BC) in agriculture has been suggested to be a win-win strategy for both climate change mitigation and increased crop production. Biochar is a carbon-rich, alkaline material, produced by heating biomass in a limited oxygen environment. In soil, BC is relatively stable and therefore it has a potential to contribute to carbon sequestration. The motivation for adoption of BC technology for use in acidic, coarse-textured soils particularly in the tropics, lies mainly in the ability of BC to increase crop production at low cost, thus contributing to food security, while the benefit of climate change mitigation remains in the background.

The increase in crop production by BC depends on the extent to which it can improve soil quality. Previous studies mainly focused on the effect of BC on soil chemistry and crop nutrition, whereas the effects on soil physical properties have received less attention. Therefore, the first part of this thesis (papers I and II) focuses on the effect of BC on soil physical properties. Previous studies, conducted mainly in laboratory and greenhouse, reported improved soil structural and hydraulic properties. Here, I report results from a field study conducted in three soils in Zambia, including a sandy loam under conservation farming amended with unsorted maize cob BC, and a loamy sand and a sand under conventional farming, both amended with maize cob BC, sorted into three particle size fractions (≤0.5 0.5–1 and 1–5 mm). Both the loamy sand and the sandy loam (Acrisols) were from Mkushi, Zambia, while the sandy soil (Arenosol) was from Kaoma, Zambia.

In planting basins in the sandy loam under conservation farming for two years, BC increased aggregate stability by 7–20% per weight percent BC added to soil ($p<0.05$). This effect was stronger under soybean than under maize, after two growing seasons. Plant available water increased by 3% per percent BC added ($p<0.05$) under both crops, whereas bulk density decreased by 3–5% per percent BC added ($p<0.01$).

After one growing season, plant available water increased significantly in response to the addition of BC with size fractions of ≤0.5 and 1–5 mm by 7–9% per percent BC in both loamy sand and sand. By contrast, BC of 0.5–1 mm had no effect on plant available water.

Biochar-induced increase in aggregation in the loamy sand resulted in a decrease in penetration resistance of the surface soil (-2.9±0.6 N cm^{-2} per percent BC added), irrespective of BC size fraction. By contrast, the penetration resistance in the sand was not significantly affected by BC addition. Reduced bulk density and penetration resistance due to BC-induced increase in soil aggregation, may aid root growth and water retention, both important for crop production. Biochar significantly reduced saturated hydraulic conductivity (K_{sat}, $p<0.05$) in the loamy sand below the surface crust by 0.17±0.07 cm hr^{-1} per percent BC added, but not in the sand two years after BC application. Since the BC amended loamy sand showed no water repellency, reduction in K_{sat} is most likely due to clogging of soil pores by BC or collapse of soil structure at near-water saturation. A crust formed at the surface of the loamy sand, irrespective of BC addition. The crust showed increased water repellency only in response to the finest BC fraction, whereas the two coarser BC fractions resulted in decreased water repellency. Increased repellency of the crust, due to fine BC, increases the risk of reduced water infiltration and increased surface runoff, which in sloping terrain may cause soil erosion.

Since BC may be lost from the root zone of the soil, either by leaching or by lateral transport through erosion, BC transport in the loamy sand and the sand was quantified (Paper III). Two size fractions of BC, produced from rice husk and maize cobs, were applied to the top 5 cm of both soils. Rice husk BC and maize cob BC (having $\delta^{13}C$ contents of -27.1‰ and -12.3‰, respectively) were traced in the loamy sand and sand, which had a $\delta^{13}C$ content of -18.9‰ and -20.8‰, respectively. The downward migration of BC after one year was confined to within 3 cm below the application depth. There was a tendency of greater downward migration of BC in loamy sand than in sand and for finer than for coarser size fractions of the BCs. Of the applied BC, 45–66% was recovered in the upper 5 cm of the soil to which it had been applied. Of the remaining BC, 10–20% was recovered in the deeper layers down to 20 cm depth, leaving 24–45% unaccounted for in the soil profile. A significant proportion of the non-recovered BC was found in the adjacent reference plots, indicating lateral transport, probably due to wind and water erosion.

Besides the potential of BC to mitigate climate change through carbon sequestration, BC has been reported to cause a reduction in the emission of N_2O, an important greenhouse gas. In Paper IV, the effect of BC on microbial denitrification, the quantitatively most important source for N_2O emissions from soils was studied. Since BC is mostly alkaline, the pH increasing effect in soil may affect the product ratio of intermediates (NO and N_2O) relative to the final product N_2, which could be an explanation for the N_2O suppressing effect of BC. Laboratory experiments were conducted with anoxic slurries in serum bottles of the acid sandy loam (Acrisol) from Mkushi, Zambia and for comparison an Acrisol from Lampung, Indonesia. Two BCs, produced from rice husk and cacao shell, respectively, were added at increasing doses. The added BCs were untreated, as well as water- and acid-leached. Water- and acid-leaching decreased its alkalizing effect. Uncharred cacao shell and sodium hydroxide (NaOH) were used for comparison. Like NaOH, non-leached BCs suppressed N_2O and NO production and increased N_2 production, irrespective of the effect on denitrification rate. The extent of N_2O and NO suppression was dose-dependent and increased with the alkalizing effect of the two BCs. Acid leaching of BC reduced or eliminated the ability of BC to suppress the net production of N_2O and NO. Although, the N_2O/N_2 product ratio was largely determined by the soil pH, increasing doses of BC resulted in sharper decline in the ratio than predicted from soil pH change alone, suggesting that BC triggers additional N_2O suppressing mechanisms, which are not yet fully identified. Addition of uncharred cacao shell stimulated denitrification due to the addition of labile carbon, but only minor effects on the N_2O/N_2 ratio were observed in accordance with its modest effect on soil pH.

Sammendrag

Klimaendring og produksjon av tilstrekkelige mengder med mat for en voksende befolkning er sentrale utfordringer verden står overfor. Disse utfordringer innebærer en avveining hvor en løsning for det ene kan være negativt for det andre. Bruk av biokull (BC) i landbruket har blitt nevnt til å være en win-win strategi som kombinerer klimaendringstiltak og økt matproduksjon. Biokull er et karbonrikt, alkalisk materiale, produsert ved å brenne biomasse ved begrenset oksygentilgang. I jord er BC relativt stabilt slik at det har potensial til å bidra til karbonbinding. Begrunnelsen for en vellykket bruk av BC i surt jordsmonn med grov tekstur, særlig i tropene, ligger hovedsakelig i BCs evne til å kunne øke avlinger ved lave kostnader, slik at det kan bidra til å bedre matvaresikkerhet. Bidraget til å motvirke klimaendring kommer ofte i andre rekke.

Økning i avlinger gjennom bruk av BC er avhengig av BCs mulighet til å forbedre jordas kvalitet. Tidligere forskning har i hovedsak fokusert på effekter av BC på jordkjemi og planteernæring, mens effekter på jordfysiske egenskaper har fått mindre oppmerksomhet. Derfor er den første delen av denne avhandlingen (papers I and II) rettet mot effekter av BC på noen viktige jordfysiske egenskaper. Tidligere undersøkelser, først og fremst gjennomført på laboratoriet og i veksthus, har funnet en forbedring i jordstruktur og hydrologiske egenskaper. Her, rapporterer jeg resultater fra et feltforsøk gjennomført i tre jordarter i Zambia under både konvensjonell og conservation (lite jordarbeiding) landbruk. De utvalgte jordtyper inkluderer en sandig lettleire som ble tilført usortert BC fra maiskolber og en siltig finsand og finsand tilført BC fra maiskolber, sortert i tre ulike størrelsesfraksjoner (≤0.5 0.5–1 and 1–5 mm). Både sandig lettleire og siltig finsand (Acrisols) er i Mkushi, Zambia, mens finsanden (Arenosol) er i Kaoma (Zambia).

I plantebassengene i sandig lettleire førte BC til økt stabilitet av jordaggregatene med 7–20% per prosent BC tilført ($p<0.05$). Effekten var større under soyabønner enn under mais, etter to vekstsesonger. Plantetilgjengelig vann økte med 3% per prosent BC tilført ($p<0.05$) for begge vekster, mens jordas tetthet minsket med 3–5% per prosent BC tilført ($p<0.01$).

Etter en vekstsesong, økte plantetilgjengelig vann signifikant med 7–9% per prosent BC i både siltig finsand og finsand som følge av tilførselen av de to minste BC fraksjonene

(≤0.5 and 1–5 mm). I motsetning til de minste BC fraksjonene hadde den grove BC fraksjonen (0.5–1 mm) ingen effekt på plantetilgjengelig vann. Den BC-indiserte økning i aggregering i siltig finsand førte til minskning av inntrengningsmotstand (penetration resistance) i overflatejord (-2.9 ± 0.6 N cm^{-2} per prosent BC tilført), uansett BCs størrelsesfraksjon. I finsand derimot ble inntrengningsmotstand ikke signifikant påvirket av BC. Den avtagende tetthet og inntrengningsmotstand i jord, som følge av BC-indusert aggregering, kan forbedre rotvekst og vannretensjon, som begge bidrar til å øke avlingene. To år etter tilførselen, førte BC til en signifikant minskning av vannledningsevnen under mettede forhold (K_{sat}) ($p<0.05$) i siltig finsand, under en tynn overflateskorpe dannet gjennom vekstsesongen, med 0.17 ± 0.07 cm hr^{-1} per prosent BC tilført. En slik effekt av BC ble ikke observert i finsand. Fordi siltig finsand, tilført BC, ikke viste vannavvisning, er den avtagende K_{sat} mest sannsynlig en følge av blokkering av jordas porer med BC partikler eller av et sammenbrudd av jordas struktur ved nær-vannmetning. Skorpedannelsen fant sted på overflate av siltig finsand, uansett BC tilførsel. Skorpen hadde økende vannavvisning bare ved den minste BC fraksjonen, mens de to grovere fraksjonene førte til mindre vannavvisning i jorda. Økt vannavvisning av skorpa øker risiko for redusert vanninfiltrasjon, som på skråninger lett kan føre til erosjon.

Siden BC etterhvert kan bli borte fra jordas rotsone, enten gjennom utvasking eller gjennom lateral transport via erosjon, undersøkte jeg transport av BC i både siltig finsand og finsand (Paper III). To størrelsesfraksjoner av BC, produsert av risskall og maiskolber, ble tilsatt de øvre 5 cm av begge jordtyper. Risskall BC og maiskolbe BC (med $\delta^{13}C$ innhold av hhv. -27.1‰ and -12.3‰) ble målt på ulik dyp i begge jordtyper, som har $\delta^{13}C$ innhold av -18.9‰ and -20.8‰ i hhv. siltig finsand og finsand. Vertikal transport av BC ett år etter tilførsel, ble begrenset til 3 cm under det sjiktet der BC hadde blitt tilsatt. Det var en tendens til litt større transport av BC i siltig finsand enn i finsand, og det fineste BC ble transportert litt lenger nedover enn det grovere BC. Av det tilsatte BC, ble 45-66% funnet igjen i de øvre 5 cm hvor det hadde blitt tilført. Videre ble 10-20% funnet tilbake mellom 5 og 20 cm dyp, slik at 24-45% hadde blitt borte. En signifikant del av dette ble målt i toppsjiktet av forsøksflatene like ved siden av. Dette tyder på lateral transport, mest sannsynlig med vind og vann.

I tillegg til BCs bidrag til karbonsekvestrering, et sluk for CO_2, finnes det antydninger til at BC kan føre til minskning av utslipp av N_2O, en viktig klimagass. I Paper IV, har jeg undersøkt mekanismene ansvarlig for den ofte rapporterte minskning av N_2O utslipp i jord etter tilførsel av BC. Siden BC er alkalisk, øker det jordas pH, noe som kan påvirke denitrifsering, en prosess der N_2O og NO dannes som produkter i tillegg til N_2. Laboratorieforsøk ble gjennomført med anoksiske suspensjoner i serumflasker med sur siltig finsand (Acrisol) fra Mkushi, Zambia og til sammenlikning med en Acrisol fra Lampung, Indonesia. To BCs, produsert av hhv. ris- og kakaoskall, ble tilført i økende mengder. Tilført BC var både ubehandlet og vasket med vann eller sterk syre. Vasking med vann og syre minsket BCs alkaliske og dermed pH-økende effekt. Ubehandlet kakaoskall og natriumhydroxid (NaOH) ble brukt til sammenlikning. På samme måte som NaOH, førte de ikke-vaskede BCs til minskning av N_2O og NO produksjon, mens den av N_2 økte, uansett effekten på selve denitrifiseringshastigheten. Minskning av både N_2O og NO produksjon var doseavhengig og økte med den alkaliserende effekten av BC. Syrebehandling av BC reduserte eller eliminerte BCs evne til å undertrykke produksjonen av N_2O and NO. Selv om N_2O/N_2 produktforholdet i hovedsak ble kontrollert av pH, hadde BC en liten tilleggseffekt, som førte til en noe skarpere avtagelse av forholdet ved økt pH enn ved NaOH tilførsel. Tilsetning av ikke-forkullet kakaoskall stimulerte denitrifisering, på grunn av det økte labile karbon, men effekten var liten på N_2O/N_2 forholdet. Dette var som forventet siden effekten på pH var liten.

List of papers

I. **In situ effects of biochar on aggregation, water retention and porosity in light-textured tropical soils**

Alfred Obia, Jan Mulder, Vegard Martinsen, Gerard Cornelissen, Trond Børresen

Soil & Tillage Research 155 (2016) 35–44

II. **Effect of biochar on crust formation, penetration resistance and hydraulic properties of two coarse-textured tropical soils**

Alfred Obia, Trond Børresen, Vegard Martinsen, Gerard Cornelissen, Jan Mulder

Under review in Soil & Tillage Research

III. **Vertical and lateral transport of biochar in light-textured tropical soils**

Alfred Obia, Trond Børresen, Vegard Martinsen, Gerard Cornelissen, Jan Mulder

Under review in Soil & Tillage Research

IV. **Effect of soil pH increase by biochar on NO, N_2O and N_2 production during denitrification in acid soils**

Alfred Obia, Gerard Cornelissen, Jan Mulder, Peter Dörsch

PLoS ONE 10(9): e0138781. doi:10.1371/journal.pone.0138781

1. Introduction

Among the key challenges facing the modern world are climate change and production of adequate amounts of food to feed the growing human population. Generally, this is considered to involve a trade-off where attempting to solve one problem, causes a negative impact on the other (Montzka *et al.*, 2011, Hasegawa *et al.*, 2015). In contrast, application of biochar (BC) to cultivated soils has been suggested to be a strategy where climate change mitigation can be combined with increased crop production (Lehmann *et al.*, 2006). The motivation of farmers for adoption of BC technology for use in acidic tropical soils lies mainly in the ability of BC to increase crop production leaving the benefit of climate change mitigation in the background.

Biochar is a carbon-rich material, produced by heating biomass in a limited or no oxygen environment, in a process called pyrolysis. Once applied to soil, BC can lock up the biologically sequestered atmospheric CO_2 (Lehmann *et al.*, 2006, Gurwick *et al.*, 2013, Kuzyakov *et al.*, 2014), thereby effectively removing carbon (C) from its active pool. Biochar has been estimated to last in soil in the range of tens to thousands of years (Schmidt *et al.*, 2011, Gurwick *et al.*, 2013). However, a small fraction of BC does not contribute to long-term C sequestration in soil, due to quick decomposition (the so-called labile fraction), which is higher for BCs produced at relatively low temperatures (Zimmerman *et al.*, 2011). This decomposition of BC involves often less than 5% of its initial mass within the first year of its application to soil and the decomposition rate of this pool decreases with time (e.g. Major *et al.*, 2010, Luo *et al.*, 2011, Zavalloni *et al.*, 2011, O'Toole *et al.*, 2013, Kuzyakov *et al.*, 2014). Because of the high stability of BC, its application to soil can contribute to curbing the increasing CO_2 concentration in the atmosphere and the associated rise in global temperature (IPCC, 2007). Besides CO_2, BC has also been reported to reduce soil emissions of nitrous oxide (N_2O) (Clough *et al.*, 2013, Cayuela *et al.*, 2014 and references therein) and methane (CH_4) (Liu *et al.*, 2011, Feng *et al.*, 2012). Mechanisms to explain these observations are not well understood (Lehmann *et al.*, 2011). Indeed, some studies even found increases in N_2O emission upon BC application to soil (Clough *et al.*, 2010, Singh *et al.*, 2010) and also with respect to CH_4 emission some studies have reported increases (Zhang *et al.*, 2010, Zhang *et al.*, 2012). N_2O and CH_4 are powerful greenhouse gases, with a radiative forcing 300 and 25 times the one of CO_2 on a 100-year basis, respectively, and their atmospheric concentrations are on the increase (IPCC, 2007). In addition, nitric oxide (NO), a gaseous intermediate of nitrogen cycling may be sensitive to BC addition. Although NO is an important regulator in many biological processes including denitrification (Nadeem *et al.*, 2013), it is a known pollutant in the lower atmosphere (Crutzen, 1970). So far, few studies, e.g. Nelissen *et al.* (2014), have examined the effect of BC on NO and found its production to be suppressed.

In addition to reducing the emission of greenhouse gases, the benefits of BC addition to soils may include a positive effect on crop production. However, these effects are not consistent, as even decreases of crop yield have been observed (Glaser *et al.*, 2002, Lehmann *et al.*, 2006, Atkinson *et al.*, 2010, Jeffery *et al.*, 2011). Increases in crop production after addition of various BCs are generally found in acidic, sandy soils with low cation exchange capacity (CEC) (Jeffery *et al.*, 2011). In this respect BC from biosolids may be an exception, probably due to the high amounts of contaminants such as heavy metal (Bridle & Pritchard, 2004). Characteristics of BC, which depend on feedstock and pyrolysis condition are important for their effect on soil properties and hence their potential to increase crop production (Sohi *et al.*, 2010, Jeffery *et al.*, 2011). The effect of BC on soil properties also depends on the soil type to which the BC is applied. Since BC is largely resistant to decomposition in soil, its potential to increase crop production may persist for a long time as compared to uncharred crop residues. This has been observed in Amazonian Anthrosols (Atkinson *et al.*, 2010) where BC has been intentionally applied to soils around 1775±325 years ago by pre-Columbian native populations (Glaser *et al.*, 2001) forming the so-called *Terra Preta* or Amazonian Dark Earth. Studies have found these soils to be more fertile with higher organic C contents than neighboring soils, which did not receive BC (Glaser *et al.*, 2001, Glaser *et al.*, 2002, Lehmann *et al.*, 2007, Sohi *et al.*, 2010). The high fertility and apparent stability of *Terra Preta* soils to date has sparked much of the current interest in BC for soil amelioration. Beyond climate change mitigation and increase in crop production, BC is also important for organic waste management and energy production, all of which add up to potentially large societal benefits (Lehmann & Joseph, 2009).

1.1 Properties of biochar

Biochar properties that determine its role in mitigating climate change and increasing crop production are of physical and chemical nature. The recalcitrant fused aromatic C, which forms large part of BC, determines the BC stability in soil, important for mitigation of climate change. Another key property of BC is its high porosity and associated high surface areas (Mukherjee & Lal, 2013). Mukherjee *et al.* (2011) and Budai *et al.* (2014) reported increases in the porosity and surface area of BC with increase in pyrolysis temperature, due to loss of volatile organic matter that would otherwise clog the BC's micro-pores with diameters in the nanometer range. The surface area reached a maximum at a temperature of around 600 °C followed by a decrease at higher temperatures, likely due to collapse of pore structures (Budai *et al.*, 2014). Braida *et al.* (2003) found that a large fraction of the total porosity of BC of up to 50% consisted of very fine pores in the nano- and micrometer range for maple-wood shavings pyrolyzed at 400 °C. The high porosity of BC resulted in low bulk density, in the range of 0.2–0.7 g cm^{-3} (Abdullah & Wu, 2009). In BCs, pores with sizes >50 nm are considered macro-pores, important in altering soil water characteristics (Atkinson *et al.*, 2010) relevant for crop production such as plant available water and hydraulic conductivity. However, in the short term, the hydrophobic nature of BC may compromise BCs' effect on soil hydraulic properties (Jeffery *et al.*, 2015). The hydrophobicity of BC, which is caused by hydrophobic compounds identified as semi-volatile organics, can be easily lost through percolating water (Yi *et al.*, 2015). These hydrophobic volatile organics are likely destroyed at higher pyrolysis temperatures and this could be the reason for lower water repellency of high temperature BCs (Jeffery *et al.*, 2015, Khanmohammadi *et al.*, 2015).

Besides the surface area, characteristics such as surface charges and functional groups are also important in determining the BCs' functions in soils. Mukherjee *et al.* (2011) found total functional group acidity in the range of 4–8 mmol g^{-1} BC made from oak, pine and grass at a temperature series of 250–650 °C, which are in the same range as those of humic substances in soil (Ritchie & Perdue, 2008). Acidic functional groups dominated by carboxylic and phenolic groups decreased with increase in pyrolysis temperature (Mukherjee *et al.*, 2011, Budai *et al.*, 2014). Decrease in acidic functional groups together with increase in ash contents caused an increase in pH of BC as pyrolysis temperature increases. The pH however, levelled off at temperature of >600 °C. Just like the pH, CEC also increases with temperature up to 400 °C, but decreases at pyrolysis temperature above 400 °C (Budai *et al.*, 2014). This may be attributed to the loss of acidic functional groups (Mukherjee *et al.*, 2011). Anion exchange capacity of BC on the other hand is very low (Mukherjee *et al.*, 2011). Surface area, CEC and pH of BC all depend on the feedstock used. Measurement of the CEC of BC is difficult, due to the presence of soluble ions in ash, which compromises the extraction of exchangeable cations by common extractants such as ammonium acetate. In a report by Verheijen *et al.* (2009), the CEC of BCs was found to vary widely in the range of nearly zero to ~40 $cmol_c$ g^{-1} while the pH of BC were found to be more homogenous, largely being neutral to basic (pH 6 to pH 10). Biochar with pH on the lower end of this range were derived from green waste and tree bark while BCs with pH on the higher end were from poultry litter feedstock.

1.2 Effect of biochar on properties of soils

Physical, chemical and biological properties of soils have been reported to change upon BC addition (Glaser *et al.*, 2002, Lehmann *et al.*, 2011, Mukherjee & Lal, 2013). These changes in soil properties have been linked to BC properties directly or indirectly and also depend on the soil type. Changes to physical properties of soil relate to both structural and hydraulic properties as reviewed by Glaser *et al.* (2002), Ogawa & Okimori (2010), Mukherjee & Lal (2013). Soil bulk density which may indicate soil structural quality has been reported recurrently to decrease along with increase in soil porosity upon BC addition (e.g. Mukherjee & Lal (2013)). Such decrease in bulk density and increase in porosity has been suggested to result from both BC's light-porous nature and from the effect of BC on soil aggregation (Verheijen *et al.*, 2009). Only recently, it has been shown that BC may increase soil aggregation, especially aggregate stability under laboratory conditions (Liu *et al.*, 2012, Awad *et al.*, 2013, Herath *et al.*, 2013, Soinne *et al.*, 2014).

Associated with an increase in soil porosity, BC has been reported to increase soil water holding capacity in loamy to sandy soils (Basso *et al.*, 2013, Cornelissen *et al.*, 2013, Herath *et al.*, 2013, Martinsen *et al.*, 2014). Despite the increase in soil water holding capacity, the effect of BC on hydraulic conductivity of soil has remained inconclusive. For example, Uzoma *et al.* (2011) observed decrease in saturated hydraulic conductivity (K_{sat}) of sandy soil due to the addition of cow manure BC, while in similar soils Jeffery *et al.* (2015) observed no effect of BC made from hay. Jeffery *et al.* (2015) suggested that the hydrophobic nature of BC could affect soil hydraulic properties. However, several laboratory studies suggest that hydrophobic nature of BC does not necessarily cause water repellency in soils (e.g. Herath *et al.*, 2013, Page-Dumroese *et al.*, 2015, Yi *et al.*, 2015).

Chemical properties of soil that are commonly altered by BC application include pH, CEC and available base cations (Glaser *et al.*, 2002, Yamato *et al.*, 2006, Verheijen *et al.*, 2009). Strong increases in soil pH, CEC and base saturation following BC application has generally been associated with acidic, low CEC soils (Glaser *et al.*, 2002, Yamato *et al.*, 2006, Martinsen *et al.*, 2015). Increases in soil pH depend on the initial pH, and CEC of the soil and the acid neutralizing capacity of the BC (Martinsen *et al.*, 2015). Since agricultural soils with low pH, CEC and base saturation are more dominant in tropical areas, soils in these areas are most likely to benefit from BC addition. Besides base cations, BC also adds other nutrients like phosphates and ammonium to soil (Glaser *et al.*, 2002, Yamato *et al.*, 2006, Hale *et al.*, 2013, Alling *et al.*, 2014). Biochar has been found to be a slow release source of nutrients with potential to supply nutrients for several seasons (Angst & Sohi, 2013). The slow release of nutrients such as ammonium and nitrate could be due to sorption to BC reducing their leaching losses (Clough & Condron, 2010, Clough *et al.*, 2013).

1.3 Effect of biochar on crop production

Biochar has been found to increase growth and yield of a number of crops in tropical cropping systems (Yamato *et al.*, 2006, Steiner *et al.*, 2007, Cornelissen *et al.*, 2013). The increase in crop yields was linked to BCs' inherent properties such as high pH, high CEC, nutrients, high specific surface area and effects on the soil's water holding capacity. In a meta-analysis by Jeffery *et al.* (2011), the grand mean increase of crop yield was only 10%. However, yield increase varied widely from -28% to 39%. Highest crop yield increases were found in acidic and coarse-textured soils, suggesting that the key mechanisms for increased yield may be a liming effect or an increased soil water retention, or both. In addition, nutrient supply may be important to explain increased yield. Most recent studies in tropical soils

(Martinsen *et al.*, 2014, Agegnehu *et al.*, 2015) seem to support the hypothesis that high yield increases are related to increased nutrients, soil pH and soil water retention.

In temperate systems, earlier evidence suggested that the effect of BC on crop yields might be small as reviewed by Biederman & Harpole (2013 and references therein). However, with increased understanding of the underlying mechanism for increased crop yields, BC may also increase yields in temperate areas (Atkinson *et al.*, 2010, Jeffery *et al.*, 2011). Some authors e.g. Karer *et al.* (2013) and Bruun *et al.* (2014) found some increases in crop yield due to BC application in temperate soil. However, since the acidic low CEC soils are not as common as in tropical areas, due to better management, increase in yields may not be widespread.

1.4 Effect of biochar on greenhouse gas emissions from soils

The effect of BC on biological activities in soil have important implications for the production and emission of greenhouse gases (CO_2, N_2O and CH_4). In an incubation study, Zimmerman *et al.* (2011) observed an increase in decomposition of soil organic matter in BC amended soil measured in term of CO_2 evolution, which varied depending on soil and BC type. In their study, the priming effect of BC on soil organic matter decomposition varied from -52 to 89% at the end of one year, but overall, positive priming dominated. Zimmerman *et al.* (2011) further showed that application of BCs, produced at low temperature, especially if applied to high C soils causes higher emission of CO_2. They attributed this to more labile C in low temperature BCs, which is utilized rapidly by copiotrophic microbes with high growth rates in the presence of labile C. There were also higher emissions of CO_2 from soil amended with BC made from grass than BC made from wood, indicating that the feedstock, from which a given BC is made, is important for the extent of the priming effect. The higher CO_2 evolution from grass BC amended soils was associated with more labile C in grass BC.

Linked to the decomposition of soil organic matter, BC has been found to alter the nitrogen (N) dynamics in soil, due to changes in N turnover processes as reviewed by Lehmann *et al.* (2011) and Clough *et al.* (2013). In these reviews, rates of mineralization and immobilization, which depend on C and N pools in soil, were found to be affected by BC application. For instance, low temperature BCs, with large amounts of labile C, were reported to cause N immobilization in soil, whereas BCs with low labile C and high pH, produced at higher temperatures, increased mineralization of nitrogen. Biochar has been reported to have no significant effect on nitrification rates in agro-ecosystems, probably because nitrification occurs commonly at high rates in agricultural soils (Clough *et al.*, 2013). Ulyett *et al.* (2014), on the other hand, observed an increase in nitrification rate in neutral pH agricultural sandy loam soils after BC application and attributed it to increased soil pH. In the nitrification process, some production and emission of NO and N_2O may occur (Firestone & Davidson, 1989), but due to the uncertainty of a BC effect on nitrification in agro-ecosystems its effect on NO and N_2O emission under aerobic condition remains unclear. In natural systems, BC addition increases nitrification rates probably due to BC's liming effect and possibly due to the removal of inhibiting substances e.g. polyphenols by BC (Clough & Condron, 2010, Clough *et al.*, 2013). The often reported suppression of N_2O emission by BC has been found mainly in soils with high moisture contents amended with nitrate (Cayuela *et al.*, 2014). This suggests that the suppression of N_2O by BC is related to the effect of BC on denitrification, which, by contrast to nitrification, requires an anoxic environment.

Denitrification is the main process that removes reactive N from soils, converting it primarily to non-reactive N_2. Unfortunately, denitrification may result in the escape of gaseous intermediates (NO and N_2O) (Firestone & Davidson, 1989). Addition of labile C in BC may stimulate denitrification and may result in more complete denitrification all the way to N_2. This is one of the many proposed mechanisms behind the often reported suppression of N_2O under both field and laboratory conditions (Clough *et al.*, 2013, Cayuela *et al.*, 2014 and references therein). The effect of BC on NO net production has only recently been included in BC research e.g. Nelissen *et al.* (2014), which apparently is the first study to consider NO.

So far, the effect of BC on CH_4 production and emission has seen relatively limited research efforts. As a result, the effect of BC addition to soil on CH_4 emission remains unclear. Increases in CH_4 emission have been observed under lowland rice (paddy field) (Zhang *et al.*, 2010, Zhang *et al.*, 2012). On the other hand, in laboratory conditions, decreases (Liu *et al.*, 2011, Feng *et al.*, 2012) or no effect (Kammann *et al.*, 2012) have been observed. Further detailed studies are highly needed to assess the effect of BC on both production and consumption of CH_4 in soil.

1.5 Rationale and hypotheses of the study

One of the key questions addressed in the present study is the impact of BC on soil physical quality. To understand the mechanisms for increased soil productivity in BC amended soils, the focus of research, so far, has been primarily on the effect of different BCs on soil chemical properties and crop nutrition, and less so on physical properties of soil (Atkinson *et al.*, 2010, Mukherjee & Lal, 2013). Yet, the effect of improved soil physical properties, due to BC amendment, could be one of the main reasons for increased crop yields especially in coarse-textured soils (Cornelissen *et al.*, 2013). A key variable among soil physical properties, important for increased crop yields, is the water holding capacity. Although BC has been found to increase soil water holding capacity (Basso *et al.*, 2013, Cornelissen *et al.*, 2013, Herath *et al.*, 2013), detailed field studies examining how BC increases water holding capacity are scarce. Increased water holding capacity is particularly important for sandy to loamy soils, such as the Acrisols and Arenosols in Zambia considered in this study. The problem of low water holding capacity of these Zambian soils is compounded by reduced and unreliable rainfall (Yatagai, 2011). Therefore, water is one of the most limiting resources affecting rain fed agriculture in Zambia, which is dominated by smallholder farmers.

The increase in water holding capacity upon BC addition in sandy and loamy soils (Mukherjee & Lal, 2013) are an indication of altered pore size distribution (Sun & Lu, 2014, Sun *et al.*, 2014). Biochar can alter pore size distribution of the soil by occupying the inter-particle pore space of soil (Barnes *et al.*, 2014), in particular in case of fine rather than coarse BC. In addition, the high porosity of BC, especially fine pores, may have a direct effect on soil pore size distribution (Mukherjee & Lal, 2013). Therefore, the use of BC of different particle sizes may provide further information of how BCs affect pore size distribution of soils. Recently, Eibisch *et al.* (2015) reported stronger increase in water retention of a loamy sand amended with fine than with coarse BCs under laboratory condition. They suggested that this could be due to filling of soil inter-particle pore space by fine BCs. Indirectly, BC may also affect pore size distribution by inducing soil aggregation, and in particular aggregate stability, as has been shown mainly under laboratory conditions (Awad *et al.*, 2013, Herath *et al.*, 2013). Under field conditions, plant roots may modify the influence of BC on aggregate stability (Reid & Goss, 1981). This is particularly so because BC has been shown to increase crop root growth and biomass (Bruun *et al.*, 2014, Abiven *et al.*, 2015). Different crop species affect soil aggregation differently due to other root structures. For example, monocots, which have more fibrous root systems have been reported to have a stronger effect on soil aggregation compared to dicots (Amézketa, 1999). Since soil aggregation can also affect pore size distribution, stronger effects of the roots of monocots on aggregation may result in a stronger positive effect on water retention.

It was hypothesized that BC, under field conditions, increases aggregate stability and water retention in sandy loam soils, while reducing bulk density. The effects are expected to be more pronounced for fine BC than for coarse BC and more so under maize (monocot) than under soybeans (dicot). It was further hypothesized that BC reduces bulk density in sandy soils, due to weight dilution rather than aggregation. These hypotheses were tested in Paper I entitled "***In situ*** **effects of biochar on aggregation, water retention and porosity in light-textured tropical soils**".

The availability of water to crops is strongly affected by the water infiltration rate. Soils with weak aggregates such as sandy Acrisols are prone to crusting (Awadhwal & Thierstein, 1985), which may reduce water infiltration into the soil. Since BC has been shown to improve soil aggregation under laboratory conditions (Herath *et al.*, 2013, Sun & Lu, 2014), stronger aggregates may prevent surface crusts (Awadhwal & Thierstein, 1985). One common way of assessing soil crusts is by measuring its strength in terms of penetration resistance (Upadhyaya *et al.*, 1995). However, to date no study has been conducted to test the possible effect of BC on soil crust formation and only few studies have reported the effect of BC on soil penetration resistance (Busscher *et al.*, 2010, Mukherjee *et al.*, 2014).

Since BC is generally water repellent, the repellency may be transferred to soils, especially coarse-textured soils, which are prone to developing water repellency (Doerr *et al.*, 2000). This is because coarse-textured soils have low specific surface areas that require only small amount of hydrophobic compounds to render their surface water repellent (Doerr *et al.*, 2000). The repellency may counteract the reported positive effect of BC on soil hydraulic properties (Jeffery *et al.*, 2015). Only recently, laboratory studies (e.g. Eibisch *et al.*, 2015, Page-Dumroese *et al.*, 2015, Yi *et al.*, 2015) have been conducted to assess the effect of BC on soil water repellency. Soil hydraulic properties that can be affected by water repellency include both water retention and hydraulic conductivity. Barnes *et al.* (2014) proposed that BC affects soil hydraulic properties through the interstitial BC-soil particle space and through pores within the BC grains themselves. Fine BC such as that used by Barnes *et al.* (2014) with size ≤0.85 mm would fit in between soil particles reducing inter-particle pore space without necessarily increasing soil volume. This may explain the reduction in K_{sat}, which they observed in sand and not in clay-rich soil. In clay-rich soil, their observed increase in K_{sat} could be due to BC-induced soil aggregation causing build-up of macro-pores. Coarser BCs on the other hand may not affect soil inter-particle space but could increase soil porosity due to its high internal porosity. Use of BC of different particle sizes may therefore aid the understanding of mechanisms behind BC effects on soil hydraulic properties.

It was hypothesized that BC reduces the penetration resistance due to BC-induced aggregation for both crusted surface and bulk soil. It was also hypothesized that the hydrophobic nature of BC, irrespective of its particle size, induces soil water repellency in BC-amended coarse-textured soils. Lastly, it was hypothesized, that BC, irrespective of particle size, increases K_{sat} in loamy soil due to BC-induced soil aggregation. By contrast, in sand, finer BC was hypothesized to reduce K_{sat} due to filling of inter particle space while coarse BC has no effect. This set of hypotheses was tested and results are presented in Paper II entitled "**Effect of biochar on crust formation, penetration resistance and hydraulic properties of two coarse-textured tropical soils**".

To sustain potential long term benefits of BC for increased crop production (Jeffery *et al.*, 2011), similar to what has been observed in *Terra Preta* soils, (Glaser *et al.*, 2001), BC must remain within the root zone of cropped soil. To date, only few experimental studies have attempted to quantify BC mobility in soil (Rumpel *et al.*, 2006, Major *et al.*, 2010, Haefele *et al.*, 2011). The rate of downward migration of BC to deeper soils varies widely ranging from <1% per year in sandy clay loam Ferralsol in cropland (Major *et al.*, 2010) to 50% in structured humic Nitisols and gleyic Acrisols cultivated with rice in one year (Haefele *et al.*, 2011). Haefele *et al.* (2011) observed that soils with higher water flow rates had greater downward migration. For lateral transport, BC has been found to undergo preferential transport during water erosion on steep slopes of slash and burn agriculture (Rumpel *et al.*, 2006). Transport of BC in soil may be aided by physical disintegration of BC to finer particles

(Spokas *et al.*, 2014). In sand, BC would not be integrated into soil aggregates unlike in loamy sand, which has a potential to undergo aggregation. Finer BC, due to its low specific weight would float in air or water and hence be more easily transported laterally. Therefore, BC particle size could be an important factor determining BC transport in soils.

It was hypothesized that downward migration of BC is greater in soils with higher K_{sat} and that this migration would be greater for finer BC fractions. This hypothesis was tested and presented in Paper III entitled "**Vertical and lateral transport of biochar in light-textured tropical soils".**

The recalcitrance of BC to biological degradation is arguably a great opportunity to curtail increasing CO_2 emissions, while providing the immediate benefit of increased crop production (Lehmann *et al.*, 2006). Another greenhouse house gas of major importance for climate change is N_2O. Here, I focused on N_2O, where BC has been recurrently reported to mostly reduce its emission from soil with only few studies reporting the opposite (Clough *et al.*, 2013, Cayuela *et al.*, 2014). Previous studies, which showed that BC suppresses N_2O emission were conducted at a range of soil moisture contents mostly on the high end (Cayuela *et al.*, 2014 and references therein), where denitrification is likely the dominant process for N_2O production, even though nitrification cannot be excluded. In most studies, it is difficult to identify the processes responsible for N_2O emission, and hence the mechanism(s) for N_2O suppression in BC amended soil, since with few exceptions, neither strict aeration nor ^{15}N technique was deployed.

Various mechanisms have been proposed to explain N_2O suppression by BC. These include, among others, increased N_2O reductase activity at raised soil pH (Cayuela *et al.*, 2014), increased electron flow to N_2O through BC-mediated electron shuttling (Cayuela *et al.*, 2013), reduced rates of denitrification through competition for electrons by BC (Joseph *et al.*, 2010), and improved soil aeration resulting in reduced denitrification (Yanai *et al.*, 2007). Since denitrification is the dominant process fueling high N_2O emissions in soil, denitrification experiments have to be conducted under strict anaerobic conditions or by applying ^{15}N technique if our understanding of the mechanisms behind the reported N_2O suppression is to be advanced. Biochar is generally alkaline and has been shown to increase soil pH (Biederman & Harpole, 2013). Several earlier studies have reported that soil pH controls the composition of gaseous products in denitrification, with greater N_2 production at neutral pH and more N_2O production under acid conditions (Liu *et al.*, 2010, Raut *et al.*, 2012, Liu *et al.*, 2014). This has been explained by impaired assembly of the N_2O reductase enzyme at low pH, restricting or delaying the efficient reduction of N_2O to N_2. Neutralizing soil acidity by BC could therefore be one of the major drivers behind the suppression of N_2O emissions observed under field conditions. Acid soils are also prone to chemical decomposition of nitrite (an intermediate of both nitrification and denitrification), resulting in chemical production of NO and N_2O (Braida & Ong, 2000, Islam *et al.*, 2008).

It was hypothesized that BC causes a suppression of NO and N_2O production relative to N_2 production during denitrification in acid soil by increasing the soil pH. This hypothesis was tested and presented in paper IV entitled "**Effect of soil pH increase by biochar on NO, N_2O and N_2 production during denitrification in acid soils**".

2. Materials and Methods

To test the hypotheses, both field and laboratory experiments were conducted. Field experiments were conducted at Mkushi and Kaoma district located in central and western Zambia, respectively, to test the impact of BC on soil physical properties and BC transport in soils. Field and laboratory measurements were then carried out one and two years after BC application. To investigate the role of soil pH increase by BC on the NO, N_2O and N_2 production during denitrification, laboratory studies were conducted using Acrisols from Mkushi and Lampung, Indonesia. The two soils were both acidic with low CEC.

2.1 Biochar production

2.1.1 Biochar used in field experiments in Zambia (Paper I, II & III)

The BCs were produced by slow pyrolysis from maize cob, which is widely available throughout Zambia and rice husk, which is available in western Zambia. Maize cob was the primary feedstock for BC implementation in Zambia (Cornelissen *et al.*, 2013, Martinsen *et al.*, 2014). Biochars were produced in two batches. The first batch was produced in 2011 from maize cob at a temperature of approximately 350 °C and a residence time of 2 days in a brick kiln at Mkushi, Zambia (Fig. 1). The second batch was produced in 2013 from maize cob and rice husk at a temperature of 350 °C and a retention time of 1 day in a drum retort kiln at Chisamba, Zambia (Fig. 1). Biochar from the first batch was used in the farmer practice experiment under conservation farming (Paper I), whereas BC from the second batch was used in experiments involving different particle sizes of maize cob and rice husk BC (Paper I, II & III) under conventional farming. The properties of the BCs are presented in Table 1.

2.1.2 Biochar used in the laboratory incubation (Paper IV)

As in the field experiment, BC from rice husk was included in the laboratory study. Cacao shell BC was also included due to its high alkalinity, to aid the study of pH-mediated effects in soil. Cacao shell BC had a ~5 times higher acid neutralizing capacity than rice husk BC (217 vs 45 $cmol_c\ kg^{-1}$) (Smebye, 2014). The two BCs used in this experiment were produced in Lampung, Indonesia in a locally fabricated metal kiln (Fig. 2) at a temperature of 400–500 °C determined using thermogravimetric analysis. The pyrolysis time was 3.5 hrs. Rice husk and cacao shell are common agricultural wastes in Lampung. The BCs were used as untreated, water-leached or acid-leached materials to study the pH effect on denitrification and its product stoichiometry (Paper IV). The properties of the BCs are presented in Table 1.

Fig. 1. Brick and drum retort kiln used in the production of BC used in field experiments. Photos taken by G. Cornelissen.

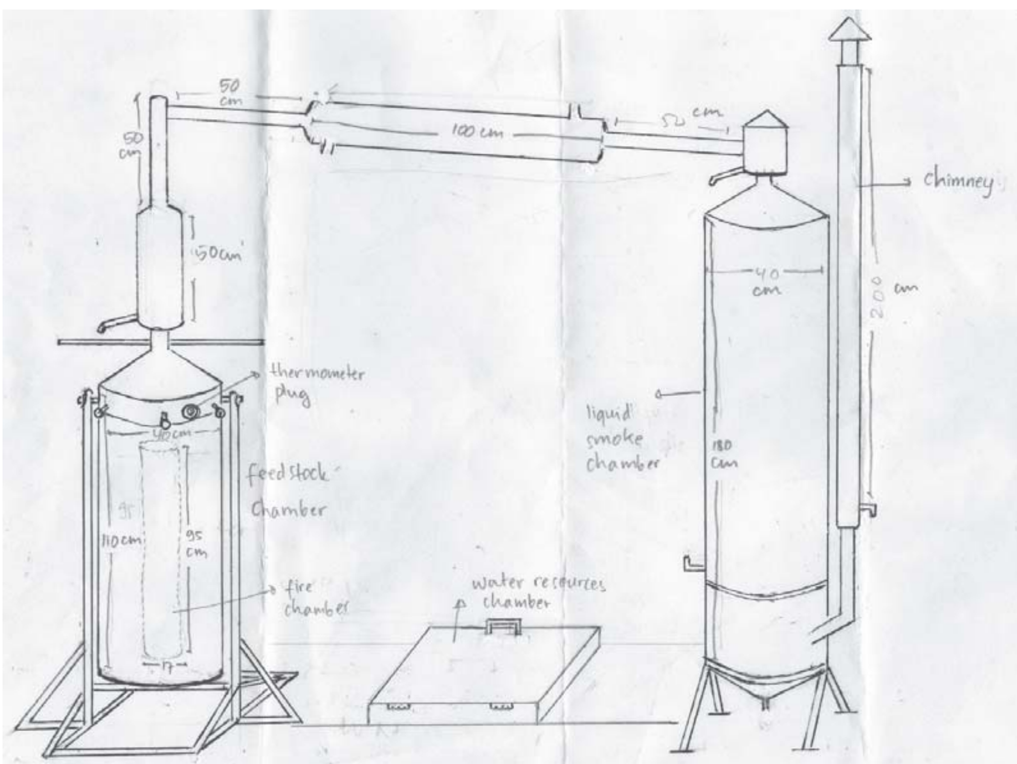

Fig. 2. Schematic drawing of the kiln used for pyrolysis in Lampung (Hale *et al.*, 2013).

Table 1. Properties of biochars

Properties	Maize cob BC 2011[a]	Rice husk BC 2013[b]			Maize cob BC 2013[b]			Incubation BCs[c]	
		≤0.5 mm	0.5–1 mm	Unsorted	≤0.5 mm	1–5 mm	Unsorted	Rice husk	Cacao shell
Total organic C (%)	81.1	39.3	42.8	47.8	44.8	60.1	53.8	44.6	54.3
Total nitrogen (%)	0.7	0.61	0.52	0.82	0.79	0.53	0.65	0.9	1.5
Total hydrogen (%)	3.0	2.33	2.41	2.37	2.09	2.63	2.36	1.9	1.4
H/C (molar ratio)	0.44	0.71	0.68	0.60	0.56	0.52	0.53	0.51	0.31
pH (H_2O)	9.7	8.3	8.3	8.3	9.0	8.6	8.8	8.4	9.8
CEC ($cmol_c$ kg^{-1})	21.1	-	-	14.0	-	-	22.2	20.0	197
K^+ ($cmol_c$ kg^{-1})	19.5	-	-	10.4	-	-	16.5	9.5	127
Ca^{2+} ($cmol_c$ kg^{-1})	0.9	-	-	2.4	-	-	4.3	3.2	37.1
Mg^{2+} ($cmol_c$ kg^{-1})	0.8	-	-	0.9	-	-	1.2	3.6	32.8
Bulk density (g cm^{-3})	-	0.37	0.27	-	0.36	0.29	-	-	-
Loss on ignition (%)	-	48.8	54.9	-	52.1	72.4	-	55.6	68.1
BET surface area (m^2 g^{-1})	-	2.4	2.3	-	10.5	4.9	-	76.4	30.9

[a] Maize cob BC produced in brick kiln at Mkushi and used in the farmer practice experiment under conservation farming at Mkushi.
[b] Rice husk and maize cob BCs produced in a drum retort kiln at Chisamba and used in experiments with different BC particle sizes in Mkushi and Kaoma.
[c] Rice husk and cacao shell BCs produced in Lampung and used in laboratory denitrification experiments.

2.2 Experiments

2.2.1 Field experiments in Zambia (Paper I, II and III)

Field experiments were established on private farms in two districts of Mkushi and Kaoma with average annual rainfall of 1220 and 930 mm and average temperature of 20.4 and 20.8 °C, respectively. The top soils at both sites are coarse-textured, acidic and have low CEC (Table 2). There were three experiments; (1) farmer practice experiment amended with maize cob BC, (2) maize cob BC particle size experiment and (3) rice husk BC particle size experiment. In the farmer practice experiment, crushed maize cob BC was added to planting basins of conservation farming while in the BC particle size experiments, maize cob and rice husk BC were sieved into different particle size fractions and added to soil under conventional farming.

Table 2. Properties of soils

Properties	Field experiment soils			Incubation experiment soils	
	Mkushi 2011[a]	Mkushi 2013[b]	Kaoma 2013[b]	Mkushi	Lampung
Sand (%)	64.4	75.1	85.4	-	-
Silt (%)	23.5	15.9	10.2	-	-
Clay (%)	12.2	9.0	4.4	-	-
Texture class	Sandy loam	Loamy sand	Sand	Sandy loam	Sandy loam
Total organic C (%)	0.67	0.74	0.62	0.5	1.2
Total nitrogen (%)	0.01	0.01	<0.01	<0.01	0.1
pH (H_2O)	6.4	5.8	5.8	4.0	4.0
CEC ($cmol_c\ kg^{-1}$)	2.7	1.7	2.8	6.4	9.7
K^+ ($cmol_c\ kg^{-1}$)	0.3	0.3	0.1	<0.1	<0.1
Ca^{2+} ($cmol_c\ kg^{-1}$)	1.4	1.1	1.2	0.1	0.3
Mg^{2+} ($cmol_c\ kg^{-1}$)	1.0	0.3	0.2	<0.1	0.1
Bulk density ($g\ cm^{-3}$)	1.26	1.27	1.47	-	-

[a] Soil used for farmer practice experiment in Mkushi.
[b] Soil used for BC particle size experiment in Mkushi and Kaoma.

Farmer practice experiment (Paper I): This experiment was established by applying crushed (unsorted) maize cob BC in the sandy loam soil under conservation farming practice at Mkushi. Here, conservation farming involved tilling about 10% of the total land by digging planting basins to conserve moisture and to minimize soil disturbance. Weeds in the rest of the land were managed through application of herbicide. The soil in the planting basins was mixed with BC at a rate of 0, 0.8 and 2.5% w/w corresponding to only 0, 2, and 6 tons ha^{-1}, respectively, since BC was concentrated in the basins. The experimental plot was divided into two, one part planted with maize and the other with soybeans. This experiment was established in October 2011 in Mkushi and soil samples were taken in April 2013. The stability of aggregates, water retention and pore size distribution on field samples were then determined in the laboratory. Aggregate stability was determined using rainfall simulation (Marti, 1984, Grønsten & Børresen, 2009). Water retention was determined by draining saturated soil in core rings (100 cm^3) and measuring moisture content at successively higher pressure using a sand box (Eijkelkamp, Giesbeek, The Netherlands) and a pressure plate apparatus (Soil moisture Equipment, Santa Barbara, CA). Water retention data was modelled using van Genuchten (1980) equation to determine continuous water retention curves. Pore size distribution was estimated from water retention curves using the capillary rise equation. Capillary equation allows conversion of matrix potential to soil pore radius.

Maize cob BC particle size experiment (Paper I & II): This experiment was established in April 2013 under conventional farming based on a split plot design by applying maize cob BC of three particle sizes prepared by crushing and dry sieving. The site was divided into three blocks, each sub-divided into three main plots amended with BC of different particle sizes (≤0.5, 0.5–1 and 1–5 mm). The main plots were divided into three sub-plots receiving BC at three doses (0, 1.7 and 3.4% w/w for Kaoma sand and 0, 2 and 4% w/w for Mkushi loamy sand). The same amounts of BC (0, 17.5, 35 tons ha^{-1}) were applied to the two sites but percentages differed due to differences in soil bulk density. The total number of sub-plots at each site was 27. From each sub-plot, the top 7 cm of soil was removed and mixed with the required amount of BC in a bucket. The soil profile from 7 cm to approx. 30 cm was loosened using a hoe to remove the compacted layer before placing it back on top, the soil-BC mixture in the bucket. The BC application is illustrated in Fig. 3. The sub-plot size was 0.5 x 0.5 m separated by vertical hard plastic sheet inserted approx. 10 cm into the soil and 10 cm remaining above the soil. Fertilizer was applied at the recommended rate (Paper 1) at the center of the sub-plots just before planting of maize (November 2013).

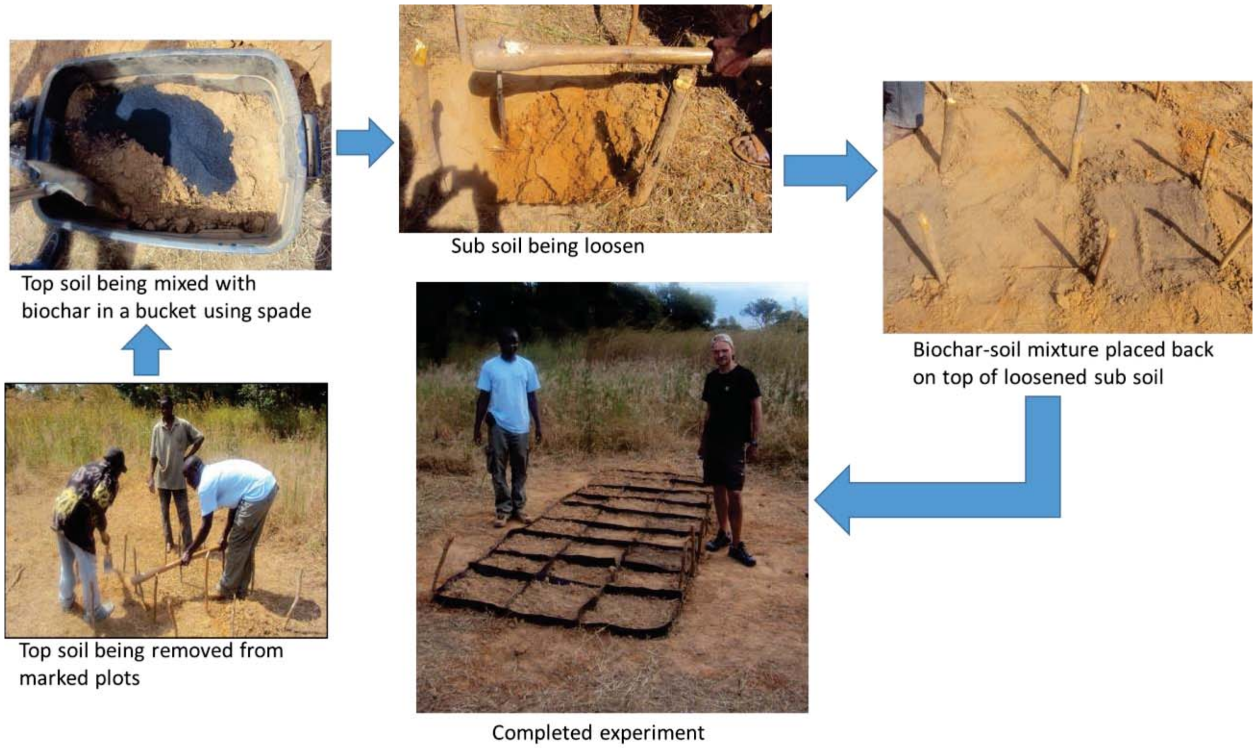

Fig. 3. Illustration of application of BC of different particle sizes in Mkushi, Zambia. Photos taken by J. Mulder in April 2013.

After one year, core ring and disturbed samples were taken for laboratory analyses. Water retention, bulk density and aggregate stability were determined as presented in paper I. In addition, after one and two years, water infiltration and penetration resistance measurements were carried out using a tension disc infiltrometer and a pocket penetrometer, respectively (Eijkelkamp, Giesbeek, The Netherlands). K_{sat} of the soil was estimated from infiltration data whereas the penetrometer was used to determine penetration resistance of the crust and bulk soils at Mkushi and Kaoma. Water repellency tests using the water drop penetration time (WDPT) and the ethanol percentage test were also carried out in year one and two after the initiation of the experiment. K_{sat}, penetration resistance and water repellency data are presented in Paper II.

Rice husk BC particle size experiment (Paper I & III): This experiment was established in Mkushi and Kaoma in April 2013. Biochar was applied to the top 5 cm of soil based on a completely randomized design. There were two BC treatments in Kaoma sand, in addition to a reference without BC. Treatments included ≤0.5 mm and 0.5–1 mm rice husk BC, both added at a rate of 3.4% w/w. In Mkushi loamy sand, the treatments included ≤0.5 mm rice husk BC, 0.5–1 mm maize cob BC and a reference. Here, BC addition rates were 4% w/w for both treatments. The same amount of BC was added per plot (625 g) to both Mkushi and Kaoma soils, but the BC contents (in %w/w) differed due to differences in soil bulk density between the two sites (Table 2). At both sites, treatments and references had three replicates resulting in nine plots per site. The experiment was established adjacent to, and using a similar approach as in the maize cob BC particle size experiment (Fig. 3). The BC and soil in both Mkushi and Kaoma had different $\delta^{13}C$ signal. This in addition to total organic C changes allowed tracing of BC in soil both laterally and vertically down to deeper soils (Paper III).

2.2.2 Laboratory experiment: effect of biochar on denitrification (Paper IV)

Soil samples used in this study were taken from Lampung, Indonesia and Mkushi Zambia. Both soils were acidic low CEC sandy loam Acrisols (Table 2). The air-dry soils were moistened prior to incubation by saturating and draining in a sand box (Eijkelkamp, Giesbeek, The Netherlands) at a suction of 50 cm until equilibration. This controlled pre-wetting was done to accommodate for the flush of microbial activity commonly observed upon rewetting of dry soil (Kieft *et al.*, 1987).

The BCs used in this experiment were from cacao shell and rice husk and were added to the soil either untreated or leached with water or acid (Fig. 4.) (Paper IV). Leaching of the BCs to partly remove their alkalizing effect before use in the experiments was done on the size fraction ≤2 mm. Prior to usage of BCs in incubation experiments, all BC were crushed further to ≤0.5 mm. The BCs were added to approx. 10 g moist soil in 120 ml serum bottles at doses of 0, 1, 2, 5 and 10% (dry weight basis). In order to prepare soil slurries, 30 ml of 2 mM KNO_3 was added to provide ample amounts of nitrate for denitrification. Uncharred cacao shell was included to study the effect of feedstock alone. Since the hypothesis of the study was that the N_2O suppressing effect of BC is mainly due to the increased pH, the pH effect needed to be separated from other potential effects of BC on denitrification. In this regard, a NaOH treatment was included, in which the pH of soil was manipulated independently of BC. pH was measured before and after the incubation to account for the unavoidable alkalization by anaerobic microbial activity. To induce anoxic condition, the headspace after sealing the bottles was flushed with helium by alternately evacuating and helium filling the bottles 5 times.

Column set up at NGI during leaching of BC

Incubation system

Fig. 4. Biochar leaching set up and incubation system. The inset in the right panel shows the bottles after incubation. Photos by A. Obia.

All incubations were carried out in a water bath at 20°C under constant stirring to maintain equilibrium of gases between the soil slurry and the bottle headspace. A robotized incubation system similar to that described by Molstad *et al.* (2007) was used to monitor the kinetics of O_2 depletion, CO_2 production and N-gas accumulation (NO, N_2O, N_2) during denitrification. The system consists of a water bath connected to a cryostat, placed under the robotic arm of an autosampler (Combi Pal, CTC, Switzerland) (Fig. 4). The water bath can accommodate up to 30 stirred bottles which are pierced repeatedly (here five-hourly) by the hypodermic needle of the autosampler which is connected to a peristaltic pump transporting the gas sample to a gas chromatograph equipped with various detectors and further to an NO-chemiluminescence analyzer.

2.3 Data analysis

Data analysis in all the four papers was conducted using R software (R Core Team, 2014). Data was analyzed using analysis of variance (ANOVA) whenever all the explanatory variables were categorical. Differences of means were determined using Tukey's test at 5% level of significance. When some of the explanatory variables had continuous data, analyses of covariance (ANCOVA), which combines both ANOVA and regression were conducted. This allowed combining both categorical and continuous data in the analysis. Mean values ± standard errors and regression coefficients (slopes and intercepts) ± standard errors are presented.

3. Main results and Discussion

3.1 Paper I and II: Impact of biochar on soil structural properties

In the sandy loam soil, the aggregate stability was higher under maize crop than under soybeans in the planting basins of the farmer practice experiment. For example, there were 42.6±2.1% stable aggregates of 2–6 mm under maize as opposed to 34.0±2.9% under soybeans in the soils without BC. The increase in percentage of stable aggregates due to maize cob BC was higher under soybeans compared to under maize. The percentage of stable aggregates increased with increase in BC derived carbon of aggregates, reaching a maximum at BC carbon of between 1 and 2% (Fig. 5). Since the C content of the BC was 81% (Table 1), the 1–2% BC carbon of aggregates coincide with BC application rate of between 1.2 to 2.5% w/w. This relatively low dose of BC producing a significant impact will be important when addressing the question of potentially large amounts of feedstock needed for BC implementation.

The greater aggregate stability under maize than under soybeans in the absence of BC is consistent with earlier report that different crops have different effect on aggregate stability (Reid & Goss, 1981). In particular, monocots such as maize have been reported to have a greater positive effect on aggregate stability than dicots such as soybeans (Amézketa, 1999). The lower impact of BC on aggregate stability under maize may be associated with maximum possible aggregate stability. Maize on its own already had a greater impact than soybeans on aggregate stability and BC could only add little to reach the maximum stability.

The bulk density of soil on the other hand was reduced upon BC application. The effect of BC on bulk density was similar under both maize and soybeans. This was likely because bulk density depend on both soil aggregation and weight dilution by BCs' light-weight as suggested by Verheijen *et al.* (2009). Results from the present study showed that soil aggregation contributed at least 20% of the decrease in bulk density (Paper I). The decrease in bulk density in the aggregating sandy loam was mainly associated with the increase in macro-porosity with pores of >0.03 mm diameter (pores that hold water at suction smaller than at field capacity) due to BC application (Fig. 6). These big pores contributing to decrease in bulk density are likely a result of soil aggregation and less so due to BCs' high porosity because BC has mainly fine pores (Braida *et al.*, 2003).

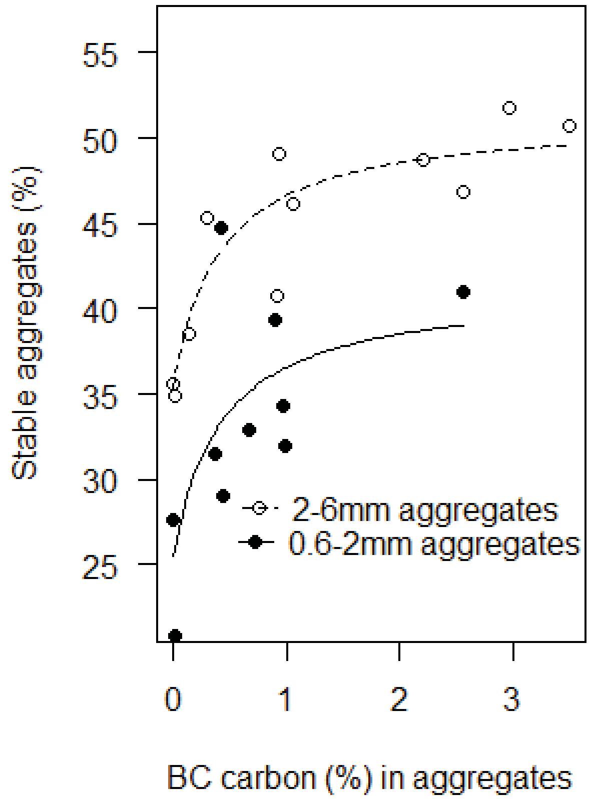

Fig. 5. Stable aggregates plotted against BC carbon in aggregates of BC amended soils from farmer practice experiment in Mkushi, Zambia. The figure shows a fitted three-parameter Michaelis-Menten model, which estimates stable aggregate (c) at zero BC, maximum stable aggregates achievable (d) and BC carbon at half d (e). For 0.6-2mm aggregates, c, d=25.5±1.9, 41.3±4.9 and for 2-6mm aggregates c, d=35.4±2.2, 51.4±3.7, respectively ($p<0.001$). Parameter e=0.4±0.4 ($p>0.05$) for both aggregate sizes tested.

In the loamy sand at Mkushi with its potential to aggregate, there were indications of BC-induced structural development but it was not consistent (e.g. aggregate stability and bulk density). This may be due to the coarser texture of the soil resulting to slow development of aggregates. Bulk density in this soil was significantly reduced only by the coarsest BC of 1–5 mm (Table 3). In non-aggregating sand at Kaoma, bulk density decreased irrespective of particle size of BC applied (Table 3). The decrease can be explained by the weight dilution effect of BC. The absence of an effect of the particle size of BC on bulk density in the sand could be linked to the fact that BC, irrespective of particle size, are light-weight (Table 1) relative to soil.

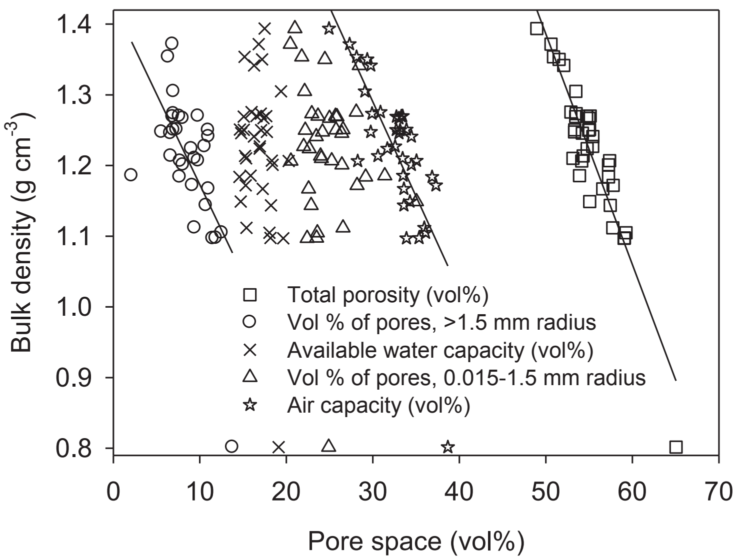

Fig. 6. Relationship of the bulk density and various components of the pore space in planting basins of farmer practice experiment. Basic BC and soil properties are in Table 1 and 2.

Biochar reduced the penetration resistance in the loamy sand at Mkushi two years after BC addition (Fig. 7). In the same soil, the bulk density measured one year earlier was reduced only for plots amended with coarsest BC. This positive correlation between penetration resistance and bulk density (at least for coarser BC amended loamy sand) has been previously reported (Hernanz *et al.*, 2000). The penetration resistance of the surface soil was higher with the crust intact than with the crust removed (33.9±1.0 vs 27.9±1.0 N cm^{-2}). However, the decrease in penetration resistance per percent BC applied were similar ($p>0.05$) with crust intact or removed. This indicate that the strength of the crust was not affected by BC application. In the sand at Kaoma, there was no effect of BC on the penetration resistance probably due to absence of aggregation. Similar to observation in the present study, Busscher *et al.* (2010) observed a decrease in penetration resistance with increasing BC dose in loamy sand. However, the magnitude of decrease was 3–5 times higher in their study (~10 vs 2–3 N cm^{-2}).

Table 3. Regression parameters (±SE) of soil quality indicators versus dose of maize cob BC of different particle sizes in the loamy sand at Mkushi and the sand at Kaoma[1]

Soil quality indicator	Site	BC sizes	Intercept (0% BC)	Slope (change per % of BC added)
Bulk density ($g\ cm^{-3}$)	Mkushi	1-5 mm	1.29 (0.03)	-0.03 (0.010)**
		0.5-1 mm	1.25 (0.05)	0.00 (0.017)
		≤0.5 mm	1.27 (0.05)	-0.02 (0.015)
	Kaoma	1-5 mm	1.41 (0.03)	-0.03 (0.008)**
		0.5-1 mm	1.45 (0.04)	-0.04 (0.011)**
		≤0.5 mm	1.52 (0.04)	-0.03 (0.011)**
Field capacity (vol. %)	Mkushi	1-5 mm	14.5 (0.3)	1.0 (0.08)***
		0.5-1 mm	15.2 (0.5)	0.2 (0.14)
		≤0.5 mm	14.3 (0.5)	0.8 (0.12)***
	Kaoma	1-5 mm	13.9 (0.6)	1.2 (0.21)***
		0.5-1 mm	14.6 (0.9)	0.6 (0.30)
		≤0.5 mm	13.9 (0.9)	1.0 (0.33)***
Permanent wilting point (vol. %)	Mkushi	1-5 mm	3.8 (0.2)	0.06 (0.07)
		0.5-1 mm	3.8 (0.3)	0.10 (0.11)
		≤0.5 mm	3.9 (0.3)	0.15 (0.09)
	Kaoma	1-5 mm	2.1 (0.2)	0.34 (0.11)**
		0.5-1 mm	2.2 (0.4)	0.26 (0.17)
		≤0.5 mm	2.9 (0.4)	-0.01 (0.15)
Plant available water (vol. %)	Mkushi	1-5 mm	10.7 (0.4)	0.9 (0.11)***
		0.5-1 mm	11.4 (0.6)	0.1 (0.13)
		≤0.5 mm	10.4 (0.6)	0.7 (0.16)***
	Kaoma	1-5 mm	11.8 (0.7)	0.8 (0.20)***
		0.5-1 mm	12.4 (0.9)	0.3 (0.28)
		≤0.5 mm	11.0 (0.9)	1.0 (0.31)***
Total porosity (vol. %)	Mkushi	1-5 mm	50.6 (1.6)	1.4 (0.50)*
		0.5-1 mm	51.9 (2.3)	0.1 (0.82)
		≤0.5 mm	51.1 (2.3)	0.7 (0.70)
	Kaoma	1-5 mm	46.9 (1.2)	1.2 (0.34)**
		0.5-1 mm	45.6 (1.8)	1.5 (0.53)**
		≤0.5 mm	42.0 (1.7)	1.4 (0.47)**

The star in the slope column indicate a significant difference from zero. *$p<0.05$, **$p<0.01$, ***$p<0.001$. [1] Basic BC and soil properties are in Table 1 and 2.

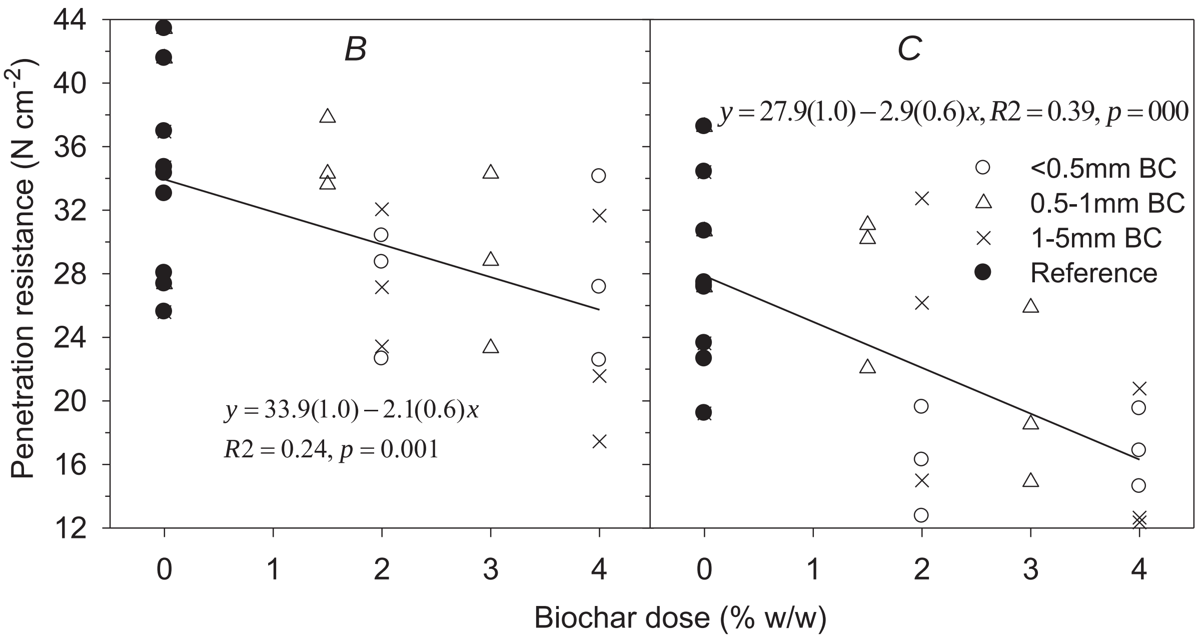

Fig. 7. Penetration resistance of soil amended with BC of different particle sizes. B = 0–6 mm Mkushi soil surface with crust intact in March 2015, C = 10–16 mm Mkushi soil layer underneath the crust in March 2015. Numbers in the bracket in regression equations are SEs. No difference between particle sizes (p>0.05).

The occurrence of the soil crust in the loamy sand at Mkushi is at least partly associated with weak soil aggregates, which was observed in this study. The aggregates were weak such that the procedure of rainfall simulation had to be adapted from routine 3 minutes duration with water pressure set at 1.5 bar to 2 minutes with water pressure at 1 bar in order for the method to become sensitive. Particularly for the loamy sand, mere draining of saturated soil caused structural collapse resulting in a decrease in soil volume of up to 20%. Awadhwal & Thierstein (1985) in there review reported that weak aggregates is one of the main precursors for formation of soil crust. Biochar did not have a consistent effect on the percentage of stable aggregates in the loamy sand at least after the first year of the experiment.

The data presented above supports the hypothesis that BC, under field conditions, increases aggregate stability in sandy loam soils, while reducing bulk density. However, the effect of BC was stronger under soybeans than under maize, which was not in accordance with the hypothesis. Biochar of different particle sizes had no consistent effect on aggregate stability in slightly coarser loamy sand probably due to slow development of aggregates. This does not support the hypothesis that finer BCs increase aggregate stability more than coarser

BCs. For the bulk density in the loamy sand, the result was opposite to the hypothesis that finer BC reduces bulk density more strongly than coarser BC. In the sand at Kaoma, BC reduced bulk density irrespective of BC particle size as hypothesized. The hypothesis that BC reduces penetration resistance in soil with potential for aggregation was supported by the data.

3.2 Paper I and II: Impact of biochar on soil hydraulic properties

Important soil hydraulic properties in the context of agricultural production are water retention and water conductivity in soil. Both can be affected by the water repellency status of the soil. These hydraulic properties depend on both soil structure and texture. Soil texture is usually stable but structure may change quite quickly in response to soil management practices. The observed changes in the aggregate stability (Fig. 5) and bulk density (Fig. 6 and Table 3) of the soils have implications for pore size distribution. Indeed the application of BC altered pore size distribution resulting in changes of water retention. Of great importance was the increase in field capacity and plant available water (Table 3) in all the soils studied, irrespective of texture. The mechanism behind increased plant available water in this study was different between the loamy soils in Mkushi and the sand in Kaoma. In the aggregating loamy soils at Mkushi, changes in pore size distribution were most likely due to a combination of both soil aggregation (Fig. 5) and BCs' internal high porosity (Table 1). In the sand on the other hand, the BCs' high porosity alone was likely the main reason for increased plant available water.

Alteration of the pore size distribution in clayey and sandy loam soils upon BC application has been reported recently (Sun & Lu, 2014, Sun *et al.*, 2014). Changes in pore size distribution was found to be the reason for increased plant available water in BC amended soil (de Melo Carvalho *et al.*, 2014). Worth noting is that BC may also increase the proportion of large pores larger than those (30 μm diameter) that hold water at field capacity. These large pores was generally not significantly increased in the present study unlike in previous studies such as Basso *et al.* (2013) and Sun & Lu (2014) where significant increases were reported. These large pores constitute, what is commonly referred to as, air capacity, which is a measure of the amount of air in the soil at field capacity. Increases in air capacity of the soil by BC might be important in clay soils, which often have aeration and drainage problems.

Macro-pores control water flow in soils, generally expressed in terms of K_{sat}. There was a small but significant decrease in K_{sat} of 0.13 cm hr^{-1} per percent BC added in the loamy sand at Mkushi ($p=0.02$) but not in sand at Kaoma. The water repellency data indicate that the decrease in K_{sat} was not due to the hydrophobic effect of BC as suggested by Jeffery *et al.* (2015). Instead, filling of the soil inter-particle pores by BC and collapse of water conducting pores in the soil at near saturation were more likely causes of the observed decrease in K_{sat}.

The soils at both Mkushi and Kaoma were non-repellent to water (Fig. 8). The absence of an effect of BC on water repellency of the bulk soil indicates that the BCs' hydrophobicity is temporary and is lost rather quickly upon mixing BC with soil. This has also been demonstrated by Yi *et al.* (2015). However, the increase in water repellency on crusted surface of the loamy sand soil due to the addition of ≤0.5 mm BC (Fig. 8) might be detrimental, as it reduces the water entry into soil resulting in surface run-off. Since the effect of BC on the hydrophobicity of soil was observed only for the crust of the Mkushi loamy sand, this effect is probably an indirect one (i.e. not due to hydrophobicity of BC per se). The dark shiny surface reflecting slimy growth (e.g. fungi) on the crusted surface of loamy sand amended with ≤0.5 mm BC might be the cause of water repellency (Doerr *et al.*, 2000). This finest BC aided fungal growth possibly by providing more nutrients than the coarse BC. Fine BC had lower loss on ignition (Table 1) indicating that it had higher amounts of inorganic compounds and nutrients.

Based on the data presented above, the hypothesis that BC increases water retention was supported. However, water retention was similar under both soybeans and maize unlike the hypothesis that the effect of BC would be stronger under maize. In addition, the hypothesis that fine BC increases water retention more strongly than coarser BCs was not supported. Finest (≤0.5 mm) and coarsest (1–5 mm) BC increased water retention while the intermediate size of 0.5–1 mm did not have any effect. The hypothesis that BC directly induces water repellency in coarse-textured soils was rejected. The hypothesis that BC increases K_{sat} in loamy sands due to BC-induced soil aggregation was not supported and instead, a decrease was observed. Biochars irrespective of particle sizes had no effect on K_{sat}

in sand at Kaoma. Therefore, the hypothesis that finest BCs reduces K_{sat} due to filling of inter particle space in sand was not supported.

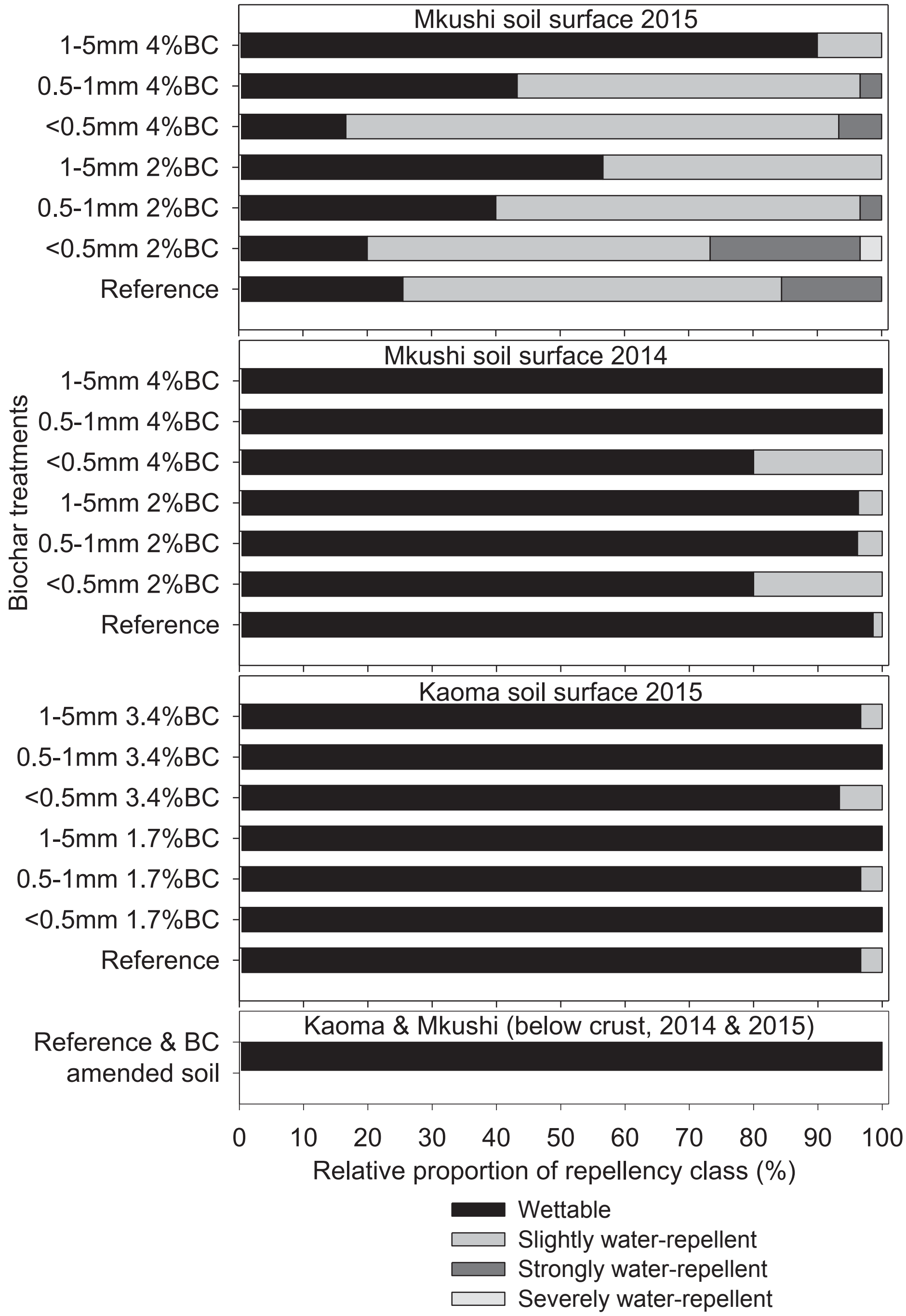

Fig. 8. Relative proportion of wettable and water repellent soil surface of a loamy sand (Mkushi) and a sand (Kaoma) for various BC treatments (n = 90 for reference plot and n = 30 for BC treatments). The bottom panel of Kaoma and Mkushi (below crust, 2014 & 2015) represents measurements conducted both in the field and laboratory irrespective of moisture content: all 100% wettable. Water repellency classes according to Dekker &

Jungerius (1990): WDPT < 5 s - wettable or non-water-repellent, 5 s < WDPT < 60 s - slightly water-repellent, 60 s < WDPT < 600 s - strongly water-repellent, 600 s < WDPT < 3600 s - severely water-repellent, WDPT > 3600 s - extremely water-repellent.

3.3 Paper III: Biochar mobility in soils

For sustained positive effect of BC on crop production, BC must remain within the root zone of the soil. The experimental data showed limited downward transport of BC in both loamy sand and sand of less than 3 cm below the BC application depth of 5 cm (Fig. 9). There was slightly more downward migration for finer than coarser BCs and more in the loamy sand than in the sand. Below the depth of 8 cm, there was no significant difference between the amount of BC recovered in BC amended and reference plots. Overall, 45–66% of the applied BC was recovered within the application depth, while 10–20% was found below the application depth down to 20 cm. This means that total recovered BC amounted to 55–76%. In other studies the downward migration was reported to be ~50% and <1% of the applied BC according to Haefele *et al.* (2011) and Major *et al.* (2010), respectively. However, the migration rate reported in those studies were observed at slightly greater depth relative to the one reported in the present study.

Between 24–45% of the applied BC was not found in the amended plots. Biochar was transported to adjacent reference plots as shown by the change in the $\delta^{13}C$ signal of their soil surface layer (0–5 cm) (Fig. 9). Slightly more BC was found in reference plot in the sand than in loamy sand. The recovery of BC in the reference plots means that lateral transport of BC was an important process and that it may account for much of the BC not found in the BC amended plots. This lateral transport was probably a result of erosion by water and/or wind. Major *et al.* (2010) reported that about 20–53% of the applied BC was not recovered in the amended plots. They suggested that erosion could be the main factor behind the observation. Interestingly, the proportion of BC not recovered in their study are in the same order of magnitude as in the present one.

The hypothesis that there is greater downward migration of finer BC in soils was supported by data. However, this was not in accordance with the hypothesis that downward transport was greater in the sand (higher K_{sat}) than in the loamy sand.

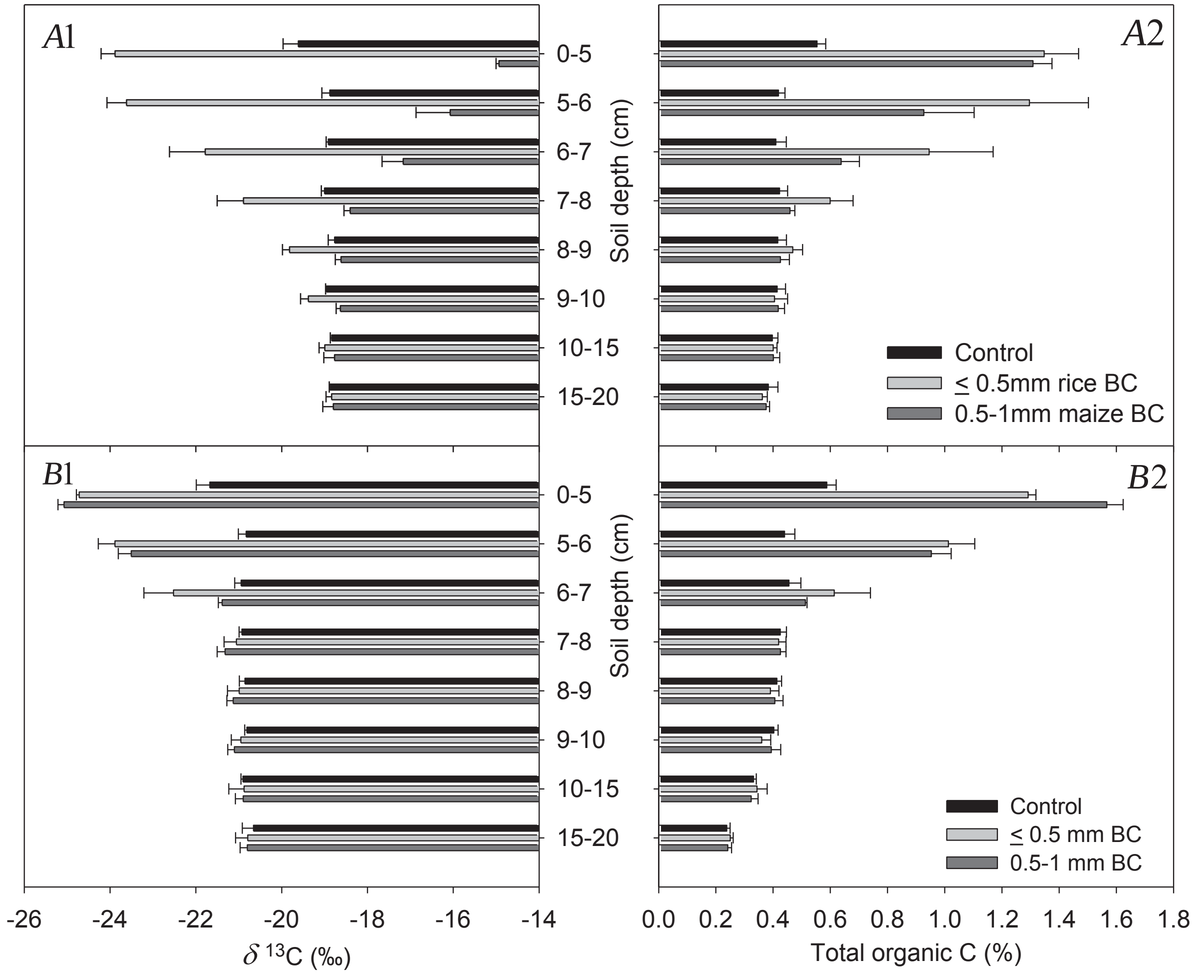

Fig. 9. Distribution of $\delta^{13}C$ and TOC in the soil profile one year after BC was applied to the surface soil (0–5 cm depth). *A1* and *A2* indicate the $\delta^{13}C$ and TOC for Mkushi soil amended with rice husk BC (with $\delta^{13}C$ = 27.3±0.03) and maize cob BC (with $\delta^{13}C$ = 12.3±0.3), whereas *B1* and *B2* indicate the $\delta^{13}C$ and TOC for the Kaoma soil (only rice BC was added). Error bar is SE.

3.4 Paper IV: Biochar pH effect on denitrification

Increasing amounts of BC (1 – 10% w/w) increased the soil pH, more so with cacao shell than rice husk BC. Concomitant with pH increase, BC reduced the net production of NO and N_2O and increased that of the final denitrification product, N_2 (Fig. 10). Suppression of N_2O in BC amended soils has been observed in a number of studies (Clough *et al.*, 2013, Cayuela *et al.*, 2014 and references therein). Since BC is very porous (Table 1) and increases soil porosity (Table 3), increased soil aeration has been proposed to be one of the main reasons for the observed suppression of N_2O (Yanai *et al.*, 2007). Suppressed denitrification by improved aeration would mean lower emission of denitrification gases. In the present study, any effect of BC on the soil matrix was effectively excluded by working with fully anoxic, continuously stirred soil slurries. Yet, a clear suppression of N_2O production (relative to N_2 production) was observed, suggesting that BC has a direct effect on the product stoichiometry of denitrification rather than suppressing denitrification as a whole. Other proposed mechanisms for reduced N_2O emission that were ruled out in the present study include reduced rates of denitrification through competition for electrons (Joseph *et al.*, 2010), as no suppression of overall denitrification was found. In addition, it is unlikely that N_2O suppression was explained by adsorption of ammonium, nitrate and N_2O (Clough *et al.*, 2010, Clough *et al.*, 2013, Cornelissen *et al.*, 2013) or by microbial N immobilization, due to labile C in BC (Bruun *et al.*, 2012). This is because the sum of N recovered in the denitrification products was more or less equal to the added amount of nitrate-N, particularly so in incubations with high denitrification rates (5 & 10% cacao shell BC addition) (Fig. 10).

In the present study, the inclusion of water- and acid-leached BCs resulted in a reduction or elimination of the BC effect on N_2O and NO. This indicates that some of the removed constituents during BC leaching were responsible for the suppression on NO and N_2O and the concomitant increase in N_2. Apart from organic C, alkalinity was the main constituent removed, resulting in a decline of the pH of BC. The lack of suppression of NO and N_2O net production in incubated systems amended with acidified rice husk BC (pH 2.5) illustrates the importance of pH for the denitrification product stoichiometry. Addition of NaOH, causing an increase in pH also resulted in suppression of NO and N_2O and a simultaneous increase in N_2. Although similar, the effect of BC was somewhat more pronounced than that of NaOH, at the same pH (Table 4). This suggests that also other factors than pH increase by BC contribute to the suppression of N_2O production. Earlier liming has been shown to suppress the net production of N_2O, while simultaneously increasing N_2 production. This was attributed to alleviating the impaired functioning of N_2O reductase at low pH (Liu *et al.*, 2010, Bakken *et al.*, 2012). The suppression of NO on the other hand seems to be due to less chemical decomposition of nitrite at higher pH (Braida & Ong, 2000, Islam *et al.*, 2008).

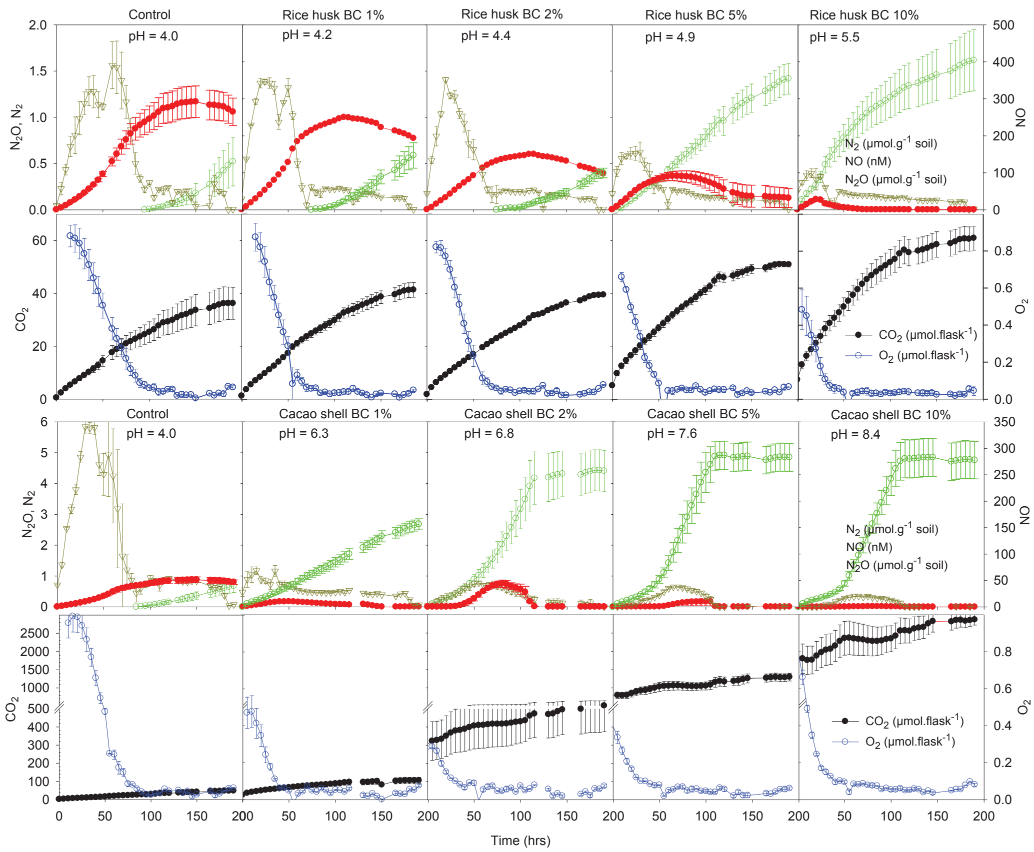

Fig. 10. Denitrification kinetics and CO_2 and O_2 concentrations in anoxic incubations of Lampung soil amended with increasing doses of untreated rice husk BC (upper 2 panels) and cacao shell BC (lower 2 panels). Shown are averages of three incubations; error bars denote SE. Approximately 6.1 µmol NO_3^--N g^{-1} was added to 9.8 g soil in the bottles. The pH values given in the figures denote the average pH 0.5 hours after adding the BC. Note the differences in the scale of y-axis.

The addition of uncharred cacao shell, which also had a pH effect suppressed N_2O net production and the suppression per unit pH increase was similar in magnitude to the NaOH addition. This implies that the stronger suppression of N_2O by BC was due to some property of the char not found in feedstock. Labile C in BC played no role in altering the proportion of denitrification gases despite playing a role in increasing the denitrification rate. The electron shuttling proposed by Cayuela *et al.* (2013) could be the additional mechanism that resulted to stronger suppression by BC than NaOH.

Overall the hypothesis that BC suppresses the net production of NO and N_2O in acid soil during denitrification was confirmed. Although pH increase was found to be the main factor responsible for the suppression, some other factors may have contributed.

Table 4. Regression coefficients of N_2O product ratios explained by dose effect (w/w %) or by pH effect of different amendments added to Lampung soil

Analysis	Amendment	Intercept	Slope	Significance of slope	R^2
Dose effect	Untreated rice husk BC	0.90 (0.05)	-0.092 (0.010)	Slope different from zero ($p<0.001$)	0.91
	Water leached rice husk BC	0.84 (0.05)	-0.083 (0.010)	Slope not different from untreated rice husk BC ($p>0.05$)	
	Acid leached rice husk BC	0.81 (0.05)	-0.004 (0.010)	Slope different from untreated rice husk BC ($p<0.001$)	
	Uncharred cacao shell	0.77 (0.05)	-0.044 (0.010)	Slope different from untreated rice husk BC ($p<0.001$)	
pH effect	NaOH	2.34 (0.27)	-0.326 (0.047)	Slope different from zero ($p<0.001$)	0.80
	Untreated rice husk BC	5.12 (0.61)	-0.856 (0.113)	Slope different from NaOH ($p<0.001$)	
	Water leached rice husk BC	4.72 (0.81)	-0.797 (0.150)	Slope different from NaOH ($p<0.01$)	
	Acid leached rice husk BC	1.77 (0.52)	0.005 (0.107)	Slope different from NaOH ($p<0.01$)	
	Uncharred cacao shell	2.66 (0.59)	-0.399 (0.110)	Slope not different from NaOH ($p>0.05$)	

Intercept = value of product ratio at 0% BC and uncharred cacao shell addition or if pH of the soil would be zero. Slope = unit decrease in product ratio per percent increase of BC or uncharred cacao shell added or per unit increase in soil pH due to amendment added. Numbers in brackets are the standard errors.

4. Conclusions and Implications

In the present study, BC was found to be beneficial to soil properties and processes in the context of agricultural production and climate change mitigation in acid coarse-textured soils. Biochar improved structural properties in sandy loams and loamy sands, including aggregate stability and bulk density. In the sandy loam soil, the effect of BC on aggregate stability was dependent on the type of crop, the effect being greater under soybeans than under maize. In loamy sand, due to its coarser texture than sandy loam, BC-induced aggregate development was weak. Biochar reduced penetration resistance in loamy soil likely due to the improvement of soil structure. The reduction in penetration resistance may aid root growth in soil. The improvement in soil structure (aggregate stability) especially in loamy sand did not reduce the formation of a soil crust.

Biochar also changed the pore size distribution of aggregating soil due to both BC-induced soil aggregation and the high porosity of BC. In sand at Kaoma, BC altered pore size distribution in addition to lowering the bulk density mainly due to the high porosity of BC itself. The change in pore size distribution of both loamy soils and sand resulted in an increased field capacity and plant available water. The water flow within the loamy sand, expressed as K_{sat}, was reduced, presumably because BC blocked some of the inter-particle pore space or because some soil pores collapsed at near saturation in loamy sand. The decrease in K_{sat} of the soil was not due to the hydrophobic effect of BC, as BC did not cause water repellency in the studied soils. Only the crusted surface in loamy sand amended with finest BC of ≤0.5 mm showed increased water repellency, albeit most likely due to BC-stimulated growth of fungi rather than a direct effect of the added BC. Increased repellency of the crust may seriously reduce water entry into soil. Coarser BCs of 0.5–1 and 1–5 mm, in turn, reduced water repellency of crusted soil surfaces compared with untreated soils. The moderate reduction in K_{sat} in the loamy sand may not have any negative effect but instead prevent water from draining rapidly from the root zone and hence increase water availability for crops. Since 1–5 mm BC reduced water repellency of the crust and increased plant available water better than finer BCs, use of 1–5 mm BC is recommended especially in soil where water holding capacity is low. Use of coarser BC also reduces energy requirement for crushing BC.

The downward migration of BC was generally slow given the high K_{sat} and fine BCs applied. This indicates that downward migration may not be an important process especially when coarser BCs are applied, at least in the short term. Lateral transport of BC through erosion is likely an important process in soil that may move large amounts of BC from crop fields where it has been applied. However, applying BC in planting basins like those used in conservation farming in Zambia, may reduce lateral transport if the basins are not opened often.

In this study, it was also shown that BC is important in suppressing NO and N_2O emission through directly controlling the product stoichiometry of denitrification, the quantitatively most important biological process for N_2O formation in soil. The increase of soil pH by BC addition was identified as the major factor mediating this suppression. The suppression of NO was likely linked to less chemical decomposition of nitrite to NO due to pH increase. N_2O suppression on the other hand was in accordance with the notion that raising pH in acid soils greatly stimulates N_2O reductase enzyme activity resulting in more complete denitrification with N_2 as the dominant end product. Other factor(s) contributing to the observed increase in N_2O reductase activity such as BC-mediated electron shuttling may play a role, but further experiments have to be done to test this.

5. Outlook

In this study, I show that BC improves soil physical conditions important for increased crop production in light-textured tropical soils in Zambia. Since the main motivation for adoption of BC technology in tropical soils lies in the capacity of BC to increase crop production at low cost, BCs should be designed to suit this purpose. Biochar has to be optimized to alleviate specific soil related problems that limit crop production (Abiven *et al.*, 2014). In Zambia, the main challenges for crop production are the high acidity of soils, their low CEC, nutrient status and plant available water. In the case of coarse-textured Zambian soils, BC with high pH, CEC and porosity prepared from feedstock with high nutrient content is ideal. Budai *et al.* (2014) reported that porosity and pH of BCs increase with pyrolysis temperature reaching a maximum at ~600 ^oC while CEC reaches a maximum at ~400 ^oC. Therefore, the optimal BC for Zambian condition should be produced within this temperature range. To meet the demand for a full-scale implementation of BC technology in tropical crop production, available crop wastes would have to be utilized as feedstock but feedstocks that give the highest pH, CEC, porosity and nutrients should be preferred. The possibility of loading the BCs with additional nutrients needs to be explored such as use of urine (Schmidt *et al.*, 2015) or co-composting (Kammann *et al.*, 2015).

Production of BC using clean technology has to be promoted. Traditional BC production technology such as earth mount in Zambia can result in large emissions of particulate matter, less volatile organic compounds, methane and carbon monoxide with negative impact on health and climate change (Sparrevik *et al.*, 2013). However, with strong increases in crop yield, Sparrevik *et al.* (2013) showed in a life cycle analysis that the negative impact of emissions from BC production are compensated for. Life cycle analysis combines all effects of BC, including mitigation of climate change, mitigation of pollutant emission, soil improvement, energy production and waste management. In Kaoma with its sandy acidic soils, application of BC in agriculture (both conventional and conservation) resulted in an overall beneficial impact (Sparrevik *et al.*, 2013). This means that BC has large potential not only in Zambia, but also in other areas around the world where crop production challenges are similar to those of Zambia.

With respect to crop production, the current study focuses on the effect of BC on soil physical properties of loamy and sandy soils. The study was relatively short-term (two cropping seasons) and more long-term experiments are required. Also, other factors controlling BC-induced soil aggregation need to be assessed in detail. These factors include soil texture, BC particle sizes and the loss and conversion of BC in the root zone of soil. Biochar particle sizes influenced several soil physical properties differently in the two soils studied. Incorporation of BC particle sizes even beyond those used in the present study need to be considered in future research. To understand the effects of BC on soil hydrology fully, field studies are required and should include use of TDR, tensiometers and water transport models. In general, we know little about other soil types and studies should incorporate a range of soils from light- to heavy-textured ones.

There was no effect of BC on the strength of soil crust. Since this is the first study to consider this soil phenomenon, a more detailed assessment is needed for a range of soil types. The occurrence of increased water repellency on crusted soil surfaces upon addition of BC, especially fine ones, needs to be considered in future research to assess whether it is a local problem or more widely occurring.

The mobility of BC in soil in the present study calls for a strategy to minimize such mobility. Research to identify ways of reducing BC mobility especially the high lateral transport need to be conducted. Application of BC in the planting basins of conservation farming and infrequently opening the basins may reduce lateral transport. Incorporation of BC during mechanical ripping of soil may also reduce lateral transport of BC but needs further assessment.

In the present study, BC application to soil suppressed NO and N_2O net production, two gases with detrimental effects in atmospheric chemistry and climate change. The mechanism for reduced N_2O emission other than pH effect need further assessment. Such studies need to identify accurately the underlying N-turnover process occurring in the soil.

In a meta-analysis of published data from both laboratory and field experiments on the effect of BC addition on N_2O emissions, Cayuela *et al.* (2014) found an overall emission suppression of 54%. Montzka *et al.* (2011) reported that 40% of global N_2O emission comes from anthropogenic sources, which amounts to 6.7±1.7 Tg N yr^{-1} (3.1±0.8 Gt CO_2-C equivalent per year). Of this, 4.1 Tg N yr^{-1} N_2O emission is from agriculture, mostly as direct soil emission. If BC is to be used in agricultural land globally, emission of N_2O from agriculture will be reduced by 54% i.e. ~2.2 Tg N yr^{-1} (1.0 Gt CO_2-C equivalent per year)! This climate change benefit will be important even without considering that BC locks up large amounts of C in soil. Currently, global anthropogenic (fossil fuel and land-use change) emission of CO_2 is about 7 Gt C yr^{-1} (Lehmann & Joseph, 2009). Woolf *et al.* (2010) estimated the maximum reduction of greenhouse gas emission by BC at 1.8 Gt CO_2-C equivalent per year, which is about 12% of current anthropogenic emission. This climate change mitigation coupled with increase in crop production is an enormous benefit from a simple technology such as BC.

References

Abdullah H & Wu H (2009) Biochar as a fuel: 1. Properties and grindability of biochars produced from the pyrolysis of mallee wood under slow-heating conditions. *Energy & Fuels* **23**: 4174-4181.

Abiven S, Schmidt MWI & Lehmann J (2014) Biochar by design. *Nature Geoscience* **7**: 326-327.

Abiven S, Hund A, Martinsen V & Cornelissen G (2015) Biochar amendment increases maize root surface areas and branching: a shovelomics study in Zambia. *Plant and Soil* 1-11.

Agegnehu G, Bass AM, Nelson PN, Muirhead B, Wright G & Bird MI (2015) Biochar and biochar-compost as soil amendments: Effects on peanut yield, soil properties and greenhouse gas emissions in tropical North Queensland, Australia. *Agriculture, Ecosystems & Environment* **213**: 72-85.

Alling V, Hale SE, Martinsen V, Mulder J, Smebye A, Breedveld GD & Cornelissen G (2014) The role of biochar in retaining nutrients in amended tropical soils. *Journal of Plant Nutrition and Soil Science* **177**: 671-680.

Amézketa E (1999) Soil aggregate stability: A review. *Journal of Sustainable Agriculture* **14**: 83-151.

Angst TE & Sohi SP (2013) Establishing release dynamics for plant nutrients from biochar. *GCB Bioenergy* **5**: 221-226.

Atkinson CJ, Fitzgerald JD & Hipps NA (2010) Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant and Soil* **337**: 1-18.

Awad YM, Blagodatskaya E, Ok YS & Kuzyakov Y (2013) Effects of polyacrylamide, biopolymer and biochar on the decomposition of ^{14}C-labelled maize residues and on their stabilization in soil aggregates. *European Journal of Soil Science* **64**: 488-499.

Awadhwal NK & Thierstein GE (1985) Soil crust and its impact on crop establishment: A review. *Soil and Tillage Research* **5**: 289-302.

Bakken LR, Bergaust L, Liu B & Frostegard A (2012) Regulation of denitrification at the cellular level: a clue to the understanding of N_2O emissions from soils. *Philosophical transactions of the Royal Society of London Series B, Biological sciences* **367**: 1226-1234.

Barnes RT, Gallagher ME, Masiello CA, Liu Z & Dugan B (2014) Biochar-induced changes in soil hydraulic conductivity and dissolved nutrient fluxes constrained by laboratory experiments. *PloS one* **9**: e108340.

Basso AS, Miguez FE, Laird DA, Horton R & Westgate M (2013) Assessing potential of biochar for increasing water-holding capacity of sandy soils. *GCB Bioenergy* **5**: 132-143.

Biederman LA & Harpole WS (2013) Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. *GCB Bioenergy* **5**: 202-214.

Braida W & Ong SK (2000) Decomposition of nitrite under various pH and aeration conditions. *Water, Air, and Soil Pollution* **118**: 13-26.

Braida WJ, Pignatello JJ, Lu Y, Ravikovitch PI, Neimark AV & Xing B (2003) Sorption hysteresis of benzene in charcoal particles. *Environmental Science & Technology* **37**: 409-417.

Bridle T & Pritchard D (2004) Energy and nutrient recovery from sewage sludge via pyrolysis. *Water Science & Technology* **50**: 169-175.

Bruun EW, Ambus P, Egsgaard H & Hauggaard-Nielsen H (2012) Effects of slow and fast pyrolysis biochar on soil C and N turnover dynamics. *Soil Biology and Biochemistry* **46**: 73-79.

Bruun EW, Petersen CT, Hansen E, Holm JK & Hauggaard-Nielsen H (2014) Biochar amendment to coarse sandy subsoil improves root growth and increases water retention. *Soil Use and Management* **30**: 109-118.

Budai A, Wang L, Gronli M, Strand LT, Antal MJ, Abiven S, Dieguez-Alonso A, Anca-Couce A & Rasse DP (2014) Surface properties and chemical composition of corn cob and miscanthus biochars: Effects of production temperature and method. *Journal of Agricultural and Food Chemistry* **62**: 3791-3799.

Busscher WJ, Novak JM, Evans DE, Watts DW, Niandou MAS & Ahmedna M (2010) Influence of pecan biochar on physical properties of a Norfolk loamy sand. *Soil Science* **175**: 10-14.

Cayuela ML, Sánchez-Monedero MA, Roig A, Hanley K, Enders A & Lehmann J (2013) Biochar and denitrification in soils: when, how much and why does biochar reduce N_2O emissions? *Scientific Reports* **3**: 1732.

Cayuela ML, van Zwieten L, Singh BP, Jeffery S, Roig A & Sánchez-Monedero MA (2014) Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis. *Agriculture, Ecosystems & Environment* **191**: 5-16.

Clough T, Condron L, Kammann C & Müller C (2013) A review of biochar and soil nitrogen dynamics. *Agronomy* **3**: 275-293.

Clough TJ & Condron LM (2010) Biochar and the Nitrogen Cycle: Introduction. *Journal of Environment Quality* **39**: 1218.

Clough TJ, Bertram JE, Ray JL, Condron LM, O'Callaghan M, Sherlock RR & Wells NS (2010) Unweathered wood biochar impact on nitrous oxide emissions from a bovine-urine-amended pasture soil. *Soil Science Society of America Journal* **74**: 852.

Cornelissen G, Rutherford DW, Arp HP, Dörsch P, Kelly CN & Rostad CE (2013) Sorption of pure N_2O to biochars and other organic and inorganic materials under anhydrous conditions. *Environmental Science & Technology* **47**: 7704-7712.

Cornelissen G, Martinsen V, Shitumbanuma V, Alling V, Breedveld G, Rutherford D, Sparrevik M, Hale S, Obia A & Mulder J (2013) Biochar effect on maize yield and soil characteristics in five conservation farming sites in Zambia. *Agronomy* **3**: 256-274.

Crutzen PJ (1970) Influence of nitrogen oxides on atmospheric ozone content. *Quarterly Journal or the Royal Meteorological Society* **96**: 320.

de Melo Carvalho MT, de Holanda Nunes Maia A, Madari BE, Bastiaans L, van Oort PAJ, Heinemann AB, Soler da Silva MA, Petter FA, Marimon Jr BH & Meinke H (2014) Biochar increases plant-available water in a sandy loam soil under an aerobic rice crop system. *Solid Earth* **5**: 939-952.

Dekker L & Jungerius P (1990) Water repellency in the dunes with special reference to The Netherlands. *Catena, Supplement* 173-183.

Doerr SH, Shakesby RA & Walsh RPD (2000) Soil water repellency: Its causes, characteristics and hydro-geomorphological significance. *Earth-Science Reviews* **51**: 33-65.

Eibisch N, Durner W, Bechtold M, Fuß R, Mikutta R, Woche SK & Helfrich M (2015) Does water repellency of pyrochars and hydrochars counter their positive effects on soil hydraulic properties? *Geoderma* **245–246**: 31-39.

Feng Y, Xu Y, Yu Y, Xie Z & Lin X (2012) Mechanisms of biochar decreasing methane emission from Chinese paddy soils. *Soil Biology and Biochemistry* **46**: 80-88.

Firestone MK & Davidson EA (1989) Microbiological basis of NO and N_2O production and consumption in soil. *Exchange of trace gases between terrestrial ecosystems and the atmosphere* **47**: 7-21.

Glaser B, Lehmann J & Zech W (2002) Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal - a review. *Biology and Fertility of Soils* **35**: 219-230.

Glaser B, Haumaier L, Guggenberger G & Zech W (2001) The'Terra Preta'phenomenon: A model for sustainable agriculture in the humid tropics. *Naturwissenschaften* **88**: 37-41.

Grønsten HA & Børresen T (2009) Comparison of two methods for assessment of aggregate stability of agricultural soils in southeast Norway. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science* **59**: 567-575.

Gurwick NP, Moore LA, Kelly C & Elias P (2013) A systematic review of biochar research, with a focus on its stability in situ and its promise as a climate mitigation strategy. *PloS One* **8**: e75932.

Haefele SM, Konboon Y, Wongboon W, Amarante S, Maarifat AA, Pfeiffer EM & Knoblauch C (2011) Effects and fate of biochar from rice residues in rice-based systems. *Field Crops Research* **121**: 430-440.

Hale SE, Alling V, Martinsen V, Mulder J, Breedveld GD & Cornelissen G (2013) The sorption and desorption of phosphate-P, ammonium-N and nitrate-N in cacao shell and corn cob biochars. *Chemosphere* **91**: 1612-1619.

Hasegawa T, Fujimori S, Shin Y, Tanaka A, Takahashi K & Masui T (2015) Consequence of climate mitigation on the risk of hunger. *Environmental Science & Technology* **49**: 7245-7253.

Herath HMSK, Camps-Arbestain M & Hedley M (2013) Effect of biochar on soil physical properties in two contrasting soils: An Alfisol and an Andisol. *Geoderma* **209-210**: 188-197.

Hernanz JL, Peixoto H, Cerisola C & Sánchez-Girón V (2000) An empirical model to predict soil bulk density profiles in field conditions using penetration resistance, moisture content and soil depth. *Journal of Terramechanics* **37**: 167-184.

IPCC (2007) Climate change 2007: The physical science basis. *Agenda* **6**: 333.

Islam A, Chen D, White RE & Weatherley AJ (2008) Chemical decomposition and fixation of nitrite in acidic pasture soils and implications for measurement of nitrification. *Soil Biology and Biochemistry* **40**: 262-265.

Jeffery S, Verheijen FGA, van der Velde M & Bastos AC (2011) A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agriculture, Ecosystems & Environment* **144**: 175-187.

Jeffery S, Meinders MBJ, Stoof CR, Bezemer TM, van de Voorde TFJ, Mommer L & van Groenigen JW (2015) Biochar application does not improve the soil hydrological function of a sandy soil. *Geoderma* **251-252**: 47-54.

Joseph S, Camps-Arbestain M, Lin Y, Munroe P, Chia C, Hook J, Van Zwieten L, Kimber S, Cowie A & Singh B (2010) An investigation into the reactions of biochar in soil. *Soil Research* **48**: 501-515.

Kammann C, Ratering S, Eckhard C & Müller C (2012) Biochar and hydrochar effects on greenhouse gas (carbon dioxide, nitrous oxide, and methane) fluxes from soils. *Journal of Environmental Quality* **41**: 1052-1066.

Kammann CI, Schmidt HP, Messerschmidt N, Linsel S, Steffens D, Muller C, Koyro HW, Conte P & Joseph S (2015) Plant growth improvement mediated by nitrate capture in co-composted biochar. *Scientific Reports* **5**: 11080.

Karer J, Wimmer B, Zehetner F, Kloss S & Soja G (2013) Biochar application to temperate soils: effects on nutrient uptake and crop yield under field conditions. *Agricultural and Food Science* **22**: 390-403.

Khanmohammadi Z, Afyuni M & Mosaddeghi MR (2015) Effect of pyrolysis temperature on chemical and physical properties of sewage sludge biochar. *Waste management & research: The journal of the International Solid Wastes and Public Cleansing Association, ISWA* **33**: 275-283.

Kieft TL, soroker E & firestone MK (1987) Microbial biomass response to a rapid increase in water potential when dry soil is wetted. *Soil Biology and Biochemistry* **19**: 119-126.

Kuzyakov Y, Bogomolova I & Glaser B (2014) Biochar stability in soil: Decomposition during eight years and transformation as assessed by compound-specific ^{14}C analysis. *Soil Biology and Biochemistry* **70**: 229-236.

Lehmann J & Joseph S (2009) Biochar for environmental management: An introduction. (Lehmann J & Joseph S, eds.), pp. 1-9. Earthscan, London, UK.

Lehmann J, Gaunt J & Rondon M (2006) Bio-char sequestration in terrestrial ecosystems – A review. *Mitigation and Adaptation Strategies for Global Change* **11**: 395-419.

Lehmann J, Kern DC, Glaser B & Woods WI (2007) Amazonian dark earths: Origin Properties Management. Springer Science & Business Media.

Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC & Crowley D (2011) Biochar effects on soil biota – A review. *Soil Biology and Biochemistry* **43**: 1812-1836.

Liu B, Frostegard A & Bakken LR (2014) Impaired reduction of N_2O to N_2 in acid soils is due to a posttranscriptional interference with the expression of nosZ. *mBio* **5**: e01383-01314.

Liu B, Morkved PT, Frostegard A & Bakken LR (2010) Denitrification gene pools, transcription and kinetics of NO, N_2O and N_2 production as affected by soil pH. *FEMS microbiology ecology* **72**: 407-417.

Liu X-H, Han F-P & Zhang X-C (2012) Effect of biochar on soil aggregates in the loess plateau: Results from incubation experiments. *International Journal of Agriculture & Biology* **14**: 975–979.

Liu Y, Yang M, Wu Y, Wang H, Chen Y & Wu W (2011) Reducing CH_4 and CO_2 emissions from waterlogged paddy soil with biochar. *Journal of Soils and Sediments* **11**: 930-939.

Luo Y, Durenkamp M, De Nobili M, Lin Q & Brookes PC (2011) Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. *Soil Biology and Biochemistry* **43**: 2304-2314.

Major J, Lehmann J, Rondon M & Goodale C (2010) Fate of soil-applied black carbon: downward migration, leaching and soil respiration. *Global Change Biology* **16**: 1366-1379.

Marti M (1984) Kontinuierlicher Getreidebau ohne Plug im Südosten Norwegens - Wirkung auf Ertrag, Physikalsche und Chemische Bodenparameter. Thesis, Agricultural University of Norway, Ås.

Martinsen V, Mulder J, Shitumbanuma V, Sparrevik M, Børresen T & Cornelissen G (2014) Farmer-led maize biochar trials: Effect on crop yield and soil nutrients under conservation farming. *Journal of Plant Nutrition and Soil Science* **177**: 681-695.

Martinsen V, Alling V, Nurida NL, Mulder J, Hale SE, Ritz C, Rutherford DW, Heikens A, Breedveld GD & Cornelissen G (2015) pH effects of the addition of three biochars to acidic Indonesian mineral soils. *Soil Science and Plant Nutrition* 1-14.

Molstad L, Dörsch P & Bakken LR (2007) Robotized incubation system for monitoring gases (O_2, NO, N_2O N_2) in denitrifying cultures. *Journal of Microbiological Methods* **71**: 202-211.

Montzka SA, Dlugokencky EJ & Butler JH (2011) Non-CO_2 greenhouse gases and climate change. *Nature* **476**: 43-50.

Mukherjee A & Lal R (2013) Biochar impacts on soil physical properties and greenhouse gas emissions. *Agronomy* **3**: 313-339.

Mukherjee A, Zimmerman A & Harris W (2011) Surface chemistry variations among a series of laboratory-produced biochars. *Geoderma* **163**: 247-255.

Mukherjee A, Lal R & Zimmerman AR (2014) Impacts of 1.5-year field aging on biochar, humic acid, and water treatment residual amended soil. *Soil Science* **179**: 333-339.

Nadeem S, Dörsch P & Bakken LR (2013) The significance of early accumulation of nanomolar concentrations of NO as an inducer of denitrification. *FEMS Microbiology Ecology* **83**: 672-684.

Nelissen V, Saha BK, Ruysschaert G & Boeckx P (2014) Effect of different biochar and fertilizer types on N_2O and NO emissions. *Soil Biology and Biochemistry* **70**: 244-255.

O'Toole A, Knoth de Zarruk K, Steffens M & Rasse DP (2013) Characterization, stability, and plant effects of kiln-produced wheat straw biochar. *Journal of Environmental Quality* **42**: 429-436.

Ogawa M & Okimori Y (2010) Pioneering works in biochar research, Japan. *Australian Journal of Soil Research* **48**: 489-500.

Page-Dumroese D, Robichaud P, Brown R & Tirocke J (2015) Water repellency of two forest soils after biochar addition. *TRANSACTIONS OF THE ASABE* **58**: 335-342.

R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raut N, Dörsch P, Sitaula BK & Bakken LR (2012) Soil acidification by intensified crop production in South Asia results in higher $N_2O/(N_2 + N_2O)$ product ratios of denitrification. *Soil Biology and Biochemistry* **55**: 104-112.

Reid J & Goss M (1981) Effect of living roots of different plant species on the aggregate stability of two arable soils. *Journal of soil science*.

Reid JB & Goss MJ (1981) Effect of living roots of different plant species on the aggregate stability of two arable soils. *Journal of Soil Science* **32**: 521-541.

Ritchie JD & Perdue EM (2008) Analytical constraints on acidic functional groups in humic substances. *Organic Geochemistry* **39**: 783-799.

Rumpel C, Chaplot V, Planchon O, Bernadou J, Valentin C & Mariotti A (2006) Preferential erosion of black carbon on steep slopes with slash and burn agriculture. *CATENA* **65**: 30-40.

Schmidt H, Pandit B, Martinsen V, Cornelissen G, Conte P & Kammann C (2015) Fourfold increase in pumpkin yield in response to low-dosage root zone application of urine-enhanced biochar to a fertile tropical soil. *Agriculture* **5**: 723-741.

Schmidt MW, Torn MS, Abiven S, *et al.* (2011) Persistence of soil organic matter as an ecosystem property. *Nature* **478**: 49-56.

Singh BP, Hatton BJ, Singh B, Cowie AL & Kathuria A (2010) Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrasting soils. *Journal of Environment Quality* **39**: 1224.

Smebye A (2014) The effect of biochar on dissolved organic matter in soil. Master Thesis Thesis, University of Oslo, Oslo.

Sohi SP, Krull E, Lopez-Capel E & Bol R (2010) Chapter 2 - A review of biochar and its use and function in soil. *Advances in Agronomy,* Vol. 105, pp. 47-82. Academic Press.

Soinne H, Hovi J, Tammeorg P & Turtola E (2014) Effect of biochar on phosphorus sorption and clay soil aggregate stability. *Geoderma* **219–220**: 162-167.

Sparrevik M, Field JL, Martinsen V, Breedveld GD & Cornelissen G (2013) Life cycle assessment to evaluate the environmental impact of biochar implementation in conservation agriculture in Zambia. *Environmental Science & Technology* **47**: 1206-1215.

Spokas KA, Novak JM, Masiello CA, Johnson MG, Colosky EC, Ippolito JA & Trigo C (2014) Physical disintegration of biochar: An overlooked process. *Environmental Science & Technology Letters* **1**: 326-332.

Steiner C, Teixeira WG, Lehmann J, Nehls T, de Macêdo JLV, Blum WEH & Zech W (2007) Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant and Soil* **291**: 275-290.

Sun F & Lu S (2014) Biochars improve aggregate stability, water retention, and pore-space properties of clayey soil. *Journal of Plant Nutrition and Soil Science* **177**: 26-33.

Sun Z, Arthur E, de Jonge LW, Elsgaard L & Moldrup P (2014) Pore structure characteristics after two years biochar application to a sandy loam field. *Journal of Soil Science*.

Ulyett J, Sakrabani R, Kibblewhite M & Hann M (2014) Impact of biochar addition on water retention, nitrification and carbon dioxide evolution from two sandy loam soils. *European Journal of Soil Science* **65**: 96-104.

Upadhyaya S, Sakai K & Glancey J (1995) Instrumentation for in-field measurement of soil crust strength. *Transactions of the ASAE* **38**: 39-44.

Uzoma KC, Inoue M, Andry H, Fujimaki H, Zahoor A & Nishihara E (2011) Effect of cow manure biochar on maize productivity under sandy soil condition. *Soil Use and Management* **27**: 205-212.

van Genuchten MT (1980) A Closed-form equation for predicting the hydraulic conductivity of unsaturated soils. *Soil Sci Soc Am J* **44**: 892-898.

Verheijen FGA, Jeffery S, Bastos AC, van der Velde M & Diafas I (2009) Biochar application to soils: A critical scientific review of effects on soil properties, processes and functions. *EUR 24099 EN, Office for the Official Publications of the European Communities, Luxembourg, 149pp*.

Woolf D, Amonette JE, Street-Perrott FA, Lehmann J & Joseph S (2010) Sustainable biochar to mitigate global climate change. *Nature Communications* **1**: 1-9.

Yamato M, Okimori Y, Wibowo IF, Anshori S & Ogawa M (2006) Effects of the application of charred bark of Acacia mangium on the yield of maize, cowpea and peanut, and soil chemical properties in South Sumatra, Indonesia. *Soil Science and Plant Nutrition* **52**: 489-495.

Yanai Y, Toyota K & Okazaki M (2007) Effects of charcoal addition on N_2O emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments. *Soil Science and Plant Nutrition* **53**: 181-188.
Yatagai A (2011) Interannual variation of seasonal rain fall in South Zambia. Vol. FR4 Project Report (Umetsu C, ed.) pp. 206-212. Research Institute For Humanity And Nature, Kyoto.
Yi S, Witt B, Chiu P, Guo M & Imhoff P (2015) The origin and reversible nature of poultry litter biochar hydrophobicity. *Journal of environmental quality* **44**: 963-971.
Zavalloni C, Alberti G, Biasiol S, Vedove GD, Fornasier F, Liu J & Peressotti A (2011) Microbial mineralization of biochar and wheat straw mixture in soil: A short-term study. *Applied Soil Ecology* **50**: 45-51.
Zhang A, Cui L, Pan G, Li L, Hussain Q, Zhang X, Zheng J & Crowley D (2010) Effect of biochar amendment on yield and methane and nitrous oxide emissions from a rice paddy from Tai Lake plain, China. *Agriculture, Ecosystems & Environment* **139**: 469-475.
Zhang A, Bian R, Pan G*, et al.* (2012) Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: A field study of 2 consecutive rice growing cycles. *Field Crops Research* **127**: 153-160.
Zimmerman AR, Gao B & Ahn M-Y (2011) Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biology and Biochemistry* **43**: 1169-1179.

PAPER I

In situ effects of biochar on aggregation, water retention and porosity in light-textured tropical soils

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In situ effects of biochar on aggregation, water retention and porosity in light-textured tropical soils

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ABSTRACT

Biochar (BC) has been reported to improve soil physical properties mainly in laboratory and greenhouse pot experiments. Here we study, under field conditions, the effect of BC and its particle sizes on soil aggregate stability, bulk density (BD), water retention, and pore size distribution in two experiments in Zambia. A) Farmer practice experiment in sandy loam with maize cob BC in conservation farming planting basins under maize and soybeans crops. B) Maize cob and rice husk BC particle size experiments ($\leq$0.5, 0.5–1 and 1–5 mm particle sizes) in loamy sand and sand. In the farmer practice experiment, BC increased aggregate stability by 7–9% and 17–20% per percent BC added under maize and soybeans crops respectively ($p < 0.05$) after two growing seasons. Total porosity and available water capacity (AWC) increased by 2 and 3% respectively per percent BC added ($p < 0.05$) under both crops, whereas BD decreased by 3–5% per percent BC added ($p \leq 0.01$). In the maize cob BC particle size experiment after one growing season, dose was a more important factor than particle size across the soils tested. Particle size of BC was more important in loamy sand than in sand, with $\leq$0.5 and 1–5 mm sizes producing the strongest effects on the measured properties. For example, BD decreased while total porosity increased ($p < 0.01$) for all BC particle sizes in sand whereas only 1–5 mm BC significantly decreased BD and increased total porosity in loamy sand ($p < 0.05$). However, AWC was significantly increased by only $\leq$0.5 and 1–5 mm BCs by 7–9% per percent BC added in both loamy sand and sand. Rice husk BC effect after one year followed similar pattern as maize cob BC but less effective in affecting soil physical properties. Overall, reduced density of soil due to BC-induced soil aggregation may aid root growth and with more water available, can increase crop growth and yields.

1. Introduction

Biochar (BC) is the charcoal product from pyrolysis of biomass and has been reported to increase crop production when applied to soils (Glaser et al., 2002). Increase in crop production has been attributed to BCs' inherent properties such as high pH, high cation exchange capacities (CEC), high specific surface area and its effects on soil properties (Steiner et al., 2007; Sun et al., 2014; Yamato et al., 2006). However, BC properties and the effect on crop production depend on feedstock, pyrolysis conditions and soil type (Jeffery et al., 2011).

The effects of BC on soil physical properties have received less attention than effects on soil chemical properties (Atkinson et al., 2010), despite the potential importance of improved physical properties in increasing crop production in light-textured soils (Cornelissen et al., 2013). One of the most important soil physical conditions supporting crop production is available water capacity (AWC), which is the difference between water content at −100 hPa matrix potential (field capacity—FC) and water content at −15000 hPa (permanent wilting point—PWP). Biochar has been shown to increase both soil water holding capacity and AWC (Basso et al., 2013; Cornelissen et al., 2013; Herath et al., 2013; Martinsen et al., 2014; Mukherjee and Lal, 2013). However, other studies found no effect of BC addition on water holding capacity (Carlsson et al., 2012). Most studies reporting increased water holding capacity involved FC measurements only, and without PWP data, it is difficult to quantify the increase in AWC. Indeed, an increase in PWP after BC addition (Carlsson et al., 2012; Herath et al., 2013) may cause an overall reduced effect on AWC despite increase in FC (Herath et al., 2013). In addition, most studies have been conducted as either laboratory incubations or pot trials in greenhouses. Reports from field studies are only now beginning to appear, e.g.,

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de Melo Carvalho et al. (2014) in which BC was found to increase AWC.

The increase in AWC upon BC addition in sandy and loamy soils (Mukherjee and Lal, 2013) are an indication of altered pore-size distribution (Sun et al., 2014). These increases could be a direct effect of BC due to its high porosity (Mukherjee and Lal, 2013) or an indirect effect due to soil aggregation. Recent incubation studies in the laboratory reported increased aggregate stability following BC addition (Awad et al., 2013; Herath et al., 2013; Liu et al., 2012; Ouyang et al., 2013; Soinne et al., 2014; Sun and Lu, 2014). Even in studies where soil aggregation was not measured, the general increase in water holding capacity and decrease in bulk density (BD) (Mukherjee and Lal, 2013) are potential indicators for increased soil aggregation in loamy soils. The reasons for stimulation of soil aggregation can be attributed to BC surface characteristics, which result in direct binding of soil particles or firstly sorption of soil organic matter, which then binds soil particles (Brodowski et al., 2006; Joseph et al., 2010). This behavior causes occlusion of BC into aggregates (Brodowski et al., 2006). In addition, BC may increase root biomass (Bruun et al., 2014) and root activity causing an increase in aggregate stability (Reid and Goss, 1981). The effect of roots on aggregate stability may depend on crop type with monocotyledonous plants having stronger effect than dicotyledonous plants (Amézketa, 1999), even under the influence of BC. Improved aggregation of loamy soils by BC may therefore cause an increase in AWC.

Soils with a sand to loamy sand texture have inherently low AWC and high air capacity. Such soils, having physical conditions not conducive for crop production, are common in large parts of western Zambia, classified mainly as Arenosols and central Zambia, classified mainly as Acrisols. Effects of adverse physical soil properties on crop growth are exacerbated by the high inter-annual variation of rainfall and the general trend of declining rainfall amount in some areas of Zambia (Yatagai, 2011). In effect, inter-annual variation of rainfall is a major factor explaining the already low production and productivity of the Zambian agricultural sector (Goverment of Zambia, 2011) dominated by smallholder farmers who rely on rain fed agriculture. Biochar produced from crop wastes such as maize cobs which is widely available, has been shown to increase crop yields in these soils (Cornelissen et al., 2013; Martinsen et al., 2014), probably partly due to BC's potential to increase AWC, as shown only under laboratory conditions.

In this study, we hypothesize that BC will improve soil physical properties (increase aggregate stability and water retention and reduce bulk density) depending on the crop type. Biochar with fine particles will improve soil physical properties, e.g., water retention, more strongly than coarse BC particles due to better mixing with soil.

The objectives of the present study were to determine the effect of (i) BC from maize cobs on soil aggregate stability, water retention and pore size distribution under conservation farming planted with maize and soybeans. (ii) particle sizes of maize cob and rice husk BC on soil aggregate stability, water retention and pore size distribution under maize in conventional farming.

To this end, two sets of field experiments were conducted in Zambia. The first experiment involved locally produced maize cob BC applied following conservation farming practices (Cornelissen et al., 2013). The second experiment involved the application of locally produced maize cob and rice husk BC of different particle sizes mixed into the soil. Water retention curves, aggregate stability and BD were then determined on the samples taken from the field experiments. This study is one of the few investigating these parameters under field conditions, under various crops, and for various "real-world" BCs (i.e., not synthesized in the laboratory) of different particle sizes.

2. Materials and methods

2.1. Biochar production

The BCs whose properties are presented in Table 1 were produced in a slow pyrolysis (2–3 days) from two feedstocks: Maize cob, which is widely available throughout Zambia, was our primary feedstock for BC implementation (Cornelissen et al., 2013; Martinsen et al., 2014) and rice husk, which is available in western Zambia. The maize cobs were complete dry cobs after removing grains. Biochars were produced in two batches and the first batch was produced in 2011 from maize cob at a temperature of approximately 350 °C and a residence time of 2 days (during most of the residence time, temperature was 300–350 °C) in a brick kiln at Mkushi, Zambia. The second batch was produced in 2013 from maize cob and rice husk in a drum retort kiln at Chisamba, Zambia at a temperature of 350 °C and a retention time of 1 day. Details of other production conditions can be found in Sparrevik et al. (2015). Biochar from the first batch was used in the farmer practice

Table 1
Soil and biochar properties.

Properties	Mkushi soil Exp. A	Mkushi soil Exp. B	Kaoma soil Exp. B	Maize cob BC Exp. A	Rice husk BC, Exp. B			Maize cob BC, Exp. B		
					≤0.5 mm	0.5–1 mm	Unsorted	≤0.5 mm	1–5 mm	Unsorted
Sand (%)	64.4	75.1	85.4	–	–	–	–	–	–	–
Silt (%)	23.5	15.9	10.2	–	–	–	–	–	–	–
Clay (%)	12.2	9.0	4.4	–	–	–	–	–	–	–
Texture class	Sandy loam	Loamy sand	Sand	–	–	–	–	–	–	–
Total organic C (%)	0.67	0.74	0.62	81.1	39.3	42.8	47.8	44.8	60.1	53.8
Total nitrogen (%)	0.01	0.01	0.00	0.7	0.61	0.52	0.82	0.79	0.53	0.65
Total hydrogen (%)	0.10	0.27	0.05	3.0	2.33	2.41	2.37	2.09	2.63	2.36
H/C (molar ratio)	0.06	0.06	0.05	0.44	0.71	0.68	0.60	0.56	0.52	0.53
pH (H_2O)	6.4	5.8	5.8	9.7	8.3	8.3	8.3	9.0	8.6	8.8
CEC ($cmol_c\,kg^{-1}$)	2.7	1.7	2.8	21.1	–	–	14.0	–	–	22.2
K^+ ($cmol_c\,kg^{-1}$)	0.3	0.3	0.1	19.5	–	–	10.4	–	–	16.5
Ca^{2+} ($cmol_c\,kg^{-1}$)	1.4	1.1	1.2	0.9	–	–	2.4	–	–	4.3
Mg^{2+} ($cmol_c\,kg^{-1}$)	1.0	0.3	0.2	0.8	–	–	0.9	–	–	1.2
Bulk density ($g\,cm^{-3}$)	1.26	1.27	1.47	–	0.37	0.27	–	0.36	0.29	–
BET surface area ($m^2\,g^{-1}$)	–	–	–	–	2.4	2.3	–	10.5	4.9	–
Loss on ignition (%)	–	–	–	–	48.8	54.9	–	52.1	72.4	–

Exp. = Experiment.

experiment (Experiment A), whereas BC from second batch was used in maize cob and rice husk BC particle size experiments (Experiment B).

2.2. Experimental sites/set up

Field experiments were established on private farms in two districts of Mkushi and Kaoma in central and western Zambia respectively. The average annual rainfall of Mkushi and Kaoma is 1220 and 930 mm and average temperature is 20.4 °C and 20.8 °C respectively. The top soils in all the sites are light-textured, acidic and have low CEC (Table 1). The experimental set up is summarized in Table 2. There were two experiments: farmer practice experiment (Experiment A) and BC particle size experiment (Experiment B). Farmer practice experiment consisted of crushed maize cob BC applied in conservation farming while BC particle size experiment consisted of maize cob and rice husk BC, respectively, sieved into different particle sizes applied under conventional farming.

2.2.1. Farmer practice experiment (Experiment A)

This was established by applying crushed (unsorted) maize cob BC in sandy loam soil under conservation farming practice as described by Cornelissen et al. (2013). Briefly, conservation farming involved tilling about 10% of the total land by digging planting basins to conserve moisture and to minimize soil disturbance. Weeds in the rest of the land were managed through application of herbicide. The soil in the planting basins, of approximately $15 \times 20 \times 40$ cm size (~10 L) was dug and mixed with crushed maize cob BC at a rate of 0, 0.8 and 2.5 w/w%. Since the BC was concentrated in the planting basins, 0, 0.8 and 2.5 w/w% was equivalent to only 0, 2, and 6 ton ha^{-1} respectively. Fertilizer (140 kg ha^{-1} N:P:K:S—10:20:10:6 followed by 140 kg ha^{-1} top dressing with urea) was applied to all planting basins every season (i.e. November 2011–March 2012 and November 2012–March 2013). The experimental plot was divided into two, one part planted with maize and the other with soybeans. The layout consisted of nine rows planted with maize and nine rows planted with soybeans, with each row having 15 planting basins. Under maize crop, three neighboring rows each received 0, 0.8, 2.5 % BC and the same arrangement follows for soybeans. Further details of the set up can be found in Martinsen et al. (2014).

2.2.2. Biochar particle size experiment (Experiment B)

This was established in April 2013 under conventional farming by applying maize cob BC of three particle sizes prepared by crushing and dry sieving based on a split plot design (Table 2). The sites were uniform with respect to soils and divided into three blocks, each sub-divided into three main plots amended with BC of different particle sizes (≤0.5, 0.5–1 and 1–5 mm). The main plots were divided into three sub-plots receiving BC at three doses (0, 1.7 and 3.4 w/w% for Kaoma sand and 0, 2 and 4 w/w% for Mkushi loamy sand). The same amounts of BC (0, 17.5, 35 ton ha^{-1}) were applied to the two sites but percentages differed due to differences in soil bulk density. The total number of sub-plots/experimental units at each site was 27. From each sub-plot, the top 7 cm of soil was removed and mixed with the required amount of BC in a bucket. The soil profile from 7 cm to approx. 30 cm was loosened using a hoe to remove the compacted layer before placing it back on top, the soil-BC mixture in the bucket. The sub-plot size was 0.5×0.5 m separated by vertical hard plastic sheet inserted approx. 10 cm into the soil and 10 cm remaining above the soil. Fertilizer at the recommended rate (see farmer practice experiment) was applied at the center of the sub-plots just before planting of maize (November 2013).

A small experiment was also established in April 2013 by applying rice husk BC of ≤0.5 and 0.5–1 mm particle sizes under conventional farming based on a completely randomized design. The experiment was established adjacent to, and using a similar approach as in the maize cob BC particle size experiment. Main findings are included only in the text of the result section. Experimental details (Description S1) and data (Table S1 and Fig. S1) are in the supplementary information.

2.3. Soil sampling

2.3.1. Farmer practice experiment

Soil was sampled in April 2013, two rainy seasons after application of BC, taking six undisturbed core ring samples and six disturbed samples randomly from each treatment. The samples were taken only from the planting basins with crops within the top 15 cm of soil, just prior to harvest in the second season.

2.3.2. Biochar particle size experiments

In April 2014, one year after BC application, we sampled the top soil from each of the sub-plots. Two undisturbed core ring samples and two disturbed samples were taken from each sub-plot.

2.4. Aggregate stability determination

Aggregate stability was assessed for disturbed soil samples from Mkushi (Experiments A and B—in maize cob BC experiments). Air-dry soil samples from the field were sieved in a shaker fitted with stacked sieves (20, 6, 2 and 0.6 mm). The stability of aggregates were tested for sizes 2–6 and 0.6–2 mm using the rainfall simulation method (Marti, 1984). The 2–6 and 0.6–2 mm

Table 2
Summary of experimental set up.

Distinguishing feature	Farmer practice - Experiment A	BC particle size - Experiment B[a]	
District	Mkushi	Mkushi	Kaoma
Site coordinate	S13 45.684, E29 03.349	S13 44.839, E29 05.972	S14 50.245, E25 02.150
Farming practice	Conservation farming	Conventional farming	Conventional farming
Soil type	Sandy loam Acrisol	Loamy sand Acrisol	Sand, Arenosol
BC type	Maize cob BC	Maize cob BC	Maize cob BC
BC particle size	Unsorted	≤0.5, 0.5–1 & 1–5 mm	≤0.5, 0.5–1 & 1–5 mm
BC dose[b] (%w/w)	0, 0.8 & 2.5%	0, 2 & 4%	0, 1.7 & 3.4%
BC application depth	15 cm	7 cm	7 cm
BC application time	October 2011	April 2013	April 2013
Soil sampling time	April 2013	April 2014	April 2014
Crop planted	Maize and soybeans	Maize	Maize

[a] Details about rice husk BC of different particle sizes are in the supplementary information.
[b] For BC particle size experiment, same amounts of BC were applied in Mkushi and Kaoma. Differences in percent BC are due to differences in bulk density of Mkushi (1.27 g cm^{-3}) and Kaoma (1.47 g cm^{-3}) soil. 0, 17.5 and 35 ton ha^{-1} BC correspond to 0, 2 and 4% BC in Mkushi and 0, 1.7 and 3.4% BC in Kaoma.

aggregates were air-dried for one week by spreading the soil on trays in the laboratory. Twenty grams of the air-dry soil aggregates were spread on the pre-wetted 0.5 mm sieves just before rainfall simulation. Pre-wetting of sieves moistened the soil and minimized slaking of aggregates. Eight sieves with moistened aggregates were placed on a circular rotating platform, 32 cm under the rain nozzles for each round. The water pressure for rain simulation was set at 1 atm. producing rain with intensity of approx. 350 mm hr^{-1} and the simulation was allowed to run for 2 min. Despite the high rainfall intensity, this method has been found to give results consistent with the more commonly used wet sieving (Grønsten and Børresen, 2009). Soil that remained in the sieves, providing a measure of stable aggregates, were removed and air-dried for 10 days before weighing. The soil weight was corrected for coarse sand and BC particles (>0.5 mm). This was done by immersing the 0.5 mm sieve having air-dry soil, that were retained in the sieve during rainfall simulation, in sodium hexametaphosphate solution (5 g l^{-1}) and washing out the fine soil particles less than 0.5 mm. The coarse fraction of sand and BC was also air-dried for 1 week. Stable aggregates (%) were calculated using a formula adapted from (Kemper and Koch, 1966):

$$\text{Stable aggregates} = \frac{\text{Dry wt soil after simulation} - \text{Dry wt coarse fraction sand \& BC}}{\text{20g dry soil} - \text{Dry wt coarse fraction sand \& BC}} \times 100 \quad (1)$$

included in the optimization of van Genuchten parameters i.e. not fixed to measured PWP and total porosity, respectively. α is related to the inverse of the air entry matrix potential, n is a measure of the pore-size distribution, and m is derived from parameter n where;

$$m = 1 - \frac{1}{n} \quad (3)$$

Soil volume in the core rings decreased as the saturated soil was drained in the sand box, especially for loamy sand from Mkushi (Experiment B). The decrease in the soil height in the core ring was measured using a ruler after equilibration at −100 hPa matrix potential and the decrease in soil volume was calculated. The water retention data generally fitted well to Eq. (2) except at high matrix potential (at saturation and −10 hPa; not shown) probably because of soil volume shrinkage. Agronomic important water retention points of FC, PWP and AWC were determined based on modelled water contents. FC and PWP are water contents at matrix potentials of −100 and −15000 hPa, respectively whereas AWC is the difference between FC and PWP. In addition, the air content of the soil at FC, commonly referred to as air capacity was measured using an air pycnometer (Torstensson and Eriksson, 1936). The presented total porosity was determined by summing up air capacity and FC.

2.5. Soil water retention and pore size distribution

Water retention and pore size distribution was determined for undisturbed soil cores of 100 cm^3 (~3.7 cm height, ~5.8 cm diameter) collected by driving steel rings into the soil vertically at a depth of ~0–5 cm. The rings were closed after cutting off with a knife both ends of the soil in the rings before transporting to the laboratory.

Standard laboratory procedure was used to measure water retention of initially saturated samples by applying various pressures to drain the soil. A sand box (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) was used to determine water retention at high matrix potentials of −10, −30, −50 and −70 hPa, by measuring the weight of the samples when in equilibrium at each of the matrix potentials. For −100 and −1000 hPa, water retention was determined on the same undisturbed core samples using pressure plates (Soil moisture Equipment, Santa Barbara, CA). Positive pressure was applied on the samples for approximately one week until no water was coming out from the device for two days before taking the weight of the samples. After −1000 hPa matrix potential, the samples in the core rings were oven-dried for two days at 105 °C to determine water content at this potential and other higher potentials. The determined soil dry weight also allowed calculation of BD. The oven dry samples were then crushed and passed through a 2 mm sieve. Sub-samples were taken and water-saturated in small PVC rings placed on −15000 hPa pressure plate to determine PWP. Upon equilibration of the samples for 10 days at 15-bar pressure, soil weight was taken, and oven-dried to determine the water content.

The gravimetric water contents at all the measured pressures were converted to volumetric water contents using the measured BD. The volumetric water contents (θ) and matrix potential (h) were fitted to the van Genuchten (1980) model.

$$\theta = \theta_r + (\theta_s - \theta_r)\left[1 + (\alpha|h|)^n\right]^{-m} \quad (2)$$

Where, θ_r and θ_s are the residual and saturated (h = 0 hPa) volumetric water content respectively. Both θ_r and θ_s were

Pore size distribution was calculated using a capillary model (Eq. (4)) based on the water content retained at all matrix potentials between −1 and −16000 hPa modelled using Eq. (2). The capillary model (Eq. (4)) assumes that all pores are cylindrical with radius r.

$$h = \frac{2\gamma.\cos\theta}{\rho g r} \quad (4)$$

where, h = matrix potential (hPa), γ = water surface tension (0.0728 N m^{-1} at 20 °C), ρ = density of the water (1000 Kg m^{-3}), g = gravitational constant (9.81 m s^{-2}) and θ = contact angle between water and solid ~0, r = pore radius (μm);

$$r \approx \frac{15}{h} \times 10^2 \quad (5)$$

2.6. Other lab analysis of soil and biochar

Soil texture was determined for Kaoma and Mkushi soils using the Pipette method. Total organic carbon, total nitrogen and total hydrogen content was determined on soil samples, on BCs after acidification to remove carbonates and on soil aggregates of sizes of 0.6–2 and 2–6 mm. The samples were milled and analyzed, using CHN analyzer (CHN-1000, LECO USA). Biochar carbon of soil aggregates was estimated by subtracting organic carbon content of aggregates of reference soil from organic carbon content of aggregates of BC amended soil. We did not expect either increase or decrease in soil carbon content after 18 months of BC amendment to affect strongly the obtained 'BC carbon'. Any increase in soil carbon would be negligible with respect to added BC carbon given the known slow build-up of soil organic carbon. Also, a decrease in soil organic carbon due to BC would be small, e.g., Luo et al. (2011) where only ~3% of the soil organic carbon was lost and mainly at the start of incubation, in very acidic and neutral pH low-carbon soils. Density of BC was determined by averaging five values of density derived from weighing five 10 cm^{-3} cups filled with BC. pH was measured in water at a ratio of 1:2.5 soil (BC):water on volume basis using a pH meter (Orion 2 Star, Thermo Fisher Scientific, Fort Collins, CO, US) after overnight sedimentation and shaking. Base cations were determined after extracting the soils and BCs with ammonium acetate pH 7 and ammonium nitrate respectively. Extractable acidity of the soil was determined by back titration of ammonium acetate extract using NaOH. CEC of the soils were determined by summing base cations and extractable acidity and CEC for BCs by summing base cations. Soil and BC characteristics are presented in Table 1.

2.7. Data analysis

All the data were analyzed using the statistical software R (R Core Team, 2014). For Experiment A, the dependent variables were BD, aggregate stability, PWP, FC, AWC, air capacity and total porosity, whereas the explanatory variables consisted of crop (categorical; maize and soybeans) and BC dose (continuous; %). The data were analyzed by analysis of covariance (ANCOVA). Experiment B for the maize cob BC particle size consisted of the same dependent variables as Experiment A and were analyzed separately for each site. These data were analyzed using mixed model ANCOVA (lme4 package in R). Biochar particle size (categorical; three levels) and dose (continuous; 0–4%) were included as explanatory variables (fixed effects) whereas block and its interaction with particle size were included as random effects. Regression coefficients of ANCOVA are tabulated, with their standard errors and R^2 included, whereas the overall water retention curves and pore size distribution are presented graphically.

The aggregate stability of the soils from planting basins (Experiment A) were also plotted against BC carbon of the aggregates as explanatory variable. This data fitted well to Michaelis–Menten model (drc package in R), a dose response model previously used for describing enzyme kinetics.

$$y = c + \frac{d - c}{1 + (e/x)} \qquad (6)$$

where, y = response variable (percent stable aggregates), c = percent stable aggregates at zero BC addition, d = maximum percent stable aggregates, e = BC carbon at half d and x = explanatory variable (BC carbon). Three-parameter Michaelis–Menten model ($c \neq 0$) was fitted to the percent stable aggregates as a function of BC carbon to determine how this factor relates to aggregate stability.

3. Results

3.1. Effect of biochar on soil aggregate stability

Biochar produced from maize cobs increased aggregate stability of the sandy loam soil in Mkushi (Experiment A, Table 3). The increase in stable aggregates under soybeans was 4.6 ± 1.9 and 6.8 ± 1.9% for the 0.6–2 and 2–6 mm aggregates, respectively, for each percent BC added. Under maize, stable aggregates increased by 2.6 ± 1.9 and 2.9 ± 1.9% for the 0.6–2 and 2–6 mm aggregates, respectively, for each percent BC added. The increase in the stability of aggregates due to BC was higher under soybeans than under maize crop but significant only for 2–6 mm soil aggregates (p = 0.05). In the absence of BC, soils under maize had higher aggregate stability than soil under soybeans (Table 3). The effect of different size fractions of maize cob BC (Experiment B) on soil aggregate stability in loamy sand at Mkushi was significant for the 0.5–1 mm BC particle size fraction on 0.6–2 mm aggregates only (Table 4). In both Experiments A and B, the 2–6 mm soil aggregates had higher stability than the smaller 0.6–2 mm soil aggregates irrespective of BC rate or particle size fraction.

The relationship between BC carbon and the stability of the 0.6–2 and 2–6 mm soil aggregates (Experiment A) was well described by the three-parameter Michaelis–Menten equation (Eq. (6)) (Fig. 1). Continuous BC carbon contents of the aggregates instead of BC doses, after merging the data of the two crop types as a factor, allowed fitting a non-linear Michaelis–Menten model as opposed to only three doses of BC under maize and soybeans fitted with a linear model. The increase in the stable aggregates with increasing BC carbon was steep at relatively low BC carbon (≤0.4%) of the aggregates. Stable 2–6 and 0.6–2 mm soil aggregates increased from 35 and 25% respectively at zero BC addition, which was greater than half of the maximum observed stability at high BC contents. The increase in stable aggregates with increasing BC carbon leveled off at a maximum of 51.4 and 41.3% for 2–6 mm and 0.6–2 mm aggregates, respectively (Fig. 1). Unlike in Experiment A, the stable aggregates of Experiment B at Mkushi increased linearly with increasing organic carbon in the aggregates (Fig. S2).

Table 3
Regression parameters (±SE) of soil quality indicators versus dose of maize cob BC in planting basins of sandy loams at Mkushi (Experiment A).[a]

Soil quality indicators	Crop	Intercept (0% BC)	Slope (change per percent BC added)	R^2
Aggregate stability 0.6–2 mm aggregates	Maize	30.2 (2.0)	2.6 (1.3)	0.33
	Soybeans	27.2 (2.8)	4.6 (1.9)*	
Aggregate stability 2–6 mm aggregates	Maize	42.6 (2.1)**	2.9 (1.4)*	0.52
	Soybeans	34.0 (2.9)**	6.8 (1.9)**	
Bulk density (g cm^{-3})	Maize	1.26 (0.04)	-0.06 (0.02)**	0.35
	Soybeans	1.29 (0.04)	-0.04 (0.03)**	
Field capacity (vol.%)	Maize	22.4 (0.5)	0.5 (0.2)*	0.20
	Soybeans	21.5 (0.5)	0.5 (0.2)*	
Permanent wilting point (vol.%)	Maize	5.5 (0.3)	0.0 (0.2)	0.01
	Soybeans	5.7 (0.3)	0.0 (0.2)	
Available water capacity (vol.%)	Maize	16.8 (0.4)	0.5 (0.2)*	0.23
	Soybeans	15.7 (0.5)*	0.5 (0.2)*	
Total porosity (vol.%)	Maize	54.3 (0.8)	1.2 (0.4)**	0.22
	Soybeans	53.2 (0.9)	1.2 (0.4)**	

Star in the slope column indicates significant difference from zero and star in the intercept column indicates significant difference between maize and soybeans for a given soil quality indicator.
* $p < 0.05$.
** $p < 0.01$.
[a] Basic soil and BC properties are in Table 1.

Table 4
Regression parameters (±SE) of soil quality indicators versus dose of maize cob BC of different particle sizes in loamy sands at Mkushi and sand at Kaoma (experiment B).[a]

Soil quality indicator	Site	BC sizes	Intercept (0% BC)	Slope (change per percent BC added)
Aggregate stability 0.6–2 mm aggregates	Mkushi	1–5 mm	25.8 (3.7)	0.3 (1.7)
		0.5–1 mm	18.8 (3.2)	3.0 (1.4)*
		≤0.5 mm	20.9 (3.7)	0.1 (1.7)
Aggregate stability 0.6–2 mm aggregates	Mkushi	1-5 mm	42.4 (5.2)*	−1.7 (2.4)
		0.5–1 mm	29.4 (3.8)	1.4 (1.9)
		≤0.5 mm	30.0 (5.2)	1.4 (2.4)
Bulk density ($g\,cm^{-3}$)	Mkushi	1–5 mm	1.29 (0.03)	−0.03 (0.010)**
		0.5–1 mm	1.25 (0.05)	0.00 (0.017)
		≤0.5 mm	1.27 (0.05)	−0.02 (0.015)
	Kaoma	1–5 mm	1.41 (0.03)	−0.03 (0.008)**
		0.5–1 mm	1.45 (0.04)	−0.04 (0.011)**
		≤0.5 mm	1.52 (0.04)	−0.03 (0.011)**
Field capacity (vol.%)	Mkushi	1–5 mm	14.5 (0.3)	1.0 (0.08)***
		0.5–1 mm	15.2 (0.5)	0.2 (0.14)
		≤0.5 mm	14.3 (0.5)	0.8 (0.12)***
	Kaoma	1–5 mm	13.9 (0.6)	1.2 (0.21)***
		0.5–1 mm	14.6 (0.9)	0.6 (0.30)
		≤0.5 mm	13.9 (0.9)	1.0 (0.33)***
Permanent wilting point (vol.%)	Mkushi	1–5 mm	3.8 (0.2)	0.06 (0.07)
		0.5–1 mm	3.8 (0.3)	0.10 (0.11)
		≤0.5 mm	3.9 (0.3)	0.15 (0.09)
	Kaoma	1–5 mm	2.1 (0.2)	0.34 (0.11)**
		0.5–1 mm	2.2 (0.4)	0.26 (0.17)
		≤0.5 mm	2.9 (0.4)	−0.01 (0.15)
Available water capacity (vol.%)	Mkushi	1–5 mm	10.7 (0.4)	0.9 (0.11)***
		0.5–1 mm	11.4 (0.6)	0.1 (0.13)
		≤0.5 mm	10.4 (0.6)	0.7 (0.16)***
	Kaoma	1–5 mm	11.8 (0.7)	0.8 (0.20)***
		0.5–1 mm	12.4 (0.9)	0.3 (0.28)
		≤0.5 mm	11.0 (0.9)	1.0 (0.31)***
Total porosity (vol.%)	Mkushi	1–5 mm	50.6 (1.6)	1.4 (0.50)*
		0.5–1 mm	51.9 (2.3)	0.1 (0.82)
		≤0.5 mm	51.1 (2.3)	0.7 (0.70)
	Kaoma	1–5 mm	46.9 (1.2)	1.2 (0.34)**
		0.5–1 mm	45.6 (1.8)	1.5 (0.53)**
		≤0.5 mm	42.0 (1.7)	1.4 (0.47)**

The star in the slope column indicate a significant difference from zero.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
[a] Basic soil and BC properties are in Table 1.

The association of BC particles of different sizes with either 0.6–2 or 2–6 mm soil aggregates differed in Experiment B (Fig. S3). Biochar particle sizes of ≤0.5 mm had an equal distribution between 0.6–2 and 2–6 mm soil aggregates. By contrast, 0.5–1 mm BC particles were mainly associated with 0.6–2 mm soil aggregates, whereas coarse 1–5 mm BC particles sizes as expected, were more (two times) strongly associated with 2–6 mm soil aggregates. The organic carbon of aggregates of the reference soil did not significantly differ between the aggregate sizes analyzed.

3.2. Effect of biochar on soil bulk density

In the planting basins at Mkushi (Experiment A), maize cob BC significantly decreased BD of the sandy loams ($p < 0.01$) by 0.04–0.06 $g\,cm^{-3}$ per percent BC applied (Table 3) under both maize and soybeans crops. The decrease in BD was more associated with increase in macro-pores (Fig. 2). In the loamy sands at Mkushi (Experiment B), only maize cob BC with particle size of 1–5 mm and not the smaller size fractions of ≤0.5 and 0.5–1 mm significantly decreased the BD (0.03 $g\,cm^{-3}$ decrease per percent BC added, Table 4). Similarly, fine rice husk BC with size of ≤0.5 mm did not significantly reduce the BD (Table S1). In the more coarse textured sand at Kaoma, BC application rate, but not particle size, affected BD causing a significant decrease of ~0.03 $g\,cm^{-3}$ per percent BC applied (Table 4 and S1).

3.3. Effect of biochar on pore size distribution of soil

Biochar altered pore size distribution of soils in the planting basins of the sandy loams at Mkushi (Experiment A), with greater alterations at the largest BC application rate (2.5%; Fig. S4). Under maize, this occurred via an increase in the proportion of pores with a radius >1 μm whereas under soybeans the alteration of pore size distribution was not as strong as under maize even though the pattern was similar.

In Experiment B, the addition of fine (≤0.5 mm) and coarse (1–5 mm) maize cob BC in the Kaoma sand decreased the proportion of pores with radius 10–100 μm, while the proportion of the bigger or smaller pore sizes increased (Fig. 3). The intermediate BC particle size fraction of 0.5–1 mm had the smallest effect in altering the pore size distribution. For the loamy sand at Mkushi (Fig. S5), smaller BC particle sizes increased the proportion of pores with

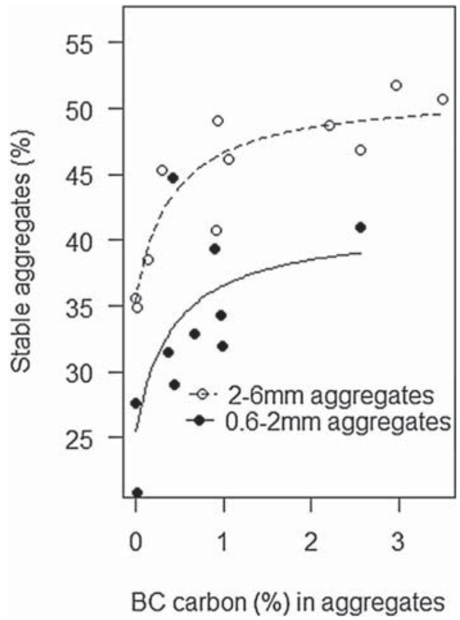

Fig. 1. Stable aggregates plotted against BC carbon in aggregates of BC amended soils from Experiment A in Mkushi, Zambia. The figure shows a fitted three-parameter Michaelis–Menten model (Eq. (6)), which estimates stable aggregate (c) at zero BC, maximum stable aggregates achievable (d) and BC carbon at half d (e). For 0.6–2 mm aggregates, c and $d = 25.5 \pm 1.9$ and 41.3 ± 4.9, respectively, and for 2–6 mm aggregates c and $d = 35.4 \pm 2.2$ and 51.4 ± 3.7, respectively ($p < 0.001$). Parameter $e = 0.4 \pm 0.4$ ($p > 0.05$) for both aggregate sizes tested. All parameters are ±SE.

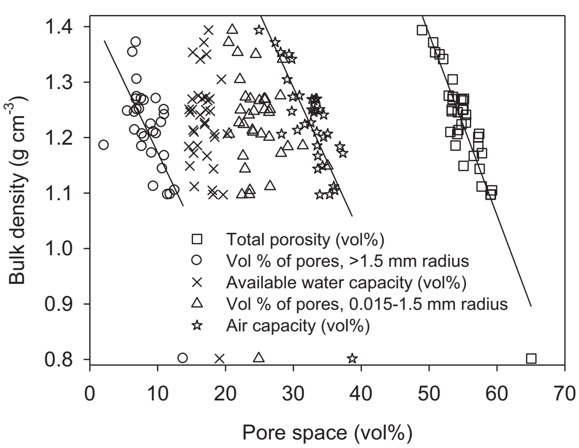

Fig. 2. Relationship of the bulk density and various components of the pore space in planting basins of Experiment A. Basic soil and BC properties are in Table 1.

radius 10–100 μm whereas coarse particle sizes of 1–5 mm followed a trend similar to that of the Kaoma soil.

Rice husk BC increased the proportion of the pores with radius 10–100 μm in the two soils, except for the ≤0.5 mm BC size fraction at Kaoma (Fig. S1). Generally, rice husk BC followed a similar pattern as maize cob BC of similar sizes in similar soils and at the same doses.

3.4. Available water capacity, air capacity and total porosity

In the planting basins at Mkushi (Experiment A), maize cob BC significantly ($p < 0.05$) increased AWC by 3% for each percent BC applied under both maize and soybeans ($p < 0.05$). In reference plots, the AWC was smaller under soybeans compared to maize ($p < 0.05$). There was also significant ($p < 0.05$) increase in FC and a non-significant effect on PWP (Table 3). Similarly, for Experiment B with controlled maize cob BC particle sizes, AWC increased for each of the size fractions, except for the intermediate (0.5–1 mm) BC particle sizes. The increase was between 7 and 9% per percent BC applied to both Mkushi and Kaoma soils ($p < 0.001$). There was also a significant increase in FC whereas PWP was not affected significantly (except BC of 1–5 mm particle size in Kaoma) for the soils at both Kaoma and Mkushi (Table 4). Rice husk BC had no significant effect on AWC in both Kaoma and Mkushi (Table S1).

The air capacity of the soil in the planting basins was 32% under soybeans and maize and was not affected by BC (Data not shown). On the other hand, BC significantly ($p < 0.01$) increased soil total porosity in the planting basins by 2% per percent BC applied (Table 3), but there was no difference between crops. Similarly, in Experiment B, there was no effect of BC on air capacity at both Kaoma (30%) and Mkushi (36%) (data not shown), whereas there was an increase in total porosity for all BC particle sizes in Kaoma soil ($p < 0.01$) and for the coarse 1–5 mm BC fraction in Mkushi ($p < 0.05$) (Table 4). Rice husk BC on the other hand increased both air capacity and total porosity (Table S1).

3.5. Soil shrinkage in core rings during water retention analysis

The soil volume in core rings decreased by 10–20% for samples taken from Experiment B at Mkushi during drainage of the saturated loamy sand soil in a water retention analysis as matrix potential decreased from zero to −100 hPa (Fig. 4). This effect depended on BC particle size and dosage (Fig. 4B). Consistent decrease in the soil volume with increasing dosage occurred in samples from 1–5 mm BC amended plots. Rice husk BC also caused an increase in shrinkage of Mkushi soil (Fig. 4C). The increase in soil volume shrinkage correlated positively with increase in soil porosity brought about by BC addition (Fig. 4A). The shrinkage was most influenced by porosity filled with water at matrix potential more than −10 hPa i.e. porosity composed of large pores with radius >150 μm (Fig. 4A).

4. Discussion

In this study, BC application changed the soil physical properties positively from an agronomic perspective. The changes in these properties, including increased soil aggregate stability and AWC in addition to reduced BD, are in line with results previously reported for soil incubated in the laboratory and greenhouse pot experiments (Mukherjee and Lal, 2013). The increase in aggregate stability may indicate that BC aids soil aggregation, which could at least partly be responsible for the increase in soil water retention and alteration of pore size distribution especially in the aggregated loamy soil at Mkushi. Besides soil aggregation, the high specific surface area (Table 1) and porosity of BCs compared to soil (Mukherjee and Lal, 2013) could have contributed to increase in water retention, particularly in the Kaoma sand (Table 4). From an agronomic perspective, the increase in AWC, generally low for the type of soils investigated in this study, is of major importance. Yields of maize crop significantly increased after application of maize cob BC in an earlier experiment established adjacent to the site of the current study at Kaoma (Martinsen et al., 2014). Low AWC with values less than 15% (v/v) and high air capacity (Section 3.4,Tables 3 and 4 and S1) render soil 'droughty or potentially droughty' (Reynolds et al., 2007). The effect of BC in increasing AWC under field conditions could also contribute to addressing the problem of uncertain rainfall patterns in Zambia (Yatagai, 2011). The increase in AWC due to maize cob BC addition in this study was more than that under laboratory condition for soils taken from the same sites at Kaoma and Mkushi (∼2.5% versus 3–9% increase per percent BC added in this study) reported by

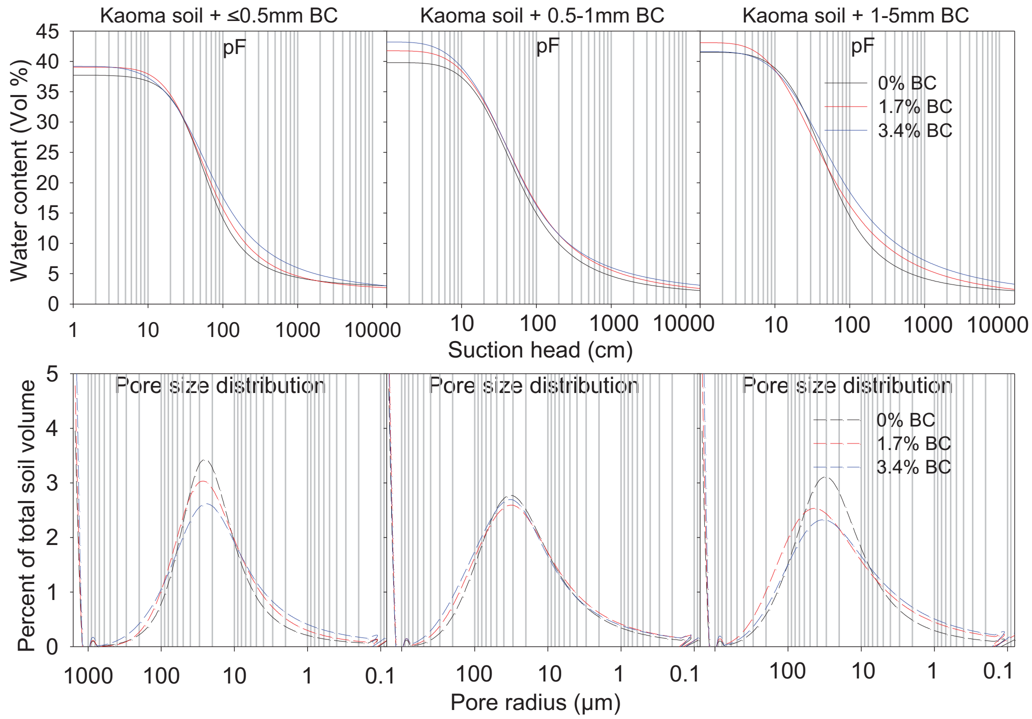

Fig. 3. Water retention curves fitted to van Genuchten equation and pore size distribution of sandy soils at Kaoma amended with maize cob BC of different particle sizes (Experiment B). Basic soil and BC properties are in Table 1.

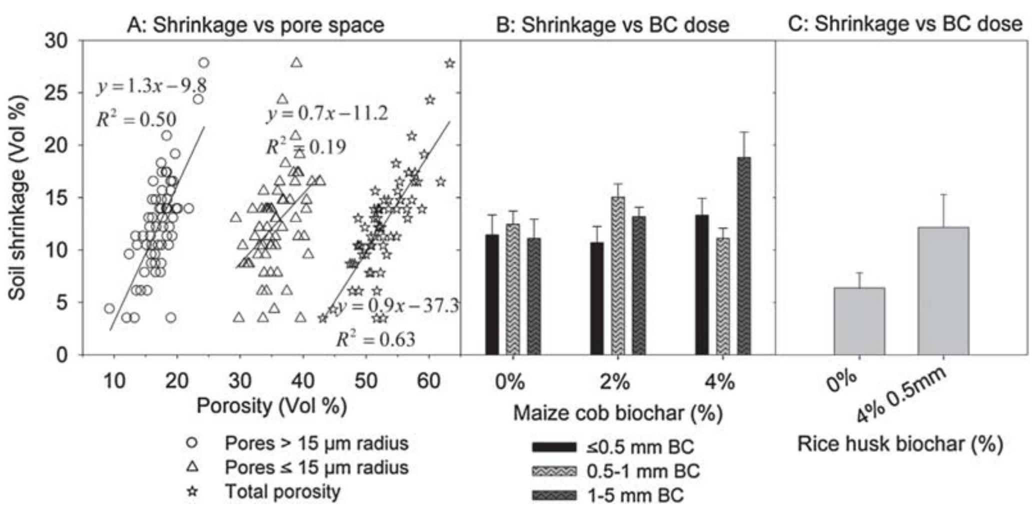

Fig. 4. Shrinkage of loamy sand at Mkushi (Experiment B) in the core rings during water retention study as a function of soil porosity. Basic soil and BC properties are in Table 1.

Martinsen et al. (2014). Maize cob BC addition to soils resulted in similar patterns but stronger net effect than rice husk BC on the soil physical properties, especially the hydraulic properties.

There was stronger increase in aggregate stability by BC under soybeans with slightly lower initial total organic carbon than under maize. In silt loam soils from New Zealand, Herath et al. (2013) also demonstrated that addition of corn stover and its corresponding BC increased aggregate stability. Stronger effects were also observed for soil with low initial total organic carbon (4%) than in soil with already high total organic carbon (10%). The increase in the stability of aggregates of Mkushi soils correlated positively with aggregate BC carbon, with strong responses at low BC carbon (Fig. 1). An optimal amount of BC carbon in aggregates occurred above which there was negligible increase in aggregate stability and this coincided with as low as 1–2% BC application rate (Fig. 1) in sandy loam soil of Experiment A. At these optimal BC carbon contents, the fraction of stable aggregates of soil increased from 25 to 35% and from 35 to 45% for 0.6–2 and 2-6 mm aggregates, respectively. This low BC carbon, which can potentially occur at low dose of BC, whilst producing a significant impact on the soil, is important given the potential difficulty in acquiring the large amount of BC for application to agricultural soil. Half the maximum stable aggregates occurred in the soil at its native state of total organic carbon and clay without BC addition (insignificant BC carbon = 0.4%, $p > 0.05$ at half the maximum stable aggregates based on Michaelis–Menten model). Probably, the native soil organic matter and the clay-size fraction (kaolinites, iron and aluminum oxides) in Mkushi soils contributed to binding of soil particles forming aggregates. The role of soil organic matter (Oades, 1984) and clay (Martin et al., 1955) in building soil aggregates are a rather well known phenomena.

The interaction between crop and BC addition on aggregate stability observed in this study (Experiment A) indicates an indirect effect of BC on soil aggregate stability. The application of BC in Experiment A enhanced root growth (Abiven et al., 2015) leading to increased root activity (e.g., releasing root exudates and moving soil particles aiding aggregate formation) in the planting basins. Root activity, together with the direct effect of BC acting as a binding agent of soil particles (Brodowski et al., 2006), could be responsible for the increase in aggregate stability relative to the reference plots. The higher root biomass of maize compared to soybeans (monocot vs dicot) (Amézketa, 1999) was probably the reason for higher organic carbon under maize than soybeans (Fig. S6). Organic carbon in the absence of BC was consistently higher for both 0.6–2 and 2–6 mm aggregates under maize than under soybeans (2–6 mm aggregates had 0.72% C under maize vs 0.53% under soybeans; 0.6–2 mm aggregates had 0.88% C under maize vs 0.56% under soybeans). Therefore, the higher aggregate stability under maize in the absence of BC compared to soybeans was most likely caused by higher root activity and organic matter e.g. root exudates as previously reviewed by Amézketa (1999). The difference in the stability of soil aggregates between maize and soybeans are also in accordance with the known effect of different plant species on aggregate stability e.g. Blanco-Canqui and Lal (2004); Reid and Goss (1981); Tisdall and Oades (1982).

The addition of BC to planting basins in sandy loams caused a reduction in soil BD, which was associated with an increase in soil porosity, particularly of the volume of macro-pores with radius >1.5 mm (Fig. 2) (Experiment A). This indicates that the build-up of soil macro-porosity, induced by BC was important, in addition to the direct weight dilution effect of BC on soil BD (Verheijen et al., 2009), which relates to BCs' light and porous nature. All BCs used in this study had a density of ~0.3 g cm^{-3} (Table 1) and weight dilution, assuming an increase in soil volume after BC addition, would result in a decrease in BD of 0.04 g cm^{-3} compared to the measured 0.05 g cm^{-3} per percent BC applied in Experiment A (maximum potential weight dilution contribution of 80%). Thus, minimum of 20% of the decrease in BD was due to increase in soil aggregation and not mere weight dilution. In fact, BD decreased with increasing stable aggregates (0.005 g cm^{-3} for every 1% increase in stable aggregates, data not shown). For the single-grained sandy soils at Kaoma (Experiment B), weight dilution was a more important factor than at Mkushi, contributing 0.05 g cm^{-3} compared to the measured 0.03 g cm^{-3} decrease in BD per percent BC applied (i.e. 160% contribution in decreasing BD; >100% means volume was not additive). In fact, in Kaoma sand, BC doses and not BC particle size played the main role in reducing BD, further pointing to the importance of a dilution effect in sandy soils. This is in contrast to the observations from the more loamy Mkushi soils (Experiments B), where both dose and BC particle size were important factors. Only coarse BC of 1–5 mm decreased BD in Mkushi, probably by creating packing voids forming weak pores.

The general lack of significant effects of BC on soil aggregate stability in BC particle size Experiment (B) (loamy sands at Mkushi) was probably caused by low stability of aggregates due to coarse texture. Only 0.5–1 mm BC in Experiment B at Mkushi resulted in a significant increase in stability of 0.6–2 mm soil aggregates (Table 4) probably because most of 0.5–1 mm BC was associated with 0.6–2 mm aggregates (Fig. S3). The low stability of soil pores in Experiment B caused structural collapse as shown by shrinkage of soil in core rings during the water retention study (Fig. 4). The tendency of increased soil shrinkage with BC addition is a potential indicator of initiation of soil structure build-up caused by BC. This collapse of soil structure in the core rings during water retention study makes it difficult to infer other soil properties (e.g., hydraulic conductivity) that rely on soil large pores from water retention curves without measuring them.

The reduced effect of 0.5–1 mm BC compared to smaller or larger BC particle sizes on soil hydraulic properties and pore size distribution (Table 4 and S1) of Experiment B was probably because 0.5–1 mm BC was within the sizes of soil particles dominating the sand and loamy sand soils. This would then result in minimal changes in the pore sizes of the soils.

5. Conclusions

In this work, we have demonstrated that BC can improve the physical condition of light-textured soils important for crop growth. Biochar increased soil aggregate stability, porosity and AWC and reduced soil bulk density. The fact that 'low dose' of BC of 1–2% impact soil properties (Experiment A) is important because large quantities of BC can be difficult to obtain. However, BC impact depends on soil texture (compare Experiments A & B in Mkushi, which was for two and one season, respectively): coarser textured loamy soils require more BC and time to produce any significant increase in aggregate stability. The BC particle size experiment (Experiment B) showed that the addition of larger particle size BCs, e.g. 1–5 mm, might result in equally strong positive effects on soil physical properties as powdery BC. Coarse BC eliminates the necessity of thorough crushing, and reduces dust formation during BC application. Maize cob BC additions resulted in stronger effects than rice husk BC on soil physical properties. Reduced density of soil due to BC-induced soil aggregation may aid root growth and with more water available, can increase crop growth and yields. Biochar application to highly weathered and sandy soils will therefore increase the soils' resilience against drought.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.still.2015.08.002.

References

Abiven, S., Hund, A., Martinsen, V., Cornelissen, G., 2015. Biochar amendment increases maize root surface areas and branching: a shovelomics study in Zambia. Plant Soil 1–11.
Amézketa, E., 1999. Soil aggregate stability: a review. J. Sustainable Agric. 14, 83–151.
Atkinson, C.J., Fitzgerald, J.D., Hipps, N.A., 2010. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. Plant Soil 337, 1–18.
Awad, Y.M., Blagodatskaya, E., Ok, Y.S., Kuzyakov, Y., 2013. Effects of polyacrylamide, biopolymer and biochar on the decomposition of14C-labelled maize residues and on their stabilization in soil aggregates. Eur. J. Soil Sci. 64, 488–499.
Basso, A.S., Miguez, F.E., Laird, D.A., Horton, R., Westgate, M., 2013. Assessing potential of biochar for increasing water-holding capacity of sandy soils. GCB Bioenergy 5, 132–143.
Blanco-Canqui, H., Lal, R., 2004. Mechanisms of carbon sequestration in soil aggregates. Crit. Rev. Plant Sci. 23, 481–504.
Brodowski, S., John, B., Flessa, H., Amelung, W., 2006. Aggregate-occluded black carbon in soil. Eur. J. Soil Sci. 57, 539–546.

Bruun, E.W., Petersen, C.T., Hansen, E., Holm, J.K., Hauggaard-Nielsen, H., 2014. Biochar amendment to coarse sandy subsoil improves root growth and increases water retention. Soil Use Manage. 30, 109–118.
Carlsson, M., Andrén, O., Stenström, J., Kirchmann, H., Kätterer, T., 2012. Charcoal application to arable soil: effects on CO_2 emissions. Commun. Soil Sci. Plant Anal. 43, 2262–2273.
Cornelissen, G., Martinsen, V., Shitumbanuma, V., Alling, V., Breedveld, G., Rutherford, D., Sparrevik, M., Hale, S., Obia, A., Mulder, J., 2013. Biochar effect on maize yield and soil characteristics in five conservation farming sites in Zambia. Agronomy 3, 256–274.
Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—a review. Biol. Fertil. Soils 35, 219–230.
Goverment of Zambia, 2011. Sixth National Development Plan 2011–2015: Sustained Economic Growth and Poverty Peduction. Government of Zambia, Lusaka, Zambia, pp. 108.
Grønsten, H.A., Børresen, T., 2009. Comparison of two methods for assessment of aggregate stability of agricultural soils in southeast Norway. Acta Agric. Scand. Sect. B 59, 567–575.
Herath, H.M.S.K., Camps-Arbestain, M., Hedley, M., 2013. Effect of biochar on soil physical properties in two contrasting soils: an Alfisol and an Andisol. Geoderma 209–210, 188–197.
Jeffery, S., Verheijen, F.G.A., van der Velde, M., Bastos, A.C., 2011. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agric. Ecosyst. Environ. 144, 175–187.
Joseph, S., Camps-Arbestain, M., Lin, Y., Munroe, P., Chia, C., Hook, J., Van Zwieten, L., Kimber, S., Cowie, A., Singh, B., 2010. An investigation into the reactions of biochar in soil. Soil Res. 48, 501–515.
Kemper, W.D., Koch, E., 1966. Aggregate stability of soils from western United States and Canada. Measurement procedure, correlation with soil constituents. Technical Bulletin. United States Department of Agriculture, Agricultural Research Service.
Liu, X.-H., Han, F.-P., Zhang, X.-C., 2012. Effect of biochar on soil aggregates in the Loess Plateau: results from incubation experiments. Int. J. Agric. Biol. 14, 975–979.
Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., Brookes, P.C., 2011. Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. Soil Biol. Biochem. 43, 2304–2314.
Marti, M., 1984. Kontinuierlicher Getreidebau ohne Plug im Südosten Norwegens—Wirkung auf Ertrag, Physikalische und Chemische Bodenparameter. Agricultural University of Norway, Ås.
Martin, J.P., Martin, W.P., Page, J., Raney, W., De Ment, J., 1955. Soil Aggregation. Academic Press.
Martinsen, V., Mulder, J., Shitumbanuma, V., Sparrevik, M., Børresen, T., Cornelissen, G., 2014. Farmer-led maize biochar trials: effect on crop yield and soil nutrients under conservation farming. J. Plant Nutr. Soil Sci. 177, 681–695.
Mukherjee, A., Lal, R., 2013. Biochar impacts on soil physical properties and greenhouse gas emissions. Agronomy 3, 313–339.
Oades, J.M., 1984. Soil organic matter and structural stability: mechanisms and implications for management. Plant Soil 76, 319–337.
Ouyang, L., Wang, F., Tang, J., Yu, L., Zhang, R., 2013. Effects of biochar amendment on soil aggregates and hydraulic properties. J. Soil Sci. Plant Nutr. 13, 991–1002.
R Core Team, 2014. R: A Language and Environment for Statistical Computing. Foundation for Statistical Computing, Vienna, Austria.
Reid, J., Goss, M., 1981. Effect of living roots of different plant species on the aggregate stability of two arable soils. J. Soil Sci. 32, 521–541.
Reynolds, W.D., Drury, C.F., Yang, X.M., Fox, C.A., Tan, C.S., Zhang, T.Q., 2007. Land management effects on the near-surface physical quality of a clay loam soil. Soil Tillage Res. 96, 316–330.
Soinne, H., Hovi, J., Tammeorg, P., Turtola, E., 2014. Effect of biochar on phosphorus sorption and clay soil aggregate stability. Geoderma 219–220, 162–167.
Sparrevik, M., Adam, C., Martinsen, V., Jubaedah Cornelissen, G., 2015. Emissions of gases and particles from charcoal/biochar production in rural areas using medium-sized traditional and improved retort kilns. Biomass Bioenergy 72, 65–73.
Steiner, C., Teixeira, W.G., Lehmann, J., Nehls, T., de Macêdo, J.L.V., Blum, W.E.H., Zech, W., 2007. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. Plant Soil 291, 275–290.
Sun, F., Lu, S., 2014. Biochars improve aggregate stability, water retention, and pore-space properties of clayey soil. J. Plant Nutr. Soil Sci. 177, 26–33.
Sun, Z., Arthur, E., de Jonge, L.W., Elsgaard, L., Moldrup, P., 2014. Pore structure characteristics after two years biochar application to a sandy loam field. J. Soil Sci..
Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33, 141–163.
Torstensson, G., Eriksson, S., 1936. A new method for determining the porosity of the soil. Soil Sci. 42, 405–414.
Verheijen, F.G.A., Jeffery, S., Bastos, A.C., van der Velde, M., Diafas, I., 2009. Biochar Application to Soils: A Critical Scientific Review of Effects on Soil Properties, Processes and Functions. EUR 24099 EN, Office for the Official Publications of the European Communities, Luxembourg 149pp.
Yamato, M., Okimori, Y., Wibowo, I.F., Anshori, S., Ogawa, M., 2006. Effects of the application of charred bark of *Acacia mangiumon* the yield of maize, cowpea and peanut, and soil chemical properties in South Sumatra, Indonesia. Soil Sci. Plant Nutr. 52, 489–495.
Yatagai, A., 2011. Interannual variation of seasonal rain fall in South Zambia. In: Umetsu, C. (Ed.), Vulnerability and Resilience of Social-Ecological Systems. Research Institute For Humanity And Nature, Kyoto, pp. 206–212.
de Melo Carvalho, M.T., de Holanda Nunes Maia, A., Madari, B.E., Bastiaans, L., van Oort, P.A.J., Heinemann, A.B., Soler da Silva, M.A., Petter, F.A., Marimon Jr, B.H., Meinke, H., 2014. Biochar increases plant-available water in a sandy loam soil under an aerobic rice crop system. Solid Earth 5, 939–952.
van Genuchten, M.T., 1980. A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. Soil Sci. Soc. Am. J. 44, 892–898.

In situ effects of biochar on aggregation, water retention and porosity in light-textured tropical soils

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Supplementary information

This document consists of one description providing details of rice husk biochar experiment, one table and six figures.

Description S1. Materials and methods - Rice husk BC particle size experiment

This was established under conventional farming by applying rice husk BC of different particle sizes based on a completely randomized design. There were three treatments: ≤0.5 and 0.5-1 mm particle size rice husk BC at 3.4 w/w% and a reference without BC at Kaoma. At Mkushi, treatments were ≤0.5 mm rice husk BC and 0.5-1 mm maize cob BC, all at 4% and a reference without BC. The same amounts of BC (0 and 25 ton ha^{-1}) was applied to the two sites but percentages differs due to differences in soil bulk density. All treatments in each site were in triplicates. From each plot, the top 5 cm of soil was removed and mixed with the required amount of BC in a bucket. The soil profile from 5 cm to approx. 30 cm was loosened using a hoe to remove the compacted layer before placing back on top, the soil-BC mixture in the bucket. The plot size was 0.5 x 0.5 m separated by vertical hard plastic sheet inserted approx. 10 cm into the soil and 10 cm remaining above the soil. Fertilizer at the recommended rate (see farmer practice experiment in the main text, section 2.2) was applied at the center of the plot just before planting of maize (November 2013). The maize cob BC treatment here (due to shortage of rice husk BC) is not discussed any further because similar treatment is covered under maize cob BC particle size experiment.

One core ring sample was taken per sub-plot in April 2014, one year after BC application from within the depth where BC was applied. The samples were then analyzed for water retention and pore size distribution as described in the main text (section 2.5). All the other analyses of the soil and BC are as described in section 2.6. The data were analyzed by one-way analysis of variance (ANOVA) in the R software. Dependent variables consisted of BD, PWP, FC, AWC, air capacity and total porosity and the explanatory variables were the three treatments (categorical). Data were analyzed for each of the dependent variable against explanatory variables for each site separately. Mean values and their standard errors are tabulated (Table S1) whereas the overall water retention curves and pore size distribution are presented graphically (Fig. S5).

Table S1. Mean values of soil quality indicators of soil amended with rice husk BC in loamy sand at Mkushi and sand at Kaoma[1]

Soil quality indicator	Site	Rice husk BC			
		Reference	≤0.5mm	0.5-1mm	SE
Bulk density (g cm^{-3})	Mkushi	1.26	1.17	-	0.06
	Kaoma	1.40	1.28 *	1.27 *	0.03
Field capacity (vol. %)	Mkushi	14.1	15.9	-	0.9
	Kaoma	15.3	15.5	14.5	1.0
Permanent wilting point (vol. %)	Mkushi	3.9	4.2	-	0.5
	Kaoma	1.7	2.1	2.7	0.5
Available water capacity (vol. %)	Mkushi	10.2	11.7	-	0.8
	Kaoma	13.5	13.5	11.8	1.3
Total porosity (vol. %)	Mkushi	51.8	56.2	-	3.1
	Kaoma	48.0	52.1 *	52.1 *	1.4
Air capacity (vol. %)	Mkushi	37.7	40.2	-	2.7
	Kaoma	32.7	36.7	37.6 *	1.9

Biochar dose was 4 and 3.4% at Mkushi and Kaoma respectively. *$p<0.05$ showing difference between reference with no BC and BC treatment, SE is standard error. [1] Basic soil and BC properties are in table 1.

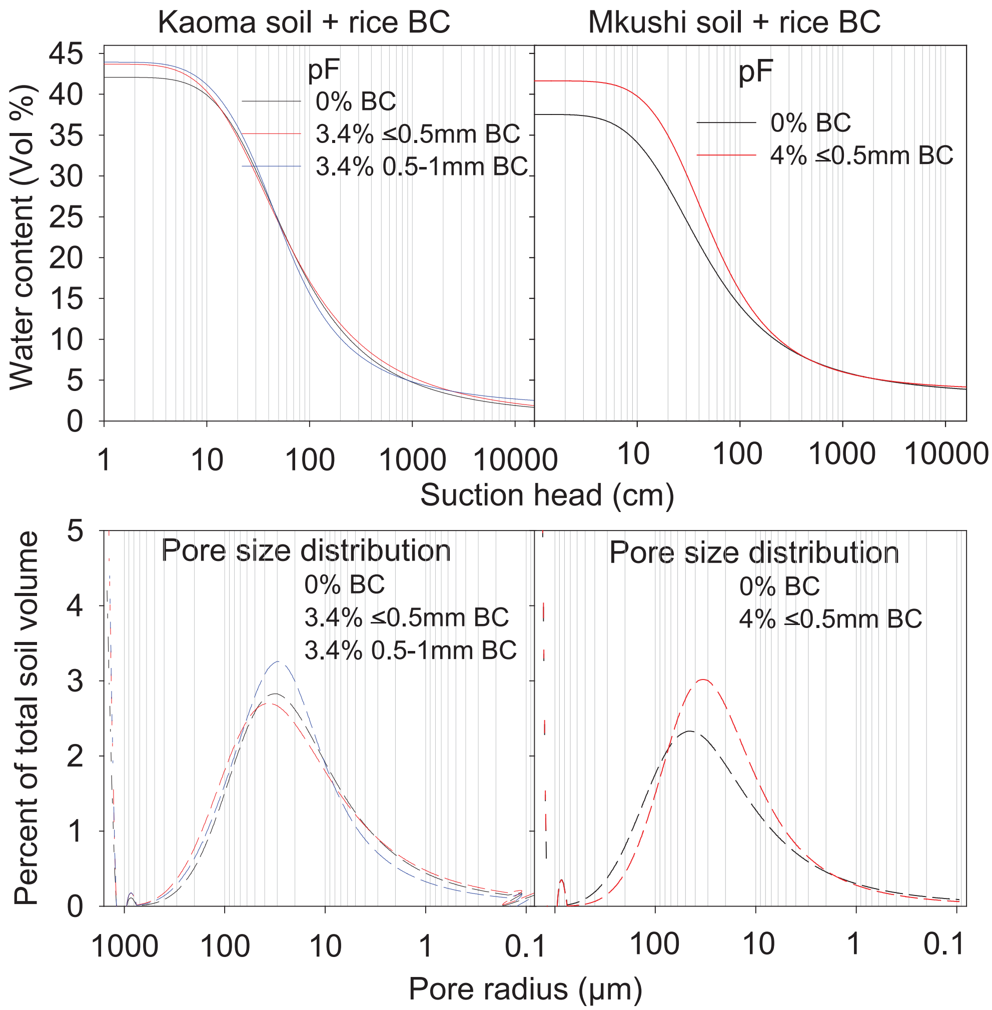

Fig. S1. Water retention curves and pore size distribution of Kaoma and Mkushi soil amended with rice husk BC of different particle sizes. Basic soil and BC properties are in table 1.

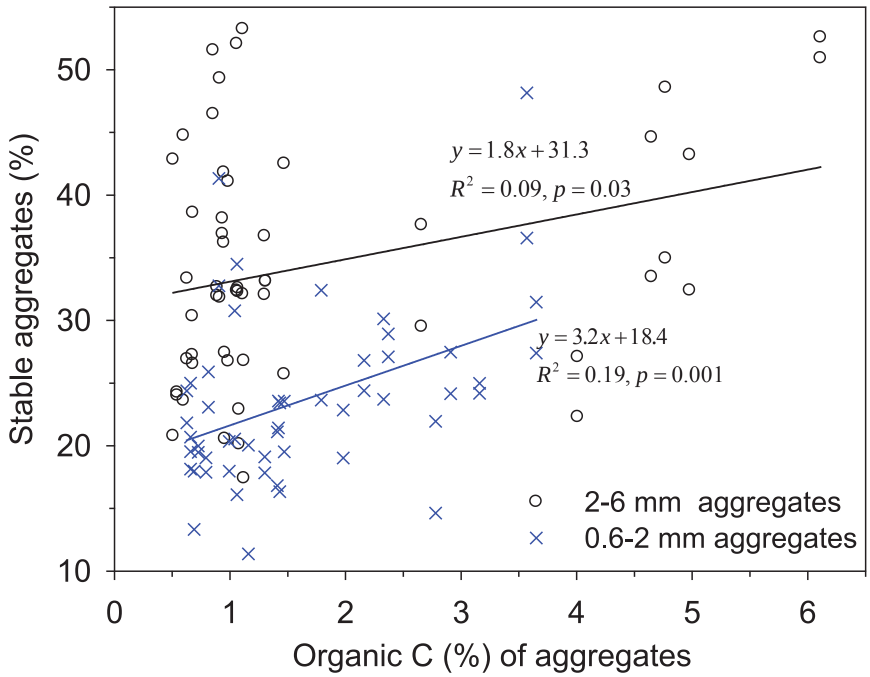

Fig. S2. Stable aggregate plotted against organic carbon of aggregates of Mkushi loamy sand (Experiment B) amended with maize cob BC. Basic soil and BC properties are in table 1.

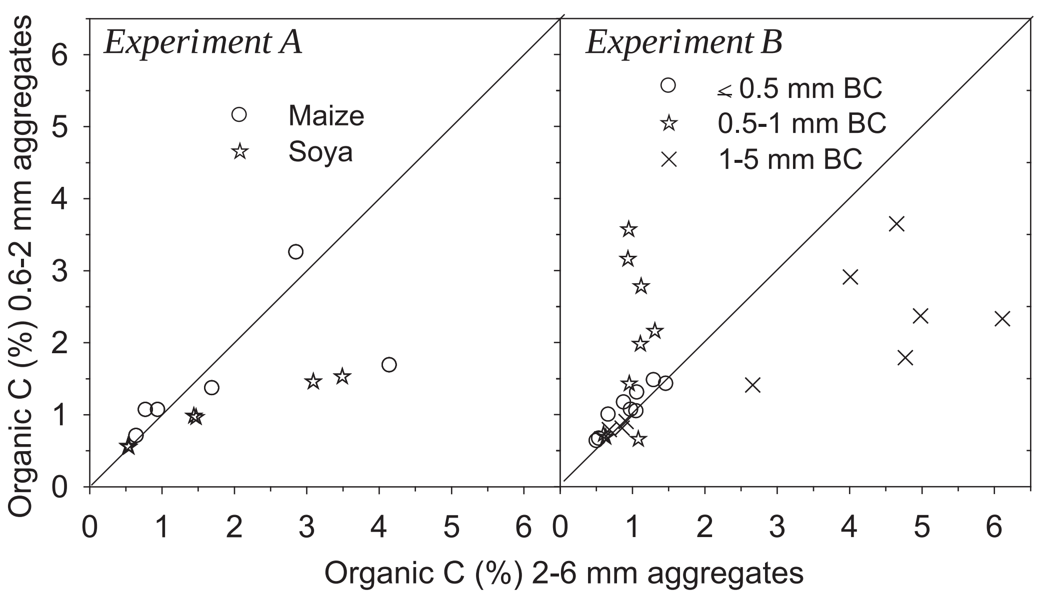

Fig. S3. Distribution of BC to aggregates reflected by carbon content in aggregates. Reference bulk soils have < 1% TOC. Diagonal line shows the 1:1 distribution of BC between 0.6-2 and 2-6 mm aggregates. Basic soil and BC properties are in table 1.

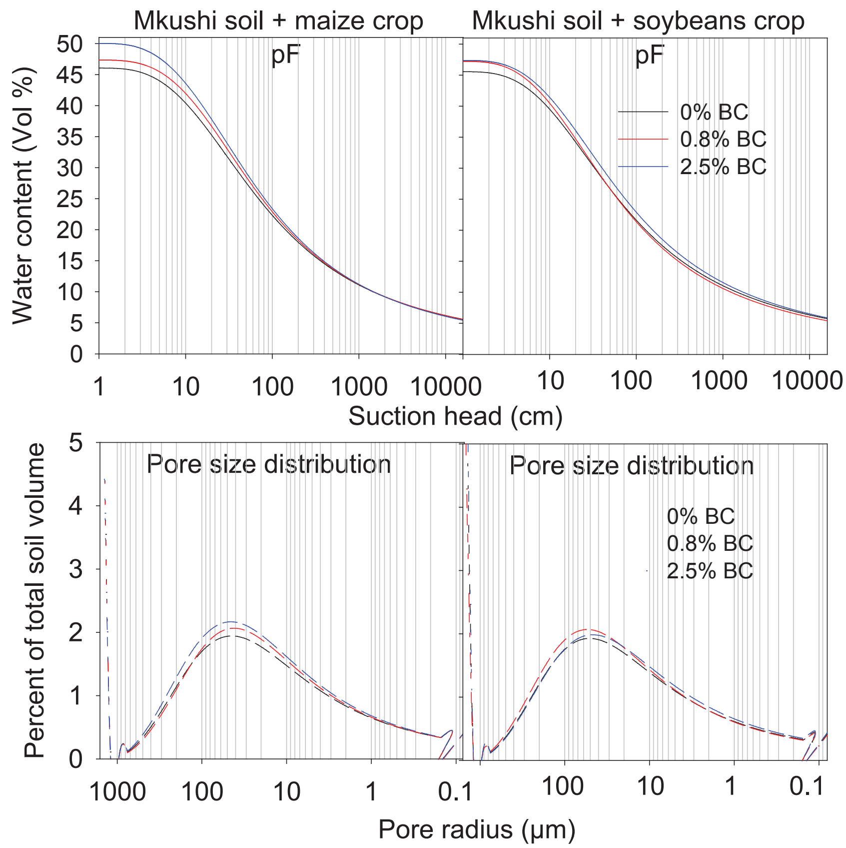

Fig. S4. Water retention curve and pore size distribution of soil amended with maize cob BC and planted with maize or soybeans (Experiment A). Basic soil and BC properties are in table 1.

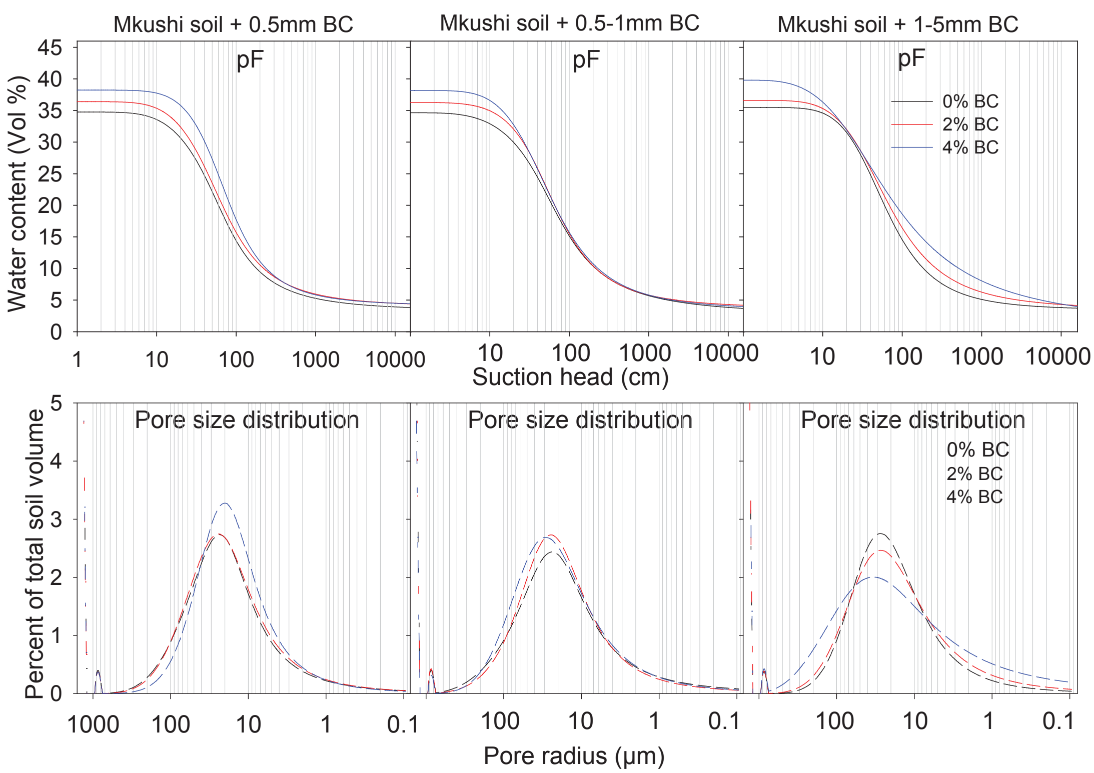

Fig. S5. Water retention curves and pore size distribution of Mkushi soil amended with maize cob BC of different particle sizes (Experiments B). Basic soil and BC properties are in table 1.

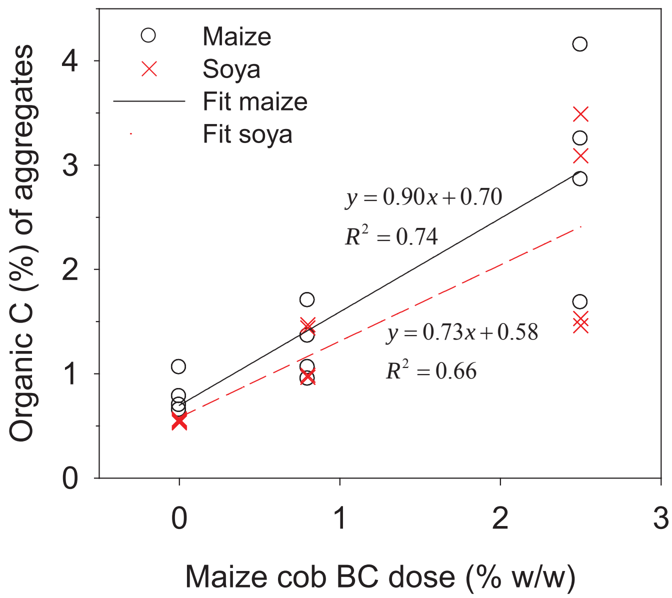

Fig. S6. Organic carbon content of 0.6-6 mm aggregates of soil from planting basins planted with maize and soybeans (Experiment A). Basic soil and BC properties are in table 1.

PAPER II

Effect of biochar on crust formation, penetration resistance and hydraulic properties of two coarse-textured tropical soils

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Effect of biochar on crust formation, penetration resistance and hydraulic properties of two coarse-textured tropical soils

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Abstract

Biochar (BC) has been reported to improve a number of soil structural and hydraulic properties. Detailed studies are scant on how BC affects crust formation, penetration resistance and soil hydraulic properties. We investigated the effect of maize cob BC (≤0.5, 0.5–1 and 1–5 mm particle sizes) on soil crusting (penetration resistance), water repellency (water drop penetration time (WDPT) and ethanol percentage test) and saturated hydraulic conductivity (K_{sat}) of sand and loamy sand in Zambia. Biochar reduced the penetration resistance of surface soil of loamy sand with both crust intact (-2.1 ± 0.6 N cm^{-2} per percent BC added; $p=0.001$ in March 2015 and slightly smaller in October 2014) and crust removed (-2.9 ± 0.6 N cm^{-2} per percent BC added; $p=0.0001$). This effect occurred irrespective of particle size ($p>0.05$). No effect of BC on penetration resistance was found in sand ($p>0.05$). The decrease in penetration resistance may aid growth of plant roots in aggregating soil and therefore may directly affect crop growth. In very dry loamy sand with moisture content <1% v/v, the proportion of wettable crusted surface was significantly smaller than in moist soil with moisture content ~10% (25% and 98%, respectively). Only fine BC of ≤0.5 mm increased WDPT of the crusted surface of loamy sand ($p<0.05$), reducing the proportion of wettable surface from 98 to 80% in moist soil and from 25 to 18% in very dry soil. Thus, fine BC may increase the risk of reduced water infiltration possibly promoting soil erosion on sloping terrain. Coarser BCs instead increased the proportion of wettable crusted surface (from 25% to 45% and 90% for 0.5–1 and 1–5 mm BCs, respectively, at 4% BC addition in very dry soil). Biochar significantly reduced K_{sat} ($p<0.05$) in loamy sand below the crust by 0.17 ± 0.07 cm hr^{-1} per percent BC added, but not in sand. Since BC amended loamy sand below the crust showed no water repellency, reduction in K_{sat} cannot be explained by water-repellent nature of BC. Instead, this may be due to clogging of soil pores by BC or collapse of soil structure at near water saturation.

Keywords

Biochar particle size, soil hydraulic properties, penetration resistance

Highlights

- Biochar had no effect on penetration resistance, water repellency and K_{sat} of sand.
- Loamy sand developed a crust whose strength was not affected by maize cob biochar.
- Fine biochar increased water repellency of crust while coarser fraction reduced it.
- Loamy sand below crust showed no water repellency regardless of biochar addition.
- Biochar reduced K_{sat} and penetration resistance of the loamy sand below the crust.

1. Introduction

Biochar (BC), a biomass pyrolysis product, has received considerable attention as a soil amendment that can increase crop growth and yield (Glaser et al., 2002; Jeffery et al., 2011). To understand the mechanisms responsible for increased productivity, research has focused on BC's effect on soil chemical properties and crop nutrition rather than on soil physical properties (Atkinson et al., 2010; Lehmann et al., 2011; Mukherjee and Lal, 2013). Only recently, a number of studies have reported the effects of BC on soil aggregation, bulk density, water retention and saturated hydraulic conductivity (K_{sat}) (de Melo Carvalho et al., 2014; Herath et al., 2013; Obia et al., 2016; Ouyang et al., 2013; Sun and Lu, 2014). Optimal soil physical characteristics are required for increased soil productivity. These include hydraulic properties, which determine water (often limiting resource in agriculture) availability to crops and structural properties that aid root growth.

Studies of the effect of BC on K_{sat} of soil are inconclusive, as increase, decrease or no effect have been observed. Increased K_{sat} in response to the addition of BC was found in silty clay and sandy loam (Ouyang et al., 2013), in silt loam (Herath et al., 2013) and in clay rich soil (Barnes et al., 2014), all incubated in the laboratory, without plants. Increase in K_{sat} was also observed in field experiments in loamy (Asai et al., 2009) and sandy clay loam (Major et al., 2010) soils. The increase in K_{sat} of loamy soils could be linked to BC-induced increases in soil aggregation (Herath et al., 2013; Ouyang et al., 2013), which is expected to require time to develop, especially coarse-textured soils (Obia et al., 2016). No effect of BC on K_{sat} has been observed in clay soil (Asai et al., 2009) and in Dutch sandy soils (Jeffery et al., 2015), both in the field. On the other hand, decreases in K_{sat} were reported for sand and organic soils in laboratory and greenhouse incubations (Barnes et al., 2014; Uzoma et al., 2011). The negative impact on K_{sat} may be due to the water repellent nature of BC (Briggs et al., 2005; Verheijen et al., 2009). The water repellent nature of BC has been reported to decrease with increase in pyrolysis temperature (Jeffery et al., 2015; Khanmohammadi et al., 2015), implying that low temperature BCs could be very water repellent. Recently, Yi et al. (2015) reported that the water repellency of poultry litter BC originated from surface coating by semi-volatile organic compounds. How the water repellent nature of BC affects soil water repellency has only recently received attention (Abel et al., 2013; Eibisch et al., 2015; Herath et al., 2013; Page-Dumroese et al., 2015; Yi et al., 2015). In general, these studies, which were all conducted in the laboratory, show that BC had little effect on soil water repellency.

Soil water repellency is known to reduce water infiltration causing increase in soil erosion (Doerr et al., 2000), which can be exacerbated by soil crusting. Soil crusting may be assessed by measuring its strength in terms of a penetration resistance (Upadhyaya et al., 1995). Penetration resistance of the crust may indicate how easy it is for water to infiltrate the soil thereby directly affecting crop growth and yield. Soil crusting occurs primarily in soils with weak aggregates and high amounts of silt (Awadhwal and Thierstein, 1985). Increasing aggregate stability of soil such as by BC (Obia et al., 2016) could potentially reduce crust formation (Awadhwal and Thierstein, 1985 and references therein). Yet, the effect of BC on soil crusting in crust-prone soils has not yet been tested. Also effect of BC on the penetration resistance of soil below the crust (or in soils without crusting) has received little attention (Busscher et al., 2010; Mukherjee et al., 2014), despite the fact that it relates directly to soil structural properties (Gao et al., 2016) that can influence plant root growth. In the laboratory, ground pecan shell BC reduced penetration resistance of bulk Norfolk loamy sand (Busscher et al., 2010). However, under field conditions, oak wood BC had no effect on the penetration resistance of bulk silt loam soil in the first year, and even increased the resistance in the second year (Mukherjee et al., 2014). Biochar has been reported to reduce bulk density and increase porosity in a range of soil types (Mukherjee and Lal, 2013), showing that BC could reduce penetration resistance of soil (Gao et al., 2016). In turn, reduction in the penetration resistance of bulk soil may reduce resistance to root growth in soils (Materechera and Mloza-Banda, 1997).

In situ studies are urgently needed to further explore the implication of the effect of BC addition on soil hydraulic properties in the field. This is all the more important in areas prone to drought, e.g. in Zambia where rainfall, the main source of agricultural water, is erratic and unreliable (Yatagai, 2011) and has negative effect on crop production. Coarse-textured soils such as the ones studied here generally have low water retention (Obia et al., 2016) and can suffer more in case of drought. Use of BC of different particle sizes may aid the understanding of mechanisms behind BC effects on soil hydraulic properties. Barnes et al. (2014) proposed that BC affects soil hydraulic properties through the interstitial BC-soil particle space and through pores within the BC grains themselves. These proposed mechanisms may depend on the particle sizes of the BC, similar to the dependence of aggregate formation on particle size of the BC added (Obia et al., 2016).

The hypotheses of the present study were that

(i) BC, irrespective of particle size, reduces the penetration resistance for both crusted surface and bulk soil in aggregating loamy sand but not in sand (single grain structure without aggregation).

(ii) hydrophobic compounds of BC induces soil water repellency in BC-amended coarse-textured soils.

(iii) BC, irrespective of particle size, increases K_{sat} in loamy sand due to BC-induced soil aggregation and in sand, finer BC reduces K_{sat} due to filling of inter particle space while coarse BC have no effect.

To investigate these hypotheses, three particle size fractions of maize cob BC (≤0.5, 0.5–1 and 1–5 mm, respectively) were applied and homogenized at three different application rates to the upper 7 cm of aggregating loamy sand at Mkushi, Zambia (crust-prone soil), and sand at Kaoma, Zambia. After one and two years (one and two cropping seasons, respectively) in the field, crusting and penetration were assessed using a penetrometer. Water repellency was quantified using water drop penetration time (WDPT) and the ethanol percentage test, and K_{sat} was measured using a tension disc infiltrometer. This study is among the first to test these hypotheses in *in situ* condition in tropical coarse-textured soils.

2. Materials and methods

2.1 Biochar and experiments

The BCs were produced from dry maize cob after removing the grains in a slow pyrolysis for one day, using a drum retort kiln at Chisamba, Zambia at a temperature of 350 ºC. Other BC production details can be found in Obia et al. (2016). Basic properties of the BC are presented in Table 1.

The experiments were established in April 2013 at Mkushi (S13 44.839, E29 05.972) and Kaoma (S14 50.245, E25 02.150) in Zambia, with the soils being classified as Acrisol and Arenosol, respectively. There is only one annual growing (wet) season in Zambia, which runs from November to March followed by a dry season from April to October. The experiments were planted with maize in the first season (Nov 2013 – Mar 2014) and under fallow in the second season (Nov 2014 – Mar 2015). Site details are as described in Obia et al. (2016). The experiments followed a split plot design, where maize cob BC of three particle sizes (≤0.5, 0.5–1 and 1–5 mm) was applied to small plots of 50 x 50 cm. The BC was applied at rates of 0, 2 and 4% (w/w) in Mkushi and 0, 1.7 and 3.4% (w/w) in Kaoma, to the top 7 cm of the soil in triplicates (at each site 27 plots in total). The amounts of BC applied to the two sites were the same (0, 17.5 and 35 t ha^{-1}) but the percentages were different because of differences in soil bulk density (Table 1). All measurements reported in the present study were conducted at the end of the growing season (April 2014 and March 2015), except penetration resistance, which had one set of measurement conducted just before the beginning of growing season (October 2014). The main measurements are summarized in Table 2.

2.2 Analyses of soil and biochar properties

The texture of the soil was determined using the Pipette method. Total organic carbon (TOC), total nitrogen and total hydrogen of soil and BC were determined using CHN analyzer (CHN-1000, LECO USA). Loss on ignition of BC was determined by burning the sample at 550 ºC in an oven (Carbolite Bamford, Sheffield, England). Soil and BC pH was measured using an Orion 2 Star pH meter (Thermo Fisher Scientific, Fort Collins, CO) in 1:2.5 soil(BC):water mixture. To measure exchangeable base cations of soils and BC, samples were extracted using ammonium acetate (buffered at pH 7) and ammonium nitrate, respectively. Base cations in the extracts were determined using flame spectrophotometry (Perkin Elmer, AAS 3300). The cation exchange capacity (CEC), at pH 7, was computed as the sum of base cations for BCs and as the sum of base cations and exchangeable acidity for soils, where exchangeable acidity was determined by back titration of the ammonium acetate extract using sodium hydroxide (0.05M NaOH). The density of BC was determined from the weight of BC in filled 10 cm^3 cups. Bulk density of the soils were derived from the dry weight (oven-drying at 105 ºC for two days) of soil in 100 cm^3 core rings taken in April 2014 from the top 0–5 cm soil depth.

2.3 Moisture content of the soil

The *in situ* soil moisture content (0–5 cm surface layer) was recorded with five replicates per plot, using hand-held time domain reflectometer (TDR) – SM150 (Delta-T Devices, Cambridge, England). Measurements were done on the same day as those of water infiltration, water repellency and penetration resistance.

2.4 Penetration resistance of the soil

Measurements of penetration resistance were carried out at the end of the growing season in March 2015 (two years after BC application) at both sites. At Mkushi, one set of measurements was also conducted in October 2014 at the end of the dry summer, just before the onset of the rainy season. A pocket penetrometer (Eijkelkamp, Giesbeek, The Netherlands) was used to quantify the penetration resistance of the soil. The penetrometer with flat tip (diameter = 6.35 mm) was gently pressed until the shaft was ~6 mm into the soil and the pressure reading on the penetrometer taken.

The penetration resistance of the soil at Mkushi in March 2015 was measured for both the crust (<6 mm) and for the soft soil underneath. The penetration resistance of the soil underneath was done following careful removal of the crust (<6 mm), with a knife. The penetration resistance of the Kaoma soil was measured only for the soil surface since no crust was observed. Ten random measurements were carried out in each plot for both soil crust and for the soil underneath, totaling 270 measurements at Kaoma and 540 measurements at Mkushi at each time point.

2.5 Effect of biochar on soil water repellency

2.2.1 Water drop penetration time

Water drop penetration time (WDPT) provides a measure of stability or persistence of soil water repellency and is normally used to detect the existence of repellency (Dekker et al., 2009). Water drop penetration time was measured in the field at Mkushi and Kaoma according to Dekker et al. (2009). In addition, WDPT was determined in the laboratory. Measurements of WDPT was carried out in April 2014 and March 2015, one and two years after BC application, respectively. Water drops were placed on the soil surface and the time to complete infiltration recorded for both Mkushi and Kaoma soils. For Mkushi soil, where surface crusting occurred, WDPT was also measured after removal of the crust. Ten drops of distilled water were placed within each of the 27 plots at each site on the soil surface both before and after carefully removing the crust (the latter for the loamy sand at Mkushi only). Measurement of WDPT at greater depth (down to 25 cm) was done on soil samples, obtained using a half cylindrical auger as previously described by Dekker et al. (2009). At least ten drops were placed on the soil sample and the water entry time registered.

In the laboratory, two core ring samples (100 cm^{-3}) per plot, taken in April 2014 were used to test for repellency after oven drying at 105 ºC. Five water drops were placed on each side of the two core ring samples, giving twenty water drops per plot.

The WDPT registered were classified according to Dekker and Jungerius (1990). The frequency of occurrence of the different WDPT classes were grouped for each of the BC treatments.

2.2.2 Ethanol percentage test

The Ethanol percentage test was used to assess the degree or severity of water repellency of crusted soil surface at Mkushi, as previously described by Buczko et al. (2002). Ethanol breaks soil water repellency by reducing the surface tension of water. If the contact angle between a water drop and the soil surface is >90º, then the soil is water repellent. Increasing ethanol concentration reduces the contact angle due to reduction of liquid surface tension. Here, we report the surface tension of the ethanol solution droplet (σ_e) rather than the volumetric content of ethanol at which drops penetrated soil at ≤5 s. The surface tension of the ethanol solution was calculated according to de Jonge et al. (1999) in eq.1 where surface tension of ethanol solution is linearly related to the volumetric content of ethanol.

$$\sigma_e = 0.0721 - 0.0502 * \left(\frac{\% \, ethanol}{100} \right) \qquad \text{eq.1}$$

Surface tension of the ethanol solution was selected as variable of interest, because it is a fundamental property in the characterization of the degree of water repellency of the soil, due to its relationship with soil-air surface tension (Letey et al., 2000; Watson and Letey, 1970).

Only the crust of the Mkushi soil was included in the test, because only here the WDPT revealed water repellency, i.e., WDPT exceeded five seconds (see results section). Solutions of ethanol ranging from 1 to 40% (v/v) were prepared by dilution with distilled water and small drops were placed using a laboratory dropper on the crusted soil surface. Solutions with higher ethanol concentrations were used until drop penetration time was ≤5 s. Ten drops were placed on the soil crust at each ethanol concentration. The ethanol concentration at which at least eight drops infiltrated the soil at ≤5 s and the other two drops at ≤10 s or nine drops at ≤5 s and one drop at >10 s was considered the concentration at which the soil water repellency was broken. This ethanol concentration was converted to surface tension and then used in further analysis.

2.6 Measurement of K_{sat} and sorptivity of soil

Saturated hydraulic conductivity and sorptivity of soil was measured in the field using tension disc infiltrometers (Eijkelkamp, Giesbeek, The Netherlands) in April 2014 and March 2015, one and two years after BC application, respectively. Two sets of measurements were performed at each plot at two different tensions (high-tension, h_2 of 15 cm water column and low-tension, h_1 of 6 cm water column). Water infiltration rate ($cm^3\ hr^{-1}$) was calculated by multiplying surface area of the disc in contact with soil with steady state reading – fall in height of water column ($cm\ hr^{-1}$). Soil sorptivity, α, the ability of the soil to absorb water was calculated from the combined equations of Wooding (1968) and Gardner (1958) in eq.4 at two suction pressures h_1 and h_2 as described in the manual of tension disc infiltrometer (Eijkelkamp).

$$\alpha = \frac{In[Q(h_2)/Q(h_1)]}{h_2 - h_1} \qquad \text{eq.4}$$

Where $Q(h_2)$ and $Q(h_1)$ were the water infiltration rates at high (h_2) and low (h_1) tensions respectively in cm^3 hr^{-1}. K_{sat} (cm hr^{-1}) was calculated by substituting for α in the combined Wooding's equation (Wooding, 1968) and Gardner' equation (Gardner, 1958) (eq.5) for either the known $Q(h_1)$ and h_1 or $Q(h_2)$ and h_2.

$$Q(h_1) = \pi r^2 K_{sat} \exp(\alpha h_1)\left[1 + \frac{4}{\pi r \alpha}\right] \qquad \text{eq.5}$$

Where r (cm) is the radius of the disc in contact with soil. K_{sat} and sorptivity α were determined for two replicates per plot.

2.7 Statistical analysis

The data were analyzed using R software (R Core Team, 2014). Ethanol percentage test, K_{sat}, sorptivity and penetration resistance of the soil were analyzed using analysis of covariance (ANCOVA). Repeated measurements from each plot were averaged before fitting the data to linear model ANCOVA for each site separately. In fitting the model, ethanol percentage test (expressed in terms of surface tension, N m^{-1}), K_{sat} (cm hr^{-1}), sorptivity (cm^{-1}) and penetration resistance (N cm^{-2}) were the dependent variables and BC particle size (categorical) and BC dose (continuous) were the independent variables. Non-significant terms in the model were removed, where such removal did not significantly affect the explanatory power of the model, in order to obtain the minimal adequate model where all terms were significant. For WDPT, the data were categorized into repellency classes and presented graphically to show the proportion of repellency classes in the soil.

3. Results

3.1 Effect of biochar on penetration resistance of the crust and soil underneath

In both April 2014 and March 2015, the loamy sand at Mkushi exhibited surface crusting, whereas the sand at Kaoma did not. Despite the fragile nature of the crust, the soil surface layer with intact crust at Mkushi had a significantly ($p<0.05$) larger penetration resistance (33.9 ± 1.0 N cm^{-2}, Fig. 1B) than the soil below the crust (27.9 ± 1.0 N cm^{-2}, Fig. 1C). The penetration resistance of the Kaoma sand was smaller (16.7 ± 1.3 N cm^{-2}, Fig. 1D) than that of the Mkushi loamy sand at comparable (low) soil moisture contents in March 2015 (Table 3).

At Mkushi, BC significantly decreased the penetration resistance of the soil surface layer with intact crust (-2.1 ± 0.6 N cm^{-2} per unit increase in percent BC; $p = 0.001$, Fig. 1B in March 2015). Likewise, BC also reduced penetration resistance after crust removal in March 2015 (-2.9 ± 0.6 N cm^{-2} per unit increase in percent BC; $p<0.0001$, Fig. 1C) and when there was no visible crust in October 2014 (-1.4 ± 0.5 N cm^{-2} per unit increase in percent BC; $p = 0.005$, Fig. 1A). There was no significant difference on the effect of BC on penetration resistance between October 2014 and March 2015. The decrease in penetration resistance in Mkushi soil was independent of the BC particle size ($p>0.05$). The penetration resistance in the Kaoma sand was not significantly affected by BC application ($p = 0.77$, Fig. 1D).

3.2 Effect of biochar on soil water repellency – WDPT and ethanol percentage test

In the field, the sand at Kaoma, down to 25 cm depth, was non-repellent (WDPT generally within 1 s; Fig. 2), even when very dry (e.g. March 2015, Table 3). Likewise, the loamy sand below the surface crust at Mkushi was non-repellent in both April 2014 and March 2015 (Fig. 2). The non-repellent behavior of the soil at Kaoma and Mkushi (below the crust) was not affected by BC, one and two years after BC amendment to the upper 7 cm of the soil. Even in the laboratory, there was immediate infiltration of water drops (within 1 s) into the oven-dry soil from both Mkushi (below the crust) and Kaoma (Fig. 2).

The crusted soil surface at Mkushi showed *in situ* water repellent behavior (Fig. 2). The water repellency of the crusted surface at Mkushi was greater during the drought in March 2015 (74% of surface was repellent) than during the wetter conditions in April 2014 (only 2% of surface was repellent) (Fig. 2). The soil moisture content was <1% in March 2015 compared to ~10% v/v in April 2014 (Table 3). In moist conditions (as in April 2014), only addition of the finest BC fraction (≤0.5 mm) caused increased repellency of crusted surface (decreased wettability from 98% to 80%; Fig. 2). When the soil was very dry (as in April 2015), the crusted surface at Mkushi was mainly classified as slightly water-repellent (60% of the surface with WDPT = 5–60 s; Fig. 2). The finest BC of ≤0.5 mm decreased proportion of wettable surface from 26% of the crusted soil surface in reference to 17% at 4% BC (Fig. 2). Addition of the coarser BC fractions (>0.5 mm) on the other hand increased wettability e.g. proportion of wettable surface increased from 26% in reference plots to 90% at BC addition rates of 4% (1–5 mm size fraction; Fig. 2). Despite water-repellent behavior of crusted soil surfaces at Mkushi, there was no case where WDPT reached the "extremely water repellent" class of >3600 s (Dekker and Jungerius (1990). In the sandy soil at Kaoma, BC addition did not affect the WDPT of the surface, which remained highly wettable (Fig. 2).

The ethanol percentage test here was expressed as surface tension of the drops of ethanol solution. The average surface tension was between 0.060-0.065 N m^{-1} and was not affected by BC addition ($p = 0.42$) (Fig. 3) in March 2015.

3.3 Effect of biochar on K_{sat} and sorptivity of the soil

In dry soils (March 2015), the average K_{sat} was smaller in the loamy sand at Mkushi (below the crust) than in the sand at Kaoma (1.7 vs 5.2 cm hr^{-1}, respectively). Both soils showed a general trend of decreasing K_{sat} with increasing BC doses (Fig. 4), irrespective of BC particle size ($p>0.05$). However, this trend was significant only for the loamy sand at Mkushi (-0.13 cm hr^{-1} per percent BC added, $p = 0.02$), but not for the sandy soil at Kaoma ($p = 0.31$) (Fig. 4). K_{sat} was not significantly affected by BC at the two sites when the soil was moist (April 2014; data not shown, $p = 0.62$ at Mkushi and $p = 0.15$ at Kaoma).

Both Mkushi and Kaoma soil had similar sorptivity (~0.08 cm^{-1}). Sorptivity showed a decreasing, albeit non-significant trend ($p>0.05$), with increasing amount of BC applied (Fig. 4).

4. Discussion

4.1 Effect of biochar on penetration resistance of soil at and below the surface

Soils with weak aggregation such as loamy sand at Mkushi (Obia et al., 2016) can develop a crust at its soil surface (Awadhwal and Thierstein, 1985). In the loamy sand at Mkushi, BC significantly reduced the penetration resistance of the soil surface layer with intact crust. Also in the soil beneath the crust, BC caused a significant decrease in penetration resistance (Fig. 1). These effects were irrespective of BC particle size. In contrast, BC had no effect on the penetration resistance in the sand at Kaoma. Thus, our hypothesis that BC irrespective of particles size reduces the penetration resistance for both crusted surface and bulk soil in aggregating loamy sand but not in sand, without aggregation (Obia et al., 2016) was confirmed.

The difference in penetration resistance of surface soil, including the crust (Fig. 1B), and the bulk soil below the crust in the loamy sand (Fig. 1C), which represents the resistance of the crust alone, is relatively small (6 to 10 N cm^{-2}) and is not significantly affected by BC (Fig. 1B and 1C). This suggests that BC addition has little effect on the strength of the soil crust, which is important in triggering surface water run-off. Besides the crust, soil texture affects the penetration resistance, with sand having a lower penetration resistance than loamy sand in the absence of BC (compare the intercept in Fig. 1C vs 1D), which is consistent with other studies e.g. (Dexter et al., 2007).

Similar to our results, a significant decrease in penetration resistance has also been reported by Busscher et al. (2010) in loamy sand amended with up to 2% pecan BC under laboratory conditions. However, in their study the magnitude of decrease in penetration resistance per percent BC added was much higher (~10 N cm^{-2}) compared to our study (<3 N cm^{-2}). Mukherjee et al. (2014) on the other hand observed no effect of BC on penetration resistance of silty clay loam but the application dose was rather low at 0.5%.

4.2 Effect of biochar on repellency and soil hydraulic properties

The crusted soil surface in the loamy sand at Mkushi showed significant water repellency, when very dry (74% of the crusted surface with WDPT >5 s in March 2015). The finest BC fraction increased the proportion of repellent surface, whereas coarse BCs reduced it (Fig. 2). Since BC affected the water repellency of the crust and not that of the bulk soil (Fig. 2), the changes in the repellency of the crust were probably not due to BCs' direct hydrophobic effect. Much of the hydrophobic compounds of BC, which have been identified as semi-volatile organics, may have been lost relatively quickly to percolating soil water (Yi et al., 2015). In their study, virtually all hydrophobic compounds were lost three days after submerging poultry litter BC in water. Such rapid loss of hydrophobic compounds from BC may explain the lack of significant effect of BC on severity of water repellency of the crust measured using ethanol solution (Fig. 3).

The dark shiny appearance of the crusted surface observed in the field probably indicated previous surface growth of microbes especially fungi, which render the surface water repellent (Doerr et al., 2000 and references therein). Shiny-crusted surfaces were more frequently observed for plots amended with ≤0.5 mm BC than for those with coarser BC. Biochar of ≤0.5 mm sizes had higher pH, lower TOC (45% vs 60% for 1–5 mm BC) and loss on ignition (52% vs 72% for 1–5 mm BC) compared to the 1–5 mm fraction of BC (Table 1). Higher pH and lower loss on ignition suggest greater alkalinity in ≤0.5 mm BCs, which may have stimulated fungal growth. In a review by Warnock et al. (2007), BC was shown in a number of studies to increase the abundance of fungi, especially mycorrhizal fungi, which was linked to greater availability of nutrients introduced by BC. The coarser BC of 0.5–5 mm, which increased the wettability of the crust of the loamy sand (Fig. 2), had smaller amounts of inorganic constituents indicated by higher loss on ignition (Table 1). In addition, due to its large sized particles, they would not fit the thin crusts in large amounts. Low amounts of coarser BCs together with its lower inorganic constituents in the crust did not facilitate surface fungal growth. The observed reduction in WDPT of the crusted soil surface amended with coarse BC (0.5–5 mm particle sizes; Fig. 2) could be related to larger pores on the crusted surface. We hypothesize that the packing of large BC and soil particles left relatively large inter-particle pores for water drops to infiltrate unlike fine BC that would fill the pores instead.

The non-repellency of the coarse-textured soils at Mkushi (underneath the crust) and Kaoma (Fig. 2) is contrary to the common occurrence of repellency in this type of soils (Doerr et al., 2000). The occurrence of repellency in coarse-textured soils has been explained by their smaller surface areas, which require small amount of hydrophobic organic compounds for coating the soil particles

(Doerr et al., 2000). Our sites had little TOC (Table 1), which may translate into small amounts of hydrophobic organic compounds. The relatively large repellency of the crusted soil surface in Mkushi in March 2015 as compared to April 2014 (Fig. 2) was due to the dry state of the soil (Table 3) and is consistent with the known transient character of water repellency with changes in soil moisture content (Doerr and Thomas, 2000).

Unlike the crusted surface in loamy sand at Mkushi, the water repellency of the bulk soils was not affected by BC (Fig. 2). This is consistent with previous studies where mixing maize BC with sand (Abel et al., 2013) and silt loam (Herath et al., 2013) did not significantly affect their water repellency at varying moisture content including oven-dried soils. This indicates that any effect of BC on soil hydraulic properties in the present study may not be related to water-repellent nature of BC. This strengthens the observation that hydrophobic compounds, initially present in BC, may be lost relatively quickly (Yi et al., 2015). Therefore, our hypothesis that the hydrophobicity of BC induces soil water repellency in coarse-textured soils was rejected.

Saturated hydraulic conductivity decreased with increasing BC amounts in loamy sand (underneath the crust) irrespective of BC particle size (Fig. 4). Since BC had no effect on water repellency of the bulk soils (Fig. 2), the decrease in K_{sat} could not be attributed to the water-repellent behavior of BC, as suggested by Jeffery et al. (2015). Eibisch et al. (2015) also reported that the wettability characteristics of their digestate and woodchip BCs played no role in the observed increase in K_{sat} of loamy sand in their study. Thus, other mechanisms probably contributed to the observed decrease in K_{sat} of soils upon BC addition in the present study. This may include the filling by BC of large water-conducting inter-particle soil pores (macro-pores >30 μm diameter), which may be further aided by disintegration of BC in soil (Spokas et al., 2014). Filling of soil inter-particle space versus inclusion of BC into soil aggregates (Herath et al., 2013;

Obia et al., 2016; Ouyang et al., 2013; Soinne et al., 2014) are potentially opposing mechanisms. Thus, the direction of the BC effect on K_{sat} is probably dependent on which of these mechanisms is dominant. For fine textured soils with the possibility of soil aggregation, such as the ones studied by Major et al. (2010) and Asai et al. (2009), the increase in water flow rates may have been due to increased BC-induced soil aggregation. However, in our study, the soils were coarse-textured such that aggregation is either not possible (sand) or very weak (loamy sand). In our earlier work (Obia et al., 2016), we observed soil structural collapse upon draining saturated Mkushi soil amended with BC, indicating that structural development was very slow. Therefore, we hypothesize that the filling of large water conducting soil pores directly by BC, or associated with structural collapse during infiltration at near saturation, caused a BC-induced decrease in soil sorptivity and K_{sat} (Fig. 4). The hypothesis that BC increases K_{sat} in loamy sand due to BC-induced soil aggregation was falsified. Similarly, the hypothesis that finer BC reduces K_{sat} in sand, due to filling of inter particle space, is not supported, as no significant effect was observed, irrespective of BC particle sizes.

5. Conclusions and implications

Independent of its particle size, BC reduced the penetration resistance of the surface of loamy sand soils both with intact crust and after crust removal. By contrast, BC had no significant effect on penetration resistance in sandy soil. The reduction of penetration resistance in response to BC addition in loamy sand was attributed to BC-induced soil aggregation, which did not occur in sand. The reduction of penetration resistance in loamy sand may aid growth of roots, which may translate into better crop growth.

Biochar affected water repellency of the crusted surface of loamy sand. This effect was related to BC particle size. Biochar with fine particle sizes promoted water repellency, whereas coarser BCs reduced water repellency. By contrast, there was no effect of BC on the water repellency of sandy soil and loamy sand soil below the crust indicating that the repellency of BC was lost in less than one year after BC application. This suggests that the reduction in hydraulic conductivity (K_{sat}) of loamy sand, due to BC, was not because of water-repellent behavior of BC per sé, but may be due to clogging of pores or to structural collapse. The coarse-textured soils studied here have a relatively high K_{sat} and its moderate reduction in K_{sat} in response to BC addition is not expected to have any detrimental effect on soil productivity. The indirect promotion of water repellency of surfaces of crusted soil by fine BC may limit water infiltration and promote soil erosion.

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References

Abel, S., Peters, A., Trinks, S., Schonsky, H., Facklam, M., Wessolek, G., 2013. Impact of biochar and hydrochar addition on water retention and water repellency of sandy soil. Geoderma 202–203, 183-191.

Asai, H., Samson, B.K., Stephan, H.M., Songyikhangsuthor, K., Homma, K., Kiyono, Y., Inoue, Y., Shiraiwa, T., Horie, T., 2009. Biochar amendment techniques for upland rice production in Northern Laos. Field Crop Res. 111, 81-84.

Atkinson, C.J., Fitzgerald, J.D., Hipps, N.A., 2010. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. Plant Soil 337, 1-18.

Awadhwal, N.K., Thierstein, G.E., 1985. Soil crust and its impact on crop establishment: A review. Soil Tillage Res. 5, 289-302.

Barnes, R.T., Gallagher, M.E., Masiello, C.A., Liu, Z., Dugan, B., 2014. Biochar-induced changes in soil hydraulic conductivity and dissolved nutrient fluxes constrained by laboratory experiments. PLoS One 9, e108340.

Briggs, C.M., Breiner, J., Graham, R., 2005. Contributions of Pinus Ponderosa charcoal to soil chemical and physical properties, The ASACSSA-SSSA International Annual Meetings Salt Lake City, USA.

Buczko, U., Bens, O., Fischer, H., Hüttl, R., 2002. Water repellency in sandy luvisols under different forest transformation stages in northeast Germany. Geoderma 109, 1-18.

Busscher, W.J., Novak, J.M., Evans, D.E., Watts, D.W., Niandou, M.A.S., Ahmedna, M., 2010. Influence of Pecan Biochar on Physical Properties of a Norfolk Loamy Sand. Soil Sci. 175, 10-14.

de Jonge, L.W., Jacobsen, O.H., Moldrup, P., 1999. Soil water repellency: effects of water content, temperature, and particle size. Soil Sci. Soc. Am. J. 63, 437-442.

de Melo Carvalho, M.T., de Holanda Nunes Maia, A., Madari, B.E., Bastiaans, L., van Oort, P.A.J., Heinemann, A.B., Soler da Silva, M.A., Petter, F.A., Marimon Jr, B.H., Meinke, H., 2014.

Biochar increases plant-available water in a sandy loam soil under an aerobic rice crop system. Solid Earth 5, 939-952.

Dekker, L., Jungerius, P., 1990. Water repellency in the dunes with special reference to The Netherlands. Catena Supp. 173-183.

Dekker, L.W., Ritsema, C.J., Oostindie, K., Moore, D., Wesseling, J.G., 2009. Methods for determining soil water repellency on field-moist samples. Water Resour. Res. 45, 10.1029/2008wr007070.

Dexter, A.R., Czyż, E.A., Gaţe, O.P., 2007. A method for prediction of soil penetration resistance. Soil Tillage Res. 93, 412-419.

Doerr, S.H., Shakesby, R.A., Walsh, R.P.D., 2000. Soil water repellency: its causes, characteristics and hydro-geomorphological significance. Earth-Sci. Rev. 51, 33-65.

Doerr, S.H., Thomas, A.D., 2000. The role of soil moisture in controlling water repellency: new evidence from forest soils in Portugal. J. Hydrol. 231–232, 134-147.

Eibisch, N., Durner, W., Bechtold, M., Fuß, R., Mikutta, R., Woche, S.K., Helfrich, M., 2015. Does water repellency of pyrochars and hydrochars counter their positive effects on soil hydraulic properties? Geoderma 245–246, 31-39.

Gao, W., Whalley, W.R., Tian, Z., Liu, J., Ren, T., 2016. A simple model to predict soil penetrometer resistance as a function of density, drying and depth in the field. Soil Tillage Res. 155, 190-198.

Gardner, W.R., 1958. Some steady-state solutions of the unsaturated moisture flow equation with application to evaporation from a water table. Soil Sci. 85, 228-232.

Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal - a review. Biol. Fert. Soils 35, 219-230.

Herath, H.M.S.K., Camps-Arbestain, M., Hedley, M., 2013. Effect of biochar on soil physical properties in two contrasting soils: An Alfisol and an Andisol. Geoderma 209-210, 188-197.

Jeffery, S., Meinders, M.B.J., Stoof, C.R., Bezemer, T.M., van de Voorde, T.F.J., Mommer, L., van Groenigen, J.W., 2015. Biochar application does not improve the soil hydrological function of a sandy soil. Geoderma 251-252, 47-54.

Jeffery, S., Verheijen, F.G.A., van der Velde, M., Bastos, A.C., 2011. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agri. Ecosyst. Environ. 144, 175-187.

Khanmohammadi, Z., Afyuni, M., Mosaddeghi, M.R., 2015. Effect of pyrolysis temperature on chemical and physical properties of sewage sludge biochar. Waste Manag Res. 33, 275-283.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota – A review. Soil Biol. Biochem. 43, 1812-1836.

Letey, J., Carrillo, M.L.K., Pang, X.P., 2000. Approaches to characterize the degree of water repellency. J. Hydrol. 231–232, 61-65.

Major, J., Lehmann, J., Rondon, M., Goodale, C., 2010. Fate of soil-applied black carbon: downward migration, leaching and soil respiration. Glob. Change Biol. 16, 1366-1379.

Materechera, S.A., Mloza-Banda, H.R., 1997. Soil penetration resistance, root growth and yield of maize as influenced by tillage system on ridges in Malawi. Soil Tillage Res. 41, 13-24.

Mukherjee, A., Lal, R., 2013. Biochar Impacts on Soil Physical Properties and Greenhouse Gas Emissions. Agronomy 3, 313-339.

Mukherjee, A., Lal, R., Zimmerman, A.R., 2014. Impacts of 1.5-Year Field Aging on Biochar, Humic Acid, and Water Treatment Residual Amended Soil. Soil Sci. 179, 333-339.

Obia, A., Mulder, J., Martinsen, V., Cornelissen, G., Børresen, T., 2016. In situ effects of biochar on aggregation, water retention and porosity in light-textured tropical soils. Soil Tillage Res. 155, 35-44.

Ouyang, L., Wang, F., Tang, J., Yu, L., Zhang, R., 2013. Effects of biochar amendment on soil aggregates and hydraulic properties. J. Soil Sci. Plant Nutr. 13, 991-1002.

Page-Dumroese, D., Robichaud, P., Brown, R., Tirocke, J., 2015. Water repellency of two forest soils after biochar addition. T. ASABE 58, 335-342.

R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Soinne, H., Hovi, J., Tammeorg, P., Turtola, E., 2014. Effect of biochar on phosphorus sorption and clay soil aggregate stability. Geoderma 219–220, 162-167.

Spokas, K.A., Novak, J.M., Masiello, C.A., Johnson, M.G., Colosky, E.C., Ippolito, J.A., Trigo, C., 2014. Physical Disintegration of Biochar: An Overlooked Process. Environ. Sci. Technol. Lett. 1, 326-332.

Sun, F., Lu, S., 2014. Biochars improve aggregate stability, water retention, and pore-space properties of clayey soil. J. Plant Nutr. Soil Sci. 177, 26-33.

Upadhyaya, S., Sakai, K., Glancey, J., 1995. Instrumentation for in-field measurement of soil crust strength. T. ASAE 38, 39-44.

Uzoma, K.C., Inoue, M., Andry, H., Fujimaki, H., Zahoor, A., Nishihara, E., 2011. Effect of cow manure biochar on maize productivity under sandy soil condition. Soil Use Manage. 27, 205-212.

Verheijen, F.G.A., Jeffery, S., Bastos, A.C., van der Velde, M., Diafas, I., 2009. Biochar Application to Soils: A Critical Scientific Review of Effects on Soil Properties, Processes and Functions. EUR 24099 EN, Office for the Official Publications of the European Communities, Luxembourg, 149pp.

Warnock, D.D., Lehmann, J., Kuyper, T.W., Rillig, M.C., 2007. Mycorrhizal responses to biochar in soil – concepts and mechanisms. Plant Soil 300, 9-20.

Watson, C., Letey, J., 1970. Indices for characterizing soil-water repellency based upon contact angle-surface tension relationships. Soil Sci. Soc. Am. J. 34, 841-844.

Wooding, R., 1968. Steady infiltration from a shallow circular pond. Water Resour. Res. 4, 1259-1273.

Yatagai, A., 2011. Interannual variation of seasonal rain fall in South Zambia., In: Umetsu, C. (Ed.), Vulnerability and Resilience of Social-Ecological Systems. Research Institute For Humanity And Nature, Kyoto, pp. 206-212.

Yi, S., Witt, B., Chiu, P., Guo, M., Imhoff, P., 2015. The origin and reversible nature of poultry litter biochar hydrophobicity. J. Environ. Qual. 44, 963-971.

Table 1. Soil and biochar properties[a]

Properties	Kaoma soil	Mkushi soil	Maize cob BC		
			≤0.5 mm	1–5 mm	Unsorted
Sand (%)	85.4	75.1	-	-	-
Silt (%)	10.2	15.9	-	-	-
Clay (%)	4.4	9.0	-	-	-
Texture class	Sand	Loamy sand	-	-	-
Total organic C (%)	0.62	0.74	44.8	60.1	53.8
Total nitrogen (%)	0.00	0.01	0.79	0.53	0.65
Total hydrogen (%)	0.05	0.27	2.09	2.63	2.36
H/C (mole ratio)	-	-	0.56	0.52	0.53
pH	5.8	5.8	9.0	8.6	8.8
CEC ($cmol_c$ kg^{-1})	2.79	1.73	-	-	22.19
K^+ ($cmol_c$ kg^{-1})	0.08	0.32	-	-	16.47
Ca^{2+} ($cmol_c$ kg^{-1})	1.20	1.09	-	-	4.30
Mg^{2+} ($cmol_c$ kg^{-1})	0.24	0.32	-	-	1.21
Bulk density (g cm^{-3})	1.27	1.47	0.36	0.29	-
Loss on ignition (%)	-	-	52.1	72.4	-

[a]All soil measurements are from samples taken from 0–7 cm depth interval. Maize cob BC of 0.5–1 mm were exhausted in the field and not characterized in the laboratory.

Table 2. Summary of measurements conducted[a].

Soil property	Measurement method	Site	Time of sampling or measurement	Number of measurements
Penetration resistance	Penetrometer	Kaoma	March 2015	10 per plot
		Mkushi	October 2014 & March 2015	10 per plot
Water repellency	WDPT - field & lab, on soil surface only	Kaoma	April 2014 & March 2015	10 drops/plot - field 20 drop/plot - lab
	WDPT - field & lab, on & below crusted surface	Mkushi	April 2014 & March 2015	10 drops/plot - field 20 drop/plot - lab
	Ethanol % - field, on crusted surface only	Mkushi	March 2015	10 per plot
K_{sat} & sorptivity	Tension disc infiltrometer	Kaoma	April 2014 & March 2015	2 per plot
		Mkushi	April 2014 & March 2015	2 per plot
Moisture content	Hand-held TDR	Kaoma	April 2014 & March 2015	5 per plot
		Mkushi	April & October 2014, March 2015	5 per plot

[a]The experiment was set up in April 2013. Penetration resistance, K_{sat} and sorptivity were measured only in the field.

Table 3. Soil moisture content and bulk density at the time of penetration resistance/water repellency measurement[a]

BC particle size	BC dose (w/w %)		Moisture (vol %) April 2014		Moisture (vol %) March 2015		Bulk density (g cm^{-3}) April 2014	
	Mkushi	Kaoma	Mkushi	Kaoma	Mkushi	Kaoma	Mkushi	Kaoma
Reference plot	0	0	11.2±0.5	0.8±0.3	0.7±0.2	0.2±0.1	1.27±0.02	1.47±0.02
≤0.5 mm	2	1.7	12.4±0.7	0.1±0.0	1.4±0.8	0.1±0.1	1.24±0.01	1.46±0.02
	4	3.4	11.8±0.9	0.5±0.3	0.5±0.1	0.2±0.1	1.21±0.03	1.42±0.01
0.5-1 mm	2	1.7	9.7±0.4	1.3±0.4	0.7±0.6	0.2±0.1	1.21±0.02	1.37±0.01
	4	3.4	9.5±0.7	0.5±0.2	0.8±0.4	0.4±0.1	1.27±0.03	1.35±0.05
1-5 mm	2	1.7	9.6±0.3	0.2±0.1	0.2±0.2	0.1±0.0	1.25±0.01	1.35±0.02
	4	3.4	9.2±0.6	0.4±0.2	0.0±0.0	0.1±0.0	1.15±0.04	1.33±0.01

[a]The moisture content in October 2014 during the measurement of penetration resistance was below detection limit of TDR. Numbers in the table are means ± standard error (n = 3). All measurements are for the bulk soil within 0–7 cm depth interval.

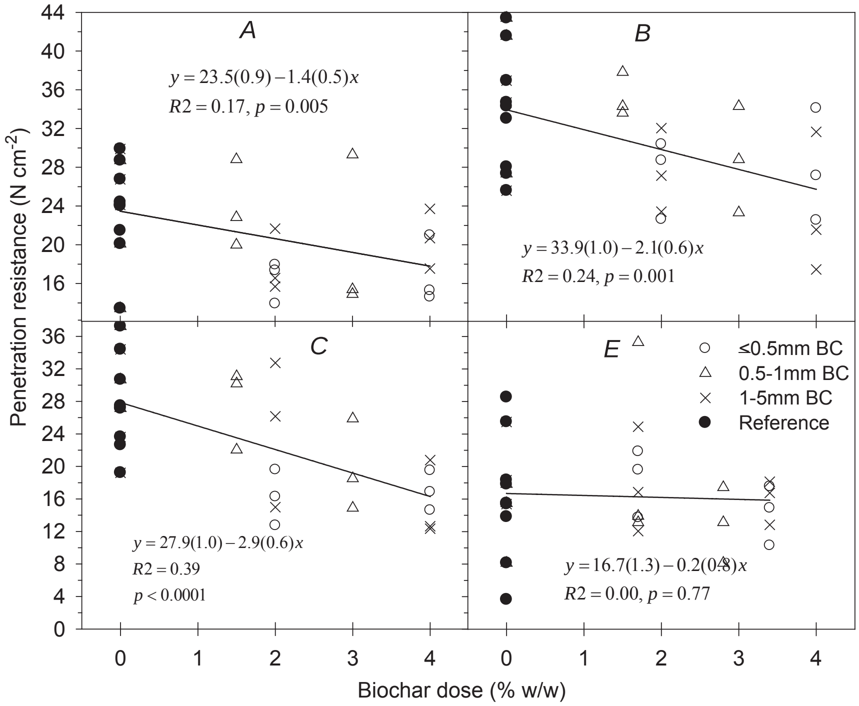

Fig. 1. Penetration resistance of soil amended with BC of different particle sizes. A = 0–6 mm Mkushi soil surface with no visible crust in October 2014, B = 0–6 mm Mkushi soil surface with crust intact in March 2015, C = 10–16 mm Mkushi soil layer underneath the crust in March 2015, D = 0–6 mm Kaoma soil surface layer in March 2015. Numbers in the bracket in regression equations are SEs. No difference between particle sizes (p>0.05).

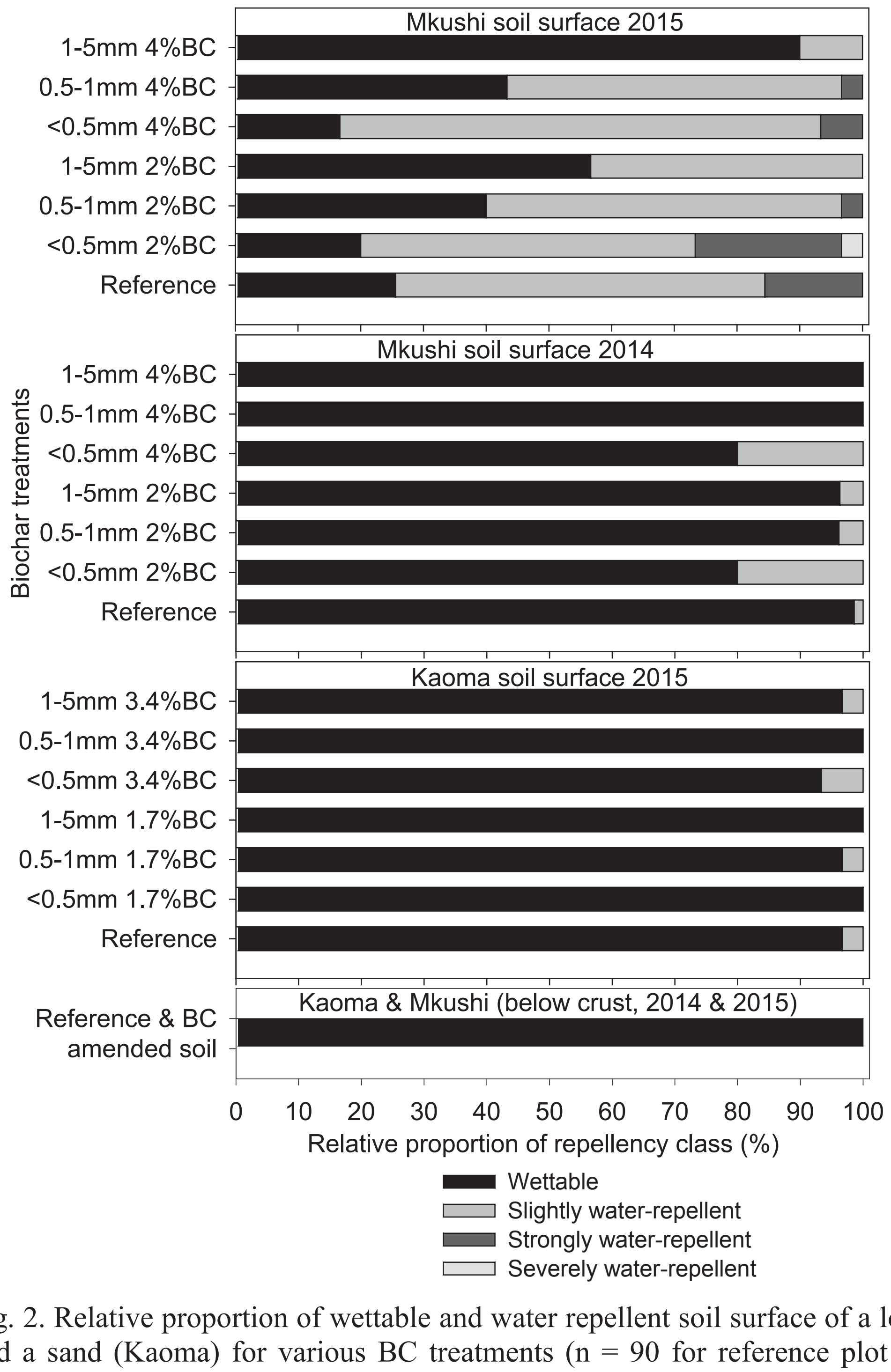

Fig. 2. Relative proportion of wettable and water repellent soil surface of a loamy sand (Mkushi) and a sand (Kaoma) for various BC treatments (n = 90 for reference plot and n = 30 for BC treatments). The bottom panel of Kaoma and Mkushi (below crust, 2014 & 2015) represents measurements conducted both in the field and laboratory irrespective of moisture content: all 100% wettable. Water repellency classes according to Dekker and Jungerius (1990): WDPT < 5 s - wettable or non-water-repellent, 5 s < WDPT < 60 s - slightly water-repellent, 60 s < WDPT < 600 s - strongly water-repellent, 600 s < WDPT < 3600 s - severely water-repellent, WDPT > 3600 s - extremely water-repellent.

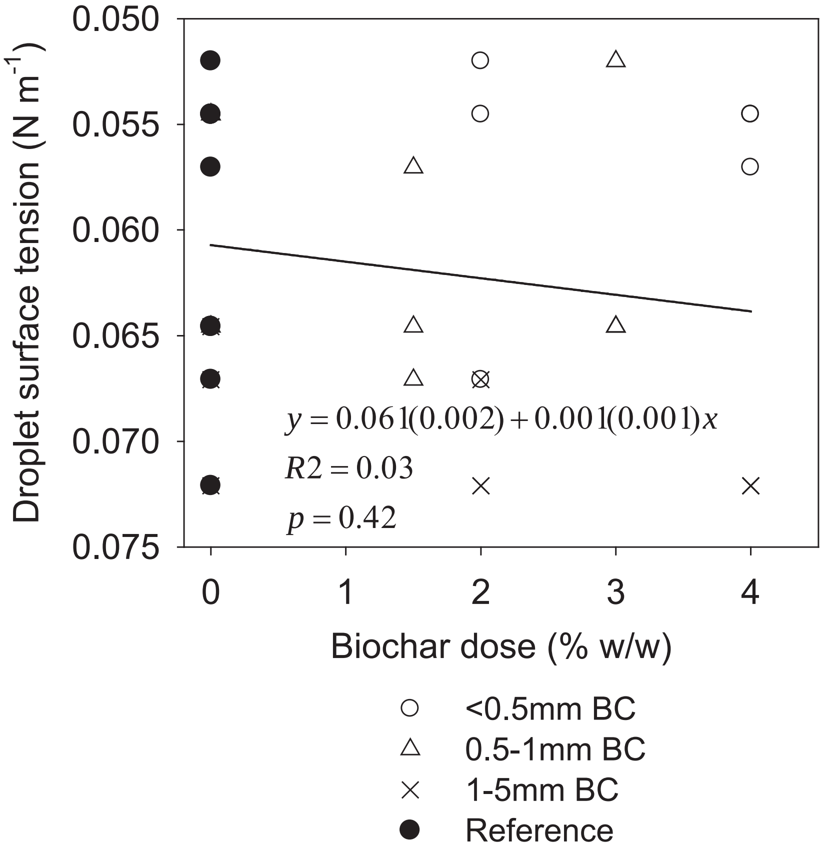

Fig. 3. Water repellency expressed as surface tension of drops of ethanol solution placed on the crusted surface of BC amended Mkushi soil measured in March 2015. Note the reversed y-axis; lower surface tension means higher alcohol concentration. Numbers in the bracket in regression equations are SEs. No difference between particle sizes (p>0.05).

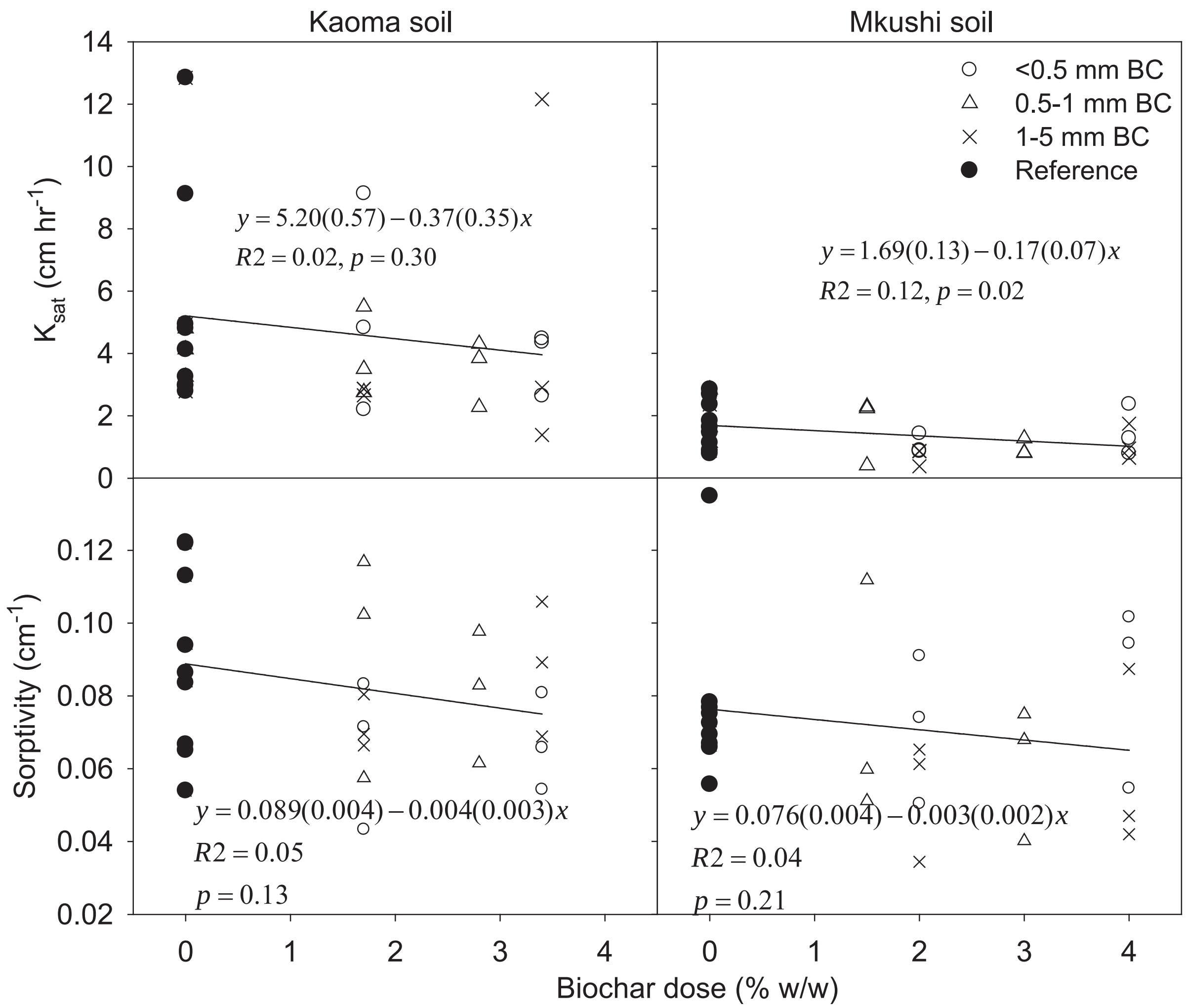

Fig. 4. Saturated hydraulic conductivity and sorptivity of sandy (Kaoma) and loamy sand soils (Mkushi, below the crust) amended with maize cob BC of different particle sizes, measured in March 2015. Numbers in the bracket within the fitted regression equation is the SE for either intercept or slope. No difference between particle sizes (p>0.05).

PAPER III

Vertical and lateral transport of biochar in light-textured tropical soils

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Vertical and lateral transport of biochar in light-textured tropical soils

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Abstract

Field experiments were conducted in Arenosols (sand) and Acrisols (loamy sand) in Zambia to quantify vertical and lateral transport of biochar (BC) of ≤0.5 and 0.5–1 mm particle sizes using the BC and soil ^{13}C isotope signatures and total organic carbon contents. The applied BCs were made from rice husk, except 0.5–1 mm BC in loamy sand, which was from maize cob. One year after mixing BC homogeneously in the 0–5 cm surface layer, soil down to 20 cm depth was sampled. The downward migration of BC was significant down to 8 cm depth in loamy sand and down to 6 cm in sand. Below these depths, there was no significant difference in BC amounts between the BC amended and the reference plots. There was a general tendency for greater downward migration for the ≤0.5 mm than for 0.5–1 mm BC. Total BC recovery at 0–5 cm depth in the BC-treated soils amounted to 45–66% of the total applied amount of BC. As only 10–20% was recovered in the deeper soil layers, 24–45% of the applied BC could not be accounted for in the soil profile. Although, decomposition and downward migration to below 20 cm depth may contribute to the loss of BC from the surface soil, much can be attributed to lateral transfer through erosion. This is the first study that explicitly focuses on the theme of BC dispersion and shows that in Arenosols and Acrisols of the tropics, the downward migration of BC is limited.

Key words

Biochar particle size, ^{13}C isotope, Biochar downward migration, Biochar recovery, Erosion

Highlight

- Rice husk biochar of ≤0.5 and 0.5–1 mm sizes were applied to soil.
- Migration rate to deeper soil was higher for finer biochar but limited to few cm.
- There was higher migration of biochar in loamy sand with lower K_{sat} than in sand.

- 55–76% of applied biochar was recovered in soil leaving 24–45% unaccounted.
- The unaccounted for biochar was transported away by erosion.

1. Introduction

Biochar (BC), which is a biomass pyrolysis product has been reported to increase crop production with the co-benefit of sequestering carbon (C) (Glaser et al., 2002; Jeffery et al., 2011). Reported increases in crop production varied widely depending on soil and BC types, but there are indications that this effect of BC might be stronger in sandy and acidic soils (Glaser et al., 2002; Jeffery et al., 2011; Martinsen et al., 2014), which are widespread in tropical regions. Some of the mechanisms for the reported increase in crop production include increase in water holding capacity, liming effect and direct addition and retention of nutrients by BC (Cornelissen et al., 2013; Glaser et al., 2002). If these BC effects on soil properties are to benefit the crops for extended periods, then BC applied to top soils should remain within the top soil where root density is high. However, any transport of BC would not affect its C sequestration potential.

There are few studies, which indicate that BC, once applied to soil, might to a certain extent be mobile within the soil profile (Foereid et al., 2011; Haefele et al., 2011; Major et al., 2010). Such transport of BC within the soil profile could be exacerbated by physical disintegration of BC to nano- and micrometer sized particles, moving with infiltrating water (Spokas et al., 2014). Haefele et al. (2011) reported that as much as 50% of BC applied to 15 cm top soil, estimated based on total C changes in the soil profile, migrated to deeper soil horizons of structured humic Nitisols and gleyic Acrisols after one year. The migration of BC to deeper soil was fast in soils with high water infiltration rate (Nitisol and Acrisol), whereas no migration was found in soils with low water infiltration rate, as heavy paddy soil. In an experiment designed to measure the fate of BC from mango prunings applied at 0–10 cm in sandy clay loam Ferralsol using $\delta^{13}C$, Major et al.

(2010) reported slow downward migration of BC at 15 cm depths at a rate of <0.5% of the BC applied to soil per year.

An additional number of studies report downward migration of black C (Hockaday et al., 2006; Leifeld et al., 2007), which is similar to BC. Black C in the environment are organic products commonly derived from incomplete combustion without intentionally limiting oxygen (soot and charcoal). In drained peatland, black C from deposited combustion residues of household waste migrated to deeper soil layers (Leifeld et al., 2007). Leifeld et al. (2007) found between 21 to 69% of black C below plough depth of 30 cm, 50 years after the last deposition of black C. The migration rate of black C was estimated to be 0.6–1.2 cm year^{-1}. Similarly, Hockaday et al. (2006) found that black C can be mobile in fire-impacted forest soil (medium sand with poorly developed Podzol), particularly the soluble organic constituents resulting from decomposition, which leach with percolating soil water.

Based on a modeling study, Foereid et al. (2011) suggested that lateral transport of BC could be a very important transport pathway of BC in soils, but limited field data are available. In their modelling work, the authors predicted that erosional transport of BC decreased with time due to incorporation of BC into soil aggregates (Awad et al., 2013; Obia et al., 2016). Due to the scarcity of experimental data on both vertical and lateral transport of BC, more studies are warranted. In addition, no study has reported the influence of BC particle size on its lateral and vertical dispersion.

Acrisols and Arenosols, characterized by low agricultural productivity, dominate central and western regions of Zambia. The productivity of these soils, which are widespread globally, has been demonstrated to increase through the application of BC (Cornelissen et al., 2013; Martinsen et al., 2014). One of the main factors proposed to explain the BC-induced increase in productivity of these soils is the increase in water holding capacity in the root zone (Obia et al., 2016) leading to better-developed root systems (Abiven et al., 2015). Migration of large amounts of BC to deeper soil horizons with low density of roots might eliminate or reduce the effect of BC on soil productivity (Haefele et al., 2011).

In a controlled field experiment in two light-textured soils in Zambia, we determined BC transport rates and their dependence on BC particle size. We hypothesized that there would be greater downward migration of BC at Kaoma (sand with higher saturated hydraulic conductivity ~5.2 cm hr^{-1}) than at Mkushi (loamy sand soil with saturated hydraulic conductivity of ~1.7 cm hr^{-1}) (Obia et al., unpublished data) and that this migration would be greater for finer BC fractions. Lateral transport of BC is also expected to be greater at Kaoma than at Mkushi and greater for finer than coarser BCs. The objective of the present study was therefore to quantify the downward and lateral transport of fine (≤0.5 mm) and slightly coarser (0.5–1 mm) BC in sand (Arenosol) and loamy sand (Acrisol).

Biochars were produced from rice husk and maize cobs with $\delta^{13}C$ signals different from that of the soil were applied. Before application, the BCs were sieved into ≤0.5 and 0.5–1 mm size fractions. Biochars were homogeneously mixed with the upper 0–5 cm of the soil. One year after BC application, soil samples were taken from different layers in the 0–20 cm depth interval and analyzed for $\delta^{13}C$ and total organic C (TOC) to quantify the amount of BC in the soil profile. This study is one of the few explicitly dedicated to studying BC mobility in soil and the first to consider the *in situ* mobility of different BC particle sizes.

2. Materials and methods

2.1 Biochars and soils

The BCs used in this study were prepared from rice husk and maize cobs after shelling the grains. Rice husk is available in western Zambia, whereas maize cobs are available throughout Zambia. Pyrolysis of the feedstocks was carried out in a drum retort kiln at Chisamba, Zambia at a temperature of 350 ºC and a retention time of one day. Other pyrolysis conditions can be found in (Sparrevik et al., 2015). The maize cob BC was used in extensive field trials (Martinsen et al., 2014) and in mechanistic studies (Alling et al., 2014; Hale et al., 2013). However, we primarily used the rice husk BC in the present study since its $\delta^{13}C$ was expected to deviate more from that of the soil than $\delta^{13}C$ of the maize cob BC. The BCs were sieved to particle sizes of ≤0.5 and 0.5–1 mm before application to soil. Field experiments were established in loamy sand Acrisol at Mkushi, central Zambia (S13 44.839, E29 05.972) and in sandy Arenosol at Kaoma, western Zambia (S14 50.245, E25 02.150) in April 2013. The annual rainfall in Mkushi and Kaoma is 1220 and 930 mm, respectively (Martinsen et al., 2014). The soil and BCs were characterized for TOC, total nitrogen, total hydrogen (CHN analyzer - CHN-1000, LECO USA), loss on ignition, pH (1:2.5 soil(BC):water (pH meter - Orion 2 Star, Thermo Fisher Scientific, Fort Collins, CO), $\delta^{13}C$ (Isotope Ratio Mass Spectrometer), texture (pipette method) and surface area (N_2-BET). Biochar was acidified to remove carbonates before determination of TOC. Easily soluble constituents of the BCs were determined by mixing 2 g of BC in 100 ml deionized water (1:50) followed by overnight shaking. The BCs were then filtered and oven dried. The soil and BC properties are presented in Table 1.

2.2 Experimental set up

Biochar was applied in the top 5 cm of soil based on completely randomized design. There were two BC treatments in Kaoma sand, in addition to a reference without BC. Treatments included ≤0.5 mm and 0.5–1 mm rice husk BC, both added at a rate of 3.4% w/w. In Mkushi loamy sand, the treatments included ≤0.5 mm rice husk BC, 0.5–1 mm maize cob BC and a reference. Here, BC addition rates were 4% w/w for both treatments. At Mkushi, the coarser (0.5–1 mm) fraction was maize cob BC (and not rice husk BC), due to shortage of coarser rice husk BC, caused by easy crumbling of rice husk BC during sieving to finer sizes. The same amount of BC was added per plot (625 g) to both Mkushi and Kaoma soils, but the BC contents (in %w/w) differed due to differences in soil bulk density between the two sites (Table 2). At both sites, treatments and references had three replicates resulting in nine plots per site. Plot sizes were 50 x 50 cm, separated by 20 cm high hard plastic sheets, inserted approximately 10 cm vertically into the soil. Layout of the experimental design can be found in supplementary information Fig. S1.

To apply the BC, we removed the top 5 cm of the soil by hand hoe and spade and mixed it with appropriate amount of BC in a bucket. The top soil was very dry when the experiment was set up, so that mixing with BC was easy. The soil layers below 5 cm down to approx. 30 cm were loosened using a hand hoe to remove any compacted layer before placing back the soil-BC mixture at the surface. Loosening the compacted subsoil is a common farmer practice to increase root volume as recommended by the conservation farming unit (CFU) of Zambia for farmers practicing conservation farming (Cornelissen et al., 2013; Umar et al., 2011). The soil in the reference plots were treated in the same way as the BC amended plots.

2.3 Soil sampling and sample preparation

Soil samples were taken at the end of March 2014, one year after BC application, to determine the amount of BC recoverable in the soil profile down to 20 cm depth. Two samples were taken from each of eight depth intervals per plot: 0–5 cm (depth of BC application), 5–6 cm, 6–7 cm, 7–8 cm, 8–9 cm, 9–10 cm, 10–15 cm, and 15–20 cm. Each sample was taken by cutting 1 cm thick vertical slices of soil across the plot through the entire layer of each of the eight depth intervals. Samples were sealed in sampling bags prior to analysis.

The field-moist soil samples were dried at 40ºC for 3 days before passing through a 2 mm sieve. Sub-samples of the sieved homogenous soils were milled for analysis of $\delta^{13}C$ and TOC. Milled samples were prepared in 8x5 mm tin capsules and sent to Stable Isotope Facility, University of California, Davis for analysis using isotope ratio mass spectrometry.

Core ring samples (100 cm^3) were taken to determine the bulk density of the soil to allow calculation of TOC stocks in each of the depth intervals and thus establishment of BC mass balances relative to the amount applied to the plots in April 2013. The bulk density (Table 2) was determined at two depths (0–5 cm and 6–10 cm) for each plot. The bulk density at 6–10 cm depth was used for calculation of TOC stocks in all depths from 6–20 cm. This is reasonable because the soils from 6–20 cm were homogenized during plot establishment (see above).

2.4 Calculation of biochar amounts and mass balance

The TOC stock (g) per soil depth interval at each 50x50 cm plot was calculated according to:

$$TOC\,(g) = 50 * 50 * Depth * Bulk\ density * \frac{TOC\,(\%)}{100} \qquad \text{eq.1}$$

Where Depth and Bulk density are in cm and g cm^{-3}, respectively.

The fraction *f* of TOC contributed by BC was calculated according to equation 2, adapted from Kocyigit (2006):

$$f = \frac{\delta^{13}C_{mixture} - \delta^{13}C_{ref.\,soil}}{\delta^{13}C_{biochar} - \delta^{13}C_{ref.\,soil}} \qquad \text{eq.2}$$

Where $\delta^{13}C_{mixture}$ = $\delta^{13}C$ of the soil-BC mixture, one year after BC application, $\delta^{13}C_{biochar}$ = $\delta^{13}C$ of the BC and $\delta^{13}C_{ref.\ soil}$ = $\delta^{13}C$ of the reference soil. The reference $\delta^{13}C$ of the 0–5 cm was determined at the time of experimental set up in April 2013 while the 5–20 cm was determined in the reference plots as average value at depth of 5–20 cm in April 2014. The $\delta^{13}C$ of the reference plots was constant with depth at the interval of 5–20 cm (Fig. 1).

The amount of BC recovered (g) from each depth interval in each plot was calculated according to:

$$BC\ recovered = TOC * f * \frac{100}{\%C\ of\ BC} \qquad \text{eq.3}$$

The two measurement values of $\delta^{13}C$ (‰), TOC (%) and BC (g) recovered per plot for each depth interval were averaged prior to statistical analysis.

2.5 Statistical analysis

The data were analyzed using the software package R (R Core Team, 2014). In order to display the distribution of BC along the soil profile, $\delta^{13}C$ and TOC (%) were plotted along the soil depth profile. The amount of BC recovered at each depth interval were analyzed using two-way analysis of variance (ANOVA). In this analysis, BC treatments (no BC, ≤0.5 mm BC, and 0.5–1 mm BC) and sites (Mkushi and Kaoma) were considered as the explanatory factors for the differences in BC recovered. This allowed comparison among treatments within each site and comparison of treatments between sites. Differences between mean values were assessed using Tukey's test at 5% level of significance. All numbers presented in tables are mean values ± standard errors.

3. Results

The $\delta^{13}C$ signal and the TOC contents of the BC amended plots changed along the depth profile in both Mkushi and Kaoma soils (Fig. 1). The $\delta^{13}C$ of the rice husk BC (-27.1±0.04‰) and maize cob BC (-12.3±0.1‰) (Table 1), which are from C3 and C4 plants respectively, were very different from those of the two soils and therefore allowed tracing of the BCs in the soils. Maize cob BC (0.5–1 mm particle size) was applied only in Mkushi soil. The reference value of $\delta^{13}C$ of Mkushi and Kaoma soil were -18.1±0.3‰ and -20.2±0.1‰, respectively, for the 0–5 cm depth and -18.9±0.03‰ and -20.8±0.03‰, respectively, for the 5–20 cm (Table 1). One year after BC application, the surface soil layer (0–5 cm) of the neighboring reference plots received BC transported laterally from the BC amended plots as indicated by their $\delta^{13}C$ values (-19.6±0.3‰ and -21.7±0.3‰, in Mkushi and Kaoma soil, respectively; Fig. 1 and Table 3 and 4).

In Kaoma sand where only rice husk BC ($\delta^{13}C$ = -27.1‰) was applied, the $\delta^{13}C$ in the top soil (0–5 cm) was -24.7±0.1‰ for the ≤0.5 mm BC and -25.1±0.1‰ for the 0.5–1 mm BC, respectively. Both values were significantly smaller than the soil reference value of -20.2±0.05‰ prior to BC addition in 2013 (Fig. 1 B1). With increasing soil depth, the $\delta^{13}C$ increased to values not significantly different from the soil's reference value of 20.8±0.03‰ below 8 and 7 cm soil depth for ≤0.5 mm BC and 0.5–1 mm BC, respectively. The TOC of 0–5 cm soil depth interval was significantly larger for the 0.5–1 mm BC (1.57±0.05%) than for the ≤0.5 mm BC (1.29±0.02%)

amended plots (Fig. 1 B2 and Table S2). However, for both treatments, TOC decreased with depth and was no longer significantly different from that of the reference soil (0.43±0.02%) at 7–8 cm soil depth interval.

In the loamy sand at Mkushi, the addition of ≤0.5 mm rice husk BC with $\delta^{13}C$ of -27.1‰ significantly reduced $\delta^{13}C$ of the 0–5 cm soil from reference value of -18.1±0.3‰ (prior to BC addition in 2013) to -23.9±0.3‰ measured in April 2014 ($p<0.05$) (Fig. 1 A1). For deeper soil layers below the application depth, the $\delta^{13}C$ of the soil in April 2014 increased gradually with depth reaching the reference soil value of -18.9±0.03‰ at the depth of 10 cm (Fig. 1). On the other hand, the 0.5–1 mm maize cob BC with $\delta^{13}C$ of -12.3‰ increased $\delta^{13}C$ of the soil from reference value prior to BC addition of -18.1±0.3‰ to -14.9±0.1‰ in the 0–5 cm soil (Fig. 1 A1). Below 5 cm depth, $\delta^{13}C$ decreased reaching the soil reference value at 9 cm soil depth. The TOC content of the soil followed a similar pattern with soil depth as $\delta^{13}C$ (Fig. 1 A2). However, the treatments with ≤0.5 mm rice husk BC and 0.5–1 mm maize cob BC reached the reference soil's TOC level at 9 and 8 cm soil depth, respectively, 1 cm depth short of that estimated using $\delta^{13}C$ (Fig. 1 A2). The TOC contents of the soil in the 5–9 cm depth interval were larger for ≤0.5 mm rice husk BC than for 0.5–1 mm maize cob BC amended plots.

We recovered greater amounts of BC of the ≤0.5 mm fraction below the application depth compared to the 0.5–1 mm fraction in Mkushi (19% vs 9%) (Table 3 and S1). Likewise, there were also greater amounts of fine BC below the application depth in Kaoma. However, the difference between the two BC particle sizes was not significant ($p = 0.41$) (13% vs 9%). Overall, the downward transport of BC was greater in Mkushi loamy sand than in Kaoma sand. The recovered BC in the top 0–5 cm of the Kaoma soil based on eq. 3 after one year was 56% and 67% for ≤0.5 mm and 0.5–1 mm BCs, respectively (Table 3 and S1). In Mkushi, since the two BCs used had different $\delta^{13}C$ signals and there was cross-transportation of BCs between the surface layers of the plots, no accurate estimate of the recovery of BC in the 0–5 cm layer, based on $\delta^{13}C$ (Table 3 and S1) was possible. Use of TOC changes alone to calculate BC recovery indicated a recovery of 53% and 45% for ≤0.5 mm rice husk and 0.5–1 mm maize cob BCs, respectively (Table S2). The overall BC recovery in the 0–5 cm at both sites after one year were therefore between 45 to 67%. The total recovery in the soil profile down to the 20 cm soil depth was 55–76% of the BC applied. Below the depth of 8 cm, less than 2% of added BC was found with no significant difference between BC content in amended and reference plots based on eq. 3 ($p>0.05$) (Table 3 and S1). The recovery of BC based on eq. 3 and based on TOC alone were similar, especially for Kaoma where only rice husk BC was used. The only difference was below the depth of 8 cm where $\delta^{13}C$ signal allowed detection of small amounts of BC. The total recovery of 0.5–1 mm maize cob BC in Mkushi soil was small (55%) (Table S2) compared to other BC treatments (69–76%) at Mkushi and Kaoma (Table 3 and S1) ($p<0.05$).

4. Discussion

The changes in both $\delta^{13}C$ signal and TOC content of the soil with depth in the 5–10 cm interval at both Mkushi and Kaoma (Fig. 1) showed that BC migrated to deeper soil horizons. The downward migration of BC one year after the application was confined to less than 3 cm below the application depth, i.e., the 5–8 cm depth interval (Fig. 1 and Table 3). The downward migration of the BCs down to 5–20 cm was in the range of 9–19% of the applied BC and was generally greater for fine BC of ≤0.5 mm than for coarser BC of 0.5–1 mm (e.g. 19% vs 9% in Mkushi). There was greater downward migration of BC in loamy sand (Mkushi) than in sand (Kaoma) (e.g. 19% vs 13% for ≤0.5 mm BC). Migration of BC in the Zambian loamy sand Acrisol and sandy Arenosol differed in magnitude from that reported by Haefele et al. (2011) who found annual downward migration rates of up to 50% of the applied rice husk BC in humic Nitisol and gleyic Acrisol in the Philippines and Thailand, respectively. Major et al. (2010) on the other hand found a very low annual downward migration rate of <1% of the applied BC in sandy clay loam Ferralsol in Colombia.

Several factors may influence migration rates of BC to lower horizons including BC particle size, tillage practice, soil texture, soil structure/aggregation, hydraulic conductivity and rainfall amount. Our results suggest that the finer the BC, the faster it will migrate to deeper horizons (TOC in Fig. 1 and recovered BC in Table 3). The TOC content of the 0–5 cm depth interval was smaller for ≤0.5 mm than 0.5–1 mm BC amended plots in Kaoma (TOC = 1.29±0.02% for the ≤0.5 mm BC vs 1.57±0.05% for 0.5–1 mm BC plots). This was not just because of smaller TOC content of ≤0.5 mm BC (Table 1) but also because of greater downward migration (Fig. 1 and Table 3). Tillage practice using planting basins, as common in conservation agriculture, may aid the increased migration rate of BC in soil by creating big soil-packing voids that may be filled, due to subsequent preferential colloidal and particle transport with percolating water to deeper soil horizon. In this study, we suspect that such packing voids were the main factor responsible for the slightly greater downward migration of BC in Mkushi loamy sand compared to Kaoma sand. The Kaoma soil lacks packing voids as the sandy soil (85% sand) does not exhibit any significant extent of aggregation, as shown in our previous work on the same sites (Obia et al., 2016). The slightly greater migration depth in the loamy sand at Mkushi can also be explained by its higher rainfall (1220 mm yr^{-1}) compared with Kaoma (930 mm yr^{-1}). The importance of water percolation for BC movement has been reported previously for sandy clay loam Ferralsol (Major et al., 2010). The water flow rate in the soil, as emphasized by Haefele et al. (2011), appeared less important in the present study in determining migration rate of BC. This was shown by the smaller migration rate in Kaoma, which had higher saturated hydraulic conductivity than Mkushi (5.2 vs 1.7 cm hr^{-1} measured using tension disc infiltrometers; Obia et al., unpublished data).

Although the downward migration of BC was mainly within a few cm below the application depth, the total BC recovery for the 0–20 cm depth interval sampled was less than the amount of BC applied. The amount of maize cob BC recovered in the application layer (0–5 cm soil depth) at Mkushi (based on the change in TOC content) was smaller (55%) than that of rice husk BC treatments (69–76%) in both Mkushi and Kaoma ($p<0.05$) (Table 3 and S1). However, the amount of maize cob BC recovered in the 5–20 cm depth profile, i.e., below the application layer, was of the same order of magnitude as that of the other treatments at both sites. For example, there was similar pattern in the recovery of maize cob BC and rice husk BC of 0.5–1 mm at Mkushi and Kaoma, respectively in 5–20 cm depth (Table 3). The TOC contents corroborated the similarity of the trends in the distribution of maize cob and rice husk BCs below application depth at both sites (Table S2). Maize cob BC had higher TOC contents than rice husk BC (Table 1) and the similar TOC stocks of these two BCs in the application layer (0–5 cm) (Table S2) indicated that more maize cob BC must have moved out of the plots. The total recovery of BC at 0–20 cm depth was 55–76%, leaving between 24–45% of the applied BC unaccounted.

The unrecovered BC of at least 24% in the top 20 cm of soil can be attributed to i) loss due to decomposition, ii) migration as solid BC or dissolved organic matter to soil layers below 20 cm depth, which were not sampled, and iii) lateral loss due to both water and wind erosion of the surface soil. The decomposition rate of BC produced within the same temperature range as our BCs has been reported to be small, with values in the range of ≤1% yr^{-1} within the first year of application (Carlsson et al., 2012; Kuzyakov et al., 2009; Luo et al., 2011). Some of the studies e.g. Kuzyakov et al. (2009) were conducted under optimal condition of temperature and moisture throughout the year, implying that under non-continuous optimal conditions in the field, the decomposition rate is expected to be smaller. This might especially be true under Zambian conditions where hardly any rainfall occurs for seven months per year. Decomposition losses are therefore expected to be <5%. Migration of BC to soil layers below 20 cm depth mainly as dissolved organic C was most likely small as well. In Table 1, the water soluble constituents of BC were ~2%, and in our earlier rice husk BC washing experiment (Obia et al., 2015), dissolved organic C consisted of only 2.4% of the total constituents of BC leachate. In the soil depth interval of 15–20 cm, the accumulated BC was as little as 0.2–0.7% (Table 3 and S1). Thus, BC transfer to below the 20 cm soil depth is at most of the same order of magnitude (<1.4% of total BC). Also Haefele et al. (2011), despite observing high extents of BC leaching, found no indication of rice husk BC migration beyond 30 cm soil depth, four years after BC application. Major et al. (2010) found very small amounts (<1%) of BC moving with percolating water in intact subsoil below the application depth (10 cm) at 15 cm depth both as dissolved and particulate organic C. Thus, overall at least 20% of the BC added to the Mkushi and Kaoma soils was not accounted for and could not be attributed to leaching to deeper soil layers or to decomposition.

Black C, which is similar to BC with respect to for example their low density relative to soil, has been shown to undergo preferential water erosion (Rumpel et al., 2006). Lateral losses of BC through erosion by water and wind, could account for the non-recovered BC in the present study as well. The importance of lateral transport at the Mkushi and Kaoma sites is supported by the change in $\delta^{13}C$ of the reference plots adjacent to the BC-treated ones. The BC in the reference plots is part of the BC not recovered in the 0–20 cm depth of the amended plots. This BC in the reference plots was probably brought by wind and water erosion from amended plots and indicate that much of the missing BC (24–45%) may have been transported outside the experiment.

There was greater lateral transport of BC in sand at Kaoma than in aggregating loamy sand at Mkushi (Table 3). The opposite was true for downward transport where there was smaller downward transport in sand than in loamy sand. Since we work with fine BCs with sizes less than 1 mm, the lateral transport through erosion and downward migration of particulate BC observed here is most likely the upper limits. Usually much coarser, hand-crushed BC will be applied, which is less vulnerable to downward migration and erosional lateral transport.

5. Conclusions

In this study, we showed that significant downward migration of fine size fractions of BC (≤0.5 mm and 0.5–1 mm) once applied to soil was mainly limited to ~3 cm below the application depth. There was a tendency for somewhat greater downward migration of the finer BC size fraction. Slightly greater downward migration of BC in the Mkushi loamy sand compared to the Kaoma sand was likely caused by more rainfall and the presence of packing voids through which BC could move downward with percolating water. In Kaoma sand, with its single-grain structure devoid of aggregates, formation of packing void does not happen. In this study, between 45–66% of BC was found within the application depth after one year. A further 10–20% moved below the application depth. This means that between 24–45% of the BC was not recovered in the upper 20 cm of the soil profile. Transportation of BC to adjacent reference plots indicates that a large part of the unrecovered BC was transported laterally through erosion.

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References

Abiven, S., Hund, A., Martinsen, V., Cornelissen, G., 2015. Biochar amendment increases maize root surface areas and branching: a shovelomics study in Zambia. Plant and Soil, 1-11.

Alling, V., Hale, S.E., Martinsen, V., Mulder, J., Smebye, A., Breedveld, G.D., Cornelissen, G., 2014. The role of biochar in retaining nutrients in amended tropical soils. Journal of Plant Nutrition and Soil Science 177, 671-680.

Awad, Y.M., Blagodatskaya, E., Ok, Y.S., Kuzyakov, Y., 2013. Effects of polyacrylamide, biopolymer and biochar on the decomposition of14C-labelled maize residues and on their stabilization in soil aggregates. European Journal of Soil Science 64, 488-499.

Carlsson, M., Andrén, O., Stenström, J., Kirchmann, H., Kätterer, T., 2012. Charcoal Application to Arable Soil: Effects on CO_2 Emissions. Communications in Soil Science and Plant Analysis 43, 2262-2273.

Cornelissen, G., Martinsen, V., Shitumbanuma, V., Alling, V., Breedveld, G., Rutherford, D., Sparrevik, M., Hale, S., Obia, A., Mulder, J., 2013. Biochar Effect on Maize Yield and Soil Characteristics in Five Conservation Farming Sites in Zambia. Agronomy 3, 256-274.

Foereid, B., Lehmann, J., Major, J., 2011. Modeling black carbon degradation and movement in soil. Plant and Soil 345, 223-236.

Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal - a review. Biology and Fertility of Soils 35, 219-230.

Haefele, S.M., Konboon, Y., Wongboon, W., Amarante, S., Maarifat, A.A., Pfeiffer, E.M., Knoblauch, C., 2011. Effects and fate of biochar from rice residues in rice-based systems. Field Crops Research 121, 430-440.

Hale, S.E., Alling, V., Martinsen, V., Mulder, J., Breedveld, G.D., Cornelissen, G., 2013. The sorption and desorption of phosphate-P, ammonium-N and nitrate-N in cacao shell and corn cob biochars. Chemosphere 91, 1612-1619.

Hockaday, W.C., Grannas, A.M., Kim, S., Hatcher, P.G., 2006. Direct molecular evidence for the degradation and mobility of black carbon in soils from ultrahigh-resolution mass spectral analysis of dissolved organic matter from a fire-impacted forest soil. Organic Geochemistry 37, 501-510.

Jeffery, S., Verheijen, F.G.A., van der Velde, M., Bastos, A.C., 2011. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agriculture, Ecosystems & Environment 144, 175-187.

Kocyigit, R., 2006. Contribution of soil organic carbon and C3 sugar to the total CO_2 efflux using ^{13}C abundance. Plant, Soil and Environment 52, 193-198.

Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by ^{14}C labeling. Soil Biology and Biochemistry 41, 210-219.

Leifeld, J., Fenner, S., Muller, M., 2007. Mobility of black carbon in drained peatland soils. Biogeosciences 4, 425-432.

Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., Brookes, P.C., 2011. Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. Soil Biology and Biochemistry 43, 2304-2314.

Major, J., Lehmann, J., Rondon, M., Goodale, C., 2010. Fate of soil-applied black carbon: downward migration, leaching and soil respiration. Global Change Biology 16, 1366-1379.

Martinsen, V., Mulder, J., Shitumbanuma, V., Sparrevik, M., Børresen, T., Cornelissen, G., 2014. Farmer-led maize biochar trials: Effect on crop yield and soil nutrients under conservation farming. Journal of Plant Nutrition and Soil Science 177, 681-695.

Obia, A., Cornelissen, G., Mulder, J., Dorsch, P., 2015. Effect of soil pH increase by biochar on NO, N_2O and N_2 production during denitrification in acid soils. PloS one 10, e0138781.

Obia, A., Mulder, J., Martinsen, V., Cornelissen, G., Børresen, T., 2016. In situ effects of biochar on aggregation, water retention and porosity in light-textured tropical soils. Soil and Tillage Research 155, 35-44.

R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rumpel, C., Chaplot, V., Planchon, O., Bernadou, J., Valentin, C., Mariotti, A., 2006. Preferential erosion of black carbon on steep slopes with slash and burn agriculture. CATENA 65, 30-40.

Sparrevik, M., Adam, C., Martinsen, V., Jubaedah, Cornelissen, G., 2015. Emissions of gases and particles from charcoal/biochar production in rural areas using medium-sized traditional and improved “retort” kilns. Biomass and Bioenergy 72, 65-73.

Spokas, K.A., Novak, J.M., Masiello, C.A., Johnson, M.G., Colosky, E.C., Ippolito, J.A., Trigo, C., 2014. Physical Disintegration of Biochar: An Overlooked Process. Environmental Science & Technology Letters 1, 326-332.

Umar, B.B., Aune, J.B., Johnsen, F.H., Lungu, O.I., 2011. Options for improving smallholder conservation agriculture in Zambia. Journal of Agricultural Science 3, p50.

Table 1. Soil and biochar (BC) properties[1]

Properties	Kaoma soil	Mkushi soil	Rice husk BC			Maize cob BC	
			≤0.5 mm	0.5–1 mm	Unsorted	≤0.5 mm	Unsorted
Sand (%)	85.4	75.1	-	-	-	-	-
Silt (%)	10.2	15.9	-	-	-	-	-
Clay (%)	4.4	9.0	-	-	-	-	-
Texture class	Sand	Loamy sand	-	-	-	-	-
Total organic C (%)	0.62	0.74	39.3	42.8	47.8	44.8	53.8
Total nitrogen (%)	0.00	0.01	0.61	0.52	0.82	0.79	0.65
Total hydrogen (%)	-	-	2.33	2.41	2.37	2.09	2.36
H/C (molar ratio)	-	-	0.71	0.68	0.60	0.56	0.53
pH	5.8	5.8	8.3	8.3	8.3	9.0	8.8
Loss on ignition (%)	-	-	48.8	54.9	-	52.1	-
Soluble constituents (%)	-	-	2.1	0.6	2.0	2.6	2.4
BET surface area (m^2 g^{-1})	-	-	2.4	2.3	-	10.5	-
CEC ($cmol_c$ kg^{-1})	2.8	1.7	-	-	14.0	-	22.2
K^+ ($cmol_c$ kg^{-1})	0.1	0.3	-	-	10.4	-	16.5
Ca^{2+} ($cmol_c$ kg^{-1})	1.2	1.1	-	-	2.4	-	4.3
Mg^{2+} ($cmol_c$ kg^{-1})	0.2	0.3	-	-	0.9	-	1.2
$\delta^{13}C$ (‰)	-20.78	-18.86	-27.05	-27.05	-27.05	-12.27	-12.27

[1]Maize cob BC with particle size 0.5–1 mm was not characterized in the lab because it was exhausted in the field. Calculation of mass balance of 0.5–1 mm maize cob BC in the soil layers was based on C content of the ≤0.5 mm BC. The $\delta^{13}C$ of the soil presented here is the average of the bulk soil at 5–20 cm depth measured in April 2014.

Table 2. Bulk density (g cm^{-3}) of the soil from biochar experiments in Zambia[1]

Site	BC particle size	BC dose (%)	Soil depth	
			0–5 cm	5–10 cm
Mkushi	Ref. soil	0	1.26±0.01	1.28±0.02
	≤0.5 mm	4	1.17±0.03	1.25±0.02
	0.5–1 mm	4	1.16±0.05	1.34±0.04
Kaoma	Ref. soil	0	1.40±0.02	1.40±0.04
	≤0.5 mm	3.4	1.28±0.01	1.38±0.01
	0.5–1 mm	3.4	1.27±0.02	1.46±0.03

[1]The bulk density of 0–5 cm soil depth was presented in our earlier work (Obia *et al.* 2016). The BCs were from rice husk except 0.5–1 mm BC in Mkushi, which was from maize cob. Values are means ± standard error (n = 3).

Table 3. Amount of biochar recovered in the 0–20 cm soil depth, one year after establishing the experiment. Computations based on δ^{13}C and TOC contents (eq. 3)

Soil depth (cm)	Mkushi sandy loam			Kaoma sand			SE
	Reference plot BC (g)	≤0.5 mm rice husk BC (g)	0.5–1 mm maize cob BC (g)	Reference plot BC (g)	≤0.5 mm rice husk BC (g)	0.5–1 mm rice husk BC (g)	
0-5	45.2a	328.1bc	224.2b	56.5a	350.3bc	416.9c	34.3
5-6	0.5a	63.8b	31.5b	0.8a	46.4b	41.4b	12.2
6-7	0.1a	30.3a	13.3a	1.2a	17.8a	4.4a	9.2
7-8	0.5a	13.5b	2.5a	0.8a	2.0a	3.2a	3.2
8-9	0.1a	4.5a	1.4a	0.8a	1.5a	2.0a	1.0
9-10	0.3a	2.0a	1.3a	0.2a	1.2a	1.9a	0.7
10-15	0.0a	2.5a	5.3a	2.4a	7.0a	4.4a	3.3
15-20	0.0a	1.2a	4.2a	2.3a	3.6a	1.9a	2.5
Total BC recovered	46.7a	445.9b	283.7c	65.0a	429.8bc	476.1b	35.9

Amount of BC are mean values (n = 3) recovered in each depth of soil with a single standard error (SE) for the three BC treatments at the two sites. Different letters following means for each soil depth indicate significant difference between BC treatments and between sites, Tukey's test, $p<0.05$. 625g of BC that was applied to plots.

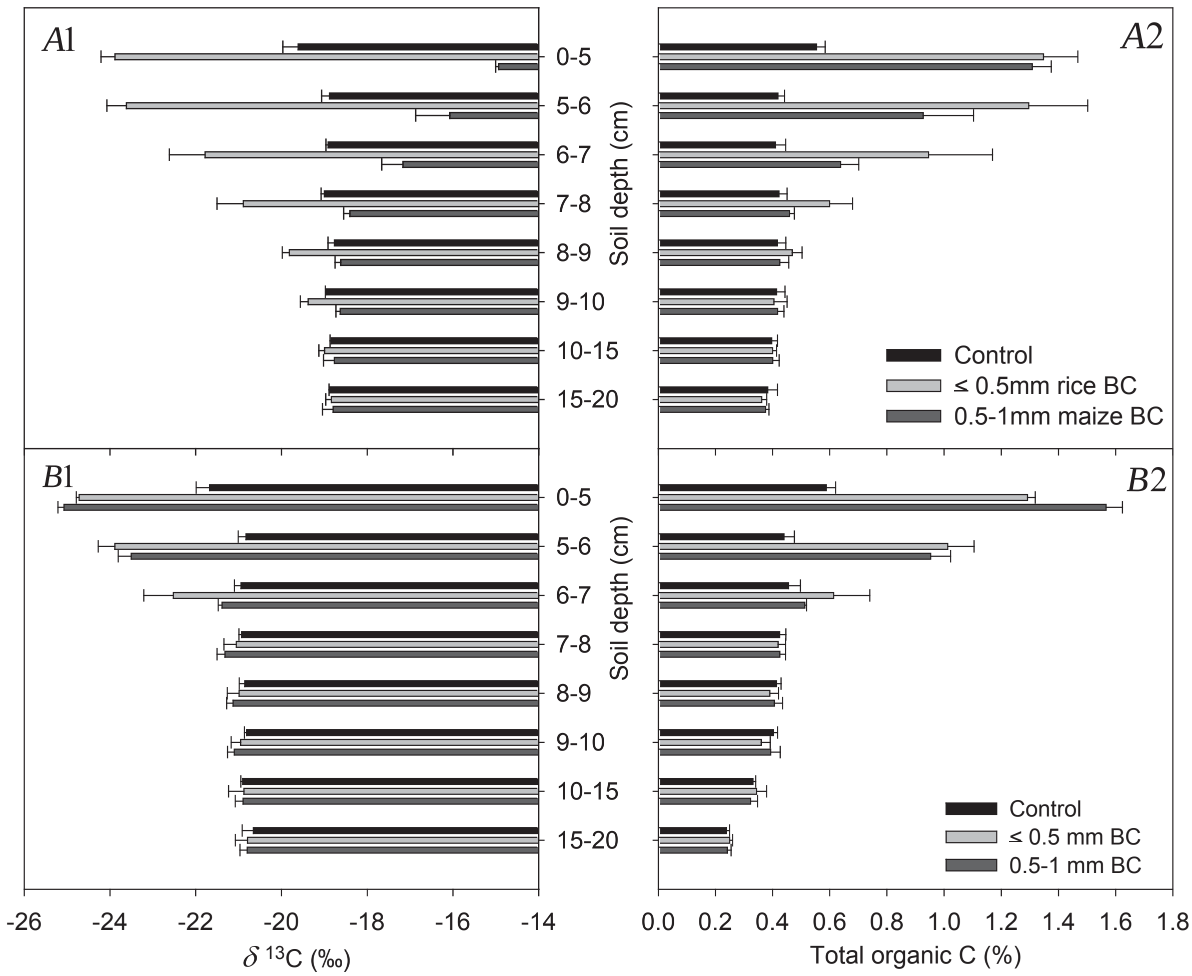

Fig. 1. Distribution of $\delta^{13}C$ and TOC in the soil profile one year after BC was applied to the surface soil (0–5 cm depth). *A1* and *A2* indicate the $\delta^{13}C$ and TOC for Mkushi soil amended with rice husk BC (with $\delta^{13}C$ = 27.3±0.03) and maize cob BC (with $\delta^{13}C$ = 12.3±0.3), whereas *B1* and *B2* indicate the $\delta^{13}C$ and TOC for the Kaoma soil (only rice BC was added). Error bar is SE.

Vertical and lateral transport of biochar in light-textured tropical soils

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Supplementary information

Reference	≤0.5mm rice husk BC	≤0.5mm rice husk BC
0.5–1 mm rice husk BC	Reference	0.5–1 mm rice husk BC
≤0.5mm rice husk BC	0.5–1 mm rice husk BC	Reference

Fig. S1. A completely randomized design layout of the experiment at Kaoma consisting of a 50 x 50 cm individual plots. In Mkushi, 0.5–1 mm rice husk BC was replaced with 0.5–1 mm maize cob BC. All the amended plots in both sites received 625 g of BC.

Table S1. Percentage of BC recovered based on $\delta^{13}C$ and TOC in the various layers of two Zambian soils in 0–20 cm depth interval.

Soil depth (cm)	Mkushi loamy sand		Kaoma sand		SE
	≤0.5mm rice husk BC (%)	0.5-1mm maize cob BC (%)	≤0.5mm rice husk BC (%)	0.5-1mm rice husk BC (%)	
0-5	52.5	35.9	56.0	66.7	3.8
5-6	10.2	5.0	7.4	6.6	0.9
6-7	4.9	2.1	2.9	0.7	0.7
7-8	2.2	0.4	0.3	0.5	0.3
8-9	0.7	0.2	0.2	0.3	0.1
9-10	0.3	0.2	0.2	0.3	0.1
10-15	0.4	0.8	1.1	0.7	0.3
15-20	0.2	0.7	0.6	0.3	0.2
Total BC recovered	71.3	45.4	68.8	76.2	4.1

Percentage of BC are mean values (n = 3) recovered in each depth of soil with a single standard error (SE) for the BC treatments at the two sites. Percentages was calculated based on recovered BC and amount applied (625g/plot).

Table S2. TOC and recovered BC based on TOC alone for different depth intervals for the 50x50 cm plot[1].

Depth cm	Mkushi site					Kaoma site					SE of TOC (g)
	Ref. plot	≤0.5 mm rice husk BC		0.5–1 mm maize cob BC		Ref. plot	≤0.5 mm rice husk BC		0.5–1 mm rice husk BC		
	TOC (g)	TOC (g)	BC (%)	TOC (g)	BC (%)	TOC (g)	TOC (g)	BC (%)	TOC (g)	BC (%)	
0-5	87.4	196.5	52.9	192.7	45.0	102.9	206.9	53.8	248.4	64.9	14.0
5-6	13.4	41.3	11.4	30.2	6.0	15.4	34.7	8.0	34.7	7.4	2.7
6-7	13.1	30.0	6.8	20.9	2.7	15.9	21.0	2.5	18.7	1.4	1.7
7-8	13.5	19.1	2.4	15.1	0.6	14.9	14.4	0.0	15.5	0.2	0.6
8-9	13.3	15.0	0.7	14.0	0.2	14.4	13.4	0.0	14.8	0.0	0.4
9-10	13.2	12.9	0	13.7	0.2	14.1	12.3	0.0	14.3	0.0	0.4
10-15	63.5	64.1	0	65.9	0	58.0	58.8	0.0	58.8	0.0	1.5
15-20	61.3	58.1	0	61.8	0	41.6	42.8	0.0	44.0	0.0	2.3
Total	278.7	437.1	74.2	414.2	54.8	277.1	404.3	64.3	449.1	73.9	17.2

[1]TOC was measured after one year. To estimate BC recovery based on TOC alone, BC carbon in the soil was determined by subtracting reference soil TOC from TOC of BC amended soil. The reference soil TOC was estimated by averaging TOC contents in reference plot at 5–10 cm depth. The BC carbon was converted to BC amount using percent carbon in BC (Table 1). The recovered BC was then calculated as a fraction of the originally applied BC (625 g/plot).

PAPER IV

Effect of soil pH increase by biochar on NO, N_2O and N_2 production during denitrification in acid soils

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RESEARCH ARTICLE

Effect of Soil pH Increase by Biochar on NO, N_2O and N_2 Production during Denitrification in Acid Soils

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Abstract

Biochar (BC) application to soil suppresses emission of nitrous- (N_2O) and nitric oxide (NO), but the mechanisms are unclear. One of the most prominent features of BC is its alkalizing effect in soils, which may affect denitrification and its product stoichiometry directly or indirectly. We conducted laboratory experiments with anoxic slurries of acid Acrisols from Indonesia and Zambia and two contrasting BCs produced locally from rice husk and cacao shell. Dose-dependent responses of denitrification and gaseous products (NO, N_2O and N_2) were assessed by high-resolution gas kinetics and related to the alkalizing effect of the BCs. To delineate the pH effect from other BC effects, we removed part of the alkalinity by leaching the BCs with water and acid prior to incubation. Uncharred cacao shell and sodium hydroxide (NaOH) were also included in the study. The untreated BCs suppressed N_2O and NO and increased N_2 production during denitrification, irrespective of the effect on denitrification rate. The extent of N_2O and NO suppression was dose-dependent and increased with the alkalizing effect of the two BC types, which was strongest for cacao shell BC. Acid leaching of BC, which decreased its alkalizing effect, reduced or eliminated the ability of BC to suppress N_2O and NO net production. Just like untreated BCs, NaOH reduced net production of N_2O and NO while increasing that of N_2. This confirms the importance of altered soil pH for denitrification product stoichiometry. Addition of uncharred cacao shell stimulated denitrification strongly due to availability of labile carbon but only minor effects on the product stoichiometry of denitrification were found, in accordance with its modest effect on soil pH. Our study indicates that stimulation of denitrification was mainly due to increases in labile carbon whereas change in product stoichiometry was mainly due to a change in soil pH.

Introduction

Denitrification, the microbially mediated, stepwise reduction of nitrogen oxides to N_2 via nitric oxide (NO) and nitrous oxide (N_2O) [1], is the dominant pathway returning reactive nitrogen to the atmosphere. NO and N_2O are gaseous intermediates of denitrification which, once escaped to the atmosphere, have adverse effects on plant and animal health [2], stratospheric ozone [3] and the radiative balance of the Earth [4]. About 45% of the total terrestrial N_2O emissions can be attributed to nitrogen (N) cycling in agriculture [5], making denitrification a primary target for greenhouse gas abatement [6].

Numerous studies have shown that biochar (BC), a biomass pyrolysis product originally devised for carbon (C) sequestration and soil amelioration [7–10] suppresses N_2O emissions ([11] and references therein) alongside with increasing crop production [12–14]. Only few studies have reported that BC leads to increased N_2O emissions [15, 16]. Thus, BC appears to be a promising agent to mitigate N_2O emissions from agroecosystems, but the mechanisms mediating the suppression are unresolved. Various mechanisms have been proposed, such as increased N_2O reductase activity at raised soil pH [11], increased electron flow to N_2O through BC-mediated electron shuttling [17], reduced rates of denitrification through competition for electrons [18], direct sorption of N_2O [19], improved soil aeration [20] and immobilization of ammonium or nitrate through adsorption or enhanced soil cation/anion exchange [15, 21, 22]. Other proposed mechanisms are ethylene production by BC resulting in temporary inhibition of nitrification [23] and microbial N immobilization due to the presence of labile organic carbon in BC [24]. Increased N_2O emission after BC application has been attributed to high N content in certain BC such as that made from poultry manure [16, 22].

Most BCs are alkaline owing to their ash content, causing release of base cations, and alkaline properties of organic functional groups [25]. Biochar addition to soils neutralizes soil acidity and may increase the cation exchange capacity (CEC) and base saturation, depending on the intrinsic properties of the soil and the BC [26, 27].

Soil pH strongly controls the $N_2O/(N_2O+N_2)$ product ratio of denitrification. This has been demonstrated for pure cultures of denitrifying bacteria [28] and for soil denitrifying communities [29–33]. The likely reason is that low pH prevents the assembly of functional N_2O reductase (N_2OR), the enzyme reducing N_2O to N_2 in denitrification [29, 34]. Since BC is generally alkaline, increased N_2OR activity due to pH rise could be one of the major mechanisms behind the observed suppression of N_2O emission in BC treated acid soils. If so, N_2O suppression by BC would be mainly a "liming effect".

The objectives of the present study were to evaluate the role of BC-induced pH change on NO and N_2O net production in soil denitrification. Although, NO is an important regulator in many biological processes including denitrification [35, 36], only few BC studies have addressed NO [37]. We carried out *ex situ* denitrification experiments in closed bottles with two acidic agricultural soils from Indonesia and Zambia. We applied increasing doses of two types of BC strongly differing in amount and type of alkalinity and studied the responses of soil pH, overall denitrification rate and gaseous reaction products (NO, N_2O, N_2). To shed light on the role of soil pH, we removed alkalinity from the BCs through leaching with water and acid prior to incubation in a second experiment. In a third experiment, sodium hydroxide (NaOH) was used as an alkali analogue to study the effect of pH *per se* in the absence of BC. Furthermore, the NO and N_2O suppressing effect of BC was compared to that of uncharred feedstock. The denitrification kinetics were studied in stirred soil slurries in helium (He) atmosphere, using a high-throughput incubation robot which monitors oxygen (O_2), carbon dioxide (CO_2), NO, N_2O and N_2 at high temporal resolution [38]. Stirring ensured homogeneous soil slurries and equilibrium of gases between headspace and liquid phase. Unlike previous studies, our investigations were carried out under fully anoxic conditions, preventing confounding effects on denitrification NO and N_2O production by other N-cycling processes.

Materials and Methods

Soils and biochars

Acidic, sandy loam Acrisols were sampled at Lampung (Sumatra, Indonesia; 05°00.406' S; 105° 29.405' E) and Mkushi (Zambia; 13°36.264′ S; 29°29.768′ E) in October 2012 and April 2013, respectively. The soils were sampled from private lands with permission of the owners during the dry season and stored air-dried. Selected soil and BC properties are presented in Table 1. Different N-forms in soils and BCs were not considered. The NH_4^+ content was deemed irrelevant because our main experiments were under anaerobic conditions ruling out nitrification. The added ample amount of NO_3^- would override any sorption effect and denitrification and its product stoichiometry, are not sensitive to small differences in NO_3^- availability [39].

The BCs were prepared from rice husk and cacao shell, two common agricultural wastes in Lampung, Indonesia. The two BCs differed in extent and type of alkalinity (Table 1); cacao shell BC had a lower ash content but a ~10 times higher CEC than rice husk BC. The exchangeable cations of cacao shell BC were dominated by potassium (K). Overall, cacao shell BC had a ~5 times higher acid neutralizing capacity (ANC) than rice husk BC (217 vs 45 $cmol_c\ kg^{-1}$) [40].

The BC pyrolysis conditions, taken from Hale, Alling [43], can be found in Description A in S1 File. Since the BCs were not produced in the laboratory, thermogravimetric analyses (TGA) was used to estimate the pyrolysis temperature, indicating that this was between 400 and 500°C. In short, during the TGA, the temperature was stepwise increased up to 900°C, and weight loss was monitored. The weight loss profile was then compared to three temperature series of laboratory-generated BCs where pyrolysis had taken place at an exactly measured temperature. Weight loss and high to low temperature weight loss ratios of our BC samples both showed pyrolysis temperature of 400–500°C.

The BCs used in this experiment were either untreated or leached with water or acid. Leaching of the BCs to partly remove their alkalizing effect before use in the experiments was done on the size fraction $\leq$ 2 mm. For leaching, columns of 5 cm diameter and 30 cm length were filled with BC. The columns were fitted with tubing at the inlet and outlet and filter paper (0.45μm) was placed on both ends of the column. Biochars were first leached with demineralized water at a 1:50 (BC:water w/w) ratio with a flow rate of 70–80 ml hr^{-1} for 4 days to produce "water-leached" BC. After removing part of the BC from the column (water-leached), leaching continued with 0.05 M HCl at a 1:10 (BC:acid w/w) ratio with a flow rate of 20–30 ml hr^{-1} for 1 day to produce "acid-leached" BC. During the leaching, water and subsequently HCl were pumped through the vertical columns from the bottom upwards. Pumping stopped temporarily when leachate appeared on the top of the column and resumed after 2 days (in the case of water) or 1 day (in the case of HCl). A peristaltic pump was used to control the flow rate. Both water- and acid-leached BCs were oven-dried at 40°C for 3 days resulting to a moisture content of 13 and 6%w/w, respectively. Prior to mixing with the soil, the BCs (both untreated and leached) were ground and passed through a 0.5 mm sieve. Despite possible release of fresh materials after grinding of leached BCs to $\leq$ 0.5 mm, the pH measured in soil-leached BC slurries before incubation (Table 2) was lower than in slurries with untreated BC, hence satisfying the purpose of reducing or removing alkalizing effect of BC. Cations, anions and dissolved organic C removed by leaching with water and acid, respectively, can be found in S2 File.

Denitrification experiments

Air-dried soils were saturated with water and equilibrated to 5 kPa suction in a sand box (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) over a 5 days period.

Table 1. Selected soil and biochar properties[1].

Soil/Biochar	pH	TN	TOC	TH	H/C	LOI	Ash	Surface area	CEC and base cations ($cmol_c$ kg^{-1} soil or char)				
	H_2O	(%)	(%)	(%)		(%)	(%)	BET m^2 g^{-1}	CEC	K^+	Na^+	Ca^{2+}	Mg^{2+}
Lampung soil	4.0	0.1	1.2	-	-	-	-	-	9.7	<0.1	<0.1	0.3	0.1
Mkushi soil	4.0	0.00	0.5	-	-	-	-	-	6.4	<0.1	0.0	0.1	<0.1
Untreated rice husk BC	8.4	0.9	44.6	1.9	0.51	55.6	51.0	76.4	20	9.5	0.2	3.2	3.6
Water leached rice husk BC	8.2	1.0	48.0	2.1	0.53	59.2	-	108.2	-	-	-	-	-
Acid leached rice husk BC	2.5	0.9	47.8	1.9	0.48	58.2	-	88.5	-	-	-	-	-
Untreated cacao shell BC	9.8	1.5	54.3	1.4	0.31	68.1	18.9	30.9	197	127	0.3	37.1	32.8
Water leached cacao shell BC	9.6	1.8	70.9	1.7	0.29	85.0	-	255.8	-	-	-	-	-
Acid leached cacao shell BC	8.0	1.7	75.9	1.8	0.28	86.7	-	274.8	-	-	-	-	-
Uncharred cacao shell	-	1.4	46.5	-	-	90.3	-	-	-	66.5	0.3	36.7	31.7

[1]TN = Total nitrogen, TOC = Total organic carbon, TH = Total hydrogen, H/C = molar ratio, LOI = Loss on ignition. Untreated BC properties (Ash, CEC & base cations) and surface area data were obtained from Martinsen, Alling [41] and Smebye, Alling [42] respectively. All the other soil and BC data were measured in sub-samples from homogenized bulk samples used in the study. Soil and BC pH was measured in a 1:2.5 v/v slurry in water (n = 2) using a pH meter (Orion 2 Star, Thermo Fisher Scientific, Fort Collins, CO, USA) after overnight sedimentation and shaking. Base cations were measured in the eluate of ammonium acetate at pH 7 for BC and ammonium nitrate for soil (n = 1), with a flame spectrophotometer (Perkin Elmer, AAS 3300). CEC was determined as sum of base cations and exchangeable acidity in ammonium acetate pH 7 and ammonium nitrate extract for BCs and soil respectively. TOC, TN and TH were determined using CHN analyzer (n = 1) (CHN-1000, LECO, USA). The TOC for BCs were determined after acidification to remove carbonates.

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Controlled pre-wetting was done to accommodate for the flush of microbial activity commonly observed upon rewetting of dry soil [44]. For the incubation assays, approx. 10 g sand box equilibrated soil was placed in 120 ml serum bottles together with a magnetic stirring bar. Treatments included BCs (untreated and leached) and uncharred cacao shell, the latter to

Table 2. Mean soil slurry pH after treatment with various doses of the amendments at the start and end of incubation.

Soil	Amendment	Soil pH at the start of incubation						Soil pH at the end of incubation					
Lampung soil	**Cacao shell BC doses (%)**	**0**	**1**	**2**	**5**	**10**	**SE**	**0**	**1**	**2**	**5**	**10**	**SE**
	Untreated	4.0	6.3	6.8	7.6	8.4	0.1	5.7	6.9	7.6	8.3	9.0	0.2
	Water leached	4.0	-	5.7	6.6	7.2	0.0	5.8	-	6.3	7.1	7.9	0.3
	Acid leached	4.0	-	5.0	6.1	6.6	0.1	5.6	-	5.3	6.4	6.9	0.2
	Rice husk BC doses (%)	**0**	**1**	**2**	**5**	**10**	**SE**	**0**	**1**	**2**	**5**	**10**	**SE**
	Untreated	4.0	4.2	4.4	4.9	5.5	0.0	5.9	5.9	6.1	6.2	6.2	0.4
	Water leached	4.0	-	4.4	4.6	5.0	0.0	5.8	-	6.2	6.5	6.0	0.3
	Acid leached	4.0	-	3.9	3.6	3.3	0.0	6.2	-	5.4	5.7	4.7	0.4
	Uncharred cacao shell doses (%)	**0**	**1**	**2**	**5**	**10**	**SE**	**0**	**1**	**2**	**5**	**10**	**SE**
	Uncharred cacao shell	3.7	-	4.4	4.8	5.9	0.0	5.4	-	6.1	5.8	5.6	0.1
	NaOH doses (ml)	**0**	**0.35**	**1.25**	**1.8**	**-**	**SE**	**0**	**0.35**	**1.25**	**1.8**	**-**	**SE**
	NaOH	3.7	4.8	7.2	8.0	-	0.1	5.4	5.9	6.9	7.3	-	0.2
Mkushi soil	**Cacao shell BC doses (%)**	**0**	**1**	**2**	**5**	**10**	**SE**	**0**	**1**	**2**	**5**	**10**	**SE**
	Untreated	3.9	-	8.1	9.3	9.8	0.0	6.2	-	8.4	9.5	9.9	0.5
	Water leached	4.0	-	5.8	6.8	7.5	0.0	5.6	-	7.9	8.8	8.2	0.4
	Acid leached	4.0	-	-	6.5	6.8	0.1	5.6	-	-	8.5	8.4	0.5

SE is standard error calculated from all doses of each amendment for either start or end pH.

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assess the effect of the feedstock alone (in Lampung soil only), at doses of 0, 1, 2, 5 and 10% (dry weight basis). Weight losses during leaching were implicitly corrected for since the same weights of the treated chars were used. To investigate if the effect of BC on denitrification and its gaseous reaction products was merely a pH effect, another set of experiments was run with Lampung soil in which soil pH was manipulated by adding 0.35, 1.25 and 1.80 ml of 0.1M NaOH prior to anoxic incubation. Dose of NaOH was decided based on the alkalizing effect of BC, e.g. 1.8 ml 0.1M NaOH was equivalent to 10% untreated cacao shell BC in Lampung soil. All treatments were prepared in triplicate. In preparation of soil slurries, 30 ml of a 2 mM KNO_3 solution were added to the bottles thereby providing ample NO_3^- for denitrification. After the amendment, the effective pH values in the soil slurries were measured by a pH meter (Orion 2 Star, Thermo Fisher Scientific, Fort Collins, CO, USA) after 0.5 hour of oxic stirring. Thereafter, bottles were tightly closed with rubber septa and aluminum crimp seals and flushed with He (99.999%, AGA Industrial Gasses, Oslo, Norway) by alternately evacuating and He-filling the bottles 5 times using an automated manifold. This was done under constant stirring to achieve close to fully anoxic conditions. Measurements of pH in the slurries were repeated at the end of the incubation. An oxic incubation was carried out independently to check for BC-induced toxicity or stimulation of microbial activity (measured as O_2 consumption) (Figure A in S3 File).

Incubation and data collection

All incubations were carried out in a water bath at 20°C (which is within optimal range for microbial activities [45]) under constant stirring to maintain equilibrium of gases between the soil slurry and the bottle headspace. We used a robotized incubation system similar to that described by Molstad, Dörsch [38] to monitor the kinetics of O_2 depletion, CO_2 production and N-gas accumulation (NO, N_2O, N_2) during denitrification. The system consists of a water bath connected to a cryostat, placed under the robotic arm of an autosampler (Combi Pal, CTC, Switzerland). The water bath can accommodate up to 30 stirred bottles which are pierced repeatedly (here five-hourly) by the hypodermic needle of the autosampler which is connected to a peristaltic pump transporting the gas sample to a gas chromatograph equipped with various detectors and further to an NO-chemiluminescence analyzer. Details of the incubation system and gas analysis can be found in Description B in S1 File.

Data handling

Rates of gas production and consumption were corrected for sampling loss and dilution as described by Molstad, Dörsch [38]. Maximum induced denitrification rate was calculated as the slope of the steepest part of the accumulation curve given by the sum of all N-gas products. The $N_2O/(N_2O+N_2)$ product ratio was calculated as the area under the curve of N_2O divided by the area under the curve of $NO+N_2O+N_2$ [29]. As a cut off, the maximum accumulation of N_2 was used, usually coinciding with the complete exhaustion of N-oxides in the bottles. In the instances where N-oxides were not exhausted, the accumulation curves were integrated over the entire experimental period. As a measure of NO net production in denitrification, maximum dissolved NO (nM) was calculated from headspace concentrations, using Henry's law.

Statistical analysis was carried out using the R software [46]. Progression of denitrification was inspected by plotting cumulative N-gas and CO_2 production as well as depletion of residual O_2 over time. Maximum induced denitrification rates for each of the amendment type across its doses were subjected to one-way ANOVA and mean values of the doses were separated using Tukey's Test at 5% significance level to establish statistically significant differences between BC doses.

To identify the possible factors explaining the effect of the amendments on maximum induced denitrification rate, $N_2O/(N_2O+N_2)$ ratio and maximum NO production, analyses of covariance (ANCOVA) were carried out. Firstly, ANCOVA was used to assess the effect of different types of untreated BC and doses, which was then followed by inclusion of BC leaching (untreated, water-leached and acid-leached) and effective pH in the statistical model as explanatory variables. Secondly, ANCOVA was used to separate the effect of labile C and other factors in BC on rate, $N_2O/(N_2O+N_2)$ ratio and maximum NO production by comparing charred and uncharred cacao shell. Furthermore, the effect of labile C and pH increase after adding BC on rate, $N_2O/(N_2O+N_2)$ ratio and maximum NO production were separated by comparing uncharred cacao shell and NaOH treatments using ANCOVA. pH, being an important explanatory variable for BC effect on $N_2O/(N_2O+N_2)$ ratio and maximum NO production, its values at the beginning and end of incubation are also presented.

Results

Effect of biochar on soil pH before and after anoxic incubation

The addition of BC increased the pH of both soils (Table 2). The dose-dependent pH rise was more pronounced in Mkushi soil than in Lampung soil, reflecting the weaker buffer capacity (lower CEC) of the former (Table 1). Biochar from cacao shell and rice husk differed vastly in alkalinity and thus in its alkalizing effect on soil. For instance, addition of 1% (w/w) cacao shell BC to Lampung soil increased the soil pH by 2.3 units, whereas adding the same amount of rice husk BC resulted in only 0.2 units pH increase. Carbonate contributed a large part to the alkalizing effect of cacao shell BC as shown by high CO_2 concentrations immediately following mixing the BC with acid soils (Fig 1).

Water leaching removed 159 $cmol_c$ of base cations kg^{-1} (S2 File) from cacao shell BC and reduced its $pH(H_2O)$ from 9.8 to 9.6. Additional leaching with acid removed another 61 $cmol_c$ of base cations and reduced its $pH(H_2O)$ to 8.0. For rice husk BC, water leaching removed 15$cmol_c$ kg^{-1} base cations and reduced the pH from 8.4 to 8.2. Acid leaching removed an additional 19 $cmol_c$ kg^{-1} and effectively acidified the BC (pH 2.5). In terms of mass, leaching with water and acid removed materials of approx. 65 and 14 mg g^{-1}, respectively, of cacao shell BC and 7 and 5 mg g^{-1}, respectively, of rice husk BC, and increased the surface area of BC (Table 1). For both BCs, base cations, in particular K^+, removed by sequential water and acid leaching exceeded ammonium acetate exchangeable amounts (Table 1). The leaching treatment removed a significant part of the alkalizing effect of both BCs in soil (Table 2) and it may have changed other properties of BC. The cacao shell feedstock increased soil pH only modestly compared to its BC, if applied at an equivalent dose of mass (Table 2).

Anoxic incubation of soil slurries caused an increase in soil pH from initial values between 4.0 and 9.8 to final values between 5.4 and 9.9 (Table 2). In control soils and acidic soil-BC slurries, the pH increased more strongly than in alkaline slurries. This increase in pH can be attributed to denitrification (an alkalizing process), continuous release of cations from the BCs and exchange reactions during stirring.

Kinetics of denitrification

Fig 1 and Figure B in S3 File show the kinetics of N-gas production and consumption together with the depletion of residual O_2 (after He-flushing) and cumulative CO_2 production (total inorganic carbon) in response to addition of untreated BC to Lampung and Mkushi soil, respectively. Controls (no BC addition) showed transient NO accumulation, instantaneous N_2O net production and measurable N_2 production after ~100 hours of incubation. Maximum

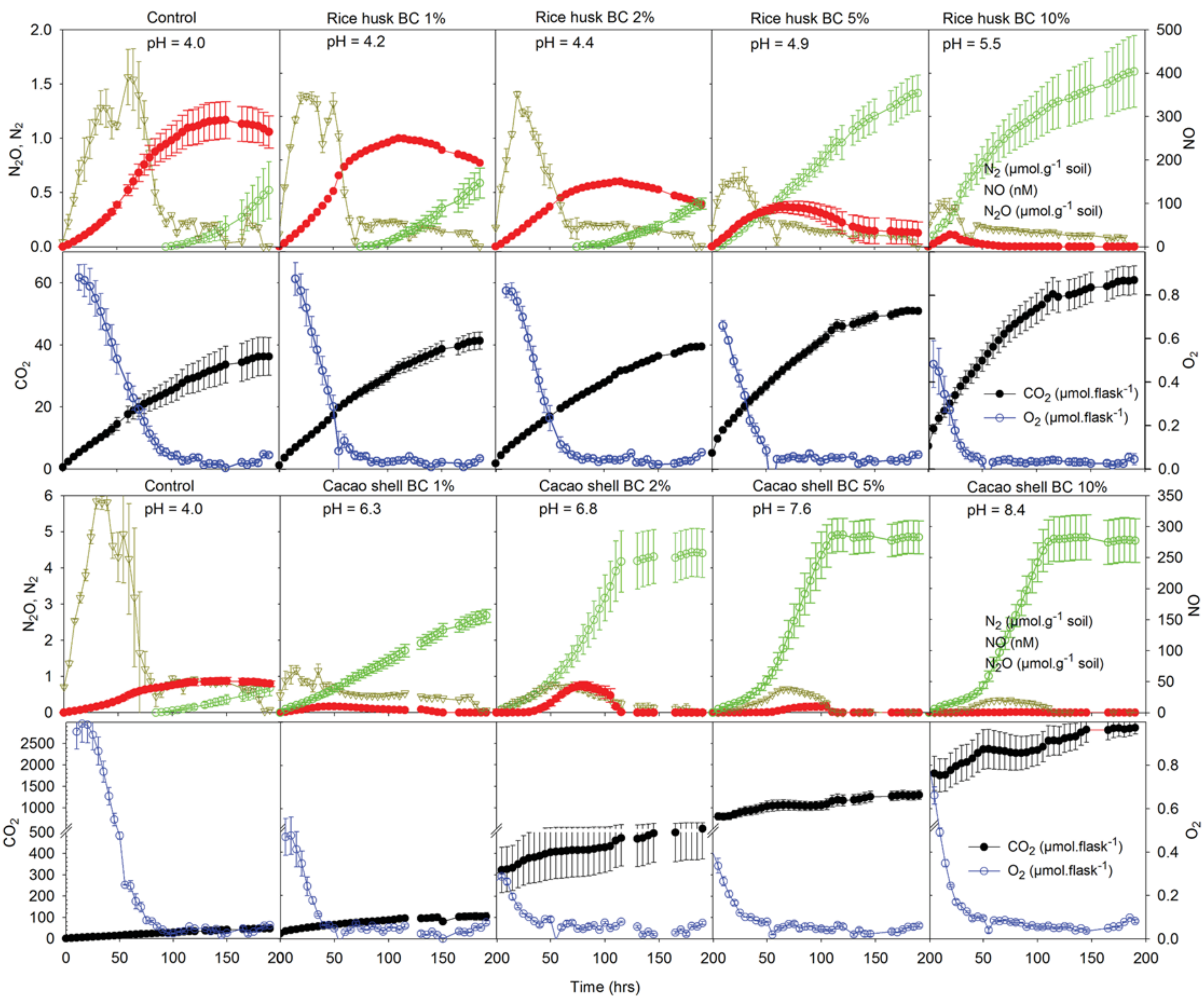

Fig 1. Denitrification kinetics and CO_2 and O_2 concentrations in anoxic incubations of Lampung soil amended with increasing doses of untreated rice husk BC (upper 2 panels) and cacao shell BC (lower 2 panels). Shown are averages of three incubations; error bars denote SE. Approximately 6.1 μmol NO_3^--N g^{-1} was added to 9.8 g soil in the bottles. Note the differences in the scale of y-axis.

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NO accumulation was one order of magnitude greater in the Lampung soil (0.3–0.5 μM, Fig 1) than in Mkushi soil (0.05 μM, Figure B in S3 File).

Both BCs suppressed the net production of NO and N_2O and increased N_2 production, but cacao shell BC (Fig 1; lower panel) stimulated overall denitrification (measured as total N_2 accumulation) more than rice husk BC (Fig 1; upper panel). With cacao shell BC doses > 2%, N_2 production reached a plateau after slightly more than 100 hours incubation, indicating that all N-oxides were exhausted. In this case, cumulative N_2 production roughly balanced the sum of initially present total soil N and added NO_3^-. Biochar also shortened the time needed to detect measurable N_2 production (except in the 10% cacao shell BC addition to Mkushi soil), indicating earlier induction of N_2O reductase (N_2OR) activity in the presence of BC. In

Lampung soil, the suppression of NO and N_2O and stimulation of N_2 as well as CO_2 production was dose-dependent irrespective of BC type. In Mkushi soil, 2% cacao shell BC addition stimulated complete denitrification resulting in high production rates of N_2 and practically eliminated N_2O accumulation (Figure B in S3 File). However, with further increases in the dose of cacao shell BC, slurry pH increased up to pH 9 in this weakly buffered soil and maximum NO accumulation and N_2 production decreased, indicating inhibition of denitrification at high pH. N_2O suppression with concomitant increase in N_2 production was also seen in the NaOH treatments of Lampung soil (Figure C in S3 File) and in incubation of BC in 2 mM KNO_3 without soil (Figure D in S3 File). In contrast, uncharred cacao shell stimulated overall denitrification strongly, while suppression of N_2O was small (Figure C in S3 File).

Water-leached rice husk BC caused only a modest decline in pH and resulted in denitrification kinetics similar to those with untreated BC in Lampung soil (compare Fig 1 and Figure E in S3 File). By contrast, addition of acid-leached rice husk BC reduced soil pH, but left the net production of N_2O and overall N-gas largely unchanged when compared with the control soil (Figure F in S3 File). Unlike acid-leached rice husk BC, acid-leached cacao shell BC retained some of its N_2O suppressing effect in Lampung soil (Figure F in S3 File) in line with its remaining alkalizing effect. However, the N_2O suppressing effect of water or acid-leached cacao shell BC was non-linear with maximum suppression already reached at 2% BC. At higher doses of leached cacao shell BC, no further N_2O suppression occurred and we observed biphasic kinetics in particular of NO accumulation showing two peaks during incubation (Figures E and F in S3 File).

Table 3 shows maximum induced denitrification rates for Lampung and Mkushi soil amended with rice husk and cacao shell BC, uncharred cacao shell and NaOH. In Lampung soil, addition of more than 2% untreated cacao shell BC significantly increased denitrification rates compared to the control ($P<0.05$), whereas rice husk BC did not. Water- and acid-leaching of the cacao shell BC removed most of the stimulating effect. Higher doses of acid-leached rice husk BC caused a small but significant decrease in denitrification rate in Lampung soil ($P<0.05$). In Mkushi soil, only 2% untreated cacao shell BC stimulated denitrification whereas leached BC did not. This contrasts findings from aerobic incubations, which showed clear stimulation of respiration by all doses of untreated BCs in both soils (Figure A in S3 File). NaOH also stimulated denitrification (Table 3) but to a much lesser extent compared to untreated cacao shell BC and uncharred cacao shell despite similar increases in soil pH (Table 2).

Possible factors contributing to the BC effect on net N_2O and NO production and denitrification rate

Linear model ANCOVA showed differences in the response of denitrification product ratio ($N_2O/(N_2O+N_2)$), maximum NO accumulation and denitrification rate to BC type (rice husk or cacao shell) and dose, total C content (at onset of the experiment) and pH of the slurry (Table 4). In particular, BC type was a very important factor ($p = 0.000$). Doses were also important ($p = 0.000$ for denitrification product ratio and maximum NO accumulation; $p = 0.01$ for denitrification rate). Upon incorporation of BC leaching (untreated, water- and acid-leached BC) and pH as factors in addition to BC type and doses in the analysis, $N_2O/(N_2O+N_2)$ ratio, maximum NO accumulation and denitrification rates were significantly affected by all the factors at $p = 0.000$ (except the effect of BC dose on denitrification rate, which was at $p = 0.003$). Several interaction terms between factors were also significant ($p<0.05$).

ANCOVA also showed that total organic C (either as cacao shell or as its BC) added to the system was important in determining denitrification rate ($p = 0.006$) and maximum NO

Table 3. Maximum inducible denitrification rates in Lampung and Mkushi soil amended with cacao shell BC, rice husk BC, uncharred cacao shell and NaOH.

Soil	Amendment	Denitrification rates[a] (nmol N g^{-1} soil hr^{-1})					
Lampung soil	**Cacao shell BC doses (%)**	**0**	**1**	**2**	**5**	**10**	**SE**
	Untreated	22.7a	38.5ab	114.1bc	157.3c	116.3bc	27.7
	Water leached	26.3a	-	25.0a	38.5b	49.5c	2.1
	Acid leached	36.9a	-	20.8b	25.2b	37.2a	2.2
	Rice husk BC doses (%)	**0**	**1**	**2**	**5**	**10**	**SE**
	Untreated	28.5a	29.58a	16.8a	29.3a	31.0a	5.4
	Water leached	26.3a	-	25.6a	20.1a	17.0a	3.5
	Acid leached	33.1a	-	25.7a	16.5b	16.6b	2.7
	Uncharred cacao shell doses (%)	**0**	**1**	**2**	**5**	**10**	**SE**
	Uncharred cacao shell	36.3a	-	146.9b	209.7c	262.0d	4.7
	NaOH doses (ml)	**0**	**0.35**	**1.25**	**1.80**	**-**	**SE**
	NaOH	36.3ab	24.9a	48.6b	94.2c	-	5.3
Mkushi soil	**Cacao shell BC doses (%)**	**0**	**1**	**2**	**5**	**10**	**SE**
	Untreated	13.8a	-	35.9b	17.6a	12.0a	4.7
	Water leached	13.8a	-	17.8a	14.3a	8.6a	4.1
	Acid leached	13.8a	-	-	11.2a	12.6a	2.2

[a]Mean rate of various doses of each amendment in a row followed by different letters denote significant difference (Tukey's test, P<0.05). SE is standard error calculated from all doses of each amendment.

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accumulation (p = 0.000) but not $N_2O/(N_2O+N_2)$ ratio (p = 0.41). In addition, a comparison of treatments with uncharred cacao shell, providing significant amounts of labile C, and NaOH, without addition of labile C, showed the strong influence of labile C on denitrification rate (p = 0.000) but not on $N_2O/(N_2O+N_2)$ ratio (p = 0.06). In this comparison, pH significantly affected both denitrification rate and $N_2O/(N_2O+N_2)$ (p = 0.000).

NO accumulation and $N_2O/(N_2O+N_2)$ product ratios

Increasing doses of both untreated rice husk and cacao shell BC, as well as NaOH, caused maximum NO accumulation to decrease (Fig 2 upper panel). Corresponding doses of leached BC reduced suppression of maximum NO accumulation. Acid leaching of rice husk BC entirely eliminated the suppression of NO accumulation. Uncharred cacao shell had weaker effect on suppression of NO accumulation than corresponding doses of cacao shell BC whether leached or not. Maximum NO accumulation decreased asymptotically with increasing pH to trace levels at pH > 6.5 (Fig 2 lower panel). The NO accumulation rate was greatest at the beginning of the incubation reaching maximum values within 72 hours (Fig 1 and Figures C, E and F in S3 File), except in Mkushi soil with > 5% cacao shell BC (Figure B in S3 File). Here NO accumulation gradually increased throughout the incubation period.

The $N_2O/(N_2O+N_2)$ product ratio decreased with increasing doses of untreated BC (Fig 3 upper panel). Rice husk BC addition to Lampung soil resulted in a decrease of the $N_2O/(N_2O+N_2)$ ratio with increase in dose, reaching values below 0.1 at 10% BC addition (Fig 3A1). Adding the same amounts of cacao shell BC to Lampung soil suppressed the denitrification product ratio much more strongly; reaching low product ratios already with 1% addition and increasing the doses did not have additional benefit in suppressing N_2O. Cacao shell BC with its strong alkalizing effect was more effective in suppressing N_2O than its feedstock at equivalent doses of

Table 4. Results from stepwise linear ANCOVA showing the importance of labile C and pH for BC effect on denitrification rate, product ratio and maximum NO accumulation.

Analysis	Factors and interactions	$N_2O/(N_2O+N_2)$	Rate	NO
BC effect (Cacao shell & rice husk BC)	BC type	***	***	***
	BC dose	***	*	***
	BC type:BC dose	*	*	ns
BC leaching (Cacao shell & rice husk BC either untreated, water-leached or acid-leached)	BC type	***	***	***
	BC leaching	***	***	***
	BC dose	***	**	***
	pH	***	***	***
	BC type:BC leaching	***	***	***
	BC type:pH	ns	***	.
	BC type:BC dose	***	ns	***
	BC leaching:BC dose	ns	ns	***
	BC leaching:pH	*	*	***
	BC dose:pH	**	ns	***
	BC type:BC leaching:BC dose	***	ns	***
	BC type:BC leaching:pH	ns	ns	ns
	BC type:BC dose:pH	*	ns	ns
	BC leaching:BC dose:pH	ns	ns	*
	BC type:BC leaching:BC dose:pH	*	ns	ns
Labile C effect (labile C vs other factors in cacao shell)	Cacao shell (BC & uncharred)	***	***	***
	C added	ns	**	***
	pH	***	***	***
	Cacao shell:pH	**	***	***
	Cacao shell:C added	*	.	***
	C added:pH	ns	ns	ns
	Cacao shell:pH:C added	ns	ns	**
pH effect (separate pH from labile C)	Material (NaOH & uncharred cacao)	.	***	**
	pH	***	***	***
	C added	ns	***	***
	Material:pH	ns	***	ns
	pH:C added	ns	***	ns

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'ns ' 1, ':' means interaction of factors.

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mass (Fig 3B1). Thus, the strong effect of cacao shell BC compared to that of rice husk BC on the N_2O product ratio could be linked to its strong alkalizing effect, resulting in greater soil pH increase at equivalent doses (Table 2, Fig 3). Due to its strong alkalizing effect, no $N_2O/(N_2O+N_2)$ data are available for cacao shell BC-amended Lampung soils in the pH range 4.8–6.6 (Fig 3B2). Therefore, our data do not allow a direct comparison of pH-related effects of the two BCs. In Mkushi soil, the $N_2O/(N_2O+N_2)$ ratio was reduced to zero even at the lowest dose (here 2%, which increased soil pH to 8.3; Fig 3C). A 10% cacao shell BC addition to Mkushi caused high, but uncertain values of product ratio probably due to suppression of overall denitrification activity (Figure B in S3 File). Thus, our data for BC-amended soils indicate that the $N_2O/(N_2O+N_2)$ ratio decreased from close to 1 at pH < 4 (no induction of N_2OR activity) to

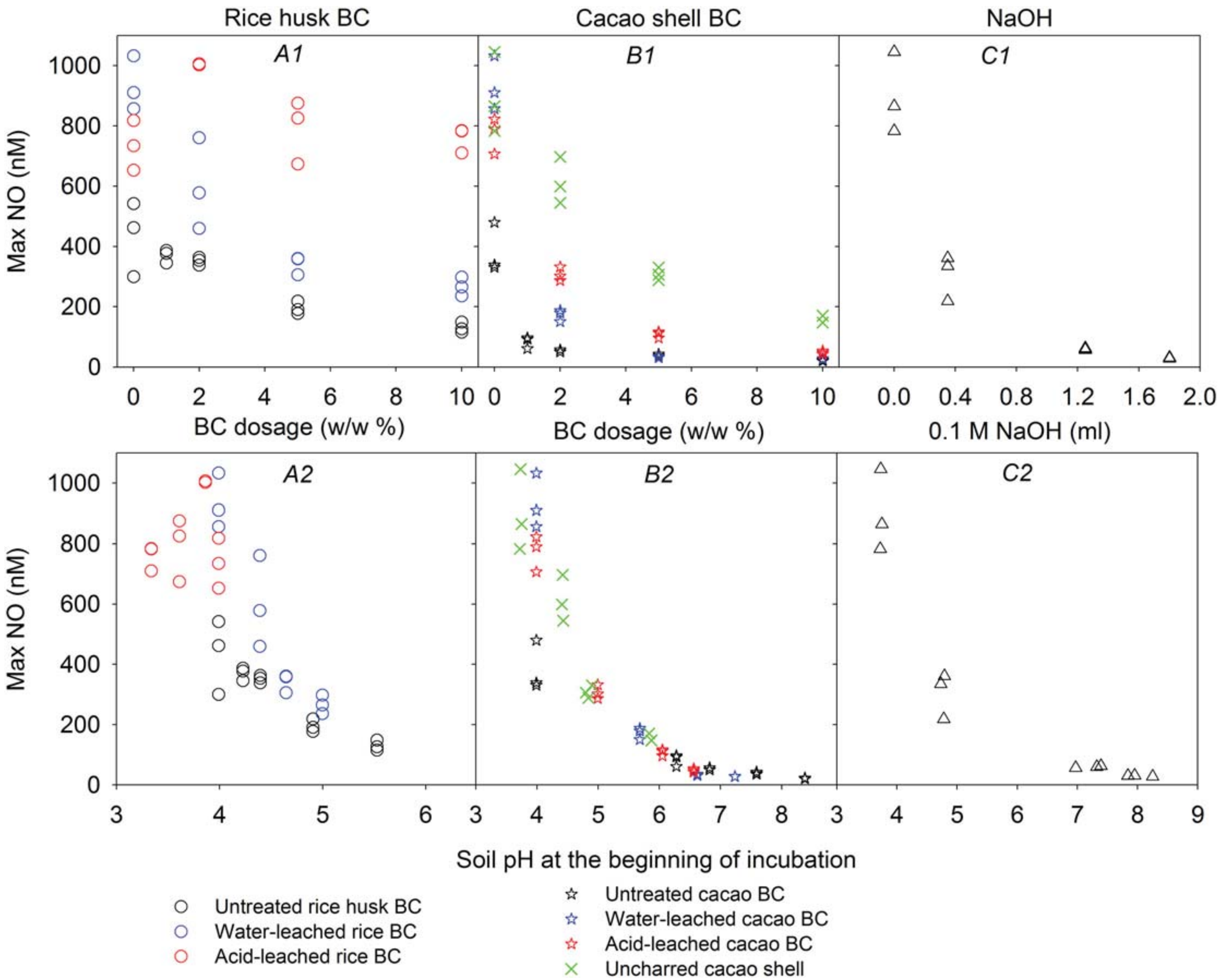

Fig 2. Maximum NO concentration in the liquid phase plotted against doses of BC, uncharred cacao shell and NaOH added to Lampung soil (upper panel—A1, B1 & C1), and against initial pH for Lampung soil amended with BC, uncharred cacao shell and NaOH (lower panel—A2, B2 & C2).

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close to zero at pH > 6 (sufficient induction of N_2OR to prevent significant net production of N_2O).

Addition of NaOH also decreased net N_2O production (Fig 3D). In the pH range 4 to 7, the relationship between pH and $N_2O/(N_2O+N_2)$ product ratio had a significantly smaller slope for NaOH-amended- than for BC-amended soils but similar to that of uncharred cacao shell (Table 5).

Applying water-leached rice husk BC to Lampung soil resulted in a similar relationship between $N_2O/(N_2O+N_2)$ ratio and dose (or pH) as observed in soils with untreated rice husk BC (Fig 3A). Addition of acid-leached rice husk BC, which had lost all its alkalizing effect, resulted in large $N_2O/(N_2O+N_2)$ product ratios independent of BC dose (Fig 3A). Water and acid leached cacao shell BC decreased the $N_2O/(N_2O+N_2)$ ratio at low dose (2%), albeit less

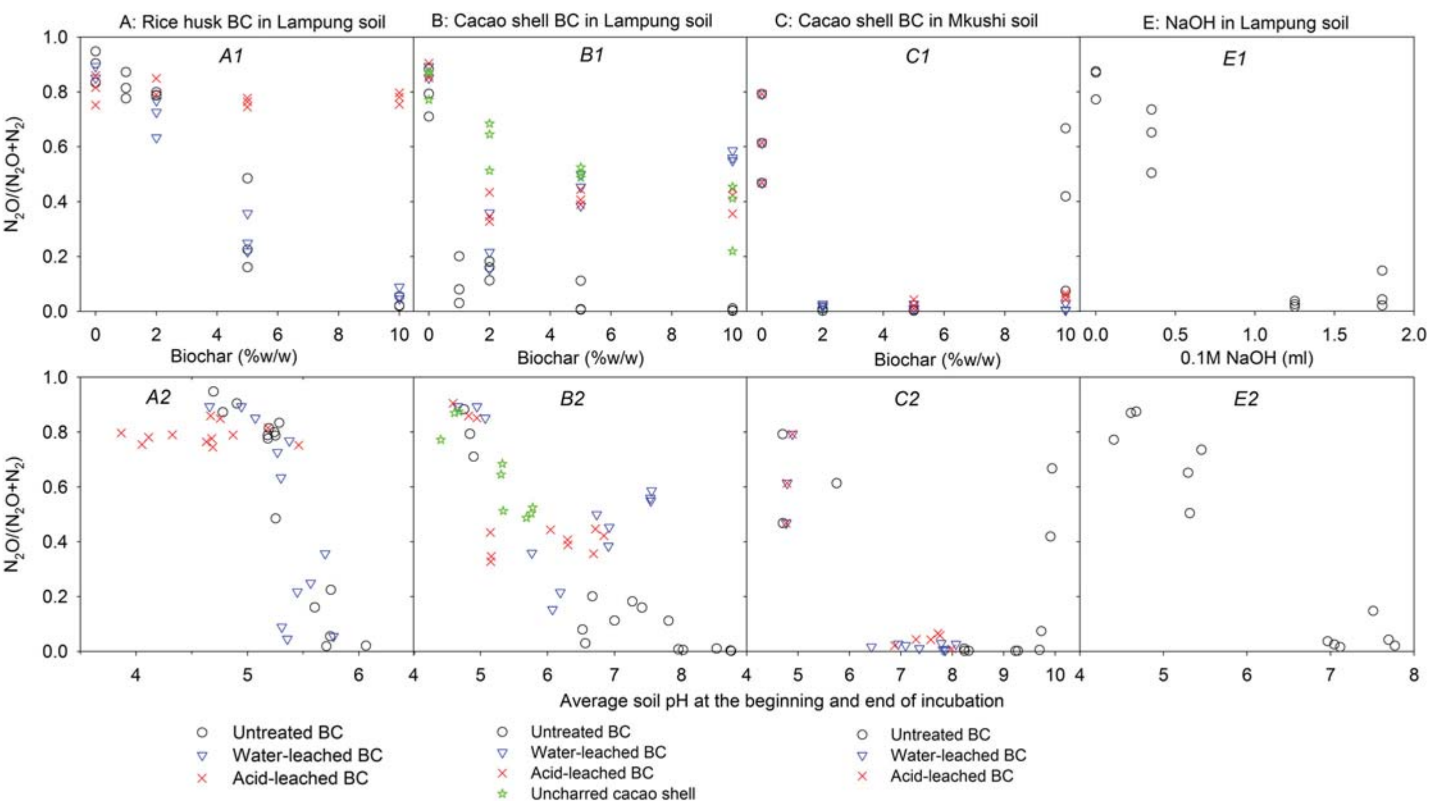

Fig 3. Plots of N_2O product ratio of denitrification against BC dose (upper panel—A1, B1, C1 & D1) and against average effective soil pH (lower panel—A2, B2, C2 & D2) of BC, uncharred cacao shell and NaOH amended soil.

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than untreated BC. At higher doses of leached BC, the ratios were relatively high compared to those of in response to untreated cacao shell BC additions at similar doses (Fig 3B).

N_2O reduction to N_2, which requires functional N_2OR, only occurred after dissolved NO concentrations decreased to values $\leq$ 100 nM (Fig 1). In addition, N_2O reduction only

Table 5. Regression coefficients of N_2O product ratios explained by dose effect (w/w %) or by pH effect of different amendments added to Lampung soil.

Analysis	Amendment	Intercept	Slope	Significance of slope	R^2
Dose effect	Untreated rice husk BC	0.90 (0.05)	-0.092 (0.010)	Slope different from zero (p<0.001)	0.91
	Water leached rice husk BC	0.84 (0.05)	-0.083 (0.010)	Slope not different from untreated rice husk BC (p>0.05)	
	Acid leached rice husk BC	0.81 (0.05)	-0.004 (0.010)	Slope different from untreated rice husk BC (p<0.001)	
	Uncharred cacao shell	0.77 (0.05)	-0.044 (0.010)	Slope different from untreated rice husk BC (p<0.001)	
pH effect	NaOH	2.34 (0.27)	-0.326 (0.047)	Slope different from zero (p<0.001)	0.80
	Untreated rice husk BC	5.12 (0.61)	-0.856 (0.113)	Slope different from NaOH (p<0.001)	
	Water leached rice husk BC	4.72 (0.81)	-0.797 (0.150)	Slope different from NaOH (p<0.01)	
	Acid leached rice husk BC	1.77 (0.52)	0.005 (0.107)	Slope different from NaOH (p<0.01)	
	Uncharred cacao shell	2.66 (0.59)	-0.399 (0.110)	Slope not different from NaOH (p>0.05)	

Intercept = value of product ratio at 0% BC and uncharred cacao shell addition or if pH of the soil would be zero. Slope = unit decrease in product ratio per percent increase of BC or uncharred cacao shell added or per unit increase in soil pH due to amendment added. Numbers in brackets are the standard errors.

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occurred at soil pH ≥ 5. At pH ≥ 5, e.g. after the amendment of rice husk BC at 5%, N_2OR activity started immediately at the beginning of incubation (Fig 1). For soils or soil-BC mixtures with initial pH < 5 (e.g. treatments with 1–2% rice husk BC), denitrification driven alkalization, increasing pH to ~5 had to take place before induction of N_2OR activity was observed. Initial delay in N_2OR activity caused high accumulation of N_2O in acidic soil or soil-BC mixtures.

Discussion

Effect of biochar on NO, N_2O and N_2 production and denitrification rate

Addition of untreated BCs to the two acidic Acrisols in this study suppressed the net production of both NO and N_2O during anoxic incubation (Fig 1), which is in line with previously reported studies ([11] and references therein). Here we show that this suppression went along with increase in N_2 production, suggesting increase in the activity of N_2OR [47] due to alkalization [29].

Leaching of the BCs except for water-leached rice husk BC reduced or eliminated the effect of NO and N_2O suppression (Figs 2 and 3), indicating that some of the BC constituents removed by leaching (S2 File) contributed to the suppression. Base cations and carbonates (shown by the high amount of CO_2 released upon mixing of acidic soil with BC—Fig 1) were the major constituents removed by leaching, thus causing a decrease in alkalizing effect. Suppression of NO and N_2O production in response to the addition of NaOH indicated that pH is an important factor contributing to the suppression. A recent study reported loss of alkalizing effect together with a loss in N_2O suppression due to field aging of BC [48], suggesting that N_2O suppression by BC might be a transient effect connected to the transiency of its alkalizing effect.

The $N_2O/(N_2O+N_2)$ product ratio decreased when the initial soil pH increased from pH 4 to 6 in response to the addition of BC (Fig 3). The rise in pH through addition of BC or NaOH removed the impairment of N_2OR, typically seen at low pH [29, 33, 49]. The relief of this impairment through pH increase is similar to what has been reported for denitrifying pure cultures and for soils from long-term liming experiments in which raised pH stimulated N_2OR and reduced N_2O production or emissions [28, 29, 31, 33]. This direct effect of pH was attributed to a threshold pH above which functional N_2OR is assembled [29, 31]. In this study, we found a threshold of pH ≈ 5 for the induction of N_2OR based on the timing of N_2 production onset (Fig 1), amount of accumulated denitrification intermediates (Fig 1) and pH at the beginning and end of incubation (Table 2). This threshold pH is close to threshold pH values for N_2OR induction around pH 6, observed through detection of measurable N_2 in earlier anoxic studies [29, 32]. The greater decrease of the $N_2O/(N_2O+N_2)$ ratio with increasing pH in rice husk BC-amended soil compared to that of previously published data [29, 32] and results from the NaOH-amended soil (Fig 3D and Table 5) suggest that BC has a somewhat stronger effect on the suppression of N_2O than explained by pH alone. However, the effectiveness of N_2O suppression seems to depend on the timing of induction of N_2OR, which is controlled by the alkalizing effect of BC. Denitrification-driven alkalization contributed to induction of N_2OR if the threshold pH for N_2OR induction was not achieved by the BC alkalizing effect alone. Recently, Harter, Krause [50] reported an increased relative abundance of *nos*Z genes encoding for N_2OR during 80 days of incubation after BC addition to soil, which is in line with the increased activity of N_2OR observed in this study.

Only few recent studies have reported BC effects on NO production. Recently, Nelissen, Saha [37] reported a decrease in NO production similar to this study. The driver behind NO suppression by BC appears to be similar to that underlying N_2O suppression because the two gases decreased with increasing doses of untreated BCs in a similar fashion (Fig 1). The concentration of the two gases increased initially and reached a peak before decreasing, although in all cases, NO reached the peak earlier than N_2O. Low NO concentrations in BC- or NaOH-amended soils (Fig 1 and Figure C in S3 File) were likely due to the pH-increasing effect (Table 4), which prevents chemical decomposition of NO_2^- to NO [51, 52], leaving only enzymatically produced NO to accumulate. Higher NO production in Lampung compared to Mkushi soil was probably due to higher microbial activities producing nitrite, part of which was decomposed chemically to NO at low pH. Our data also suggest that induction of N_2OR is linked to low NO concentration, as N_2OR activity was not initiated before NO concentration dropped to values below 100 nM. NO has been proposed to play an important role in the regulation of denitrification enzyme regulation [53], but little is known how reactive gaseous N species like NO react with BC.

In general, both aerobic and anaerobic respiration were stimulated by BC addition to soil (Fig 1 and Figure A in S3 File). Suppression of anaerobic respiration was only found at high doses of cacao shell BC added to Mkushi soil resulting in soil pH values > 9 (Figure B in S3 File). Anoxic incubation of untreated BC in 2 mM KNO_3 solution without soil revealed that BC themselves carried out some denitrification activity which was expressed when residual O_2 was fully exhausted (Figure D in S3 File). Interestingly, no N_2O accumulated, suggesting full N_2OR induction at high pH. Denitrification activity was clearly greater with rice husk (pH 8.4) than cacao shell BC (pH 9.8). This might reflect the inability of the denitrifier community to thrive when too much BC is added driving soil pH to high values at which NO_2^- may accumulate to toxic levels [54]. Additionally, the osmotic effect of salts due to high dose (10%) BC in poorly buffered Mkushi soil may have inhibited microbial activity. Other than at high dose, our BC did not have any direct inhibitory effect on microbial activities such as shown for BC-mediated ethylene production [23].

BC is a complex material, which may alter many soil variables besides pH. In particular, BC increased bioavailable carbon (C) (Figure D in S3 File; residual O_2 was consumed and CO_2 was produced during incubation of BC without soil) [55, 56] and nutrients (S2 File) which could stimulate microbial growth [56] and affect the regulation of denitrification. Addition of bioavailable C clearly affected denitrification rate as seen after adding uncharred cacao shell (Tables 3 and 4), but it did not affect the product ratio (Table 4). The decrease in product ratio with increase in BC dose applied was better explained by pH increase than by C-addition in our ANCOVA. The contribution of bioavailable organic C and/or nutrients of cacao shell BC to increased denitrification rates is clearly seen when comparing cacao shell BC treatments with NaOH treatments at similar soil pH.

Leaching of BC, which mimics field aging, affected both its alkalinity and surface chemistry (Table 1 and S2 File). Changes to BC surface chemistry may occur through alterations of surface functional organic groups. The leaching experiments showed that certain BC types such as cacao shell BC may be more resistant to aging presumably through release of base cations and secondary carbonation, which would explain the relatively minor effect of acid leaching on cacao shell BC's alkalinizing effect (Table 1 and S2 File). Denitrification experiments with leached cacao shell BC did not show ordinary dose response. Instead, higher doses of leached cacao shell BC resulted in conspicuous biphasic NO kinetics with two peaks in Lampung soil, a delayed peak of N_2O production as well as delayed production of N_2 by either enzymatic or chemical pathways (Figures E and F in S3 File) [57]. This went along with higher $N_2O/(N_2O+N_2)$ ratios at high doses as compared with untreated BC (Fig 3B). This may point at some chemical interaction of newly exposed BC surfaces with denitrification intermediates. Initially, leached cacao shell BC may have acted as electron sink [11, 18], competing with denitrification reductases for electrons. However, there was no indication of chemical reaction such as sorption and desorption between BC and N-compounds in an anoxic incubation of BC (untreated or leached) without soil (Figure D in S3 File).

Factors determining NO and N_2O suppression by biochar

In this study, we found that the pH effect of BC in acid soil played a major role in the suppression of both NO and N_2O under anoxic conditions. However, any extrapolation of our data beyond acidic soils needs to be done with caution. Cayuela, Sánchez-Monedero [17] also observed reduced $N_2O/(N_2O+N_2)$ ratios during N_2O peak emission in wet soils amended with brush BC but a direct pH effect was not clearly captured probably because of the small pH increase (0.1 pH units). Instead, Cayuela, Sánchez-Monedero [17] could show that the observed reduction in $N_2O/(N_2O+N_2)$ ratios were positively correlated to the buffer capacities of the added BC. Earlier, Yanai, Toyota [20] had concluded that suppression of N_2O emissions (which they believed originated from denitrification) by BC was not the result of changes in soil chemical properties. Cayuela, Sánchez-Monedero [17] and the present study clearly show that BC can affect the soil chemical properties with consequences for the product stoichiometry of denitrification. In this study, we used controlled anoxia with direct quantification of N_2 production to study the effect of BC on denitrification stoichiometry. Yanai, Toyota [20] did not separate N cycling processes and their study could have been confounded by nitrification, an acidifying process, as suggested by the decrease in pH at the end of their incubations. We did not account for dissimilatory nitrate reduction to ammonium (DNRA) in this study; however, it is unlikely that this process played a major role as we recovered the added nitrate quantitatively as N_2.

The steeper slopes of $N_2O/(N_2O+N_2)$ versus pH in BC treatments compared to NaOH and uncharred cacao shell treatments (Table 5) indicate that some other factors may have contributed to the suppression of N_2O in addition to the pH effect. The similarity of the slopes for uncharred cacao shell and NaOH suggests that stronger suppression of N_2O by BC was not due to cacao shell itself or to labile C but to some other BC property. Biochar redox behavior (electron shuttling), where the electron-conductance of BC serves as a catalyst in denitrification as suggested by Cayuela, Sánchez-Monedero [17] could be one of these factors. The reduction or elimination of BC suppression of N_2O after leaching of BC in this study raises questions about how leaching affects electron shuttling and how important electron shuttling is, in suppressing N_2O.

Conclusions

This study is the first of its kind assessing BC effects under full denitrification conditions, simultaneously quantifying NO, N_2O and N_2 production at high temporal resolution. We found compelling evidence that BC strongly suppresses relative NO and N_2O net production from denitrification in two acid soils, resulting in a reduced propensity for NO and N_2O emissions. Increase of soil pH by BC addition was identified as a major factor mediating this suppression. NO suppression was linked to less chemical decomposition of NO_2^- to NO due to pH increase. N_2O suppression on the other hand was in accordance with the notion that raising pH in acid soils greatly stimulates N_2OR activity resulting in more complete denitrification with N_2 as the dominating end product. Other factor(s) contributing causally to the observed increase in N_2OR activity cannot be excluded and need further testing.

Supporting Information

S1 File. Description of biochar production, incubation system operation and gas chromatograph detectors. Biochar production (Description A). Incubation system operation and gas

chromatograph detectors (Description B).
(DOCX)

S2 File. Constituents removed from BC through leaching. Constituents removed from BC through leaching with water and strong acid (HCl) (Table A).
(DOCX)

S3 File. Mean oxygen consumption during oxic incubations and kinetics of gas production (N_2, N_2O, NO, CO_2) and consumption (O_2) during anoxic incubations. Mean oxygen consumption in BC amended soils during oxic incubations (Figure A). Denitrification kinetics and CO_2 and O_2 concentrations in incubations of Mkushi soil amended with untreated cacao shell BC (Figure B). Denitrification kinetics and CO_2 and O_2 concentrations in incubations of Lampung soil amended with uncharred cacao shell (upper 2 panels) and 0.1M NaOH (lower 2 panels) (Figure C). Denitrification kinetics and CO_2 and O_2 concentrations in anoxic incubations of 2.36 g BC without soil in 30 ml 2mM KNO_3 (Figure D). Denitrification kinetics and CO_2 and O_2 concentrations in incubations of Lampung soil amended with water-leached rice husk BC (upper 2 panels) and cacao shell BC (lower 2 panels) (Figure E). Denitrification kinetics and CO_2 and O_2 concentrations in incubations of Lampung soil amended with acid-leached rice husk BC (upper 2 panels) and cacao shell BC (lower 2 panels) (Figure F).
(DOCX)

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Author Contributions

Conceived and designed the experiments: AO GC JM PD. Performed the experiments: AO PD. Analyzed the data: AO PD. Contributed reagents/materials/analysis tools: AO GC JM PD. Wrote the paper: AO GC JM PD.

References

1. Zumft WG. Cell biology and molecular basis of denitrification. Microbiol Mol Biol R. 1997; 61(4):533–616.
2. Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, et al. Human alteration of the global nitrogen cycle: Sources and consequences. Ecol Appl. 1997; 7(3):737–750.
3. Ravishankara AR, Daniel JS, Portmann RW. Nitrous Oxide (N2O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. Science. 2009; 326(5949):123–125. doi: 10.1126/science.1176985 PMID: 19713491
4. IPCC. Climate change 2007: The physical science basis. Agenda. 2007; 6(07):333.
5. Reay DS, Davidson EA, Smith KA, Smith P, Melillo JM, Dentener F, et al. Global agriculture and nitrous oxide emissions. Nature Clim Change. 2012; 2(6):410–416.
6. Montzka SA, Dlugokencky EJ, Butler JH. Non-CO_2 greenhouse gases and climate change. Nature. 2011; 476(7358):43–50. doi: 10.1038/nature10322 PMID: 21814274
7. Spokas KA, Koskinen WC, Baker JM, Reicosky DC. Impacts of woodchip biochar additions on greenhouse gas production and sorption/degradation of two herbicides in a Minnesota soil. Chemosphere. 2009; 77(4):574–581. doi: 10.1016/j.chemosphere.2009.06.053 PMID: 19647284
8. Lehmann J. A handful of carbon. Nature. 2007; 447.

9. Lehmann J, Gaunt J, Rondon M. Bio-char Sequestration in Terrestrial Ecosystems—A Review. Mitig Adapt Strategies Glob Chang. 2006; 11(2):395–419.
10. Lehmann J. Bio-energy in the black. Front Ecol Environ. 2007; 5(7):381–7.
11. Cayuela ML, van Zwieten L, Singh BP, Jeffery S, Roig A, Sánchez-Monedero MA. Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis. Agr Ecosyst Environ. 2014; 191:5–16. doi: 10.1016/j.agee.2013.10.009
12. Zhang A, Cui L, Pan G, Li L, Hussain Q, Zhang X, et al. Effect of biochar amendment on yield and methane and nitrous oxide emissions from a rice paddy from Tai Lake plain, China. Agr Ecosyst Environ. 2010; 139(4):469–475. doi: 10.1016/j.agee.2010.09.003
13. Cornelissen G, Martinsen V, Shitumbanuma V, Alling V, Breedveld G, Rutherford D, et al. Biochar Effect on Maize Yield and Soil Characteristics in Five Conservation Farming Sites in Zambia. Agronomy. 2013; 3(2):256–274. doi: 10.3390/agronomy3020256
14. Jeffery S, Verheijen FGA, van der Velde M, Bastos AC. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agr Ecosyst Environ. 2011; 144(1):175–187. doi: 10.1016/j.agee.2011.08.015
15. Clough TJ, Bertram JE, Ray JL, Condron LM, O'Callaghan M, Sherlock RR, et al. Unweathered Wood Biochar Impact on Nitrous Oxide Emissions from a Bovine-Urine-Amended Pasture Soil. Soil Sci Soc Am J. 2010; 74(3):852–860. doi: 10.2136/sssaj2009.0185
16. Singh BP, Hatton BJ, Singh B, Cowie AL, Kathuria A. Influence of Biochars on Nitrous Oxide Emission and Nitrogen Leaching from Two Contrasting Soils. J Environ Qual. 2010; 39(4):1224–1235. doi: 10.2134/jeq2009.0138 PMID: 20830910
17. Cayuela ML, Sánchez-Monedero MA, Roig A, Hanley K, Enders A, Lehmann J. Biochar and denitrification in soils: when, how much and why does biochar reduce N2O emissions? Sci Rep. 2013; 3:1732. doi: 10.1038/srep01732 PMID: 23615819
18. Joseph S, Camps-Arbestain M, Lin Y, Munroe P, Chia C, Hook J, et al. An investigation into the reactions of biochar in soil. Soil Res. 2010; 48(7):501–515.
19. Cornelissen G, Rutherford DW, Arp HP, Dörsch P, Kelly CN, Rostad CE. Sorption of pure N_2O to biochars and other organic and inorganic materials under anhydrous conditions. Environ Sci Technol. 2013; 47(14):7704–7712. doi: 10.1021/es400676q PMID: 23758057
20. Yanai Y, Toyota K, Okazaki M. Effects of charcoal addition on N_2O emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments. Soil Sci Plant Nutr. 2007; 53(2):181–188. doi: 10.1111/j.1747-0765.2007.00123.x
21. Clough TJ, Condron LM. Biochar and the Nitrogen Cycle: Introduction. J Environ Qual. 2010; 39 (4):1218–1223. doi: 10.2134/jeq2010.0204 PMID: 20830909
22. Clough T, Condron L, Kammann C, Müller C. A Review of Biochar and Soil Nitrogen Dynamics. Agronomy. 2013; 3(2):275–293. doi: 10.3390/agronomy3020275
23. Spokas KA, Baker JM, Reicosky DC. Ethylene: potential key for biochar amendment impacts. Plant Soil. 2010; 333(1–2):443–452. doi: 10.1007/s11104-010-0359-5
24. Bruun EW, Ambus P, Egsgaard H, Hauggaard-Nielsen H. Effects of slow and fast pyrolysis biochar on soil C and N turnover dynamics. Soil Biol Biochem. 2012; 46(0):73–79. doi: 10.1016/j.soilbio.2011.11.019
25. Yuan J-H, Xu R-K. Effects of biochars generated from crop residues on chemical properties of acid soils from tropical and subtropical China. Soil Res. 2012; 50(7):570–578. http://dx.doi.org/10.1071/SR12118.
26. Biederman LA, Harpole WS. Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. GCB Bioenergy. 2013; 5(2):202–214. doi: 10.1111/gcbb.12037
27. Verheijen FGA, Jeffery S, Bastos AC, van der Velde M, Diafas I. Biochar Application to Soils: A Critical Scientific Review of Effects on Soil Properties, Processes and Functions. EUR 24099 EN, Office for the Official Publications of the European Communities, Luxembourg, 149pp. 2009.
28. Bergaust L, Mao Y, Bakken LR, Frostegard A. Denitrification response patterns during the transition to anoxic respiration and posttranscriptional effects of suboptimal pH on nitrous oxide reductase in Paracoccus denitrificans. Appl Environ Microb. 2010; 76(19):6387–6396.
29. Liu B, Morkved PT, Frostegard A, Bakken LR. Denitrification gene pools, transcription and kinetics of NO, N_2O and N_2 production as affected by soil pH. FEMS Microbiol Ecol. 2010; 72(3):407–417. doi: 10.1111/j.1574-6941.2010.00856.x PMID: 20370831
30. Dörsch P, Braker G, Bakken LR. Community-specific pH response of denitrification: experiments with cells extracted from organic soils. FEMS Microbiol Ecol. 2012; 79(2):530–541. doi: 10.1111/j.1574-6941.2011.01233.x PMID: 22093000

31. Liu B, Frostegard A, Bakken LR. Impaired reduction of N2O to N2 in acid soils is due to a posttranscriptional interference with the expression of nosZ. mBio. 2014; 5(3):e01383–14. doi: 10.1128/mBio.01383-14 PMID: 24961695

32. Qu Z, Wang J, Almøy T, Bakken LR. Excessive use of nitrogen in Chinese agriculture results in high N2O/(N2O+N2) product ratio of denitrification, primarily due to acidification of the soils. Glob Change Biol. 2014; 20(5):1685–1698. doi: 10.1111/gcb.12461

33. Raut N, Dörsch P, Sitaula BK, Bakken LR. Soil acidification by intensified crop production in South Asia results in higher N2O/(N2 + N2O) product ratios of denitrification. Soil Biol Biochem. 2012; 55:104–112.

34. Bakken LR, Bergaust L, Liu B, Frostegard A. Regulation of denitrification at the cellular level: a clue to the understanding of N2O emissions from soils. Philos T Roy Soc B. 2012; 367(1593):1226–1234. doi: 10.1098/rstb.2011.0321

35. Nadeem S, Dörsch P, Bakken LR. Autoxidation and acetylene-accelerated oxidation of NO in a 2-phase system: Implications for the expression of denitrification in ex situ experiments. Soil Biol Biochem. 2013; 57:606–614. doi: 10.1016/j.soilbio.2012.10.007

36. Nadeem S, Dörsch P, Bakken LR. The significance of early accumulation of nanomolar concentrations of NO as an inducer of denitrification. FEMS Microbiol Ecol. 2013; 83(3):672–684. doi: 10.1111/1574-6941.12024 PMID: 23035849

37. Nelissen V, Saha BK, Ruysschaert G, Boeckx P. Effect of different biochar and fertilizer types on N_2O and NO emissions. Soil Biol Biochem. 2014; 70:244–255. doi: 10.1016/j.soilbio.2013.12.026

38. Molstad L, Dörsch P, Bakken LR. Robotized incubation system for monitoring gases (O2, NO, N2O N2) in denitrifying cultures. J Microbiol Meth. 2007; 71(3):202–211. doi: 10.1016/j.mimet.2007.08.011

39. Wang R, Feng Q, Liao T, Zheng X, Butterbach-Bahl K, Zhang W, et al. Effects of nitrate concentration on the denitrification potential of a calcic cambisol and its fractions of N2, N2O and NO. Plant Soil. 2012; 363(1–2):175–189. doi: 10.1007/s11104-012-1264-x

40. Smebye A. The effect of biochar on dissolved organic matter in soil. Master Thesis. University of Oslo. 2014. Available: https://www.duo.uio.no/handle/10852/42266?show = full

41. Martinsen V, Alling V, Nurida NL, Mulder J, Hale SE, Ritz C, et al. pH effects of the addition of three biochars to acidic Indonesian mineral soils. Soil Sci Plant Nutr. 2015:1–14. doi: 10.1080/00380768.2015.1052985

42. Smebye A, Alling V, Vogt RD, Gadmar TC, Mulder J, Cornelissen G, et al. Biochar amendment to soil changes dissolved organic matter content and composition. Chemosphere. 2015. doi: 10.1016/j.chemosphere.2015.04.087

43. Hale SE, Alling V, Martinsen V, Mulder J, Breedveld GD, Cornelissen G. The sorption and desorption of phosphate-P, ammonium-N and nitrate-N in cacao shell and corn cob biochars. Chemosphere. 2013; 91(11):1612–1619. doi: 10.1016/j.chemosphere.2012.12.057 PMID: 23369636

44. Kieft TL, Soroker E, Firestone MK. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biol Biochem. 1987; 19(2):119–126. doi: 10.1016/0038-0717(87)90070-8

45. Saad OALO, Conrad R. Temperature dependence of nitrification, denitrification, and turnover of nitric oxide in different soils. Biol Fert Soils. 1993; 15(1):21–27. doi: 10.1007/BF00336283

46. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2014.

47. Holtan-Hartwig L, Dörsch P, Bakken LR. Comparison of denitrifying communities in organic soils: kinetics of NO3- and N_2O reduction. Soil Biol Biochem. 2000; 32(6):833–843.

48. Spokas KA. Impact of biochar field aging on laboratory greenhouse gas production potentials. GCB Bioenergy. 2013; 5(2):165–176. doi: 10.1111/gcbb.12005

49. Simek M, Cooper JE. The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. Eur J Soil Sci. 2002; 53:345–354.

50. Harter J, Krause HM, Schuettler S, Ruser R, Fromme M, Scholten T, et al. Linking N_2O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. ISME J. 2013:1–15. doi: 10.1038/ismej.2013.160

51. Islam A, Chen D, White RE, Weatherley AJ. Chemical decomposition and fixation of nitrite in acidic pasture soils and implications for measurement of nitrification. Soil Biol Biochem. 2008; 40(1):262–265. doi: 10.1016/j.soilbio.2007.07.008

52. Braida W, Ong SK. Decomposition of nitrite under various pH and aeration conditions. Water Air Soil Poll. 2000; 118(1–2):13–26.

53. Spiro S. Regulators of bacterial responses to nitric oxide. FEMS Microbiol Rev. 2007; 31(2):193–211. doi: 10.1111/j.1574-6976.2006.00061.x PMID: 17313521

54. Bollag J-M, Henninger NM. Effects of nitrite toxicity on soil bacteria under aerobic and anaerobic conditions. Soil Biol Biochem. 1978; 10(5):377–381.

55. Bruun EW, Hauggaard-Nielsen H, Ibrahim N, Egsgaard H, Ambus P, Jensen PA, et al. Influence of fast pyrolysis temperature on biochar labile fraction and short-term carbon loss in a loamy soil. Biomass Bioenergy. 2011; 35(3):1182–1189. doi: 10.1016/j.biombioe.2010.12.008

56. Luo Y, Durenkamp M, De Nobili M, Lin Q, Brookes PC. Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. Soil Biol Biochem. 2011; 43 (11):2304–2314. doi: 10.1016/j.soilbio.2011.07.020

57. Dail DB, Davidson EA, Chorover J. Rapid abiotic transformation of nitrate in an acid forest soil. Biogeochemistry. 2001; 54(2):131–146.

Effect of soil pH increase by biochar on NO, N_2O and N_2 production during denitrification in acid soils

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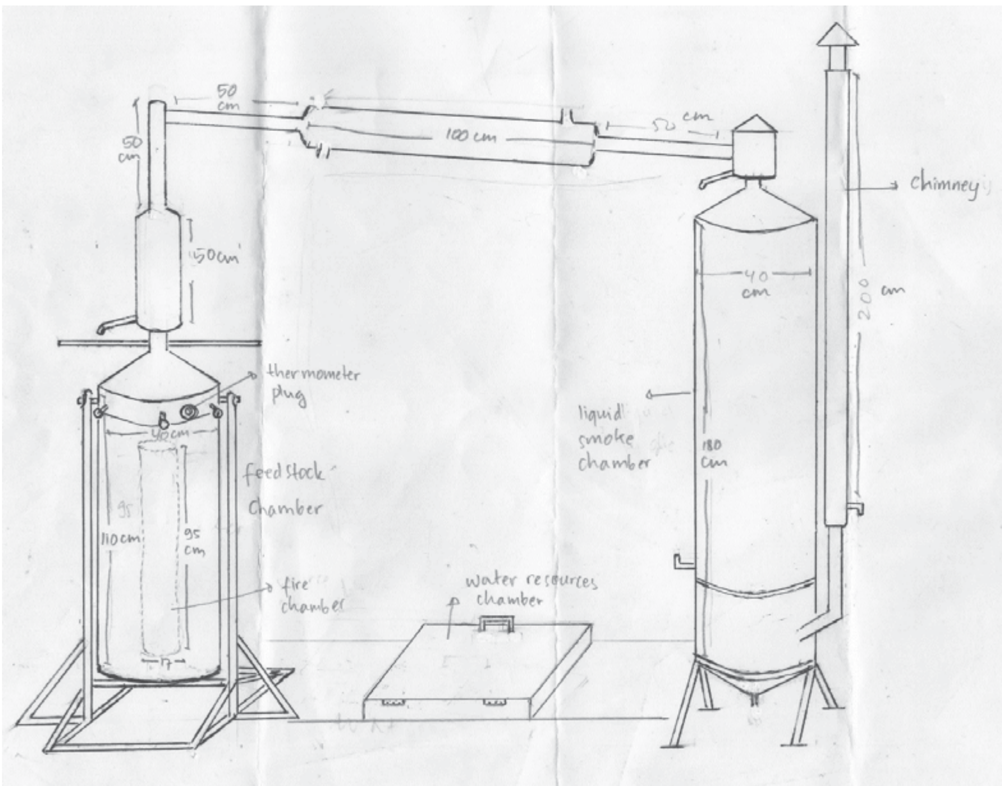
Supplementary information

S1 File. Description of biochar production, incubation system operation and gas chromatograph detectors

Description A. Biochar production

The biochars (BCs) were produced in a locally fabricated metal kiln with a volume of 30-40L at a temperature of 250-350ºC for 3.5hours. The BC yield was 22% and 30.4% per unit weight of biomass. The schematic drawing of the kiln and a photograph from Hale et al., Chemosphere 2013, 57(11), 1612-1619 is shown below.



Estimation of charring temperature by use of thermogravimetric analysis (TGA)

Stepwise TGA Analysis: The degree of charring has been studied by measuring the weight loss of a char sample as it undergoes additional pyrolysis under a nitrogen atmosphere in a thermogravimetric analyzer (TGA). Each sample was heated sequentially to 8 increasing temperatures, varying by 100-degree increments, between 200 and 900^{o}C, with a hold time of one hour at each temperature. The weight loss occurring at 200^{o}C was taken as the moisture content of the sample. The weight loss at each temperature increment above 200^{o}C, after correction for moisture and ash content, was used to determine the cumulative weight loss (total weight loss), as well as incremental loss profiles at each temperature step.

Estimation of degree of charring: The analysis of the data attempts to rank the chars by degree of charring using three measures taken from the stepwise analysis. The first measure is the total weight loss (sum of weight loss at each step from 300 to 900^{o}C) after correction for moisture and ash content. The second measure is the ratio of high temperature weight loss (sum of weight loss from 600 to 900^{o}C) to low temperature weight loss (sum of weight loss from 300 to 500^{o}C). The third measure is the temperature of maximum weight loss. This should indicate that much of the sample has effectively seen a maximum charring temperature lower than this temperature.

The "degree of charring" is not meant to be an estimate of the highest heat treatment temperature seen by the char, since the degree of charring is a factor of both highest heat treatment temperature and the duration of charring. The comparison with the reference chars is meant to provide a scale to compare the test chars to and give an estimate of the "effective charring temperature".

Reference chars: The reference series of chars was produced by pyrolysis in a muffle furnace under inert (nitrogen) atmosphere for eight hours at the designated charring temperature. Temperatures were carefully controlled and varied by 50 or 100°C increments from 250°C to 900°C. The reference materials consisted of pine wood (*Pinus ponderosa*), switch grass (*Panicum virgatum*), and purified cellulose.

Cacao and rice hull chars: The cacao shell and rice hull char samples from Indonesia were provided by Gerard Cornelissen, Norges Geotekniske Institutt.

Total weight loss

The total weight loss of reference chars (in percentage) after correction for moisture and ash content are shown below. The numbers in parenthesis are standard deviations. (n=3 for pine wood char and n=2 for switchgrass chars)

Charring Temperature (°C)	Pine wood char	Switch grass char	Cellulos e char
250	64.3 (0.7)	61.2 (2.90)	48.9
300	48.7 (2.2)	45.5 (2.03)	44.8
350	37.0 (1.8)	38.0 (2.04)	43.1
400	34.0 (2.3)	32.9 2.04)	37.4
450	28.4 (2.6)	25.5 (0.37)	21.6
500	18.0 (0.6)	22.0 (0.06)	15.8
600	17.0 (2.0)	18.2 (0.80)	10.7
700	14.0 (1.9)	14.7 (0.96)	
800	14.5 (3.0)	14.5 (2.23)	
900	12.1 (1.8)	13.1 (2.93)	

The total weight loss of the cacao shell char was 32.9% (Standard Deviation 3.6, n=3), which would indicate a charring temperature of about 400°C.

The total weight loss of the rice hull char was 33.1% (Standard Deviation 0.66, n=3), which would be the equivalent of an 8 hour charring temperature of about 400°C.

High to Low temperature weigh loss ratio

The ratio of high temperature weight loss (sum of weight loss from 600 to 900ºC) to low temperature weight loss (sum of weight loss from 300 to 500ºC) for the reference chars are shown below. The numbers in parenthesis are standard deviations. (n=3 for pine wood char and n=2 for switchgrass chars)

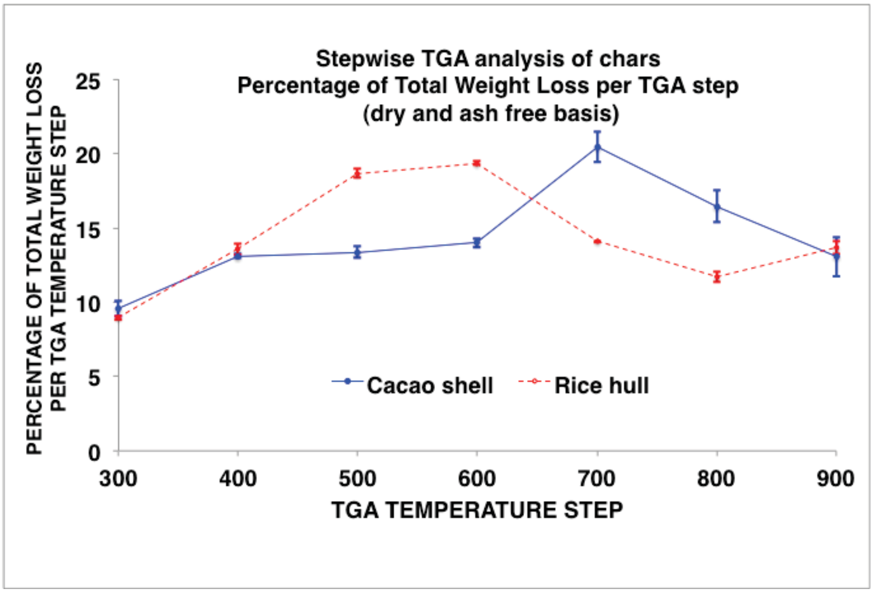
Charring Temperature (ºC)	**Pine wood char**	**Switch grass char**	**Cellulose char**
250	0.25 (0.02)	0.23 (0.03)	0.03
300	0.64 (0.11)	0.46 (0.02)	0.11
350	0.78 (0.11)	0.64 (0.00)	0.32
400	1.06 (0.23)	0.96 (0.06)	0.65
450	1.57 (0.24)	1.42 (0.17)	0.97
500	2.51 (0.10)	2.20 (0.04)	2.87
600	2.85 (0.18)	2.65 (0.07)	6.70
700	2.84 (0.05)	2.48 (0.02)	
800	3.05 (0.15)	2.23 (0.01)	
900	2.90 (0.08)	2.04 (0.15)	

The ratio of high temperature weight loss to low temperature weight loss of the cacao shell char was 1.8 (Standard Deviation 0.08, n=3), which would indicate a charring temperature of between 450 and 500ºC.

The ratio of high temperature weight loss to low temperature weight loss of the rice hull char was 1.4 (Standard Deviation 0.04, n=3), which would be the equivalent of an 8 hour charring temperature of between 450 and 500ºC.

Incremental loss profiles

The incremental loss profile of the cacao shell char is shown below. The temperature of maximum weight loss of the cacao shell occurs at 700°C, indicating that the char has seen a maximum charring temperature lower than this temperature. The temperature of maximum weight loss of the rice hull char occurs at 600°C, indicating that the char has seen a maximum charring temperature lower than this temperature.



The TGA step with the maximum weight loss (degrees Celsius) of reference chars are shown below:

Charring Temperature (°C)	**Pine wood char**	**Switch grass char**	**Cellulose char**
250	300	300	400
300	500	400	500
350	500	500	600
400	500	500	600
450	600	600	600
500	600	600	600
600	700	700	700
700	700	900	
800	900	900	
900	900	900	

Description B. Incubation system operation and gas chromatograph detectors

The incubation system consists of a water bath connected to a cryostat, placed under the robotic arm of an autosampler (Combi Pal, CTC, Switzerland). Submersible magnetic stirrers (Variomag HP 15; H+P Labortechnik GmbH, Germany) accommodate up to 30 continuously stirred bottles and additional bottles with calibration gasses, which are pierced at selected intervals by the hypodermic needle of the programmable autosampler. For each sampling, an aliquot of the headspace gas (ca. 1.2 ml) is removed by a peristaltic pump (Gilson Minipuls 3) and transferred to a gas chromatograph (Model 7890A, Agilent, Santa Clara, CA, US) and an NO chemiluminescence analyzer (model 200A; Advanced Pollution Instrumentation) via dedicated sampling loops. He 5.0 was used as carrier gas. CO_2 and N_2O were separated by a 20 m wide-bore (0.53 mm diameter) Poraplot Q capillary column run at 38ºC and the bulk gases (N_2, O_2 and Ar) on a 30 m 5Å molsieve capillary column run at 25ºC. A packed Heysept column was used for back-flushing water. The analytical columns were connected to a thermal conductivity detector (TCD) and an electron capture detector (ECD) for quantification of N_2O. The ECD was run at 375ºC with 17 ml min^{-1} Ar/methane (CH_4) (90/10 vol%) as a make-up gas. The chemiluminescence NOx analyzer was programmed to analyze NO only and coupled to a dedicated sampling loop via an open split. After each sampling, the pump was automatically reversed, pumping back an equal amount of He into the incubation bottles to maintain atmospheric pressure. The resulting dilution (approx. 1.0% per injection) and the leakage of O_2 and N_2 into the bottles were estimated based on empty flasks with air and He, respectively. Bottles filled with certified standards were included into the sampling sequence for calibrating the incubation system.

S2 File. Constituents removed from BC through leaching

Table A. Constituents removed from BC through leaching with water and strong acid (HCl)

Leaching procedure	**Biochar constituents ($cmol_c$. kg^{-1} dry BC)**								**% (w/w)**	
	Ca^{2+}	K^{+}	Mg^{2+}	Na^{+}	SO_4^{2-}	NO_3^{-}	Cl^{-}	PO_4^{3-}	DOC	TN
Water leaching of cacao shell BC	0.52	153.64	4.57	0.69	5.76	0.01	1.75	2.50	0.02	0.00
Acid leaching of cacao shell BC	17.54	22.39	20.89	0.21	0.30	0.00	3.19*	0.24	0.00	0.00
Total	**18.06**	**176.02**	**25.46**	**0.90**	**6.06**	**0.01**	**1.44***	**2.75**	**0.02**	**0.01**
Water leaching of rice husk BC	0.17	14.04	0.62	0.35	0.85	0.00	0.92	2.19	0.02	0.00
Acid leaching of rice husk BC	7.93	6.92	5.10	0.11	0.11	0.00	14.85*	0.73	0.00	0.00
Total	**8.09**	**20.95**	**5.72**	**0.45**	**0.95**	**0.00**	**13.92***	**2.92**	**0.02**	**0.00**

For details on water and acid leaching of BCs, see Materials and Methods. Total constituent is what was removed from acid leached BC. DOC – Dissolved organic carbon, TN – Total nitrogen. Cations were measured using ICP-OES (Perkin Elmer, USA), anions using Lachat IC5000 Ion Chromatograph w/XYZ Autosampler (Zellweger analytics, Inc., IL USA), DOC was analyzed using a Total Organic Carbon Analyzer (Shimadzu Corp., Japan), TN was analyzed using a CHN analyzer (CHN-1000, LECO USA). * means that Cl^{-} was added to BC from HCl during leaching experiment.

S3 File. Mean oxygen consumption during oxic incubations and kinetics of gas production (N_2, N_2O, NO, CO_2) and consumption (O_2) during anoxic incubations

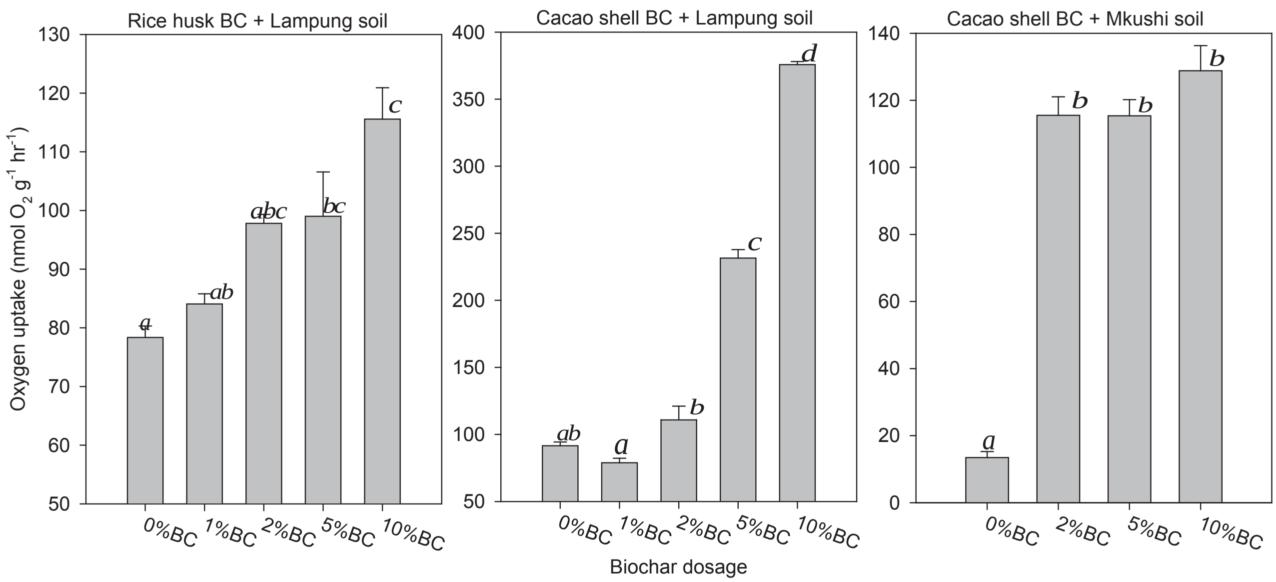


Figure A. Mean oxygen consumption in BC amended soils during oxic incubations. Treatment mean is the average of 3 replicates with standard error. Treatment means followed by different letters are significantly different (P<0.05). Note different scaling of y-axis.

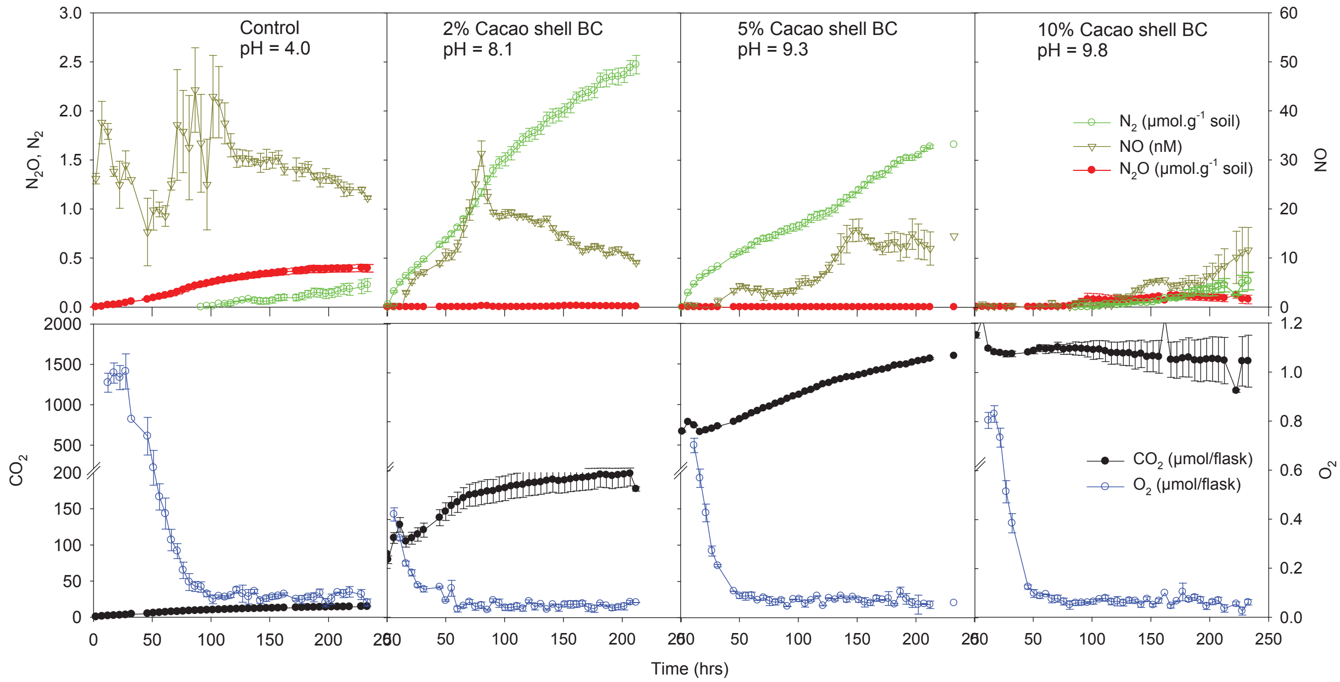


Figure B. Denitrification kinetics and CO_2 and O_2 concentrations in incubations of Mkushi soil amended with untreated cacao shell BC. Shown are averages of three incubations; error bars denote SE. Approximately 7.2 µmol NO_3^--N g^{-1} was added to 8.3 g soil in the bottles.

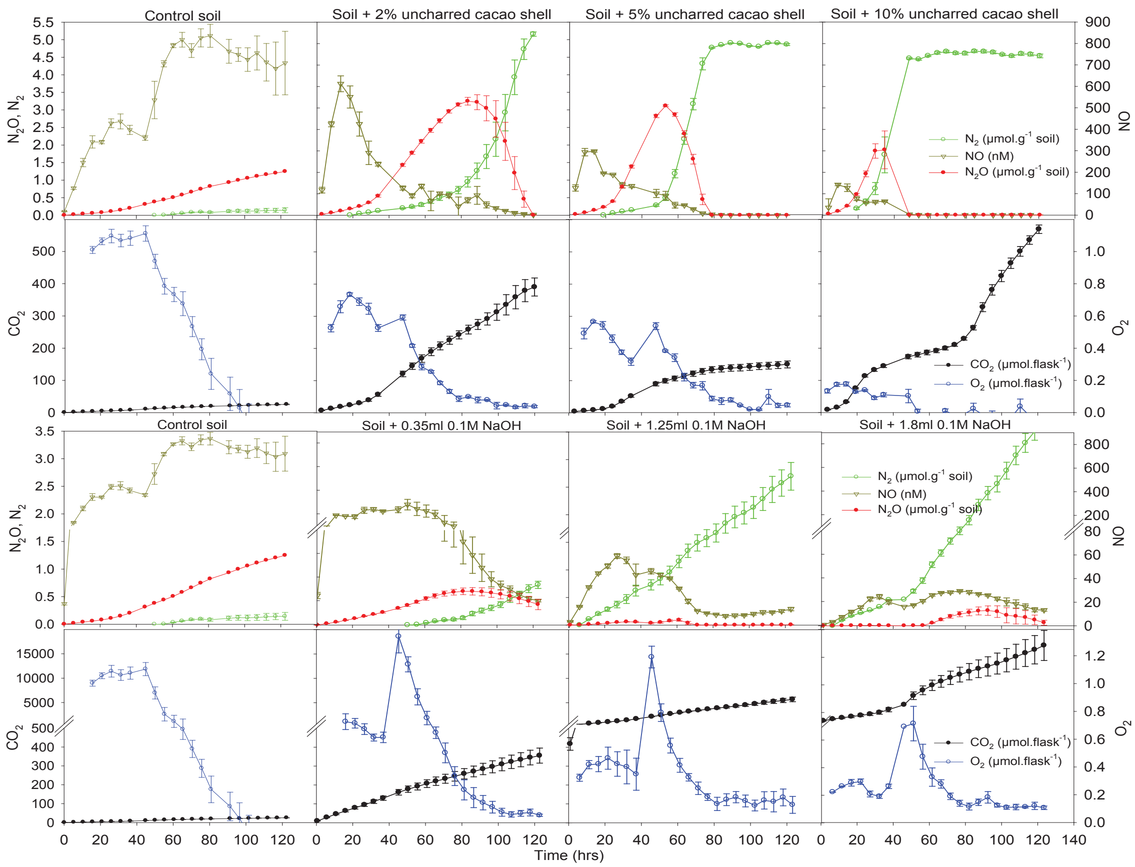


Figure C. Denitrification kinetics and CO_2 and O_2 concentrations in incubations of Lampung soil amended with uncharred cacao shell (upper 2 panels) and 0.1M NaOH (lower 2 panels). Shown are averages of three incubations; error bars denote SE. Approximately 7.1 μmol NO_3^--N g^{-1} was added to 8.4 g soil in the bottles.

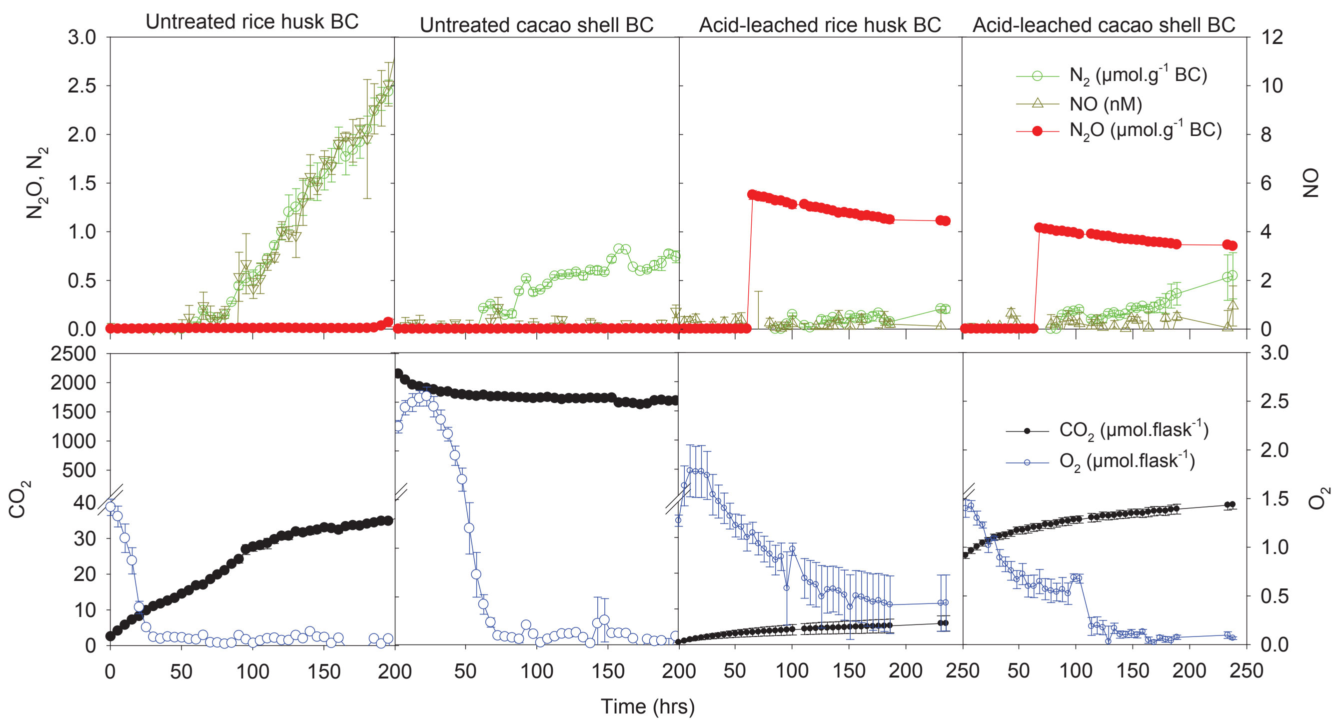


Figure D. Denitrification kinetics and CO_2 and O_2 concentrations in anoxic incubations of 2.36 g BC without soil in 30 ml 2mM KNO_3. Acid-leached BCs were spiked with N_2O gas (0.1 ml at 1 atm pressure) at 65 hrs of incubation. Shown are averages of three incubations; error bars denote SE. Approximately 25.38 µmol NO_3^--N g^{-1} BC was added to the bottles.

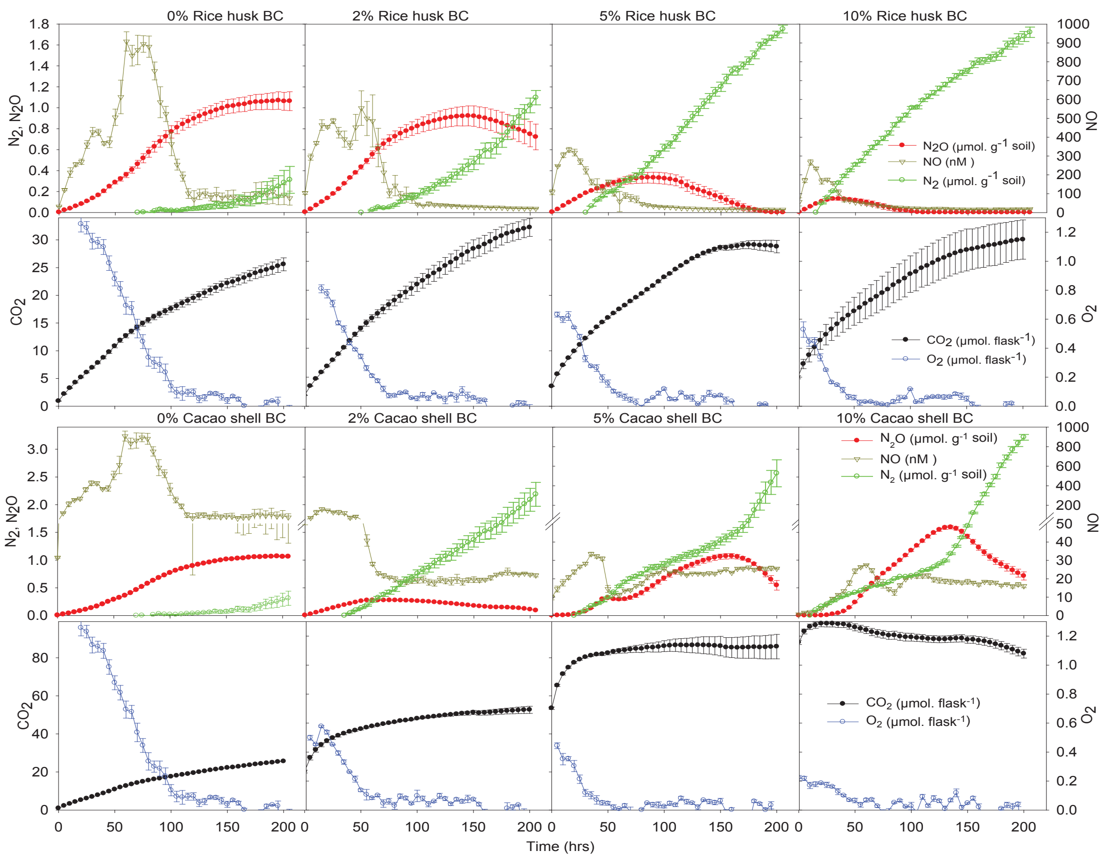


Figure E. Denitrification kinetics and CO_2 and O_2 concentrations in incubations of Lampung soil amended with water-leached rice husk BC (upper 2 panels) and cacao shell BC (lower 2 panels). Shown are averages of three incubations; error bars denote SE. Approximately 7.4 µmol NO_3^--N g^{-1} was added to 8.1 g soil in the bottles.

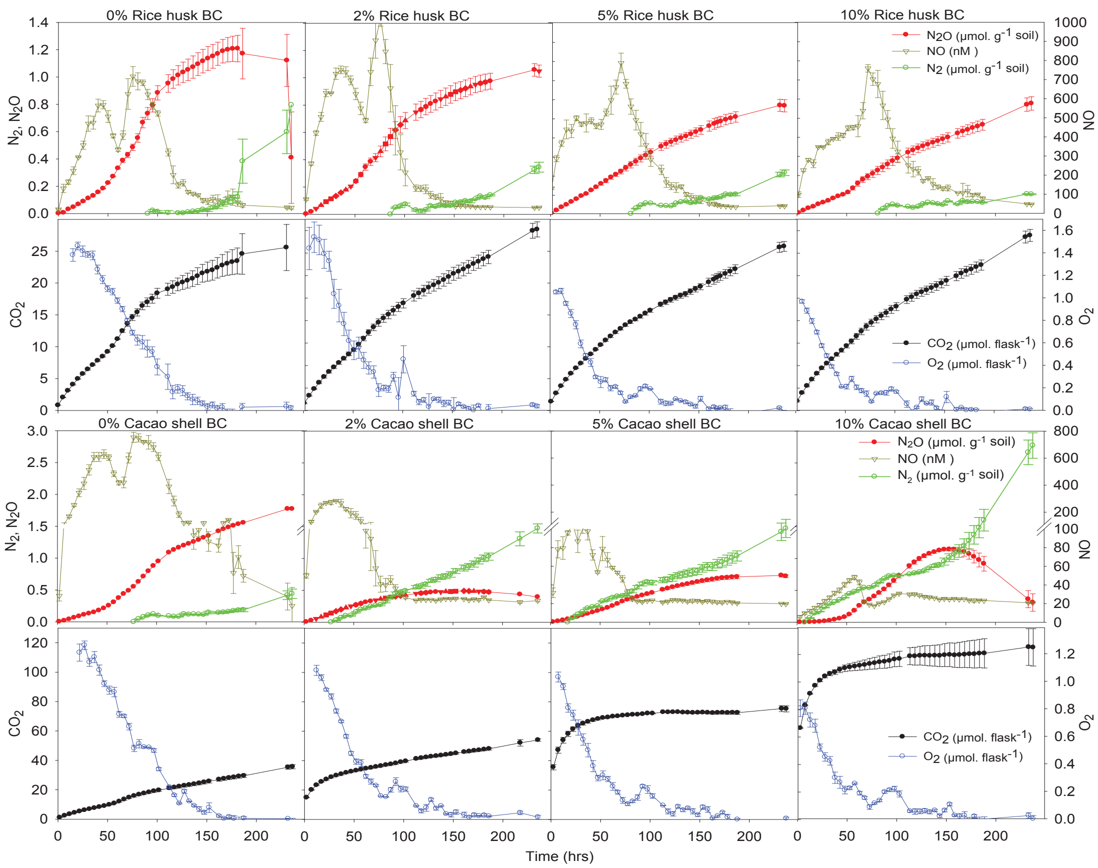


Figure F. Denitrification kinetics and CO_2 and O_2 concentrations in incubations of Lampung soil amended with acid-leached rice husk BC (upper 2 panels) and cacao shell BC (lower 2 panels). Shown are averages of three incubations; error bars denote SE. Approximately 7.6 µmol NO_3^--N g^{-1} was added to 7.9 g soil in the bottles.