Norwegian University of Life Sciences
Faculty of Environmental Sciences
and Natural Resource Management

Philosophiae Doctor (PhD)
Thesis 2021:84

The agronomic and environmental effects of biochar under field conditions in Norway

Effekten av biokull på agronomi og klimagassutslipp under feltforhold i Norge

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Abbreviations and definitions

BC – Biochar

Paper I

MC8 – Micanthus straw applied at 8 t C ha^{-1}

BC8 – Miscanthus biochar applied at 8 t C ha^{-1} (11.6 t BC ha^{-1})

BC25 – Miscanthus biochar applied at 25 t C ha^{-1} (31.5 t BC ha^{-1})

Paper II

BC-Aged – Miscanthus biochar applied at 25 t C ha^{-1} (31.5 t BC ha^{-1}) in 2010

BC-New – Miscanthus biochar applied at 25 t C ha^{-1} (31.5 t BC ha^{-1}) in

in 2012 and 2014

Paper III

BC-Low - Miscanthus biochar applied at 8 t C ha^{-1} (11.6 t BC ha^{-1})

BC-High – Miscanthus biochar applied at 25 t C ha^{-1} (31.5 t BC ha^{-1})

Paper IV

BC-Low – Wood biochar mixed with anaerobic digestate at 20% V/V

BC-High – Wood biochar mixed with anaerobic digestate at 40% V/V

List of papers

I. O'Toole, A., Moni, C., Weldon, S., Schols, A., Carnol, M., Bosman, B., Rasse, D.P., 2018. Miscanthus Biochar had Limited Effects on Soil Physical Properties , Microbial Biomass , and Grain Yield in a Four-Year Field Experiment in Norway. Agriculture 8, 171. https://doi.org/10.3390/agriculture8110171 (published)

II. O'Toole, A., Rasse, D.P., Silvennoinen, H., Krogstad, T. The effect of aged vs new biochar on fluxes of N_2O and CH_4 over 2 field seasons in a silty clay loam (manuscript submitted)

III. O'Toole, A., Uzinger, N., Plessis, C., Rasse, D.P. The transport, fate, and recovery of biochar 5 years after application to a flat-terrain cereal crop field (manuscript submitted)

IV. O'Toole, A., Weldon, S., Rasse, D.P., Joseph, S., Taherymoosavi, S., Budai, A. Biochar improves the nitrogen fertilization effect of anaerobic digestate in spring onions. (manuscript submitted)

Abstract

Biochar is emerging as a promising solution for increasing carbon in soil, improving the fertility of selected soils, and mitigating N_2O emissions from agricultural soils. In Norway, biochar is high on the agenda of potential actions to address climate change. Farmers, farmer organizations and authorities wish to know more about effects of biochar on Norwegian soils and environmental impacts. This Ph.D. thesis reports results from four field based studies, which document both agronomic and environmental impacts of biochar application in grain and vegetable production systems in Norway. Paper I:III report results from a 4 year field in a silty clam loam in flat terrain in Ås, Norway. In Paper I, I assessed the impact of applying 11.6 and 31.5 t ha^{-1} miscanthus biochar on soil physical properties, soil microbial biomass and oat and barley yield. Soil volumetric water content was significantly higher and bulk density was significantly lower at the higher biochar application rate. However, there was no significant effect of biochar on soil aggregate stability, pore size distribution, penetration resistance, microbial biomass, basal respiration and barley and oat yields over four years. At the same field site in Ås, Paper II assessed the impact of new vs aged biochar on N_2O and CH_4 fluxes in two field seasons. New biochar additions at 31.5 t ha^{-1} significantly reduced N_2O by 80% in a single peak event post-harvest in 2012 whereas 2-year aged biochar measured 41% less N_2O flux compared to the control (not significant). In 2014, new biochar application induced more N_2O emission than the control whereas aged biochar did not differ. However, the magnitude of N_2O emissions in 2014 was 2-6 times less than in 2012 due to much drier weather, so the net effect over two field seasons was that new biochar mitigated N_2O more than it stimulated it. Analysis of aged vs new biochar shows that biochar loses its alkalinity over 2-4 years and this may explain the weaker N_2O mitigation effect in aged compared to new biochar. In contrast to previous studies, I found that aged biochar did not reduce the soil CH_4 sink capacity but instead improved the soil sink capaicty during peak CH_4 emission events. New biochar did not differ from the control in CH_4 flux. Cumulative emissions of CO_2, N_2O and CH_4 did not differ between treatments in 2012 and 2014 due to the high standard deviation recorded within treatments, which is common in GHG field studies using closed chamber measurements. In Paper III, we documented the mobility and fate of biochar 5 years after application. We measured the vertical (0-60cm) and lateral (9 m from plot edge) transport of the biochar in the field at Ås. After 5 years, we accounted for 92-107% ±6 of the applied biochar. Forty-five to seventy two percent of biochar was present in the 0-23 cm plough layer within plot boundaries, 22-

31% vertically transported to 23-60 cm depth, 0-21% had moved laterally within 9 m of the plot boundary and 4% was mineralized as CO_2. Under laboratory tests, I found that biochar was easily released from soil aggregates when exposed to water slaking. However, considering the high recovery rates we achieved after 5 years we conclude that erosive loss of biochar via slaking was not a significant risk factor in this flat field site where biochar was well incorporated into the soil. Due to the moderate amount of biochar that is vertically transported below the plough layer I recommend that future soil sampling strategies designed to document biochar C stocks considers this vertical movement. In the final study, Paper IV, we cooperated with a commercial farmer to test the synergistic fertilization effect of biochar added as 20% and 40% (V/V) to liquid anaerobic digestate and applied at 7 cm depth under spring onion planting rows in a coarse sandy soil. We measured both soil NH_4-N, NO_3-N, and N_2O measurements in first month of plant establishment. Although differences in yield were not significant, biochar digestate mixture increased spring yield by up to 37% compared to the NPK-Control treatment while digestate alone performed similar to the Control. Soil mineral N significantly increased up to 305% in the digestate-biochar treatment compared to the control, while digestate did not significantly differ from the control. This finding suggests that biochar addition to digestate buffers the loss of digestate nutrients under irrigated conditions in sandy soils and supports a more balanced supply of NO_3 and NH_4 from digestate. In addition to the results and discussion in these papers, I give a thorough summary on the topic of biochar agronomy and impacts on GHGs and offer suggestions for new research topics that fill identified research gaps.

Norsk sammendrag

Biokull vokser fram som en lovende løsning for å øke karbon i jord, forbedre fruktbarheten i utvalgte jordarter og redusere N_2O -utslipp fra jordbruksjord. I Norge står biokull høyt på dagsordenen som et klimatiltak i landbruket. Bønder, bondeorganisasjon, og myndigheter uttrykker økende interesse for mer kunnskap om forventede effekter på agronomi og miljøvirkninger. Denne doktorgradsoppgaven rapporterer resultater fra 4 feltbaserte studier som dokumenterer både agronomiske og miljømessige konsekvenser av biokullapplikasjon i korn- og grønnsaksproduksjonssystemer i Norge. Artiklene I- III rapporterer resultater fra et 4 -årig felt i en siltig lettleire i flatt terreng i Ås, Norge. I Artikkel I testet jeg effekten av tilførsel av 11,6 og 31,5 t ha^{-1} miscanthus biokull på jordas fysiske egenskaper, jordmikrobiell biomasse og havre- og byggavling mellom 2011-14. Jordvolumetrisk vanninnhold var betydelig høyere og jordtetthet ble signifikant redusert med den høyere biokull mengde. Det var ingen signifikant effekt av biokull på jordaggregatstabilitet, porestørrelsesfordeling, penetrasjonsresistens, mikrobiell biomasse, basal respirasjon og bygg- og havreavling over 4 år. På samme felt på Ås, vurderte papir II virkningen av ny versus gammel biokull på N_2O og CH_4 fluks over to festsesonger. Ny biokull tilført med 31,5 t ha^{-1} reduserte N_2O signifikant med 80% i en enkelt fluksepisode høsten 2012, mens 2-årig biokull målte 41% mindre N_2O-flux sammenlignet med kontrollen i samme episode (ikke signifikant). I 2014 slapp ny biokull ut mer N_2O enn kontrollen, mens eldre biokull ikke var forskjellig. Imidlertid var størrelsen på N_2O-utslipp i 2014 2-6 ganger mindre enn i 2012 på grunn av mye tørrere vær. Nettoeffekten over 2 festsesonger var at ny biokull reduserte N_2O mer enn den stimulerte den. Analyse av eldre versus ny biokull viser at biokull mister sin alkalitet over 2-4 år, og dette kan forklare reduksjonen i N_2O-reduserende effekt fra eldre sammenlignet med ny biokull. I motsetning til tidligere studier, fant jeg ut at eldre biokull ikke reduserte kapasiteten på tilbakeholdelse av CH_4 i jorda, men i stedet hadde en større tilbakeholdelse enn kontrollen ved høye CH_4 utslipp. Ny biokull avvek ikke fra kontrollen når det gjaldt CH_4 -fluks. Akkumulertutslipp mellom behandlingene var ikke forskjellige i 2012 og 2014 på grunn av høyt standardavvik registrert innen behandlinger, noe som er vanlig i feltstudier ved bruk av lukkede kammermålinger. Flere høyfrekvente målinger ved bruk av automatisert kammer og måling over en hel feltsesong er anbefalt for å gi sikrest mulig resultat av effekten av biokull på klimagassreduksjonen fra jorda. I papir III dokumenterte vi transport og skjebnen til biokull 5 år etter at den ble påført. Vi målte vertikalt (0-60cm) og lateralt (9 m fra rutekanten)

transport av biokull i Ås-feltet. Etter 5 år gjenfant vi 92-107% ± 6 av påført biokull. Førtifem til syttito prosent av biokull ble målt i 0-23 cm ploglaget innenfor rutegrensene, 22-31% ble transportert nedover til 23-60 cm dybde, 0-21% beveget seg horisontalt innenfor 9 m av rutegrensen og 4% av biokull-C ble mineralisert som CO_2. Jeg fant ut at biokull som ble utskilt fra jordaggregater under forseglingstesten, men på grunn av den høye innsamlingsgrad som ble funnet i dette flate terrenget etter 5 år, konkluderte jeg at tap av biokull ved forsegling er sannsynligvis ikke en stor risikofaktor i dette jordsmonnet, særlig når biokullet er godt innarbeidet i jorda. Jeg anbefaler at fremtidige forsøk på å dokumentere lagrene av biokull C tar hensyn til den vertikale bevegelsen av biokull ved utforming av prøvetakingsstrategier. I den siste studien, Artikkel IV, samarbeidet vi med en kommersiell grønnsaksbonde for å teste den synergistiske gjødslingseffekt av biokull tilsatt som 20% og 40% (V/V) til flytende biorest fra biogass fabrikk. Vi påført blandingen i 7 cm dybde under planteradene til vårløk i en grov sandjord. Vi målte både mineral N og N_2O utslipp i første måned etter såing. Vårløk avling med biokull + biorest var 37% høyere enn kontrollbehandlingen med NPK-gjødsel, men ikke signifikant forskjellig. Biorest alene var ikke forskjellig fra kontrollen. Mineral-N økt betydelig ved 305% i behandlingen med biorest + biokull sammenlignet med kontrollen, mens biorest alene hadde ikke noen signifikant effekt. Jeg mener at innblanding av biokull med biorest bufrer tap av plantenæringsstoffer under spiringsperiode når vanning av sandjord er en nødvendighet for god spring. Biokull bidrar med retensjon av plantenæringsstoffer i sandjord og sørge for en mer balanse av NO3:NH4 til plantene sammenlignet med biorest uten biokull. I tillegg til fremlegging og diskusjon av egne funn og resultatene gir denne Ph.D. oppgaven leseren en grundig innføring i temaet om biokull effekt på agronomi og klimagasser og på slutten av avhandlingen peker jeg på nye forskningsspørsmål som kan tette kunnskapshull.

1 Introduction

Healthy soils are an essential requirement for our ability to grow food now and in the future and are also recognized as critical for maintaining biodiversity, reducing pollution, and mitigating and adapting to climate change (Paustian et al., 2007; Rojas et al., 2016; Smith et al., 2015). One of the key elements to sustainable soils is the management of soil organic matter (Lal, 2010). Organic matter provides multiple benefits to the functioning of soil including improving tilth, increasing soil water storage and availability, buffering changes in soil pH, increasing soil aggregate stability, supporting the energy needs of soil biota, and as a sink for atmospheric CO_2 (need multiple refs here). In the wake of climate change, the role that soil plays as a sink for atmospheric C is receiving increasing attention with the most notable example being the 4 per mille initiative launched in France in 2015. This initiative encourages participating countries to build or protect soil organic carbon at a rate of 4 per mille per year as a method for neutralizing anthropogenic greenhouse gas (GHG) emissions (Minasny et al., 2017) .

However, increasing or even maintaining SOC in cultivated soils is not an easy task. Results from a 30 year long term field trial at NIBIOs Apelsvoll field station show that only grain fields with at least 50% grass rotation were able to maintain original SOC levels from 30 years ago (Riley et al. in prep). A farmer would need to cycle ~9 t ha^{-1} yr^{-1} of materials such as plant residues, roots, manure etc. to maintain SOC levels and even more to increase it (ibid). With advancements in technology and techniques which enable *in situ* characterization of soil organic matter, it has been shown that the longevity of C in soil is not just due to the molecular structure of biomass and its biochemical stability (e.g. Lignin vs Sugar) but the complex biogeochemical conditions which interact with those molecules (Schmidt et al., 2011). This means that even a sugar molecule protected within a soil aggregate may reside longer in the soil than an unprotected lignin molecule, despite its lower biochemical stability (Grandy and Neff, 2008). The exception to this rule is pyrogenic C (i.e. charred biomass). The archeological record across multiple ecosystems show that pyrogenic C is the most refractory fraction present in soils and marine sediments and is dated from decades to thousands of years ago depending on fire severity and soil storage conditions (Schmidt and Noack, 2000). A global estimate averaged from 55 studies attributed ~14% of SOC as being of pyrogenic origin (Reisser et al., 2016) .

In the wake of climate change, soil scientists and environmentalists have proposed the idea of intentionally producing pyrogenic C in larger quantities and adding it to soil as a way to sequester greater amounts of biogenic C in the soil and thereby reduce equivalent amounts of atmospheric CO_2 (Lehmann et al., 2006). One of its early proponents, the late Dr. Peter Read, coined the term 'Biochar' at a conference in Exeter in 2005 (Read and Lermit, 2005). Biochar is defined as the solid product remaining from the heating of biomass under oxygen-depleted conditions and is made with the intention of improving soils and agronomic productivity and storing biogenic carbon (Lehmann and Joseph, 2015). Biochar has been extensively researched in the last 15 years with the aim to evaluate its efficacy to sequester soil carbon(Smith, 2016; Woolf et al., 2010), remediate polluted soil and water bodies (Ahmad et al., 2014) and improve agronomic productivity of soils (Jeffery et al., 2017). Conversion of biomass to biochar and its deposition in soil can sequester ~50% of the biomass-C which is a much higher stabilization ratio than when crop residues are burnt in the field (~ 3%) or via biological degradation of biomass (<10-25% after 5-10 years)(Lehmann et al., 2006). Biochar is produced by heating biomass in an O_2-free- or low atmosphere at temperatures > 370 ° C (Budai et al., 2016; Mašek et al., 2013). Pyrolysis transforms the molecular arrangement of biomass C into aromatic C structures that have high bonding strength and require a substantial enzymatic investment for bacteria and fungi to break down. For this reason, only a minor amounts of biochar is mineralized by soil biota and the majority remains in the soil as a long term C sink.

Research on the intentional use of biochar in agriculture started in Norway in 2009. NIBIO made preliminary assessments of the potential for biochar production and GHG mitigation in Norway based on available feedstocks of straw and forestry residues (Norwegian Environment Department, 2010). As a masters student at NMBU at the time, I cooperated with Dr. Daniel Rasse and Dr. Kathrin de Zarruk to conduct the first pot and laboratory experiments in 2008-2010, assessing the effects of wheat straw biochar on soil quality parameters and yield of perennial ryegrass (O'Toole et al. 2013). In 2010, I was employed at NIBIO and took responsibility for carrying out the first biochar field trial in Norway. The main purpose of the field trial was to assess the stability of biochar C under Norwegian conditions. We measured the mineralization of biochar C using natural abundance isotopic methods in 2011-2012 and confirmed that the biochar was indeed stable compared to unmodified biomass and we estimated a mean residence time in soil exceeding 100 years (Rasse et al., 2017).

Confirming the stability of biochar-C in soil was an important step towards assessing its potential towards mitigating GHGs in Norwegian agriculture; other questions are also important. What are the agronomic effects? and how does biochar affect other GHGs such as nitrous oxide (N_2O)? In other words, what are the overall agronomic and environmental effects of biochar?

1.1 Biochar: Is it safe to use?

In Norway, the Fertilizer Product Regulation [*Gjødselvareforskriften*] regulates the quality criteria for fertilizers and soil amendments from organic origin. Although biochar is not mentioned directly in the regulations, the text refers to "products from combustion", which could be interpreted to include biochar. The level of heavy metal content is the main criteria which determines whether, where and by how much a fertilizer or soil amendment from organic origin can be used. Special regulations apply for the use of biosolids from waste water treatment plants and biogas plants, and these we assume would also apply if biochar is produced from biosolids. Biochar made from construction waste wood has been shown to contain high levels of Zinc even after magnet separation of metals. This is due to the small zinc laden nails and staples which can be lodged inside wood chips (Sørmo et al., 2020). For agricultural purposes, feedstocks with low levels of heavy metals are required to produce clean biochar that is suitable for applying to food producing soils.

Polyaromatic hydrocarbons (PAH) are a potential contaminant in biochars and this should be analyzed before determining whether a particular biochar is suitable for application to agricultural soil. PAHs are a group of persistent organic contaminants produced from the incomplete combustion of fossil fuels or biomass. Source include industrial facilities, wood burning stoves and forest fires. Long term or high exposure to PAHs can increase the risk of cancer or birth defects. Biochar PAH content is influenced by a combination of factors including: feedstock type, feedstock moisture, reactor temperatures, pyrolysis type (batch vs continuous) (Hale et al., 2012; Rollinson, 2016). Biochar PAH content can be drastically reduced by increasing the carrier gas flow in the pyrolysis reactor (Buss et al., 2016) with the authors concluding that reactor design and its influence on solid/gas contact and reactions were important for PAH formation. There is no regulation on PAHs for biochar under Norwegian law, however the Norwegian pollution regulation [*forurensingsforskriften*] considers a soil "polluted" if it contains more than 2 mg kg^{-1} DM. Researchers and industry representatives have been proactive to develop voluntary industry standards for the

sustainable production of biochar. This is described in detail in the European biochar certificate (EBC, 2012), which sets strict voluntary standards for biochar depending on the intended use such as animal feed and soil amendment. The limit for PAH for agricultural biochar in the EBC is 6 mg kg-1 and for animal feed biochar the limit is 4 mg kg-1 (EBC, 2012) .

To put into context the risk for biochar to PAH accumulation we can consider how much biochar would be needed to take a "clean" soil and raise its PAH level until it was considered "polluted soil". The norm value for ∑16 PAHs in polluted soil under Norwegian regulation is 2 mg kg^{-1}. However, there is no purely "clean" soil. PAHs can travel long distances and can travel from heavy fossil fuel sites in Europe and are deposited on Norwegian territory. An assessment of background ∑16PAHs in Norwegian grassland soils found on average 0.06 mg kg-1 in grassland soils and 0.24 - 0.5 mg kg^{-1} in forests. We calculate that it would take 970 years of annual applications of biochar in amounts equivalent to that we applied in Paper IV (3 t ha^{-1})and 97 years of annual applications of amounts given in Paper 1:III (31.6 t ha^{-1}) before the soil was considered polluted with PAH laden biochar. And this is with the unrealistic assumption that the PAHs are not biologically degraded in that time. In other words if only clean biochar is used in agriculture as described in voluntary regulations such as the EBC, there is very little risk for undue PAH pollution of soil.

1.2 The agronomic effects of biochar

The following section gives a short introduction to the agronomic effects of biochar including effects on yield and soil physical, chemical and biological parameters. The reader should note that when we refer to biochar, it includes the pyrolysis of a wide variety of plant and animal based feedstocks, which results in products with a variety of physical strength, porosity, surface area, pH, nutrient content, etc. and therefore results may vary accordingly.

1.2.1 Biochar effects on soil physical parameters

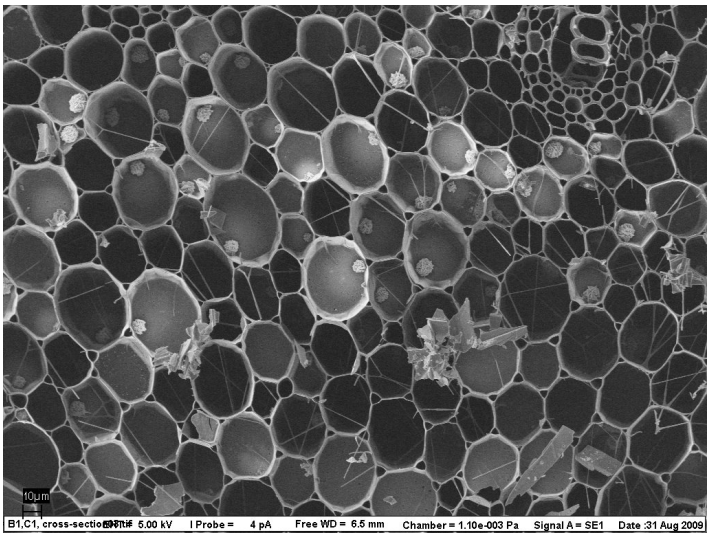

Fig. 1. A Scanning Electron Microscopy (SEM) image of a cross section of wheat straw biochar. The porosity is inherited from the cellular structure of the plant material biochar is made from. (Photo: Adam O'Toole, NIBIO)

Biochar is a highly porous material (Fig. 1), with low bulk density and can adsorb several times its own weight with water. The extensive body of biochar research conducted in the last decade shows that biochar can increase the plant available water in coarse-texture soils and increase the saturated hydraulic conductivity of heavy clay soils (Omondi et al., 2016a). For coarse sandy soils, the addition of biochar can increase plant available water via several mechanisms. Firstly, biochar particles smaller than sand grains can fill larger pore spaces. The smaller soil pores created by biochar can hold more water against gravity compared to larger pores between sand particles (Masiello et al., 2015). Secondly, larger (>1mm) and irregular shaped biochar particles can make the path of water through the soil more tortuous, thereby slowing down water percolation (Jing et al., 2017; Lim et al., 2016). Finally, water can fill macro pores of the biochar structure itself. These biochar macro-pores, when larger than ~5 µm, can hold plant available water (Brewer et al., 2014) or allow water to diffuse into surrounding soil during drying periods (Wang et al., 2019). However, high biochar application rates of ~10-20 t ha^{-1} are required before significant differences in plant available water are observed (Günal et al., 2018; Omondi et al., 2016b; Wang et al., 2019). For compacted and heavy clay soils, biochar can increase saturated hydraulic conductivity but requires high application rates (10% w/w or >100 t ha^{-1}) (Barnes et al., 2014; Wong et al., 2018).

Soil aggregation and structure

A soil that is well aggregated and structured facilitates O_2 diffusion to respiring plant roots and microbes, allows water to flow and be stored in pore spaces, and protects soil from erosion (Troeh and Thompson, 2005). Soil aggregation is fostered by the decomposition of organic matter by bacteria and fungi with their microbial products acting as binding agents for soil particles (Martin et al., 1955). Considering that biochar-C is mostly recalcitrant one would assume that biochar would not contribute to greater aggregate stability. However, a review of literature (34 studies) shows that biochar increased wet aggregate stability in 70% of studies (Blanco-Canqui, 2017). Biochar-C can form organo-mineral bonds with soil minerals and adsorb root exudates (Joseph et al., 2021) and biochar can thus form a "seed" for micro-aggregate development ((Han) Weng et al., 2017). Biochar can mobilize dissolved organic carbon (DOC) from acidic soils (Smebye et al., 2015) that may stimulate microbial activity and help with aggregate formation.

1.2.2 Biochar effects on soil chemical parameters

Biochar is an alkaline material due to the content of base cations K, Ca, Mg, and sometimes Si. Depending on the feedstock and pyrolysis temperature biochar will usually have a pH between 7 and 10 (Ippolito et al., 2015). Modifications of pH and electrical conductivity are the most notable effect of biochar on soil chemistry. The same elements that make biochar alkaline can also be a direct source of nutrients for plants, notably K, Ca, Mg, Si. Biochar also contains a moderate amount of P, and can even be a form of P fertilizer if made from animal bones (Vassilev et al., 2013) . Biochar made from plant materials is low in N, while biochar made from animal manures and biosolids have a higher content of N and P, but less than unpyrolyzed manure and biosolids.

Biochar effects on N cycling

Retention of water in sandy soil has benefits for a reduction in NO_3 leaching, which is a major source of N loss in agricultural landscapes and creates pollution problems for drinking water and marine ecosystems, including the Oslo fjord in Norway (Staalstrøm et al., 2021). A meta-analysis of 88 laboratory and field studies reported no overall change in soil NO_3 content but a 13% reduction in NO_3 leaching (Borchard et al., 2019). The meta- analysis found that biochar made from grass and agricultural residue feedstocks at temperatures >500 ◦C had the best effect on reducing NO_3 leaching (ibid). Biochar can increase nitrification rates which has been linked to its capacity to adsorb nitrification-inhibiting

phenolic compounds (DeLuca TH, MacKenzie MD, 2009) and stimulate the growth of a greater diversity of nitrifying bacteria (Ye et al., 2016). Stimulatory effects on nitrifying bacteria can be due to biochar ash content increasing soil pH towards alkaline conditions (7.8-8.5) which are optimal for nitrification (Antoniou et al., 1990; Havlin et al., 2005) and improved O_2 diffusion (Li et al., 2019). Alkaline biochar can lead to higher NH_3 volatization especially when added to acidic soils, but has also been shown to decrease NH_3 when applied together with organic fertilizers (Sha et al., 2019). Ageing of biochar results in a loss of its alkalinity (Spokas, 2013) so the effect on NH_3 volatization may be a short term effect. Also when biochar ages in soil or is included in composting its surface can be coated with organo-mineral plaque, which has been shown to improve biochar N retention capacity (Hagemann et al., 2017).

1.2.3 Biochar effects on soil biological parameters

The effect of biochar on soil biota has received less attention than other aspects, but is quickly becoming a research focus with the aim to understand mechanisms behind biochar modifications to nutrient cycling and greenhouse gas emission dynamics. A review of studies shows that, in most cases, microbial biomass increase is due to biochar additions, with significant alteration of microbial diversity and enzyme activity (Lehmann et al., 2011). Biochar pores are assumed to provide a habitat for microorganisms but studies so far show conflicting results when attempting to confirm or reject this hypothesis (Pietikäinen et al., 2000; Quilliam et al., 2013). A shift in microbial community composition and functioning towards a slower organic C turnover was found in China in a field where biochar was added 3 years earlier (Chen et al., 2017).

1.2.4 Biochar yield effects

Biochar is currently an expensive product[1] and therefore farmers want to know what the expected yield effects are before they purchase or invest in production equipment. Early biochar research was inspired by the fertility of biochar rich soils known as *Terra preta* found in the Amazon rainforest in Brazil. Research experiments have now been conducted on every continent in the last 10 years to verify the yield effects of biochar and impacts on

[1] Estimate market price of 8000 kr TS tonne^{-1} (Strøm Prestvik and Lilleby, 2021)

soil quality parameters. The most recent meta-analysis conducted on biochar yield effects shows that biochar boosts yields in tropical soils but has no consistent effect in temperate soils (Jeffery et al., 2017). Strategic use of biochar combined with fertilizer in maize planting holes increased yield by 3-4 fold in low-CEC and acidic sandy soil in tropical climate in Zambia, although the same biochar did not improve yield in fertile silty clay and loams (Cornelissen et al., 2013). Increases in plant available water and increases in base saturation were considered the reasons for yield increases (ibid). In long term field trial in Kenya, application of 50 t ha^{-1} acacia derived biochar led to a persistent annual yield increase of 1.2 Mg ha^{-1} for maize and 0.4 Mg for soybean (Kätterer et al., 2019). Increases in N use efficiency, water holding capacity, and possible stimulation of N fixation due to extra biochar derived Mo were reasons given to explain yield benefits (ibid).

In Mediterranean and temperate soils the yields effects of biochar have been mixed. In Italy, biochar application increased yield of durum wheat by 30% over 2 years (Vaccari et al., 2011) and in Wales hay grass responded positively during the last two years of a 3-year trial (Jones et al., 2012). In the Italian study, yield increases were attributed to faster soil warming due to biochar, which allowed for earlier germination and crop development. In the Welsh study, yield increase was attributed to increased water holding capacity from the biochar during a dry spring planting season. Other trials in temperate regions have found no significant differences. A two year European ring trial carried out in 7 different countries in Northern Europe (which we participated in) found that application of a wood biochar at 20 t ha^{-1} had no significant effect on grain crop yields over two years (Ruysschaert et al., 2016). In general, biochar is expected to increase agricultural productivity where it can amend a limiting factor to plant growth e.g. alleviating water and nutrient deficiencies, correcting pH levels or reducing the availability of toxic elements etc. The extent to which biochar can do this depends on the type of biochar and the precise conditions of the plant-soil system it is being added to.

1.3 Biochar impact on GHG emissions

One of the main arguments against strategies to increase soil organic carbon as a climate measure is a potential trade-off effect with increasing emissions of N_2O (Guenet et al., 2021). One of the key arguments for the use of biochar is that this tradeoff does not exist and in fact one can expect reductions in N_2O emissions (Guénet et al, 2021), as now explained.

1.3.1 Effects of biochar on soil N_2O emissions

The first meta-analysis on biochar N_2O effects found an average reduction in N_2Oemissions across studies of 49%, but these studies were mostly at laboratory scale and with high application rates (Cayuela et al., 2014). A more recent meta-analysis considering only field studies, showed that biochar reduced N_2O emissions by 9.2% (Verhoeven et al., 2017). Biochar reduces N_2O emissions via multiple biotic and abiotic mechanisms including: adsorption of N_2O in biochar pores (Oleszczuk et al., 2012; Cornelissen et al., 2013), abiotic redox reactions (Quin et al., 2015), improved metabolic conversion of N_2O into N_2 as biochar increases pH (Bakken et al., 2012; Weldon et al., 2019), and abiotic/biotic synergies that hold N_2O transiently in biochar pores giving more time for denitrifiers to reduce N_2O to N_2 (Harter et al., 2016). While there is enough data available to conclude that biochar can mitigate N_2O in most cases (Schmidt et al., 2021), there is a lack of research on how long the mitigating effect will endure. The N_2O mitigation effect of biochar appears significantly reduced in studies longer than 120 days (Borchard et al. 2019). Meta-analyses of N_2O mitigation also report lower biochar effect in field studies alone (-10.6% , Verhouven et al. 2017) as compared to field and laboratory incubations combined (-54%, Cayuela et al. 2014). This can be due to field trial artefacts and heterogeneity of biochar distribution at field (Kammann et al. 2017), but may also be due to biochar weathering and interactions with soil minerals and organic matter.

Nitrification is a less potent source of N_2O than denitrification (Bakken et al., 2012), however still a consistent source over a wide soil moisture range in most aerobic soils. There is disagreement in the literature regarding to which extent biochar inhibits or stimulates nitrification rates, and nitrification mediated N_2O emissions (Verhoeven et al., 2017; Wells and Baggs, 2014) with evidence on both sides for increased and decreased N_2O . Sánchez-García et al. (2014) showed that the dominant soil microbial community in a given pedo-climatic soil strongly influences the dominant N_2O pathway (denitrification vs nitrification) and that a given biochar can both increase and decrease N_2O emissions depending on the soil type where it is applied.

1.3.2 Biochar effects on CH_4 emissions and uptake

Compared to N_2O, the effects of biochar on methane emissions and uptake are more uncertain. This area of research was identified as a knowledge gap in a recent and still to be published report for the EJP-Soil financed SOMMIT project, which I co-authored. As

described in the report, I identified three meta-analyses Jeffery et al. (2016) (42 studies), Cong et al. (2018) (40 studies), and Ji et al. (2018) (61 studies) dealing with the effect of biochar on CH_4 emissions and uptake in soil. In Jeffery et al. (2014), biochar was found on average to reduce CH_4 emissions from flooded paddy and acidic soils, while reducing the CH_4 sink capacity of neutral and non-flooded soils. Biochar was more likely to reduce CH_4 emissions in studies where N application was >120 kg ha^{-1}. This is related to the inhibition effect of N fertilization on methanotrophic bacteria population (J. Chen et al., 2021). However, in the Cong et al. (2018) meta-analysis, a stricter criterion was placed on the non-independence of multiple biochar treatments within individual studies and this resulted in no effect of biochar on CH_4 flux in either paddy or upland soils. In contrast, the Ji et al., 2018 meta-analysis found a 12% and 72% reduction in CH_4 emissions in paddy and upland soils, but a 84% reduction in CH_4 uptake in upland soils. Given the predominance of upland soils in Europe, the potential reduction in CH_4 uptake due to biochar is a cause for concern and warrants more research on biochar effects on CH4 uptake dynamics (Ji et al. 2018).

2 Objectives and aims

The general objective of the thesis was to assess the agronomic impact of biochar under field conditions and assess effects on non-CO_2 emissions (N_2O and CH_4). I focused on this aspect of biochar, because realizing the potential of biochar C sequestration will likely depend on its ability to improve soil conditions or at least do no harm. I did not focus on the potential soil contamination risks of using biochar e.g. PAH content, as this had been well documented by other Norwegian researchers (Hale et al., 2012). However, all biochar used in the field studies were screened for heavy metal content in order to make sure it was within the legal limits defined for bio-based soil amendments under the Norwegian fertilizer regulation [*gjødselvareforskriften]*.

In 2010 when the field trial was set up, our first questions were centered on the stability of biochar C, as there was still some contention about how stable it actually was and to what extent one could measure the stability of biochar carbon. The results from that work were published in Rasse et al. 2017, which is not included in the present thesis. However, I co-authored the work, as well as conducted the field CO_2 and ^{13}C measurements. In this Ph.D thesis, I focus on the agronomic effects and environmental effects beyond carbon sequestration. Therefore, in **Paper-I the objective** was to investigate both the soil quality aspects (including microbial biomass, and soil physical measurements) and plant yields over 4 years. Due to the high surface area and porosity of the miscanthus biochar and the relatively high amounts that were applied, **we hypothesized in Paper I** that biochar could improve soil water retention and alleviate short-term soil water deficits as are common in early summer in Norway. This we proposed would indirectly lead to increased plant growth and crop yield. **Furthermore, we hypothesized** that the relative stability of the biochar carbon would mean that relatively large amounts of biochar could be added to improve soil physical conditions, without leading to microbial N-immobilization, which is usually the case with addition of high C:N organic materials.

After confirming the stability of the biochar C under field conditions (Rasse et al. 2017), our next question was whether there were also additional benefits in reducing N_2O. By 2012, a number of studies had already been published, showing that biochar could reduce N_2O emissions (Bruun et al., 2011; Case et al., 2012; van Zwieten et al., 2010). Our question was

how long this effect would last. Therefore, the next experiment described in **Paper II had the objective** to compare N_2O and CH_4 flux from soil where new biochar was applied in 2012 and 2014 (BC-New) and compared this with GHGs from soil where biochar was applied in 2010 (BC-Aged). **In Paper II, we hypothesized** that BC-New would mitigate N_2O more than BC-Aged. Methane was also measured but we did not have a clear hypothesis for CH_4 at the outset. However, the data can serve as a basis for developing new hypotheses for future research.

After 5 years in 2015, the interest surrounding biochar and its role as a climate change solution were growing, and Norwegian agricultural and environmental authorities were taking a greater interest in financing research and supporting demonstration projects. Our question at this point was how biochar would be accounted for in an eventual soil C sequestration program where farmers are rewarded for storing biochar-C in soil. Here, the fate of biochar physically in the soil becomes an important consideration, because one needs to know whether changes in biochar content in soil samples over time are due to C mineralization or physical transport. Thus, the **objective of Paper III** was to document the vertical and lateral transport of biochar 5 years after application. We pursued this study as an exploratory study and there were no hypotheses at the outset.

Parallel to our scientific investigations a small number of farmers were taking interest in the use of biochar. Two of these were Bjørge Madsen and Kristen Stenersen from Skjærgaarden gartneri in Vestfold. Skjærgaarden grow vegetables on irrigated sandy soils in Vestfold, and see the potential in using biochar to improve soil quality, reduce irrigation demand, and become less reliant on fertilizers. I was able to include them in a research project "Capture +" and we cooperated in order to install the first biochar pilot plant on their farm in 2017. In 2018 we followed up with a field trial to test innovative methods for combining biochar in two doses together with biogas digestate from a nearby biogas plant in Tønsberg ('*Den Magiske Fabrikken*'). The results from the field trial in 2018 are reported in Paper IV. **The objective of Paper IV** was to investigate whether biochar-AD mixture could be a sustainable fertilizer solution for spring onion production in a sandy soil in Norway. **In Paper IV we hypothesized** that AD could substitute NPK as a basal fertilizer for spring onions and the addition of biochar to AD would improve the retention and N use efficiency of AD-derived N, leading to increased plant yield and a reduction in soil N_2O emissions.

3 Materials and Methods

In this section, I present the experimental sites, and summarize the main methods used. More detail is given on each method in the respective papers.

3.1 Study sites and experimental designs

Fig. 2. Study site 1: NMBU field station, Ås, Norway.

Study site 1 – Ås.

The study site for Papers I, II, and III was located at NMBUs field station, Ås, Norway (Fig. 2). The soil type is a silty clay loam Albeluvisol (WRB classification) with an average content of 27% clay, 43% silt and 30% sand.

The experiment was set up in a randomized block design with 4 treatments and 4 replicated plots (32 m^2). The treatments were:

1. Control – No biochar
2. 8 t C ha^{-1} Micanthus Straw (unpyrolyzed) (MC8)
3. 8 t C ha^{-1} Micanthus Biochar (BC8)
   (Equivalent to 11.6 t biochar ha^{-1} or 0.40% w/w soil)
4. 25 t C ha^{-1} Miscanthus Biochar (BC25)
   (Equivalent to 31.5 t biochar ha^{-1} or 1.12% w/w soil)

The experiment was designed to assess the carbon stability of biochar under field conditions and therefore treatments were dosed according to C content. The stability of the biochar C is

not one of the research questions of this thesis but the results are discussed later and available in Rasse et al 2017.

For Paper 2, where we assessed N_2O and CH_4 effects, new biochar plots were added adjacent to the original field and one plot was added per block in 2012 and 2014 (Fig. X). Treatments tested in Paper II were:

1. Control – No biochar
2. BC-Aged (which is the same as 'BC25' treatment in Paper I)
3. BC-New – New biochar applied at the same application rate as BC-Aged in 2012 and 2014 (Fig. 3A and B)

For Paper III, we assess the fate of biochar vertically and laterally in the soil. Here we refer to "BC8" as "BC-Low" and "BC25" as "BC-High". The different terminology used for the same treatments was done to better reflect on the research questions of each study.

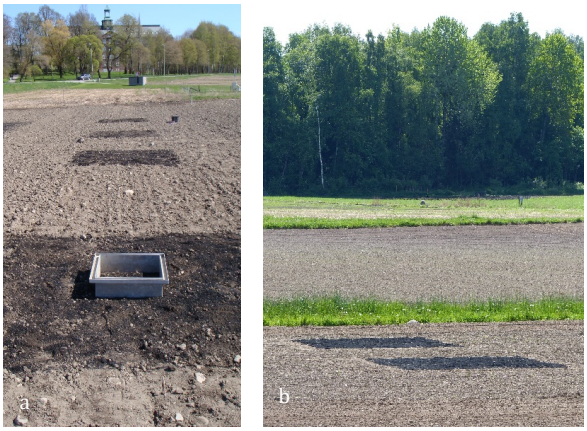

Fig. 3. New biochar plots added in (a) 2012 and (b) 2014 for assessing N_2O effects (Paper II). The pictures are taken just before the biochar was rototilled into the soil.

Study site 2 - Åsgårdstrand [Paper IV]

Fig. 4. Study site for Paper IV was a commercial vegetable farm 'Skjærgaarden', located at Åsgårdstrand in Vestfold County, Norway.

The field experiment for Paper IV was conducted on a commercial vegetable farm 'Skjærgaarden' in Vestfold county, Norway (59°21′14″N, 10°26′51″E) (Fig. 4). The farm was the first in Norway to purchase a biochar reactor and aims to be more sustainable, notably by substituting chemical fertilizers with organic alternatives, such as biogas digestate, compost, and biochar.

The experimental design was a randomized complete block design with the following 4 treatments arranged in 4 blocks:

1. Control-NPK: Standard mineral fertilization
2. AD: Anaerobic digestate liquid slurry
3. AD + BC-Low: A mixture AD and 20% V/V biochar
4. AD + BC-High: A mixture of AD and 40% V/V biochar

Further details on N application rate and biochar mass concentrations are described in Paper IV.

3.2 Biochar production and properties

There was no commercial biochar plant in operation in Norway when our field trials were set up and therefore we imported biochar for both field studies. In Ås, the biochar was produced from *Miscanthus giganteous* straw and at Skjærgaarden the biochar was made from a mix of wood chips, mostly spruce. Both were made in Germany in a Pyreg™ slow

pyrolysis auger reactor. The highest heating point of a Pyreg reactor was reported by the biochar contractor to be between 500-750 °C. The biochar was cooled and moistened with water and arrived on site with approximately 25-35% moisture content. For Paper II, a second batch of miscanthus biochar was sourced from the same supplier in 2014, to compare the effects of field aged vs new applications of biochar on N_2O emissions. A description of the physio-chemical properties of the biochars are given in Table 1.

3.3 Soil sampling and biochar addition to soil [Paper I:IV]

For soil sampling to determine soil chemical properties, a 2 cm D soil auger was used to take 10 sub samples per plot in the plough layer. Bulk density (BD) was measured in 2012 and 2014 with 4 x 100 cm^3 metal rings. Intact 250 cm^3 soil cores were used for determining the water retention curve via the evaporation method. When applying biochar to the soil, the moisture content was measured and preliminary tests were conducted to measure the bulk density of biochar for dosing purposes. With knowledge of the bulk density and its moisture content, biochar was applied to field plots at the desired rate (Fig. 5), and spread evenly with a rake before being tilled in.

Fig. 5 Miscanthus biochar and Miscanthus chipped straw was dosed out according to its C content to allow for comparison of C mineralization rates

The reader should note that for Paper II where we established new plots. Though the same amount of biochar was applied, the distribution of the biochar in the new plots was more

concentrated in the top 10-15cm (Fig. 6), compared to the original biochar which had more years and tillage events to mix it into the topsoil layer (0-23 cm)

Fig. 6. Incorporation depth of biochar in new plots that were added in 2014. Biochar was highly concentrated in the top 15 cm of soil.

For paper IV where biochar was applied as part of a digestate slurry, biochar was mixed thoroughly with digestate with a mechanical mixer for 8 hours (Fig. 7a). This allowed me to mix the biochar homogenously with the slurry. In Ås, the amount applied was approximately 30 t ha^{-1} whereas in Åsgårdstrand the mixtures of 20 and 40% (v/v) biochar in digestate were equivalent to pure biochar application rates of 1.5 and 3 t ha^{-1} respectively. Liquid mixtures of AD and biochar were banded manually using watering cans in 7 cm deep furrows (Fig.7b). A rake was used to immediately cover the AD fertilizers with soil to minimize ammonia volatilization. After one week, spring onion seeds were sown in four lines per planting bed (1.5 m D) located directly over the banded AD treatments. More details on the method is described in Paper IV.

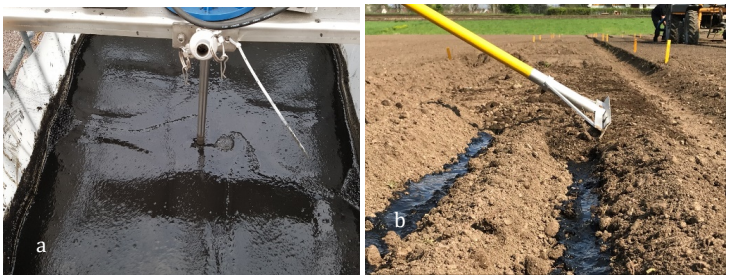

Fig. 7 (a) Mixing of biochar and biogas digestate in Paper IV and (b) application of Biochar-Digestate 40% mix applied 7 cm under spring onion rows one week before sowing in spring 2018.

3.4 Retrieving and quantifying biochar in soil

In Paper IV, biochar in soil was quantified via the $\delta^{13}C$ method, where the $\delta^{13}C$ signal of biochar derived from C4 photosynthesizing miscanthus is analytically discernible from the $\delta^{13}C$ of the predominately C3 plant derived native soil organic carbon (SOC). The concentration and ^{13}C isotopic composition of soil C were measured by combusting 6-20 mg of prepared soil in a combustion module CHN Elemental Analyzer, VARIO model, coupled to an IRMS, PRECISION model (Elementar Analysensysteme GmbH, DE). The following mixing model was used to calculate the proportion of soil C derived from biochar:

$$\text{biochar derived C (\%)} = \frac{\delta^{13}C_{Measured} - \delta^{13}C_{soil/}}{\delta^{13}C_{biochar} - \delta^{13}C_{soil}} x\ 100 \qquad 1)$$

The following equation was used to calculate biochar concentration in soil samples:

$$Biochar\ concentration\ (g\ kg^{-1} soil) = A * B * (1/C) \qquad 2)$$

A= $soil\ C\ (g\ kg^{-1} soil)$

B= $Biochar\ derived\ C\ (\%)\ (Eq. 1)$

C= $Fraction\ of\ C\ in\ biochar$ determined by dry combustion method

*Biochar recovered per sample depth (g m^{-2}) = Eq.2 * soil mass per sample depth (kg m^{-2})* (3)

3.5 Soil physical methods

Aggregate stability tests (Paper I)

Aggregate size distribution was determined by dry sieving 2.5 L of air-dried soil for 3 min using a mechanical sieving apparatus. Wet sieving was performed on aggregates from the 2–6 mm size class and by using a wet sieving apparatus (Eijkelkamp, Giesbeek, The Netherlands) we followed the method described by Kemper and Rosenau, 1986. After wet sieving, aggregates remaining on the sieve were dried, weighed, and then passed through a

set of nested sieves to determine aggregate size classes and Mean Weight Diameter (MWD). To elucidate how biochar containing aggregates respond to different aggregate breakdown mechanisms, we subjected aggregates to following three stress test methods as described by Le Bissonnais, 1996; fast wetting, slow wetting, and shaking. Fast wetting of aggregates simulates the compression of trapped air (slaking), slow wetting simulates the process of differential clay swelling, and the shaking test simulates the process of mechanical breakdown by raindrop impact and physio-chemical dispersion. To evaluate results from all aggregate tests, a comparison was made with an MWD stability index developed by Le Bissonnais, 1996.

Soil water content – Time domain reflectance (TDR) sensors

Soil moisture content was measured every hour in the growth seasons of 2012 and 2014 using Time Domain Reflectance (TDR) soil moisture sensors (5TM model, Decagon Devices, [now Meter]). One TDR was inserted horizontally in undisturbed soil at 5 cm and 15 cm depth of each plot. The TDRs were calibrated in the lab using dried soil repacked in 1 L containers with and without biochar at known gravimetric and volumetric water contents.

Soil water retention curve – evaporation method

Soil water retention characteristics (h) were measured between pF 1–3.2 with the evaporation method and by using a Ku-pF apparatus (Umwelt-Geräte-Technik GmbH, Müncheberg, Germany). More details on this method are described in Paper I.

Soil penetration resistance

In Paper I, soil penetration resistance (PR) was measured in October 2015 using an electronic penetrometer (2 cm^{-2} cone tip) that continuously logs depth and soil resistance upon probe insertion (Eijkelkamp Soil and Water, NL). Ten measurements were conducted per plot (5 between tractor wheel tracks and 5 within tractor wheel tracks) to assess to what extent biochar moderated soil compaction.

3.6 Soil chemistry methods

Mineral Nitrogen and pH

For soil NH_4 and NO_3, 10-15 g of field moist soil was extracted with 2M KCl and immediately frozen until they were ready to be sent to the lab, where concentrations were determined using an automated segmented flow analyzer (Seal Analytical Ltd, UK). Plant-available P, Ca, K, and Mg in both soil and biochar were measured by Eurofins Environment Testing Norway AS (NO) using the Egners AL (ammonium lactate) method (Egner et al., 1960).

Soil greenhouse gas (GHG) measurements

Soil GHG measurements were taken only during the growing season in Paper II and IV as the purpose of the measurements was to assess in-between treatment effects on GHG and not to monitor annual emissions. The static chamber method was used (Fig. 8a) according to Pumpanen et al., 2004 and we followed the same methods for measuring GHG field emissions which has been carried out via the nitrogen group at NMBU in several studies. These are described in detail in Paper II, IV. The flux calculation methods used in this study are described in more detail by Nadeem et al., 2012. Soil pore GHGs were also measured at 10 and 20 cm depth (Fig. 8b). This was done by inserting a PVC pipe into the soil, which had a sampling point at the end of it connected to a Teflon tube that was accessible from the surface. A syringe was used to draw gas samples from the tubes and then inserted into evacuated vials.

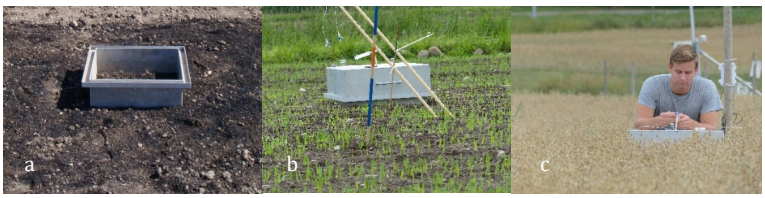

Fig 8. (a) Closed chambers and (b) soil gas probes used for taking (c) GHG measurements

Scanning Electron Microscopy – Energy Dispersive X-ray spectroscopy (SEM-EDX)

Surface morphology and changes in surface elemental content in new and aged biochar particles were carried via Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM–EDX). The equipment used was a Zeiss EVO—50—EP equipped with X-ray Type INCA 450 Xstream/Mic located at NMBU imaging center. Three crushed and 3

intact particles were analyzed from both new and aged biochar. Surface chemical analysis using X-ray photoelectron spectroscopy was conducted by co-authors Pr. Stephen Joseph and Dr. Sara Taherymoosavi at the University of NSW, Australia and is described in detail in (Taherymoosavi et al., 2017)

3.7 Microbial analysis

Soil microbial analysis in Paper 1 was conducted by co-authors Monique Carnol, Bernard Bosman from the University of Liège, in Belgium. The methods they used for each measurement are described as follows: Soil microbial biomass C (Cmic) was determined by the chloroform fumigation extraction method (Beck et al. 1997, Vance, Brookes, and Jenkinson 1987). Soil microbial biomass-C was calculated by dividing the difference of total extract between fumigated and non-fumigated samples with a Cmic extraction efficiency factor of 0.45 (Sparling and West 1988). Respiration potential (Robertson et al. 1999) was measured as CO2 accumulation in the headspace (250 ml) of an amber bottle (Supelco, USA) from 20 g fresh soil, at 15 °C in the dark after an overnight pre-incubation. For more details regarding microbial analysis refer to Paper I methods.

3.8 Statistical methods

Statistical analyses for all four papers were carried out using R software (R Core Team 2020) and SigmaPlot v.13 for repeated measures analysis in Paper I. The general approach taken with statistical analysis was to first to first make a visual assessment of the distribution of the data using histograms and boxplots. Here it can be determined the influence of outliers, and whether the data is skewed and general trends in the data. ANOVA was used to assess differences in treatment means with statistical significance set at $\alpha=0.05$. Post-hoc multiple comparison of treatments was done via the Tukey test, if comparison between all groups were of interest. If comparison with the Control treatment was more relevant, it was done via the Dunnett's test. For Paper II and IV the cumulative GHG emissions were calculated by linear interpolation between flux measurements. For measurements where repeated measures were taken e.g. N_2O, soil NO3, I transitioned after Paper 1 from using repeated measures ANOVA in Sigmaplot to the use of mixed models in R. Mixed models have the advantage over repeated measures ANOVA that they tolerate unbalanced data sets and missing values. They are also able to separate the influence of independent variables of interest e.g. Treatment from random variables of non-interest e.g. Plot, block. Both linear models and repeated measure ANOVA assume that model residuals

are normally distributed. The models were built in a stepwise manner to test explanatory strength of independent variables. Candidate models were selected according to the lowest corrected Akaike information criteria (AICc) and a ΔAICc <2 (Burnham and Anderson, 2002). For all models, post-hoc tests using QQ plots and the Shapiro-Wilk test were conducted to assess normality of model residuals. Where outliers affected residual normality, outlier detection and removal was conducted iteratively with the Grubb's test (Gubbs, 1950). In cases where we had non-normal data such as N_2O we employed a generalized linear mixed effects model (GzLMM). More information on the specific statistical method and R packages used for each measurement are given in the individual papers.

4 Main Results and Discussion

4.1 Effects of biochar on soil physio-chemical properties (Paper I, II, III)

In the Ås experiment, we wanted to find out if there were any soil improving and yield benefits from applying biochar that would potentially motivate farmers to use it. At the time (2010) there was a high level of optimism about the agronomic potential of biochar, also in well managed fertile soils such as in Norway and Northern Europe. Therefore, we measured a number of soil physio-chemical parameters (Table 1). The influence of biochar on soil physical properties as described in Paper 1 had also ramifications on GHG impact (Paper II) and the extent to which biochar is mobilized and transported in the soil (Paper III). We did not assess soil physical changes due to biochar in the Skjærgaarden experiment (Paper IV) because biochar was used in much smaller amounts and our experience to that date suggested differences would not be detectable. In the Ås experiment we observed with the use of high frequency TDR sensors that the higher dose of biochar (BC25) increased soil water content at compared to the control in both 2012 and 2014 (Fig. 9). To investigate this effect in more detail we took 4 intact 250 cm^3 soil cores from each treatment and measured water retention characteristics via the evaporation method (Schindler, 1980). This analysis revealed that BC25 was able to store greater amounts of water when the soil was saturated (Fig. 10), which we hypothesize is due to water filling in macro pores created by the irregular shaped biochar particles altering the packing arrangement of mineral soil particles, as demonstrated by Liu et al., 2017 in a controlled experiment. Biochar additions did not significantly influence the amount of plant available water. Water retention at field capacity and at soil saturation was significantly positively correlated with SOM content and significantly negatively correlated with bulk density (Paper I). Biochar was not present in the same amount in each intact core due to the heterogeneous distribution of biochar in field soil. This was confirmed at our field site by colleagues in a short study where they mapped 2D biochar distribution on a planar surface of a soil monolith via hyperspectral analysis (Burud et al., 2016).

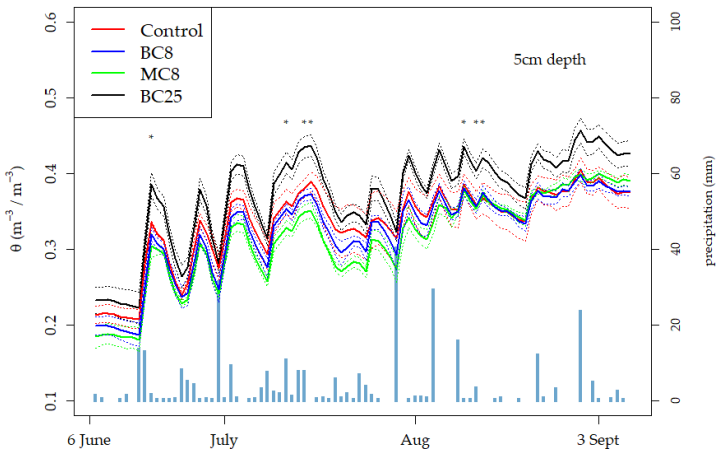

Fig. 9. Soil volumetric water content (VWC) in 2012 at 5 cm depth. Solid lines depict treatment mean and dotted lines standard error. Asterix indicates days when BC25 only (in black) was significantly higher VWC than the Control (from Paper I) BC25 = Biochar 25 t ha^{-1}, BC8= Biochar 8 t C ha^{-1}, MC8= Miscanthus straw 8 t C ha^{-1}, Control – soil without organic amendments

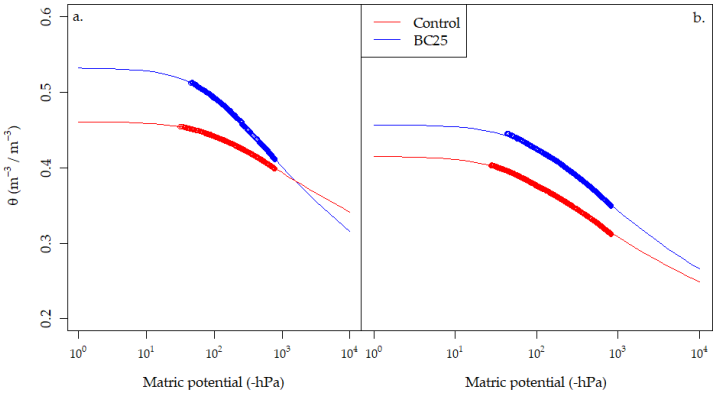

Fig. 10. Soil water retention curve from selected intact cores with (a) high SOM (5.27-7.9%) and (b) moderate (5%) SOM content. Dots are measured values via evaporation method and lines are model fits to the van genuchten-maulem model (from Paper I). BC25 = Biochar 25 t ha^{-1}, Control = soil without organic amendments

Bulk density is a soil property which has relevance for all of the topics covered in the present thesis with respect to agronomy (Paper I), GHGs (Paper II), and the mobility of biochar (Paper III). In 2014 bulk density was significantly reduced by 7% in BC25 (1.21 g cm-3) compared to the control (1.30 g cm^{-3}). This finding agrees with meta-analysis by

Omondi et al., 2016 where across studies, biochar on average, reduced bulk density by 7.6%. Bulk density in the lower biochar dose BC8 (1.29 g cm^{-3}) did not differ from the control. Penetration resistance (PR) is a proxy indicator for a plants ability to penetrate the soil matrix and agronomists use it to assess soil compaction. The results did not confirm our hypothesis that the lower bulk density in BC25 would translate to a reduction in soil penetration resistance (PR) in the silty clay loam. The soil was not excessively compacted with PR values of <2 MPa recorded across the field, which has been observed as a critical limit for root growth in soil (Martino and Shaykewich, 1994), and thus compaction was unlikely to be a growth limiting factor in this soil.

The aggregation of soil particles is important for structural stability, resistance to erosion and soil sealing/crusting. Improved aggregation fosters water infiltration into the soil and better soil aeration, which is essential for root extension, nutrient accessibility and respiration (Nimmo and Perkins, 2002). In Paper I, I assessed aggregate stability as the percentage of water stable 2-6 mm aggregates remaining after 2 minutes of wet sieving. The treatments BC8, MC8, or BC25 recorded higher water stable aggregates (%) than the control but the difference was not statistically significant (Fig. 11)

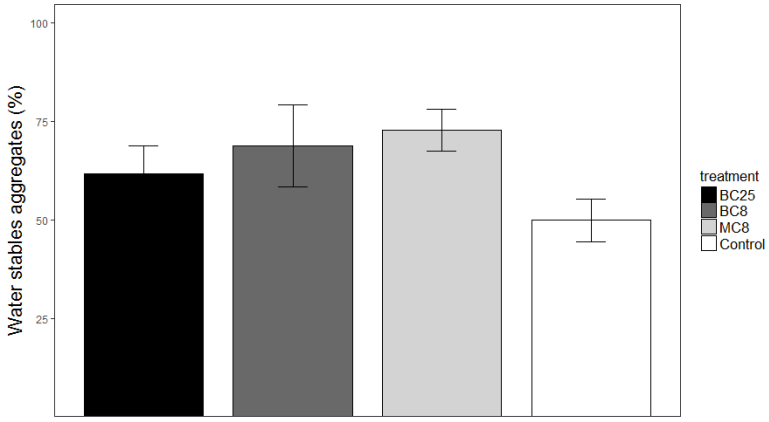

Fig. 11. Percentage of water stable aggregates in 2-6mm left on the sieve after wet sieving. Error bars indicate standard error, n=4. There were no significant differences between treatments (from Paper I). BC25 = Biochar 25 t ha^{-1}, BC8= Biochar 8 t C ha^{-1}, MC8= Miscanthus straw 8 t C ha^{-1}, Control – soil without organic amendments

Table 1. Biochar effects on soil physical and chemical properties in Ås experiment (Paper II). Soil pH, NO_3 and NH4 values are averages of multiple measurements taken throughout the season.

Year	Treatment	Bulk density (g cm-3)	Soil Porosity (%)	Biochar pH (H_2O)	Soil pH (H_2O)	Soil NO_3-N (mg kg^{-1})	Soil NH_4-N (mg kg^{-1})
2010	BC-Aged	-	-	10	-	-	-
2012	Control	1.30	49.05	-	6.3 ±0.2[a]	8.1 ± 7.3[a]	1.5 ± 2.7[a]
	BC-Aged	1.22		7.86 *	6.4 ± 0.1[a]	9.5 ± 6.9[a]	0.7 ±0.3[a]
	BC-New	-			6.4 ± 0.2[a]	6.6 ± 7.8[a]	1.2 ± 1.3[a]
2014	Control	1.32	49.81	-	6.1 ± 0.2[a]	27 ± 15[a]	5.4 ± 6.4[a]
	BC-Aged	1.23	53.94	5.5	5.9 ± 0.2[a]	38 ± 7[a]	8.4 ± 8.9[a]
	BC-New	1.17	51.31	8.75	5.9 ± 0.3[a]	40 ± 23[a]	5.8± 5.5[a]

(Mean ± Standard Deviation, n=3 in 2012, n=4 in 2014), * BC-Aged pH from stored sample

Microbial biomass C and N and basal respiration were significantly higher in MC8 (straw) compared to the control, whereas neither BC8 nor BC25 differed from the Control. This confirmed our hypothesis in Paper I that the microbes did not appear to use biochar C as a substrate in any significant amount. As the growth of the microbes was not fostered by biochar, the microbes did not require more N to grow, and therefore biochar addition did not lead to N immobilization. Surprisingly the increased microbial C and N immobilization in the MC8 straw treatment did not negatively affect grain yield over 4 years. Also, the fact that MC8 had slightly higher aggregate stability (Fig. 10) than the biochar treatments is probably a result of the increased microbial activity. Other studies have reported that biochar application can stimulate microbial activity in soil, however this happened at high application rates for example >5% (w/w soil) (Gomez et al., 2014; Kolb et al., 2009), suggesting that large amounts of biochar are needed to compensate for the low fraction of labile carbon.

In the Skjærgaarden experiment (Paper IV), we applied biochar in much lower amounts per hectare (1.5-3 t ha^{-1}) compared to the Ås experiment (11 and 31 t ha^{-1}). However, the Skjærgaarden experiment involved applying biochar and digestate together in concentrated bands directly in the root zone. This resulted in high concentration of biochar in the

rhizosphere, which may explain why we observed higher NO_3 soil concentrations (indicative of higher nitrification rates) in the Skjærgaarden trial (Fig. 12) but not significant treatment difference in N_2O in the Ås experiment. In order to maximize agronomic benefits, it is now commonly recommended to add biochar to either composts or mix it with N rich organics such as digestate, manure, or urine (Kammann et al., 2016) and our results in the Skjærgaarden trial support this view.

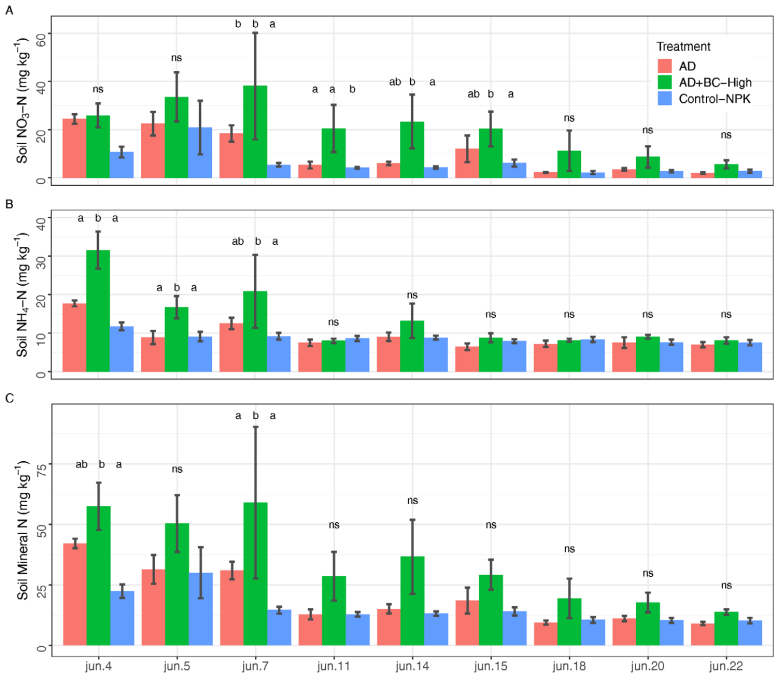

Fig. 12. A. Soil NO_3-N B. Soil NH_4-N and C. Soil Mineral-N levels at the start of the growing season on the days when N_2O measurements were taken. Standard error bars for mean values indicated (from Paper IV – Skærgaarden spring onion experiment). AD= Anaerobic digestate, AD+BC-High = Anaerobic Digestate mixed with biochar at 40% V/V, AD+BC-Low = Anaerobic digestate mixed with biochar at 20% V/V. Different letters above bars indicate statistical significance ($p<0.05$) between treatments at each date, ns = not significant.

4.2 Yield effects of biochar in temperate region agricultural soils

In the Ås experiment (Paper 1) neither BC8, BC25, nor MC8 had significant influence over barley and oat yields over four cropping seasons 2011-2014 (Fig. 13). Even in 2014 when conditions were drier than normal, our hypothesis that "*increased soil water holding capacity [due to biochar] would lead indirectly to increased yields*" was not supported. In that

year, oat yield was less than half of what is normal, and there were no differences between treatments (Fig. 13).

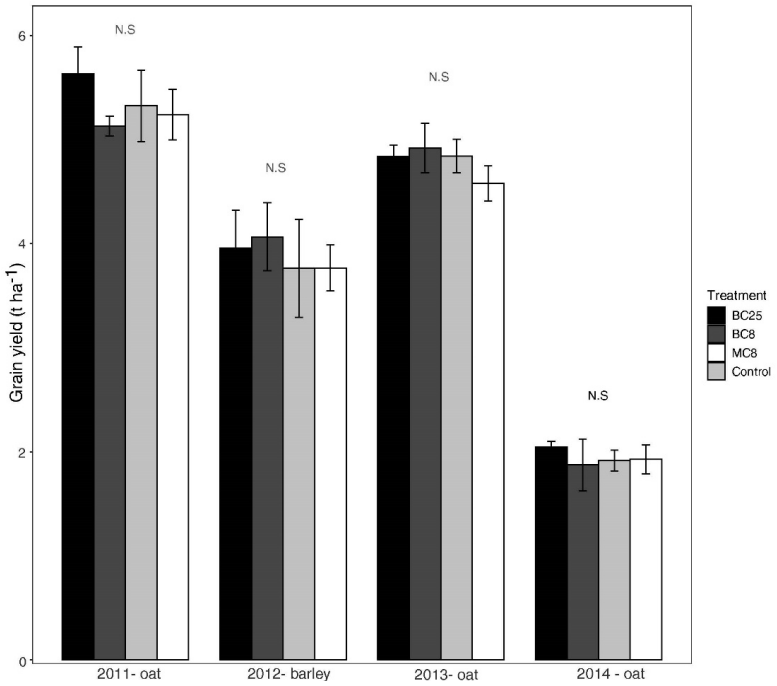

Fig. 13. Grain yield (Mean, ±SE, n=4) over 4 years in Ås trial (Paper I). BC25: Biochar 25 t C ha^{-1}, BC8: Biochar 8 t C ha^{-1}, MC8 – Uncarbonized Miscanthus straw 8 t ha^{-1}, Control- unamended soil. Different letters above bars indicate statistical significance ($p<0.05$) between treatments at each date, N.S = not significant.

Insignificant effects of biochar on crop yield in temperate regions was one of the main conclusions from a global meta-analysis by Jeffery et al., 2017. The authors found crop yields were boosted in tropical regions by 25% mostly due to the liming and fertilizer effect of biochar in acidic and nutrient poor soils. By contrast, soils in temperate regions generally tend towards neutral pH and are sufficiently fertilized. Therefore, they are in less need of soil amendments such as biochar to perform well. Recently, Kalu et al., 2021 reported that a one-time application of different rates of spruce and pine biochar (5, 10, 20, 30 t $ha^{-1)}$ to a fertile fine textured Stagnosol and a nutrient poor coarse textured Umbrisol in Finland, had overall no significant effect on the yield of wheat, barley, grass, oat, and pea over 8 cropping seasons from 2011-18. However, they found a significant interaction between biochar and fertilizer, leading to increased yields in barley and peas in 2013 and 2016 (ibid). They also found an enduring effect for the biochar to suppress N_2O emissions over the 8 years.

Another Finnish 2 year field trial found no effect of 30 t ha^{-1} of forest residue derived biochar on oat yield in a clay (64%) Stagnosol (Soinne et al., 2020). Similarly to our results, the authors observed that the biochar increased water holding capacity in the clay soil but did not result in better yields, even during a dry year (ibid). In 2012 and 2013, we also participated in a 7 country Northern European ring trial (Ruysschaert et al., 2016) where we applied 20 t ha^{-1} of a mixed wood biochar to a silty sand adjacent to the Ås field experiment (Paper 1). We found no significant change in barley grain yield in 2012, but a significant 14% increase in barley straw yield. Grain and straw yields were unaffected in Belgium, Denmark, Netherlands, while Sweden and the UK had significantly higher yield (5% and 33%) and Germany lower yield (11%) (ibid). In Norway, a 3-year biochar field trial was conducted between 2016-18 on a newly converted forest to agriculture site in Våler, Hedmark. In this study, a wood based biochar was applied at 5 and 10 t ha-1 with 3 N levels (70%, 85% and 100% of standard N fertilization) to a sandy soil to assess agronomic effects in oat and potato. Biochar had no significant effect on oat yield over 2 years or potato in one year. The experiment was highly influenced however by high rates of degrading forest residue left over after forest conversion. This led to fertilizer N immobilization and made it difficult to assess the biochar effect in this soil (Erstad et al., 2019). In summary, the results from my thesis together with meta-analysis results and results from selected studies in Nordic countries show that in most cases application of biochar in quantities of 5-50 t ha^{-1} to well-managed agricultural soils in boreal and temperate climates is unlikely to provide sufficient cereal and grass yield benefits.

In our study, the lack of yield benefits from biochar led us to conclude in Paper I that:

> "*in order to realize biochar's climate mitigation potential* , we suggest *future research should focus on improving the agronomic utility of biochar in engineered fertilizer and soil amendment products*" *p. 33*.

Therefore, in Paper IV, we focused our efforts on the combination of biochar and digestate as a combination fertilizer/soil amendment for commercial spring onion production as earlier described in section 5.4. The yield results in 2018 for spring onions due to these amendments are given in Fig. 14. Mean total yield was 13, 24, and 37% higher in AD, AD+BC-High and AD+BC-Low compared to the Control with NPK fertilization, but none of

these differences were statistically significant.

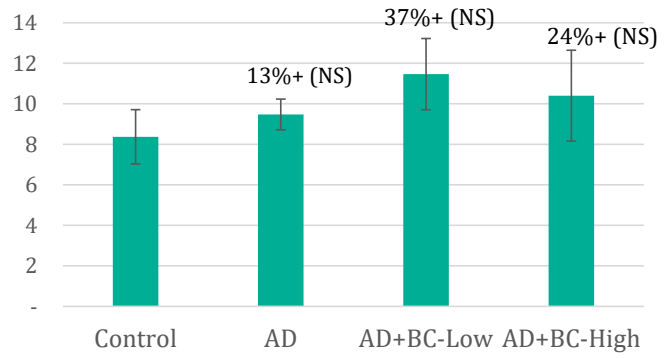

Fig. 14. Total plant yield (tonn ha^{-1}) of Spring onions from Paper IV % differences compared to the Control. **NS** = Not statistically significant, AD= Anaerobic digestate, AD+BC-High = Anaerobic Digestate mixed with biochar at 40% V/V, AD+BC-Low = Anaerobic digestate mixed with biochar at 20% V/V

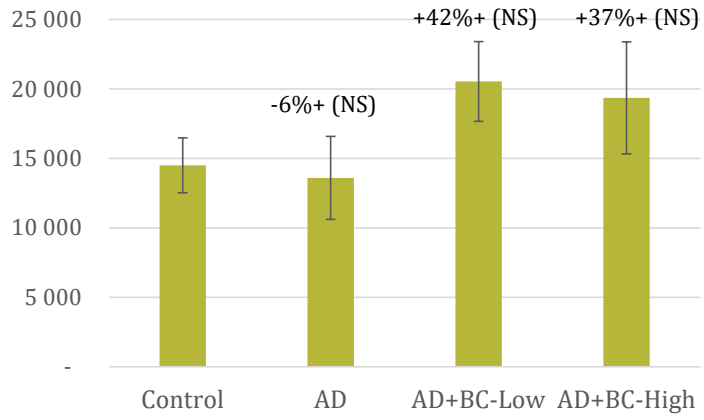

Fig. 15. Marketable yield (bunts ha^{-1}) of spring onion from Paper IV % differences compared to the Control. NS = Not statistically significant. AD= Anaerobic digestate, AD+BC-High = Anaerobic Digestate mixed with biochar at 40% V/V, AD+BC-Low = Anaerobic digestate mixed with biochar at 20% V/V

Marketable yield was reduced with AD compared to the control but not so with the addition of biochar to digestate (Fig. 15). The main reason for this effect appeared to be that that the AD treatment had a reduced germination rate compared to AD+BC-Low and AD+BC-High. Previous research suggests that the germination protection effect of biochar comes from its

ability to adsorb NH_3 (Taghizadeh-Toosi et al., 2012). In our case, this resulted in fewer plants that grew to maturity in the AD treatment but larger plants, which negatively affected marketable yield for AD. In contrast, more plants germinated in the biochar treatments and thus there were a greater number of optimally sized plants to choose from to make commercial bunts.

4.3 Biochar impact on GHG emissions

In 2012, BC-New and BC-Aged reduced N_2O by 80% ($p<0.001$) and 41% ($p=0.48$) respectively at one peak emission in late September. Otherwise there were minimal differences between treatments at other measurement dates and cumulative N_2O emissions in 2012 were not significantly different between treatments. Soil NO_3 was positively correlated with N_2O emissions in 2012 while soil pH was negatively correlated. Peak N_2O emissions occurred in a period when 7 day accumulated precipitation (~40mm) and soil moisture (>75% WFPS) were high. At soil moisture >70% WFPS denitrification is the dominant process responsible for N_2O emissions (Bateman and Baggs, 2005). One of the mechanisms identified for biochar reduction of N_2O under dentrification is increased soil pH. Under controlled laboratory conditions where anoxic biochar soil slurries were monitored for N_2O emissions Obia et al., 2015 found that biochar's alkalinizing effect influenced directly the product stoichiometry of denitrification resulting in less N_2O being emitted per unit of denitrified N_2 compared to non biochar amended soil. The liming effect of biochar is linked to its ash content which can be leached from the biochar over time (Buss et al., 2018), leading to less suppression of N_2O in aged biochars than fresh biochar (Spokas, 2013). In paper II, our hypothesis that the mitigation effect of biochar on N_2O emissions decreases with time was confirmed. In 2014, N_2O emissions were 2-6 times lower than in 2012 (Fig. 16c) and therefore treatment differences in 2012 had a greater influence on the aggregated results for both years.

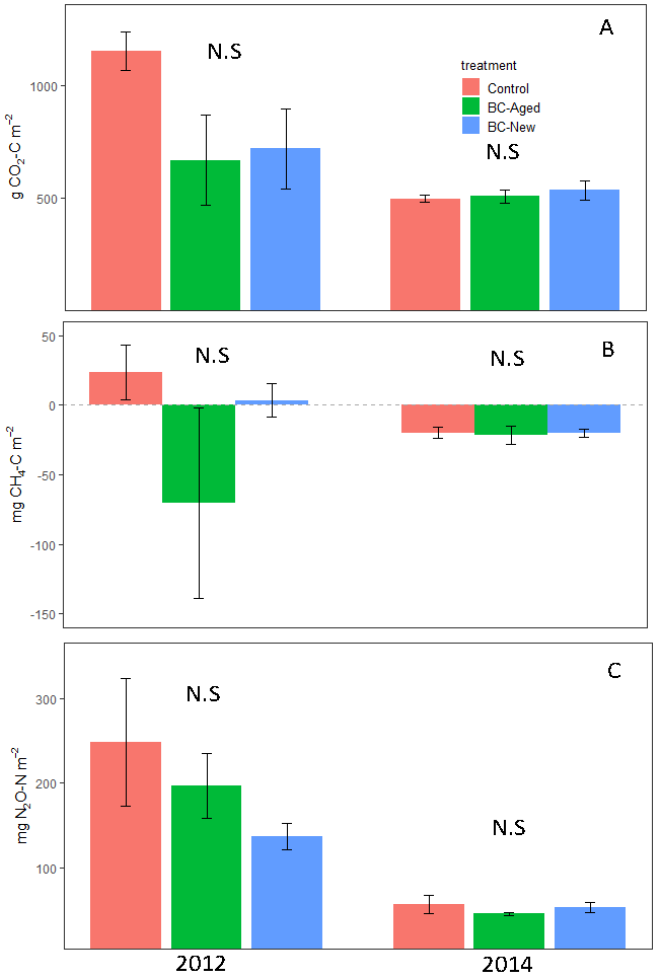

Fig. 16 Cumulative GHG emissions in 2012 and 2014 for (A) Carbon Dioxide (Dark respiration) (B) Methane (C) Nitrous oxide emissions from soil. Error bars are standard error. n= 3 (2012), n=4 (2014). N.S = No significant difference between treatments.

In the Skjærgaarden experiment (Paper IV), N_2O flux and cumulative N_2O emissions were not significantly different between treatments. Analysis via the generalized linear model indicated that N_2O flux was significantly increased by soil temperature ($p<0.001$) and NH_4 soil content ($p=0.03$), but with no effect for NO_3 soil content ($p=0.19$) or water filled pore space ($p=0.35$). There was a significant interaction effect with soil mineral N and treatments AD and AD+BC-High. Both AD and AD+BC-High had less N_2O production per unit of soil mineral N compared to the Control (Fig.17). Our findings corroborate with Martin et al., 2014, who also found that soil NO_3 concentrations increased and N_2O decreased when

biochar was mixed with digestate and they speculated that this may have been due to the known phenomena of adsorption of NO_3 in biochar pores, as recently demonstrated in Haider et al., 2020.

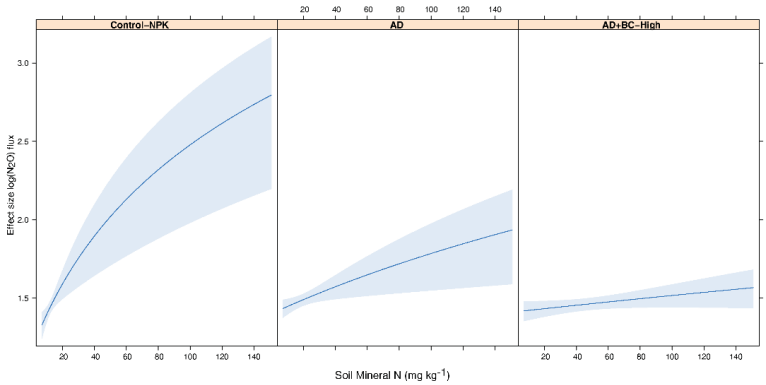

Fig. 17. GLM output from Paper IV showing the N_2O emissions as influenced by concentrations of soil mineral N in each treatment (Control, AD= Anaerobic digestate , AD+BC-High = Anaerobic digestate mixed with biochar at 40% V/V)

4.4 Biochar mobility in the soil

Based on ^{13}C measurements, we found that both BC-High and BC-Low were detectable in the plough layer (0-23cm), only BC-High was detectable at 23-45 cm, and no biochar-C was detectable at 45-60 cm (Fig.18 A). A significant difference in soil C could only be detected between BC-High and Control in the 0-15 cm depth with no difference between treatments at other depths (Fig. 18.B).

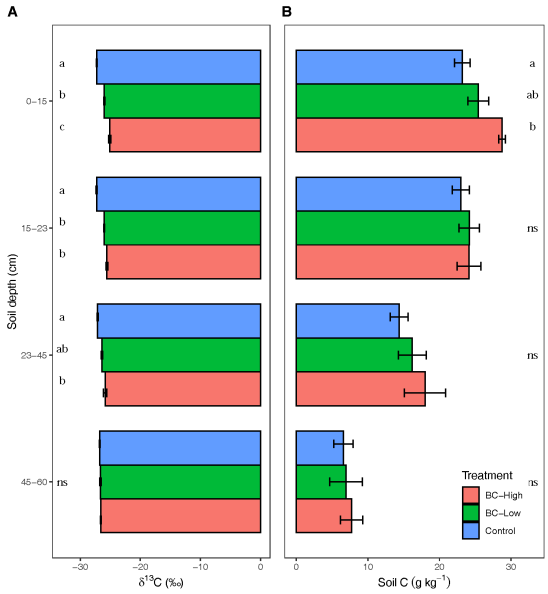

Fig. 18. Mean (±SE, n=4) (A) Soil δ^{13} C and (B) Soil C content (g kg^{-1}) from soil samples taken at 4 depths in 2015. Plough depth is 23 cm. **BC-High**=31.5 t biochar ha-1, **BC-Low**= 11.6 t biochar ha^{-1} Different letters above bars indicate statistical significance ($p<0.05$) between treatments at each date, ns = not significant.

Using the results of Fig. 18 and soil bulk density measurements, I calculated the amount of biochar present in the soil at each depth and per m^2. These results are described in more detail in Paper III. After 5 years, I accounted for 92-107% ±6 of the biochar that was originally applied, including 4% biochar C mineralization rate over the same period. A recovery rate that exceeds the original application amount can be explained by the heterogeneous distribution of biochar which can result in high concentrations of biochar in individual soil samples if the sampling auger hits a concentrated patch of biochar. Forty-five to seventy two percent of biochar was found in the 0-23 cm plough layer within plot boundaries, 22-31% vertically transported to 23-60 cm depth, 0-21% had moved laterally within 9 m of the plot boundary and 4% was mineralized as CO_2 (Table 2). The latter value is a 5 year extrapolation of an annual biochar mineralization rate of 0.8% calculated from δ^{13}C-CO_2 soil respiration field measurements taken in the first two years of our experiment (Rasse et al., 2017). This means we accounted for 92% of the biochar in BC-High and 107 of biochar BC-Low (Table 2).

Table 2. Biochar fate and recovery (%) at two biochar (BC) application rates after 5 years in a flat terrain silty clay loam in temperate climate

Where did the char go?	BC-High (31.5 t BC ha^{-1})	BC-Low (11.6 t BC ha^{-1})
Retained within plot (0-23 cm)	45% ± 2	72% ± 6
Transported vertically (23-60 cm)	22% ± 7	31% ± 7
Moved laterally <2 m (due to tractor)	12%	NA
Moved laterally (due to plough) <1m	9%	NA
Biochar-C mineralization to CO_2 after 5 years (ref: Rasse et al. 2017)	4%	4%
Biochar accounted for	92% ± 5	107% ± 7

There is a large variability in biochar recovery rates in published literature. Dong et al., 2017 recovered approximately 60% of rice husk and sunflower hull biochar in the 0-20 cm depth 5 years after application to a flood irrigated flat alluvial plain soil. The authors attributed vertical or lateral transport of biochar to biannual flood irrigation events, but lacked data to confirm this. In a 9 year study in 4 different field sites in sub-humid Kenya in flat or gently sloping terrain, Kätterer et al., 2019 reported that 32-96% of the biochar applied in the beginning of the experiment was recovered in the 0-20cm depth. Loss pathways were not measured but were assumed to be a combination of mineralization, erosion or vertical translocation. Major et al., 2010 could not account for 20-53% of biochar after 2 years in a flat sloped field where biochar was applied at 11.6, 23.2, and 116.1 t C ha^{-1} to the top 0-15 cm of a sandy clay loam Oxisol and assumed the unaccounted loss to lateral surface runoff during intense rain events. Twelve months after application Singh et al., 2015 recovered 82% of biochar in a Arenosol, 101% in a Cambisol and 104% in a Ferrosol one year after application. Our biochar recovery rate of 45-66% in the plough layer is within the range reported by Obia et al., 2017, who retrieved 55-76% in the 0-20 cm layer after 1 year.

The mobility of biochar creates a challenge for future soil sequestration programmes if verification of biochar-C stocks are required. Unrecovered biochar-C that has vertically migrated into the soil transported can be mistakenly assumed to have mineralized to CO_2. For example, from our study a vertical transport of biochar of 22-31% below the plough layer is 5.5-7.75 times higher than the estimated C mineralization rate. Already after 1 year, Singh et al., 2015 found that the vertically migrated biochar was 1.7-2.2 times higher than the amount of mineralized biochar C in an Arenosol and Ferrosol. This suggests that the fraction of vertically migrated biochar will increase with time and that sampling of the subsoil is essential to gain an accurate assessment of biochar C stocks. Furthermore, lateral transport on sloped soils, which are common in Norway, would reduce recovery rates regardless of how precise the GPS positioning of where the biochar was originally applied.

5 Outlook and research needs

i) Biochar and BCFs

As described in Paper 1 and literature cited in section 5.5.2, I have shown that the application of pure biochar to fertile fields in Norway and other temperate regions is unlikely to result in yield increases. In contrast, in Rasse et al. (*in prep.*) I reviewed 19 studies where biochar was used as an ingredient in engineered fertilizers and found that biochar based fertilizers can increase median crop yield by 17% compared to a mineral fertilizer control with the same amount of N. Biochar based fertilizers are already being used in China to increase nutrient use efficiency (Dong et al., 2020), reduce plant uptake of Cd (Chen et al., 2021), and improve the yield of rice (Chew et al., 2020). In Norway, research is underway on this topic via the NIBIO led research project CarboFertil (www.nibio.no/carbo-fertil). Using biochar in smaller amounts in fertilizer products may be a way to increase the nutrient use efficiency of fertilizers while also contributing with annual amounts of biochar C to the soil. This topic of research is still in its infancy and requires more study to understand how biochar will function in engineered fertilizer products.

ii) Biochar and digestate

There is an ambition in Norway to deliver 30% of animal manure to biogas plants in order to reduce CH_4 from animal manure storage and produce CH_4 based biofuels (Ålund and Weeghel, 2020). The end bi-product from this process is a C-poor digestate that is rich in plant nutrients. As demonstrated in Paper IV, biochar is a promising material to mix with digestate to stimulate nitrification and to hold onto more nutrients under irrigated conditions. Research has also been conducted with the addition of biochar to biogas reactors during the anaerobic digestion process. Studies show that this can increase CH_4 output due to: biochar buffering effects on inhibiting substances (Masebinu et al., 2019) and due to biochar increasing the surface area available for CH_4 producing methanogens too (Cooney et al., 2015; Pant and Rai, 2021). In this context, future studies would be useful to explore the effects of biochar through the whole value chain to determine at what point in the biogas system it is most advantageous to add biochar.

iii) Assessment of aged vs new biochar effects on N_2O during freeze thaw events and in controlled laboratory conditions

Measurements in all four papers were taken during the growing-season period. As the biochar effect of denitrification appeared as the key driver for reductions in N_2O emissions, we can hypothesize that this effect might be greater in the cold season when denitrification processes are fostered by high soil moisture contents. More research is needed to confirm this hypothesis. Due to the spatial and temporal variability of N_2O emissions, a laboratory study where aged biochar is retrieved from the field and compared to fresh biochar under controlled conditions will assist understanding of underlying mechanisms.

iv) Gaseous emission balance when mixing biochar with manure

As more farmers have started to trial the use of biochar in Norway, I have received repeated requests to recommend how much biochar should be added to slurry manure and what expected results will be from biochar and manure mixtures. The results from Paper IV, where we mixed biochar with digestate, may not translate directly to how biochar should be mixed with manure due to the differing elemental content of manure vs digestate. Other factors such as timing may need to be considered for adding biochar to manure, due to the long periods that manure is stored in farm lagoons. More research is needed on biochar effects on gaseous emissions and N cycling when combined in animal manure storage. Of the few studies conducted so far, Meiirkhanuly et al., 2020 found that a 6 mm thin layer of biochar applied to the surface of swine manure can reduce NH_3 40-52% but the mitigation effect wears off over 3 weeks. In the first 1-2 weeks of this study, biochar initially decreased CH_4 emissions from swine manure by 5-50%, but by the third week increased CH_4 emissions by 26-68% after the biochar sank in the manure. This is not surprising considering that biochar has been shown to increase CH_4 output in biogas digesters (Cooney et al., 2015; Pant and Rai, 2021). Biochar GHG mitigation in manure may possibly have its greatest impact when applied immediately before manure mixing and removal from lagoons. This is when store manure emission peak and if added just before mixing the biochar will still be able to float (Chen et al., 2021). This area of research should be a priority to find biochars that can be made from Norwegian feedstocks with optimal floatability and pH to reduce both NH_3 and CH_4 emissions from stored animal manure.

6 Conclusion

The four field based studies presented in this Ph.D thesis document the performance of biochar under field conditions in a fertile silty clay loam and an intensively cultivated sandy soil in Norway. Covering 10 years of research, the Ph.D started with answering basic questions regarding the safety and agronomic effects of biochar and concluded with a farm based study where the aim was to find a best practice for application. In a recent conversation with the farmer Bjørge Madsen at Skjærgaarden (Paper IV), he reported that their farm has continued after our experiment with the practice in fertilizing their fields with biochar and digestate mixes and is on track by next year to replace 50% of their synthetic fertilizer consumption. His example and results have also inspired four of the largest commercial vegetable producers in the country to adopt the practice from season 2022. This illustrates that my thesis project and research has played a role in the adoption of more sustainable cultivation practices in Norwegian horticulture.

In summary, I give the following advice that can assist further policy development and implementation of biochar in Norway and elsewhere:

1. Despite some soil improvement benefits such as water retention this is not likely to translate to yield improvements in grain production in well managed soils in Norway

2. Biochar can improve the N fertilization effect of biogas digestate, especially under irrigated conditions in sandy soils which are prone to leaching

3. That biochar is likely to reduce N_2O under Norwegian field conditions, but the suppressive effect will be less with aged biochar compared to fresh biochar

4. Emerging soil carbon accounting schemes where biochar is included should design soil-sampling regimes to take into account the vertical and lateral movement of biochar over time.

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8 Errata

In Paper I, published in 2018, it was stated that an application rate of 25 t biochar-C ha^{-1} is equivalent to 35 t ha^{-1} biochar. This is a typographic error and has been corrected in Paper II and III as 31.5 t ha^{-1}.

Paper I

agriculture

MDPI

Article

Miscanthus Biochar had Limited Effects on Soil Physical Properties, Microbial Biomass, and Grain Yield in a Four-Year Field Experiment in Norway

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Abstract: The application of biochar to soils is a promising technique for increasing soil organic C and offsetting GHG emissions. However, large-scale adoption by farmers will likely require the proof of its utility to improve plant growth and soil quality. In this context, we conducted a four-year field experiment between October 2010 to October 2014 on a fertile silty clay loam Albeluvisol in Norway to assess the impact of biochar on soil physical properties, soil microbial biomass, and oat and barley yield. The following treatments were included: Control (soil), miscanthus biochar 8 t C ha^{-1} (BC8), miscanthus straw feedstock 8 t C ha^{-1} (MC8), and miscanthus biochar 25 t C ha^{-1} (BC25). Average volumetric water content at field capacity was significantly higher in BC25 when compared to the control due to changes in BD and total porosity. The biochar amendment had no effect on soil aggregate (2–6 mm) stability, pore size distribution, penetration resistance, soil microbial biomass C and N, and basal respiration. Biochar did not alter crop yields of oat and barley during the four growing seasons. In order to realize biochar's climate mitigation potential, we suggest future research and development efforts should focus on improving the agronomic utility of biochar in engineered fertilizer and soil amendment products.

Keywords: biochar; miscanthus; Norway

1. Introduction

The challenge of producing more food for a growing world population while also mitigating climate change demands new solutions for managing agricultural systems [1]. The application of biochar to soil has received increasing attention as an alternative method for increasing long-term soil carbon levels while potentially improving soil quality and crop productivity [2]. Biochar is the term given to charcoal or carbonized biomass when it is used for the purpose of soil carbon sequestration and for improving soil fertility [3]. Meta-analyses confirm that biochar can improve soil physical and hydrological functioning [4] and can reduce N_2O and CH_4 emissions [5,6]. Across multiple studies, biochar has been shown to increase crop yields on average by 25% in the tropics but has had no effect in temperate regions [7]. In Norway, biochar application is recognized as one of several methods with

the potential to significantly reduce the carbon footprint of the agricultural sector [8]. Our previous investigations using the same biochar and soil used in this study confirm a low annual C mineralization rate of 0.8% and estimated mean residence time of >100 years for biochar in the soil [9].

While climate benefits of biochar application appear promising, adoption by farmers requires a demonstration of its safety and, ideally, its benefit in terms of soil quality and crop yield. Previous biochar agronomic studies have shown that biochar can enhance water retention [10,11], which is often attributed to the large surface area and intra-porosity of biochar and its ability to alter inter-pore porosity between mineral soil particles [12]. Biochar has been shown to both increase saturated hydraulic conductivity (K_{sat}) in clay soil and decrease it in sandy soil [13,14] due to biochar either filling pore spaces in sand or opening pore channels in clay [15]. However, the results from studies on biochar effects on K_{sat} vary widely according to the soil, type of biochar used, and amendment rate [4]. Several studies report improvements in soil structure, which is indicated by increased aggregate stability [11,16,17] and reduced penetration resistance (PR) [18,19]. Once again, the mechanisms involved rely upon interactions between biochar and soil properties. In clay soils, biochar has been shown to reduce soil tensile strength and the plasticity index (degree of swelling/shrinkage), but usually requires large application rates [20–22].

The impact of biochar on soil biota has received less attention, but studies to date show increases in microbial abundance in the short to medium term [23,24]. The effects seem to be more pronounced in weathered soils where soil organic matter is often a limiting factor [25]. Mechanisms for increases in microbial biomass and changes in microbial community diversity include direct effects from labile C fractions present in fresh biochar and/or indirect effects brought about by short-term changes to soil physio-chemical conditions such as pH [26].

Our study was the first field trial testing of biochar in Norway. The objectives were to assess the agronomic effects of miscanthus biochar under field conditions over four years and to give farmers and authorities in Norway insight for the suitability of biochar as a climate change mitigation method. Our results provide an agronomic context to Reference [9] (where we previously investigated biochar C stability from the same field site). In the present study, we hypothesized that biochar could improve soil water retention and alleviate short-term soil water deficits as are common in the early summer in Norway. This, we proposed, would indirectly lead to increased plant growth and crop yield. Furthermore, we hypothesized that the relative stability of the biochar carbon would mean that relatively large amounts of biochar could be added to improve soil physical conditions without leading to microbial N-immobilization, which is usually the case with the addition of high C:N organic materials.

2. Materials and Methods

2.1. Field Description and Experimental Design

A field experiment was conducted from September 2010 to October 2014 at the Norwegian University of Life Sciences (NMBU) field station in Ås, Norway (59°39′51″ N 10°45′40″ E) (Figure 1). The field had been used for field research for grain and grass production since the 1950s. Weather data (Table S1) collected 1.3 km from the field site at the NMBUs weather station.

Figure 1. Biochar field site in Ås, Norway, September 2010. The darkest plots visible are the BC25 treatment.

The soil is a silty clay loam Albeluvisol (WRB classification) with an average content of 27% clay, 43% silt, and 30% sand. The biochar was produced from *Miscanthus giganteous* straw by Pyreg Gmbh (DE) in a continuous slow pyrolysis machine, which is operated on a commercial basis. Pyreg reported a working temperature range between 500 and 750 °C during the production of the biochar. The biochar was cooled and moistened with water to approximately 35% moisture content after exiting the pyrolysis reactor.

The experiment was of randomized complete block design with 4 treatments × 4 blocks. Plots were 8 m × 4 m and buffer areas between blocks were 6 m wide. The four treatments consisted of: Control (no organic amendments), *Miscanthus* biochar 8 t C ha^{-1} (BC8), *Miscanthus* straw (unpyrolyzed) 8 t C ha^{-1} (MC8), and *Miscanthus* biochar 25 t C ha^{-1} (BC25). Dose units are given in tons C to show that equivalent amounts of carbon were added in the MC8 and BC8 treatments. Corresponding BC application rates and mass percent concentrations in the 23 cm Ap soil horizon were 11.4 t BC ha^{-1} or 0.38% (w/w) for BC8 and 35 t BC ha^{-1} or 1.16% (w/w) for BC25. *Miscanthus* was chosen as a feedstock because it is a C3 plant with contrasting $\delta^{13}C$ to the C4 soil, which was relevant for the primary objective of the field experiment as reported in Rasse et al. [9] (i.e., biochar-C stability over two years under field conditions). Biochar or *Miscanthus* straw were applied and raked out on the surface of the plots in September 2010 and all plots (including controls) were then mouldboard ploughed to a depth of 23 cm. Mouldboard ploughing resulted in the biochar and straw being distributed in concentrated diagonal seams in the Ap horizon in 2011 (Figure S1A). Further ploughing and harrowing in 2012–2014 resulted in more evenly distributed biochar and resulted in a more even distribution throughout the Ap (Figure S1B) even though biochar patchiness persisted to some degree, which we discovered by visual inspection of soil during soil sampling campaigns. Tillage operations were the same across all treatments over the experiment period and consisted of autumn ploughing and spring harrowing. The field was sown with oat on 9 May 2011, barley on 19 May 2012, and oat on 3 June 2013 and 27 May 2014. Fertilizer (Yaramila ™ NPK 22-3-10, Yara Norge AS, Oslo, Norway) was applied at a rate of 550 kg ha^{-1} (110 kg N, 16.5 kg P, and 55 kg K ha^{-1}) on an annual basis at the time of seeding.

2.2. Soil and Biochar Analysis (Table 1)

For soil pH, 11 g of field moist soil was shaken for 1 hour in 50 mL of distilled water and left to stand for 3 h before measurement with a pH electrode. Biochar pH was measured with distilled water using a 1:5 (*w*/*w*) ratio. Shaking time was increased to 1.5 h to increase equilibration between biochar surfaces and the solution [27]. Proximate and elemental analyses and heavy metal content of biochar were conducted by Eurofins Ost Gmbh (Aschheim-Dornach, Germany), according to standards DIN5178 (H_2O), DIN5179 (Ash), DIN51720 (VM), DIN51734 (FC), DIN51732 (C,H,N), DIN51733 (O), 51724-3 (S), NS EN ISO 11885 (As, Pb, Cr, Ni), NS 4768 (Hg), and NS 4781-1 (Cd). Total N and P, NO_3, and NH_4 in soil were measured by the ALS labs, Norway, according to EN-ISO standards. Plant-available P, Ca, K, and Mg in soil and biochar were measured in-house using the Egners AL (ammonium lactate) method [28]. The extraction fluid (pH 3.75) was a mixture of ammonium lactate (0.1 mol L^{-1}) and acetic acid (0.4 mol L^{-1}). Specific surface area for biochar was measured by N adsorption–desorption isotherms at 77 K using a Micromeritics Tri Star 3000 instrument (Micromeritics Instrument Corp., Norcross, GA, USA). Before analysis, the samples were dried at 120 °C and degassed overnight in a VacPrep 061 Degasser (Micromeritics Instrument Corp.) at 0.05 mbar and 393 K. The Brunauer–Emmet–Teller equation was used to calculate the specific surface area [29]. Particle size distribution of biochar was determined via sieving 285 g and by using a Retsch AS200 (Retsch GmbH, Haan, Germany) nested machine sieve with eight size fractions between 63 µm and 4 mm. Sieving was done initially for 3 min at 55 amplitude and then the largest fraction (2–4 mm) was redistributed into two sieves to make sure that smaller particles were not floating on top of a mass of larger biochar particles and being prevented from passing through. Then a second 3 min of sieving was repeated.

Table 1. Properties of miscanthus biochar, miscanthus straw (biochar feedstock), and the soil.

	Unit	Miscanthus Biochar	Miscanthus Straw (Biochar Feedstock)	Soil (Spring 2011)
Fixed C	%DM	81.10	-	-
Volatile matter	%DM	7.40	-	-
Ash	%DM	11.50	3.50	-
Total C	%DM	80.00	46.73	2.45
H	%DM	1.2	-	-
N	%DM	0.6	0.20	0.23
O	%DM	6.6	-	-
S	%DM	0.10	0.05	-
C:N	Ratio	256.77	233.65	17.45
Total P	mg kg^{-1}	1300	80	2900
P-AL	mg kg^{-1}	1100	-	106
K-AL	mg kg^{-1}	7500	-	86
Ca-AL	mg kg^{-1}	4600	-	2058
Mg-AL	mg kg^{-1}	640	-	116
Na-AL	mg kg^{-1}	360	-	28
Si	mg kg^{-1}	-	3.40	-
NO_3	mg kg^{-1}	3.32	-	12.10
NH_4	mg kg^{-1}	-	-	1.50
Fe	mg kg^{-1}	1100	-	-
Mn	mg kg^{-1}	160	-	-
Mo	mg kg^{-1}	<1.1	-	-
Zn	mg kg^{-1}	39	-	-
Cl	mg kg^{-1}	477	-	-
B	mg kg^{-1}	5.10	-	-
BET-N_2	m^2 g^{-1}	348	-	-
pH (±SD, *n* = 9)	(H_2O)	7.86 ± 0.05 (*n* = 3)	-	6.39 ± 0.2
EC	mS/m	130	-	4.10
Δ13C (±SD, *n* = 3)	‰	−13.60 ± 0.2	−12.38 ± 0.1	−27.13 ± 0.1
H:C (atomic)		0.18	-	-
O:C (atomic)		0.06	-	-

2.3. Sampling and Analysis Methods

2.3.1. Soil Sampling

For soil chemical analysis, soil sampling was done using a 2 cm wide soil auger to take 10 sub-samples per plot to a depth of 23 cm. The soil sub-samples were mixed to form one composite sample per plot. The same method was used for taking soil samples for microbial analysis and were taken in the summer of 2012.

Bulk density (BD) was measured in 2012 and 2014 with 4 × 100 cm^3 metal rings in each plot. Intact 250 cm^3 soil cores were taken (2–9 cm and 12–19 cm) in 2014 for the water retention experiment and BD. Total porosity was calculated according to the formula below.

$$Porosity = \left(1 - \frac{BD}{MD}\right) \times 100\ (vol\%) \quad (1)$$

We assumed a material density (MD) of 2.65 g cm^{-3} for mineral soil and skeletal density of 1.5 g cm^{-3} for biochar [30,31] and by that adjusted biochar/soil mix density to 2.646 g cm^{-3} and 2.637 g cm^{-3} in BC8 and BC25 to account for the lighter biochar particles present. Soil aggregates used for aggregate stability tests were taken from the Ap horizon of the field in 2015 by using a shovel to collect a representative 2.5 L bulk sample from each plot. Plant roots, organisms, and soil that was compacted from the spade were excluded from the sample. Soil aggregates >30 mm were carefully broken into smaller aggregates by hand and air-dried at 20 °C for one week.

2.3.2. Soil Water Content in the Field

Soil moisture content was measured every hour in the growth seasons of 2012 (n = 3) and 2014 (n = 4) using Time Domain Reflectance (TDR) soil moisture sensors (5TM model, Decagon Devices, Inc., Pullman, WA, USA). One TDR was inserted horizontally in undisturbed soil at 5 cm and 15 cm depth of each plot. The TDRs were calibrated in the lab using dried soil repacked in 1 liter containers with and without biochar at known gravimetric and volumetric water contents. Linear regressions were derived between probe output (mV) and VWC for the control and biochar amended soils BC8 and BC25 and these equations (S.8) were used to correct field measured TDR data.

2.3.3. Soil Water Retention and Pore Size Distribution from Intact Cores

Soil water retention characteristics θ(h) were measured between pF 1–3.2 with the evaporation method [32] and by using a Ku-pF apparatus (Umwelt-Geräte-Technik GmbH, Müncheberg, Germany). In the lab, soil cores were saturated with water from below after which two micro tensiometers were inserted horizontally in pre-drilled holes before being placed on the Ku-pF apparatus. The cores were weighed automatically every 10 min over 2 weeks whereby the tensiometer reading and sample weight loss were converted to matric potential (cm head) and volumetric water content (θ) values at each time step. Available water for the plants was calculated as the difference of θ between field capacity (FC) (−33 kPa) and the permanent wilting point (PWP) (−1500 kPa). Due to the limited pressure range of the tensiometers at the dry end of the soil water retention curve (<1000 kPa), θ and matric potential at the dry end were estimated by fitting observed data to the van Genuchten-Maulem model (VGM) [33] in R (R Core Development Team, Vienna, Austria). The permanent wilting point was estimated by the use of a pedotransfer function developed by Reference [34], which calibrated the function based on the SOM (%), gravel (%), and BD of 192 silty clay loam soil samples from South East Norway.

The high frequency measurements of matric potential and soil water content during evaporation can be directly related to emptying of water from soil pores of different sizes. Pore size distribution in

the core samples was estimated by taking the derivative of Theta (pF) and converting pF in pore size following the equation from Reference [35].

$$d = \frac{3000}{10^{pF}} \quad (2)$$

where d represents the equivalent pore diameter in μm corresponding to a given level of matric potential.

Using Equation (2), we further estimated the proportion of the soil volume occupied by pores characterized by $d \leq 3.5$ μm and by pores with 3.5 μm < d < 300 μm, noted respectively $P < 3.5$ and $P = 3.5$ to 300.

$$P_{<3.5} = \theta\left(\log(h)\left(\frac{3000}{3.5}\right)\right) \quad (3)$$

$$P_{<300} = \theta\left(\log(\mathrm{h})\left(\frac{3000}{300}\right)\right) \quad (4)$$

$$P_{3.5-300} = P_{<300} - P_{<3.5} \quad (5)$$

where θ is the volumetric soil water content as a function of the matric potential (pF).

2.3.4. Aggregate Stability

Aggregate size distribution in size classes: <0.6, 0.6–2, 2–6, 6–20, and >20 mm were determined by dry sieving 2.5 L of air-dried soil for 3 min using a mechanical sieving apparatus, which is described by Reference [36].

Wet sieving was performed on aggregates from the 2–6 mm size class and by using a wet sieving apparatus (Eijkelkamp, Giesbeek, The Netherlands) following the method described by Kemper and Rosenau (1986). The procedure involved placing 4 g of 2–6 mm aggregates (×4 replicates/treatment) in small sieves and exposing them to intermittent submerging in distilled water for 3.75 min. To avoid the artefact of premature aggregate breakdown, which can occur when trapped air in dry aggregates is expelled upon rapid exposure to water [37]. Samples were pre-wetted by exposure to a mist produced from a consumer electronic humidifier. After wet sieving, aggregates remaining on the sieve were dried, weighed, and then passed through a set of nested sieves to determine aggregate size classes >2 mm, 1–2 mm, 0.5–1 mm, 250–500 μm, 125–250 μm, 63–125 μm, and <63 μm to determine the Mean Weight Diameter (MWD), which was calculated by using the equation below.

$$MWD = \sum_{i=1}^{n} D \times W \quad (6)$$

where D is the mean diameter of each size fraction (mm) and W is the proportion of the sample mass in the corresponding aggregate size fraction. The percentage of water stable aggregates (WSA) was also calculated as the percentage of aggregates in the 2–6 mm size range remaining on the sieve after the 3.75 min wet sieving exposure.

To elucidate upon how treatments affected the different aggregate breakdown mechanisms, further tests were conducted by using the methods of Le Bissonnais, 1996 [38]. In this case, the main mechanisms of aggregate breakdown, namely breakdown by compression of trapped air (slaking), breakdown by differential swelling, and mechanical breakdown by raindrop impact and physio-chemical dispersion are simulated by three tests: fast wetting (for slaking), slow wetting (for differential swelling), and shaking (for mechanical and physio-chemical dispersion). Aggregates were also pre-wetted with mist for the slow wetting test following the method mentioned previously. To evaluate results from all aggregate tests, a comparison was made with an MWD stability index developed by Le Bissonais [38].

2.3.5. Soil Penetration Resistance

Soil penetration resistance (PR) was measured in October 2015 from 0–40 cm by using an electronic penetrometer (2 cm^2 cone tip) that continuously logs depth and soil resistance upon probe insertion (Eijkelkamp Soil and Water, Giesbeek, The Netherlands). Ten measurements were conducted per plot (5 between tractor wheel tracks and 5 within tractor wheel tracks) to assess to what extent biochar moderated soil compaction. Soil BD and moisture were also measured (2–7 cm depth) alongside PR measurements, since these are known to influence PR [39].

2.3.6. Soil Microbial Biomass C and Respiration Potential

Soil microbial biomass C (Cmic) and N were determined by the chloroform fumigation extraction method [40,41]. Fumigations were carried out for three days in vacuum desiccators with alcohol-free chloroform. 15 g of soil of both fumigated and unfumigated field moist soils were extracted with 0.5 M K_2SO_4 (1:5, *w:v*). After filtration (Whatman n° 42, GE Healthcare Life Sciences, Buckinghamshire, UK), extracts were analyzed for organic C using a Total Organic Carbon analyzer (LabToc, Pollution and Process Monitoring limited, Kent, UK). Soil microbial biomass C and N were calculated by dividing the difference of total extract between fumigated and unfumigated samples with a Cmic extraction efficiency factor of 0.45 [42] and 0.54 for microbial biomass N [43].

Respiration potential [44] was measured as CO_2 accumulation in the headspace (250 mL) of an amber bottle (Supelco, Merck KGaA, Darmstadt, Germany) from 20 g fresh soil at 15 °C in the dark after an overnight pre-incubation. Gas samples (4 mL) were taken at 0, 120, 150, and 180 min with an air-tight syringe (Hamilton Model 1005, The Hamilton Company, Reno, NV, USA) and analyzed with an infrared absorption gas analyzer (EGM-4, PP-Systems, Hitchin, UK). The respiration potential was estimated by linear regression of CO_2-C against time.

2.3.7. Plant Grain and Straw Yields

Grain and straw yields were measured in 2011–2014 by using a field station harvester (Wintersteiger Nurserymaster elite). The harvested area of each plot measured 1.5 m × 6 m (9 m^{-2}) and was located in the middle of the 32 m^{-2} plots so that plot edges were avoided. Grain quality, as measured by protein and fat content and 1000 grain weight, were measured in 2012 (Barley) and 2014 (Oat) for BC25 and control treatments only. For this, a subsample of 200 g was taken from the harvested grain from each plot for protein and fat content (via NIR spectrometry using an Infratec™ Grain Analyzer (Foss Analytics, Hilleroed, Denmark) and 1000 grain weight analysis.

2.4. Statistical Analysis

Statistical analyses were carried out by using packages from R software (The R Foundation for Statistical Computing, Vienna, Austria) [45] and SigmaPlot v.13. Statistical (Systat Software Inc., London, UK) significance was set at $\alpha = 0.05$ for all analyses. Statistical differences between treatment means for grain and straw yield, aggregate stability tests, Cmic, Nmic, and respiration potential were tested with ANOVA and post-hoc multiple comparison of treatments vs. control via the Dunnett's test if $p < 0.05$. The Welch two sample *t*-test was used to test differences between the mean grain protein and 1000 grain weight in BC25 and Control. Soil PR was summarized at 5 cm range intervals until 25 cm depth for both within and outside tractor tracks and treatment averages within these range intervals used for ANOVA. Hourly soil water content measurements from the TDR sensors were averaged for each day and daily averages were statistically analyzed via two-way repeated measures ANOVA with treatment and time as factors and Dunnett's test for post hoc multiple pair comparison of treatments vs. the control by using SigmaPlot v.13 software. Daily averages were used for statistical analysis due to our observation that, within day soil, water content did not differ greatly and, therefore, the use of daily averages represented a simplified approach, which reduced data size and analysis complexity. For analysis of pore-size distribution, we used the lme4 package from R to perform a

linear mixed effects analysis of the relationship between the volume of pores of various size and the manipulated parameters of the experiment (i.e., Biochar amendments and depth). As random effects, we had intercepts for the plot.

3. Results

3.1. Effects of Biochar on Bulk Density

There were no significant differences between the treatments in soil bulk density and porosity in 2012. However, bulk density was significantly lower by 7% in BC25 in 2014 compared to the control while the lower dose treatment (BC8) did not differ from the control (Table 2). There was a reduced standard error in BC25 and BC8 in 2014 compared to 2012. Total porosity was higher in BC25 compared to the control in 2014 while BC8 did not differ.

Table 2. Bulk density (g cm^{-3}) and total porosity.

	BD (1–8 cm)	BD (12–19 cm)	Total Porosity (%)
2012			
Control	1.30 ± 0.02 [a]	-	50.87 ± 0.84 [a]
MC8	1.19 ± 0.04 [a]	-	54.99 ± 1.32 [a]
BC8	1.16 ± 0.05 [a]	-	56.33 ± 2.03 [a]
BC25	1.22 ± 0.07 [a]	-	53.87± 2.56 [a]
2014			
Control	1.30 ± 0.02 [a]	1.36 ± 0.02 [a]	49.93 ± 0.66 [a]
BC8	1.29 ± 0.02 [a]	1.38 ± 0.03 [a]	49.67 ± 1.04 [a]
BC25	1.21 ± 0.03 [b]	1.26 ± 0.02 [b]	53.27 ± 0.81 [b]

Bulk density (BD) ± SE, Sampling density, 2012: n = 4 for each treatment, 2014: BC8 (n = 8), BC25 (n = 13), control (n = 10), different letters denote statistically significance difference between the treatment and the control within each depth and year.

3.2. Soil Aggregate Distribution and Stability

Dry aggregate size distribution was not significantly different between treatments (Figure S4). The air-dry soil aggregates did not separate easily into smaller fractions via machine sieving possibly due to a moderately high clay content (27%) and hardening during air-drying. There was no significant differences among treatments for the percentage of 2 to 6 mm water stable aggregates remaining on the sieves after exposure to wet sieving (p = 0.19).

The addition of unpyrolyzed feedstock (MC8) significantly increased soil aggregate stability in the clay swelling test (slow wetting) compared to the control (p = 0.049) while biochar treatments had no significant effect (Figure 2). Across all treatments, only slaking (fast wetting) caused unstable soil aggregates (Figure 2, Table S2). Neither biochar nor its feedstock buffered the impact of slaking (fast wetting test) (Figure 2).

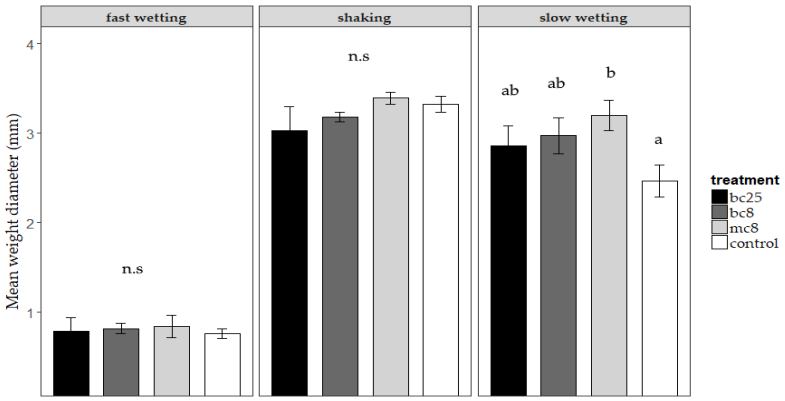

Figure 2. Aggregate mean weight diameter as influenced by slaking (fast wetting), mechanical force (shaking), and clay swelling (slow wetting). Error bars = SE for n = 4. Different letters indicate significant differences between treatments within each test ($p < 0.05$). n.s. = not significant.

3.3. Soil Resistance to Penetration

Biochar amendments did not significantly moderate soil compaction as measured via the PR outside wheel tracks ($p = 0.4$) (Figure 3a) or inside the wheel tracks ($p = 0.2$) (Figure 3b). Volumetric soil water content and BD outside of the wheel tracks on the day of PR measurements were 34% ± 2% and 1.25 g cm^{-3} for BC25 and 31% ± 2% and 1.30 g cm^{-3} for the control (not significant). Volumetric water content and BD were not measured for BC8 and MC8.

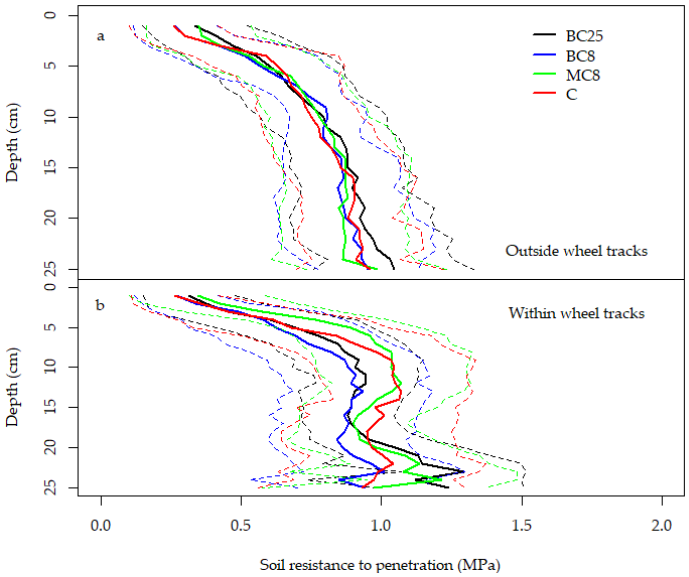

Figure 3. Soil resistance to penetration outside (**a**) and within (**b**) harvester wheel tracks on harvested plots in autumn 2015 (Solid lines are averages from four plots from where five subsamples were averaged to one measurement per plot, dotted lines are SD). There are no statistical differences between treatments for measurements taken outside and within wheel tracks.

3.4. Soil Water Content and Retention

In the 2012 growth season, average volumetric water content (VWC) was significantly higher in BC25 compared to the control (p = 0.025) while MC8 and BC8 did not differ from the control (Figure 4). Average volumetric water content for the treatments in 2012 were as follows: BC25 37.10% (±1.05%), Control 33.42% (±1.21%), BC8 32.37% (±1.21%), and MC8 31.43% (±1.21%). In 2014, only BC25 and the control were measured and the data revealed only a few days in the growth season (Figure 5) where VWC was significantly higher in BC25 than in the control plots. This coincided with precipitation events. A dry period in June and July 2014 (Table S1, Figure 5) caused soil moisture in the top 5 cm of the control plots to dip below the permanent wilting point (PWP) of 15% VWC while the biochar plots on average retained moisture above PWP and were approximately 5% points higher than the control (although not statistically significant). Soil moisture in 2014 remained above the PWP at a 15 cm soil depth and, therefore, the plant roots had probably enough water reserves at this depth to avoid wilting (Figure 5).

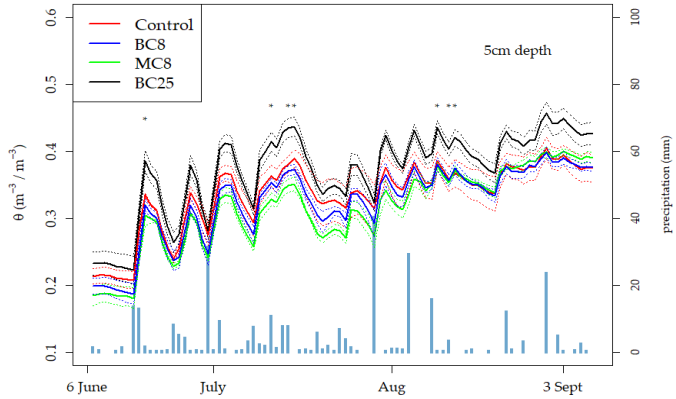

Figure 4. Soil volumetric water content in 2012 at 5 cm depth. Solid lines depict the treatment mean and dotted lines SEM, n = 3. Blue bar graph is daily precipitation in the measurement period. An asterix (*) above the lines indicates dates when there was a significant difference between BC25 and the control (only). Otherwise, treatment means were not significantly different at other dates.

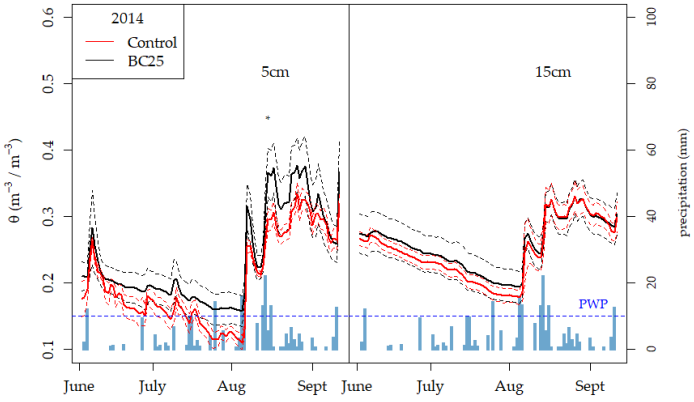

Figure 5. Soil volumetric water content in 2014 for BC25 vs. the control at 5 and 15 cm depth. Solid lines depict the treatment mean and the dotted lines SEM, n = 4. An asterix (*) above lines depicts dates when there was a significant difference between treatments. Otherwise, treatment means were not significantly different at other dates.

For the soil water retention measurements carried out on intact soil cores, there was a significant effect from SOM content across treatments on plant available water ($p < 0.001$) (Figure 6) and a significant positive correlation between SOM and both FC and PAW specifically in the BC25 treatment (Figure 6). Across treatments, θ_{sat} was positively correlated with SOM and negatively correlated with BD (Figure 7). Variation in the water retention curve start-points and end-points were greater in BC25 when compared to BC8 and the control (Figure S7). In individual samples where biochar content was high, the wet end of the soil retention curve was observably influenced (Figure 8). There was no significant difference between treatments for pore volume in the <3.5 µm or the 3.5–300 µm range.

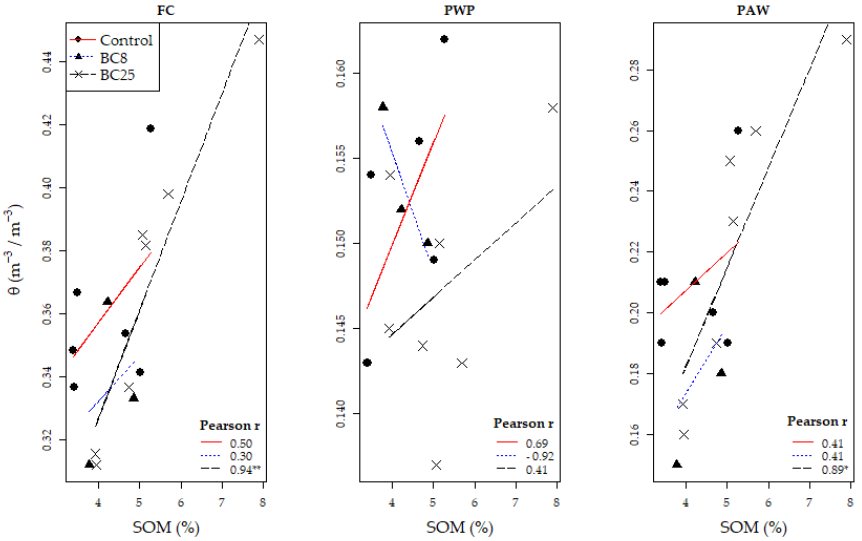

Figure 6. Field capacity (FC), Permanent Wilting Point (PWP), and Plant available water (PAW) as a function of SOM content (%) in intact soil cores from the water retention experiment.

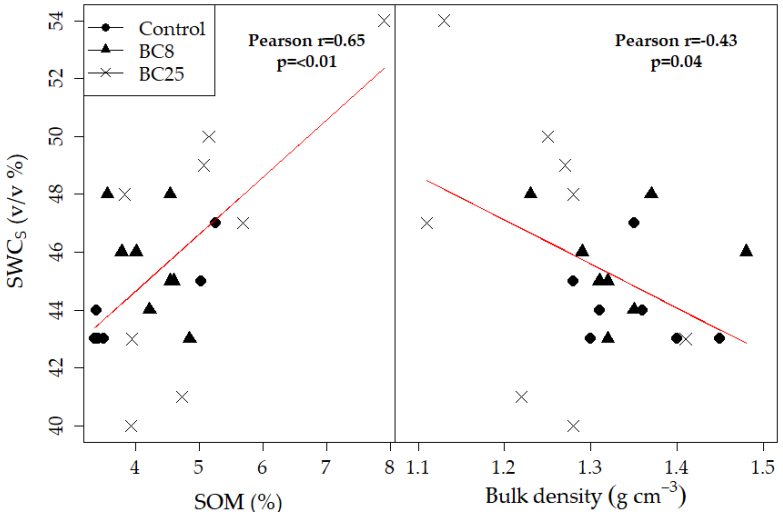

Figure 7. Pearson correlation coefficient between soil water content at saturation (SWC_s) and SOM% (left) and Bulk Density (right).

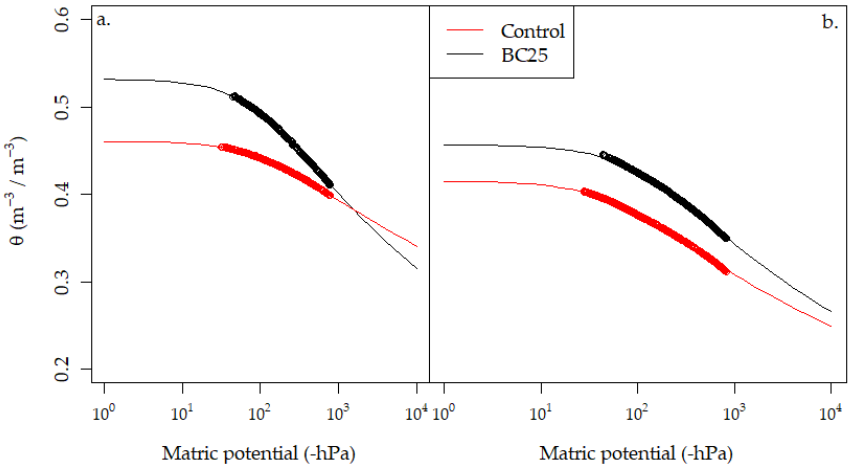

Figure 8. Soil water retention curve from selected cores with (**a**) high (5.2–7.9%) and (**b**) moderate (5%) SOM content (n = 1). Points are measured values and lines are model fits to the van Genuchten-Maulem model.

3.5. Microbial Biomass C and N and Basal Respiration

Twenty-two months after the treatment application in the field, microbial biomass C, N, and basal respiration were significantly higher in the MC8 treatment when compared to the control (p = 0.05 and p = 0.005, respectively) (Figure 9). Biochar treatments (BC8 and BC25) did not differ when compared to the control for these three measurements.

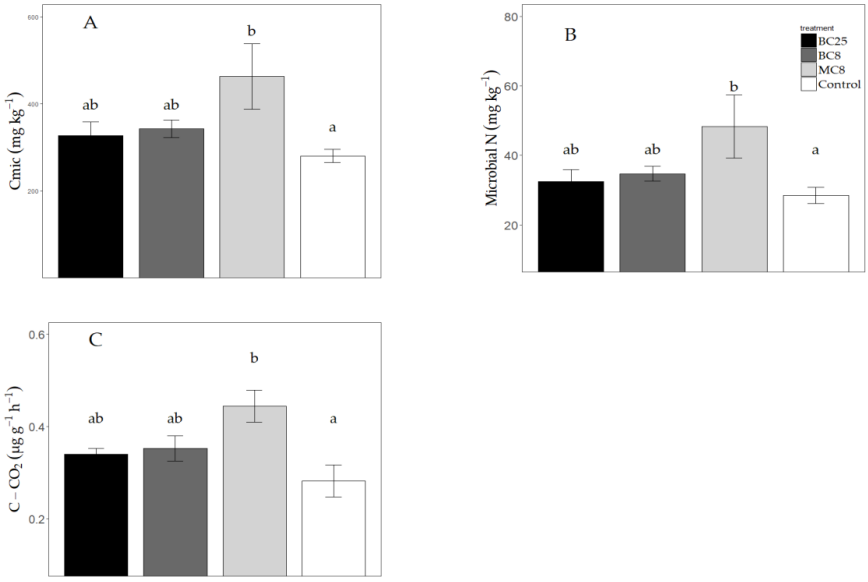

Figure 9. Microbial biomass C (**A**), microbial biomass N (**B**), and basal respiration (**C**) measured in soil samples from each treatment in 2012. ± SE, n = 4. Different letters denote significance $p < 0.05$ between treatments and the control.

3.6. Grain and Straw Yield

Grain yields from MC8, BC8, or BC25 were not significantly different compared to the control in any of the four years (2011–2014). Straw yields were also not significantly different across all years (Table 3). The reduced oat yield in 2014 compared to previous years was due to late planting, which coincides with a dry period (Table S3) and further delays plant growth and establishment. The quality of the grain, as measured by the weight of a 1000 grains and the protein content, were also not significantly modified by the biochar treatment (Table 3).

Table 3. Grain and straw yield and grain quality (2011–2014). Values are mean ($n = 4$) ± SE. No significant differences between treatment means within each year.

	2011—Oat	**2012—Barley**	**2013—Oat**	**2014—Oat**
Grain	t ha^{-1}	t ha^{-1}	t ha^{-1}	t ha^{-1}
Control	5.33 ± 0.36	3.76 ± 0.94	4.84 ± 0.16	1.92 ± 0.10
MC8	5.24 ± 0.24	3.77 ± 0.45	4.58 ± 0.17	1.93 ± 0.14
BC8	5.13 ± 0.09	4.07 ± 0.65	4.92 ± 0.24	1.88 ± 0.25
BC25	5.64 ± 0.27	3.96 ± 0.73	4.84 ± 0.11	2.04 ± 0.50
Straw	t ha^{-1}	t ha^{-1}	t ha^{-1}	t ha^{-1}
Control	3.10 ± 0.20	1.38 ± 0.19	-	0.99 ± 0.12
MC8	2.65 ± 0.22	1.66 ± 0.05	-	1.01 ± 0.86
BC8	2.96 ± 0.98	1.47 ± 0.25	-	0.94 ± 0.14
BC25	3.06 ± 0.18	1.68 ± 0.12	-	1.03 ± 0.09
1000 grain weight		g		g
Control	-	37.04 ± 0.37	-	32.56 ± 0.54
BC25	-	37.74 ± 0.16	-	33.11 ± 0.31
Grain protein		%		%
Control	-	9.85 ± 0.23	-	9.80 ± 0.44
BC25	-	10.33 ± 0.25	-	9.02 ± 0.33

4. Discussion

4.1. Biochar Suitability as a Soil C Amendment in Reference to Regulations and Standards

Miscanthus biochar was low in heavy metals and complied with the highest quality class (class 0) set for organic soil amendments under the Norwegian law (Table S3). Total C content was high (80%) and H:C and O:C ratios (Table 1), and Reference [9] were well below the respective thresholds (H:C 0.6, O:C 0.4) that characterize biochars thought to be suitable for long term soil carbon sequestration [46,47]. This biochar fulfills the minimum requirements for safety and utility as a material to increase carbon in agricultural soil. However, this cannot be generalized for other biochars and, thus, future producers in Norway are recommended to have their products tested in a like manner and compare values against the industry standards such as the European Biochar [48].

4.2. Biochar Effects on Soil Physical Properties

A biochar application rate of 1.16% (*w*/*w*) (BC25) reduced the BD of this silty clay loam by 7% while 0.38% (BC8) did not differ from the control. Our results concur well with the meta-analysis of 463 studies by Reference [4] where they calculated an average reduction in BD of 7.6% due to the biochar application. Between 2012 and 2014, variability in BD among intact BC25 cores decreased, which suggests that the biochar became more evenly distributed in the soil over time. Evidence of this can be seen from photographs taken in 2015 compared to 2011 (Figure S1A–C). Reduction in BD after biochar addition is reported to be due to more than just the dilution of the soil with lower density biochar [49] and is likely attributable to the irregular-shaped biochar particles altering the

packing arrangement of mineral soil particles where macropores are formed, which was shown in Reference [12]. From BD and MD data, we attribute 7% of the reduction in BD to mass dilution and 93% to increased intra-particle and inter-particle porosity caused by a biochar addition in our study. The increased porosity is the likely explanation for observed increases of θ in BC25 as measured by TDR in the field in 2012. While there were no statistical differences between treatments for PAW, we did find that SOM content influenced PAW and that samples high in biochar content increased θ at the higher matric potential levels. An obvious benefit of greater soil water retention in dry conditions is an extension of time before which plants experience water deficit stress. Under wetter conditions, increased water retention can also help to reduce water surface runoff and erosion [50]. However, one disadvantage may also be a delay in soil drying, which can put soils at risk of compaction if tractors are driven on wet soils [51].

Biochar did not affect penetration resistance as expected. Penetration resistance tests are used frequently by agronomists to assess the degree of soil compactness, impediments to root growth, and surface crusting. Soil compaction is an increasing concern in Norway since it is estimated to reduce yields by 6% to 20% when compared to optimal soil conditions [52]. Compaction problems have intensified in recent years due to the wetter soils under a wetter climate [53], outdated drainage systems, and the increasing use of heavier tractors [54]. In our study, the soil was not excessively compacted with PR values below 2 MPa, which has been observed as a critical limit for root growth in a variety of soils [55]. Previous studies such as Reference [56] and Reference [20] have observed reductions of up to 66% in PR with biochar additions, but these were both in repacked soil cores at a lab scale and where two to 10 times more biochar was applied when compared to our study. These amounts may be unrealistic and uneconomic for farmers to apply. Biochar may have more utility in ameliorating compaction in subsoils where it has been shown to support greater root growth and water retention [57] provided practical methods are developed to incorporate biochar at lower depths.

Biochar had a limited effect on aggregate stability in our experiment probably due to the fact that this soil was well aggregated from before with sufficient levels of soil organic matter (~5%), moderate clay content (27%), and abundant earthworm activity, which are all factors known to improve soil aggregation [50]. Only the MC8 treatment significantly increased MWD under slow wetting (Figure 2). This suggests that the labile C in *Miscanthus* straw and the subsequent promotion of microbial activity was the primary driver of increased aggregate stability. Similarly, labile C sources from crop residues have been reported to increase soil aggregation more than changes in soil properties induced by mulching (Rasse, Smucker, and Santos, 2000) [58]. By contrast, the miscanthus biochar used for the field experiment is highly stable [9], which probably explains the absence of effect on microbial biomass and, therefore, the absence of significant effect on soil aggregate stability.

We later identified that some of the 2–6 mm aggregates that were wet sieved from the BC25 treatment were clay encrusted biochar particles appearing to be soil aggregates in the 2–6 mm range (Figure S4). These washed biochar particles were removed (the washed and ejected biochar from BC25 weighed an average of 1.2% of the aggregate weight and, therefore, did not overly influence the end MWD result for BC25) from the sample after sieving and, thus, lowered the MWD of BC25. In future experiments, selecting a larger aggregate size class e.g., 2 to 20 mm would allow for greater occlusion of larger biochar particles in aggregates. The MWD of aggregates in clay swelling and mechanical breakdown tests remained over the 1.3 mm threshold of Le Bissonnais' MWD stability index (Table S2). Slaking had the greatest impact on aggregate breakdown and was not moderated by the addition of biochar (Figure 2). Slaking was also reported as the main aggregate breakdown mechanism in biochar studies conducted by References [11,17]. In the study by Sun and Lu, 2014 [11] very high gravimetric concentrations of 4% to 6% were needed in order to improve resistance to slaking while a lower biochar dose (2%) actually increased slaking compared to the control.

4.3. Effects on Microbial C and N and Basal Respiration

Straw significantly increased microbial biomass and basal soil respiration (lab incubation) while biochar had no effect. These results are consistent with soil respiration measurements (field chamber measurements) taken from the same experimental site as this study in 2011 and 2012 and which showed no significant difference between biochar and control plots [9]. In another study under temperate conditions, microbial abundance was also unaffected three months after the addition of 30 t ha^{-1} (approx. 1.2% *w*/*w*) Miscanthus biochar [59]. Gomez et al. [24] report that microbial abundance as estimated by phospholipid fatty acids (PLFAs) only differed from the control at biochar rates >5% (*w*/*w*) and not at 1%, which suggests that high application rates of biochar are needed to affect microbial biomass in soils. A similar trend was observed by Reference [60] where biochar applications between 5–10% *w*/*w* in soil significantly increased microbial activity (substrate induced respiration and basal respiration) but not so for 1% and 2.5%. A 5–10% *w*/*w* concentration in laboratory soil incubation equates to field application rates of 150–300 t ha^{-1}, which is unrealistically high for a single application and would be too expensive for farmers to apply without significant carbon subsidies [61]. The limited microbial response from the addition of significant amounts of carbon to the soil supports the claim that biochar-C is difficult for microbes to use as an energy source and provides a means to store more carbon in soil without having to simultaneously add extra amounts of N to satisfy plant and microbial needs. Increasing biochar C application by three times (BC8 vs. BC25) did not lead to any difference in Microbial N, which confirms the high stability of this biochar C as reported in Rasse et al. [9].

4.4. Grain Yield and Quality

As biochar is a material with high C content, some farmers may be concerned that adding large amounts to their soils (the amount of biochar in BC25 applied in our experiment is 10 times that of yearly straw produced from the same land area) may lead to a microbial immobilization of N and, thus, reduce plant yields. While some studies have shown short-term N immobilization that restricts plant growth [62,63], we did not observe this under field conditions over four years where even the higher BC treatment did not reduce grain, straw yields, and grain protein or increase Microbal C or N. A lack of yield response after the biochar amendment concurs with other studies in boreal and temperate regions. Reference [64] found no yield differences in wheat or faba bean after applying spruce and pine biochar at 10 t ha^{-1} in a three-year field experiment in Finland and a similar absence of yield effect was found in a two-year field ring trial carried out in seven different countries in Northern Europe [65]. However, increased hay grass yield was observed in the two last years of a three-year field trial in Wales [66]. Jones reports that yield increases were attributed to increased water holding capacity from the biochar during a dry spring planting season. Short-term dry periods are also common in the Norwegian spring and we hypothesized that the extra water holding capacity of biochar would translate to higher crop yields. This, however, was not the case for this loamy soil, which had a sufficient amount of available water for plants throughout the season, which was observed from TDR measurements at 15 cm. In general, biochar is expected to increase agricultural productivity when it alleviates a limiting factor for plant growth such as water stress or soil acidity [67]. The extent to which biochar can do this depends on the type of biochar, the soil environment, and the cropping system. The absence of a yield response to biochar in our study suggests that none of these factors were a constraint to production in our field. However, other soils in Norway with reduced water holding capacity might benefit from the addition of biochar. Sandy soils characterize 10–15% of the agricultural soils in the main grain growing counties and up to 30% in irrigated vegetable growing counties in Norway [68] and could be a potential target area for future biochar applications and testing. With regard to grain quality, the unchanged levels of grain protein in our study concurs with the results from Reference [65] where six of the seven countries participating in a two-year field trial reported no significant differences in grain protein in biochar amended plots (20 t ha^{-1}) when compared to the control. We participated as one of the seven countries in a field adjacent to the one used in the current study and, in 2012, the barley grain yield or quality did not differ between biochar and control.

5. Conclusions

Application of pure *Miscanthus* biochar at 8 and 25 t ha^{-1} to a clay loam did not increase crop yields over four years in a temperate climate even in the 2014 season when biochar contributed to greater soil water content during a prolonged dry spell. One of the aims of our study was to see whether there were sufficient soil improvement benefits of applying biochar (apart from carbon sequestration), which would encourage farmers to use it. We observed that there was higher plant available water with greater amounts of added biochar but no differences in aggregate stability. Microbial activity or Microbial N were not significantly stimulated by either biochar at low (BC8) or high (BC25) application rates, as compared to straw, which confirms that microbial immobilization due to a high-C substrate was not a concern with this *Miscanthus* biochar. This means that it is technically possible to add significant amounts of biochar to agricultural soil to increase soil C and mitigate climate change without it negatively affecting grain yields. However, without yield increases or subsidies for carbon sequestration, there would be little incentive for farmers to use biochar until revenues exceed costs. Therefore, in order to realize the biochar's climate mitigation potential, we suggest future research and development efforts should focus on improving the agronomic utility of biochar in the engineered fertilizer and soil amendment products.

Supplementary Materials: The following are available online at http://www.mdpi.com/2077-0472/8/11/171/s1, Figure S1: (**A**) Images of concentrated seams of biochar in soil profile caused by inverse ploughing of surface applied biochar. (**B**) Image of biochar distribution on soil surface in April 2012; Figure S2: Aggregate size distribution from machine dry sieving; Figure S3: SEM images of fresh Miscanthus biochar showing the porous surface and irregular shape; Figure S4: SEM image of weathered Miscanthus biochar collected after 5 years of field incubation. Evidence of partial clogging of surface micropores with soil particles; Figure S5: Approximately 15 g of 2–6 mm sized aggregates placed in small sieves prior to pre-wetting and wet sieving; Figure S6: Illustrative evidence of "Biochar patchiness" or "hot spots" in field samples; Figure S7: Water retention curves for each intact soil core in each treatment and depth with corresponding SOM content for each soil core; Figure S8: Linear regression equations for calibrating the TDR sensors; Table S1: Mean air temperature (°C) and monthly precipitation (mm) in Ås, Norway for four growing seasons (2011–2014) compared with normal (1961–1990) monthly averages; Table S2: Classes of stability and crustability, according to MWD values (reprinted with permission from [38]); Table S3: Maximum limit for heavy metals under Norwegian law for soil improvement materials of organic origin.

Author Contributions: A.O. and D.P.R. designed the experiment. A.O. collected field data, A.S. conducted Aggregate Stability tests, and M.C. and B.B. conducted microbial analysis. C.M. assisted with field work and pore-size distribution calculations and statistical analysis. S.W. assisted with water retention curve data and analysis. A.O. wrote the paper with input and comment from authors especially D.P.R. who co-supervised this work.

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Conflicts of Interest: There were no conflicts of interest between the authors and the subject of study in this article.

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Paper II

Paper II: The effect of aged vs new biochar on fluxes of N_2O and CH_4 over 2 years in a silty-clay loam

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Highlights

- *New biochar had twice the suppressive effect of aged biochar at peak N_2O emission events compared to the control during wet denitrifying conditions*
- *Aged biochar lost its alkalinity over 4 years which likely contributed to its lower suppression of N_2O*
- *CH_4 flux was not significantly affected by biochar addition in two field seasons*

Abstract

Biochar can reduce N_2O emissions but there is still uncertainty about the duration of this effect. We compared the effect of aged (BC-Aged) vs new miscanthus biochar (BC-New) on N_2O and CH_4 fluxes over two field seasons in a temperate silty clay loam. BC-New significantly reduced N_2O by 80% and BC-Aged reduced N_2O by 41% (not significant) compared to the control during an emission peak in 2012. Cumulative N_2O emissions in 2012 growing season were 45% ±9 lower in BC-New and 21% ±7 lower in BC-Aged compared to the control, but the differences were not significant. BC-Aged decreased in pH from 10 to 5.5 over 4 years, which may explain why it had less N_2O mitigation effect than BC-New in wet conditions. In the growing season of 2014, N_2O emissions were 2-6 times lower than in the growing season of 2012 most likely due to drier conditions which decrease denitrification-related N_2O production. In a dry period after fertilization in 2014 where nitrification related N_2O was likely dominant, BC-New soil emitted about 3 times as much N_2O as the control, while BC-Aged had no significant effect. We also measured N_2O at 10 cm and 20 cm depth in the soil. Soil N_2O

concentration at 10cm was more positively correlated with surface N_2O flux in the control compared to biochar treatments, suggesting that physical sorption of N_2O to biochar also played a role in N_2O mitigation. Biochar did not significantly affect CH_4 flux in our study, contrastingly to some studies reporting increased CH_4 uptake rates in mineral soils. Overall, the general trend was towards lower GHG emissions in biochar amended soil. Our findings give greater confidence to current agricultural policy initiatives in Norway which support the use of biochar as a climate change mitigating practice.

Keywords: Biochar and N_2O emissions, aged biochar

Abbreviations: BC=Biochar

1. Introduction

Biochar has been the focus of considerable research effort in the last decade with the main aims to assess its efficacy as a climate change solution (Smith, 2016; Woolf et al., 2010) and to improve the productivity of soils (Jeffery et al., 2017). Biochar is the solid fraction produced after heating of biomass in an O_2-free or O_2-depleted atmosphere and is intended for improving soils or used in environmental applications (Lehmann and Joseph, 2015). Biochar C is more resistant to microbial decomposition compared to its parent feedstock and thus persists longer in the soil (Lehmann et al., 2015). In addition to soil C sequestration benefits, multiple meta-analyses report that biochar can reduces N_2O flux by -9 to -54% (Borchard et al., 2019; Cayuela et al., 2014; Verhoeven et al., 2017). This mitigation effect is due to a variety of mechanisms, including: N_2O adsorption in biochar pores (Cornelissen et al., 2013), N_2O abiotic reduction on organo-mineral biochar surfaces (Quin et al., 2015), improved metabolic conversion of N_2O into N_2 as biochar increases pH (Weldon et al., 2019), and increases in microbial C efficiency which frees up energy for denitrifiers to reduce N_2O (Zhang et al., 2021). Parallel to the question of why biochar reduces N_2O emission is the question of how long this suppression may last. Indeed, most studies to date use freshly produced biochar and measure N_2O over short periods of weeks or months, and several reviews have highlighted the knowledge gap regarding the permanence of biochar GHG

mitigation potential in soil (Kammann et al., 2017; Kuppusamy et al., 2016, Verheijen et al., 2014).

Biochar effects on CH_4 fluxes have been less studied than those of N_2O fluxes. The meta-analyses carried out to date report contradictory effects of biochar on CH_4 over different soil types and agricultural systems. In the meta-analysis by Jeffery et al. (2016), biochar reduced CH_4 emissions in flooded paddy and acid soils (Hedges d=-0.87), while reducing CH_4 uptake rate of neutral-pH and upland soils (Hedges d = 0.62). Methane-flux reductions were more pronounced with high-temperature biochars (>600 °C), which have greater surface area and less labile carbon (ibid). In another meta-analysis, Cong et al. (2018) found no effect of biochar on CH_4 emissions and uptake in neither paddy nor upland soils. In contrast, the meta-analysis by Ji et al. (2018) found 12% and 72% decrease in CH_4 emissions in paddy and upland soils, but 84% decrease in CH_4 uptake in upland soils.

We previously confirmed the high C stability of a miscanthus biochar C under temperate conditions over two seasons (Rasse, et al 2017). In the present study we provide further information on the climate impacts of biochar by measuring N_2O and CH_4 from the same field site 2 and 4 years after the biochar was applied and compared this with fresh applications of the same biochar in two field seasons in order to assess overall GHG impacts of new vs aged biochar. Based on results from previous studies, we hypothesized that the new biochar would mitigate GHG emissions more than the aged biochar. Our study reports the first field data collected in Norway to assess the effect of biochar on non-CO_2 emissions and thus provides important information to assess its GHG abatement potential in Norway and other countries with similar pedo-climatic conditions.

2. Materials and Methods

2.1 Field site description and experimental design

The field experiment was set up in September 2010 at the Norwegian University of Life Sciences (NMBU) field station in Ås, Norway (59° 39' 51" N 10° 45' 40" E). The mean average temperature (1991-2020) is 6.3 ◦C and the average annual rainfall

(1991-2020) is 886 mm. The soil is a silty clay loam Albeluvisol (WRB classification) with an average content of 27% clay, 43% silt and 30% sand. The biochar was produced from *Miscanthus giganteous* straw by Pyreg Gmbh (DE). Further characterization of the biochar and soil is reported in O'Toole et al. (2018) and included in Table S1. The experimental design consisted of a randomized complete block design with 3 treatments and 4 replicate plots per treatment with an area of 32 m^2. For this study measurements were taken in 2012 and 2014. The treatments were:

(1) ***Control*** (no biochar)
(2) ***BC-Aged***: *Miscanthus* biochar, 31.5 t ha^{-1} or 1.12% (w/w) in 0-23 cm depth in soil, applied in 2010
(3) ***BC-New:*** *Miscanthus* biochar, 31.5 t ha^{-1} or 1.8% (w/w) in 0-15cm depth in soil, applied in new plots in both 2012 and 2014

BC-New had higher biochar soil concentrations than BC-Aged because incorporation depth of BC-New was 0-15cm, whereas BC-Aged incorporated within 0-23 cm plough layer due to subsequent tillage operations. Miscanthus was chosen as a feedstock because it is a C4 plant with contrasting $\delta^{13}C$ to the C3 plant dominated soil carbon and enabled quantifying biochar content in soil based on stable isotopic methods, as previously reported in Rasse et al. (2017). Biochar was applied manually to plots in September 2010 and ploughed into the soil to a depth of 23 cm. In 2012 and 2014 new plots were established and miscanthus biochar was applied again using the same amount and method as in 2010. The 2012 plots used the same biochar which had been stored in a bag since 2010, and the 2014 plots used a new batch from the same supplier. Barley 'Heder' was grown in 2012 and Oat 'Belinda grown in 2014. In both years the field was fertilized with 550 kg ha^{-1} of (Yaramila Fullgjødsel ™ NPK 22-3-10, Nitrate 10%, Ammonium 11.6%) corresponding to 120 kg N ha^{-1}. In 2014, an additional 30 kg N ha^{-1} (16-19 $CaNO_3$ Yara Tropicote ™) was applied as top dressing fertilizer on the 05.07.14.

2.2 Greenhouse gas (GHG) flux measurements

Greenhouse gas flux measurements were taken between May and September of 2012 and (10 measurement days) and 2014 (16 measurement days) from closed static

chambers. The static chamber method was used according to Pumpanen et al. (2004). Aluminium collars (60 cm x 60 cm x 20 cm) were driven into the soil in each plot. At the time of measurement, aluminium chamber tops (60 cm x 60 cm x 20 cm) were installed on the collars and 4 gas samples were taken at time points 1, 15, 30 and 45 minutes, using a syringe and injected into evacuated vials. Temperature inside and outside the chamber was recorded with a digital thermometer after 45 minutes. Measurements were conducted block wise so that each treatment plot was included within a 1 hr time window, in order to reduce differences in flux due to diurnal variations. Chamber height extensions were used from July-September to allow for chamber tops to be placed over mature grain stand. Gas vials were analysed for CO_2, N_2O, and CH_4 with a gas chromatograph (GC) equipped with an automatic sampler that facilitated high throughput measurements. The GC (Model 7890A, Agilent, Santa Clara, CA, US) was equipped with 20 m wide-bore (0.53 mm diameter) Poraplot Q column, 30m5 Å mol sieve (0.53mmdiameter), 2 HayeSep columns for backflushing water, a thermal conductivity detector (ECD) for analysing N_2O, a flame injection analyser (FID) for analysing CH_4. Gas production rates were computed from raw data following Molstad et al. (2007). Flux calculation methods used in this study are described in more detail by Nadeem et al. (2012).

2.3 GHG concentration in soil profile

Two soil air probes were installed at 10 and 20 cm depth and <30 cm distance from each flux chamber to measure N_2O concentrations in the soil as described in Nadeem et al. (2012) and Russenes et al. (2019). In brief, the air probe consisted of a PVC electrical conduit tube with a polyethylene porous cup glued to its end. A Teflon tube was fed down the PVC tube and connected to the porous cup, and glued to make an air tight fit. On the end of the Teflon tube a 3- way stop cock was connected to the Teflon tubing for taking soil gas samples with a 50 ml syringe. Probes were inserted at into the soil on a 60° angle in order to minimize preferential vertical gas flow along the tube wall and to prevent water running along the tube and directly into the sampling hole.

2.4 Soil *sampling and chemical analysis*

Soil samples were collected on the same day as flux measurements were conducted using a 2 cm D soil auger. Ten sub-samples were taken to a depth of 23 cm and aggregated to make one sample per plot. Soil NH_4 and NO_3 were determined by Flow Injection Analysis (FIAstar5000, SoFIA) after extracting 10 g field moist soil with 50 ml 2 M KCl. Parallel 40 g soil samples were oven dried to determine soil N concentrations per dry weight basis. Ammonium and NO_3 levels which were below the limit of detection (LOD) were replaced with estimates given as $LOD/\sqrt{2}$ (Tekindal et al., 2017). Soil pH and electrical conductivity (EC) were measured with 10 g of field moist soil which was shaken for 1 hour in 50 mL of distilled water and left to stand for 3 h before measurement with EC followed by pH electrode connected to a pH meter (Orion Dual Star pH/ISE benchtop, Thermo Scientific).

2.5 Characterization of aged vs fresh biochar

New and Aged biochar pH was measured in distilled water using a 1:5 (w/w) ratio. Aged biochar was retrieved from the field in March 2014, and separated from soil via floatation in water and sieving. Soil bulk density at 0-10 cm and 10-20 cm was measured 3 times in each plot during 2012-2014. Surface morphology and changes in surface elemental content in new and aged biochar particles were carried via Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM–EDX). The equipment used was a Zeiss EVO—50—EP equipped with X-ray Type INCA 450 Xstream/Mic. The voltage was set to 30 kV, probe current 320 pA. Three crushed and 3 intact particles were analysed from both new and aged biochar. Only sampled area regions (and not specific points of interest) were used to obtain particle averages. Surfaces were not coated prior to analysis.

2.6 Soil Water Content in the Field

Soil water content was measured every hour between April-October of 2012 and 2014 using Time Domain Reflectance (TDR) soil moisture sensors (5TM model, Decagon Devices, Inc., Pullman, WA, USA [now Meter Environment]). One TDR was inserted horizontally in undisturbed soil at 5 cm and 15 cm depth of each plot. Further description and calibration method for sensors is described previously in O'Toole et al., 2018. Volumetric water content was converted to water filled pore space (WFPS),

which is a more commonly used soil moisture unit for explaining variation in N_2O. The material density for control soil was assumed to be 2.65 g cm^{-3}, and the material density of biochar-soil treatments were down-adjusted to 2.64 g cm^{-3} for BC-Aged, and 2.63 g cm^{-3} for BC-New to account for the lower material density of the biochar. We assumed a material ("skeletal") density of biochar of 1.6 g cm^{-3}, as reported by Brewer et al. (2014) for miscanthus biochar produced at 450° C in an auger reactor, which is very similar to biochar and its production method. Bulk density and material density were used to derive soil porosity

2.7 Data analysis and statistics

All statistical analyses were carried out using R Software v.4.0.4 (R Core Team, 2020). The cumulative emissions were calculated by linear interpolation between flux measurements and then the final cumulative measurements were analysed with one-way ANOVA and post hoc Tukey tests ($p < 0.05$) to assess difference between treatment means. Due to the variability in N_2O and CH_4 fluxes in time and space, mixed model analysis was used to better isolate explanatory independent variables of interest from random variables such as plot location. Nitrous oxide fluxes and N_2O concentrations at 10 cm and 20 cm depth were initially tested with a linear mixed model but did not conform to assumptions for linear regression, and thus a generalized linear mixed effects model (GzLMM) was used and implemented via the 'glmer' function from the 'lme4' package (Bates et al., 2015). The model was built up in a step-wise manner to test explanatory strength of independent variables. Candidate models were selected according to the lowest corrected Akaike information criteria (AICc) and a $\Delta AICc < 2$ (Burnham and Anderson, 2002). Independent variables included in model optimization were treatment, NO_3, NH_4, pH, WFPS, soil temperature, electrical conductivity, 2 and 7 day accumulated precipitation, and CO2 flux (dark respiration). Random variables were plot and sampling day. The output of the glmer models were exponentiated to derive odds ratios (OR) of the predictor variables and p values were derived from Wald Z tests. A quasi R^2 developed for testing the goodness of fit of mixed models (Nakagawa and Schielzeth, 2013) was used via the 'r.squaredGLMM' function: 'MuMIn' R package (Barton, 2019). For WFPS and CH_4 data a linear mixed effects model (lmer function: lme4 package) was used and for NO_3 data a GzLM (glmer function: lme4

package) was used. Soil pH data, which had less observations, were combined per plot per season and one way ANOVA was done to assess treatment differences. For all models, post-hoc tests were conducted to assess residual normality using QQ plots and the Shapiro-Wilk test. Where outliers affected residual normality, outlier detection and removal was conducted iteratively with the Grubb's test (Gubbs, 1950) and carried out with the 'grubbs.test' function: 'outliers' package (Komsta, 2006).

3. Results

3.1 Biochar effect on N_2O fluxes in 2012 and 2014

Mean N_2O fluxes in 2012 ranged from 11-271, 16-160, and 6-96 μg m^{-2} hr^{-1} in Control, BC-Aged and BC-New respectively (Fig. 1). At one peak emission date (Sept 28th), BC-New significantly reduced N_2O by 80% ($p<0.001$) compared to the control while BC-Aged reduced N_2O by 41% ($p=0.48$, not significant) compared to the Control (Fig.1). Cumulative N_2O emissions in 2012 growing season were 45% ±9 lower in BC-New and 21% ±7 lower in BC-Aged compared to the Control, but the differences were not significant (Fig. 2). In 2014, N_2O emissions were 2-6 times lower than in 2012, ranging from 2-41, 2-33, and 2-44 μg m^{-2} hr^{-1} for Control, BC-Aged and BC-New respectively (Fig. 3). On the 2nd and 3rd measurement taken within a week after fertilization, BC-New soil emitted 258% and 350% more N_2O than the control. However, a reverse effect was found on the 2nd and 3rd last measurement days of the season where BC-New emitted 60% and 61% less N_2O than the control. At the end of the 2014 season, cumulative N_2O emissions were relatively unchanged between treatments with BC-Aged 5% less and BC-New 0.62% less then Control (Fig. 2).

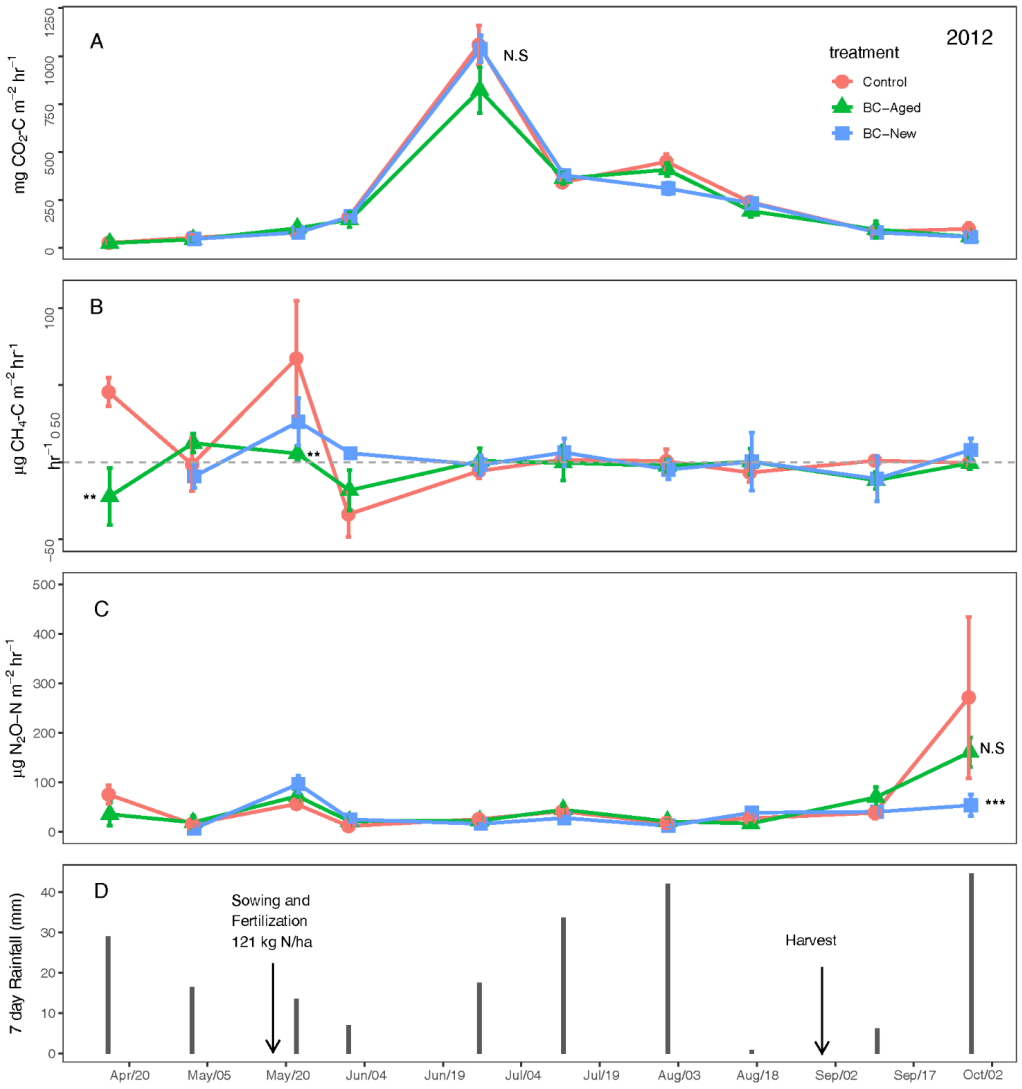

Fig. 1. Soil greenhouse gas emissions (Mean,±SE, n=3) A. Carbon Dioxide B. Methane C. Nitrous Oxide and D. 7 day accumulated precipitation at measurement days in the growing season of 2012. *Statistically significant ($p<0.05$) treatment compared to the control. N.S = denotes no statistical significance at any measurement date.

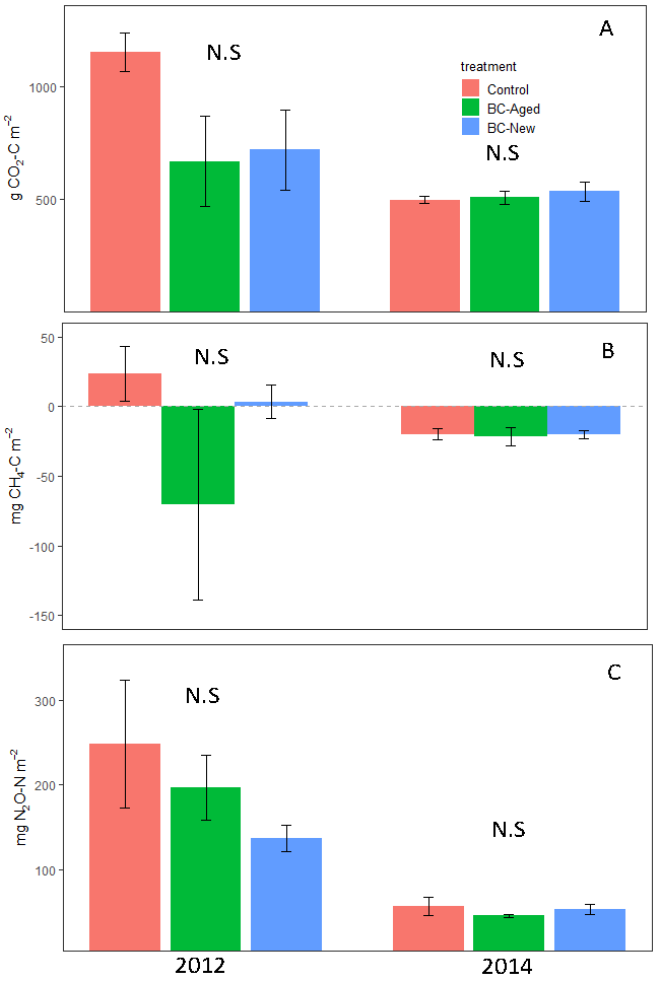

Fig. 2 Cumulative GHG emissions in 2012 and 2014 for (A) Carbon Dioxide (Dark respiration) (B) Methane (C) Nitrous oxide emissions from soil. Error bars are standard error. n= 3 (2012), n=4 (2014). N.S = No significant difference between treatments.

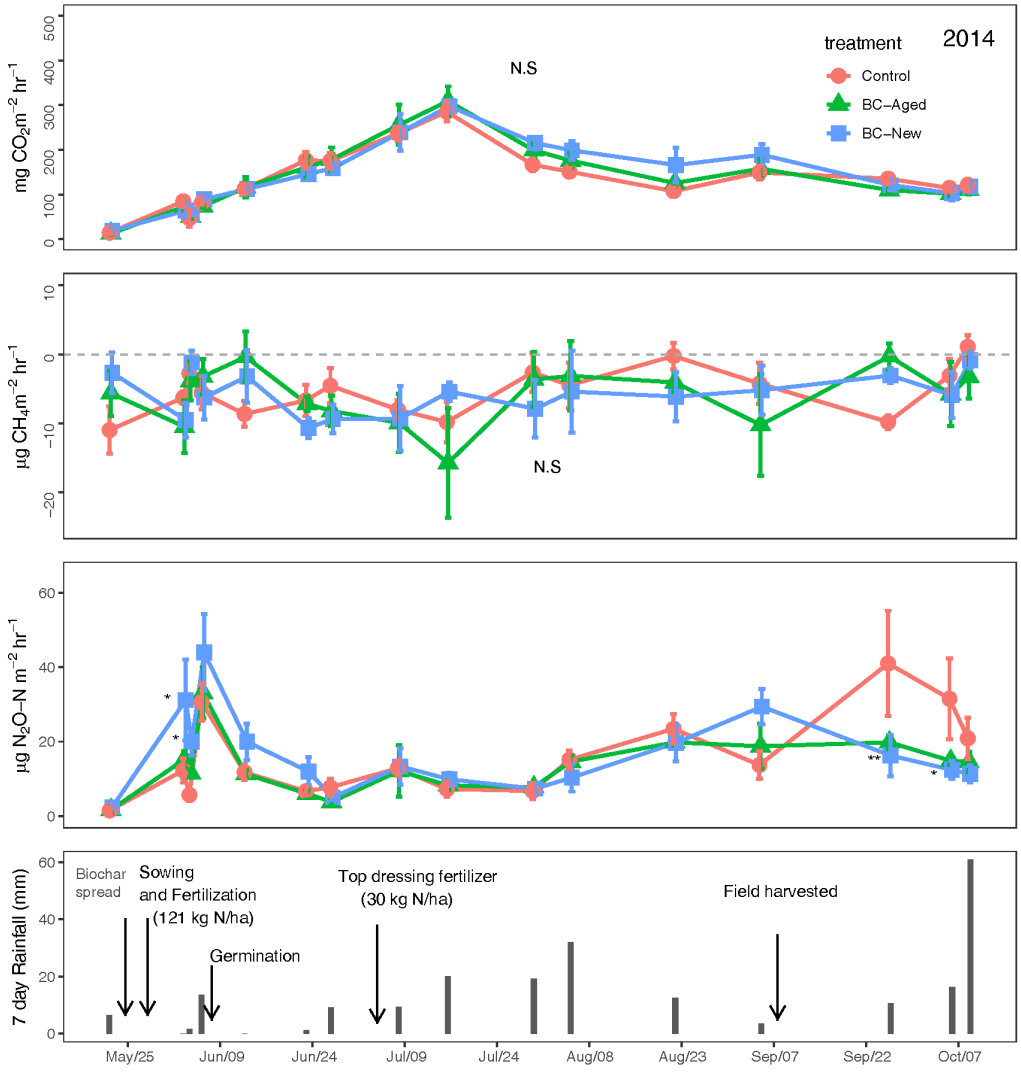

Fig. 3. Soil greenhouse gas emissions (Mean,±SE, n=4) and 7 day accumulated precipitation at measurement days in the growing season of 2014. *Statistically significant ($p<0.05$) treatment compared to the control. N.S = denotes no statistical significance between treatments at any measurement date

3.2 Soil N_2O concentrations at 10 cm and 20 cm depth

There was no significant difference between treatments in pore space N_2O at 10 and 20 cm depths (Fig. S1). However, there was a strong positive correlation between N_2O produced in the soil and that which was emitted from the surface in the Control

(Pearson R 0.74, p<0.001), which was not the case for BC-Aged (Pearson R 0.15, p=0.55) or BC-New (Pearson R 0.29, p=0.30) (Fig. 4).

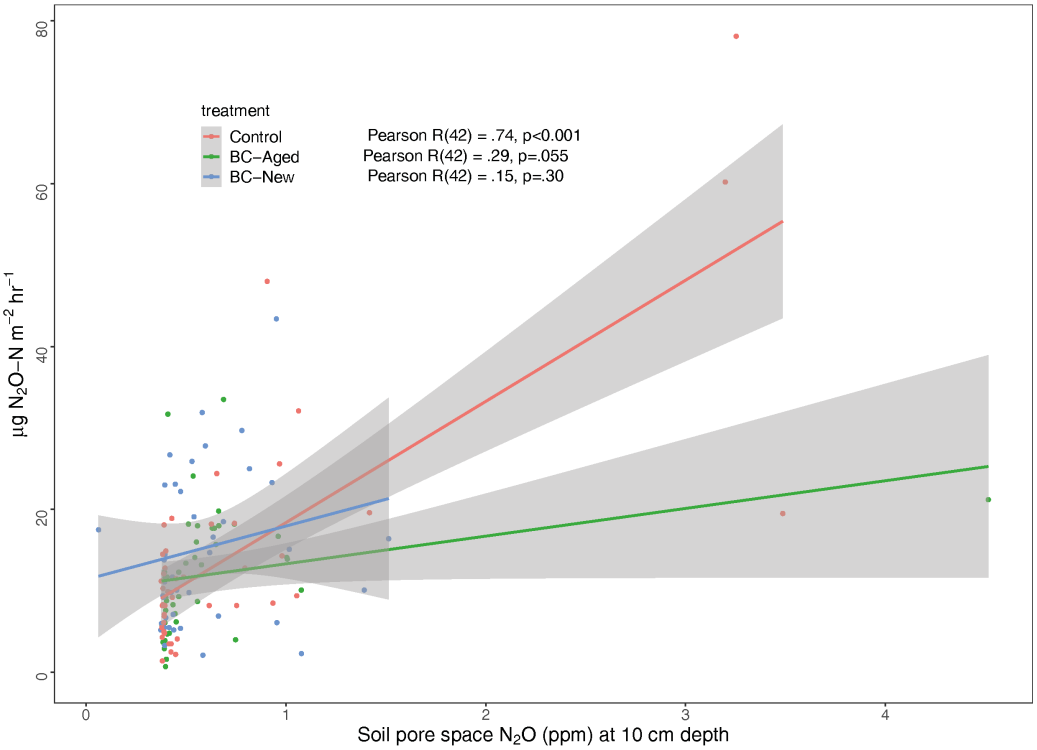

Fig. 4. Correlation between N_2O concentration at 10 cm depth in the soil and N_2O flux at the soil surface.

3.3 Controls of N_2O flux

The GzLM had a R^2_{GLMM} of 0.32 in 2012 and 0.47 in 2014 indicating that the best fitting independent variables (soil NO_3, NH_4, pH, WFPS, and treatment) had a weak to moderate explanatory power of the variation in N_2O response. In 2012, GzLM predicted that N_2O emissions approximately tripled as NO_3-N increased from 0-40 mg kg^{-1} (Fig. 5, Table S.2). Conversely, modelled N_2O flux significantly decreased (p=0.02) by 17% as soil pH increased by 1 pH unit from 5.8 to 6.8 (Fig. 5). Despite the model predicting a 15% reduction in N_2O flux in 2012 due to the presence of both BC-Aged (p=0.29) and BC-New (p=0.28), the reduction was not significant. In 2014, N_2O fluxes were mostly influenced by WFPS at 5cm and the presence of BC-New. As WFPS increased from 10-

60 %, N_2O was predicted to approximately double (p=0.035) (Fig. 6). The model predicted that BC-New increased N_2O emissions by 151% in 2014 (p<0.001) whereas BC-Aged did not significantly affect N_2O emission (+13%) (p=0.465) (Fig. 6, Table S.1). Ammonium and pH were not significant explanatory factors for N_2O in 2014 (Fig. 6).

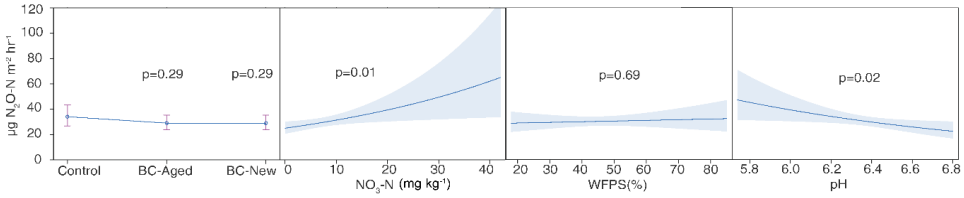

Fig. 5. Predicted response of N_2O fluxes to treatment, NO_3-N, WFPS (%) at 5cm depth and pH in 2012 (by GzLM model).

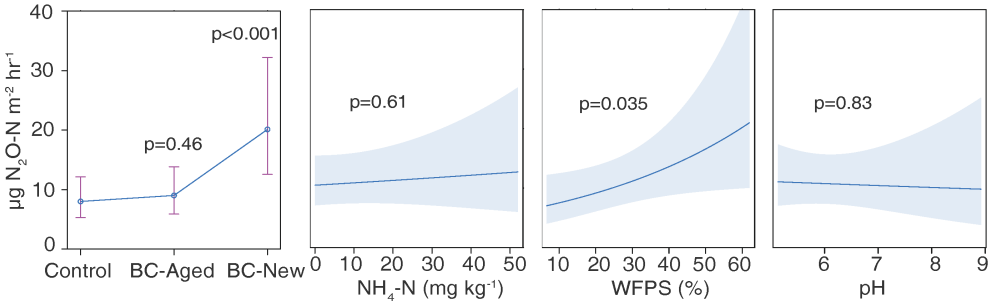

Fig. 6. Predicted response of N_2O fluxes to treatment, NH_4-N, WFPS (%) at 5cm and pH in 2014 (by GzLM model)

3.4 Biochar effect on CH_4 fluxes in 2012 and 2014

In 2012, mean CH_4 fluxes ranged from -33 to 67, -22 to 12, and -18 to 26 μg CH4-C m^{-2} hr^{-1} for Control, BC-Aged, and BC-New respectively (Fig. 1). Repeated measure analysis using the mixed model approach, found only significant treatment differences on measurement day 1 and 3, when BC-Aged emitted 148% and 91% less CH_4 than the control respectively (Fig. 1). There were no significant treatment difference for mean season cumulative CH_4 flux in 2012. On average, BC-Aged, acted as a sink for CH_4 (-70 ± 68), while Control (23 ± 33) and BC-New (3 ± 20) were sources of CH_4 (Fig. 3B). In 2014, the soil acted mostly as a sink for CH_4, with mean CH_4 uptake ranging from -11 to 1, -16 to -0.3, and -11 to -0.8 μg m^{-2} hr^{-1} for Control, BC-Aged, and BC-New (Fig. 1).

There was no significant differences between treatments for CH_4 cumulative flux in 2014 (Fig. 3B).

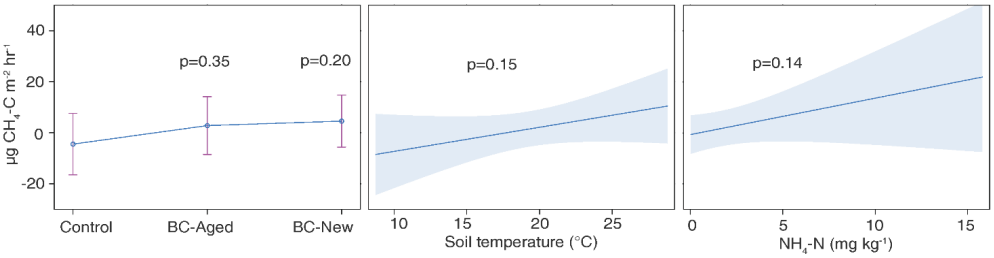

Fig. 7. Predicted response of CH_4 fluxes to treatment, soil temperature and soil NH_4 in 2012 (by Linear mixed effects model)

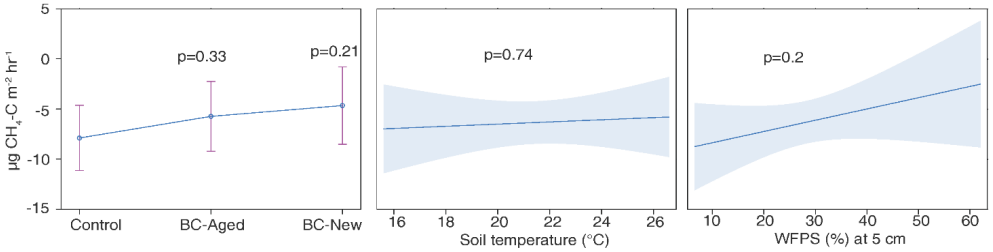

Fig. 8. Predicted response of CH_4 fluxes to treatment, soil temperature and WFPS (%) in 2014 (by Linear mixed effects model)

3.5 Controls on CH_4 flux

The linear mixed effects model used for CH_4 flux analysis had a R^2 of 0.2 both in 2012 and 2014, indicating that the variation in CH_4 response was poorly explained by the key independent variables, i.e. treatment, soil temp (p=0.15), and soil NH_4 (p=0.14) (Fig. 7, Table S3). In both years the general trend was in the direction of greater CH_4 emissions at higher soil temperatures and elevated concentrations of soil NH_4 (Fig. 7 and 8).

3.6 Biochar effect on soil CO_2 flux

Carbon dioxide flux (heterotrophic and dark respiration from soil and plants in a closed chamber) was 4 times higher in 2012 than in 2014. There were no significant

treatment differences for CO_2 flux on individual measurements or in cumulative CO_2 fluxes in either 2012 or 2014 (Fig. 1).

3.7 Biochar effects on soil physical and chemical properties

In the growing season (Apr.-Aug.) of 2012, WFPS in the 0-15cm depth was in the range of 25-75%. In the post-harvest period of 2012 precipitation was high and WFPS was in the range of 50-90% range. Mixed model analysis revealed only one measurement date (17. Aug. 2012) when BC-New had significantly higher WFPS (73%), compared to BC-Aged (53%) and Control (48%) (Fig. 9). In 2014, BC-New had the opposite effect with significantly lower WFPS in the 0-15 cm depth in the first half of the season compared to BC-Aged and Control (Fig. 9). There was no significant treatment effects on soil NH_4 in neither 2012 nor 2014. For soil NO_3, there was generally few treatment differences in 2012 and 2014 except for a significant spike in NO_3 in BC-Aged and BC-New in June 2014 (Fig. 9). No significant treatment effect was observed in either year for soil pH, which ranged from 6.3-6.4 in 2014 and 5.9-6.1 in 2014 (Table 1, Fig. S2).

Table 1. Biochar effects on soil physical and chemical properties during the experiment. Soil pH, NO_3 and NH_4 values are averages of the time series measurements taken together with flux measurements.

Year	Treatment	Bulk density (g cm-3)	Soil Porosity (%)	Biochar pH (H_2O)	Soil pH (H_2O)	Soil NO_3-N (mg kg^{-1})	Soil NH_4-N (mg kg^{-1})
2010	BC-Aged	-	-	10	-	-	-
2012	Control	1.3 ± 0.02	49.05	-	6.3 ± 0.2[a]	8.1 ± 7.3[a]	1.5 ± 2.7[a]
	BC-Aged	1.22 ± 0.14		7.86 ± 0.1[α]	6.4 ± 0.1[a]	9.5 ± 6.9[a]	0.7 ± 0.33[a]
	BC-New	-			6.4 ± 0.2[a]	6.6 ± 7.8[a]	1.2 ± 1.3[a]
2014	Control	1.32 ± 0.02	49.81	-	6.1 ± 0.2[a]	27 ± 15[a]	5.4 ± 6.4[a]
	BC-Aged	1.23 ± 0.10	53.94	5.5 ± 0.2	5.9 ± 0.2[a]	38 ± 7[a]	8.4 ± 8.9[a]
	BC-New	1.17 ± 0.10	51.31	8.75	5.9 ± 0.3[a]	40 ± 23[a]	5.8± 5.5[a]

(Mean ± Standard Deviation, n=3 in 2012, n=4 in 2014), [α] BC-Aged pH from stored sample

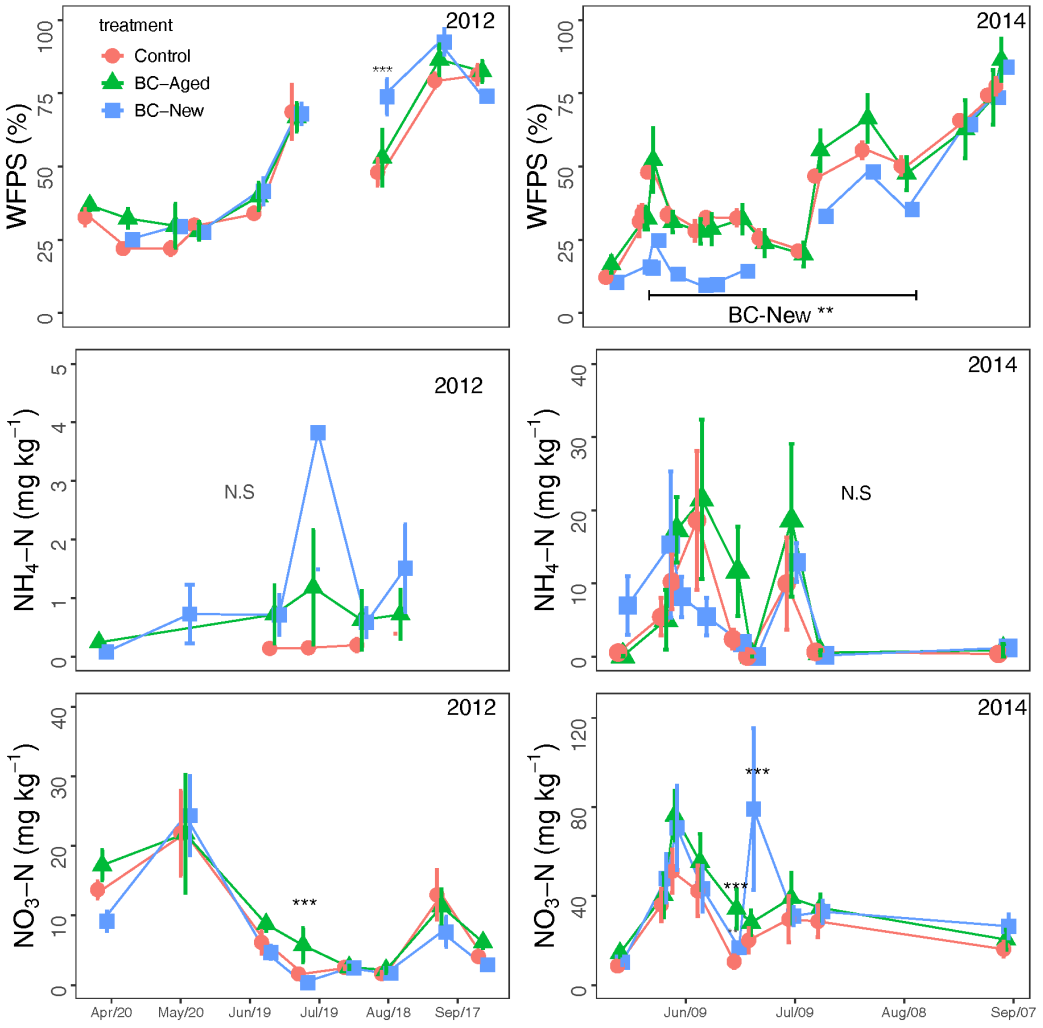

Fig. 9 Water filled pore space (WFPS) (%), and NO_3 and NH_4 soil concentrations taken on N_2O measurement days in 2012 and 2014. Statistically Significant, *($p<0.05$) ** ($p<0.01$), ***($p<0.001$), on specific measurement dates. NS= denotes no significant treatment differences at any measurement date.

3.8 Changes in biochar properties with ageing

The miscanthus biochar had originally a pH_{H2O} of 10 before it was applied to the field in 2010. In 2014, pure biochar stored in plastic bags since 2010 had a pH_{H2O} of 7.86 ± 0.1 (n=3), and aged biochar collected from the field had a pH_{H2O} of 5.5 ± 0.2 (Table 1). SEM-EDX analysis of biochar particle surfaces showed that Al content significantly increased

while K, Mg, P contents significantly decreased with biochar ageing. No significant differences were found for C, Ca, O, S or Si (Fig. 10). SEM images reveal partial clogging of biochar pores with mineral soil particles on aged biochar surfaces (Fig. 11)

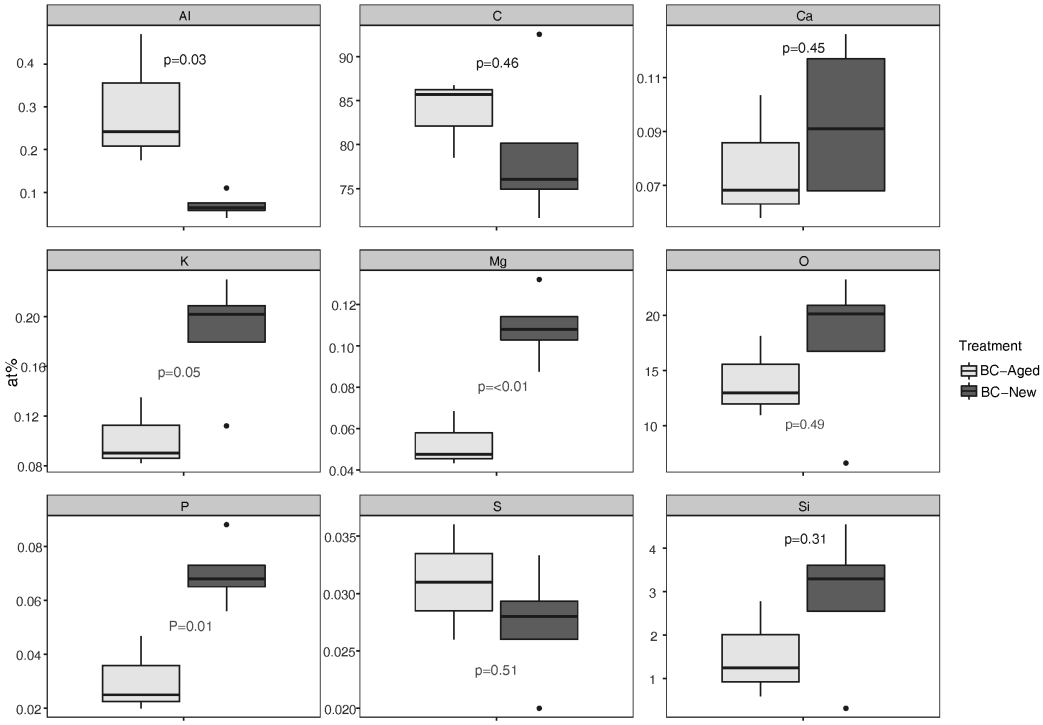

Fig. 10. Box plots (n=3) of SEM-EDX elemental content on particles surfaces from BC-Aged (collected from field) and BC-New (stored for one year). Corresponding SEM pictures are featured in Supplementary materials.

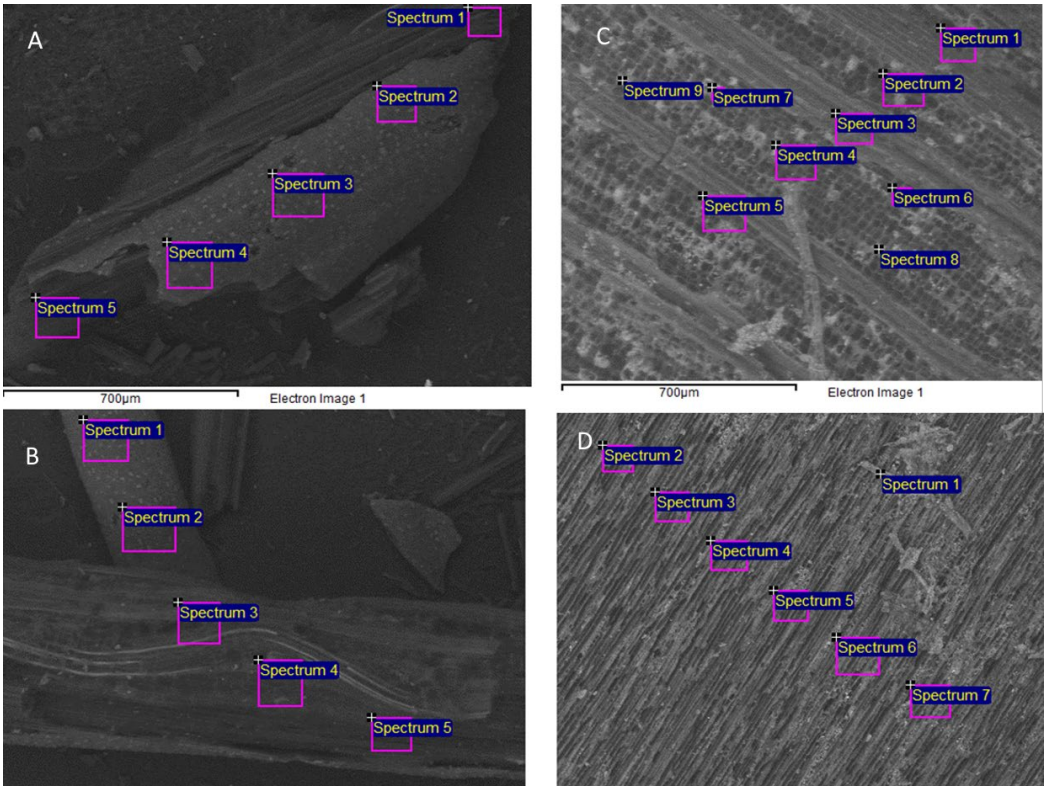

Fig. 11. Examples of BC-New (A, B) and BC-Aged (C, D) particles under SEM analysis. The pink boxes were where EDX elemental surface analysis was taken

4. Discussion

4.1 Effect of Aged vs New biochar on N_2O emissions

In our study BC-New mitigated N_2O significantly by 60-80% compared to the control at specific peak emissions events during wet periods at the end of the 2012 and 2014 growing seasons (Fig 1 and 3). However, BC-New also stimulated N_2O by 258-350% compared to the control at two measurement dates directly after fertilization in 2014. The wet period emissions in 2012 were a greater contributor to total emissions than at fertilization in 2014 and therefore the net effect over two years was a suppression of N2O emission by BC-New. Despite BC-Aged decreasing N_2O emissions compared to the control by 40% at peak emission events in 2012 and by 16% and 21% at peak emission events in 2014, the differences were not statistically significant. Reduced efficacy of

aged biochar was also observed by Spokas (2013) who reported that new biochar mitigated N_2O emission by 30% but had no effect 3 years after application.

We assume that denitrification processes were mostly responsible for N_2O production in 2012. We base this assumption on the fact that soil NO_3 was positively correlated with N_2O emissions, soil pH levels were negatively correlated with N_2O emissions, and N_2O emission peaks occurred at periods of high WFPS (>75%). Our miscanthus biochar fitted the criteria from Weldon et al. (2019) for biochars with higher likelihood to suppress N_2O under denitrifying conditions, namely high degree of carbonization (low H/Corg), high initial pH and high surface area (Table S1). Noticeably, the pH of our biochar dropped from 10 initially to 5.5 after 4 years. A similar finding was reported by Spokas (2013), who observed a drop in pH from 10.1 to 5.7 in a soft-wood pellet biochar after 3 years field ageing. Fresh biochars often have a high content of soluble ash, comprised of alkaline salts such as KOH, NaOH, $MgCO_3$ and $CaCO_3$ which can increase the pH of soil (Ippolito et al., 2015). Buss et al. (2018) demonstrated that fresh miscanthus biochar increases the pH of surrounding soil to ~5 mm distance from biochar particles in the first day after exposure to moist soil. In our study, BC-New increased soil pH by ~0.5 units immediately after application but thereafter was within the pH range of the control and BC-Aged soil (pH 5.4-6.2) for the remainder of the experiment (Fig. S2). Our SEM-EDX data also showed that original levels of K and Mg were reduced by 50% in aged biochar (Fig. 10). This agrees with the findings of Obia et al. (2015), who after leaching cacao shell with demineralized water at a 1:50 (BC:water w/w) ratio with a flow rate of 70–80 ml hr^{-1} for 4 days, found that the main leached elements to consist of K^+ (76%), Mg^{2+} (10%) and Ca^{2+} (8%). When applying this biochar to two acidic (pH 4) tropical soils subject to denitrifying conditions, the authors found that the leached biochar suppressed N_2O emissions to a lesser extent than the untreated biochar did.

In 2014, BC-New stimulated N2O emission after fertilization. This period was dry with WFPS ranging from 20-50%, and thus N_2O was mostly likely attributable to nitrification (Bateman and Baggs, 2005). BC-New had significantly lower WFPS in this period than Control and BC-Aged (Fig.9). This could be because the newly incorporated biochar decreased bulk density in the soil from 1.32 to 1.17 (Table 1) and may have led

to accelerated evaporation compared to BC-Aged and Control whose tilth was less disturbed. Sánchez-García et al. (2014), showed that biochar can both suppress and stimulate N_2O emissions in soil depending on the dominant pathway for N_2O production in a particular soil. In our study, N_2O emission peaks were 2-6 times lower during drier nitrifying dominated periods than during wetter denitrifying dominated periods. Thus, denitrification was the dominant N_2O pathway in our soil, and was the one most alleviated by biochar application.

4.2 Effect of Aged vs New biochar on CH_4 flux

In two field seasons, the soil acted generally as a sink for CH_4, except for the first and third measurements of 2012 when there was a spike in CH_4 emissions in the control. On these dates BC-Aged had significantly less flux than the control but this was not the case for BC-New. In contrast, Karhu et al. (2011) found in a silt loam that also newly applied biochar decreased CH_4 flux. In our study, there was no significant difference in NH_4 between treatments on the few occasions when higher CH_4 emission peaks occurred. Smaller amounts of applied N are known to stimulate methanotrophic bacteria population, while higher amounts can inhibit their activity (Chen et al., 2021). At higher rates of applied N, ammonia oxidizing bacteria can outcompete methanotrophs for N, and high N rates can acidify the soil below optimal pH conditions for methanotrophs (ibid). In a global meta-analysis of 42 studies, Jeffery et al. (2016) found that, under 120 kg N ha^{-1} fertilization rate, biochar applications generally increase CH_4 oxidation. Our field trial was fertilized with ammonium nitrate (45% : 55%) at 120 kg N ha^{-1} and we observed no significant effect on CH_4 uptake or emissions overall. Jeffery et al. (2016) also report biochars made at over 600 °C generally reduce CH_4 emissions while lower temp chars have the opposite effect. This could be because biochar produced at low temperature often have a higher cation exchange capacity (Ippolito et al., 2015) and can adsorb more NH_4 which would thereby inhibit CH_4 oxidation. Biochar with higher BET surface area had greater CH_4 sink effect (Jeffery et al., 2016). Our biochar was made at a maximum temperature of 550°C and a had a high BET surface area of 348 and 189 m^2 g^{-1} for BC-Aged and BC-New respectively (Tables S1), and thus fulfilled some of this criteria.

5. Conclusion

In this study, we documented the effect of miscanthus straw biochar on emissions of N_2O and CH_4 and examined how new biochar applications perform compared to aged biochar. Our field observations obtained in a clay-loam in a temperate climate showed that BC-New but not BC-Aged significantly reduced peak N_2O emissions during wet denitrifying periods. We attributed this to the high alkalinity of BC-New. However, BC-New also stimulated N_2O emissions in smaller amounts during drier periods. This is likely due to a combination of decreased bulk density / higher aeration and higher pH in new biochar amended soil, which stimulated nitrification mediated N_2O after fertilization. The mitigating effect of new biochar during wetter periods outweighed stimulation of N_2O in drier periods and outperformed aged biochar for N_2O mitigation. We observed that biochar suppression of N_2O emission weakens with time. Biochar did not significantly change the uptake and oxidation of CH_4, except for a few measurement dates when aged biochar significantly reduced CH_4 flux compared to the control and new biochar. As the biochar effect on denitrification appeared as the key driver for reductions in N_2O emissions, we hypothesize that this effect might be greater in the cold season when denitrification processes are fostered by high soil moisture contents and freeze/thaw events. More measurements in this period of the year would help determine the annual GHG mitigation impact of biochar addition. The apparent mitigation effects of biochar on N_2O or CH_4 emissions reported in this study support current agricultural policy initiatives in Norway, which are seeking to promote the use of biochar as a soil carbon sequestration practice.

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Competing interests

The authors declare no competing interests.

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Supplementary data for Paper II, O'Toole et al. The effect of aged vs new biochar on flux of N_2O and CH_4 over 2 years in a silty-clay loam

Table S1. Properties of miscanthus biochar added to the soil in 2010 (BC-Aged plots) and in 2014 (BC-New plots). The BC-New added in 2012 was stored biochar from the same 2010 batch.

	Unit	Biochar (BC-Aged) 2010	Biochar (BC-New) 2014
Fixed C	%DM	81.10	75.1
Volatile matter	%DM	7.40	9.7
Ash	%DM	11.50	15.20
Total C	%DM	80.00	73.65
H	%DM	1.2	-
N	%DM	0.6	0.70
O	%DM	6.6	-
S	%DM	0.12	0.14
C:N	Ratio	133	106
Total P	g kg^{-1}	1.30	5.9
P (Ammonium lactate extr.)	g kg^{-1}	1.10	1.1
K (Ammonium lactate extr.)	g kg^{-1}	7.50	13
Ca (Ammonium lactate extr.)	g kg^{-1}	4.60	2.1

Mg (Ammonium lactate extr.)	g kg^{-1}	0.60	0.49
Na	g kg^{-1}	0.38	
Si	g kg^{-1}	-	17
NO_3	mg kg^{-1}	3.32	7.74
NH_4	mg kg^{-1}	<LOD	<LOD
Fe	mg kg^{-1}	1100.00	4500
Mn	mg kg^{-1}	160.00	340
Mo	mg kg^{-1}	<1.1	-
Zn	mg kg^{-1}	39.00	160
Cl	mg kg^{-1}	477.00	-
B	mg kg^{-1}	5.10	-
BET-N_2	m^2 g^{-1}	348	189
pH ((±SD, n=9)	(H_2O)	7.86 (±0.05 n=3)	
EC	mS/m	130	
Δ13C (±SD, n=3)	‰	-13.60 ± 0.2	
H:C (atomic)		0.18	-
O:C (atomic)		0.06	-
Total pore volume	cm^3 g^{-1}	-	0.11
Average pore diameter	nm		2.28

Table.S2. GzLM outputs estimating the relationship between treatment and edaphic variables on N_2O fluxes

	N_2O flux 2012			**N_2O flux 2014**		
Model predictors[a]	*Estimates*[b]	*CI*	*p*	*Estimates*[b]	*CI*	*p*
(Intercept)	33.44	26.43 – 42.30	**<0.001**	2.83	0.12 – 68.51	0.523
pH	0.83	0.71 – 0.97	**0.022**	0.97	0.73 – 1.29	0.831
WFPS (%) - 0-5cm	1.04	0.85 – 1.28	0.691	1.02	1.00 – 1.04	**0.035**
Precipitation <2day	-	-	-	0.98	0.97 – 0.99	**<0.001**
Soil Temp ◦C	-	-	-	1.05	0.95 – 1.16	0.367
NH_4	-	-	-	1	0.99 – 1.02	0.608
NO_3	1.21	1.04 – 1.40	**0.014**	1	0.99 – 1.00	0.132
BC-Aged	0.85	0.63 – 1.15	0.29	1.13	0.82 – 1.54	0.465
BC-New	0.85	0.63 – 1.15	0.287	2.51	1.64 – 3.84	**<0.001**
Observations	44			75		
R^2_{GLMM}	0.32			0.47		

[a]Values given for only predictors that contributed to the best model fit n

[b] coefficients are multipliers of the Intercept

Table.S3. Linear mixed model outputs estimating the relationship between treatment and edaphic variables on CH_4 fluxes

	CH_4 flux 2012			**CH_4 flux 2014**		
Model predictors[a]	*Estimates*[b]	*CI*	*p*	*Estimates*[b]	*CI*	*p*
(Intercept)	-4.06	-15.66 – 7.53	0.49	-6.84	-10.24 – -3.45	<0.001
pH	-	-	-	0.18	-1.21 – 1.58	0.79
WFPS (%) - 0-5cm	-	-	-	2.57	-1.38 – 6.53	0.2
Soil Temp ◦C	6.01	-2.26 – 14.29	0.15	0.54	-2.71 – 3.79	0.74
NH_4	3.76	-1.30 – 8.81	0.14	-	-	-
BC-Aged	7.26	-8.06 – 22.57	0.35	2.16	-2.21 – 6.53	0.33
BC-New	9.01	-4.97 – 22.99	0.2	3.24	-1.80 – 8.28	0.21
Observations	35			74		
R^2	0.2			0.2		

[a]Values given for only predictors that contributed to the best model fit

[b] coefficients are +/- to the Intercept

Table S4. Bulk density of biochar soils in 2012 and 2014 as influenced by the volume of the sample cylinder

		Soil core volume				
		100 cm3		250 cm3		1178 cm3
		5-10cm	12-17cm	5-10cm	12-17cm	0-15cm
Control	2012	1.30 ± 0.04	NA	NA	NA	NA
Control	2014	1.28 ± 0.04	1.34± 0.02	1.30 ± 0.05	1.37 ± 0.04	1.30 ±0.03
BC-Aged	2012	1.22 ± 0.14	NA	NA	NA	NA
BC-Aged	2014	1.20 ± 0.07	1.26 ±0.04	1.19 ± 0.1	1.24 ± 0.11	1.17 ± 0.04
BC-New	2012					
BC-New	2014	1.09 ± 0.18		1.23 ± 0.09		1.19 ± 0.04

Table S5. Mean air temperature (°C) and monthly precipitation (mm) in Ås, Norway for four growing seasons (2012 and 2014) compared with normal (1961-1990) monthly averages

	Mean daily air temperature (°C)			Total precipitation (mm)		
Month	*Normal (1961-1990)*	2012	2014	*Normal (1961-1990)*	2012	2014
April	*4*	4	7	*39*	31	62
May	*10*	11	11	*60*	14	40
June	*15*	13	15	*68*	84	25
July	*16*	16	20	*81*	110	46
Aug.	*15*	15	15	*83*	83	123
Sept.	*11*	10	12	*90*	83	31

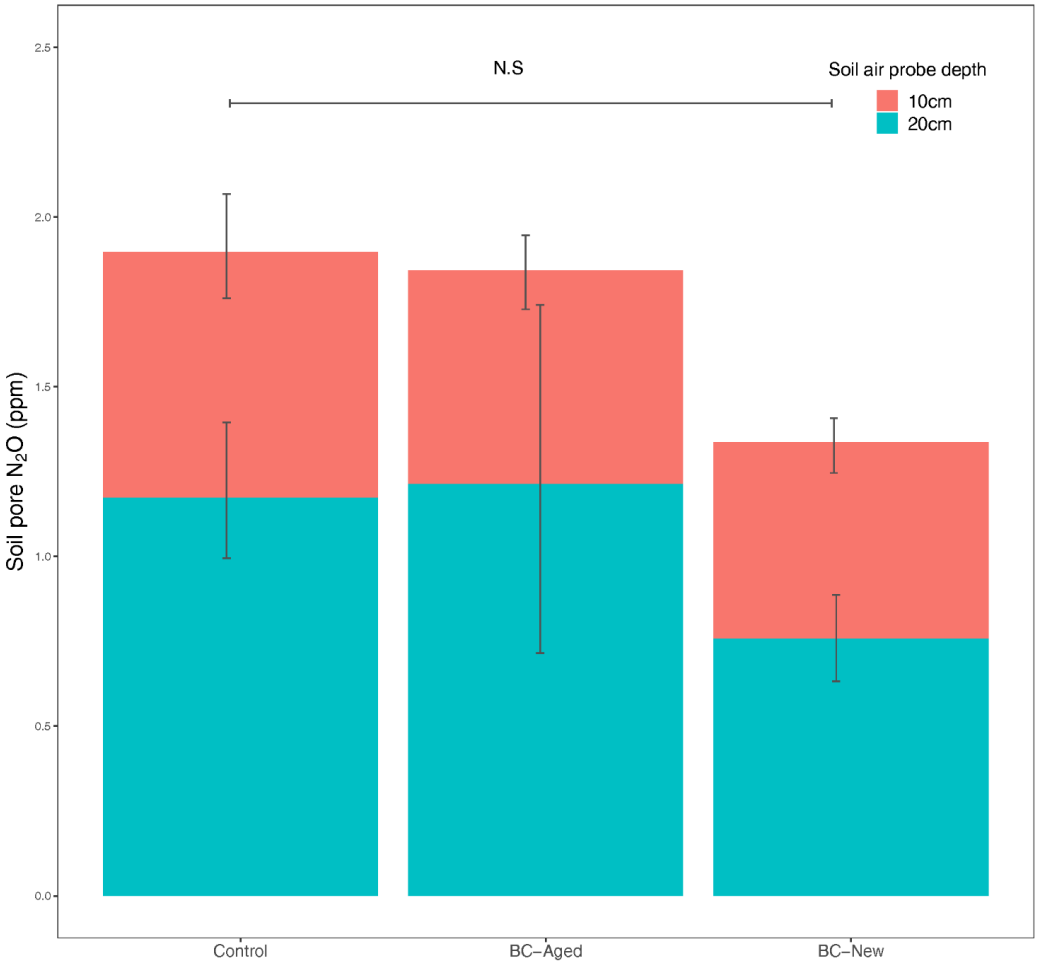

Fig. S1. Mean N_2O concentration (ppm) in soil pore solution at 10 and 20 cm in 2014. Outlier removed for Control 20cm (52 ppm).

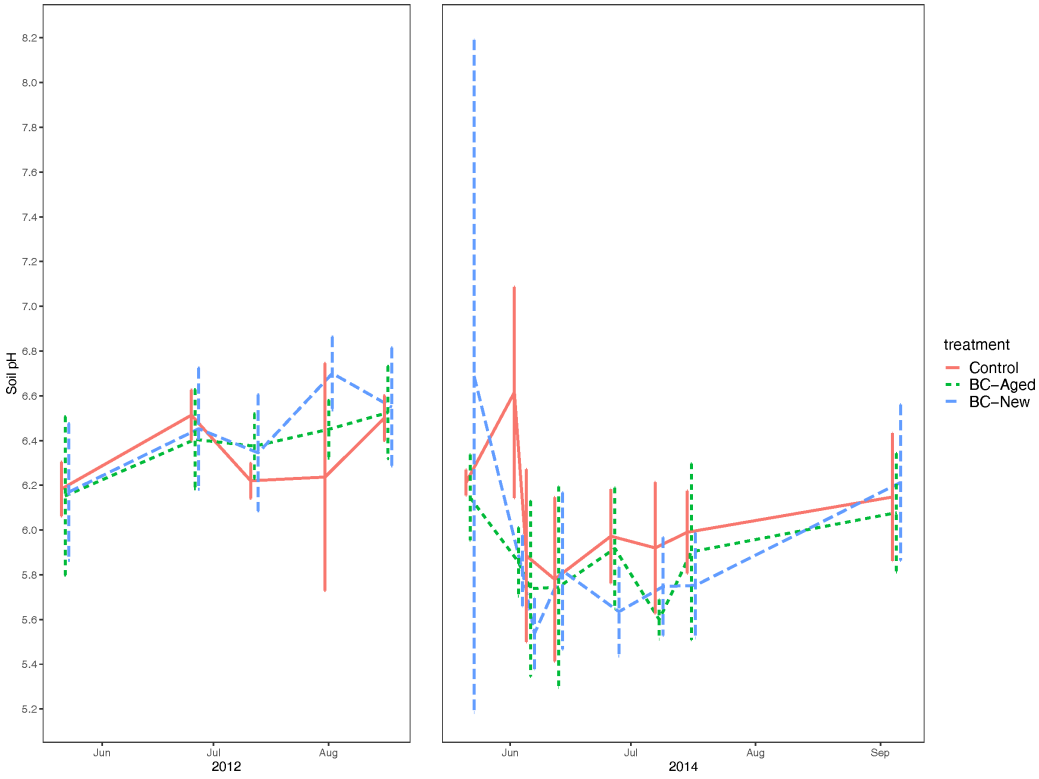

Fig. S2. Soil pH as influenced by biochar treatments in 2012 and 2014 growing season

Paper III

Paper III: The transport, fate and recovery of biochar 5 years after application to a flat-terrain cereal crop field

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Highlights

- 92%-107% of biochar was accounted for 5 years after application
- 45%-72% was retained in the plough layer
- 22%-31% was transported vertically below the plough layer
- <21% was transported laterally mostly within 2 m of plot boundaries
- Understanding the physical movement of biochar in the soil is important to accurately assess biochar C stocks

Abstract

Biochar is a promising method for increasing long-term soil C stocks and improving soil quality and productivity. However, biochar has a low density and can be easily transported after application, potentially reducing its agronomic benefits. Documenting the fate of biochar after application is important to inform farmers about best application practices and to assess methods for accurately reporting biochar C stocks in national soil C inventories. For these purposes, we investigated the vertical (0-60 cm) and lateral (9 m from plot edge) transport of 11.6 or 31.5 t ha^{-1} miscanthus biochar 5 years after it was applied to a flat terrain, silty clay loam under boreal climate in Norway. Biochar was quantified in soil samples via loss on ignition and $\delta^{13}C$ isotopic tracing and the two methods compared. Similar amounts of biochar were recovered by loss-on-ignition and

$\delta^{13}C$ methods at the lower biochar dose, but loss-on-ignition overestimated the amount of recovered biochar at the higher dose. This discrepancy is likely related to the heterogeneous distribution of biochar in soil. Based exclusively on the isotopic tracing data, we estimate that after 5 years between 45% and 72% of biochar was retained within the plough layer inside the plots, 22-31% was transported vertically below the plough layer to the 23-60 cm depth, 21% was transported laterally within 2 m of the plot edge and 4% mineralized to CO_2 (estimated from an earlier study on biochar C stability). The relative proportion of biochar that was vertically transported was similar for both the low and high biochar application rates. On average, the isotopic tracing allowed us to account for 92-107% ±5-7% of the biochar initially applied. To elucidate on transport mechanisms, we quantified the amount of biochar occluded in or ejected from water stable 2 - 6mm aggregates during a series of aggregate stability tests. Biochar ejection from aggregates was mostly affected by slaking of dry aggregates in water (79-83%) followed by differential clay swelling (63%), and mechanical forces (59%). However after 5 years, biochar concentration in aggregates was very close to the theoretical bulk soil biochar concentration. Therefore, we conclude that biochar present in particulate form in the soil matrix pore space was the likely source of transportable biochar.

Keywords: Biochar ^{13}C isotope, Biochar transport in soil, Biochar recovery, Biochar aggregate stability

Abbreviations: BC=Biochar, LOI=Loss-on-ignition, fPOM= free Particulate Organic Matter

1. Introduction

Increasing soil-C levels by the addition of biochar is a promising carbon negative solution for reducing atmospheric CO_2 levels and limiting global warming (Smith, 2016). Biochar is produced by heating biomass in the absence of oxygen (pyrolysis) > 370 °C whereby approximately 50% of the biomass C remains as biochar and 50% can be used for bioenergy purposes (European Biochar Certificate, 2020). Biochar-C has a half-life 60 times longer than its parent feedstock (Budai et al. 2016; Rasse et al., 2017), and a review of incubation and modelling studies indicate that approximately 70% of biochar C remains in soil > 100 years (Lehmann et al., 2015). In addition to its utility towards C sequestration,

meta-analyses have shown that on average biochar increases crop yields 0-25% across different climates zones (Jeffery et al., 2017), increases aggregate stability by 8%, available water holding capacity by 15% and saturated hydraulic conductivity by 25% (Omondi et al., 2016), increases microbial biomass C by 18% (Liu et al., 2015) and reduces N_2O emissions by 54% (Cayuela et al., 2014). However, in a four year field trial in Norway, where 10-30 t ha^{-1} of biochar was applied, we observed no significant effect on grain yields and microbial biomass despite increases in water holding capacity (O'Toole et al., 2018). Therefore, results are likely dependent on a variety of factors including the type of biochar used, and the soil/plant/climate system it is applied to.

With the majority of scientific research validating the positive benefits of biochar for soil C sequestration and soil improvement, this has given more confidence to policy makers to include biochar in plans for improving the CO_2 footprint of the agricultural sector. Recently the IPCC included biochar as a draft method in its refinement of methods for the Land Use, Land Use Change, and Forestry (LULUCF) sector (IPCC, 2019), and the Norwegian government has included the use of biochar as one of the strategies to reduce CO_2 in the Norwegian agricultural sector (Miljødirektoratet et al., 2020). The draft Tier-1 method of the IPCC relies on the amount of biochar produced nationally with corresponding information about C content and chemical indicators related to its C stability over a 100 year period (IPCC, 2019). Individual countries reporting to the IPCC can also develop a Tier-2 or 3 method which involves documenting and/or modelling country specific emission data. This could include the mitigating effect of biochar on N_2O (Cayuela et al., 2014; Borchard et al., 2019). However, a Tier-3 method might also require a physical verification of biochar C stocks in soil. This may pose a challenge to soil sampling campaigns considering the mobility of biochar in soil.

Biochar, due to its low density and initial hydrophobic nature can be mobilized with the forces of wind and water following application. Dong et al. 2017 were unable to account for 40% of biochar that was applied to 0-20cm depth and speculated that biochar loss due to vertical or lateral transport related to biannual flood irrigation in winter wheat. In a light textured soil in Zambia, Obia et al., 2017 were unable to account for 25-45% of applied biochar after one year. They attributed losses to lateral surface erosion during

heavy rain episodes. After 9 years, Kätterer et al., 2019 were unable to account for 4-68% of applied biochar in the 0-20cm depth from 4 field sites located on flat or gently sloping terrain in sub-humid Kenya. They attributed loss to a combination of mineralization, erosion or vertical translocation. Major et al., 2010 were unable to account for 20-53% of biochar after 2 years in a flat sloped field where biochar was applied at 11, 23, and 116 t C ha^{-1} to the top 0-15 cm of a sandy clay loam Oxisol. They attributed losses to surface run-off. In summary, there is a lack of understanding about how far biochar moves, in which direction, and by which forces. The majority of studies still speculate upon the fate of biochar and none of the above studies were able to account for 100% of the biochar that was originally applied.

In the present study, we quantify biochar content in the Ap horizon of a loamy soil 2 to 5 years after application using two methods, namely: ^{13}C isotope method, and loss on ignition (LOI). We further use the isotopic method to estimate the lateral and vertical transport of biochar outside the plot Ap horizon where it was originally applied. Further, in order to explain better the processes that lead to biochar retention or loss from the soil matrix we also quantify the extent to which biochar is retained in 2-6mm soil aggregates after their exposure to wet sieving and stability tests.

2. Materials and Methods

2.1 Field site description and experimental design

Measurements of biochar transport in the soil were conducted on a field experiment that was set up in September 2010 at the Norwegian University of Life Sciences (NMBU) field station in Ås, Norway (59° 39' 51" N 10° 45' 40" E). The field was relatively flat with a slope gradient of <1°. Mean average temperature (1991-2020) is 6.3 °C (Fig. S1). The average annual rainfall (1991-2020) is 886 mm (Fig. S2). The soil can experience freeze/thaw events between December-March. The most common erosion events occur during early spring due to snow melt. The soil is a silty clay loam Albeluvisol (WRB classification) with an average content of 27% clay, 43% silt and 30% sand. The biochar was produced from *Miscanthus giganteous* straw by Pyreg Gmbh (DE). Further characterization of the biochar and soil is reported in O'Toole et al., 2018, and also in Table S1. The experimental design consists of a randomized complete block design with 3 treatments and 4 replicate plots per treatment with an area of 32 m^2. The treatments are:

(1) ***Control*** (no biochar)

(2) ***BC-Low***: *Miscanthus* biochar (11.6 t ha^{-1} or 0.40% (w/w) in soil)

(3) ***BC-High***: *Miscanthus* biochar (31.5 t ha^{-1} or 1.12% (w/w) in soil)

Miscanthus was chosen as a feedstock because it is a C4 plant with a contrasting $\delta^{13}C$ to the C3 plant dominated native soil carbon. Thus, C4 derived biochar can be detected separately to the C3 native soil organic C (SOC).

Biochar was manually applied to the surface at the appropriate rate in September 2010 and ploughed into the soil to a depth of 23 cm. Ploughing resulted in the distribution of biochar in concentrated diagonal seams in the ploughed A horizon (Ap) in the following growth season in 2011 (O'Toole et al., 2018). Annual tillage operations from 2011-14, which involved autumn ploughing followed by spring harrowing and bed preparation, resulted in more evenly distributed biochar in the Ap soil for the rest of the experiment (as confirmed by visual inspection of soil horizon in the 2012-2014).

2.2 Soil sampling and biochar content measurement

Quantification of biochar content was carried out by two methods: (i) tracing of $\delta^{13}C$ signature to derive proportions of C4 biochar in C3 soil (ii) Quantification of biochar content from loss-on-ignition (LOI) measurements done on soil samples collected in year 2 and 4 after incorporation. Bulk density was measured at 0-10, 10-20, and 25-40 cm depths using 100 cm^{-3} steel rings to convert biochar-C soil concentration (g kg^{-1} soil) into biochar-C-stock (g m^{-2}). Proximate and elemental analyses of biochar were conducted by Eurofins Ost Gmbh (DE), according to standards DIN5178 (H_2O), DIN5179 (Ash), DIN51720 (VM), DIN51734 (FC), DIN51732 (C,H,N), DIN51733 (O), 51724-3 (S). More details on biochar characterization were previously reported in O'Toole et al. 2018. A further description of the methods for determining biochar-C soil concentration follows:

2.3 Delta ^{13}C method

Biochar in soil was quantified via the $\delta^{13}C$ method, where the $\delta^{13}C$ signal of biochar derived from C4 photosynthesizing miscanthus is analytically discernible from the $\delta^{13}C$ of the predominately C3 plant derived native soil organic carbon (SOC). In October 2015, 4 replicate soil samples were taken from each plot from within the plough layer (2 cm -15 cm, 15 cm-23 cm) and below it (23 cm - 45 cm and 45 cm – 60 cm), using a pneumatic tractor-mounted 3 cm D steel auger. Collected soil samples were air-dried at 25 ˚C for one week, sieved at 2 mm to remove roots and gravel, dried at 105 ˚C and finally ball milled at 250 rpm for 2 minutes. The concentration and ^{13}C isotopic composition of soil C was measured by combusting 6-20 mg of prepared soil in a combustion module CHN Elemental Analyzer, VARIO model, coupled to an IRMS, PRECISION model (Elementar Analysensysteme GmbH, DE).

The following mixing model was used to calculate the proportion of C from biochar:

$$\text{biochar derived C (\%)} = \frac{\delta^{13}C_{Measured} - \delta^{13}C_{soil/}}{\delta^{13}C_{biochar} - \delta^{13}C_{soil}} x\ 100 \quad (1)$$

The following equation was used to calculate biochar mass content in soil samples:

$$Biochar\ content\ (g\ kg^{-1} soil) = A * B * (1/C) \quad (2)$$

A= $soilC\ (g\ kg^{-1} soil)$

B= $Biochar\ derived\ C\%\ (Eq.1)$

C= $Fraction\ of\ C\ in\ biochar$ determined by dry combustion method

The amount of biochar (BC) recovered within the plot from all measured depths after 5 years was calculated using the following equation:

$$Recovered\ BC\ in\ plot\ \ (g\ m^{-2}) = Biochar\ content * SM \quad (3)$$

Where:

$SM\ is\ the \sum soil\ mass\ \ (kg\ m^{-2})$

2.4 Sampling of lateral movement of biochar outside of plots

After 5 years, we quantifed lateral movement of biochar due to annual ploughing, or physical displacement due to wind and/or rain. One plot (due to resource constraints) from the BC-High treatment and soil samples were taken along two transects. In transect one (12.9 m), we took 37 soil samples every 20 cm in the direction of tractor traffic. In transect two, we took 10 soil samples along a 4.5 m transect perpendicular to transect one (Fig. S3). All soil samples were taken to 23 cm depth. Quantification of biochar content in the soil samples were conducted using the $\delta\ ^{13}C$ method and calculations as described in Eq. 1-3.

We estimated the amount of biochar that moved outside the plot boundary by calculating the proportion of area under concentration curves (Area A + B) to the total area under the combined concentration curves (Areas A+B+C) (Fig. S4.A).

Area A and B were calculated according to (Eq. 4) which had been fitted to the biochar content data.

$$Area\ under\ the\ curve = \int_{x1}^{x0} f(x)\,dx \qquad (Eq.4)$$

Where:

x0 = 1 cm outside plot edge

x1 = 900 cm outside plot edge

f(x) dx = the integral of the function to describe the change in concentration of biochar from x0 to x1.

Biochar found outside the plot perpendicular to the tractor direction did not follow an exponential decay curve and thus we averaged values outside the plot to find a rectangular area concentration (Fig. S4.B). We measured only biochar movement from 2 of 4 plot edges because ploughing direction was alternated every year and thus we assume that biochar movement was symmetrical on opposing sides of the plot.

2.5 Loss-on-ignition method (LOI)

The LOI method is commonly used for quantifying soil organic matter. The method involves combusting a dry soil sample at temperatures >550 °C, whereby the mass loss from the soil is attributed to the combustible organic matter (and also clay bound H_2O) (Hoogsteen et al., 2015). In this study, biochar content was estimated from LOI measurements following the method described by Koide et al., 2011, which involves calculating biochar content in soil based on the difference in LOI between the biochar treated soils versus the unamended control soil. The method is dependent upon a reliable estimate of the LOI of the control soil, pure biochar added to the soil, and soil/biochar mixtures. A correction factor was applied to LOI measurements to account for clay-bound water that is not removed in the first drying step of 105 °C (Riley, 1996). Muffle oven temperature was set to 550°C for 12 hours. Seventeen soil sampling campaigns were carried out between April and October in 2012 (8 samples), and 2014 (9 samples). For these samples a 3 cm D soil corer was used to take 10 sub-samples to a depth of 23 cm and pooled to make one soil sample per plot per date (n=4).

Biochar mass content g (BC^{LOI}) of the soil was calculated from LOI (after correcting for clay bound water) using the equation from Koide et al. 2011:

$$BC^{LOI} = (LOI - qW)/(y-q) \quad (5)$$

Where LOI is the loss on ignition (g) of the mixed soil and biochar sample, q is the proportion of LOI from control soil without biochar content, W is the dry weight (g) of the soil sample, y is proportion of LOI of pure biochar.

2.6 Quantification of biochar in soil aggregates after exposure to aggregate stability tests

Soil aggregates were taken from the Ap horizon of the field in autumn 2015 using a spade to collect a representative 2.5 L bulk sample from each plot. Plant roots, macro fauna, and soil compacted by the spade were excluded from the sample. Soil aggregates >30 mm were carefully broken apart by hand into smaller aggregates. All soil aggregates were air-dried for one week. Soil aggregates were sieved and a sub-samples of the 2-6 mm fractions were subjected to 3 aggregate stability tests: slaking (fast wetting), mechanical force (shaking), clay swelling (slow wetting) as described by Le Bissonnais, 1996. In

addition, samples of 2-6 mm aggregates were exposed to 2 minutes treatment with a wet sieving apparatus (Eijkelkamp, Giesbeek, The Netherlands) following the method described by Kemper and Rosenau, 1986.
Further detail on the methods we used are described in O'Toole et al. 2018 where we reported on the effect of biochar on the mean weight diameter of aggregates after wet sieving and the Le Bissonnais tests.

In the present study, we report on the fate of biochar content from the same 2-6 mm aggregates, which were exposed to the above mentioned aggregate stability tests. After stability tests and post drying, the remaining soil was split into two fractions: 1) free particulate biochar and 2) stable aggregates, which remained after stability tests. Free biochar particles were collected with tweezers, and biochar particles <250 μm were separated from soil by gently air-blowing the biochar out of the sample into a surrounding plastic bowl (Fig. S5). Biochar content in the free particulate (expelled) and aggregate fraction (occluded) were prepared and quantified using the $\delta^{13}C$ method as earlier described. Particle size distribution of pure biochar was measured by sieving 276 g of air dry biochar for 3 minutes through a series of nested sieves (4-8 mm, 2-4 mm, 1-2 mm, 0.5-1mm; 250-500 μm, 125-250 μm, 63-125 μm) mounted on a Retsch AS-200 sieving machine, and weighing the amount of biochar in each fraction.

2.7 Data analysis and statistics

All statistical analyses were carried out using R Software v3.5.0 (The R Foundation for Statistical Computing, Vienna, Austria). Analysis of LOI data was done using a mixed-model repeated-measures analysis of variance using the linear mixed effects (lme) function within the 'nlme' package, with treatment as a fixed factor and plot as a random factor nested within block and year of sampling. This method was chosen over repeated measures ANOVA because it can allow for unbalanced repeated measure data sets. Treatment differences for $\delta^{13}C$ measurements were done with ANOVA, with post-hoc multiple comparison of treatments vs. control done via the Dunnett's test if $p < 0.05$. (multcomp package in R). Student's t-test was used to assess treatment differences in mean biochar content at each soil depth. Pearson R test was done to assess correlation

between biochar particle size distribution and the fraction of biochar in occluded in different sized aggregates after wet sieving.

3. Results

3.1 Vertical and lateral distribution and transport of biochar

There was a significant enrichment (i.e. less negative) in $\delta^{13}C$ for BC-High at 0-15 cm, 15-23 cm, and 23-45 cm depths ($p<0.001$) but not 45-60 cm (Fig.1A). BC-Low was also significantly enriched in $\delta^{13}C$ compared to Control at 0-15 cm, but not at lower depths. A significant difference in soil C could only be detected between BC-High and Control in the 0-15 cm depth with no differences between treatments at other depths (Fig. 1.B)**.** Biochar content (g m^{-2}) using the ^{13}C method was quantified for each depth both above and below the plough layer (Fig. 2). Due to the higher application rate, significantly more biochar was found in BC-High compared to BC-Low plots at 0-15cm depth however significant differences in biochar content between treatments were not observable at lower depths (Fig. 2). Biochar was distributed at depth proportional to application rate i.e. Biochar at the higher application rate did not preferentially migrate downwards compared to the low application rate. For BC-Low and High, 72 and 45% of the applied biochar remained in the 0-23cm plough layer after 5 years respectively (Table 1).

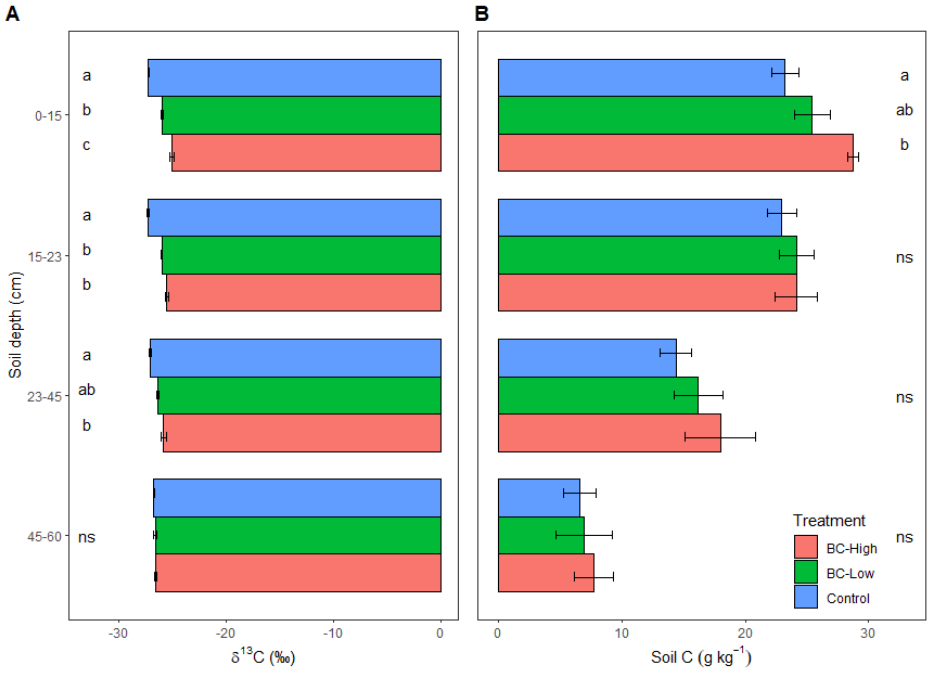

Fig. 1. Mean (±SE, n=4) (A) Soil δ^{13}C and (B) Soil C content (g kg^{-1}) from soil samples taken at 4 depths in 2015. Plough depth is 23 cm.

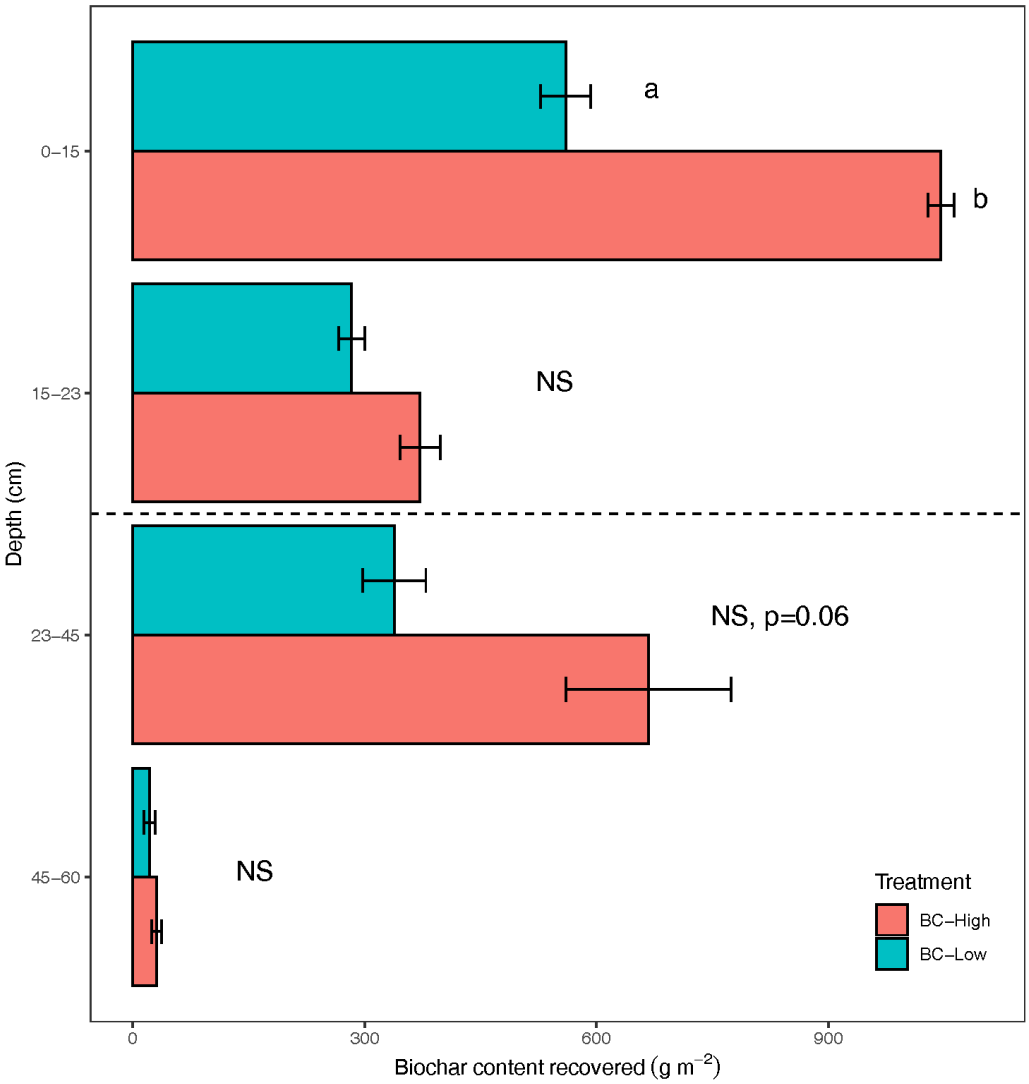

Fig. 2. Mean (±SD, n=4) biochar content (g m^{-2}) at 4 depths from 0-60 cm depth. The bottom of the plough layer is indicated with the dotted line (23 cm). Different letters denote signficance between treatments. Data derived from ^{13}C measured samples only.

We calculated that 12% of biochar had moved outside the plot (blue line, Fig. 3A) in the direction of tillage (Fig. 5A) and 9% moved outside the plot perpendicular to tillage (Fig. 3B) (Table 1), and thus including both directions lateral transport was estimated to account for 21% of applied biochar. In summary, we were able to account for 92% ±5 of the biochar in BC-High and 107% ±7 of biochar BC-Low (Table 1). Biochar C mineralization over 5 years was as estimated to 4%, which is a simple linear extrapolation of an annual biochar mineralization rate of 0.8% calculated from $\delta^{13}C$-CO_2 soil respiration field measurements taken in the first two years of our experiment (Rasse et al., 2017).

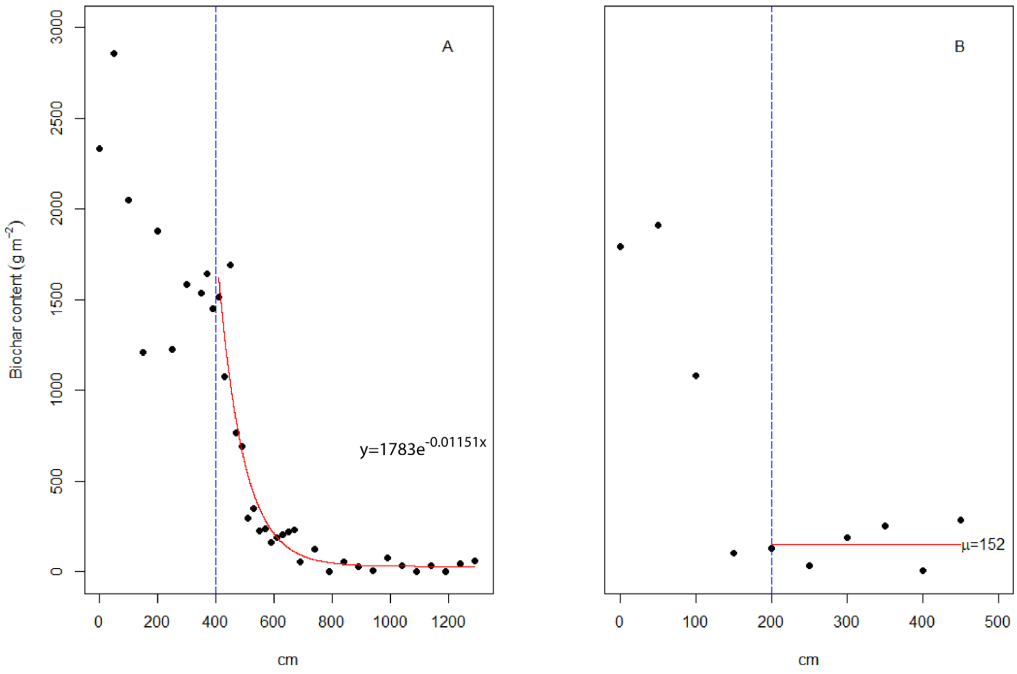

Fig. 3. Biochar-C content in soil (0-23 cm) measured along two transects from the middle of one BC-High plot (8 x 4 m) in two directions: (A) in the direction of tractor driving and (B) perpendicular to tractor driving. The blue dotted line indicates the plot boundary and 0 cm is the middle of the plot. The red line in (B) is the average.

Table 1. Biochar recovery after 5 years (derived from ^{13}C method data only)

	BC-High		BC-Low	
	g m^{-2}	%	g m^{-2}	%
Original amount applied	3150	100	1164	100
Retained within plough layer 0-23 cm	1418 ±61	45 ±2	843 ±73	72 ± 6
Transported vertically (to 23-60 cm depth)	698 ±213	22 ±7	360 ±83	31 ± 7
Moved laterally (due to tractor)	387	12	NA*	NA*
Moved laterally (due to plough)	276	9	NA*	NA*
C-Mineralization after 5 years†		4		4
Biochar accounted for$^{\beta}$		92 ± 5		107 ±7

*Lateral movement not measured in BC-Low

†4% mineralization of Biochar C calculated from ~0.8% annual mineralization rate as measured in Rasse et al. 2017 from same field experiment multiplied by 5 years

3.3 Biochar occlusion in and ejection from soil aggregates

Isotopic ^{13}C analysis of aggregate samples showed that 2-6 mm aggregates contained 1.13% and 0.24% biochar before and after their exposure to wet sieving. Thus 79% of the biochar was ejected after wet sieving (Table 2). The fast wetting test, which tests slaking, resulted in the highest biochar ejection rate with 83%, whereas the shaking test, which tests the effect of mechanical forces independent of trapped air in dry aggregates, ejected significantly lower amounts of biochar than fast wetting (59%, p=0.04). Slow wetting, which tests aggregate breakdown forces from the differential swelling of

clay, was also lower with 63%, but not different to fast wetting or wet sieving (Fig. 4). Of the biochar remaining in aggregates after wet sieving, 11 and 16 mg were present in 1-2, and 2-6 mm fractions, corresponding to 30% and 43% of occluded biochar respectively (Table 2). There was no significant difference in the percentage of biochar occluded in different size fractions after wet sieving (Table 2). There was a high correlation (Pearson R 0.98, n=6, $p<0.001$) between the original biochar particle size at application and the presence of biochar in similar sized aggregate fractions determined after wet sieving (Fig. 5 A and B). We observed a 5% increase in 1-2 mm and 4% reduction in 2-6 mm sized biochar particles after 5 years in soil (Fig. 5A and B).

Table 2. Mean and SD± amount and % of Biochar (BC) occluded in aggregates before and after wet sieving (WS). Wet sieving conducted only for BC-High treatment. Different letters denote statistical significance between BC % content in different aggregate size fractions

Aggregate fractions	**Aggregate weight (g)**	**Aggregate BC content (mg)**	**Aggregate BC content (%)**
Before wet sieving			
2-6mm	15.87 ±0.19	179 ±29	1.13
After wet sieving (aggregate occluded biochar in water stable aggregates)			
2-6 mm	9.01 ±0.67	16.02 ±5	0.18 ±0.06[a]
1-2 mm	3.27 ±0.36	11.11 ±4	0.34 ±0.13[a]
0.5-1 mm	1.84 ±0.23	4.57±1	0.27 ±0.06[a]
250-500 µm	0.98 ±0.12	2.64 ±1	0.31 ±0.06[a]
125-250 µm	0.58 ±0.36	2.76 ±3	0.52 ±0.47[a]
63-125 µm	0.12 ±0.03	0.37 ±0.1	0.33 ±0.14[a]
Sum of biochar occluded in aggregate fractions after WS		39 ±14	
Sum of biochar ejected from aggregates after WS		142 ±26	

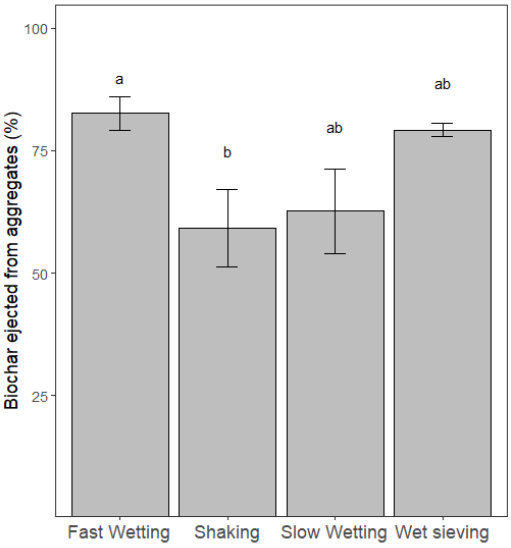

Fig. 4. Mean (±SE, n=4) Amount of biochar ejected (%) from BC-High aggregates after tests to simulate: Slaking (fast wetting test), mechanical breakdown (shaking test), clay swelling (slow wetting test), and the combination of slaking and mechanical breakdown (Wet sieving test).

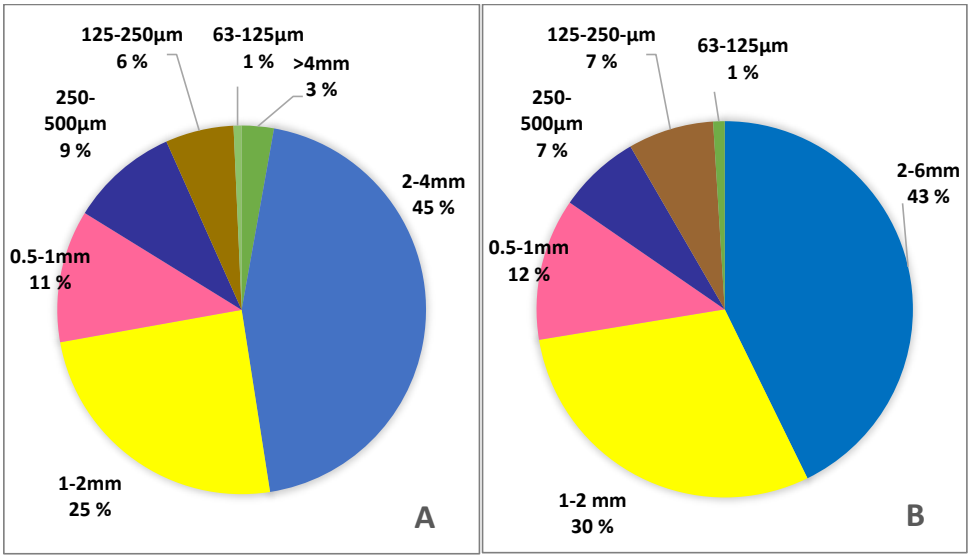

Fig. 5: (A). Particle size distribution of miscanthus biochar before application in 2010, and (B) Biochar content in aggregate size fractions determined after the wet sieving test in 2015.

3.4 Biochar content quantification using LOI vs 13C method

Comparing methods of biochar recovery, we find that in BC-Low the LOI method recovered 100% ±11 at 0-23 cm versus 103% ±6 recovery at 0-60 cm using the ^{13}C method. For BC-High, the LOI method recovered 106% ±8 at 0-23cm versus 70%±4 at 0-60cm using the ^{13}C method (Fig. 6).

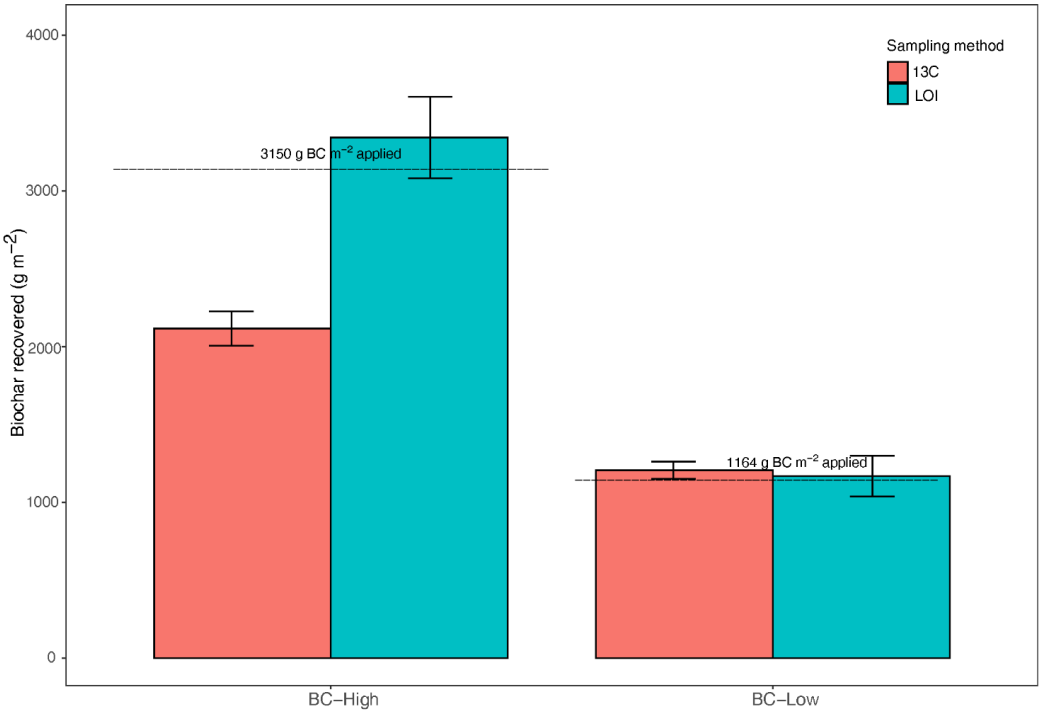

Fig. 6. Mean (±SE, n=4) amount of biochar (g m^{-2}) recovered after 5 years from soil samples taken inside the plot (0-60 cm depth for ^{13}C method and 0-23cm depth for LOI method). The amount of biochar applied at the start of the experiment is indicated by the dotted line.

4. Discussion

4.1 Vertical distribution and transport of biochar

Vertical transport below the 23 cm plough layer made up 22-31% of recovered biochar and was the dominant loss pathway in our study. Biochar application rate did not alter the proportion of biochar distributed vertically, thus we conclude that processes for biochar vertical transport acted similarly for BC-Low and BC-High. Vertical movement of biochar was likely accelerated by tillage operations. At the start of the experiment, incorporation of biochar via ploughing resulted in the presence of high concentrations of biochar at the bottom of the plough layer (23 cm) in 2011 (Fig. S6). Despite subsequent ploughing inverting the soil again and bringing most of the biochar to the surface (Fig. S7), some of the biochar may have remained at the bottom of the plough layer where it can be mobilized to lower depth with percolating water. We did not measure the particle size of biochar that had migrated below the plough layer, but previous research suggests that that biochar particles in the nano and micron size exhibit colloidal properties, can be suspended in water, and are preferentially transported downwards via percolation (Rumpel et al., 2015). This was also confirmed by Obia et al., 2017 who found that two times more <0.5 mm than 0.5 – 1 mm sized biochar particles had vertically migrated below the biochar application depth. Similarly, Wang et al. 2013 studied the transport of differently sized biochar particles in 20 cm deep saturated sand columns and found that 56 - 81% of nano-sized biochar particles were leached, compared to 12 - 52% of micron sized chars. The biochar that was vertically transported to 23 - 60 cm is still potentially beneficial to crop growth because cereal crop roots can often reach down to 1 m depth (Fan et al., 2016), and additions of biochar to subsoil can improve root growing conditions. This was evidenced by Bruun et al., 2014 who observed a 22% increase in root density and 23% increase in grain yield after amendment of 1% w/w wheat straw biochar amended to a compacted sandy subsoil. However, in our field trial, biochar did not significantly affect barley and oat yields compared to the control over four seasons (O'Toole et al. 2018).

4.2 Lateral transport of biochar

Lateral transport was responsible for 21% of biochar loss from plots in our study. Biochar was found in higher concentration outside the plot in the direction of tillage (12%) and to a lesser extent in the direction perpendicular to tillage (9%) (Fig. 3b). Thus biochar lateral movement behaves similarly to soil particles in general, which have been shown to move and be eroded to a greater extent in the direction of tillage than perpendicular to it (Lindstrom et al., 1990). Our field slope was almost flat and the biochar was well incorporated into the soil, so we expect little biochar erosion from the field site. Moreover, the field was mouldboard ploughed each autumn, and the plough ridges appeared to remain stable over winter (Fig. S7) with no visible signs of erosion on the field in the 5 year experiment period. Tillage operations exposed biochar to the surface from October to April and it is probable that a certain amount of biochar became ejected from soil aggregates due to slaking and clay swelling during these annual periods. However, regardless of the ejection of biochar from aggregates, after 5 years most of the biochar was still recovered mostly within 2 m from the plot boundaries in both directions (Fig. 3). Therefore, we conclude that biochar lateral transport was minimal in silty clay loam Albeluvisol located on flat terrain. Lateral transport of 21% in our study was higher than that reported by Obia et al. 2017 who retrieved 6.6 - 9% of applied <1 mm sized biochar in reference plots located <1 m distance from biochar plots. However, in their study, plot dimensions were only 50 x 50 cm, and measurement of lateral transport only extended to 50 cm outside the biochar plot boundaries. In the end, they were unable to account for 24 - 45% of biochar and attributed this loss to lateral transport the 2.25 m^2 experimental area. Most of the studies to date on lateral transport of biochar relate to the study of erosion of biochar produced and remaining on the soil surface after forest fires (referred to as Pyrogenic-C). In one such study, Bellè et al., 2021 found that soil type was the main predictor variable to whether surface applied biochar was lost via runoff or splash erosion after a 30 min. simulated rainfall event, with 61% lost from a sandy silt Luvisol and 11% lost in a clay-loam Cambisol. However, while our study found limited lateral transport in a silty clay loam on flat terrain, we acknowledge that both slope and soil type are likely to highly influence biochar transport in the landscape. Under Norwegian climate conditions, we expect that biochar will be more prone to erosion and transport in silt soils, which are more sensitive to aggregate breakdown under the freeze-thaw cycles commonly happening during the Norwegian winter and spring (Kværnø and Øygarden, 2006).

4.3 Biochar occlusion in aggregates

We found biochar in all of the six water-stable aggregate fractions <6 mm but there was no preferential enrichment of biochar in any one particular fraction (Table 2). Our results contrast to Brodowski et al., 2006, who found an enrichment of biochar in the <53 μm fraction and a smaller amount of biochar in the >2mm fraction. The difference with our study can be due to time, with the Brodowski study conducted in a long term field site with historical pyrogenic C content present in aggregates. Biochar is highly friable and is expected to physically disintegrate over time due to weathering processes (Spokas et al., 2014), where it is then more likely to be found in smaller aggregate fractions. After 5 years, we observed considerable amounts of elongated biochar particles >4 mm present as fPOM (not quantified) (Fig. S8). This was also observed by Herath et al., 2014, who recovered >64% of the total biochar content in the free particulate organic matter (fPOM) fraction (>250 μm) after 295 days. Paetsch et al., 2017 also found that 52% of biochar was present in the free particulate organic matter (fPOM) fraction while 33% was occluded in aggregates 1 year after application to a loam Dystric Cambisol under temperate grassland.

Macro aggregates undergo accelerated breakdown in tilled soil, such as ours, compared to non-tilled soil (Six et al., 1999), and we can reasonably expect that biochar will be temporarily dislodged due to tillage and slaking. In the present study under laboratory settings, 59-83% of biochar was expelled from aggregates when exposed to either slaking, differential swelling of clay, or mechanical forces (Fig. 4). However, after 5 years in the field, we measured a biochar concentration of 1.13% ±0.18 in 2-6 mm soil aggregates, which was remarkably close to the original biochar soil concentration in 2010 of 1.16% (Table 2). This suggests that under our field conditions biochar is readily re-occluded in aggregates after dislodgement by aggregate breakdown events. We do not have any data to quantify aggregate breakdown episodes at the field scale, so results from aggregate stability tests done in the laboratory should be regarded as a potential outcome when aggregates and biochar are exposed to erosive forces. We observed a 5% increase in 1 - 2 mm and a 4% decrease in 2 - 4 mm aggregates in 2015, which we speculate is due to a combination of weathering processes, among them the bioturbation by soil organisms, freezing and thawing forces, and mechanical breakdown due to contact with soil tillage equipment. Spokas et al., 2014 argue that the process of physical disintegration of biochar

and its subsequent transport has been overlooked, and that contact with water, freezing and thawing, and weathering can quickly result in the physical breakdown of 5 - 35% of biochar, followed by its vertical migration to deeper soil depths which are seldom sampled. This mechanism has been hypothesized to explain why pyrogenic C is not found in greater amounts in the terrestrial environment given its refractory nature; a question referred to as the "black carbon paradox" (Czimczik and Masiello, 2007). Biochar purposefully applied in agricultural landscapes via tillage will allow it to be more readily protected by mineral soil compared to pyrogenic C produced from vegetation fire , which is preferentially lost after exposure to post-fire rain (Rumpel et al., 2009)

4.4 Biochar recovery after 5 years

After 5 years, we accounted for 92-107% of the biochar originally applied, which includes 4% attributed to mineralization of biochar-C to CO_2. This means in practice were able to account for all of the biochar which was originally applied, with variation in recovery rates between BC-Low and BC-High likely due to the heterogeneous distribution of biochar in soil as has been earlier demonstrated in our field (Burud et al., 2016). Forty-five to seventy two percent of biochar was found in the 0-23 cm plough layer within plot boundaries, 22-31% vertically transported to 23-60 cm depth, and 0-21% had moved laterally within 9 m of the plot boundary and the majority located within 2 m of the plot boundary (Table 1). There is a large variability in biochar recovery rates in published literature and a lack of measurements to discern loss pathways. Dong et al., 2017 recovered approximately 60% of rice husk and sunflower hull biochar in the 0-20 cm depth 5 years after application to a flat alluvial plain soil which received annual flood irrigation. The authors speculated that biochar loss due to vertical or lateral transport related to biannual flood irrigation, but did not take measurements to confirm. After 9 years, Kätterer et al., 2019 report that 32 - 96% of applied biochar was recovered in the 0 - 20cm depth from 4 different field sites in sub-humid Kenya in flat or gently sloping terrain. They assumed biochar losses to a combination of mineralization, erosion or vertical translocation. Major et al., 2010 were not able to account for 20-53% of biochar after 2 years in a flat sloped field where biochar was applied at 11.6, 23.2, and 116.1 t C ha^{-1} to the top 0-15 cm of a sandy clay loam Oxisol. They assumed the unaccounted loss to be due to lateral surface runoff during intense rain events. The only study we could find which

retrieved 100% of the applied biochar was in the comparitive field study in a Cambisol an Ferrasol by Singh et al., 2015, who were took samples down to 50 cm and also prevented lateral loss with plot walls. Our biochar recovery rate of 45-72% in the plough layer is within the range reported by Obia et al., 2017, who retrieved 55-76% in the 0-20 cm layer after 1 year. The authors reported that vertical transport was limited and speculated that the main cause for 24-45% of unaccounted biochar was via lateral loss with wind and rain erosion. In our field trial, we expect that recovery rates will continue to decline over time as a result of annual tillage operations, bioturbation such as by earthworms and invertebrates (Maaß et al., 2019) and eluviation, wind and water erosion and percolation (Schiedung et al., 2020).

4.5 Implications of biochar transport and accounting for biochar C stocks

The high variability in recovered biochar reported across studies as mentioned above is a reflection of the variable site specific factors such as topography, rainfall, wind, temperature, tillage operations, bioturbation which are known to affect the transport and loss pathways of applied biochar (Rumpel et al. 2015). This creates a challenge for future soil sequestration programmes that include biochar and where soil sampling may be required to physically verify biochar C stocks. Vertical transport of biochar as found in the present study (22-31%) is equivalent to 5.5 - 7.75 times the C mineralization rate over 5 years (Table 1). Already after 1 year, Singh et al., 2015 found that the vertically migrated biochar was 1.7 - 2.2 times higher than the amount of mineralized biochar C in an Arenosol and Ferrosol. This suggests that the fraction of vertically migrated biochar will increase with time and that sampling of the subsoil is essential to gain an accurate assessment of biochar C stocks. Furthermore, lateral transport of 21% up to 9 m from plot boundaries found in the present study implies that even if the precise GPS location is known about where biochar was applied future soil sampling campaigns will need to sample a wider surrounding area in order to assess accurately the fate of biochar and to avoid the possible error of attributing unrecovered biochar to C mineralization. However if biochar is used under commercial farm conditions it may be spread over a whole field and a 9 m lateral movement of biochar would not be considered as “lost”. In any case, based on the topography of the field the lateral movement over time should be taken into account when designing a sampling regime.

4.6 Evaluation of methods for quantifying biochar content and recovery

The LOI method overestimated the biochar content in soil, with recovery rates from soil samples taken within plots (0-23 cm) exceeding original amounts of biochar applied to plots (Fig. 6). There are two possible explanations for this. Firstly, we suspect that the use of a hand held auger for taking soil samples related to LOI results could have overestimated biochar content compared to ^{13}C soil samples which were taken with a tractor mounted hydraulic soil sampler. When using a hand-held sampler for LOI samples in dry conditions, it was not always possible to drive the auger down to 23 cm depth due to hardened clay and in some sub-samples the auger only reached a maximum of 15 cm depth. Considering we found higher concentrations of biochar in the 0-15 cm layer (Fig. 4), preferential sampling at 0-15 cm may have contributed to an overestimate of biochar content in the 0-23 cm layer. Secondly, the heterogeneous distribution of biochar in the field soil may result in variable recovery rates, regardless of which laboratory method is used to quantify biochar in soil. The spatial heterogeneity of biochar in our field trial was reported by Burud et al., 2016, who mapped the spatial distribution of biochar in an undisturbed 30 cm soil monolith using a combination of ^{13}C and hyperspectral NIR methods. The authors found that biochar distribution was heterogeneous and patchy. We found evidence of the biochar "hot spots" in the soil even 4 years after application in spite of the multiple opportunities for the biochar to mix with soil under annual tillage operations (ploughing and harrowing) (Fig. S9). Finally, when applying large amounts of biochar to field plots there can be inaccuracies in estimating the exact amount of biochar applied to each plot, because in our case we found variation in biochar moisture content of up to 10% at different depths in a 1.5 m^3 big-bag of commercial biochar.

5. Conclusion

Our results show that biochar was highly mobile in the soil with 45-72% of biochar recovered in the 0-23cm plough layer within plot boundaries, 22-31% vertically transported to 23-60 cm depth, and 0-21% transported laterally within 9 m of the plot boundary after 5 years. Biochar recovery rates were within a similar range of a number of other field studies, but transport mechanisms appear to be highly site specific and more

field studies will be required before robust predictions can be made of biochar transport mechanisms under different pedo-climatic conditions. We found that 59-83% of biochar can be expelled from aggregates in laboratory based aggregate stability tests, but we observe that after 5 years of field incubation approximately the same concentration of biochar is found in 2-6 mm aggregates (1.13%) as was applied originally in 2010. Therefore, we conclude that biochar is readily occluded in aggregates but can also be easily dislodged. Further efforts should focus on optimal incorporation methods and possibly co-amendments with other materials which may extend the residence time of biochar in the depth of the soil and where it can provide maximum agronomic benefits. Future efforts to quantify biochar C stocks should take into account the vertical and lateral movement of biochar over time.

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Supplementary data for Paper III, O'Toole et al., *The transport, fate and recovery of biochar 5 years after application to a flat-terrain cereal crop field*

Table S1. Properties of miscanthus biochar and the soil

	Unit	Biochar	Soil (spring 2011)
Fixed C	%DM	81.10	-
Volatile matter	%DM	7.40	-
Ash	%DM	11.50	-
Total C	%DM	80.00	2.50
H	%DM	1.2	-
N	%DM	0.6	0.23
O	%DM	6.6	-
S	%DM	0.12	-
C:N	Ratio	256.77	10.80
Total P	g kg^{-1}	1.30	2.90
P (Ammonium lactate extr.)	g kg^{-1}	1.10	10.60
K (Ammonium lactate extr.)	g kg^{-1}	7.50	8.60
Ca (Ammonium lactate extr.)	g kg^{-1}	4.60	205.80
Mg (Ammonium lactate extr.)	g kg^{-1}	0.60	11.60
Na	g kg^{-1}	0.38	2.80
Si	g kg^{-1}	-	-
NO_3	mg kg^{-1}	3.32	12.10
NH_4	mg kg^{-1}	-	1.50
Fe	mg kg^{-1}	1100.00	-

Mn	mg kg^{-1}	160.00	-
Mo	mg kg^{-1}	<1.1	-
Zn	mg kg^{-1}	39.00	-
Cl	mg kg^{-1}	477.00	-
B	mg kg^{-1}	5.10	-
BET-N_2	m^2 g^{-1}	348	-
pH ((±SD, n=9)	(H2O)	7.86 (±0.05 n=3)	6.39 ±0.2
EC	mS/m	130	4.10
Δ13C (±SD, n=3)	‰	-13.60 ± 0.2	-27.13 ±0.1
H:C (atomic)		0.18	-
O:C (atomic)		0.06	-

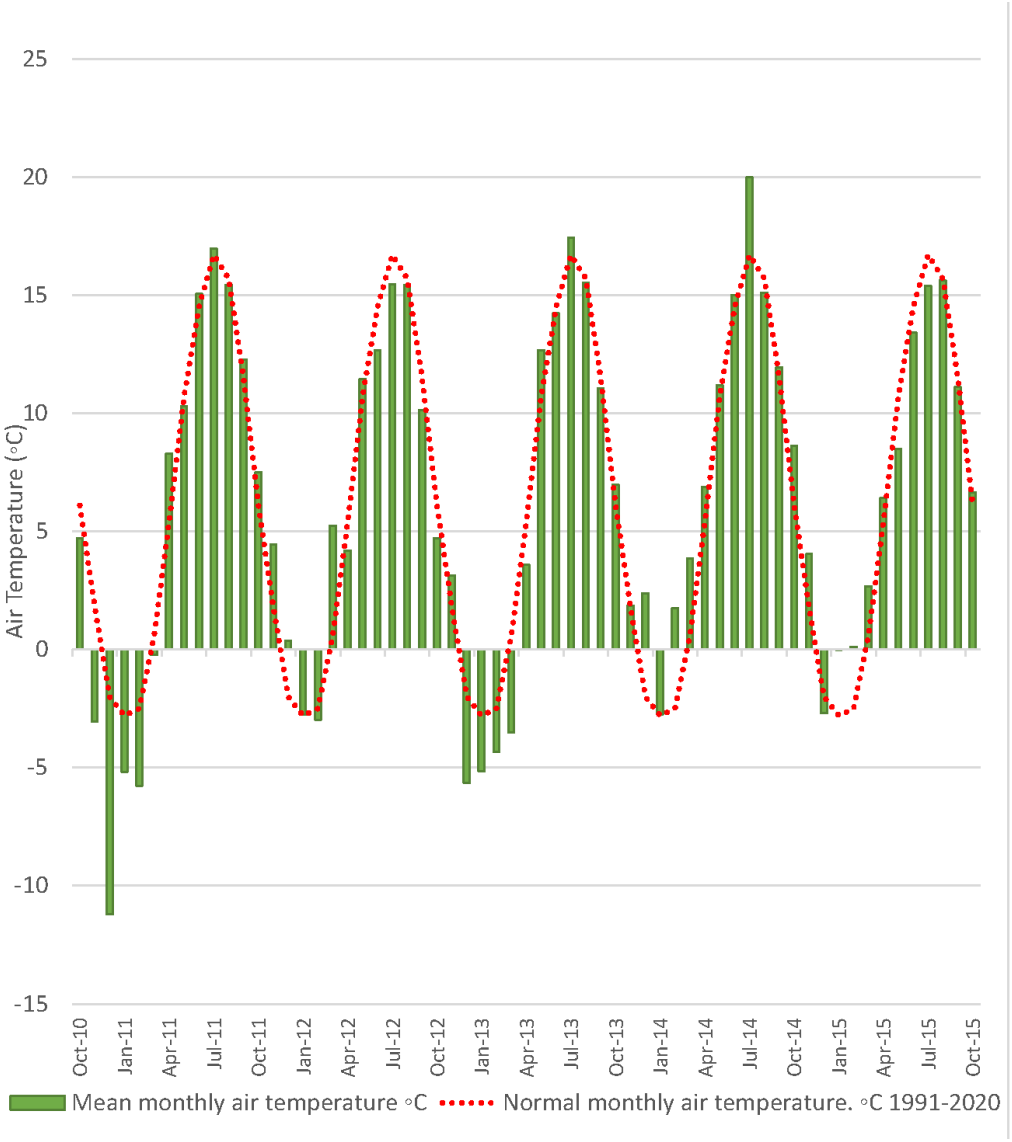

Fig. S1. Monthly mean air temperature ˚C during the experiment and Normal monthly air temperature for the period 1991-2000.

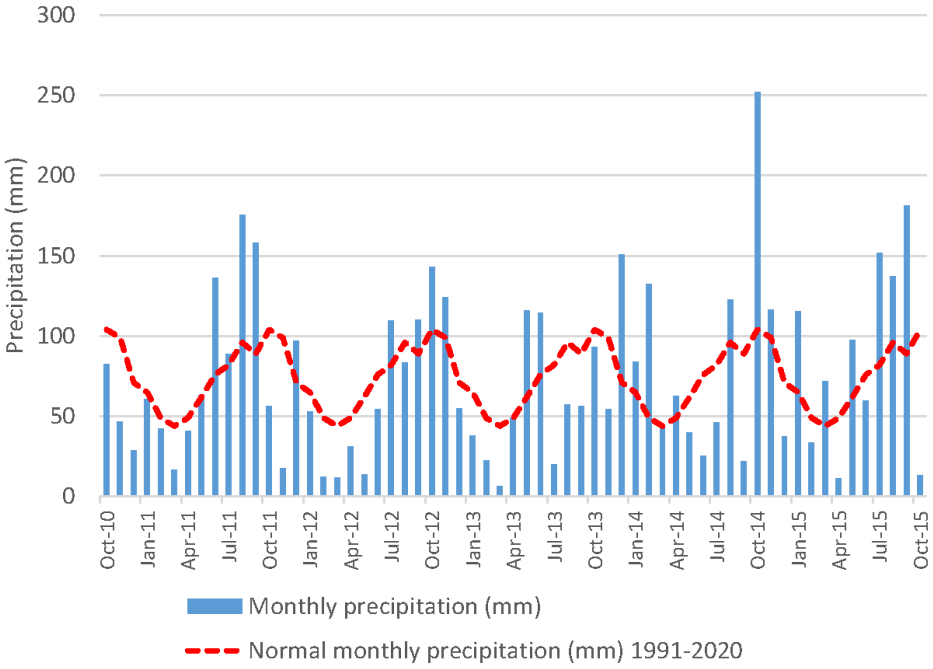

Fig. S2. Monthly precipitation (mm) during the experiment and Normal monthly precipitation (mm) for the period 1991-2000.

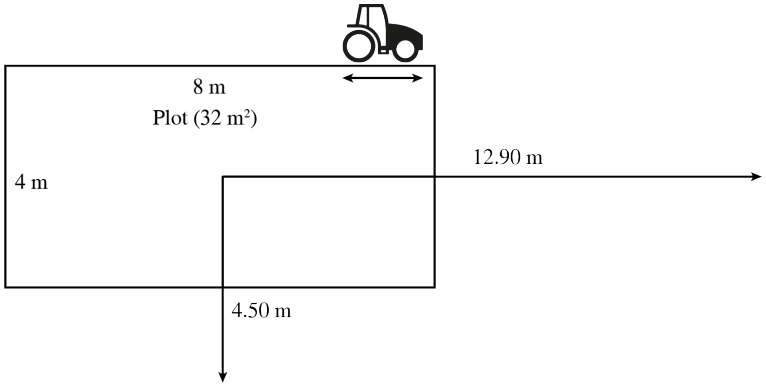

Fig. S3. Position of the two transects for sampling soil to estimate biochar lateral movement in two directions beginning from the middle of the plot and extending beyond the plot borders. Tractor icon indicates ploughing direction.

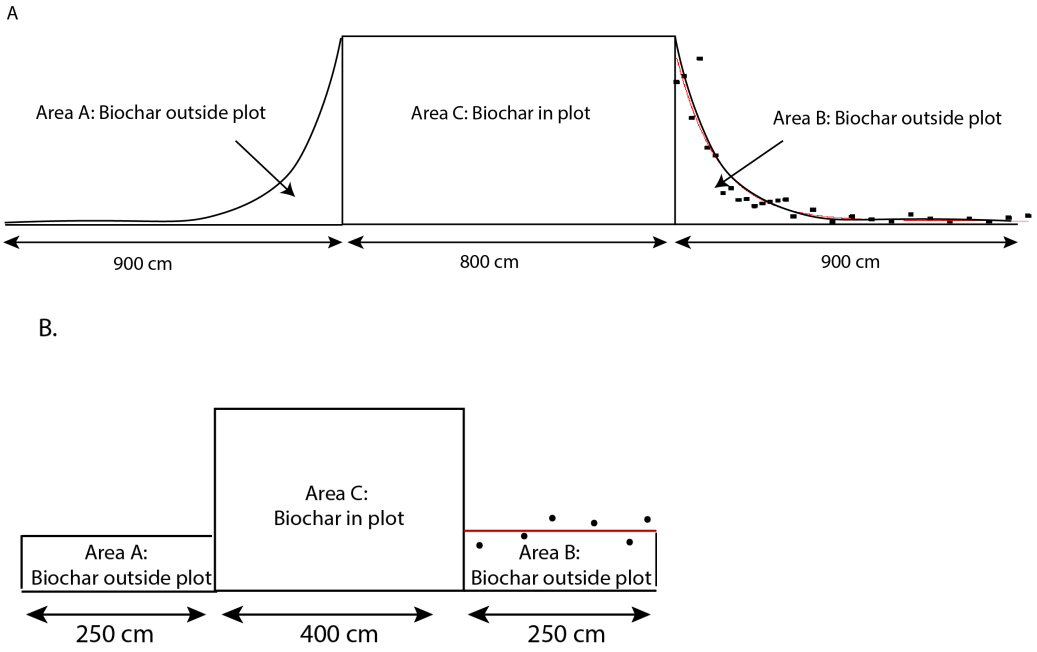

Fig. S4. Theoretical concentration area for biochar inside and outside plots in (A) the direction of tractor driving and (B) perpendicular to the direction of tractor driving. Red lines indicate concentration curves.

Fig. S5. In-house method for separating heavier soil particles from lighter biochar particles, whereby air is blown gently on to the soil sample (in the plastic dish) to expel biochar into the surrounding bowl.

Fig. S6. High Biochar concentrations located at bottom of the plough layer (23 cm depth) in October 2011 due to ploughing in of surface applied biochar at the start of the experiment in October 2010.

Fig. S7. Biochar after tillage in early winter 2011. This is the second ploughing event where biochar, which was buried the year before with ploughing and flipped back to the surface in the second year.

Fig. S8. Soil aggregates and biochar particles in the 2-6 mm range. Sample taken in 2015, showing that after 5 year, a lot of the biochar is still present in the free POM fraction.

Fig. S9. Anecdotal evidence of "Biochar patchiness" from field samples. This sample is from 4 years after biochar addition. Originally published in supplementary file for O'Toole et al. 2018

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Paper IV

Paper IV: Biochar improves the nitrogen fertilization effect of anaerobic digestate in spring onions

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Keywords: Anaerobic digestate, biochar, onion, nitrogen fertilization

Abstract

Mixing biochar with anaerobic digestate is a promising method for organic fertilization of crops and substitution of chemical fertilizer. Anaerobic digestate is the by-product from biogas production that is rich in macro and micro nutrients suitable for plant fertilization. Biochar, on the other hand, can increase soil carbon level, reduce N_2O emission, while improving nutrient use efficiency. In this study, we tested the synergistic fertilization effect of biochar added as 20% or 40% (V/V) to liquid digestate and applied at 7 cm depth under planting lines for spring onions in a coarse sandy soil on a commercial vegetable farm in Norway. The treatments were compared to standard NPK fertilization, broadcasted on the surface in granular form. We hypothesized that digestate could substitute NPK as a basal fertilizer for spring onions (*Allium fistulosum*) and that the addition of biochar to digestate would improve N-use efficiency, leading to increased plant yield and reduced N_2O emissions. SEM/EDS microscopy was used to examine the spatial distribution of elements in the biochar, digestate and its mixture. X-ray photoelectron spectroscopy analysis indicated that biochar-digestate mix inherited

oxidized functional carbon groups and amino acid N coatings from digestate, which may have contributed towards further nutrient retention. Although differences in yield were not significant, biochar-digestate treatments increased spring onion yield by up to 37% compared to the NPK-control treatment while digestate alone performed similar to the control. At select sampling times, soil mineral N was significantly increased by up to 305% in the digestate-biochar treatment compared to the control, while digestate alone had no significant effect. Nitrous oxide emissions were largely unaffected by either digestate or biochar-digestate treatments despite higher concentrations of soil mineral N. This study confirms that digestate can be used to replace NPK fertilizer for growing spring onions in sandy soil where biochar contributes to improved N fertilization effect without stimulating additional N_2O emissions.

1. Introduction

Combining biochar (BC) and anaerobic digestate (AD) is attracting interest as an organic fertilizer, which can provide nutrients for plant growth and sequester more carbon in soil (Oh et al. 2014, Glaser et al. 2015, Elbashier et al. 2018, Greenberg et al. 2019, Ronga et al. 2020). Anaerobic digestate includes the solid and liquid residue remaining after the extraction and utilization of CH_4 as a biofuel from biogas facilities, while biochar is a carbon rich solid co-product produced by pyrolysis of biomass in the absence of oxygen. Biogas plants are a solution for the production of biofuels but their long term viability is conditional to AD being accepted and used by farmers as a fertilizer. The features that make AD a suitable soil fertilizer are the availability of macronutrients, most importantly N (mostly in the form of NH_4) and P, and stimulation of greater soil microbial diversity compared to mineral fertilizer (Sapp et al. 2015). Application of AD as a substitute for chemical fertilizer has been previously demonstrated in several studies. Haraldsen et al. (2011), found that liquid AD with a total-N content of 2200 ml L^{-1} gave a comparable fertilization to Yara Fulljgødsel® NPK 21-4-10 for barley. Sogn et al. (2018) tested 5 different ADs made from different manure, food waste and sewage sludge mixes and found that all 5 ADs were able to produce a similar yield of wheat compared to when using mineral fertilizer. They also found that ADs had less NO_3 leaching post-harvest compared to mineral fertilizer. Similar findings, with commensurate yield of grass and reduced NO_3 leaching after applying AD were found by

Walsh et al. (2012). Less work has been done on the use of AD in horticulture, where N fertilization is high. In this study, we focus on the use of AD for vegetable crops on a sandy soil in Vestfold county, Norway.

Vegetable farming often requires a significant amount of fertilizer to improve the yield and the quality of the product. Sandy soil is often chosen for growing vegetables because it is free draining, quicker to warm up in spring than clay soils, and thereby more suitable for cultivating early season vegetables (Ulfeng, 2020). However, the risk of nutrient leaching is higher on sandy soil compared to loam and clay soils due to its lower ion exchange capacity (Gaines and Gaines, 1994). In addition, the water holding capacity of sand is lower than clay and it requires frequent irrigation. This can increase the risk of NO_3 leaching if N amounts exceeds plant uptake (Quemada et al. 2013). In addition, 10% of N leaching can occur in the form of NH_4 leaching due to the low cation exchange capacity of sand (Pathan et al. 2002) and this means that also NH_4 rich AD is liable to loss from these soils. For example, during a one-time simulated rain episode conducted on a sand soil, barley pot trial, Haraldsen et al. 2011 observed 3600% higher NH_4 leaching from liquid AD compared to Yara Fulljgødsel® NPK 21-4-10 when both were applied at 160 kg N ha^{-1}. Therefore, combining liquid AD with porous materials such as biochar could be one way to sorb AD and increase its retention time in the root zone.

Biochar has gained significant research attention in the last decade as a means to store soil organic carbon and improve soil physicochemical properties, while reducing soil greenhouse gas emissions (Lehmann and Joseph, 2015). Meta-analysis of previous research showed that biochar application boosts yields in acidic tropical soils, but with little effect in temperate soils (Jeffery et al. 2017). Grain yields were mostly unchanged in a ring trial in Europe, where 20 t ha^{-1} of unmodified (pure) biochar was applied (Ruysschaert et al. 2016). In Norway, miscanthus biochar had limited effects on soil physical properties, microbial biomass, and grain yield in a 4-year field experiment (O'Toole et al. 2018), although its carbon sequestration benefits were confirmed (Rasse et al. 2017). Thus, to improve the agronomic effect of biochar, combining biochar with other materials such as manure, minerals and clay, have been recommended (Joseph et al. 2013). Other authors suggest adding biochar to composting piles (Kammann et al. 2017), where it has been shown to adsorb nutrients (Hagemann et al. 2017) and increase N availability by stimulating the growth of nitrifying bacteria (Ye et al. 2016).

Combining biochar and AD increased maize yields by 26-42% compared to AD without biochar (Glaser et al. 2015). However, a similar treatment on winter rye produced no significant yield effect (Greenberg et al. 2019). A series of 13 farm trials in Nepal found that biochar enriched with cow urine and blended with compost resulted in a 123% yield increase (cabbage, tea, pumpkin and maize) compared with a similar treatment without biochar. The authors attributed the positive effect to enhanced nutrient retention in the root zone (Schmidt et al., 2017). In a 32-week, plant-free, incubation experiment, where 3% (w/w) wood biochar and 5% (w/w) maize silage AD were added to a sandy loam, biochar increased soil NO_3 concentration by approximately 180% compared to AD and soil alone, while at the same time reducing N_2O flux by 50% (Martin et al. 2014). In another study, application of biochar mixed with AD decreased cumulative N_2O emissions by 17-40% compared to biochar and AD applied separately (Dicke et al., 2015). A recent meta-analysis conducted by Borchard et al. (2019) showed that biochar reduced N_2O emissions by 38% on average with the greatest effect on horticulture, where N use is high and with particular effect in Anthrosols and Arenosols.

The aim of the current study was to investigate whether biochar-AD mixture could be a sustainable fertilizer solution for spring onion production in a sandy soil in Norway. We hypothesized that AD could be a substitute for NPK as a basal fertilizer for spring onions and that biochar combined with AD would improve the retention and N use efficiency of AD-derived N, leading to increased plant yield and a reduction in soil N_2O emissions.

2. Materials and Methods

2.1 Site description

The field experiment was conducted on a commercial vegetable farm in Vestfold county, Norway (59°21′14″N, 10°26′51″E). The farm aims to be more sustainable, notably by substituting chemical fertilizers with organic alternatives, such as AD, compost, and biochar.

2.2 Treatments and experimental design

The experimental design was a randomized complete block design (1.5 x 10m plots) with the following 4 treatments arranged in 4 blocks:

1. **Control-NPK:** Standard basal fertilization with 400 kg ha^{-1} of 12:4:18 NPK *YaraMila Fullgjødsel micro*™. N present as 5% NO_3-N and 7% NH_4-N.
2. **AD:** Anaerobic digestate liquid slurry dosed to match N basal fertilization in the Control treatment
3. **AD + BC-Low:** A liquid mixture of anaerobic digestate and biochar (20% Vol. or 6.25% w/w of BC in AD),
4. **AD + BC-High:** A liquid mixture of anaerobic digestate and biochar (40% Vol. or 12.5% w/w of BC in AD)

The treatments above refer to differences in basal fertilization only. All treatments received additional amounts of top-dressing fertilizer later during the peak vegetation stage and after N_2O and soil sampling had been completed. AD+BC-Low and AD+BC-High received higher dose applications than the AD-alone treatment to compensate for the volume dilution of NH_4 after addition of 20 or 40% BC. One week after application of biochar and digestate, the field was sown with spring onions '*Allium fistulosum*'. See Table S1 for a chronology of soil and plant management during the experiment.

2.3 Soil, Biochar, and Digestate analysis

The field experiment was conducted on a silty course sand Arensol (90% sand, 8% silt and 2% clay). Chemical properties of the soil (before start) are given in Table 1. Soil NH_4 and NO_3 were measured according to ISO 14256-2(2005). 10-15 g of field moist soil was extracted with 1M KCl using an automated segmented flow analyzer (Seal Analytical Ltd, UK). Plant-available P, Ca, K, and Mg in both soil and biochar were measured by Eurofins Environment Testing Norway AS (NO) using the Egners AL (ammonium lactate) method (Egner et al., 1960). The extraction fluid (pH 3.75) was a mixture of ammonium lactate (0.1 mol L−1) and acetic acid (0.4 mol L−1).

The biochar was made from a mixture of spruce(*Picea abies*) and pine (*Pinus sylvestris*) wood chips in a Pyreg 500 continuous slow pyrolysis reactor operated by NovoCarbo

Gmbh (DE) where reactor temperatures ranged between 500-600℃. Biochar chemical properties (Table 1) were determined by Eurofins Umwelt Ost Gmbh (DE). Ash content was determined by combusting biochar in a muffle oven at 550°C according to DIN EN 51719. Total organic Carbon (TOC), H, N, and O were determined according to DIN 51732, TIC by DIN51726, and S by DIN51724-3. Calcium, Fe, K, Mg, Na, P, Si were determined according to DIN EN ISO 11885. Arsenic, Pb, Cd, Cu, Ni, Hg, Cr, Zn, B, and Mn were analyzed after a microwave pressure digestion according to DIN EN ISO 17294-2. Biochar pH was measured in 1:5 volume of biochar in 0.01 mol/l $CaCl_2$ solution according to DIN ISO 10390: 2005-12. Conductivity was carried out according to a method used for compost in Germany (BGK III. C2: 2006-09). A toluene extraction was used to determine poly-aromatic hydrocarbon (PAH) content according to DIN EN 15527. Heavy metals, PAHs, and other elements are listed in Table S3.

Chemical properties of AD and AD+BC-High are shown in Table 1. The liquid fraction of AD was sourced from Greve Biogas AS biogas facility in Tønsberg, Vestfold county, Norway, where the feedstock for the biogas plant is a mixture of municipal food waste and cow and pig slurry from the surrounding region. Elemental analysis of AD and AD+BC-High mixture was conducted by Eurofins Environment Testing Norway AS (NO). The following standards were used for elemental analysis: Total Carbon: EN 13137, Total P: EN ISO 11885:2009, NO3: SS028133, Total N: EN13654-1. An extended chemical analysis of the soil, AD, and biochar is described in supplementary materials (Tables S2-4).

Table 1.

Chemical properties of Soil before the experiment, and the materials that were added to the soil: Biochar (BC), Digestate (AD), and AD+BC-High

			Soil	**BC**	**AD**	**AD+BC-High mix**
TOC	%	DM	2.83	72.00	41.00	62.00
TN	%	DM	1.8	1.21	3.40	-
NH_4-N	mg kg^{-1}	FW	-	-	2800	2500
NO_3-N	mg kg^{-1}	DM	-	-	14	10
P-AL	mg kg^{-1}	DM	600	-	-	-
Total P	mg kg^{-1}	DM	-	2000	8800	5000
K-AL	mg kg^{-1}	DM	200	-	-	-
Total K	mg kg^{-1}	DM		8000	63000	31000
pH	in H2O	-	6.97	8.00	7.70	8.50
H/C_{org} (calculated)	Molar	-	-	0.44	-	-

2.4 Microscopic and surface chemical analysis

Both AD and AD+BD-high slurry liquids were freeze dried and the finely ground powders along with the biochar were analyzed using different microscopic and spectroscopic techniques. Scanning electron microscopy (SEM) was performed, using either a NanoSEM 230 or a NanoSEM 450 SEM configured with energy dispersive X-ray analysis (EDX), to examine the microstructure and chemical composition of the samples. The specimens were coated with chromium to improve conductivity. Spectra from EDS were analysed using proprietary software "Quantax", Esprit 1.9. Surface chemical analysis was performed on finely ground particles using X-ray photoelectron spectroscopy (XPS). Details on the instrument and the procedures used are explained by Taherymoosavi et al. (2017). Data was analysed using Avantage software.

2.5 AD and Biochar mixture preparation and fertilizer application

In preparation for the experiment, biochar was added to AD and mixed mechanically in a plastic tank using an FBSX-110 electrical agitator (Frank Berg Industrial Supplies, NL).

Two mixtures were made, one with 20% vol. biochar (6.25% w/w) and one with 40% vol. (12% w/w) for treatment AD+BC-Low and AD+BC-High respectively. The biochar initially floated when added to the tank filled with AD, but became gradually submerged after 30 minutes of mixing. Mixing continued for 7.5 hours which further reduced the particle size via exposure of the biochar to the steel mixing blade and mixture viscosity was sufficiently low to allow for unrestricted flow of the mixture from a watering can.

The field site was set up on 13.05.2018. Liquid mixtures of AD and biochar were banded manually using watering cans in 7 cm deep furrows (Fig. S1). A rake was used to immediately cover the AD fertilizers with soil to minimize ammonia volatilization. After one week, spring onion seeds were sown in 4 lines per planting bed (1.5 m D) located directly over the banded AD treatments. The three AD treatments as described earlier were dosed to match the N content in the NPK-Control treatment. As AD also includes organic N, which is not immediately available to plants, NH_4 content of AD was used to match mineral N in the NPK-Control treatment as suggested by Sogn et al., 2018.
For the control treatment, NPK fertilizer was applied in granulate form and broadcast on the soil surface, as per current farmer practice.

2.6 Soil sampling

Soil samples used for N analysis were taken to a depth of 15 cm in each plot on the same days that N_2O measurements were taken. Ten subsamples were taken per plot and aggregated for one soil sample per date per plot. Soil samples were frozen on the same day of sampling for later analysis of NH_4 and NO_3. Soil samples for AD+BC-Low treatment were not taken because this treatment was not included in the N_2O measurement campaign, due to financial and time constraints.

*2.7 Harvesting of plant*s and p*lant sap analysis*

All spring onion plants were harvested by hand and the weight (yield) and number of plants (plant population density) were recorded per plot. In the commercial farm packing house, the number of marketable bunts were recorded. Marketable bunt yield, is

defined here as the number of 150 g spring onion bunts prepared from the total harvested yield which were of sufficient quality to be sold in Norwegian supermarkets.

On the morning of harvesting day, 25 old and new leaves were sampled from each plot, and sent for plant sap analysis at Nova Crop Control (www.novacropcontrol.nl). Leaf sap was analyzed for Sugar%, pH, EC, K, Ca, K/Ca, Mg, Na, NH_4, NO_3, Total-N, Cl, S, P, Si, Fe, Mn, Zn, B, Cu, Mo and Al. Plant sap analysis was chosen over dry leaf analysis due to this sap analysis method being already used as an on-farm plant quality diagnostic tool.

2.8 Greenhouse gas emission measurements

Emissions of N_2O were measured 8 times between 05-22 June 2018, i.e. following basal fertilization and prior to top dressing application. The static chamber method was used according to Pumpanen et al. (2004). Aluminum collars (60 cm x 60 cm x 20 cm) were driven into the soil in each plot. At the time of measurement, aluminium chamber tops (60 cm x 60 cm x 20 cm) were installed on the collars and 4 gas samples were taken at time points 1, 15, 30 and 45 minutes, using a syringe and injected into gas tight vials. Gas vials were analyzed with a gas chromatograph fitted with an ECD for measurements of N_2O, and an automatic sampler that facilitated high throughput measurements. Flux calculation methods used in this study are described in detail by Nadeem et al. (2012). The AD+BC-Low treatment was left out of the measurements for N_2O.

2.9 Soil temperature and moisture

Soil temperature and moisture were recorded each time N_2O flux were measured (Fig. S2). Soil moisture time domain reflectance (TDR) sensors (GS3 model, Decagon Devices [now Meter Environment]) were installed in each plot at a depth of 5 cm within one meter of the GHG measurement chamber in the soil lines where BC and AD were applied. Measurements of volumetric water content (VWC) were used to calculate water filled pore space (WFPS)(Eq. 1), which is commonly used soil moisture unit for explaining variation in N_2O.

$$WFPS = 100 - \left(VWC - \frac{Bulk\ density\ [1.3\ g\ cm^{-3}]}{Material\ density\ [2.65\ g\ cm^{-3}]}\right) \quad \textbf{(Eq.1)}$$

2.91 Statistical Analysis

All statistical analyses were carried out using R Software v3.5.0 (The R Foundation for Statistical Computing, Vienna, Austria). Statistical differences between treatment means for plant yield, plant sap nutrient content, content were analyzed using analysis of variance (ANOVA) from the multcomp package in R (Hothorn et al. 2008), with post-hoc multiple comparison of treatments vs. control done via the Tukey test if $p < 0.05$. Repeated measure analyses of soil NO_3, NO_4 and Mineral N were conducted using a linear mixed effects model from the nlme package in R (Pinheiro et al., 2020) with treatment and measurement day (and their interaction) as fixed effects and block as a random effect. Non-normally distributed data, e.g. soil nitrate concentrations, were log transformed. A Generalized Linear Model (GLM) from R "stats" package (R Core Team, 2020) was used to evaluate how N_2O emissions were influenced by environmental variables (mean soil temperature, mean VWC, Soil NO_3-N and NH_4-N content), experimental treatments and interactions between treatments and environmental variables. Candidate models were selected according to the lowest corrected Akaike information criteria (AICc) and a ΔAICc <2 (Burnham and Anderson, 2002). Residuals and predicted random effects were plotted (QQ plot) to assess deviation of residuals from normality.

3. Results

3.1 Spring onion yield and plant density

Mean total yield was 13, 24, and 37% higher in AD, AD+BC-High and AD+BC-Low compared to control-NPK treatment, with none of the treatment differences being statistically significant (Table 2). Mean marketable bunt yield was 42% and 33% higher in AD+BC-Low and AD+BC-High compared to control-NPK treatment, while AD had -6%, with none of the treatment differences being statistically significant (Table 2). Total number of plants surviving to maturity were 26% higher in AD+BC-Low, 37% lower in

AD, and 4.3% lower in AD+BC-High compared to the control, with none of the treatment differences being statistically significant (Table 2).

Table 2.

Total and marketable yield and plant population density

	Total Yield (tonn ha^{-1})		Marketable yield (nr. bunts ha^{-1})		Plant population density (nr. plants surviving to harvest maturity ha^{-1})	
	Mean	±SE	Mean	±SE	Mean	±SE
Control	8.37	1.34	14499	1976	162268	28468
AD	9.47	0.76	13598	2987	102051	20026
AD+BC-Low	11.46	1.76	20535	2870	203963	30876
AD+BC-High	10.40	2.24	19356	4032	155192	12650

**No statistical significance between treatments for total yield (p=0.55), marketable yield (p=0.16), or harvestable plant numbers (p=0.07)*

3.2 Soil Nitrogen levels

Repeated measure analysis indicated there were significant treatment differences in at least one of the 8 measurements of soil NO_3, NH_4, and cumulative soil mineral N. For these measurements, concentrations of soil NO_3 were 200-600% higher in AD+BC-High compared to control (4^{th} and 7^{th} June, Fig. 1A). For Soil NH_4, AD+BC-High was 80-170% higher than Control-NPK and 66-88% higher than AD (Fig. 1B). For Mineral N, AD+BC-High was 150-300% higher than in Control-NPK and 91% higher than in AD (Fig. 1C). Differences in NO_3 appeared one week later in the season as compared to differences in NH_4 content.

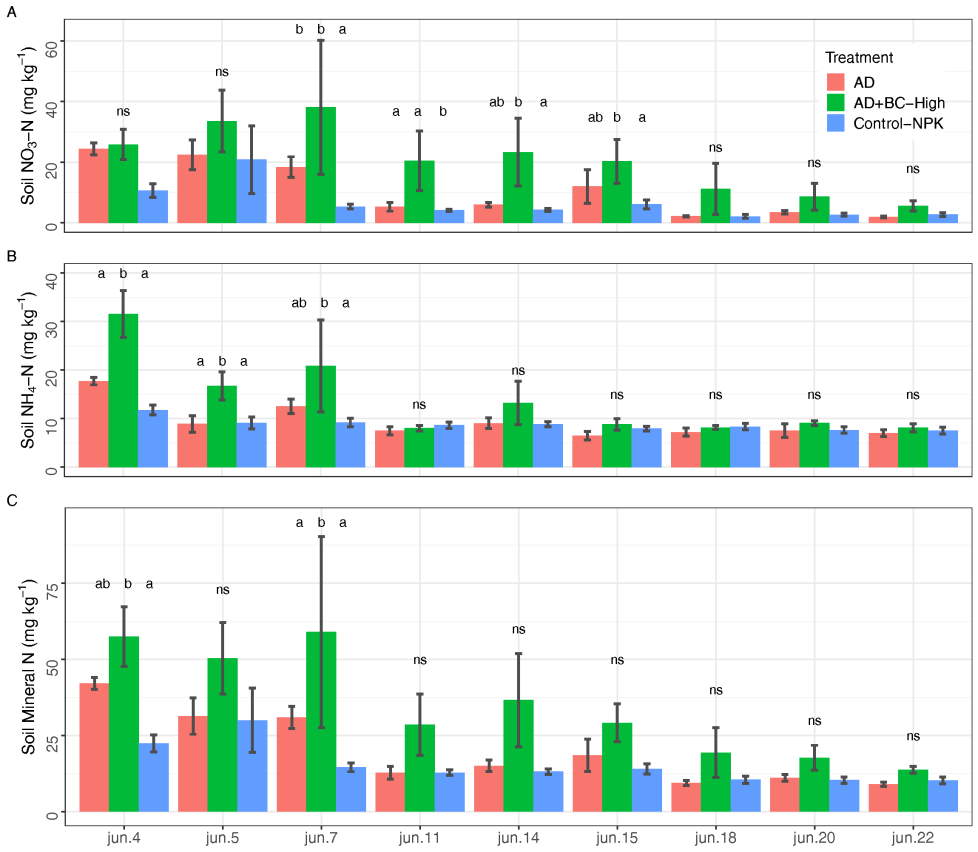

Fig. 1. A. Soil NO_3 **B.** Soil NH_4 and **C.** Soil mineral-N levels at 0-15 cm depth at the start of the growing season on the days when N_2O measurements were taken. Standard error bars for mean values indicated.

3.3 Microscopic and surface chemical analyses of biochar, AD and AD modified biochar

The low magnification secondary electron image of a unmodified biochar particle (Fig. 2) along with EDS spectrum, collected from the whole area, shows a high concentration of C, O, Si, Ca, K, with a low concentration of Mg, Al and P. Cr is from the coating. The high magnification SEM image of an AD + BC High particle shows the porous structure of the biochar, with a pore size less than 10 µm (Fig. 3A) and indicates the presence of Mg/P rich phases (possibly $Mg_3(PO_4)_2$) on the surface of the biochar (point a). Figure 3.B reveals the formation of C/Ca rich clusters, possibly $CaCO_3$, mixed with a low concentration of Na, AL, K, Si, Cl and S, on the surface of an AD+BC-High particle (point

b). Figure 3.C (point d) also indicates a high concentration N, Mg, O, and P, indicating possible struvite precipitation on the biochar surface.

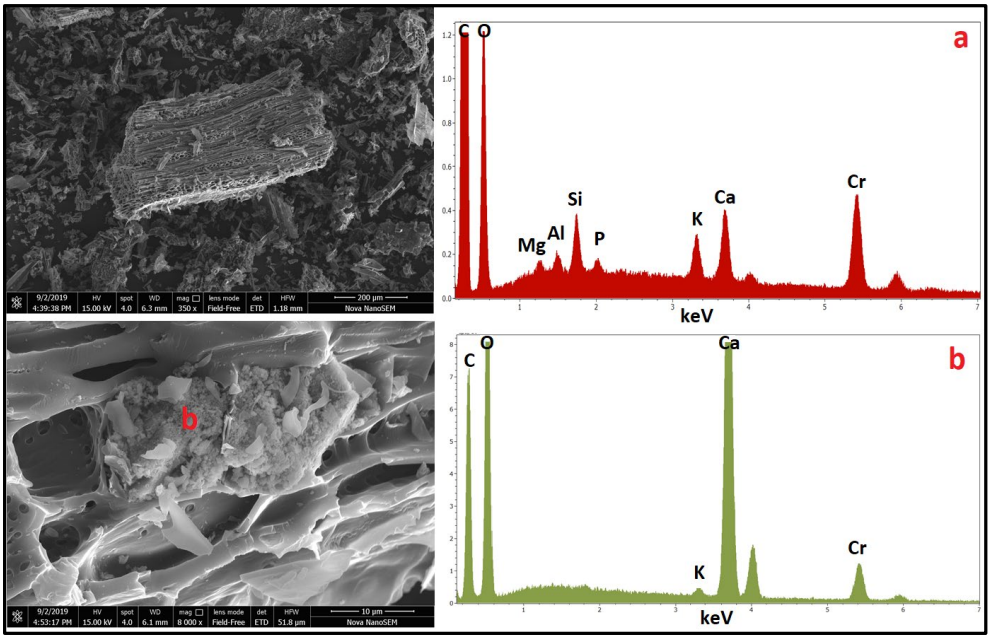

Fig. 2. Low magnification SEM image of an unmodified biochar particle along with EDS spectrum, collected from the whole area (a). Point b shows the presence of Ca-rich clusters within the biochar structure

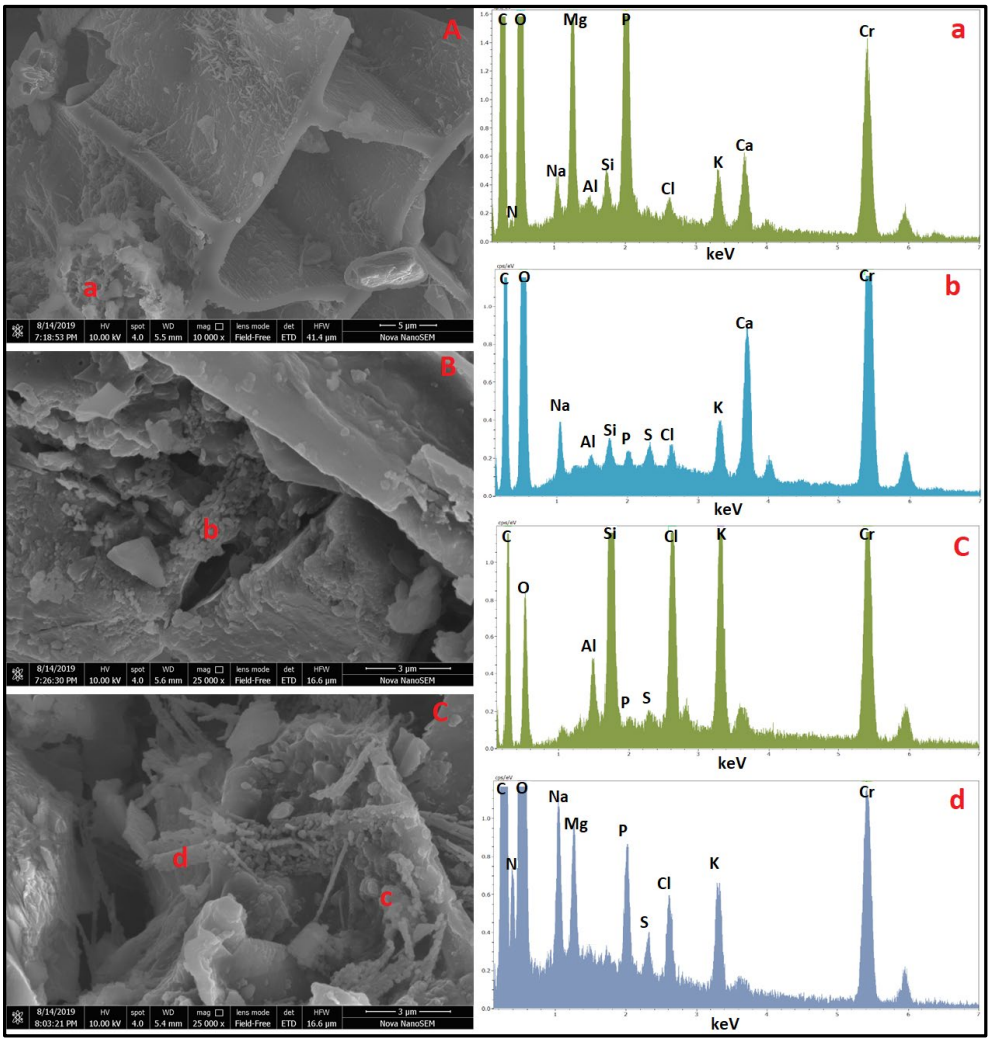

Fig. 3. High magnification images of the AD+BC-High and EDS spectra of specific points of interest where minerals are adsorbed and precipitated on the biochar surface.

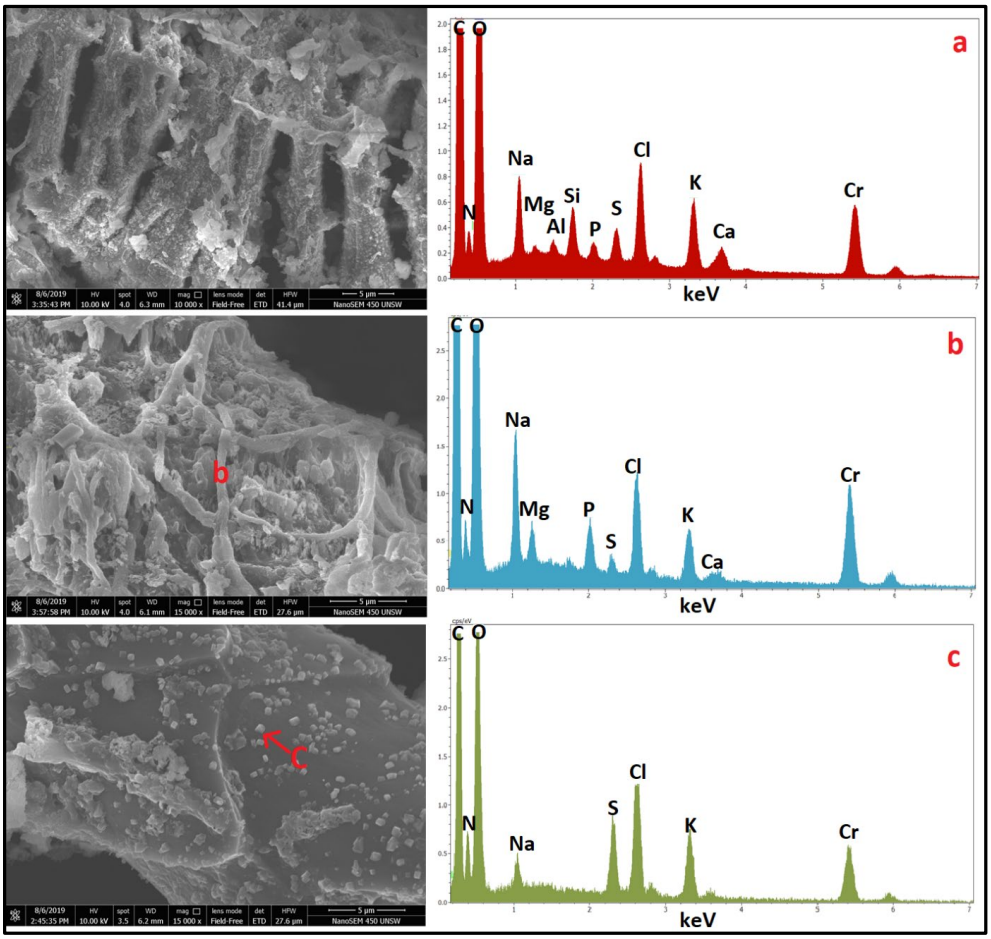

Fig. 4. Secondary electron images and associated EDS spectrum of an AD particle, collected from the (a) whole area. Point b indicates the presence of either NaCl and/or KCl mixed with high C, N, Mg and P on the surface of an AD particle; point c shows the presence of S/Cl/K/N rich nano-particles mixed with C on the surface.

The macro and microstructure of different AD particles examined were different (Fig 4), but the chemical composition was broadly similar. AD sample (Fig 4, a) was rich in C, N, O, Na, Cl, K, Si and S with lower concentrations of P, Ca, Mg and Al. EDS analysis (Fig 4, point b) indicates the presence of possibly bacteria and a high concentration of NaCl and/or KCl on the surface of an AD particle. A high magnification image of another AD particle also revealed the formation of organo-mineral nano-particles rich in C, N, O, Cl, S

and K. As can be seen in the Figure S.3 AD+BC-High had a high concentration of C, O, Si, Ca, K, Cl, with a low concentration of Al, P, Mg and N.

The changes in surface chemical bonds and functional groups are shown in Table 3. XPS analysis indicates significant changes in the surface functionality of both the biochar and the AD when they are combined. Biochar has a high concentration of surface aromatic saturated aliphatic hydrocarbons (C-C) and carboxylic and carbonate groups compared with the AD which in turn has a higher concentration of C-O (hydroxy/phenol/ether) groups. When the two substances are combined the C-C concentration becomes very similar to the pure AD indicating that the surface of the biochar is coated with the organics from AD. As can be seen in Table 3 AD contained a high concentration of surface N (4.55 at%). Nitrogen bond on the surface of AD+BC-High were in the form of amino acid N (N-C-COOH).

Table 3.

The concentration of surface functional groups, measured by XPS.

Region scans	Functional groups	Peak BE	BC (at.%)	AD (at.%)	AD+BC-High (at.%)
C1s A	C-C/C-H	284.80	60.23	42.54	43.69
C1s B	C-O/C-OC	286.40	12.20	17.81	21.48
C1s C	C=O	288.00	3.65	5.25	7.01
C1s D	O=C-O/Carboxylic	289.20	3.27	1.25	
C1s E	Carbonate	290.40	2.69		0.28
N1s A	NH2/amine	399.00	0.41	4.55	
N1s A	N-C-COOH /Pyridone	400.7	0.47		4.27
O1s A		531.85	6.62	10.97	9.32
O1s B		533.52	7.16	10.58	9.69
Ca2s		438.55	0.71	0.50	0.38
Mg1s		1304.88	0.34	0.13	0.13
Si2p		103.71	0.66	0.34	0.34
K2p3 A		293.53	1.26	1.79	1.28
Cl2p		199.90	0.20	1.71	0.66
S2p A		169.46		0.15	0.10
S2p B		163.45		0.26	0.14
Na1s A		1071.36		1.94	1.02
P2p		133.46	0.13	0.23	0.20

3.4 Greenhouse gas emissions

Nitrous oxide flux and cumulative N_2O emissions were not significantly different between treatments (Fig. 5a and 5b). Flux peaks coincided with irrigation and rainfall events (Fig. S2). Analysis via the generalized linear model (Table 4) indicated that N_2O flux was significantly increased by soil temperature ($p<0.001$) and NH_4 soil content ($p=0.03$) but with no effect for NO_3 soil content ($p=0.19$) or water filled pore space ($p=0.35$)(Table 3). There was a significant interaction effect with soil mineral N and treatments AD and AD+BC-High. Both AD and AD+BC-High had less N_2O production per unit of soil mineral N compared to the Control (Fig. 6.)

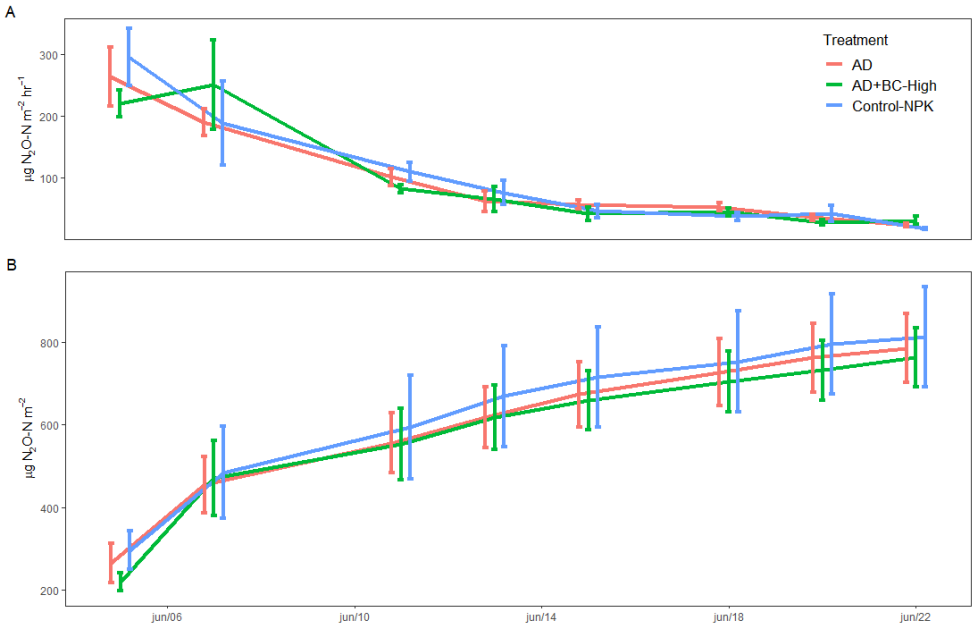

Fig. 5. A. Soil N_2O flux and **B.** Cumulative N_2O emissions in the first month of the growing season. Points are means with standard error bars. No significant difference between treatments.

Table 4.

Generalized Linear Model of N_2O emissions as explained by experimental treatments, mean daily soil temperature, volumetric soil water content and soil mineral N content

	Estimate	**Std. error**	***t* value**	**Pr(>[t])**
Intercept	5.05	0.24	20.30	<0.0001 ***
Soil temperature	0.57	0.07	7.98	<0.0001 ***
Soil Min-N	1.74	0.52	3.31	0.002**
Soil water content	0.15	0.05	2.66	0.01*
Treatments and interactions				
AD	-0.55	0.26	-2.11	0.04*
AD+BC+High	-0.82	0.28	-2.89	0.006**
Min-N*AD	-1.37	0.51	-2.70	0.01*
Min-N*AD+BC-High	-1.65	0.52	-3.20	0.003**

Null deviance: 29.47 on 47 degrees of freedom, Residual deviance: 4.41 on 40 degrees of freedom, AIC: 449.65

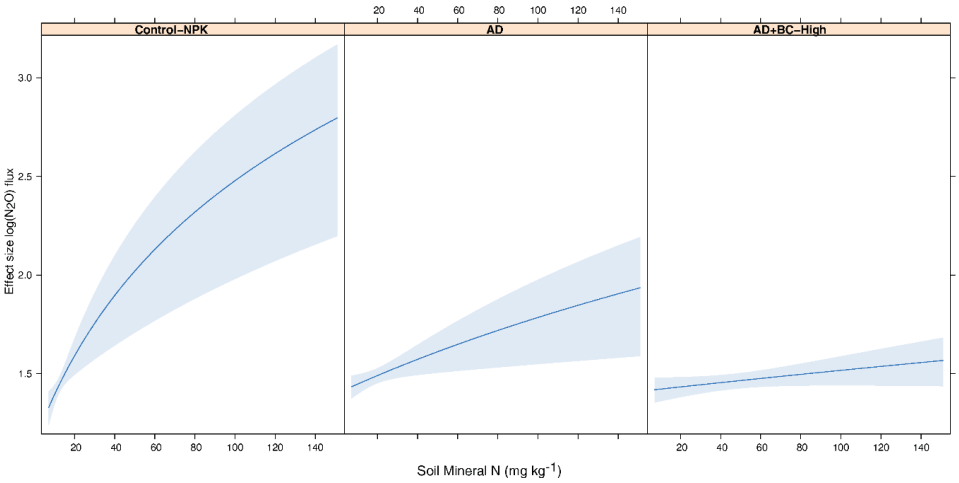

Fig. 6. Modelled effect size of N_2O flux from the interaction of soil mineral N content and treatment

3.5 Plant sap analysis

Compared to Control-NPK, AD+BC-Low had a 30% reduction in total-N ($p<0.05$) in the sap of older leaves, while AD and AD+BC-High had 21% and 7.4% less respectively (both not significant). Similarly, for K content in older leaf sap, AD+BC-Low had a significant reduction of 15% while AD and AD+BC-High both had 11% less (not significant) compared to the control. Otherwise for sugar content, pH, and all other macro and micro nutrients there were no significant differences between treatments for neither older leaves nor newer leaves (Table S5).

4. Discussion

4.1 Yield and fertilization effect of AD and Biochar

Total yield was not reduced in the treatments receiving AD basal fertilizer compared to the NPK-control, which confirms that AD can be a suitable substitute for NPK basal fertilizer for spring onion. Our finding agrees with earlier studies including for fertilization of barley (Haraldsen et al. 2011), wheat (Sogn et al. 2018), and greenhouse tomatoes (Stoknes et al. 2018). Despite marketable yield increasing by 33-42% with AD-

BC-Low and AD-BC-High compared to the control, standard deviation was high within treatment and differences were not significant. Nevertheless, the increased soil NO_3 concentration due to biochar addition to AD is a positive development for the use of AD as a fertilizer. Onion roots will preferentially take up NO_3 even when NH_4 is the dominant N species (Abbès et al. 1996) and therefore a more balanced NH_4-NO_3 fertilization was provided in the AD + BC-High treatment. Increasing the biochar amount in the AD from 20% to 40% did not alter yield (Table 2), and we do not have enough data currently to recommend an optimal mixing rate of biochar and AD. The biochar added to the soil via the AD + BC mixes are equivalent to 0.05% w/w soil in the AD+BC-Low and 0.10% w/w soil in the AD+BC-High. This is an order of magnitude under what is typically tested in biochar pot and field trials (>1% concentration) (Jeffery et al. 2011). In a previous field experiment we observed no significant change in grain yields over 4 years by addition of pure biochar applied at 1% w/w or 30 t ha^{-1} (O'Toole et al. 2018). In contrast, Glaser et al. (2015) demonstrated that combining as little as 1 t ha^{-1} of biochar in combination with either NPK or AD increased yield in maize in one field season in a sandy soil, with commensurate increases found in soil Total-N concentrations in biochar-AD mixes. Increasing the dose to 40 t ha^{-1} further stimulated yield and increased soil water holding capacity but may not be net profitable. There is growing evidence that the pre-incubation of biochar with nutrient-rich liquids or inclusion in composting processes is determinant in the extent to which biochar improves N cycling and yield effects. Mixtures of biochar and urine increased average pumpkin yield by 306% across 8 farmer field sites in Nepal compared to urine or biochar alone (Schmidt et al. 2015). The authors attributed the effect to organic coatings developed on urine soaked biochar which could have enhanced anion and cation exchange. This theory was later confirmed by Hageman et al. (2017), where adsorptive organic coatings were identified on biochar outer and inner (pore) surfaces after inclusion of biochar in composting. Annual additions of smaller amounts of biochar to liquid fertilizers could be an economically efficient way of using biochar in agriculture.

4.2 Biochar and digestate synergies for improved fertilization

AD+BC-High showed significantly higher levels of both NH_4 and NO_3 in the soil during the first month of the growing season compared to Control-NPK (Fig. 1). There are

several possible explanations for this finding. Biochar is known to act as a sorbent for nutrient such as NH_4 (Yang et al. 2018) and its sorption capacity is influenced by surface area, pH, CEC, and acidic surface functional groups. Surface area of biochar was not measured but we can imply that the mechanical mixing of the biochar with the AD would have decreased particle size and increased exposure of the biochar surface area to the AD. Several studies have shown that reducing biochar particle size via ball milling or crushing is a simple method for increasing surface area and potential adsorption sites (Peterson et al., 2012., Qin et al 2019, Fahmi et al. 2018). Enhanced sorption of NO_3 into biochar pores is also likely related to the extended contact time between AD and biochar during 8 hours of mixing. Hafshejani et al. (2016), observed that a modified sugar cane bagasse biochar achieved maximum NO_3 adsorption after 60 minutes contact time. For NH_4 adsorption to biochar, Sumaraj et al. (2020), found that adsorption reached a maximum after 24 hours. Biochar can be used as adsorbent to remove nutrients from AD, or the two materials can be simply mixed together as in our case. One advantage of our approach is that there is no left over liquid that needs to be disposed of, and thus can be a preferable approach for biogas facilities if it is deemed economical to transport AD liquids to farms.

Recent research suggests that acidic surface functional groups, pH ~8, and a higher ash content are important parameters that control the adsorption of NH_4 from aqueous media (Sumaraj et al. 2019). These findings are highly relevant for understanding the mechanism for beneficial synergies between AD and biochar, because these conditions are often met when mixing the two materials. In our study, surface functional group analysis revealed that after mixing biochar and AD, C-O and the C=O increased, and the carbonates were reduced on the biochar surface. COOH were not detected which could indicate that the biochar surface has catalyzed some organic reactions in the AD. A similar pattern was observed for the N functional groups where the biochar had a small concentration and AD had no N-C-COOH (amino acid N)/pyridone groups. However, when the two were combined the amino acid groups increased significantly. Similarly, the AD had a high concentration of NH_2/amine groups but these were not detected in the combined mixture. In summary, biochar inherited functional groups from the AD but N compounds took on a different form. Lin et al. (2012) suggest that amino acid

adsorption on biochar surfaces can seed further adsorption sites with metal oxides and help to form nano-organo mineral complexes.

The high magnification SEM images of the AD+BC-High (Fig. 3) and EDS spectra, indicate high levels of Ca, Mg, P, O, K, Cl, Na and N at selected areas of interest where mineral deposits appear to be precipitating on the surface. These mineral precipitates could take a number of chemical forms including struvite, magnesium phosphate, calcium phosphate, sodium and potassium nitrate. It is probable that the thorough mixing of AD with biochar in our study and its application as a thick slurry in the root zone of the soil allowed more time for surface reactions to occur between AD and BC compared to when AD was applied alone as banded liquid to the sandy soil. The ability for biochar surfaces to catalyze precipitation of salts from solutions supported in the literature. Marshall et al. (2017) observed that calcium phosphate was precipitated from an aqueous solution in the presence of biochar. Moreover several studies where biochar is doped with MgO have shown to precipitate struvite on biochar surfaces (Xu et al. 2018, Muhmood et al. 2019, Fang et al. 2014). Struvite stored on the biochar could act as a long term slow release source of both P and N (Talboys et al., 2016).

While not significant, there appeared a trend towards higher NO_3 in the AD+BC-High compared to AD alone (Fig. 1.A), suggesting that stimulation of nitrification took place, because the only source of N in these treatments were from the NH_4 rich AD. Nitrification can take place over a wide range of pH (4.5-10) but is optimal at 8.5 (Havlin et al. 2005), which was the pH of the AD+BC-High (Table 3). Stimulation of nitrification was observed by Wang et al. (2017) who reported both reduced NO_3 and NH_4 leaching as well as reduced N_2O and increases of rice yield (>10%) in an irrigated rice paddy. In contrast, Marchetti and Castelli (2013) reported that a wood biochar was unable to sequester min-N from AD when co-applied to soil. A lack of nitrification stimulation from biochar in the study from Marchetti and Castelli (2013) may be due to the fact that the biochar was mixed with soil before exposure to AD, compared to our study where the biochar and AD were thoroughly mixed before addition to the soil.

Finally, mixing of AD and biochar may lead to greater retention of N and reduced leaching by biochar holding onto more water containing soluble NO_3. Greater N retention and reduced leaching was also observed in a ^{15}N tracing field experiment with maize after addition of 30 t ha^{-1} biochar. The authors found a 140% increase in ^{15}N fertilizer retention in the top soil, and 300% increase of ^{15}N content in microbial biomass from the biochar treated soil (Güereña et al. 2013). Also, in the study by Schmidt et al. (2017), the superior performance of biochar-urine over urine alone was also attributed to the leaching of half of the urine-N below the rhizosphere during irrigation and rain events. We suggest that the same phenomena occurred in our study, where biochar helped to offset the leaching of nutrients during irrigation events. The depleted levels of NO_3 and NH_4 in Control and AD compared to AD+BC-High suggest that without biochar, nutrients were more readily leached from the rooting zone during irrigation early in the growing season. While pure biochar does not have a high adsorption capacity for NO_3 due to its low anion exchange capacity, reduced NO_3 leaching is thought to occur by the enhanced retention of NO_3 laden water in biochar pores (Kammann et al. 2015). This was evidenced by Bell and Worall (2011) where biochar reduced NO_3 leaching by 41%, and where over half the effect was attributed to reductions in leachate volumes.

4.3 Plant sap analysis

Plant sap analysis indicates the sugar, pH, and nutrient concentration status in a plant at the time of sampling and is increasing used by farmers for adjusting fertilization requirements during the season. The analysis of old versus new leaves gives an indication of possible nutrient deficiencies due to the fact that some macro nutrients such as N, P, K, and Mg are mobile and are translocated to new leaves and leave old leaves deficient, while others such as S, Ca, and Fe are immobile in the plant and deficiencies become visible in the new leaves. From our analysis, leaf sap levels were fairly similar across treatments except for Total N and K. Potassium reduction in the AD treatments is likely caused by the high concentration of NH_4 which has the potential to inhibit K uptake if found in high concentrations in the soil solution. Nitrate levels were somewhat reduced in the leaves of the AD treatments compared to NPK which could likely due to the moderate levels of NaCl- in the AD (Table S.4) which can displace NO_{3-}

for plant uptake. Salt levels in AD stem from municipal food waste, and could be a limiting factor for AD use as a fertilizer if levels are too high. Onions are known to be sensitive to saline conditions (Regessa, 2010).

4.4 Soil N_2O emissions

The early growing season was relatively dry and thus N_2O emissions in general were low. Peak N_2O flux range of 200-300 μg N_2O N m^{-2} hr^{-1} in our study were similar in range to peak flux range 171-300 μg N_2O N m^{-2} hr^{-1} observed in another field trial (Nadeem et al. 2012). In our study, emissions were mostly influenced by soil temperature and moisture content. Flux of N_2O was relatively unchanged by the treatments, -3.4% in the AD treatment and -6.1% in AD+BC-High compared to the control, despite much higher levels of NO_3 and NH_4 in the AD + BC-High treatment. The results of the GLM showed there was a significant interaction between soil Mineral N and treatment, (Table 3). The AD treatment, and to a greater extent AD+BC-High produced less N_2O per unit of mineral nitrogen (Fig. 3). Our result agree with with Martin et al., 2014 who also found that soil NO_3 concentrations increased and N_2O decreased when biochar was mixed with digestate and speculated that this may have been due to the known of phenomena of adsorption of NO3 in biochar pores, as recently demonstrated by Haider et al., 2020.

. In a ^{15}N incubation study by Case et al. 2014, biochar suppressed cumulative N_2O emissions by 91% in near saturated conditions while increasing nitrification by 34%.In a 2 year field trial in a sandy soil in Germany, a biochar-AD mixture reduced N_2O by 40% compared to AD alone. Here biochar was also pre-incubated with AD before application to enhance interactions (Dicke et al. 2015). While our experiment did not allow us to discriminate between N_2O produced from nitrification or denitrification, we assume it was mostly caused as a by-product of nitrification due to the drier weather and that the soil was a highly aerated sandy soil. Nitrification, that is the oxidation of NH_4 to NO_3, is the main pathway for N_2O production after the addition of ammonium based fertilizers, especially in sandy soils which are more aerated or where O_2 levels are above 1% (Zhu et al. 2013). Nitrous oxide emissions have been shown to be mostly attributable to nitrification when WFPS is between 35 and 70% (Bateman and Baggs, 2005) which was the predominate range measured in our study (Fig. S.2). Therefore, while previous

research suggests that biochar stimulates N_2O via nitrification (Sánchez-García, 2014) we did not observe this in our experiment. We cannot discount, that we did not capture all of the N_2O flux spikes that may have occurred on days when we did not measure. In summary, the combined results of our study and the studies mentioned gives a positive signal that biochar can contribute in making nitrogen available to plant growth and microbial processes while at the same time not stimulating more N_2O loss, and at best even reducing it.

5. Conclusion

Pre-mixing of biochar with AD showed to be a promising method for increasing the fertilizer effect of AD rendering a mixture which appeared to have greater ability to provide nutrients to spring onions and is less easily leached during irrigation than AD alone or mineral fertilizer. An additional co-benefit was that for per unit of soil mineral N there was less N_2O emissions when biochar was mixed with AD. This study focused only on the use of AD as a basal fertilizer and more work needs to be done in further studies to make AD suitable for top dressing and fertigation of vegetable crops such that progress can continue on closing nutrient cycles and reducing dependency on less sustainable mineral fertilizers.

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Supplementary materials, Paper IV, O'Toole et al. Biochar improves the nitrogen fertilization effect of anaerobic digestate in spring onions)

Table S1. Chronology of soil and plant management activities during the field experiment

Date	Activity
13.05.2018	Field trial set up – application of AD and biochar and NPK fertilizer
	Standard basal fertilization with 400 kg ha-1 of 12:4:18 NPK YaraMila
	Fullgjødsel micro™
21.05.2018	Sowing of spring onions
	Irrigation applied by farmer according to soil and plant need throughout
	the season
05-22.06.18	N_2O measurement period (8 measurements – see flux data)
29.6.2018	Additional top dress fertilizer applied to all plots 200 kg /ha of 12-4-18
	Yara "fullgjødsel"
8.8.2018	Additional top dress fertilizer applied to all plots 200 kg /ha of 12-4-18
	Yara "fullgjødsel"

Table S2. Soil properties (n=3)

Kjeldahl N	mg kg^{-1}	1800	±800
P-AL	mg kg^{-1}	600	±100
K-AL	mg kg^{-1}	200	±<100
Ca-AL	mg kg^{-1}	2400	±100
Mg-AL	mg kg^{-1}	200	±<100
Na-AL	mg kg^{-1}	100	±<100
pH	(in H_2O)	6.97	±0.06
Dry matter	%	92.00	±1.73
Loss on Ignition	% DM	5.97	±0.67
Bulk density	g/cm^3	1.30	±<0.01
Porosity	%	51	±<1.00

Table S3.

Biochar properties

Ash content (550°C)	% (w/w)	16.1
Total organic carbon	% (w/w)	72.3
Total nitrogen	% (w/w)	1.21
Hydrogen	% (w/w)	2.6
Oxygen	% (w/w)	8.7
Total inorganic carbon (TIC)	% (w/w)	0.7
H/C_{org} ratio (calculated	Molar	0.44
O/C ratio (calculated)	Molar	0.089
pH	in H2O	8.0
Conductivity	µS/cm	615
Phosphorus	mg kg^{-1}	2000
Potassium (K)	mg kg^{-1}	8000
Sulphur (S), total	mg kg^{-1}	400
Calcium (Ca)	mg kg^{-1}	26000
Magnesium (Mg)	mg kg^{-1}	3000
Iron (Fe)	mg kg^{-1}	3000
Boron (B)	mg kg^{-}	24
Manganese (Mn)	mg kg^{-}	2320
Silicon (Si)	mg kg^{-}	32000
Sodium (Na)	mg kg^{-}	1000
<u>Heavy metals (as regulated in Norway)</u>		
Arsenic (As)	mg kg^{-1}	1.1
Lead (Pb)	mg kg^{-1}	11
Cadmium (Cd)	mg kg^{-1}	0.3
Copper (Cu)	mg kg^{-1}	16

Nickel (Ni)	mg kg^{-1}	11
Mercury (Hg)	mg kg^{-1}	< 0.07
Zinc (Zn)	mg kg^{-1}	229
Chromium (Cr)	mg kg^{-1}	12
Poly Aromatic Hydrocarbon (PAH) content		
Naphthalene	mg kg^{-1}	2
Acenaphthylene	mg kg^{-1}	< 0.1
Acenaphthene	mg kg^{-1}	< 0.1
Fluorene	mg kg^{-1}	< 0.1
Phenanthrene	mg kg^{-1}	< 0.1
Anthracene	mg kg^{-1}	< 0.1
Fluoranthene	mg kg^{-1}	< 0.1
Pyrene	mg kg^{-1}	< 0.1
Benz(a)anthracene	mg kg^{-1}	< 0.1
Chrysene	mg kg^{-1}	< 0.1
Benzo(b)fluoranthene	mg kg^{-1}	< 0.1
Benzo(k)fluoranthene	mg kg^{-1}	< 0.1
Benzo(a)pyrene	mg kg^{-1}	< 0.1
Indeno(1,2,3-cd)pyrene	mg kg^{-1}	< 0.1
Dibenz(a,h)anthracene	mg kg^{-1}	< 0.1
Benzo(g,h,i)perylene	mg kg^{-1}	< 0.1
Total 16 EPA-PAH excl. LOQ	mg kg^{-1}	2

Table S4.

Chemical properties of Anaerobic Digestate(AD) and AD+Biochar-High mixture

	Unit	Anaerobic Digestate (AD)	AD + Biochar-High
DM	%	3.70	8.40
NH_4-N	mg kg^{-1} FW	2800	2500
NH_4	mg kg^{-1} DM	77000	30000
P	mg kg^{-1} DM	8800	5000
P	mg kg^{-1} FW	238	60
K	mg kg^{-1} DM	63000	31000
Ca	mg kg^{-1} DM	23000	
Mg	mg kg^{-1} DM	4100	
Na	mg kg^{-1} DM	30000	
NO3-N	mg kg^{-1} DM	<14	9.90
S	mg kg^{-1} DM	8200	
TOC	% DM	40.50	62.10
pH (in H2O @ 23 +/- 2°C)		7.70	8.50
Electrical Conductivity 25°C (23 +/- 2°C)	mS/m	-	270.00

Table.S5

Chemical analysis of spring onion leaf sap (n=4)

		Control				AD				AD+BC-Low				AD+BC-High			
		old leaves		*young leaves*		*old leaves*		*young leaves*		*old leaves*		*young leaves*		*old leaves*		*young leaves*	
		mean	*±SE*	*mean*	*±SE*	*mean*	*±SE*	*mean*	*±SE*	*mean*	*±SE*	*mean*	*±SE*	*mean*	*±SE*	*mean*	*±SE*
Sugar	%	1.9	0.1	3.1	0.1	2.0	*0.3*	3.0	*0.4*	2.4	*0.1*	3.3	*0.4*	2.3	0.2	3.1	*0.5*
ph	in H2O	5.7	0.1	5.6	0.2	5.7	*0.0*	5.6	*0.1*	5.6	*0.0*	5.7	*0.1*	5.7	0.0	5.8	*0.1*
EC	**ms/m**	**9.75 b**	**0.3**	6.8	1.1	**9.13 ab**	***0.3***	7.2	*0.6*	**8.45 b**	***0.2***	6.4	*0.5*	**9.37 ab**	**0.2**	6.0	*1.3*
K	**ppm**	**3194 b**	**96.5**	1 970	213.5	**2855 ab**	***43.7***	2 215	*331.0*	**2714 a**	***148.3***	1 928	*190*	**2852 ab**	**51.0**	1 776	*395*
Ca	ppm	1 178	134.9	338.3	139.2	1 208.3	*118.7*	470.8	*98.9*	968.8	*94.5*	335.5	*29.4*	1 178	173.9	323	*53.2*
K/Ca		2.8	0.3	6.4	2.1	2.4	*0.2*	4.8	*0.7*	2.9	*0.4*	5.8	*0.8*	2.6	0.3	5.5	*0.6*
Mg	ppm	172.0	18.0	90.0	10.5	175.8	*15.9*	119.8	*26.9*	155.5	*5.0*	99.3	*12.1*	187.0	16.7	96.3	*9.3*
Na	ppm	32.0	8.3	56.0	19.5	29.8	*3.1*	38.0	*2.4*	21.5	*3.3*	40.5	*8.7*	20.0	2.0	44.7	*13.8*
NH_4	ppm	96.8	8.3	94.3	7.1	98.5	*7.0*	86.0	*7.7*	84.8	*6.4*	84.8	*7.5*	93.8	9.2	85.7	*31.3*
NO_3	ppm	1 686	330.6	506	645.3	1 099.3	*75.1*	487	*308.0*	749.8	*207.2*	842.3	*225*	1 581.0	218.7	931	*692*
N-NO3	ppm	380.8	74.7	340	145.9	248.5	*17.0*	335.8	*69.4*	169.0	*46.7*	190.3	*50.7*	357.0	49.2	210	*156*

		1051		1				1								
Tot-N	**ppm**	**b**	**119.5**	214	213.4	**829 ab**	***42.9***	048	*142.0*	**736 a**	***86.3***	901.3	*133*	**973 ab**	**34.0**	942	*424*
Cl	ppm	826.5	65.2	317	62.4	805.5	*86.4*	434.3	*82.8*	747.8	*77.3*	366.8	*24.1*	803.0	43.7	324	*45.6*
S	ppm	247.5	14.3	251	17.5	287.8	*12.3*	262.3	*29.0*	257.5	*18.2*	242.0	*23.5*	287.3	19.3	253	*54.0*
P	ppm	118.0	6.9	192	18.1	113.0	*13.6*	153.5	*28.3*	124.8	*10.3*	174.5	*34.2*	112.5	9.0	166	*64.4*
Si	ppm	3.8	0.4	1.4	0.4	4.4	*0.5*	1.6	*0.5*	3.4	*0.5*	1.5	*0.3*	4.2	0.9	1.4	*0.1*
Fe	ppm	0.8	0.1	0.4	0.1	0.5	*0.0*	0.5	*0.1*	0.8	*0.1*	0.6	*0.2*	0.6	0.0	0.5	*0.1*
Mn	ppm	2.6	0.3	1.0	0.3	3.6	*1.0*	1.2	*0.4*	2.7	*1.0*	1.1	*0.1*	2.3	0.7	1.0	*0.0*
Zn	ppm	0.9	0.0	0.9	0.1	1.1	*0.1*	1.1	*0.1*	1.0	*0.1*	1.4	*0.6*	0.9	0.1	1.1	*0.2*
B	ppm	1.5	0.3	0.9	0.3	1.9	*0.7*	0.9	*0.8*	0.8	*0.1*	0.7	*0.1*	1.9	0.6	0.9	*0.3*
Cu	ppm	0.2	0.0	0.1	0.0	0.2	*0.0*	0.2	*0.0*	0.2	*0.0*	0.2	*0.0*	0.2	0.0	0.2	*0.0*
Mo	ppm	1.0	0.2	0.2	0.1	1.0	*0.2*	0.3	*0.1*	1.0	*0.2*	0.3	*0.0*	0.7	0.2	0.2	*0.1*
Al	ppm	0.2	0.0	0.1	0.0	0.2	*0.0*	0.1	*0.1*	0.3	*0.1*	0.1	*0.1*	0.2	0.1	0.1	*0.0*

*Measurements in bold indicate where treatment means are significantly different from one another and are marked with different letters.

Otherwise no significant difference between treatments where letters do not appear

Fig. S1. AD, AD+BC-Low, and AD+BC-High were applied to 7 cm deep furrows. The picture shows immediate raking over of soil after application of AD+BC-High

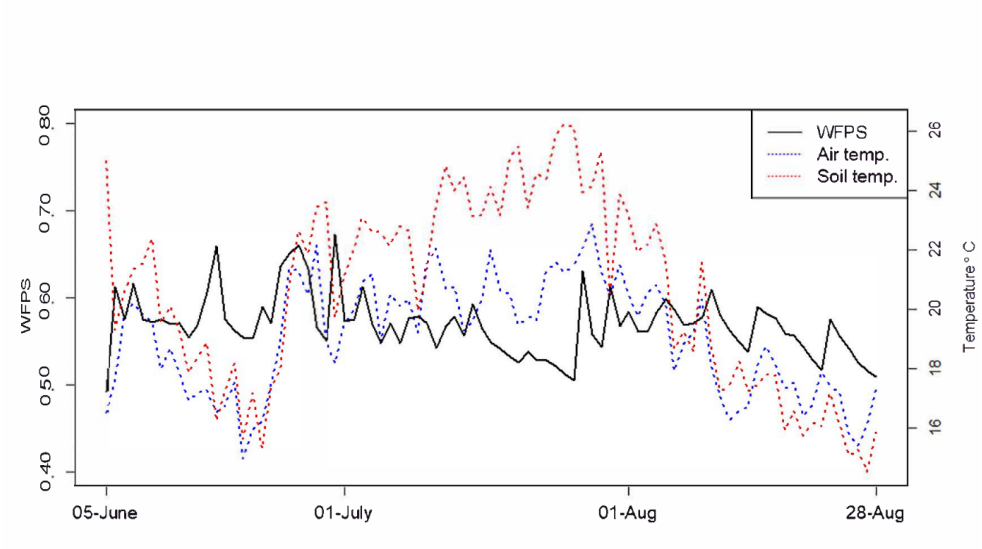

Fig.S2. Water filled pore space (WFPS) (averaged across treatments), soil temperature (averaged across treatments) and air temperature (from nearest weather station) during growth season.

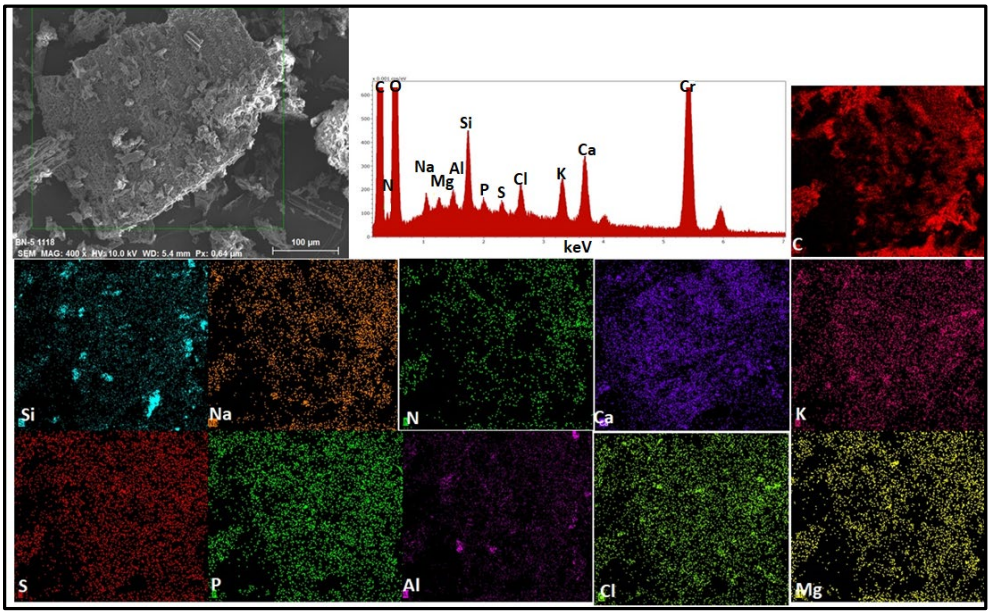

Fig. S3. Secondary electron image of the AD+BC-High along with EDS spectrum, collected from the green square, and EDS elemental maps. Cr is the coating used for spectroscopy.

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