SOFTWOOD BIOCHAR
AS A SOIL AMENDMENT MATERIAL FOR BOREAL AGRICULTURE

DOCTORAL THESIS
PRIIT TAMMEORG

ACADEMIC DISSERTATION

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CONTRIBUTIONS

The following table presents the contributions of the authors to the original articles of the dissertation:

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ABSTRACT

Biochar is a porous carbonaceous solid material produced by pyrolysis, the thermochemical conversion of biomass in an anoxic atmosphere. The condensation of aromatic compounds during pyrolysis produces a carbon (C) form that is more resistant to microbial degradation, so the application of biochar to soils is considered as an efficient way of C sequestration. Furthermore, depending on soil conditions, it may enhance soil fertility and the yields of agricultural crops. The effects of biochar as a soil amendment have mostly been studied in (sub-) tropical conditions where the soil and climatic conditions differ notably from those prevailing in the boreal zone. In this dissertation, the effects of softwood biochar added to two boreal soils, a fertile Stagnosol and a nutrient deficient Umbrisol, were explored in southern Finland. The research focused on the effects of biochar on 1) the mineralisation of nitrogen (N) from organic fertilisers, 2) on the physicochemical properties of soil, 3) on earthworm abundance and behaviour, and 4) on the morpho-physiological traits and yields of wheat (*Triticum aestivum* L. emend Thell.), turnip rape (*Brassica rapa* L., ssp. *oleifera* (DC.) Metzg.) and faba bean (*Vicia faba* L.).

The effects of biochar on the N mineralisation dynamics from organic fertilisers and on earthworm behaviour were studied in the laboratory. Further, the biochar effects on soil physicochemical properties, earthworm abundance and the morpho-physiological traits and yields of crops were explored in field experiments where biochar was applied in combination with different fertilisers, two inorganic and one based on meat bone meal (MBM).

Biochar application to soils caused initial reduction in the N availability, probably by N immobilisation due to increased microbial biomass. The effect was greater when the biochar application was combined with an organic fertiliser with a high C:N ratio than when one with a low C:N ratio was used. In the field experiments, the N immobilisation was, however, moderate as the N uptake of crops was not affected and in the Umbrisol, the highest nitrate-N content of soil was found in the biochar treatments in the second year (probably because of the turnover of microbial biomass).

Biochar application increased the contents of C and exchangeable potassium (K) in the soil, but had no significant effects on other soil chemical properties within the first two to three years of the experiments. Biochar effects on soil physical properties were varied. In the Stagnosol with a sandy clay loam texture, the application was associated with slightly increased soil moisture contents in the field, but no effects on water retention characteristics (WRC) or soil porosity were observed. In the Umbrisol with a loamy sand texture, biochar increased the plant-available water content (AWC) of the topsoil in the first year and increased soil porosity in the second year after application but had no effects on the moisture content of the soil.

In the laboratory, biochar had no effect on the habitat choice of earthworms when the test lasted for 2 days, but after 2 weeks, biochar-

treated soil was avoided. The avoidance effect was associated with a slight decrease in soil water potential. This avoidance effect was not detected under field conditions, where there was even an indication of increased abundance and biomass of earthworms in biochar-added soil.

The effects of biochar application on the plant growth dynamics and N uptake of turnip rape and wheat were not significant, but the enhanced accumulation of biomass and N uptake of faba bean during the initial N immobilisation phase can be taken as an indication of enhanced biological N fixation via increased abundance of N-fixing bacteria. In dry years, biochar addition affected the yield formation of crops, as it was associated with decreased plant density and increased number of reproductive units (pods, siliques or ears) per plant. The latter was attributed to two additive mechanisms, namely the compensation for decreased plant density and relieved moderate water deficit. The effects of biochar on crop yields were, however, not significantly different from the control, irrespective of the fertiliser treatments or the soil types studied.

It can be concluded that the application of biochar in combination with inorganic fertilisers or with MBM to boreal soils with near neutral pH and relatively high original soil organic matter (SOM) content may reduce deficits of water and K but should not be expected to significantly affect yields of faba bean, turnip rape and wheat during the first few years. As added biochar had no negative effects on crop yields or earthworms, it can be suggested that softwood biochar application is an agriculturally safe way of sequestering C. Considering the longevity of biochar in soils, future studies are needed for monitoring the long-term effects of biochar under field conditions, including the changes in soil microbiology.

LIST OF ABBREVIATIONS

AGB	Above-ground plant biomass
AM	Arbuscular mycorrhiza
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AWC	Plant-available water content
CCM	Composted cattle manure
CEC	Cation exchange capacity
DM	Dry matter
GHG	Greenhouse gases
GS	Growth stage
ICP-OES	Inductively coupled plasma - optical emission spectrometry
LAI	Leaf area index
MBM	Meat bone meal
PAH	Polycyclic aromatic hydrocarbons
PWP	Permanent wilting point
SOC	Soil organic carbon
SOM	Soil organic matter
SPAD	Single photon avalanche diode reading (a relative index of leaf chlorophyll content)
SSA	Specific surface area
TDR	Time domain reflectrometry
TSW	1000 seed weight
v/v	Volume to volume
VM	Volatile matter
w/w	Weight to weight
WFPS	Water filled pore space
WRC	Water retention characteristics
X_{avoid}	Earthworm avoidance, %

KEY DEFINITIONS

Biochar: a carbonaceous porous solid material, produced by thermochemical conversion of biomass in anoxic atmosphere (pyrolysis), that has physiochemical properties suitable for the safe and long-term storage of carbon (C) in the environment and, possibly, soil improvement (Shackley and Sohi 2010). The pyrolysis process additionally produces bio-oil, syngas and heat energy; the relative yields of the different product components depend on the raw materials and the pyrolysis conditions (Vamvuka 2011).

Carbon sequestration: transfer of atmospheric carbon dioxide (CO_2) into long-lived pools and secure storage of it (Lal 2004). The term includes terrestrial C sequestration (conversion of a part of the net primary production into stable humic substances and secondary carbonates), oceanic C sequestration (burial of CO_2 into oceanic ecosystems) and geologic C sequestration (engineering techniques for the injection of industrially emitted CO_2 into geologic strata; Lal 2010).

Soil amendment: a material that, when applied to soils, improves the physical, chemical, or biological properties of the soil. The term thus includes both soil conditioners that improve mostly the physical structure of soil and fertilisers that supply nutrients to soil and improve plant productivity.

1 INTRODUCTION

1.1 Biochar as a tool for carbon sequestration

Soils of the world contain 2500 Gt of C, which is 3.3 times more than the amount of C stored in the atmosphere (760 Gt) and almost five times more than that in the biotic pool (560 Gt; Lal 2004). During 1850–1998 around 78 Gt of C has been emitted from soils to the atmosphere as CO_2 (Lal 2004), mainly through mineralisation, soil degradation and accelerated erosion. The historic loss in most agricultural soils ranges from 30–75% of their initial soil organic carbon (SOC) pool, adversely affecting soil fertility, crop yields and water quality (Stavi and Lal 2013). In Finland, the annual loss of existing topsoil C content between 1974 and 2009 was estimated at 0.2–0.4% (Heikkinen et al. 2013).

The emission of CO_2 from soils to the atmosphere may, together with the use of fossil fuels, intensify global warming (Lal 2004; Lal 2010). The increased atmospheric concentrations of greenhouse gases (GHG) resulted in radiative forcing of atmosphere to increase by 23% between 1990 and 2006 (Stavi and Lal 2013). The stabilisation of the GHG concentrations in the atmosphere could be achieved by both radically curbing anthropogenic GHG emissions and, more importantly, continuously increasing the proportion of CO_2 drawn down from the atmosphere to a level that future net anthropogenic emissions would approach zero (Matthews and Caldeira 2008; Woolf et al. 2010). Differently from the fossil fuel pool, the soils, depending on land use and management practices, can be turned from a source into a sink of atmospheric CO_2 (Lal 2010; Stavi and Lal 2013). When such a sink is long-lived, the practice is called “carbon sequestration” (Lal 2004).

The maximum C-sink capacity in the soils is probably about the size of the past depletion of C since the dawn of settled agriculture (Lal 2010), and numerous soil management practices have been suggested for reaching this capacity (Lal 2004; 2010; Woolf et al. 2010; Stavi and Lal 2013). They include agroforestry, cover cropping, minimised tillage, use of organic fertilisers (manures, composts, sludges) and enhanced management of grazing and forestry (Lal 2004; Stavi and Lal 2013). The worldwide application of these practices could sequester 0.4–1.2 Gt of C annually (Lal 2004). The C sequestration through these practices is, however, relatively short-lived because much of what is added to soils is easily degradable by soil microbial communities (Stavi and Lal 2013).

A significantly longer-term C sequestration practice that has received considerable interest during the last decade is the use of biochar as a soil amendment (Glaser et al. 2002; Lehmann et al. 2003; Woolf et al. 2010; Stavi and Lal 2013). The thermal treatment of the biomass during pyrolysis results in a high proportion of aromatic compounds in biochar (Cheng et al. 2008). As a result, biochar is relatively recalcitrant against microbial degradation, slowing down the rate at which C fixed by photosynthesis is returned to the atmosphere. The mean residence times of C in biochars produced from plant biomass have been estimated to range from 300 to 4000 years (Cheng et al. 2008; Lehmann et al. 2008; Singh et al. 2012; Kuzyakov et al. 2014). The sustainable use of biochar as a soil amendment delivers a technical potential to sequester 1.8 Gt C annually, while preserving food security, biodiversity and ecosystem stability (Woolf et al. 2010). This potential is considerably higher than that of the aforementioned strategies based on increasing SOC content of soils as well as the strategies involving combustion of the biomass for bioenergy (1.5 Gt per year; Woolf et al. 2010). Further, depending on the soil conditions, the application of certain biochars to soils has been associated with additional agricultural and environmental benefits, such as the liming effect on acid soils (Major et al. 2010; Van Zwieten et al. 2010a; Vaccari et al. 2011), decreased leaching of nutrients (Brockhoff et al. 2010; Laird et al. 2010b; Güereña et al. 2013; Major et al. 2012) and improved water retention of the soil (Eastman 2011; Liu et al. 2012). These benefits could contribute to overcoming the economic, social and cultural caveats limiting the realisation of biochar's technical potential.

1.2 Agricultural and environmental effects of soil-applied biochar

1.2.1 The historical use of biochar

Some of the oldest biochar application sites known are located in central Amazonia, where anthropogenic soils with exceptionally high SOM contents exist in spite of the dominant humid tropical conditions and rapid mineralisation rates (Smith 1980; Glaser et al. 2002; Lehmann et al. 2003). These highly fertile *terra preta* or "dark earth" soils were apparently created by pre-Columbian populations starting from 2930 years before the present (Smith 1980) by the input of charred organic materials (biochar), bones and organic wastes (Smith 1980; Glaser et al. 2002; Lehmann et al. 2003). These soils have relatively high cation exchange capacity (CEC; Smith 1980; Liang et al. 2006) and are richer in nutrients (especially phosphorus (P), but also calcium (Ca), manganese (Mn) and zinc (Zn)) than adjacent soils, which is why they are still highly valued by local farmers for their high production potential (Lehmann et al. 2003).

Similar cultivation practices have been historically used also in boreal cropping systems (Ahokas 2012). For example, in southern Finland, the char produced by anaerobic burning of woodpiles, peat or common reed was applied to soils (*kytö*) to continue cultivation on slash-and-burn fields after the soil became infertile (Ahokas 2012). The *kytö* practice increased the availability of nutrients in soil, raised soil pH and reduced plant diseases, pests and weeds (Ahokas 2012). The practice has been dated back to at least 1460 years before the present and it was widely adopted in Finland and neighbouring areas until the late 19th century (Ahokas 2012).

In addition to the C sequestration potential of biochar, the fertility of the historical Anthrosols has inspired research on biochar during the past decade. Consequently, numerous experiments have been conducted in pursuit of replication of the *terra preta* phenomenon with new biochar additions to arable soils.

1.2.2 Effects on soil chemical properties

The effects of biochar application on soil chemical properties include increased CEC of the (sub-) tropical soils (Liang et al. 2006; Major et al. 2010; Peng et al. 2011; Jien and Wang 2013), associated with increased soil specific surface area (SSA; Liang et al. 2006; Jien and Wang 2013) and oxidation of biochar leading to more negatively charged functional groups on biochar surface (Cheng et al. 2006; Liang et al. 2006; Jien and Wang 2013). Additionally, certain biochars with liming value may decrease soil acidity (Chan et al. 2007; Chan et al. 2008; Major et al. 2010; Van Zwieten et al. 2010a; Vaccari et al. 2011; Alburquerque et al. 2013; Jien and Wang 2013; Xu et al. 2013). Further, the increased pH due to the incorporation of biochar can improve the availability of P in acid soils (Uzoma et al. 2011; Xu et al. 2013).

Depending on its raw material, biochar can release nutrients to soil solution, as has been reported for P (Chan et al. 2007; Uzoma et al. 2011; Alburquerque et al. 2013; Xu et al. 2013), potassium (K; Chan et al. 2007; Laird et al. 2010a; Major et al. 2010; Jones et al. 2012; Liu et al. 2012; Quilliam et al. 2012; Jien and Wang 2013; Xu et al. 2013), Mn (Laird et al. 2010a), Na (Xu et al. 2013) and Ca and magnesium (Mg; Laird et al. 2010a; Jien and Wang 2013; Xu et al. 2013). In addition, biochars may increase nutrient (N, P, Ca and Mg) retention in soil (Steiner et al. 2008; Laird et al. 2010a, b; Major et al. 2010) via electrostatic adsorption or immobilisation of N to microbial biomass. The immobilisation of N to microbial biomass is probably related to the decomposition of a small portion of biochar C within the first months by microbial and abiotic oxidation (Hamer et al. 2004; Cheng et al. 2008; Jones et al. 2011; Singh et al. 2012). Nutrient retention in soil may be beneficial for reducing the nutrient burden on the watercourses: decreased leaching from soils of N (Brockhoff et al. 2010; Laird et al. 2010b; Güereña et al. 2013; Major et al. 2012), P and Mg (Laird et al. 2010b) have been reported after biochar application. On the other hand, negative effects on plant development and yield may follow if N is immobilised during the growing season (Garabet et al. 1998; Asai et al. 2009; Lentz and Ippolito 2012; Nelissen et al. 2014).

Biochar may also increase the decomposition of the native SOM (positive priming effect), particularly after application of low temperature (250–450 °C) biochars containing more than 411 g kg^{-1} of volatile matter (VM; Zimmermann 2010; Zimmermann et al. 2011). The positive priming effect may be caused by increased abundance of microbes remineralising soil nutrients and co-metabolising SOM, e.g. soil humic materials (Zimmermann et al. 2011). The negative priming effect, however, is expected to prevail in the long-term, as SOM is increasingly sorbed within biochar pores and onto surfaces where it is protected from degradation (Zimmermann et al. 2011).

Additionally, biochar may adsorb pesticides, with high (242 m^2 g^{-1}) specific surface area biochars having greater effect than low (4 m^2 g^{-1}) specific area biochars (Graber et al. 2012). Such an adsorption would be useful if the pesticide residues interfere with growth of a sensitive crop, but, if the reduced pesticide efficacy would increase the pesticide application dose required for appropriate pest protection, the effect would be undesirable (Graber et al. 2012).

Lastly, the possibility of chemical pollution should be carefully assessed before biochars are applied to soils. For example, gasification biochars may contain high contents of polycyclic aromatic hydrocarbons (PAH; Hale et al. 2012) and sewage sludge biochars may contain high contents of heavy metals (Hossain et al. 2011).

1.2.3 Effects on soil physical properties

Effects of biochar application on soil physical properties include increased soil SSA (Liang et al. 2006; Laird et al. 2010a; Mukherjee and Lal 2013), increased porosity and decreased bulk density (Oguntunde et al. 2008; Laird et al. 2010a; Masulili et al. 2010; Eastman 2011; Abel et al. 2013; Hardie et al. 2013; Herath et al. 2013; Jien and Wang 2013), and improved soil aggregate stability and erosion resistance (Herath et al. 2013; Jien and Wang 2013; Soinne et al. 2014). Additionally, the dark colour of biochar may reduce soil albedo and thus increase the adsorption of heat by the soil surface (Genesio et al. 2012). The application of hardwood biochar to silty loam in Mediterranean conditions increased the temperatures of the topsoil by up to 2°C and was consequently associated with faster emergence of durum wheat in spring (Vaccari et al. 2011).

The increase in soil SSA has been attributed to the SSA of the added biochar being higher than that of the soil (Liang et al. 2006; Laird et al. 2010a; Mukherjee and Lal 2013*)*. Additionally, it has been proposed that the interactions between biochar-amended soil and microbes could increase the SSA of soil in the longer term (Mukherjee and Lal 2013), but this hypothesis needs to be tested in experiments.

The improved aggregate stability and increased proportion of soil macroaggregates (> 250 μm) after biochar application is partly caused by increased microbial biomass contributing microbial mucilage that binds microaggregates to macroaggregates (Herath et al. 2013; Jien and Wang 2013). The formation of cation bridges between surfaces of oxidised biochar and soil particles may contribute to the formation of macroaggregates (Jien and Wang 2013; Soinne et al. 2014).

The enhanced soil structure after biochar treatment is measured as the increased soil porosity and decreased bulk density and penetration resistance of soils. The increased soil porosity and decreased bulk density may be caused by the increased formation of macroaggregates (Herath et al. 2013; Jien and Wang 2013) or the dilution effect of the added low bulk density biochar to higher bulk density soils (Eastman 2011; Abel et al. 2013; Herath et al. 2013; Jien and Wang 2013). Furthermore, the earthworm burrowing in biochar-containing soils may add macroporosity (Hardie et al. 2013). The effects of biochar on decreasing soil bulk density may be beneficial for reducing soil compaction: reduced soil penetration resistance has been reported under laboratory (Busscher et al. 2010; Masulili et al. 2010) and greenhouse (Chan et al. 2007) conditions.

Biochar particles packing in between of the soil matrix may also change the pore size distribution of the soils, depending on the original soil properties. Application of coarse (≥ 0.5 mm) biochar increased the macroporosity (higher volumetric water content at tensions ≥ –0.3 bar) of a Typic Fragiaqualf but in a Typic Hapludand, the increase was in mesoporosity (higher volumetric water content at tensions from −1 to −0.1 bar); the effect was attributed to the higher proportion of fine silt and clay particles in the former soil (Herath et al. 2013).

The increases in soil porosity and aggregate stability have been associated with increased saturated soil hydraulic conductivity (Uzoma et al. 2011; Herath et al. 2013, Jien and Wang 2013; Hardie et al. 2013) and retention of plant-available water (Eastman 2011; Liu et al. 2012; Abel et al. 2013; Herath et al. 2013), which together improve the soil water conditions for plants. Under field conditions, the improving effect of biochars on the plant-available water content (AWC) is generally greater in soil macropores than in micropores (Eastman 2011; Liu et al. 2012; Baronti et al. 2014).

Alterations in soil physical properties may also explain the reported decreases in the emissions of nitrous oxide (N_2O) from the soils (Yanai et al. 2007; Case et al. 2012; Kammann et al. 2012; Angst et al. 2013). Possible mechanisms include improved soil aeration (Yanai et al. 2007), retention of nitrate (NO_3^-) within biochar pores in dissolved form (Van Zwieten et al. 2010c; Prendergast-Miller et al. 2011; Case et al. 2012, Felber et al. 2014), and biological immobilisation of NO_3^- (Bruun et al. 2012; Case et al. 2012; Angst et al. 2013; Zheng et al. 2013). Additionally, the decreased N_2O emissions from soils may be due to biochar facilitating the transfer of electrons to denitrifying microbes in soil, which could enhance the reduction of N_2O to N_2 (Cayuela et al. 2013). On the other hand, when biochars with a high N content are added to soils, N_2O production may also increase because of added nutrients and labile organic matter (Spokas and Reicosky 2009).

1.2.4 Effects on soil biota

Biochar application to soils may increase the activity of soil microbes and change the composition of soil microbial community due to the improved availability of water and nutrients in soils, increased pore space providing protection for grazers, and sorption and inactivation of growth-inhibiting substances after biochar application (Warnock et al. 2007; Thies and Rillig

2009; Liang et al. 2010; Lehmann et al. 2011; Güereña et al. 2013). From the agronomical perspective, perhaps one of the most relevant responses in soil microbiology is the increased abundance of arbuscular mycorrhiza (AM; Blackwell et al. 2010; Solaiman et al. 2010) and endomycorrhizal fungi (Husk and Major 2010) that could assist uptake of nutrients (P and Zn) and water by plants. The increased abundance of AM has been attributed to biochar pores improving habitat for the fungi (Blackwell et al. 2010).

The effects of biochar on soil fauna have been less studied, but from laboratory studies there is some evidence for earthworm preference of biochar-amended soil and positive effects of biochar on earthworm activity, mostly because of decreased soil acidity (Topoliantz and Ponge 2003, 2005; Van Zwieten et al. 2010a; Busch et al. 2011). If earthworms ingest biochar particles together with soil particles, the higher pH in the gut could assist the assimilation of other resources (Weyers and Spokas 2011). Similarly, the microbial biomass in ingested biochar particles might add microbial enzymes to the earthworm’s digestive system (Topoliantz and Ponge 2003), or be an energy source itself.

Negative effects of biochar addition on soil biota include weight loss and avoidance of treated soil by the earthworm *Eisenia fetida* Sav., attributed to desiccation triggered by high water retention of the biochar (Li D. et al. 2011). Furthermore, the application of poultry manure biochar decreased the survival of *E. fetida*, the effect was associated with the toxic effects of ammonia, salinity or with a rapid increase in soil pH (Liesch et al. 2010).

1.2.5 Effects on crop growth

Due to the improved physical, chemical and biological conditions caused by the addition of biochar to soil, increased crop growth and yields have been reported under tropical and sub-tropical climatic conditions (Steiner et al. 2007; Kimetu et al. 2008; Asai et al. 2009; Blackwell et al. 2010; Major et al. 2010; Solaiman et al. 2010; Van Zwieten et al. 2010b; Vaccari et al. 2011; Zhang et al. 2012; Cornelissen et al. 2013). According to the most extensive literature review available on biochar effects to crop yields (including 103 publications and 880 pairs of data; Liu et al. 2013), the mean crop productivity improvement following biochar application to soils was 11% over the no-biochar control (including both yields and biomass). The 57 field experiments covered by the study were conducted under tropical, subtropical and temperate conditions and lasted for one to four years; and the mean yield increase of 9% was reported across all crop species tested (including cereals, legumes and vegetables; Liu et al. 2013). The main mechanisms suggested as responsible for the increase were the liming of acid soils, the improved soil aggregation, and the increased availability of moisture and nutrients to the crop (Liu et al. 2013).

The increased activity of mycorrhizal fungi may also affect plant growth. For example, the increased colonisation of roots with AM after the application of *Eucalyptus* wood biochar probably caused improved water availability to wheat on drought-prone Australian soils (Blackwell et al. 2010; Solaiman et al. 2010). Similarly, the increase in forage biomass after the application of 3.9 t ha^{-1} hardwood biochar to a temperate clay loam in Canada was attributed to higher root colonisation by endomycorrhizal fungi that may have improved zinc (Zn) availability (Husk and Major 2010).

In addition to the improved crop yields, nutrient uptake efficiency of the crops may also increase after biochar application. For instance, uptake of N by different crops including radish (*Raphanus sativus)*, sorghum (*Sorghum bicolor* L. Moench), maize (*Zea mays* L.) and wheat significantly improved in tropical soils under both greenhouse (Chan et al. 2007; Van Zwieten et al. 2010a; b) and field conditions (Steiner et al. 2008; Major et al. 2010). The improvement has been attributed to enhanced friability and water holding capacity of the soil (Chan et al. 2007), reduced gaseous N losses (Yanai et al. 2007) and diminished N leaching (Brockhoff et al. 2010).

Crop growth is not always improved significantly after biochar application as the effects depend on the soil and the biochar in question. For example, when biochar was applied to fertile soils in temperate (Güereña et al. 2013; Jones et al. 2012) and boreal climates (Karhu et al. 2011), no effects on the crop yields were seen one to four years after the start of the studies, despite the increased water holding capacity (Karhu et al. 2011) and availability of K (Jones et al. 2012).

Decreased crop biomass or yields have also been reported (Kishimoto and Sugiura 1985; Van Zwieten et al. 2010a; Lentz and Ippolito 2012; Alburquerque et al. 2013; Nelissen et al. 2014). This effect was attributed to decreased availability of N (Asai et al. 2009; Lentz and Ippolito 2012; Alburquerque et al. 2013; Nelissen et al. 2014) and sulphur (S; Lentz and Ippolito 2012). The decrease in N availability was probably caused by initial N immobilisation by microbial biomass (Deenik et al. 2010; Novak et al. 2010; Nelson et al. 2011; Bruun et al. 2012; Angst et al. 2013; Zheng et al. 2013), while the lower availability of S in calcareous soil may be due to reduced mineralisation of soil C (negative priming effect; Lentz and Ippolito 2012).

1.3 Research needs

The past few years have witnessed a remarkable increase in the studies reporting biochar effects on soil properties and plant growth under field conditions (Jeffery et al. 2011; Liu et al. 2013; Mukherjee and Lal 2013). However, few peer-reviewed field-scale studies have focused on biochar effects on soil and plant growth in temperate (Güereña et al. 2013; Jones et al. 2012; Liu et al. 2012; Quilliam et al. 2012) and boreal cropping systems (Karhu et al. 2011). These soils are generally less constrained by low SOM content, nutrient deficiencies and acidity than soils in the tropics and subtropics. Furthermore, boreal soils are commonly affected by freeze-thaw cycles, high moisture content and wide seasonal variation in solar radiation and air temperatures, which may alter the processes interacting with the applied biochars. Considering the high availability of biomass suitable for biochar production in the boreal region (e.g., crop and forestry residues), it is crucial to target the gap of knowledge on the effects of biochar under these pedo-climatic conditions.

Because of depleting resources for inorganic fertiliser production (e.g. P rocks (Cordell et al. 2009) and fossil fuels) and recent fluctuations in the prices of inorganic fertilisers (Silva 2011; USDA 2013), the significance of nutrient recycling via augmented use of organic fertilisers is widely recognized (Roy et al. 2002; Römer 2009; Fischer and Glaser 2012). Hence, information is needed about the interactions between organic fertilisers and biochar. Such interactions may include the augmented contents of C moieties in soils improving the cation availability through increased CEC (Glaser et al. 2002; Schulz and Glaser 2012), and higher sorption capacity of phytotoxic substances (Hille and den Ouden 2005; Schulz and Glaser 2012).

Previous studies combining biochar and organic fertilisers have studied the treatment effects on soil properties and plant growth in combination with manures (Lehmann et al. 2003; Laird et al. 2010a; b; Lentz and Ippolito 2012) and composts (Steiner et al. 2007; 2008; Schulz and Glaser 2012; Schulz et al. 2013). No information exists about the potential interactions between biochar and meat bone meal (MBM), a by-product of the rendering industry used as an organic fertiliser. The MBM has relatively low (4-5) C:N ratio, which facilitates faster N mineralisation compared with manures (Salomonsson et al. 1994). As with biochar, the MBM additions have been reported to enhance the activity of soil micro-organisms (Mondini et al. 2008), increase N and P use efficiencies (Ylivainio et al. 2008, Jeng and Vagstadt 2009) and improve crop yields (Salomonsson et al. 1994; Jeng et al. 2004; 2006, Chen et al. 2011). Knowledge of the agronomic effects following the combination of biochar with MBM would be especially valuable for organic farming systems.

Furthermore, little is known about the effects of biochar on soil biota, with the exception of the relatively well established increase in microbial biomass following biochar application under most conditions (Liang et al. 2010; Lehmann et al. 2011; Güereña et al. 2013). In particular, biochar-induced effects on earthworms in soils have seldom been studied on the field scale. Neither of the two studies available (Husk and Major 2010; Weyers and Spokas 2011) were replicated, which prevented the statistical comparison of treatment effects. Earthworms may modify and transport biochar particles in the soil (Topoliantz and Ponge 2003, 2005; Eckmeier et al. 2007) and consequently affect the soil microbial activity (Lehmann et al. 2011). Considering the importance of earthworms in modifying the soil physical structure (Lavelle 1988; Blouin et al. 2013) and nutrient availability (Lavelle et al. 1998; Chaoui et al. 2003; Blouin et al. 2013), it is important to examine the effects of biochar on earthworms to unveil any unwanted changes in their ecology.

Once applied, biochar cannot be removed from the soil (Jones et al. 2012). Thus, it is of utmost importance to explore both the positive and negative effects of different biochars on the soil-plant-atmosphere system before making any recommendations about large-scale biochar application to agricultural soils.

2 AIMS OF THE RESEARCH

This dissertation explored the effects of softwood biochar application on soil physicochemical properties, earthworms and yield formation of common agricultural crops in boreal conditions. The benefits and drawbacks of using biochar as a soil amendment were studied both in the laboratory and in the field.

The research questions for this dissertation were:

1. How does applied biochar affect the N mineralisation dynamics of organic fertilisers incubated in a laboratory (**I**) and do the effects persist in a two-year field experiment (**III**)?
2. What are the effects of biochar application on the chemical and physical properties of two distinctive soils under field conditions: a fertile sandy clay loam (Stagnosol, **II**) and a nutrient-deficient loamy sand (Umbrisol, **III**)?
3. How does biochar application affect the common earthworms in arable soil under both laboratory and field conditions (**IV**)?
4. How does biochar application affect the morpho-physiological traits and yield of faba bean (*Vicia faba* L.), spring turnip rape (*Brassica rapa* L., ssp. *oleifera* (DC.) Metzg.) and spring wheat (*Triticum aestivum* L. emend Thell.) in a fertile sandy clay loam field (**II**)?
5. How does biochar application affect the morpho-physiological traits and yield of spring wheat in a nutrient deficient loamy sand, and do the effects depend on the type of fertiliser used (**III**)?

3 MATERIALS AND METHODS

3.1 Experimental sites and soils

This research was conducted on two different boreal soils. The first soil was a nutrient-deficient Endogleyic Umbrisol (WRB 2007) with a loamy sand texture in the topsoil layer (2% clay; pH 6.4; SOC content 31.7 g kg^{-1}; field location 60°13’42" N 25°2'34" E; **I**; **III**; **IV**). The second soil was a fertile Luvic Stagnosol (WRB 2007) with a sandy clay loam texture in the topsoil layer (24% clay; pH 6.6; SOC content 34.4 g kg^{-1}; field location 60°13’27’’ N 25°1’38’’ E; **II**). The initial content of soil organic matter (SOM) was 68.8 g kg^{-1} for the Stagnosol and 63.4 g kg^{-1} for the Umbrisol, assuming a 50% C content for the SOM (Pribyl 2010). Both fields were part of the Viikki Research and Experimental Farm, University of Helsinki, Finland, and were cropped for the preceding five years with small grains with conventional mouldboard ploughing up to 25 cm depth. The Stagnosol had no nutrient deficiencies (**II**), whereas the Umbrisol contained insufficient levels of exchangeable Ca, K, Mg and S prior to the experiments (**III**).

The air temperatures during the growing seasons 2010–2011 were markedly higher than the long-term means, especially in July–August, whereas the temperatures in 2012 were similar to the long-term means (Table 1). In June and July 2010, precipitation was 26% (29 mm) below the long-term means, whereas in May–June 2012, the precipitation was 88% (72 mm) above the long-term mean. The precipitation in summer 2011 was similar to the long-term mean, except for the considerably (95 mm) wetter August.

Table 1. Mean air temperature (ºC) and monthly precipitation (mm) in Helsinki of growing seasons 2010–2012 (FMI 2012, 2014), compared with the long-term (1971–2000) mean of the weather station at Kaisaniemi, Helsinki (FMI 2012).

Month	**Mean air temperature (ºC)**				**Monthly precipitation (mm)**			
	1971–2000	2010	2011	2012	1971–2000	2010	2011	2012
May	9.9	11.5	9.9	10.9	32	59	27	65
June	14.8	14.6	16.7	13.7	49	33	49	88
July	17.2	21.7	20.6	17.7	62	49	56	54
August	15.8	18.1	17.5	16.0	78	97	173	39
September	10.9	12.2	13.6	12.5	66	50	88	160

3.2 Biochars

The three biochar batches used in this experiment were produced by pyrolising dried chips of debarked softwood from Southern Finland: either spruce (*Picea abies* (L.) H. Karst.) or a mixture of spruce and pine (*Pinus sylvestris* L.; Table 2). The chips were pyrolised in a continuously pressurized carboniser (Preseco Oy, Lempäälä, Finland) at 550–600 °C for 10–15 minutes, and the differences in biochar properties (Table 2) are likely due to the different proportions and origin of the spruce and pine chips in the raw material. The chips were transported to the reactor tube through an airtight feeding system and then moved through the hot region of reactor tube by a screw conveyor. The process produced about 50% biochar, 30% gaseous products, and 20% bio-oil. The biochars were cooled overnight in an airtight silo and ground with a roller mill. After grinding, more than 88% of each dry biochar was in particles less than 5 mm in diameter. The methods used for analysing the biochars are presented in Table 3 (p. 27).

Table 2. Selected physicochemical properties of the biochars.

Property[a]	**Biochar batch**			**Unit**
	1 (**I**)	2 (**II**)	3 (**III**; **IV**)	
Raw material	Spruce and pine	Spruce and pine	Spruce	
SSA	12	34	265	m^2 g^{-1}
pH_{H2O}	8.9	10.8	8.1	
Liming value	0.19	0.62	0.18	mol kg^{-1}
Ash	23	56	27	g kg^{-1}
VM	105	268	122	“
Ca	4.8	10	4.7	“
Fe	0.4	2.9	0.3	“
K	2.8	4.0	4.5	“
Mg	0.8	1.7	0.9	“
Mn	0.3	0.5	0.3	“
Na	0.1	0.3	0.2	“
P	0.2	1.1	1.8	“
S	0.2	0.6	0.2	“
Zn	0.1	0.1	0.1	“
C	903	878	883	“
N	6.1	6.2	3.5	“
C:N	148	142	251	

[a] n = 6 for volatile matter (VM), n = 2 for pH, total C and N and n = 3 for other analyses. SSA = specific surface area.

3.3 Design of the experiments

3.3.1 The N mineralisation experiment (I)

A 133-day laboratory experiment with incubation time, biochar, and fertiliser as experimental factors was conducted to study the effects of biochar on the net N mineralisation dynamics of two organic fertilisers in the infertile sandy loam soil taken from the top 0–25 cm layer of the Umbrisol field. The soil was homogenised and passed through a 2-mm sieve. Biochar (batch 1; Table 2) was added at rates of 0, 4.6, 9.1 and 13.6 g kg^{-1} soil corresponding to 0, 10, 20 and 30 t ha^{-1}, either with or without organic fertilisers. The fertilisers were meat bone meal-based organic fertiliser Aito-Viljo (MBM; N content 8%, C:N ratio 4.7) and composted cattle manure (CCM; N content 1.1% and C:N ratio of 19.7). Both fertilisers were applied at 306 kg N ha^{-1} and the experiment was set up in a completely randomized design with six batches of samples. The samples were prepared by mixing 24.3 g fresh soil (20.6 g dry weight) with biochar and fertilisers in 100-ml open-top beakers. The trays with the beakers were kept in a constant-temperature room at 15 ± 1°C in separate polyethylene bags, and deionised water was added weekly to maintain the field capacity moisture content (240 g kg^{-1}; 45% water filled pore space (WFPS). The soil temperature and moisture conditions as well as the duration of incubation were chosen to approximate the typical environmental conditions in the topsoil and the duration of growing season in the boreal climate of southern Finland.

3.3.2 The Stagnosol field experiment (II)

To explore the effects of biochar addition on the soil properties and plant growth under field conditions, three identically designed split-plot field experiments with four replicates were conducted over three successive growing seasons (2010–2012) in the fertile Stagnosol field. The experiments were set up in the same field as three adjacent parcels next to each other, with each parcel having a different crop (wheat, turnip rape and faba bean). The annual rotation of crops between the parcels every year resulted in a three-year crop rotation where each crop was sown once in each parcel by the end of the experiments. The main plot factor was the biochar (batch 2; Table 2) application rate (0, 5 and 10 t dry matter (DM) ha^{-1}), and the application rate of the compound NPK fertiliser was the subplot factor. The compound NPK fertilisers were Agro 16-7-13 for faba bean, and Agro 28-3-5 for both wheat and turnip rape, applied at three rates (30%, 65% and 100% of the N level recommended for the individual crop, 100% being 150, 130 and 40 kg N ha^{-1} for wheat, turnip rape and faba bean, respectively). The single biochar application was conducted in May 2010 with a sand spreader (Fig. 1), followed by its incorporation into the uppermost 10 cm soil layer by two opposite passes with a rotary power harrow.

Figure 1. Spreading of the biochar by a sand spreader (on left), and a view of the Stagnosol field (**II**) at flowering in 2010 (on right).

The neighbouring main plots were separated by buffer plots of the same crop for minimizing biochar carryover. Both the experimental and buffer plots had the dimensions of 2.2 x 10 m. Two days after biochar application, the plots were sown with wheat cv Amaretto, turnip rape cv Apollo, and faba bean cv Kontu at 650, 200 and 60 viable seeds m^{-2}, respectively. The crop management followed integrated practices including the use of herbicides and pesticides used for crop protection as needed. The field was tilled with a disc harrow to the depth of 12 cm after each growing season (except for the wet autumn in 2011), followed by rotary power harrowing to the same depth in spring.

3.3.3 The Umbrisol field experiment (III)

A two-year field experiment (2011–2012) was conducted in the nutrient deficient Umbrisol field to study the effects of biochar application with or without organic and inorganic fertiliser on soil physicochemical properties and wheat yield formation. The experiment was a split-plot design with four replicates, the main plot factor being the application rate of biochar (batch 3; Table 2; applied at 0, 5, 10, 20 and 30 t DM ha^{-1}) and the sub-plot factor being the fertiliser treatment (unfertilised control, MBM and inorganic fertiliser). The single biochar application was conducted in May 2011. The plot size, use of buffer plots and the biochar application were similar to those in the Stagnosol field experiment (**II**; section 3.3.2). The day after biochar application, spring wheat cv Amaretto was sown and fertilised with a combine seeder at 650 viable seeds m^{-2}.

The MBM-based fertiliser was Aito-Viljo 8-5-2 and the inorganic fertiliser was Agro 28-3-5. Both fertilisers were applied at 100 kg N ha^{-1}, as the plant availability of N in MBM has been reported to be comparable with that of inorganic N fertilisers (Jeng et al. 2004, Chen et al. 2011). Assuming that 18% of total MBM-P was water soluble in the first growing season (Ylivainio and Turtola 2009), the applied fertilisation delivered equal amounts of plant-available P in the first year. To even out the residual effect of MBM-P, Yara Fosforiravinne (9% P) was added to the inorganic fertiliser in 2012. Similarly, to equalize the higher K application with MBM, K_2SO_4 (K content 41.5%) was added to the inorganic fertiliser treatment. Thus, both fertiliser treatments provided 10.8 kg P ha^{-1} and 19.5 kg K ha^{-1} in easily soluble form in 2011, as well as 14 kg P ha^{-1} and 19.5 kg K ha^{-1} in 2012.

Integrated crop management practices were used, including the use of chemical herbicides, fungicides and pesticides when necessary. The field was tilled with a disc harrow to the depth of 12 cm after the first growing season, followed by rotary power harrowing to the same depth in spring 2012.

3.3.4 The earthworm studies (IV)

In order to determine the short-term effects of biochar (batch 3; Table 2) application on earthworm species typical for arable soils, the density and biomass of earthworms was studied in the Umbrisol field (section 3.4.2), and the biochar avoidance by earthworms was investigated in the laboratory. For the avoidance test, soil from the Umbrisol site (sampled in spring 2011) was first heated at 60°C for 4 days to eradicate earthworms, passed through a 2-mm sieve, thoroughly mixed and moistened to 300 g kg^{-1} DM moisture content. Cylindrical closed-bottom polyvinyl chloride (PVC) vessels divided into the control and treatment parts were used as experimental units. The two parts were filled with the soil to the same volume (of height 15 cm) either alone (control) or mixed with 16 g biochar kg^{-1} corresponding to 30 t DM ha^{-1}. The biochar was passed through a 2-mm sieve before mixing with soil. The control and the biochar treatments were separated with a vertically introduced 3 mm wide divider (Makroclear® polycarbonate, Etra Oy, Helsinki, Finland) during the setup of the experiment. After the separator was removed, eight randomly chosen, mature individuals of *Aporrectodea caliginosa* Sav. (the most common earthworm species in Finnish agricultural soils; Nieminen et al. 2011) were placed on the separating line of each test vessel. The earthworms had been collected from the immediate vicinity of the Umbrisol field experiment. A perforated plastic wrap was installed over the vessels in order to prevent the escape of the earthworms.

The vessels were kept upright next to each other in a dark, temperature-controlled (15 °C) room on a shelf, and their positions and orientation on the shelf were randomised. Half of the vessels were sampled after 2 days, and the other half after 14 days with moisture replenished after one week (n = 8 for both durations). At the end of the experiment, the separator was swiftly introduced into its original position, the contents of different sides of the vessel emptied separately and the number of earthworms from each side recorded. Additionally, the effects of biochar on the soil pH and water potential were monitored with tensiometers and pH measurements over 11 days in an additional arrangement with similar experimental setup but without earthworms.

3.4 Sampling and analyses

3.4.1 Soils

In the N mineralisation experiment (**I**), the six identical batches of samples were destructively sampled and analysed for soil mineral N (NO_3^- and NH_4^+) content on days 0, 14, 28, 56, 84 and 133 (analytical methods are given in Table 3).

In the field experiments (**II**; **III**; **IV**), soil samples for chemical analyses (0–20 cm depth; 10 samples from each plot pooled for a composite sample) were taken before starting the experiment and then after each growing season. Soil moisture content was measured weekly by TDR in the field experiments from a selection of treatment plots (from four treatments in the Stagnosol field (**II**) and six treatments in the Umbrisol field (**III**)). The moisture content was measured in three layers: at 0–18, 0–30 and 0–58 cm depth in the Stagnosol field (**II**) and at 0–15, 0–28 and 0–58 cm depth in the Umbrisol field (**III**). To determine the WRC of surface soil (2.5–7.5 cm depth to avoid the superficial plant residues), four undisturbed soil samples per plot were taken at the end of the growing seasons from the same treatments as used for measurements of soil moisture content. The samples were taken into steel cylinders and used for the determination of WRC (Table 3) as well as for the calculation of the dry bulk density and the total porosity.

3.4.2 Earthworms

To study the effects of biochar on earthworms under field conditions, earthworms were sampled from the Umbrisol field experiment in September 2011 after harvest and before the autumn tillage (**IV**). From the four experimental treatments (0 and 30 t ha^{-1} biochar with or without inorganic fertilisation), three soil samples (with an area of 25 x 25 cm and a depth of 28 cm) were taken from each replicate plot at regular intervals and earthworms were hand-sorted from these in the field. The number of earthworms collected was recorded in the field and the individuals were stored in 3.7% formaldehyde solution for 1.5 months, after which they were transferred to 85% ethanol solution, weighed and their species identified according to Timm (1999). As the density of deep burrowing *Lumbricus terrestris* L. was probably low at the site due to the frequent ploughing, no chemical extraction of the earthworms was included.

The earthworm samples were taken from two layers (at 0–15 cm and 15–28 cm depths) and the sampling was completed within a 10-day period. Although the soil was rather wet during the sampling, the weather conditions were generally suitable for earthworm sampling: the volumetric soil moisture content was 28.1 ± 4.5% and the topsoil temperature was 15 ± 1.3 °C (mean ± standard deviation). The temperature and moisture content of soil were not significantly different between the biochar and fertiliser treatments.

3.4.3 Plants

In both field experiments (**II**; **III**), the plant stand density was measured by counting the number of plants in the representative 3 x 30 cm, 3 x 50 cm and 3 x 100 cm sowing row lengths of wheat, turnip rape and faba bean, respectively, at the leaf development growth stage (GS 12, as described by Meier 2001). Above-ground plant biomass (AGB) was sampled at three growth stages: before stem elongation (GS 29), at full flowering (GS 65) and at the end of ripening (GS 85–89). The AGB was sampled within a 2 m x 2 m area at one end of the plot by cutting at 2 cm above the soil surface from three randomly chosen 50 cm row lengths for turnip rape and faba bean (**II**), and from three 30 cm row lengths for wheat (**II**; **III**). After drying the plant samples at 60°C for 72 h, the dry weight was recorded. From the first two samplings, the total C and N contents were analysed by dry combustion after grinding (Table 3) and the N uptake was calculated by multiplying the AGB by the N content.

The last AGB sample (GS 85–89) was divided into the yield components by recording the number of plants in each sample and dividing the plants into vegetative mass and reproductive organs (siliques, pods or ears), which were counted. Next, the samples were threshed and the number and weight of seeds were recorded. At crop maturity, an area of 11.25 m^2 (1.5 m x 7.5 m) of the plots was harvested with a combine harvester and the seed yield was dried, cleaned and weighed before quality analyses (**II**; **III**; Table 3).

Table 3. Measurements and methods used in the experiments

	Variable	Method	Reference	Publication
Biochar	pH	Standard combination electrode, 1:5 (w/w) in water		**I–IV**
	Liming value	Reaction with 1 M HCl and titration with 0.1 M NaOH		**III; IV**
	Ash content	Dry combustion at 500 °C for 3 hours	Miller (1998)	**II–IV**
	VM content	Weight loss after heating at 910 ± 30 °C for 7 minutes	ASTM (2002)	**II–IV**
	Total elemental composition	Treating ash with 0.2 M HCl, ICP-OES	Miller (1998)	**I–IV**
	Total C and N content	Dumas dry combustion		**I–IV**
	SSA	N adsorption with a single point method		**I–IV**
				I–IV
Soil	pH	Standard combination electrode, 1:2.5 (w/w) in water	Vuorinen and Mäkitie (1955)	**I–IV**
	Soluble Ca, K, Mg, S	Acid ammonium acetate extraction (1:10 v:v), ICP-OES	Vuorinen and Mäkitie (1955)	**I–IV**
	Soluble P	Acid ammonium acetate extraction, colorimetry	Vuorinen and Mäkitie (1955)	**I–IV**
	Total C[a] and N content	Dumas dry combustion		**I–IV**
	NH_4^+-N and NO_3^--N content	2 M KCl extraction, colorimetry (I) or spectrophotometry (III; IV)		**I; III; IV**
	Moisture content	Time domain reflectrometry (TDR)		**II–IV**
	Particle size distribution	Pipette method	Elonen (1971)	**I–IV**
	Temperature	Platinum resistance (Pt100 probes)		**IV**
	WRC	Sandbox at matric suctions 3 and 6 kPa; pressure plate at 10, 50, 250 and 1500 kPa	Dane and Hopmans (2002)	**II; III**
Plant	Leaf area index (LAI)	SunScan SS1 ceptometer bar		**II; III**
	SPAD	SPAD-502 portable chlorophyll meter		**II; III**
	Total C and N content	Dumas dry combustion		**II; III**
Yield quality	1000 seed weight	Samples counted by a semi-automated counter, gravimetry		**II; III**
	Protein and starch content of wheat grains	Near infrared spectrophotometer		**II; III**
	Protein and oil content of turnip rape seeds	Near infrared spectrophotometer		**II**
	Total N content of faba bean seeds	Dumas dry combustion		**II**

[a] The soil C was assumed organic as the carbonate content in the soils was known to be negligible.

3.5 Statistical analyses

The data from the laboratory incubation experiment (**I**) was first analysed for the fertiliser effects on N availability with a three-way analysis of variance (ANOVA) with fertiliser type, biochar level, time and their interactions as fixed effects. Biochar effects at a given time within each fertiliser treatment were compared with *post-hoc* tests using the Tukey HSD multiple comparison procedure.

In the field experiments (**II**; **III**), the effects of biochar and fertiliser treatments and the interaction of these factors on the changes in soil chemical properties from the initial conditions prior to the experiments were tested with ANOVA, followed by *post-hoc* testing with Tukey HSD multiple pair-wise comparison. In the three-parcel field experiment on Stagnosol (**II**), the soil chemical properties were analysed over all three sub-experiments to increase the statistical power of the analyses, resulting in 3 sub-experiments x 4 replicates = 12 replicates, while the soil physical properties as well as plant morpho-physiological traits and yields were tested by crop (4 replicates) in each year.

The Umbrisol field experiment (**III**) had a highly variable initial soil C content, so the effects of treatments on soil physical properties and plant morpho-physiological traits were tested with two-way analysis of covariance (ANCOVA), with the initial C content of soil as the covariate. The adjusted least-square means were compared after Bonferroni correction in the *post-hoc* tests. In addition, Pearson correlation coefficients were estimated between biochar application rates, chemical properties of soil and parameters of wheat growth and yield (**III**).

The earthworm avoidance test data (**IV**) was used as Tally data and analysed by assuming the Bernoulli distribution with the probability of individual earthworms in avoiding biochar-amended soil = 0.5 (binomial test). Furthermore, the preference/avoidance percentage was calculated as proposed by Busch et al. (2011):

$$X_{\mathrm{avoid.}} = -100\,\frac{(n_c - n_t)}{\mathrm{n}} \qquad \text{Eq. 1.}$$

where X_{avoid}. is the avoidance in percent, n_{c} is the number of worms in the control soil (mean of all eight replicates), n_{t} is the number of worms in the test soil (as above), and n is the total number of earthworms. The statistical significance of the X_{avoid} was analysed with Fisher's least significant difference test.

The effects of biochar and fertiliser addition on the total density and biomass of earthworms under field conditions (**IV**) was first analysed for the two sampling depths (0–15 cm and 15–28 cm) and for the combined data (0–28 cm) with two-way ANCOVA, with the original soil C content as the covariate and comparing the adjusted least-square means. Next, a

Generalised Linear Mixed Model with the sampling depth as a correlated factor was used for detecting the interactions of sampling depth with the biochar and fertiliser treatments. All statistical analyses were carried out with software packages PASW/SPSS v.18.0–21.0 (SPSS Corp., Chicago, USA) at $p < 0.05$ level of significance.

4 RESULTS AND DISCUSSION

4.1 Biochar and N mineralisation dynamics

Increasing biochar application rate both with and without organic fertiliser addition was associated with increased N immobilisation in soil in the laboratory incubation (**I**) and in the field (**III**) during the first growing season. In the incubation, the net N mineralisation after biochar addition decreased with the increasing C:N ratio of the added fertiliser: the net N mineralisation was highest for MBM (C:N ratio 4.7), followed by CCM (C:N ratio 19.7) and it was the lowest for the unfertilised control (**I**). Considering that after two weeks from the beginning of the incubation, more than 97% of the mineral N pool in soil consisted of NO_3^-, the decreased net N mineralisation could be attributed either to gaseous N losses by denitrification or to N immobilisation to microbial biomass, as leaching loss and plant uptake could not have occurred in the 133-day incubation without plants (**I**). In earlier studies, denitrification was increased by biochar application to wet soils (water contents > 73% of WFPS; Yanai et al. 2007; Cayuela et al. 2013), whereas decreased denitrification was recorded in drier soil (64% WFPS; Yanai et al. 2007). In this experiment, the soil water content of 45% WFPS with the soil air-filled porosity above 31% support the conclusion that the reduced nitrate-N contents can be mainly contributed to the microbial N immobilisation rather than to denitrification (**I**).

The immobilisation of N in microbial biomass after biochar addition is consistent with the results from previous laboratory incubations (Deenik et al. 2010; Novak et al. 2010; Nelson et al. 2011; Bruun et al. 2012; Angst et al. 2013; Zheng et al. 2013; Nelissen et al. 2014). The effect has been attributed to a small portion (< 1% of total C, including dissolved organic C and carbonates) of wood biochars decomposing within the first months by microbial and abiotic oxidation (Hamer et al. 2004; Cheng et al. 2008; Jones et al. 2011; Singh et al. 2012), and to the high C:N ratio of biochars (Rajkovich et al. 2012).

Such a biochar-induced N immobilisation seems, however, transient, as the portion of C readily available for microbial assimilation is used up in a few months, and the turnover of microbial biomass starts releasing mineral N (Novak et al. 2010; Bruun et al. 2012). The decreasing reductions in the net N mineralisation from organic fertiliser after two months of incubation (**I**) are in concordance with this mechanism. Furthermore, the results from the two-year field experiment on the same soil provide further support for this conclusion, as the initial biochar-induced decrease in the NO_3^--N content of soil was followed by a significantly increased NO_3^--N content of the soil 1.5 years after the biochar application (**III**; Fig. 2). Moreover, the protein contents of wheat grains were higher in the 20 and 30 t ha^{-1} biochar treatments than in the control in the unfertilised treatments in 2012. Both the positive and negative effects of biochar on the mineral N content of soil were smaller in the field than in the laboratory incubation, mainly because of plant uptake of N in the field experiment.

The short-term N immobilisation by added biochar has beneficial effects to the environment, such as reduced leaching of mineral N from soil to the waterways (Güereña et al. 2013; Angst et al. 2013; Zheng et al. 2013) and decreased N_2O emissions from the soils (Yanai et al. 2007; Case et al. 2012; Angst et al. 2013). On the other hand, if N is immobilised during the growing season, negative effects on plant development and yield may follow (Garabet et al. 1998; Asai et al. 2009; Lentz and Ippolito 2012; Nelissen et al. 2014), especially when the soil has initially low (less than 25 g kg^{-1}) SOM content (Asai et al. 2009; Lentz and Ippolito 2012; Nelissen et al. 2014). Nevertheless, in numerous other studies on soils with higher SOM content (32–68 g kg^{-1}), the biochar-induced N immobilisation effect, whether or not initially present, did not significantly affect plant N uptake or grain yields (Steiner et al. 2007; Major et al. 2010; Vaccari et al. 2011; Güereña et al. 2013; Jones et al. 2012; **II**; **III**). The effects of biochar on the N mineralisation and immobilisation dynamics are specific to individual combinations of soil, fertiliser and biochar (Clough et al. 2013) and generalisations to other conditions should be made with caution. However, the temporary N availability problems to plants could possibly be reduced by applying biochar several months before the next growing season, e.g., in the autumn, allowing for the N immobilisation effect to pass before sowing crops in the spring (Novak et al. 2010; Bruun et al. 2012; Nelissen et al. 2014). An alternative solution would be providing additional N fertiliser to biochar treatments in the first growing season (Lentz and Ippolito 2012; Nelissen et al. 2014).

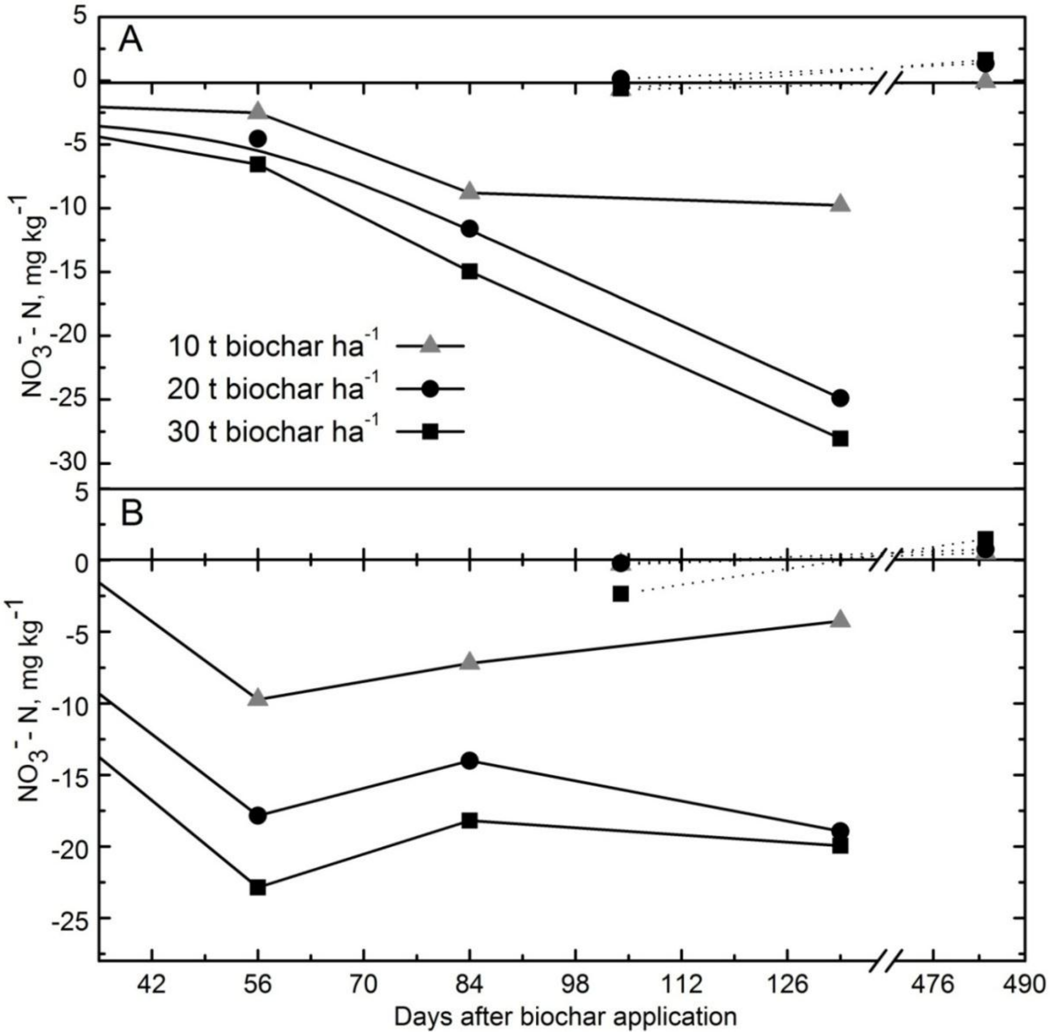

Figure 2. The temporal changes of biochar effects on the nitrate concentrations in soil with no fertiliser (A) and with MBM (B). In both panels the control (no biochar addition) is provided as the baseline (the x-axis at 0 mg NO_3^--N kg^{-1} soil). Solid lines: data from laboratory incubation (**I**); dotted lines: data from field experiment with plants (**III**). The mean values presented have been converted from volume base to mass base assuming a soil bulk density of 1.08 g cm^{-3}.

4.2 Effects of field-scale application of biochar on the chemical and physical properties of boreal mineral soils

4.2.1 Soil chemical properties

Adding biochar to two distinctive boreal soils increased the contents of C and exchangeable K in topsoil (0–20 cm), but had no significant effects on other soil chemical properties (electrical conductivity, pH, content of exchangeable Ca, P, Mg, S and total N) within the first 2–3 years (**II**; **III**). This is consistent with results from field experiments in non-weathered temperate soils (Jones et al. 2012; Liu et al. 2012) and may be partly attributed to the originally high SOM content (63–69 g kg^{-1}), neutral pH (**II**; **III**) and high initial levels of nutrients (**II**) in the soil. Moreover, the biochars used in our experiments contained low proportions of P, Mg and S and had relatively low liming values (**II–IV**). Thus it is likely that the previously reported biochar effects on neutralising acidity (Major et al. 2010; Van Zwieten et al. 2010b; Vaccari et al. 2011) and increasing nutrient retention via increased CEC of low SOM content (sub-) tropical soils (Liang et al. 2006; Major et al. 2010; Peng et al. 2011; Jien and Wang 2013) were not relevant. The increased K content of soil is attributable to the high amount of K applied with the biochar (Major et al. 2010; Quilliam et al. 2012; Xu et al. 2013) rather than to any long-term shifts in soil nutrient dynamics, such as that due to increased soil CEC reported for (sub-) tropical soils (Liang et al. 2006; Major et al. 2010; Peng et al. 2011).

Even though the Umbrisol was deficient in both exchangeable K and Ca (**III**), and biochar added comparable amounts of these elements, no significant effect on soil exchangeable Ca content was found. As the soil contained originally 20 times more exchangeable Ca than K, the biochar-added K may have displaced some of the exchangeable Ca from soil particle surfaces. In agreement with this, Jones et al. (2012) reported only 30% loss in exchangeable Ca compared to 90% loss of the exchangeable K of the biochar particles recovered from a fertile sandy clay loam after a 3-year experiment in Wales.

Addition of 10 (**II**) or 20 t biochar ha^{-1} (**III**; corresponding to 8.8 or 17.6 t C ha^{-1}) was enough to cause a detectable increase in the C content of the soil above background variation. In the Stagnosol field, the effect in the 0–20 cm layer persisted for all three years, while in the Umbrisol field the increase was significant only in the first year. Considering the difference from the control, all the biochar-applied C in the Stagnosol was detected in the topsoil samples (at 0–20 cm depth) after the first growing season (**II**), while in the subsequent years and throughout the Umbrisol experiment (**III**), the C recovery was much smaller. The C recovery was only 70% in the 10 t biochar ha^{-1} treatment in the Stagnosol in 2012. Similarly, in the Umbrisol, only 18% of the added C was detected in the 0–20 cm soil layer at the highest biochar application rate (30 t ha^{-1}) two years after the application (**III**).

The reduced recovery of C of these fine biochars (88–94% smaller than 5 mm) is consistent with previous studies (Jones et al. 2012; Petter et al. 2012; Felber et al. 2014) and can mainly be attributed to downward movement of biochar by tilling, earthworm activity and leaching. The differences in the downward movement of biochar in the soil profile probably also explain the higher loss in C in the topsoil of the coarse-textured Umbrisol in comparison with the finer-textured Stagnosol. The contribution of the breakdown of biochar to the C loss is likely to be rather low, as the biochars with VM contents similar to ours (122–268 g kg^{-1}) have been estimated to lose less than 10% of their C in 100 years (Zimmermann 2010). This estimate is given by a model based on laboratory incubation data. Similarly, wind erosion during the fallow period probably played only a minor role, as the soils were frozen and under snow cover during the winter, and were wet, when unfrozen in the autumn and spring. Nevertheless, if biochar application to soils becomes more widespread in the future, the translocation of dissolved charcoal to marine systems could increase, affecting there microbial dynamics (Jaffé et al. 2013).

4.2.2 Soil physical properties

In the Umbrisol field (**III**), the 30 t ha^{-1} application of biochar increased the porosity and decreased the bulk density of the loamy sand topsoil (at 2.5–7.5 cm depth) in the second growing season, but these effects were not detected in the first year, or in the Stagnosol field (**II**). The effects of biochar on soil physical structure are likely to be dose-dependent, as the application of 10 t biochar ha^{-1} did not affect significantly the WRC in the topsoil of the Stagnosol (**II**) or the Umbrisol (**III**), while the 30 t ha^{-1} rate was enough to increase the water retention at 6 kPa matric suction and the AWC after the first growing season (**III**). Such a dose-dependent effect of biochar on WRC, being more pronounced in soil macropores than in micropores (no significant changes in soil water content at 1500 kPa potential) is concordant with previous studies with undisturbed soil samples from field (Eastman 2011; Liu et al. 2012). No significant changes in WRC and AWC (difference between the soil water contents at 33 kPa and 1500 kPa potentials) were reported with the application of 5 t biochar ha^{-1} (Eastman 2011). Similarly, the application of 20 t biochar ha^{-1} had no effects on the WRC of a Columbian clay soil (Major et al. 2012). On the other hand, the application of 25 t ha^{-1} (Eastman 2011) or 10 and 25 g biochar kg^{-1} soil (Abel et al. 2013; rates corresponding to 13 and 33 t ha^{-1} if applied to the top 12 cm layer of the Umbrisol) increased the AWC of those soils. This suggests that depending on soil and biochar properties, a certain minimum biochar application rate exists for the increase in soil porosity and in the water retention to become significant.

This may also be true for the soil moisture content, as the increasing effect of biochar application was seldom significant in the Stagnosol (**II**), and in the Umbrisol, the differences from the control were not significant ($p > 0.05$) at any depth in any of the time points (**III**). The significant ($p < 0.01$) increase in the moisture content of topsoil (0–18 cm) observed in the Stagnosol during the wet post-harvest period in 2011 (**II**) is in agreement with the previously reported effects of biochar on WRC caused by changes in macropores rather than micropores (Busscher et al. 2010; Eastman 2011; Liu et al. 2012; Abel et al. 2013; Hardie et al. 2013).

In the Umbrisol field, the soil moisture content down to a depth of 28 cm was decreased by the application of inorganic fertiliser, possibly because the increased plant AGB was accompanied by more intense root growth and higher transpiration than in the unfertilised control. Likewise, increased root biomass (Kammann et al. 2011; Carter et al. 2013) and incremented AM colonisation (Blackwell et al. 2010; Solaiman et al. 2010) may have reduced water content in the biochar-amended soil via higher transpiration than in the control. However, as neither root growth nor AM colonisation were measured in the present studies (**II**; **III**), their contributions as mechanisms affecting the soil water content remains open.

Furthermore, the initial SOM content seems to play a key role in determining the effects of biochar on soil water retention. Earlier, significantly increased water retention and AWC of laboratory-packed soil columns were reported in soils with low SOM contents (1–15 g kg^{-1}), whereas no significant effect was found when biochar was applied into soil with a high SOM content (91 g kg^{-1}; Abel et al. 2013). Similarly, the application of 10 or 20 t biochar ha^{-1} together with compost to a loamy sand with a low SOM content (16 g kg^{-1}) significantly improved the soil water retention compared to compost addition alone (Liu et al. 2012). It is therefore plausible that the application of biochar to soils with SOM contents lower than the range of 63–69 g kg^{-1} in the present studies would have greater effects on soil moisture content and water retention.

4.3 Response of earthworms to added biochar in soil

4.3.1 Avoidance test

In the avoidance test, the distribution of *A. caliginosa* individuals did not differ between the soil treated with 16 g kg^{-1} biochar and the control treatment after 2 days incubation, but after 14 days, earthworms avoided the biochar-side of vessels ($p = 0.033$; **IV**). According to the preference/avoidance value (X_{avoid}), the avoidance effect was, however, not significant ($p = 0.174$). These results are in agreement with no significant effect on the avoidance of 10 g biochar kg^{-1} by *Eisenia fetida* in a 2-day avoidance test (Li D. et al. 2011). However, when Li D. et al. (2011) increased the biochar application to 100 g kg^{-1}, a significant avoidance effect was observed unless the biochar was previously wetted to its field capacity. This suggests that biochar-induced desiccation was the probable cause for the avoidance reaction.

The avoidance reaction of earthworms observed in present study was most likely related to the increased water retention caused by the biochar in the packed soil columns. Biochar induced a greater decline in the matric potential of the soil, the matric potentials in the biochar-treated soil being 0.2–0.5 kPa lower than in the control soil during the 11-day follow-up arrangement with tensiometers. In wet soils (i.e. matric potential higher than –5 kPa), the sensitivity of the response to changes in soil matric potentials varies between earthworm species and soils (Kretzschmar and Bruchou 1991; Doube and Styan 1996; Holmstrup 2001). Considering that Holmstrup (2001) reported lower growth of *A. caliginosa* at –6 kPa than at –2 kPa in loamy sand (7% clay), a related negative effect could play a role in our low clay content (2%) soil.

The increased water retention may have caused the biochar avoidance effect of earthworms because of desiccating earthworms (Li D. et al. 2011) and increased soil strength (Chan and Barchia 2007). The increased soil strength might have been caused by the packing of the biochar-soil mixture into the same volume as the control soil and thus possibly causing the fine biochar particles to fill some of the large pores and decrease the soil porosity. This may have led to increasing contacts between particles, increased amount of small pores, and the degree of water saturation in the soil. When both the degree of water saturation and water suction in soil increase, the effective stress between soil particles determining the soil strength also increases (Fredlund et al. 1995; Baumgartl and Köck 2004). However, as neither the increased soil strength nor the desiccation of earthworms was directly measured in our study, future work is needed for exploring the contributions of the mechanisms causing the earthworm avoidance effect.

The importance of other mechanisms explaining the avoidance, such as biochar–induced increase in soil pH (Van Zwieten et al. 2010a; Busch et al. 2011), heavy metal content and PAH content was presumably rather low, as the biochar had a low liming equivalence and the pH of soil was not much changed during the experiment. The contents of heavy metals and PAHs were similarly low in the biochar (less than the legislative limits for soil in Finland; **IV**). Nevertheless, other undetermined factors (e.g. biochar-contained volatile organic compounds; Spokas et al. 2011) may have been effective in the experiment, and more work is needed to elucidate the mechanisms affecting biochar avoidance of earthworms.

4.3.2 Field experiment

In the loamy sand Umbrisol, a significantly higher density and biomass of earthworms was found in the topsoil (0–15 cm) compared to the underlying soil layer (15–28 cm), most likely because of the higher SOM content in topsoil supporting the activity of endogeic *A. caliginosa,* the dominant species in this field *(***IV**). Biochar and fertiliser treatments did not affect significantly the earthworm density and biomass. A trend of higher values in the biochar treatments over the control was, however, present. For instance, in the 0–28 cm soil layer the density of earthworms was +112% higher compared to the control ($p = 0.077$). Such an effect is consistent with one of the two previous field studies available: repeated sampling during a two-year period following the application of 3.9 t ha^{-1} hardwood biochar to a temperate clay loam in Canada revealed the increased abundance of earthworms (Husk and Major 2010). In the other previous field experiment, there were no notable differences in the earthworm density between the biochar and control treatments after the application of 22.5 t biochar ha^{-1} to a silt loam in Minnesota, USA (Weyers and Spokas 2011). The comparison of the present results from the Umbrisol field, with its loamy sand topsoil, with those from previous studies is, however, complicated by the fact that the earlier studies were unreplicated and the statistical significance of the treatment differences remains in doubt (Husk and Major 2010; Weyers and Spokas 2011).

Biochar may affect the earthworm density by changing the physicochemical properties of soil and by increasing microbial biomass and microbial metabolites. Biochar particles found in earthworm guts (Topoliantz and Ponge 2003; 2005), have been suggested to assist the assimilation of other resources by increasing gut pH (Weyers and Spokas 2011), and enhancing gut microbial communities favouring the production of digestive enzymes (Topoliantz and Ponge 2003). In future studies, the earthworm sampling in the field should be coupled with the data on soil matric potential and microbial biomass, in order to reveal the mechanisms behind the changes in the earthworm populations.

4.4 Impacts of biochar on the growth dynamics and yield formation of crops

4.4.1 Growth dynamics of crops

Biochar treatment decreased the SPAD values of wheat and the N content of wheat and turnip rape AGB in the first growing season in the Stagnosol field, probably because of initially reduced N availability (**II**). Apart from that, biochar application had no significant effects on the biomass growth dynamics or N uptake of non-leguminous crops in the field experiments irrespective of fertiliser treatments (**II**; **III**). The latter is in contrast with previous studies where biochar increased crop N uptake in (sub-) tropical

(Steiner et al. 2008; Major et al. 2010) and temperate soils (Jones et al. 2012) and suggests that there was no change in the N use efficiency of turnip rape and wheat in these boreal soils. Similarly, no change in N uptake by maize, but reduced N leaching through N retention to microbial biomass, was reported in a four-year field experiment in a temperate climate (Güereña et al. 2013). Decrease in the plant N uptake follows only when the N immobilisation is severe enough such as that reported for maize in alkaline (pH 7.6) soil by Lentz and Ippolito (2012).

The temporarily decreased N availability to wheat and turnip rape in the Stagnosol field can most likely be attributed to the N immobilisation in microbial biomass (see section 4.1), following the application of biochar with a relatively high VM content (268 g kg^{-1}) and C:N ratio (142:1). In contrast, no discernible signs of lower N availability to wheat were seen during the first two growing seasons in the Umbrisol field (**III**) after the application of biochar with a lower VM content (122 g kg^{-1}) but higher C:N ratio (251:1) than that added to the Stagnosol field. It can thus be that under boreal conditions, the N immobilisation potential by different wood-based biochars increases with increasing VM contents in the added biochar. The C:N ratio of biochars may be less important in predicting the potential N immobilisation effect, as most of the C and N in the biochars are stable against decomposition. Nonetheless, the N immobilisation by added biochar in the Stagnosol experiment was certainly not severe, as the AGB and LAI were not affected during the first experimental year (2010), and no significant effects on the N content of AGB were found during the subsequent growing seasons (2011 and 2012; **II**).

Simultaneously with the N immobilisation by added biochar in the first growing season, a significant increase in the AGB and a tendency of increased N uptake was found at the flowering time of faba bean (**II**). This may have been associated with the reduced N availability in soil increasing the abundance of N-fixing bacteria (Rondon et al. 2007; Anderson et al. 2011) and causing increased biological N fixation.

4.4.2 Yield formation

In the Stagnosol experiment, biochar application was associated with decreased plant density of faba bean and turnip rape in the first two growing seasons, and a similar tendency was noticed for wheat in the second year. The decrease took place between the leaf development and the seed ripening (GS 85) growth stages (**II**). Considering that no plant diseases were observed, the introduced chemicals, e.g. ethylene, either from biochar or from the microbial communities that develop after biochar is applied (Graber et al. 2010), remain possible explanations for the effect. The decreased growth of maize after application of macadamia (*Macadamia integrifolia* Maiden and Betche) nut shell biochar, containing amounts of VM (225 g kg^{-1}) comparable to our biochar, was attributed to the phytotoxic compounds of biochar volatiles (Deenik et al. 2010).

Almost always when the plant density decreased with biochar application in the Stagnosol experiment, the number of reproductive units (pods, siliques or ears) per plant (**II**) increased, demonstrating the well established ability of these crops to compensate one yield component with another (McGregor 1987; Whaley et al. 2000; López-Bellido et al. 2005). Since the topsoil moisture content was below or close to the permanent wilting point (PWP) during flowering in the dry years of 2010 and 2011 (**II**), the relief of water deficit by biochar may have contributed to this compensation. All three crop species used in the present studies are sensitive to water deficit during flowering and grain setting (Passioura 2004; Porter and Semenov 2005; Rajala et al. 2009; Fábián et al. 2011). The ability of biochar to slightly increase soil moisture content (**II**) and its association with increased root growth (Kammann et al. 2011; Carter et al. 2013), may have alleviated the moderate water deficit of plants. The reduced water deficit following biochar application has been reported to support the ear formation of wheat (Blackwell et al. 2010) and to increase the grain number per ear (Solaiman et al. 2010) in field experiments in Australia. Similarly, addition of 22 or 44 t ha^{-1} of biochar produced from orchard pruning biomass augmented the AWC of a sandy clay loam and subsequently increased the leaf water potential and the leaf stomatal conductance of grape (*Vitis vinifera* L.) during drought in central Italy (Baronti et al. 2014). However, it seems that when the heat stress and water deficit became severe (below PWP soil moisture contents during seed filling in 2010 and 2011 in the Stagnosol field), the ability of biochar to reduce stresses was no longer sufficient, limiting the potential for yield improvements (**II**).

The effects of biochar on reducing the plant water deficit have previously been associated with the improved colonisation of wheat roots by AM (Blackwell et al. 2010; Solaiman et al. 2010). The colonisation rate of roots by AM is negatively affected by high soil P content (Jensen and Jakobsen 1980; Ryan and Angus 2003). Thus, the relatively high P content of the Umbrisol may have reduced the AM colonisation of wheat roots, possibly explaining why no signs of reduced water deficit were observed in the Umbrisol (**III**). Nevertheless, a) the highest application rates of biochar in these studies accounted for only a minor fraction (0.6–4 g biochar kg^{-1} soil) of a typical depth of water extraction of the crops (Entz et al. 1992; Nielsen 1997) and b) the root growth, colonisation with AM and plant water status were not directly monitored in these experiments (**II**; **III**). Therefore, no conclusive evaluation of the relative importance of the alleviated water deficit by biochar and the compensation of the reduced plant number by the increased number of reproductive yield components can yet be made.

4.4.3 Interactions of biochar with fertilisers

The effects of biochar on the plant morpho-physiological traits and yields were only seldom significantly different between NPK fertilisation rates (**II**) or between the organic and inorganic fertilisers (**III**), as can be concluded from the predominantly nonsignificant interactions between biochar and fertiliser treatments during the field experiments (**II**; **III**). In the Umbrisol field, when 10 and 20 t ha^{-1} biochar was combined with MBM treatments, the N immobilisation initially increased over that found in combinations with inorganic fertiliser treatments (lower N content of the AGB at tillering in 2011; **III**). Apart from that, no significant differences in wheat growth and yield formation were present between the inorganic and organic fertiliser treatments (**III**). Thus it can be concluded that when biochar is used as a soil amendment in boreal conditions combined with low C:N ratio organic fertilisers (such as MBM) or with inorganic fertilisers, no negative effects on the yield formation of spring wheat should be expected at rates up to 30 t ha^{-1}. On the other hand, the lack of synergy effect of MBM and biochar in comparison to mineral fertiliser (**III**) suggests that the previously proposed mechanisms of increased nutrient retention capacity and sorption of allelopathic substances (Schulz and Glaser 2012) were not relevant under these conditions.

4.4.4 Crop yield and yield quality

Biochar application had no significant effects on the yields of wheat, turnip rape and faba bean in the fertile Stagnosol field in the first three years (**II**), or on the amount or quality of yields of wheat grown in the nutrient deficient Umbrisol field in the first two years (**III**). This contrasts with previous studies in (sub-) tropical soils, where biochar applications have caused yield improvements attributed to neutralised soil acidity (Major et al. 2010; Van Zwieten et al. 2010b; Vaccari et al. 2011), improved nutrient availability (Steiner et al. 2007; Kimetu et al. 2008; Asai et al. 2009; Blackwell et al. 2010; Major et al. 2010; Van Zwieten et al. 2010b) or relieved water deficit (Kimetu et al. 2008; Blackwell et al. 2010; Solaiman et al. 2010).

Similarly, in a recent literature review covering 57 field experiments on biochar across all continents, a significant mean yield increase of 9% was reported across all crop species tested, while the mean increase was as high as 30% for legumes and 11% for wheat (Liu et al. 2013). The increase was associated with the reduced acidity of soils as no significant yield improvement was reported in neutral soils (pH 6.5–7.5). Furthermore, the biochar increased the SOM content, supporting the retention of nutrients and water. The highest increase in crop productivity was found in sandy soils having a low SOC content (7 g kg^{-1} on average, corresponding to a SOM content of 14 g kg^{-1}; Liu et al. 2013).

In the present studies, slight improvements were observed in soil water status, SOC content and K content (**II**; **III**). These effects, together with the indications of increased activity of microbes and earthworms in soil (**II**; **III**; **IV**) were not sufficient to affect crop yields in these boreal soils with near neutral pH and high SOM content. This is consistent with the results from the few available field experiments with fertile, neutral pH (pH 6.4–7.4) soils in temperate (Güereña et al. 2013; Jones et al. 2012) and boreal (Karhu et al. 2011) climates.

Biochar addition seldom affected the quality of crops (**II**; **III**). In the Stagnosol field, biochar addition was associated with increased starch and lower protein content of wheat grains, and with increased oil and decreased protein content of turnip rape seeds over the control in the first two growing seasons (**II**). As the synthesis of starch (Ugalde and Jenner 1990; Altenbach et al. 2003; Li P. et al. 2011) and oil (Champolivier and Merrien 1995) are more sensitive to water deficit than protein synthesis, these effects may be taken as indications of the water deficit-alleviating effect of the biochar.

4.5 Future perspectives

Considering that the effect of biochar on crop yields has been reported to increase over time (Steiner et al. 2007; Major et al. 2010; Vaccari et al. 2011), possibly via long-term changes in soil fertility, there is a need for a long-term follow-up of the field experiments including observations of soil microbiology and macro-faunal communities. Furthermore, as the main constraints in the broad-scale implementation of the C sequestration by biochar application are economic by nature (Woolf et al. 2010; Liu et al. 2013), various options to increase the feasibility of the practice should be explored. For example, as already suggested by Schmidt (2012), benefits from biochar use could be cascaded when biochar is first used as a feed additive for cattle, after which it becomes a manure additive possibly decreasing gaseous N losses to the atmosphere, and finally it will be used as a soil amendment.

5 CONCLUSIONS

1. The initial decrease in the mineral N content of soil after biochar application is probably caused by immobilisation of N to microbial biomass. The N immobilisation potential of different wood-based biochars in boreal soils can be concluded to increase with increasing volatile matter contents of the added biochar. Nevertheless, the N immobilisation effect in soils with high initial organic matter content is considered only moderate and short-term. The application of biochar to fields together with organic fertilisers in the autumn could prevent leaching of N to waterways during the fallow period. When the short-term N immobilisation effect has ended in the next spring, the N availability to plants would no longer be reduced.
2. Biochar contributes exchangeable K to the soil. When softwood biochar is used as a soil amendment, part of the K fertilisation could thus be compensated. The negligible effects of biochar on soil pH may be attributed to the originally neutral soil pH and the low liming value of these softwood biochars. Considering the high total amounts of Ca, Mg, Fe, Mn and Zn in biochars, long-term field studies are needed for detecting the potential of biochar for use as a long-term slow-release fertiliser.
3. The recovery of the C applied with biochar in the topsoil may reduce after the first year, probably due to the downward movement of biochar by tilling, earthworm activity and leaching. The decrease in C recovery is greater in coarser than in finer-textured soils.
4. The effects of biochar on soil porosity and water retention characteristics are dose-dependent and more relevant for increasing the macroporosity than microporosity of soil.
5. The biochar avoidance effect by the earthworms observed after a two-week laboratory incubation was likely caused by reduced soil matric potential after biochar application. This points to the need to consider the impact of biochars on the water retention and strength of soils in future avoidance tests. Under field conditions, however, no signs of such avoidance were observed, as biochar addition did not affect the density and biomass of earthworms. Future earthworm studies in the field, combined with measurements of soil water potential and microbial biomass, are needed for exploring the underlying mechanisms for the trend of increased earthworm density and biomass by added biochar.
6. The initial N immobilisation after biochar addition may cause enhanced biological N fixation in legumes, as shown by improved accumulation of biomass and N uptake of faba bean.
7. The biochar application may increase the number of reproductive units per plant in faba bean, turnip rape and wheat. This can be

caused by the combination of relieved water deficit of plants with compensation for the decreased plant density. Future research exploring the effects of biochar in reducing water deficit of crops should include measurements of root growth and colonisation by arbuscular mycorrhiza, as the latter may play a key role in plant water and nutrient uptake.

8. The application of biochar together with meat bone meal or inorganic fertilisers to boreal soils with high initial SOM content and neutral pH should not be expected to affect significantly the yields of wheat, turnip rape and faba bean in the first few years after application. The fact that no evidence of biochar-facilitated negative effects on crop yields or on earthworms was found during these multi-year field experiments suggests that softwood biochar application is an environmentally and agriculturally safe option for sequestering C.
9. In order to follow the long-term changes in soil fertility, there is a need for a follow-up of the field experiments, including observations of soil microbiology and faunal communities.

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