THE EFFECTS OF BIOCHAR AND FERTILISATION STRATEGIES ON SOIL FERTILITY AND BARLEY GROWTH ON NUTRIENT DEFICIENT SOIL EIGHT YEARS AFTER BIOCHAR APPLICATION

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Master’s thesis
Agroecology
Department of Agricultural Sciences
University of Helsinki
2022

ABSTRACT

HELSINGIN YLIOPISTO — HELSINGFORS UNIVERSITET — UNIVERSITY OF HELSINKI

Tiedekunta/Osasto — Fakultet/Sektion — Faculty	Laitos — Institution — Department
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Työn nimi — Arbetets titel — Title

The effects of biochar and fertilisation strategies on soil fertility and cereal growth on nutrient deficient soil eight years after biochar application

Oppiaine — Läroämne — Subject

Agroecology

Työn laji — Arbetets art — Level	Aika — Datum — Month and year	Sivumäärä — Sidoantal — Number of pages
M.Sc. Thesis	May 2022	42 p.

Tiivistelmä — Referat — Abstract

Biochars are produced from organic materials using pyrolysis and are used as a soil amendment to improve soil fertility and plant growth. Biochars are particularly beneficial to soils with high acidity, low carbon (C) content, nitrogen (N) content and other nutrient contents. However, it is not well-studied whether the beneficial effects of a biochar exist for the long-term.

The aim of this study was to examine the effects of one-time application of spruce biochar on soil and barley (*Hordeum vulgare* L.) properties in the long-term. For this purpose, soil and plant properties were measured from the biochar field experiment in a boreal nutrient deficient Umbrisol where spruce biochar was applied eight years earlier. The experiment had a split-plot design with biochar application rates (0, 5, 10, 20 and 30 t ha^{-1}) as the main-plot factor. The effects of fertilisers alone and their interaction with biochar were studied with three treatments (control, mineral fertiliser and meat bone meal (MBM)) as the sub-plot factor. Soil moisture content at 0–15 cm depth, as well as pH, plant available nutrients, total C and N content and C/N ratio of the soil were measured. Barley growth was assessed indirectly by measuring leaf chlorophyll content (SPAD), leaf area index (LAI), plant stand density, biomass, C and N content, C/N ratio, grain yield and weight of 1000 grains (TGW).

No consistent significant effects of biochar on soil moisture content or soil chemical properties were observed. Biochar application did not have significant effects on leaf chlorophyll, leaf area index, plant density or biomass of barley. The highest biochar application rate of 30 t ha^{-1} tended to increase grain yield and TGW but the increases were not statistically significant. Mineral and MBM fertilisers produced similar grain yields although N was likely less available from MBM earlier in the growing season. Biochar and fertiliser interaction did not have significant effects on any of the measured properties.

The lack of effects of biochar may be explained by the high amount of initial soil organic matter as well as low liming effect and low nutrient content of the used biochar. In addition, the lack of significant effects of biochar also suggests loss of biochar from topsoil due to weathering and downward displacement of biochar over the period of eight years.

In this study, biochar application did not have negative consequences on the measured soil and crop properties. Therefore, it should be safe to use. Biochars may provide a viable option to sequester carbon in boreal agriculture. Further research on this is still needed to investigate the long-term effects of different types of biochars on different types of soils.

Avainsanat — Nyckelord — Keywords

biochar, barley, *Hordeum vulgare* L, mineral fertiliser, meat bone meal, yield

Säilytyspaikka — Förvaringsställe — Where deposited

HELDA - Digital repository of the University of Helsinki

Muita tietoja — Övriga uppgifter — Further information

Supervisors Priit Tammeorg and Subin Kalu

TIIVISTELMÄ

HELSINGIN YLIOPISTO — HELSINGFORS UNIVERSITET — UNIVERSITY OF HELSINKI

Tiedekunta/Osasto — Fakultet/Sektion — Faculty Maatalous-metsätieteellinen tiedekunta	Laitos — Institution — Department Maataloustieteiden laitos

Tekijä — Författare — Author

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Työn nimi — Arbetets titel — Title

Biohiilen ja lannoituskäsittelyiden vaikutuksia maaperän viljavuuteen ja viljan sadonmuodostukseen vähäravinteisessa maaperässä kahdeksan vuotta lisäyksen jälkeen

Oppiaine —Läroämne — Subject

Agroekologia

Työn laji — Arbetets art — Level Maisterintutkielma	Aika — Datum — Month and year Toukokuu 2022	Sivumäärä — Sidoantal — Number of pages 42 s.

Tiivistelmä — Referat — Abstract

Biohiili on maanparannusaine, joka valmistetaan orgaanisesta aineesta pyrolyysillä. Biohiiliä käytetään maaperän viljavuuden parantamiseen ja kasvien kasvun edistämiseen. Biohiilen käytön hyödyt tulevat esille erityisesti vähähiilisillä, happamilla ja vähäravinteisilla mailla. Biohiilen pitkäaikaisia vaikutuksia ei ole kuitenkaan paljoakaan tutkittu.

Tämän tutkimuksen tavoitteena oli selvittää kuusesta valmistetun biohiilen pitkäaikaisvaikutuksia maaperän ominaisuuksiin ja ohran (*Hordeum vulgare* L.) kasvuun boreaalisessa vähäravinteisessa karkeassa hietamaassa. Maaperän ja ohran ominaisuuksia mitattiin kenttäkokeessa, jossa kuusen biohiiltä oli levitetty kahdeksan vuotta aiemmin. Koemalli oli osaruutukoe, jossa pääruututekijänä oli biohiilen levitysmäärä (0, 5, 10, 20 ja 30 t ha^{-1}). Lannoitteiden ja biohiilen yhteisvaikutusta tutkittiin osaruututekijänä olleen lannoituskäsittelyn (kontrolli, mineraalilannoite ja lihaluujauho) avulla. Maaperästä mitattiin kosteuspitoisuus 0–15 cm:n syvyydestä, pH, kasvien saatavilla olevien ravinteiden määrä, kokonaishiili ja -typpi sekä C/N-suhde. Ohran kasvua arvioitiin epäsuorasti mittaamalla lehtien lehtivihreäpitoisuutta, lehtialaindeksiä, kasvitiheyttä, biomassaa, C- ja N-pitoisuutta, C/N suhdetta, jyväsatoa sekä 1000 jyvän painoa (TJP).

Biohiilellä ei havaittu olevan tilastollisesti merkittäviä vaikutuksia maaperän kosteuteen ja kemiallisiin ominaisuuksiin eikä ohran lehtivihreäpitoisuuteen, lehtialaindeksiin, kasvitiheyteen tai biomassaan. Suurin biohiilen levitysmäärä, 30 t ha^{-1}, lisäsi suuntaa antavasti jyväsatoa ja TJP:tä, mutta lisäykset eivät olleet tilastollisesti merkitseviä. Mineraali- ja lihaluujauholannoitteilla saatiin samansuuruisia jyväsatoja, vaikka typen saatavuus lihaluujauhosta oli todennäköisesti heikompaa aiemmin kasvukaudella. Biohiilen ja lannoitteen yhteisvaikutuksia ei havaittu.

Tuloksia selittävät maaperän orgaanisen aineksen suuri määrä sekä käytetyn biohiilen alhainen kalkitusvaikutus ja alhainen ravinnepitoisuus. Myös biohiilen rapautumista ja kulkeutumista syvemmälle maaperään on oletettavasti tapahtunut kahdeksan vuoden aikana.

Tässä tutkimuksessa käytetyn biohiilen levityksellä ei ollut negatiivisia vaikutuksia maaperän ominaisuuksiin eikä ohran kasvuun ja sen käytön voidaan katsoa olevan turvallista. Biohiilet voivat tarjota varteenotettavan vaihtoehdon hiilensidonnalle peltomaihin pohjoisissa olosuhteissa. Lisää tutkimusta tarvitaan erityyppisten biohiilten pitkäaikaisvaikutusten selvittämiseksi erilaisissa peltomaissa.

Avainsanat — Nyckelord — Keywords

biohiili, ohra, *Hordeum vulgare* L, kivennäislannoite, lihaluujauho, sato

Säilytyspaikka — Förvaringsställe — Where deposited

HELDA - Helsingin yliopiston digitaalinen arkisto

Muita tietoja — Övriga uppgifter — Further information

Ohjaajat Priit Tammeorg ja Subin Kalu

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ABBREVIATIONS AND CONCEPTS

AGB	Above ground biomass
BET SSA	Brunauer-Emmet-Teller Specific Surface Area
$CaCO_3$	Calcium carbonate
CEC	Cation exchange capacity
C/N ratio	Carbon-to-nitrogen ratio
GHG	Greenhouse gas
LAI	Leaf area index
MBM	Meat bone meal
NH_4^+-N	Ammonium
NO_3^--N	Nitrate
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
SOC	Soil organic carbon
SOM	Soil organic matter
SPAD	Single photon avalanche diode, relative leaf chlorophyll content
TDR	Time domain reflectometry
TGW	Thousand grain weight

1 INTRODUCTION

Better practices for sustainable food production are needed as the world is facing climate change and population growth. Loss of carbon (C) from arable land is a continuous problem, also in Finland (Heikkinen et al. 2013), and the carbon losses are expected to increase by the climate change (Heikkinen et al. 2022). Agricultural sector is responsible for 20% of the global greenhouse gas (GHG) emissions (FAO 2020). The use of biochars in agriculture is one of the promising alternatives for sequestering more carbon in arable soils and decreasing the GHG emissions from the soils. Biochars are carbonaceous substances that are produced in a pyrolysis process at high temperatures in anaerobic conditions using different organic materials as feedstock. Biochars are highly resistant to microbial decomposition due to their aromatic structure and can remain in soil from hundreds to thousands of years, differently from non-pyrolyzed biomass (Wiedner and Glaser 2015, Lehmann et al. 2021). Application of certain biochars can increase soil carbon storage (Gross et al. 2021), decrease GHG emissions (Schmidt et al. 2021) and improve crop yields (Singh et al. 2022).

The ability of biochars to improve the sustainability of food production has received much attention recently. While there are numerous studies available about biochar effects in tropical soils, less studies have been conducted in temperate and boreal climate. Biochars have been reported to improve the fertility of low fertile tropical soils, but controversial results have been reported in boreal and temperate conditions (Jeffery et al. 2017, Schmidt et al. 2021). In addition, many of the results obtained were from short-term studies lasting less than five years. While short-term studies can give indications on the effects of biochar application, field aging of biochar often changes its properties (Mia et al. 2017) and therefore, also long-term studies in field conditions are needed. Differences in climates, used biochars, soil types and agricultural production systems make it difficult to generalise the results from studies (Verheijen et al. 2010). It is therefore important to study the effects of biochars in representative conditions.

The studies of biochar effects on barley production are common from tropical and temperate climate regions but only few studies exist from boreal climate. Barley is one of the most important crops in Europe and in Finland. In Finland, barley is currently the most popular cereal with cultivation area of 455 000 ha in 2019, that is about 1/5 of all cultivated area (Luke Tilastotietokanta 2021a). It is important to study the effects of biochar on growth of barley in boreal conditions since these conditions differ in many respects from other climates. For example, the boreal growing season is short and freeze-thaw cycles in the winter and night frosts during the growing season may occur (Peltonen-Sainio et al. 2016). Boreal agricultural soils usually also have higher C contents than soils in other climatic zones (Heikkinen et al. 2021).

The aim of this study was to assess the long-term effects of spruce biochar addition in boreal field conditions. Biochar effects on soil moisture content, soil nutrient content and barley growth were evaluated eight years after biochar application. The effects of different biochar application rates were studied with five different biochar application rates. The effects of fertiliser use along with biochar was studied using unfertilised control, mineral fertiliser and organic meat bone meal (MBM). This study was part of the long-term biochar research conducted in the AgriChar research group at the University of Helsinki.

2 EFFECTS OF BIOCHARS ON SOIL PROPERTIES AND CROP YIELD FORMATION

2.1 Biochars

2.1.1 The production and use of biochars

Biochars are produced from organic materials such as wood, manure and crop residues using a low-oxygen pyrolysis process at temperatures between 350 °C – 1000 °C (Lehmann and Joseph 2015, EBC 2019). Biochars can be used for many purposes such as soil amendment, climate change mitigation and remediation of soils polluted with heavy metals (Lehmann and Joseph 2015). Traditionally, biochars have been used for soil improvement in agriculture throughout the world (Wiedner and Glaser 2015). Different guidelines define requirements for sustainable biochar production. For example, European Biochar Foundation (EBC) defines that gases and heat produced in the pyrolysis must be recovered and the used biomass needs to be sustainably obtained (EBC 2019).

The properties of biochars are affected by feedstock, pyrolysis temperature and pyrolysis duration. Biochars produced from woody biomasses are usually more stable against microbial decomposition and typically have lower pH, nutrient content and higher specific surface area (SSA) than other types of biochars (Enders et al. 2012). Pyrolysis temperature also affects the biochar properties (Figure 1), specially biochars’ stability in the soil, C content and SSA are increased with temperatures over

500 °C (Ippolito et al. 2020). Although duration of the pyrolysis affects biochar properties less than feedstock and temperature (Ippolito et el. 2020), slower pyrolysis duration (> 30 min) has increased surface area, cation exchange capacity (CEC), nutrient content and C content, especially the stable forms of C (Mohanty et al. 2013).

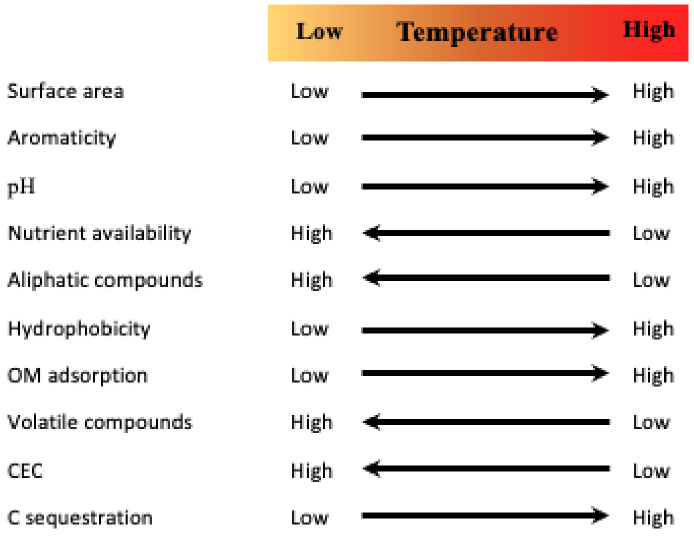

Figure 1. Effects of pyrolysis temperature on biochar properties. Adapted from Ippolito et al. 2015 and El-Naggar et al. 2019.

Since the properties of biochars vary, standards from International Biochar Initiative (IBI) and European Biochar Foundation (EBC) give guidelines for certification of biochars. For example, pH, liming effect and carbon content must be analysed (IBI 2015, EBC 2019). To ensure the safe use of biochars, standards also provide positive lists of allowed raw materials and thresholds for harmful compounds like heavy metals, polycyclic aromatic hydrocarbons (PAH), dioxins and polychlorinated biphenyl (PCB) (IBI 2015, EBC 2019).

2.1.2 Environmental effects of the use of biochars

Due to high aromaticity, the carbon from biochar mineralises slower than uncharred biomass (Kuzyakov et al. 2014) and can remain in soil for hundreds or thousands of years (Wiedner and Glaser 2015). Therefore, biochars can increase soil carbon stocks and help to mitigate climate change (Gross et al. 2021). Biochars can also help to decrease the GHG emissions from soil. Biochar application can reduce nitrous oxide emissions through capturing nitrate in biochar particles and reducing denitrification processes (Kammann et al. 2017, Yang et al. 2022). Reduced methane emissions have been attributed to improved soil aeration and increased methane diffusion to soil (Karhu et al. 2011, Yang et al. 2022). But as biochar and soil interactions are complex (Kammann et al. 2017), nitrous oxide or methane emissions can also increase in wet conditions (Kammann et al. 2017, Abacandura et al. 2022) or biochars may not have any effects on these emissions (Karhu et al. 2011). In some cases, carbon dioxide emissions have decreased after biochar application due to reduced mineralisation of C in eroded soils (Abacandura et al. 2022) but increased carbon dioxide emissions have also been observed because biochar increased available C in soil (Yang et al. 2022).

There are also other environmental benefits that can be achieved by biochar use. Some biochars can reduce the bioavailability of heavy metals and other toxic compounds in soils (Beesley et al. 2015). Nitrate leaching can be reduced because of increased capture of nitrate by the biochar (Haider et al. 2017, Karhu et al. 2021). Controversial results have been reported in the case of ammonium and phosphate leaching losses after the application of biochar. For example, Haider et al. (2017) observed reduced ammonium leaching, probably due to increased nitrification of ammonium by the biochar. In contrast, no effects on ammonium were observed by Karhu et al. (2021), probably because of lack of effect of biochar on soil CEC. Similarly, Soinne et al. (2014) stated that biochars increased aggregate stability that may reduce the loss of phosphate from soil whereas Saarnio et al. (2018) found that biochar application increased phosphate leaching probably due to increased soil pH leading to increased solubility of P (Paasonen-Kivekäs et al. 2016, p. 185).

2.2 Effects of biochars on soil properties

2.2.1 Soil water

In their meta-analysis, Strunecký et al. (2022) concluded that biochar application has generally increased water retention in soils with lower organic matter content. This has also been observed in temperate and boreal field studies. After four years of application, biochars at rates exceeding 8 t ha^{-1} have increased soil moisture content and available water content in soils with soil organic carbon (SOC) contents less than 2.5% for woody (Haider et al. 2017) and grassy (O’Toole et al. 2018) biochars. On the other hand, no significant effects have also been observed for woody biochars 2–7 years after application in soils with SOC content varying from 0.8% to 3.2% at biochar application levels of 9.3–30 t ha^{-1} (Tammeorg et al. 2014a, Blanco-Canqui et al. 2020, Kalu et al. 2021). In addition to the biochar application rate and the initial SOC content of the soils, the effects of biochars on soil water properties can be affected by soil texture. In field studies, the effects of soil texture have been contrasting. Haider et al. (2017) observed increase in soil moisture in coarse structured silty sand with low SOC content (0.6%) but in contrast no significant effects were observed in loamy sand with higher SOC content (3.2%) (Tammeorg et al. 2014a) for the same application rate of 30 t ha^{-1} of woody biochars. Observations on finer structured soils have been similar, O'Toole et al. (2018) observed increased moisture content for silty clay loam (SOC 2.5%), but Soinne et al. (2020) observed no effects on a clay soil with SOC content of 5.1%.

Biochar addition can alter soil hydrology by changing soil bulk density, porosity in the soil and aggregation of soil particles as well as soil and biochar surface chemistry (Masiello et al. 2015). Soil water storage can be impacted by both the porosity of the biochar itself and biochar interaction with soil particles causing changes in soil pore space (Liu et al. 2017). These changes can improve both soil water retention and infiltration (Masiello et al. 2015).

2.2.2 Soil carbon content

Biochars produced from wood at application levels of 9–31.5 t ha^{-1} have increased soil C in boreal short-term studies (Tammeorg et al. 2014c, Soinne et al. 2020) and 4–6 years after biochar application (Blanco-Canqui et al. 2020, Abagandura et al. 2022). In some cases, soil C increases have been short-term (lasting only a year) (Tammeorg et al. 2014a) or no increases have been observed (Farkas et al. 2020). In some cases, this increase has been dose dependent, in an eight-year study 30 t ha^{-1} of biochar from rice husks decreased SOC but 60 t ha^{-1} and 90 t ha^{-1} increased SOC, probably because the lower application rate of 30 t ha^{-1} decreased the proportion of larger soil aggregates that can have higher SOC content (Sun et al. 2020).

Biochar C decomposes more slowly than fresh biomasses and may lead to increased SOC in long-term (Dong et al. 2017). Biochar induced plant growth, especially root growth, can lead to increased soil C (Schmidt et al. 2021). Biochars can also protect native SOC from decomposition. Improved aggregation of soil via biochar application can bind native C within soil aggregates (Ameloot et al. 2014, Sun et al. 2020) or native C can be bound on biochar pores (Chen et al. 2021) making C inaccessible to decomposers.

2.2.3 Soil nutrient status

Biochars can improve soil nutrient status by increasing soil pH and CEC. In boreal conditions biochars made from wood have increased the soil pH of acidic soil from 4.4 to 6.1-7.2 (Saarnio et al. 2018) but no effects on effects have been found when soil pH was close to neutral (pH 6.4–6.6) (Tammeorg et al. 2014a, Tammeorg et al. 2014c). In temperate studies biochar made from grain husk and paper fibre sludge has increased the soil pH by 24% in an acidic soil (pH 4.4) but no effects were observed for calcareous soil (pH 7.9) for the same biochar (Farkas et al. 2020). Soils with high SOC and clay content can buffer against the pH changes, and this can also explain the lack of biochar effects on soil pH (Soinne et al. 2020). While biochars generally have basic pH, the liming efficacy of biochars is quantified by biochar carbonate ($CaCo_3$) equivalency rather than the biochar pH (Ippolito et al. 2015). Liming efficacy of wooden biochars is typically lower than biochars from other materials (Ippolito et al. 2020). Biochar induced CEC increases have been observed in soils with low SOC (Blanco-Canqui et al. 2020, Farkas et al. 2020).

Biochars can also be a source of nutrients, but the nutrient content varies for different feedstocks. Biochars produced from manure and biosolids contain more nutrients and the availability of nutrients is higher than biochars produced from woody materials or straw (Jeffery et al. 2017, Ippolito et al. 2020). Nonetheless, addition of woody biochars can increase soil K, Ca and P contents (Tammeorg et al. 2014a, Tammeorg et al. 2014c, Saarnio et al. 2018, Soinne et al. 2020), consequently increasing the plant availability of these nutrients (Kalu et al. 2021).

Biochars as materials with high C/N ratio can alter soil nutrient transformation processes such as nitrification by increasing microbial activity (Zhang et al. 2020). In field studies nitrate contents of topsoil have increased (Haider et al. 2017) or no effects have been observed (Farkas 2020). Ammonium content has decreased for biochar produced from grain husk and paper fibre sludge in an acidic soil, probably due to increased nitrification rates and CEC (Farkas et al. 2020). In some cases, ammonium content has been unaffected for woody biochars (Haider et al. 2017). Short-term N immobilisation has been reported in laboratory experiments (Tammeorg et al. 2012) and in field conditions (Tammeorg et al. 2014a).

2.2.4 Biochars and fertilisers

In the past, many of the biochar studies have not included a fertilisation treatment, but the use of biochars to enhance the effects of fertilisers has received more attention lately (Schmidt et al. 2021). Kalu et al. (2021) observed some longer-term effects on biomass yield and nutrient contents of plants in boreal conditions with the combined use of biochar and fertiliser, but the effects depended on the pre-crop effects and weather conditions. In their meta-analysis Bai et al. (2022), concluded that both organic and mineral fertilisers used together with biochar have increased crop yields. In field studies, the combined effects of biochars with mineral or organic fertilisers have been contrasting. Schulz and Glaser (2012) reported that compost together with biochars increased oat yield over mineral fertiliser along with biochar. Contrary to this, Steiner et al. (2007) observed that mineral fertiliser with biochar increased rice and sorghum grain yields over organic amendments together with biochar. In boreal conditions biochar along with meat bone meal (MBM) did not differ from biochar with mineral fertiliser for biomass yield or soil nutrient content (Tammeorg et al. 2014a). On the other hand, N and K use efficiency of plants were increased by biochar with mineral fertiliser compared to biochar with MBM (Kalu et al. 2021).

2.2.5 Effects of aging of biochars

Weathering of biochar can cause both physical and chemical changes in biochars (Mia et al. 2017). As biochar ages, the total mass of biochar is diminished but remaining C fractions are more stable and may provide means for longer-term increase of C sequestration (Dong et al. 2017). In some cases, hydrophilicity of biochar has increased with aging (Mia et al. 2017). Aging of biochars may also change the plant availability of nutrients in soil (Burgeon et al. 2022). In addition to weathering, biochars are likely to move downwards in soil and leach from soil (Ameloot et al. 2014) and therefore dilute the beneficial effects of biochars on soil properties and plant growth.

2.3 Effects of biochars on cereal properties

While grain yields, biomass, weight of 1000 grains (TGW) and leaf chlorophyll content have been reported to increase in tropical soils (Jeffery et al. 2017, Farhangi-Abriz et al. 2021), no grain yield increases have generally been observed in temperate and boreal conditions for barley and other cereals (Table 1).

Table 1. Biochar effects on barley, wheat, rye and oat grain yields in field studies in temperate and boreal climates for different soil types.

Reference	Length of study	SOC %	Soil texture	Climate	Crop	BC type	BC rate (t ha^{-1})	Effect on grain yield
Short-term studies (1–3 years)								
Liang et al. 2014	3 years	1.0	Silt loam	Temperate	Winter wheat	Rice husk and shell of cotton seed	30, 60 and 90	No significant effects
Tammeorg et al. 2014a	2 years	3.2	Loamy sand	Boreal	Wheat	Spruce	5,10, 20 and 30	No significant effects
Tammeorg et al. 2014c	3 years	3.4	Sandy clay loam	Boreal	Wheat	Spruce and pine	5 and 10	No significant effects
Sänger et al. 2017	3 years	3.7	Sandy loam	Temperate	Wheat and rye	Wood	7.7	No significant effects
Soinne et al. 2020	2 years	5.1	Clay	Boreal	Oat	Forest residue	30	No significant effects
Long-term studies (> 3 years)								
Haider et al. 2017	4 years	0.6	Silty sand	Temperate	Wheat and barley	Spruce and beech	15 and 30	Decreased 1st year by 1–11% and 3rd year by 5-26%, otherwise no significant effects
Aydin et al. 2020	4 years	0.9	Loamy silt	Temperate	Barley	Paper fibre sludge and grain husks	10 and 20	No significant effects
Hangs et al. 2021	4 years	1.5	Loam	Temperate	Barley and wheat	Willow	8 t C ha^{-1}	No significant effects
O'Toole et al. 2018	4 years	2.5	Silty clay loam	Boreal	Barley and oat	Grass	8 and 25	No significant effects
Hangs et al. 2021	4 years	5.3	Clay	Temperate	Barley and wheat	Willow	8 t C ha^{-1}	No significant effects

For boreal climate all studies are included and for temperate climate, only studies lasting > 2 years are included. Soil organic C content indicated when available. If SOM content was reported, SOC conversion was made assuming 50% of C in SOM to be SOC (Pribyl 2010).

Contrasting results for other crop properties have also been reported in temperate and boreal field studies. For example, woody biochars increased barley grain weight due to improved water utilisation (Hood-Nowotny et al. 2018) and plant K content as a result of long-lasting K fertilisation effect of the biochar (Kalu et al. 2021). In contrast no significant effects on grain nutrient content or leaf chlorophyll of barley have been found due to lack of effects on pH or soil moisture content for woody or grassy biochars (Haider et al. 2017, O'Toole 2018).

Biochar application can affect yield formation through several indirect mechanisms. In a meta-analysis, Jeffery et al. (2017) concluded that biochar use can increase yields because of reduced acidity of soils and fertilisation effect from biochar. Increased plant productivity by biochar has also been attributed to improved root growth (Schmidt et al. 2021) and increased soil microbial activity (Ding et al. 2016) leading to enhanced nutrient uptake by plants. Lack of effects on plant growth have been explained by several factors. In soils with more than 1% initial SOC, effects of biochars on SOC or soil moisture are probably insufficient to increase yields (Tammeorg et al. 2014a). No effects on yields were observed in already fertile soils, even though biochars increased soil nutrient contents (Tammeorg et al. 2014c, Soinne et al. 2020). In some cases, biochar did not alter soil pH or any of the other yield limiting factors (O'Toole et al. 2018). Haider et al. (2017) attributed decreases in yield the in first and third year to reduced N availability and drought stress that was not alleviated by biochar.

Although there are many studies on the effects of biochars on cereal yield formation, long-term research is still lacking specially in boreal field conditions (Table 1). Long-term research is needed as field-aging changes the properties of biochars, and it is impossible to remove the biochar once applied. In some cases, the effects of biochar have been dose-dependent and it is therefore important to study different biochar application rates. There are only a few studies on the combined use of biochar with fertilisers, specially with the inclusion of mineral and organic fertilisers in the same study. Further research on these topics is still needed.

3 RESEARCH OBJECTIVES

The aim of this study was to determine how different biochar application rates, fertilisation treatments and their interactions affect the soil properties and barley yield formation after eight years of biochar application.

Research questions were:

What effects do the different biochar application rates, fertilisation treatments and their interactions have on:

i. soil moisture content
ii. soil nutrient status, soil organic carbon content and pH
iii. growth and grain yield of barley

4 MATERIALS AND METHODS

4.1 Site and site history

All the measurements for this study were collected in the growing season of 2019 from the field experiment in the Vadelmakallio field at the Viikki Research Farm of University of Helsinki. The experimental site is situated in Helsinki, Southern Finland (60° 13' 42" N 25° 2' 34" E). The experiment was setup in 2011 (Tammeorg et al. 2014a) on a Endogleyic Umbrisol (WRB 2007). Texture of the soil was classified as loamy sand with 83% of sand, 15% of silt and 2% of clay (Tammeorg et al. 2014a). The wilting point moisture content of the top 2.5–7.5 cm of soil was determined to be 8% (Tammeorg et al. 2014a). The soil properties (0–20 cm depth) were analysed prior to the application of the experimental treatments (Table 2).

Table 2. Soil properties of the experimental field before biochar addition in 2011 (Tammeorg et al. 2014a).

Soil property	Value	Unit
Electrical conductivity	75.8	mS cm^{-1}
SOM	63.4	g kg^{-1}
pH	6.35	
Ca	1127	g m^{-3}
P	21	g m^{-3}
K	62	g m^{-3}
Mg	100	g m^{-3}
S	5.2	g m^{-3}
NH_4^+-N	6.2	g m^{-3}
NO_3^--N	5.5	g m^{-3}
N_{min}	11.7	g m^{-3}
Total N	2.4	g kg^{-1}
C org	31.7	g kg^{-1}

The field was disc-tilled in autumn and rotary power harrowed in spring before sowing annually, with the exceptions of 2013–2015 when the grass was growing without disturbance. Cereals, grasses and legumes were grown since the start of the experiment (Table 3).

Table 3. Cropping history of the study site from the start of the experiment.

Year	**Crop**	**Reference**
2011–2012	Wheat (*Triticum aestivum* L.)	Tammeorg et al. 2014a
2013	Barley (*Hordeum vulgare* L.), timothy-grass (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.)	Kalu et al. 2021
2014–2015	Grasses (timothy-grass and red clover)	Kalu et al. 2021
2016	Oat (*Avena sativa* L.)	Kalu et al. 2021
2017	Pea (*Pisum sativum* L.)	Kalu et al. 2021
2018	Barley	Kalu et al. 2021

4.2 Biochar

The biochar was produced in a continuously pressurised carboniser (Preseco Oy, Lempäälä, Finland) by pyrolyzing chips of debarked spruce (*Picea abies* (L.) H. Karst.) anaerobically at 550–600°C for 10–15 min (Tammeorg et al. 2014b). After the pyrolysis the biochar was cooled overnight in an airtight silo and then ground into < 10 mm particles in a roller mill (Tammeorg et al. 2014b). Biochar was applied to experimental plots in 2011 with a sand spreader and it was mixed into soil at 0–10 cm depth with a rotary harrow. The biochar was wetted to 25% (w/w) to avoid dust problems during application.

Chemical and physical properties of the biochar were analysed (Table 4). Analysis of ash content and elemental composition of the biochar was done using dry ashing method by Miller (1998). Total C and N contents were measured using Dumas dry combustion method in VarioMax CN analyser (Elementar Analysensysteme GmbH, Hanau, Germany). H content was analysed using combustion in CHN-1000 elemental analyser (LECO Corp. St. Joseph, MI, USA). Volatile matter was determined using the ASTM D3175-02 standard (2002). The pH of the biochar was measured in 1:5 (w/w) suspension using deionized water. The liming effect of biochar compared to calcium carbonate ($CaCO_3$) was determined by the method of Rowell (1994). Carbonate-C content was determined using gas chromatographic analysis. The amount of organic C was obtained by subtracting carbonate-C from the total amount of C. PAH concentration was analysed using the methods of Hale et al. (2012) and Hilber et al. (2012). The Brunauer–Emmett–Teller specific surface area (BET SSA) of the biochar was analysed using the N_2 absorption technique (Micromeritics Co., Norcross, USA).

Table 4. Properties of biochar analysed in 2011 (Tammeorg et al. 2014b).

Measurement	**Value**	**Unit**
Surface area	265	$m^2\ g^{-1}$
pH	8.1	
C/N ratio	251	$g\ g^{-1}$
H/Corg	0.34	$mol\ mol^{-1}$
Ash content	26.6	$g\ kg^{-1}$
Volatile matter	121.6	$g\ kg^{-1}$
$CaCO_3$ equivalence	9.0	$g\ kg^{-1}$
Carbonate-C	1.2	$g\ kg^{-1}$
$C_{organic}$	881.3	$g\ kg^{-1}$
Al	0.09	$g\ kg^{-1}$
Ca	4.66	$g\ kg^{-1}$
Fe	0.34	$g\ kg^{-1}$
K	4.5	$g\ kg^{-1}$
Mg	0.9	$g\ kg^{-1}$
Mn	0.33	$g\ kg^{-1}$
Na	0.21	$g\ kg^{-1}$
P	1.83	$g\ kg^{-1}$
S	0.22	$g\ kg^{-1}$
C	882.5	$g\ kg^{-1}$
N	3.52	$g\ kg^{-1}$
Cd	0.01	$mg\ kg^{-1}$
Co	0.25	$mg\ kg^{-1}$
Cu	11.46	$mg\ kg^{-1}$
Ni	3.56	$mg\ kg^{-1}$
Pb	2.51	$mg\ kg^{-1}$
Sr	25.57	$mg\ kg^{-1}$
Zn	64.79	$mg\ kg^{-1}$
Total PAH	10.06	$mg\ kg^{-1}$

4.3 Experimental setup

Experimental design was a factorial split-plot design with four replicates (Figure 2). Main plot factor was the biochar application level with five application levels: 0, 5, 10, 20, 30 t of dry matter ha^{-1}. Subplot factor was the fertiliser treatment with three different treatments: control, meat bone meal (MBM) and mineral fertiliser. Main treatment plot size was 6.6 m ×10 m with 3 subplots each sized 2.2 m × 10 m.

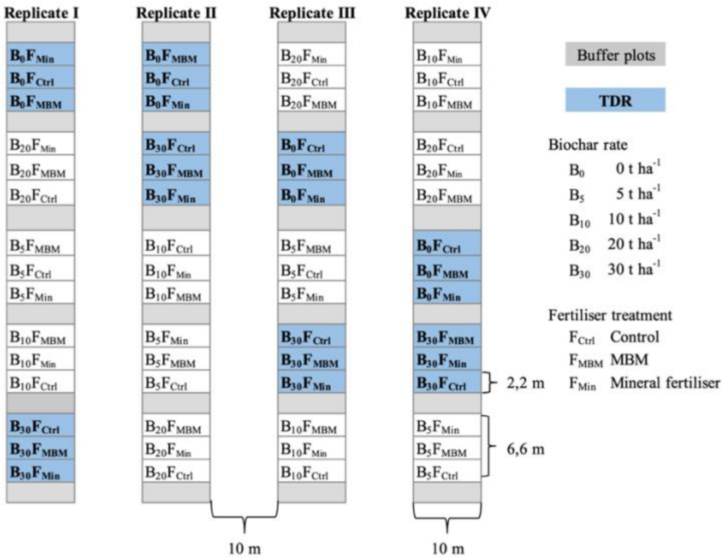

Figure 2. Experimental setup in the Vadelmakallio field. Main plot factor was biochar application level (B_0-B_{30}), subplot factor was fertiliser treatment (F_{Ctrl}, F_{MBM}, F_{Min}). Grey plots were buffer plots. In 2019 soil moisture (TDR) measurements were taken from the plots marked with blue colour and bold font.

In 2019, all the plots were sown with barley (*Hordeum vulgare* L.), ryegrass (*Lolium spp.*) and red clover (*Trifolium pratense* L.). The fertiliser treatments were applied at the time of sowing. Mineral fertiliser treatment consisted of Yara Mila HeVi 6 N-P-K 14-3-15 (Yara International ASA) as the main fertiliser and Yara Starttiravinne N-P 12-23 (Yara International ASA) and Patenttikali K 25 (Lantmännen Agro) as supplementary fertilisers. For the MBM treatment Erikois-Viljo N-P-K 8-4-8 (Honkajoki Oy) was used as the main fertiliser and Patenttikali K 25 and YaraBela Suomensalpietari N-K 26,8-1 (Yara International ASA) as supplementary fertilisers to deliver same amount of NPK between mineral and MBM fertiliser treatments (Table 5). The aim of the fertilisation treatments was to cover all the nutrient needs based on Viljavuuspalvelu (2000) and for delivering the 20 kg ha^{-1} rate of Mg to the plots by spraying Yara Magtrac on the 23rd of May. As the spraying treatment had to be conducted to all the experimental plots, including the control plots, negligible amount (about 2.67 kg ha^{-1}) of mineral N that was part of the Magtrac solution was also applied to the control plots.

Table 5. Plant available nutrients (kg ha^{-1}) from different fertiliser treatments in 2019.

	N	P	K	S	Mg	B	Cu	Mn	Ca	Zn
Control	3	-	-	-	20.0	-	-	-	-	-
MBM	108	45	101	48.6	30.0	3.43	0.00	10	112.5	10.06
Mineral	108	45	101	71.9	36.7	2.23	0.32	10	0.0	0.00

Sowing and fertiliser application was done on the 17th of May with a combine seeder. Barley was sown to 4–5 cm depth and the fertilisers were added to 5–6 cm depth. The mixture of ryegrass and red clover was later sown to 2–3 cm depth. Manual harrowing was done after sowing to cover any uncovered seeds with soil. Red clover did not germinate, probably because of drought after sowing.

4.4 Weather in 2019

The average temperatures in May, July, August and September in 2019 were close to long-term average 1980-2010 but temperatures in June were 3.1 °C higher in 2019 (Figure 3) (Finnish Meteorological Institute 2020). May and September were wetter than the long-term average, with 28.1 mm and 12.7 mm higher precipitation, respectively. In June, the precipitation was notably lower (19.8 mm) than the long-term average (57.3 mm). In July, precipitation was close to the long-term average. In August the precipitation was 9.5 mm lower than the long-term average. Rain was not distributed evenly during the growing season. In June, almost half of the monthly precipitation (9.7 mm of the total 19.8 mm) occurred on the June 3rd during leaf development stage. In July, most rain was received at the beginning of the month during inflorescence emergence before flowering. Although the precipitation in August (70.5 mm) was close to the long-term average (80 mm), most of the rainfall occurred on one day, on the August 22nd (40.6 mm) during ripening.

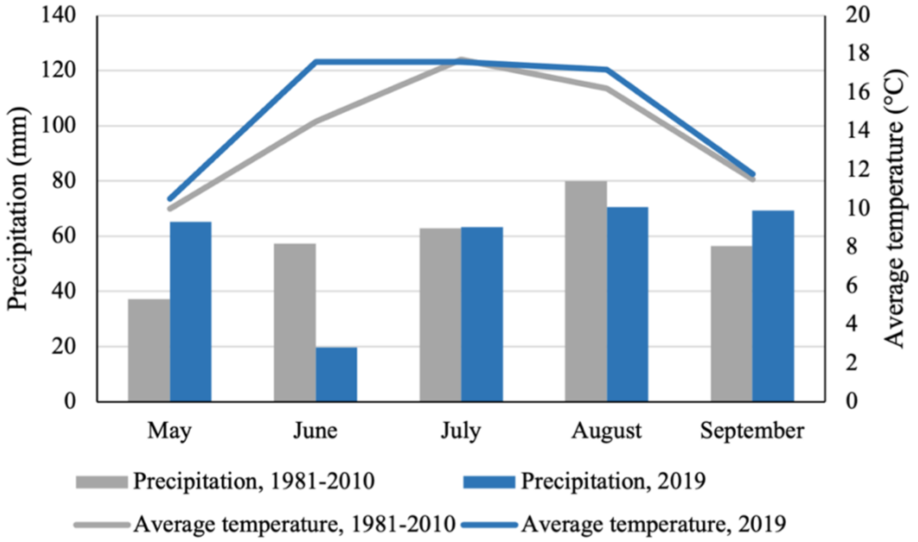

Figure 3. Average temperatures (°C) and precipitation (mm) in 1981-2010 and in 2019 in Kumpula weather observation station 5 km from the study site (Finnish Meteorological Institute 2020).

4.5 Sampling and measurements in 2019

Sampling and measurements were carried out in the field from May to September (Table 6). Plant development stages of barley and ryegrass were evaluated weekly using the BBCH-scale (Meier 2001).

Table 6. Measurements and sampling in 2019.

Measurement	Date	Details
Plant development stage	11.6.-27.8.2019	BBCH-scale
Soil moisture content	11.6.-27.6.2019	TDR (Trime), depth of 0-15 cm
Soil moisture content	4.6.2019, 8.7.-27.8.2019	TDR (MiniTrase), depths of 0-15 cm, 0-30 cm and 0-60 cm
Plant tiller density	11.6.2019	Number of tillers in 30 cm, 3 samples/each plot
Plant samples	16.7.2019	3 samples/each plot, 30 cm of row in a sample, plants cut at 3-4 cm height
Leaf chlorophyll (SPAD)	17.7.2019	20 samples/each plot
Leaf area index	17.7.2019	4 measurements/each plot
Harvesting	29.8.2019	Each plot separately with a combine harvester
Soil sampling	25.9.2019	Depth of 25 cm with an auger, 8 subsamples combined/plot

Soil moisture content was measured weekly during the growing season from 4th of June and 27th of August. Between 11th of June and 27th of June, soil moisture was measured from the depth of 0–15 cm with Trime (TRIME-FM TDR, IMKO, Ettingen, Germany). On 4th of June and between 8th of July and 27th of August, soil moisture was measured from the same depth with another TDR (MiniTrase 6050X3, Soilmoisture Equipment, Santa Barbara, USA).

Plants were randomly sampled from within 1 m of the one end of a plot by avoiding the first two rows from either side and non-representative areas. Plant stand density of barley was determined during tillering by counting the number of plants in 30 cm length in three sowing rows. Plant samples of barley were collected once during full flowering stage. Plants were cut 3-4 cm above ground at the length 30 cm in three sowing rows. The collected plant samples were dried in an oven at 60 °C for 72 hours. After drying, the samples were weighed to determine the dry matter weight to calculate above ground biomass. Then, the plant samples were ground using a hammer mill (screen size 1 mm; Koneteollisuus Oy, Helsinki, Finland). The C and N contents of the ground plant samples were analysed using the CN828 carbon-nitrogen analyser (Leco Corporation, St. Joseph, MI, USA). Dry matter content of the ground samples was determined weighing the water loss by the small 1 g subsamples drying the samples in an oven at 105 °C for 16-18 h for mass correction for calculating plant C and N contents.

Relative leaf chlorophyll content (SPAD) was measured once during the flowering stage using a SPAD-502 device (Soil-Plant Development, Minolta Camera Co. Ltd., Osaka, Japan). Average SPAD was calculated from 20 measurements taken from the uppermost fully developed leaves from different parts of the plot. Leaf area index (LAI) was measured once during the flowering stage using the SunScan SS1-device (Delta-T Devices Ltd, Cambridge, United Kingdom). Four measurements were taken from each plot and the average of the measurements was calculated.

The plants were harvested on the 29th of August from an area of 9.9 m^2 using a combine harvester from the middle of each plot, leaving 2 m and 1 m from the ends of the plots unharvested. Grain was dried and sorted. The moisture content of the grain was determined by drying the samples in an oven at 105 °C for 16-18 h and weighing the water loss. Afterwards, 1000 g weight of grains (TGW) was determined.

Soil sampling was carried out on 25th of September with an auger up to 25 cm depth. Eight samples of each plot (one from each corner and four from the centre of the plot) were pooled to form a composite soil sample. The soil samples were sieved through 2 mm and ground. A subsample of fresh soil was dried in the oven at 105 °C for determining dry matter content. Another subsample of soil was dried at 40 °C for 72 hours and ground to determine the C and N contents as described above for plant samples. The soil nutrient contents and pH were analysed from fresh soil samples using the Finnish soil testing methods (Vuorinen and Mäkitie 1955, Viljavuuspalvelu 2019).

4.6 Statistical analyses

The effects of biochar application rates, fertiliser treatments and their interactions were examined using a linear mixed model. Fixed factors were the biochar application rate, fertiliser treatment and their interactions. Soil C value measured in spring 2011 was used as a covariate and replicate was used as a random factor. Bonferroni post-hoc testing was used for pairwise comparisons. The normality of the data was checked using Shapiro-Wilk test and Q-Q plot. Homogeneity of variance was tested using Levene's test. When the assumption of normality or homogeneity of variance was violated, Box-Cox transformation (Box and Cox 1964) was used. Analyses were performed using the IBM SPSS Statistics software (version 25.0, IBM Corp., Armonk, NY, USA). Statistical significance level used for testing was $p < 0.05$.

5 RESULTS

5.1 Soil moisture content

The application of softwood biochar at rate of 30 t ha$^{–1}$ increased soil moisture content at 0–15 cm depth by 14% ($p = 0.013$) compared to the control at tillering on the 11th of June (Table 7 and Figure 4). The effect of biochar application did not have significant effects on any other measurement day. On 13th of August and 20th of August, some effects of biochar application were notable when 30 t ha^{-1} decreased soil moisture compared to control ($p = 0.060$ and $p = 0.055$, respectively). Soil moisture was close to the wilting point (8%) on 27th of June during tillering for both treatments, 8.5% for control and 8.1% for 30 t ha$^{–1}$. Moisture was below the wilting point between 30th of July and 13th of August, between flowering and grain filling, for both biochar treatments. On the 30th of July, soil moisture was as little as 5.8% for 0 t ha^{-1} and 4.9% for 30 t ha^{-1}. Soil moisture was on the 13th of August 7.7% for 0 t ha^{-1} and 6.3% for 30 t ha^{-1}. On 20th of August soil moisture was still below the wilting point for 30 t ha^{-1} (7.7%) while soil moisture was above wilting point for control (9.6%).

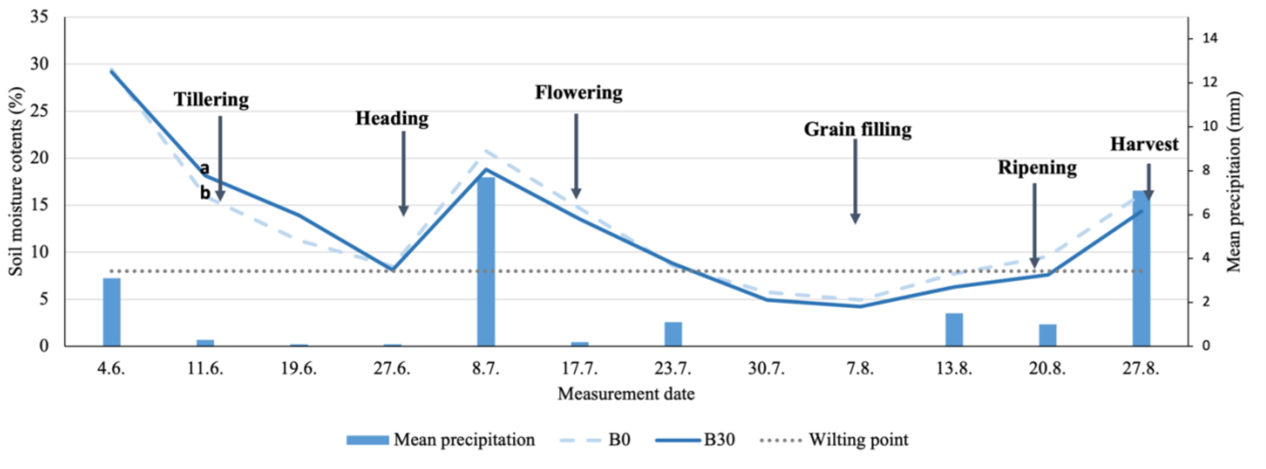

Figure 4. Soil moisture content at 0–15 cm depth with barley development stages for biochar treatments of 0 t ha^{-1} and 30 t ha^{-1} with mean weekly precipitation during the growing season 2019 ($n = 4$). Blue bars indicate weekly mean precipitation from the previous seven days before the measurement. Different lowercase letters indicate statistical difference between Bonferroni corrected treatment means (Tukey HSD, $p = 0.05$). Mean weakly precipitation data was recorded in a weather station of Finnish Meteorological Institute at Kumpula that is five km west from the field site (Finnish Meteorological Institute 2020).

Similarly, the fertiliser treatments did not affect the topsoil moisture content significantly throughout the growing season 2019 except for two cases where the mineral fertilised treatments resulted in drier soils than the unfertilised control [27th of June ($p = 0.032$) and on 30th of July ($p = 0.011$)] (Table 7 and Figure 5). The fertilisers tended to decrease soil moisture compared to control also on 17th ($p = 0.095$) and 23rd of July ($p = 0.098$). No interaction effects between biochar and fertiliser treatments were observed in regards of topsoil moisture content.

On 27th of June soil moisture content was below wilting point for mineral fertiliser treatment (5.9%) and close to the wilting point for MBM (8.7%). On 23rd of July, soil moisture was slightly below the wilting point for mineral (7.9%) and MBM (7.9%) fertilisers. Between 30th of July and 13th of August, i.e., during flowering and grain filling stage, the soil moisture contents were below wilting point for all fertiliser treatments. On 20th of August, soil moisture content was distinctly above the wilting point for the control (9.0%) while MBM (8.2%) and mineral fertilisers (8.5%) still remained closer to the wilting point.

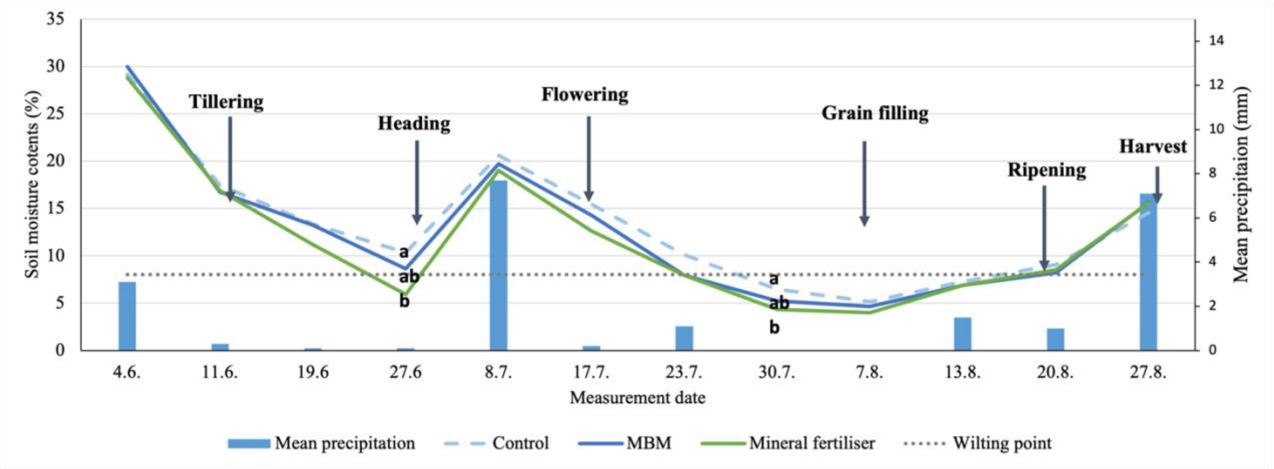

Figure 5. Soil moisture content at 0–15 cm depth with barley development stages for fertiliser treatments with mean weekly precipitation during the growing season 2019 (n=4). Blue bars indicate weekly mean precipitation from the previous seven days before the measurement. Different lowercase letters indicate statistical difference between Bonferroni corrected treatment means (Tukey HSD, $p < 0.05$). Mean weakly precipitation data was recorded in a weather station of Finnish Meteorological Institute at Kumpula that is five km west from the field site (Finnish Meteorological Institute 2020).

Table 7. Soil moisture content (%) at 0–15 cm depth in 2019 (n=4). Biochar (B) application rates are B_0 = 0 t ha^{-1} and B_{30} = 30 t ha^{-1}. Fertilisation (F) treatments are Control, MBM and Mineral fertilisers.

Treatment		**4.6.**	**11.6.**[1]	**19.6.**	**27.6.**	**8.7.**[1]	**17.7.**	**23.7.**[1]	**30.7.**	**7.8.**	**13.8.**	**20.8.**	**27.8.**[1]
B_0		29.5	15.9b	11.3	8.5	20.7	14.7	8.6	5.8	5.0	7.7	9.6	16.2
B_{30}		29.2	18.1a	13.9	8.1	18.8	13.5	8.8	4.9	4.2	6.3	7.6	14.4
F_{Control}		29.2	17.5	13.4	10.3a	20.6	15.4	10.1	6.5a	5.1	7.3	9.0	14.6
F_{MBM}		30.0	16.7	13.2	8.7ab	19.7	14.3	7.9	5.2ab	4.6	6.9	8.2	15.6
F_{Mineral}		28.8	16.9	11.2	5.9b	19.0	12.6	7.9	4.3b	4.0	6.9	8.5	15.6
	df	**p-values**											
B	1	0.834	**0.013**	0.111	0.811	0.202	0.406	0.881	0.201	0.210	0.060	0.055	0.220
F	2	0.646	0.621	0.315	**0.033**	0.427	0.095	0.098	**0.013**	0.161	0.838	0.687	0.634
B × F	2	0.252	0.966	0.530	0.140	0.695	0.892	0.692	0.965	0.986	0.766	0.629	0.316

[1] Data residuals were not normally distributed and Box-Cox transformation was used. Bonferroni correction was used for pairwise comparisons and lowercase letters indicate significant ($p < 0.05$) differences between means.

5.2 Soil chemical properties

Soil C, N and C/N ratio were not significantly affected by the biochar application (Table 8). Biochar application significantly affected soil Ca ($p = 0.044$) and Na ($p = 0.042$) contents (Table 9). However, post-hoc analysis did not show statistical differences between the application rates. The lowest Ca content was observed for biochar treatment of 10 t ha^{-1} (947 g kg^{-1}) and this was 21.5% lower than control (1151 g kg^{-1}) which had the highest Ca content. There were no clear trends between nutrient content and biochar application rate. For example, Ca content was lower for 30 t ha^{-1} treatment (994 g kg^{-1}) than for 20 t ha^{-1} treatment (1094 g kg^{-1}). Similar results were observed for Na content, 5 t ha^{-1} treatment had highest Na content (8.3 mg kg^{-1}) while higher biochar treatments had lower Na contents [10 t ha^{-1} (7.9 mg kg^{-1}), 20 t ha^{-1} (7.5 mg kg^{-1}), 30 t ha^{-1} (7.9 mg kg^{-1})]. Although there was not a significant difference, all biochar treatments had slightly lower soil pH in comparison to control.

Table 8. Soil C, N and C/N for biochar and fertiliser treatments (n=4). Biochar (B) application rates are B_0 = 0 t ha^{-1}, B_5 = 5 t ha^{-1} B_{10} = 10 t ha^{-1}, B_{20} = 20 t ha^{-1} and B_{30} = 30 t ha^{-1}. Fertilisation (F) treatments are Control, MBM and Mineral fertilisers.

Treatment		**C**[1] %	**N**[1] %	**C/N**[2]
B_0		2.88	0.19	15.25
B_5		2.85	0.19	15.27
B_{10}		2.77	0.18	15.42
B_{20}		2.87	0.18	15.96
B_{30}		2.99	0.19	15.90
$F_{Control}$		2.80	0.18	15.41
F_{MBM}		2.92	0.19	15.60
$F_{Mineral}$		2.89	0.18	15.66
	df			
B	4	0.673	0.592	0.321
F	2	0.107	0.365	0.258
B × F	8	0.457	0.405	0.713

[1]One measurement was missing for treatment type $B_{10}F_{MBM}$. [2]Data residuals were not normally distributed and Box-Cox transformation was used.

Fertilisation treatments did not have significant effects on soil C, N and C/N ratio (Table 8). Mineral fertiliser and MBM treatments significantly increased electrical conductivity ($p < 0.001$) and decreased pH ($p < 0.001$) compared to control (Table 9). Both the mineral and MBM fertiliser treatments had significantly higher soil P, K, S, B, and Mn contents compared to the control. MBM had significantly higher soil Ca, P and Na contents but significantly lower soil K content than the mineral fertiliser treatment. The soil Ca content in the mineral fertiliser was also significantly less than the control. No interaction effects of biochar and fertilisation treatments were observed on any of the measured soil chemical properties.

Table 9. Results of soil analysis for biochar and fertiliser treatments (n=4). Abbreviations as in Table 8.

Treatment		**EC**	**pH**	**Ca**^{1}	**P**	**K**	**Mg**	**S**^{1}	**B**^{2}	**Cu**	**Mn**^{1}	**Zn**^{1}	**Na**
		(μS cm^{-1})		(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
B_0		0.76	6.00	1151	19.0	66.4	105.3	8.9	0.6	11.3	17.4	18.1	7.4
B_5		0.81	5.82	953	20.4	69.7	90.3	14.1	0.8	11.9	18.0	21.5	8.3
B_{10}		0.74	5.90	947	23.2	71.4	93.2	10.3	0.9	10.1	16.9	18.2	7.9
B_{20}		0.79	5.98	1094	20.4	64.9	103.5	10.9	0.6	9.9	16.3	16.6	7.5
B_{30}		0.78	5.88	994	22.7	73.0	90.8	11.4	0.7	11.5	19.6	20.7	7.9
$F_{Control}$		0.53a	6.00a	1050a	17.7c	46.1c	94.3	4.1b	0.4b	11.1	14.7b	19.0	6.9b
F_{MBM}		0.90b	5.92b	1060a	24.4a	75.7b	97.9	14.4a	0.8a	10.7	18.8a	18.9	9.2a
$F_{Mineral}$		0.90b	5.84c	973b	21.4b	85.4a	97.6	14.9a	0.8a	11.1	19.4a	19.1	7.3b
	df	**p-values**											
B	4	0.780	0.202	**0.044**	0.418	0.652	0.167	0.157	0.127	0.764	0.661	0.634	**0.042**
F	2	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.069	**<0.001**	**<0.001**	0.100	**0.001**	0.512	**<0.001**
B × F	8	0.884	0.946	0.472	0.814	0.105	0.289	0.326	0.605	0.424	0.685	0.603	0.233

[1] Data residuals were not normally distributed and Box-Cox transformation was used. [2] Data residuals were not normally distributed even after Box-Cox transformation, so original values were used.

5.3 Barley growth and grain yield

Biochar application did not have significant effects on leaf chlorophyll (SPAD), LAI, plant density, above ground biomass (AGB), grain yield, TGW, C content, N content and C/N ratio of barley (Table 10). Although not significant, biochar application rate 30 t ha^{-1} increased grain yield by 31.8% compared to other treatments and variation of data in this treatment was lower compared to other treatments (Figure 6). Some statistically insignificant but trend wise effects were observed for TGW ($p = 0.081$) where highest TGW was observed for 30 t ha^{-1} (45.0 g) and lowest for 20 t ha^{-1} (39.1 g).

Table 10. SPAD, LAI, plant density, biomass (AGB), grain yield, TGW, C and N content and C/N ratio of barley for different treatments (n=4). Grain yield and TGW values are presented at 14% moisture content. Abbreviations as in Table 8.

Treatment	**SPAD**	**LAI**[1]	**Plant density**[2] (plants m^{-2})	**AGB** (t ha^{-1})	**Grain yield** (t ha^{-1})	**TGW** (g)	**C** %	**N**[2] %	**C/N**
B_0	34.9	2.3	513.7	4.8	2.2	41.3	45.45	1.53	30.32
B_5	32.9	1.9	480.1	4.1	2.2	42.6	45.28	1.46	31.52
B_{10}	32.0	2.1	477.8	4.1	2.2	39.8	45.25	1.54	30.20
B_{20}	34.2	2.1	476.4	3.8	2.2	39.1	45.33	1.52	30.72
B_{30}	33.4	2.4	506.6	4.7	2.9	45.0	45.26	1.49	30.76
$F_{Control}$	31.6b	1.7b	493.6	2.8c	1.5b	40.1b	45.19b	1.40b	32.40a
F_{MBM}	32.5b	2.2a	501.8	4.6b	2.7a	42.3a	45.27b	1.39b	32.84a
$F_{Mineral}$	36.3a	2.5a	477.1	5.5a	2.8a	42.3a	45.48a	1.73a	26.87b

	df	**p-values**								
B	4	0.675	0.129	0.633	0.540	0.415	0.081	0.349	0.934	0.934
F	2	**<0.001**	**<0.001**	0.428	**<0.001**	**<0.001**	**0.005**	**<0.001**	**<0.001**	**<0.001**
B × F	8	0.267	0.802	0.079	0.954	0.964	0.296	0.982	0.622	0.608

[1] Data missing for treatment type $B_{10}F_{Mineral}$. [2] Data residuals were not normally distributed and Box-Cox transformation was used. Bonferroni correction was used for pairwise comparisons and lowercase letters indicate significant ($p < 0.05$) differences between means.

Fertiliser treatment had significant effects ($p < 0.001$) on both SPAD and LAI values (Table 10). SPAD values were significantly higher ($p < 0.001$) for mineral fertiliser treatment compared to the control and MBM treatments. MBM and mineral fertiliser treatments increased LAI significantly ($p = 0.004$ and $p < 0.001$, respectively) over control treatment. Plant stand density was not significantly affected by fertiliser treatments. Both fertilisers increased biomass yield and grain yield in comparison to control ($p < 0.001$). Mineral fertiliser increased biomass yield by 96.4% over control ($p < 0.001$) and by 19.6% over MBM ($p = 0.022$). MBM increased biomass yield by 64.3% compared to control ($p < 0.001$). Mineral fertiliser increased grain yield by 86.7% over control ($p < 0.001$) and MBM by 80.0% over control ($p < 0.001$). Both MBM and mineral fertiliser increased TGW by 5.5% compared to control ($p = 0.013$ and 0.014, respectively). Grain yields were similar for MBM and mineral but there was greater variation in MBM plots (Figure 6). Fertiliser treatments had significant effects ($p < 0.001$) on plant C- and N-contents and plant C/N ratio. Mineral fertiliser treatment significantly increased the C and N content of plant samples compared to control ($p < 0.001$ and $p < 0.001$, respectively) and MBM treatments ($p = 0.002$ and $p < 0.001$, respectively). Since plant N content was clearly increased by the mineral fertiliser, the C/N ratio was lowest for this treatment. No interaction effects of biochar and fertiliser treatments were observed for any of the measured plant properties.

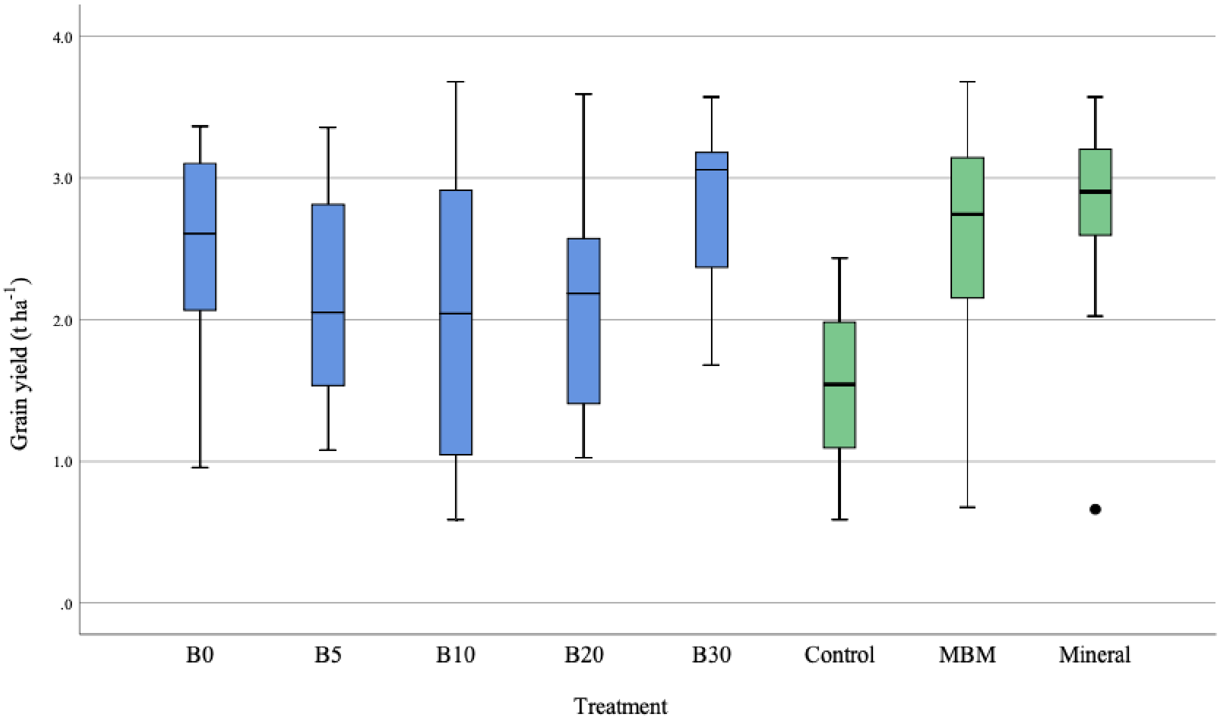

Figure 6. Barley grain yields (t ha^{-1}) for different treatments. Biochar (B) application rates are $B_0 = 0$ t ha^{-1}, $B_5 = 5$ t ha^{-1} $B_{10} = 10$ t ha^{-1}, $B_{20} = 20$ t ha^{-1} and $B_{30} = 30$ t ha^{-1}. Fertilisation treatments are Control, MBM and Mineral fertilisers. The line inside the box is the median, upper and lower boundaries of the box indicate third (Q3) and first (Q1) quartiles, respectively. Top whisker represents Q3 + 1.5 IQR and bottom whisker Q1-1.5 IQR, dots indicate values beyond the range of whiskers.

6 DISCUSSION

6.1 Soil moisture content

In this study, significant effects of spruce biochar application on soil moisture content were observed only on one measurement day. This is consistent with other studies, where lack of significant effects of woody biochars have been reported on soil moisture content in short-term studies (Karhu et al. 2011, Tammeorg et al. 2014a, Soinne et al. 2020) and 6–9 years after biochar application (Blanco-Canqui et al. 2020, Kalu et al. 2021). Even though biochars have been suggested to have potential to improve soil water content in coarse textured soils (Ibrahimi and Algamdi 2022), contrasting effects of biochar have been reported in the literature. In this study, even the higher application rate of 30 t ha^{-1} was unable to significantly affect the soil moisture content in the sandy soil and similar results have been obtained from the same site earlier (Tammeorg et al. 2014a, Lehti 2015, Hämäläinen 2018, Härkönen 2019, Kalu et al 2021). On the other hand, Haider et al. 2017 reported that biochar application rate of 15 t ha^{-1} increased soil moisture in a sandy soil. The effect of biochar on soil moisture content seems to be linked with initial soil organic matter content of a soil. In field studies increased soil moisture has been observed in soils with lower SOC (0.6%–2.5%) (Haider et al. 2017, O’Toole et al. 2018). The soil in the experimental site of this study had a relatively high initial SOC content (3.2%) before the biochar application (Tammeorg et al. 2014a) and this may explain the lack of biochar effects on soil moisture.

In the first year of this experiment plant available water increased for 30 t ha^{-1} treatment (Tammeorg et al. 2014a) but the effects had disappeared after six years (Kalu et al. 2021). Since this study was conducted eight years after biochar application, both physicochemical changes from weathering of biochar (Dong et al. 2017) and downward displacement of biochar particles from topsoil because of tillage (Ameloot et al. 2014) might have occurred. Such weathering and downward movement of biochar particles might have diluted the biochar effects on topsoil, consequently leading to the insignificant results.

Soil moisture content was significantly decreased by the mineral fertiliser treatment on two measurement days over control and soil moisture was longer below the wilting point in the fertilised plots. Mineral fertilisation has decreased the moisture earlier at the same site (Tammeorg et al. 2014a, Lehti 2015, Hämäläinen 2018, Härkönen 2019). Increased plant water uptake may explain the results since plant biomass, yield and TGW were higher for mineral and MBM treatments than control. No moisture measurements were taken after harvesting so the effects of removing the vegetation could not be assessed in 2019. In previous years, after harvest measurements have been made and in some years no effects of fertilisation have been found (Hämäläinen 2018) or mineral fertilisation has decreased soil moisture content compared to control (Tammeorg et al. 2014a, Lehti 2015, Härkönen 2019).

6.2 Soil chemical properties

The non-significance of increased soil C content for the biochar treatment of 30 t ha^{-1}, after eight years of biochar application, is likely due to physical movement of biochar particles farther down in the soil profile because of intensive tillage annually. This is in line with observations at the beginning of this experiment where already in the second year after biochar addition, the difference between control and 30 t ha^{-1} biochar application rate was not significant anymore (Tammeorg et al. 2014a). In contrast, SOC was increased six years after application of 9–31.5 t ha^{-1} of woody biochar (Cooper et al. 2020) but continuous and steady yearly loss of biochar C from soil has also been observed. In fact, de la Rosa et al. (2018) observed that 11–27% of biochar C was lost after biochar was aged in field for two years. Downward movement (Mia et al. 2017), leaching of biochar from soil and degradation of biochar (Dong et al. 2017) may explain loss of biochar C in the biochar treatments, leading to no long-term effects of biochar application on soil C content at 0–25 cm depth. Increases in SOC content due to biochar application are generally observed in soils with lower C content (Chagas et al. 2022). Woody biochar has increased SOC in soils with initial SOC content between 0.8–2.4% six years after biochar application (Blanco-Canqui et al. 2020), but at this experimental site with the initial SOC of 3.2%, increases in SOC were observed only in the first year of biochar application (Tammeorg et al. 2014a).

Similarly, the soil pH was not significantly affected by biochar application. Through the years the pH of soil has been declining from the original mean value of 6.35 measured in spring 2011 (Tammeorg et al. 2014a, Hämäläinen 2018, Härkönen 2019) and the decline continued in 2019 also. This is as expected since the liming efficacy of the used biochar is low ($CaCo_3$ equivalence 9.0 g kg^{-1}) (Tammeorg et al. 2014b), pH of the experimental site soil is near neutral and no significant biochar effects on pH were observed at the beginning of this experiment (Tammeorg et al. 2014a).

Biochar effects on soil nutrients were observed only for Ca and Na in this study. In this study, highest soil Ca content was observed for the control. At the time of the application, the used biochar contained significant amounts of total Ca (4.66 g kg^{-1}) but plant-available soil Ca content was not affected in the first years of this experiment (Tammeorg et al. 2014a). This suggests that over the eight years' period, the Ca contained in biochar has not been converted into plant available but rather has been absorbed by the soil. Although Na content of the soil was higher for the biochar treated plots than control, the Na content was still within safe limits according to the Finnish soil testing standards (Viljavuuspalvelu 2019). In this study K content of soil was not affected by biochar application but K content of plants was increased four years after biochar application at the same experimental site due to the fertilisation effect from biochar (Kalu et al. 2021). Soinne et al. (2020) observed in their 2-year experiment that P increased for application rate of 30 t ha^{-1} of woody biochar, but no biochar effects on P was observed in this study. It is probable that after eight years of application some of the available nutrients contained in biochar have been removed from soil with crop (Dong et al. 2017). Due to the weathering of biochars, they are expected to increase CEC (Mia et al. 2017) and withhold more nutrients from fertilisers. However, any major significant effects of biochar on soil nutrient contents were not observed in this study. This probably due to the loss of biochar from topsoil either due to mineralisation of biochar or downward movement in the soil profile (Mia et al. 2017).

Both MBM and mineral fertilisers decreased pH compared to control. This can be expected, since fertilisers contain ammonium and nitrification of ammonium causes acidification of soils (Paasonen-Kivekäs et al. 2016, p. 194). Fertilisers increased P, K, S, B and Mn compared to control as expected because both fertilisers contained these nutrients. MBM increased the Ca content of the soil compared to mineral fertiliser because mineral fertilisation treatment did not receive Ca from the fertiliser. Mineral and MBM fertilisers contained same amounts of soluble P but P content in the soil was significantly higher for MBM. It may that P from MBM has accumulated to soil due to residual effect. This has been observed earlier too, both Chen et al. (2011) and Nogalska et al. (2017) observed higher soil P levels for MBM compared to mineral fertilisers. P from MBM can remain in soil for 3–5 years (Chen et al. 2011). Although the continuous application of MBM over the years had increased the accumulation of P in MBM treatment, the amount was not problematically high according to the Finnish soil testing standards (Viljavuuspalvelu 2019).

6.3 Barley growth and grain yield

In this study barley grain yields (2.33 t ha^{-1}on average for all treatments) were below the average grain yield of 3.94 t ha^{-1} in 2019 in the Uusimaa region of the Southern Finland (Luke Tilastotietokanta 2021b). At the beginning of growing season 2019, temperatures were higher, and precipitation was lower than the long-term average and soil moisture content was close to the wilting point during heading stage. Low soil moisture content before heading stage, a critical development stage for barley yield determination (Arisnabarreta and Miralles 2008), may decrease yields. Uneven distribution of rain in July and August caused the soil moisture to be below wilting point between flowering and ripening. Drought stress during grain filling stage reduces grain yields and grain weight (Samarah 2005). Optimal soil pH for barley growth in this type of soil would be between 6.4-6.8 (Farmit 2021) but the soil pH varied between 5.6 and 6.2 in different plots. Dry weather during the critical development stages of barley and low soil pH may explain the relatively low grain yields in this study.

Grain yield and TGW were increased for the biochar application of 30 t ha^{-1} but the increases were statistically insignificant. This is consistent with the earlier findings at the same site, when statistically insignificant increases in grain yields for the 30 t ha^{-1} treatment have been observed or wheat (Tammeorg et al. 2014a) and barley (Härkönen 2018). Biochar addition did not affect any of the other measured plant properties (SPAD, LAI, C contents, N contents, C/N ratio or biomass) in this study. Similar results have been obtained in other studies too, where biochar application did not increase barley grain yield, TGW or leaf chlorophyll in temperate and boreal conditions four years after biochar application (Haider et al. 2017, O’Toole et al. 2018, Hangs et al. 2021). In contrast, barley grain weight increased two years after biochar addition in a temperate climate (Hood-Nowotny et al. 2018). The biochar used in this study seemed to be safe in terms of contaminants because the total PAH content and heavy metal contents (Cd, Ni, Pb, Cu and Zn) were under the limit defined by EBC (2019) and Finnish legislation (MMM 1994), respectively. Indeed, the highest biochar treatment of 30 t ha^{-1} was not significantly different in any of the measured plant properties over other treatments.

Increases in yields have been attributed to increased soil pH, fertilisation effect of biochar and improved nutrient availability (Jeffery et al. 2017, Singh et al. 2022). However, no biochar effects on soil pH were observed in this study. The used biochar had low fertilisation effect (Tammeorg et al. 2014a) and biochar application did not increase K, P or any of the measured micronutrients in soil. Most of the soil nutrients analysed in this study were classified between sufficient or poor except for

P and Zn that were classified as good (Viljavuuspalvelu 2019). This suggests that biochar did not improve nutrient status of this nutrient deficient soil either through fertilisation effect or improved nutrient availability. Some studies have reported that biochar application increased crop yield in soils with low SOC (< 2%) (Hood-Nowotny et al. 2018). The soil in this experimental site has relatively high SOC (3.2%) (Tammeorg et al. 2014a) and this also may explain the lack of biochar effects on any of the measured crop properties. Majority of barley roots are in the topsoil (0–15 cm) although some roots may extend to 75 cm in sandy soils (Dwyer et al. 1988). Lateral and vertical movement of biochar and degradation of biochar (Mia et al. 2017) may have decreased the amount of biochar in topsoil where barley roots obtain water and nutrients.

Mineral fertiliser treatment increased leaf chlorophyll, plant C content, plant N content and biomass yield, and decreased plant C/N ratio compared to MBM treatment. Low precipitation before the measurements may have decreased the availability of N from MBM. Although the availability of N in MBM has been found to be similar as in mineral fertilisers (Chen et al. 2011), dry conditions slow down the mineralisation of N from MBM (Borowik and Wyszkowska 2016). It is therefore probable that drought during the growing season decreased the availability of N from MBM. Although there was difference in the crop properties measured earlier in the growing season, grain yield and TGW did not differ for mineral and MBM. This is in line with other studies, similar grain yields for mineral and MBM fertilisers have been observed for barley (Chen et al. 2011, Nogalska 2016) and oat (Chen et al. 2011). Kivelä (2015) observed that when using MBM, N content of the soil was low at the beginning of the growing season but increased later. It could be that N availability from MBM was increased later in the growing season and therefore no differences on grain yield and TGW were observed between fertilisers. In this study mineral fertiliser and MBM treatments increased the LAI, grain yield and TGW value compared to the control. This is as expected since nutrients from fertilisers improve barley growth.

7 CONCLUSIONS

The aim of this study was to determine the long-term effects of spruce biochar application combined with fertiliser treatments on soil and crop properties. The biochar application did not consistently improve soil moisture content, soil nutrients or barley growth. On the other hand, no negative impacts of the biochar were observed. This indicates that the used biochar should be safe to use in the long-term. Low fertilisation effect of the used biochar, lack of biochar effects on soil pH and high initial

SOC may explain the lack of significant effects on the measured properties. As this study was conducted eight years after the biochar application, loss of biochar particles in plant root zone through physical and chemical breakdown of biochar as well as downward movement might also have contributed to the lack of significant effects of biochar.

The mineral fertiliser increased plant N content over the MBM during the flowering stage of barley. This suggests that the availability of N was delayed from the MBM. Nevertheless, mineral and MBM fertilisers produced similar grain yields. The continuous application of MBM has accumulated P in soil. It should be considered that with the continued use of MBM there is a risk of leaching of P from soil.

Direct agronomical benefits of biochars use are usually lacking in boreal agriculture but there is potential for climate change mitigation through C sequestering and lowered GHG emissions. Although biochar and fertilisation interaction effects were not observed in this study, combined use of biochars and fertilisers may enhance plant availability of nutrients and reduce nutrient leaching. Heterogeneity of biochars and soils make it difficult to generalise the results from different studies. Further research is still needed on the long-term effects of biochars with the inclusion of various types of biochars and soils in boreal field conditions.

8 ACKNOWLEDGEMENTS

I wish to thank my supervisors Priit Tammeorg and Subin Kalu for all their help during the various stages of my work. I am grateful to my opponent Suvi Tikka for her help on the measurements and comments on my writing. Many thanks also to the AgriChar research group members for their help on field work and making me feel welcome as part of the group. I would also like to thank my family and friends for their emotional support.

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