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Biochar as a Beneficial Soil Amendment in Sandy Soils

A long term field and greenhouse study to improve the mechanistic understanding

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Dedicated

to

my parents, family

&

beloved son, Saihan Haider

with love and gratitude

If we knew what it was we were doing, it would not be called research, would it?

(Albert Einstein)

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List of abbreviations

Al	Aluminum
BC	biochar
BC_{fresh}	Fresh biochar
BC_{aged}	Field aged biochar
CEC	Cation exchange capacity
C	Carbon
Ca	Calcium
CH_4	Methane
CO_2	Carbon dioxide
Cu	Copper
EC	Electrical conductivity
EUF	Electro-ultrafiltration
GC	Gas chromatograph
GHG	Greenhouse gas
GHGs	Greenhouse gases
Gt	Gigaton
GWP	Global warming potential
HAP	Humic acid product
IPCC	International Panel on Climate Change
K	Potassium
KCL	Potassium chloride
KNO_3	Potassium nitrate
M	Molar
Mg	Magnesium
MRT	Mean residence time
N	Nitrogen
Na	Sodium
N_2O	Nitrous oxide
NH_3	Ammonia
NH_4^+	Ammonium
NH_4Cl	Ammonium chloride
NO_3^-	Nitrate

OC	Organic carbon
P	Phosphorous
PAH	Polycyclic aromatic hydrocarbons
pH	potentia Hydrogenii
SBD	Soil bulk density
SOC	Soil organic carbon
SOM	Soil organic matter
TOC	Total organic carbon
VOCs	Volatile organic compounds
WHC	Water holding capacity
Zn	Zinc

Summary

The growing awareness about increaseing human population numbers and demands and the concomitant global climate change is an all-embracing subject for humankind. Therefore, it has triggered significant research efforts to meet food security and enable climate change mitigation. Biochar (BC) production and soil application has been proposed as a potential means to improve soil quality and crop production whilst serving carbon sequestration in the face of global climate change. In particular, crop water and nutrients supply is a limiting factor to crop production, even in temperate regions as climate warms, and as the frequency and severity of drought spells increases. Meanwhile, many BC studies have been conducted under greenhouse and field conditions. However, there is a dearth of investigations of BC effects on a soil-plant-atmosphere continuum, nutrient supplies and crop production under temperate field conditions.

Therefore, the major aims of this thesis were to quantify the effects of BC amendment (alone or in combination with humic acid product (HAP)) on (a) soil-plant-water relations, (b) nutrients availability (both, macro- and micronutrients), (c) growth and yield of cereals under drought conditions. Furthermore, to identify the impact of BC addition on mineral nitrogen (nitrate (NO_3^-) and ammonium (NH_4^+)) retention and greenhouse gas (GHG) emission were investigated. In order to accomplish these goals, laboratory, greenhouse and field studies were carried out during 2012 to 2015, using two levels of biochar amendment (15 and 30 t ha^{-1}) in combination with two watering treatments (60 and 25 - 30% soil water holding capacity WHC in greenhouse and irrigated versus rainfed under field conditions).

The WHC and greenhouse gas (GHG) emissions of BC-amended soil were carried out under controlled conditions. The impact of BC/HAP addition on water and nutrients supply was determined by monitoring the following ecophysiological parameters: water relations (leaf osmotic potential, relative water content, stomatal resistance, and leaf transpiration), leaf photosynthesis (photosystem II photochemistry) and final growth and yield of plants.

The results of growth and yield improvement (as observed in the greenhouse) were tested on diverse crops under field conditions for four years. In addition, following the initial observations of greater NO_3^- retention in BC amended soil under greenhouse study, the type and strength of mineral N retention in soil, fresh and field aged biochar were investigated with different mineral N extraction methods.

The results of preliminary greenhouse studies revealed that BC addition has the potential to enhance soil WHC and to mitigate soil GHG emissions. The increased moisture supply with BC amendments was able to maintain soil nutrient supplies under both water supply conditions, thereby increasing plant growth and biomass yields. The loading of HAP on BC had no significant effect on soil-plant-water relations, photosynthesis, and final dry matter yield.

However, the results of the greenhouse study could not be transferred directly under field conditions during 2012 - 2015. In the year 2012, freshly incorporated BC increased soil moisture, caused manganese (Mn) deficiency and reduced N uptake thereby reducing grain yield of the first crop. Supplying micronutrients in addition with N fertilization (2013) did not increase N uptake but soil N content and had no significant effect on grain yield of the second crop. Interestingly a higher NO_3^- retention was observed in topsoil (0 - 15 cm) of BC amended plots since the start of the study.

Furthermore, the higher NO_3^- retention of BC amended soils was confirmed by deep (0 - 90 cm) soil sampling during 2014. To test the availability of the stored soil N the subsequent third crop was not supplied with N fertilizer. Unfortunately, even though N concentrations were higher in BC amended soils they were not available to plants and caused a reduction in N uptake and grain yield.

In the last year of field study (2015), there was no significant effect of BC addition on N uptake or grain yield. However, a prolonged drought spell (32% less precipitation compared to 396 mm as the 30-year average precipitation) during crop growth season (April - October 2015) caused reduced soil moisture, N uptake and grain yield of the fourth crop. Contrary to the expectations from the greenhouse study, the BC application did not alleviate drought impact on grain yield of the crop under field conditions.

The increased NO_3^- retention of the BC amended soil is in strong contrast to the hypothesis that there will be higher NH_4^+ retention in BC amended soil where the positive charge of NH_4^+ enables sorption on negatively charged BC surfaces. The latter hypothesis could not be confirmed. Instead, subsequent investigations revealed that the increase of NO_3^- retention was associated with BC particles. In addition, the binding of NO_3^- by BC particles was so strong that standard extraction methods (2 M KCl and electro-ultrafiltration (EUF)) were inefficient to extract all captured NO_3^-. Therefore, new N extraction approaches were developed in this thesis.

Surprisingly, the greenhouse and field studies gave inconsistent results. It suggests that the BC application has a potential to improve plant growth and yield under ideal greenhouse conditions but these results could not be reproduced under complex field conditions. The reduced availability or retention of macro and micronutrient nutrients due to BC addition, shown only under field condition, should be studied intensely in further investigations. Based on the results of the present study, it would also be desirable to study the effects of mixed biochar/compost applications. In this work, only one type of biochar was tested and it is therefore not possible to generalize the results shown.

The considerable binding strength of the BC for NO_3^- shown in this thesis appears to be rather counterproductive from the point of view of N availability for better crop growth and yield. However, the NO_3^- retention property of BC may reduce NO_3^- leaching while increasing C sequestration. Thus BC can have the potential to mitigate the effects of global climate change and may act as a long-term N buffer.

Taken together, the results of this study suggest that a further optimization of BC application (in combination with other additives or using small doses every year rather than a single high dose application at once) may be more beneficial to improve its use for ecological and economical purposes.

Zusammenfassung

Die Auswirkungen des Bevölkerungswachstums und der globalen Klimaveränderungen rückt zunehmend in das Bewusstsein der Weltöffentlichkeit. Eine Folge davon ist eine signifikante Erhöhung der Forschungsaktivitäten um die Nahrungsmittelsicherheit zu gewährleisten und generell globale Klimaveränderungen abzumildern. Als Möglichkeit zur Verbesserung der Bodenqualität, der Ernteerträge und der C-Sequestrierung wird u.a. die Produktion bzw. die Verwendung von Biokohle (BC) als Bodenzusatz diskutiert. Das gilt speziell auch für die Verbesserung der pflanzlichen Wasserversorgung, die nach Klimaerwärmung und in Folge zunehmender Trockenperioden auch in gemäßigten Breiten einen begrenzenden Faktor bei den Ernteerträgen darstellt. Inzwischen wurden diverse teilweise widersprüchliche BC Studien unter Labor-, Gewächshaus- und Feldbedingungen. Zur Ermittlung der Eignung und Wirkungsweise von Biokohle erscheinen besonders wichtig vertiefende Untersuchungen der Auswirkung von BC auf das Boden-Pflanze-Atmosphäre Kontinuum, auf Ernteerträge unter gemäßigten Feldbedingungen und auf das ökologische Gleichgewicht.

Deshalb bestanden die Hauptziele dieser Dissertation darin (a) die Auswirkung der BC Zugabe (einzeln oder in Kombination mit Huminsäure (HAP)) bei Trockenheit auf die a) Boden-Pflanze-Wasserverhältnisse, b) Nährstoffverfügbarkeit (sowohl Makro- als auch Mikronährstoffe) c) Wachstum und agrarwirtschaftlichen Gewinn zu quantifizieren. Weiterhin sollten d) die Auswirkungen von BC auf die Stickstoffretention im Boden und die Emission von Treibhausgasen untersucht werden. Um diese Ziele zu erreichen wurden zwischen 2012 bis 2015 umfangreiche Gewächshaus-, Labor- und Felduntersuchungen durchgeführt mit zwei BC-Stufen in Kombination mit zwei Bewässerungsstufen (im Gewächshaus: 60 % und 25 - 30 % Wasserhaltekapazität WHC, im Feld: Bewässerung und Regen).

Die Bestimmung der WHC von biocharhaltigen Böden und der klimarelevanten Spurengase (GHG) erfolgten unter idealisierten Gewächshausbedingungen. Die Auswirkung von Biokohle- und Huminsäurezugabe auf die Wasser- und Nährstoffverfügbarkeit im Boden und die pflanzliche Reaktion wurde dort mit Hilfe der folgenden ökophysiologischen Parameter bestimmt: Wasserhaushaltsparameter (Blattosmotisches Potential, relativer Wassergehalt, stomatärer Widerstand und Transpiration), Blattphotosynthese (Photosystem II Photochemie) und Wachstum sowie Ertrag.

Die Ergebnisse der Gewächshausstudie zeigten, dass die BC Zugabe zu einer Verbesserung des Wasserangebots sowohl bei begrenzter als auch optimaler Bewässerung und zu

verminderten GHG Emissionen beiträgt. Das durch BC Zugabe erhöhte Wasserangebot ermöglichte unter Gewächshausbedingungen auch eine kontinuierliche Nährstoffversorgung bei Trockenheit, was maßgeblich zu einer Verbesserung der pflanzlichen Leistung, sowie Wachstum und Ertrag führte.

Die im Gewächshaus beobachte Zunahme von Wachstum und Ertrag wurden in den folgenden vier Jahren mit verschiedenen Nutzpflanzen unter Feldbedingungen überprüft. Anlässlich der höheren NO_3^- Retention in biocharhaltigen Böden unter Gewächshausbedingungen wurde zusätzlich der Typ und die Stärke der mineralischen N- Retention in Boden sowie in frischer und im Feld gealterter Biokohle mit drei verschiedenen N- Extraktionsmethoden untersucht.

Die Ergebnisse der vorausgehenden Gewächshausstudien zeigten, das Biocharzugabe zu einer Erhöhung der WHC und Abnahme der GHG Emissionen beiträgt. Das hohe Feuchtigkeitsangebot in biocharhaltigen Böden sorgte außerdem auch bei Trockenheit für ein ausreichendes Mineralstoffangebot und bewirkte somit höheres Wachstum und höheren Biomasseertrag. Die Zugabe von HAP zu biocharhaltigen Böden hatte keine signifikanten Auswirkungen auf den Boden-Pflanze-Wasserhaushalt, die Photosynthese und den Trockenmasseertrag.

Die Ergebnisse der Gewächshausstudie konnten nicht problemlos auf Feldbedingungen übertragen werden, die in den Jahren 2012 bis 2015 erfolgten. Im ersten Jahr der Feldstudie (2012) bewirkte die BC-Zugabe niedrigere Kornerträge bei der ersten Nutzpflanze und es traten sogar Mn- Mangel Erscheinungen auf. Die verbesserte Mikronährstoffgabe bei BC Applikation im Jahr 2013 bewirkte keine Zunahme der N-Verwertung jedoch des Boden-N-Gehalts und hatte keine signifikante Auswirkung auf den Kornertrag der zweiten Nutzpflanze.

Interessanterweise wurde seit Beginn der Studie eine erhöhte NO_3^- Retention in der Oberschicht (0 - 15 cm) biocharhaltiger Böden beobachtet. Eine erhöhte NO_3^- Retention biocharhaltiger Böden wurde im Jahr 2014 durch tiefere Bodenprobenahmen im 0 - 90 cm Bodenprofil bestätigt. Zur Überprüfung der Verfügbarkeit von gespeichertem Boden-N wurde bei der folgenden dritten Nutzpflanze auf die Zugabe von N Dünger verzichtet. Bedauerlicherweise war das in biokohlehaltigen Böden erhöhte N Angebot nicht pflanzenverfügbar und führte sogar zu einer reduzierten N Aufnahme und verringertem Kornertrag.

Im letzten Jahr der Feldstudie (2015) verminderte eine lang anhaltende Trockenheit (32 % weniger Niederschlag zum langjährigen Mittel von 396 mm) während der Vegetationsperiode

(April bis Oktober 2015) nachhaltig die Bodenfeuchte, die N-Akkumulation und den Kornertrag der vierten Nutzpflanze. Entgegen der Erwartung aus den Gewächshausstudien milderte die BC Applikation bei Wassermangelbedingungen nicht deren Auswirkungen auf den Ernteertrag im Freiland.

Die Hypothese, dass eine Beladung der BC mit NH_4^+ eine erhöhte Sorption von negativ geladenen NO_3^- Molekülen bewirkt, konnte ebenfalls nicht bestätigt werden. Stattdessen konnte in weiteren Studien nachgewiesen werden, dass eine erhöhte NO_3^- Retention direkt an die BC Partikel erfolgt Die NO_3^- Bindung an den Biokohlepartikeln war sogar dermaßen stark das Standard Extraktionsmethoden (2 M KCl und Elektro-Ultrafiltration (EUF)) nicht ausreichten um das gebundene NO_3^- freizusetzen. Letztendlich mussten aus diesem Grund neue N-Extraktionsmethoden im Rahmen dieser Thesis entwickelt werden.

Erstaunlicherweise ergaben die Gewächshaus- und Freilanduntersuchungen sehr unterschiedliche Ergebnisse (s.o.). Kernaussage der Gewächshausuntersuchungen ist, dass die Biokohlezugabe unter kontrollierten bzw. idealisierten Bedingungen potentiell wachstums- und ertragsfördernd wirkt. Dieses Ergebnis lässt sich leider nicht ohne weiteres auf komplexere Systeme im Freiland übertragen. Die Verfügbarkeit bzw. Bindungsstärke von Makro- und Mikroelementen an BC in Abhängigkeit von den Boden- und Klimabedingungen im Freiland bietet ein weites Feld für weitere Untersuchungen z.B. über die Vermeidung von Nährstoffmangel. Aufgrund der vorliegenden Studie wäre es erstrebenswert dabei die Auswirkungen von gemischten Biokohle/Kompostapplikationen mit einzubeziehen.

In dieser Arbeit wurde außerdem nur ein Biokohletyp getestet und es ist deshalb nicht möglich die aufgezeigten Ergebnisse zu verallgemeinern. Möglicherweise führen andere Biokohlen zu besseren Produktionsbedingungen im Freiland als die verwendete.

Die in dieser Thesis nachgewiesene erhebliche NO_3^- Bindungsstärke von BC und reduzierte N-Verfügbarkeit ist aus dem Blickwinkel der Wachstumsförderung zunächst eher kontraproduktiv. Die Zurückbehaltung des NO_3^- durch BC vermindert jedoch auch die NO_3^- Auswaschung aus dem Boden und erhöht die C-Sequestrierung. Diese Eigenschaften haben das Potential die Auswirkungen globaler Klimaveränderungen zu vermindern.

Auf Basis dieser Studie scheint es zwingend notwendig die Applikation von BC (im Gemisch mit anderen Additiven oder auch einzeln) zu optimieren und anhand von Vergleichsuntersuchungen oder Reihenuntersuchungen im Freiland nutzbringend für ökologische und ökonomische Zwecke einzusetzen.

1 Synopsis

1.1 Introduction

One of the most pressing global challenges is to mitigate anthropogenic influences on nature and concomitant global climatic changes. For instance, the ever-growing human population has reached to a level where a demand for the “second green revolution” has recently emerged to ensure food security, particularly in the face of global climate change (Smith and Gregory 2013; Foyer et al. 2016). Over-exploitation of land or water resources and industrialization are emerging as the major trade-offs to cope with food security (Glaser et al. 2002; Lal 2009; Schulz and Glaser 2012; Zabel et al. 2014). Furthermore, industrialization and agriculture contribute a significant part (21 and 14% of total, respectively) to the total GHGs emission (Bott 2014), specifically carbon dioxide (CO_2), thereby increasing global warming (Lal 2009; Lal 2010). Global warming, on the other hand, can cause unprecedented effects on the hydrological cycle causing weather extremes such as droughts or devastating floods (Ramanathan and Feng 2009; Wang et al. 2011).

Exhausted agricultural soils are mainly deficient in soil organic matter (SOM), lack biodiversity, and have a low WHC due to low SOM, compaction or salinization, and thus have low fertility and vitality for crop production (Cerda et al. 2009; Vaccari et al. 2011; Bruun et al. 2014; Colazo and Buschiazzo 2015; de Oliveira et al. 2015; Stanchi et al. 2015). Therefore, carbon (C) sequestration in degraded soils has been widely considered as an opportunity to improve soil productivity and at the same time to mitigate global climate change (Lal 2004; Spokas et al. 2010; Vaccari et al. 2011; Smith and Gregory 2013). Green manuring, mulching, residue incorporation, composting, introducing cover crops and decreasing tillage practices have frequently been suggested and practiced to enhance soil fertility (West and Post 1997; Glaser et al. 2002; Six et al. 2004; Stavi and Lal 2013). However, fresh organic residues decompose quickly (Tiessen et al. 1994; Bot and Benites 2005) specifically, under tropical climates (Tiessen et al. 1994) and only a small part of the biomass organic carbon (OC) can be sequestered in soils, the soil OC is quickly decomposed and successively escapes to the atmosphere via CO_2 emission (Fearnside 2000).

Biochar, the product of the process of pyrolysis or charing (incomplete combustion of biomass under limited to zero oxygen supply at temperatures over 250 - 300°C) (Lehmann and Joseph 2015) has been proposed as a soil amendment to sequester recent, photosynthetically fixed atmospheric CO_2-C (Lehmann et al. 2006; Lehmann 2007; Woolf et al. 2010; Karhu et al. 2011). The process of pyrolysis converts approximately 20 - 50% of the biomass-C into polycondensed aromatic C, which is highly recalcitrant against microbial degradation and can persist in the soil for decades to centuries (Kuzyakov et al. 2009; Lehmann et al. 2015). The interest in BC research was triggered by the findings of greater SOM and high soil fertility of anthropogenic soils in Brazilian Amazon basin, the so-called, Terra Preta do Indio (Terra Preta). These soils are famous for having a huge amount of black carbon (a chemical marker of carbonized organic matter), likely due to the addition of recalcitrant carbon, either because of charcoal production, forest fires or due to deliberate practices of slash-and-burn agriculture (Fearnside et al. 1999; Fearnside 2000). However, not all of the biochars may exhibit similar recalcitrance properties (as the charcoal found in Terra Preta) in soils because biochars can vary greatly in their properties, depending on the feedstock, pyrolysis temperature, and other process conditions (Czimczik and Masiello 2007; Lehmann et al. 2015). The low-temperature biochars contain relatively greater amounts, and high-temperature biochars contains relatively low amounts, of labile carbon (Zimmerman et al. 2011). These volatile organic compounds (VOCs) are quickly decomposed by microbes and results in to enhanced soil respiration for a shorter period of time following addition (Smith et al. 2010; Major et al. 2010a; Jones et al. 2011; Mukherjee and Zimmerman 2013). Besides C sequestration, BC amendment has been reported to reduce other GHG (methane and nitrous oxide, CH_4 and N_2O respectively) emissions from agricultural soils (Roberts et al. 2010; Kammann et al. 2012; Clough et al. 2013). Although the exact mechanisms of GHGs emission reduction are still in debate, the recent meta-studies revealed that BC amendments could significantly reduce N_2O and CH_4 emission (Van Zwieten et al. 2015; Jeffery et al. 2016).

Furthermore, several studies have reported positive effects of BC amendment on physiochemical properties (Cation exchange capacity (CEC), the measure of soil’s water and nutrient retention capacity), electrical conductivity (EC), WHC, porosity, bulk density (BD) and pH of agricultural soils (Kimetu et al. 2008; Spokas and Reicosky 2009; Zhang et al. 2010; Basso et al. 2013; Cornelissen et al. 2013). Nutrient transformations in soils play an important role in plant nutrient availability and productivity. Evidence shows that BC amendments can play a significant role in soil nutrients turnover specifically N, phosphorous (P) and potassium (K), directly due to its inherited nutrient concentrations (ashes), or indirectly by influencing soil properties and microbial population growth and activity. Therefore, there is a great interest in BC technology because of its significant impacts on nutrient transformation and crop production (Glaser et al. 2002; Lehmann et al. 2003; Atkinson et al. 2010; Jeffery et al. 2011; Deluca et al. 2015). For instance, BC amendment (30 - 60 Mg ha^{-1}) in a silty soil under Mediterranean field conditions increased wheat yield by 30% compared to the control (Vaccari et al. 2011). Another recent study with two different treatments of BC and NPK fertilizers applications (BC + balanced NPK and BC + imbalanced NPK ratios) revealed that BC + balanced fertilizer (NPK) produced 23% greater maize yield under rainfed conditions compared to BC + imbalanced (NPK) fertilization (Zhang et al. 2016a). Crop yield improvements with biochar amendments are usually associated with better nutrients availability (Lehmann et al. 2003), nutrient transport due to enhanced soil moisture availability and improved plant ecophysiological traits (Rogovska et al. 2014; Haider et al. 2015). However, there are also several examples of no yield improvement following BC amendment (e.g. Tammeorg et al. 2014a). Several studies have reported greater N (NO_3^- and NH_4^+) retention, capture or reduced N availability following BC amendment (Mizuta et al. 2004; Zheng et al. 2013; Kammann et al. 2015; Yuan et al. 2016) with sometimes no yield improvement or little negative effects. However, the exact underlying mechanisms are still under investigations.

Properties of biochars can be engineered in order to achieve desired agronomic and environmental benefits (Yao 2013). For instance, a BC produced from magnesium (Mg) enriched tomato tissues increased phosphorus (P) sorption either by precipitation of P due to chemical reaction with Mg particles or by surface deposition (Yao 2013). Likewise, biochars may produce multiple beneficial effects when used in combination with commercially available humic acid products (HAP). Because the so-called HAP have been reported for improving plant physiological traits (Orlov and Sadovnikova 2005), soil biological activity, CEC, pH buffering, soil water supply and C sequestration (Piccolo et al. 1996; Drozd et al. 1997; Schnitzer 1999).

Various studies have reported that the beneficial effects of biochar may become more visible in degraded, nutrient poor, less fertile soils (van Zwieten et al. 2010; Zhang et al. 2012). For instance, Kimetu et al. (2008) found a 100% increase in maize yield following eucalyptus biochar amendment in degraded soil, while Cornelissen et al. (2013) observed up to 300% yield increase in degraded tropical soil using conservation farming (i.e. direct application of biochar plus fertilizer in planting holes, reducing the amount of biochar needed). The potential effects of biochar amendment on fertile soils usually remain uncertain (Deenik et al. 2010; Gaskin et al. 2010) The major positive influences of BC amendments were generally found in tropical environments, while the effects under temperate conditions are mostly not too positive (e.g. Ruysschaert et al. (2016) and poorly understood (Borchard et al. 2014). Furthermore, a recent review of 768 peer-reviewed publications revealed that only 26% of total biochar studies were performed under field conditions (Zhang et al. 2016b). Field studies often show contrasting results compared to the results obtained in greenhouse studies (Liu et al. 2013; Glaser et al. 2015).

Thus, there are both, a lack of mechanistic understanding of BC amendment-associated processes in soils as well as a lack of reliable field evidence. Therefore, a comprehensive set of greenhouse study under controlled conditions, including mechanistic investigations, along with a long-term field experiment with similar biochar inputs, soil type, and treatments would be ideal to provide basic results for long-term use of BC technology for better crop production and C sequestration.

1.2 Literature overview

1.2.1 Biochar effects on soil properties

The soil physicochemical, hydrological and biological properties and their responses to agricultural production inputs and extreme climatic conditions are the indicators of soil health and fertility. Soil chemical properties including, soil pH, CEC, base saturation percentage, plant nutrient availability and soil organic matter play an important role in soil fertility and crop production. Biochar, generally being alkaline in nature has great potential to neutralize the soil pH (Atkinson et al. 2010; van Zwieten et al. 2010; Jien and Wang 2013). For instance, Castaldi et al. (2011) found a pH change from 5.2 to 6.7 following biochar amendments (3 and 6 kg m^{-2}) in a field soil, sown with wheat during two growing seasons. When applied to soil, the oxidation of aromatic carbon and formation of carboxyl groups on biochar surface may increase the CEC of amended soil (Homburg 2007 Glaser et al. 2003). Eucalyptus biochar amendment significantly increased soil CEC of a degraded soil (Kimetu et al. 2008) and in another study, Laird et al. (2010b) found a 20% increase in CEC and 69% increase in soil organic carbon (SOC) of a Clarian loam soil 500 days after biochar addition.

Soil BD is one of the most important physical characteristics affecting water infiltration, air capacity, and root growth. Biochar, being highly porous in nature (Downie et al. 2009; Lehmann and Joseph 2009; Atkinson et al. 2010; Sohi et al. 2010; Verheijen et al. 2010) and having a high surface area can decrease soil BD after soil amendment (Oguntunde et al. 2008;

Laird et al. 2010b). Decrease in soil BD due to biochar amendment may enhance soil aeration and porosity thereby improving soil WHC (Busscher et al. 2010; Githinji 2014). Tammeorg et al. (2014b) found improved plant available water content in the topsoil (20 cm) and decreased soil BD during the first and second years, respectively, following biochar amendment. Jien and Wang (Jien and Wang 2013) found a significant decrease in soil BD from 1.4 to 1.1 Mg m^{-3}, saturated hydraulic conductivity and soil aggregation by using (*Leucaena leucocephala* (Lam.) de Wit) biochar at 0 and 5% in acidic Ultisol. Biochar amendment in soils can improve soil stability and aggregation (Piccolo et al. 1996; Novotny et al. 2009; Obia et al. 2016).

1.2.2 Biochar effects on soil hydrology

Biochar amendment in the soils has the potential to alter soil hydrology and thereby cause significant changes in soil-plant-water supply and associated ecosystem processes (Lei and Zhang 2013; Peake et al. 2014; Masiello et al. 2015; Liu et al. 2016b). Biochar amendments may directly influence the hydrological cycle by altering soil infiltration and drainage (Basso et al. 2013), soil water content/WHC (Jeffery et al. 2011; Basso et al. 2013), plant available water content *i.e* the amount of water held in soil between field capacity and permanent wilting point (Laird et al. 2010b; Cornelissen et al. 2013; Mulcahy et al. 2013; Hardie et al. 2014) and soil hydrophobicity (Cheng et al. 2008; Joseph et al. 2010; Basso et al. 2013). Biochar effects on soil infiltration and drainage may vary with both (i) the pore size distribution of BC, variations in BC pore size after oxidation, exposure to field conditions or by field ageing (Brewer et al. 2014; Sorrenti et al. 2016) and (ii) soil porosity, soil texture varying from pure sandy to clayey which may cause clogging of biochar pores (Masiello et al. 2015; Sorrenti et al. 2016). Improvement in soil WHC following biochar amendment is considered as the major benefit of biochar use for rainfed agriculture (Jeffery et al. 2011; Sorrenti et al. 2016) as indicated by its capacity to hold up to the tenfold water content than its weight depending on its feedstock and production (Brockhoff et al. 2010; Kinney et al. 2012). However, with woody biochar the WHC is more in the range of 1 - 3 times its own weight, which is still much more than sandy soils (Fig. 4). Since, pure biochars have very high WHC, therefore, it is more important to consider the WHC of soil plus biochar mixtures rather than that of pure biochars only (Masiello et al. 2015). Hence, it is both, biochar and soil properties, which determine the potential for WHC improvement in specific soils following the addition of specific biochars. Likewise, not all biochar amendments may always show improved WHC (Major et al. 2012; Hardie et al. 2014; Jeffery et al. 2015; Rex et al. 2015) or plant available water content (Hardie et al. 2014). There are not many studies concerning the effect of biochar on plant available water content (e.g. Cornelissen et al. (2013), however, Atkinson et al. (2010) predicted that biochar amendments in sandy soil might potentially improve soil available water content. Recently, it has been confirmed by some studies that biochar amendment in soils enhanced the plant available water content (Kameyama et al. 2012; Sun and Lu 2014; Castellini et al. 2015). Soil WHC, or better plant available water content, may directly be influenced by hydrophobic properties of biochar (Gray et al. 2014; Jeffery et al. 2015). Some biochars, particularly low-temperature biochars, may need additional treatments to oxidize their tars and other hydrophobic compounds (Pakdel and Roy 1991; Cornelissen et al. 2005). Jeffery et al. (2015) reported no improvement in plant available water content and attributed the effect to biochar’s hydrophobicity. To summarize, biochar effects cannot be generalized with regard to soil moisture effects, since different biochars have different effects on the hydrology of various soil in terms of infiltration and drainage, WHC, plant available water content and soil hydrophobicity. The ultimate test is plant growth in biochar-amended soil under conditions of water shortage when other factors such as nutrient supply can be ruled out.

1.2.3 Biochar effects on soil nitrogen dynamics

Nitrogen is the most limiting nutrient for crop production (Vitousek and Howarth 2007; Clough et al. 2013; Prommer et al. 2014). It exists in four major forms in the soil such as (i) soil organic matter (humus and plant material), (ii) soil microbes and other organisms, (iii) ammonium ions held by soil organic matter and clay minerals and (iv) freely available NO_3^- and NH_4^+ and (low) concentrations of nitrite (NO_2^-) in the soil solution. Biochar has shown significant influences on almost all aforementioned forms of N, either directly or indirectly (Clough and Condron 2010; Pan et al. 2013).

For instance, Prayogo et al. 2014) showed that Willow (*Salix viminalis*) BC (470°C) reduced N mineralization when added (0.5 - 2%) to a clay loam soil having 2.87 and 0.24% C and N, respectively. Likewise, a woody BC (Oak, 400°C) amendment in a loamy soil decreased plant residues decomposition (Awad et al. 2016). In another study, Dempster et al. (2012b) found decreased SOM decomposition and N mineralization following Jarrah (*Eucalyptus marginata*) BC application in a coarse-textured soil under incubation conditions. In contrast, the poultry litter BC amended in two different soils with relatively greater SOM (~ 9 and 16 g C kg^{-1}) significantly increased N mineralization (Ameloot et al. 2015). Furthermore, in the same study, authors found significantly greater N immobilization following pine chip BC addition in both soils. Moreover, the N immobilization further increased with increasing pyrolysis temperature (Ameloot et al. 2015). Therefore, BC effects on SOM turnover may largely depend on feedstock, pyrolysis conditions and physicochemical properties of BC and soil under investigation (Stewart et al. 2013).

Biochars can possess a large surface area due to their highly porous structure including micropores (specifically when produced at higher temperature) compared to a sandy soil. After soil amendment, it may provide labile organic carbon (Liang et al. 2010) as a substrate for microbial nutrition thereby increasing microbial populations (Lehmann et al. 2003). For instance, Xu et al. (2014) found a significant influence of rice straw biochar (500°C) on soil microbes (as indicated by increased bacterial diversity and relative changes of the taxa), likely due to changes in soil chemical properties influencing soil nutrient cycling (C and N) in an acidic (pH 4.48) acrisol in a greenhouse study. Likewise, in another study, woody BC (Oak, 0.5, 1 and 2%) amendment in a degraded red soil improved soil microbial biomass and enzyme activity (Demisie and Zhange 2015). The *Miscanthus* straw BC (550 - 600°C, 9.3 Mg ha^{-1}) increased about >50% of total soil microbial biomass following 31 months of application in a permanent grassland (Rex et al. 2015). On the contrary, in a more detailed and a long-term (6 years) field study, BC amendment alone showed no effect on soil microbial population. However, the same BC amendment in combination with NPK fertilizers significantly altered the soil microbial community structure (Tian et al. 2016).

The bioavailability of inherited nutrient concentrations of biochars cannot be properly predicted because feedstocks and pyrolysis conditions strongly influence final BC properties (Gaskin et al. 2008; Kloss et al. 2011). Gaskin et al. (2008) found that only 27.4 and 89.6% of total feedstock N were conserved in plant- and manure-based (pine chip and poultry manure) biochars, respectively. Therefore, feedstock and pyrolysis temperature determine the final N concentration of biochars, which

is not necessarily plant available (Ippolito et al. 2015). A number of biochars produced under different experimental conditions were reported to cause nitrate sorption (Mizuta et al. 2004; Mishra and Patel 2009; Yao et al. 2012; Dempster et al. 2012b; Kameyama et al. 2012; Haider et al. 2015; Jassal et al. 2015; Kammann et al. 2015; Haider et al. 2016; Han et al. 2016; Yuan et al. 2016). However, there are some studies reporting no NO_3^- retention with BC amendment. For instance, Gai et al. (2014) reported no adsorption of NO_3^- following amendment with crop residues derived biochars (400 - 700°C, peanut shell, wheat and corn straw). Whilst, there are several studies reporting NH_4^+ sorption/reduced leaching following a variety of biochars amendment under field or laboratory conditions (Lehmann et al. 2003; Güereña et al. 2013; Yuan et al. 2016), but also with no sorption/retention with BC amendment under field conditions (Haider et al. (Chapter 4 under revision); Han et al. 2016).

Hence, soil amendment with various biochars can enhance soil N (total, NO_3^-, NH_4^+) concentration most likely due to following underlying mechanisms (although the exact mechanism(s) is unknown):

(i) Electrostatic adsorption or entrapment (Lehmann et al. 2003).

(ii) Higher pore space and surface area may promote nutrient retention (Ippolito et al. 2015).

(iii) Increased CEC (Taghizadeh-Toosi et al. 2012; Zheng et al. 2013).

(iv) Oxygen-containing functional groups due to short and long-term oxidation (Borchard et al. 2012; Quilliam et al. 2012).

(v) Sorption due to ion exchange (i.e) NH_4^+ sorption via chemo-sorption ammonia fixation, ion exchange, with columbic forces or an association with S-functional groups (Hina et al. 2010; Ding et al. 2010; Yao et al. 2012; Morales et al. 2013).

(vi) Chemistry of surface functional groups may be involved in NH_4^+ sorption (reacting with acid functional groups to form amides and amines) (Spokas et al. 2012) and chemical adsorption of NH_3 to the carboxyl acid functional group on the charcoal surface (Asada et al. 2002).

(vii) Physical entrapment of NO_3^- via anion exchange capacity (Laird et al. 2008; Lawrinenko 2014).

(viii) NO_3^- retention via solution mass flow into BC particles (Prendergast-Miller et al. 2011; Felber et al. 2014; Haider et al. 2015).

(ix) Base functional groups on biochars (woody) produced at 800°C causing NO_3^- adsorption (Kameyama et al. 2012; Kameyama et al. 2016).

(x) The π-π electron donor-acceptor interactions generated through fused aromatic C structures (Swiatkowski et al. 2004; Zhu and Pignatello 2005).

(xi) Physical adsorption (van der Waals adsorption) of NH_4^+ onto BC surface (Zhang et al. 2015).

(xii) Non-conventional ion-water bonding may cause NO_3^- capture in BC particles (Conte et al. 2014; Conte 2014; Kammann et al. 2015).

(xiii) Nitrate capture due to bonding between negatively charged NO_3^- with surface functional groups or positively charged cationic salts on BC surface (Haider et al. 2016).

1.2.4 Biochar effects on crop production

Improved crop productivity is one of the most important and most wanted effects of biochar amendment in agriculture soils (Jeffery et al. 2011; Biederman and Harpole 2013; Jeffery et al. 2015). However, still, there are some uncertainties of BC effects on crop production because the reported results to date vary widely under certain conditions. For instance, several studies have reported an increase in biomass/grain yield of various crops following biochar amendment under greenhouse (Joseph 2007; Rondon et al. 2007; Kammann et al. 2011; Wang et al. 2012; Zhao et al. 2014; Haider et al. 2015), or field conditions (Glaser et al. 2002; Kimetu et al. 2008; Cornelissen et al. 2013; Genesio et al. 2015; Glaser et al. 2015; Zhang et al. 2016a). Biochar addition (30 - 60 Mg ha^{-1}) in a silty loam under field conditions increased wheat biomass (30%) and grain yield with no difference among biochar addition rates (Vaccari et al. 2011). Beechwood biochar (550°C, at 24 and 72 Mg ha^{-1}) addition in temperate soils increased barley grain yield by 10% over control (Karer et al. 2013). The most frequent reasons for such biomass and grain yield improvements of various crops under different soil and environmental conditions were attributed to improved WHC and nutrient availability.

Whilst, there are several studies reporting no effect of biochar addition on crop production. For example, eucalyptus or corn stalk biochar (550°C, 10 Mg ha^{-1}) additions to a fine sandy loam soil showed no effect on corn yield (Free et al. 2010). Likewise, Tammeorg et al. (2014a) found no yield improvement of wheat, faba bean (*Vicia faba*) or turnip rape (*Brassica rapa*) following biochar application in a fertile sandy clay loam soil under field conditions. Therefore, effects of pure biochar application under fertile soil remain usually uncertain, while positive effects of biochar are mainly associated with highly weathered and sandy soils (Jien and Wang 2013). Furthermore, biochar amendment may even negatively influence crop growth and productivity (Asai et al. 2009). Rice straw biochar addition (450°C, 5 Mg ha^{-1}) in a sandy soil reduced rice grain yield by 27 - 35% (Ly et al. 2014). In another study with two different biochars (peanut hull and pine chip biomass, pyrolysis at 400°C) applied at 0, 11 and 22 Mg ha^{-1} with or without nitrogen fertilization, authors found varying results. For instance, peanut hull biochar reduced maize yield at the higher rate of application even with nitrogen fertilization, while pine chip biochar linearly decreased maize yield with increasing rate of addition only during the first year of application (Gaskin et al. 2010).

Furthermore, some studies have reported complex effects of biochar on crop production. For instance, (i) increase in biochar addition resulted in decreasing crop yield (Asai et al. 2009); (ii) negative effects were observed after the first year of addition (Borchard et al. 2014); (iii) negative effects waned and were gone after the first year of addition (Gaskin et al. 2010) and (iv) sometimes lower addition rates of same biochar resulted in decreasing, and higher rates resulted in improving crop yields (Chan et al. 2007). Hence, the BC amendments may produce complex effects, which mainly ascribed to its influence on soil plant nutrient and water interactions leading to different results under varying conditions.

1.2.5 Biochar and plant nutrition

Biochar has the potential to influence plant nutrition either due to several reasons. For instance, *Lantana camara* L. biochar produced at 300°C inherited available P, K, Ca, Mg and Na at 0.64, 711, 5880, 1010 and 1145 mg kg^{-1} respectively (Masto et al. 2013). Moreover, fresh biochar can potentially release approximately 23 - 635 and 46 - 1664 mg kg^{-1} N and P respectively (Mukherjee and Zimmerman 2013). However, the total nutrient concentration in biochar may not necessarily be plant-available and changes in production temperature further influence biochar nutrient concentrations. This applies particularly for the nutrient "nitrogen". For example, Lang et al. (2005), while investigation the effect of pyrolysis temperature (100 - 1100°C) on retention behaviour of carbon, hydrogen, oxygen and N; found that reduction in charred material started at 400°C leading to 50% loss at 750°C of four herbaceous and three wood-based biochars. The reduction in biochar N concentration and availability is attributed to its conversion into heterocyclic N with increasing pyrolysis temperature (Zhang et al. 2013). Hence, biochar produced from different feedstocks may carry different concentrations of nutrients, varying with pyrolysis conditions, resulting ultimately in different plant availability.

The physicochemical properties of biochars play an important role in influencing soil properties thereby improving soil fertility. Evidence show that, the porous structure of BC support greater water retention and thereby improving the most prominent factor (WHC) involving greater crop production (Lal 2008; Sohi et al. 2010; Lehmann and Joseph 2015). Biochar can significantly influence soil pH (Laird et al. 2010a) leading to changes in the forms of soil nutrients (reviewed by Ding et al. (2016)), thereby increasing or decreasing the availability of nutrients. For instance, soybean and maize yield were significantly reduced using charcoal in volcanic ash soil and the yield reduction attributed to charcoal induced changes in soil pH leading to reduced micronutrient availability. Increased CEC due to BC amendment may enhance extractable nutrient elements like Ca, Mg, Na and K (Laird et al. 2010b; Ding et al. 2016). Nonetheless, the improved soil physicochemical properties leading to improve soil fertility and plant nutrition are not necessarily true for all biochar (Ding et al. 2016).

Furthermore, BC has the potential to reduce nutrient (N, P, Mg and silicon (Si)) leaching (Laird et al. 2010b), specifically N in the form of nitrate (Haider et al. 2016; Mandal et al. 2016) thereby increasing chances for N availability to plants. For instance, the availability and plant uptake of P, K, Ca, Cu and Zn were found to increase with charcoal amendment (Lehmann et al. 2003; Major et al. 2010b; Major et al. 2010a; Deluca et al. 2015). Moreover, BC amendment in acidic soils can neutralize pH thereby increasing P and decreasing aluminum (Al) availability (respective toxicity) to plants (Hammes and Schmidt 2009). In recent studies with BC-compost combinations or the use of BC-compost (BC co-composted) it was shown that BC served as a nutrient carrier after co-composting; furthermore, the use in combination with organic fertilizers enhanced plant productivity (Glaser et al. 2015; Kammann et al. 2015). There are serval studies reporting increased N retention or reduced N availability, uptake limitations or immobilization following BC amendment (Lehmann et al. 2003; Knowles et al. 2011; Major et al. 2012; Güereña et al. 2013; Dong et al. 2014; Guo et al. 2014; Haider et al. 2015) Wood-based BC produced at high temperature (>600°C) have been mainly reported for N immobilization or NO_3^- sorption/capture. A full picture of N cycling and soil N transformations with biochar use has not yet emerged.

1.2.6 Biochar and plant ecophysiology

Only a few studies have evaluated the effect of BC amendment on plant ecophysiological parameters (Kammann and Graber 2015). Most of the studies using BC amendments mainly focused on the agronomic yields of crop plants, or on soil parameters indicative of soil fertility, as this usually is the ultimate goal. However, this is a quite human-centric perspective since improved yield may be of great interest for humans, but may not necessarily be helpful for plants regarding their long-term survival in their environment (Kammann and Graber 2015). A greater biomass yield came for example at the expenses of lower defense in Arabidopsis and lettuce (*Lactuca sativa* L.) following BC amendment (Viger et al. 2014).

Thus, investigating plant ecophysiological responses may be helpful to understand biochar effects at the soil-plant interface. Chapter 2 of this dissertation, Haider et al. (2015), is one of the few studies to investigate the effect of BC alone or loaded with HAP on photosynthesis, water and osmotic relations of maize under frequent and limited water supply under controlled conditions. Significant improvements were observed in plant ecophysiological parameters, leading to greater biomass yield with BC amendment. However, the positive yield increases and ecophysiological effects are thought to be mainly regulated by the water saving property of BC (Baronti et al. 2014; Haider et al. 2015). In another study, Akhtar et al. (2014) found improved physiology, yield and quality of tomato under deficit irrigation with more pronounced effects in particular under partial root-zone drying irrigation in BC amended soil under controlled conditions. Rice husk BC (900 - 1100°C) amendment in combination with P in a silty loam soil enhanced rice photosynthetic efficiency and water use efficiency over that of the control under high day or night temperature treatments (Fahad et al. 2016).

Biochar amendment can influence plant physiology not only by interacting via soil-plant-water relations but also by influencing nutrition (excess/deficiency). For instance, wheat Na uptake was reduced due to its adsorption onto BC surface, thereby alleviating the salt stress on wheat (Akhtar et al. 2015). Biochar amendment may not always produce similar positive effects on the physiology of all species. For instance, *Fagus grandifolia* sawdust BC (378°C) amendment did not improve water use efficiency, photosynthetic carbon gain and chlorophyll fluorescence in two broadleaf herbaceous plants *Abutilon theophrasti* and *Prunella vulgaris*, despite sufficient sorption of salts (Thomas et al. 2013). In another study, BC was found to reduce phenolic acid concentrations in a replant soil probably due to sorption mechanisms (Wang et al. 2014a) and to increase plant photosynthesis, height, and fresh biomass. Furthermore, BC amendment in the same study improved the activity of antioxidants (peroxidase, catalase, superoxide dismutase and ascorbate peroxidase) in seedlings (Wang et al. 2014a). Thus, the BC amendment in various soils may produce different physiological effects with respect to the BC used, to plant species, to the soil nutritional status, soil moisture, microbial population and to other ecological aspects.

1.2.7 Biochar effects on carbon sequestration and greenhouse gas emission

Anthropogenic GHGs emission is the leading entity of global climate change, which is growing widely since the pre-industrial era because of fossil fuel use and land use change, due to escalating economic and population growth (IPCC 2014). Historic records suggest that several-thousand-year-old agriculture may have been the first man-made driver of increasing GHG emissions (Crucifix et al. 2005). Currently, agriculture is contributing ~14% to global GHG emissions (IPCC 2014). Of the GHGs, the CO_2 emissions stand first in terms of contribution to radiative forcing, and last in the global warming potential (GWP) compared to other GHGs. One kg of CO_2 has per definition a GWP of 1. One kg of methane (CH_4) and nitrous oxide (N_2O) have GWPs of 25 and 298 times the GWP of CO_2, respectively, over a period of 100 years after the release of the gas to the atmosphere (Myhre et al. 2013). The most important reservoir of the fast-responding terrestrial global C cycle is the soil organic carbon (SOC) pool (otherwise, the largest fast-responding pool is the CO_2 dissolved in ocean water). Therefore, SOC may have the potential to be the major source or sink of GHGs because of its size and fast interaction with the atmosphere (Lal 2004). Arable agricultural lands, particularly the land-use change from native to managed ecosystems, are major sources or of CO_2 emissions from the SOC pool; on the other hand, arable lands can be C sinks with adapted management practices. Therefore, several practices including increasing crop rotation, reduced tillage, introducing cover crops, mulching, and recently biochar amendment to sequester C, improve soil fertility and crop production have been suggested and tested (West and Post 2002; Six et al. 2004; Lehmann et al. 2006; Johnson et al. 2007; Lal 2008). Hence, BC due to its recalcitrant nature and great potential for sequestering C in the soil, and to mitigate other GHG emissions, has been suggested as a potential strategy for climate change mitigation (Woolf et al. 2010).

1.2.7.1 Carbon sequestration

Carbon sequestration is the set of deliberate or natural processes whereby CO_2 can be removed from the atmosphere (vegetation) or diverted from emission and stored in geologic formations, terrestrial environments (i.e soils, sediments, and vegetation) and oceans. Soils are the largest terrestrial C pool (after oceans) having approximately, 1500 - 2400 Gt C (1 Gt (gigaton) = 1 billion tons) at the depth of 1 - 2 m, respectively (Batjes 2014). Moreover, such SOC pool is threefold of current atmospheric CO_2 (~830 Gt C) and 240 folds of CO_2 emission from annual fossil fuel consumption (~10 Gt) on a global scale (Ciais et al. 2013). Thus, even a few percent augmentation of soil C storage may represent a substantial C sink potential (Stockmann et al. 2013; Paustian et al. 2016). For instance, only a 10% change (increase or decrease) in SOC pool would be equivalent to 30 years of anthropogenic emission (and vice versa), and may substantially influence atmospheric CO_2 concentration (Kirschbaum 2000). There are two generalized possibilities of increasing the soil C sink potential. For instance, (a) increasing organic matter inputs and (b) decreasing their decomposition rate, to increase soil C, and reduce atmospheric C stocks, respectively (Paustian et al. 1997). However, incorporation of fresh organic materials (manures and sludge) does not immediately turn into higher soil C stocks as these materials decompose quickly (Bot and Benites 2005), due to their availability as an active organic matter for soil biota. Also, a global scale reduction in mean annual temperature (most unlikely to date) may generally enhance SOC pools (Post et al. 1982). However, it is rather the opposite that is underway at the moment.

Biochar amendment in carbon-poor soils emerged as a promising technology for carbon sequestration due to its recalcitrant nature towards microbial degradation and gaseous emission (Glaser et al. 2002; Lehmann et al. 2006; 2007; Kuzyakov et al. 2009). On an average, about 40% of biomass C is lost during pyrolysis and about 10% during the first years via initial mineralization of BC following an amendment to soil but at least 50% of biomass C regarded as recalcitrant towards degradation (Stavi and Lal 2013). For instance, different biochars, in terms of feedstock and pyrolysis temperature *i.e*, (Pine (Loblolly pine, *Pinus taeda* L.), Oak (Laurel oak, *Quercus laurifolia*), Eastern Gamma grass *(Tripsacum dactyloides* L.*),* Ceader (Eastern red cedar, *Juniperus virginiana* L.), sugarcane bagasse and Bubinga (the tropical hardwood, *Guibourtia demeusei*)) and (250, 400, 525, 650 and 650°C for 72 hours) respectively, were incubated for one year in a laboratory study. The percent carbon loss (modeled for 100 years) via CO_2 emission of these biochars was investigated, which revealed that Cedar BC (400°C) lost a minimum (7%) and Eastern Gamma grass BC (400°C) lost a maximum (27%) carbon. While both (Cedar and Eastern Gamma grass) BC at 650°C lost 3 and 37% carbon in 100 years, respectively (Zimmerman 2010). However, there are some studies showing almost no significant effect on C loss following BC amendment to soils. Wood BC amendment (30 and 60 Mg ha^{-1}) in a wheat field soil for about 420 days showed no significant effects on carbon loss via CO_2 emission (Castaldi et al. 2011). Several studies have reported that BC was highly recalcitrant in soils even under extreme weathering conditions (Schneider et al. 2011; Lehmann et al. 2015).

Soil organic amendments (e.g, BC addition) may cause priming or apparent priming effects on native SOC as observed by Wardle et al. (2008). These include direct and indirect effects on increases or decreases in SOC mineralization following BC amendment (Whitman et al. 2015). For instance (directly), BC may contain energy-rich, degradable organic compounds which, on an amendment to the soil, may trigger increased soil microbial growth and increased enzyme activity leading to all types of native organic matter mineralization (Luo et al. 2013; Whitman et al. 2015). Biochar amendment may indirectly affect SOC mineralization by influencing soil pH (Luo et al. 2013), posing nutrient (e.g, N, P) constraints (Mukherjee and Zimmerman 2013) and changes in the soil faunal and microbial habitat (Pietikäinen et al. 2000). At the same time, BC may induce changes in substrate leading to direct decrease in SOC mineralization (Kuzyakov et al. 2000). Biochar amendment may also decrease SOC mineralization indirectly due its unique properties and effects on the amended soil. For example, (1) sorption of labile native SOC to BC surface or inside BC pores thereby reducing its availability for microbial degradation (Kasozi et al. 2010; Heitkotter and Marschner 2015); (2) influencing the enzyme activity by sorption to BC thereby influencing SOC mineralization (Zimmerman and Gao 2013), (3) influencing organomineral interactions by ligand exchange leading to stable soil aggregation causing less availability of SOC for microbial degradation (Brodowski et al. 2006; Liang et al. 2010) and (4) inducing reduction in nutrient availability may cause reduction in microbial activity.

Thus, there are several possibilities of an increase or decrease in SOC with BC amendment with respect to specific biochars, soils, and environmental conditions. Therefore, intensive research work is needed to identify the specific BC for specific soils for increasing SOC and avoiding priming. This paragraph examined more or less short term responses of SOC stocks to biochar amendment over a few months to a few years. Nonetheless, it has been widely documented that biochars contain recalcitrant carbon which can be sequestered in the soil for 100 of years (Lehmann et al. 2006; Kuzyakov et al. 2009; Kuzyakov et al. 2014; Lehmann et al. 2015).

1.2.7.2 Carbon dioxide emission

Although, CO_2 has relatively less GWP than N_2O and CH_4, but still it is one of the major anthropogenic greenhouse gasses due to huge atmospheric concentration (391ppm), which is approximately 40% higher than pre-industrial era (IPCC 2014). The conversion of organic residues to BC and then soil incorporation, itself can substantially offset net CO_2 emission by converting biomass to highly recalcitrant pyrogenic carbon (Woolf et al. 2010; Thomazini et al. 2015; Liu et al. 2016a); which otherwise may potentially be decompose quickly and escape to atmosphere as CO_2 (Ameloot et al. 2013b). Furthermore, BC amendment in soils may influence CO_2 emission due to various biotic and abiotic factors, such as environmental conditions *i.e* temperature (Hilscher and Knicker 2011), soil types and their disturbance intensity following BC addition, BC type and amendment rates (Singh et al. 2010; Ameloot et al. 2013b). There is a possibility that CO_2 produced (microbial and plants roots respiration or by roots associated mycorrhizal respiration) may precipitate on biochar due to its highly alkaline pH and a plenty of alkaline metals thereby decreasing net CO_2 emission (Lehmann et al. 2011; Xu et al. 2016). On the other hand, BC amendments in soil may also enhance CO_2 emission due to priming of native SOM pool (Kuzyakov et al. 2009; Ameloot et al. 2013a). In addition, the decomposition of abiotically released C from BC associated carbonates or other C containing chemicals sorbed on BC surface may enhance soil CO_2 emission (Cheng et al. 2006; Kuzyakov et al. 2009; Zimmerman 2010; Jones et al. 2011; Bruun et al. 2014). For instance, a recent meta-analysis of 46 studies revealed a 28% increase in CO_2 emission following BC amendments (Sargoli et al. 2015), further, they classified that the biochars produced at <350°C caused greater CO_2 emission independent of the ratios of pyrogenic and soil organic carbon contents. While the biochars produced at >350 to up to 550°C exhibited greater CO_2 emission only when the ratios of pyrogenic and soil organic carbon contents were greater than two (Sagrilo et al. 2015). However, Schimmelpfennig et al. (2014) recently found a negligible effect of BC amendment on soil CO_2 emission and it was attributed to the negligible concentration of carbonates on their BC, which may otherwise be able to shortly enhance CO_2 emission on decomposition. Thus, there are possibilities to optimize the conditions in terms of biochars production and selection of soils for an amendment to enable reduced CO_2 emission.

1.2.7.3 Nitrous oxide emission

Nitrous oxide is one of the most potent GHGs with a largest global warming potential (298 compared to CO_2) and ozone depleting potential (Myhre et al. 2013). Intensive agricultural land use and fertilization, industrialization and fossil fuel combustion are the major sources of atmospheric N_2O (IPCC 2014; Xiang et al. 2015). The atmospheric concentration of N_2O has been increased to 324 (p.p.b.v.) parts per billion by volume compared to its pre-industrialization atmospheric concentration 270 p.p.b.v. (IPCC 2014). Thus, several studies addressed its formation in soils and sediments, its emissions and impacts, as well as mitigation measures. Biochar often been observed to reduce agricultural N_2O emissions (Cayuela et al. 2013; Felber et al. 2014; Sánchez-García et al. 2014) or had no effect (Schimmelpfennig et al. 2014). Recently, Rose et al. (2016) found a cumulative reduction in N_2O emission with poultry litter + wood biochar (4 + 11 Mg ha^{-1}, respectively) amendment under field conditions. The giant reed (*Arundo donax* L.), a perennial grass BC (200 - 400°C) amendment in a silty clay soil reduced N_2O emission when used after removing phenolic compounds

(Wang et al. 2013). Moreover, in the same study polyaromatic hydrocarbons (PAHs) played an important role in reducing N_2O emission with low (300 - 400°C) temperature BC (Wang et al. 2013). In another study, Kang et al. (2016) found a significant reduction in N_2O emission following BC (barley straw BC) amendment in Chinese cabbage fields in an upland soil. Application of seven different biochars in a Luvisol in the presence of urea, NH_4Cl and KNO_3 caused 52 - 84% reduction in N_2O emission (Nelissen et al. 2014). Likewise, Kammann et al. (2012), using various biochars (peanut hull, maize, wood chip and charcoal) in three different laboratory incubation studies found an overall ~60% reduction in N_2O emission. In another recent study, rice husk BC (0, 15 and 30 Mg ha^{-1}) significantly reduced N_2O emission in two of the three different subtropical soils (Nguyen et al. 2016). However, there are also some studies showing no or even increased N_2O emission following different BC amendments (Suddick and Six 2013; Troy et al. 2013; Van Zwieten et al. 2013; Xiang et al. 2015). Nonetheless, a recent meta-study revealed that BC amendments can significantly reduce N_2O emissions (reviewed by Cayuela et al. (2014), and (Van Zwieten et al. 2015). In general, both biotic and abiotic mechanisms of reduced N_2O emission (Cayuela et al. 2013; Clough et al. 2013) may likely be the function of both soil and BC properties (Van Zwieten et al. 2015). Various underlying mechanisms behind reduction or increase in N_2O emission following BC amendment are proposed. These included reduced N mineralization thereby reducing N availability for denitrification (Aguilar-Chávez et al. 2012), BC-induced changes in soil water relation (Yanai et al. 2007), sorption of NH_4^+ to BC surface reducing N availability for nitrification and denitrification and thereby reducing N_2O emission (Berglund et al. 2004; Lehmann et al. 2006). The most prominent mechanism'(s) of N_2O emission reduction with biochar addition to soils have not yet been identified.

1.2.7.4 Methane emission and consumption in soils

Methane (CH_4) is the second most potent GHG with 25 times greater GWP than CO_2 (Myhre et al. 2013). Evidence shows that anthropogenic activities including land use changes, increased N fertilization, deforestation and soil degradation have reduced the soil CH_4 sink potential in the past few decades (Ojima et al. 1993; Galloway et al. 2008; Hansen et al. 2012; Van Zwieten et al. 2015). Recently, Jeffery et al. (2016) reported a 150% increase in CH_4 emission since 1750 and it was mainly contributed by the agriculture sector. Under the anaerobic environments CH_4 produced by methanogenic activity (Feng et al. 2012) of about 50 known species of methanogenic archaea. At the same time, there are methanotrophic proteobacteria, which consume CH_4 to acquire energy. For instance Joye (Joye 2012), reported that a bacterial spp. "*Methoxymirabilis oxyfera*" get oxygen from conversion of nitrite to nitric oxide and further splitting it to oxygen and N to get oxygen for CH_4 oxidation under anaerobic environments. Such methanotrophic bacteria can potentially oxidize about 90% of total CH_4 production (Bosse and Frenzel 1997). Thus, there are two possibilities to reduce CH_4 emission either (1) reducing methanogenic activity to reduce CH_4 production or (2) enhancing a number of methanotrophs to increase CH_4 consumption (Feng et al. 2012). Biochar has been reported to reduce CH_4 emission from a paddy soil (Liu et al. 2011; Feng et al. 2012) and suggested for potentially reducing CH_4 emission from paddy and acidic soils (Jeffery et al. 2016). Dong et al. (2013) found a 47 - 87% reduction in CH_4 emission by adding rice straw BC (22.5 Mg ha^{-1}) in a paddy field compared to direct incorporation of rice straw. Wheat straw BC (500°C) amendment at increasing rates (24 and 48 Mg ha^{-1}) in a paddy field soil resulted in 33 - 40% reduction in CH_4 emission with increasing rate of BC addition, respectively (Liu et al. 2014). The general mechanism of reduction in CH_4 emission following BC amendments has been mainly attributed to inhibition of methanogenic growth or increased methanotrophs (Liu et al. 2011; Feng et al. 2012; Liu et al. 2014). However, in some BC studies, there were increased methanogenic growth contrary to expectations but still reduced CH_4 emission was observed (Feng et al. 2012). This was attributed to the release of dissolved organic carbon from low-temperature biochars (300 - 400°C), which supported microbial growth, but methanotrophs grow relatively better than methanogens (Feng et al. 2012). On the other hand, under aerobic conditions activity of methanotrophs play a significant role for CH_4 consumption. For instance, Khan et al. (2013) reported a 78 - 61% increase in the uptake of CH_4 following sewage sludge BC (500°C) amendment at 50 and 100 Mg ha^{-1}, respectively, in an acidic soil. In another recent study, pigeon pea (*Cajanus cajan* L.) stalks BC (slow pyrolysis, 450°C) enhanced CH_4 consumption in a tropical vertisol (Kollah et al. 2015). Hence, BC amendment in aerobic soils may potentially increase CH_4 consumption via (i) increasing soil porosity and aeration; (ii) decreasing bulk density; (iii) influencing soil water holding capacity with impact on soil diffusivity (Van Zwieten et al. 2015).

1.3 General Objectives and Hypothesis

We chose a rainfed, carbon-poor sandy soil at the experimental station of Justus Liebig University Giessen situated in Groß-Gerau, south of Hessia, Germany. Rainfed soils around the world are facing fertility constraints, occupy about 75% of global cropland area and still contribute about 58% to total food production (Rosegrant et al. 2002; Portmann et al. 2010). Therefore, in this context, the Giessen - Gross-Gerau field study in combination with parallel greenhouse/laboratory investigations was initiated in April 2012. At this time there was a lack of field studies in temperate soils, however, several field experiments started during this time so that today a lack comparable to 2012 can no longer be claimed (e.g. Ruysschaert et al. 2016, a compilation of >30 European field studies).

The general objective of the study was to test how biochar can be used in a temperate sandy soil under irrigated and rainfed conditions (hoping for a drought spell in the field) to improve soil plant water and nutrient relations to achieve better crop production and carbon sequestration.

BC can affect the soil-plant system to influence agronomic yield and provide environmental benefits in following ways:

A. Soil water supply to plants

B. Soil nutrients (especially N) supply and long-term retention (reduced leaching)

Therefore, this study focused on evaluating the behavior of BC in soil ecosystem under field or greenhouse conditions with the following research questions:

1. Does

   a. biochar amendment in a sandy soil increase plant water supply under limited (specifically) and frequent water supply conditions thereby increasing plant ecophysiological traits and biomass yield under greenhouse conditions? (Chap. 2)

   b. humic acid product (HAP) amendment alone or when loaded onto BC produce complementary or synergistic advantages on plant water supply, growth, photosynthesis and biomass yield of maize under frequent and limited water supply under greenhouse conditions? (Chap. 2)

2. Can BC amendment in soil cause N retention? If yes, which form of N (NO_3^- or NH_4^+) is retained? is the N retained in BC-amended soil (e.g with humus formation) or captured in the BC particles? If such a retention exists, does it increase with field aging (of BC or the amended soil), and is it easily extractable with standard extraction methods? (Chap. 3)

3. How can a single application dose of BC influence the agronomic yield of cereals over the subsequent four years? If it causes greater N and water retention and supply to crops, will it increase the grain yield? Theoretically, biochar amendment should improve plant water supply. To what extent will this influence crops yield under prolonged natural drought spells (if they occur)? and finally, does it induce greater soil respiration by a priming effect? (Chap. 4)

To answer and evaluate the **research question 1 a** and **b**, we hypothesized that:

I. BC amendment will enhance the water retention capacity of the sandy soil, thereby improving the soil-plant-water relations and photosynthesis, leading to greater biomass yield compared to the control soil.

II. HAP loading on BC will produce synergistic effects of improving soil-plant-water relations and biomass yield of maize, i.e. HAP loading will improve biochar functions.

III. Any beneficial effect of BC, HAP or their combination will be more prominent under limited than frequent water supply.

To answer and evaluate the research **question 2**, we hypothesized that:

IV. Freshly incorporated BC will initially reduce N supply due to microbial immobilization or NH_4^+ adsorption onto BC particles.

V. There will be no effect of BC aging on N retention and standard methods would be well efficient to extract mineral N from BC amended soil or BC_{aged} particles.

To answer and evaluate the research **question 3**, we hypothesized that:

VI. BC amendment in sandy soil under field conditions will enhance soil water retention properties and plant water supply. By increasing water retention, N retention will also be increased. This will cause an improved, more constant supply of water and nutrients leading to increased crop yields especially under drought conditions.

VII. BC amendment will increase soil respiration only for short period following amendment due to the release of labile organic matter thereby increasing microbial activity. Over time (weeks or months), this effect will vanish.

In order to elucidate the main objective of the study and key research questions, the above-mentioned hypotheses were tested with three sets of experimentation. For instance, questions

1, 2 and 3 were investigated in a greenhouse study provided in this dissertation as "***Chapter 2. Biochar but not humic acid product amendment affected maize yields via improving plant-soil moisture relations***" (Haider et al. 2015), as a field plus laboratory study in "***Chapter 3. Standard Extraction Methods May Underestimate Nitrate Stocks Captured by Field-Aged Biochar***" (Haider et al. 2016), and as the results of the long-term field study "***Chapter 4. Biochar reduced nitrate leaching and improved soil moisture content without yield improvements in a four-year field study***" (Haider et al. 2017). The respective material and methods used in the published papers are given in the respective chapters. However, the general field site description, experimental material, and methods are also provided in more detail below.

Study site and experimental setup

The study site is the experimental research station of the Institute of Plant Breeding and Agronomy I, Justus Liebig University Giessen. It is located in the in the upper Rhine Valley at Gross-Gerau (49°45′N and 8°29′E, 90 - 145 m above sea level) Germany, with the river Main to the North, River Rhine to the west and Odenwald mountains to the east. The aerial overview of the experimental site is shown in Fig. 1.

The climate of the area falls under warm-temperate region with an average (over 56 year) temperature and precipitation sum of the region of 9.8°C and 600 mm, respectively. The average temperature and precipitation of the experimental years (2012 - 2015) are given in Fig. 2. The soil of experimental field is silty sand formed from sand deposits of River Rhine. The soil is characterized as a carbon-poor sandy soil due to very low OC contents (the detailed soil characteristics are shown in Table. 1.

The biochar used in the study was produced at PYREG® GmbH in Dörth, Germany, from a mixture of wood chip shavings of Norway spruce (*Picea abies* L., 70%) and European Beech (*Fagus sylvatica L.,* 30%). The total feedstock comprised a mixture of wood chips, needles, bark, and twig pieces with roughly 30% needles of total feedstock. These sievings are the leftover of wood chip production. The pyrolysis was done at the highest heating temperature of 550 - 600°C. The detailed chemical characteristics and size distribution of BC are shown in Table 1, and scanning electron microscopic pictures of fresh and field aged BC are provided in Fig. 3.

Table 1. Elemental composition, main nutrients, chemical and physical characterization of fresh (BC_{fresh}) and field aged (BC_{aged}) biochar (retrieved from the field after ~ three years of field aging) and soil at the experimental site.

	Soil properties	
Parameter	**Unit**	**Value**
CAL-P	mg kg^{-1}	92.2
CAL-K	mg kg^{-1}	124.5
Mg	mg kg^{-1}	35.5
Organic carbon	%	0.592
Total N	%	0.057
Soil pH	-	6.31
Sand	%	85.2
Silt	%	9.6
Clay	%	5.2
Soil textural class	Silty sand	
	Properties of fresh biochar	
Parameter	**Unit**	**Value**
Carbon	g kg^{-1}	744
Nitrogen	g kg^{-1}	5.6
Hydrogen	g kg^{-1}	<10
Oxygen	g kg^{-1}	100.6
Phosphorous	g kg^{-1}	1.63
Sodium	g kg^{-1}	3.3
Potassium	g kg^{-1}	6.07
Calcium	g kg^{-1}	19.07
Magnesium	g kg^{-1}	2.09
Iron	g kg^{-1}	2.59
Copper	g kg^{-1}	0.02
Zinc	g kg^{-1}	0.17
CEC (K, Na, Ca, Mg) (BC_{fresh})	cmol$_c$ kg^{-1}	19.11
pH (BC_{fresh})	-	9
	Properties of field aged biochar	
CEC (K, Na, Ca, Mg)	cmol$_c$ kg^{-1}	5.54
pH	-	6
	Biochar particle size fraction	
Fraction size	**Unit**	**Values †**
>6.3 mm	%	0.5
3.15 - 6.3 mm	%	24.2
2 - 3.15 mm	%	7.1
1.6 - 2 mm	%	20.9
1 - 1.6 mm	%	4.9
0.63 - 1 mm	%	17
0.1 - 0.63 mm	%	25.3
<0.1 mm	%	0.1

† Values are given in percent of dry mass

Fig. 1. The aerial view of the experimental site. The green highlighted area specifies the main experimental plot. The red line is separating rainfed and irrigated plots. The picture was taken from google maps (https://www.google.de/maps; last accessed: 13.06.2016).

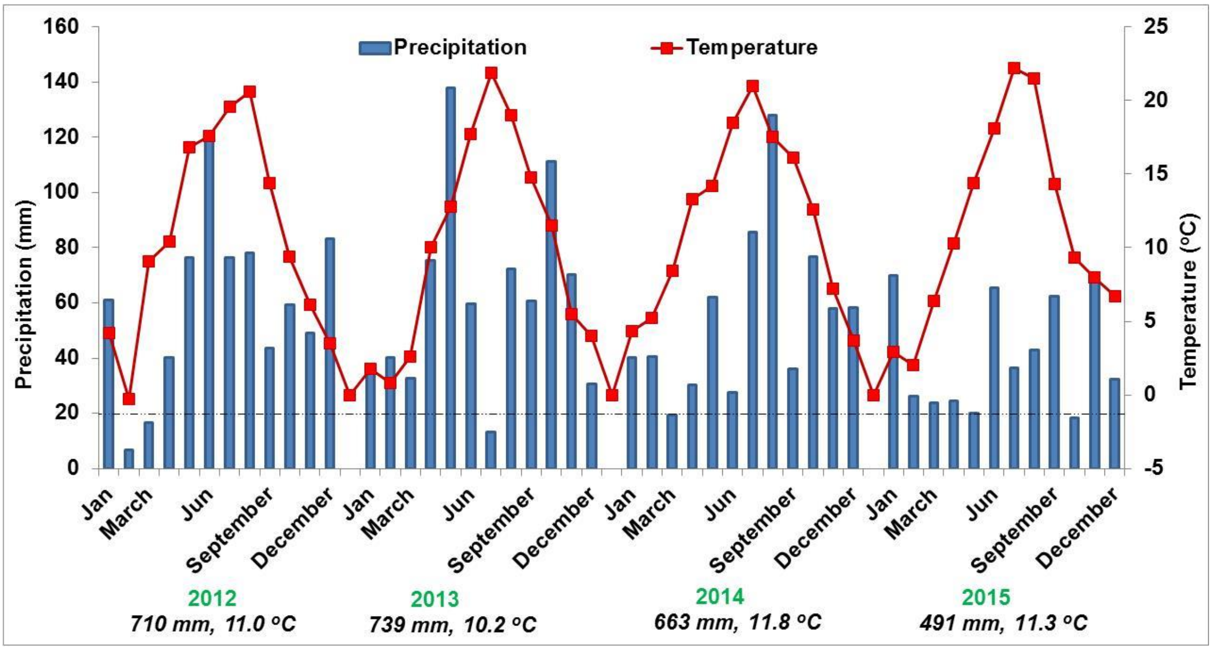

Fig. 2. Mean monthly precipitation (mm) and temperature (°C) during the experimental period. Given values under respective years are total sum and average per annum of precipitation and temperature respectively.

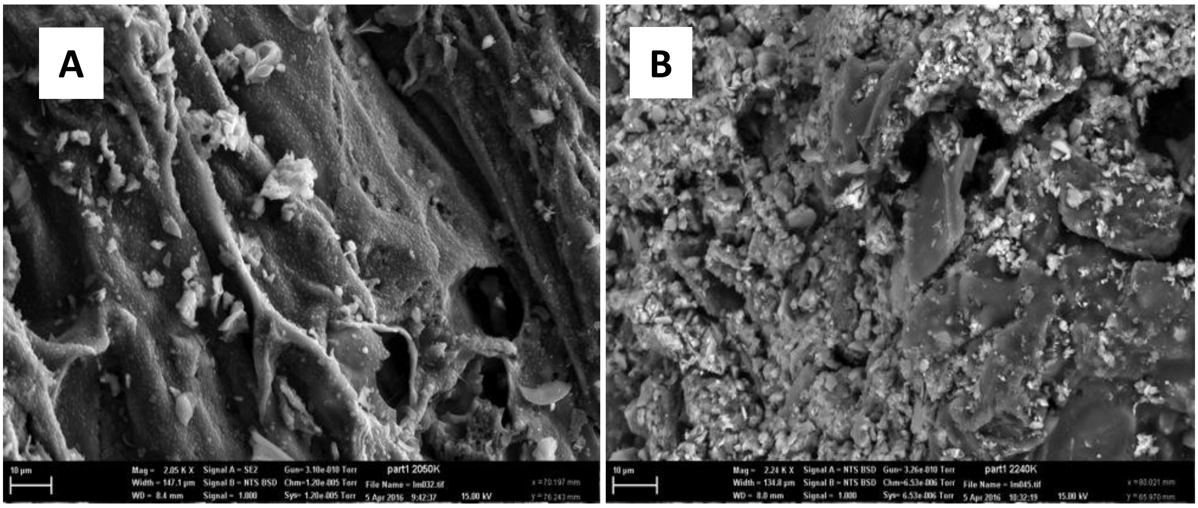

Fig. 3. The electron microscopic picture of fresh (A) and field aged biochar (B) surface taken during April 2016 in China by Prof. Stephen Joseph. Both (A and B) pictures were taken at the same scale (10 µm).

The soil for the greenhouse study was taken from the plow layer of field experiment plots before the application of BC. The soil was passed through 5 mm sieve to remove large crop residues, soil macrofauna, and stones. Initial soil water holding capacity (WHC) and greenhouse gasses emissions were investigated in the laboratory by adding 0, 1, 2, 4 and 8% BC (w/w) in soil. Soil WHC was determined by following the procedure described by Kammann et al. (2011) (results: Fig. 4). The GHG emissions were determined by laboratory incubations of BC amended (0, 1, 2, 4 and 8% BC (w/w)) soil in 1100 ml glass jars (WECK GmbH u. Co. KG, Wehr, Germany).

The WHC of soil plus substrate in the incubation jars (*n*=20) was adjusted to 50% of the mixture maximum, respectively, and then incubated at room temperature (20 ± 2°C) in the laboratory. The GHG samplings were carried out at times 0, 30 and 60 minutes following jar closure. The gas samples were analyzed within 24 hours after sampling at GC (HP 6890 or Shimadzu 14B, Japan).

Greenhouse gas fluxes were calculated by linear regression and related to the total soil (or a soil-BC mixture) weight or to the ground area (pot surface) over the incubation time by using ideal gas law (Equ. 1).

$$\text{GHG flux} = \frac{d[\text{GHG}]}{dt} \times 10^{x} \times \frac{V_{\text{head}} \times P \times 100 \times \text{Mwt}}{R \times T} \times 10^{y} \times \frac{1}{A}$$

Where, d[GHG]/d*t* is the rate of GHG concentration change (ppm h^{-1}; ppb h^{-1}); 10^{x} is a conversion factor (mL $m^{-3} = 10^{-6}$ m^3 m^{-3} or μL $m^{-3} = 10^{-9}$ m^3 m^{-3}); V_{head} is the volume of head space (m^3) of pot under use; P is the atmospheric pressure (hPa); Mwt is the molecular weight of the respective gas (g mol^{-1}); R stands for universal gas constant (= 8.314 J mol^{-1} K^{-1}); T is the temperature in Kelvin; 10^{y} is the conversion factor (g in mg, 10^3 or g in μg, 10^6) and A is the covered soil area (m^2) or soil weight in (kg). The GHG fluxes were expressed in ng and μg of the species or their CO_2 equivalents emitted or consumed per kg of soil (or a soil-BC mixture) per hour. The initial GHG fluxes of all three gasses, CO_2, N_2O and CH_4 from the experimental soil were significantly reduced with BC amendments (Fig. 5).

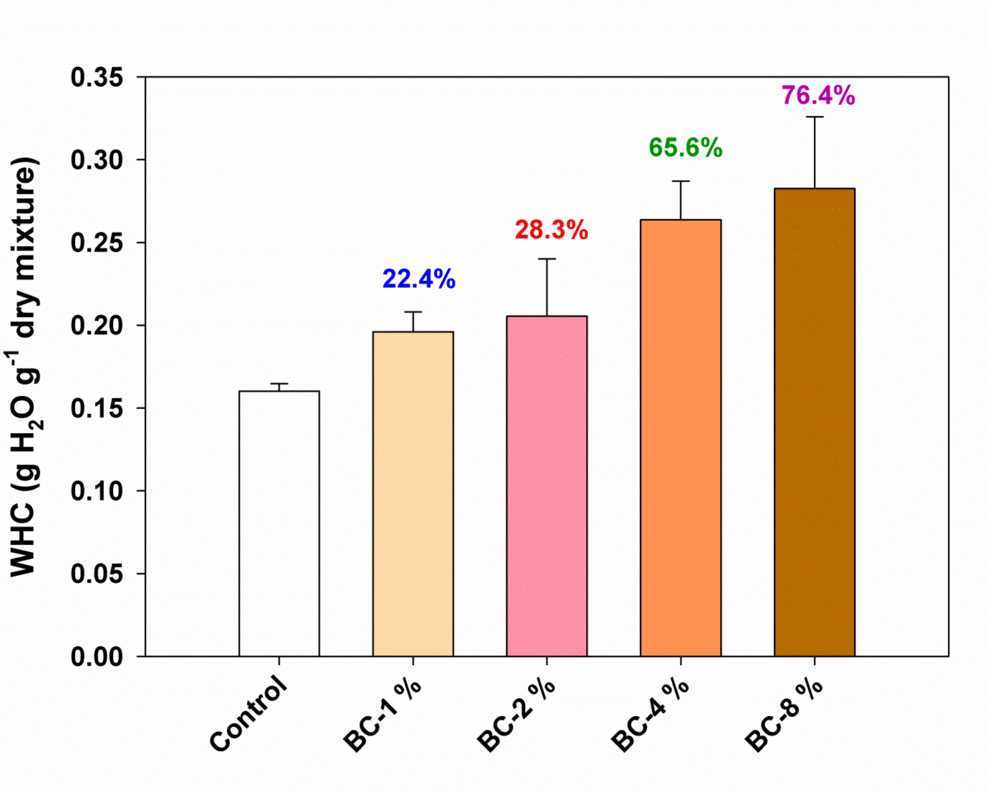

Fig. 4. Water holding capacity of experimental soil following BC amendments (BC, 0, 1, 2, 4 and 8%). The y-axis scale is based on mean values and error bars indicate standard deviations (*n*=3).

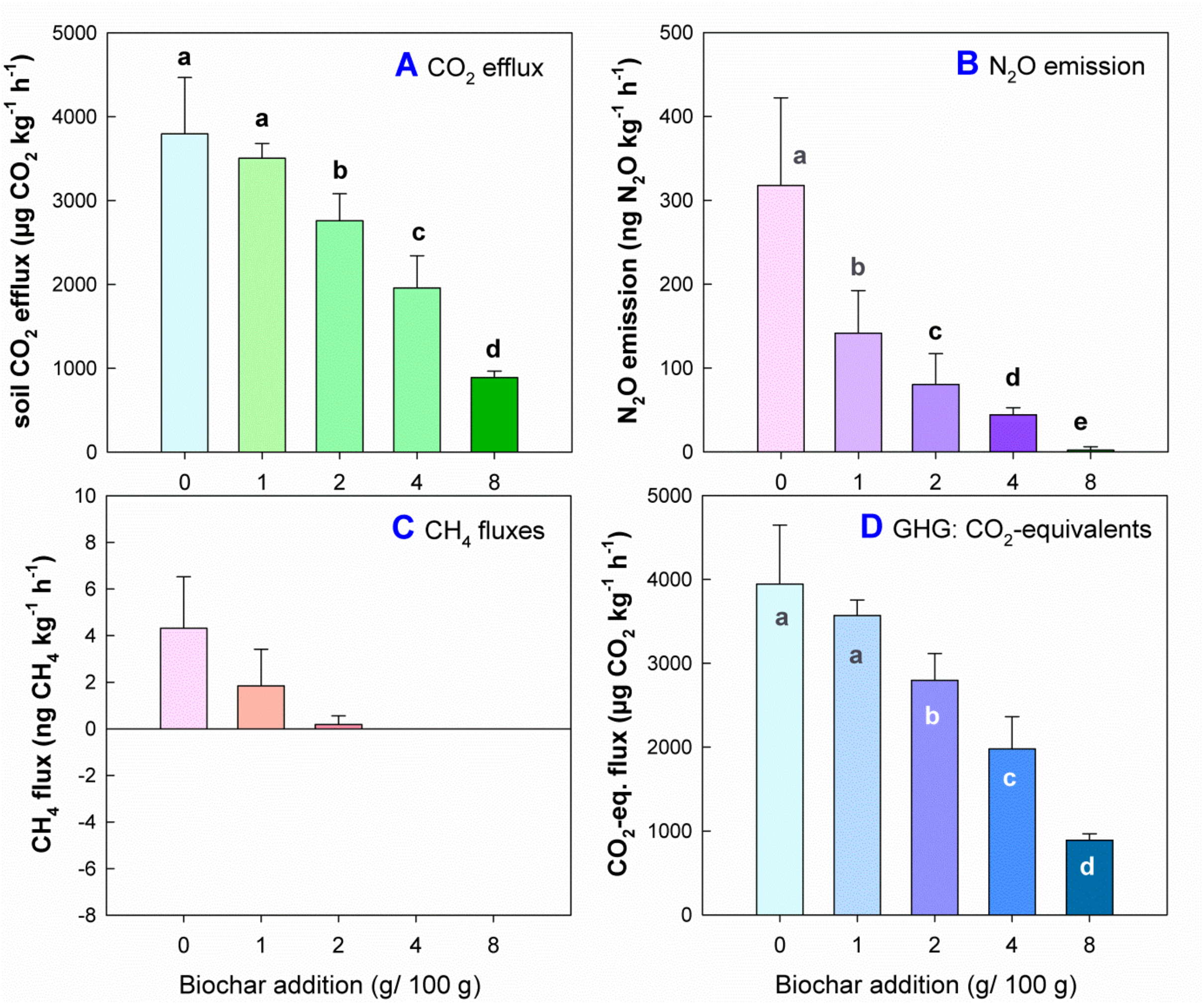

Fig. 5. Greenhouse gas emissions from the experimental soil in Groß-Gerau following BC amendments (0, 1, 2, 4 and 8% w/w) adjusted to 50% WHC_{max}. The y-axis scales of A, B, C and D are based on the respective mean values of the largest gas flux (A: CO_2, B: N_2O; C: CH_4; D: CO_2-equivalents of all three GHGs). The error bars indicate standard deviations, and different letters on bars indicate significant differences due to BC treatment following one-way ANOVA (Tukey HSD test, $p<0.05$, $n=20$).

The field experiment was initiated on April 12, 2012, with a single application of moisture rich BC (with known dry matter weight at 0, 15 and 30 Mg ha^{-1}) in respective plots (4.5 x 7 m). The biochar had been mixed thoroughly on a large foil before the application was started. The layout was a split-plot design where BC treatment was randomized within the irrigation treatments. Two irrigation treatments, rainfed (no irrigation) and irrigation (when needed) were separated by an interspace row (4.5 m wide). The BC was manually spread and incorporated superficially in respective plots (Fig. 6); it was incorporated in 0 - 15 cm depth

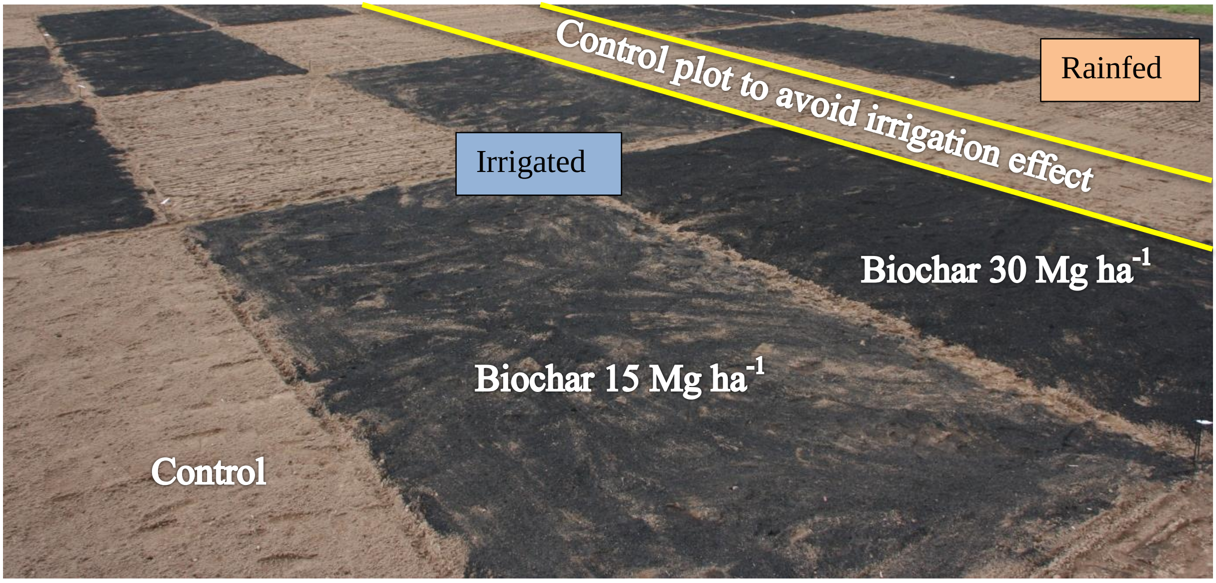

Fig. 6. Picture of experimental plots after BC application according to respective treatments (BC, 0, 15 and 30 Mg ha^{-1}) and watering regimes (irrigated and rainfed).

on the following day by machinery. The crop rotation during four years of experimentation is shown in Table 2.

Table 2. Crops were grown during four consecutive growth seasons during 2012 - 2015.

Crops	**Season**
Winter wheat (Triticum aestivum L.)	Winter - 2012
Peas (*Pisum sativum* L.)	Winter - 2013
Summer barley (*Hordeum vulgare* L.)	Summer - 2014
Peas (*Pisum sativum* L.)	Winter - 2014
Maize (*Zea mays* L.)	Summer - 2015

A detailed description of crop fertilization and irrigation regimes are given in Chapter 4.

2 Biochar but not humic acid product amendment affected maize yields via improving plant-soil moisture relations

"Paper published in the journal of Plant and Soil. 395:141-157 (2015). doi:10.1007/s11104-014-2294-3"

2.1 Publication outline

The publication in the following chapter describes the detailed outcomes of a controlled glasshouse experiment to monitor the effects of BC/HAP on growth, ecophysiological traits and biomass yield of maize under different treatment combinations. The BC/HAP were used alone or in combination under two watering regimes (i.e.) 60% WHC throughout the experiment and limited water supply (gradual reduction in WHC down to 30 - 25% imposed after the 28th day of normal growth at 60% WHC. Plant water and osmotic relations (relative water content, leaf osmotic potential and leaf gas exchange) being the potential players to reveal drought impacts on the plant were monitored. Furthermore, the plant photosynthesis traits like electron transport rate (ETR) of photosystem (PSII), effective photochemical quantum yield (Y(II)), regulated heat dissipation Y(NO), and non-photochemical quenching (Y(NPQ)) were gathered to assess the treatment effects. Finally, plant biomass yield, water and N use efficiency; soil respiration and mineral N (NO_3^- and NH_4^+) were measured after harvest. The main aim of this study was to find out the complementary or synergistic effects of BC and HAP amendment on maize growth under controlled conditions. Besides this, the study enabled to compare the results of BC effects on maize grown under natural rainfed or irrigated field conditions (chapter 4) to those obtained under controlled greenhouse conditions.

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REGULAR ARTICLE

Biochar but not humic acid product amendment affected maize yields via improving plant-soil moisture relations

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Abstract

Aims Biochar (BC) and humic acid product (HAP) soil amendments may improve plant performance under water-limited conditions. Our aim was to investigate if BC and HAP amendments, alone or in combination, will have positive and synergistic effects.

Methods A three-factorial fully randomized study was carried out in the greenhouse for 66 days, including the factors 'BC', 'HAP' and 'water regime'. Maize (*Zea mays* var. 'Amadeo' DKC-3399) was grown in pots (6 kg sandy soil pot^{-1}) amended with/without BC (0, 1.5 and 3 %; *w/w*) and with/without HAP (0 or an equivalent of 8 kg ha^{-1}). Two water regimes, limited and frequent (H_2O_{limit}, H_2O_{frequ}), were applied after day 28 following seedling establishment at 60 % water holding capacity (WHC). In the H_2O_{limit} treatment, the soil water content was allowed to drop until wilting symptoms became visible (25–30 % WHC) while in H_2O_{frequ} the WHC was brought to 60 % of the maximum on a daily basis

Results BC but not HAP, added alone or in combination with BC, significantly increased the biomass yield and the water and N use efficiency of plants at both water regimes. The BC-mediated relative increase in the yield was equal with both watering regimes, refuting initial hypotheses. BC had generally a stimulating effect on water relations and photosynthesis, it increased the relative water content and the leaf osmotic potential, decreased the stomatal resistance and stimulated the leaf gas exchange (transpiration). Both, BC and pure HAP addition, stimulated photosynthesis by increasing the electron transport rate (ETR) of photosystem II (PSII) and of the ratio between effective photochemical quantum yield to non-photochemical quenching (Y(II)/Y(NPQ), revealing reduced heat dissipation.

Conclusions Biochar use in poor sandy soils can improve plant growth by improving soil-plant water relations and photosynthesis under both H_2O_{frequ} and H_2O_{limit} conditions. HAP loading, however, did not improve the effect of biochar or vice versa.

Keywords Biochar · Eco-physiology · Photosynthesis · Humic acid products · Maize · Sandy soil · Water holding capacity · Water stress · Osmotic potential

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Introduction

It is likely that climate change-induced hydrological variations will threaten water resources for both rainfed and irrigated agriculture (FAO 2008). Agricultural drought may impair food security and economic prosperity in number of countries in the world (Schewea et al. 2014). All types of drought (meteorological, hydrological and agricultural) are interrelated, but agronomic drought is reported to be the most frequent (Lal 2009). It is affected mainly by the available water capacity, which depends on the soil properties, especially organic carbon contents and aggregation (Reich and Eswaran 2004; Bot and Benites 2005; Lal 2009), and approaches to alleviate water scarcity in agricultural production usually include soil organic matter (SOM) increases (Lal 2008).

Biochar (BC) has recently been proposed as an option for improving soil fertility, for carbon sequestration and greenhouse gas emission reductions (e.g. Woolf et al. 2010; Lehmann 2007a; Jeffery et al. 2011). BC increases soil organic carbon stocks, i.e. the stable organic matter fraction, and thus may have the potential to alleviate climate change problems (Lehmann 2006, 2007b; Laird 2008; Sohi et al. 2010; Atkinson et al. 2010). It may significantly improve nutrient availability, either by nutrient delivery from the BC itself, or by changes in nutrient retention and cycling, and thus the growth of plants (Glaser et al. 2002; Chan et al. 2007; Renner 2007; Lehmann and Joseph 2009). It has been shown to reduce nutrient leaching (Ventura et al. 2013) and greenhouse gas emissions (Kammann et al. 2012; Cayuela et al. 2013), and it may stimulate microbial activity (Singh et al. 2010). However, positive effects are not always guaranteed (Jeffery et al. 2011). With regard to water supply, it was reported that biochar improved the structure and water holding capacity (WHC) (Brodowski et al. 2006; Clough et al. 2013; Laird et al. 2010; Kammann et al. 2011) and soil hydraulic conductivity of the soil (Steiner et al. 2007; Karhu et al. 2011). Novak et al. (2009) reported an increase in WHC from 6.7 % in control to 15.9 % due to the addition of switchgrass biochar in loamy sand. An increase in the water supply to plants grown on sandy soils amended with BC has been reported by Buss et al. (2012) and Kammann et al. (2011), while others reported improvements in soil characteristics pertinent to soil-plant water relations, including the structure and development of micro-pores (Cheng et al. 2006; Bornemann et al. 2007; Major et al. 2009; Liu et al. 2012).

So-called "humic acid" can be bought as commercial products. They are complex organic molecules mostly generated from Leonardite, a brown coal precursor, as sodium or potassium salts. Such products have been shown to lead to changes in the surface chemistry of soil solids and to improve soil fertility (Amirbahman and Olson 1995). Humic compounds in general can have multiple beneficial effects on soil functions including biological activity, nutrient availability, cation exchange capacity, pH buffering, carbon sequestration, and soil-water relations (Drozd et al. 1997; Piccolo et al. 1996; Schnitzer 2000). They are reported to improve stress tolerance of plants by exerting hormone-like effects and stimulating the activity of microorganisms including those that produce growth promoting hormones (Zhang et al. 2005), e.g. root growth promotion (Vaughan and Malcom 1985; Trevisan et al. 2010). Therefore, theoretically, humic acid products could add to the beneficial effects of fresh BC additions on soil fertility and productivity, particularly under water limited conditions.

Drought stress can severely influence the plant metabolism such as physiological, biochemical, and molecular components of photosynthesis. It primarily causes stomatal closure at the whole-plant level to minimize further water loss (Cornic 1994; Lawlor 1995), ultimately reduces inflow of CO_2 into mesophyll tissue (Flexas et al. 2006) and therefore decreases photosynthesis (Mwanamwenge et al. 1999; Yordanov et al. 2000). Water stress also inhibits the photosynthetic electron transport rate through photosystem II 'PSII' (Chakir and Jensen 1999), reducing the photosynthetic efficiency of plants while increasing non-photochemical quenching (heat dissipation). Thus it can be expected that plant-physiological reactions may detect the effects of BC or HAP on plant-soil water relations before the effects are detectable in the whole-plant biomass yield.

The main aim of the study was to evaluate the complementary or synergistic advantages of biochar and a commercial humic acid product on growth, water relations and photosynthesis of maize at limited and frequent (H_2O_{limit}, H_2O_{frequ}) water supply. We hypothesised that (1) BC amendments to sandy soil will improve the water retention capacity, and thereby increase photosynthesis and plant-soil water relations, resulting in higher biomass yields in a "the more BC the better" manner; that (2) BC loaded with HAP will further improve soil and plant water relations compared to BC alone, and that (3) any beneficial BC and/or HAP effects will, in relative terms, be more pronounced when the water supply is limiting and plants experience drought stress than with frequent water supply.

Material and methods

Experimental setup and growth conditions

The sandy soil used in this study was obtained from the plough layer of the agricultural experimental station of the Institute for Plant Breeding and Agronomy I, Justus Liebig University Giessen at Gross-Gerau, Germany. The site is located (49°45′N and 8°29′E, 90–145 m above sea level) in the upper Rhine Valley with the river Main to the North, River Rhine to the west and Odenwald mountains to the east. The soil was formed from Rhine sand deposits and the agricultural area is frequently irrigated during hot spring or summer dry spells. The soil was silty sand that consisted of 85.2 % sand, 9.6 % silt, and 5.2 % clay. It contains a low amount of organic carbon (0.592 %) and total N (0.057 %), CAL-P 92.2 mg kg^{-1}, CAL-K 124.5 mg kg^{-1}, Mg 35.5 mg kg^{-1} and a pH (0.01 M $CaCl_2$) of 6.31. Before use, the soil was air-dried, thoroughly mixed, and sieved (≤5 mm). Prior to the start of the experiment, the water holding capacity (WHC) was determined for soil or soil-BC mixtures as described by Kammann et al. (2011): the entire Mitscherlich pot was submersed in distilled water for 24 h and then allowed to drain for another 24 h with the soil surface covered. Pre- and post-pot weights were then compared to calculate the WHC in g H_2O per g of dry soil. For seed germination and early plant growth, the WHC of the respective soil/soil-BC mixtures was adjusted to 60 % by daily watering. Growing plant weight in pots was accounted for by harvesting additional replicates that were grown for this purpose.

Biochar was produced from wood-chip sievings at 550–600 °C (Pyreg GmbH, Dörth, Germany). Collectively, the feedstock was comprised of wood chip sievings (needles, bark, twig pieces and small wood chips) of *Picea abies* (70%) and deciduous wood sievings of *Fagus sylvatica* (30%); needles roughly contributed 30% to the total feedstock. It contained 74.4 % C, <1 % H, 10.6 % O, 0.56 % N, 0.163 % P, 0.607 % K, 0.33 % Na, 1.907 % Ca, 0.209 % Mg, 0.259 % Fe, 0.002 % Cu and 0.017 % Zn. The particle size fractions were as follows: >6.3 mm 0.5 %, 3.15–6.3 mm 24.2 %, 2–3.15 mm 7.1 %, 1.6–2 mm 20.9 %, 1–1.6 mm 4.9 %, 0.63–1 mm 17 %, 0.1–0.63 mm 25.3 % and <0.1 mm 0.1 %. The BC, was sieved (≤2 mm) to get a 100 % mixture of particle size between <0.1 and 2 mm. It was oven dried at (105 °C) before use. The humic acid product (HAP; granulated potassium salt, 100 % water soluble) is a commercial product of Humintech GmbH, Germany, marketed as POWHUMUS® WSG 85.

In this three-factorial completely randomized greenhouse study, each of the 36 Mitscherlich pots (0.30 m in diameter and 0.175 m in height; 3 replicates per treatment) was filled with 6 kg of soil, or soil-BC mixture according to the following factors (1) “biochar” including i) 0 BC, ii) 1.5 % BC or 34.26 Mg ha^{-1}, iii) 3 % BC or 68.53 Mg ha^{-1}, (2) “humic acid product”, including iv) HAP, v) 1.5 % BC + HAP, and vi) 3 % BC + HAP. In all cases HAP was added at a rate equivalent to 8 kg ha^{-1} as recommended by the manufacturers. (The third factor, “water”, applied frequently or limited, is explained below.) HAP was applied in solution either directly to the soil (HAP-control) or after loading onto the required amount of biochar. To provide similar conditons, BC was moistened to 40 % of its WHC with the HAP solution to deliver an amount equivalent to 8 kg ha^{-1} when the respective amount of BC was added. The HAP-loaded BC was dried and applied to the soil during mixing and pot-filling as described above.

Soil in each pot was fertilized with 13 g of a compound fertilizer (Nitrophoska special blue) that contained 12 % nitrogen as NH_4NO_3, 5.2 % phosphorous as $Ca(H_2PO_4)_2.2H_2O$), 14.1 % potassium as K_2SO_4 and KCl, 1.2 % magnesium as $MgSO_4 \times 7 H_2O$, 6 % sulphur, 0.02 % boron as H_3BO_3 and 0.01 % zinc as $ZnSO_4 \times 7 H_2O$. In addition, 20 ml of micronutrient solution was added to each pot and thoroughly mixed. One litre of this solution contained 6.4 g copper ($CuSO_4 \times 5 H_2O$), 14.3 g zinc ($ZnSO_4 \times 7 H_2O$), 8.2 g manganese ($MnSO_4 \times H_2O$), 0.86 g boron (H_3BO_3), and 0.06 g molybdenum (ammonium molybdate).

Five seeds of maize (cv. DKC-3399) were sown into each pot on May 29, 2012. After emergence the two relatively weaker seedlings were removed to maintain three healthy plants per pot. For the first 4 weeks of the experiment, soil water was maintained at 60 % WHC (as optimum watering) of soil or soil-BC mixtures by daily watering. On the 29th day after sowing (DAS), 3 replicates from each treatment were picked at random for frequent (H_2O_{frequ}) or limited (H_2O_{limit}) watering regimes, respectively. For H_2O_{frequ}, soil moisture was maintained at 60 % WHC by daily adjustment on a balance to the desired target weight. However, in the H_2O_{limit} treatment, the supply of water was reduced to 25–30 % WHC (e.g. not a sudden decrease from 60 to 25–30 % WHC was imposed but it was done be gradual decrease in terms of 2–3 days with the plants' water consumption) until wilting symptoms became visible; wilting symptoms first occurred in the controls without biochar/HAP amendment. For the H_2O_{limit} treatment the pure control treatment (no BC/HAP) was the benchmark: The same amount of water that was daily provided to the control (with the first wilting symptoms visible) was applied to the BC, HAP and BC-HAP treatments, no matter if BC, HAP or BC-HAP treatments may have needed more water than the benchmark control treatment to reach the WHC of 25–30 %. In this way a moderate drought stress was imposed, with equal rainfall/water supply for all H_2O_{limit} treatment pots.

Chlorophyll content, photosynthesis, transpiration, and relative water content

Relative chlorophyll contents were measured with the SPAD-502 device (Minolta, USA) on the first fully developed leaf at the leaf base, middle and tip on 29th and 66th DAS (day after sowing) for all three plants in a pot; values were averaged per pot. Measurements of leaf transpiration and/or chlorophyll fluorescence were carried out on the same day for all treatments after achieving visible symptoms of water stress in the H_2O_{limit} treatments. Chlorophyll *a* fluorescence imaging techniques were used to monitor photosynthetic performance of plants (Schurr et al. 2006; Baker 2008). These techniques allow the estimation of the relative quantum efficiency of the electron transport through the photosynthesis apparatus, photosystem II (PSII) which reacts to environmental stresses (Ort and Baker 2002). The operating efficiency of PSII is characterized by two factors: a) the efficiency by which excitation energy is transferred to photo-synthetically active (open) PSII reaction centres, which can be estimated by the rate of heat dissipation in PSII antennae (non-photochemical quenching); and b) the electron transport efficiency of PSII to acceptors (photochemical quenching); the latter depends on the availability of CO_2 or suitable electron sinks in the chloroplasts (Baker et al. 2001). A Junior-PAM, i.e. a miniaturised Pulse-Amplitude-Modulated photosynthesis yield analyser (Company Walz, Effeltrich, Germany), was used to image chlorophyll fluorescence kinetics parameters. Measurements were performed according to Schreiber et al. (1986) at 62th DAS on the adaxial side of same leaf on which transpiration measurements were made. The plants were dark-adopted for a minimum of 30 min prior to the measurements and the value of minimum fluorescence (Fo) was obtained by applying a modulated light (<0.1 μmol photon m^{-2} s^{-1}) and that of maximum florescence (Fm) after imposing a saturating pulse of 10,000 photons (μmol $m^{-2}s^{-1}$) for 0.6 s (Pfundel 2007). The photochemical utilization, Y(II) or effective photochemical quantum yield, was calculated as:

$$Y(II) = (F'm - F)/F'm \tag{1}$$

where F is steady-state fluorescence in the light and F'm is maximum fluorescence in the light when saturating light imposed (Genty et al. 1989). The non-regulated heat dissipation Y(NO) and non-photochemical heat dissipation Y(NPQ) were calculated according to Kramer et al. (2004). We also calculated apparent photosynthetic electron transport rate (ETR) by using Y(II) and photosynthetic active radiation (PAR, μmol photons $m^{-2}·s^{-1}$). The ETR calculation was made according to Schreiber et al. (1994) as:

$$ETR = 0.5 \times Y(II) \times PAR \times 0.84\, \mu mol\ m^{-2}s^{-1} \tag{2}$$

where 0.5 is the fraction of excitation energy distributed to PSII and 0.84 is a standard factor representing the fraction of incident light absorbed by a leaf.

Stomatal resistance (S. cm^{-1}) and transpiration (mmol m^{-2} s^{-1}) were measured between 9 a.m. and 12 p.m. in the last week prior to harvesting, i.e. 60th DAS, on the first fully developed leaf using a steady-state porometer LI-1600 (LI-COR, Inc. LTD., Lincoln, USA).

Before harvesting the plants on 66th DAS, the relative water content (RWC) of the first fully developed leaf was determined by taking leaf discs of 0.013 m diameter (3 leaves of 3 plants per pot). After noting the fresh weight (FW), leaf discs were floated overnight on well watered filter paper in glass petri plates for rehydration at 4 °C. Turgid weight (TW) was then taken after gently blotting water from the surface of the leaf discs using tissue paper. Leaf samples were oven-dried at

70 °C for 48 h to obtain the dry weight (DW) and RWC computed by using the equation:

$$RWC(\%) = [(FW\text{–}DW)]\Big/[(TW\text{–}DW)] \times 100 \qquad (3)$$

Osmotic potential (Ψπ)

Leaf samples (first fully developed leaf) were frozen at −80 °C just after excision from intact plants. For measurements, frozen leaf samples were brought to room temperature, cut into small pieces, put in Eppendorf tubes, and incubated at 100 °C in a water bath for 15 min. Leaf sap was collected for Ψπ (osmotic potential) determination (in MPa). The 50 μl of leaf sap was taken in eppendorf tubes and Ψπ was measured by using the freeze-point depression method with a cryo-osmometer (type, 030 Gonotec, Germany).

Quantification of sugars

Water soluble sugar contents were determined by the Ludwig and Goldberg (1956) method after drying and grinding of first fully developed leaf. A 0.5 g of dry, ground leaf material was taken in 20 ml screw cap glass tubes. Subsequently, 10 ml of deionised water was added and final weight of the glass tubes was recorded. The tubes were incubated in a water bath at 100 °C for 1 h and deionised water was added where needed. Extract was filtered (Rotilabo-activated carbon filter papers round, Ø 185 mm) and stored in a refrigerator at 4 °C. One ml of hot water extract (diluted as necessary) was pipetted in another screw capped tube and 2 ml of anthrone reagent was added. The mixture was again incubated for 11 min in a water bath at boiling temperature. Afterwards the reaction was terminated by rapidly cooling the glass tubes in an ice bath. The observations were taken at 630 nm by Beckman photometer '(Beckman Coulter inc., Fullerton, USA) using deionised water as blank and final sugar concentration was calculated on the basis of a glucose standard curve (12.5–100 mg L^{-1}).

Final harvest

At 66th DAS, the plants were clipped at the soil surface and data on plant height and fresh weight of leaves and stems were recorded. Dry mass was recorded after drying at 70 °C for 48 h. Root biomass was collected by sieving the soil from each pot through a 2 mm mesh sieve and gently but thoroughly washing the sieved roots with tap water. Roots were blotted and dried at 70 °C.

Soil respiration

Soil respiration was measured within 30 min after removing the plant tops from pots using a LI-8100 soil efflux chamber system (LICOR, Nebraska, USA). The large survey chamber (0.2 m diameter) fitted exactly to the brim of the Mitscherlich pots that were used in the experiment. The offset (height between soil surface and pot brim) of each pot was entered into the LI-8100-driving software for calculation of the correct system volume and thus of the soil CO_2 efflux. Measurement time and observation delay were set to 60 and 20 s, respectively, to provide sufficient time for chamber-volume mixing and CO_2 concentration increase. The increase in CO_2-concentration always showed a linear slope with $R^2>0.99$. The flux was calculated automatically by the LI-8100 software that used the ideal gas law and linear regression. The respiration rate is given as CO_2 flux in μmol m^{-2} s^{-1}.

Carbon and nitrogen content of biomass, NUE and WUE

The leaf and stem biomass of the three plants from each pot was milled using a Retsch mill type SM300 (Hahn, Germany) with a 0.5 mm sieve. An aliquot of the plant material (~200 mg) was combusted in a CN analyzer (Vario MAX, Elementar Analysensysteme Gmbh, Hanau, Germany) for the determination of the N concentration. Nitrogen use efficiency (NUE) was calculated as above ground biomass dry matter produced per unit of fertilizer-N applied. Water use efficiency of productivity (WUE_P) was calculated on the basis of dry matter produced (g) per unit of water consumed.

Soil mineral nitrogen and moisture contents

Gravimetric soil moisture content was measured after completely removing roots from the soil. Soil mineral nitrogen ($NO_3^-$and NH_4^+) was quantified using the methods of Keeney and Nelson (1982). A 20-g portion of soil was mixed with 80 ml 2M KCl, shaken for 1 h at 100 rpm and filtered (Round filter ø 70 mm S and S type 595). Concentrations of NH_4^+-N and NO_3^--N were determined colorimetrically using an auto-analyzer (Seal, Germany).

Statistical analyses

The effects of all three factors (BC, HAP and water regime) were determined using three way analysis of variance (ANOVA) unless stated otherwise. Means were separated at the $P \leq 0.05$ level with the Tukey HSD test. Data were occasionally log-transformed to ensure normal distribution (Komolgorov-Smirnov test) or homogenous variances (Levene median test). Linear regression analyses were also performed to describe the relationship among different parameters. All statistical tests were performed using Sigma Plot 11.0 (Systat, Inc., Richmond, USA).

Results

Plant growth and yield response

Plant growth and productivity was significantly enhanced (6.5 to 7.9 %) by addition of BC which was largely the result of greater stem heights and weights. Water limitation reduced the plant biomass by 35 % while the root:shoot ratio was increased (Fig. 1a, Table 1, Table S1). No difference was found between 1.5 and 3 % BC addition compared to the respective control in any parameter. Addition of HAP had no significant effect on yield parameters (Fig. 1a, Table 1, Table S1) with the exception of a significant negative effect ($p \leq 0.033$) of the BC x HAP interaction on the root mass.

Water use efficiency of productivity and plant soil N dynamics

Biochar addition at 1.5 % was best to improve WUE_P in the H_2O_{frequ} treatment while in the H_2O_{limit} treatment, BC addition did not significantly improve WUE_P (Fig. 1b; Table 1). This is reflected in a significant BC x H_2O interaction (Table 1). Frequent watering generally reduced the WUE_P significantly. HAP addition had no effect on WUE_p.

Tissue N concentrations decreased significantly by 23.4 to 22.9 % due to BC addition (Fig. 2a). Since the biomass increased, the NUE was significantly improved by 6.5 to 7.8 % (Fig. S1; Table S1); the 1.5 and 3 % addition results did not differ from each other (Fig. S1). Limited water supply resulted in significantly higher (53 %) tissue N concentrations as compared to frequent water supply (Fig. 2a; Table S1). This reduced in turn the NUE by 35.5 % in the limited compared to frequent water supply (Fig. S1; Table S1). Both, tissue N concentration and NUE were not significantly influenced by HAP addition (Table S1).

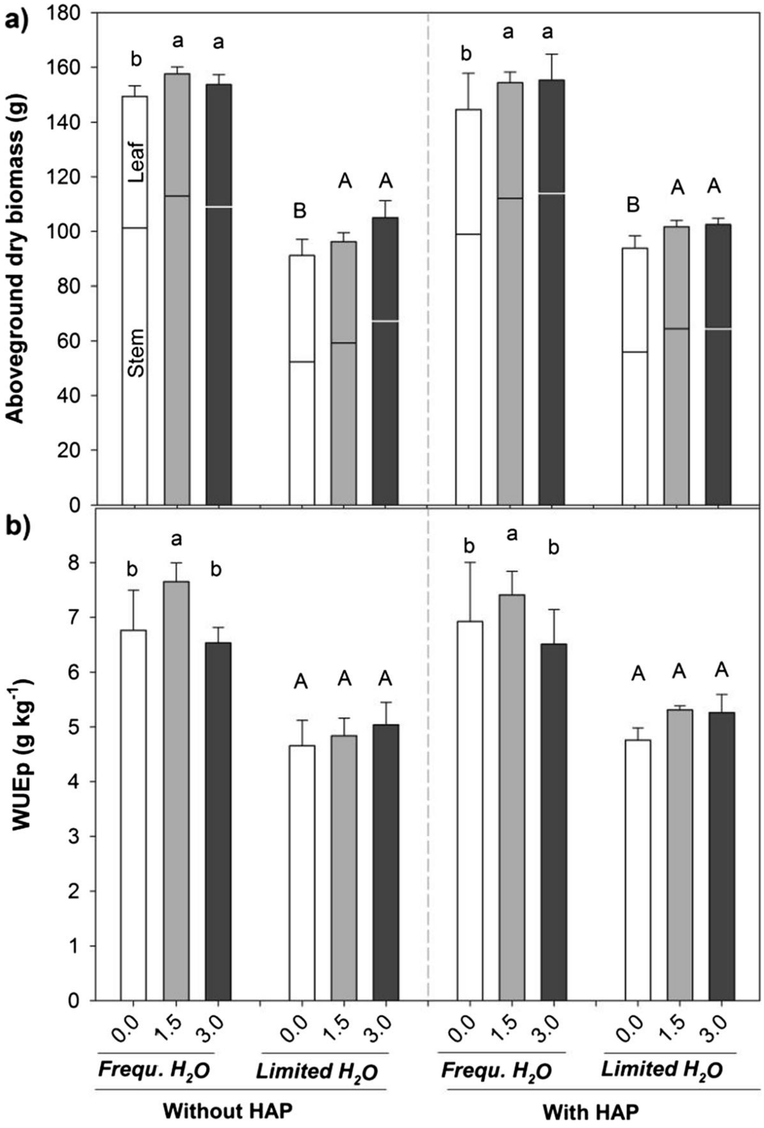
After the plant harvest, the NO_3^--N concentration in the control soils was zero, either in the H_2O_{frequ} or H_2O_{limit} treatment (Fig. 2b, Table S1). However, with BC, significant NO_3^--N concentrations were detected, and significantly more NO_3^--N was retained in the H_2O_{limit} than H_2O_{frequ} treatment. NH_4^+-N amounts were negligible, with no significant effect of the treatments (not shown). HAP addition did not affect mineral N concentration.

Plant water relations and photosynthetic response

Limited soil water always significantly impacted the plant physiological parameters including the osmotic potential. However, BC addition supported the plants at both water regimes. Biochar addition significantly increased the relative water content (RWC) and the osmotic potential of the leaves ($\Psi\pi$) and generally enhanced transpiration due to significant decreases in stomatal resistance (Tables 2 and 3). The BC-induced improvements (most probably plant water availability) increased $\Psi\pi$ while decreasing sugar concentrations compared to the control (Tables 2 and 3). This is further indicated by a significant negative correlation between the $\Psi\pi$ and sugar concentration (Fig. 3a). HAP significantly decreased RWC, $\Psi\pi$ and stomatal resistance but had no effect on transpiration (Tables 2 and 3). Biochar addition in the H_2O_{limit} treatments significantly decreased the chlorophyll content (Tables 2 and 3).

As expected, water limitation negatively influenced photosynthesis: it decreased the photosynthetic electron transport rate (ETR) and the effective quantum yield (Y(II)), at the costs of increased heat dissipation Y(NO) and non-photochemical quenching Y(NPQ) in PSII (Tables 2 and 3). This resulted in a significant decrease of Y(II)/Y(NPQ), a ratio between the effective photochemical quantum yield and non-photochemical quenching. Biochar addition did not increase ETR and Y(II), but it increased Y(NO), reduced Y(NPQ), and thus increased the Y(II)/Y(NPQ) ratio (Tables 2 and 3). Also, HAP increased the Y(II)/Y(NPQ) ratio

Fig 1 Impact of biochar application (BC 0, 1.5 and 3 %) with or without humic acid product (HAP) addition under two water regimes (frequent or limited supply) on **a**) aboveground dry matter yield (*bars* show means of stem (*lower bar part*) plus leaves (*upper bar part*); *error bars* give the standard deviation of the aboveground biomass; *n*=3), **b**) water use efficiency of productivity, (*error bars* = stdev. of means, *n*=3); means with similar *letters* are not significantly different. *Lower-case letters* show differences due to the BC treatment within "Frequent H_2O" while *upper case letters* show differences within "Limited H_2O" when the water treatment effect was significant, respectively; the factor HAP was not significant, see statistical results, Table 1



(Tables 2 and 3). The significant positive influence of BC on photosynthesis was coupled with better water supply by the BC amended treatments, as indicated by a significant negative correlation between ETR and stomatal resistance (Fig. 3b).

The HAP addition, in the absence of BC, appeared to have a positive influence on photosynthetic parameters (Table 2). Therefore, the effect of HAP was further investigated by two way ANOVAs with the factor water regime, excluding the data sets with the factor BC (Table 3, bottom). This confirmed that pure HAP addition indeed had a significantly positive effect on photosynthetic parameters which was masked by the effects of BC when the factor BC was included in the three way ANOVAs (Table 3).

Soil moisture contents and respiration (CO_2 efflux) at harvesting

Gravimetric soil moisture content measured after the harvest was significantly higher in BC amended soil and higher in the H_2O_{frequ} than H_2O_{limit} treatment, respectively (Fig. S2b). In the H_2O_{frequ} treatment addition of 1.5 and 3 % BC increased the soil moisture by 48 and 129 %, respectively (Fig. S2b; Table S1) while HAP had no impact. Soil respiration (CO_2 efflux) measured

Table 1 Results of three way ANOVA's with factors biochar (BC 0, 1.5 and 3 %) and humic acid product (HAP) addition under frequent and limited water supply on Leaf DW = leaf dry weight (g), Stem DW = stem dry weight (g), Total DM = total aboveground dry biomass (g) and WUEp = Water use efficiency of productivity (g kg^{-1})

Factors	Leaf DW		Stem DW		Total DM		WUEp	
	F	p	F	p	F	p	F	p
BC	2.04	0.152	15.44	<0.001	5.59	0.010	3.82	0.036
H_2O	38.8	<0.001	649.7	<0.001	487.7	<0.001	135	<0.001
HAP	1.97	0.173	0.56	0.463	0.00	0.962	0.45	0.509
BC×H_2O	0.80	0.461	0.78	0.471	0.56	0.576	3.54	0.045
BC×HAP	0.04	0.958	0.06	0.946	0.07	0.934	0.00	0.997
H_2O×HAP	1.48	0.236	0.14	0.716	0.66	0.425	0.77	0.389
BC×H_2O×HAP	0.10	0.907	1.52	0.238	0.69	0.511	0.43	0.654

directly after cutting the plant tops (with the roots still in the soil) was significantly increased with BC addition in the H_2O_{frequ} treatment. Water shortage reduced soil respiration on average by 39 % (Fig. S2a; Table S1). HAP addition significantly ($p \leq 0.039$) decreased the CO_2 efflux relative to the treatment with no HAP (Fig. S2a, Table S1).

Discussion

Hypotheses revisited: expected and unexpected effects

Biochar addition clearly improved plant-soil water relations and plant eco-physiological traits, resulting in significantly increased maize biomass as observed earlier (Kammann et al. 2011; Yamato et al. 2006; Sukartono et al. 2011; Uzoma et al. 2011a, 2011b). However, increasing the BC amendment rate did not have linearly positive effects; responses at 1.5 and 3 % addition were mostly identical. We also hypothesized that HAP loading would improve the performance of BC, and that the beneficial effects will be more pronounced at limited compared to frequent water supply which was not the case.

Biochar effects on plant water relations and dry matter yield

Many studies report that biochar addition can considerably promote the water holding capacity (disturbed soil samples/mixtures), or field capacity (undisturbed soil cores) of sandy soils in particular (Abel et al. 2013; Artiola et al. 2012; Belyaeva and Haynes 2012; Case et al. 2012; Kammann et al. 2011, 2012; Kinney et al. 2012; Liu et al. 2012; Novak et al. 2012 and Rajkovich et al. 2012), but also in other soil types (Chan et al. 2007; Glaser et al. 2002 and van Zwieten et al. 2010c). Indeed significant increases of 12.5 and 24.7 % in the WHC were observed with 1.5 and 3 % BC addition to the sandy, SOC-poor soil, respectively. Although the available water capacity (AWC) (soil moisture between field capacity and permanent wilting point) was not determined, it was very likely enhanced. In other studies, the permanent wilting point was increased slightly with biochar addition (Abel et al. 2013; Cornelissen et al. 2013; Utomo 2013 or Brecht 2012) and interestingly, the amount of water held at field capacity increased to a larger extent than that held at the permanent wilting point, i.e. increase of AWC. Therefore, in the current study, the significantly increased WHC is taken as indication for an overall increase in the plant-available water that the BC-amended soil is able to deliver.

Improved biomass yields with biochar addition in greenhouse (Buss et al. 2012; Kammann et al. 2011; Mulcahy et al. 2013) as well as in field studies (Liu et al. 2012; Major et al. 2010; Vaccari et al. 2011; Baronti et al. 2014); were often attributed to an improved soil water supply. The two water regimes applied here were chosen to differentiate between growth-promoting effects caused by higher water availability (the H_2O_{frequ} treatment: WHC increase with biochar provided by daily adjustment to 60 % WHC), and positive effects

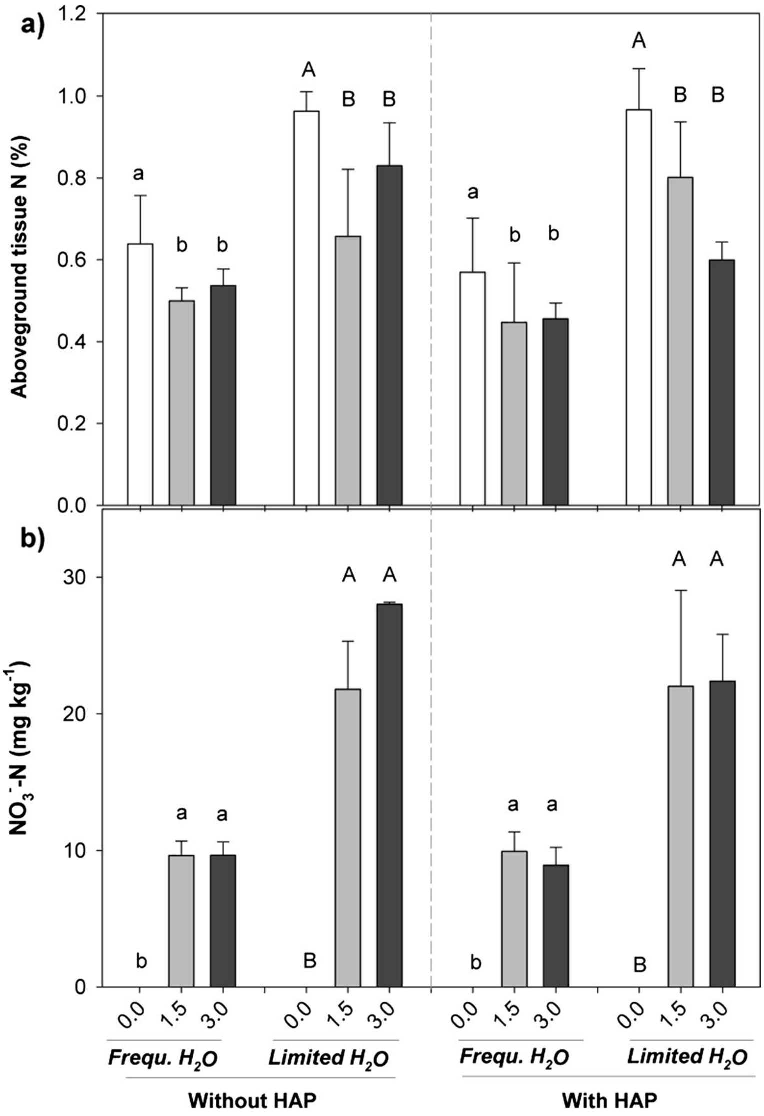


Fig 2 Impact of biochar application (BC 0, 1.5 and 3 %) with or without humic acid product (HAP) addition under two water regimes (frequent or limited supply) on **a** aboveground tissue N concentration, **b** soil NO_3^--N left at harvesting; (*bars* show means + stdev., $n=3$); means with similar *letters* are not significantly different. *Letters* as described in Fig. 1

"beyond more water supply" (the H_2O_{limit} treatment at the verge of drought stress, with equal reduced daily water supply to all treatments).

The biomass production results clearly show that biochar caused not only an improvement effect at higher WHC (H_2O_{frequ} treatment), but also when this surplus water supply was not allowed (H_2O_{limit} treatment). However, it was unexpected that in both water treatments the biomass increases due to BC amendment had the same relative magnitude; and that the positive biomass response was not linearly increasing with increasing biochar additions. The relationship followed a saturation curve with no difference between 1.5 and 3 % BC additions for most of the measured parameters. In the H_2O_{frequ} treatment, the water use efficiency of productivity, WUE_P, was significantly increased only with 1.5 % but not 3 % BC amendment which was surprising. Therefore other, competing mechanisms may have ameliorated linear water-related effects of BC addition, such as phyto-hormonal signalling (Graber et al. 2010; Jaiswal et al. 2014), or nitrate capture (Ventura et al. 2013). We argue that biochar may have immobilised/adsorbed mineral-N which was therefore be unavailable for plant uptake because (i) significantly larger nitrate amounts were extracted from the biochar but not control treatments at the end of the study, and because (ii) a reduced N uptake into the plant biomass was observed. However, other reasons for the lack of a direct

Table 2 Influence of biochar (BC 0, 1.5 and 3 %) and humic acid product (HAP) addition under frequent and limited water supply on photosynthesis and water/osmotic relations of maize; means ± stdev., *n*=3; Chlorophyll (SPAD values) ETR = electron transport rate (μmol m^{-2} s^{-1}), Y(II) = effective photochemical quantum yield, Y(NO) = regulated heat dissipation, Y(NPQ) = non-regulated heat dissipation, $Y_{(II)}/Y_{(NPQ)}$ (ratio), Ψπ = leaf osmotic potential (MPa), RWC = leaf relative water content (%), Rs = stomatal resistance (S. cm^{-1}) and Transpiration (mmol m^{-2} s^{-1})

Treatment factors			Photosynthesis						Water and osmotic relations			
HAP	H_2O	BC	Chlorophyll	ETR	$Y_{(II)}$	$Y_{(NO)}$	$Y_{(NPQ)}$	$Y_{(II)}/Y_{(NPQ)}$	Ψπ	RWC	Rs	Transpiration
Without HAP	Frequ. H_2O	0.0	36.31±1.05	35.5±1.66	0.45±0.02	0.27±0.00	0.29±0.02	1.55±0.15	−1.48±0.13	75.7±6.85	20.7±2.7	0.69±0.12
		1.5	33.07±0.99	37.8±0.98	0.47±0.01	0.28±0.00	0.24±0.01	1.95±0.17	−1.35±0.10	82.3±0.16	15.1±3.9	1.07±0.23
		3.0	31.06±1.14	38.7±1.53	0.49±0.02	0.28±0.01	0.23±0.02	2.13±0.31	−1.29±0.02	78.7±6.68	12.4±0.8	1.09±0.07
	Limit. H_2O	0.0	32.66±0.80	30.2±2.55	0.38±0.03	0.28±0.02	0.34±0.05	1.13±0.23	−1.72±0.04	60.4±4.31	96.8±16.5	0.14±0.02
		1.5	30.56±2.90	35.5±0.57	0.45±0.01	0.28±0.01	0.27±0.01	1.64±0.07	−1.74±0.16	64.7±1.32	53.5±5.6	0.25±0.02
		3.0	28.61±1.17	32.0±2.89	0.40±0.04	0.31±0.00	0.29±0.03	1.40±0.27	−1.58±0.08	69.7±4.70	49.6±10.1	0.26±0.05
With HAP	Frequ. H_2O	0.0	33.71±1.37	37.1±1.34	0.47±0.02	0.27±0.02	0.27±0.02	1.76±0.18	−1.57±0.20	72.0±9.91	13.1±0.5	0.96±0.04
		1.5	32.36±1.01	36.1±2.41	0.45±0.03	0.27±0.02	0.28±0.04	1.66±0.37	−1.37±0.15	85.6±5.08	15.6±0.9	0.74±0.04
		3.0	29.46±0.56	38.8±0.73	0.49±0.01	0.29±0.01	0.22±0.00	2.19±0.07	−1.37±0.05	77.9±2.61	10.6±0.4	1.20±0.06
	Limit. H_2O	0.0	33.81±0.33	34.7±0.66	0.43±0.01	0.31±0.00	0.26±0.00	1.69±0.06	−2.28±0.38	72.7±4.87	55.7±17.9	0.25±0.06
		1.5	31.73±2.03	33.2±2.21	0.42±0.03	0.31±0.01	0.28±0.02	1.52±0.21	−1.83±0.12	75.5±0.93	43.6±12.5	0.30±0.07
		3.0	27.58±1.89	34.3±1.04	0.43±0.01	0.32±0.02	0.26±0.01	1.69±0.07	−1.62±0.15	77.6±2.91	50.0±11.4	0.25±0.07

Table 3 Results of three and two way ANOVA's with factors biochar (BC 0, 1.5 and 3 %) and humic acid product (HAP) addition under frequent and limited water supply on photosynthesis and water/osmotic relations of maize; deviation = F-value, p = p-value; Chlorophyll (SPAD values) ETR = electron transport rate (μmol m^{-2} s^{-1}), Y(II) = effective photochemical quantum yield, Y(NO) = regulated heat dissipation, Y(NPQ) = non-regulated heat dissipation, Ψπ = leaf osmotic potential, RWC = leaf relative water content (%), Rs = stomatal resistance (S. cm^{-1}) and Transpiration (mmol m^{-2} s^{-1})

Factors	Photosynthesis												Water and osmotic relations							
	Chlorophyll		ETR		$Y_{(II)}$		$Y_{(NO)}$		$Y_{(NPQ)}$		$Y_{(II)}/Y_{(NPQ)}$		Ψπ		RWC		Rs		Transpiration	
	F	p	F	P	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
ANOVA three way (BC, H_2O, HAP) with 'F' and 'P' values																				
BC	23.8	0.001	8.43	0.174	1.90	0.171	4.81	0.018	4.62	0.020	4.90	0.016	7.12	0.004	4.38	0.024	7.26	0.003	8.99	0.001
H_2O	9.77	0.005	146.4	<0.001	32.8	<0.001	19.2	<0.001	6.94	0.015	19.0	<0.001	36.0	<0.001	18.0	<0.001	132	<0.001	381	<0.001
HAP	1.05	0.315	4.84	0.309	1.11	0.302	2.94	0.099	3.10	0.091	1.90	0.181	5.14	0.033	5.95	0.023	6.82	0.015	0.71	0.408
BC×H_2O	0.09	0.916	7.03	0.229	1.53	0.236	0.06	0.944	1.09	0.352	2.36	0.116	0.92	0.412	1.80	0.186	4.44	0.023	5.52	0.011
BC×HAP	0.59	0.561	19.49	0.024	4.40	0.024	0.55	0.587	4.60	0.020	4.36	0.025	1.85	0.179	0.28	0.762	3.72	0.039	6.52	0.005
H_2O×HAP	3.11	0.09	5.44	0.281	1.22	0.28	4.20	0.051	3.10	0.060	2.17	0.154	1.66	0.210	7.10	0.014	3.37	0.079	0.21	0.652
BC×H_2O×AHP	0.62	0.546	2.45	0.585	0.57	0.574	1.05	0.365	0.26	0.776	0.09	0.913	1.44	0.257	0.42	0.662	1.91	0.17	5.53	0.011
ANOVA two way (H_2O, HAP) with 'F' and 'P' values																				
H_2O	6.77	0.032	10.49	0.012	10.6	0.012	9.13	0.017	1.41	0.270	4.30	0.072	8.95	0.017	2.29	0.169	46.9	<0.001	6.19	0.039
HAP	1.12	0.321	6.33	0.036	6.47	0.035	3.55	0.096	7.68	0.024	10.4	0.012	4.19	0.075	0.79	0.399	7.90	0.023	0.19	0.679
H_2O×HAP	7.55	0.025	1.46	0.261	1.52	0.253	2.22	0.174	2.54	0.149	2.06	0.189	2.24	0.173	2.73	0.137	3.76	0.089	0.48	0.508

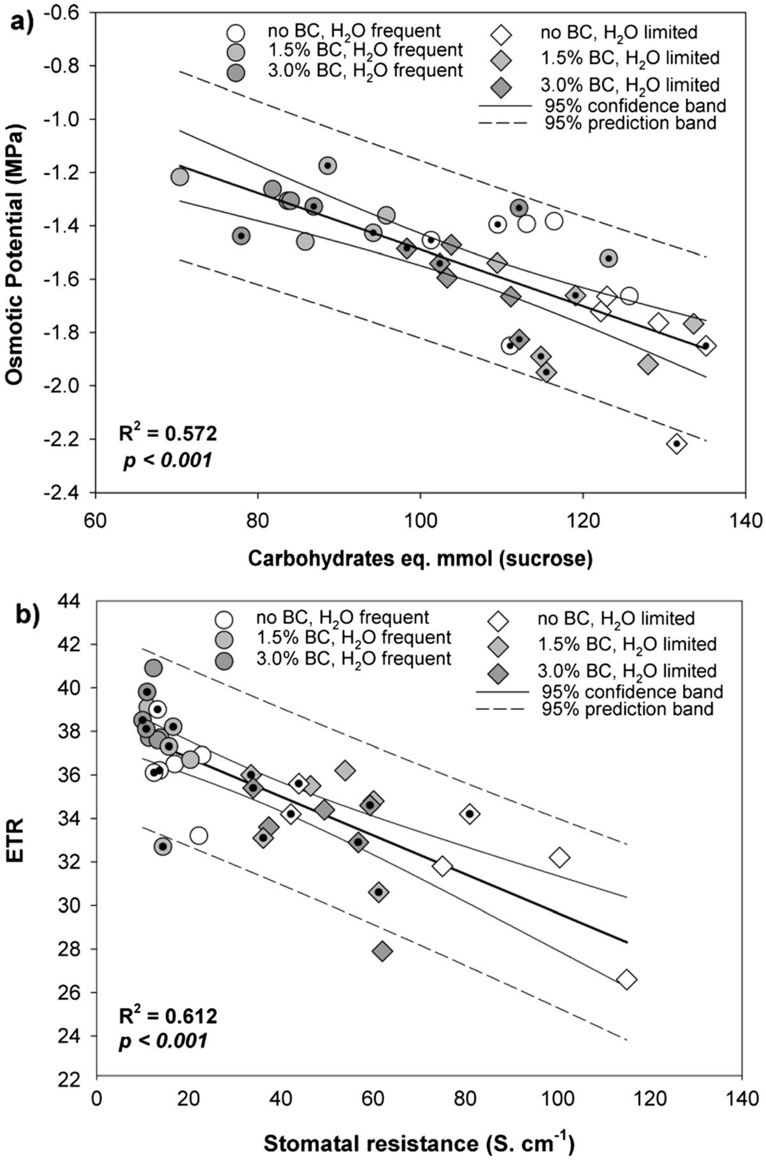


Fig 3 Impact of biochar application (BC 0, 1.5 and 3 %) with or without humic acid product (HAP) addition under two water regimes (frequent or limited supply) on **a** correlation between osmotic potential and leaf sugar concentrations **b** correlation between stomatal resistance and electron transport rate, ETR (means ± stdev., n=3). *Dots* within symbols indicate HAP treatment. (Treatment means and statistical results see Tables 2 and 3)

correlation to biochar addition cannot be ruled out and deserves further study.

Biochar effects on plant physiology

Generally, water limitation impairs photosynthesis by increasing stomatal resistance or through metabolic limitations (Cornic 2000; Lawlor 2002). However, plants have evolved not only osmotic and stomatal regulation mechanisms (Jones and Sutherland 1991) to cope with water shortages, but also defence strategies (xanthophyll cycle, photorespiration etc.) to alleviate the harmful effects of excessive energy under such stress conditions (Ort and Baker 2002). Biochar amendments improved the leaf osmotic potential $\Psi\pi$ of *Chenopodium quinoa* plants which grew significantly better with addition of peanut hull biochar, either at sufficient water supply or drought (Kammann et al. 2011); the same was observed here with maize, and a woody biochar. The accumulation of sugars or other osmotically active substances lowers $\Psi\pi$ under drought stress to maintain turgor, stomatal opening, photosynthesis and growth to a certain extent (Bolaños and Edmeades 1991; Kakani et al. 2011). In our study BC improved the osmotic potential which closely correlated to lower accumulations of soluble sugars. This corresponded to reduced stomatal resistance, larger transpiration rates, and higher relative water contents of the leaves at the harvest with BC additions.

The PAM chlorophyll fluorometer permits the assessment of excitation energy fluxes at PSII in three different pathways, termed Y(II), Y(NO) and Y(NPQ), which adds up to an unity. Any one or two of these can increase or decrease at the rate of the remaining one(s) in PSII (Kramer et al. 2004). Moreover, Genty et al. (1989, 1990) reported that Y(II) is directly related to the rate of CO_2 assimilation in the leaf. In this study, BC amendment (without HAP) caused a relative increase in the electron transport rate (ETR) and Y(II) in PSII. Thus the ratio of the effective photochemical yield to the non-photochemical quenching Y(II)/Y(NPQ) significantly increased with BC addition so that more excitation energy was directed into the photosynthetic yield instead of energy loss. The more efficient photosynthetic energy gain finally resulted in higher biomass with BC amendment.

Stresses generally reduce photosynthetic efficiency and CO_2 fixation. For example Qu et al. (2013) reported that combined salt and potassium stress significantly decreased Y(II) and increased Y(NPQ) or Y(NO) in maize. Other researchers have also reported lower photosynthetic CO_2 gain due to declined Y(II) under severe drought stress e.g. in cucumber (Li et al. 2008). In the only other study where BC was applied to herbaceous plant species under salt stress (*Abutilon theophrasti* Medik. and *Prunella vulgaris* L.), and where photosynthetic performance was measured, Thomas et al. (2013) found no significant influence of BC amendments on photosynthetic carbon gain (A_{max}), chlorophyll fluorescence (Fv/Fm) or on water use efficiency. The authors amended BC at rates of 5 and 50 Mg ha^{-1}, the higher rate of which is in between the BC application we used in this study (1.5 and 3 % correspond to 34.26 and 68.53 Mg ha^{-1}, respectively). Their findings are in contrast to this study where significant improvements were observed with biochar addition, which is the first report of its kind to our knowledge, in both water treatments.

Taken together, the results indicate that the yield improvements were not only caused by an improved water supply (as evidenced by the results of the H_2O_{limit} treatment), but rather by subtle improvements of the plant water status and stomatal conductance, and thus changes in the performance of the photosynthetic apparatus (PSII photochemistry). Thus, biochar amendment increased the overall potential for photosynthetic carbon gain. The results clearly demonstrate that PSII photochemistry was positively impacted by BC soil amendment, even despite reductions observed in the relative chlorophyll content (see below). Biochar therefore acted dominantly along the 'water-effect route' of plant physiology.

Biochar effects on nitrogen dynamics

Lehmann et al. (2003) observed lower N uptake by cowpea in an Anthrosol due to charcoal addition. Similarly, in this study, BC addition decreased maize N uptake and decreased the leaf chlorophyll content. The reduced N uptake was likely not the result of N losses, as NO_3^--N was still present in BC treated soils even after the harvest.

If the amount of N removed with the aboveground plant material and the amount of mineral N left in the (BC-amended) soil is summed up, no differenc exist between treatments. Soil N retention was also observed by van Zwieten et al. (2010a); Taghizadeh-Toosi et al. (2011); Rajkovich et al. (2012) or Prendergast-Miller et al. (2011). However, there are studies where the N uptake was increased by increasing rates of BC addition, depending on plant species, soil bio-chemical properties and type of biochar (van Zwieten et al. 2010b; Chan et al. 2007). Lehmann et al. (2003) reported that plant productivity was increased even by 50 % less foliar N uptake; in our study the NUE was also increased at lower foliar N contents. It is unclear if (and if so, how) the remaining mineral N, mostly NO_3^--N, was bound to the biochar particles. Furthermore, it is unclear if the plants were unable to retrieve the mineral N that was extractable with KCl at the end of the study; or if there was no need for the plants to take up the remaining soil mineral N. Thus the question remains if the increase in NUE was a genuine physiological response of the maize plants, or if the plants were not able to take up the N. In the latter case the improved NUE with biochar would rather be a demonstration of their physiological plasticity. In line with Clough et al. (2013), our results suggest that the nature of the mineral N retention in the biochar-amended soil is more complex than we know so far and deserves further investigations.

Biochar and water treatment effects on soil respiration

The argument that water effects dominated the measured responses is backed up by the soil respiration measurements taken directly after the harvest. Effect of BC addition on soil respiration and CO_2 efflux can vary considerably depending on biochar feedstock, soil type and moisture conditions, long-term land use and other factors that impact the soil microbial community (Bamminger et al. 2013; Kammann et al. 2012; Kolb et al. 2009; Spokas and Reicosky 2009; Ulyett et al. 2014; van Zwieten et al. 2010c; Zimmerman et al. 2011 and Hilscher et al. 2009). When plant roots were included as done here, soil respiration increased with BC addition, concomitantly with the root mass (Major et al. 2010). Here, the root mass was unchanged, but in the H_2O_{frequ} treatment soil respiration significantly increased with BC addition, whereas with limited water supply soil moisture and soil respiration were unchanged which is in line with the results of Zhang et al. (2012) or Kammann et al. (2011). The soil CO_2 efflux was largely predicted by soil moisture with an exponential rise function (R^2=0.83; p≤0.0001) but not by root mass (not shown). Therefore, this study showed that the most important effect of biochar was on the improvement of the water supply.

Effect of the added humic acid product

Humic acid products have been found to improve respiration and photosynthetic performance of plants before, by modifications in mitochondria functioning and chloroplasts (Orlov and Sadovnikova 2005); HAP amendment is often discussed for growth improvement (Trevisan et al. 2010). In our study, however, beneficial HAP effects were restricted only to small improvements in PSII photochemistry, increased stomatal conductance and Ψπ. This became only visible when the factor HAP was tested alone, omitting BC amendments. Either the stronger BC effects masked the smaller HAP effects; or, alternatively, HAP as complex organic molecules were adsorbed onto the BC surfaces and thus unavailable for interaction with the plant roots, since BC is a known strong adsorber of a variety of organic compounds such as PAHs (Smernik 2009; Schimmelpfennig and Glaser 2012; Hilber et al. 2012; Quilliam et al. 2013).

Conclusions

Global climate change has strong impacts on precipitation patterns and thus soil water resources. Therefore effective and farmer-friendly countermeasures are urgently needed. We observed that biochar can improve soil-plant water relations. The beneficial biochar effect enrolled via positive (i.e. self-reinforcing) feedback loops within the plants' eco-physiological response capabilities: BC addition (1) increased Ψπ, RWC and transpiration while decreasing soluble sugars and stomatal resistance; (2) decreased chlorophyll contents with higher N leftover in soil, but still improved NUE and PSII photochemistry efficiency, as indicated by increased Y(II)/Y(NPQ) ratios; (3) improved WUEp even with daily adjustment to optimal WHC, and finally (4) these improvements resulted in a higher plant biomass yield. Biochar and HAP amendments both had positive effects on plant water and photosynthetic parameters (with BC > HAP). However, when used in combination, BC overruled the smaller positive effect of HAP, presumably due to sorption of HAP. Thus, HAP loading on BC, or their combined use, did not provide an improvement. Nitrogen retention in BC-amended soils deserves further investigations because the results suggested restrictions for plant N uptake, which may have been the cause for the U-shaped or saturation-type responses in WUE_p or biomass production to increasing amounts of biochar, respectively. For future research the use of well-designed watering regimes may be helpful to identify and develop best-suited (designer) biochars for improving crop water relations.

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Supplementary material

Title: Biochar but not humic acid product amendment affected maize yields via improving plant-soil moisture relations

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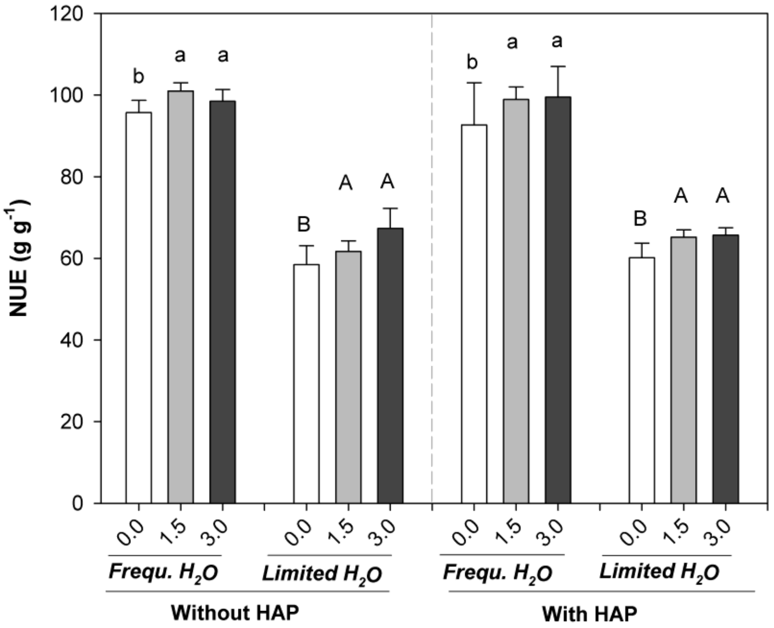

Fig S1 Impact of biochar application (BC 0, 1.5 and 3%) with or without humic acids product (HAP) addition under two water regimes (frequent or limited supply) on nitrogen use efficiency; (bars show means + stdev., *n*=3); means with similar letters are not significantly different. Lower-case letters show differences due to BC treatment within “Frequent H_2O” while upper-case letters show differences on the basis of BC within “Limited H_2O”, respectively; statistical results see Table S1.

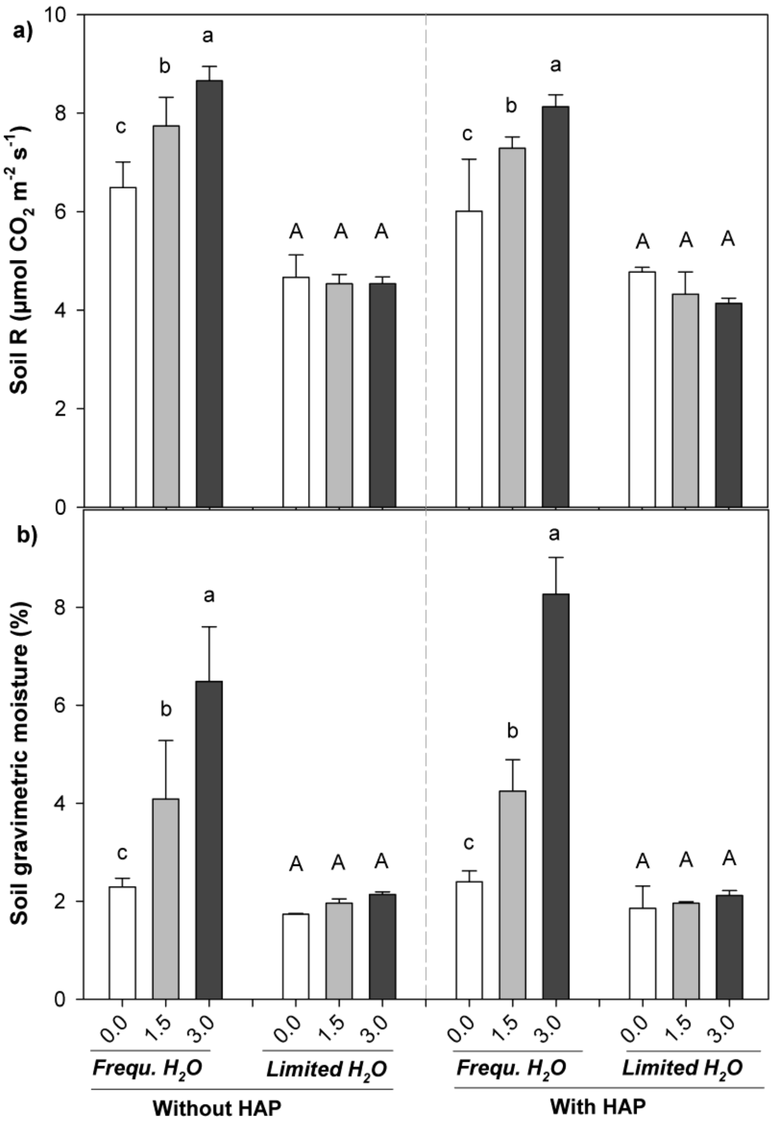

Fig S2 Impact of biochar application (BC 0, 1.5 and 3%) with or without humic acids product (HAP) addition under two water regimes (frequent or limited supply) on (a) Soil respiration and (b) Soil gravimetric moisture contents; (bars show means + stdev., *n*=3); means with similar letters are not significantly different. Lower-case letters indicate significant difference due to BC within “Frequent H_2O” while upper-case letters show significant difference due to BC within “Limited H_2O”, respectively; Statistical results see Table S1.

Table S1 Results of three-way ANOVAs with the factors biochar (BC 0, 1.5 and 3% addition), humic acid product (HAP) addition, and water supply (frequent, limited) on NUE = nitrogen use efficiency (shown in Fig 2); aboveground tissue nitrogen; mineral nitrogen concentration (NO_3^--N) at harvesting (shown in Fig 2) and gravimetric soil moisture and soil respiration (shown in supplementary Fig S2); Plant height, Root dry matter and Root:Shoot.

Factors	**NUE** $g\ g^{-1}$		**Tissue N** %		**NO_3^--N** mg kg^{-1} soil		**Soil moisture** %		**Soil R** µmol $CO_2\ m^{-2}\ s^{-1}$		**Plant height** cm		**Root DM** g		**Root:Shoot**	
	F	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
BC	5.59	*0.01*	12.4	*<0.001*	167	*<0.001*	67.2	*<0.001*	11.6	*<0.001*	11.3	*<0.001*	0.08	*0.919*	1.58	*0.227*
H_2O	488	*<0.001*	65.5	*<0.001*	119	*<0.001*	195	*<0.001*	375	*<0.001*	191.5	*<0.001*	95.89	*<0.001*	5.04	*0.034*
HAP	0.00	*0.962*	1.93	*0.177*	1.28	*0.27*	3.5	*0.074*	4.8	*0.039*	1.00	*0.327*	0.94	*0.342*	0.53	*0.474*
BC x H_2O	0.56	*0.576*	1.56	*0.231*	31.5	*<0.001*	51.9	*<0.001*	24.3	*<0.001*	0.52	*0.601*	2.77	*0.083*	1.56	*0.231*
BC x HAP	0.07	*0.934*	2.91	*0.074*	1.66	*0.212*	1.9	*0.176*	0.3	*0.751*	1.26	*0.302*	3.94	*0.033*	3.85	*0.035*
H_2O x HAP	0.66	*0.425*	0.33	*0.571*	0.94	*0.342*	2.9	*0.102*	1.1	*0.295*	0.02	*0.881*	2.93	*0.1*	1.29	*0.268*
BC x H_2O x HAP	0.69	*0.511*	2.18	*0.135*	0.89	*0.423*	2.3	*0.124*	0.2	*0.804*	1.21	*0.316*	2.83	*0.079*	2.96	*0.071*

3 Standard Extraction Methods May Underestimate Nitrate Stocks Captured by Field-Aged Biochar

"Paper published in Journal of Environmental Quality 45:1196-1204 (2016). doi:10.2134/jeq2015.10.0529".

3.1 Publication outline

The publication in the following chapter describes the results of detailed methodological investigations on mineral N retention and extraction from BC amended soil, and from fresh and field aged BC (BC_{aged}) under laboratory conditions. The study was initiated following (1) the observation of greater NO_3^- retention in BC amended pots at the harvest of maize in the greenhouse study and greater NO_3^- retention in topsoil (0 - 15 cm, BC amended zone) in the parallel field study (chapter 2 and 4). It was surprising that the amount found as nitrate in the soil being equivalent to the lower N amount (reduced N concentrations) in the harvested biomass (chapter 2). BC_{aged} particles retrieved from the field soil samples saved after each soil sampling for mineral N extraction were investigated for mineral N extraction with different standard and modified approaches. This study aimed to disclose the fact that either (a) greater NO_3^- in BC amended pots (chapter 2) or plots (chapter 4) were due to NO_3^- retention in BC amended soil or (b) in BC particles itself. Furthermore, it had to be clarified (c) if the freshly incorporated BC can capture just a maximum soil mineral N, or if this changes with field aging of BC over time. In addition, (d) it was unclear if the standard extraction methods, for instance, 2 M KCl or Electro-ultrafiltration (EUF), can really extract all captured NO_3^- in BC_{aged} particles, or (e) if other approaches (i.e.) water washing or prolonged shaking in water/KCl at room or at high temperature were better suited to retrieve all nitrate.

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Standard Extraction Methods May Underestimate Nitrate Stocks Captured by Field-Aged Biochar

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Abstract

Biochar (BC) has been shown to increase the potential for N retention in agricultural soils. However, the form of N retained and its strength of retention are poorly understood. Here, we examined if the N retained could be readily extractable by standard methods and if the amount of N retained varied with BC field ageing. We investigated soil and field-aged BC (BC_{aged}) particles of a field experiment (sandy soil amended with BC at 0, 15, and 30 t ha^{-1}) under two watering regimes (irrigated and rain-fed). Throughout the study, greater nitrate than ammonium retention was observed with BC addition in topsoil (0–15 cm). Subsoil (15–30 cm) nitrate concentrations were reduced in BC treatments, indicating reduced nitrate leaching (standard 2 mol L^{-1} KCl method). The mineral-N release of picked BC_{aged} particles was examined with different methods: standard 2 mol L^{-1} KCl extraction; repeated (10×) extraction in 2 mol L^{-1} KCl at 22 ± 2°C and 80°C (M_0); electro-ultrafiltration (M_1); repeated water + KCl long-term shaking (M_2); and M_2 plus one repeated shaking at 80°C (M_3). Nitrate amounts captured by BC_{aged} particles were several-fold greater than those in the BC-amended soil. Compared with M_0, standard 2 mol L^{-1} KCl or electro-ultrafiltration extractions retrieved only 13 and 30% of the total extractable nitrates, respectively. Our results suggest that "nitrate capture" by BC may reduce nitrate leaching in the field and that the inefficiency of standard extraction methods deserves closer research attention to decipher mechanisms for reactive N management.

Core Ideas

- Biochar reduced nitrate leaching from temperate sandy soil in the field.
- Biochar particles captured nitrate amounts several-fold greater than found in soil.
- Standard extraction methods may not retrieve all biochar-captured nitrate.
- Field-aged biochar captured much more nitrate than ammonium.
- Captured nitrate amounts were independent of the biochar particle size.

The rapid increase in human population and living standards over the last decades have increased the demand for food, feed, fiber, and raw material resources (Erisman et al., 2011; Uzoma et al., 2011). Therefore, humans have dramatically altered the nitrogen (N) cycle and doubled the amount of fixed reactive N used for intensified agricultural production over the past century (Galloway et al., 2008; Schlesinger, 2009; Erisman et al., 2011; Steffen et al., 2015). Extensive N fertilizer production and application in agricultural soils causes increasing N leaching losses to surface and ground waters (Donner and Kucharik, 2008). Nitrogen loss depletes soil fertility and causes water pollution and thereby has adverse impacts on environmental and human health (Cameron et al., 2013). It is therefore mandatory to develop effective agricultural management strategies to reduce N leaching losses.

One option for this may be the use of biochar (BC), "the product of heating biomass in the absence of or with limited air to above 250°C, a process called charring or pyrolysis" (Lehmann and Joseph, 2015). Biochar has been shown to reduce nutrient leaching from agricultural soils in some studies (Lehmann et al., 2003; Novak et al., 2009; Laird et al., 2010; Yoo et al., 2014) but not in all. Biochar use can have additional benefits, such as atmospheric carbon sequestration in soil (Lehmann, 2007), reduced greenhouse gas emissions (Kammann et al., 2012; Cayuela et al., 2014; Van Zwieten et al., 2015), and improved water retention capacity (Glaser et al., 2002; Abel et al., 2013) resulting in improved soil–plant–water relations and crop production (Haider et al., 2015).

Recent studies on BC amendments, mostly under laboratory conditions, have reported varied results regarding N sorption, retention, or leaching. Biochar produced at 600°C from peanut hull effectively reduced nitrate (NO_3^-), ammonium (NH_4^+), and phosphate leaching from a sandy soil under laboratory conditions by 34, 35, and 21%, respectively (Yao et al., 2012). Hardwood BC applied at 20 g kg^{-1} to an agricultural soil amended with swine manure reduced NO_3^- leaching by 10%

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Abbreviations: BC, biochar; BC_{aged}, field-aged biochar; BC_{comp}, composted biochar; DOC, dissolved organic carbon; EUF, electro-ultrafiltration; M_0, 10× extraction in 2 mol L^{-1} KCl at 22 ± 2°C and 80°C; M_1, electro-ultrafiltration; M_2, repeated water + KCl long-term shaking; M_3, M_2 plus one repeated shaking at 80°C; N_{min}, mineral nitrogen.

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(Laird et al., 2010). In another study, a 94% reduction in the leaching of NO_3^- and NH_4^+ was observed from a BC-amended ferralsol in a 37-d soil column leaching experiment (Lehmann et al., 2003). Biochars produced from poultry litter and softwood chips of spruce-pine-fir at pyrolysis temperatures of 400, 500, and 600°C sorbed 5% N by mass, irrespective of the N source (NH_4NO_3 or urea NH_4NO_3), in a laboratory study (Jassal et al., 2015). Sugarcane filter-cake BC, produced at >500°C, reduced dissolved organic carbon (DOC) but not NO_3^- leaching from a sugarcane field treated with vinasse (nutrient-dense effluent containing 0.08 g N L^{-1}) (Eykelbosh et al., 2015). Biochar from woodchips significantly increased mineral nitrogen (N_{min}) (NO_3^-, NH_4^+) and phosphate retention in a laboratory study, whereas the same BC lost 60 to 80% of adsorption capacity after 7 mo of field incubation (Gronwald et al., 2015).

The studies discussed above used standard extraction methods. They discussed possible mechanisms of N_{min} or N (total) retention/adsorption that can be grouped into three frequently reported hypotheses/assumptions: (i) BC amendment may alter soil cation and/or anion exchange capacity, which leads to reduced N leaching (Liang et al., 2006; Cheng et al., 2008; Laird et al., 2010; McElligott, 2011); (ii) BC amendment may alter the soil microbial community influencing N transformation (Neill, 2007; Liang, 2008); and (iii) N retention in BC-amended soil may occur due to improved soil water holding capacity and NH_4^+ or N immobilization (Knowles et al., 2011; Zheng et al., 2013).

Leaching of NO_3^- to groundwater from agricultural landscapes is of concern worldwide (Di and Cameron, 2002; Stout, 2003). Therefore, many methods have been used to try and quantify NO_3^- leaching in field soils, such as suction cups and lysimeters (Williams and Haynes, 1994; Di and Cameron, 2002; Decau et al., 2003). Nitrate, the readily water-soluble and highly mobile form of N, can be extracted with a number of chemical solutions (0.35% saturated $CaSO_4{\cdot}2H_2O$ solution; 0.03 mol L^{-1} NH_4F; 0.015 mol L^{-1} H_2SO_4; 0.01 µL $CaCl_2$; 0.5 mol L^{-1} $NaHCO_3$ [pH 8.5]; 0.01 mol L^{-1} $CuSO_4$ containing Ag_2SO_4 and 2 mol L^{-1} KCl solution) or simply with deionized water (Maynard and Kalra, 1993). In addition, attempts have been made to develop a single method to extract a number of plant-available nutrients along with NO_3^- in soil samples simultaneously, such as electro-ultrafiltration (EUF) based on electrochemistry (Nemeth, 1979). The EUF technique to extract available N in soil has been widely used in Western Europe (Barekzai et al., 1992; Appel and Mengel, 1993), and it can be programmed (duration and electrical current) according to requirements for different fractions of soil nutrients (Nemeth, 1979). However, the most widely used chemical extraction method for N_{min} determination is 2 mol L^{-1} KCl (Keeney and Nelson, 1982; Li et al., 2012), which is also used for N fertilization recommendations (Velthof and Oenema, 2010).

Most of the recent studies on BC amendment and N retention have used standard methods of N_{min} extraction. In contrast, Jassal et al. (2015) and Kammann et al. (2015), who reported that NO_3^- may be retained in BC pores and will probably not be completely extracted using standard extraction methods. Moreover, most studies have used fresh BC; only a few studies have investigated field-aged BC (BC_{aged}) from longer-term (>1 yr) field experiments.

The aims of our study were to evaluate the potential of fresh (BC_{fresh}) and BC_{aged} to capture NO_3^- in situ by examining repicked aged particles and by monitoring N_{min} concentrations in the field as an indication of NO_3^- retention. More specifically, we evaluated (i) if freshly incorporated BC affected the concentrations of the N_{min} species in a temperate sandy soil; (ii) if the BC_{aged} particles captured NO_3^- and, if so, if the NO_3^- concentration within the BC particles varied with time of exposure in the field and with particle size; and (iii) if standard methods of N_{min} extraction (2 mol L^{-1} KCl, EUF) could retrieve all extractable N_{min}, particularly NO_3^-, from BC_{aged} particles or if other approaches were more efficient.

Materials and Methods

Study Site and Soil Quality

A long-term field study (2012–2014) was conducted at the research station (Gross-Gerau 49°45′ N and 8°29′ E, 90–145 m above sea level) of the Institute of Plant Breeding and Agronomy I, Justus Liebig University Giessen, Germany. The experimental site is located in the upper Rhine Valley with the river Main to the north, the river Rhine to the west, and the Odenwald mountains to the east. The average (over 56 yr) temperature and precipitation of the region are 9.8°C and 600 mm, respectively, with the experimental years being warmer and wetter than average (Supplemental Fig. S1). The soil was silty sand formed from Rhine sand deposits and consisted of 85.2% sand, 9.6% silt, and 5.2% clay. The top 0- to 25-cm soil layer is poor in organic carbon (5900 mg kg^{-1}) and total nitrogen (600 mg kg^{-1}). It contains CAL-P 92.2 mg kg^{-1}, CAL-K 124.5 mg kg^{-1}, and Mg 35.5 mg kg^{-1} and has a pH (0.01 mol L^{-1} $CaCl_2$) of 6.31. There are some secondary calcareous precipitations of different thicknesses and depths up to 1 m.

Feedstock and Properties of Biochar

The BC was produced from wood chip shavings (including needles, twig pieces, bark, and small wood chips) of *Picea abies* (L.) Karst. and *Fagus sylvatica* L., 70 and 30% by weight, respectively; needles comprised almost 30% of total feedstock. The pyrolysis temperature was approximately 550 to 600°C (Pyreg GmbH). The detailed chemical and physical characterizations of BC are given in Table 1.

Experimental Design

The experimental set-up was a randomized complete block design (split plot arrangement). Six treatment plots (4.5 × 7 m) with four replicates (*n* = 24) were installed to investigate the effects of the following main factors: BC application level (control, 15 t ha^{-1}, and 30 t ha^{-1}) and watering regime (rain-fed plus optimum irrigation [i.e., irrigation if needed, in case of drought spells] and rain-fed only). Watering regime plots were separated by a 4.5-m-wide row to avoid irrigation effects in the nonirrigated treatment.

An initial soil sampling (0–30 cm) for nutrient analysis was performed before the incorporation of BC. Biochar was incorporated into the top 15-cm layer on 12 Apr. 2012. The experimental field was planted with the following crop sequence: maize (*Zea maize* L.) in 2012, winter wheat (*Triticum aestivum* L.) in 2012–3013, and pea (*Pisum sativum* L.) as a cover crop in winter 2013–2014. Maize was supplied with 150 kg N ha^{-1} on 2 May 2012 as granulated ammonium sulfate nitrate with sulfur having total N of 26% and was provided as NH_4^+ and NO_3^- plus water soluble S at amounts of 19, 7, and 13%, respectively. Winter wheat was supplied with 190 kg N ha^{-1} as ammonium sulfate nitrate with sulfur in three splits of 60, 80, and 50 kg N ha^{-1} on 6

Mar., 26 Apr., and 3 June 2013, respectively. Maize had to be supplied with additional irrigation of 70 mm twice during drought spells (11 and 25 July 2012). The wheat crop was supplied with only 30 mm irrigation (25 June 2013) because no drought occurred (Supplemental Fig. S1). Maize and wheat stalks and the complete pea crop were likewise incorporated in at the 0- to 15-cm depth.

Soil Sampling and Biochar Extraction from Soil

Soil samplings were performed at depths of 0 to 15 and 15 to 30 cm on a monthly basis from five locations randomly selected within each plot. During the third year of the study (February 2014), sampling was extended down to 90 cm depth in four layers (0–15, 15–30, 30–60, and 60–90 cm) to investigate soil NO_3^- concentrations in the top and deeper soil layers as a proxy for N leaching/retention. Soil samples were immediately stored in cooling boxes and carried to the laboratory for N_{min} extraction. The remaining soil from each sample was oven dried at 40°C and stored at room temperature.

For extracting BC particles, the stored soil (from selected sampling dates: 8 June 2012, 2 mo after BC addition; October 2012; September 2013, after the harvest of maize and winter wheat, respectively; and February 2014, after the cover crop) was sieved, and BC particles were removed with forceps. Two differently sized BC fractions were separated for N_{min} extraction (≤2 and ≥2 mm).

Soil Extraction

Fresh soil samples were extracted for N_{min} with the standard 2 mol L^{-1} KCl extraction method (Keeney and Nelson, 1982). Field-fresh soil (20 g) was placed in 80 mL of 2 mol L^{-1} KCl (1:4 w/v soil/KCl), shaken for 1 h at 100 rpm, and filtered (round filter ø 70 mm; S&S type 595). Concentrations of NO_3^- and NH_4^+ were quantified colorimetrically using an auto-analyzer (Seal).

Table 1. Elemental composition, main nutrients, and chemical and physical characterization of fresh biochar.

Element	Unit	Value
Carbon	$g\ kg^{-1}$	744
Nitrogen	$g\ kg^{-1}$	5.6
Hydrogen	$g\ kg^{-1}$	<10
Oxygen	$g\ kg^{-1}$	100.6
Phosphorous	$g\ kg^{-1}$	1.63
Sodium	$g\ kg^{-1}$	3.3
Potassium	$g\ kg^{-1}$	6.07
Calcium	$g\ kg^{-1}$	19.07
Magnesium	$g\ kg^{-1}$	2.09
Iron	$g\ kg^{-1}$	2.59
Copper	$g\ kg^{-1}$	0.02
Zinc	$g\ kg^{-1}$	0.17
	Particle size fraction	
Fraction size	**Unit**	**Value†**
>6.3 mm	%	0.5
3.15–6.3 mm	%	24.2
2–3.15 mm	%	7.1
1.6–2 mm	%	20.9
1–1.6 mm	%	4.9
0.63–1 mm	%	17
0.1–0.63 mm	%	25.3
<0.1 mm	%	0.1

† Values are estimated in percentage by dry mass.

Extraction of Field-Aged Biochar Particles

The particles were extracted for N_{min} with different methods as follows.

Standard 2 mol L^{-1} KCl extraction was based on Keeney and Nelson (1982), except a greater dilution was used (1:10 instead of 1:4 w/v BC/KCl).

With the modified standard 2 mol L^{-1} KCl method (M_0), BC particles were repeatedly shaken (100 rpm) 10 times, each time for 1 h (the first though fifth times at room temperature [20 ± 2°C] and the sixth through tenth times in a hot water bath at 80°C) and each time in new KCl (1:10 w/v BC/KCl) solution. Any N_{min} extracted with the first extraction was used as a standard to compare with other modified methods (M_0–M_3). After each 1-h shaking, extractants were filtered using the same type of filter paper as used above and analyzed for N_{min}, and the BC particles were passed on to the next extraction until the last one was finished. Concentrations of N_{min} were determined colorimetrically as described above.

The next method (M_1) used electro-ultrafiltration (EUF) based on the use of an electrical field (Nemeth, 1979). A sample of 1 g of finely ground (≤1 mm) BC and 4 g of a sandy soil of known negligible N concentration were weighed into an EUF cell; 4 g of the same soil plus 1 g quartz sand (≤1 mm) were used as a blank in a separate extraction. Distilled water was allowed to cover the electrode and temperature gauge to extract the sample at 20°C, 200 V, and 15 mA; elutes (representing the fraction of readily plant-available N_{min}) were collected every 5 min (six times). After collecting these elutes over 30 min, the voltage was increased to 400 V and the temperature to 80°C for the next six elutes every 5 min (representing the fraction of potentially plant-available N_{min}), thereby increasing the force for desorption of nutrients from soil/BC particles (Nemeth, 1979; Mengel and Uhlenbecker, 1993). The concentration of extractable N_{min} was quantified colorimetrically as described above.

For the modified standard method (water + KCl long-term shaking, M_2), biochar (1:10 BC/deionized water, w/v) was repeatedly shaken in water (100 rpm) for 1 and 24 h and subsequently in 2 mol L^{-1} KCl, again for 1 and 24 h (1:10 w/v of BC/2 mol L^{-1} KCl). After each shaking period, samples were filtered to determine N_{min} concentrations in the extractant, and BC particles were passed on for the next extraction to new water or KCl solution. The N_{min} concentration was determined colorimetrically as described above.

The next method used water + KCl long-term extraction plus (M_3). This method was similar to M_2 but with an additional fifth step of 24-h shaking of BC particles (1:10 w/v of BC/2 mol L^{-1} KCl) in a hot water bath at 80°C. We adopted this method to further investigate if BC still had some extractable N_{min} after the observations made with the M_2 method.

Statistical Analysis

According to the experimental field study design, we used a two-way ANOVA for complete data sets (e.g., NO_3^- concentrations) from the field experiment. Initially, data were tested for normality (Kolmogorov–Smirnov test) and for homogeneous variances (Levene's mean test) and were log transformed where needed (indicated with the respective results). Data were analyzed with SigmaPlot 11.0 (Systat, Inc.) an evaluated at a significance level of $p < 0.05$. If significant differences existed within BC application rates, Tukey's HSD test was performed to identify treatment levels that were different from each other ($p < 0.05$).

Results

Mineral N Retention in Sandy Soil under Field Conditions

Soil N_{min} concentrations after BC application, about 5 wk after fertilization of the first crop (BC residence time, 2 mo), and at the harvest of first and second crops (after 6 and 16 mo of BC residence time in soil, respectively) are given in Fig. 1 and Supplemental Table S1. Results revealed that the presence of BC increased NO_3^- concentrations (by 99–389%) in the topsoil (0–15 cm) above the control ($p < 0.001$), with a significant increase of NO_3^- after increasing BC application rates (Fig. 1a). The subsoil showed elevated NO_3^- concentrations in the control and 15 t ha^{-1} BC treatment compared with the 30 t ha^{-1} BC treatments ($p < 0.001$), indicating reduced NO_3^- leaching with a high rate of BC addition (Fig. 1b). Concentrations of NH_4^+ were very small and further reduced (60–95%) in the topsoil with increasing rates of BC addition, whereas there was no significant effect of BC addition on NH_4^+ in the subsoil (Fig. 1a, b). The effect of BC on NH_4^+ was reversed after 16 mo of field ageing (Fig. 1e, f). After 6 and 16 mo, BC amendment consistently caused higher NO_3^- concentrations in the topsoil compared with the control (Fig. 1c, e). The "rain-fed only" treatment only had greater NO_3^- concentrations than the "rain-fed + irrigation" treatment at the harvest of the first crop (Fig. 1c). Prolonged field ageing altered the response of BC in such a way that NO_3^- and NH_4^+ concentrations increased with increasing rates of BC addition in the topsoil (Fig. 1e).

The deep soil sampling (0–90 cm depth during the third year) revealed that BC amendments significantly reduced NO_3^- concentrations below the BC horizon (Supplemental Fig. S2; Supplemental Table S2). A significant effect of BC amendment on NH_4^+ retention in the topsoil (0–15 cm) was not observed. However, the 15- to 30-cm and 30- to 60-cm soil layers showed significantly greater NH_4^+ concentrations due to BC addition (Supplemental Fig. S2). The rain-fed + irrigation treatment generally showed less N_{min} retention in the BC-amended topsoil than the rain-fed only treatment.

Mineral N Extraction from Fresh and Aged Biochar Particles with Different Methods

The standard 2 mol L^{-1} KCl method extracted only 174 mg NO_3^- kg^{-1} BC, which was 13% of the total (1349 mg N kg^{-1} BC) extracted using repeated extractions (M_0) (Fig. 2a). Similarly, only a quarter of the total amount of NH_4^+ (27% of 51 mg kg^{-1} BC) was extracted with the standard 2 mol L^{-1} KCl extraction (Fig. 2a).

The M_1 method (EUF) extracted only 404 mg NO_3^- kg^{-1} BC (152 mg in readily plant-available and 252 mg in potentially plant-available form; sum of six low and six high temperature fractions, respectively). This was approximately a third of the NO_3^- amount extracted by M_0 (Fig. 2a, b).

The M_2 method extracted significantly greater amounts of total NO_3^- (1011.6 mg kg^{-1} BC) than the standard 2 mol L^{-1} KCl or than M_1 (EUF), but it was smaller than the total sum of NO_3^- extracted with M_0 (Fig. 2c).

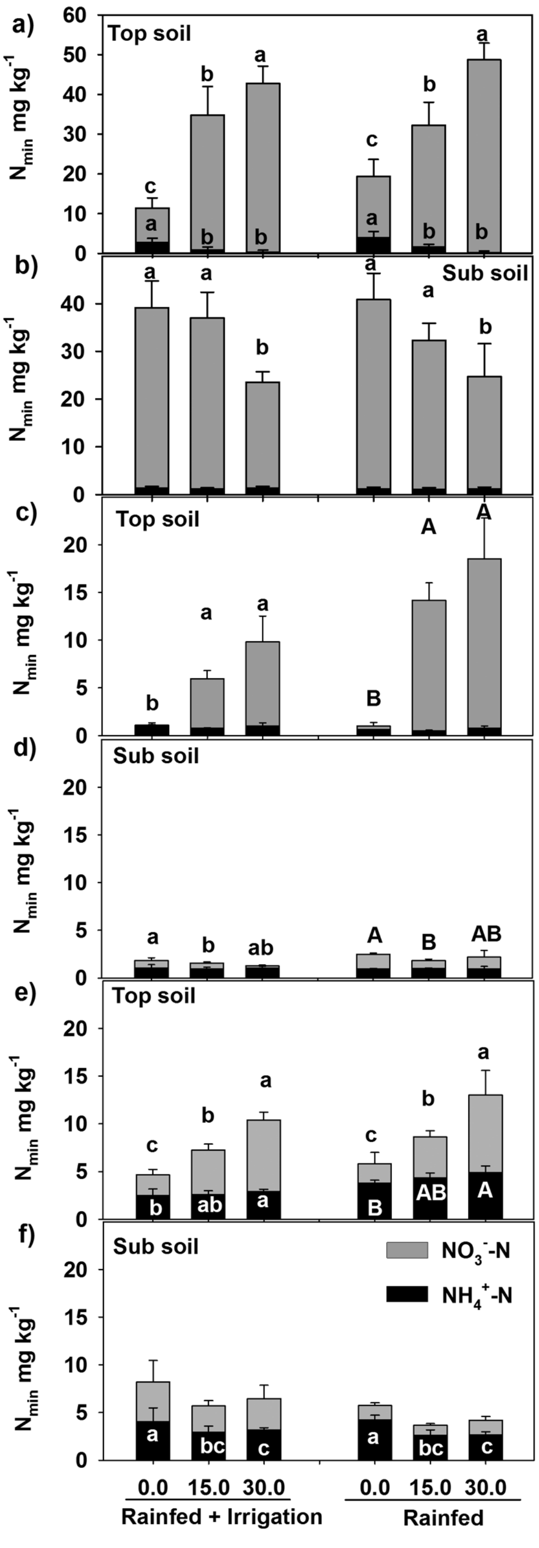

Fig. 1. Effect of biochar (BC) addition (0, 15, and 30 t ha^{-1}) and watering regime (rain-fed + irrigation and rain-fed only) on mineral nitrogen (N_{min}) (NO_3^- and NH_4^+) retention in sandy soil under field conditions (means and SD; $n = 24$). (a, b) The N_{min} results of 2 mo after BC addition and 5 wk after the first N fertilization; (c, d and e, f) The results of 6 and 16 mo after BC addition, after the first (maize) and second crop (wheat) harvest, respectively. The y axis scales of a, b, and c through f are based on the respective mineral-N retention in the topsoil. Different letters on NO_3^- and in the bars (NH_4^+) indicate significant differences in the respective mineral N species due to BC treatments; lowercase versus uppercase letters indicate a significant irrigation effect, respectively, after two way ANOVA (Tukey test, $P < 0.05$).

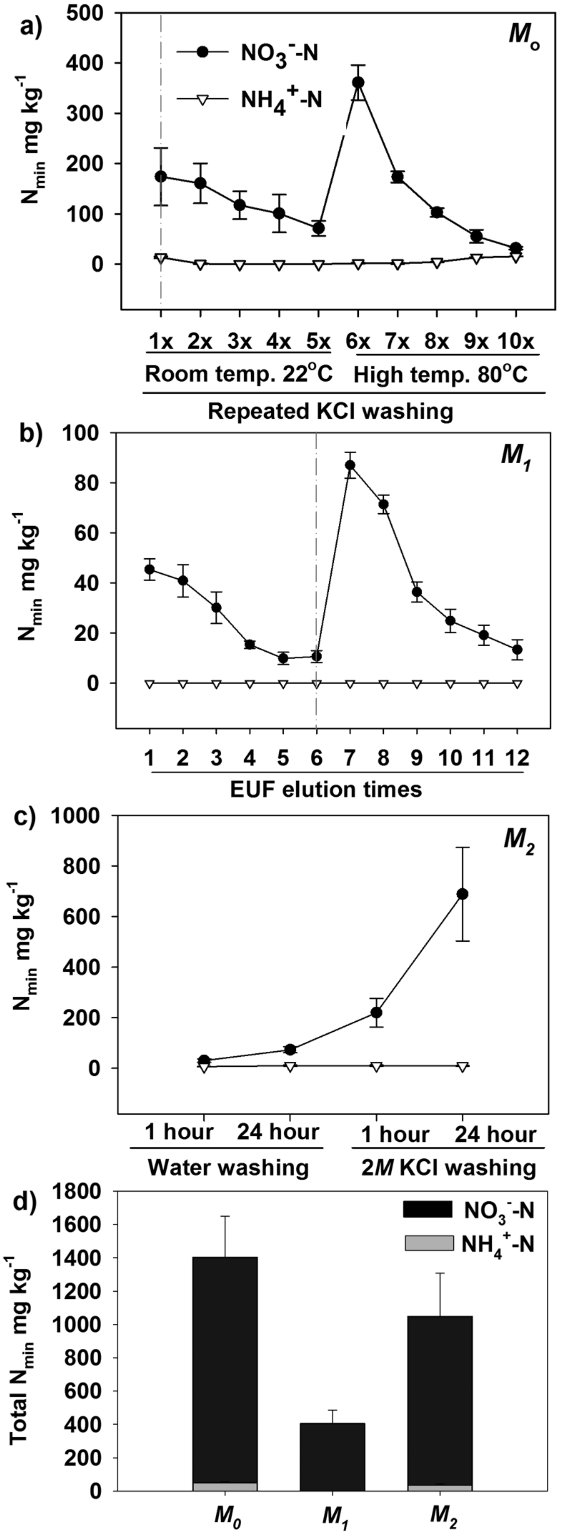

Fig. 2. Comparison of different extraction methods. (a) M_0, repeated extraction in 2 mol L^{-1} KCl (extractions 1–5 at room [22 ± 2°C] and extractions 6–10 at high temperature [80°C]). (b) M_1 (electro-ultrafiltration). (c) M_2 (repeated water + KCl long-term shaking). (d) Total mineral nitrogen (N_{min}) of all three methods for N_{min} (NO_3^- and NH_4^+) extraction from 16-mo field-aged biochar particles (size fraction, 2–5 mm). The lines and bars indicate means and SD.

The M_3 method revealed that the BC particles still captured some NO_3^- to be delivered, which was invisible with M_2 (Supplemental Fig. S3).

Effects of Biochar Particle Size on Mineral N Retention

The BC_{fresh} did not carry a considerable amount of N_{min} compared with N released from BC_{aged} (Fig. 3). Increasing the temperature to 80°C for repeated extractions initiated more N_{min} release from BC_{aged} (but not BC_{fresh}) even if the BC seemed to have released all captured N during first five extractions at room temperature (20 ± 2°C) (Fig. 3a, b). Overall there was no significant difference in the total N_{min} sum released from different particle size fractions (>2 or <2 mm) within BC_{fresh}, BC_{aged}, or the two water treatments (Fig. 3c).

Discussion

Improved N retention, reduced NO_3^- leaching, and reduced N uptake limitations in plants after BC addition have been demonstrated in a number of studies (Lehmann et al., 2003; Knowles et al., 2011; Major et al., 2012; Güereña et al., 2013; Haider et al., 2015; Guo et al., 2014; Zhang et al., 2015; Dong et al., 2015). High-temperature (500–700°C) biochars usually carry a negative charge that further increases with weathering (Cheng et al., 2008); hence, it is considered unlikely that nitrite or NO_3^- sorption will occur due to repulsive forces between the negative surface of BC and anions such as NO_3^- (Iqbal et al., 2015). Therefore, it has often been assumed that N immobilization is the cause of increased retention, reduced leaching, or reduced availability of NO_3^- to plants after BC addition (Knowles et al., 2011; Major et al., 2012; Güereña et al., 2013; Zheng et al., 2013; Guo et al., 2014; Zhang et al., 2015). Nitrogen immobilization is usually attributed to the labile organic C contained in the applied BC, stimulating microbial growth. This is in contrast to the observation that higher-temperature BCs with a higher inner surface area are better at retaining NO_3^- (Clough et al., 2013; Dempster et al., 2012; Jassal et al., 2015), although they carry lower fractions of labile carbon than low-temperature BCs. Anion exchange capacity may cause NO_3^- sorption (Chintala et al., 2013), but this is usually very low in freshly produced BC and is lost quickly after addition to soil due to oxidation (Cheng et al., 2008; Laird, 2008; Hollister et al., 2013). Thus, reduced NO_3^- leaching or increased extractable amounts of total N in BC-amended soils are often related to direct NH_4^+ adsorption (Lehmann et al., 2003; Güereña et al., 2013) because of higher cation than anion exchange capacity of BC (Hale et al., 2013) or due to NH_4^+ binding via electrostatic exchange with other negatively charged species on BC surfaces (Mukherjee et al., 2011; Mukherjee and Zimmerman, 2013; Hale et al., 2013).

In this study we observed significantly increased NO_3^- retention in the BC-amended topsoil either shortly after the start of the study after N fertilization or after the first- and second-year harvest when N_{min} (mostly NO_3^-) was supposed to have been taken up by the crop or leached out of the topsoil (Fig. 1). This indicates that some of the NO_3^- extracted with the 2 mol L^{-1} KCl method in the BC treatments was likely not plant available, as observed for the same BC in a pot study with maize (Haider et al., 2015) and in a pot study with activated BC and Italian ryegrass (*Lolium multiflorum* ssp. *italicum*) (Borchard et al.,

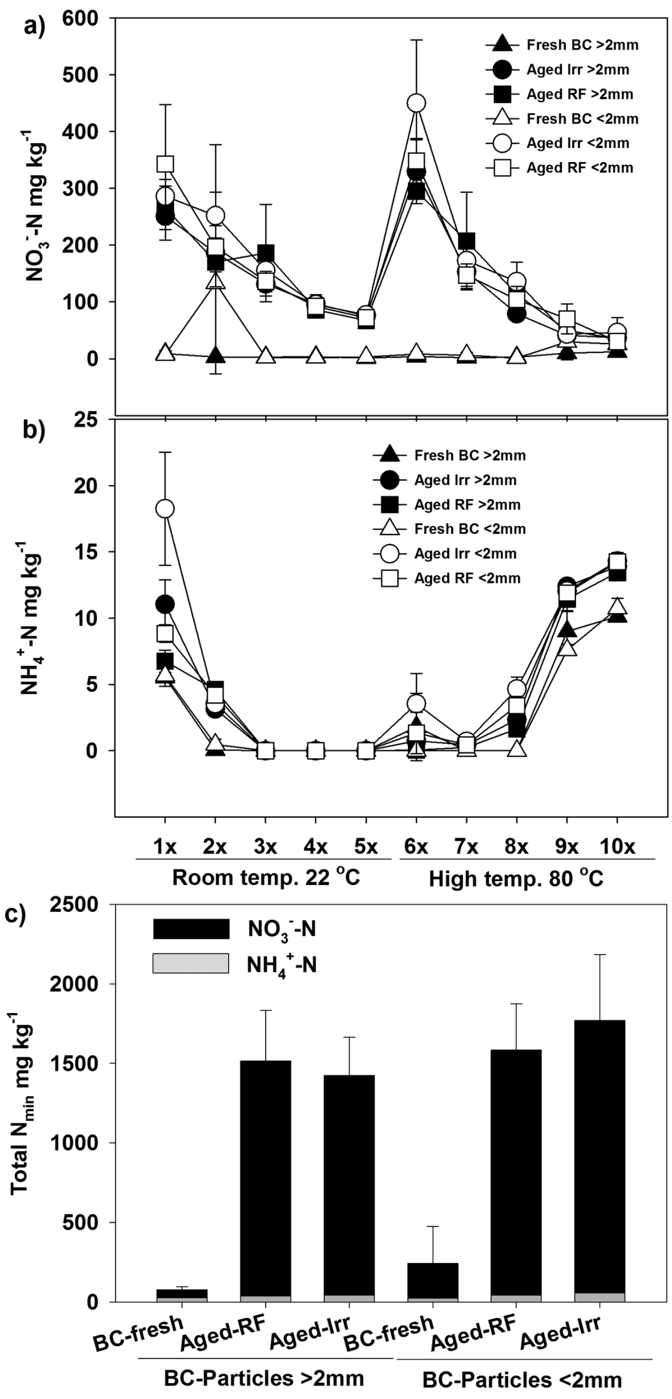

Fig. 3. Effect of biochar (BC) size fraction (>2 mm vs. <2 mm) and ageing (either under field conditions (BC_{aged} under rain-fed + irrigation and irrigation only treatments) or fresh BC (BC_{fresh}) stored under laboratory conditions for 22 mo on the captured mineral nitrogen (N_{min}) (NO_3^- and NH_4^+). The BC particles were extracted with the M_0 method (repeated extraction in 2 mol L⁻¹ KCl; extractions 1–5 at room [22 ± 2°C] and extractions 6–10 at high [80°C] temperature). The lines and bars indicate means and SD (*n* = 24).

2012). However, the NO_3^- retained in the BC-amended soil was obviously also protected against leaching. At the time of deep soil sampling after plenty of precipitation since the start of the experiment, BC amendment elevated NO_3^- concentrations in the topsoil (0–15 cm; i.e., the BC horizon), accompanied by significantly reduced NO_3^- concentrations in the subsoil (30–90 cm) (Supplemental Fig. S2). This translated into significantly higher total NO_3^- stocks in the BC plots than in the control (Supplemental Fig. S4; Supplemental Table S3). This was due to topsoil accumulation balancing significantly lower subsoil stocks below 30 cm (Supplemental Fig. S4) even when a lower bulk density due to BC addition is assumed (Supplemental Table S5). Therefore, BC amendment most likely reduced NO_3^- leaching in the poor sandy soil under field conditions. Ventura et al. (2013) also reported significantly reduced cumulative NO_3^- leaching by 75% in an apple orchard, quantified using ion exchange resins over 1 yr. Rizhiya et al. (2015) reported higher NO_3^- concentrations in the BC-amended topsoil, accompanied by reduced N_2O emissions. A number of laboratory studies have also frequently reported reduced NO_3^- leaching (Lehmann et al., 2003; Laird et al., 2010; Yao et al., 2012; Zheng et al., 2013; Jassal et al., 2015), sometimes accompanied by reports of reduced amounts of extractable NH_4^+ and particularly NO_3^- (Sika and Hardie, 2014) and sometimes accompanied by reports of increased amounts of extractable NO_3^- (Van Zwieten et al., 2010; Cayuela et al., 2013; Martin et al., 2015). However, some field studies show no reduction in NO_3^- leaching (Castaldi et al., 2011; Eykelbosh et al., 2015) or even greater NO_3^- leaching than NH_4^+ due to BC addition (Güereña et al., 2013).

We suggest that BC may not only cause microbial immobilization of N but that it may also capture NO_3^-, with the NO_3^- being sometimes retrieved by KCl extraction and sometimes not, depending on BC pore size distribution and the capturing mechanism(s). In the first case, higher mineral N concentrations are reported in soils with BC (Kameyama et al., 2012; Kammann et al., 2015; Rizhiya et al., 2015). This leads to the further questions of if, and to what extent and how strong, BC particles may capture mineral N, particularly NO_3^-, and if the extent of "capturing" varies over time in the field.

The BC_{aged} particles released surprising amounts of NO_3^-, but not so much NH_4^+ (0, 36.2, and 51.35 mg kg⁻¹ BC with the M_1, M_2, and M_0 methods, respectively), only when repeatedly extracted (Fig. 2 and 3). The same was observed by Kammann et al. (2015) for co-composted BC. However, Jassal et al. (2015) reported an even higher capture of NO_3^- (34–39 g kg⁻¹ BC) when either woody or poultry litter BCs were treated with ammonium nitrate solution. In their study, both BC types rereleased little of the captured NO_3^- (mostly <1%; but the authors did not try repeated extractions); BCs produced from a blend of both feedstocks captured only 10 to 20% of the NO_3^- amount captured by the woody or poultry litter BCs, respectively; however, the mixed-blend BCs released a higher percentage (10–12%) of the amount they had captured (Jassal et al., 2015).

Standard mineral N sorption mechanisms such as CEC may play a role for the tiny amounts of NH_4^+ detected in the BC particles (Lehmann et al., 2003; Güereña et al., 2013). Clough et al. (2013) suggested that NH_4^+ adsorbed to negatively charged functional groups (carboxylic acid and hydroxyl groups) should be easily extractable with KCl. Here about half of the NH_4^+ was extractable only by repeated extractions or at higher temperatures. Saleh et al. (2012) also observed very low desorption of NH_4^+ from peanut BC with the standard KCl method. Lower NH_4^+ than NO_3^- extraction suggests that BC addition to soil accelerated nitrification of NH_4^+ to NO_3^- (Steiner et al., 2008;

Kuzyakov et al., 2009; Prommer et al., 2014; Foereid, 2015), thus providing more NO_3^- for capture than NH_4^+.

Taken together, the greater NO_3^- capture and sorption in BC-amended soil or on BC particles (Supplemental Fig. S5) may be attributable to three different mechanisms: (i) solution mass flow into BC particles, where the hydrated, asymmetric NO_3^- ions are physically entrapped within the BC pores of the "right" size (Prendergast-Miller et al., 2011; Kameyama et al., 2012; Felber et al., 2014) particularly when the soil dries; (ii) bonding between negatively charged NO_3^- and some functional groups or positively charged cationic salts on BC surfaces; and (iii) nonconventional H bonding, which may additionally hold NO_3^-, as indicated by slow movement of water through poplar BC at room temperature, which markedly increased with temperature (Conte et al., 2014; Felber et al., 2014). More investigations that go beyond batch sorption experiments in solution (or 100% soil moisture; e.g., leaching) are needed to unravel the mechanisms and quantify their relative contributions to the observed phenomenon under real-world field conditions.

Thus, it is highly likely that BCs, depending on their properties, can vary in their ability to capture, retain, and subsequently release NO_3^- to soil solution or plants. Also, post-treatment effects may further modify this ability (Zhou and Butterbach-Bahl, 2014; Iqbal et al., 2015), as investigated in waste water treatment research. Although we lack a complete mechanistic understanding of how BC retains NO_3^-, it is clear that BC properties with regard to NO_3^- capture may be either modified by feedstock or feedstock blending before production (Clough et al., 2013; Jassal et al., 2015) or after production (e.g., organic coating, composting, and/or metal enrichment [Kammann et al., 2015; Prost et al., 2013; Zhang et al., 2012]).

We used different approaches to extract N_{min} from the BC particles, with varying effectiveness (Fig. 2). The release of NO_3^- from the BC_{aged} particles was incomplete after a regular 1-h shaking with 2 mol L^{-1} KCl. Although the electric forces used by the EUF method did not succeed in retrieving the largest amount of NO_3^- and NH_4^+, it was evident from the methods used here that (i) the duration of shaking (equilibration time in the water or KCl extraction solution) and (ii) the extraction temperature (80°C vs. room temperature) played central roles in retrieving NO_3^- captured within BC particles. Mechanistically this suggests at least partial physical entrapment of the asymmetrically shaped, hydrated NO_3^- molecules in the BC–pore continuum, which warrants future research.

We recommend extracting BC-amended soils or BC_{aged} particles by long-duration shaking at higher temperatures (see Supplemental Fig. S3 and S5). The extraction temperature should be above 50°C because Conte et al. (2014) reported that the 2-dimensional surface water flow in BC particles changes to a 3-dimensional inner-pore water flow and becomes much faster when the temperature rises above 50°C, likely shortening equilibration times.

Further questions addressed in this study were (i) if the field-residing BC would capture more NO_3^- over time, (ii) if the smaller BC particles would capture more NO_3^- than larger particles (Spokas et al., 2012; Kizito et al., 2015), and (iii) if the relative extractability of captured NO_3^- (i.e., the "ease of release") would change over time. The studies of Kammann et al. (2015) and Prost et al. (2013) with co-composted BCs have suggested a relationship between DOC and NO_3^- sorption and capture, with NO_3^- capture increasing over time with DOC adsorption to BC. However, the answer to the first two of these questions was "no": The amount of NO_3^- in the BC_{aged} particles was almost identical in the first and second cropping years (i.e., after 6 and 16 mo of soil residence, both times after crop soil NO_3^- depletion) (Supplemental Fig. S5). There was no significant effect of BC fraction size on N_{min} capture in BC_{aged} particles. Borchard et al. (2012) also found no significant effect of BC particle size on greater total P and N retention in topsoil. Furthermore, the relative extractability did not change over time: When extracted with water for 1 and 24 h, the largest relative release of NO_3^- (8%) occurred with the more or less freshly applied BC (2 mo field ageing and closer to N fertilization), whereas after 6 and 16 mo and after the harvests, the fraction retrieved by 1-h water shaking was only 3% (Supplemental Fig. S5). The additional extraction by M_3 at 80°C compared with M_2 still extracted more NO_3^-, but no increase over time was found (Supplemental Fig. S3). However, in the present study we cannot exclude the possibility that the closeness of N fertilization (after 2 mo) or the different crops in the first and second year, as well as climatic conditions, may influence the absolute amount and relative fractions of NO_3^- captured in and released from the BC particles. The only statement that can be made with some certainty is that the amount of NO_3^- captured in BC particles, and its re-extractability, did not markedly increase over time.

Is it possible to explain the observed NO_3^- retention in the topsoil (i.e., in the BC horizons, with 15 and 30 t ha^{-1}) by NO_3^- capture in the BC particles? Is it possible that normal soil KCl extraction does not deliver all NO_3^- in the BC-amended soil when perhaps some NO_3^- remains captured in the BC particles? We addressed these questions by a back-of-the-envelope approach whereby we calculated theoretical topsoil NO_3^- stocks (control soil NO_3^- plus BC particle NO_3^-) and compared these with the soil NO_3^- stocks extracted from the field soil samples with standard 2 mol L^{-1} KCl extraction (Supplemental Fig. S5; Supplemental Table S4). This revealed that NO_3^- capture by BC particles can indeed account for the surplus NO_3^- held in the topsoil but only if the amount of NO_3^- obtained by repeated extractions (M_0) is used in the calculations (compare Supplemental Fig. S6a with b and c, respectively). Moreover, these theoretical calculated amounts of NO_3^- in the BC horizons tended to be greater than the measured NO_3^- stocks with the standard 2 mol L^{-1} KCl method. This suggests the possibility of underestimating NO_3^- stocks in BC-amended soils; however, the underestimation may not be as great as initially thought (compare Supplemental Fig. S6a and b and Fig. S6a and c).

A recent meta-analysis of 114 published studies reported that BC amendments improved plant growth and yield on average while accumulating total N in the soil (Biederman and Harpole, 2013). However, there are some studies showing improved mineral N accumulation in BC-amended soils with no yield stimulation (Borchard et al., 2012; Güereña et al., 2013). Due to our findings of NO_3^- capture in BC particles, we put forward the following hypotheses: (i) The ability of BCs for capturing NO_3^- (rather than adsorbing NH_4^+) may explain many of the "strange" results concerning plant and soil N availability and also N_2O emission reductions in BC-amended soils reported in the literature. Repeatedly extracting BC_{aged} particles in these studies may elucidate previous findings. (ii) The ability of BCs to capture NO_3^- may be purposefully optimized, depending on the intended use, into two directions: either toward "capture and retention of NO_3^- against leaching" (as observed here) or toward "NO_3^- capture and release to plants" (as observed by Kammann et al. [2015], with a higher fraction of water-extractable NO_3^- from BC_{comp} particles). In any case, the "NO_3^- capture" option by BC deserves closer research attention to decipher mechanisms for enabling beneficial (surplus) reactive N management by BC use in agriculture and waste treatment.

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Supporting Information

Title: Standard extraction methods may underestimate nitrate stocks captured by field aged biochar

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Supplementary Figures	**6**
Supplementary Tables	**5**

Table S1. Results of two-way ANOVA's showing the effects of factors biochar (BC, 0, 15 and 30 t ha^{-1}) and watering regime (WR, rain-fed + optimum irrigation and rain-fed only) on top and subsoil N$_{min}$ (NO$_3^-$ and NH$_4^+$) concentration at three different dates of sampling. Statistics accompanying figure 1 and SI figure S5.

Factors	**2 months after BC addition**							
	Topsoil 0-15 cm				**Subsoil 15-30 cm**			
	NO$_3^-$		**NH$_4^+$**		**NO$_3^-$**		**NH$_4^+$**	
	F	***p***	**F**	***p***	**F**	***p***	**F**	***p***
BC	69.6	*<0.001*	18.2	*<0.001*	15.1	*<0.001*	0.30	*0.746*
WR	1.82	*0.194*	2.3	*0.146*	0.03	*0.860*	0.82	*0.377*
BC x WR	1.92	*0.176*	0.79	*0.470*	0.75	*0.487*	0.04	*0.961*
	6 months after BC addition							
BC	129.1	*<0.001*	3.22	*0.064*	5.70	*0.012*	0.07	*0.936*
WR	29.8	*<0.001*	13.2	*0.002*	26.4	*<0.001*	0.30	*0.591*
BC x WR	4.81	*0.021*	0.26	*0.771*	4.25	*0.030*	0.24	*0.793*
	16 months after BC addition							
BC	38.0	*<0.001*	3.70	*0.045*	2.43	*0.116*	6.60	*0.007*
WR	0.40	*0.538*	52.1	*<0.001*	43.2	*<0.001*	0.40	*0.536*
BC x WR	0.28	*0.756*	0.74	*0.490*	1.24	*0.312*	0.37	*0.694*

Table S2. Influence of BC (BC, 0, 15 and 30 t ha^{-1}) and watering regime (H_2O, rain-fed + optimum irrigation and rain-fed only) on mineral N_{min} at four different depths in amended soil. Statistics accompanying figure S2.

Factors	**Approximately 2 years after BC addition**															
	0-15 cm				**15-30 cm**				**30-60 cm**				**60-90 cm**			
	NO_3^-		**NH_4^+**		**NO_3^-**		**NH_4^+**		**NO_3^-**		**NH_4^+**		**NO_3^-**		**NH_4^+**	
	F	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***
BC	7.40	*0.005*	0.25	*0.778*	0.19	*0.829*	7.37	*0.005*	4.49	*0.026*	5.07	*0.018*	15.3	*<0.001*	2.29	*0.130*
WR	0.15	*0.706*	3.47	*0.079*	0.18	*0.679*	17.7	*<0.001*	1.46	*0.242*	7.63	*0.013*	3.67	*0.071*	0.05	*0.812*
BC x WR	0.21	*0.817*	0.82	*0.455*	0.26	*0.773*	8.00	*0.003*	0.44	*0.649*	0.36	*0.704*	2.67	*0.096*	0.15	*0.857*

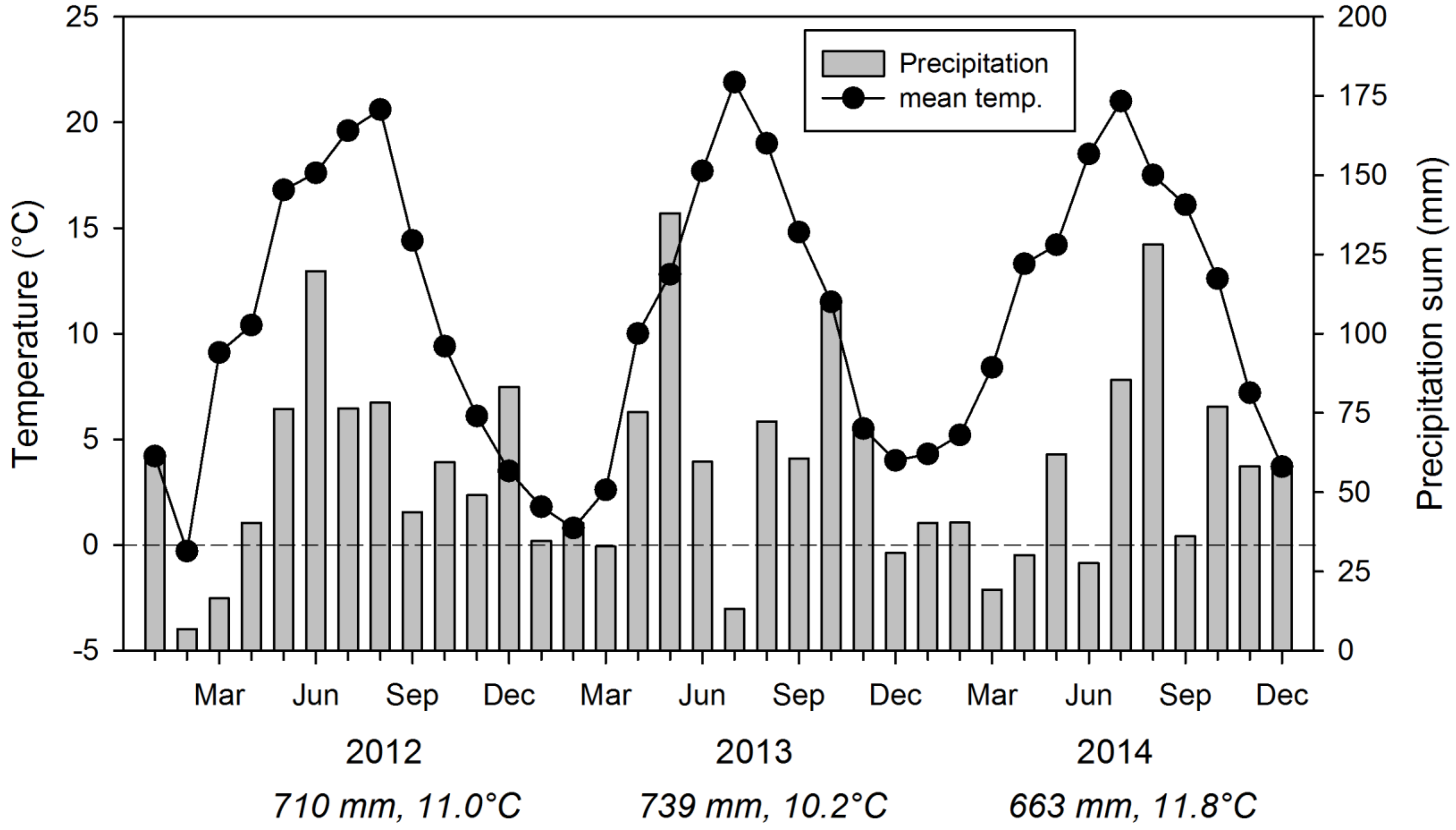

Fig. S1. Weather data of mean monthly precipitation (mm) and temperature (°C) for an experimental period.

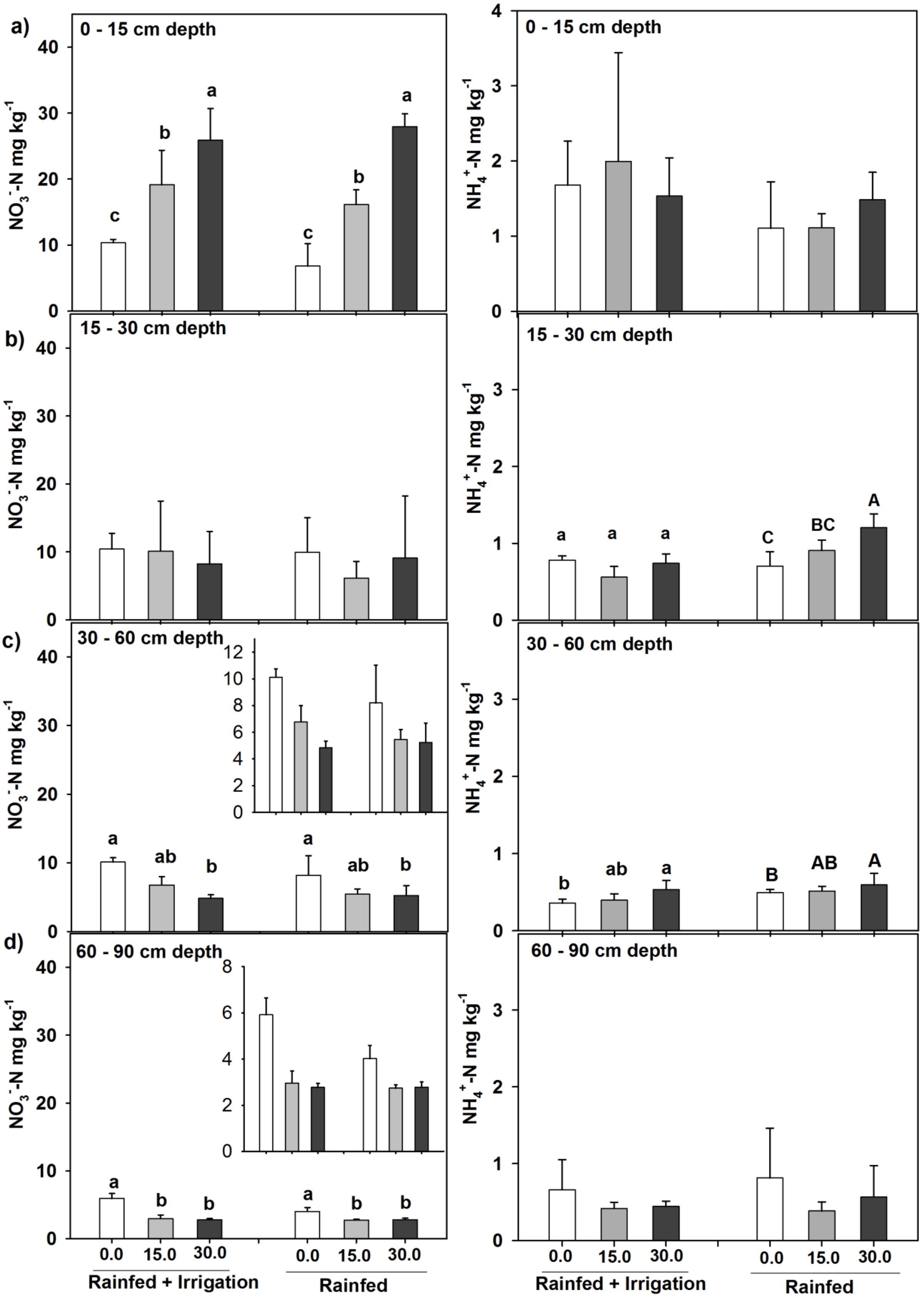
a)
0 - 15 cm depth
b)
15 - 30 cm depth
c)
30 - 60 cm depth
d)
60 - 90 cm depth
NO3--N mg kg-1
NH4+-N mg kg-1
0
10
20
30
40
1
2
3
4
12
8
6
c
b
a
c
b
a
a
a
a
C
BC
A
a
ab
b
a
ab
b
b
ab
a
B
AB
A
a
b
b
a
b
b
0.0
15.0
30.0
Rainfed + Irrigation
Rainfed

Fig. S2. Effect of biochar addition (BC 0, 15 and 30 t ha^{-1}) and watering regime (rain-fed + irrigation, and rain-fed only) on N_{min} (NO_3^- and NH_4^+) retention within the soil profile (0-90 cm; means + s.d., *n*=24), measured after nearly two years (22 months) of field ageing. The soil was extracted with the standard 2 *M* KCl method (1:4 w/v, 1 h shaking at 100 rpm). Note that the y-axis scales for NO_3^- and NH_4^+ are different. Different letters indicate significant differences due to BC treatments, lower- vs. upper-case letters indicate significant differences due to irrigation, respectively, following two-way ANOVA's (Tukey test, $p<0.05$) presented in table S3.

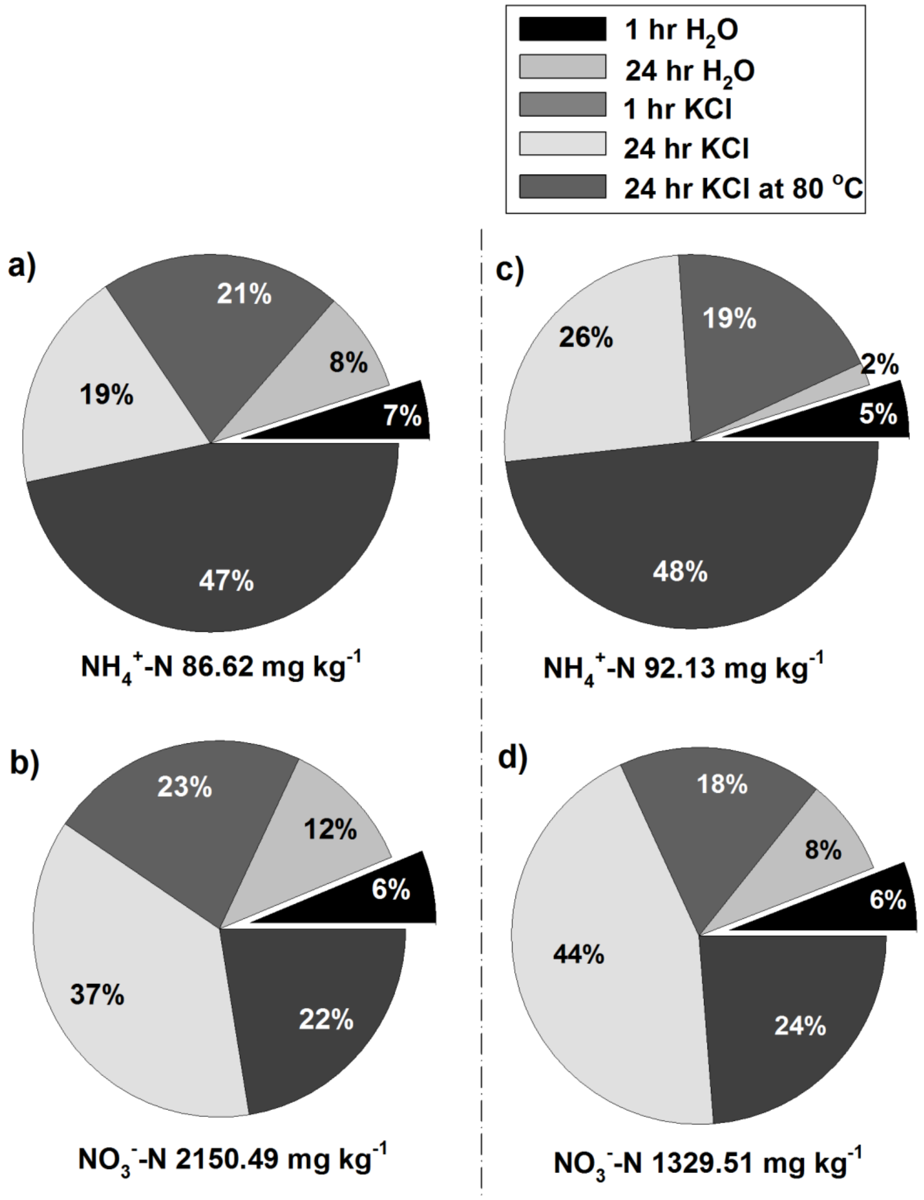

Fig. S3. Effect of field ageing on the potential of biochar (BC) to retain and deliver N_{min} (NO_3^- and NH_4^+) when extracted with modified method M_3. Figure parts a, b and c, d represent the N_{min} extracted from field-aged BC particles retrieved after the first N-fertilization (when maize was growing in the field) and at the maize-crop harvest (i.e. after 2 or 6 months of field ageing). The slices of the pie chart show the percentage of the total extracted NO_3^- or NH_4^+ of BC; the total amount that was extracted is given below each pie chart.

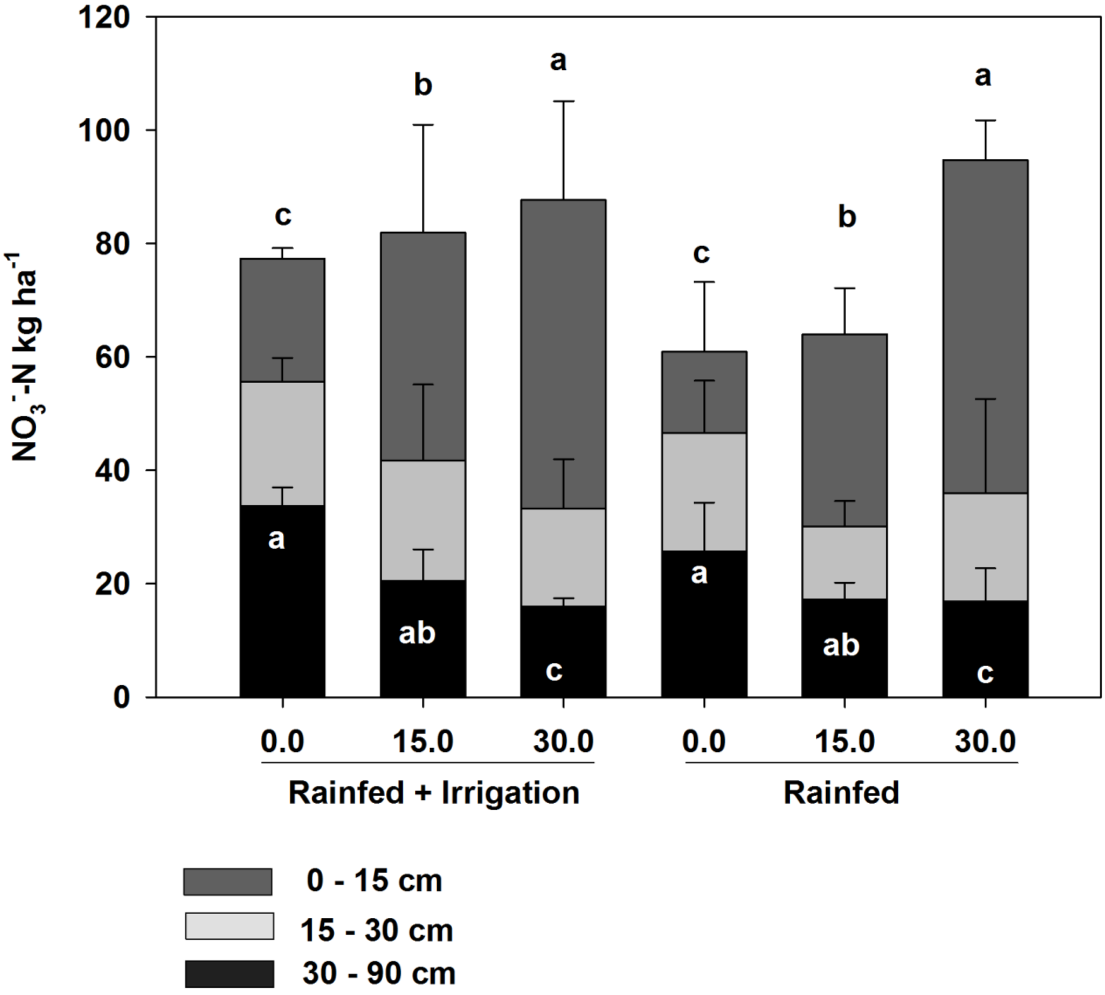

Fig. S4. Influence of biochar amendment (BC 0, 15 and 30 t ha^{-1} under rain-fed plus irrigation or rain-fed only treatment) on total NO_3^- stocks in the topsoil (0-15 cm, BC amended layer), upper subsoil (15-30 cm), and deeper subsoil (30-90 cm). Different letters on the bars indicate significant differences in the NO_3^- stocks due to BC treatments following two-way ANOVA's (Tukey test, $p<0.05$) presented in table S4. Letters above stacked bar: differences between the entire nitrate amount in 0-90 cm; letters within the 30-90 cm bar part: differences in the 30-90 cm layer.

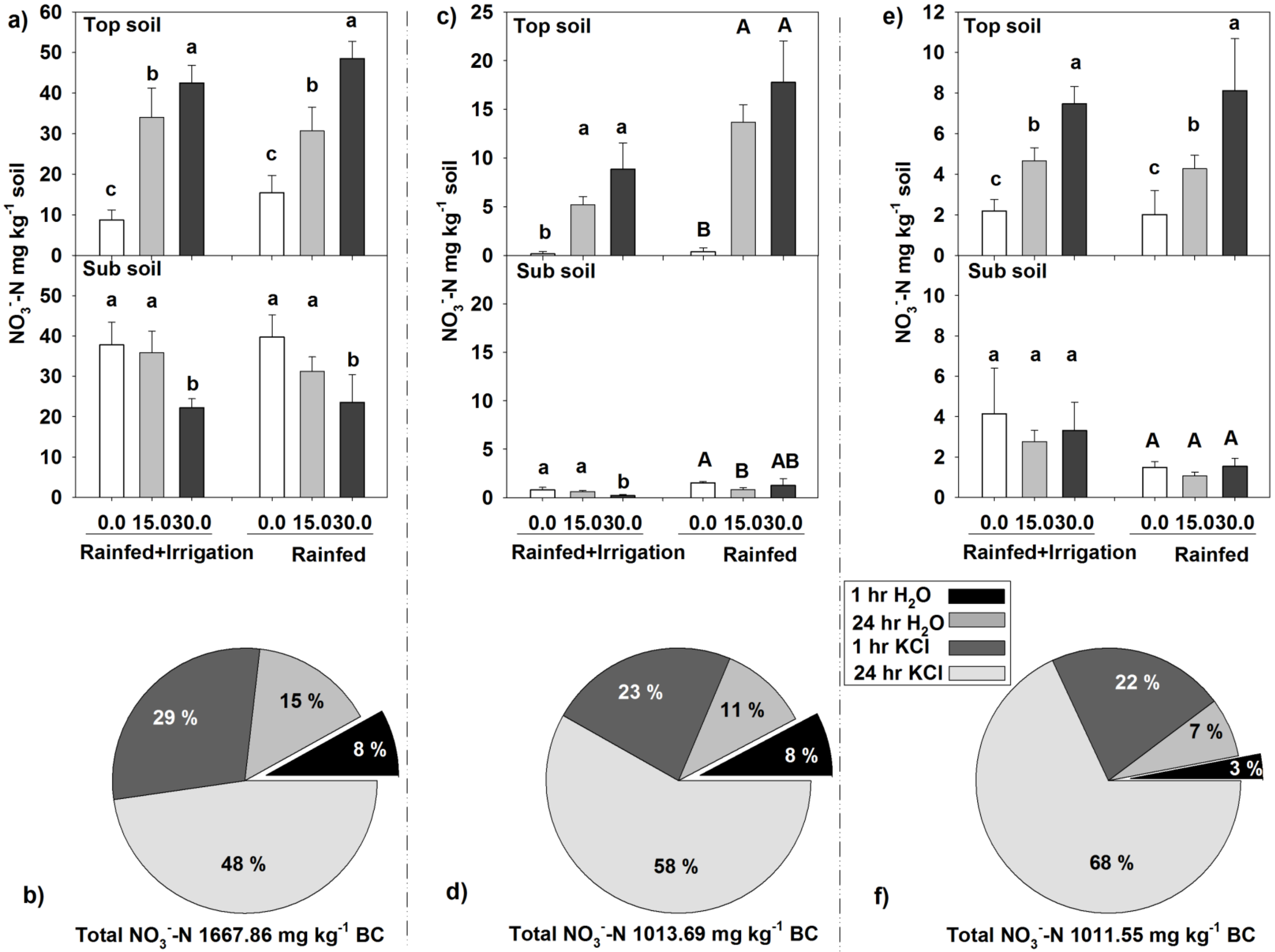
a)
Top soil
Sub soil
NO3--N mg kg-1 soil
0
10
20
30
40
50
60
c
b
a
c
b
a
a
a
b
a
a
b
0.0 15.0 30.0
0.0 15.0 30.0
Rainfed+Irrigation
Rainfed
c)
Top soil
Sub soil
NO3--N mg kg-1 soil
0
5
10
15
20
25
b
a
a
B
A
A
a
a
b
A
B
AB
0.0 15.0 30.0
0.0 15.0 30.0
Rainfed+Irrigation
Rainfed
e)
Top soil
Sub soil
NO3--N mg kg-1 soil
0
2
4
6
8
10
12
c
b
a
c
b
a
a
a
a
A
A
A
0.0 15.0 30.0
0.0 15.0 30.0
Rainfed+Irrigation
Rainfed
1 hr H2O
24 hr H2O
1 hr KCl
24 hr KCl
29 %
15 %
8 %
48 %
b)
Total NO3--N 1667.86 mg kg-1 BC
23 %
11 %
8 %
58 %
d)
Total NO3--N 1013.69 mg kg-1 BC
22 %
7 %
3 %
68 %
f)
Total NO3--N 1011.55 mg kg-1 BC

Fig. S5. Effect of biochar amendment (BC 0, 15 and 30 t ha^{-1} under rain-fed plus irrigation or rain-fed only treatment) on NO_3^- retention in top and subsoil (after 2, 6 and 16 month after BC addition = subfigures a, c and e, respectively) in comparison with NO_3^- retention in field-aged BC particles (retrieved from same soil samples taken after 2, 6 and 16 month after BC addition = subfigures b, d and f, respectively). Soil samples were analyzed with the standard 2 *M* KCl method, while BC particles were extracted with method M_2 (biochar (1:10 w/v BC/deionized water) where the particles were repeatedly shaken in water (100 rpm) for 1 and 24 hours, and subsequently in 2 *M* KCl again for 1 and 24 (1:10 w/v of BC/2 *M* KCl). Note that the x-axis scale for each date (a, c or e) is different. Slices of the pie charts show percentages of the total amount of NO_3^- extracted; the total amount is given below the respective figure. Different letters on the bars indicate significant differences in the NO_3^- retention due to BC treatments, lower- vs. upper-case letters indicate a significant irrigation effect, respectively, following two-way ANOVA's (Tukey test, $p<0.05$) presented in table S1.

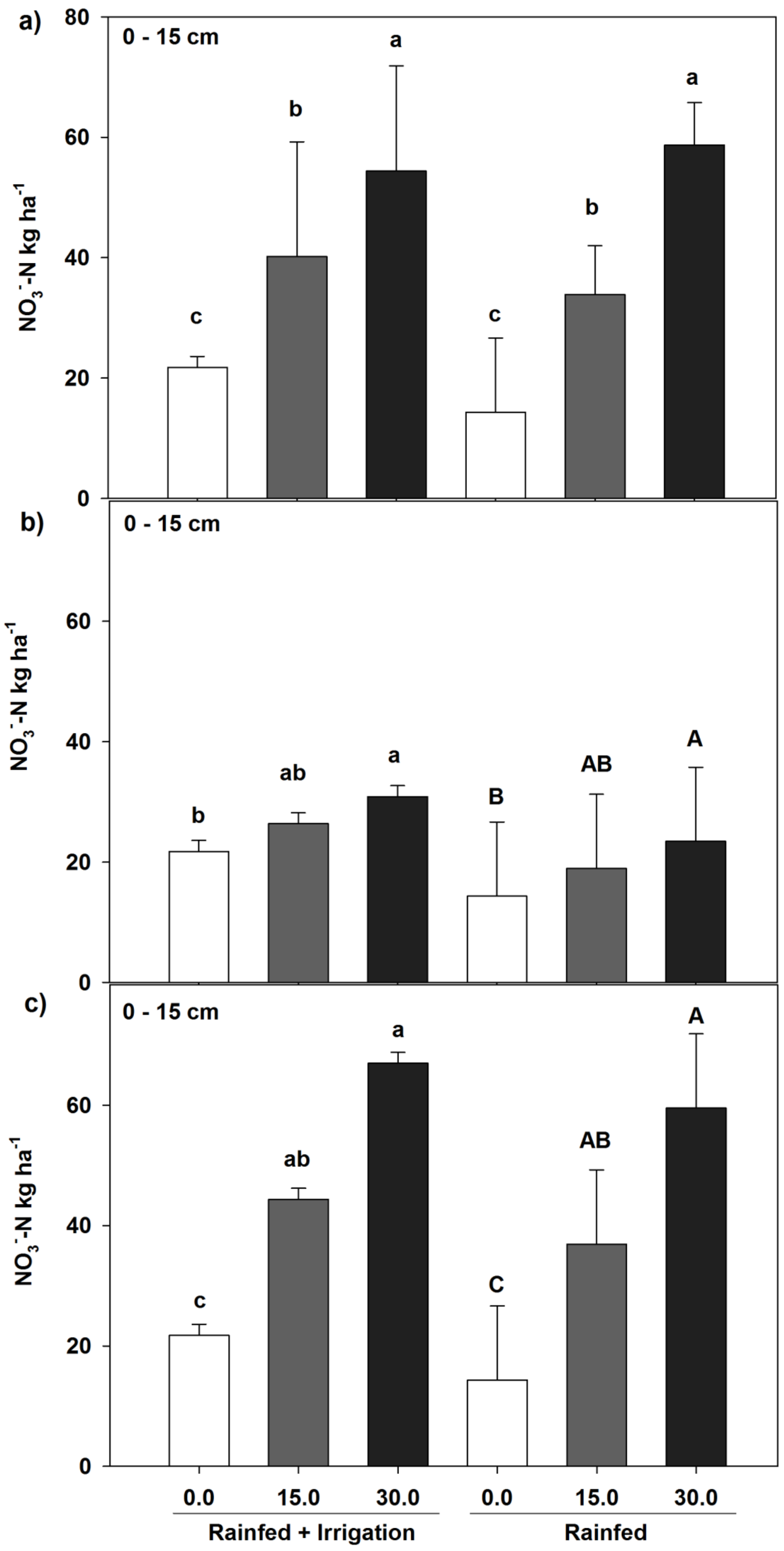
a)
0 - 15 cm
b)
0 - 15 cm
c)
0 - 15 cm
NO_3^--N kg ha^{-1}
0
20
40
60
80
c
b
a
c
b
a
b
ab
a
B
AB
A
c
ab
a
C
AB
A
0.0
15.0
30.0
0.0
15.0
30.0
Rainfed + Irrigation
Rainfed

Fig. S6. Influence of biochar amendment (BC 0, 15 and 30 t ha^{-1} under rain-fed plus irrigation or rain-fed only treatment) on total NO_3^- stocks from topsoil (BC amended layer 0-15 cm), a) extracted with 2 *M* KCl, b) calculated as NO_3^- stock of the control soil (standard 2 *M* KCl), plus NO_3^- stocks extracted from BC_{aged} particles in the BC treatments (standard 2 *M* KCl) and c) calculated as NO_3^- stock of the control soil (2 *M* KCl), plus the NO_3^- stocks repeatedly extracted from field-aged BC particles with the M_0 method (repeated (10x) extraction in 2 *M* KCl at room and high temperature). Different letters above the bars indicate significant differences in the NO_3^- stocks due to BC treatments, lower- vs. upper-case letters indicate a significant irrigation effect, respectively, following two-way ANOVA's (Tukey test, $p<0.05$) presented in Table S4.

Table S3. Effect of biochar (BC, 0, 15 and 30 t ha^{-1}) and watering regime (H_2O, rain-fed + optimum irrigation and rain-fed only) on total NO_3^- retention in BC amended soil layer (0-15 cm), subsoil layer (15-30 cm) and deep subsoil layer (30-90 cm), statistics accompanying Figure S4.

Factors	**Total NO_3^- in 0-90 cm soil profile**					
	0-15 cm		**15-30 cm**		**30-90 cm**	
	F	***p***	**F**	***p***	**F**	***p***
BC	14.225	*<0.001*	0.275	*0.763*	10.953	*<0.001*
WR	0.282	*0.602*	0.257	*0.618*	2.174	*0.158*
BC x WR	0.403	*0.674*	0.378	*0.691*	0.757	*0.483*

Table S4. Measured and calculated (theoretical) stocks of NO_3^- retained in topsoil (0-15 cm) as influenced by BC amendment (0, 15 and 30 t ha^{-1}) and watering regime (H_2O, rain-fed plus irrigation or rain-fed only treatment); statistics accompanying figure S6.

Factors	**Total NO_3^- in 0-15 cm soil profile**					
	Soil extracted with 2 *M* KCl		**Control soil + BC_{aged} extracted with 2 *M* KCl**		**Control soil extracted with 2 *M* KCl + BC_{aged} extracted with M_0 method**	
	F	***p***	**F**	***p***	**F**	***p***
BC	14.225	*<0.001*	40.055	*0.024*	97.344	*0.010*
WR	0.282	*0.602*	175.879	*0.006*	20.117	*0.046*
BC x WR	0.403	*0.674*	-	-	-	-

Table S5. Theoretical effect of biochar (BC, 0, 15 and 30 t ha^{-1}) and watering regime (H_2O, rain-fed + optimum irrigation and rain-fed only) amendments on the bulk density of BC amended soil layer (0-15 cm) and its ultimate effects on NO_3^- kg ha^{-1} retention. Table column "Homogeneous bulk density assumed (1.4 kg L^{-1}) for all treatments" presents the results of two-way ANOVA's for the amount of NO_3^- kg ha^{-1} retained in BC-amended soil layer when no change in soil bulk density due to BC addition was assumed. The second column "Changed bulk density assumed (control = 1.4, BC- 15 = 1.35 and BC-30 = 1.3 kg L^{-1})" represent the results for the amount of NO_3^- kg ha^{-1} retained in BC-amended soil layer when BC amendment would have reduced soil bulk density accordingly.

Factors	**0-15 cm (NO_3^- kg ha^{-1})**			
	homogenous bulk density assumed (1.4 kg L^{-1}) for all treatments		**Changed bulk density assumed (control = 1.4, BC- 15 = 1.35 and BC-30 = 1.3 kg L^{-1})**	
	F	***p***	**F**	***p***
BC	14.225	*<0.001*	12.446	*<0.001*
WR	0.282	*0.602*	0.314	*0.582*
BC x WR	0.403	*0.674*	0.409	*0.671*

4 Biochar reduced nitrate leaching and improved soil moisture content without yield improvements in a four-year field study

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4.1 Publication outline

The publication in the following chapter describes the overall effects of BC amendment (single application, 0, 15 and 30 Mg ha^{-1}) and watering regime (irrigated or rainfed) on crops growth, yield, soil-plant-nitrogen interactions, soil respiration and moisture contents over four consecutive growth periods (2012 - 2015). Following BC amendments, cereals (maize, winter wheat, summer barley, and again maize (the year I - IV, respectively) were grown in sequence and grain yields were accounted. This study mainly aims to improve the understanding of intrinsic factors involved in determining the BC effects on a) plant macro- and micronutrient concentration, b) straw and grain nitrogen concentration, N uptake and use efficiency of crops, c) changes in soil mineral N concentration (NO_3^- and NH_4^+) and moisture contents throughout the study period and d) soil respiration following BC amendments.

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Biochar reduced nitrate leaching and improved soil moisture content without yield improvements in a four-year field study

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ABSTRACT

The use of biochar (BC) is discussed as a strategy to sequester carbon in soils, to reduce GHG emissions and improve soil fertility. However, the responses of crop yields to biochar amendments in agricultural ecosystems, specifically under temperate field conditions, are still uncertain. Furthermore, results obtained under field conditions are often differing from laboratory studies. Therefore, the establishment of long-term studies under field conditions is mandatory to provide the base for recommendations. We carried out a two-factorial split-plot field experiment over four years (2012–2015, *still in progress*) to compare the effects of BC on crop yields, mineral nitrogen (NO_3^- and NH_4^+) dynamics, soil moisture and initial soil CO_2 efflux. A temperate sandy soil was amended with BC (0, 15 and 30 Mg ha^{-1}) with the second factor being watering regime (irrigated or rainfed). The soil CO_2 efflux was increased only for a short time following BC amendments. Freshly incorporated BC (30 Mg ha^{-1}) initially induced manganese (Mn) deficiency at the vegetative stage of the first crop maize (*Zea maize* L.). Biochar amendments significantly reduced NO_3^- leaching, as indicated by greater NO_3^- stocks in the topsoil and reduced stocks in the subsoil (0–15, BC amendment zone and 60–90 cm respectively). In BC treatments a higher soil moisture and higher NO_3^- amount was observed, however, this did not translate into higher yields. Rather, grain yields of maize (year I) and summer barley (*Hordeum vulgare* L., year III, no nitrogen (N) fertilization) were significantly reduced (1–11 and 5–26% respectively) due to N deficiency with BC amendment or (non-alleviated) drought stress. A prolonged drought spell in 2015 (year IV) drastically reduced the grain yield of maize (5 and 0.7 Mg ha^{-1}) and N uptake (96 and 11 kg ha^{-1}) in the irrigated and rainfed treatments respectively, without any alleviating effects of biochar amendment. We conclude that application of large amounts of pure, non-nutrient-loaded biochar to temperate sandy soils may provide environmental benefits, such as carbon sequestration and reduction of nitrate leaching, but without an economic incentive for implementing biochar use, at least for the initial few years of application.

1. Introduction

Global population is continuously increasing and is expected to reach 9 billion by 2050 (Food and Agriculture Organization (FAO), 2011). Therefore, the pressure on natural resources (land and freshwater) will continue to rise because of increasing demands for higher caloric food, feed, fiber and energy (Pfister et al., 2011; Zabel et al., 2014). On the other hand, the total current cropland area which is already intensively used (Wani et al., 2015), and which is likely to decrease will have to provide the food for the growing population (FAO, 2009). This might lead to overexploitation of land resources in terms of monoculture and heavy use of mineral fertilizers (Zuo and Zhang, 2009). Specifically, soil quality degradation and greenhouse gas (GHG) emissions (agriculture contributing 13.5% of global GHG emissions) are likely to increase (FAO, 2011; Lal, 2009, 2010).

Global warming itself may further increase soil aridity/water scarcity problems due to shifts in global precipitation patterns and increase in the frequency of extreme weather events including

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heat waves (IPCC, 2015; Giorgi et al., 2004; Lenderink et al., 2007; Mulcahy et al., 2013; Sheffield and Wood, 2008). Hence, the identification and implementation of adaptation measures aimed to enhance the resilience of agroecosystems to climate change impacts are mandatory.

One potential option is to enhance the productivity of degraded, carbon-poor, arid and sandy soils under changing climate, via an increase of the soil carbon content (Lal, 2004; Smith and Gregory, 2013). Particularly the fertility of sandy soils will benefit from an increase in soil organic carbon (SOC) mainly via the improvement in soil aggregation and water holding capacity (WHC; Mulcahy et al., 2013). In addition, sandy soils can exhibit lower crop productivity due to limited nutrient and water retention capacity. As a consequence, they can show rapid N leaching losses especially via nitrate (Jovanovic et al., 2009; Liu et al., 2012). Therefore, improving the WHC of sandy soils may enhance their water use efficiency and agricultural productivity (Basso et al., 2013; Oki and Kanae, 2006).

In recent years, biochar "the product of heating biomass in the absence of or in limited air to above 250 °C, a process called charring or pyrolysis" (Lehmann and Joseph, 2015) has gained interest in public and private sectors because of its potential benefits as an option to improve degraded land resources (Beesley et al., 2011; Glaser, 2007; Zhang et al., 2016b). It has been claimed that depending on feedstock properties and pyrolysis conditions (Antal and Grønli, 2003; Chan and Xu, 2009) biochar amendments may achieve various sustainability goals in terms of carbon sequestration (Glaser et al., 2002; Lehmann et al., 2006; Lehmann, 2007; Sombroek et al., 2003; Stavi and Lal, 2013), reductions in GHG emissions (Cayuela et al., 2014; Harter et al., 2014; Sohi et al., 2010; Kammann et al., 2012), reduced nutrient leaching (Haider et al., 2016; Kammann et al., 2015; Lehmann et al., 2011; Oram et al., 2014; Ventura et al., 2013; Yuan et al., 2016), enhanced nutrient uptake (Lehmann et al., 2003), improved soil fertility (Glaser et al., 2002; Liu et al., 2014; Sombroek et al., 2003; Spokas et al., 2012; Zhang et al., 2016b), agronomic performance (Biederman and Stanley Harpole, 2013; Liu et al., 2013), energy production and climate change mitigation (Woolf et al., 2010). Biochar possesses the potential to ameliorate soil moisture conditions and hence ecosystem functioning by increasing the amount of plant available water (i.e. the amount stored in a soil between the permanent wilting point and field capacity; Basso et al., 2013; Jeffery et al., 2011; Masiello et al., 2015). Improved WHC is often but not always an indicator for an increase in the plant-available water (Cornelissen et al., 2013; Kammann and Graber, 2015; and citations therein).

A potential benefit of BC in temperate sandy/coarse textured soils could be increased WHC and plant available water (Rogovska et al., 2014). For instance, biochar applied at 96 Mg ha^{-1} in Midwestern Mollisol improved readily plant available water content (i.e. available −10 kpa and −100 kpa) and improved maize grain yield by 11–55% over the control during the first year of application (Rogovska et al., 2014). A 10% yield increase of barley (*Hordeum sativum*) with biochar amendment in a chernozem region under prolonged drought stress was attributed to increased water availability (Karer et al., 2013). Similarly, Basso et al. (2013) found in a soil column study with hardwood biochar a significant increase in soil WHC and predicted that the increased WHC may enhance available water capacity (AWC = available water between field capacity and permanent wilting point) for crops. Tomato seedlings were protected from wilting due to improved soil moisture content with higher (30% v/v) rates of biochar amendments in sandy soil (Mulcahy et al., 2013). Structural properties of biochar can help to retain more water and nutrient concentrations under drought conditions (Major et al., 2010; Novak et al., 2009) in sandy soils. It has been frequently demonstrated that biochar amendments may enhance crop productivity via improving soil water and nutrients availability especially under sandy soil conditions (Jeffery et al., 2011; Tryon, 1948). However, there are some reports where crop yield did not improve after biochar amendments. For instance, a soft wood biochar applied at 5–10 Mg ha^{-1} increased relative moisture contents of amended soil but the increased water availability was not sufficient to cause yield improvements of wheat, turnip and faba bean (Tammeorg et al., 2014).

Thus, the effects of BC amendments on the soil-plant system may vary widely depending on BC or soil properties, crop species and climatic and environmental conditions. For instance, biochar has been found to show its beneficial effects in the presence of balanced fertilization, especially nitrogen, as indicated by a 10% increase in grain yield of barley with 72 Mg ha^{-1} biochar amendment in combination with the standard required nutrients for the crop (Karer et al., 2013). The findings of Asai et al. (2009) further support the idea that biochar amendments without additional fertilization may even decrease crop yields, most probably due to N immobilization (Bruun et al., 2012; Novak et al., 2010; Tammeorg et al., 2014). Haider et al. (2015) found increased maize biomass yield and nitrogen use efficiency with wood chip sieving biochar at addition rates of 1.5 and 3% (w/w). However, the yield increase was rather attributed to soil moisture improvement than increased N availability to plants. In this study a retention of nitrate in biochar-amended soils has been observed (Haider et al., 2015) which was understood only later when studied in more detail (Haider et al., 2016). A substantial increase in quinoa (*Chenopodium quinoa* L.) yield was observed by Kammann et al. (2015) when co-composted biochar was used in a greenhouse pot study; the co-composted biochar was clearly nutrient-loaded and had captured considerable amounts of nitrate. However, it is unclear how field aging of biochar may influence its yield improvement potential, because there was no yield improvement of maize during the second year of biochar addition under drought stress conditions (Rogovska et al., 2014).

Hence, there are varying results of BC amendments in soil-plant systems observed under greenhouse, laboratory or tropical-environment conditions (Jeffery et al., 2011). A recent review of 798 biochar studies until August 2015 revealed that only 26% of all biochar studies were performed under field conditions (Zhang et al., 2016b), and temperate regions particularly lack in biochar field trials (Hammond et al., 2013). Moreover, field studies often showed contrasting findings compared to greenhouse studies (Glaser et al., 2015; Liu et al., 2012). In particular results from long-term field studies are needed to predict effects and likeliness of biochar use on a global scale (Woolf et al., 2010) and to provide insights into the effect of changing soil properties or plant growth processes after biochar amendment (Ernsting and Smolker, 2009; Lehmann et al., 2015).

In this study a four-year field experiment was conducted to quantify the effects of biochar on cereal crop production in a rainfed sandy soil managed according to conventional standard practices. Sandy soils – relying on rainfall for water supply – are about 75% of global cropland area and are usually less fertile but still contributing 58% to global food production (Portmann et al., 2010; Rosegrant et al., 2002). Cereals were used in this study because of their economic importance in global food security. Biochar was applied at increasing rates up to 0, 15 and 30 Mg ha^{-1} following the findings of Vaccari et al. (2011) who found (∼31%) grain yield improvement of wheat either with biochar application of 30 or 60 Mg ha^{-1}. Likewise, Liu et al. (2012) found substantial crop growth improvement in a sandy soil with biochar amendments in East Germany (but using a combination of biochar and compost). Therefore we used BC application rates below or up to 30 Mg ha^{-1} of Vaccari et al. (2011) who did not achieve further benefits with using higher rate than that. Two watering regimes were set: a) irrigated (additional irrigation in rainfed plots only during the seasonal dry crop growth period (if needed)), b) completely rainfed (no additional irrigation even during drought spells). The following hypotheses have been tested during the four consecutive growing seasons 2012–2015. Biochar amendments (compared to control sites): i) improve soil hydraulic properties, and hold and deliver more water to crops, thereby increase the crop production (Haider et al., 2015), ii) enhance nitrogen use efficiency and crop growth (Haider et al., 2015), and iii) will only initially increase soil respiration due to the release of small amounts of labile carbon, but thereafter be unchanged, confirming biochar's overall low SOC-priming potential and high recalcitrance in soil (Kerré et al., 2016; Kuzyakov et al., 2009). Finally, biochar effects are dependent on climatic conditions during the course of study such as prolonged seasonal drought spells that reduce water and nutrient availability particularly in the non-irrigated treatment, with biochar alleviating these limitations.

2. Materials and methods

2.1. Experimental site and soil description

A field experiment over four consecutive growing seasons (2012–2015) was conducted at the experimental station of Justus-Liebig University Giessen, Germany. The research area is at Gross-Gerau, 49°45′N and 8°29′E, 90–145 m above sea level, which is located in the upper Rhine Valley with the river Main to the North, River Rhine to the west and Odenwald mountains to the east. The climate of the area is characterized as the warm-temperate climate with average (56 years) temperature of 9.8 °C and annual precipitation of 600 mm. The detailed weather data of the entire experimental period are given as supplementary Fig. S1. The soil was formed from river (Rhine) sand deposits and it is characterized as silty sand (particle size, sand 2.0–0.5, silt 0.05–0.002 and clay <0.002 mm) having sand, silt and clay as 85.2, 9.6 and 5.2% respectively). It is characterized as a carbon-poor soil due to very low organic carbon contents (5.92 g kg^{-1}). It contains total N (0.57 g kg^{-1}), CAL-P (0.092 mg g^{-1}), CAL-K (0.125 mg g^{-1}), Mg (0.36 mg g^{-1}) and a pH (0.01 M $CaCl_2$) of 6.31, which is prevailing in the experimental region.

2.2. Biochar production and characterization

The feedstock for the biochar production was comprised of wood chip sieving (needles, bark, twig pieces and small wood chips) of Norway spruce (*Picea abies* L., 70%), and a deciduous tree, European Beech (*Fagus sylvatica* L., 30%); needles roughly contributed 30% to the total feedstock. Biochar was produced at 550–600 °C (Pyreg GmbH, Dörth, Germany) and physicochemical properties were determined (Table 1). The moisture rich (256%) biochar was thoroughly mixed before field application.

2.3. Experimental setup

The field experiment was established in April 2012 and initiated with a single application of biochar to the experimental plots. The experiment was laid out in a randomized complete block design with two factors (BC, 0, 15 and 30 Mg ha^{-1}; watering regime, rainfed or irrigated) in split plot arrangement. The biochar was applied at 0, 15 and 30 Mg ha^{-1} (on dry weight basis) and spread manually with hand spreader in the respective plots (4.5 × 7 m) with treatments being four-fold replicated (n = 24). The irrigated and rainfed plot areas were separated with a same sized (as experimental plot) buffer area to avoid irrigation side effects. The replicated blocks were separated by 2 m wide pathways to avoid cross contamination and treatments effects. The day after biochar application (on April 13, 2012), it was incorporated to the top 15 cm soil depth with a rotary power harrow. Four major and two cover crops were grown in consecutive growing seasons from 2012 to 2015 in the following sequence: maize (*Zea maize* L.), winter wheat (*Triticum aestivum* L.), peas (*Pisum sativum* L.), summer barley (*Hordeum vulgare* L.), peas (*Pisum sativum* L.) and again maize. The irrigation (in irrigated treatment plots) was supplied only during prolonged natural dry spells with no/very less rainfall in the experimental area. The time and amount of additional irrigation was decided on the basis of past 30 year's rainfall data of the region. When there was ~30% less rainfall and symptoms of water shortage become visible on plants the additional irrigation was supplied. Therefore, in the "irrigated" treatment, the first crop, maize, was supplied with only two irrigations of 35 mm each on two dates (July 11 and 25, 2012), winter wheat and summer barley were supplied with one additional irrigation each of 30 mm on June 25, 2013, and April 15, 2014, respectively. The last maize crop in 2015, however, faced a severe drought spell starting in early summer. Here, the "irrigated" treatment was supplied with additional irrigations of 30 mm each on June 12, July 03, 14 and August 04, 2015 (see Metrological data Fig. S1). Apart from the biochar or watering treatments crops were supplied with same amount of fertilizers and similar management practices. Crops were supplied with macronutrients according to the conventional requirements of this soil and of the respective crops sown. Maize was supplied with 150 kg N ha^{-1} as granulated fertilizer (ASN) "ammonium sulfate nitrate" containing 26% N (19% NH_4^+ and 7%

Table 1
Chemical characterization, elemental composition, main nutrients, and particle size distribution of biochar.

Chemical Characterization	Content (g kg^{-1})	Particle size fraction (mm)	[a]Values (%)
Carbon (C)	744	<0.1	0.1
Hydrogen (H)	<10	0.1–0.63	25.3
Oxygen (O_2)	100.6	0.63–1	17
Nitrogen (N)	5.6	1–1.6	4.9
Phosphorous (P)	1.63	1.6–2	20.9
Potassium (K)	6.07	2–3.15	7.1
Calcium (Ca)	19.07	3.15–6.3	24.2
Magnesium (Mg)	2.09	>6.3	0.5
Iron (Fe)	2.59	[b]CEC (fresh biochar)	19.11 (cmole kg^{-1})
Copper (Cu)	0.02	[b]CEC (>3 year field aged biochar)	5.54 (cmole kg^{-1})
Zinc (Zn)	0.17	pH (fresh biochar)	9
Sodium (Na)	3.3	pH (>2.5 year field aged biochar)	6

[a] Values are estimated in percent of soil dry mass following sieving procedure.
[b] Cation exchange capacity (K, Na, Ca, Mg).

NO_3^-) and 13% water soluble sulfur (SO_4-S). The wheat crop was supplied with 190 kg N ha^{-1} in three splits of 60, 80 and 50 kg N on March 6, April 26 and June 3, 2013, respectively as ASN fertilizer. Summer barley was supplied with only phosphorous (single superphosphate), potassium (muriate of potash, 60% K_2O) and Mn ($MnSO_4$) at the rate of 25, 200 and 4 kg ha^{-1} respectively. No nitrogen was supplied to summer barley because of surplus (average, ~108 kg N ha^{-1} in 0–90 cm soil profile) mineral N (mainly NO_3^-) reserves of the soil after previous crops (see Fig. S2, Table S1). In addition it was evident that the nitrate concentration was higher in the biochar treatments (see Haider et al., 2016) so that a growth increase was expected. The last maize crop during 2015 was supplied with 50 kg P as triple super phosphate (46% P_2O_5) and 100 kg N as ASN plus 200 kg Potassium (K_2O) ha^{-1} on April 17 and May 05, respectively. All four crops were harvested for grain yield from measured central area (by leaving 1 m area from the borders of experimental plots) of the plots to avoid border effects and then yield was converted (on dry mass basis) to kg ha^{-1}. The straw of each crop and complete cover crops were subsequently incorporated into the top 15 cm soil depth.

2.4. Soil sampling and measurement of mineral N and soil moisture contents

The plots were laid out after the seedbed preparation following the experimental design. Subsequently one initial soil sampling down to 30 cm depth was carried out. Five samples were taken from each plot at random positions, mixed, carried to the lab in cooling boxes and extracted for initial nutrient concentration with two different methods. A) The fresh soil was analyzed for mineral N (NO_3^- and NH_4^+) with standard 2 M KCl extraction (Keeney and Nelson (1982). B) The dried soil samples at 40 °C were thoroughly sieved to remove stones and crop residues and then ground to analyze with the electro-ultra-filtration (EUF) method for macro and micro nutrients. Subsequent samplings throughout the study were carried out on two separate depths, top 0–15 and sub 15–30 cm, mostly on a monthly basis (soil sampling dates are given in respective tables and figures). Two deep soil samplings down to 90 cm depth were done on February 20, 2014 (before summer barley sowing) and April 2015 (before last maize crop sowing) to investigate the nutrient stocks and potential nitrate leaching to deeper soil layers. The gravimetric soil moisture content was measured from the soil samples taken at every sapling date.

2.5. Measurement of soil CO_2 effluxes

The measured CO_2 effluxes represent the soil respiration (including below ground root respiration and carbon decomposition of the soil organic carbon by the microbial population) of the soil system. The CO_2 effluxes were measured with a LI-8100 automated soil flux system (LI-COR, Nebraska, USA) by using the 20 cm survey chamber. The 20.3 cm (8″) soil collars (about 11 cm high) of PVC pipe with sharper edges were anchored in the soil in plots between maize rows at 20th day after the first crop (maize) sowing. First CO_2 efflux measurements were performed three days after collar installation. The offset (height) of the soil collars from soil surface (± 1 cm) was entered into the LI-8100-analylsis software to correct flux calculations (i.e. the total chamber volume). The measurement time and observation delay were set to 120 and 60 s, respectively to allow sufficient chamber-volume mixing time and enough time to move from one plot to another after starting the measurement. The soil CO_2 efflux was expressed in $\mu mol\ CO_2\ m^{-2}\ s^{-1}$; the linear flux calculation was used because fluxes always showed a linear slope of $R^2 > 0.9$.

2.6. Plant biomass nutrient analysis and nitrogen use efficiency

The total plant biomass/grain nutrients concentrations of P, K, Na, Ca, Mg, Cu, Mn, Zn and Fe were analyzed using an acid digestion method (Rosopulo et al., 1976). The full spectrum of all macro and micronutrients was analyzed only in the first maize crop (2012) biomass at the vegetative stage, at the harvest, and from the harvested maize grains. Plant height (cm) of randomly selected 10 plants and chlorophyll contents from their first fully developed leaf was measured (SPAD values) at 12 leaf stage. Plant biomass carbon (C) and nitrogen (N) concentrations of all four crops (maize, winter wheat, summer barley and the last maize crop) were analyzed by using an elemental analyzer (Vario Max, Hanau, Germany). The nitrogen use efficiency (NUE) of the crops was calculated as the ratio of grain yield produced relative to nitrogen application (kg kg^{-1} in one hectare). Total N uptake (kg ha^{-1}) of the crops was calculated as a sum of N uptake by plant straw and grain yield. Note, that the total N uptake for the last maize crop (2015) was based on total N uptake by grain yield only because we could not obtain the total straw yield data for this crop.

2.7. Statistical analysis

A field experiment was designed and performed, based on a randomized complete block design (split plot arrangement) to investigate the effect of each, BC level or watering regime, on response variables (mainly crop yields and soil-plant N concentrations), thus one-way and a two factor split-plot ANOVA's were used for complete data sets of the study. To find out the realistic results of all those data sets which were taken before the onset of real additional irrigation were analyzed with one way ANOVA, because the second factor 'watering regimes' of study was not initiated at that time. Initially, the data were tested for normality (Kolmogorov-Smirnov test) and homogeneous variances (Levene median test). The data were log transformed where needed (indicated in the respective results). Data were analyzed using Sigma Plot 11.0 (Systat, Inc., Richmond, USA) and R program version 3.3.1 at a significance level of $p < 0.05$. If significant differences existed within a factor, i.e. biochar application rates and watering regimes, the Tukey's Honest Significant Difference (Tukey's HSD) test was performed to identify treatment levels that were different from each other ($p \leq 0.05$).

3. Results

3.1. Effect of biochar and watering regime on soil-plant system during the first year of experimentation

Freshly incorporated biochar negatively influenced ($p < 0.001$) the first maize crop (year I) and reduced plant height (7–14%) with increasing rates of biochar addition (15 and 30 Mg ha^{-1}) (Fig. 1a, Table S1). Plant biomass nutrient analysis at an early stage (12 leaf stage) revealed that there was no significant effect on N, P, Ca, Mg, Na, Fe, Cu and Zn concentrations while K concentration was increased up to 16% with BC amendments, with no difference between 15 or 30 Mg ha^{-1} BC addition (Fig. 1b, Tables 2 , S1, S2). The plant Mn concentration was reduced by 25 and 42% with 15 and 30 Mg ha^{-1} BC addition, respectively, compared to the control (Fig. 1c, Tables 2, S1, S2). Notably, there was significantly greater nitrate and soil moisture content in the topsoil (0–15 cm) at that stage, and even a month afterwards, increasing with increasing rate of BC addition (Fig. 2a–d, Table S1).

Biochar amendment effects on plant nutrient concentrations differed at crop maturity and BC aging, compared to the nutrient concentrations at vegetative stage. For instance increases in K and decreases in Mn concentration due to BC amendment at the

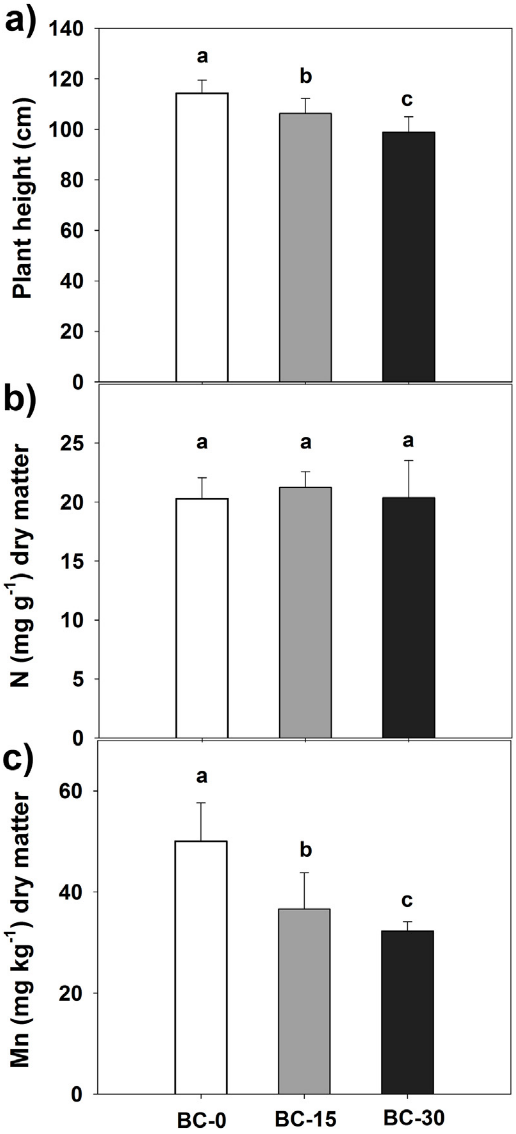

Fig. 1. Plant height and biomass nitrogen (N) and manganese (Mn) concentrations of first crop maize (year I) at the vegetative 12-leaf stage as influenced by biochar amendments (BC, 0, 15 and 30 Mg ha^{-1}). The y axis scales of a, b and c are based on the respective mean values. The error bars indicate standard deviations, and different letters on bars indicate significant differences due to BC treatments following one way ANOVA (Tukey HSD test, $p < 0.05$, $n = 24$). See the accompanying statistical results in Table S1.

vegetative stage were no longer visible in plant straw or in grains at the harvest date (Tables 2, S2). The concentrations of P, K and Zn in plant straw and only Ca in grain were increased, while the N and Ca concentration was significantly reduced with BC and water treatments in both grain and straw yield at the harvest of first crop (Tables 2, S2).

3.2. Grain yield, straw and grain N concentration, N uptake and NUE of four crops (year I–year IV)

The results are presented in Tables 3, 4, S3. The grain yield of first crop maize (year I) was reduced ($p = 0.003$) due to BC amendment and water deficiency. Likewise, the total N uptake and NUE of maize was also reduced ($p = < 0.001$ and $p = 0.003$, respectively) with BC amendment (mainly with 30 Mg ha^{-1}) and watering treatments. However, there was no significant effect on grain yield of the second crop, winter wheat (year I–II) due to BC amendment or watering regiems. The total N uptake of wheat was also significantly reduced with BC addition, but there was no effect on straw or grain N concentration (Tables 3, 4, S3). The third crop summer barley (year III) was not supplied with mineral N fertilizer because of sufficient amount of reserved mineral N (from last two years following BC amendment), mainly NO_3^-, in the 0–90 cm soil profile (more in BC plots, in the top rooting zone, see Fig. S2, Table S4). The results revealed that, the grain yield of summer barley was significantly reduced due to higher rate of BC (30 Mg ha^{-1}) addition and water defficiency (3, S3). However, the negative effects of BC addition on total N uptake were not visible during third crop summer barley or last crop maize (year IV), (Tables 4, S3). However, the last crop maize (year IV) faced a prolonged seasonal drought in summer 2015 with a drastic reduction in grain yield due to severe drought leading to N uptake limitations (Tables 3, 4, S3). There was no significant effect on NUE of last three crops wheat, summer barley and maize (year II, III and IV) due to BC amendment (Tables 4, S3).

3.3. Changes in soil moisture contents following BC amendments and watering regime

Generally, BC amendment improved soil moisture contents in the topsoil (0–15 cm where BC was incorporated) during the experimental period (nine out of thirteen sampling dates) (Fig. 3, Tables S1, S5). However, not as frequent as expected, a significantly higher moisture content increasing with increasing rate of BC application for every date of soil sampling. However, the observed improvements in soil moisture contents did not promote a significant yield increase during the study period (Table 2). Moreover, the soil moisture in the subsoil (15–30 cm, below the BC amendment zone) was unaffected (data not shown).

3.4. Effect of BC and watering regime on soil mineral N (NO_3^- and NH_4^+) concentrations

Biochar amendments significantly influenced the soil mineral N concentrations (Figs. 2 a–d, 4 a; Tables S1, S6), starting with the first nitrogen fertilization (02-05-2012) in the first crop maize (year I, soil sampling date 15-05-2012) until after the harvest of the fourth maize crop (year IV). Soil NO_3^- concentrations in topsoil (0–15 cm) remained significantly higher, generally increasing with increasing rate of BC addition, throughout the study period (Fig. 4a; Tables S1, S6). However, there was a little or no significant change in the soil NH_4^+ concentration in the topsoil throughout the study period (Fig. 4b; Table S6). Generally, there was a significantly greater NO_3^- concentration in control subsoils, compared to BC treatments in the subsoil (15–30 cm) indicating reduced nitrate leaching from topsoil with BC amendment (Fig. S3a; Table S6). However, there was no significant effect of treatments on NH_4^+ concentration in the subsoil (Fig. S3b; Table S6). Deep soil samplings during the year III and IV indicated a significant reduction in nitrate leaching with BC (0–15 vs 60–90 cm soil during 2014 and 0–15 vs 30–60 cm during 2015) amendments (Fig. S2; Table S4).

3.5. Changes in soil respiration (μmol CO_2 m^{-2} s^{-1}) due to BC amendment and watering regime

Soil respiration was significantly increased with BC amendment (30 Mg ha^{-1}) on the very first date of soil sampling after BC application (BC application: 12-4-2012) or after first irrigation in first cropping season (July-2012) Tables 5 , S7. First soil respiration measurement was performed 5 weeks after the onset (18-5-2012) of the experiment. There was a trend of high soil respiration during the entire first season (first crop maize 2012), mostly increasing

Table 2
MacA reviewop (year I) as influenced by biochar (BC, 0, 15 and 30 Mg ha^{-1}) and watering regime (irrigated or rainfed).

Factors		N (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	Na (mg g^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
Biochar	Water										
Maize 2012 straw nutrient concentrations at vegetative stage (One way ANOVA)											
Control		20.29 ± 1.66a	3.91 ± 0.19a	44.69 ± 2.44b	2.80 ± 0.92a	2.0 ± 0.17a	0.01 ± 0.01a	50.01 ± 7.12a	*nd*	4.79 ± 0.5a	30.84a
BC-15		21.23 ± 1.25a	4.09 ± 0.26a	51.99 ± 4.43a	2.94 ± 1.09a	2.01 ± 0.21a	0.0 ± 0.00 a	36.62 ± 6.71b	*nd*	4.39 ± 0.3a	27.09a
BC-30		20.36 ± 2.95a	4.04 ± 0.18a	51.24 ± 2.67a	2.26 ± 0.50a	1.88 ± 0.13a	0.0 ± 0.01 a	32.29 ± 1.74c	*nd*	4.69 ± 0.3a	26.12a
Maize 2012 straw nutrient concentrations at harvest (split-plot ANOVA)											
Control	Irrigated	3.68 ± 0.33a	1.19 ± 0.11c	18.23 ± 1.80a	3.42 ± 0.28a	1.14 ± 0.04a	0.06 ± 0.00a	42.64 ± 4.27a	146.40 ± 11.46a	4.38 ± 1.10a	5.26 ± 2.89b
BC-15		3.35 ± 0.36b	1.28 ± 0.07b	19.25 ± 1.39a	2.85 ± 0.30b	1.05 ± 0.05a	0.06 ± 0.00a	37.83 ± 6.49a	185.19 ± 23.82a	4.29 ± 0.83a	10.89 ± 0.78a
BC-30		3.25 ± 0.57b	1.50 ± 0.10a	21.00 ± 1.28a	2.64 ± 0.56b	1.03 ± 0.07a	0.06 ± 0.01a	40.89 ± 12.52a	200.83 ± 74.57a	4.30 ± 0.74a	10.55 ± 2.07a
Control	Rainfed	4.75 ± 0.68A	0.76 ± 0.11C	22.78 ± 3.13A	3.06 ± 0.09a	0.95 ± 0.26a	0.06 ± 0.01a	41.80 ± 6.10a	187.11 ± 46.05a	3.19 ± 0.71A	11.94 ± 1.79B
BC-15		3.98 ± 0.66B	0.98 ± 0.07B	22.89 ± 1.14A	2.41 ± 0.62b	0.97 ± 0.06a	0.06 ± 0.01a	36.46 ± 9.56a	251.15 ± 62.38a	3.19 ± 0.92A	15.05 ± 2.84A
BC-30		3.53 ± 0.25B	1.10 ± 0.17A	23.26 ± 2.49A	2.67 ± 0.38b	0.98 ± 0.13a	0.05 ± 0.00a	34.48 ± 1.54a	254.00 ± 39.98a	3.30 ± 0.44A	12.93 ± 3.65A
Maize 2012 grain nutrient concentrations (split-plot ANOVA)											
Control	Irrigated	10.90 ± 0.62a	4.47 ± 0.24a	5.15 ± 0.42a	0.09 ± 0.02b	1.61 ± 0.06a	0.048 ± 0.00a	15.29 ± 1.82a	42.19 ± 3.18b	2.04 ± 0.28a	13.41 ± 0.50a
BC-15		10.55 ± 0.38a	4.39 ± 0.16a	5.39 ± 0.40a	0.14 ± 0.01a	1.53 ± 0.15a	0.046 ± 0.01ab	14.74 ± 5.44a	63.73 ± 5.33a	1.30 ± 0.29b	16.98 ± 2.13a
BC-30		9.55 ± 0.17b	3.94 ± 0.16a	4.91 ± 0.52a	0.08 ± 0.03b	1.30 ± 0.10a	0.043 ± 0.00b	13.13 ± 3.29a	53.18 ± 8.03a	1.98 ± 0.40a	18.56 ± 3.50a
Control	Rainfed	12.10 ± 0.25A	4.50 ± 0.37a	4.81 ± 0.34a	0.03 ± 0.01B	1.56 ± 0.14a	0.050 ± 0.01a	11.75 ± 3.74a	54.38 ± 6.41A	3.56 ± 0.69A	22.58 ± 1.15A
BC-15		11.68 ± 0.46A	4.17 ± 0.31a	4.61 ± 0.32a	0.11 ± 0.02A	1.54 ± 0.14a	0.048 ± 0.0ab	8.21 ± 3.20a	30.48 ± 7.32b	2.79 ± 0.44B	19.88 ± 2.01A
BC-30		10.80 ± 0.40B	4.41 ± 0.29a	4.96 ± 0.20a	0.09 ± 0.01b	1.53 ± 0.10a	0.040 ± 0.00b	10.03 ± 1.56a	30.39 ± 7.17b	3.88 ± 0.33A	20.45 ± 1.22A

Values in the columns are means ± standard deviation ($n = 4$). Different letters indicate significant differences due to BC treatments, while lower vs upper case letters indicate a significant irrigation effect following the one way ANOVA (straw nutrient concentration at vegetative stage), split-plot ANOVA (straw and grain nutrient concentration at harvest) and Tukey HSD test, respectively ($p < 0.05$, $n = 24$). See the accompanying statistical results in Table S2.

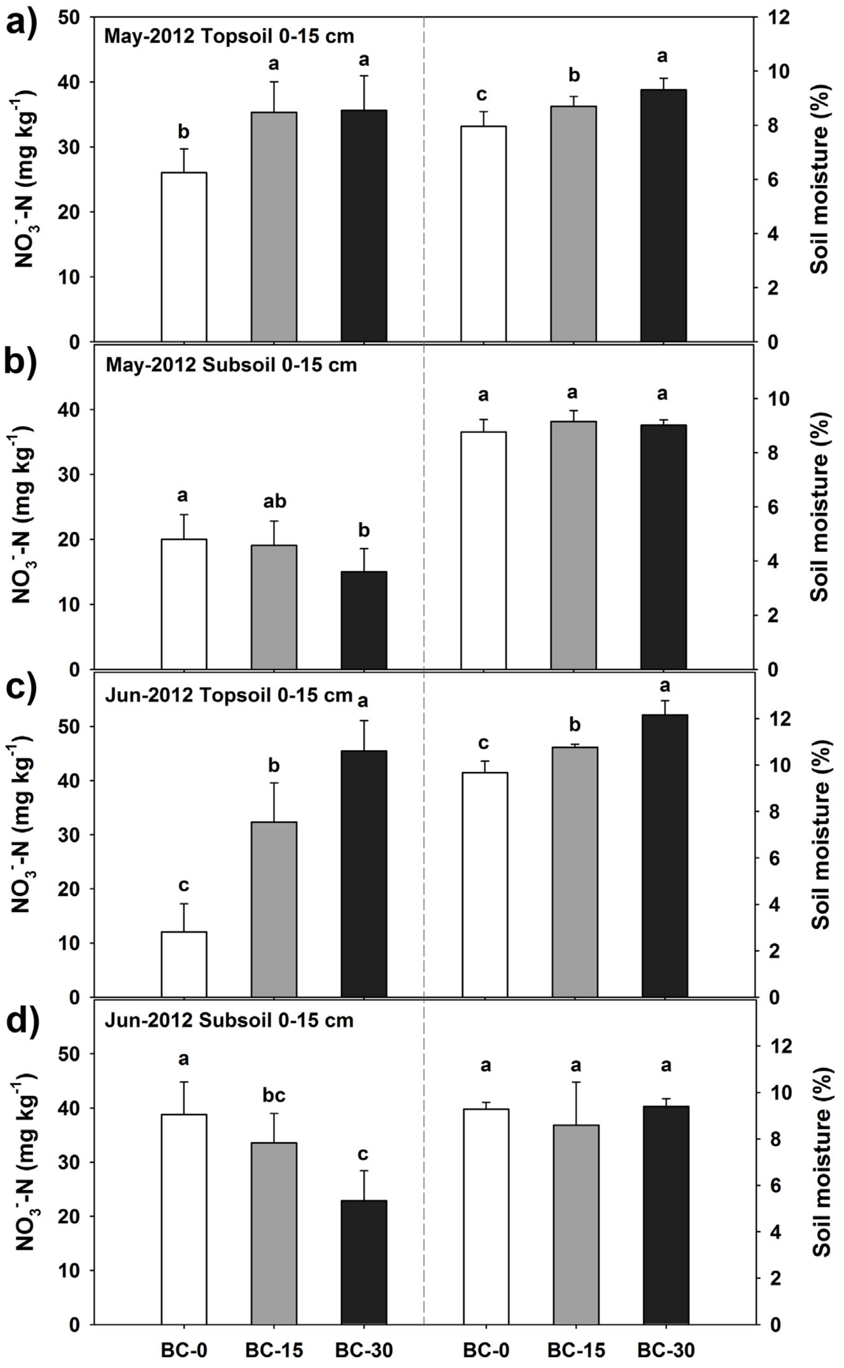

Fig. 2. Top and subsoil nitrate (NO_3^-) concentration and moisture contents during first crop maize (year I) at vegetative stage as influenced by biochar amendments (BC, 0, 15 and 30 Mg ha^{-1}). The y axis scales of A, B and C through D are based on the respective mean values. The error bars indicate standard deviations and different letters on bars indicate significant differences due to BC treatments following one way ANOVA (Tukey HSD test, $p < 0.05$, $n = 24$). See the accompanying statistical results in Table S1.

with increasing rate of BC amendments, but with no significant difference in any other measurements during that season in 2012 (5, S7).

4. Discussion

In our study no yield improvements were observed over four consecutive growing seasons with different crops and with different climatic conditions. However, crop growth and yield improvements following biochar amendments have been frequently reported, particularly in greenhouse studies (Akhtar et al., 2014; Haider et al., 2015; Kammann et al., 2011; Mulcahy et al., 2013; Wang et al., 2012) but also in field studies (Agegnehu et al., 2016a,b,c; Glaser et al., 2015; Rogovska et al., 2014; Vaccari et al., 2011; Zhang et al., 2016a). The possible routes behind such positive influences of BC amendments can be classified into two major pathways: i) biochar amendment presumably improves soil physical and hydraulic properties by decreasing bulk density, thereby increasing soil porosity and soil water retention. Reduced bulk density may e.g. ease root penetration and development as shown by Bruun et al. (2014) for hard-setting subsoils. When the plant available water content is increased, this can positively influence plant growth and development (Agegnehu et al., 2016a; Cornelissen et al., 2013; Haider et al., 2015; Liu et al., 2012; Novak

Table 3
Grain yield (on dry mass basis) and straw or grain N concentration of cereals as influenced by biochar amendments (BC, 0, 15 and 30 Mg ha^{-1}) and irrigation regimes (irrigated or rainfed).

Factors		Yield (Mg ha^{-1})			
Biochar	Water	Maize 2012	Winter Wheat 2013	Summer Barley 2014	Maize 2015
Control	Irrigated	10.9 ± 0.40a	6.08 ± 0.31a	4.89 ± 0.27a	5.7 ± 0.47a
BC-15		10.7 ± 0.22b	6.00 ± 0.17a	4.02 ± 0.18a	5.5 ± 0.13a
BC-30		9.7 ± 0.07b	5.95 ± 0.18a	3.59 ± 0.26b	5.7 ± 0.50a
Control	Rainfed	9.8 ± 0.73A	6.13 ± 0.38a	2.92 ± 0.38A	0.9 ± 0.39B
BC-15		8.9 ± 0.25B	6.11 ± 0.30a	2.97 ± 0.33A	0.6 ± 0.28B
BC-30		8.9 ± 0.28B	6.03 ± 0.19a	2.77 ± 0.33B	0.6 ± 0.16B

		Plant straw and grain N concentrations (mg g^{-1}) dry matter							
straw	grain	straw	grain	straw	grain	straw	grain	straw	grain
Control	Irrigated	3.68 ± 0.33a	10.90 ± 0.62a	3.25 ± 0.40a	17.10 ± 1.13a	3.10 ± 0.23a	10.55 ± 0.42a	7.78 ± 1.08a	17.30 ± 0.34a
BC-15		3.35 ± 0.36b	10.55 ± 0.38a	2.63 ± 0.35a	16.15 ± 0.71a	3.23 ± 0.31a	10.75 ± 0.47a	8.75 ± 0.22a	16.83 ± 0.41a
BC-30		3.25 ± 0.57b	9.55 ± 0.17b	2.90 ± 0.24a	15.75 ± 0.72a	3.28 ± 0.49a	11.05 ± 0.59a	8.25 ± 0.42a	16.75 ± 0.27a
Control	Rainfed	4.75 ± 0.68A	12.10 ± 0.25A	3.05 ± 0.18a	17.35 ± 2.13a	3.55 ± 0.23A	12.53 ± 0.71A	14.93 ± 1.12A	14.48 ± 0.65A
BC-15		3.98 ± 0.66B	11.68 ± 0.46A	3.10 ± 0.49a	17.15 ± 0.73a	4.33 ± 0.50A	13.00 ± 0.43A	15.20 ± 0.81A	15.18 ± 0.38A
BC-30		3.53 ± 0.25B	10.80 ± 0.40B	3.05 ± 0.36a	15.65 ± 0.56a	3.90 ± 0.25A	15.58 ± 2.53A	14.25 ± 0.80A	15.23 ± 0.41A

Values in the columns are the means ± standard deviations ($n = 4$). Different letters indicate significant differences due to BC treatments, while lower vs upper case letters indicate a significant irrigation effect following the split-plot ANOVA and Tukey HSD test, respectively ($p < 0.05$, $n = 24$). See the accompanying statistical results in Table S3.

Table 4
Nitrogen use efficiency (NUE) and total nitrogen uptake (i.e. sum of N concentration in straw and grain kg N ha^{-1}) of cereals as influenced by biochar amendments (BC, 0, 15 and 30 Mg ha^{-1}) and irrigation regimes (irrigated or rainfed).

Factors		Maize-2012		Winter Wheat-2013		Summer barley-2014		Maize-2015	
Biochar	Water	NUE	N-uptake	NUE	N-uptake	NUE	N-uptake	NUE	N-uptake
Control	Irrigated	72.81 ± 2.68a	395.80 ± 31.96a	32.02 ± 1.64a	245.60 ± 15.66a	41.40 ± 6.53a	109.96 ± 6.04a	57.11 ± 4.74a	98.95 ± 10.21a
BC-15		71.20 ± 1.45a	362.75 ± 30.67b	31.57 ± 0.89a	225.98 ± 12.71ab	40.95 ± 12.82a	104.88 ± 2.37a	55.24 ± 1.29a	92.92 ± 2.63a
BC-30		64.80 ± 0.47b	327.10 ± 32.48c	31.33 ± 0.95a	214.94 ± 7.48b	34.11 ± 9.57a	96.67 ± 8.52a	56.86 ± 4.96a	95.24 ± 8.51a
Control	Rainfed	65.21 ± 4.85A	409.54 ± 43.11a	32.25 ± 2.01a	259.74 ± 26.95a	35.27 ± 9.56a	93.19 ± 11.24a	9.04 ± 3.92A	13.30 ± 6.29A
BC-15		59.24 ± 1.66A	375.83 ± 19.47b	32.16 ± 1.55a	246.32 ± 15.47ab	34.68 ± 9.28a	102.61 ± 11.49a	6.40 ± 2.77A	9.73 ± 4.28A
BC-30		59.12 ± 1.85B	320.77 ± 22.77c	31.73 ± 1.00a	229.07 ± 7.37b	26.73 ± 9.23a	106.62 ± 19.53a	6.25 ± 1.62A	9.54 ± 2.53A

Values in the columns are means ± standard deviation ($n = 4$). Different letters indicate significant differences due to BC treatments, while lower vs upper case letters indicate a significant irrigation effect following the split-plot ANOVA and Tukey HSD test, respectively ($p < 0.05$, $n = 24$). See the accompanying statistical results in Table S3.

et al., 2012; Yu et al., 2013; Zhang et al., 2016a). Biochar may improve soil fertility, either by enhanced nutrient supply e.g. with the ashes (K, Ca, Mg), or an increased nutrient use efficiency (e.g. Kammann et al., 2011) via enhanced N and P supply due to reduced leaching (Agegnehu et al., 2016a; Zhang et al., 2016a). Biochar can also improve the cation exchange capacity 'CEC' (Cornelissen et al., 2013; Glaser et al., 2002) in low-CEC soils, and reduce soil acidity with its negative effects such as aluminum toxicity due to alkaline BC amendments (Han et al., 2016). No effects (Borchard et al., 2014; Lugato et al., 2013; Major et al., 2010; Nelissen et al., 2015) or even negative effects (Biederman and Stanley Harpole, 2013; Jeffery et al., 2011, 2015; Uzoma et al., 2011) of BC amendment on plant growth, yield and nitrogen availability are reported as well (Hangs et al., 2016; Lehmann et al., 2003). A negative effect may be explained by: (a) phytotoxic effects of the released organic compounds from flash-pyrolysis biochars (i.e. polyaromatic hydrocarbons, PAHs, Hilber et al., 2012) which can depress germination and plant growth (Deenik et al., 2010; Spokas et al., 2011). However, this can be excluded for the biochar that was used in the current field study since it was certified according to the European Biochar Certificate, i.e. it had negligible PAH contents; (b) reduced N availability due to immobilization of N (Lehmann et al., 2003; Tammeorg et al., 2014) associated with the small labile fraction of biochar which is usually quickly mineralized (as seen in this study by the increased soil respiration). It now emerges that reduced N availability may also be associated with the complex nitrate capture/retention behavior of biochar (Haider et al., 2016; Kammann et al., 2015; Kanthle et al., 2016), as discussed below.

4.1. Effect of biochar on first crop maize at vegetative stage

There are few reports of short-term or minor negative impacts of biochar on crop growth and yield depending on crop species, soil type, and biochar properties in terms of feedstock and pyrolysis temperature (Biederman and Stanley Harpole, 2013; Cantrell et al., 2012; Deb et al., 2015; Spokas et al., 2011). For instance, Kishimoto and Sugiura (1985) found a negative crop growth response of soy bean and maize after charcoal amendment (5 Mg ha^{-1}) in volcanic ash soil. Such an effect was attributed to the alkaline nature of charcoal increasing the soil pH and thus it likely caused a pH-induced micronutrient deficiency. In the present study, we also found a decreased first-year maize growth with BC amendment, as indicated by reduced plant height at the 12 leaf stage. The tissue Mn concentration measured at this stage was significantly reduced in the BC treatments. This is in agreement with Smider and Singh (2014), who also found reduced Mn concentrations in shoot tissues but with no yield reduction in a greenhouse study with sweet corn grown in ferralsol. We assume that the BC application decreased

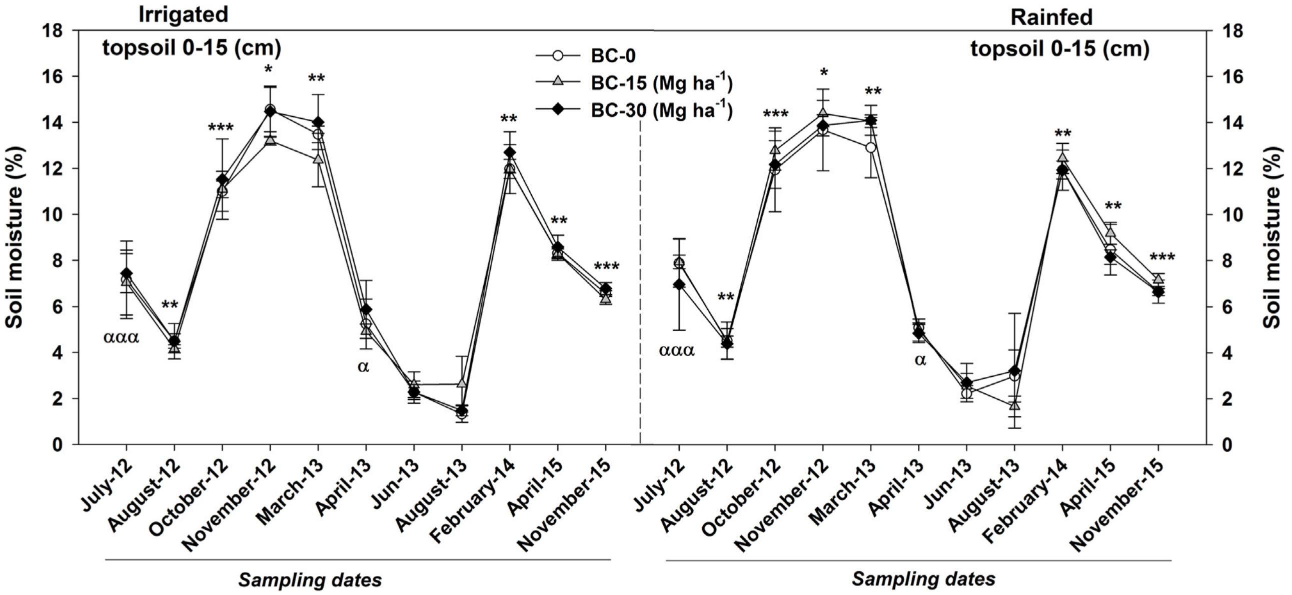

Fig. 3. Topsoil (0–15 cm) moisture contents during the study period (year I–IV) as influenced by biochar amendments (BC, 0, 15 and 30 Mg ha^{-1}) and watering regime (irrigated, left; or rainfed, right). The y axis scales is based on the mean values and error bars indicate standard deviations ($p < 0.05$, $n = 24$). The symbols (*, +, α) indicate significant treatment effects at single sample dates at different significance levels (e.g. *** = p < 0.001, ** = p < 0.01,* = p < 0.05). * indicates significant BC effects, + indicates significant effects of both BC and watering regime, and α indicates significant watering regime only. See the accompanying statistical results in Table S5.

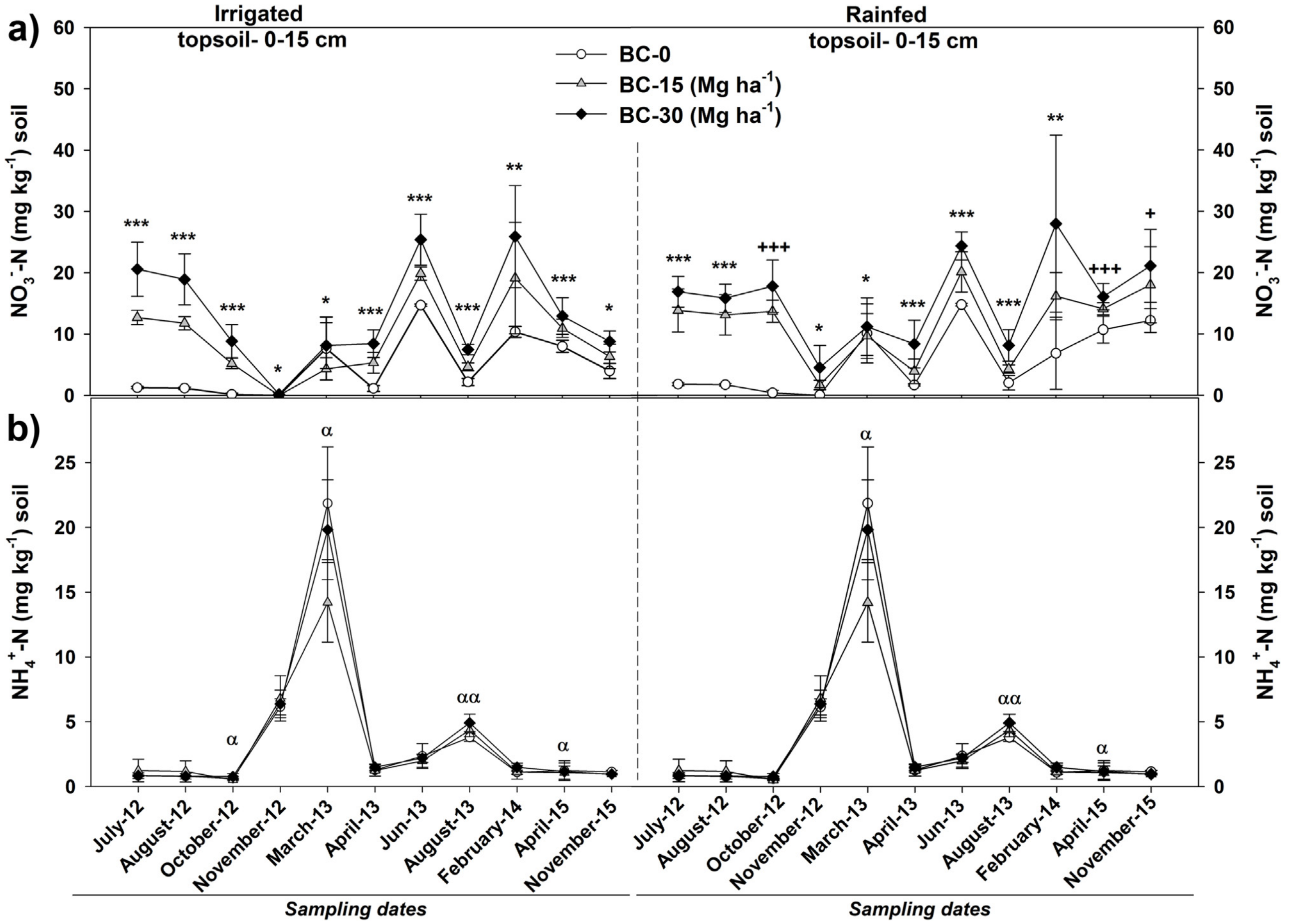

Fig. 4. Topsoil (0–15 cm) nitrate and ammonium (NO_3^- and NH_4^+) concentrations during the study period (year I–IV) as influenced by biochar amendments (BC, 0, 15 and 30 Mg ha^{-1}) and watering regime (irrigated or rainfed). The y axis scales of a and b are based on the mean values, and error bars indicate standard deviations ($p < 0.05$, $n = 24$). The symbols (*, +, α) indicate significant treatment effects at single sample dates at different significance levels (e.g. *** = p < 0.001, ** = p < 0.01,* = p < 0.05). * indicates significant BC effects, + indicates significant effects of both BC and watering regime, and α indicates significant watering regime only. See the accompanying statistical results in Table S6 and sub soil NO_3^- and NH_4^+ concentration results in Fig. S3.

Table 5
Soil respiration during year I (crop maize) as influenced by biochar amendments (BC, 0, 15 and 30 $Mg\,ha^{-1}$) and irrigation regimes (irrigated or rainfed).

Treatments	Soil respiration ($\mu mol\ CO_2\ m^{-2}\,s^{-1}$) (One way ANOVA)[a]		
	May-2012	Jun-6-2012	Jun-28-2012
Control	1.01 ± 0.27b	2.94 ± 0.81a	4.08 ± 0.69a
BC-15	1.40 ± 0.19b	3.39 ± 1.48a	3.99 ± 0.33a
BC-30	1.57 ± 0.27a	3.05 ± 0.98a	4.33 ± 0.45a

Factors		Soil respiration ($\mu mol\ CO_2\,m^{-2}\,s^{-1}$) (Split-plot ANOVA)		
Biochar	Water	July-2012	August-2012	September-2012
Control	Irrigated	1.84 ± 0.21b	2.71 ± 0.68a	1.29 ± 0.34a
BC-15		1.93 ± 0.28a	2.95 ± 0.36a	1.99 ± 0.61a
BC-30		2.95 ± 0.34a	3.33 ± 0.30a	1.83 ± 0.46a
Control	Rainfed	1.61 ± 0.15A	2.66 ± 0.35a	1.23 ± 0.28a
BC-15		2.01 ± 0.25A	2.93 ± 0.93a	1.12 ± 0.51a
BC-30		1.90 ± 0.50A	2.84 ± 0.16a	1.62 ± 0.17a

Values in the columns are the means ± standard deviation ($n = 4$). Different letters indicate significant differences due to BC treatments, while lower vs upper case letters indicate a significant irrigation effect following one way and split-plot ANOVA and Tukey HSD test, respectively ($p < 0.05$, $n = 24$). See the accompanying statistical results in Table S7.

[a] The dates of sampling in May-2012, June 6 and 28, 2012 were before first additional irrigation as watering regime. Therefore, the results of these dates were analyzed with one-way ANOVA.

the reduction of $Mn^{4+}O_2$ to Mn^{2+} because of an aeration effect of BC and because of the locally around BC particles increased alkalinity in the BC amended plots. The reduced plant height due to BC treatments in our field study, observed at no reduction in tissue N concentration and at the presence of increased soil nitrate concentrations, suggests that Mn deficiency may have impaired plant photosynthesis PS II (Marschner, 1995) leading to reduced plant growth. In addition, we found no difference ($p = 0.519$) in chlorophyll contents (SPAD values, data not shown) which is in agreement with Marschner (1995) and Nable et al. (1984), who reported that Mn deficiency had very small effects on plant chlorophyll content, but that it may reduce photosynthetic oxygen evolution by more than 50% (i.e. reducing the plants' carbohydrate gains and thus growth). Manganese deficient plants have also been observed to hamper their reduced N (NO_3^-) demands because of higher nitrate accumulation in leaves which is mainly due to a shortage of reducing equivalents in the chloroplast and of sugars in the cytoplasm.

4.2. Soil respiration and gravimetric moisture contents

Generally biochar amendment effects on the soil CO_2 efflux (bulk soil + root respiration) span a broad variability of results from stimulation to suppression (Thomazini et al., 2015; Zimmerman et al., 2011). Reactions range from (i) reductions in the CO_2 efflux from BC amended soils (Harvey et al., 2012; Lentz et al., 2014; Zimmerman et al., 2011); to (ii) no significant differences, particularly with high temperature biochar with minimum labile organic matter (Case et al., 2012; Karhu et al., 2011; Kuzyakov et al., 2009; Spokas and Reicosky, 2009; van Zwieten et al., 2010), to (iii) an initial increase in the CO_2 efflux and then stabilization or even a decrease compared to the control treatment (Smith et al., 2010; Yuan et al., 2014). A biochar-induced net release of soil CO_2 (Jones et al., 2011) has been observed in other studies which was probably due to biological breakdown of biochar released organic carbon (Jones et al., 2011) or to some extent by mineralization of added biochar (Kuzyakov et al., 2009). However, the contributing sources are hard to separate if only net rates of both, soil-C and biochar-C decomposition are measured. In this study, soil CO_2 efflux initially increased (one month after BC application) with BC amendments but the effect quickly declined with field residence of biochar. Our results are in accordance with Smith et al. (2010) who used stable carbon isotopes and reported increased CO_2 efflux with BC amendment only up to the 6th day of a laboratory incubation study, while later on it was diminished. Such responses of BC amendment on soil CO_2 production at initial stages has been attributed to labile organic carbon fractions from volatiles adsorbed on biochar surfaces during a condensation period after pyrolysis (Smith et al., 2010; Yuan et al., 2014). It is likely that the pattern observed here was due to initial labile fractions of the biochar. However, some ongoing higher labile biochar-C decomposition may have been masked by slightly lower root respiration contributions since aboveground growth and biomass were slightly reduced with biochar (Table 3).

Biochar amendments also improved soil moisture contents which is in accordance with other studies under different soils, BC and environmental conditions (Agegnehu et al., 2016a; Karer et al., 2013; Liu et al., 2012; Mulcahy et al., 2013; Rogovska et al., 2014; Zhang et al., 2016a). Soil moisture improvement due to biochar addition was mainly attributed to changes in soil bulk density (SBD) and WHC due to porous structure with high adsorption capacity of biochar particles. Moreover, even mere changes in SBD with BC amendment can significantly alter soil water retention (Castellini et al., 2015). However, the moisture improvement in the current field study was not as consistently higher with increasing rates of biochar as expected from our greenhouse study with the same BC and soil (Haider et al., 2015). In the greenhouse study there was a continuous replenishment of water loss from pots on a daily basis up to target WHC values. On the other hand, under natural field conditions, the precipitation (or additional irrigation) events were not as frequent and controlled as they were in our greenhouse study. Therefore, the soil moisture increment in BC amended plots was not continuous, and probably too small to have positive effects on the yield. Another possibility is that the increased water supply was probably not in the range of pore sizes that really increase water availability to plants (pF > 4.2).

4.3. Effect of biochar on soil mineral N concentrations

Biochar application may influence the soil nutritional status, specifically due to its directly plant available nutrient concentrations (i.e. cations, Sohi et al., 2010; Yuan et al., 2016). However, Wu et al. (2011) for instance reported that only up to 2% N, 10–60% P and 15–20% Ca in terms of total inherent concentration in an oil-malee biochar (slow pyrolysis at 300, 500 and 750 °C) was readily leachable with Milli-Q water (highly pure water with > 16 ohm resistivity). It suggest that BC derived nutrients may not be sufficient to have a significant impact on soil nutrients following amendment. Therefore, there must be some other process like retention, capture or sorption of synthetic nutrients (e.g. fertilizer derived) on to biochar which may be involved in producing significant changes in soil nutritional status following BC addition (Haider et al., 2016).

Ammonium adsorption and nitrate retention following BC amendment have been observed in some field studies (Bruun et al., 2012; Haider et al., 2016; Lehmann et al., 2003; Nelson et al., 2011; Novak et al., 2010) and in laboratory or greenhouse studies (Asai et al., 2009; Güereña et al., 2013; Haider et al., 2015; Han et al., 2016; Major et al., 2012). Mainly the soil NH_4^+ immobilization, sorption or retention have been reported, and are usually attributed to increased CEC following BC application (Yao et al., 2012; Yuan et al., 2016; Zheng et al., 2013). Ammonium with its positive charge may potentially sorb to soil colloidal particles or onto the negatively charged functional groups on BC particles (Angst et al., 2014; Clough and Condron, 2010; Clough et al., 2013).

Hence, surprisingly, in the present study, we found a significantly greater NO_3^- concentration in topsoil (BC amended zone) rather than NH_4^+ throughout the study period over four years. There was a slight or no significant difference in soil NH_4^+, either in top or subsoil; only the lower NH_4^+ concentrations after a fertilization event with biochar treatment indicate accelerated net nitrification as described by Sánchez-García et al. (2015) or Nelissen et al. (2015). The development of enhanced soil nitrate stocks started in the biochar-amended plots after the first N fertilization (02-05-2012) with the first crop maize (year I). At the first sampling date after biochar amendment (15-05-2012) there was only a 0.75–4% NO_3^- concentration increment over that of the control (i.e. virtually no difference, Table S1) which increased to a maximum 5908% over control after N fertilization (Table S8). The varying absolute nitrate concentration in the top soil horizon throughout the study and also in the biochar treatments (Table S8) suggests that biochar indeed possesses the potential of nitrate capture and release. The fact that biochar can capture nitrate, particularly when co-composted in nutrient-rich organics, but also in the field, was described recently (Haider et al., 2016; Kammann et al., 2015). It is even not unlikely that the "true" amount of nitrate, being captured in biochar particles in the field was underestimated with the standard 2 M KCl extraction for 1 h and may have been higher (Haider et al., 2016).

4.4. Grain yield, N uptake and NUE of crops

In contrast to many other studies (Chan et al., 2007; Lehmann et al., 2003; Rondon et al., 2007; Widowati et al., 2014; Zhang et al., 2016a) biochar amendments significantly reduced the grain yield of the maize crop (year I), consistent with the results of Namgay et al. (2010). However, there are some BC studies conducted under European field/greenhouse conditions with no significant effects on crop yield (Baronti et al., 2010; Jones et al., 2012). It was observed that potential negative effects of biochar amendment may diminish/alter with field experimental duration, biochar oxidation and aging or with crop maturity. Reduced Mn concentrations observed at the vegetative stage of the first crop maize (year I) were no longer present in straw or grain at the harvest date, but reduced Mn supply may have delayed or retarded plant development (reducing N demands, Marschner, 1995). Thus, in the first year, increased nitrate concentrations in the top soil in the presence of biochar could theoretically be explained by reduced plant N uptake. However, this option is unlikely to last year (year IV) when no biomass reductions or retarded development occurred due to BC amendment. It is therefore likely that nitrate capture by biochar was involved in the initial N-deficiency symptoms as well (Haider et al., 2016), not only Mn deficiency at the vegetative stage alone.

Short-term N immobilization, N retention, reduced nitrate leaching and reduced N uptake following BC application have been previously reported in greenhouse/laboratory incubation experiments (Bruun et al., 2012; Haider et al., 2015; Lehmann et al., 2003; Nelson et al., 2011; Novak et al., 2010) as well as in field studies (Asai et al., 2009; Güereña et al., 2013; Haider et al., 2016; Han et al., 2016; Major et al., 2012). Based on recent results of nitrate capture by biochar (Haider et al., 2016; Kammann et al., 2015), as well as on the observations made here, we provide evidence that "nitrate capture" and related decline in N availability may occur more frequently and may be the cause for negative growth/yield responses (provided that PAHs, contaminants or pH effects can be excluded).

There was no significant effect on grain yield of the second crop (winter wheat, year II) due to BC treatment, even though nitrate concentrations measured by 2 M KCl extraction were initially higher in biochar amended plots. However, reduced total N uptake was observed, which might be well explained by nitrate capture of biochar, indicating that the captured nitrate was only partly available to the wheat plants. Theoretically also N immobilization or NH_4^+ adsorption on biochar particles may have occurred (Ducey et al., 2013; Haider et al., 2016; Ippolito et al., 2015; Ventura et al., 2013). In contrast to our study, biochar amendments have been shown to enhance wheat ear, grain per ear, and grain yield under drought conditions (Blackwell et al., 2010; Solaiman et al., 2010). A 10% grain yield improvement of wheat was observed by Baronti et al. (2010) with 10 Mg ha^{-1} BC amendment.

The grain yield of summer barley (year III) was significantly reduced due to greater rate of BC (30 Mg ha^{-1}) addition and water deficiency, while BC amendment had no significant effect on grain yield of last crop maize (year IV). Interestingly, the straw and grain N concentration or total N uptake of last two crops was not influenced by BC, rather only water deficiency caused N uptake limitations. It suggest that field aging or oxidation of BC may contribute to alleviate the N uptake limitations either by minimising nitrate capture property of BC, or the nitrate capture and holding capacity of BC may fulfil with the passage of time in the field. Taken together, results of year I and III show that the higher KCl-extractable nitrate concentrations in the top soil with biochar were not entirely plant-available, otherwise the initial soil concentration differences would have caused higher yields in the BC treatment plots.

The fourth season 2015 was exceptionally dry so that irrigation was frequently provided, causing a drastic difference to the (low) yield in the non-irrigated plots. Drought affects yields not just by lack of water but also via reduced nutrient availability (He and Dijkstra, 2014; Li et al., 2009), as indicated by a strong reduction in the total N uptake and grain yield of the fourth crop maize (year IV). However, although a significantly higher gravimetric soil moisture content in BC amended plots was observed (Basso et al., 2013; Liang et al., 2014) it was not enough to alleviate the drought impact on plant growth. Tammeorg et al. (2014) also found no significant alleviation with BC amendment at the time of a severe water supply deficit and high temperature in a field study. In contrast, Karer et al. (2013) found a 10% grain yield increment of barley under prolonged drought in a chernozem soil and attributed the effect to the greater water supply with BC amendment.

The NUE of all crops, except that of the first maize crop (year I), was not influenced by BC treatments. This contradicts our results of the greenhouse study with the same BC, where we found increased NUE with decreased tissue N concentration (Haider et al., 2015). Based on an N balance the missing N in the greenhouse experiment might have been fixed in the biochar particles (Haider et al., 2015), thus the improved NUE might just reflect the limitation in soil volume (to be explored by roots) and the plasticity of the maize plants to respond to suboptimal N supply. The difference between the pot and field study may be due to soil water availability, micronutrient supply or both. In the greenhouse study there was continuous daily replenishment of water to a target WHC, which improved the plant water status; moreover, a micronutrient solution including Mn was initially supplied in the greenhouse, which might have alleviated initial pH-induced micronutrient (manganese) deficiencies. Indications for the latter were observed in the field in the first year. With the severe drought spell that occurred in the fourth year, BC was unable to alleviate drought effects in the rainfed as well as in the irrigated plots, despite increases in the gravimetric soil moisture with BC. Hence, we conclude that the increase in the soil water supply may have largely been driven by small biochar pores, too tiny to provide plant-available water (i.e. > pF 4.2). Furthermore, the (irrigation) water supply was not as continuous in the field as in the greenhouse study. Hence, taken together, the results of both studies (greenhouse and field) suggest that biochar effects observed under controlled conditions in the greenhouse may not always be reproducible under field conditions.

5. Conclusions

Our results clearly demonstrated that wood chip sievings biochar used in this study did not promote positive effects on yields over four years with different crops and climatic conditions, neither under irrigated nor rainfed field conditions. Biochar induced Mn deficiency in the first months and in subsequent years limitations in the N uptake even in the presence of available N (NO_3^-). Despite soil gravimetric water content increases in the topsoil (0–15 cm) no alleviation of drought impacts were observed in terms of crop yields. However, no significant effect on crop yield (year II and IV) after BC field aging indicated that at least the selected biochar may need longer (>10 years) residence time in soil to produce beneficial effects on crop growth and yield. Furthermore, the initial increase followed by non-significant effect on CO_2 efflux and greater nitrate retention in top soil suggest that minimal yield loss observed here, cannot offset the environmental benefits of carbon sequestration and reduced nitrate leaching via adding recalcitrant carbon (biochar) in a carbon poor sandy soil.

The results observed under field conditions were in contrast to the initial results of our greenhouse pot study. It suggests that biochar effects on crop growth and nutrition are highly complex and vary widely in different agroecosystems. Furthermore, results from greenhouse experiments are not necessarily compareable with the results from field studies. Biochar, once applied, cannot be purged from the field again and economic benefits to farmers are necessary to address the food security. Thus we suggest to either use a) lower rates of biochar addition, because 1 or 10 Mg ha^{-1} biochar addition showed minor impacts on soil properties, WHC and plant growth (Glaser et al., 2015) and BC application at 10 or $<$20 Mg ha^{-1} resulted in greater positive effects on crop yield (Hammond et al., 2013; Ruysschaert et al., 2016), or to use b) nutrient enrichment of biochar before field application e.g. low-dose enriched biochars as root zone fertilizer (Schmidt et al., 2015) or compound fertilizers (Qian et al., 2014), to use c) composted biochar (Kammann et al., 2015) or to use d) compost-biochar mixtures (Glaser et al., 2015; Liu et al., 2012). Nonetheless, more long-term and process oriented field studies are essential to estimate complex biochar effects on soil-biochar-plant nutrient interface under realistic agro-climatic conditions, preferentially using nutrient-enhanced biochars.

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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.agee.2016.12.019.

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Supplementary material

Title: **Biochar reduced nitrate leaching and improved soil moisture content without yield improvements in a four-year field study**

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MS Type	**Regular research article**
Figures	**(3)**
Tables	**(8)**

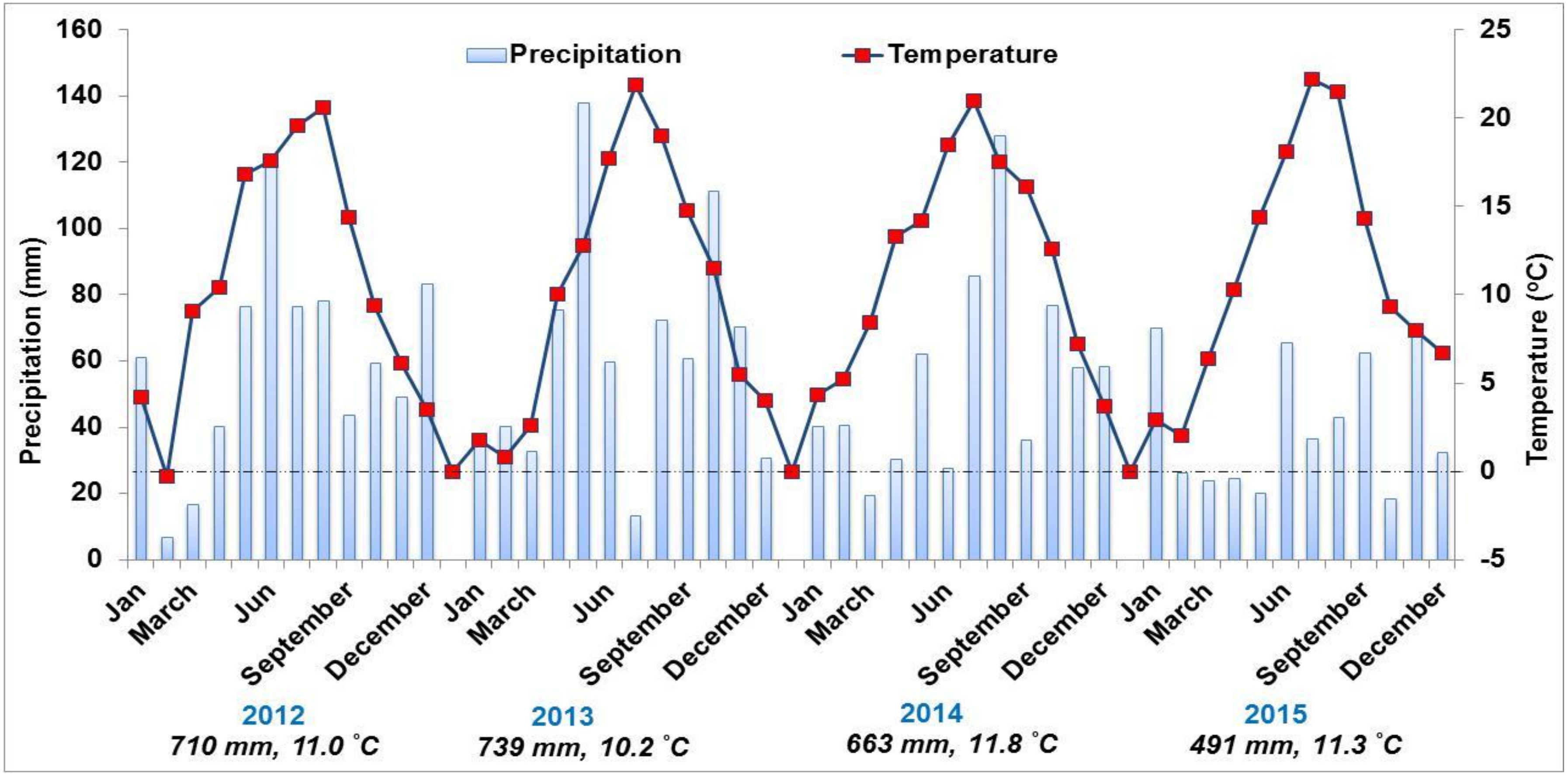

Fig. S1. Meteorological data for the experimental period. Bars and line above bars indicate total monthly precipitation and mean monthly temperature, respectively. The dotted line in the figure differentiates between above and below zero degree temperature. The values given under each year, show total annual precipitation (mm) and average annual temperature (°C).

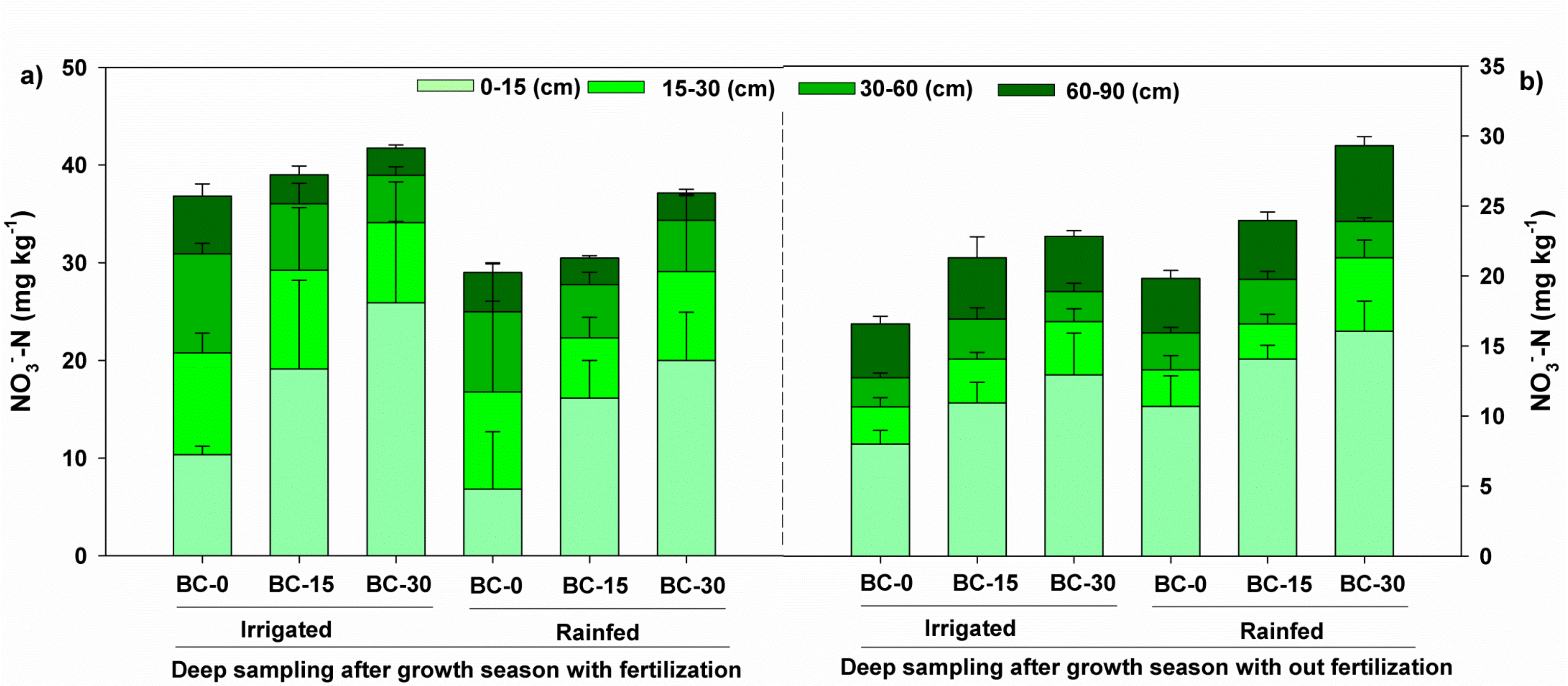

Fig. S2. Soil nitrate (NO_3^-) concentration in the soil profile (0 - 15, 15 - 30, 30 - 60 and 60 - 90 cm depth) on a) 20-02-2014 and b) 21-04-2015 approximately after two and three years of biochar amendment (BC, 0, 15 and 30 Mg ha^{-1}) and watering regime treatment (irrigated or rainfed), respectively. The soil samplings took place before fertilization events. Note that the y-axis scales of a and b are different. The error bars indicate standard deviations of each respective piece of stacked bars (e.g, 0 - 15, 15 - 30, 30 - 60 and 60 - 90 cm depth; *n*=4). See accompanying statistical results for Table S4.

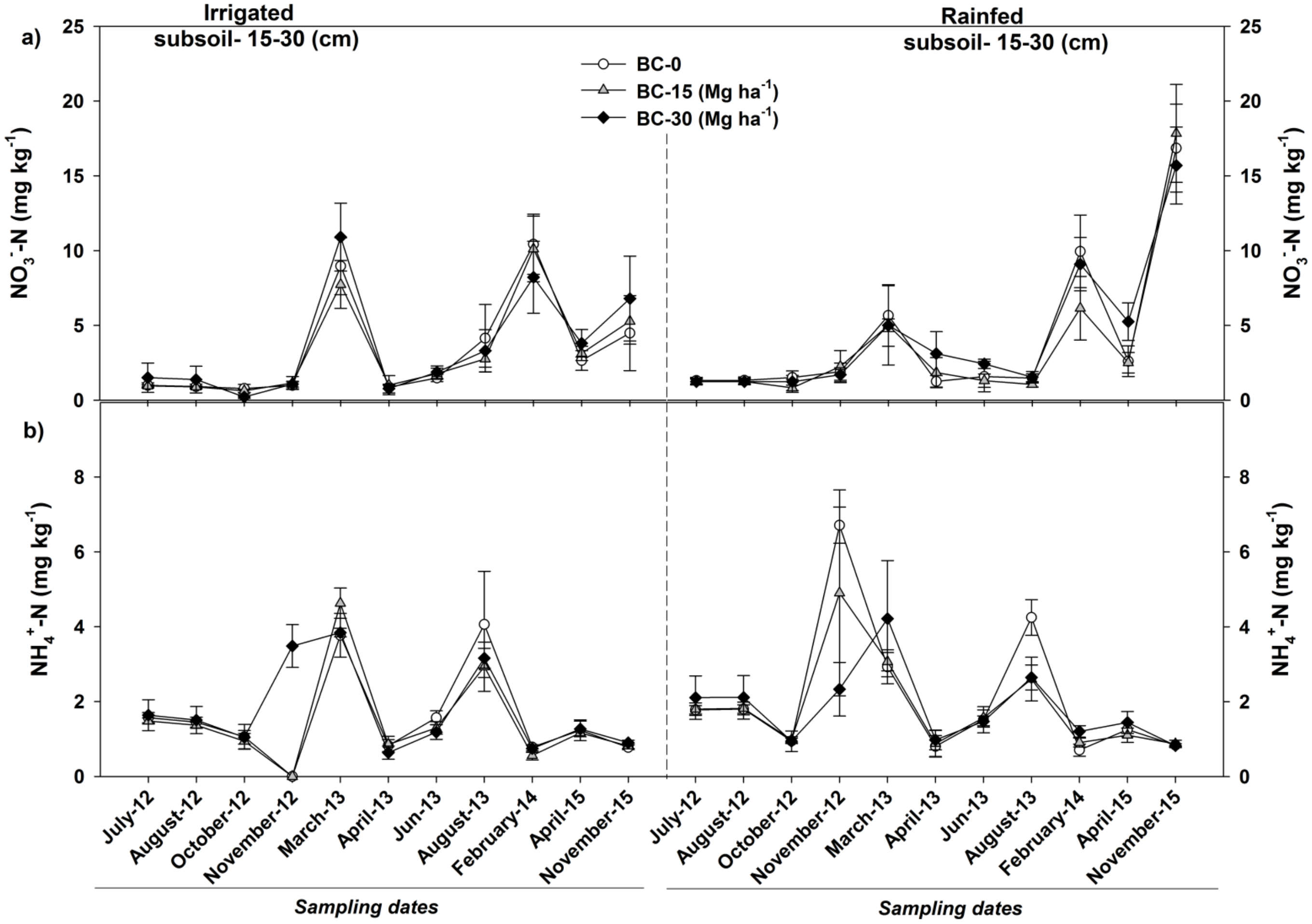

Fig. S3. Subsoil (15 - 30 cm) nitrate and ammonium (NO_3^- and NH_4^+) concentrations during the study period (the year I - IV) as influenced by biochar amendments (BC, 0, 15 and 30 Mg ha^{-1}) and watering regime (irrigated or rainfed). Note that the y-axis scales of a and b are different. Error bars indicate standard deviations (*n*=4). See accompanying statistical results for Table S6.

Table S1

Results of one-way ANOVA analyses showing the effects of biochar (BC, 0, 15 and 30 Mg ha^{-1}) on plant height, biomass nitrogen (N) and manganese (Mn) concentrations, and top and subsoil NO_3^- concentrations at the vegetative stage of first crop maize (the year I).

Factor	**Pl. Height (cm)**		**Biomass N (mg g^{-1})**		**Biomass Mn (mg kg^{-1})**		**Topsoil-May NO_3^- (mg kg^{-1})**		**Subsoil-May NO_3^- (mg kg^{-1})**		**Topsoil-Jun NO_3^- (mg kg^{-1})**		**Subsoil-Jun NO_3^- (mg kg^{-1})**		**Topsoil- May Mois. (%)**		**Subsoil-May Mois. (%)**		**Topsoil-Jun Mois. (%)**		**Subsoil-Jun Mois. (%)**	
	F	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***
Biochar	14.18	***<0.001***	0.44	*0.649*	15.46	***<0.001***	11.05	***<0.001***	4.06	***0.032***	61.82	***<0.001***	16.29	***<0.001***	17.81	***<0.001***	2.27	*0.128*	58.90	***<0.001***	*1.24*	*0.311*

Statistics, accompanying Fig. 1 and 2. One-way ANOVA was used since no irrigation had been performed up to these sampling dates, and the factor water was not significant in split-plot ANOVA tests. Significant effects are marked by bold P values ($p<0.05$). The data presented in this table was taken before first additional irrigation as the watering regime. Therefore, the results of these data were analyzed with one-way ANOVA, because at that time only single factor BC was logically functioning.

Table S2

Results of one and split-plot ANOVA showing the effects of biochar (BC, 0, 15 and 30 Mg ha^{-1}) and watering regime (irrigated or rainfed) on macro- and micronutrients of straw and grain of the first crop maize (the year I).

Factors	Nitrogen N (mg g^{-1})		Phosphorous P (mg g^{-1})		Potassium K (mg g^{-1})		Calcium Ca (mg g^{-1})		Magnesium Mg (mg g^{-1})		Sodium Na (mg g^{-1})		Manganese Mn (mg kg^{-1})		Iron Fe (mg kg^{-1})		Copper Cu (mg kg^{-1})		Zinc Zn (mg kg^{-1})	
	Maize 2012 straw nutrient concentrations at vegetative stage (One way ANOVA)†																			
	F	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***
BC	0.44	*0.649*	1.33	*0.287*	10.344	***<0.001***	3.136	*0.064*	1.259	*0.304*	0.759	*0.480*	15.460	***<0.001***	nd	*nd*	1.514	*0.243*	1.11	*0.348*
	Maize 2012 straw nutrient concentrations at harvest (split-plot ANOVA)																			
BC	7.20	***0.009***	10.44	***0.002***	0.72	*0.384*	4.78	***0.030***	0.33	*0.728*	0.91	*0.428*	0.68	*0.526*	1.21	*0.331*	0.01	*0.990*	5.59	***0.019***
W	40.09	***0.008***	969.8	***<0.001***	219.2	***<0.001***	2.58	*0.207*	1.39	*0.324*	0.46	*0.55*	1.42	*0.319*	2.10	*0.243*	18.8	***0.023***	543.1	***<0.001***
BC x W	1.64	*0.235*	0.42	*0.665*	0.35	*0.712*	0.65	*0.541*	0.88	*0.442*	3.52	*0.063*	0.21	*0.816*	0.05	*0.956*	0.02	*0.979*	1.11	*0.351*
	Maize 2012 grain nutrient concentrations (split-plot ANOVA)																			
BC	21.37	***<0.001***	3.34	*0.070*	0.100	*0.906*	21.07	***<0.001***	3.87	***0.051***	4.25	***0.040***	0.56	*0.586*	1.30	*0.309*	6.21	***0.014***	0.67	*0.530*
W	33.73	***0.010***	0.323	*0.609*	1.31	*0. 335*	61.02	***0.004***	0.66	*0.477*	0.01	*0.915*	9.05	***0.057***	18.76	***0.023***	150.3	***0.001***	10.97	***0.045***
BC x W	0.05	*0.956*	4.12	***0.043***	3.80	***0.053***	4.45	***0.036***	2.97	*0.091*	0.604	*0.563*	0.37	*0.701*	15.30	***<0.001***	0.36	*0.708*	4.28	***0.040***

Significant effects are marked by bold P values ($p<0.05$). Accompanying statistical results for Table 2.

†The straw nutrient concentration of Maize 2012 was measured before first irrigation to experimental plots. Therefore, the results of this data were analyzed with one-way ANOVA, because at that time only single factor BC was logically functioning.

Table S3

Results of split-plot ANOVA analyses showing the effects of biochar (BC, 0, 15 and 30 Mg ha^{-1}) and watering regime (irrigated or rainfed) on grain yield (on dry mass basis), straw or grain nitrogen (N) concentration and nitrogen use efficiency (NUE) or total N uptake (i.e. sum of N content in straw and grain) of cereals grown during the study (year I - IV).

Factors	**Maize-2012**				**Winter Wheat-2013**				**Summer barley-2014**				**Maize-2015**			
Yield (Mg ha^{-1})																
	F		***p***		**F**		***p***		**F**		***p***		**F**		***p***	
BC	10.2		***0.003***		0.34		*0.717*		6.23		***0.014***		0.65		*0.538*	
W	78.7		***0.003***		0.79		*0.349*		1654		***<0.001***		447.7		***<0.001***	
BC x W	2.10		*0.165*		0.03		*0.971*		4.38		***0.037***		0.211		*0.812*	
Plant straw and grain N concentration (mg g^{-1})																
	Straw		**Grain**		**Straw**		**Grain**		**Straw**		**Grain**		**Straw**		**Grain**	
	F	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***
BC	7.20	***0.009***	16.9	***<0.001***	1.37	*0.292*	2.66	*0.110*	2.28	*0.145*	3.84	*0.0514*	1.16	*0.347*	0.10	*0.910*
W	40.1	***0.008***	33.7	***0.010***	2.75	*0.196*	0.49	*0.536*	59.9	***0.005***	29.3	***0.012***	583.5	***<0.001***	284.2	***<0.001***
BC x W	1.64	*0.235*	0.05	*0.965*	1.85	*0.199*	0.36	*0.709*	1.26	*0.318*	2.12	*0.163*	0.62	*0.554*	3.22	*0.076*
	NUE		***N-uptake***		***NUE***		***N-uptake***		***NUE***		***N-uptake***		***NUE***		***N-uptake***	
BC	10.2	***0.003***	19.1	***<0.001***	0.34	*0.717*	4.91	***0.028***	1.20	*0.336*	0.06	*0.945*	0.65	*0.540*	0.95	*0.414*
W	78.5	***0.003***	1.64	*0.29*	0.77	*0.444*	4.84	*0.115*	7.97	*0.067*	0.81	*0. 435*	448.4	***<0.001***	399.9	***<0.001***
BC x W	2.11	*0.164*	0.44	*0.657*	0.03	*0.971*	0.07	*0.936*	0.01	*0.993*	1.68	*0.228*	0.207	*0.816*	0.077	*0.926*

Significant effects are marked by bold P values (p<0.05). Accompanying statistical results for Table 3

Table S4

Results of split-plot ANOVA showing soil nitrate (NO_3^-) concentration in the soil profile (0 - 15, 15 - 30, 30 - 60 and 60 - 90 cm depth) on a) 20-02-2014 and b) 21-04-2015 approximately after two and three years of biochar (BC, 0, 15 and 30 Mg ha^{-1}) and watering regime (irrigated or rainfed) amendments respectively.

Factors	**During Feb. 2014 after two crops (maize and winter wheat) which were fertilized**							
	0-15 (cm) **NO_3^- (mg kg^{-1})**		**15-30 (cm)** **NO_3^- (mg kg^{-1})**		**30-60 (cm)** **NO_3^- (mg kg^{-1})**		**60-90 (cm)** **NO_3^- (mg kg^{-1})**	
	F	***p***	**F**	***p***	**F**	***p***	**F**	***p***
BC	7.04	***0.009***	0.24	*0.794*	6.70	***0.011***	14.2	***<0.001***
W	0.57	*0.506*	0.20	*0.686*	0.70	*0.465*	5.02	*0.111*
BC x W	0.20	*0.822*	0.32	*0.731*	0.53	*0.600*	2.47	*0.126*
	During April 2015 after third crop summer barley which was not fertilized							
BC	11.1	***0.002***	12.2	***0.001***	4.13	***0.043***	2.86	*0.096*
W	62.6	***0.004***	0.15	*0.722*	4.39	*0.127*	5.10	*0.109*
BC x W	0.03	*0.974*	3.16	*0.078*	0.11	*0.897*	3.56	*0.061*

Significant effects are marked by bold P values ($p<0.05$). Accompanying statistical results for Fig. S2.

Table S5

Results of split-plot ANOVA showing the effects of biochar (BC, 0, 15 and 30 Mg ha^{-1}) and watering regime (irrigated or rainfed) on topsoil (0 - 15 cm) moisture content (%) during the study period (2012 – 2015).

Factors	**July-2012**		**Aug-2012**		**Oct-2012**		**Nov-2012**		**Mar-2013**		**Apr-2013**		**Jun-2013**		**Aug-2013**		**Feb-2014**		**Apr-2015**		**Nov-2015**	
	Topsoil 0 - 15 (cm)																					
	F	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***
BC	2.15	0.160	9.81	***0.003***	20.9	***<0.001***	4.64	***0.032***	9.19	***0.004***	0.07	*0.933*	2.23	*0.150*	2.26	*0.147*	7.70	***0.007***	6.98	***0.010***	*19.0*	***<0.001***
W	279	***<0.001***	2.12	*0.242*	6.09	*0.090*	0.65	*0.479*	2.90	*0.187*	17.6	***0.025***	3.75	*0.148*	2.75	*0.196*	3.23	*0.17*	1.106	*0.37*	*0.02*	*0.909*
BC x W	1.36	*0.294*	0.51	*0.616*	0.414	*0.670*	0.03	*0.971*	0.89	*0.436*	0.989	*0.400*	2.15	*0.159*	0.02	*0.985*	1.99	*0.179*	1.78	*0.211*	*1.01*	*0.392*

Significant effects are marked by bold P values (p<0.05).

Table S6

Results of split-plot ANOVA analyses showing the effects of biochar (BC, 0, 15 and 30 Mg ha^{-1}) and watering regime (irrigated or rainfed) on soil mineral N concentrations (NO_3^- and NH_4^+) in top and subsoil (0 - 15 and 15 - 30 cm, respectively) during the study period (2012 - 2015).

Factors	July-2012		Aug-2012		Oct-2012		Nov-2012		Mar-2013		Apr-2013		Jun-2013		Aug-2013		Feb-2014		Apr-2015		Nov-2015	
Topsoil 0-15 (cm) NO_3^- (mg kg^{-1})																						
	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
BC	63.1	***<0.001***	62.3	***<0.001***	52.2	***<0.001***	3.83	***0.052***	5.27	***0.023***	19.7	***<0.001***	43.9	***<0.001***	22.7	***<0.001***	7.04	***0.009***	11.05	***0.001***	4.00	***0.046***
W	7.90	*0.067*	2.83	*0.191*	23.6	***0.017***	6.58	*0.083*	1.54	*0.303*	0.08	*0.786*	0.15	*0.724*	0.004	*0.954*	0.57	*0.506*	62.6	***0.004***	55.2	***0.005***
BC x W	1.42	*0.281*	1.29	*0.31*	6.99	***0.009***	3.24	*0.075*	1.70	*0.224*	0.39	*0.684*	0.21	*0.812*	0.203	*0.819*	0.20	*0.821*	0.03	*0.974*	0.41	*0.68*
Subsoil 15-30 (cm) NO_3^- (mg kg^{-1})																						
BC	0.62	*0.552*	0.56	*0. 587*	3.38	*0.068*	0.77	*0.486*	0.79	*0.478*	0.32	*0.731*	3.26	*0.074*	2.60	*0.116*	0.24	*0.794*	12.2	***0.001***	0.22	*0.81*
W	0.24	*0.66*	0.97	*0.398*	11.03	***0.045***	6.45	*0.085*	25.51	***0.015***	2.68	*0.20*	0.11	*0.760*	7.47	*0.072*	0.20	*0.686*	0.15	*0.722*	34.3	***0.010***
BC x W	0.82	*0.464*	0.86	*0.446*	2.50	*0.123*	0.35	*0.710*	0.91	*0. 429*	0.44	*0.657*	1.68	*0.228*	0.92	*0.422*	0.32	*0.731*	3.16	*0.079*	1.16	*0.347*
Topsoil 0-15 (cm) NH_4^+ (mg kg^{-1})																						
BC	0.03	0.968	0.05	*0.956*	3.28	*0.073*	1.68	*0.228*	1.74	*0.218*	1.85	*0.199*	0.44	*0.654*	3.34	*0.070*	0.09	*0.918*	0.16	*0.858*	3.17	*0.079*
W	0.02	*0.897*	0.07	*0.812*	10.2	***0.050***	5.20	*0.107*	13.5	***0.035***	0.01	*0.917*	6.13	*0.090*	106.2	***0.002***	54.7	***0.005***	16.8	***0.026***	1.56	*0.301*
BC x W	1.82	*0.204*	1.77	*0.212*	0.210	*0.813*	1.88	*0.195*	1.61	*0.239*	0.39	*0.686*	0.15	*0.864*	0.67	*0.529*	0.53	*0.601*	0.005	*0.995*	4.23	***0.041***
Subsoil 15-30 (cm) NH_4^+ (mg kg^{-1})																						
BC	1.24	*0.324*	1.12	*0.360*	0.12	*0.890*	0.16	*0.851*	1.27	*0.317*	0.12	*0.887*	2.58	*0.117*	11.0	***0.002***	5.62	***0.019***	2.19	*0.154*	1.18	*0.340*
W	37.0	***0.008***	60.5	***0.004***	0.32	*0.61*	39.0	***0.008***	3.94	*0.141*	1.36	*0.327*	2.22	*0.233*	0.91	*0.412*	28.8	***0.013***	0.10	*0.768*	0.21	*0.68*
BC x W	0.33	*0.728*	0.33	*0.726*	0. 42	*0.67*	3.42	*0.067*	3.33	*0.139*	1.06	*0.376*	2.02	*0.176*	0.63	*0. 547*	6.11	***0.015***	0.64	*0.546*	2.48	*0.126*

Significant effects are marked by bold P values ($p<0.05$).

Table S7

Results of split-plot ANOVA analyses showing the effects of biochar (BC, 0, 15 and 30 Mg ha^{-1}) and watering regime (irrigated or rainfed) on Soil respiration during the year I (crop maize) of study.

	Soil respiration (μmol CO_2 m^{-2} s^{-1}) (One way ANOVA)†					
	May-2012		**Jun-6-2012**		**Jun-28-2012**	
Treatments	**F**	***p***	**F**	***p***	**F**	***p***
Biochar	11.16	***<0.001***	0.66	*0.530*	3.75	*0.400*
	Soil respiration (μmol CO_2 m^{-2} s^{-1}) (Split-plot ANOVA)					
	July-2012		**August-2012**		**September-2012**	
Factors	**F**	***p***	**F**	***p***	**F**	***p***
BC	5.92	***0.016***	1.17	*0.344*	2.26	*0.147*
W	11.7	***0.042***	1.27	*0.342*	3.28	*0.168*
BC x W	4.08	***0.045***	0.51	*0.612*	1.90	*0.192*

Significant effects are marked by bold P values ($p<0.05$). †The dates of sampling in May-2012, June 6 and 28, 2012 were before first additional irrigation as watering regime. Therefore, the results of these dates were analyzed with one-way ANOVA, because at that time only single factor BC was logically functioning.

Table S8

Results of relative (%) increase or decrease in soil NO_3^- concentrations during the study period (2012 - 2015) as influenced by biochar (BC, 0, 15 and 30 Mg ha^{-1}) and watering regime (irrigated or rainfed) amendments.

Factors	May-2012		Jun-2012	
	Topsoil 0-15 (cm) NO_3^- (mg kg^{-1}) and relative increase or decrease in NO_3^- concentration (One way ANOVA)			
	Mean	**(%) Δ**	**Mean**	**(%) Δ**
Control	31.77	0.0	23.80	0.0
BC-15	32.01	0.8	33.21	39.5
BC-30	33.13	4.3	32.87	38.1

Factors	**July-2012**		**Aug-2012**		**Oct-2012**		**Nov-2012**		**Mar-2013**		**Apr-2013**		**Jun-2013**		**Aug-2013**		**Feb-2014**		**Apr-2015**		**Nov-2015**	
	Topsoil 0-15 (cm) NO_3^- (mg kg^{-1}) concentration and relative increase or decrease compared to control during the study period (2012-2015)																					
	Mean	**(%) Δ**	**Mean**	**(%) Δ**	**Mean**	**(%) Δ**	**Mean**	**(%) Δ**	**Mean**	**(%) Δ**	**Mean**	**(%) Δ**	**Mean**	**(%) Δ**	**Mean**	**(%) Δ**	**Mean**	**(%) Δ**	**Mean**	**(%) Δ**	**Mean**	**(%) Δ**
											Irrigated											
Control	1.26	*0.0*	1.17	*0.00*	0.15	*0.00*	0.00	-	7.65	*0.0*	1.13	*0.0*	14.71	*0.0*	2.18	*0.0*	10.36	*0.0*	8.00	*0.0*	3.97	*0.0*
BC-15	12.70	*909.2*	11.78	*903.6*	5.19	*3430.2*	0.00	-	4.31	*-43.6*	5.32	*370.0*	19.86	*35.0*	4.66	*113.3*	19.13	*84.8*	10.94	*36.8*	6.34	*59.6*
BC-30	20.57	*1534.5*	18.91	*1511.7*	8.83	*5908.2*	0.18	-	8.14	*6.4*	8.42	*644.4*	25.39	*72.6*	7.48	*242.3*	25.90	*150.1*	12.94	*61.8*	8.79	*121.3*
											Rainfed											
Control	1.79	*0.00*	1.70	*0.00*	0.38	*0.00*	0.00	-	10.08	*0.0*	1.64	*0.0*	14.77	*0.0*	2.01	*0.0*	6.82	*0.0*	10.69	*0.0*	12.18	*0.0*
BC-15	13.81	*671.5*	13.09	*668.1*	13.67	*3542.3*	1.67	-	9.64	*-4.4*	3.90	*137.9*	20.09	*36.2*	4.27	*111.9*	16.13	*136.4*	14.08	*31.7*	18.00	*47.8*
BC-30	16.86	*841.8*	15.82	*828.1*	17.76	*4633.8*	4.49	-	11.16	*10.7*	8.34	*409.2*	24.33	*64.8*	8.11	*303.0*	27.96	*309.7*	16.06	*50.3*	21.08	*73.1*

The dates of sampling in May-2012 and June-2012 were before first additional irrigation (setting up of a real split block) as watering regime. Therefore, the results of May and Jun were analyzed with one-way ANOVA.

5 General conclusions and implications

The results obtained in this study have advanced the understanding of some basic effects of biochar (BC) amendment under greenhouse or temperate field conditions. We conducted laboratory, greenhouse, and field studies using two levels of wood chip BC and two watering treatments (limited and frequent or rainfed and irrigated) to quantify soil-plant-atmosphere continuum, plants water and nutrients availability, growth and yield responses of different cereals, soil N dynamics (specifically N retention) and GHGs emissions.

Biochar improved soil-plant-water relations in terms of increasing soil WHC, leaf relative water content (RWC), osmotic potential ($\Psi\pi$) and transpiration while decreasing soluble sugars and stomatal resistance of maize in limited or in frequent water supply under greenhouse conditions. In addition, the positive BC effects on soil-plant-water interactions caused better photosynthetic performance, improved nitrogen and water use efficiency (NUE and WUE, respectively) thereby increasing final biomass production under greenhouse conditions (Chapter 2). Loading of humic acid product (HAP) on BC could not produce synergistic results on the functioning of BC. Both BC and HAP have shown similar effects on soil-plant-water relations and photosynthetic performance with more pronounced effect by BC. It can be concluded that the HAP effect was masked by the greater effect of BC.

Biochar amendments also increased soil moisture during most of the study period under field conditions but could not show a significant improvement in crop’s yield (Chapter 4). It was expected that BC will show its positive effects (increased WHC) more pronouncedly in natural drought spell (if any occur) under field conditions, and that it will enhance crop yield better under rainfed than irrigated treatments. However, this did not take place, even during the last year of the field study (2015), when maize crop faced severe drought conditions (only 491 mm annual precipitation as compared to 606 mm (30-year average)) among the total experimental duration (2012 - 2015). The same BC used in the greenhouse and under field conditions produced different results of water supply, which suggest that BC may need a greater (than control soil) base amount of saved water in its porous structure and any water beyond these limits can be supplied frequently to plants as shown in the greenhouse (Chapter 2) with daily water adjustments. It suggests that BC has potential to improve soil plant water relations, but pre-investigations of water holding and supply potential of BC under extremely limited water conditions are necessary for future field studies.

Biochar amendments caused reduced N supply with no adverse effects on biomass yields under greenhouse conditions, but still improved NUE, probably due to better water supply and photosynthetic efficiency of maize (Chapter 2). The BC treatments caused greater N (NO_3^-) retention in BC amended pots at harvest (Chapter 2) and throughout the field study in BC amendment zone (topsoil 0 - 15 cm) (Chapter 3 and 4). Many other studies have reported greater NH_4^+ retention in BC amended soil, but here we found substantially greater NO_3^- retention rather than NH_4^+. Moreover, such a huge NO_3^- retention was associated with nitrate being captured within BC particles rather than in the (BC amended) soil (Chapter 3). Furthermore, BC amendments significantly reduced NO_3^- concentrations in the subsoil (in 30 - 60 or 60 - 90 cm) suggesting reduced NO_3^- leaching. Results revealed that standard extraction methods like 2 M KCl and Electro-ultrafiltration (EUF) failed to extract all captured NO_3^- from BC_{aged} particles. These findings of our comprehensive studies for the first time opens new research horizons and puts forward future research goals to confirm these results with different BC, soil combinations, and climatic conditions to shape biochar use for future reactive N management where surplus nitrate amounts occur.

Crops under field study were managed conventionally and fertilized according to basic requirements. However, there were no consistent results regarding total N uptake, NUE and N concentration in straw and grain yield of all four crops under field conditions (Chapter 4). Biochar amendments also influenced other nutrients. Most importantly, BC amendments caused manganese (Mn) deficiency at the vegetative stage of the first crop following BC application in 2012. The grain yields of the first and third crop were decreased, likely due to N deficiency associated with BC NO_3^- capture in top soil. Therefore, there are possibilities of negative agronomic effects of BC application due to reduced nutrients supply (if PAHs, contaminants or pH effects can be excluded as was the case here).

Biochar amendment under laboratory conditions reduced GHGs emission. In addition, freshly incorporated BC under field conditions initially increased soil CO_2 efflux but in the following months, it declined to no significant differences among BC and control treatments. It suggests that BC is rather stable or does not lead to accelerated C losses. However, the slightly negative effects on crop N availability need further research. Some recent studies suggest that it may be a way forward to use the biochar in nutrient-rich waste stream management first (co-composting, loading with liquid animal manures etc.) and then use it as underfoot fertilizer. This has the advantage that only small amounts of biochar are needed (<1 - 2 t ha^{-1}). Taken together, the results of the greenhouse, laboratory, and field studies suggest that it is possible to use BC for agronomic and environmental benefits with some prerequisites. The potential NO_3^- capture property of BC urgently needs mechanistic investigations because it could be both beneficial, in terms of reducing nitrate leaching or harmful by reducing crop yields.

Outlook: Need for future research work

This study has highlighted several potential BC effects on soil-plant-water relations, nutrient interactions and specifically NO_3^- retention. Since the major aim of adopting BC, technology is to improve crop production while mitigating climate change. Therefore, the following research questions need to be addressed with future research.

- Identify the exact underlying mechanism of BC NO_3^- capture or enhanced retention, particularly in relation to properties, which can vary considerably.
- Our study showed very interesting data on the inefficiency of standard extraction methods (for mineral N e.g, 2 M KCl and EUF) for the first time from a field aged biochar. Therefore, it is necessary to investigate the extraction procedures developed in this study under different climatic conditions. We have used only one type of BC and soil in this study, which should be increased in number to get more insights in soil-biochar-N interactions for future reactive N management.
- Bioavailability of the biochar-captured N needs to be investigated with other fine rooted crops than those used in this study.
- A thorough investigation of the surface chemistry of BC_{aged} particles is needed to understand the chemical changes governing NO_3^- rather than NH_4^+ retention.
- A detailed investigation of GHGs emission from NO_3^- rich BC_{aged} particles at different moisture levels is necessary to understand if the GHG (markedly N_2O) emission potential increases when biochar becomes nitrate-enriched.
- In the present study, we incorporated BC in topsoil (0 - 15 cm) in the field experiment (Chapter 4), while in greenhouse study (Chapter 2) it was thoroughly mixed in entire pot soil (6 kg, in a 30 x 17.5 cm (diameter x height) pot). Plants roots in pot experiment may have had better access to captured N in BC, while under the field conditions, BC resided in topsoil 0 - 15 cm and roots may not entirely access the captured nitrate. Therefore, deep incorporation (0 - 30 or 45 cm) of nutrient rich BC in field studies should be investigated where plants roots may have ample access to BC captured nutrients.

- More beneficial ways of economic biochar use need to be established because, despite environmental benefits, biochar will only be used by farmers, if yield increases can be realized that give farmers a return on investment in biochar production and use. Therefore, the possibilities of amending BC properties, compost mixing, pre-use nutrient loading, and small dose applications should be investigated in future studies.

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Affidavit

I declare that this dissertation submitted is a work of my own written without any illegitimate help by any third party and only with materials indicated in the dissertation. I have indicated in the text where I have used texts from already published sources, either word for word or in substance and where I have made statements based on oral information given to me. At any time during the investigations carried out by me and described in the dissertation, I followed the principles of good scientific practice as defined in the "Statutes of the Justus Liebig University Giessen for the Safeguarding of Good Scientific Practice".

Ghulam Haider

Giessen, October 2016

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