Linking soil functional biodiversity and processes to soil ecosystem services: biochar application on two New Zealand pasture soils

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Abstract

Sheep and beef farming and dairying are an important part of the New Zealand economy, occupying about 40% of land area used for the livestock. Maintenance of that land is an essential part of sustainable agriculture. For a long time, biochar has been used and considered as a multifunctional soil amendment adding to the natural capital stocks of the soils and contributing to a wide range of soil ecosystem services, provision of nutrients (soil fertility) through the increasing nutrient availability, neutralising acidity through liming, and mitigating climate change through carbon (C) storage.

In this thesis I investigate the effects of biochar, made from willow at 350°C and added as an amendment, on soil ecology and biochemistry-based processes within an ecosystem services modelling framework. In the literature review (**Chapter 2**) I draw links between the importance of soil ecosystem services, including soil biodiversity and human needs. The potential role of biochar application in improving soil productivity and mitigating the negative impact of land management are also discussed.

To evaluate the impact of biochar, added as an amendment, on the chemical and biological properties and processes in soil as it influences soil processes underpinning ecosystem services, and to explore any synergistic interactions between biochar, soil, functional groups of soil fauna and plants, two experiments were conducted: (i) a six-month mesocosm experiment in the glasshouse and (ii) a field-based mesocosm experiment that ran for 12 months. In both experiments two contrasting soils were used – an Andosol (Allophanic) and a Cambisol (Brown). Both soils cover extensive areas of New Zealand. In the mesocosm experiment in the glasshouse (**Chapter 3**) biochar had a significant positive effect on clover growth and biomass, and this effect was more pronounced in the presence of earthworms and in one soil type. On their own, biochar and earthworms increased clover growth more in the Cambisol, while the positive synergistic effect of biochar and earthworms on soil biochemical processes and clover

growth was more evident in the Andosol The synergistic effect of biochar and earthworms was also observed in an increase in the abundance of Collembola and in soil fungal biomass.

The field mesocosm experiment investigated how adding biochar as an amendment to a grazed pasture affects the soils biological and physico-chemical properties. The experiment was conducted at four locations with different livestock systems (dairy and sheep) and soils (Andosol and Cambisol) under contrasting management practices (two pastures, with or without dairy shed effluent addition on the Andosol, and two pastures with either low or high phosphorus (P) fertilizer input in the Cambisol) over 12 months. The three treatments were: (i) willow biochar produced at 350 °C (1% w/w); (ii) lime, added at the liming equivalence of the biochar application (positive control); (iii) no amendments (negative control). Results of the field experiment are reported in three chapters. **Chapter 4** reports how adding biochar affected biological and physico-chemical properties and the plant root biomass at each of the four grazed pasture locations on Andosol and Cambisol. Biochar addition had a positive ($P<0.005$) effect on total nitrogen (N), organic C, Olsen P contents, bacterial (Cb) and fungal (Cf) C biomass, and Collembola abundance, compared with the control and lime treatments 12 months after addition. At all four locations, the increases in N, C and P in the biochar treatment were greater than the amount of N, C or P added in the biochar. On average, root biomass was 6.9 Mg ha^{-1} higher ($P<0.005$) in all four soils to which biochar was added, when compared with the other two than the other two treatments. Biochar addition also lowered ($P<0.005$) the bulk density of the soil, on average by 7% across the four sites, compared with the control. Earthworm abundance in lime-treated soils was higher ($P<0.01$) than in the negative control. In the presence of biochar, earthworm abundance was only higher ($P<0.05$) than the control in the Andosol without effluent. In biochar-amended soils,

Collembola abundance was higher ($P<0.005$) than the controls in all soils, while there was no effect on Oribatida and Gamasina populations.

Chapter 5 investigated the effect biochar addition had on the biochemical activity (soil enzymes) in the soils after 12-months. Dehydrogenase activity, which is strongly correlated with soil microbial biomass, was higher in the soils to which biochar had been added. Cellulase activity was also higher in the soil to which biochar had been added, reflecting the increased amounts of plant detritus entering the soil, from the greater root biomass following biochar application. When the geometric mean of all the enzyme activities was summed, biochar had a more pronounced effect than lime. An exception was peroxidase, which in contrast to dehydrogenase and cellulase, had higher activity in the soil treated with lime (positive control) and was positively correlated with earthworm abundance, which also was higher in the lime-treated soil. Biochar had less of an effect on both pH and earthworm abundance. There was a positive correlation between nitrate reductase and earthworm abundance, as earthworms increase nitrate concentration in soil.

In **Chapter 6** I attempted to assess the long-term impact of biochar on soil potential to provide ecosystem services and investigated the influence of the biochar application on the time dynamics of physicochemical and biological properties. Soil samples were collected at 6 and 12 months after the start of the field experiment. Except for mineral N (NO_3^--N and NH_4^+-N), the effect of sampling time was similar across sites. Biochar had a long-term positive effect on OC, TN and Olsen P in all sites. Reduced by biochar, soil acidity and BD remained at the same level after 6 and 12 months in all four sites. The effect of biochar on mineral N was not constant in time, and mostly depended on the soil order and management practices rather than on treatments. Soil biological and biochemical properties had patterns which can be interpreted as seasonal. Biochar increased bacterial and fungal biomass as well as abundance of arthropods and earthworms; these changes in soil biota were reflected in soil enzymatic activities. It was

shown that biochar has a persistent effect on soil natural capital stocks and functions and showed itself as an effective amendment able to enhance the soil over time.

In the **Chapter 7** the results of the analysis of the effects of biochar and lime addition on soil physicochemical and biological properties (**Chapter 4**) and enzymatic activity (**Chapter 5**) were used to semi-quantify the effects and potential benefits of biochar and lime amendments application for the delivery of specific soil ecosystem services. In comparison with the control treatments, there was a significant positive impact of biochar on soil properties, including soil microflora, earthworms, OC, soil BD, pH and overall soil enzyme activity, associated with C sequestration. In comparison with control and lime, biochar increased components of soil natural capital stocks responsible for food and fibre production ecosystem service. There was also significant positive impact of biochar on soil properties associated with fertility maintenance. Biochar and lime had similar positive effect on water regulation and disease and pest control services.

The thesis shows that application of willow wood biochar produced at low temperature has a significant positive effect on a number of the chemical and biological properties and processes in soils (up to 12 months) that extend to the rooting characteristics of the plant, and this might contribute to the productivity of pasture land, while increasing health and resilience to the impact of land management. Biochar, through its effect on soil properties contributes to dynamic interactions between soil, plant and functional groups of soil biota. As a result, biochar positively impacts on the dynamical links between components of soil natural capital and ecosystem services provided by the soil.

In summary, biochar produced from willow wood at low temperature may be an effective tool in the pasture systems/soils investigated here as a part of sustainable farming practices, which can increase plant productivity, improve soil physical properties and fertility, reduce disease and pest risks, and at the same time might be used as an instrument to mitigate climate change.

Preface

This dissertation is submitted for the degree of Doctor of Philosophy at the Massey University. I represent that my thesis is my original work. The research described herein was conducted under the supervision of Dr Maria Minor and Prof Marta Camps-Arbestain at the School of Agriculture and Environment, Massey University, Palmerston North, and Dr Alec Mackay at AgResearch, Grasslands Research Centre, Palmerston North. The thesis consists of eight chapters, which have been written in the form of journal articles; consequently, there is some repetition between the chapters. Some chapters have been published or are currently being submitted for publication. Part of this work was presented at the New Zealand Society of Soil Science Conference 2018 in Napier, NZ and at the 2020 New Zealand Agriculture Greenhouse Gas Inventory Workshop.

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I also would like to acknowledge the Massey University Doctoral Scholarship and the Ministry of Business, Innovation and Employment funded research project (C09X1613) *Soil health and resilience – a pathway to prosperity and well-being*, for funding this project. Many thanks to AgResearch at Palmerston North, and School of Agriculture and Environment, School of Food & Advanced Technology, and School of Fundamental Sciences at Massey University, for providing facilities for conducting my research. The research required to produce this thesis would not have been possible without the support, guidance, and help from many people: Dr Peter Bishop, Dr Roberto Calvelo Pereira, Dr James Hanly, Associate Professor David Horne, Ian Furkert, Paul Barrett, Bob Toes, Ross Wallace. Thanks Sharon Wright, Fiona Bardell, Denise Stewart, Sandra Dunkinson, Tara Penketh, Sherryl Curtis, and Ali Hagen for all administrative work.

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Table of Contents

List of Figures

List of Tables

Chapter 1

Introduction

1.1 Background

Sheep, beef and dairy farming is an important part of the New Zealand economy, thus in 2017 about 40% of land area was used for livestock (Journeaux et al., 2017). The maintenance of that lands is an essential part of sustainable agriculture. Dairy, beef and sheep farming receive the major amount of fertiliser in comparison with other sectors of agriculture - 91% of nitrogen and 93% of phosphorus (Fertiliser Association of New Zealand, 2019). The loss of carbon and nutrients due to failings in land management can cause soil degradation, freshwater pollution and climate change through increasing greenhouse (N_2O) emissions.

For quite a long time, biochar has been considered as a multifunctional soil amendment which provides a wide range of soil ecosystem services, such as improving soil fertility through the increasing amount of nutrients, liming acid soils, and mitigating climate change through C storage (Hardie et al., 2014; Lorenz & Lal, 2014). A significant number of publications, including NZ studies (Anderson et al., 2011; Wang et al., 2015a), show an effect of biochar on the soil microbial community and related biochemical properties. This effect can reflect the provision of available nutrients to microflora as well as changing the physical conditions of microbial habitats. In addition to the effect on microflora, biochar affects soil fauna (Lehmann et al., 2011; Hale et al., 2013). The interactions between biochar and earthworms were most extensively summarised by Conti et al. (2015), but there is scarce information on interactions with soil arthropods. Both of these groups of soil organisms have influence on biochemical processes,

including organic matter decomposition and effect on soil physical properties. This is why it is important to understand the effect that biochar can have on these soil organisms.

Considering the soil as a key provider of ecosystem services, it should be taken into account that besides providing agricultural products, pastures and crop fields provide regulatory ecosystem services, and so the total economic value of provided services should include all constituent parts. It is essential to recognise the effects of biochar amendment on the soil as a complex ecological and economic system which provides crop production, C storage, biochemical function and habitat for soil organisms.

My work focuses on investigating the influence of biochar on physicochemical properties, functional biodiversity and biogeochemical processes in pasture soil, in relation to soil ecosystem services.

1.2 Thesis objectives

- To characterize soil biochemistry and functional biodiversity associated with different amendments, including biochar made from willow at 350°;
- To explore synergistic interactions between biochar (produced from willow at low temperature), soil, plants, functional groups of soil fauna, and the rates of soil processes
- To quantify and value the contribution of these processes to the provision of soil ecosystem services using the existing framework

The experiments quantify changes in soil biochemical processes following application of biochar made from willow at 350°. The results can form the basis for a better understanding of the net effects that soil functional diversity and organic amendments have on the soil ecosystem services. Findings can be used in NZ agricultural systems, helping land managers to better address emerging environmental and productivity issues.

1.3 Thesis structure

This thesis consists of eight chapters.

Chapter 1 gives a brief introduction to the potential benefits of biochar application for soil ecosystem services in the context of New Zealand pastures. This chapter also includes the objectives of this PhD thesis.

Chapter 2 provides detailed synopsis of the available information regarding soil ecosystem services and its components, soil biodiversity, and applying biochar as a part of sustainable agriculture.

Chapter 3 presents the results of 6-month glasshouse experiment conducted with two contrasting New Zealand soils (Andosol and Cambisol) with three treatments (control, biochar, and lime as positive control) in the presence or absence of earthworms. The values of OC, TN, pH, mineral nitrogen, soil biological properties (bacterial and fungal biomass C, Collembola abundance, plant above- and below-ground biomass) and activity of nitrate reductase have been compared between treatments. It was shown that biochar and earthworms had significantly increased plant growth rate and had positive impact on soil properties; in some cases there was synergetic interaction between biochar and earthworms.

Chapter 4 presents the result of the 12-month field-based mesocosm experiment conducted on the two contrasted New Zealand soils (Andosol and Cambisol), each soil with two pastures under two different management regimes, and with three treatments (control, biochar, and lime as positive control). The measurement of soil physicochemical properties (OC, TN, pH, BD, Olsen P, mineral nitrogen) and soil biological properties (bacterial and fungal biomass C, arthropod and earthworm abundance, root biomass) have been compared between treatments. The results provide evidence that adding biochar to the soil can positively affect soil food web and soil structure.

Chapter 5 presents the data on soil enzymatic activities in the 12-month field-based mesocosm experiment described in **Chapter 4**. The activity values of cellulase, peroxidase, dehydrogenase, urease, nitrate reductase and acid/alkaline phosphatases activities have been compared. The results show that biochar and lime had various effects on soil enzymatic activities, which indicates different mechanisms of their interaction with soil biological processes.

Chapter 6 presents results of the time dynamics study, included in the 12-month field-based mesocosm experiment described in **Chapter 4**. The dynamics of soil physicochemical properties, biological properties and enzymatic activities measured after 6 (autumn) and 12 months (spring) of the field trial have been compared. It was shown that biochar had long-term stable impact on soil properties.

Chapter 7 presents an evaluation of potential benefits of biochar application for soil ecosystem services, including provisioning, supporting and regulating services, based on experimental results. It was shown that biochar could be considered as a forward-looking, innovative amendment which can increase plant productivity, reduce disease and pest risks, improve soil physical properties, and at the same time might be used as an instrument to mitigate climate change.

Chapter 8 is the overall summary of outcomes of the experiments conducted during the study, and the conclusions based on obtained results. Future research directions are also suggested.

Chapter 2

Literature review

2.1 Soil ecosystem services

Soil is one of the fundamental components of life on Earth. Being a source of ecosystem services (ES), the soil creates conditions to support living organisms, implements geochemical processes, and regulates atmospheric composition (Dominati et al., 2010; Braat & de Groot, 2012; Dominati et al., 2014b), furthermore, soil serves as a carbon storage pool (Crowther et al., 2016; Zhu et al., 2016). According to the literature reviewed by Dominati at al. (2010), the soil ecosystem service framework is identified by several roles: fertility role, filter and reservoir role, structural role, climate regulation role, biodiversity conservation role, and resource role (**Fig. 2.1**).

While previously the attitude towards the soil was in a context of needs (structural support, resource base, etc), the modern approach to soil management is striving to include all environmental and economic aspects (Costanza, 1993; Braat & de Groot, 2012).

Existing frameworks aim at evaluation of contributions of soil properties (as a natural capital stock provider) to the ability of soil to implement ecosystem services. This evaluation can be used to understand which and how soil properties can be managed, and what consequences would follow, which in turn is essential when assessing the economic value of a specific agricultural land use.

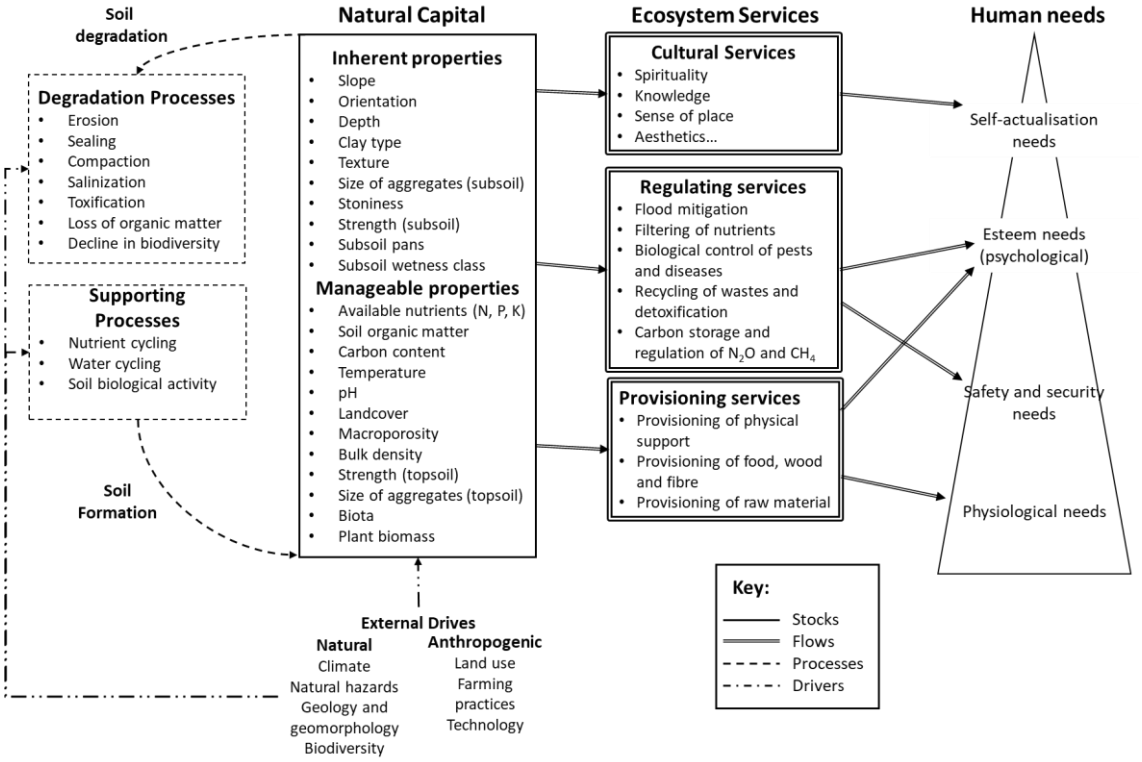


Figure 2.1 Conceptual framework linking soil natural capital, soil processes, the provision of ecosystem services and human needs (Dominati et al., 2010). Reproduced with the kind permission of the authors.

For the comprehensive assessment of soil as the natural capital, the frameworks should fulfil such terms as characterising soil properties, identifying soil formation, maintenance and degradation processes, as well as drivers of these processes (both natural and anthropogenic). Also, frameworks should include a review of soil as a source of provisioning, regulating and cultural services. The last condition is an application of mentioned above terms in the context of human needs (**Fig. 2.1**).

When addressing the ecosystems services provided by the soil, inherent and manageable soil properties (excluding cultural) can be used (Dominati et al., 2014b). Thus, soil physical properties could point to the ability of soil to provide regulating and provisioning services such as flood mitigation and physical support, respectively. Carbon and nitrogen content, in turn, indicates the capability of soil to provide food and raw

materials as provisioning services, and carbon storage and regulation of N_2O and CH_4 emission as regulating services.

2.2 Soil biodiversity

Soil is the habitat for many different organisms, from microorganisms (Christensen et al., 1999) to large earthworms (*Oligochaeta*), leaving aside plants. The biodiversity of soil organisms provides the continuously effective functioning of the soil (Wall, 2012) through the turnover of nutrients (primarily carbon and nitrogen) and maintaining soil physical properties. Soil organisms consume organic matter (plant litter, dead bodies of other organisms) and excrete enzymes, therefore changing chemical and physical characteristics of the organic matter and impacting on its components' availability. Arbuscular mycorrhizal fungi can influence soil aggregate stability by physical clutching of solid particles, by gluing the particles with extracellular secretions, and by stimulating the growth of bacteria, which in turn secrete adhesive polysaccharides (Boivin & Kohler-Milleret, 2011). Soil organisms differ in species diversity as well as functionally, which makes it important to study individual groups of soil organisms with respect to soil ecosystem services and human impact on the environment. The ability of the soil to continuously sustain the biogeochemical processes is supported by functional biodiversity (Khaziev, 2011; Pascual et al., 2015).

2.2.1 Soil microorganisms

The role of microorganisms in the soil cannot be overstated. Microbial community is an inherent component of a soil living system and is involved in all aspects of soil functional system.

The structure of soil microbial community can temporarily change due to season, temperature and moisture fluctuations, and other factors, but common characteristics are always inherent to the specific soil type (Kaiser et al., 2016; Siles et al., 2016). Significant and long-term changes such as environmental (climate) and anthropogenic (agricultural

and waste pollution) will influence diversity and density of soil microbial community. It is essential to understand the effect of managed (anthropogenic) and unmanaged (environmental) impacts on the structure of soil microbial community (Drenovsky et al., 2010; Zhang et al., 2016), as this effect can be positive or negative in regard to soil functions and ecosystem services – including carbon and nitrogen turnover and storage, or decomposition (Balser et al., 2010; Gunina et al., 2017).

An increase in temperature can stimulate microbial community (Schindlbacher et al., 2011); excess moisture and drying can influence the ratio of aerobic and anaerobic microorganisms as well as fungi:bacteria ratio. Both temperature and water regimes, which change following climate fluctuation and anthropogenic impact, can affect density and diversity of soil microorganisms (Castro et al., 2010; Zhang et al., 2016).

Agricultural soil practices affect the soil microbial community through a number of physical and chemical changes (Ross et al., 1995; Busari et al., 2015). Tillage – mechanical treatment to improve agricultural quality of soil – destroys soil structure and impacts on microorganisms by changing their environment (Mathew et al., 2012). In addition to soil structure deterioration and carbon losses (Haddaway et al., 2016), tillage can decrease microbial diversity with the subsequent increase of plant pathogenic organisms (Almeida et al., 2001; van Elsas et al., 2002). Grassland management without annual tillage also has a significant effect on diversity and spatial distribution of microorganisms (Clegg et al., 2003; Sayer et al., 2013). Agriculture involves the use of fertiliser (either organic or inorganic), which in turn has an enormous effect on soil microbial community, this effect can be different depending on the type and application rate of fertiliser and soil properties (Treonis et al., 2010; Pan et al., 2014).

Soil microorganisms' density and diversity can be used as indicators of land use and climate change impact on soil functions and provision of ecosystem services.

2.2.2 Soil Fauna

Soil fauna contribute to a wide range of services and processes in soils, such as water infiltration, organic matter incorporation and storage, and nutrient supply to plants. Soil arthropods are one of the most abundant groups of soil invertebrates and have a significant influence on soil biocenosis. Soil arthropods range in size from 200 μm to over 15 cm (Wallwork, 1970) and participate in soil processes at all levels. Arthropods that inhabit the soil contribute to soil physical and chemical properties by litter fragmentation and nutrient mineralisation, as well as through mixing the soil and developing pores (Culliney, 2013). Arthropods feed on living plant parts, plant and animal residues, faecal matter, soil microorganisms, or they hunt for other invertebrates (Culliney, 2013). Due to their wide diversity and varying feeding ecology, soil arthropods participate in nutrient cycling and regulate soil biodiversity (e.g., through predation). Moreover, many soil arthropods engineer their habitat, which increases porosity and improves water infiltration in the soil (Bagyaraj et al., 2016).

Soil arthropods inhabit the top horizons of forest and grassland soils, rich in organic matter (Wallwork, 1970). However, many are also found in agricultural soils (pastures and crop fields) (Hadjicharalampous et al., 2002), where they play an important role in maintaining soil fertility (Culliney, 2013; Bagyaraj et al., 2016). Due to high variability and diversity of soil arthropods, their community structure can be used as an indicator of soil quality, and on an equal basis with the microbial community, it can signal about chemical or physical disturbances to the soil (Blair et al., 1996; Stork & Eggleton, 2009).

Earthworms are imperative regulators of soil processes (Haimi & Huhta, 1990; Derouard et al., 1997; Bernard et al., 2012). Through their extensive burrowing, earthworms change physicochemical properties of soil and break down plant residues (Mackay & Kladivko, 1985), creating a range of favourable environments for different groups of soil organisms (Scheu, 2003; Migge-Kleian et al., 2006; Mudrák et al., 2012),

as well as influencing bioavailability of vital and trace elements, such as P, Fe, Mn, Zn, and Cu (McColl et al., 1982; Parfitt et al., 2005; Bityutskii & Kaidun, 2008; Sizmur & Hodson, 2009; Vos et al., 2014). Earthworms pass litter and soil through their gut, digest available nutrients, and excrete casts replete with enzymes and microorganisms, which continue organic matter transformation (Bernard et al., 2012). Also, earthworms dig and mix soil layers, changing soil physical properties (McColl et al., 1982). This makes earthworms one of the major components of the soil biological community – they are ecosystem engineers, able to drive chemical processes in a particular direction.

Earthworms are divided into three major ecological groups: epigeic, dwelling in top organic horizons; endogeic, living in the upper mineral horizons; and anecic, which inhabits deeper horizons but feed in litter horizons (Bouche, 1977). Each group performs different functions in the soil: epigeic earthworms process fresh litter without translocating it, endogeic improve the structure of mineral horizons, and anecic mix organic and mineral horizons (Bouche, 1977). On par with microorganisms and arthropods, earthworms are a key part of the soil ecosystem, and can be used as bio-indicators of soil quality, responding to physicochemical, biochemical and biological proportions (Fründ et al., 2010; Kim et al., 2014). Agricultural practices frequently act as a decisive factor of earthworm abundance. Application of mineral and organic amendments can contribute to either the growth or decline of earthworm populations (Mainoo et al., 2008).

2.3 Biogeochemical processes

Major soil ecosystem services are provided through biogeochemical processes, which cycle mineral and organic components. On this basis, it is essential to understand the role of soil biota as one of the main drivers of these transformations (Subke et al., 2012; Ho et al., 2016). It is especially important in the context of the economic value of soil

ecosystem services (Dominati et al., 2014b), as most frequently the soil is seen as the provider of resources.

Decomposition of plant residues is the first stage of organic matter transformation processes. The significance of these processes arises from the crucial function of removing dead plant material and consequential effect on nutrient availability, plant productivity, and C sequestration (Berg & McClaugherty, 2014) as well as further nutrients included in the soil food web (**Fig. 2.2**).

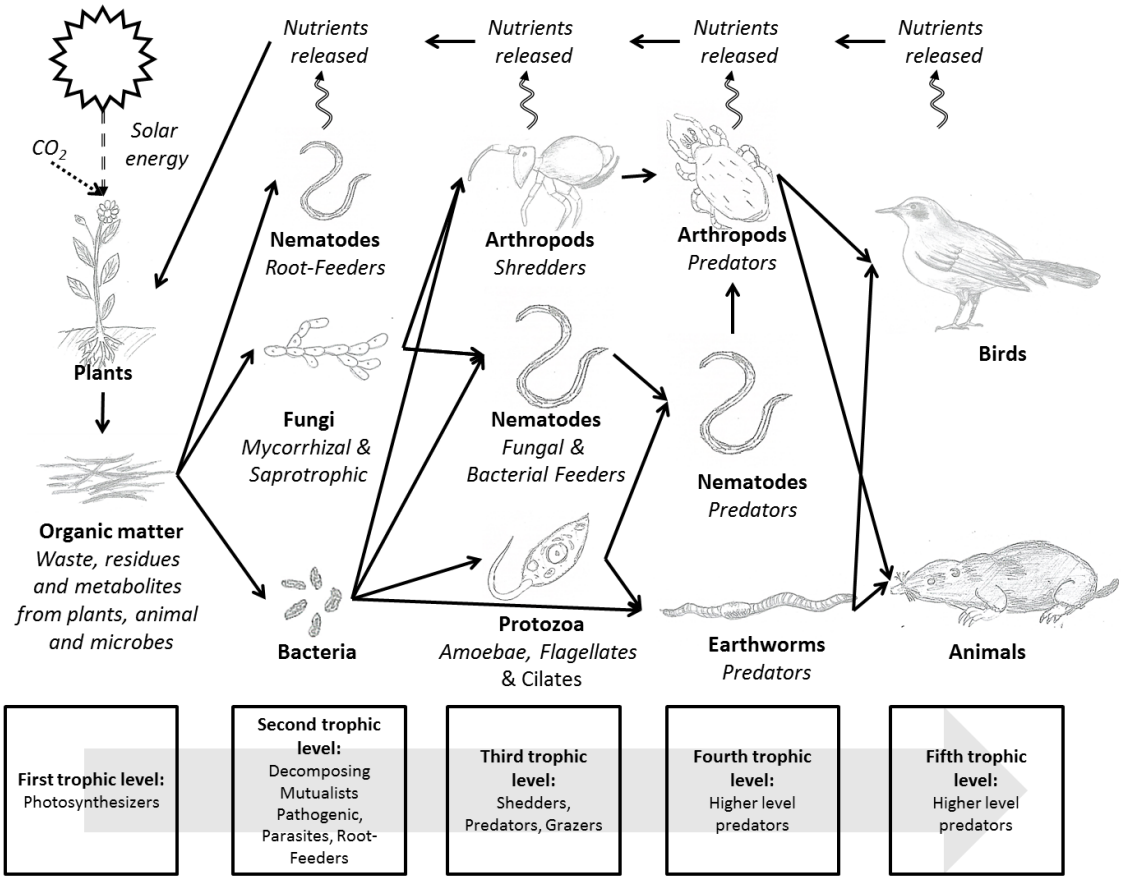


Figure 2.2 Explanatory model of soil food web, showing relationship (arrows) between plants, microorganisms, micro-, meso- and macro-fauna and energy flow. Author's modification of diagram from Wikimedia Commons.

In addition to decomposition, denitrification, a process that causes significant gaseous losses of nitrogen, combines a chain of reactions with molecular nitrogen as a final product (**Fig. 2.3**) (Hofstra & Bouwman, 2005), and influences the amount of available

nitrogen in the soil. Denitrification also drives the emission of N_2O – a potent greenhouse gas. Denitrification is linked to the decomposition process, as nitrate is used as an electron acceptor during the oxidation of organic matter in the absence of oxygen (Martens, 2005).

Both decomposition and denitrification are critical processes in the soil which affect the role of soil as the provider of ecosystem services. Farm lands are most exposed to changes in decomposition and denitrification, which can impact on soil fertility (Burges, 1967; Rheinbaben, 1990).

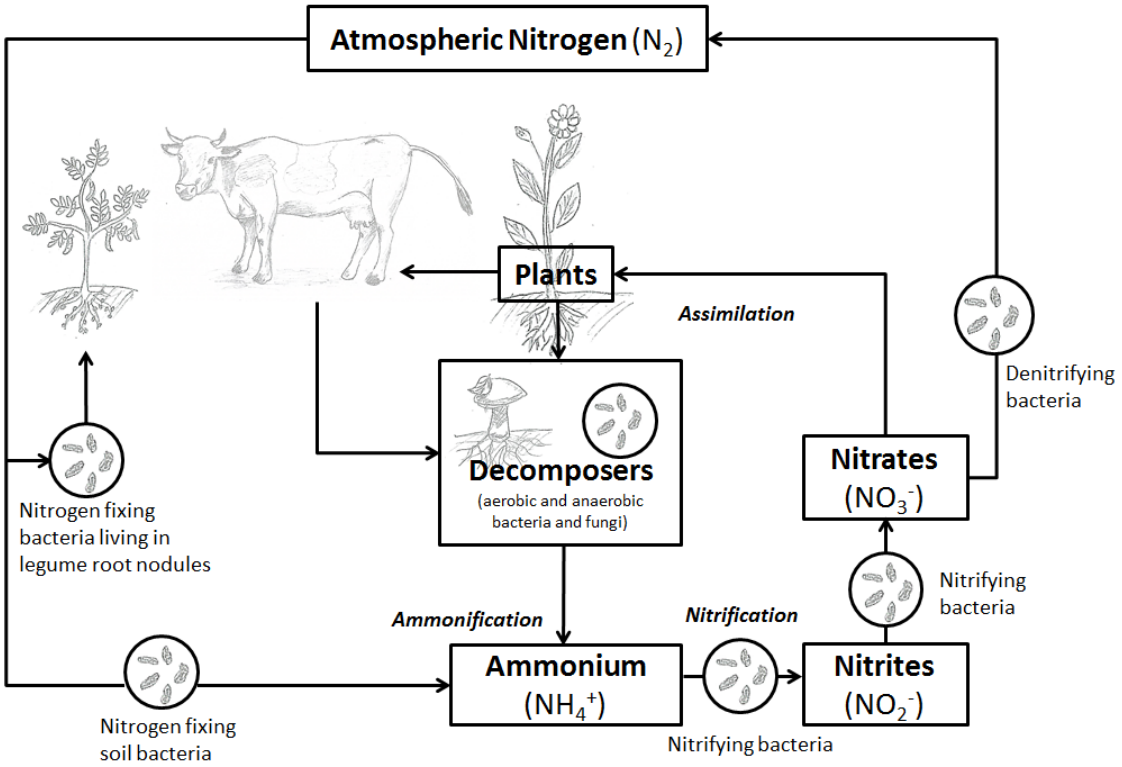


Figure 2.3 Explanatory model of the nitrogen cycle – the flow of nitrogen through the ecosystem. Author's modification of diagram from Wikimedia Commons.

2.3.1 Enzymes as promoters of biogeochemical processes and indicators of soil quality

Enzymes are an integral part of soil biosystems; as catalysts of chemical transformation in the soil, enzymes play a key role in decomposition processes and soil self-purification (Khaziev & Gul'ko, 1991; Sinsabaugh et al., 1994; Burns et al., 2013).

There are two sources of enzymes in the soil: enzymes excreted by organisms into the external environment – extracellular enzymes; and enzymes released after the death of soil organisms – endocellular enzymes (Sinsabaugh et al., 1994). Soil properties, such as pH, temperature, and chemical composition (presence or absence of some substrates) can influence the presence and activity of enzymes (Sinsabaugh et al., 1994). Soil enzymes are divided into two types: constitutive – which are permanently present in the soil and are not affected by addition of a substrate; and inducible – these are enzymes found in small amounts, but their synthesis rises in presence of a substrate (Das & Varma, 2011).

When released into the soil, some enzymes are bound to soil particles by immobilization on clay minerals and organic matter, whereas non-immobilised enzymes become more exposed to irreversible denaturation under adverse conditions (pH, temperature, etc.) (Skujins, 1978; Khaziev & Gul'ko, 1991; Burns et al., 2013).

The role of enzymes in maintaining soil functions is regularly emphasized in the literature (Dick, 1994; Khan et al., 2007; Shukla & Varma, 2011; Kalembasa & Symanowicz, 2012; Cardoso et al., 2013). The level of soil enzyme activity (increase or decrease in the activity of group of enzymes or individual enzymes) can influence specific soil parameters, such as soil fertility and biological activity (Karaca et al., 2011; Piotrowska, 2014), or reveal the existence of contamination with heavy metals or organic pollutants, such as pesticides (Riah et al., 2014; Kandziora-Ciupa et al., 2016).

Decomposition processes are carried out by enzymes. Fresh organic matter undergoes enzymatic transformation, and through these, bioavailable nutrients are released into the soil and CO_2 into the atmosphere. Therefore, the study of the enzymatic nature of litter decomposition is essential to the framework of ecosystem services.

Cellulose, for instance, is a widely presented organic component in the biosphere; it cannot be digested by soil organisms directly but is a great energy and nutrient source for organisms. To be consumed, cellulose from plant debris needs to be broken down by

cellulase to D-glucose units, of which it consists. This also applies to chitin (component of cell walls in fungi and exoskeletons of arthropods), which needs to be degraded by chitinase before it can be digested by soil organisms (Rodriguez-Kabana et al., 1983; Sinsabaugh et al., 1994; Das & Varma, 2011).

It is also important to study soil enzyme activities in the context of the soil organic matter formation. Measuring soil enzyme activity can be informative in the case of agricultural management, which mostly affects the biologically active upper soil horizon (Abramyan, 1992). A vast number of publications show the effect of tillage and amelioration on enzyme activities, particularly due to physical impacts on soil structure which in turn leads to a decrease in agronomically valuable aggregates and deterioration of their water stability (Tisdall & Oades, 1982; Pagliai et al., 2004; Kogut et al., 2012), as well as the loss of soil organic matter content. As soil enzymes are primarily associated with organic matter, agricultural impact can shift activities of certain enzymes, both positively and negatively (Pancholy & Rice, 1973; Khaziev & Gul'ko, 1991; Ross et al., 1995; Garbuz et al., 2016).

Processes of nitrogen transformation in the soil are also due to enzymes. When arriving into the soil, urea is transformed into bioavailable ammonia and CO_2 under the influence of urease (Lloyd & Sheaffe, 1973; Pancholy & Rice, 1973). Nitrates in the soil are reduced into non-available nitrogen through the chain of reactions that are carried out by soil enzymes; this is also important in the context of the fertiliser efficiency, impact on soil quality, and environmental consequences (Martens, 2005; Szajdak & Gaca, 2010).

Phosphorus is an important element in the biosphere, second after nitrogen in its effect on plant growth. Hydrolysis of a phosphoric acid monoester into a phosphate ion is an essential reaction for the soil fertility status (Dick et al., 2000).

As soil enzyme activities and abundance are affected by soil properties and conditions, in turn, the soil enzymes status can be used as an indicator of soil health (Dick, 1994;

Alkorta et al., 2003). Studying of soil enzyme activities can be used as a tool to understand influence of human impact, climate change and landscape alteration on soil quality (Karaca et al., 2011; Burns et al., 2013).

2.4 Biochar

The application of amendments, such as biochar or effluent, as a part of soil management practices, has an impact on soil physicochemical properties as well as biochemical and biological conditions.

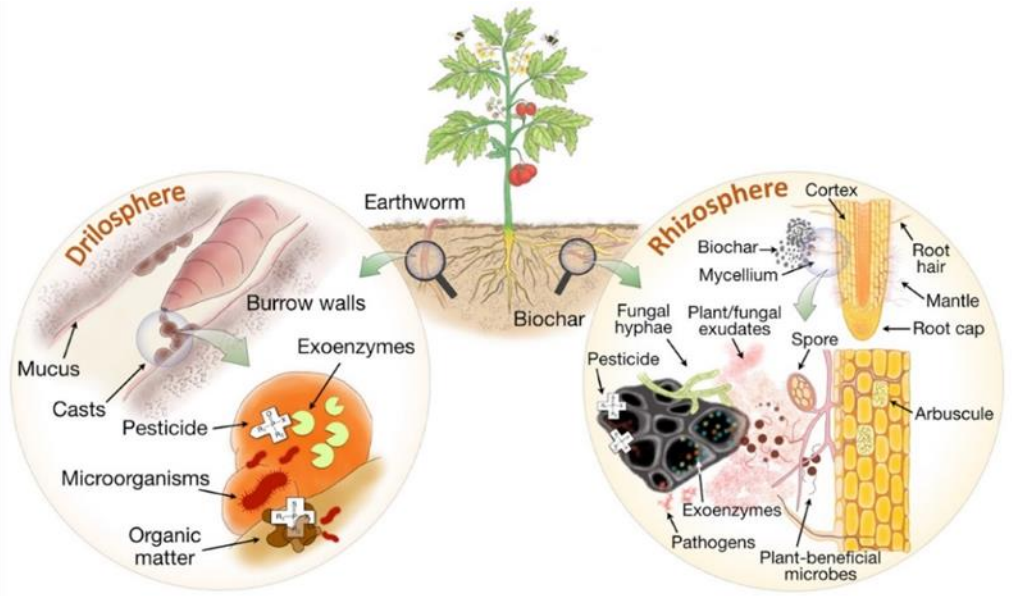
It was shown (Jindo et al., 2014) that biochar with high adsorption properties, surface area and porosity in long-term application can positively affect soil bulk density, as well as increase soil aggregate stability, soil water retention capacity and, for some types of soils, it can affect plant available water (Herath et al., 2013; Burrell et al., 2016). Most significantly, biochar can contribute to available nutrients, especially when produced from human and animal wastes (Mukherjee et al., 2014; Qayyum et al., 2014; Shen et al., 2016). Furthermore, biochar is considered as a suitable technology for C storage, as a part of climate change mitigation (Lorenz & Lal, 2014).

Biochar produced at high temperature tends to have a considerable liming equivalence and can be used as a liming agent in acidic soils, decreasing their exchangeable acidity and aluminium saturation (Chintala et al., 2013; Wang et al., 2014). As phosphorus is a critical element for plant growth, it is very important to manage its bioavailability. In this case, biochar may be considered as an effective instrument for increasing efficiency of applying phosphate fertilizer, as well as for solving the problem of phosphorus losses from agricultural fields (Soinne et al., 2014; Zhang et al., 2016).

Besides obvious impacts on soil physicochemical properties, biochar has an effect on the biological and biochemical status of the soil. Through changing soil physical and chemical characteristics, biochar indirectly affects the living environment of soil microorganisms. Changes in soil bulk density, water retention, soil nutrients availability

and soil pH caused by biochar has a great effect on soil microorganisms (Masto et al., 2013; De Tender et al., 2016). Different types of biochar can differently influence bacterial and fungal activities, shifting the microbial community structure (Pandian et al., 2016). Soil enzymatic activity has also been shown to be sensitive to biochar addition (Masto et al., 2013).

It is worthwhile to pay special attention to the interactions between biochar and earthworms, since both have a significant impact on soil properties (**Fig. 2.4**). This interaction can vary due to the different species of earthworms, weather conditions, as well the type of biochar, which is mostly dependent on type of feedstock, conditions of pyrolysis, and its particle size. In some cases biochar has a negative effect on earthworms; however, more often the effect is positive (Weyers & Spokas, 2011). It was shown that biochar and earthworms have a collaborative effect on decreasing soil carbon dioxide and nitrogen dioxide emissions (Augustenborg et al., 2012). The significant synergistic effect of biochar and earthworms was found for soil microbial community, changing abundance and activity of microorganisms, as well as enzymatic activity, which together, in turn, affected plant growth rate (Bamminger et al., 2014; Paz-Ferreiro et al., 2014; Paz-Ferreiro et al., 2015). Also, a positive effect of biochar and earthworms was detected on the content of toxic polyaromatic hydrocarbon compounds in the soil (Gomez-Eyles et al., 2011; Shan et al., 2014).



Soil quality improvement

Earthworms	Biochar
Increase water infiltration and soil aeration, alter soil aggregate distribution, and reduce water runoff.	Stimulates soil microbial proliferation and extracellular enzyme (or exoenzymes) production.
Stimulate microbial activity, nutrient cycling, and soil C mineralization and stabilization.	Reduces soil greenhouse gas emissions.
Burrow system represents hotspots for microbial activity because of deposition of nutrient-rich material (mucus, cast, and litter).	Decreases nutrient losses and triggers priming effect on soil C mineralization.
Middens created by anecic species are hotspots for microbial activity and faunal diversity.	Increases soil water holding capacity, thus favouring plant growth and development.
Stimulate plant growth and development, with potential benefits for resistance against herbivore damages, and plant pathogens. Also alter plant community by feeding and dispersion of seeds, and promote agriculture sustainability.	Some types of biochar exert a phytohormone-type effect on plants, and stimulate phytohormone production.
	Reduces soil-borne disease indirectly by binding extracellular pathogenic enzymes implied in the breakdown of cell walls.

Figure 2.4 Main beneficial effects of earthworms and biochar on soil quality in the drilosphere (soil of the earthworm burrow) and rhizosphere (Sanchez-Hernandez et al., 2019). Reproduced from Science of The Total Environment (Elsevier) with the kind permission of the editor.

There are few studies focusing on interactions between biochar and soil arthropods. Mostly, biochar has a positive effect on soil arthropods community (Godfrey et al., 2014; Conti et al., 2015), however the acting mechanisms are not clear. Biochar might supply nutrients to arthropods, or it is also possible that arthropods consume fungal hyphae colonizing biochar (Lehmann et al., 2011). The soil arthropods community can be affected by the liming effect of biochar, directly – even though the arthropods have a wide

range pH optima (van Straalen & Verhoef, 1997), or indirectly – through the pH influence on microorganisms, as these are food sources for some arthropods, and on decomposition rate (Marks et al., 2014). Hale et al. (2013) showed a positive effect of adding a 2% of biochar on Collembola reproduction, which in turn increased the overall soil biodiversity, as Collembola are food for the wide range of soil meso- and macro-arthropods (Coleman & Crossley, 2004).

Any application of amendments causes changes in soil properties, influencing physical and chemical processes, which in turn influence soil biochemical processes. Also, amendments affect the functional and structural diversity of soil biota, which are the key drivers of soil functions. Consequently, there is a need to explore the dynamic links between the functional diversity of soil biota, soil biochemical processes, and provision of ecosystem services in soils treated with biochar.

Chapter 3

The interactions between biochar and earthworms, and their influence on soil properties and clover growth: a 6-month mesocosm experiment

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Abstract

A six-month mesocosm experiment was conducted to investigate the joined effect of biochar and earthworms on soil properties and plant (white clover) growth in two contrasting soils – a dystric Cambisol and a sil-andic Andosol, both soils with pH-H_2O < 6. Treatments were (i) biochar amendment (1% weight basis), (ii) a positive control (lime added at the liming equivalence of the biochar application), and (iii) a negative control (no amendment). Each treatment had two variants: with or without earthworms (*Aporrectodea caliginosa*). Soil chemical and biological properties were measured before the start of the experiment and after 6 months of incubation. Earthworms were associated with higher ammonium-N and nitrate-N concentrations, lower pH, higher fungi:bacteria ratio, higher abundance of Collembola, and higher clover biomass in mesocosms. The influence of biochar on plant productivity was overshadowed by earthworm activity, yet a significant positive effect of biochar on clover biomass was observed in the absence of earthworms; this effect was not related to the liming potential of biochar. Synergistic effects of biochar and earthworms were observed for increasing abundance of Collembola and soil fungal biomass. The interaction between biochar and earthworms was soil-type specific – for example, on their own, biochar and earthworms increased clover growth more in the Cambisol, while the positive synergistic effect of biochar and earthworms on soil biochemical processes and clover growth was more evident in the Andosol. Combined use of biochar and earthworms has good productivity potential for acidic soils and can be part of sustainable soil management.

Keywords: *Aporrectodea caliginosa*, biochar, Andosol, Cambisol, carbon, nitrogen, nitrate reductase, productivity

3.1 Introduction

Since the industrial revolution, anthropogenic activity has caused the raising of CO_2 concentration in the atmosphere, with the associated warming effect on the global climate. A potential way to mitigate increasing atmospheric CO_2 is to boost soil carbon (C) storage. Biochar can contribute to this objective (Lehmann et al., 2010; Brassard et al., 2016) as it contains condensed aromatic carbon for which soil microbes generally lack the set of enzymes needed for its decomposition, which allows biochar to persist over time (Lehmann et al., 2015a). Moreover, biochar can provide benefits as a soil amendment, given that it influences soil chemical and physical properties, yet this is highly dependent on the type of feedstock, conditions of pyrolysis, biochar application rate, biochar particle size, and type of soil (Jones et al., 2012; Jaafar et al., 2015; Lehmann et al., 2015b). Biochar can contribute to the provision of soil nutrients, especially if produced from animal and human residues (Wang et al., 2012b; Qayyum et al., 2014) and increase soil aggregate stability, soil water and nutrient retention, and plant-available water (Herath et al., 2013; Burrell et al., 2016; Mahmud et al., 2018). Some types of biochar have a considerable $CaCO_3$-liming equivalence, and can be used as a liming agent, decreasing soil acidity and aluminium (Al) concentration in solution and at exchangeable sites (Chintala et al., 2013; Wang et al., 2014). The liming properties of biochar are mostly related to its inorganic alkalinity, but organic structural and other organic alkalinity can also contribute to it (Fidel et al., 2017). Moreover, the response of soil pH to the addition of a biochar with liming properties is influenced by the pH buffering capacity (pH-BC) of the soil (Singh et al., 2017).

Besides the impacts on soil physical and chemical properties, biochar has an indirect effect on the biological and biochemical status of the soil, as it affects the soil living environment. Changes in soil bulk density, water retention, soil nutrients availability and soil pH associated with biochar application have been associated with a positive effect on

soil microorganisms (Lehmann et al., 2011; Masto et al., 2013; De Tender et al., 2016) and arthropods (Conti et al., 2015; Reibe et al., 2015). Soil enzymatic activity has also been shown to be sensitive to biochar addition (Masto et al., 2013). The effect of biochar on earthworms activity have been shown to be generally neutral or positive (Van Zwieten et al., 2010; Weyers & Spokas, 2011), yet negative effects have also been reported, such as a decrease in earthworms biomass in some experiments using urban or artificial soil (Gomez-Eyles et al., 2011; Li et al., 2011). Interactions have been found between the type of biochar, its application rate and particle size, and species of earthworms (Noguera et al., 2010; Weyers & Spokas, 2011; Paz-Ferreiro et al., 2014). Biochar has also been shown to mitigate the CO_2 and N_2O emissions commonly associated with earthworm activity (Augustenborg et al., 2012; Liu et al., 2018b). Increases in crop productivity due to positive interaction between biochar and earthworms have been reported (Noguera et al., 2010; Paz-Ferreiro et al., 2014). The combined effect of biochar and earthworms has been shown to have an impact on soil microbial community, changing abundance and activity of soil microorganisms, as well as soil enzymatic activity and plant growth (Noguera et al., 2010; Elmer, 2012; Bamminger et al., 2014; Paz-Ferreiro et al., 2015). Yet there are still gaps in the understanding of the interactions between biochar, functional groups of soil biota, biochemical processes, and plant growth.

The objective of this study was to investigate the interactions between biochar and earthworms as they influence soil chemical, biochemical and biological properties, and plant growth in two contrasting soils, a dystric Cambisol and a sil-andic Andosol (both soils with pH-H_2O < 6 but with contrasting physicochemical properties), in a 6-month mesocosm experiment. We hypothesized that 1) biochar produced from willow and applied at ca. 12 Mg ha^{-1} will have an influence on soil biological processes and plant productivity beyond its liming value; 2) biochar and earthworms will interact in regard to their influence on soil biological processes and plant productivity.

3.2 Materials and Methods

3.2.1 Soils in This Study

Two soils belonging to different soil orders were used in this study: 1) a dystric Cambisol (IUSS Working Group WRB., 2015), Brown soil in the New Zealand soil classification system (Hewitt, 2010), from the experimental site of AgResearch Ballantrae Hill Country Research Station, Manawatu, New Zealand (40°18'35"S 175°49'41"E); 2) a sil-andic Andosol (Allophanic soil (Hewitt, 2010)), from Hawera, Taranaki, New Zealand (39°36'28"S 174°16'30"E). The top 15 cm of both soils were collected in April 2017. Two sets of 20 g samples of each soil were separated for analysis, one sample was air-dried, another frozen at -30 °C.

3.2.2 Mesocosm Experiment

The experiment used a Latin Square design. The treatments included: (i) a negative control (no amendment), (ii) a positive control (0.88 Mg ha^{-1} for the Andosol and 0.91 Mg ha^{-1} for the Cambisol of lime was added, equivalent to the liming value of 1% biochar), and (iii) a biochar treatment (1% biochar w/w, equivalent to 12 Mg ha^{-1} for the Andosol and 12.5 Mg ha^{-1} for the Cambisol). Each treatment had two variants: with and without earthworms. Treatments without earthworms had five replicates; treatments with earthworms had six replicates (the extra replicate was added in case of earthworms escaping from the pots). Planter bags PB10 (height 48 cm, Ø 15 cm) were used as pots. Two layers of mesh were placed in the bottom of each pot (bag) to prevent earthworms from escaping through drainage holes. Two rings of adhesive Velcro “hook” tape were placed on the top of each pot on the inside surface to prevent earthworms from escaping (Lubbers and van Groenigen, 2013). Please see photos of the mesocosm experiment in **Appendix 3.1**.

The soil was sieved to 3 mm without drying. Air-dried ground sheep dung (in proportion equivalent to 8.8 g per pot) was added to the soil as a feed for earthworms

(Greig-Smith, 1992), and thoroughly mixed. A subsample of the soil amended with sheep dung was taken and was considered the "initial soil". Then amendments (i.e., either biochar or lime) were added to all treatments except for the negative control. Each pot was filled with either 2.12 kg of the Andosol (bulk density – 0.80 g cm^{-3}) or with 2.20 kg of the Cambisol (bulk density – 0.83 g cm^{-3}); the volume of soil in each pot was 2650 cm^3; average height of soil column at the start of the experiment was 15 cm, however, natural settling of the soil had occurred during the incubation and after 6 months average soil column height reduced to 10 cm.

Five plants of white clover (*Trifolium repens* L.) were planted in each pot (four around the edges and one in the centre). On average, the oven-dried weight of a plant at the time of planting was 0.05 g. Adult endogeic earthworms *Aporrectodea caliginosa* (Savigny, 1826) were collected by hand-sorting the soil from a paddock at Massey University (40°23'25"S, 175°37'12"E) in April 2017. Adult earthworms were weighed (the average weight of an earthworm was 0.31 g) and four earthworms were placed in each of the pots receiving the "earthworms" treatment. The pots were arranged in a Latin Square design in the glass house. Moisture was controlled using a capillary mat, configured to maintain moisture in the pots at 30% w/w (Lowe & Butt, 2005). The temperature inside the glasshouse was maintained within 15-20 °C range.

The experiment started in May 2017. At the end of months 2 and 4 of the experiment, the clover was cut at 2 cm height above the soil. Cut clover was oven-dried (50 °C), weighed, and reported as dried biomass. After 6 months, in November 2017, the experiment was terminated. The soil in each pot was radially divided into four parts. Earthworms from all soil sections were hand sorted, counted and weighed. Clover (separately roots and above-ground biomass) from all four parts was collected, oven-dried and weighed. Total clover biomass was calculated by summing weights of all cuttings and roots. The above-ground biomass and root biomass at the end of the experiment (final

harvest) was used to calculate the root-to-shoot ratio. Out of the four radially cut sections of soil, one was used for soil biological measurements (microbial biomass), another for soil arthropods extraction, another was split into two depth levels (0-5 cm and 5-10 cm), air-dried and used for chemical analyses, and another was frozen at -30 °C and kept for future analyses.

3.2.3 Biochar Production and Characterisation

Biochar used in this experiment was produced from willow (*Salix matsudana* L.) chips. Air-dried feedstock (< 12% moisture content) was pyrolysed at a highest heating temperature of 350 ℃ and residence time of 4 h. Biochar was then ground and sieved (< 2 mm). A subsample of biochar was further ground by a ring mill to obtain a particle size < 0.3 mm for chemical analysis. Please see photos in **Appendix 3.1**.

Biochar pH and EC were measured in a suspension of biochar in deionised water at a 1:20 (w/v) ratio (Singh et al., 2017a). The ash content was measured by dry combustion at 650 °C until constant weight (Singh et al., 2017a). The liming equivalence (% $CaCO_3$-eq) was determined according to Singh et al. (2017) by titrating with a 1 M HCl suspension of biochar (1:20, w/v ratio) with 0.5 M NaOH to pH 7.0. The total C, N and H contents were determined by high temperature combustion followed by thermo-conductivity detection (TCD) using Vario Macro Cube (Elementar Analysensysteme GmbH, Germany). Inorganic C was measured by titration with 0.2 M HCl a NaOH solution in which CO_2 was trapped during a 5-days incubation (Singh et al., 2017a). Organic C (OC) was calculated by subtraction of inorganic C from the total C. Available phosphorus (2% formic acid extractable P) and nitrogen (6 M HCl hydrolysable N) were measured following Camps-Arbestain et al. (2017) after Wang et al. (2012a,b). Available SO_4-S, K, Mg, Na and Ca were determined following the method proposed by Camps-Arbestain et al. (2017), through an extraction of 1 g of biochar in 20 mL 1 M HCl. The concentration of SO_4-S in the extracts was measured by segmented flow auto-analysis

using Technicon AA-II (Technicon, USA) and that of K, Mg, Na and Ca were determined using a Microwave Plasma-Atomic Emission Spectrometer (MP-AES, Agilent Technologies, USA). The biochar was classified according to Camps-Arbestain et al. (2015) as having a liming class of 1, a C storage class of 2, and a fertiliser class of 0. The apparent bulk density of the biochar was determined by mixing sand with biochar (of known mass) in a measuring jug and looking at the corresponding increases in volume. Particle size distribution was measured by dry sieving and was as follows: 42.9, 18.9, and 38.2% for particles sizes of >1000 μm, 500-1000 μm, and <500 μm, respectively. Properties of the biochar are reported in **Table 3.1**.

Table 3.1. Properties of the biochar (willow chips feedstock) used in the experiment.

Parameter	**Unit**[a]	
Pyrolysis temperature/residence time		350 ºC/4 h
pH, 1:20		7.75
EC[b]	μS cm^{-1}	263.3
Ash	g kg^{-1}	102
Liming equivalence	% $CaCO_3$-eq	7.3
Corg	g kg^{-1}	703
N	g kg^{-1}	11
Atomic H/C_{org}		0.63
Available P	mg kg^{-1}	296.3
Available N	mg kg^{-1}	485.6
Available SO_4-S	mg kg^{-1}	125.5
Available K	mg kg^{-1}	512.3
Available Mg	mg kg^{-1}	86.0
Available Na	mg kg^{-1}	33.5
Available Ca	mg kg^{-1}	483.4
Apparent BD	g cm^{-3}	0.24

All concentrations are expressed on an oven dry weight basis.

[b]Electrical conductivity

Table 3.2. Properties of the soil (mixed with ~1.2 g kg^{-1} ground sheep dung) used in the experiment – the "initial soil".

	Andosol	**Cambisol**
Soil texture	Loamy	Clayey
pH	5.65	5.55
OC, g kg^{-1}	77.4	35.1
TN, g kg^{-1}	6.73	2.58
NO_3^--N, mg kg^{-1}	27.6	19.6
NH_4^+-N, mg kg^{-1}	14.4	8.2
Nitrate reductase, μg NO_2^--N g^{-1} $24h^{-1}$	25.2	2.4
Si_o, g kg^{-1}	6.49	0.3
Fe_o, g kg^{-1}	8.09	4.17
Al_o, g kg^{-1}	23.26	2.44
Fe_{pyr}, g kg^{-1}	3.1	4.7
Al_{pyr}, g kg^{-1}	8.525	2.89

3.2.4 Soil Chemical Properties

Soil pH was measured in a ratio of soil:deionised water = 1:2.5 (w/v). Total C (TC) and total nitrogen (TN) contents were determined by high temperature combustion followed with thermo-conductivity detection (TCD) using Vario Macro Cube (Elementar Analysensysteme GmbH, Germany). Inorganic C was negligible (< 0.05%), even in lime-treated soils after 6 months of incubation, and thus total C was considered to be all organic (OC). Nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) were determined following the method in Blakemore et al. (1987). For this, 1 g of soil was extracted with 20 mL of 2 M KCl, and NO_3^--N and NH_4^+-N were measured by segmented flow auto-analysis using Technicon AA-II (Technicon, USA). Aluminium, iron and C extractable with 0.1 M sodium pyrophosphate at pH 10 (Al_p, Fe_p, C_p) were measured following Blakemore et al. (1987). Aluminium, iron, and silicon extractable with 0.2 M ammonium oxalate-oxalic acid at pH 3 were determined in the dark following Blakemore et al.

(1987); concentrations of Al_p, Fe_p, Al_o, Fe_o, and Si_o in the extracts were determined using MP-AES as above. Concentration of C_p was determined using TOC Analyzer (Shimadzu Corporation, Japan). The main properties of the two soils are described in **Table 3.2**.

3.2.5 Soil Biological Properties

Fungal (C_f) and bacterial (C_b) biomass C were measured by substrate-induced respiration (SIR) method with selective inhibition (Nakamoto & Wakahara, 2004). Briefly, 2 g of fresh soil to which glucose (2 mg g^{-1}) was added, was incubated for 5 h, and concentration of CO_2 released by microorganisms was measured using a CO_2 analyser. Fungal and bacterial respiration was measured by adding with glucose chloramphenicol (1 mg g^{-1}) and cycloheximide (2 mg g^{-1}) respectively. Fungal and bacterial biomass C were calculated according to Anderson and Domsch (1978).

Nitrate reductase (EC 1.7.99.4) activity (NR) was determined following the Kandeler method (Schinner et al., 1996): 1 g of air-dry soil was incubated with 0.8 ml of 2,4-dinitrophenol solution (0.9 mM), 0.2 ml potassium nitrate solution (25 mM) and 1 ml distilled water for 24 h at 25 °C. After incubation 2 ml of potassium chloride (4 M) solution were added and the mixture was centrifuged for 10 min at 5,000g. The supernatant (2.5 ml) was mixed with 1.5 ml of ammonium chloride buffer (0.19 M, pH 8.5) and 1 ml of colour reagent, and allowed to stand for 15 min at room temperature. Optical density was measured with a spectrophotometer at 520 nm against the reagent blank. An external calibration curve was made using sodium nitrite.

Collembola (springtails) were extracted using the Tullgren funnels (Southwood & Henderson, 2009). The animals were stored in 70% ethanol and counted using a binocular microscope.

3.2.6 Statistical Analysis

Normality of data sets was evaluated by Shapiro-Wilk test. Analysis of variance (ANOVA) with contrast statements and Tukey HSD test were used to investigate the effect of factors: soil order (Cambisol and Andosol); treatment (control, biochar, and

lime); earthworms (with and without); and depth (0-5 cm and 5-10 cm) on variables: total clover biomass, clover root-to-shoot ratio, and soil properties (pH, OC, TN, NO_3^--N and NH_4^+-N concentration, activity of NR). ANOVA was also used to compare soil properties at the start of the experiment and at the end of experiment. Principal Component Analysis (PCA) was performed for soil chemical properties (combined 0-5 and 5-10 cm layers), biological properties and clover biomass, grouping them by factors (amendments and earthworms). Statistical analysis was done using R version 3.3.3. Because soil type has a significant effect on almost all experimental variables, the results for Cambisol and Andosol are reported separately, unless indicated otherwise.

3.3 Results

3.3.1 Effect of Treatments on Soil Chemical Properties

As expected, at the end of the experiment, the pH values of the soils that received alkaline amendments were significantly larger ($P<0.001$) than those of the control soils (**Table 3.3, SI Fig. 3.1** in **Appendix 3.2**), and this was more apparent in the Cambisol. Changes in pH were even more evident when comparing the final soil pH values with that of the "initial soil", that is, prior to the addition of either biochar or lime (e.g., pH increase in 0-5 cm layer was by 0.5 units in the Cambisol, but only by 0.2 units in the Andosol). The increases in soil pH were higher in the 5-10 cm layer than in the top 0-5 cm ($P<0.001$ for both soil types, all treatments). The difference in pH between soil layers was less noticeable in the presence of earthworms. Soil pH tended to be lower in earthworm treatments (**Table 3.3, SI Fig. 3.1**), particularly in the Cambisol.

In soils without biochar addition, total OC concentration generally decreased after 6 months of experiment (combined 0-10 cm depth, both soil types, $P<0.001$), this trend was more accentuated in the Andosol (mean OC decrease of 12.4 g kg^{-1}) than in the Cambisol (2.1 g kg^{-1}) (**Table 3.3 SI Fig. 3.2**). As expected, soils to which biochar was added had, on average, larger OC concentrations (by 2.0-5.3 g kg^{-1} in the Cambisol and 2.3-11.0 g kg^{-1} in the Andosol) (significant at $P<0.001$). Lime addition caused, in general,

a significant decrease in OC concentration (P<0.001 across both soil types, 0-10 cm depth), this being, on average, of 1.5 g kg^{-1} (Cambisol) and 2.0 g kg^{-1} (Andosol) smaller than in non-limed soils. With few exceptions, the loss of OC tended to be greater in the absence of earthworms, while their presence increased C_p at 5-10 cm depth (data not shown). In a trend opposite to OC concentrations, TN concentrations significantly increased over time, on average by 0.7 g kg^{-1} across all treatments and both soil types (P<0.001) (**Table 3.3, SI Fig. 3.3**). Soils with earthworms had significantly higher soil TN values (P<0.001 across both soil types).

Table 3.3 Experimental parameters (means) at the end of the mesocosm experiment. EW – earthworms.

	Depth	Control	Control + EW	Biochar	Biochar + EW	Lime	Lime + EW
Andosol							
pH	0-5 cm	5.6 bc†	5.6 c	5.8 a	5.7 b	5.8 a	5.8 a
	5-10cm	5.8 b	5.7 d	6.0 a	5.7 c	5.9 a	5.8 b
OC, g kg^{-1}	0-5 cm	65.0 d	69.1 c	75.9 a	71.3 b	63.4 c	67.8 d
	5-10cm	65.2 cd	68.7 b	72.6 a	70.8 a	64.2 d	66.3 c
TN, g kg^{-1}	0-5 cm	6.6 c	7.2 a	7.2 a	7.1 a	6.7 bc	6.8 b
	5-10cm	6.7 c	6.9 b	6.9 b	7.2 a	6.5 c	6.9 b
NO_3^--N, mg kg^{-1}	0-5 cm	22.1 d	34.0 b	24.6 d	37.9 a	22.1 d	27.8 c
	5-10cm	17.2 c	26.9 b	25.9 b	32.2 a	18.3 c	26.3 b
NH_4^+-N, mg kg^{-1}	0-5 cm	12.2 d	17.0 b	14.5 c	21.0 a	18.1 b	20.5 a
	5-10cm	13.1 d	18.5 b	11.9 d	21.5 a	15.8 c	18.3 b
NR, μg NO_2^--N g^{-1} 24 h^{-1}	0-5 cm	19.4 d	26.2 c	23.6 bc	27.5 b	27.7 b	39.8 a
	5-10cm	21.5d	26.5 c	27.8 bc	30.1 b	29.8 b	41.1 a
Cambisol							
pH	0-5 cm	5.7 b	5.4 c	6.1 a	5.8 b	6.1 a	6.1 a
	5-10cm	6.0 c	5.5 e	6.3 a	5.9 d	6.3 a	6.2 b
OC, g kg^{-1}	0-5 cm	32.6 c	33.5 bc	34.6 b	39.3 a	30.1 d	29.9 d
	5-10cm	28.9 d	32.9 c	36.1 b	40.8 a	29.1 d	29.9 d
TN, g kg^{-1}	0-5 cm	3.0 bc	3.2 a	2.8 d	3.1 ab	2.9 cd	3.2 a
	5-10cm	2.8 c	3.2 a	2.8 c	3.2 a	3.0 bc	3.1 ab
NO_3^--N, mg kg^{-1}	0-5 cm	9.0 c	12.9 b	8.2 c	13.3 b	9.2 c	14.5 a
	5-10cm	7.9 c	10.7 b	8.1 c	13.2 a	8.3 c	14.1 a
NH_4^+-N, mg kg^{-1}	0-5 cm	15.7 b	20.1 a	14.5 b	19.8 a	15.6 b	18.9 a
	5-10cm	17.3 b	21.6 a	14.7 c	21.0 a	16.7 b	20.3 a
NR, μg NO_2^--N g^{-1} 24 h^{-1}	0-5 cm	2.8 b	6.3 a	2.4 b	6.7 a	4.6 ab	6.8 a
	5-10cm	5.3 cd	8.1 ab	2.8 d	6.3 bc	3.0 d	9.8 a

† Values within a row followed by the same letter are not significantly different, $P<0.05$.

At the end of the 6-month experiment, NH_4^+-N concentrations almost tripled in the presence of earthworms in the Cambisol (**Table 3.3, SI Fig. 3.4**), compared to the start of the experiment. In the Andosol, the increase in NH_4^+-N concentrations was observed in all treatments with earthworms, and also in lime-only treatment; the order of magnitude for the increase was similar in both soils (average increase was 8.2 mg NH_4^+-N kg^{-1}). In the Cambisol, NO_3^--N concentration decreased significantly during the experiment (all treatments and depths, $P<0.001$), with concentrations halved in the presence of earthworms and decreasing even further in their absence (**Table 3.3, SI Fig. 3.5**).

3.3.2 Effect of treatments on soil biological properties

Across experimental treatments, the mean number of earthworms per pot decreased by 15%, the mean weight of individual earthworms did not change (data not shown) and no earthworm cocoons were detected. C_f was significantly higher in biochar and lime treatments ($P<0.001$ in both soils), and this effect was amplified in the presence of earthworms (interaction $P<0.001$ in both soils) (**Fig. 3.1**). C_b was also significantly higher in biochar and lime treatments in the Cambisol (biochar/lime vs. control $P<0.001$), but in the Andosol bacterial biomass was higher only in the soil with lime ($P<0.001$). Earthworms had no effect on the bacterial populations (**Fig. 3.1**). Fungi-to-bacteria ratio (**Fig. 3.1**) was significantly higher ($P<0.001$) in the presence of earthworms in both soils, whereas biochar and lime had either no effect or a slightly negative one (i.e., Cambisol with lime; $P<0.05$).

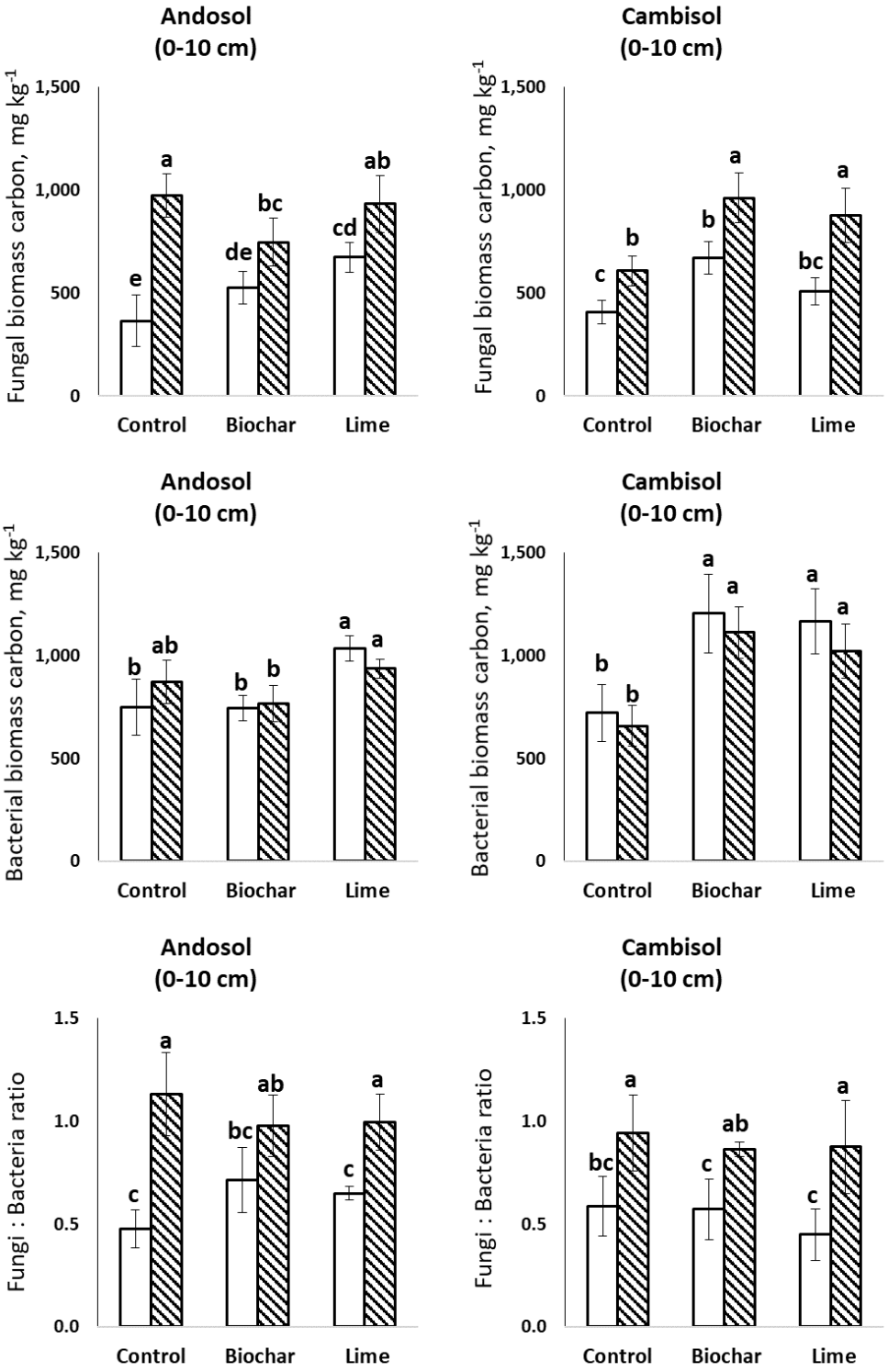

Figure 3.1 Fungal biomass, bacterial biomass and fungi to bacteria ratio at the end of the 6-month experiment. Values represent mean ± SE. Empty bar – treatments without earthworms, striped – treatments with earthworms. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between treatments within the soil.

NR activity was about ten times higher in the Andosol than in the Cambisol (significant at $P<0.001$) (**Table 3.3, SI Fig. 3.6**). Compared to the "initial soil", the NR activity in the Cambisol increased in the presence of earthworms (range of increase 3.9 to 7.4 µg NO_2^--N g^{-1} 24 h^{-1} across three treatments; $P<0.001$), but was not affected by the amendments. In the Andosol, NR activity was higher ($P<0.001$) in the presence of amendments, on average exceeding control values by 5.3 µg NO_2^--N g^{-1} 24 h^{-1} (biochar) and by 8.3 µg NO_2^--N g^{-1} 24 h^{-1} (lime). NR activity was even higher in the presence of earthworms, exceeding control by 3.1 µg NO_2^--N g^{-1} 24 h^{-1} for biochar ($P<0.005$) and by 11.7 µg NO_2^--N g^{-1} 24 h^{-1} for lime ($P<0.001$), with a significant positive interaction between lime and earthworms ($P<0.001$).

Abundance of Collembola was also higher in presence of earthworms ($P<0.001$ in both soils). In the absence of earthworms, Collembola abundance was higher in biochar treatments in both soils, whereas lime had no influence (**Fig. 3.2**).

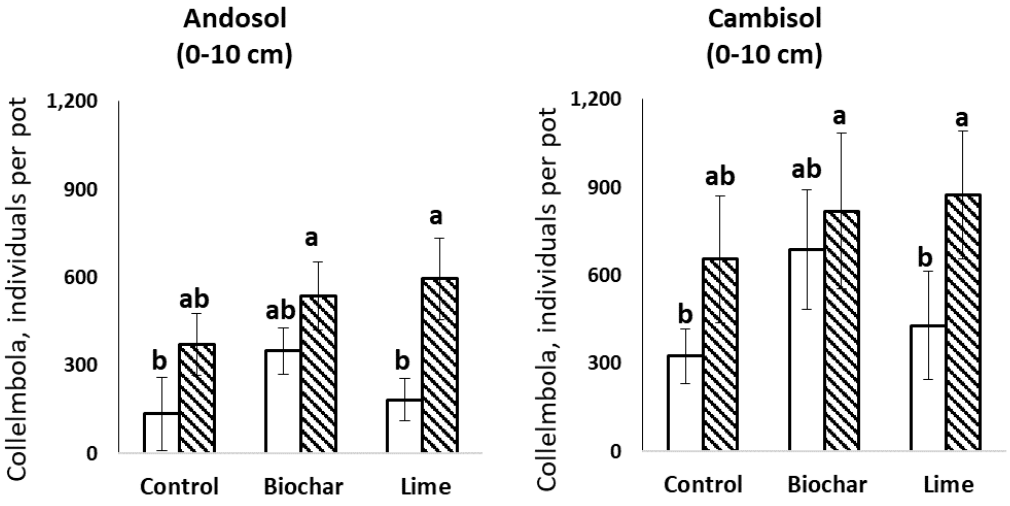

Figure 3.2 Collembola abundance at the end of the 6-month experiment. Values represent mean ± SE. Empty bar – treatments without earthworms, striped – treatments with earthworms. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between treatments within the soil.

3.3.3 Effect of treatments on clover biomass

At the end of the 6-month experiment, total clover biomass in the control Andosol was significantly higher ($P<0.05$) than in the control Cambisol (**Fig. 3.3**). In the absence of earthworms, clover biomass was higher in biochar treatments in both the Cambisol ($P<0.001$) and the Andosol ($P<0.001$), while lime had small negative effect on clover biomass in the Andosol ($P<0.05$) and no effect in the Cambisol ($P=0.331$). Presence of earthworms had a strong and significant positive effect on clover biomass, which was more pronounced in the Cambisol than in the Andosol ($P<0.001$ in both soils). In the Cambisol the highest clover biomass was observed in the control with earthworm treatment, while in the Andosol it was in the biochar with earthworms treatment (**Fig. 3.3**). Clover root-to-shoot ratio in the Andosol was four times greater than that in the Cambisol ($P<0.001$) (**SI Fig. 3.7**). There was no significant effect of amendments on the root-to-shoot ratio in either soil. The effect of earthworms was only noticeable in the Cambisol, where clover root-to-shoot ratio was three times higher in the presence of earthworms ($P<0.001$).

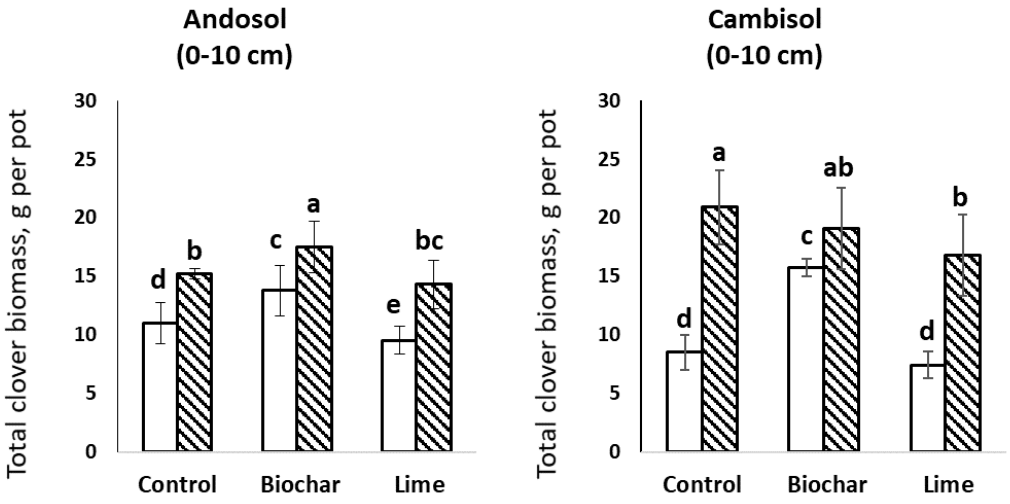

Figure 3.3 Total clover (*Trifolium repens* L.) biomass (dry weight) at the end of the 6-month experiment. Values represent mean ± SE. Empty bar – treatments without earthworms, striped – treatments with earthworms. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between treatments within the soil.

3.3.4 Principal Component Analysis (PCA) of the soil properties and clover biomass

For the Andosol, the first four principal components accounted for 84.5% of the total variability, with PC1 and PC2 accounting for 44.6% and 22.0%, respectively. The PC1 was mostly driven by the presence of earthworms, which were associated with higher ammonium-N and nitrate-N concentrations, higher total N and C_f/C_b ratio, higher abundance of Collembola, and higher clover biomass. Earthworms + biochar samples were associated with high nitrate concentrations, high total N, and high clover biomass. The PC2 reflected the effect of the amendments, with biochar associated with high values of OC and C_p, and lime with high C_b and nitrate-reductase, and to a lesser extent, with high pH values (**Fig. 3.4**).

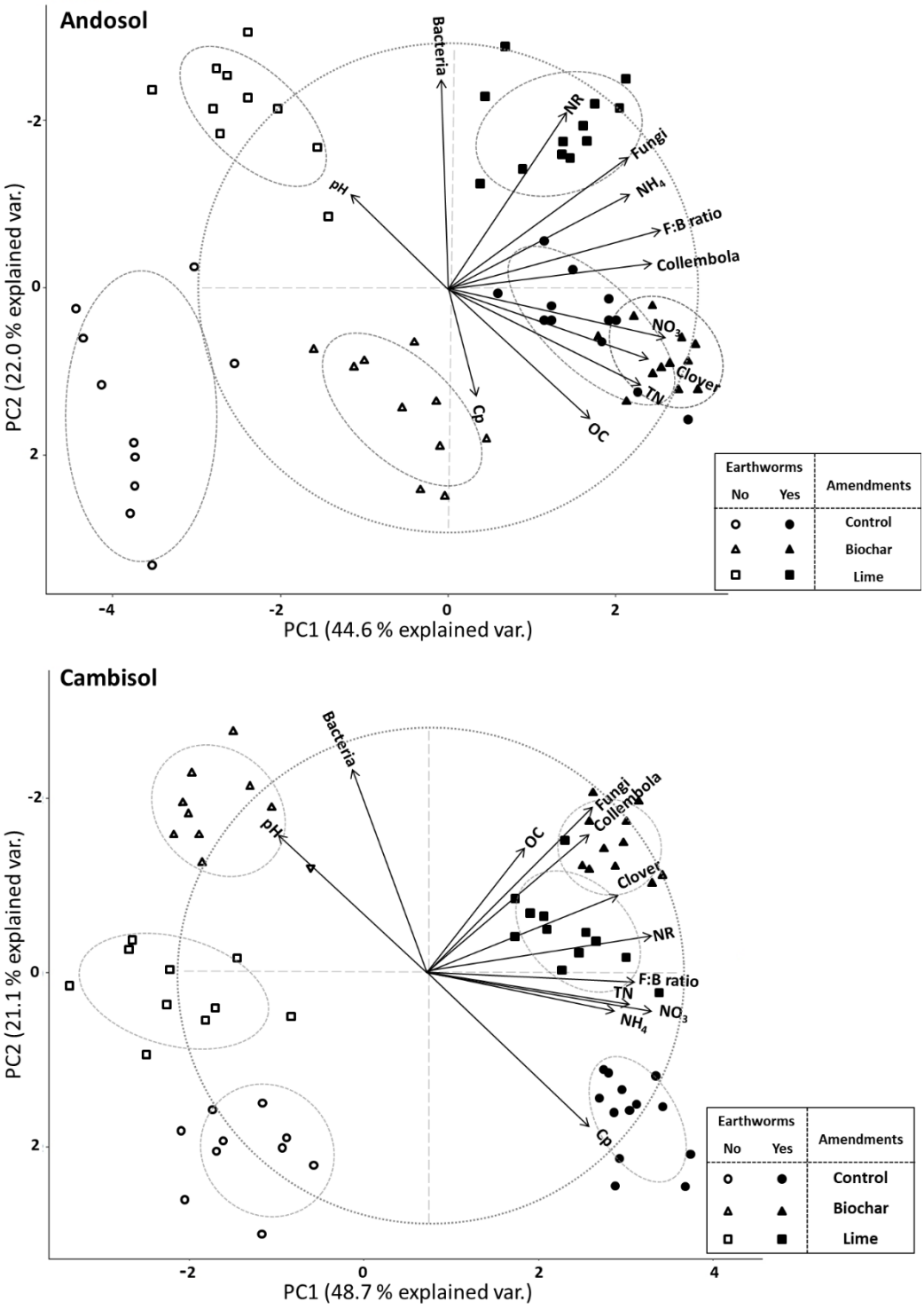

Figure 3.4 PCA bi-plot (PC1 vs. PC2) for the soil properties and total clover biomass for the Andosol and the Cambisol under different experimental treatments. OC – organic carbon, Cp – pyrophosphate extractable carbon, TN – total nitrogen, NO_3 – nitrate nitrogen, NH_4 – ammonium- nitrogen, NR – nitrate reductase activity, pH – soil pH, Bacteria – bacteria biomass carbon, Fungi – fungal biomass carbon, F:B ratio – fungal to bacteria ratio, Clover – dry clove biomass (roots and shoot), Collembola – Collembola abundance.

For the Cambisol, the first four principal components accounted for 85.7% of total variability, with PC1 and PC2 accounting for 48.7% and 21.1%, respectively. Similar to Andosol, the PC1 was driven by the presence of earthworms, while PC2 was influenced by the amendments (biochar, lime). Samples with earthworms had high ammonium-N, nitrate-N, total N, C_f/C_b ratio, nitrate reductase, and clover biomass. Lime and biochar treatments were associated with high pH and bacterial biomass C_b. Synergistic effects of biochar and earthworms on increasing abundance of Collembola and fungal biomass C_f were reflected in the PCA bi-plot (**Fig. 3.4**).

3.4 Discussion

3.4.1 Effect of Treatments on Soil Chemical Properties

Soil types. The two soils used in this experiment have different physicochemical properties (i.e., the Andosol, as per definition, is rich in short-range order constituents, which offer high OC protection), and responded differently to the treatments investigated (biochar, lime, earthworms), as reported in the literature (Wheeler et al., 1997; Biederman & Harpole, 2012; Paz-Ferreiro et al., 2014). The reduced response of the Andosol to the addition of alkalinity (lime, biochar) is consistent with its higher pH-buffering capacity. Counter-intuitively, despite the higher protected OC content of the Andosol than the Cambisol (Kov et al., 2018; Shen et al., 2018), the Andosol suffered a larger loss of OC during incubation (12.4 g kg^{-1}), which can be attributed to the fact that this soil also has a larger content of labile OC, more susceptible to decomposition upon disturbance, than the Cambisol (Shen et al., 2018). It should be noted that the amount of OC in dung added to each soil was ca. 1.2 g kg^{-1} and thus only a small fraction of the OC loss could be attributed OC in dung. This loss in labile OC was only balanced, if so, by the added OC in biochar (14.9 g kg^{-1}). In the Cambisol, an increase in OC above initial values was detected.

The abundance of short-range order constituents in the Andosol favours the formation of very fine aggregates that may remain saturated with water in a wet climate, further favouring anoxic conditions (Buurman et al., 2007a). This might explain the 10-fold higher nitrate reductase (NR) activity in the Andosol compared to that in the Cambisol. The Andosol also has higher fertility than the Cambisol, reflected in the greater total clover biomass in the control treatments, yet under the influence of earthworms (with or without biochar) the greatest total clover biomass was in the Cambisol.

Earthworms. The well-known influence of earthworms on soil fertility and the N cycle (van Groenigen et al., 2018) was evident in this study, with greater clover biomass and larger mineral N concentrations in soil with earthworms, as compared with the corresponding treatments without earthworms. The increase in nutrient availability in earthworm casts – and thus in nutrient fertility – has been mostly seen as the result of "(bio) chemical transformation processes" within the earthworm (Araujo et al., 2004; Bityutskii et al., 2007; van Groenigen et al., 2018). A recent meta-analysis has reported that earthworms, on average, increase soil mineral N concentrations by 241%, and those of available P by 84% (van Groenigen et al., 2018). Benefits of earthworms on above- and below-ground plant biomass have been reported to be more noticeable in soils with pH < 7 (van Groenigen et al., 2014), as are the soils in our study (pH ≤ 5.7). Soil texture also plays an important role in the response of plants to the presence of earthworms, with clayey soils showing a more pronounced response in plant yield (more than two-fold) compared to loamy soils (van Groenigen et al., 2014).

In our experiment, the gain in total N (TN) observed at the end of the incubation in the presence of earthworms may be attributed to the enhanced clover root growth and associated *Rhizobium* activity. Earthworms have been reported to positively affect microbial plant symbiosis (Bolan et al., 1991; Yan et al., 1996). An increase of TN associated with N_2 fixation may have contributed to the observed increases in NH_4^+-N

and NO_3^--N concentrations in the treatments with earthworms, in addition to the mineral N generated by earthworms through enhanced N mineralization (Barley & Jennings, 1959; Bityutskii et al., 2007). Moreover, an increase in N_2 fixation in the presence of earthworms may have caused the observed drop in pH, which was especially evident in the 0-5 cm soil layer, where most of the clover roots were found. This effect is opposite to what is observed with earthworms in the presence of non-legumes (Burtelow et al., 1998; Vos et al., 2014). The acidification of soils under legumes is partly explained by the increase in carboxylic groups of amino acids, which causes the release of H^+ into the rhizosphere (Nyatsanga & Pierre, 1973; Israel & Jackson, 1978).

In earthworm treatments, the larger NO_3^--N concentration in the presence of earthworms was paralleled by an increase in NR activity, which in the Andosol was especially pronounced when combined with lime. Earthworms casts have been shown to modify the microbial community by increasing denitrifying bacteria numbers (Knight et al., 1992; Parkin & Berry, 1994) and soil denitrification (Svensson et al., 1986; Elliott et al., 1990; Depkat-Jakob et al., 2010a), and thus NR activity (Burtelow et al., 1998). The formation of casts may also explain the smaller C losses experienced in the treatments with earthworms, and could be associated with the physical and chemical protection of organic matter within the casts (Zhang et al., 2013).

Biochar. The biochar used in our experiment had a low nutrient content (Fertilizer class - 0) and thus no direct fertiliser effect was expected. The influence of biochar on plant productivity was overshadowed by earthworm activity, yet a considerable effect of biochar on plant biomass was observed in the absence of earthworms, the effect being especially evident in the Cambisol. Given that in our experiment the lime itself did not have an effect on plant productivity, other properties/processes, such as changes in physical properties or in biological activity, probably had a role. Water retention, along

with that of nutrients, have been reported to be larger in biochar-treated soils (Haider et al., 2017; Mahmud et al., 2018), which may have favoured plant growth.

Herath et al. (2013) investigated the physical properties of an Andosol from the same region as the soils in our study, and found that biochar of similar particle size (< 2 mm) increased the water retention at water pressures < -0.1 bar.

Lime. The acidifying effect observed in presence of earthworms was better buffered by lime than by biochar, especially in the less pH-buffered Cambisol, which reflects a faster dissolution of the liming material there. This may also explain the larger loss of organic C in limed soils, given that as hydroxyl concentration increases, the stability of organo-mineral complexes in the soil decreases (Shen et al., 2018). As the optimum pH for NR is 7 (Abdelmagid & Tabatabai, 1987), lime and biochar both increase the activity of this enzyme by raising pH. The synergic effect of lime and earthworms combination on NR activity in the Andosol could be explained by the joined effect of pH (directly on the enzyme and indirectly on availability of organic ligands, as described above), the abundance of microaggregates in this soil, and the additional casts produced by the earthworms.

3.4.2 Effect of Treatments on Soil Biological Properties

The addition of earthworms led to greater C_f than the amendments without earthworms, whereas no effect – and, if any, a negative effect – was observed on C_b. Zhang et al. (2000) observed an overall decrease in microbial biomass after soil incubation in the presence of earthworms, with an increase in the fungal-to-bacterial ratio. Earthworms can reduce the number of bacteria in soil passing through their digestive system, whereas for fungi they can provide a positive effect by dispersing their propagules through casts (Hutchinson & Kamel, 1956; Tiwari & Mishra, 1993). Dempsey et al. (2013) have shown that earthworms can stimulate both bacterial and fungal abundance, however, in presence of biochar this effect can be inconsistent (Paz-Ferreiro et al., 2015).

With few exceptions, biochar and lime applications have a positive effect on both fungal and bacterial population. It is well known that an increase in pH stimulates bacterial and fungal activity in acidic soils (Shah et al., 1990; Mühlbachová & Tlustoš, 2006). Biochar produced at low temperature, such as the one used in our experiment, has a considerable fraction of labile C (Calvelo Pereira et al., 2011; Zhao et al., 2018), which can be used by soil microbial community for their C and energy needs (Cleveland et al., 2007; de Graaff et al., 2010; Dai et al., 2017). In addition, biochar has been reported to increase fungal abundance by providing physical growth matrix for arbuscular mycorrhizal fungi (Hammer et al., 2014). Similar to our study, Paz-Ferreiro et al. (2015) found that the effect of biochar on fungi was independent of the soil type, while the effect on bacteria was soil-specific.

Collembola are often used as model organisms in ecotoxicological tests to assess different soil amendments. In our study, biochar had a significantly positive effect on their abundance whereas lime did not, suggesting that the effect of biochar was not just due to an increase in soil alkalinity (Mueller et al., 1993), but might be related to other reasons such as improved soil porosity. Some studies report a negative effect of biochar on the reproduction of Collembola, as well as their avoidance of soils to which high concentrations of biochar (>5% w/w) were added (Amaro, 2013; Conti et al., 2018). Other authors have reported no effect of biochar type or application rate on Collembola populations (Domene et al., 2015; Reibe et al., 2015). Moreover, some studies indicate that Collembola may consume biochar without negative effect (Hale et al., 2013; Salem et al., 2013; Marks et al., 2014). Earthworms also stimulated Collembola populations, which might be related to an improvement of Collembolan food sources, as well as increased soil porosity (Brown, 1995; Wickenbrock & Heisler, 1997).

3.5 Conclusions

Our results showed that biochar applied in 1% ratio has a potential to benefit soil fertility and plant productivity in acidic soils, even without having a direct nutrient fertilizing effect. The use of positive control (lime) helped to prove that the beneficial effect of biochar on plant productivity was unrelated to the liming potential of biochar, but probably linked to its influence on soil biological communities, enhancing nutrient cycle and nutrient availability.

As expected, the presence of earthworms stimulated soil chemical and biological processes. Earthworms were associated with higher NH_4^+-N, NO_3^--N and mineral N concentrations, higher fungi:bacteria ratio, higher abundance of Collembola, and higher clover biomass. The influence of biochar on plant productivity was overshadowed by earthworm activity, yet a considerable positive effect of biochar on clover biomass was observed in the absence of earthworms, especially evident in the Cambisol. In the Andosol, a synergistic effect of earthworms-biochar combination on clover growth and soil biochemical processes exceeded the effect of each factor separately. The marked differences in treatment effects seen in the two soils indicate the complexity of processes influenced by biochar addition and earthworms activity. The two soil types differ in OC quantity and quality, texture, nutrients content and biological properties, which is reflected in their different response to experimental treatments.

Interactions between biochar, functional groups of soil biota, plants, and soil biochemical processes contribute to the regulatory and provisioning soil ecosystem services. Combined use of biochar and earthworms has good productivity potential for acidic soils and can be part of sustainable soil management.

Appendix 3.1

Biochar production

Mesocosm experiment

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Appendix 3.2

SI Table 3.1 Effect of experimental factors on clover (*Trifolium repens* L.) growth and on soil properties in the Andosol (ANOVA, α= 0.05). dfn - degrees of freedom in the numerator, dfd - degrees of freedom in the denominator.

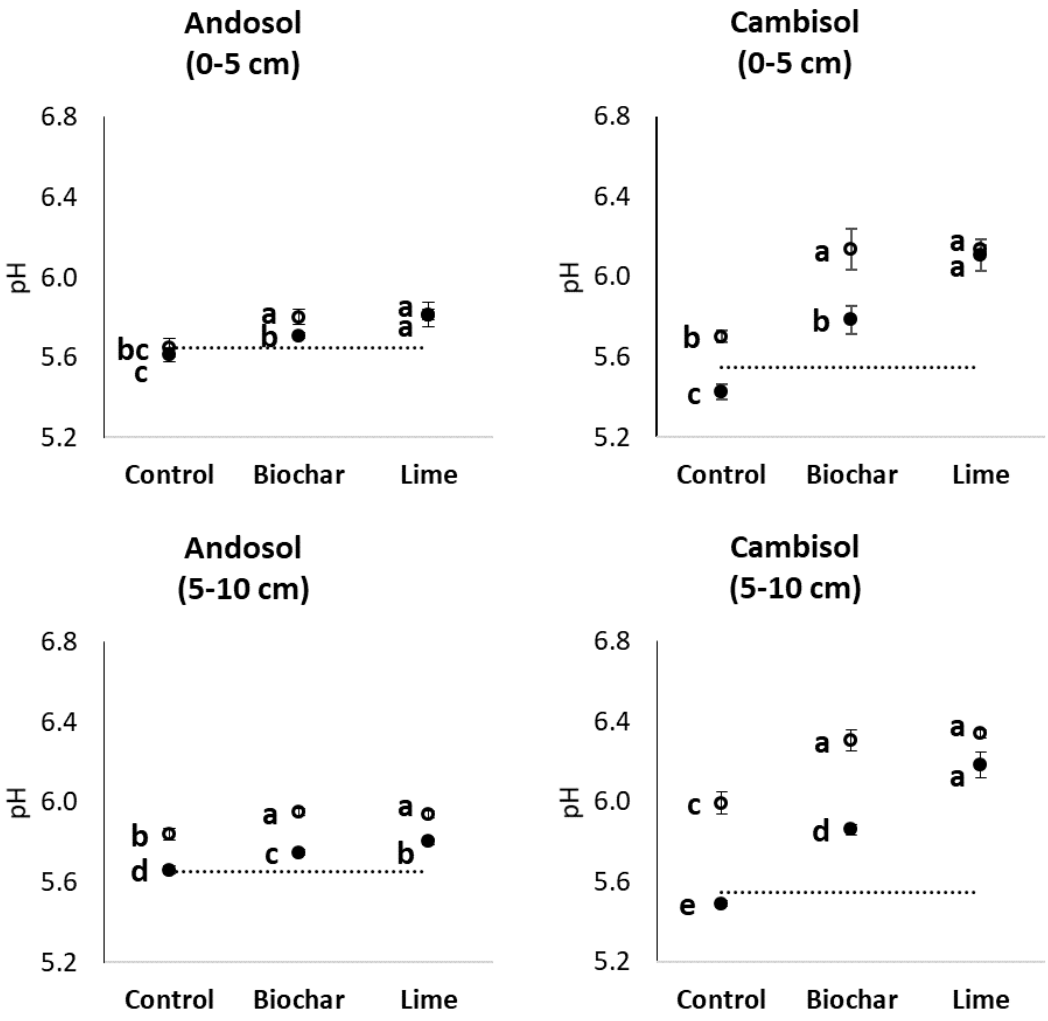
Effect	dfn	dfd	Effect size	F value	*P*
Total clover biomass					
Amendments	2	27	0.251	12.514	**<0.001**
Earthworms	1	27	0.473	47.093	**<0.001**
Amendments*Earthworms	2	27	0.005	0.239	0.789
Root to shoot ratio					
Amendments	2	27	0.039	0.575	0.569
Earthworms	1	27	0.006	0.179	0.676
Amendments*Earthworms	2	27	0.050	0.746	0.484
Fungi : Bacteria ratio					
Amendments	2	27	0.002	0.089	0.915
Earthworms	1	27	0.649	71.373	**<0.001**
Amendments*Earthworms	2	27	0.104	5.692	**<0.01**
Collembola					
Amendments	2	27	0.172	6.156	**<0.01**
Earthworms	1	27	0.412	29.494	**<0.001**
Amendments*Earthworms	2	27	0.039	1.376	0.269
pH					
Amendments	2	54	0.380	149.802	**<0.001**
Earthworms	1	54	0.260	204.850	**<0.001**
Depth	1	54	0.159	125.289	**<0.001**
Amendments*Earthworms	2	54	0.023	9.212	**<0.001**
Amendments*Depth	2	54	0.015	5.949	**<0.005**
Depth*Earthworms	1	54	0.092	72.736	**<0.001**
Amendments*Earthworms*Depth	2	54	0.001	0.474	0.625
Total Nitrogen					
Amendments	2	54	0.360	57.654	**<0.001**
Earthworms	1	54	0.311	99.481	**<0.001**
Depth	1	54	0.017	5.489	<0.05
Amendments*Earthworms	2	54	0.058	9.204	**<0.001**
Amendments*Depth	2	54	0.002	0.267	0.767
Depth*Earthworms	1	54	0.005	1.602	0.211
Amendments*Earthworms*Depth	2	54	0.005	12.716	**<0.001**
Nitrate nitrogen					
Amendments	2	54	0.211	143.44	**<0.001**
Earthworms	1	54	0.571	742.72	**<0.001**
Depth	1	54	0.095	123.18	**<0.001**
Amendments*Earthworms	2	54	0.019	12.75	**<0.001**

Amendments*Depth	2	54	0.019	12.49	**<0.001**
Depth*Earthworms	1	54	0.009	12.21	**<0.001**
Amendments*Earthworms*Depth	2	54	0.024	15.65	**<0.001**
Ammonium nitrogen					
Amendments	2	54	0.134	52.529	**<0.001**
Earthworms	1	54	0.606	473.413	**<0.001**
Depth	1	54	0.010	7.948	<0.01
Amendments*Earthworms	2	54	0.116	45.381	**<0.001**
Amendments*Depth	2	54	0.044	17.396	**<0.001**
Depth*Earthworms	1	54	0.009	7.189	<0.01
Amendments*Earthworms*Depth	2	54	0.010	4.021	<0.05
Nitrate reductase					
Amendments	2	54	0.552	296.577	**<0.001**
Earthworms	1	54	0.285	306.195	**<0.001**
Depth	1	54	0.025	27.252	**<0.001**
Amendments*Earthworms	2	54	0.078	41.910	**<0.001**
Amendments*Depth	2	54	0.005	2.784	0.0717
Depth*Earthworms	1	54	0.031	3.287	0.075
Amendments*Earthworms*Depth	2	54	0.001	0.174	0.084
Organic carbon					
Amendments	2	54	0.720	368.374	**<0.001**
Earthworms	1	54	0.043	43.866	**<0.001**
Depth	1	54	0.012	12.495	**<0.001**
Amendments*Earthworms	2	54	0.125	63.846	**<0.001**
Amendments*Depth	2	54	0.001	0.290	0.749
Depth*Earthworms	1	54	0.001	1.387	0.244
Amendments*Earthworms*Depth	2	54	0.011	23.096	**<0.001**

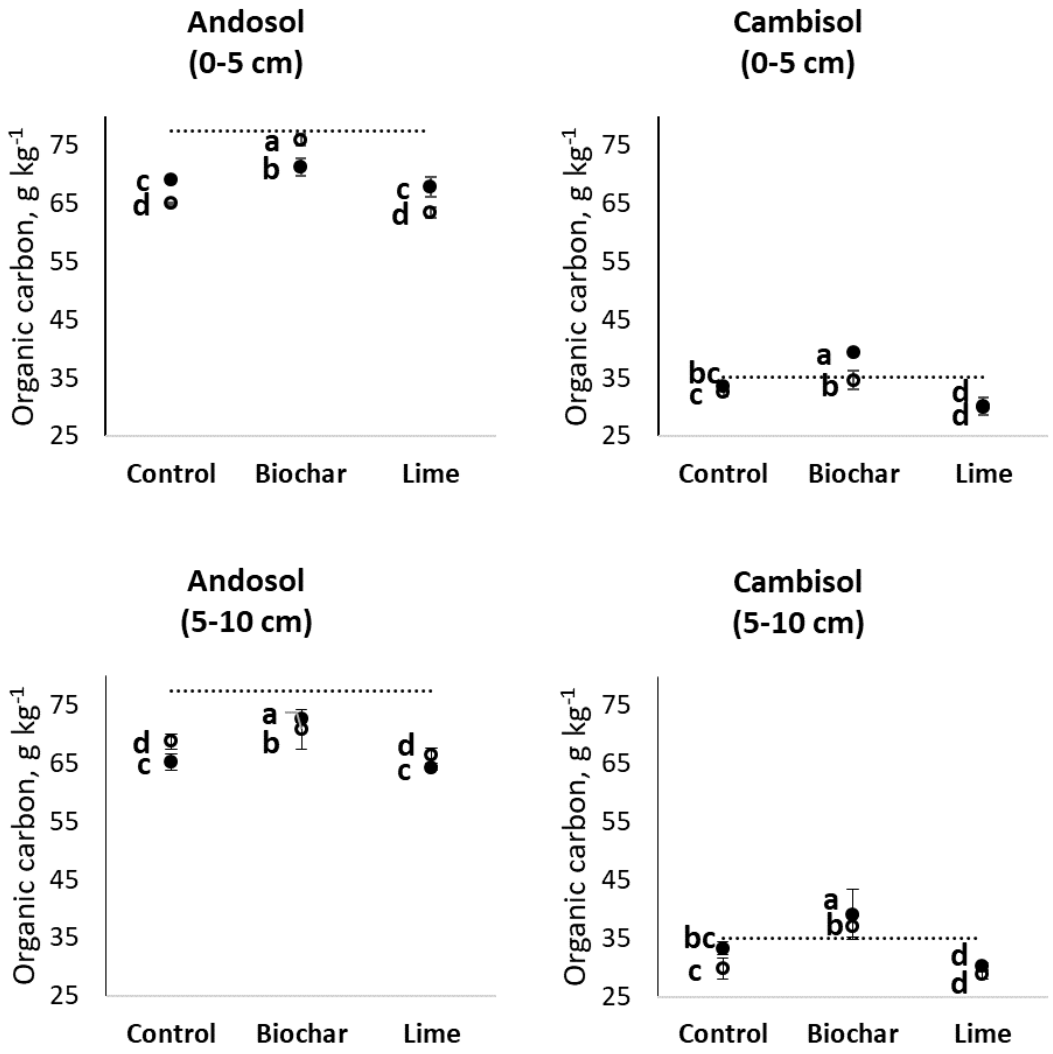
SI Table 3.2 Effect of experimental factors on clover (*Trifolium repens* L.) growth and on soil properties in the Cambisol (ANOVA, α= 0.05). dfn - degrees of freedom in the numerator, dfd - degrees of freedom in the denominator.

Effect	dfn	dfd	Effect size	F value	P
Total clover biomass					
Amendments	2	27	0.138	10.19	**<0.001**
Earthworms	1	27	0.565	83.227	**<0.001**
Amendments*Earthworms	2	27	0.113	8.339	<0.005
Root to shoot ratio					
Amendments	2	27	0.0293	0.635	0.538
Earthworms	1	27	0.346	14.999	**<0.001**
Amendments*Earthworms	2	27	0.936	0.066	0.936
Fungi : Bacteria ratio					
Amendments	2	27	0.029	1.066	0.359
Earthworms	1	27	0.587	42.789	**<0.001**
Amendments*Earthworms	2	27	0.140	0.512	0.605
Collembola					
Amendments	2	27	0.146	4.279	<0.05
Earthworms	1	27	0.355	20.777	**<0.001**
Amendments*Earthworms	2	27	0.036	1.057	0.361
pH					
Amendments	2	54	0.600	590.790	**<0.001**
Earthworms	1	54	0.242	477.253	**<0.001**
Depth	1	54	0.055	108.878	**<0.001**
Amendments*Earthworms	2	54	0.057	55.638	**<0.001**
Amendments*Depth	2	54	0.001	1.272	0.288
Depth*Earthworms	1	54	0.015	29.840	**<0.001**
Amendments*Earthworms*Depth	2	54	0.002	2.154	0.126
Total Nitrogen					
Amendments	2	54	0.040	4.732	<0.05
Earthworms	1	54	0.630	148.093	**<0.001**
Depth	1	54	0.001	0.294	0.589
Amendments*Earthworms	2	54	0.005	0.597	0.554
Amendments*Depth	2	54	0.039	4.528	<0.05
Depth*Earthworms	1	54	0.003	0.736	0.395
Amendments*Earthworms*Depth	2	54	0.053	6.207	<0.005
Nitrate nitrogen					
Amendments	2	54	0.058	37.439	**<0.001**
Earthworms	1	54	0.819	1061.72	**<0.001**
Depth	1	54	0.023	29.948	**<0.001**
Amendments*Earthworms	2	54	0.035	22.806	**<0.001**
Amendments*Depth	2	54	0.019	12.172	**<0.001**
Depth*Earthworms	1	54	0.001	0.493	0.486
Amendments*Earthworms*Depth	2	54	0.004	2.447	0.096

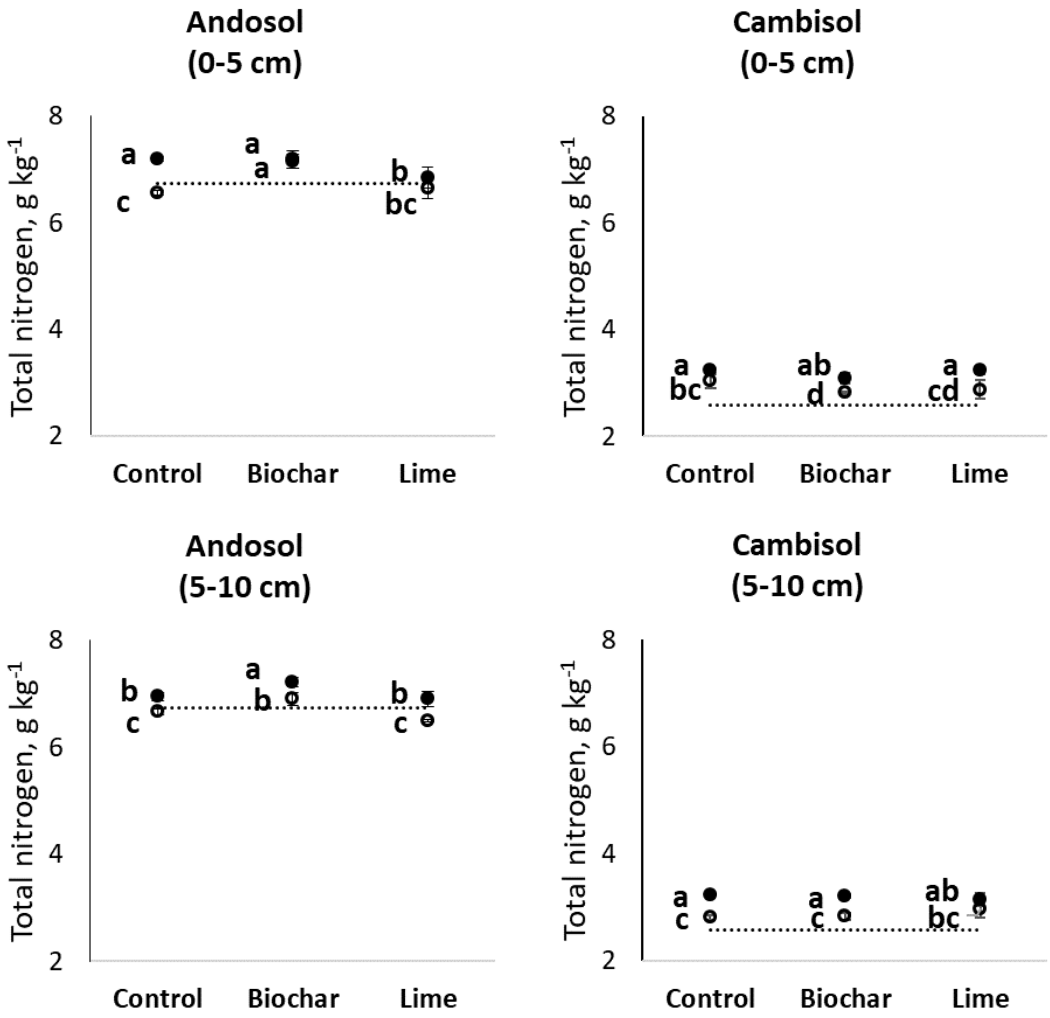
Ammonium nitrogen					
Amendments	2	54	0.032	7.998	**<0.001**
Earthworms	1	54	0.765	384.528	**<0.001**
Depth	1	54	0.055	27.748	**<0.001**
Amendments*Earthworms	2	54	0.034	8.536	**<0.001**
Amendments*Depth	2	54	0.004	0.975	0.384
Depth*Earthworms	1	54	0.001	0.681	0.413
Amendments*Earthworms*Depth	2	54	0.002	0.483	0.619
Nitrate reductase					
Amendments	2	54	0.061	13.47	<0.005
Earthworms	1	54	0.543	240.23	**<0.001**
Depth	1	54	0.037	16.314	0.007
Amendments*Earthworms	2	54	0.011	2.41	0.320
Amendments*Depth	2	54	0.030	6.72	<0.05
Depth*Earthworms	1	54	0.011	2.268	0.138
Amendments*Earthworms*Depth	2	54	0.056	5.940	<0.005
Organic carbon					
Amendments	2	54	0.766	586.996	**<0.001**
Earthworms	1	54	0.986	151.075	**<0.001**
Depth	1	54	0.002	2.357	0.131
Amendments*Earthworms	2	54	0.052	39.530	**<0.001**
Amendments*Depth	2	54	0.033	25.409	**<0.001**
Depth*Earthworms	1	54	0.007	10.888	<0.005
Amendments*Earthworms*Depth	2	54	0.001	4.808	<0.05



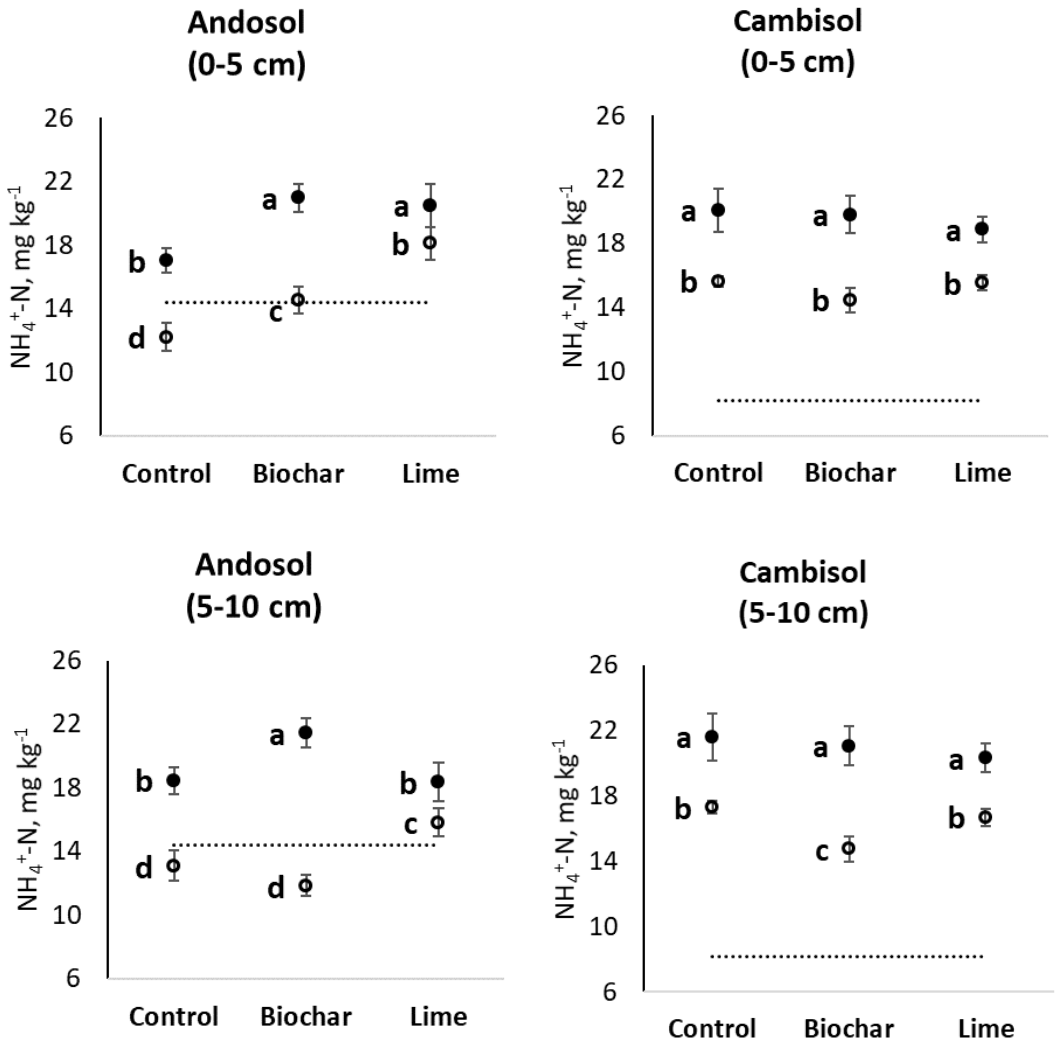
SI Figure 3.1 Soil pH in experimental treatments at 0-5 cm and 5-10 cm depths at the end of the 6-month experiment. Values represent mean ± SE. Empty circles – treatments without earthworms, filled circles – treatments with earthworms. Lowercase letters indicate significant differences (Tukey HSD test, $\alpha<0.05$) between treatments within soil layer. Dashed line shows the value of the "initial soil".



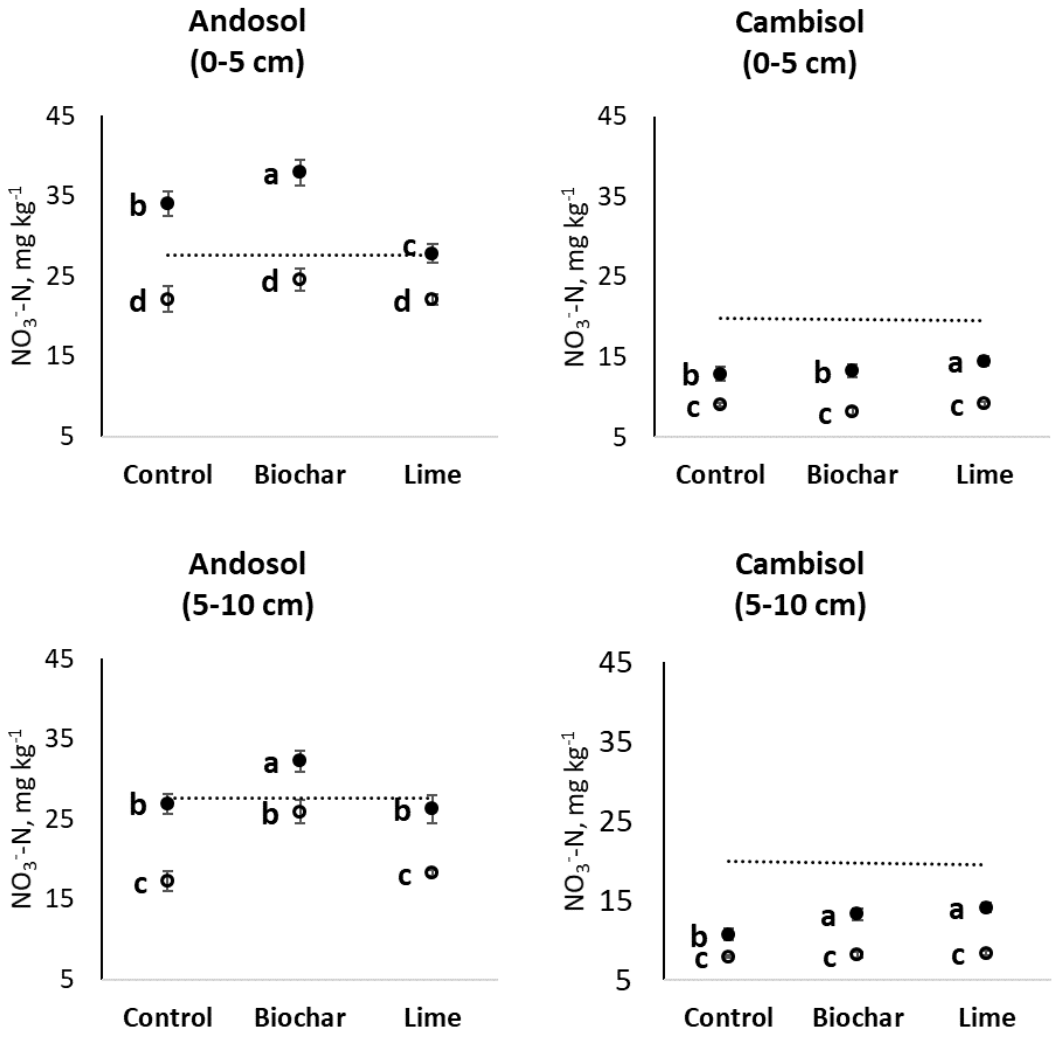
SI Figure 3.2 Organic carbon in experimental treatments at 0-5 cm and 5-10 cm depths at the end of the 6-month experiment. Values represent mean ± SE. Empty circles – treatments without earthworms, filled circles – treatments with earthworms. Lowercase letters indicate significant differences (Tukey HSD test, $\alpha<0.05$) within soil layer. Dashed line shows the value of the "initial soil".



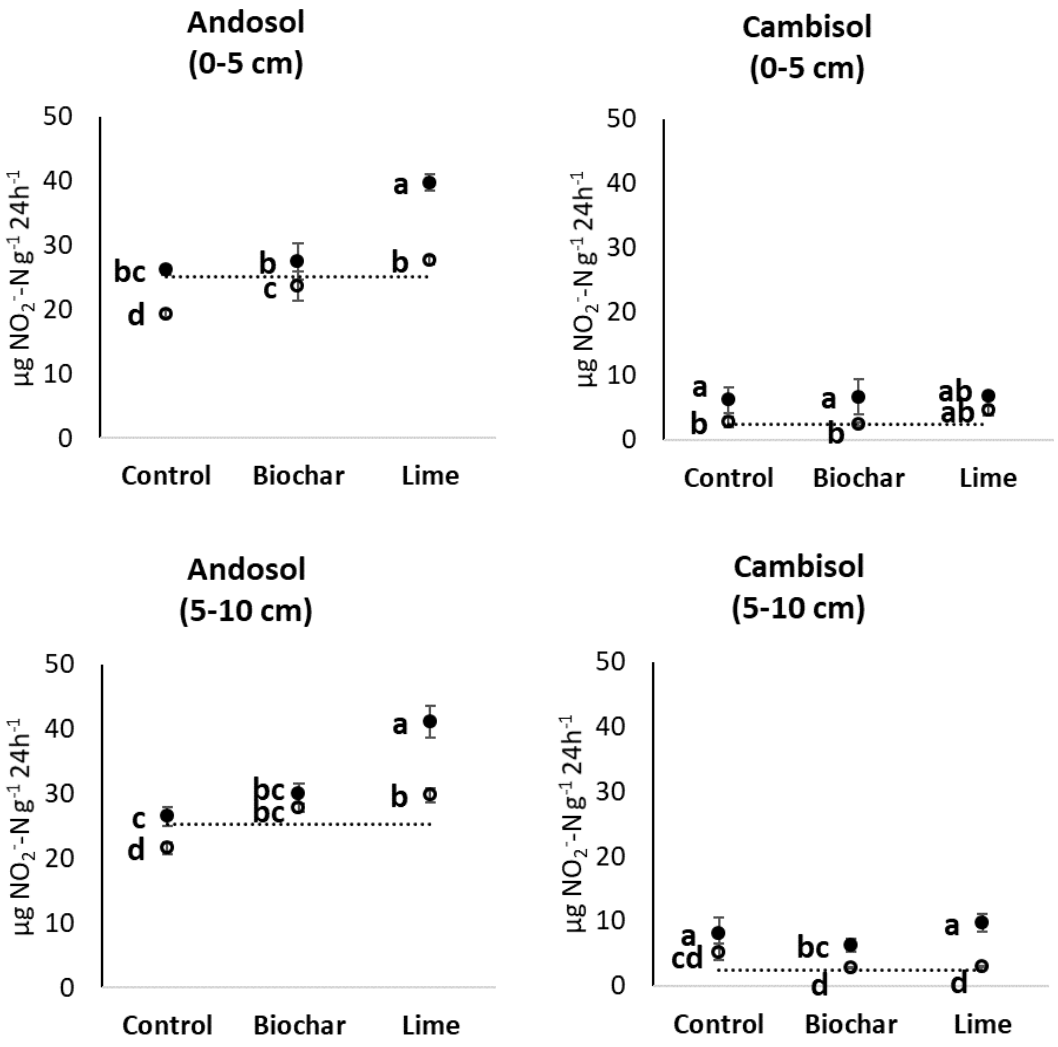
SI Figure 3.3 Total nitrogen in experimental treatments at 0-5 cm and 5-10 cm depths at the end of the 6-month experiment. Values represent mean ± SE. Empty circles – treatments without earthworms, filled circles – treatments with earthworms. Lowercase letters indicate significant differences (Tukey HSD test, $\alpha<0.05$) within soil layer. Dashed line shows the value of the "initial soil".



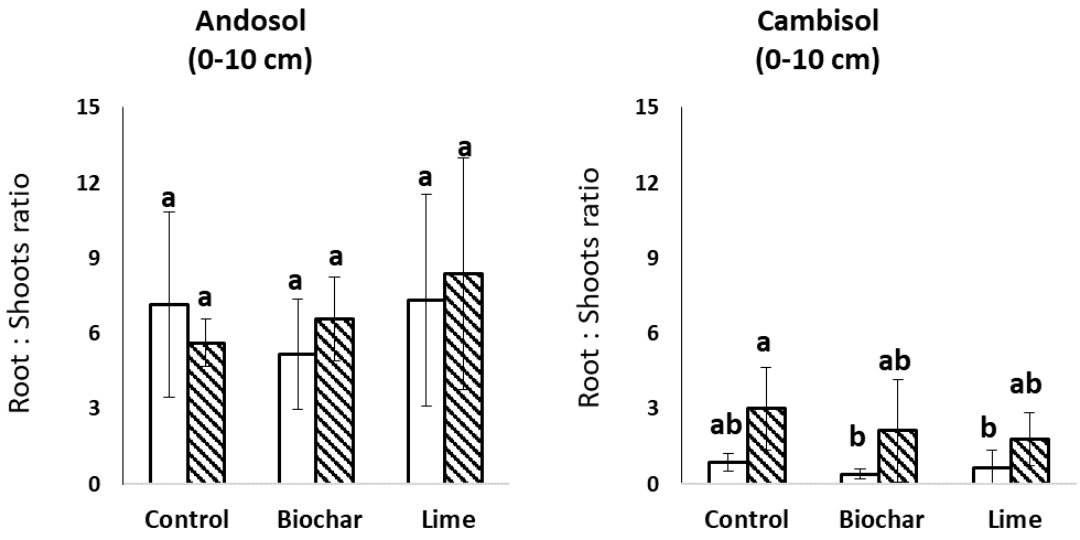
SI Figure 3.4 Ammonium nitrogen in experimental treatments at 0-5 cm and 5-10 cm depths at the end of the 6-month experiment. Values represent mean ± SE. Empty circles – treatments without earthworms, filled circles – treatments with earthworms. Lowercase letters indicate significant differences (Tukey HSD test, $\alpha<0.05$) within soil layer. Dashed line shows the value of the "initial soil".



SI Figure 3.5 Nitrate nitrogen in experimental treatments at 0-5 cm and 5-10 cm depths at the end of the 6-month experiment. Values represent mean ± SE. Empty circles – treatments without earthworms, filled circles – treatments with earthworms. Lowercase letters indicate significant differences (Tukey HSD test, $\alpha<0.05$) within soil layer. Dashed line shows the value of the "initial soil".



SI Figure 3.6 Nitrate reductase activity in experimental treatments at 0-5 cm and 5-10 cm depths at the end of the 6-month experiment. Values represent mean ± SE. Empty circles – treatments without earthworms, filled circles – treatments with earthworms. Lowercase letters indicate significant differences (Tukey HSD test, $\alpha<0.05$) within soil layer. Dashed line shows the value of the "initial soil".



SI Figure 3.7 Root to shoots ratio at the end of the 6-month experiment. Values represent mean ± SE. Empty bar – treatments without earthworms, striped – treatments with earthworms. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between treatments within the soil.

Chapter 3

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Stanislav Garbuz
Name/title of Primary Supervisor:	Dr Maria Minor
Name of Research Output and full reference:	
Garbuz, S. A., Camps Arbestain, M., MacKay A. D., DeVantier, B., & Minor, M. A. (2019). The interaction between biochar and earthworms, and their influence on soil properties and clover growth: a 6-month mesocosm experiment. Applied Soil Ecology	
In which Chapter is the Manuscript /Published work:	Chapter 3
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	65%
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
The candidate executed the experiment, collected all data, analysed the results and discussed findings. Brian DeVantier assisted with experimental work. The supervisors (M. Camps-Arbestain, A.D. MacKay, M. Minor) contributed to the design and planning of the experiment, provided advice on analysis, comments on the results, and contributed to manuscript writing.	
For manuscripts intended for publication please indicate target journal:	
Candidate's Signature:	Stanislav Garbuz 2020.02.26 11:53:47 +13'00'
Date:	26/02/2020
Primary Supervisor's Signature:	Maria Minor Digitally signed by Maria Minor Date: 2020.02.25 15:25:52 +13'00'
Date:	25/02/2020

(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)

Chapter 4

Effect of biochar on soil biological and physicochemical properties in two New Zealand pastures under livestock grazing: a field-based mesocosm experiment

Garbuz, S. A., Camps Arbestain, M., MacKay A. D., DeVantier, B., & Minor, M. A. Effect of biochar on soil biological and physicochemical properties in two New Zealand pastures under livestock grazing: a field-based mesocosm experiment. To be submitted to *Agriculture, Ecosystem and Environment.*

Abstract

Biochar application has been recognized as an effective way to improve soil functions. In this study, we investigated how adding biochar affects soil biological and physico-chemical properties in grazed pastures in a one-year field-based mesocosm experiment conducted on two sites with contrasting soils – a sil-andic Andosol and a dystric Cambisol. Each site had two paddocks managed under different agricultural practices: with and without effluent in the Andosol, and with either low or high P fertilizer input in the Cambisol. The soil amendment treatments were: (i) willow biochar produced at 350 °C (1% w/w); (ii) lime, added at the liming equivalence of the biochar application (positive control); (iii) no amendments (negative control). After 12 months, soil TN, OC and Olsen P contents significantly increased (for each $P<0.005$) in biochar-amended soils compared with initial values and controls. Changes in mineral N were site-specific. Biochar addition significantly ($P<0.005$) lowered soil BD compared with the control soil by, on average, 7% across all paddocks. Compared with the control and lime treatments, biochar-treated soils had significantly ($P<0.005$) higher values of bacterial (C_b) and fungal (C_f) biomass C. Earthworm abundance in lime-treated soils was significantly higher ($P<0.01$) than in the control. In the presence of biochar, earthworm abundance was only significantly higher ($P<0.05$) than the control in the Andosol without effluent. In biochar-amended soils, Collembola abundance was significantly higher ($P<0.005$) than the controls in all paddocks, while there was no effect on Oribatida and Gamasina populations. In all paddocks, root biomass was significantly higher ($P<0.005$; by 6.9 Mg ha^{-1} on average) in biochar-treated soils compared with the controls. Site*amendments interaction effect was significant ($P<0.005$) for C_f, C_b, Collembola abundance, and root biomass. The results provide evidence that adding biochar to the soil can positively affect soil food web and soil structure. Biochar from willow wood produced at low temperature may be an effective amendment in pasture soils as a part of sustainable farming practices.

4.1 Introduction

Applications of organic amendments, such as manure, compost, or effluent to soils as part of agricultural management practices, add nutrients and provide a source of energy and carbon (C) for heterotrophic biota, affecting soil biological conditions as well as physicochemical properties (Piqueres et al., 2006; Cleveland et al., 2007). A less widely used but promising organic amendment is biochar, a charcoal produced from biomass pyrolysis. Because biochar is rich in condensed aromatic C for which most microbes lack the required set of enzymes (Lehmann et al., 2009), it can persist in the soil over time, which offers an option for sequestering C, contributing to greenhouse gas emissions abatement (Lehmann et al., 2010; Lorenz & Lal, 2014). Understanding how adding biochar may affect soil biota and soil physicochemical properties is critical for ensuring that soil ecosystem services are maintained.

Soil biota are important in the functioning of soils and the provision of ecosystem services (Altieri, 1999; Khaziev, 2011; Yang et al., 2018a). Soil macro-, meso- and micro-fauna contribute to the effective functioning of soils (Wall, 2012) through (i) the fragmentation, incorporation, and decomposition of organic detritus, (ii) the cycling of nutrients, (iii) the formation and maintenance of microaggregates, (iv) the creation of habitats for sustaining diversity, and (v) the control of pests and disease. Microorganisms (bacteria and fungi) form the foundation of soil food webs and are the main drivers of decomposition and preservation of soil organic matter, with up to 50% of soil organic matter representing microbial-processed material (Aislabie & Deslippe, 2013; Liang et al., 2019). Extracellular microbial polymeric substances (e.g., glomalin from arbuscular mycorrhizal fungi) also contribute to particle binding and soil organic matter preservation (Tisdall, 1994; Boivin & Kohler-Milleret, 2011; Costa et al., 2018). Among soil macro- and meso-fauna, earthworms and arthropods have a key role in soil functions. Earthworms bury and incorporate plant litter and animal dung, pass litter and soil through

their gut, digest organic residues, and excrete casts; these are enriched in nutrients (van Groenigen et al., 2019), along with enzymes and microorganisms that contribute to organic matter transformation in soil (Bernard et al., 2012; Schon et al., 2012a). Through their burrowing, earthworms also modify soil porosity, which influences soil water regime and storage (McColl et al., 1982; Mackay et al., 1983; Shuster et al., 2002; Frouz et al., 2006). Arthropods that inhabit the soil contribute to litter fragmentation and nutrient mineralisation, as well as to soil mixing and pore construction (Culliney, 2013).

The nutrient content, amount of labile C, liming value, particle size, and application rate of biochar all interact to influence the soil microbial community (Pandian et al., 2016). The specific properties of a biochar depend on the type of feedstock and pyrolysis conditions. Biochar can increase the abundance of bacteria involved in the nitrogen (N) cycle, including denitrifiers (Anderson et al., 2011), and has a potential to reduce N_2O emission from the soil (Shi et al., 2019), although this is very dependent on biochar characteristics, particle size, application rate, method of application, and soil properties (Cayuela et al., 2014; Schirrmann et al., 2017). Many biochars have also liming properties that decrease exchangeable acidity and aluminium saturation in the soil (Chintala et al., 2013; Wang et al., 2014). Biochar has been shown to have a positive influence on mycorrhizal fungi abundance (Hammer et al., 2014; Shen et al., 2016), while the response of soil bacteria to biochar application can be variable depending on bacterial family (Gao et al., 2017) and the composition of native organic matter (Wang et al., 2015a). By impacting the soil microflora, biochar application can affect the entire soil food web (McCormack et al., 2013a).

Biochar has been reported to have a postive impact on earthworms activity (Topoliantz & Ponge, 2005; Van Zwieten et al., 2010), although Weyers and Spokas (2011) reported a decrease in density, weight, and reproduction of earthworms in soils amended with biochar. Ingestion of biochar might be beneficial to earthworms (Lehmann et al., 2011)

through (i) the additional grinding of litter by biochar particles in the earthworms gut, (ii) providing microbes that grow within biochar pores as a feed source, (iii) stimulating digestion enzymes, and (iv) reducing the availability of pollutants that put earthworms at risk. Biochar has been also shown to have a positive effect on soil mesofauna abundance (Hale et al., 2013; Godfrey et al., 2014; Conti et al., 2015). However, the mechanisms through which these effects occur, beyond the additional microbial food source, and/or enhanced physical stability of the soil, are not clear.

Our recent glasshouse mesocosm study (Garbuz et al., 2019) showed that biochar made from willow wood at a highest heating temperature of 350 °C, in association with earthworms, increased plant (white clover) growth. The positive effect of biochar on plant growth was not attributed to the liming properties of biochar, but rather to the positive impact of biochar on soil biota, N cycling, and nutrient availability. To establish if similar positive interactions between biochar and soil biota would occur under field conditions, we established a 12-month field mesocosm experiment in grazed pastures on two contrasting soils (a clayey dystric Cambisol and a loamy sil-andic Andosol). The objective of this experiment was to investigate whether biochar applied at ca. 12 Mg ha^{-1} would influence soil biological properties (earthworm abundance, fungal and bacterial C, arthropod abundances, and plant root biomass), physical properties (bulk density), and chemical properties (pH, organic C, total N, available phosphorus, mineral N), irrespective of the effect of biochar on soil pH.

4.2 Materials and Methods

4.2.1 Soils in this study

Two soils belonging to different soil orders described in **Chapter 3** were used in this study.

At Hawera, two paddocks grazed by dairy cows throughout the year were selected: one receiving dairy shed effluent (And-EF) and one not receiving effluent (And-NE). Both paddocks receive 160 kg of N as fertiliser N ha^{-1} yr^{-1}, 300 kg of 20% potash superphosphate ha^{-1} yr^{-1}, and 1 kg selenium prills ha^{-1} yr^{-1}. At the Ballantrae Research Station, two paddocks grazed by sheep throughout the year were selected: one (Cam-LF) had received no superphosphate since 1980 and the other (Cam-HF) receives 375 kg superphosphate ha^{-1} yr^{-1} since 1980.

4.2.2 Field-based mesocosm experiment

The field-based mesocosm experiment was conducted using large soil cores enclosed into sections of PVC cylinder pipe with a 15 cm Ø and a 30 cm length. Four holes (5.1 cm Ø) were made in the wall of each cylinder to allow the free movement of soil organisms (see **SI Fig. 4.1** and photos of the field experiment in **Appendix 4.1**). There were three treatments: (i) no amendments (the negative control), (ii) 1% of biochar application by weight (equivalent to approximately 12 Mg ha^{-1} for the Andosol and 12.5 Mg ha^{-1} for Cambisol), and (iii) lime applied at a rate corresponding to the liming equivalent of biochar (the positive control). Each treatment was replicated six times in each of the four paddocks.

During the southern hemisphere spring of 2017, 18 cores were excavated from each of the four paddocks. At the same time, the baseline samples for soil microorganisms, arthropods, bulk density and chemistry were collected. The cores were wrapped in a mesh for transporting to the laboratory. At the laboratory, the turf layer (ca. 2 cm) was split off, and the top 15 cm of soil below the turf layer was removed from all cores. All earthworms from the top soil were removed, identified, counted, labelled with the core code, and cold-stored. The soil of biochar and lime treatments was mixed with either biochar or lime, respectively. Particle size distribution of lime was as follows: 0.8, 20.3, and 78.9% for particles sizes of >1000 μm, 500-1000 μm, and <500 μm, respectively. The preparation

of biochar is described below. The soil of the negative controls was also removed and mixed. The mixed soil was placed back into the core, the earthworms were returned to the original core, and each core was covered with its original turf. The prepared cores were kept under controlled moisture (30% v/v) and temperature (approx. 20 ºC during the day and 10 ºC during the night) in the glasshouse for two weeks, at which point the cores were returned to their respective paddocks and placed in the ground close to where they had been sourced. Cores were installed in blocks of three. At each field, six pins (each corresponding to one block) were marked at least 1 m apart. Near each pin, three holes 15 cm Ø and 30 cm deep were dug using core cutter in a trianglular formation. The mesocosm cores were installed into the holes, with all three treatments in each block. The field experiment started on 24th October 2017 at the Ballantrae location (Cambisol) and on the 15th November 2017 at Hawera (Andosol), i.e., during the southern hemisphere spring. The experiment ran for ca.12 months until November 2018, when the cores were collected from the fields and sampled as described below.

4.2.3 Biochar Production and Characterisation

Biochar production and some characteristics are described in **Chapter 3.** Properties of the biochar are reported in **Table 3.1** from **Chapter 3**.

4.2.4 Soil Physical and Chemical Properties

Soil samples for chemical analysis were collected with a corer (3 cm Ø) from five depths: 0-2 cm (the turf), 2-9.5 cm, 9.5-17 cm, 17-20 cm, and 20-30 cm. All soils were air-dried. Soil bulk density (BD) was calculated by dividing the weight of soil oven-dried at 105° C by the core volume. Soil pH was measured in a 1:2.5 (w/v) ratio of soil:deionised water. Total C and total nitrogen (TN) contents were determined using a Vario Macro Cube (Elementar Analysensysteme GmbH, Germany). Inorganic C was negligible (< 0.05%), even in the lime-treated soil after 12 months of incubation, and thus total soil C was all organic (OC). Nitrate-nitrogen (NO_3^+-N) and ammonium-nitrogen

(NH_4^+-N) were determined following the method of Blakemore et al. (1987). For this, 1 g of soil was extracted with 20 mL of 2 M KCl, NO_3^--N and NH_4^+-N were measured by segmented flow auto-analysis using Technicon AA-II (Technicon, USA). Available phosphorus (Olsen P) was determined by the molybdenum-blue method using sodium bicarbonate extraction (Olsen et al., 1954). The initial physical and chemical properties of the two soils are described in **Table 4.1**.

Table 4.1 Initial physicochemical properties of the soils in four experimental pastures at 2-9.5 cm and 9.5-17 cm depths. Within a row, lowercase letters denote significant differences between fields, Tukey HSD post-hoc test, $P<0.05$, all global F-tests significant.

Soil	Depth, cm	Andosol NE	Andosol EF	Cambisol LF	Cambisol HF
TN, g kg-1	**2-9.5**	6.2 b	7.3 a	4.9 d	5.4 c
	9.5-17	5.4 b	6.5 a	4.0 d	4.8 c
Olsen P, mg kg-1	**2-9.5**	35.5 a	19.5 b	4.5 c	20.9 b
	9.5-17	31.4 a	15.8 b	4.3 c	16.8 b
OC, g kg-1	**2-9.5**	68.4 b	80.4 a	54.3 d	60.4 c
	9.5-17	55.1 b	68.3 a	44.2 d	50.1 c
BD, g cm^{-3}	**2-9.5**	0.68 a	0.69 a	0.71 a	0.71 a
	9.5-17	0.74 a	0.75 a	0.77 a	0.76 a
pH	**2-9.5**	5.6 b	5.9 a	5.1 c	5.2 c
	9.5-17	5.5 b	5.9 a	5.2 c	5.2 c
NO_3^-–N, mg kg^{-1}	**2-9.5**	21.5 b	35.3 a	2.3 d	10.8 c
	9.5-17	17.8 b	31.9 a	2.2 d	8.9 c
NH_4^+–N, mg kg^{-1}	**2-9.5**	22.6 a	24.5 a	8.6 c	15.6 b
	9.5-17	18.5 a	18.8 a	7.7 b	10.6 b

4.2.5 Soil Biological Properties

Samples (20 g) for soil microbial measurements were taken from the 2-17 cm depth and frozen at -30°C until analysed, but not longer than 2 weeks, as it was shown that freeze-storage for short time cannot influence on microbial respiration and acceptable for comparison between samples (Stenberg et al., 1998; Meyer et al., 2019). Fungal (C_f) and bacterial (C_b) biomass C were measured by the substrate-induced respiration (SIR) with selective inhibition. Briefly, 2 g of defrosted soil were incubated for 5 h immediately after the addition of glucose (2 mg g^{-1}). The concentration of CO_2 released by microorganisms was measured using a CO_2 analyser. Fungal and bacterial respiration was measured by

adding to glucose chloramphenicol (1 mg g^{-1}) and cycloheximide (2 mg g^{-1}), respectively. Fungal and bacterial biomass C was calculated according to Anderson and Domsch (1978).

Table 4.2 Initial soil biological properties in four experimental pastures. Within a row, lowercase letters denote significant differences between fields, Tukey HSD post-hoc test, $P<0.05$, all global F-tests significant.

Soil	Andosol NE	Andosol EF	Cambisol LF	Cambisol HF
Earthworms, ind m^{-2}	391.7 a	489.2 a	368.1 a	116.4 b
Bacterial biomass carbon, mg kg^{-1}	1049.1 b	1101.0 ab	1097.2 ab	860.7 a
Fungal biomass carbon, mg kg^{-1}	1016.3 a	555.2 b	417.1 b	556.3 b
Collembola, ind x 10^3 m^{-2}	29.4 a	12.6 b	9.1 c	28.1 a
Oribatida, ind x 10^3 m^{-2}	1.1 b	1.7 b	22.3 a	22.9 a
Gamasina, ind x 10^3 m^{-2}	2.6 b	4.2 b	5.8 a	9.7 a

Arthropods were extracted from fresh samples (collected by corer 5 x 5 cm) of the turf (0-2 cm) plus the soil depth mixed with amendments (2-17 cm) using the Tullgren funnels (Southwood & Henderson, 2009). The animals were stored in 70% ethanol, counted, and identified to order for Collembola and to suborder (Oribatida and Gamasina) for Acari using binocular microscope. Earthworms (from whole core) were hand sorted, identified to species when possible, and counted. Initial soils biological properties are described in **Table 4.2.** Plant roots from each core were collected, washed, oven-dried (40°C) and weighed.

4.2.6 Statistical Analysis

Normality of data sets was evaluated by the Shapiro-Wilk test. Analysis of variance (ANOVA) with contrast statements and Tukey HSD tests was used to investigate the

effect of factors: soil order (Andosol vs. Cambisol), fertilisation history (Andosol NE and EF; Cambisol LF and HF), amendment treatment (control, biochar, and lime), and soil depth (2-9.5 cm and 9.5-17 cm) on the following variables: soil biological properties (earthworm abundance, fungal and bacterial C, arthropod abundances, and plant root biomass), physical properties (bulk density), and chemical properties (pH, OC, TN, Olsen P, NO_3^--N and NH_4^+-N). Soil depth was used as a repeated measure in this analysis.

Principal Component Analysis (PCA) was performed for soil physico-chemical properties (BD, pH, OC, TN, NO_3^--N, NH_4^+-N, Olsen P, combined 2-9.5 and 9.5-17 cm depths) and biological properties (bacterial and fungal biomass, dry roots biomass, abundance of earthworms, Collembola, Oribatida and Gamasina), grouping them by factors (fertilisation history and treatment). Prior to PCA, the data was normalized by z-score standardization technique. Statistical analysis was carried out using R software version 3.3.3.

Table 4.3 Soil physical and chemical properties (mean) of the 2-9.5 cm and 9.5-17 cm depths after 12 months. Within a row, lowercase letters denote significant differences between treatments within a specific site, Tukey HSD post-hoc test, $P<0.05$, all global F-tests significant.

		Andosol NE			Andosol EF			Cambisol LF			Cambisol HF		
	Depth, cm	**Control**	**Biochar**	**Lime**	**Control**	**Biochar**	**Lime**	**Control**	**Biochar**	**Lime**	**Control**	**Biochar**	**Lime**
TN, g kg-1	**2-9.5**	6.5 b	7.3 a	6.3 c	7.4 b	7.9 a	7.4 b	5.12 b	5.8 a	5.1 b	5.6 b	6.01 a	5.5 b
	9.5-17	5.7 b	6.6 a	4.9 c	6.7 ab	6.9 a	6.4 b	4.2 b	4.7 a	4.1 b	4.5 c	5.3 a	4.9 b
Olsen P mg kg-1	**2-9.5**	38.7 b	41.7 a	39.3 ab	23.4 b	25.4 a	22.8 b	4.6 a	5.1 a	4.5 a	23.1 b	27.8 a	23.2 b
	9.5-17	31.1 b	37.4 a	31.7 b	15.5 b	17.9 a	15.5 b	4.1 b	4.6 a	4.2 b	14.2 b	18.1 a	14.4 b
OC, g kg-1	**2-9.5**	67.2 b	73.1 a	67.7 b	78.2 b	85.0 a	74.2 c	54.4 b	60.1 a	50.8 c	60.5 a	62.7 a	55.5 b
	9.5-17	56.6 b	65.8 a	56.2 b	69.4 b	80.1 a	65.8 b	44.8 b	51.3 a	44.9 b	48.6 b	53.1 a	50.5 b
BD, g cm^{-3}	**2-9.5**	0.65 ab	0.62 b	0.66 a	0.66 a	0.60 b	0.65 ab	0.68 a	0.63 b	0.68 a	0.69 a	0.65 b	0.68 a
	9.5-17	0.70 a	0.66 b	0.68 a	0.70 a	0.65 b	0.69 ab	0.73 a	0.67 b	0.72 a	0.72 a	0.67 b	0.71 a
pH	**2-9.5**	5.59 b	6.08 a	6.13 a	5.98 b	6.21 a	6.27 a	5.15 b	5.16 b	5.47 a	5.23 b	5.41 a	5.46 a
	9.5-17	5.43 c	5.68 b	5.84 a	5.91 b	6.12 a	6.20 a	5.14 c	5.24 b	5.49 a	5.18 b	5.38 a	5.46 a
NO_3^--N, mg kg^{-1}	**2-9.5**	21.9 b	25.2 a	23.2 b	35.9 a	36.0a	36.5 a	2.6 a	2.7 a	2.4 a	10.5 b	11.2 a	10.4 b
	9.5-17	18.1 b	21.5 a	21.7 a	30.2 a	31.1 a	29.9 a	2.4 ab	2.5 a	2.2 b	9.4 b	10.3 a	9.4 b
NH_4^+-N, mg kg^{-1}	**2-9.5**	23.1 a	24.5 a	23.2 a	24.4 a	20.7 b	24.9 a	8.3 a	8.6a	8.5 a	16.4 b	18.4 a	16.1 b
	9.5-17	20.1 b	22.4 a	19.6 b	20.8 a	18.9 b	20.8 a	6.9 a	7.3 a	7.0 a	9.0 a	9.7 a	9.01 a

Table 4.4 Soil biological properties (means) after 12 months. Within a row, lowercase letters denote significant differences between treatments within a specific field, Tukey HSD post hoc test, $P<0.05$, all global F-tests significant.

	Andosol NE			Andosol EF			Cambisol LF			Cambisol HF		
	Control	**Biochar**	**Lime**	**Control**	**Biochar**	**Lime**	**Control**	**Biochar**	**Lime**	**Control**	**Biochar**	**Lime**
Earthworms, ind m^{-2}	151.0 c	330.3 b	481.2 a	273.6 b	339.7 ab	462.4 a	325.5 b	500.1 ab	632.2 a	188.7 b	349.1 ab	519.0 a
Bacterial biomass carbon, mg kg^{-1}	1105.6 b	1574.3 a	1248.9 b	833.0 c	1390.4 a	1141.8 b	1927.1b	2252.9 a	1954.2 b	1411.4 b	1687.7 a	1508.2 ab
Fungal biomass carbon, mg kg^{-1}	372.3 b	701.4 a	485.1 b	451.0 c	1016.2 a	614.1 b	1121.0 b	1681.0 a	1180.2 b	468.7 b	980.7 a	616.9 b
Collembola, ind x 1000 m^{-2}	25.1 b	53.7 a	48.3 a	10.6 b	26.3 a	12.5 b	7.2 b	17.1 a	10.9 ab	28.0 b	49.5 a	41.6 ab
Oribatida, ind x 1000 m^{-2}	1.0 ab	2.0 a	0.5 b	1.8 a	2.0 a	1.4 a	24.2 a	33.1 a	67.3 a	28.7 a	39.9 a	30.2 a
Gamasina, ind x 1000 m^{-2}	2.3 a	5.9 a	2.6 a	3.3 a	5.3 a	2.9 a	7.1 a	12.1 a	8.9 a	11.9 b	16.7 a	15.2 b

4.3 Results

Neither biochar nor lime addition had affected the physical or chemical properties of the deeper soil (17-30 cm; results not shown). Below we only present results for the soil physical and chemical properties in the 2-9.5 cm and 9.5-17 cm soil depths. Where soil order*amendments interaction was significant, the results for Cambisol and Andosol are reported separately.

4.3.1 Soil physical and chemical properties

Bulk densities (BD) of the undisturbed Andosol and Cambisol were similar and increased with depth in all sampling sites (**Table 4.1**). Initial soil pH, OC, TN and total mineral N (NO_3^--N and NH_4^+-N) were higher in the Andosol than in the Cambisol. Olsen P was higher in And-NE soil than in And-EF soil, and higher in Cam-HF soil than in Cam-LF soil **(Table 4.1)**.

At the end of the experiment, the influence of the amendments was significant for TN, Olsen P, OC, and BD across all tested sites and depths. After 12 months, TN concentration increased in all amended soils ($P<0.005$) compared with initial values (**Table 4.1**, **Table 4.3**, **SI Fig. 4.2**), with the highest increase being in the soil to which biochar had been added. Biochar addition increased Olsen P ($P<0.005$, **Table 4.1**, **Table 4.3**, **SI Fig. 4.3**) in all paddocks. Soil disturbance caused some loss of soil OC concentration as is evident when comparing OC content of the initial soil with the control mesocosms at the end of the experiment (**Table 4.1**, **Table 4.3**, **SI Fig. 4.4**). Biochar significantly increased soil OC content (**Table 4.1**, **Table 4.3**, **SI Fig. 4.4**) compared with the initial soil ($P<0.005$), on average by 4.4 g kg^{-1} in 2-9.5 cm soil depth and 8.2 g kg^{-1} in the 9.5-17 cm soil depth. Lime-amended soil had significantly less OC at the 2-9.5 cm soil depth than the control ($P<0.001$), on average by 5 g kg^{-1}. At the end of the experiment, the BD of the sieved and repacked soil for the different mesocosms (at both depths) in all four sites was lower ($P<0.005$) than under undisturbed conditions (**Table 4.1**, **Table 4.3, SI Fig. 4.5**).

Addition of biochar further significantly lowered soil BD compared with the control soil ($P<0.005$), on average by 7% across all paddocks and both depths.

Soil order*amendments interaction was significant for soil pH and mineral N. As expected, pH values (**Table 4.1**, **Table 4.3**, **SI Fig. 4.6**) of the soil receiving lime were significantly higher ($P<0.005$) at all sites and at both depths. In both Andosol paddocks, soil amended with biochar had a higher soil pH than the control ($P<0.005$). In the Cambisol the liming effect of biochar was significant in Cam-HF but not in Cam-LF soil. Changes in mineral N (NO_3^--N and NH_4^+-N) were site-specific (**Table 4.1**, **Table 4.3**, **SI Fig. 4.7, SI Fig. 4.8**). Biochar-amended soil had higher ($P<0.005$) NO_3^--N and NH_4^+-N content in the And-NE and Cam-HF soils compared with the control. The And-EF soil amended with biochar had significantly less NH_4^+-N ($P<0.005$) than the control.

4.3.2 Biological properties

The initial earthworm abundance was lower in the Cam-HF soil than in all other paddocks (**Table 4.2**). C_b was highest in the Cam-HF soil and lowest in the And-NE soil, whereas the C_f in the And-NE soil was twice that of the other three sites, which all had similar C_f values (**Table 4.2**). The Collembola abundance was lowest in Cam-LF soil. Oribatida and to a lesser degree Gamasina populations were higher in the Cambisol paddocks (**Table 4.2)**.

Soil order*amendments interaction effect was significant for C_f, C_b, Collembola abundance, and root biomass. By the end of the experiment, C_b significantly increased ($P<0.005$) in all Cambisol treatments when compared to the corresponding values at the start of the experiment, whereas C_b values in the negative control of the two Andosols did not change over time.

After 12 months of field incubation, earthworm abundance (**Table 4.2**, **Table 4.4**, **SI Fig. 4.9**) in lime-treated mesocosms was higher than in the control of all soils ($P<0.01$). In the presence of biochar, earthworm abundance was only significantly higher in the

And-NE soil ($P<0.05$), although abundance values tended to be higher in all biochar-amended soils. Compared with control and lime treatments, soils treated with biochar had higher values of bacterial ($P<0.005$) and fungal ($P<0.005$) biomass C in all four paddocks (**Table 4.2**, **Table 4.4**, **SI Fig. 4.10**). Lime mesocosms had higher C_b and C_f relative to the control only in the And-EF soil ($P<0.05$). Similarly, root biomass was higher (by 6.9 Mg ha^{-1} on average) in biochar-treated soils at all sites, compared with the control and the lime treatment ($P<0.005$, **Fig. 4.1**). Assuming a root C percentage of 40%, this equates to an increase in the amount of standing root C of 1.2 - 4.0 Mg ha^{-1}. Collembola abundance in biochar-amended soil was significantly higher than the control soil at the all sites ($P<0.005$), and in lime-amended soil only in And-NE ($P<0.005$, **Table 4.2**, **Table 4.4**, **SI Fig. 4.11**). In general, amendments had no significant effect on Oribatida and Gamasina populations (**Table 4.2**, **Table 4.4, SI Fig. 4.12**).

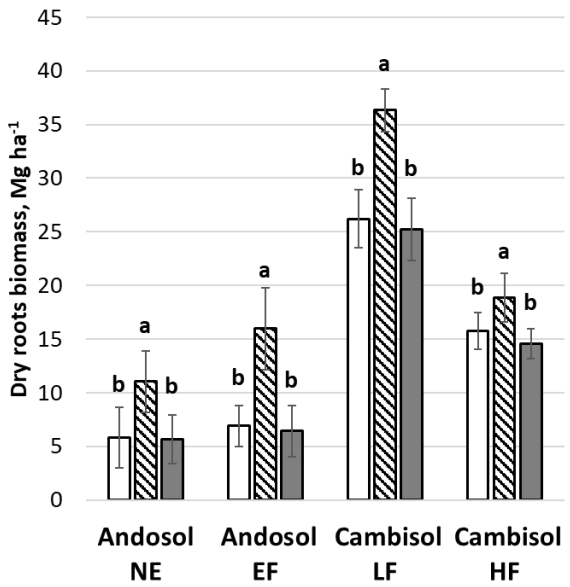


Figure 4.1 Pasture root biomass (oven-dry, 40°C) at the end of the experiment. Empty bar – Control, striped – Biochar, grey – Lime. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between treatments within a specific field.

4.3.3 Principal Component Analysis (PCA) of the soil properties

For the two Andosol paddocks, the first four principal components accounted for 81% of the total variability, with PC1 and PC2 accounting for 34.8% and 25.0%, respectively. The first principal component was mostly driven by the differences in soil chemical properties (OC, TN, pH, Olsen P, NO_3^--N) between the two Andosol paddocks (And-EF and And-NF), reflecting the influence of management history prior to the biochar or lime addition. The higher TN, pH, NO_3^--N and earthworm abundance values in And-EF soil are probably related to the effluent application (Kov et al., 2018). The second principal component was driven by the effect of amendments, with biochar application positively correlating with higher abundance of bacteria, fungi, earthworms, and springtails, and negatively correlating with bulk density and NH_4^+-N concentration (**Fig. 4.2**).

For the two Cambisol paddocks, the first four principal components accounted for 87.2% of total variability, with PC1 and PC2 accounting for 47.4% and 20.7%, respectively. Similar to what was seen in the Andosols, PC1 was driven by the differences in underlying characteristics of the two paddocks, attributed to the combination of fertiliser input and sheep stocking rate over the previous 37 years. Cam-HF paddock, with its history of high fertiliser application, had higher nutrient levels (Olsen P, NH_4^+-N, NO_3^--N). The PC2 was driven by the effect of amendments. Biological properties were again influenced by biochar addition, but while in the Andosol the effect was similar in both paddocks, in the Cambisol, the increase in root, fungi and bacteria biomass was more pronounced in the low-fertility Cam-LF pasture (**Fig. 4.2**).

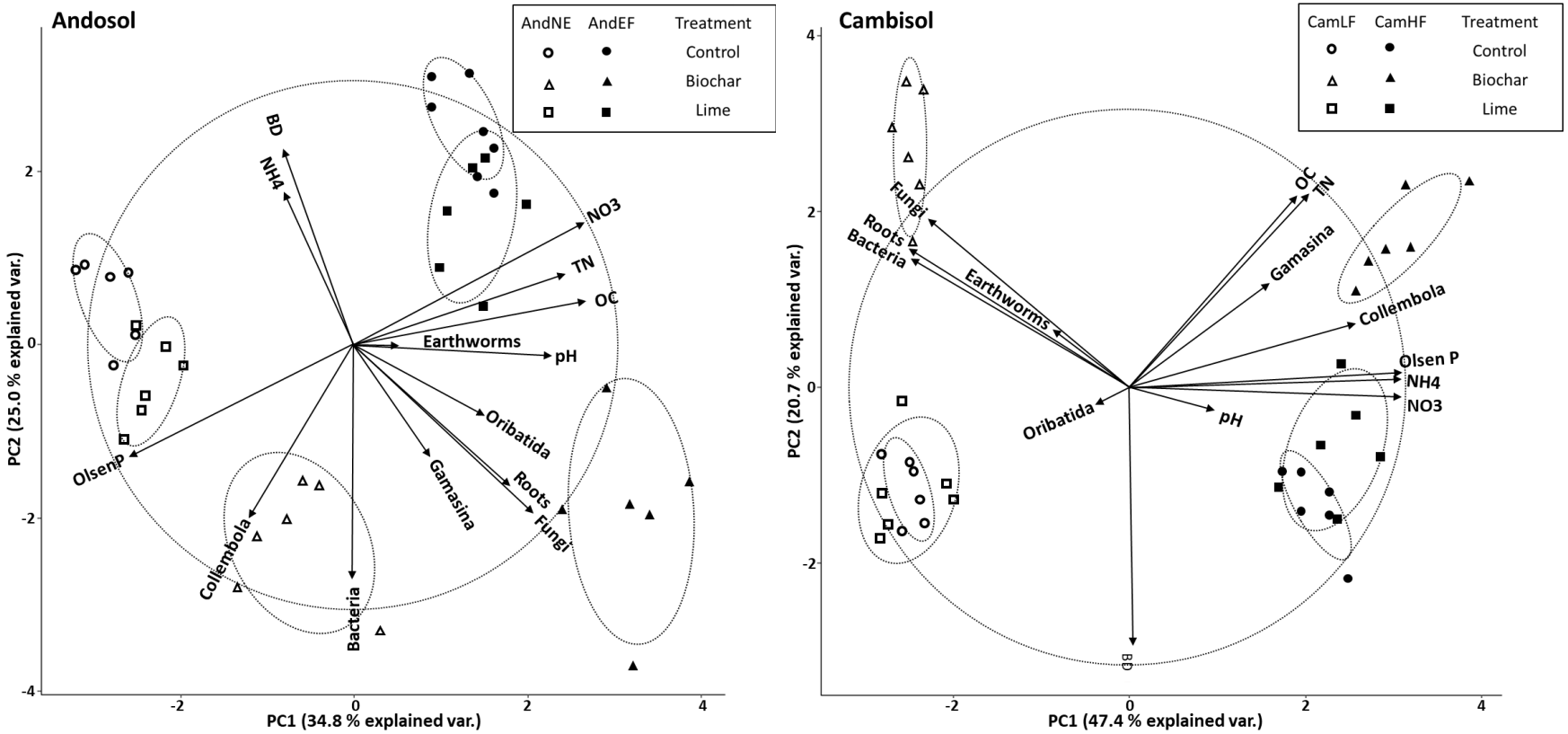


Figure 4.2 PCA bi-plot (PC1 vs. PC2) for the soil physicochemical and biological properties and oven-dry roots biomass for the Andosol and the Cambisol under different fertiliser application and experimental treatments within average of two depths. OC – organic carbon, TN – total nitrogen, NO3 – nitrate-nitrogen, NH4 – ammonium-nitrogen, Bacteria – bacterial biomass carbon, Fungi – fungal biomass carbon, Roots – dry roots biomass.

In the PCA bi-plots of the two soil orders, the scores of the biochar treatments clearly plot away from those of the other treatments, while those of lime do not (**Fig. 4.2**).

4.4 Discussion

The Andosol in this study is derived from volcanic ash, and is rich in short-range order constituents, which offer high organic matter protection, low BD, high anion retention, good physical properties and resilience to treading pressure (Molloy, 1998). The Cambisol is derived predominantly from sedimentary rock, specifically from loess materials that could contain some volcanic ash, and has a low anion storage capacity and limited physical resilience to treading pressure. Despite the higher levels of primary production of the two paddocks on the Andosol, particularly when compared with the Cambisol site that has not received any fertiliser inputs for 37 years (Cam-LF), the overall abundance of biota was generally similar across the two soil orders, with the exception of the Oribatida mites, which were several times less abundant in the Andosol than in the Cambisol. Orbatida can be a useful indicator of the amount of physical damage caused by grazing animal treading in pastures (Schon et al., 2012b); lower densities of Oribatida in the Andosol paddocks under dairy grazing vs. the Cambisol paddocks under sheep grazing could reflect differences in treading pressure, but in the current study, more research would be needed to prove this. The chemical, biological, and physical properties of both soils were affected by the addition of biochar and lime, despite the differences in soil mineralogy and management.

The biochar used in the present study significantly added to the soil carbon stocks. Mass balance indicates that at the end of the experiment the OC content of soil amended with biochar at 2-9.5 cm depth was higher (on average by 1.3 g kg^{-1} across all soils) than the expected content based on the amount of C added with the biochar (**Error! Reference source not found.1** in **Appendix 4.3**). We hypothesise that the effect of biochar on root growth contributed to this increase in soil OC stocks, as discussed in more detail further

in the text. The loss of OC from soils under lime treatment could partly be explained by the weakening of bonds of organic ligands and reactive mineral surfaces as alkalinity of the system increases (Kleber et al., 2015; Aye et al., 2016), thus increasing labile C. In the biochar-treated soils, this effect could have been compensated by the additional OC input generated from enhanced root growth.

Biochar amendment also contributed to the increase in TN (on average by 0.65 g kg^{-1} across all soils, **Error! Reference source not found.1**), which could be partly explained by the addition of N contained in biochar (a total of 0.21 g kg^{-1} soil, although N in biochar is mostly poorly available; Wang et al. 2012a), but also by biochar stimulating the N_2 fixation process in either the free-living bacteria in the soil or in the legume component of the sward (Rondon et al., 2007; Mia et al., 2014; de Assumpção, 2017). The latter is a reasonable assertion, given the significant increase in root growth in biochar treatments in all four paddocks (**Fig. 4.1**). The drop in NH_4^+-N in And-EF soil could be attributed to greater adsorption reactions at the surface of biochar particles (Ro et al., 2015; Park et al., 2019) in this soil with a pH value of 6.3 (pH values of the rest of biochar-amended soils were ≤ 6.1), yet other processes such as the direct interaction between biochar and N-containing organic compounds added with the effluent cannot be disregarded (Kameyama et al., 2012; Sarkhot et al., 2012).

At the end of the experiment the actual values of Olsen P in the soil amended with biochar were higher in both Andosol pastures and in the Cambisol with high fertility (Cam-HF) than the Olsen P values estimated based on available P added with biochar (**Error! Reference source not found.1**). Biochar added to the soil, besides contributing to P in soil, can increase available P through an enhanced P mineralization (Makoto et al., 2011; Gao et al., 2019) and P solubilisation/desorption caused by its liming properties (Gao et al., 2019) – although it should be noted that lime did not cause any significant increase in available P in our experiment.

The greater increase in soil pH caused by lime compared with biochar, despite both amendments having an identical liming equivalent, is attributed to the fact that the lime used in the present study was very fine (78% of particle sizes <500 μm), whereas biochar particles were coarser (38% particle sizes <500 μm and 42.9% particle sizes >1000 μm) and thus had a lower contact surface with soil (Sigua et al., 2014; Chen et al., 2017). The increasing liming effect of biochar as its particle size decreases has been well reported (Zaccheo et al., 2013; Liao & Thomas, 2019).

The addition of 1% biochar also resulted in a 4.6-9.1% decrease in bulk density in comparison with the control, well beyond the predicted 1.9% decrease estimated using the BD values of the biochar and soil, and their ratios in the mixture (**Fig. 4.3**). This suggests that biochar can influence factors contributing to the soil structural stability and pore structure (e.g., through interactions with soil biota), beyond the impact of an inert low BD material. The decrease in BD cannot be attributed to the stimulation of earthworm activity by the biochar, as earthworms also increased in the lime treatment, without an effect on soil BD. Other studies (Jien & Wang, 2013; Hardie et al., 2014; Kätterer et al., 2019) found a similar decrease in soil BD following biochar addition, and in one instance this decrease in BD was up to 17% (Jien & Wang, 2013).

Biological properties across all four fields showed significant but contrasting responses to biochar and lime application. Biochar showed a strong beneficial effect on root growth, with a two-fold increase in standing root biomass. The overall increase in root C in the biochar-amended soils compared with the negative control represents 1.2 to 4.0 Mg C ha^{-1}, in addition to the OC added with the biochar itself. This finding aligns with the meta-analysis of Xiang et al. (2017) who reported that biochar addition had a positive effect on root biomass, length, surface area, and morphology, with the associated increases in microbial activity in the rhizosphere and in the number of root nodules.

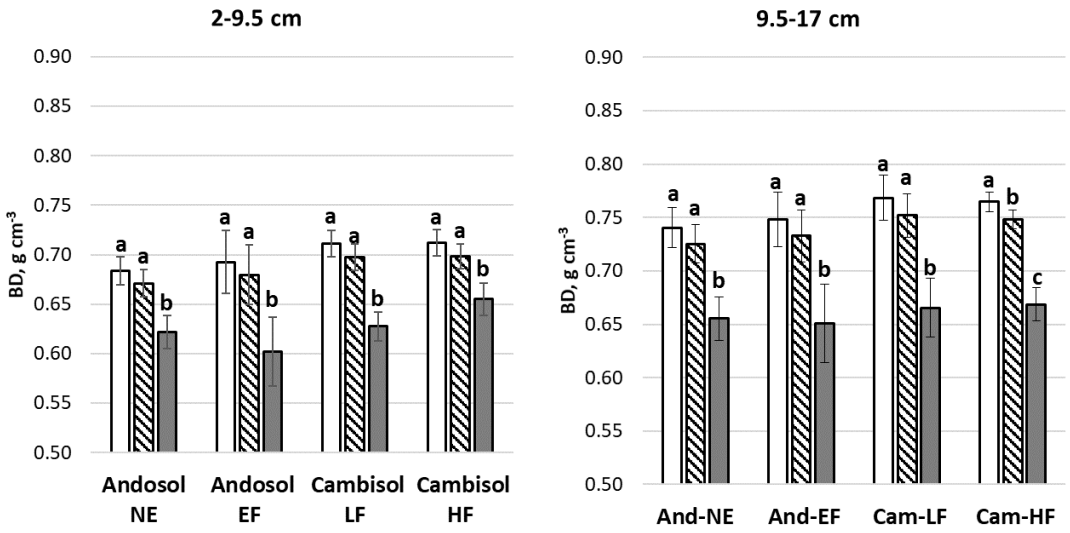


Figure 4.3 Mean soil bulk density of undisturbed soil (white), and the same soil treated with biochar - predicted (striped) and observed (grey) values. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between initial, predicted and actual values within a specific field.

In our study, the significant increase in root biomass in the presence of biochar could be related to the labile OC fraction in biochar, as this was produced at low temperature (a willow biochar made at 400° C contains about a volatile fraction on ash-free bases of 49%; Calvelo Pereira et al. (2011). Addition of labile C might have caused short-term N immobilization (Nguyen et al., 2017; Gao et al., 2019) and forced swards to allocate more OC into roots (Hill et al., 2006). Differences in mineral N were not detected after 6 months of experiment (refer to **Chapter 5**), indicating that N immobilization caused by the amendment was short term. The increase in root growth further decreased soil BD.

Biochar stimulated bacterial and fungal activity in all four paddocks (**SI Fig. 4.10**), whereas lime only stimulated the microbial community in some**.** The limited microbial response to lime as opposed to the meso- and macro-fauna response is an interesting finding in our study. In a brown Podzol in United Kingdoms and a typic Cambisol in Czech Republic (soils pH < 7), both biochar (with liming value) and lime addition have been shown to have positive effect on microbial (bacterial and fungal) activity (Shah et

al., 1990; Mühlbachová & Tlustoš, 2006). In the present study, the impact of biochar on the microbial community was not limited to its liming effect but appears to be the product of additional factors. The most obvious one, as it was mentioned before, is related to the contribution of labile organic C in this low temperature biochar, which provides an energy and a C source to microbes, as well as its indirect effect on root growth and the associated root detritus. Moreover, the micro-intra-particle structure of biochar can provide a physical growth matrix for arbuscular mycorrhizal fungi (Hammer et al., 2014). Biochar, besides labile C, can also provide soil microorganisms with nutrients (Anderson et al., 2011; Camps-Arbestain et al., 2017; Gao et al., 2017), yet the nutrient content of the biochar used in this study was low.

The increase in earthworms numbers with lime addition was expected, as they are sensitive to soil pH, with preference for neutral soils rather than either acid or alkaline, and to Ca availability, provided by lime (Nielson, 1951; Piearce, 1972; Opper et al., 2010). This is especially true in acidic soils (Satchell, 1955), as it is the case of the soils in our experiment, which had initial pH values $\leq$ 5.9. In biochar-amended soils, earthworms were less abundant than in the lime treatment, despite lime being applied to the liming equivalent of the biochar. Unlike lime, the biochar used in this study contains little Ca compared with lime (in our experiment each kg of soil received 4.8 mg Ca from biochar, and 295 mg Ca from lime).

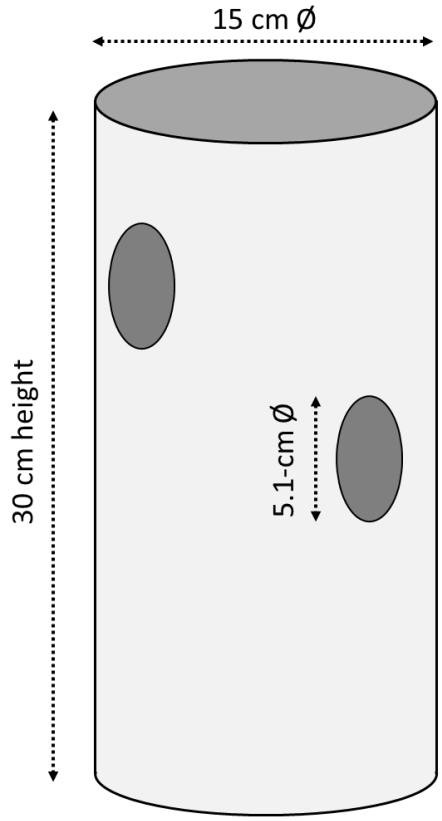
The increase in Collembola abundance in the biochar-treated soil (**SI Fig. 4.11**) as well that of earthworms could be explained by the positive effect of biochar on microbial biomass and root detritus, which would increase the food supply available for the soil food web (Hale et al., 2013; Conti et al., 2015). In this context, the absence of a clear effect of biochar on mites (Oribatida and Gamasina) abundance is hard to explain. It could be either a primary negative effect of biochar on the mites population that offsets the

positive food web effect (Domene, 2016), or the fact that seasonal dynamics overshadow the influence of the amendment. More research is needed in this regard.

4.5 Conclusion

The biochar from willow wood at an application rate of 12 Mg ha^{-1} had a positive overall effect on the soil environment and sampled soil biota of grazed pastures on two contrasting soils – it enhanced root growth, decreased soil BD, and stimulated soil biota. We hypothesize that the increase in root biomass was related to a N deficiency caused by N immobilization soon after the addition of this low-temperature biochar. We also propose that the greater microbial populations are the result of a combination of factors, including a direct effect on soil pH, labile C in biochar, bulk density, as already observed by De Tender et al. (2016) and Masto et al. (2013), and the additional root detritus. The associated increase in the microbial population created additional food sources and stimulated other trophic levels, such as Collembola. The enhanced root growth and soil biota might have contributed to the decrease in soil BD, although more research is needed in this regard.

Appendix 4.1



SI Figure 4.1 Scheme of PVC tube used in field based mesocosm experiment

1. The cores extraction

2. The cores extraction

3. The core preparation

4. Cutting the turf

5. Soil excavation

6. Soil mixing

7. Earthworms returning in the core

8. Soil returning in the core

9. The turf returning

10. Prepared cores

11. Prepared cores after 2 weeks

12. Drilling the hole

13. The core instalation

14. The core installation

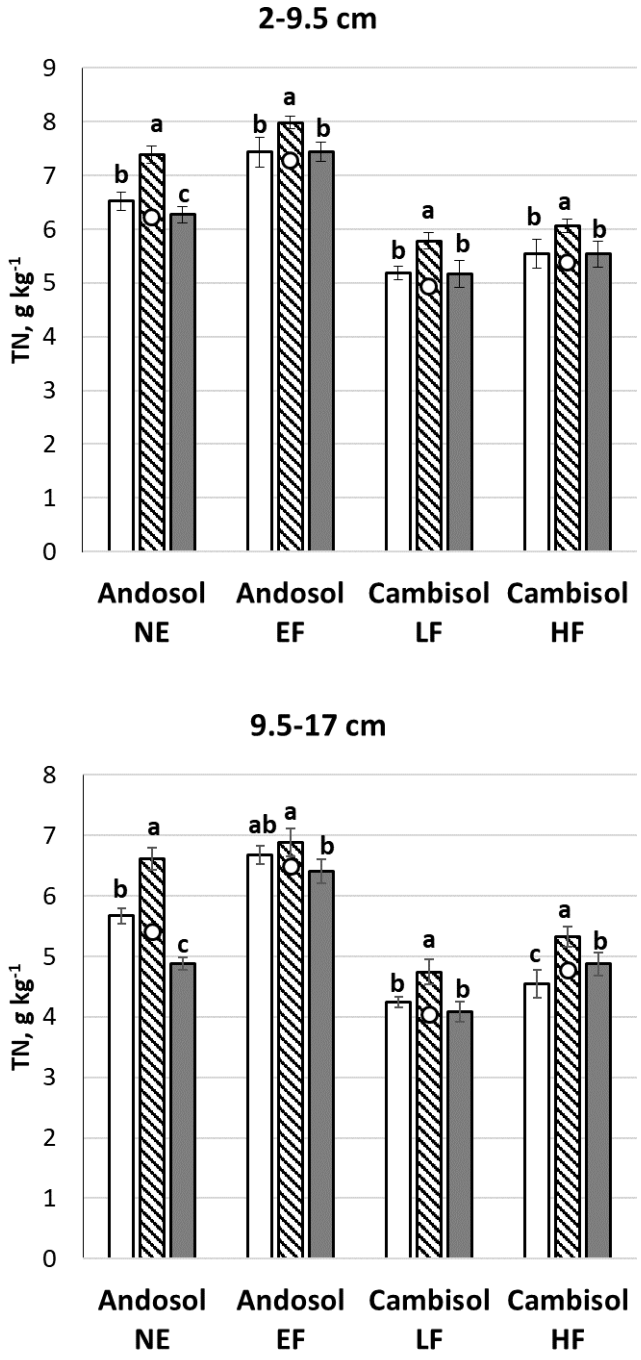
15. Installed core

16. Core cutting (extracted after 6/12 month)

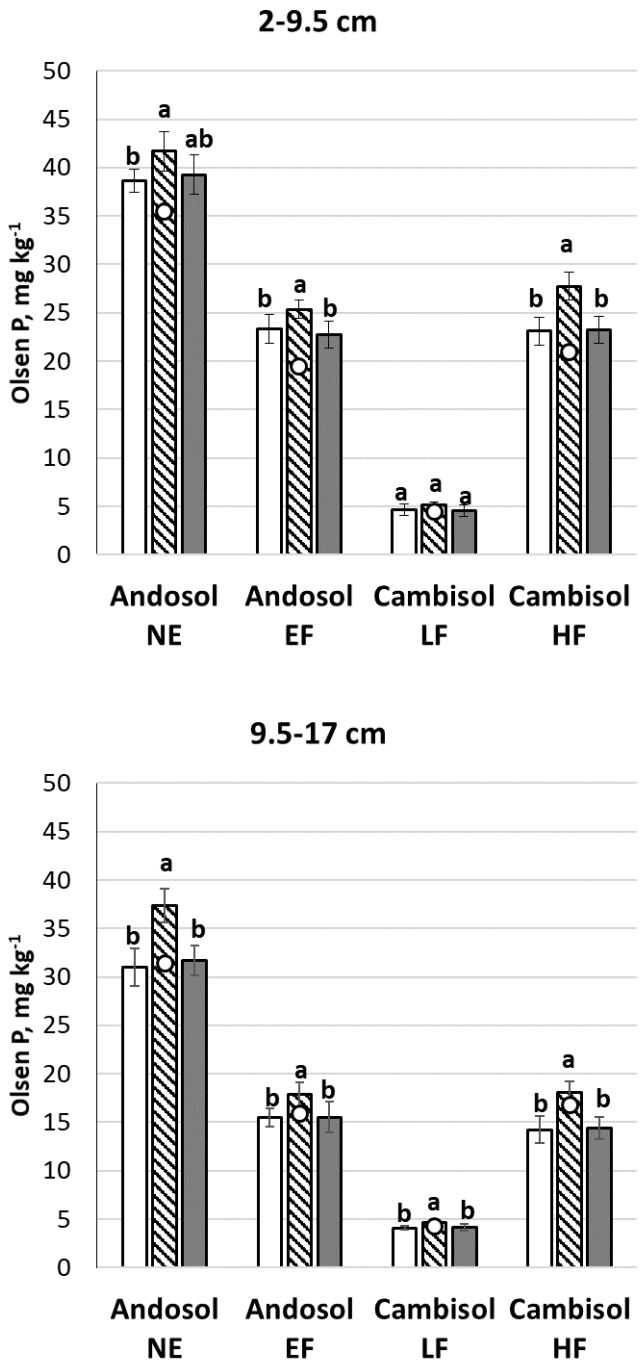
17. Core layer (extracted after 6/12 months)

18. Core layers (extracted after 6/12 months)

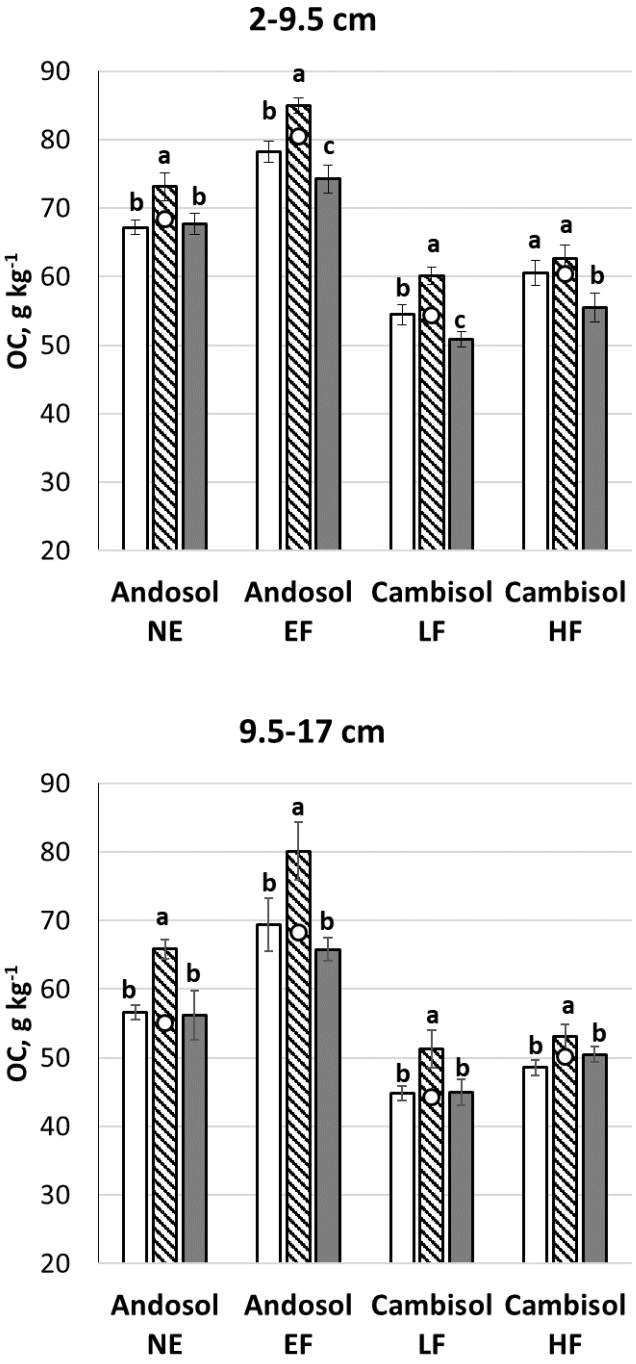
Appendix 4.2



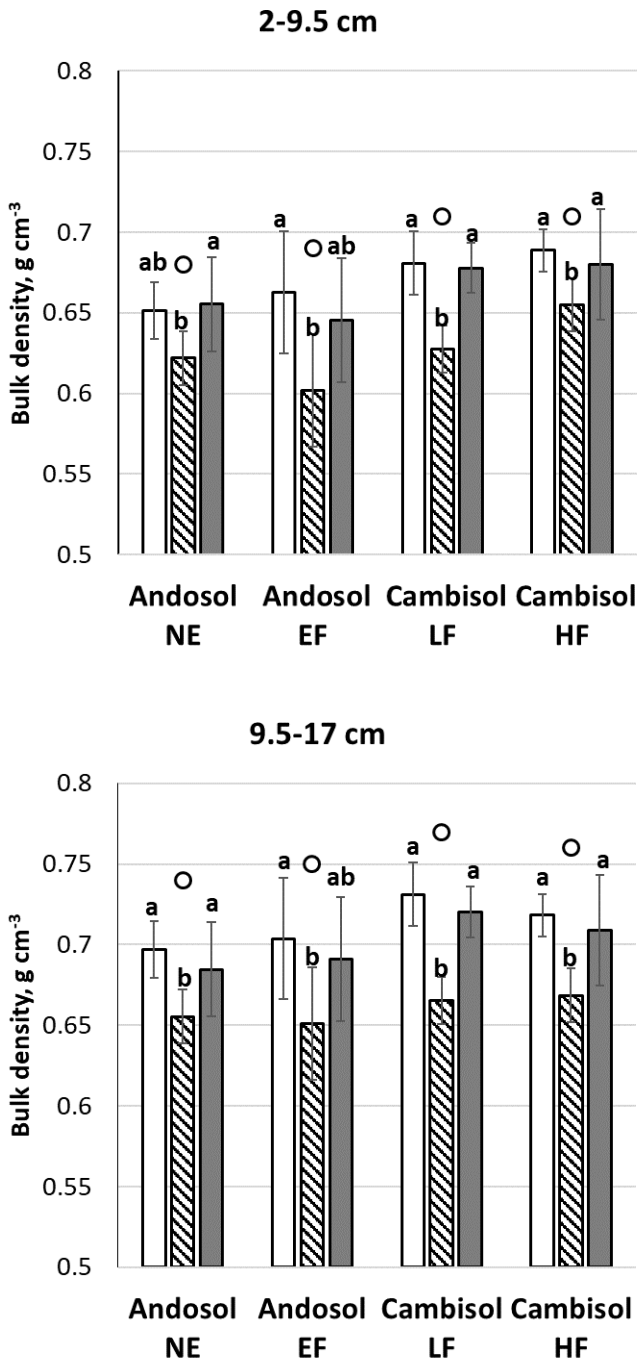
SI Figure 4.2 Soil total nitrogen concentration in experimental treatments at 2-9.5 cm and 9.5-17 cm depths at the end of the 12-month experiment. Values represent mean ± SE. Empty bar – Control, striped – Biochar, grey – Lime. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between treatments within a specific site. Circles represent the initial value in soil within the site.



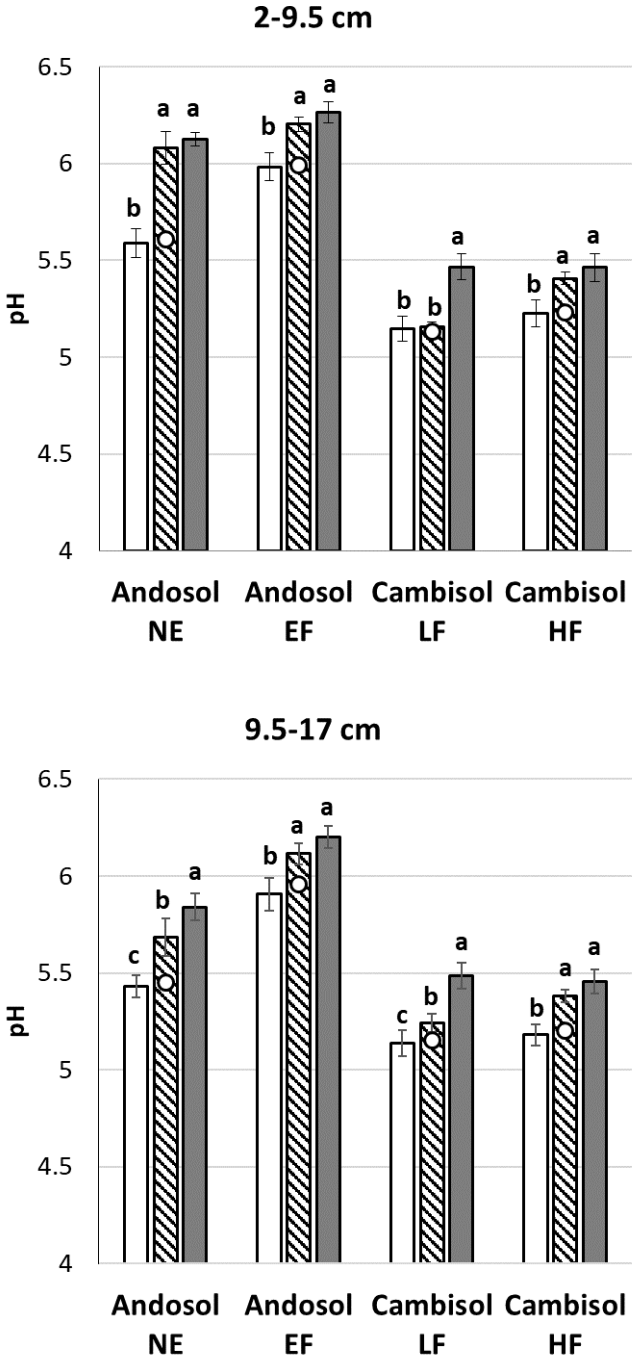
SI Figure 4.3 Soil available phosphorus (Olsen P) concentration in experimental treatments at 2-9.5 cm and 9.5-17 cm depths at the end of the 12-month experiment. Values represent mean ± SE. Empty bar – Control, striped – Biochar, grey – Lime. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between treatments within a specific site. Circles represent the initial value in soil within the site.



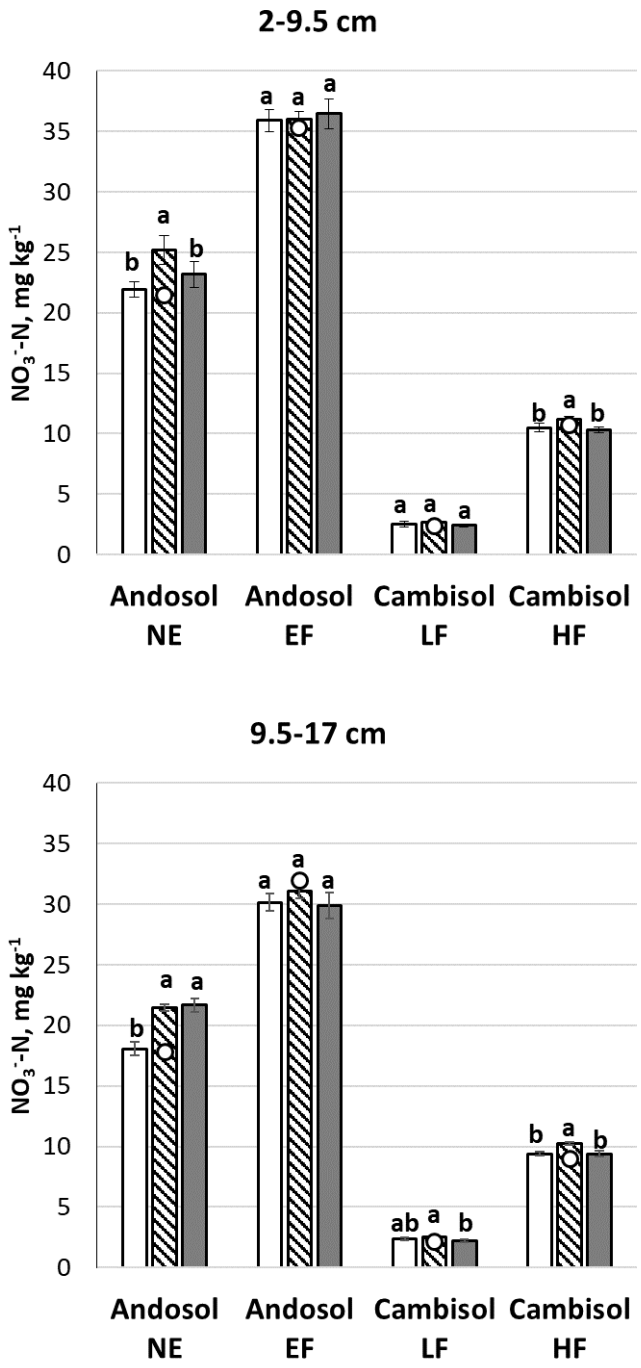
SI Figure 4.4 Soil organic carbon concentration in experimental treatments at 2-9.5 cm and 9.5-17 cm depths at the end of the 12-month experiment. Values represent mean ± SE. Empty bar – Control, striped – Biochar, grey – Lime. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between treatments within a specific site. Circles represent the initial value in soil within the site.



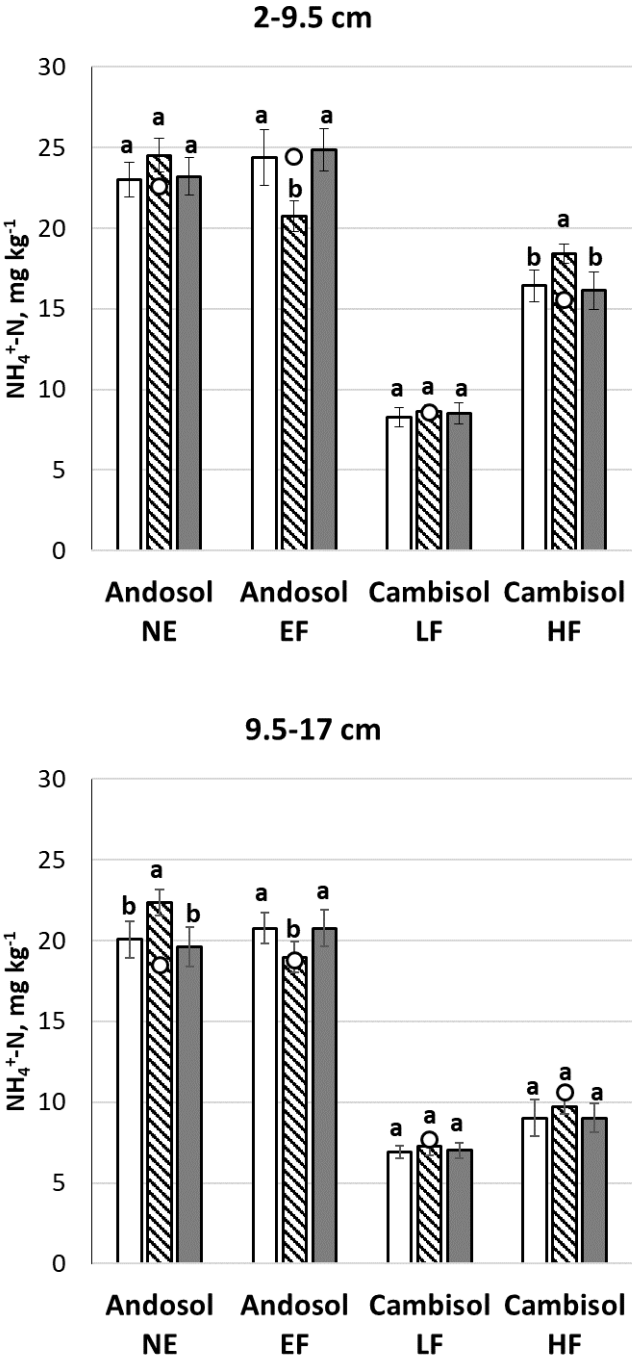
SI Figure 4.5 Soil bulk density in experimental treatments at 2-9.5 cm and 9.5-17 cm depths at the end of the 12-month experiment. Values represent mean ± SE. Empty bar – Control, striped – Biochar, grey – Lime. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between treatments within a specific site. Circles represent the initial value in soil within the site.



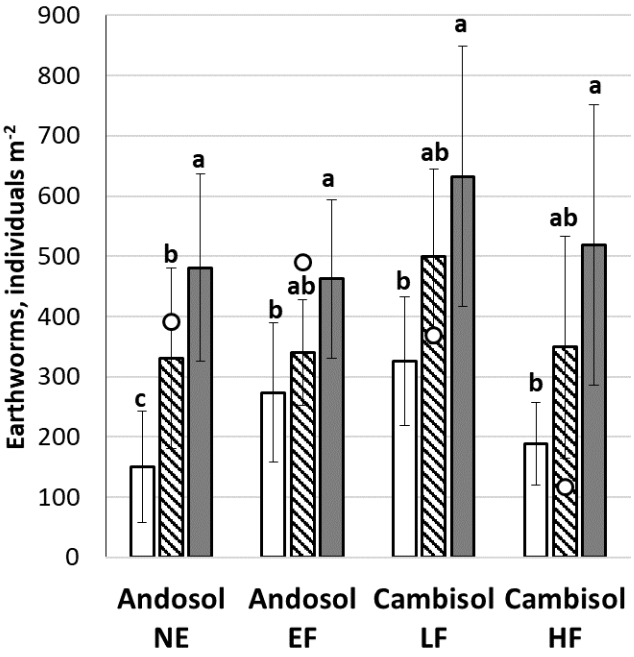
SI Figure 4.6 Soil pH in experimental treatments at 2-9.5 cm and 9.5-17 cm depths at the end of the 12-month experiment. Values represent mean ± SE. Empty bar – Control, striped – Biochar, grey – Lime. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between treatments within a specific site. Circles represent the initial value in soil within the site.



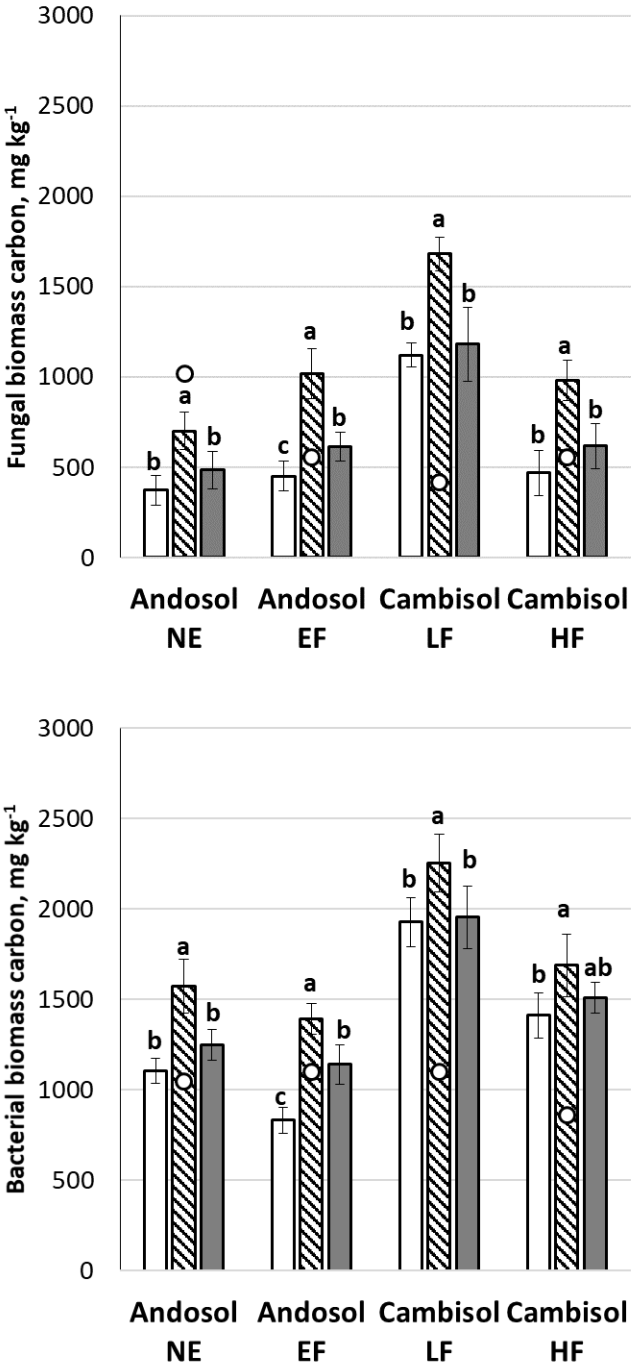
SI Figure 4.7 Nitrate-nitrogen concentration in experimental treatments at 2-9.5 cm and 9.5-17 cm depths at the end of the 12-month experiment. Values represent mean ± SE. Empty bar – Control, striped – Biochar, grey – Lime. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between treatments within a specific site. Circles represent the initial value in soil within the site.



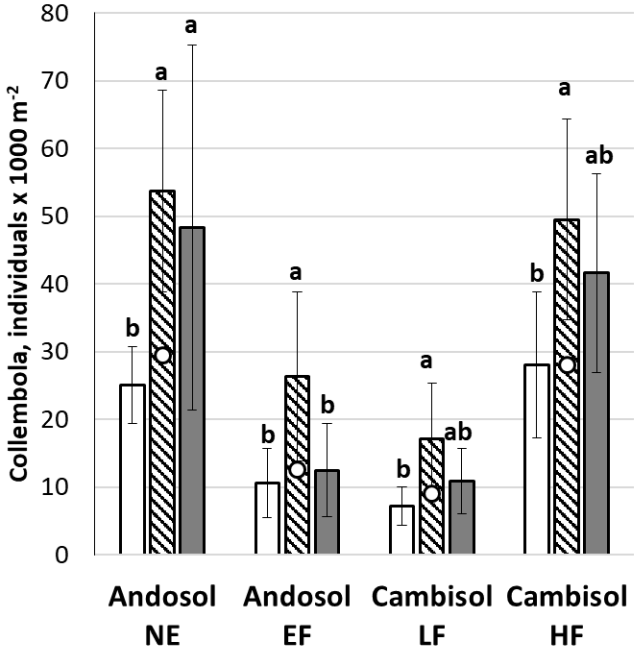
SI Figure 4.8 Ammonium-nitrogen concentration in experimental treatments at 2-9.5 cm and 9.5-17 cm depths at the end of the 12-month experiment. Values represent mean ± SE. Empty bar – Control, striped – Biochar, grey – Lime. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between treatments within a specific site. Circles represent the initial value in soil within the site.



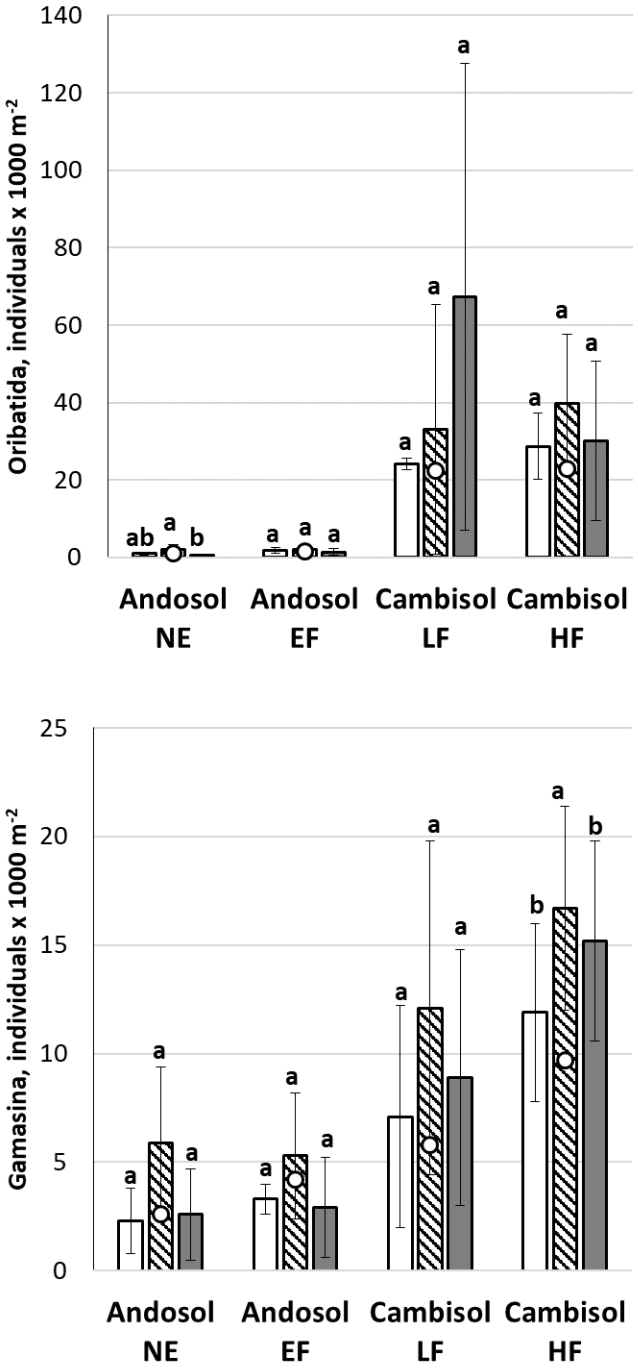
SI Figure 4.9 Soil earthworm abundance in experimental treatments at the end of the 12-month experiment. Values represent mean ± SE. Empty bar – Control, striped – Biochar, grey – Lime. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between treatments within a specific site. Circles represent the initial value in soil within the site.



SI Figure 4.10 Soil fungal and bacterial biomass carbon in experimental treatments at the end of the 12-month experiment. Values represent mean ± SE. Empty bar – Control, striped – Biochar, grey – Lime. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between treatments within a specific site. Circles represent the initial value in soil within the site.



SI Figure 4.11 Soil Collembola abundance in experimental treatments at the end of the 12-month experiment. Values represent mean ± SE. Empty bar – Control, striped – Biochar, grey – Lime. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between treatments within a specific site. Circles represent the initial value in soil within the site.



SI Figure 4.12 Soil Oribatida and Gamasina abundances in experimental treatments at the end of the 12-month experiment. Values represent mean ± SE. Empty bar – Control, striped – Biochar, grey – Lime. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between treatments within a specific site. Circles represent the initial value in soil within the site.

Appendix 4.3

Calculation of predicted effect of biochar on soil physical and chemical properties

The predicted resultant bulk density of the biochar and soil mixture was estimated using the formula:

$$BDpred. = \frac{100}{(1/BDinital * 99) + (1/BDbiochar * 1)}$$

where:

BDinitial is the BD of soil and *BDbiochar* is BD of biochar

The predicted soil pH was calculated according to Singh et al. (2017b)

$$pHpred. = \left(\frac{Rate * LE * 2}{BCsoil}\right) + pHsoil$$

where:

Rate is biochar application ratio (g biochar per 100 g of soil), *LE* is liming equivalence – % $CaCO_3$-eq, *BCsoil* is buffering capacity of soil (method not included here, for both Andosol is 25 cmolc kg^{-1} and for both Cambisol is 11 cmolc kg^{-1}), *pHsoil* is initial soil pH.

The estimated OC, TN and Olsen P of the biochar-amended soil at initial is provided in **SI Table 4.1**.

Predicted effect of biochar on soil physical and chemical properties

The bulk density of the soil upon biochar addition, was estimated to have caused an average decrease of soil BD of 2% (average across the two soils and depths) (**Fig. 4.3**). Soil pH should have increased with biochar addition 0.3 pH units in the Andosol and 0.7 pH units in the Cambisol. OC should have increased by 6.78 g kg^{-1} average (from 7.6 to 13.5%) with biochar addition and TN by 0.11 g kg^{-1} (from 1.6 to 2.6%) and Olsen P 7.8 % in And-NE, 13.7% in And-EF and Cam-HF and 40% in Cam-LF.

SI Table 4.1 Predicted soil properties after adding biochar, in brackets number showed changes of the properties in percentage from the initial.

	Depth, cm	Andosol NE	Andosol EF	Cambisol LF	Cambisol HF
BD, g cm^{-3}	**2-9.5**	0.67 (1.8)	0.68 (1.8)	0.70 (1.9)	0.70 (1.9)
	9.5-17	0.73 (2.0)	0.73 (2.1)	0.75 (2.2)	0.75 (2.1)
pH	**2-9.5**	5.9 (5.4)	6.3 (5.1)	5.8 (12.0)	5.9 (11.8)
	9.5-17	5.8 (5.6)	6.28 (5.1)	5.9 (12.0)	5.9 (11.9)
OC, g kg^{-1}	**2-9.5**	75.0 (8.9)	87.1 (7.6)	61.2 (11.3)	67.3 (10.2)
	9.5-17	61.7 (10.8)	74.9 (8.9)	51.1 (13.5)	57.0 (12.1)
TN, g kg^{-1}	**2-9.5**	6.3 (1.6)	7.4 (1.4)	5.0 (2.1)	5.5 (2.0)
	9.5-17	5.5 (1.9)	6.6 (1.6)	4.1 (2.6)	4.9 (2.2)
Olsen P, mg kg^{-1}	**2-9.5**	38.3 (7.3)	22.3 (12.6)	7.4 (39.4)	23.9 (12.2)
	9.5-17	34.2 (8.2)	18.7 (15.0)	7.2 (40.6)	16.7 (14.8)

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Stanislav Garbuz
Name/title of Primary Supervisor:	Dr Maria Minor
Name of Research Output and full reference:	
Garbuz, S. A., Camps Arbestain, M., MacKay A. D., DeVantier, B., & Minor, M. A. Effect of biochar on soil biological and physicochemical properties in two New Zealand pastures under livestock grazing: a field-based mesocosm experiment	
In which Chapter is the Manuscript /Published work:	Chapter 4
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	70%
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
The candidate executed the field trial, collected samples, analysed data and interpreted the laboratory results. The supervisors (M. Camps-Arbestain, A.D. MacKay, M. Minor) contributed to the design and planning of the experiment, provided advice on analysis, comments on the results, and contributed to manuscript writing. Brian DeVantier assisted with planning and conducting the experimental work and samples collection.	
For manuscripts intended for publication please indicate target journal:	
Agriculture, Ecosystems and Environment	
Candidate's Signature:	Stanislav Garbuz 2020.02.26 11:56:07 +13'00'
Date:	26/02/2020
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Date:	26/02/2020

(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)

Chapter 5

Enzyme activities and the influence of biochar addition on soil biochemical processes in two New Zealand pasture soils

Garbuz, S. A., Camps Arbestain, M., MacKay A. D., DeVantier, B., & Minor, M. A. Enzyme activities and the influence of biochar addition on soil biochemical processes in two New Zealand pasture soils. To be submitted to *Geoderma.*

Abstract

Many studies indicate that biochar application influences soil biochemical activity – e.g., enzymes – through changes in chemical and biological properties. In this study, we investigated how adding biochar alters activities of seven enzymes involved in the C, N and P cycles of two contrasted soils – a sil-andic Andosol and a dystric Cambisol – both under grazed pastures in a one-year field-based mesocosm experiment. Each site had two paddocks managed under contrasting agricultural practices: with and without effluent in the Andosol (And-EF and And-NE, respectively), and with either low (Cam-LF) or high P fertilizer input (Cam-HF) in the Cambisol. The soil amendment treatments were: (i) willow biochar produced at 350 °C (1% w/w); (ii) lime, added at the liming equivalence of the biochar application (positive control); (iii) no amendments (negative control).

Following 12 months of field incubation, soil amendments – biochar and lime – significantly affected enzymes activities, but the effect was variable. With the exception of acid phosphatase, all other enzymes were associated with the addition of biochar or lime amendments and showed strong correlation with soil biota. Biochar addition was the driver for urease (in the Cambisol), cellulase, and dehydrogenase activity. Higher peroxidase activity (in both soils), and nitrate reductase (in the Andosol) were associated with lime application. Both biochar and lime had a negative effect on acid phosphatase, but increased activity of alkaline phosphatase. The Geometric Mean of Enzyme Activity (GMea) was higher in the biochar- and lime-amended soils in comparison to the control, and was highest in the soil treated with biochar in all paddocks except Cam-LF. In both soils the enzymes activities, with few exceptions (e.g., peroxidase), declined with the depth, and the effects of biochar or lime addition were observed primarily in the 2-9.5 cm and 9.5-17 cm soil depths.

There were marked differences in enzyme activity between soil orders and between management practices (paddocks) within a soil order. Phosphatases, urease,

dehydrogenase, and especially nitrate reductase activities were higher in the Andosol, which also had higher pH, OC, TN, mineral N, and Olsen P (see Chapter 2), while cellulase activity was higher in the Cambisol. There was no significant difference in peroxidase activity between the two soil orders. The GMea values were about two times higher in the Andosol than in the Cambisol. The paddocks with effluent addition (And-EF) and with high P fertiliser input (Cam-HF) had higher phosphatases, nitrate reductase, and dehydrogenase activities than their low-fertility equivalents. In the Andosol, the soil receiving effluents (And-EF) had higher urease and cellulase, but lower peroxidase activity than the same soil without effluent (And-NE). In the Cambisol the opposite was found – the soil with high P fertilizer input (Cam-HF) had higher peroxidase activity and lower cellulase activity than the low fertility soil (Cam-LF).

The results suggest that biochar and lime have different mechanisms of their interaction with soil biological processes. For example, dehydrogenase activity was strongly correlated with soil microbial biomass, which was increased by biochar application. Cellulase had higher activity in soil with highest root biomass, and we hypothesise that the biochar, through stimulation of root growth and, consequently, of the amount of plant detritus entering the soil, increased the activity of cellulase. Peroxidase, unlike dehydrogenase and cellulase, had higher activity in the soil treated with lime, and was positively correlated with earthworm abundance which also was higher in the lime-treated soil. There was a positive correlation between nitrate reductase and earthworm abundance, as earthworms increase nitrate concentration in soil. Both biochar and lime had significant effect on the GMea, with biochar having a more pronounced effect.

This chapter discusses how interactions between amendments (biochar and lime), soil, plants, and functional groups of soil organisms influence particular enzymes and the enzymatic activity of the soil, and in that way affect the carbon, nitrogen and phosphorus cycles.

Chapter 5

5.1 Introduction

When considering soil ecosystem services, most attention is focused on soil natural capital stocks, especially on those that constitute the so-called “manageable properties”. For example, soil porosity, and stocks of soil carbon and nutrients play a key role in the ability of soils to provide regulating and provisioning services (Adhikari & Hartemink, 2015) – studying ways to manage these properties is important in order to maintain or increase the soil capability to meet human needs (Dominati, 2013).

However, to gain a full understanding of soil ecosystem services, dynamic soil processes (flows) should be considered in addition to soil natural capital stocks. Soil enzymes (**Table 5.1**) are an integral part of the soil nutrient and energy exchange, as they provide the link between soil biotic and abiotic components (Yang & Wang, 2002; Sinsabaugh et al., 2008). At the same time, enzymes allow the quantification of soil processes (flows) that underpin ecosystem services such as carbon sequestration (Makoi & Ndakidemi, 2008; Chen et al., 2018), soil detoxification and self-purification (Rao et al., 2010) and nutrient cycling, which enables plant growth (Shi, 2011; Jog et al., 2012).

Table 5.1 Sources and functions of selected soil enzymes

Enzyme	Source	Soil function	Process	Product	Factors influencing enzyme activity
Cellulase (Cel) [1]	Fungi, bacteria, protozoans	C-cycling	Decomposition of cellulose	Glucose	Temperature, pH, water, quality and location of organic matter
Peroxidase (PO) [2, 3]	Fungi, bacteria, plants, invertebrates	C-cycling	Decomposition of lignin	C compounds	Soil pH, soil aeration, temperature, SOM content, management practices
Dehydrogenase (DHG) [4]	Mostly microorganisms	C-cycling	Oxidation of organic compounds	Transfer of H to NAD or NADP	Soil water content, soil aeration, temperature, management practices
Nitrate reductase (NR) [5,6]	Bacteria, fungi, plant root	N-cycling	Nitrate reduction to nitrite	Nitrite (NO_2^-)	Soil pH, temperature and water content
Urease (Ure) [3,7]	Microorganisms, plants, some invertebrates	N-cycling	Hydrolysis of urea	Ammonia (NH_3) and CO_2	Organic matter content, management practices, temperature, pH
Acid phosphatase (AcP) / Alkaline phosphatase (AlP) [8,9]	Plants, fungi, bacteria	P-cycling	Hydrolysis of esters and anhydrides of phosphoric acid	Phosphate (PO_4)	Organic matter content, pH, management practices

[1] Deng and Tabatabai (1994a); [2] Sinsabaugh (2010); [3] Das and Varma (2011); [4] Wolińska and Stepniewska (2012); [5] Firestone (1982); [6] Abdelmagid and Tabatabai (1987); [7] Lloyd and Sheaffe (1973); [8] Eivazi and Tabatabai (1977); [9] Nannipieri et al. (2011).

Soil enzymes (**Table 5.1**) are involved in multiple processes including mineralization of organic materials, as well as carbon (C), nitrogen (N), and phosphorus (P) cycles (Stevenson & Cole, 1999; Sardans et al., 2008; Das & Varma, 2011). For example, cellulase (Deng & Tabatabai, 1994a), peroxidase (Sinsabaugh, 2010) and dehydrogenase (Wolińska & Stepniewska, 2012) are involved in organic matter (OM) decomposition and further transformation of organic polymers, which are important for maintaining soil aggregate stability. Nitrate reductase and urease are involved in the N cycle and can be used to assess the soil N transformation rate (Lloyd & Sheaffe, 1973; Firestone, 1982). Phosphatases transform organic P (phosphoric acid monoester) into inorganic form (PO_4) available for plants and microorganisms, and consequently, play a key role in plant P nutrition (Eivazi & Tabatabai, 1977).

As a product of biological activity, enzymes are closely linked to abundance, community structure and activity of soil microorganisms and soil micro- and meso-fauna (Caldwell, 2005). Large soil animals, such as earthworms and some arthropods, influence concentration and activity of soil enzymes in three ways (Moldenke et al., 2000; Kizilkaya et al., 2011): by releasing their own gut enzymes; by changing the microbial community inside their intestine and in their excreta; by changing physicochemical properties of the soil through their burrowing.

Persistence of enzyme activities in the soil is influenced by soil temperature, pH, nutrient content, ionic conditions, and inhibitors (Sinsabaugh et al., 1994; Burns et al., 2013). Enzymes released into the soil can become bound to clay minerals and OM (**Fig. 5.1**), which affords some protection from irreversible denaturation under adverse conditions (such as extreme pH, temperature, etc.), but which can influence their efficacy (Zimmerman & Ahn, 2011; Yang et al., 2019). The level of soil enzymes activity (increase or decrease in activity of group of enzymes or individual enzymes) can influence specific soil functions, such as soil fertility and biological activity (Karaca et al., 2011;

Piotrowska, 2014). Enzymes, therefore, can be used as indicators to assess the influence of land use practices and soil management on soil ecosystem functions (Chang et al., 2007; Garbuz et al., 2016; Holík et al., 2019).

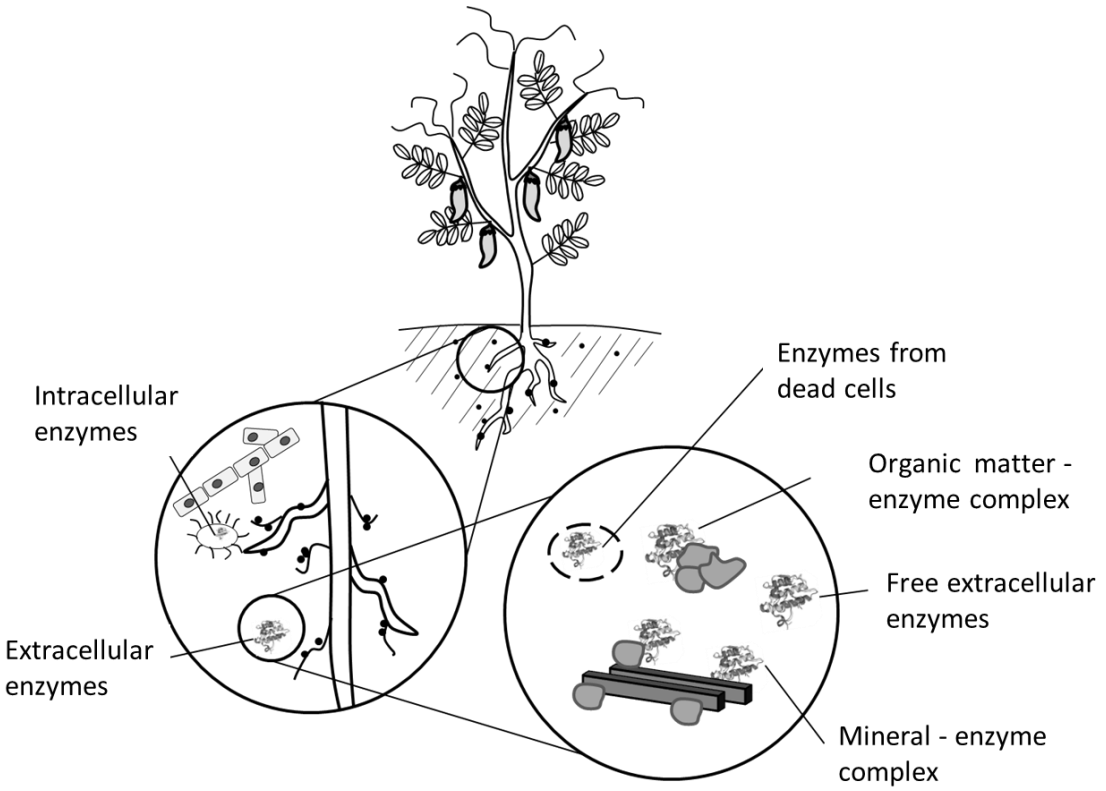


Figure 5.1 The origin and locations of enzymes in the soil. Author's drawing based on Skujiņš and Burns (1976) and Burns et al. (2013).

Biochar, a product of pyrolysis of organic materials, has long been used as an amendment in soil management practices. Depending on feedstock and pyrolysis conditions, biochar has the potential to contribute to increase soil nutrient availability (Wang et al., 2012a; Qayyum et al., 2014), to improve soil physical properties (Herath et al., 2013; Burrell et al., 2016), and also act as a liming agent, decreasing exchangeable acidity (H^+) and aluminium saturation in acidic soils (Chintala et al., 2013; Wang et al., 2014). Changes in soil bulk density, water retention, soil pH, and soil nutrient content and availability caused by biochar addition affect soil microbial communities (Masto et al., 2013; De Tender et al., 2016). Different types of biochar can influence bacterial and

fungal activities differently, in some cases shifting the microbial community structure, for example, changing fungi:bacteria ratio or changing abundance of specific groups of soil bacteria (Pandian et al., 2016; Gao et al., 2017). Generally, biochar application increases the abundance of soil microorganisms (Lehmann et al., 2011; Paz-Ferreiro et al., 2015; Palansooriya et al., 2019), whereas the influence of biochar on soil enzyme activity is more variable, being highly dependent on biochar type, and varying enormously with soil order (Ouyang et al., 2014; Paz-Ferreiro et al., 2014; Khadem & Raiesi, 2017).

Our previous results (**Chapter 4**) show that willow biochar addition (1% w/w ratio) significantly increased soil C and nutrients stocks (beyond the increased that would be expected based on biochar composition), the abundance of soil organisms (microorganisms, arthropods and earthworms) and plant root biomass in two New Zealand pasture soils. In the present Chapter, we investigate how these changes in soil properties are reflected in the activities of soil enzymes involved in the C, N and P cycles.

5.2 Materials and methods

5.2.1 Biochar production and characterisation. Soil properties. Field-based mesocosm experiment

Biochar production and characteristics are described in **Chapter 3**. The field sites, and the design and layout of the field-based mesocosm experiment are described in **Chapter 4.** Soil analysis and soil properties (physicochemical and biological) are also described in **Chapter 4**.

5.2.2 Soil enzymes analysis

Alkaline and acid phosphatases, nitrate reductase, urease, cellulase, peroxidase and dehydrogenase activities were measured in the top 30-cm depth of two contrasting soils, to which biochar and lime were added to a depth 2-17 cm (the top 2 cm depth comprised the turf). Soil samples for enzymatic activity measurement were collected from five depth

layers – 0-2 cm, 2-9.5 cm, 9.5-17 cm, 17-20 cm, and 20-30 cm; the soil was sieved (<2 mm) and air-dried.

Cellulase (EC 3.2.1.4) activity in the soil was determined by the Pancholy and Rice method (Pancholy & Rice, 1973). For this, 0.5 g of air-dry soil (<0.25 mm) was pre-treated for 15 min with 0.05 mL toluene and further incubated with 1 mL of sodium acetate buffer (pH 5.9) and 1mL carboxymethylcellulose (1%) at 30 °C for 24 h. After incubation, 8 mL DI H_2O was added and mixed well. The suspension was then centrifuged for 10 min at 5,000g. The concentration of reducing sugars in 1 mL of supernatant was measured by Somogyi-Nelson method (Deng & Tabatabai, 1994b) with a spectrophotometer at 520 nm, with glucose as a standard for making the calibration curve.

Soil urease (EC 3.5.1.5) activity was determined by the Shcherbakova method (Shcherbakova, 1983). For this, 0.25 g of air-dry soil (<0.25 mm) was incubated with 0.3 M urea in 0.2 M phosphate buffer (pH 6.5) with 0.02 mL of toluene at 37 ºC for 4 h. After incubation, the reaction was stopped by adding 20% trichloroacetic acid and 5 mL of 1 M KCl. The suspension was centrifuged for 10 min at 5,000g. The 0.2 mL of supernatant was dissolved in 4.4 mL of DI H_2O, and 0.2 mL of 50% potassium sodium tartrate (Seignette reagent) and 0.2 mL potassium tetraiodomercurate (Nessler reagent) were added. The concentration of released NH_4^+-N was measured with a spectrophotometer at 400 nm. Ammonium chloride standard solutions were used the make the calibration curve.

Alkaline (EC 3.1.3.1) and acid (EC 3.1.3.2) phosphatase activities in the soil were determined by the Tabatabai and Bremner method (Tabatabai & Bremner, 1969). A 0.1 g sample of air-dry soil (<0.25 mm) was incubated with 2 mL of modified universal buffer (pH 6.5 for the acid phosphatase and 11.0 for the alkaline phosphatase) and 0.5 mL of 0.115 M p-nitrophenyl phosphate solution for 1 hour at 37ºC. After incubation, 0.5 mL

of 0.5 M $CaCl_2$ and 2 mL of 0.5 M NaOH were added, and the mixture was centrifuged for 10 min at 5,000g. The concentration of released p-nitrophenyl was measured with a spectrophotometer at 400 nm. Calibration curve was made with standard solutions of p-nitrophenol.

Soil nitrate reductase (EC 1.7.99.4) activity was determined by Kandeler method (Schinner et al., 1996). A 1 g sample of air-dry soil (<0.25 mm) was incubated with 25 M KNO_3 solution and 1 mL water with added 0.9 mM 2,4-dinitrophenol solution as inhibitor of nitrite reductase at 25 ºC for 24 hours. After incubation 1.5 mL of 4 M potassium chloride was added. The suspension was centrifuged for 10 min at 5,000g. Then, 2.5 mL of the supernatant was mixed with 1.5 mL of ammonium chloride buffer (0.19 M, pH 8.5) and 1 mL of colour reagent (sulfanilamide and 0.1 g of N-(1-naphthyl) ethylenediamine dihydrochloride). The concentration of released NO_2^- was measured with a spectrophotometer at 520 nm. Sodium nitrite standard solutions were used for making the calibration curve.

Peroxidase (EC 1.11.1.7) activity was determined by the Karyagina–Mikhailovskaya method (Khaziev, 2005). The 0.5 g air-dried soil (< 0.25 mm) was incubated with 25 mL of freshly prepared 0.1 M hydroquinone and 0.25 mL of 0.5% hydrogen peroxide. The mixture was thoroughly mixed and kept at 30°C in a thermostat for 30 min. The reaction was stopped by the addition of 10 mL of 96% ethanol, and the reaction mixture was centrifuged for 10 min at 5,000g. The content of the formed 1,4-benzoquinone was measured with a spectrophotometer at 450 nm; 1,4-benzoquinone standard solutions were used for making the calibration curve.

Dehydrogenase (EC 1.1.1.x) activity was measured by the Thaimann method (Alef, 1995). The 0.8 g of air-dried soil (<0.25 mm) was incubated with 1.8 ml triphenyl tetrazolium chloride solution (7.5 mg/ml) in Tris-HCl buffer (pH 7.6) for 24 h at 30°C. The reaction was stopped by the addition of 5 ml acetone and kept at room temperature

for 2 h in the dark. Then the solution was centrifuged for 10 min at 5,000g. The content of the formed product was measured with a spectrophotometer at 546 nm. Triphenyl formazan standard solutions were used for making the calibration curve.

5.2.3 Statistical Analysis

Statistical analysis was carried out using R software version 3.3.3. Normality of data sets was evaluated by the Shapiro-Wilk test. Analysis of variance (ANOVA) with contrast statements and Tukey HSD test was used to investigate the effect of factors: soil order and paddock (Andosol NE and EF; Cambisol LF and HF); treatment (control, biochar, and lime); and depth on soil enzyme activities.

Principal Component Analysis (PCA) was performed for biological properties (using soil biology data from **Chapter 4**) and soil enzymes (combined 2-9.5 and 9.5-17 cm depths), grouping them by factor (treatment). Prior to PCA, the data was normalized by z-score standardization technique.

Ordinary least squares regression was conducted to investigate relationships between some enzymes (average of values for 2-9.5 and 9.5-17 cm) and functional groups of soil biota.

The geometric mean of soil enzyme activities (GMea) has been considered as a soil quality index (García-Ruiz et al., 2008). For each soil sample, the GMea was calculated as:

$$GMea = \sqrt[1/7]{\text{AlP x AcP x NR x Ure x Cel x PO x DHG}}$$

5.3 Results

There were significant differences in enzyme activity between soil orders and different paddocks. Because soil order had a significant effect on almost all experimental variables (enzymes), the results for Cambisol and Andosol are reported separately, unless indicated otherwise. Phosphatases, urease, dehydrogenase, and especially nitrate reductase

activities were higher in the Andosol ($P<0.005$), whereas cellulase activity was higher in the Cambisol ($P<0.005$). There was no significant difference in peroxidase activity between the two soil orders. In both soils, with few exceptions (e.g., peroxidase), enzyme activities declined with the depth ($P<0.005$).

The effects of biochar or lime addition were observed primarily in the 2-9.5 cm and 9.5-17 cm soil depths (see **SI Figs. 5.1-5.7** in **Appendix 5.1**). The influence of the treatments on the turf (0-2 cm) and 17-20 cm soil depth was less obvious. Subsoil (20-30 cm) was not affected by the amendments (**SI Figs. 5.1-5.7**). Soil amendments – biochar and lime – significantly affected enzymes activities, but the effect was very variable. Both biochar and lime had a negative effect ($P<0.005$) on acid phosphatase (**SI Fig. 5.1**). On the contrary, alkaline phosphatase activity in the soil with biochar or lime addition was higher than control ($P<0.005$) (**SI Fig. 5.2**). Nitrate reductase activity was higher in the soil with amendments than in control ($P<0.005$), with the effect of lime being more pronounced than that of biochar (**SI Fig. 5.3**). Urease activity was higher in biochar treatments ($P<0.005$) in all soils (**SI Fig. 5.4**). Cellulase activity was highest ($P<0.005$) with biochar addition; lime had no effect on cellulase activity (**SI Fig. 5.5**). Peroxidase activity was higher in lime-treated soil ($P<0.005$, **SI Fig. 5.6**). Dehydrogenase activity (**SI Fig. 5.7**) was higher in biochar-treated soil than in control or in lime treated soil ($P<0.005$).

There were significant paddock-level effects of soil management on enzyme activities. The paddocks with effluent addition (And-EF) and with high P fertiliser input (Cam-HF) had higher phosphatases, nitrate reductase, and dehydrogenase activities (all $P<0.005$) than their low-fertility equivalents. In the Andosol, the soil receiving effluents (And-EF) had higher urease and cellulase (all $P<0.005$), but lower peroxidase activity ($P<0.005$) than the same soil without effluent (And-NE). In the Cambisol the opposite was found – the soil with high P fertilizer input (Cam-HF) had higher peroxidase activity ($P<0.005$)

and lower cellulase activity ($P<0.005$) than the low fertility soil (Cam-LF). There was no difference in the urease activity between the two Cambisol paddocks with different input levels of P fertiliser (Cam-LF had received no superphosphate since 1980, while Cam-HF receives 375 kg superphosphate ha^{-1} yr^{-1} since 1980).

Table 5.2 Activities of soil enzymes in experimental treatments. Values represent means. Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between the treatments within a specific paddock (And-NE: Andosol, no effluent; And-EF: Andosol, effluent input; Cam-LF: Cambisol, low fertility; Cam-HF: Cambisol, high fertility).

		And-NE			And-EF			Cam-LF			Cam-HF		
	Depth, cm	**Control**	**Biochar**	**Lime**	**Control**	**Biochar**	**Lime**	**Control**	**Biochar**	**Lime**	**Control**	**Biochar**	**Lime**
Cellulase, mg Glucose g^{-1} $24h^{-1}$	2-9.5	3.3 b	4.9 a	3.1 b	3.9 b	5.2 a	4.1 b	7.3 b	10.2 a	7.3 b	4.1 b	6.2 a	4.1 b
	9.5-17	2.1 b	3.2 a	2.1 b	2.6 b	3.4 a	2.3 b	5.0 b	8.7 a	4.8 b	3.1 b	5.0 a	3.4 b
Peroxidase, µmol p-Benzoquinone g^{-1} h^{-1}	2-9.5	45.3 b	47.3 b	66.1 a	28.3 b	29.5 b	45.0 a	17.8 b	19.9 b	43.4 a	69.6 c	60.5 b	93.7 a
	9.5-17	38.5 b	39.2 b	64.1 a	32.2 c	27.8 b	44.5 a	20.0 b	21.9 b	37.5 a	61.5 b	60.1 b	83.7 a
Dehydrogenase, µg TPF g^{-1} $24h^{-1}$	2-9.5	1.7 b	2.1 a	2.1 a	2.6 b	2.9 a	1.9 c	1.5 b	2.0 a	1.6 b	1.6 c	2.3 a	2.0 b
	9.5-17	1.2 b	1.6 a	1.2 b	1.7 b	2.2 a	1.3 c	1.0 b	1.5 a	1.3 ab	1.0 c	2.0 a	1.7 b
Nitrate reductase, µg NO2--N g-1 $24h^{-1}$	2-9.5	28.4 c	32.4 b	37.0 a	82.8 a	85.2 a	87.9 a	1.8 c	2.0 b	2.8 a	3.8 b	4.2 ab	4.0 a
	9.5-17	20.0 b	21.1 b	25.0 a	41.8 b	45.7 b	60.1 a	1.5 c	1.8 b	2.7 a	2.6 b	2.9 a	2.9 a
Urease, mg NH_4^+-N g^{-1} $4h^{-1}$	2-9.5	67.3 b	70.2 a	70.7 a	100.0 a	103.6 a	101.1 a	32.7 b	34.9 a	33.2 b	27.0 b	29.5 a	27.5 b
	9.5-17	54.2 b	59.1 a	58.7 a	67.3 a	69.9 a	70.4 a	17.1 b	20.0 a	17.7 b	18.2 b	20.3 a	17.9 b
Alkaline phosphatase, µg 4-nitrophenol g^{-1} h^{-1}	2-9.5	143.4 b	157.2 a	150.3 b	198.6 a	204.0 b	209.7 b	94.2 b	103.7 ab	106.7 a	105.2 b	121.1 a	120.0 a
	9.5-17	90.8 b	103.5 a	96.4 b	231.2 a	240.1 b	254.8 b	59.9 b	70.0 b	73.0 a	58.2 b	70.1 a	67.0 a
Acid phosphatase, µg 4-nitrophenol g^{-1} h^{-1}	2-9.5	374.1 a	351.4 b	330.6 c	460.8 a	442.1 b	435.4 b	113.8 a	94.6 b	92.0 b	160.1 a	128.1 b	119.9 b
	9.5-17	278.3 a	256.2 b	236.6 c	369.4 a	335.9 b	325.9 b	79.8 a	60.1 b	52.6 c	107.1 a	90.8 b	72.9 c

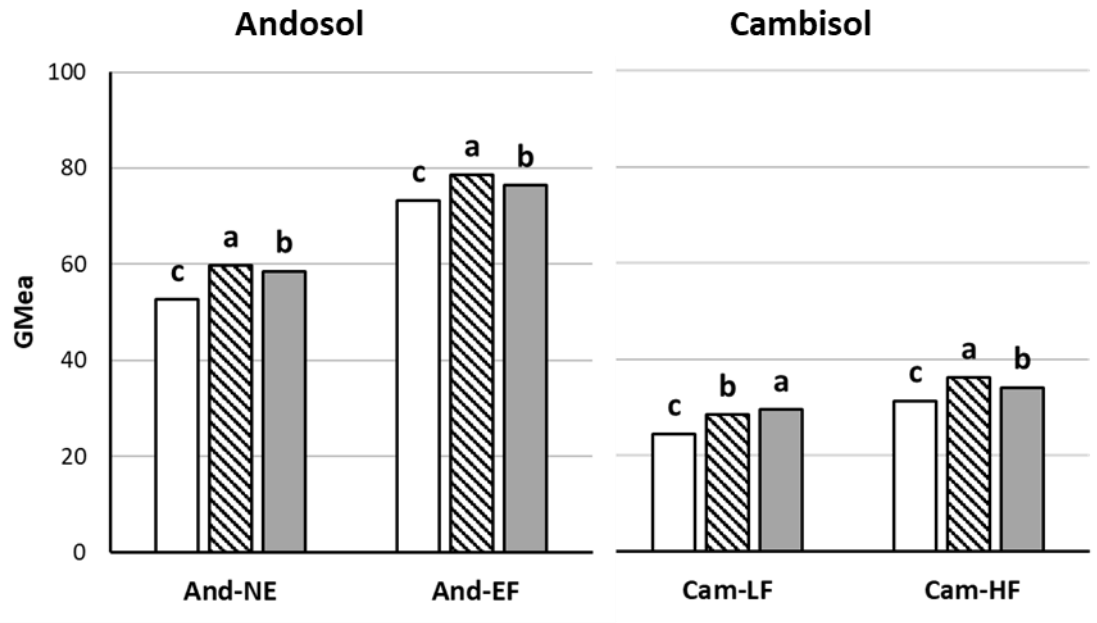


Figure 5.2 Geometric mean of enzyme activity (GMea) in experimental treatments averaged for two soil depths (2-9.5 and 9.5-17 cm), as a soil quality indicator at 12 months after of the start of the experiment. Empty bar – Control, striped – Biochar, grey – Lime. Lower case letters indicate significant differences (Tukey HSD test, $\alpha<0.05$) between treatments within a paddock. Abbreviations for paddocks here and in all other figures: And-NE and And-EF – the Andosol without and with effluent, respectively, Cam-LF and Cam-HF – the Cambisol with low and with high fertility, respectively.

Values of GMea (**Fig. 5.2**) were about two times higher ($P<0.005$) in the Andosol than in the Cambisol. The Andosol with effluent (And-EF) had highest GMea ($P<0.005$), while the Cambisol with low fertility had lowest values of GMea ($P<0.005$). Values of GMea were higher in the biochar- or lime-amended soils in comparison to control ($P<0.005$), and were highest in the soil treated with biochar in all paddocks except Cam-LF (**Fig. 5.2**).

5.3.1 Principal Component Analysis (PCA) of the soil enzymes activities

Because of paddock-level differences, the PCA results are presented for individual paddocks. For And-NE, the first four principal components accounted for 89.5% of total variability, with PC1 and PC2 accounting for 48.6% and 27.0%, respectively. For And-

EF, the first four principal components accounted for 91.2% of total variability, with PC1 and PC2 accounting for 46.1% and 35.6%, respectively (**Fig. 5.3**). For Cam-LF, the first four principal components accounted for 93.3.5% of total variability, with PC1 and PC2 accounting for 44.6% and 34.2%, respectively. For Cam-HF, the first four principal components accounted for 86.6% of total variability, with PC1 and PC2 accounting for 52.1% and 21.4%, respectively (**Fig. 5.3**).

The PCA bi-plots (**Fig. 5.3**) show a clear

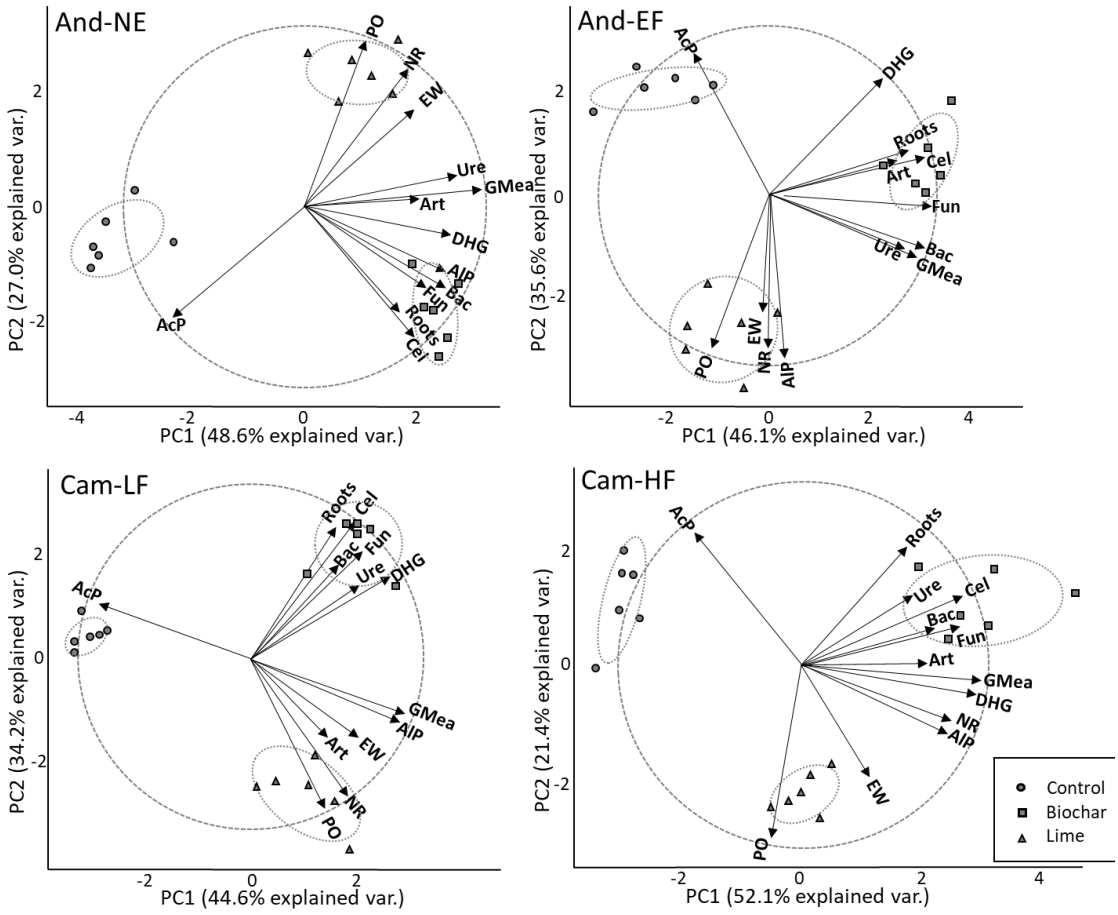


Figure 5.3 PCA bi-plots (PC1 vs. PC2) for the soil enzyme activities (average for 2-9.5 and 9.5-17 cm soil depths) and soil biota for the Andosol (And-NE and And-EF) and the Cambisol (Cam-LF and Cam-HF) under experimental treatments. AlP – alkaline phosphatase, AcP – acid phosphatase, NR – nitrate reductase, Ure – urease, Cel – cellulase, PO – peroxidase, DHG – dehydrogenase, GMea – geometric mean of enzyme activities, Bac – bacterial biomass C, Fun – fungal biomass C, EW – earthworm abundance, Art – microarthropod abundance (combined Collembola, Oribatida and Gamasina), Roots – root biomass.

separation between biochar/lime-amended soils and their respective controls. With the exception of acid phosphatase, all other biotic and enzyme variables were associated with the addition of biochar or lime amendments. In all four paddocks, biochar addition was the driver for root biomass, microbial biomass (fungi and bacteria), cellulase and dehydrogenase activity. In the Cambisol paddocks, urease activity was also associated with biochar. Higher abundance of earthworms and higher peroxidase activity (in both soils), and nitrate reductase (in the Andosol) were associated with lime application.

5.4 Discussion

This Chapter provides insights into the activity of seven enzymes in pasture paddocks under different management practices on two contrasting soil orders, after addition of either biochar or lime. The following discussion is limited to the effects of biochar and lime on enzyme activities in 2-17 cm soil depth (**Table 5.2**); data for other depths are available in the **Appendix 5.1**.

There were marked differences in enzymes actives between soil orders and between management practices (paddocks) within a soil order. Short-range order inorganic constituents (e.g., allophane) abundant in Andosols have the capacity to immobilize phosphatase (Chatterjee et al., 2014; Jordanova, 2017), and in this form protect it from adverse conditions (Shindo et al., 2002); this explains the fact that phosphatase activity was greatest in the Andosol. At the same time, allophane aggregates in the Andosol can remain saturated with water during long periods (Buurman et al., 2007b), which creates favourable anaerobic conditions for nitrate reductase (Abdelmagid & Tabatabai, 1987), which was most abundant in this soil. High urease activity in the Andosol paddocks probably reflects higher urine input from the grazing cattle (up to 55 L urine cow^{-1} day^{-1}, Betteridge et al. (1986) comparing to up to 3 L urine $sheep^{-1}$ day^{-1} (Ledgard et al., 2008) from sheep grazing in Cambisol). Urease activity has been reported to be strongly correlated with soil bacterial biomass (Amini Kiasari et al., 2018). In the present study,

bacterial biomass increased with the addition of biochar (**Chapter 4**), but did not correlate with urease activity. This may be explained by the fact that the bacteria-urease correlation relies on a specific group of bacteria, ureolytic bacteria, but not on the whole bacterial community (Lloyd & Sheaffe, 1973). In other studies, urease has shown an inconsistent response to biochar application. For example, rice husk biochar had both negative and positive effect on urease activity in two different acidic soils (Ultisol and Alfisol) (Huang et al., 2017; Oladele, 2019).

Both biochar and lime had significant effect on enzyme activities, which are best explained in conjunction with soil biological variables, as shown in **Figs 5.4-5.7** below.

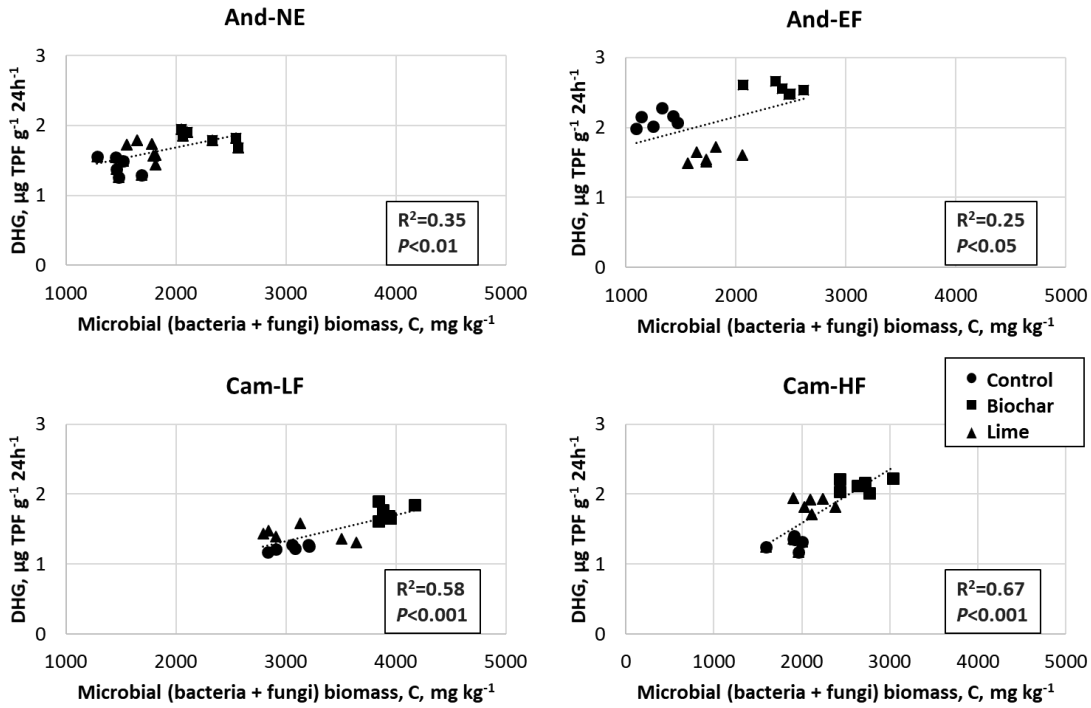


Figure 5.4 Ordinary least squares (OLS) regressions between microbial biomass C and dehydrogenase activity (2-17 cm soil depth) in four experimental sites.

Both amendments, but especially biochar, had positive effect on dehydrogenase (DHG) activity, which is often used as an indicator of soil microbial response to land use practices (Watts et al., 2010; Järvan et al., 2014). There was a strong positive correlation

between microbial biomass carbon and DHG activity (**Fig. 5.4**), which was expected, as biochar had positive effect on microbial community (see **Chapter 4**), and dehydrogenase is an important component of microbial metabolic functions (Casida, 1977). Ouyang et al. (2014) reported that biochar application (5% w/w) had positive effect on DHG activity and had increased C mineralization in the soil in the short-term, which can be related to some extent to the presence of labile C in biochar; however, most of the C in biochar remained in the soil over time, as expected. A mass balance calculation showed the loss of native OC in the soils treated with biochar and lime in our experiment (**Chapter 4**); however, the soil treated with biochar accumulated OC over time, which was mostly associated with enhanced root growth (**Chapter 4**).

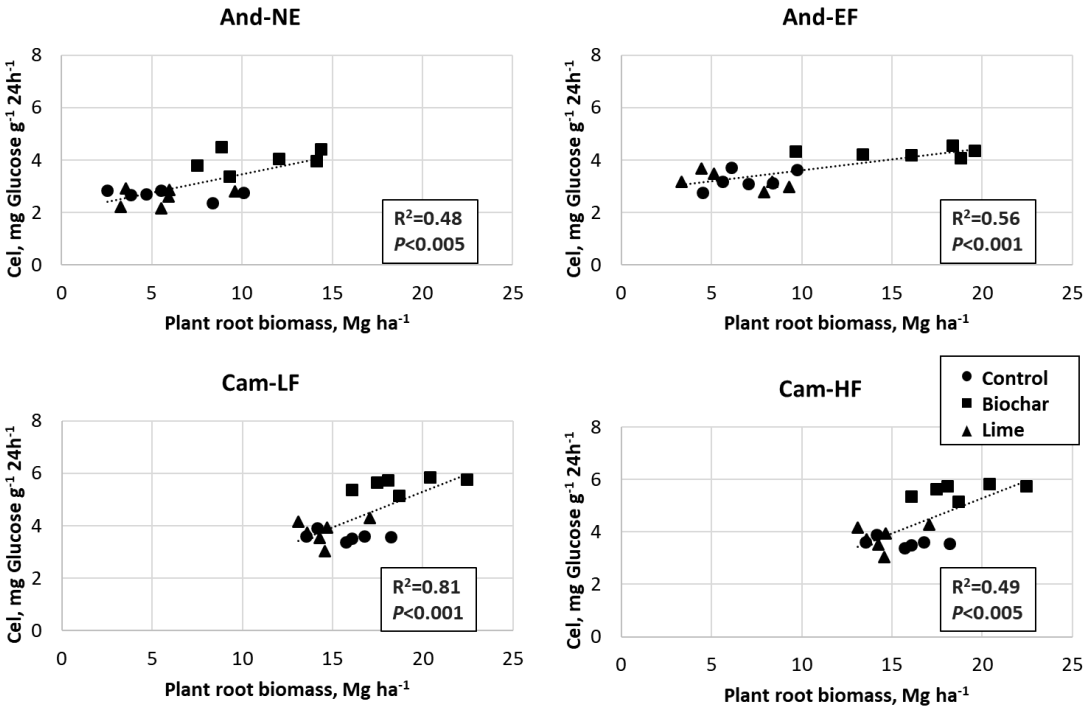


Figure 5.5 OLS regressions between dry root biomass and cellulase activity (2-17 cm soil depth) in four experimental sites.

Cellulase activity was not related to soil order, but was correlated to root biomass (**Fig. 5.5**). As shown in **Chapter 4**, there was enhanced root biomass in the biochar treatment at the end of the experiment, which would increase plant necromass – the substrate for cellulase activity. Cellulose is fully carbonised from 240°C (Demirbaş, 2004) and thus its presence was probably negligible in our biochar which was produced at 350°C. The highest activity of cellulase was observed in the Andosol receiving effluent (And-EF) and in the Cambisol with low fertility (Cam-LF), both of which had higher root biomass (regardless of the treatments) than their counterparts (And-NF and Cam-HF). An additional stimulating effect of biochar on cellulase activity could be associated with the positive effect of biochar on soil water holding capacity, which provides more favourable conditions for enzymatic activity (Peng et al., 2019).

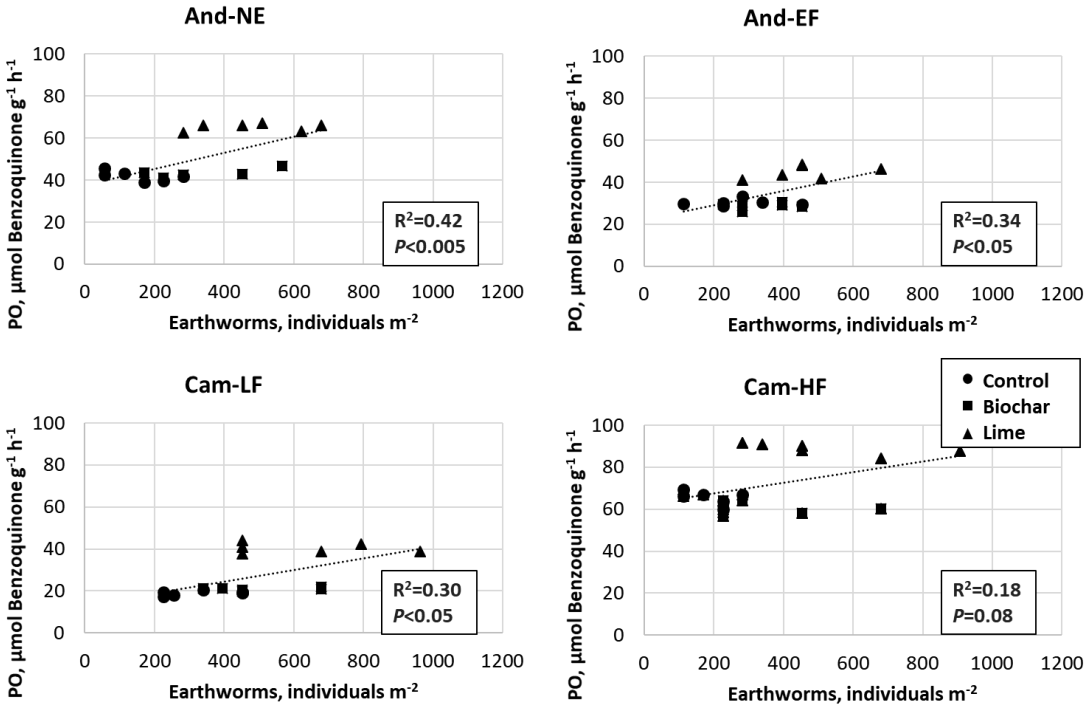


Figure 5.6 OLS regressions between the density of earthworms and peroxidase activity (2-17 cm soil depth) in four experimental sites.

The positive correlation between earthworms (which were more abundant in the presence of lime, see **Chapter 4**), and peroxidase (PO) activity (**Fig. 5.6**) could be related to the fact that soil peroxidases are very sensitive to soil pH, and it was shown that lime application substantially increases peroxidase activity (Sinsabaugh, 2010). With an increase in pH, the bonds of organic molecules (ligands) with mineral surface become weaker, and freed OM becomes more easily degraded and oxidised by peroxidases (Sinsabaugh, 2010; Tian & Shi, 2014). Also, peroxidases exist in the earthworm tissues to protect its body against the harmful effect of peroxide, and are released into the soil with the mucus from the earthworm body and with castings (Hartenstein, 1982; Hassett et al., 1988). Thus, a larger number of earthworms could contribute to increasing PO activity in the soil.

Nitrate reductase activity is affected by factors such as nitrate concentration and soil pH, with an optimum at pH 7 (Abdelmagid & Tabatabai, 1987). This is consistent with the trends observed in this study: (i) NR activity was highest in the Andosol that received effluents (And-EF) and in the Cambisol with high fertility paddock (Cam-HF); (ii) both lime and biochar increased NR activity. Yet, while lime and biochar had similar effect on soil pH, lime had a more pronounced effect on NR. Jha et al. (2016) showed that lime had strong positive effect on abundance of microbial genes encoding the denitrification process, including narG gene responsible for the reduction of nitrate. At the same time, biochar has been reported to increase the abundance of nosZ genes, encoding the nitrous-oxide reduction (Harter et al., 2017). Also to have diverse effects on other genes (Weldon et al., 2019) and being able to reduce narG abundance (Bai et al., 2015). Apparently, biochar and lime affect different groups of soil bacteria responsible for denitrification. Additional comparative research is required to find the mechanisms through which these amendments influence N cycle.

In addition, the larger earthworm abundance observed in the treatments with lime (see **Chapter 4** and **Fig. 5.7**) may have to some extent influenced the activity of NR, as the link between earthworms and denitrifying bacteria has been suggested by some authors (Burtelow et al., 1998; Depkat-Jakob et al., 2010b) through nitrate concentration increase by earthworms. Garbuz et al. (2019) (**Chapter 3**) also showed that synergistic interaction between lime and earthworms increased NR activity.

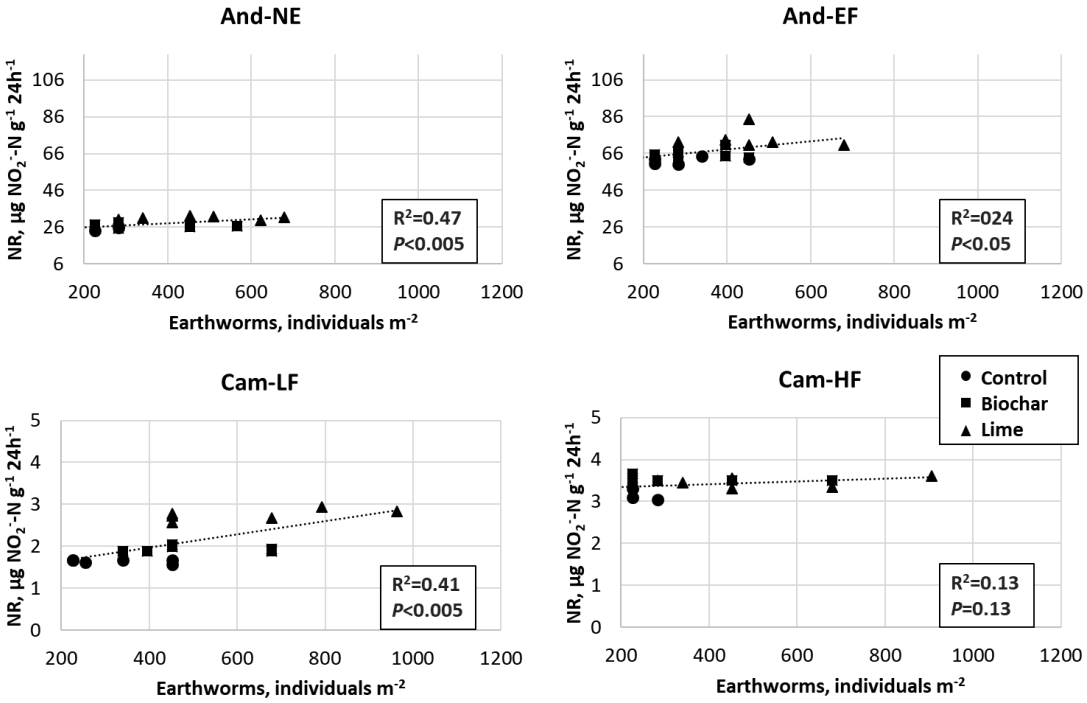


Figure 5.7 OLS regressions between the density of earthworms and nitrate reductase activity (2-17cm soil depth) in four experimental sites.

Phosphatases are very sensitive to soil pH, and activity of acid phosphatase decreases as soil pH increases, as opposed to that alkaline phosphatase (Juma & Tabatabai, 1978). Therefore, the addition of alkaline material (lime or biochar) is the driver for the increase in the AlP/AcP ratio (Acosta-Martínez & Tabatabai, 2000), as observed in our experiment. Halstead (1964) suggested that a reduction in acid phosphatase activity is related to the decline in fungal biomass after lime application. However, in **Chapter 4** it

was shown that lime amendment did not affect fungal biomass and that biochar had a positive effect on both bacterial and fungal biomass, which suggests that another underlying mechanism might explain these changes. Phosphatases (AcP and AlP) activities are found bound to OM, which enhances the stability of these enzymes, and so are related to OC and TN (Bonmati et al., 1991).

Biochar can stimulate biochemical processes in the soil, but the underlying mechanisms can be different. The biochar used in the present study has a clear influence on soil physicochemical properties, which in turn affected plants and soil biota, including microorganisms and invertebrates (see Garbuz et al. (2019) and **Chapter 4**). In **Chapter 4** we showed that, after 12 months of the experiment under field conditions, biochar significantly increased OC, TN and Olsen P concentration, and reduced soil BD and acidity in both the Andosol and the Cambisol. At the same time, biochar increased plant root biomass, which stimulated soil microbial biomass, arthropods and earthworm abundance through the soil food web. Similar effects of biochar on soil physicochemical and biological properties in the same soils were observed in the glasshouse mesocosm experiment (Garbuz et al., 2019). In addition, we found a synergistic interaction between biochar and the presence of earthworms acting upon soil microbial biomass and specific biochemical processes (C and N cycles, including activity of nitrate reductase) (Garbuz et al., 2019). Similarly, synergistic effects of biochar and earthworms on soil microbial community have been reported in other studies (Bamminger et al., 2014; Paz-Ferreiro et al., 2015), including the increase in abundance and activity of microorganisms (Paz-Ferreiro et al., 2015), increase in enzymatic activities (Paz-Ferreiro et al., 2014), and higher plant growth rate (Paz-Ferreiro et al., 2014; Sanchez-Hernandez et al., 2019).

Depending on the soil type and biochar type, the effects of biochar on enzyme activities can be positive or negative (Bailey et al., 2011). More often, the addition of biochar causes an increase in enzyme activities through changing soil physicochemical

properties (changing porosity, water regime and pH, providing labile organic C and nutrients) and, as a result, stimulating soil biota, producers of enzymes (Vázquez et al., 2000; Paz-Ferreiro et al., 2014; Mierzwa-Hersztek et al., 2019). On the other hand, biochar can adsorb enzymes, and therefore, reduce their activity (Lammirato et al., 2011; Foster et al., 2018; Primožič et al., 2019). Wang et al. (2015b) showed that small application rate of maize biochar produced at 450°C (0.5% w/w) increased the activity of enzymes involved in the C cycle, while higher application rates (>0.5%) had a negative effect on the activities of these enzymes; at the same time, enzymes involved in the N cycle increase with biochar application rate (Wang et al., 2015). Overall, the effect of biochar on selected enzymes appears to depend on soil chemical properties, available nutrients and OC content, as well as biochar properties (Paz-Ferreiro et al., 2014; Irfan et al., 2019; Oladele, 2019).

The geometric mean of enzyme activities (GMea) based on enzymes involved in C and nutrient cycles allows to summarize the total direction of soil biochemical processes and has been used as an index of soil quality (García-Ruiz et al., 2008; Piotrowska, 2014) and as a fast-acting indicator for effects of management practices (including biochar application) on soil fertility and sustainability (Paz-Ferreiro et al., 2012; Mierzwa-Hersztek et al., 2019). An increase in GMea values as a result of biochar application points to the general positive effect of biochar application on soil biological conditions and biochemical processes (Khadem & Raiesi, 2017; Mierzwa-Hersztek et al., 2019). However, the GMea does not evaluate the separate contribution of each enzyme to the soil functioning. Therefore, depending on the choice of enzymes, the GMea value may vary and may not reflect fully the effect of a land use practice such as effluent or fertilizers application on soil biochemical processes. For example, in the case of the Andosol, which is considered a highly productive soil (Parfitt, 1990) and has a higher GMea than the Cambisol, our results show that the application of dairy farm effluent to this soil increases

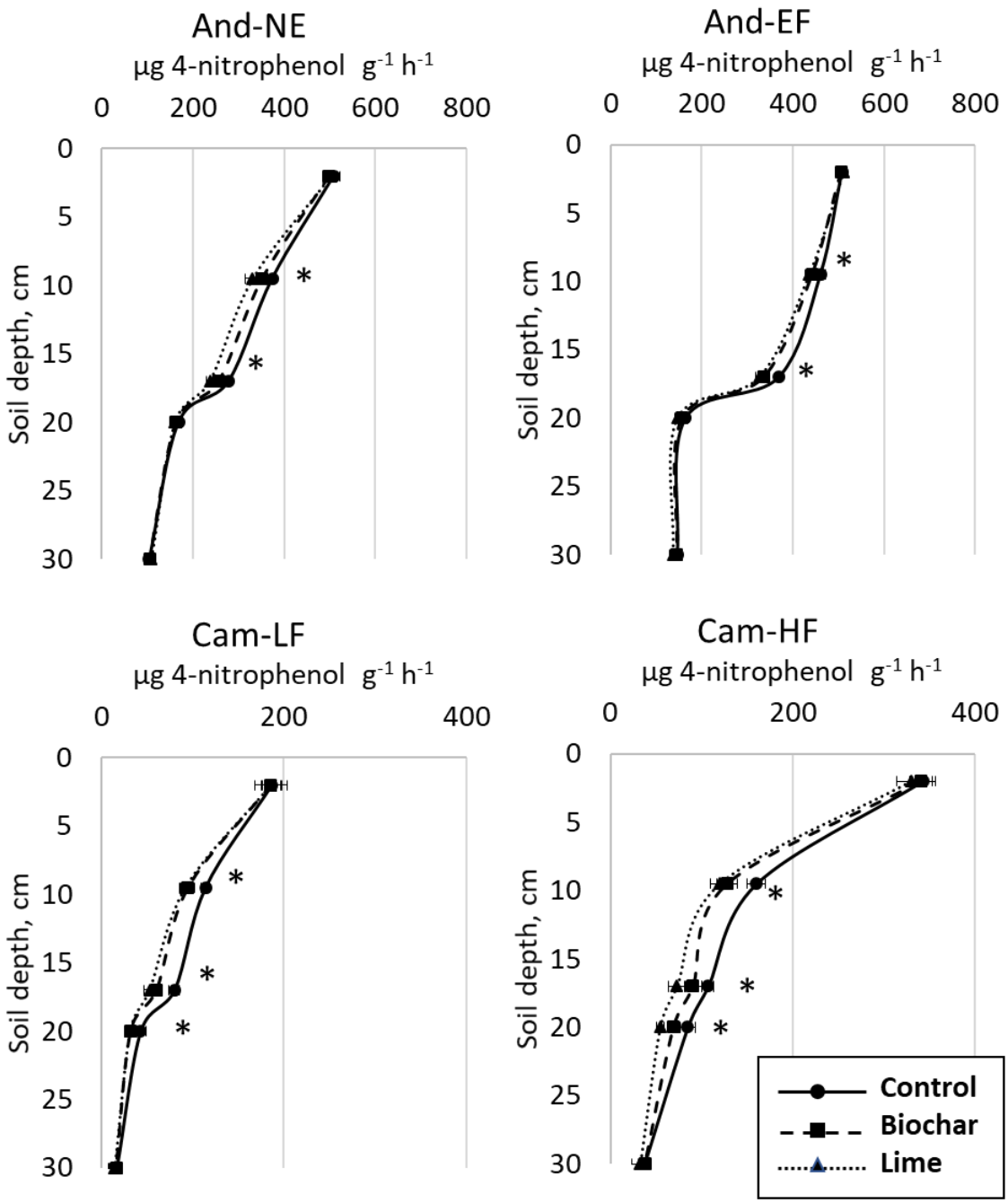
pasture productivity, biological activity, and, therefore, enzymatic activity. However, the organo-mineral complexes of the Andosol receiving effluent become unstable in the long-term (Kov et al., 2018) as more alkalinity is added to the system. This indicates that the effluent application practice could bring risk to the sustainability of this soil. Effluent application could also be a reason of environmental problems, such as reducing water quality in neighbouring waterways, as well as nutrients imbalance both in the soil and in animal diet (Hawke & Summers, 2006).

5.5 Conclusions

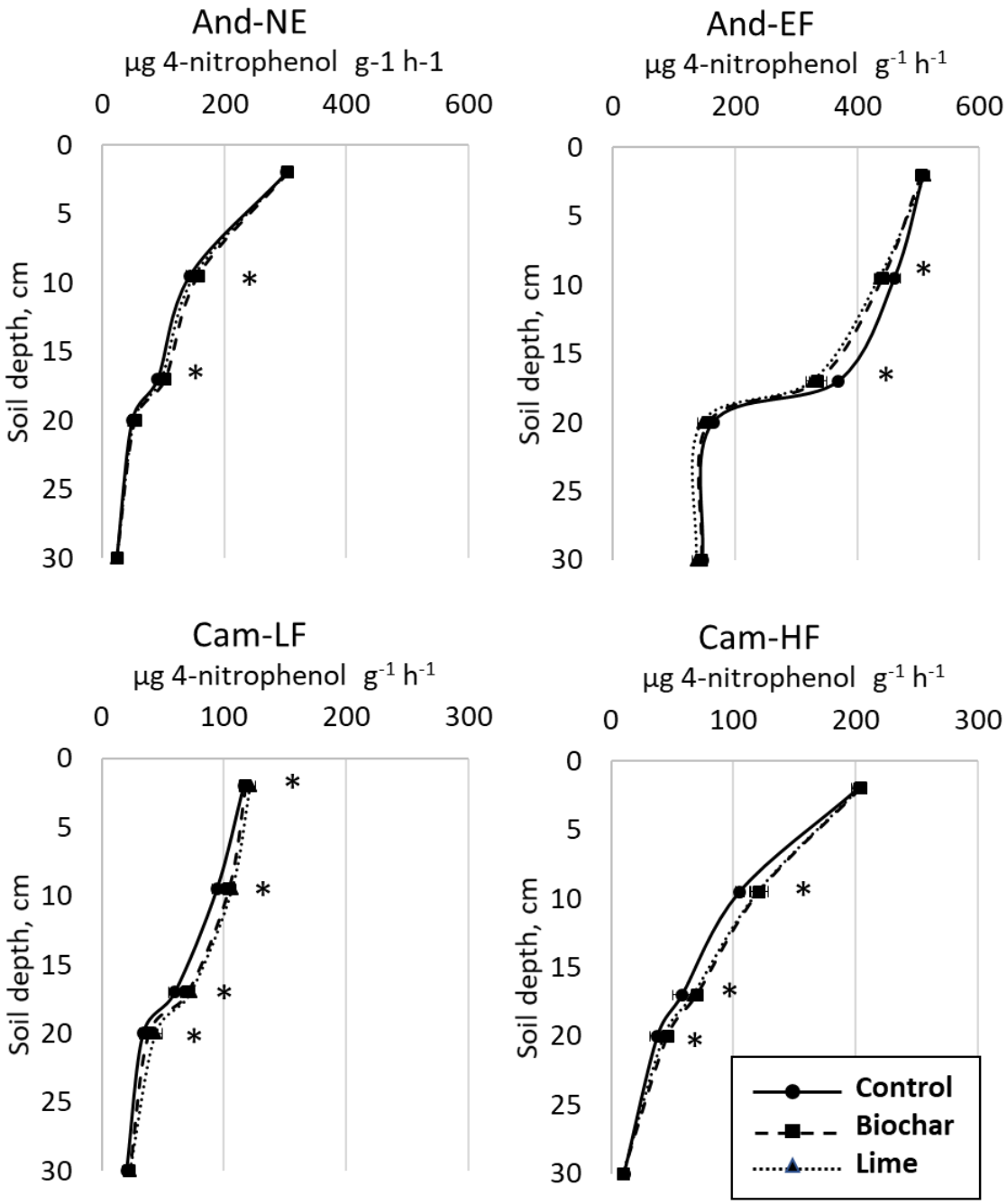
Soil amendments, such as lime and willow biochar used in the present study at an application rate of 12 Mg ha^{-1}, had a significant influence on the chemical and biological status of the soil and, consequently, on the activities of selected soil enzymes. However, this effect was diverse and enzyme-dependent. The different response of enzyme activities to biochar and lime addition could be explained by the different mechanisms through which these amendments influence soil biochemical processes. Lime increased pH and, since neutral pH is more favourable for most enzymes, it significantly increased their activity in general (e.g., peroxidase). Lime had more pronounced effect than biochar on the activity of nitrate reductase, which might to some extent be related with the effect of lime on earthworm abundance. This might have also contributed to the increased activity of other enzymes, such as peroxidase. Biochar, in addition to its liming potential, had generated more favourable conditions for root growth, which along with its labile C fraction contributed to biological processes. The overall increase in labile OM associated with biochar application (either directly from the charred material or indirectly from enhanced root growth) caused an increase in dehydrogenase and cellulose activity. Both biochar and lime, due to the liming effect on soil pH, decreased activity of acid phosphatase, and increased activity of alkaline phosphatase. Urease activity also increased with biochar application, but the mechanism through which biochar influences

urease is not clear. The higher GMea values in biochar-treated soil suggest that there is an overall positive effect of biochar application on soil quality and biochemical processes. However, the GMea may not fully reflect the long-term effects of a land use practice such as effluent, fertilizers or other amendments application on soil biochemical processes. Future research should focus on interactions between biochar, plants, and functional groups of soil organisms, and the way in which these influence on particular enzymes and total enzymatic activity affects the C, N and P cycles.

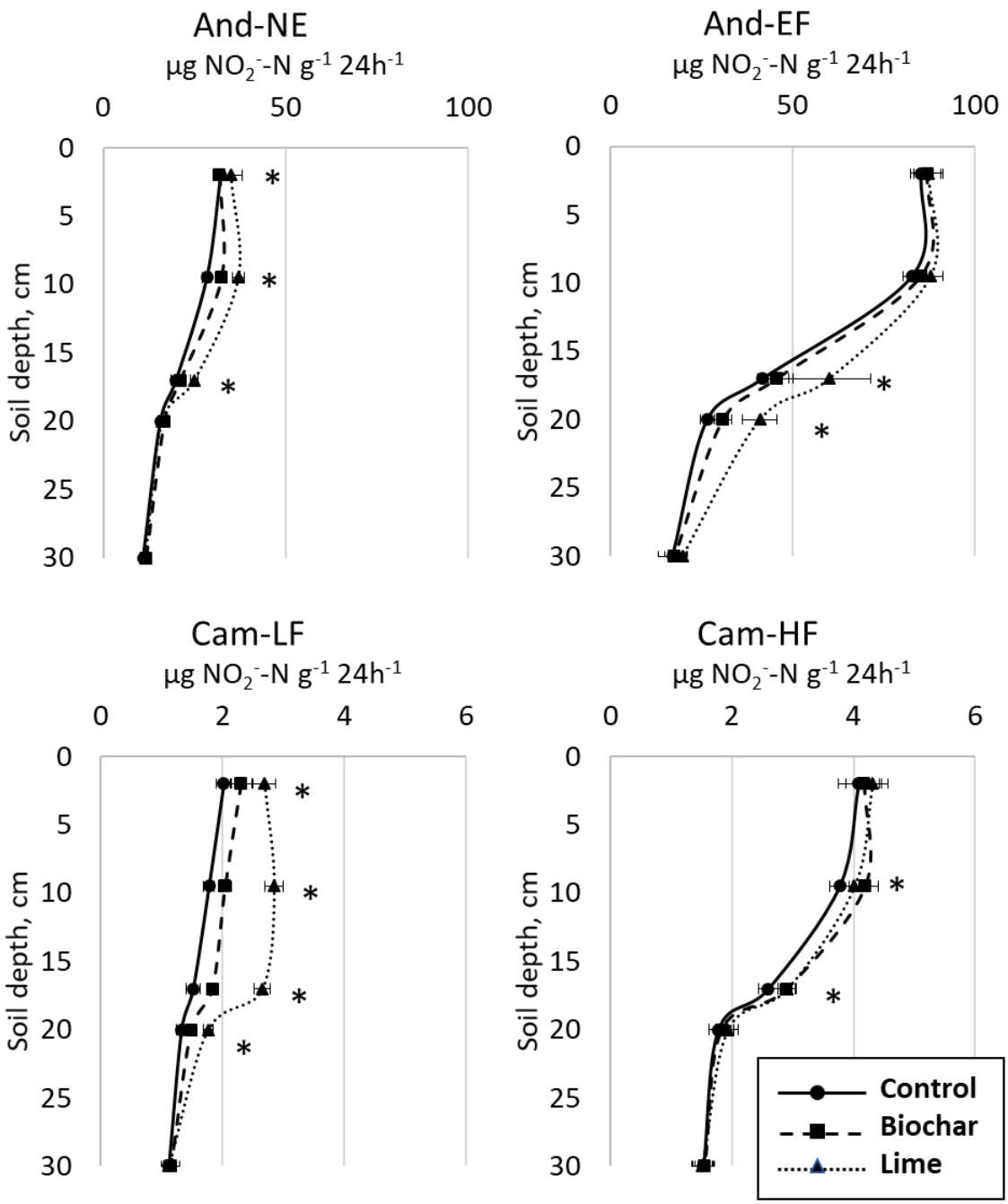
Appendix 5.1



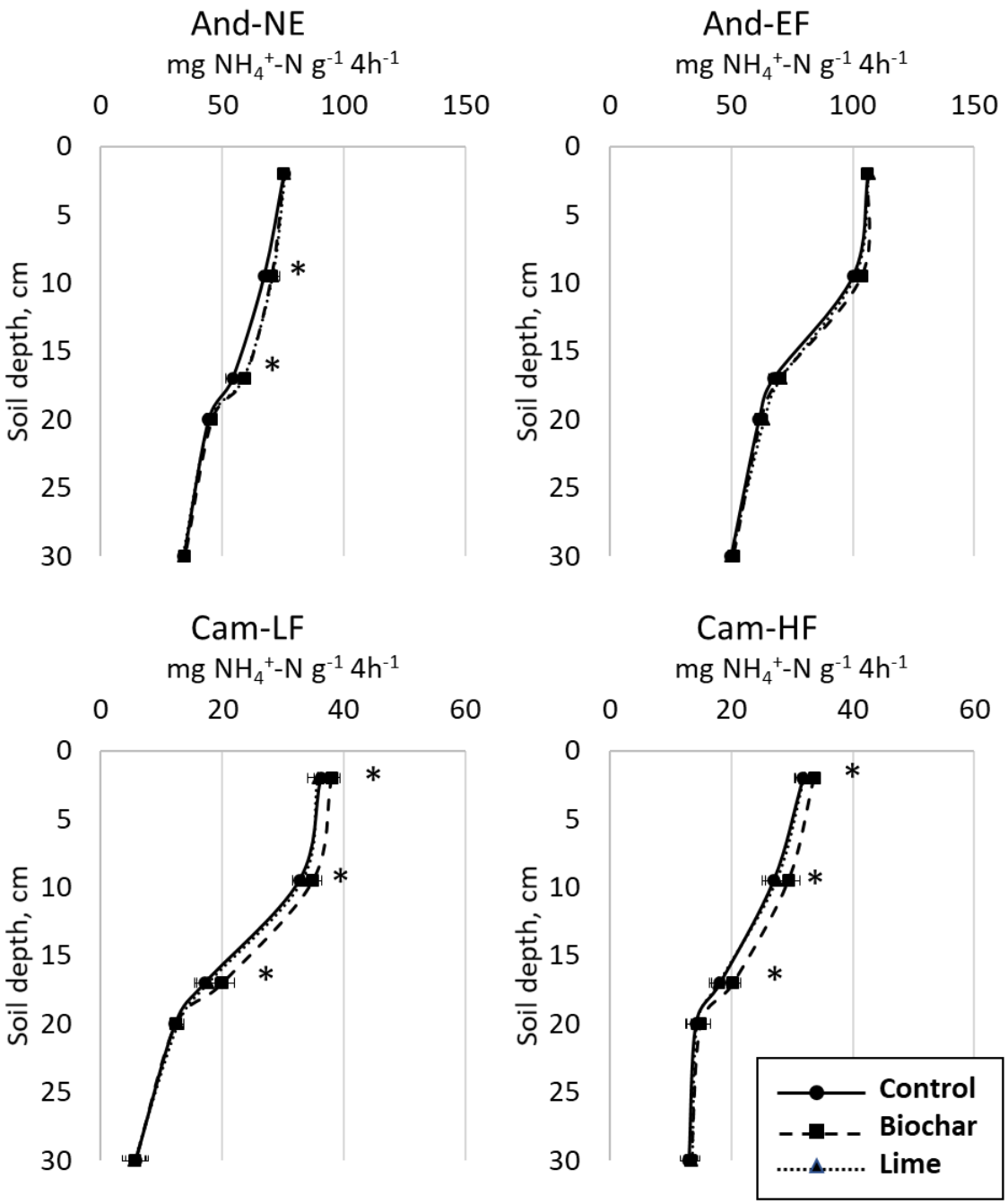
SI Figure 5.1 Acid phosphatase activity in experimental treatments within soil profile in experimental treatments after 12 months of the experiment. Values represent mean ± SE. Asterix represent the layers with significant ($P<0.05$) differences in the enzyme activities between treatments.



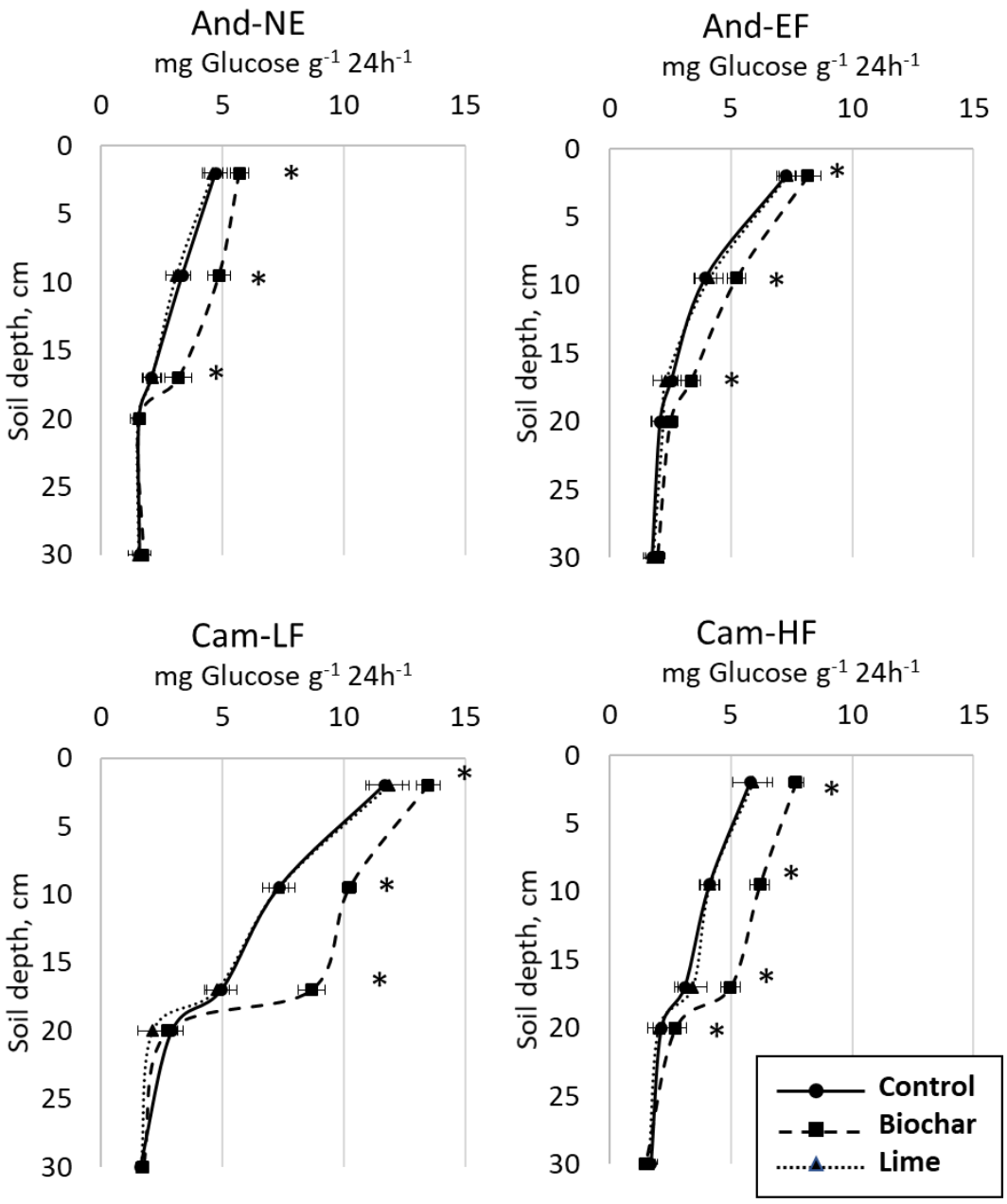
SI Figure 5.2 Alkaline phosphatase activity within soil profile in experimental treatments after 12 months of the experiment. Values represent mean ± SE. Asterix represent the layers with significant ($P<0.05$) differences in the enzyme activities between treatments.



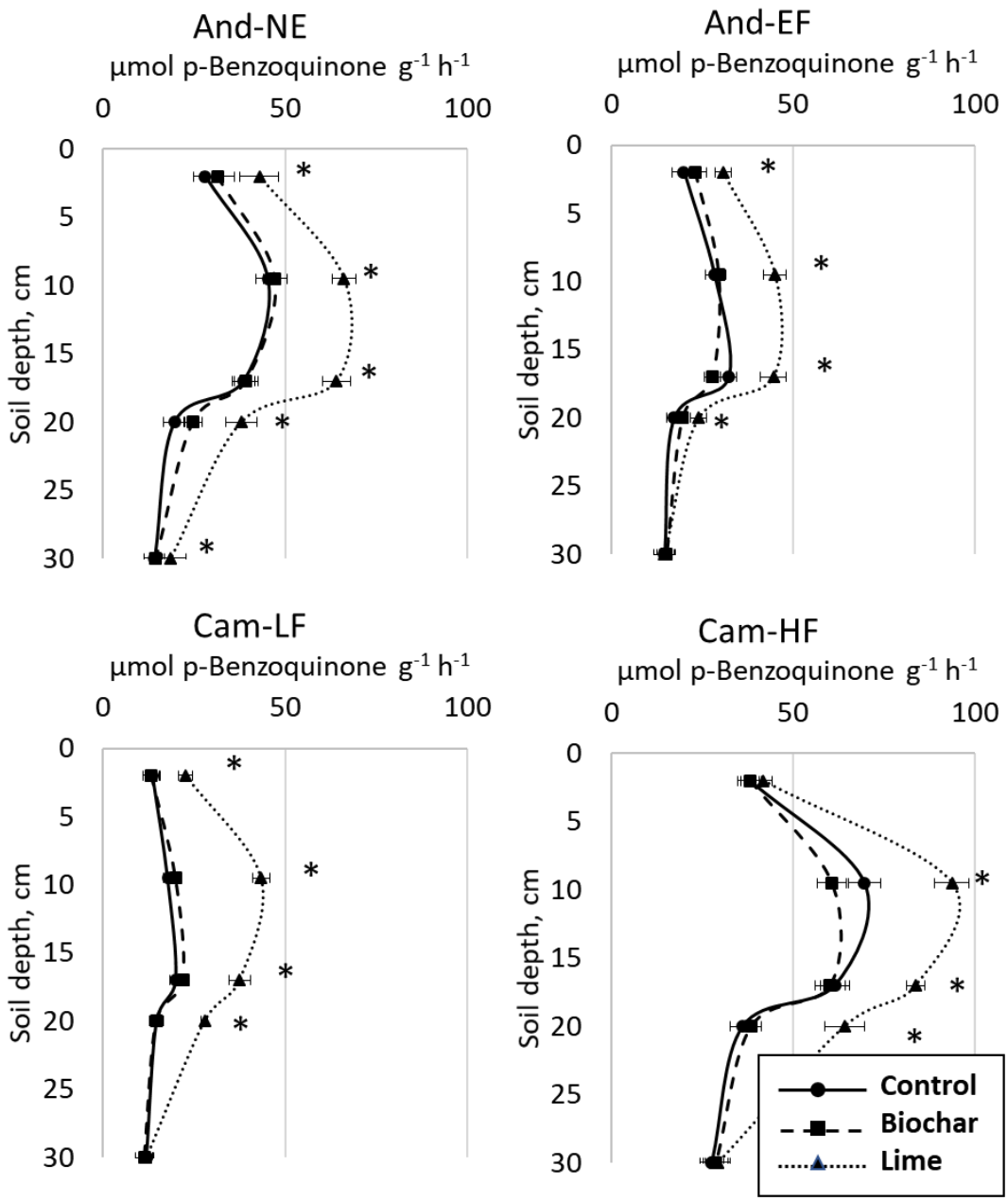
SI Figure 5.3 Nitrate reductase activity in experimental treatments within soil profile in experimental treatments after 12 months of the experiment. Values represent mean ± SE. Asterix represent the layers with significant (P<0.05) differences in the enzyme activities between treatments.



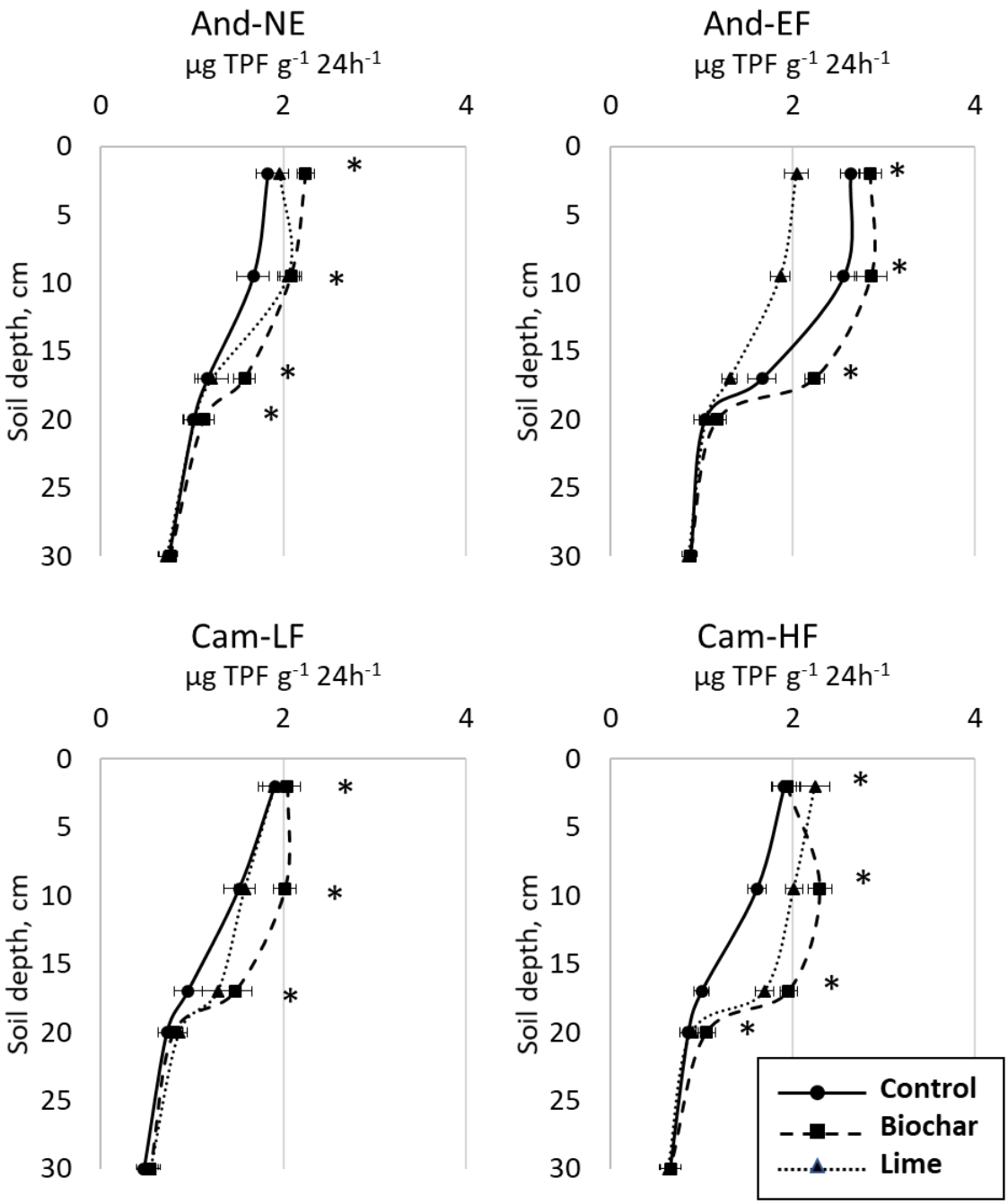
SI Figure 5.4 Urease activity in experimental treatments within soil profile in experimental treatments after 12 months of the experiment. Values represent mean ± SE. Asterix represent the layers with significant (P<0.05) differences in the enzyme activities between treatments.



SI Figure 5.5 Cellulase activity in experimental treatments within soil profile in experimental treatments after 12 months of the experiment. Values represent mean ± SE. Asterix represent the layers with significant (P<0.05) differences in the enzyme activities between treatments.



SI Figure 5.6 Peroxidase activity in experimental treatments within soil profile in experimental treatments after 12 months of the experiment. Values represent mean ± SE. Asterix represent the layers with significant (P<0.05) differences in the enzyme activities between treatments.



SI Figure 5.7 Dehydrogenase activity in experimental treatments within soil profile in experimental treatments after 12 months of the experiment. Values represent mean ± SE. Asterix represent the layers with significant (P<0.05) differences in the enzyme activities between treatments.

Chapter 5

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Stanislav Garbuz
Name/title of Primary Supervisor:	Dr Maria Minor
Name of Research Output and full reference:	
Garbuz, S. A., Camps Arbestain, M., MacKay A. D., DeVantier, B., & Minor, M. A. Enzyme activities and the influence of biochar addition on soil biochemical processes in two New Zealand pasture soils	
In which Chapter is the Manuscript /Published work:	Chapter 5
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	70%
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
The candidate used soil samples from the field experiment described in chapter 4, performed laboratory analysis and interpreted the results. The supervisors (M. Camps-Arbestain, A.D. MacKay, M. Minor) provided advice on analysis, comments on the results, and contributed to manuscript writing. Brian DeVantier assisted with samples collection.	
For manuscripts intended for publication please indicate target journal:	
Geoderma	
Candidate's Signature:	Stanislav Garbuz 2020.02.26 11:57:02 +13'00'
Date:	26/02/2020
Primary Supervisor's Signature:	Maria Minor Digitally signed by Maria Minor Date: 2020.02.26 12:23:01 +13'00'
Date:	26/02/2020

(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)

Chapter 6

Dynamics of natural capital stocks and enzyme activities in a field-based mesocosm experiment under livestock grazing 6- and 12-months after the addition of biochar

Abstract

To assess the impact of biochar on the potential of soils to provide ecosystem services, we investigated the influence of the addition of 1% w/w willow biochar (12 Mg ha^{-1}) on time dynamics of physicochemical and biological properties of two contrasting pastoral soils – a sil-andic Andosol and a dystric Cambisol. Each research area had two sites (paddocks) managed under different agricultural practices: with and without effluent in the Andosol, and with either low or high P fertilizer input in the Cambisol. Soil samples were collected 6 and 12 months after the start of the experiment.

Except for mineral N (NO_3^--N and NH_4^+-N), the effect of sampling time was similar across sites. Soil biological and biochemical properties showed time dynamics which we speculate reflect seasonal patterns. Changes in soil biota were reflected in soil enzyme activities. The positive effect of biochar on the soil natural capital stocks (organic carbon, total N and Olsen P) was apparent at both sampling times at all sites. Both soil acidity and bulk density were reduced by biochar and remained at the same level after 6 and 12 months in all four sites. The effect of biochar on mineral N was not constant over time, and mostly depended on the soil type and management practices rather than on treatments. Biochar increased bacterial and fungal biomass as well as abundance of arthropods and earthworms. Overall, the effects of biochar were consistent over time, and did not depend on the time of sampling over a period of 12 months. During such time period, biochar had a persistent effect on soil natural capital stocks and functions and showed itself as an effective amendment able to enhance the provisioning of ecosystem services over time.

6.1 Introduction

The seasonal patterns and dynamics of soil properties and biological activity help to identify the main drivers of the carbon (C), nitrogen (N) and phosphorus (P) biogeochemical cycles (Harrison, 2016; Macdonald et al., 2018). Seasonal changes in

temperature and water regime, and associated changes in biological activity (e.g., of plants and microorganisms) affect the input of organic C (Wuest, 2014) and nutrients (Baars et al., 1990; Ross et al., 1995; Lal, 2002) from decaying roots.

Application of organic amendments, such as biochar, may have a long-term impact on soil biota and soil processes (Jones et al., 2012), and on the dynamics of soil biogeochemical cycles (Sarathchandra et al., 1988; Teutscherova et al., 2018; Holík et al., 2019). In the previous chapters (Garbuz et al. (2019), **Chapter 4**, **Chapter 5**) we considered the soil natural capital stocks (C, N, nutrients, soil biota) of four pasture systems and looked at the effect of biochar on these stocks. Twelve months after the application of willow biochar application (1% w/w), there was a significant effect on soil physical, chemical and biological properties. Biochar reduced soil bulk density (BD) and soil acidity (at the same level as did lime), increased non-labile (i.e., protected from decomposition) organic C, soil N stocks, and soil available phosphorus (Olsen P). Soil biological properties were also affected by biochar addition with (i) soil microbial C (bacteria and fungi), (ii) plant root biomass, and (iii) soil meso- and macro-fauna being significantly higher in biochar-treated soils.

To better understand the long-term impact that biochar may have on soil ecosystem services, in the present study we investigate the effect of applying willow biochar at a rate of 1% w/w on the time dynamics of physicochemical and biological properties of two contrasting pastoral soils.

6.2 Materials and methods

6.2.1 Field-based mesocosm experiment

The field sites and the design and layout of the field-based mesocosm experiment are described in **Chapter 4**. The experiment at the Ballantrae location (two Cambisol paddocks) started on October 24th 2017, and at Hawera (two Andosol paddocks) on November 15th 2017, during the southern hemisphere spring (climatological information

is provided in **Fig. 6.1**). The first destructive sampling (18 cores from each paddock) was done 6 months after the start of the experiment during the southern hemisphere autumn – on May 6 2018 for the Cambisol and on May 21 2018 for the Andosol. The second sampling (the remaining 18 cores) was collected 12 months after the start of the experiment (southern hemisphere spring – on November 6 2018 for the Cambisol and on November 20 2018 for the Andosol).

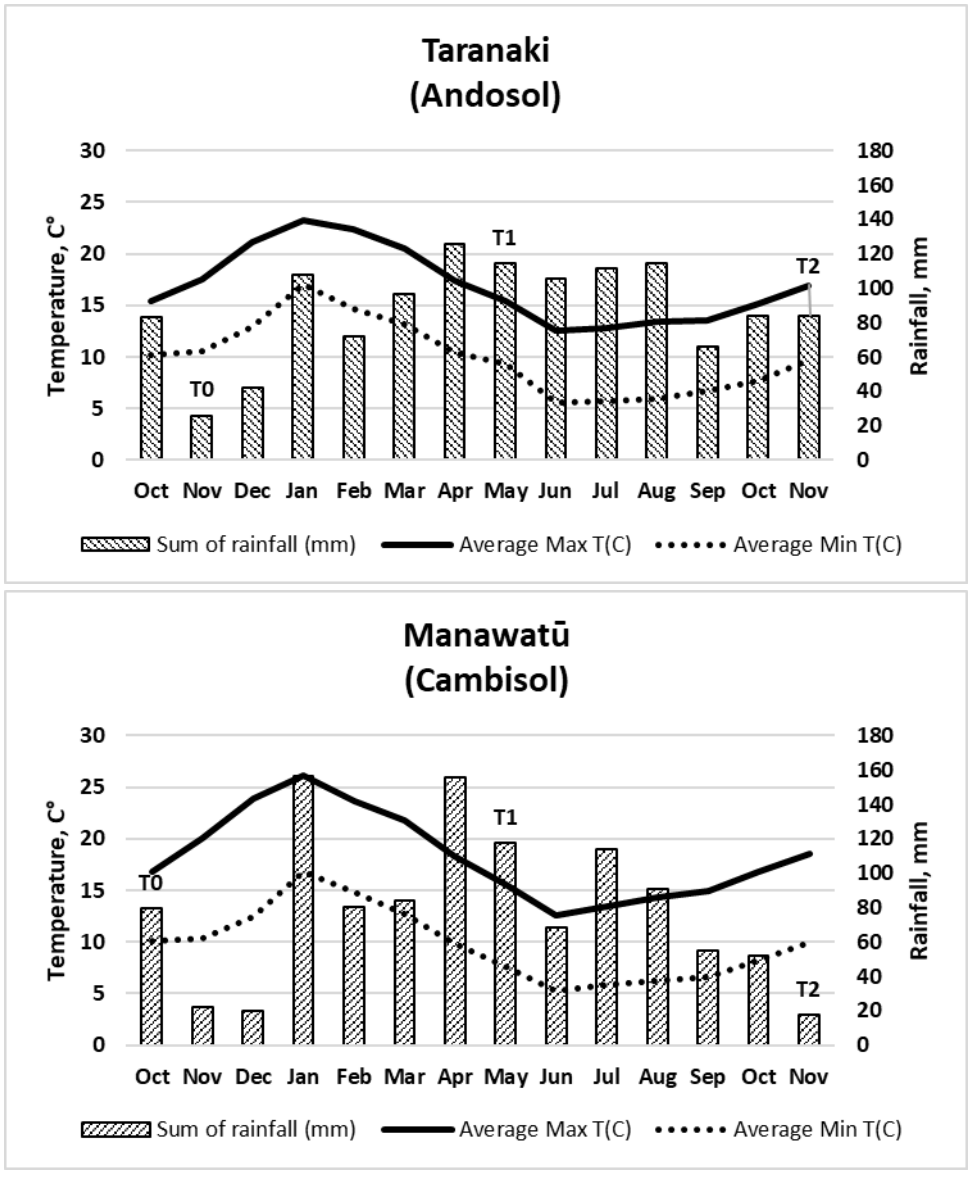

Figure 6.1 Average monthly temperatures (max and min) and precipitation during the time of the experiment (source: Meteorological Services of New Zealand Limited). T0, T1 and T2 represent the start of the experiment (T0, "initial" soil), sampling at 6 months (T1) and at 12 months (T2).

6.2.2 Biochar Production and Characterisation, and Soil Properties

In the present study (as in the previous Chapters), we used biochar produced from willow wood at relatively low temperature 350°C. This biochar had a low nutrient content (Fertilizer class - 0) and a relatively low liming potential (Liming class 1), but had a high C_{org} content (>70%) and an intermediate C storage potential (Carbon storage class 2). Biochar production and characteristics are described in **Chapter 3**.

Soil properties (physicochemical and biological) are described in **Chapter 4**. Methods for quantifying soil enzymatic activities are detailed in **Chapter 5.** Initial soil physicochemical properties at five depths are presented in **Table 6.1**.

6.2.3 Statistical Analysis

Statistical analysis was carried out using R software version 3.3.3. Normality of data sets was evaluated by the Shapiro-Wilk test. Analysis of variance (ANOVA) with contrast statements and Tukey HSD test were used to investigate the effect of factors: paddocks (And-NE, And-EF, Cam-LF, Cam-HF), sampling time (after 6 months and after 12 months), paddock*time and treatments*time interactions (where treatments were control, biochar, and lime) on variables: soil biological properties (earthworms, microbes and arthropods), physical (bulk density) and chemical properties (pH, OC, TN, Olsen P, NO_3^--N and NH_4^+-N) and enzyme activities (dehydrogenase, cellulase, peroxidase, urease, nitrate reductase, acid and alkaline phosphatases).

For all variables for which the paddock*time interaction was not significant, only global effects were reported, while for variables for which the paddock*time interaction was significant, the four paddocks were considered separately (**Table 6.2**).

6.3 Results

6.3.1 Soil BD, and soil C and N stocks

Bulk densities (BD) of the undisturbed soil were similar within each soil order (**Table 6.1**). After 6 months, the soil BD in all paddocks decreased (P<0.005) in

comparison with the initial undisturbed soil. However, no differences were found between the 6-months and 12-months sampling (P=0.66) (**Fig. 6.2**, **Table 6.2**).

For soil OC and TN concentrations, both time effects and site (paddock) effects were significant (**Table 6.2**). Initial soil OC and TN concentrations were higher in the Andosol than in the Cambisol, with the highest values in the Andosol receiving effluent (And-EF) (**Table 6.1**). At 6 months, the negative controls had a lower OC concentration in comparison with their initial values (P<0.005), but these differences were mitigated by the end of the experiment (at 12 months) (**Fig. 6.3**). The OC concentration of the positive controls (soil treated with lime) also decreased after 6 months of the experiment (P<0.005) to the same level as the negative control, but did not recover to its initial value at 12 months (P<0.005). As expected, the biochar-treated soil had higher OC concentration than the control soils at month 6 (P<0.005) and also higher than the soil at the start of the experiment – on average by 3 g kg^{-1} for all four paddocks (biochar provided 7 g OC to each kg of soil). Interestingly, at month 12, OC concentration values in biochar-treated soil increased further (P<0.005) (**Fig. 6.3**), and were, on average, 6.3 g kg^{-1} OC above the initial values (before the addition of the biochar) for all four paddocks.

Table 6.1 Initial soil physicochemical properties at five depths. Within a row, lowercase letters denote significant differences between paddocks (Tukey HSD, $\alpha<0.05$, all global F-tests significant).

Soil	Depth, cm	Andosol NE	Andosol EF	Cambisol LF	Cambisol HF
BD, g cm^{-3}	**0-2**	0.86 a	0.87 a	0.71 b	0.70 b
	2-9.5	0.68 b	0.69 b	0.71 a	0.71 a
	9.5-17	0.74 b	0.75 b	0.77 a	0.76 ab
	17-20	0.76 b	0.78 ab	0.80 a	0.81 a
	20-30	0.95 a	0.96 a	0.96 a	0.96 a
pH	**0-2**	6.1 a	6.0 a	5.2 c	5.3 c
	2-9.5	5.6 b	5.9 a	5.1 c	5.2 c
	9.5-17	5.5 b	5.9 a	5.1 c	5.2 c
	17-20	5.6 b	5.9 a	5.2 c	5.3 c
	20-30	5.7 b	6.1 a	5.3 c	5.4 c
OC, g kg^{-1}	**0-2**	86.1 b	92.5 a	79.6 c	65.2 d
	2-9.5	68.4 b	80.4 a	54.3 d	60.4 c
	9.5-17	55.1 b	68.3 a	44.2 d	50.1 c
	17-20	44.7 b	50.7 a	33.7 d	40.8 c
	20-30	27.3 b	33.5 a	20.6 d	34.0 c
TN, g kg^{-1}	**0-2**	8.3 a	8.3 a	5.8 d	6.7 c
	2-9.5	6.2 b	7.3 a	4.9 d	5.4 c
	9.5-17	5.4 b	6.5 a	4.0 d	4.8 c
	17-20	4.5 b	5.1 a	3.1 d	4.0 c
	20-30	3.1 b	3.5 a	2.2 c	3.4 a
Olsen P, mg kg^{-1}	**0-2**	51.1 a	35.4 c	6.1 d	40.6 c
	2-9.5	35.5 a	19.5 c	4.5 d	20.9 c
	9.5-17	31.4 a	15.8 c	4.3 d	16.8 c
	17-20	13.1 a	7.3 c	3.3 d	9.3 c
	20-30	8.2 a	4.7 b	2.4 c	4.8 b
NO_3^-–N, mg kg^{-1}	**0-2**	23.5 b	39.1 a	3.2 d	12.2 c
	2-9.5	21.5 b	35.3 a	2.3 d	10.8 c
	9.5-17	17.8 b	32.0 a	2.2 d	8.9 c
	17-20	13.2 b	25.2 a	2.0 d	9.5 c
	20-30	12.2 a	10.1 b	2.0 d	5.3 c
NH_4^+–N, mg kg^{-1}	**0-2**	28.3 b	31.2 a	11.0 d	20.2 c
	2-9.5	22.6 b	24.8 a	8.6 d	15.6 c
	9.5-17	18.5 a	18.8 a	7.7 c	10.6 b
	17-20	12.2 b	17.2 a	2.1 d	4.9 c
	20-30	7.3 a	6.8 b	1.3 d	3.6 c

Table 6.2 F- and *P*-values for analysed soil properties and effects of paddock ("site"), time, and their interaction.

	Site		Time		Site*Time
	$F_{3,136}$	*P*	$F_{1,136}$	*P*	$F_{3,136}$
BD	8.98	**<0.005**	0.197	0.66	0.111
pH	231.23	**<0.005**	0.94	0.33	0.15
OC	281.64	**<0.005**	18.09	**<0.005**	0.96
TN	319.41	**<0.005**	5.62	**<0.05**	2.29
NO3	5523.78	**<0.005**	12.84	**<0.005**	16.71
NH4	1203.58	**<0.005**	144.94	**<0.005**	21.19
OlsenP	2080.33	**<0.005**	0,00	0.99	0.443
Earthworms	9.966	**<0.005**	0.961	**<0.005**	2.54
Bacteria	56.55	**<0.005**	137.33	**<0.005**	29.64
Fungi	36.41	**<0.005**	9.47	**<0.005**	11.37
Arthropods	52.05	**<0.005**	39.78	**<0.005**	10.68
AlP	2093.3	**<0.005**	12.73	**<0.005**	43.74
AcP	2269.71	**<0.005**	832.5	**<0.005**	40.7
PPO	370.48	**<0.005**	57.59	**<0.005**	8.32
DHG	66.91	**<0.005**	75.26	**<0.005**	10.86
Cel	306.5	**<0.005**	253.4	**<0.005**	22.9
Ure	11309.37	**<0.005**	53.68	**<0.005**	100.43
NR	5247.96	**<0.005**	16.78	**<0.005**	43.77

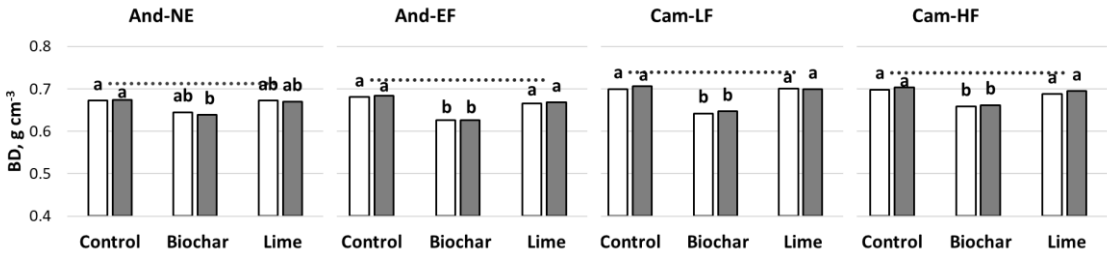

Figure 6.2 Soil BD in experimental treatments (mean for 2-9.5 and 9.5-17 cm depths) at the 6 months (white) and 12 months (grey) of the experiment. Dashed line shows the value in the initial soil. Lowercase letters indicate significant differences between treatments and between sampling times within a specific site (paddock), Tukey HSD test, $\alpha<0.05$.

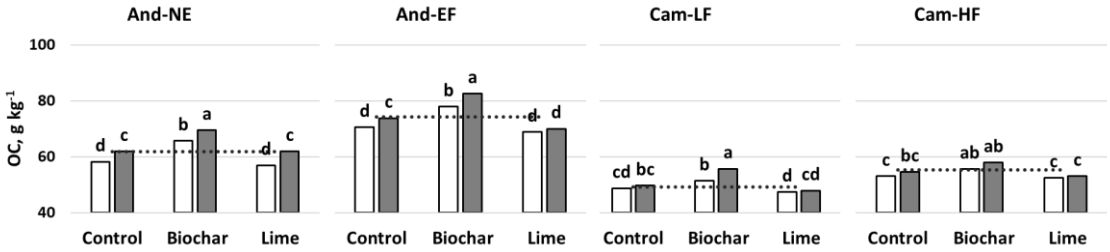

Figure 6.3 Soil OC in experimental treatments (mean for 2-9.5 and 9.5-17 cm depths) at the 6 months (white) and 12 months (grey) of the experiment. Lowercase letters indicate significant differences between treatments and between sampling times within a specific site (Tukey HSD test, $\alpha<0.05$). Dashed line shows the value of the initial soil.

In all sites, at month 6, TN concentration values of the control soils and of the lime-treated soils were similar to that of the initial soil and did not change by month 12 (**Fig. 6.4**). In the biochar-treated soils, TN values at month 6 were similar to their initial level, but by month 12 they increased ($P<0.005$) in all paddocks except for And-NE. In And-NE at month 6 the biochar-treated soil had a TN concentration already higher than the initial soil ($P<0.005$) and it remained so thereafter (**Fig. 6.4**).

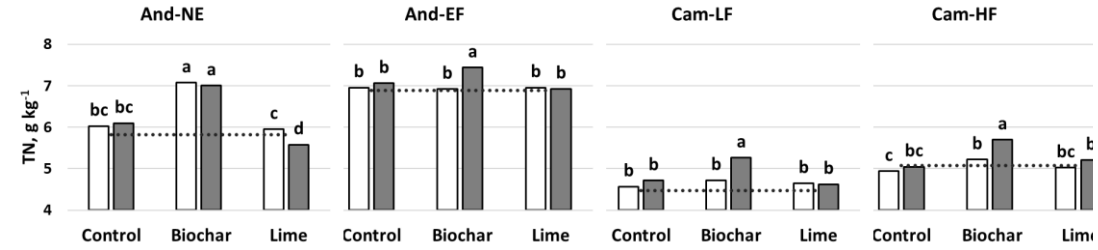

Figure 6.4 Soil TN in experimental treatments (mean for 2-9.5 and 9.5-17 cm depths) at the 6 months (white) and 12 months (grey) of the experiment. Lowercase letters indicate significant differences between treatments and between sampling times within a specific site (Tukey HSD test, $\alpha<0.05$). Dashed line shows the value of the initial soil.

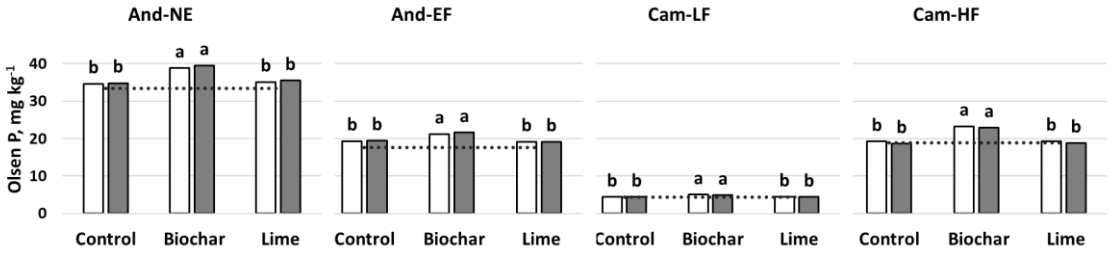

Figure 6.5 Soil Olsen P in experimental treatments (mean for 2-9.5 and 9.5-17 cm depths) at the 6 months (white) and 12 months (grey) of the experiment. Lowercase letters indicate significant differences between treatments and between sampling times within a specific site (Tukey HSD test, $\alpha<0.05$). Dashed line shows the value of the initial soil.

Olsen P did not change over time in any paddock or treatment ($P<0.005$, **Fig. 6.5**). In biochar-treated soils, Olsen P was higher than in the other treatments at both sampling times ($P<0.005$). Biochar application added 2.96 mg of available P to each kg of soil; however, the observed increase in Olsen P was on average by 3.83 mg kg-1 across all paddocks. In all paddocks, soils that received biochar and lime had higher ($P<0.005$) soil pH (**Fig.6.6**); there was no effect of sampling time on pH ($P=0.33$, **Table 6.2**).

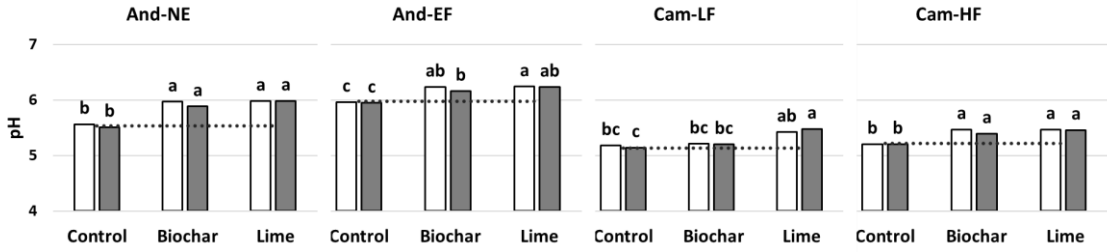

Figure 6.6 Soil pH in experimental treatments (mean for 2-9.5 and 9.5-17 cm depths) at the 6 months (white) and 12 months (grey) of the experiment. Values represent mean ± SE. Lowercase letters indicate significant differences between treatments and between sampling times within a specific site (Tukey HSD test, $\alpha<0.05$). Dashed line shows the value of the initial soil.

For NO_3^--N and NH_4^+-N, the effects of time, site, and site*time interactions were significant (**Table 6.2**). In the Cambisols, concentration of NO_3^--N for all treatments increased ($P<0.005$) after 6 months (autumn) (**Fig. 6.7 a**), with soils that received biochar having higher concentration of NO_3^--N ($P<0.005$) than the other treatments. After 12 months (spring), NO_3^--N concentration values decreased down to their initial levels ($P<0.005$). In the Andosol sites, NO_3^--N concentration of the control and lime-treated soils remained the same as at the time 0, except for the And-EF soil that received lime, where NO_3^--N concentration increased over time ($P<0.005$). Biochar-treated Andosols had higher NO_3^--N concentration at month 6 than the initial soil ($P<0.005$), as did the biochar-treated Cambisols. After 12 months (spring), NO_3^--N concentrations in the And-EF soils returned to their initial level ($P<0.005$), whereas those in the And-NE soil remained high (**Fig. 6.7 a**).

Concentrations of NH_4^+-N in the And-EF and Cam-LF paddocks 6 months after of the start of the experiment were much lower than their initial values ($P<0.005$). However, by month 12, the NH_4^+-N increased back to the initial levels ($P<0.005$, **Fig. 6.7 b**). In the other two sites (And-NE and Cam-HF) there was no noticeable differences between the initial NH_4^+-N concentrations and those at month 6. In all sites and treatments, concentration values of NH_4^+-N were higher ($P<0.005$) at month 12 (spring) than at month 6 (autumn).

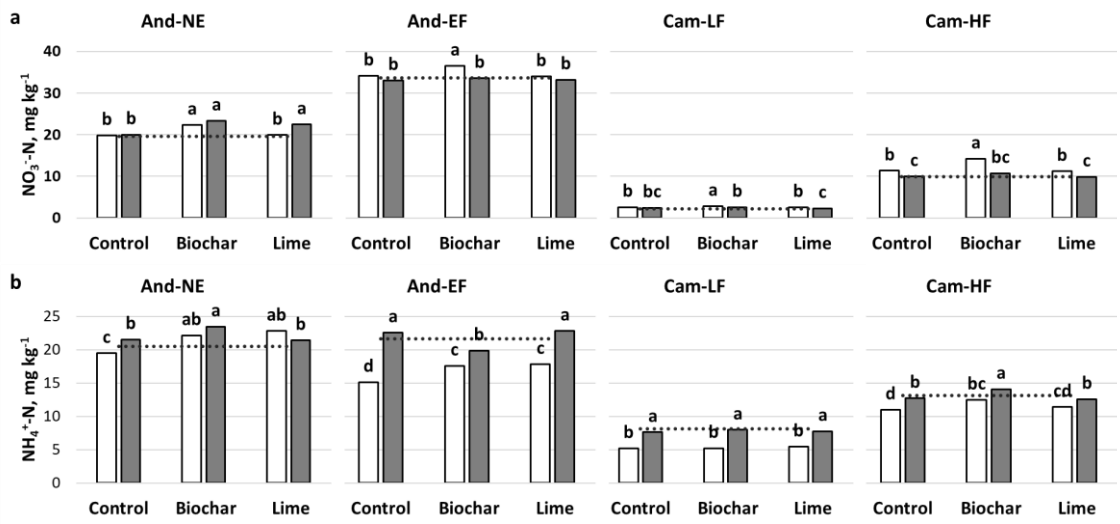

Figure 6.7 Soil nitrate-N (a) and ammonium-N (b) in experimental treatments (mean for 2-9.5 and 9.5-17 cm depths) at the 6 months (white) and 12 months (grey) of the experiment. Lowercase letters indicate significant differences between treatments and between sampling times within a specific site (Tukey HSD test, $\alpha<0.05$). Dashed line shows the value of the initial soil.

6.3.2 Biological properties

For all biological variables the effect of sampling time was significant; the site*time interaction was significant as well (**Table 6.2**), so the four paddocks (sites) are described separately.

Initial C_b was within a similar range (860 – 1101 mg kg^{-1}) across all four sites, whereas the C_f of the And-NE soils (1016 mg kg^{-1}) was twice that of the other three sites, which had a similar C_f (417-556 mg kg^{-1}). At month 6, both C_b and C_f were higher than control in both biochar-treated Andosols (all $P<0.005$), while in Cambisol paddocks the effect was less consistent (**Fig. 6.8 a, b**). At month 12 (spring), C_b values in the Andosol paddocks remained at the same level as at month 6, whereas in the Cambisol they increased further ($P<0.005$). The C_f values in And-NE and Cam-HF stayed at the same level at months 6 and 12, but in And-EF and Cam-LF they increased at month 12 ($P<0.005$). The most significant increase in C_b and C_f over time was observed in the Cam-

LF soil (**Fig. 6.8 a, b**). At month 12, both C_b and C_f were higher than the control in all biochar-treated soils.

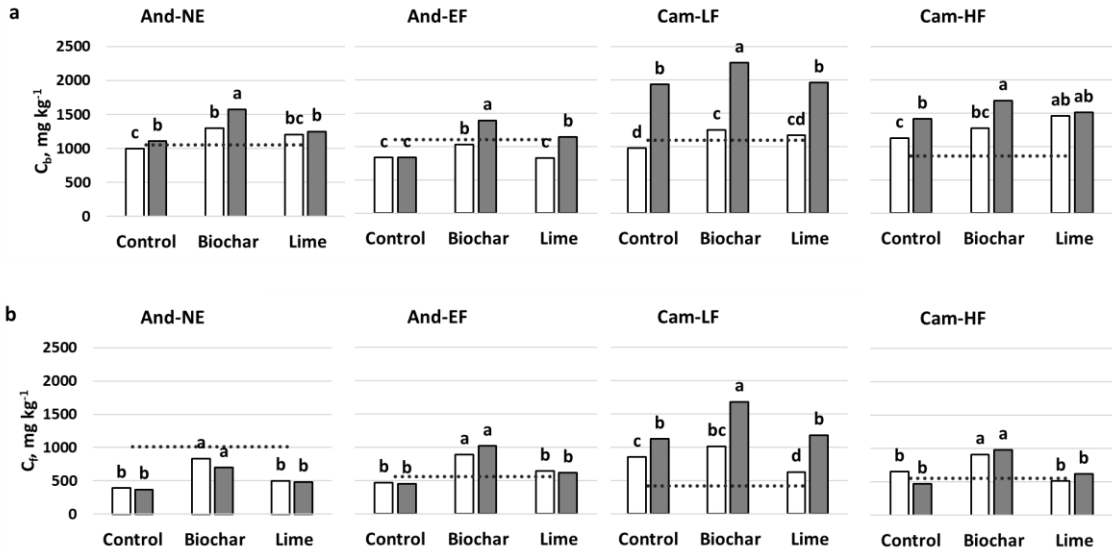

Figure 6.8 Soil bacterial (a) and fungal (b) biomass C in experimental treatments (mean for 2-9.5 and 9.5-17 cm depths) at the 6 months (white) and 12 months (grey) of the experiment. Lowercase letters indicate significant differences between treatments and between sampling times within a specific site (Tukey HSD test, α<0.05). Dashed line shows the value of the initial soil.

Earthworm abundance was lower in the Cam-HF than in all other paddocks (P<0.005, **Fig. 6.9**). Earthworms abundance after 6 months (spring) decreased (P<0.005) in the Andosol, and increased (P<0.005) in the Cam-HF soil (**Fig. 6.9**). At month 12 (autumn), there were no changes in earthworm abundance in the Andosol, whereas in the Cambisol they slightly decreased (P<0.005). Biochar did not affect earthworms (except in the Cam-LF, where earthworm abundance was higher in the presence of biochar, P<0.005), whereas their abundance was significantly higher in all lime-treated soils. At month 12, the effect of biochar and lime on earthworm abundance was less pronounced.

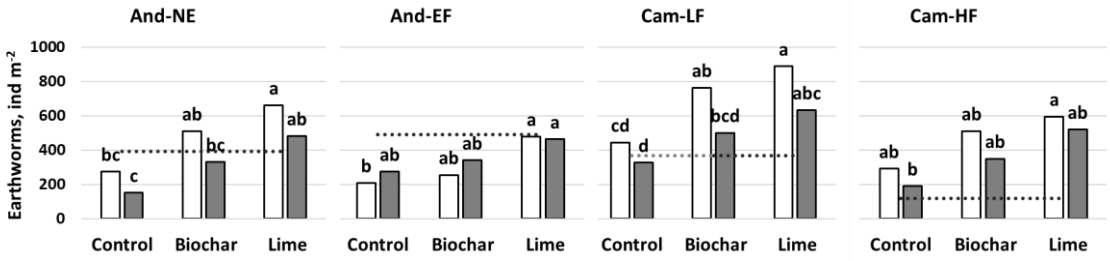

Figure 6.9 Earthworms abundance in experimental treatments (mean for 2-9.5 and 9.5-17 cm depths) at the 6 months (white) and 12 months (grey) of the experiment. Lowercase letters indicate significant differences between treatments and between sampling times within a specific site (Tukey HSD test, $\alpha<0.05$). Dashed line shows the value of the initial soil.

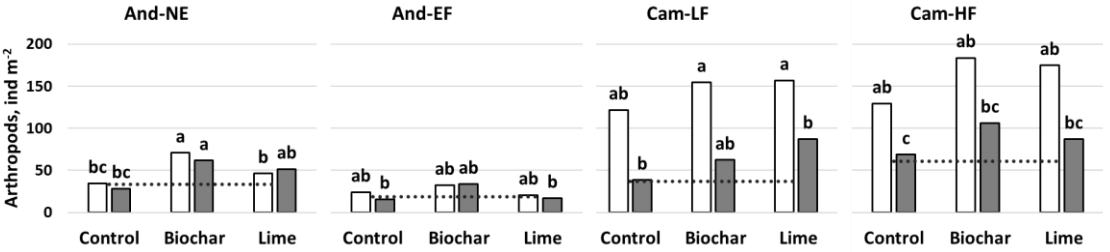

Figure 6.10 Arthropods abundance in experimental treatments (mean for 2-9.5 and 9.5-17 cm depths) at the 6 months (white) and 12 months (grey) of the experiment. Lowercase letters indicate significant differences between treatments and between sampling times within a specific site (Tukey HSD test, $\alpha<0.05$). Dashed line shows the value of the initial soil.

The arthropods population in the Andosol paddock receiving effluent (And-EF) was less than half that of the paddock without effluent (And-NE), while in the high fertility Cam-HF arthropods were more abundant than in low fertility Cam-LF. Arthropods abundance (**Fig. 6.10**) did not change over the time in the Andosol, while in the Cambisol, at month 6, the arthropod abundance increased ($P<0.005$) in comparison with the initial soil, and at month 12 it returned to the initial value ($P<0.005$). In the Andosol treated with biochar, arthropod abundance was higher ($P<0.005$) than control at both sampling times. In the Cambisol, there was no effect of biochar on arthropods at month 6, while at month 12, their abundance was higher in the Cam-HF with biochar ($P<0.005$).

6.3.3 Soil enzyme activities

Similar to soil biota variables, all soil enzymes were significantly influenced by sampling time and paddock ("site"); the site*time interactions were significant for all enzymes (**Table 6.2**).

At month 6 (autumn), cellulase (Cel) and peroxidase (PO) activities in all four paddocks were higher ($P<0.005$) than the initial values, but returned to levels similar to the initial values at month 12 (spring); the dehydrogenase (DHG) had the opposite trend (**Fig. 6.11 a-c**). Cellulase activity (**Fig. 6.11 a**) was similar across all sites, except in the Cambisol with low fertility (Cam-LF) where Cel activity was significantly higher than the rest ($P<0.005$). After 6 months, there was no effect of treatment on Cel activity, whereas after 12 months, its activity was higher ($P<0.005$) in soils with biochar. Peroxidase activity (**Fig. 6.11 b**) at month 6 was higher than the negative control in soils with biochar and lime, whereas at month 12, PO activity was higher ($P<0.005$) than the negative control only in lime-treated soils. In biochar-treated soils, the activity of DHG was higher ($P<0.005$) than that of negative controls for all paddocks and at both sampling times (**Fig. 6.11 c**). In lime-treated soils, DHG activity was higher ($P<0.005$) than that in the negative control in the And-EF soil at both sampling times, and in the And-NE and Cam-LF soils only at month 12. In the Cam-HF soil with lime, DHG activity was lower ($P<0.005$) than that of the negative control at month 12.

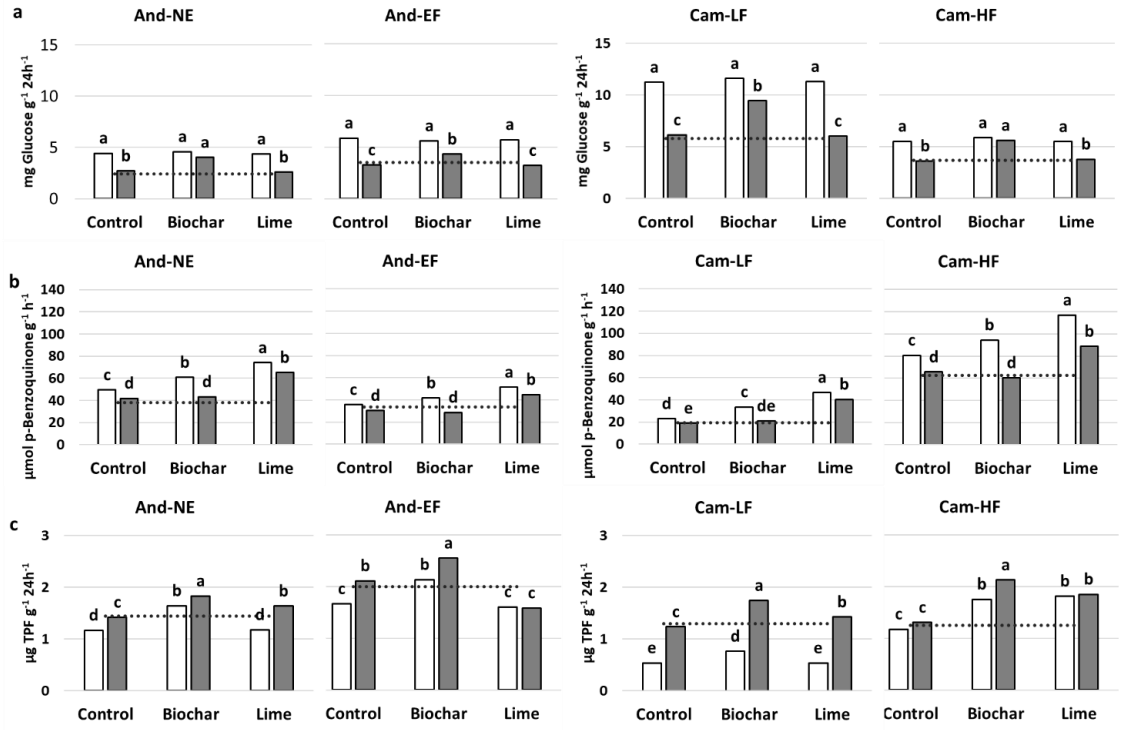

Figure 6.11 Activities of cellulase (a), peroxidase (b), and dehydrogenase (c) in experimental treatments (mean for 2-9.5 and 9.5-17 cm depths) at the 6 months (white) and 12 months (grey) of the experiment. Lowercase letters indicate significant differences between treatments and between sampling times within a specific site (Tukey HSD test, $\alpha<0.05$). Dashed line shows the value of the initial soil.

In all paddocks, the nitrate reductase (NR) activity in the negative control did not change over time, although in the Cam-HF soil it was lower ($P<0.005$) than in the initial soil at both sampling times (**Fig. 6.12 a**). In the low fertility Cambisol (Cam-LF) with biochar, the NR activity was not different from the negative control at month 6 (autumn) but increased ($P<0.005$) at month 12 (spring). In the Cam-HF soil with biochar, the NR activity was higher ($P<0.005$) than control at both sampling times. In the Andosol soils with biochar, the NR activity was higher than in control ($P<0.005$) after 6 months (autumn), whereas at month 12 (spring), it was at the same level as in the initial soil. Nitrate reductase activity in soils with lime was higher ($P<0.005$) than in the negative

control at both sampling times and in all four paddocks, and the effect of lime mostly was more pronounced (P<0.005) than that of biochar.

Urease (Ure) activity (**Fig. 6.12 b**) in all four sites was lower at month 6 (autumn) than in the initial soil, but returned to the initial level at month 12. In biochar-treated soil of all four paddocks, Ure activity at month 6 was higher (P<0.005) than in the negative control, and at month 12 this effect became even more pronounced (P<0.005).

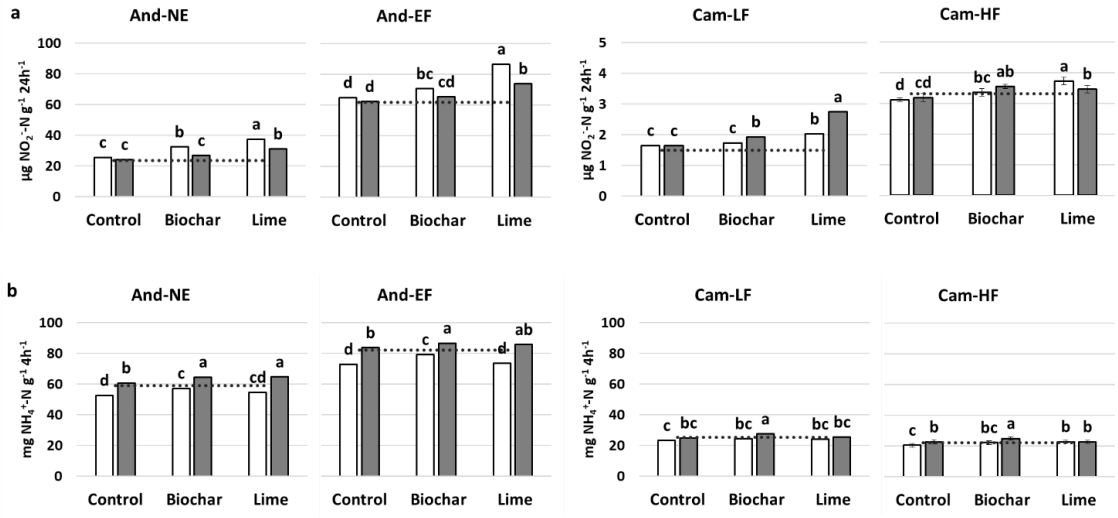

Figure 6.12 Activities of nitrate reductase (a) and urease (b) in experimental treatments (mean for 2-9.5 and 9.5-17 cm depths) at the 6 months (white) and 12 months (grey) of the experiment. Lowercase letters indicate significant differences between treatments and between sampling times within a specific site (Tukey HSD test, α<0.05). Dashed line shows the value of the initial soil.

At month 6 (autumn), the alkaline phosphatase (AlP) activity was higher (P<0.005) than the initial values. At 12 months (spring), AlP activity returned to the initial values (P<0.005) in all sites except in the And-NE, where it had an opposite trend (**Fig. 6.13 a**). Biochar did not have any significant effect on AlP activity at month 6, but at month 12 activity of AlP in soil with biochar was higher than in the negative control. Across all sites, acid phosphatase (AcP) activity (**Fig. 6.13 b**) was higher (P<0.005) at month 6 (autumn) than in the initial soil, and at month 12, the activity was similar to that of the

initial soil. The soils that received amendments (biochar and lime) had lower AcP activity than the control (P<0.005), this effect was more pronounced at month 12 (P<0.005).

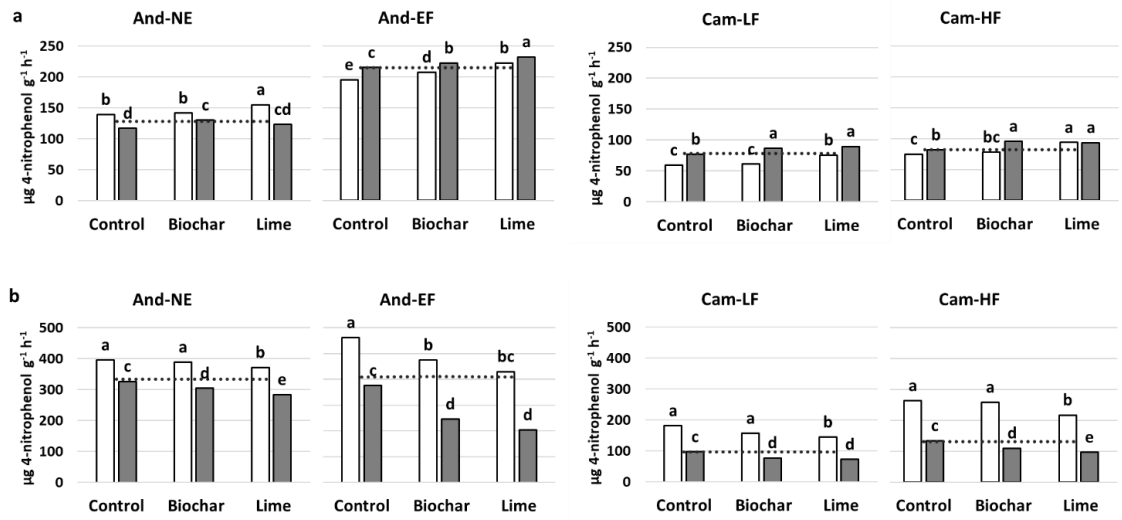

Figure 6.13 Activities of alkaline (a) and acid (b) phosphatases in experimental treatments (mean for 2-9.5 and 9.5-17 cm depths) at the 6 months (white) and 12 months (grey) of the experiment. Lowercase letters indicate significant differences between treatments and between sampling times within a specific site (Tukey HSD test, α<0.05). Dashed line shows the value of the initial soil.

6.4 Discussion

Due to the mild winters in the North Island of New Zealand, the biological activity does not have winter gaps. The lowest activity is observed during the dry and hot summer season (Wever et al., 2001). Despite the small temperature oscillation throughout the year, there is a noticeable effect of seasons on soil chemical properties (Ross et al., 1995; Luo et al., 1999), and in New Zealand pastures, soil biota and plant productivity have clearly outlined seasonal patterns. These pastures are characterized by a relatively low plant species diversity and high intensive grazing which, in turn, influences chemical, biological and biochemical processes (Chen et al., 2003; Wakelin et al., 2013). Further, and importantly, plant biological activity is much higher in spring season (Radcliffe,

1979; Baars et al., 1990). Plant growth rate depends not only on temperature and precipitation, but also on solar radiation, which is maximal in late spring and early summer, which is when all conditions are favourable (Anslow & Green, 1967; Barbhuiya et al., 2004). Plants stimulate microbial activity and biochemical processes through the trophic chains (Moore et al., 2004), and through symbiotic relationships (Santos-González et al., 2007). Seasonal abundance of soil invertebrates is linked to plant and microbial productivity (George Eni et al., 2014), as well as favourable soil moisture and temperature conditions (Barbhuiya et al., 2004). Accumulation of soil organic matter in New Zealand pastures is highest in late autumn and early winter, once the active plant growth ends. At that stage, the increase in organic C concentration could be up to 7 g kg^{-1} C larger than the lowest value in late spring (Ross et al., 1995).

In our experiment, there were no noticeable differences in average temperature between the autumn (at 6 months) and the spring (at 12 months) sampling times, but in autumn there was higher rainfall prior to the sampling, in comparison to the drier spring. Also, it should be taken into account, that the timeframe of our experiment does not allow to separate linear time effects from cyclic seasonal effects; in our study both would be confounded as a combined factor – the length of field incubation.

6.4.1 Soil physicochemical properties

The major differences in the soil physicochemical properties between the two soil orders can be explained by the difference in parent materials – the Andosol is derived from volcanic ash, while the Cambisol is derived from sedimentary material. Specifically, the Andosol can better resist compaction in lower horizons (Molloy, 1998), it has a higher OC content (Percival et al., 2000) and associated labile OC (Shen et al., 2018). The latter may explain why in our experiment the Andosol suffered from more pronounced drop in OC concentration at month 6, following soil disturbance during the experiment preparation. In addition, the soils could have experienced different pressure from

livestock, given that the Andosol paddocks were under dairy grazing and the Cambisol paddocks under sheep grazing. It is well known that grazing also has an impact on soil biochemical processes (Manas et al., 2000; Ingram et al., 2008) and on C and N stocks (Schipper et al., 2014).

After 6 months of the experiment, the soil BD of the negative control soil was lower than that of the undisturbed soil, which probably reflects soil structure breakdown during the preparation of the soil columns. This effect persisted over the entire duration of the experiment, which indicates that 12 months is not enough time for BD recovery. The soils that received biochar had even lower BD and this effect remained after 12 months of experimental duration. In the meta-analysis by Blanco-Canqui (2017), it was shown that under field conditions, application of biochar at even a small rate (1-2%) significantly decreased soil BD, and this effect could persist for 4 years.

Soil pH did not change over time (from month 6 to month 12); other authors observed limited seasonal dynamics of soil acidity in a 1-year experiment (Martins et al., 2016). As expected, soil with both biochar and lime amendments had higher pH, except Cam-LF where only lime had effect on soil pH. The liming effect of biochar and lime was still evident by the end of the experiment.

After 6 months from the start of the experiment, the biochar-amended soil had more Olsen P compared with the initial soil; the higher level of Olsen P remained present at 12 months. Gao et al. (2019) and Makoto et al. (2011) have shown that biochar has potential to stimulate P mineralization and, in this way, provides soil with more available P that the biochar itself may add. The dynamics of P are closely associated with that of microbial biomass, which in New Zealand pastures has seasonal patterns (Roberts, 1987; Chen et al., 2003; Edmeades et al., 2006).

Addition of alkaline amendments (lime, biochar) can cause a decline in OC by enhancing mineralization of labile organic C (Chan & Heenan, 1999; Shen et al., 2018;

Garbuz et al., 2019). Increasing of OC over time, observed in the present study, indicates the processes of C accumulation. Simultaneously, biochar can replace lost C with recalcitrant C present in its structure, which has a very slow decomposition (Lehmann et al., 2009). The significant increase in root biomass caused by biochar application observed the end of the experiment (see **Chapter 4**) (roots added 1.2 to 4.0 Mg C ha^{-1} to the soil C pool) is supported by our observations in the 6-month glasshouse experiment (Garbuz et al., 2019). In **Chapter 4** we speculated that the increase in root biomass was related to the mineral N deficiency in a first weeks/months of the experiment, as labile C from biochar enhanced the growth of soil microbial biomass, which lead to immobilization of mineral N. Mineral N has a very dynamic nature (Ellis, 1974; Ruz-Jerez et al., 1991) and, it is likely that the effect of amendments was overshadowed by the seasonal changes. Plants, especially in rhizosphere, and soil biota seasonal activity can have an impact on the dynamic of soil properties. In this study, the effect of biochar on soil TN (which increased) was only evident at the end of the experiment (spring). This could be explained by the fact that biochar stimulates roots growth (Xiang et al., 2017; Garbuz et al., 2019) and thus N_2 fixation in legumes, and has an effect on bacteria involved in N cycling (e.g., it can reduce N_2O emissions; Shi et al. (2019)); so, it can be assumed that biochar enhances N_2 fixation and protects soil from N loss; this effect is more pronounced during the active growth season (spring). Nguyen et al. (2017) have shown that biochar can reduce available N in short-term experiments, however, over time this effect disappears once labile C levels in biochar decline (Gao et al., 2019).

6.4.2 Biota

In the present study, bacterial biomass fluctuated over time, while fungal biomass tended to remain constant. The effect of sampling time was especially pronounced in the Cambisol with low fertility (Cam-LF). Biochar effect on bacterial and fungal biomass was more pronounced at month 12 (spring season). The microbial community is closely

linked to soil processes and nutrient transformation (Perrott et al., 1992), and the fluctuations of NH_4^+-N and OC over time observed in this study, especially in the Cam-LF soil, influenced the microbial biomass.

Despite the relatively long life of earthworms, their abundance changed during the time of the experiment. The highest earthworm abundance was at month 6 (autumn), while at the start of experiment and at 12 months (both in spring) the numbers were lower. Redmond et al. (2014) have shown that different ecological groups of earthworms have their particular seasonal dynamics. In New Zealand pastures earthworms had higher population density in autumn than in spring (Prestidge et al., 1997), which can in part be explained by accumulation of food resources and increase of root biomass during the summer and autumn (Eisenhauer et al., 2009; Wedderburn et al., 2010). As indicated above, in our experiment, autumn had approximately the same mild (15-20 ^oC) temperatures as in spring, but also had higher rainfall (**Fig. 6.1**), which would make it more favourable for earthworms, which prefer adequate moisture and mild temperatures (Curry, 2004; Ivask et al., 2006).

Total arthropods abundance in the Cambisol had the same time dynamics as the earthworms, while in the Andosol there was no time effect. There is little information on seasonal or time dynamics of arthropods in New Zealand pastures. Elsewhere, studies have shown a trend of increasing soil arthropod abundance during autumn (George Eni et al., 2014; Duyar & Makineci, 2016). The absence of seasonal effect in the Andosol can be perhaps explained by the permanently distressed conditions of soil arthropods under cattle treading pressure (Schon et al., 2012b).

6.4.3 Soil enzymes

Dynamics of soil enzyme activities are related to the activity of soil biota and their seasonal needs (Sarathchandra et al., 1984; Ross et al., 1995). Depending on the seasonal needs, plants and soil biota produce a variety of enzymes at different concentrations

(Steinweg et al., 2013; Yang et al., 2018b; Song et al., 2019). Data on the seasonal patterns of enzyme activities in New Zealand soils is very limited. In one of the published studies, Ross et al. (1995) measured the dynamics of three enzymes in a pasture soil (Ballantrae, the same area with the Cambisol as used in the present study) and found that each enzyme had a different pattern: invertase and sulphatase activities had small fluctuations during a year, whereas phosphodiesterase activity varied over time and had maximum in autumn and winter.

In the present study, there were no evident seasonal/time trends in soil enzymatic activities. Elsewhere, seasonal dynamics of soil enzymes have been observed. Lemanowicz (2018) showed that phosphatases (acid and alkaline) in a forest soil in Poland had the highest activities during the spring season. In a cultivated soil and a soil under a shelterbelt (also in Poland), nitrate reductase activity was boosted with increasing moisture content (Szajdak and Gaca (2010). High cellulase and peroxidase activities in the autumn season have been explained by the increase in root dieback at the end of summer, and increased plant residue inputs (Vardavakis, 1989; Sajjad et al., 2002; Sinsabaugh, 2010). Dehydrogenase activity has been associated with microbial activity (Casida, 1977). In the present study, the DHG activity was higher at month 12 (spring), when microbial biomass also tended to be highest (**Fig. 6.8 a, b**).

6.5 Conclusions

Studying the long-term effect of biochar on soil properties (C, N, P and etc) and biological processes is important in order to understand the potential benefits of biochar application to the provision of ecosystem services. The present study showed that even small application rates of biochar made from willow wood have large impacts on soil chemical and physical properties, and biological processes. These impacts remain apparent for up to 1 year.

Application of alkaline material (biochar and lime) as well as soil disturbance during the preparation of the soil columns resulted in the loss of OC. However, biochar compensated this loss, not only because of its content of condensed aromatic C, but also by enhancing root growth and biological activity. Overall, biochar favoured the accumulation of OC. The decrease in BD and soil acidity caused by biochar were still apparent at the end of the experiment. The positive effect of biochar on Olsen P was also stable after 1 year.

With the increase in root biomass, there was an accumulation of TN in pasture with clover, which can be attributed to the stimulation of N_2 fixation. This effect may be season-dependent, as it was more pronounced at the end of the experiment, in spring. Mineral N had a very noticeable (seasonal) pattern, with the highest nitrate concentration found in autumn and highest ammonium concentration detected in spring, although the timeframe of our experiment does not allow to separate linear time effects from cyclic seasonal effects.

Soil biota (bacteria and fungi, arthropods and earthworms) also had a time dynamics pattern which we interpret as seasonal. Soil enzymes, as products of biological activity, reflected the time dynamics of soil biota. Biochar, through the influence on soil chemical properties (providing some labile C and nutrients) and roots growth stimulation, generally stimulated soil trophic chains at various levels for different groups of soil biota; this effect was stable during the experiment. Biochar had a significant positive effect on cellulase, dehydrogenase and peroxidase and urease activities, which for cellulase, dehydrogenase and cellulase remained stable over time.

The effects of biochar on soil natural capital stocks and functions were stable within a one-year timeframe. This suggests that biochar could be an effective amendment to enhance the soil provisioning of ecosystem services over time.

Chapter 7

Effect of biochar addition as an amendment on the natural capital stocks and soil processes as they influence the provision of services in two contrasting soils

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Abstract

With increasing demands for food and fibre provided by the soil, there is more pressure on the environment. Biochar as a soil amendment could increase soil productivity and improve soil sustainability as the soil faces anthropogenic impacts. In this **Chapter** we (i) build an inventory of the effects that biochar addition, as an amendment, has on the quantity of soil natural capital (NC) stocks, the condition of the stocks, and on soil processes in two contrasting soils (Andosol and Cambisol) and (ii) explore the influence this has on five ecosystem services (ES), including provisioning services (food and fibre), regulating services (carbon sequestration, water regulation, disease and pest control), and a supporting process (fertility maintenance). For the investigation of each ecosystem service, specific soil properties (components of a soil NC) responsible for this service were chosen based on the literature, as were the key soil processes. The potential effect of biochar overall on soil ES was estimated following the applied approach developed by Hewitt et al. (2015). For this, the data on the effects of biochar addition on soil physicochemical and biological properties (**Chapter 4**) and enzymatic activity (**Chapter 5**) were used to calculate the percentage difference of these properties between control treatments and biochar/lime treatments. The sum of the percentage differences to control showed the overall effect of biochar or lime on soil natural capital stocks relevant to specific ecosystem services.

In comparison with the negative and the positive (lime) controls, biochar increased components of soil natural capital responsible for food and fibre production. In comparison with control and lime, there was a significant positive impact of biochar on soil properties, including soil microflora, earthworms, OC, soil BD, pH and overall soil enzyme activity, associated with carbon sequestration. Both biochar and lime had positive effect on water regulation and disease and pest control ecosystem services.

Results suggest that biochar could be considered as a forward-looking amendment which can increase plant productivity, reduce disease and pest risks, improve soil physical properties, and at the same time be used as an instrument to mitigate climate change.

7.1 Biochar as a soil amendment for sustainable agriculture – its effects on soil natural capital stocks and processes

The growing World population constantly requires more food and energy, which translates into more pressure on the planet Earth. One of manifestations of this pressure is global warming. Climate change is becoming increasingly acute each and every year, forcing the scientific community, industry and public authorities to search for ways to mitigate rising carbon dioxide levels in the air (IPCC, 2018). Sustainable agriculture, which includes both time-tested and advanced technologies, recognises both the needs of the human population and the importance of maintaining the environment (Pretty, 2008; Delgado et al., 2011). Biochar has shown itself as a technology that offers promise as a suitable component of sustainable agriculture (Woolf et al., 2010; Rodrigues & Horan, 2018; Peiris et al., 2019). Biochar applied as a soil amendment has been shown to offer a wide range of potential benefits to the soil, to agriculture and to the wider environment, but this effect depends on biochar feedstock, pyrolysis procedure and properties of soil it was applied to (Paz-Ferreiro et al., 2014; El-Naggar et al., 2018; Dai et al., 2020). Biochar addition to the soil has been shown to have an effect on the physical properties of the soil, including decreasing bulk density and improving water holding capacity (Herath et al., 2013; Burrell et al., 2016). In **Chapter 4** and **Chapter 6** we showed that the bulk density of two pasture soils to which biochar (1% w/w) was added as an amendment was 4.6-9.1% lower than control soils after 6 and 12 months in a field experiment using intact cores. The observed decrease in soil bulk density was much higher than the 1.9% decrease predicted from the bulk density values for the biochar and the soil, and their ratios in the soil mixture. It was suggested that biochar, in addition to its impact as an inert low bulk

density material, influences factors contributing to the soil aggregate structural assemblage and pore structure (e.g., through interactions with soil biota).

Biochar has the potential to retain nutrients in the soil through adsorption on its active surfaces (Zheng et al., 2013; Park et al., 2019), increasing nutrient retention and availability for plants, including phosphorus (Shen et al., 2016), and reducing the risk of nutrient losses by leaching. This improves nutrient use efficiency and, at the same time, reduces the risk of nutrient losses to the environment by reducing the amount of nutrients applied as fertilisers. In the present study (**Chapters 3, 4** and **6**) it was found that biochar application contributed positively to soil TN and Olsen P stocks. Total N had a dynamic increase which amounts to 11.2% (**Chapter 6**), as a result of the positive effect of biochar on N_2 fixation by legumes.

Biochar has proven to be a good liming agent to reduce soil acidity (Chintala et al., 2013). In **Chapters 3, 4** and **6** it was shown that biochar application had a similar effect on soil pH as lime application. The slightly lower liming effect of biochar appears to relate to its slightly larger particle size, while lime had very fine texture (**Chapter 4**).

Biochar addition has also been shown to have a positive influence on soil biota. In many cases this is seen as an increase in the size of the microbial community (Lehmann et al., 2011; Paz-Ferreiro et al., 2015). The stimulation of the microbial community translates into an increases in organic matter decomposition rates (Schinner, 1982), nutrient availability (Saccá et al., 2017) and aggregate stability (Boivin & Kohler-Milleret, 2011), all of which have the potential to enhance plant growth (Biederman & Harpole, 2012; Rawat et al., 2019). In the present study (**Chapters 3, 4 and 5**) we found that biochar application significantly increased both the fungal and bacterial components of the microbial community, as well as the abundance of soil meso- (Collembola, mites) and macro-fauna (earthworms).

As a consequence of its effect on nutrient supply, soil physical properties, available water, and biota, biochar addition has been found to improve plant growth and the quality of harvested parts of a wide range of crops (Biederman & Harpole, 2012; Paz-Ferreiro et al., 2014). Paz-Ferreiro et al. (2014) found that biochar addition increased millet production. Akhtar et al. (2014) and Petruccelli et al. (2015) both showed that biochar application positively influenced fruit quality of tomatoes. In this study (**Chapter 3** and **Chapter 4)** biochar addition had a positive effect on legume growth and pasture root growth, respectively. In the glasshouse experiment (**Chapter 3**) biochar application increased dry clover biomass on average by 22.7 Mg ha^{-1}. In the field-based mesocosm experiment (**Chapter 4**) we observed a 49.7%n increase in pasture grass root biomass following biochar application, on average by 6.9 Mg ha^{-1} (the above ground biomass was not measured). It was postulated that the increase in root biomass was a function of increased fungal and bacterial microbial activity in weeks following the biochar application. The hypothesis proposed in **Chapter 4** was that the increased microbial activity at the root surface resulted in N immobilization, creating a short-term deficiency of available N to which plants responded with increased root growth.

Biochar by nature has a high percentage of carbon resistant to degradation, so it can be used as an instrument to mitigate climate change by contributing to carbon sequestration (Lehmann et al., 2010; Biederman & Harpole, 2012). The use of biochar as a soil amendment adds directly to the soil carbon stocks. Brassard et al. (2016) in their meta-analysis have characterised 76 biochars from 40 research studies and showed that pyrolysis temperature during biochar production affects the C sequestration potential of the biochar. Calvelo Pereira et al. (2011) showed that with increase in pyrolysis temperature resulting biochar contains less labile C (available for soil microbes) and more recalcitrant C (resistant to microbial decomposition). Biochar used in present study was pyrolyzed at a relatively low temperature (350°C) and thus still had a considerable

fraction of labile C (about 49 % out of total C based on the study of (Calvelo Pereira et al., 2011) using the same feedstock and similar temperature of pyrolysis). In the present study (**Chapter 4**) the addition of 1% biochar, despite some initial loss of OC, increased soil OC stocks on average by 6.5 g kg^{-1} (10.6%) relative to control.

Addition of biochar as an amendment to the soil has the potential to modify existing soil natural capital (NC) stocks (e.g., soil microbiota and fauna, soil structure, bulk density, water holding capacity, pH, soil carbon stocks, etc.), and the potential to modify a range of soil processes (e.g. mineralisation, nutrient availability and attenuation), all of which influence the soil ability to sustain its overall stocks and the flow of services. Soils not only underpin food and fibre production, but also provide a wider range of other services or benefits to humans, which are often not recognised and only become of value when their supply becomes limited. These other services include, for example, flood mitigation through water regulation, filtering of nutrients and contaminants through exchange processes, carbon storage and greenhouse gases regulation through a range of carbon and nitrogen processes in soils, detoxification and the recycling of wastes through biological processing of dung and litter, regulation of pests and diseases through the provision of habitat for predators, in addition to the provision of a wide range of social and cultural services (Dominati et al., 2010).

In this Chapter we describe and quantify the impact of biochar addition on soil NC stocks, their condition, and on soil processes, as they influence the provision of ES in two contrasting pasture soils. Drawing on the results from **Chapter 4**, **Chapter 5** and **Chapter 6** and on the published literature, this Chapter aims to explore the influence of biochar, as an amendment to the soil, on five ecosystem services (ES), including provisioning (food and fibre), regulating services (carbon sequestration, water regulation, disease and pest control), and supporting services (fertility maintenance).

7.2 Methodology

7.2.1 Framework for evaluation of soil ecosystem services

The framework used for soil ES (Dominati et al., 2010; Adhikari & Hartemink, 2015; Su et al., 2018) (**Fig. 7.1**) allows for the concurrent evaluation of cultural, environmental and production benefits from land management. Further, by linking ES to natural capital (inherent and manageable properties of the soil) and their condition in the framework, the influence of a change in the stock (quantity or condition) or process has on the capability of a soil to provide ecosystem services can be explored.

The framework of Dominati et al. (2010) consists of four main components: NC stocks (quantity and condition), drivers of the capital and of key processes, and ES supported by the capital in response to human needs. The NC stocks include both inherent and manageable properties, with the latter the focus of an intervention. Inherent properties, such as soil depth, clay type and texture reflecting the properties of the parent material, landscape position (slope, elevation, aspect) cannot be changed by human activity, while properties, such as available nutrients content, bulk density, and pH, can be manipulated and/or managed to varying degrees (Dominati, 2013). Liability to control soil ES provisioning through the soil management makes it very important to clearly separate inherent and manageable properties of soil NC (Maseyk et al., 2017).

The drivers can be natural (e.g. climate, geology) and anthropogenic (e.g. land use, practices, technologies); both types of drivers can add to or degrade the soil NC stocks. The external drivers affect the provision of ES by impacting on the quantity or condition of the soil NC stocks, or by influencing key soil processes or functions. The soil NC stocks underpin the delivery of ES to meet human needs. The soil functions are the mechanisms or process through which soil delivers ES. Despite the similarity of terms “function” and “services”, there is a fundamental difference between them when used in the framework. Soil functions, along with stocks, underpin the provision of all services.

Soil services have a more anthropocentric meaning, and are derived through the soil functions (Baveye et al., 2016). Depending on the fulfilment of existing or new human needs, land owner (stakeholder) can make decisions about the ways in which to control soil processes and properties (Smith et al., 2013); these decisions could have both positive and negative consequences.

In the framework developed by Dominati et al. (2010) a distinction is also made between processes and services. Dominati et al. (2010) argued that the supporting services are in fact processes that underpin the other three, as supporting services do not directly benefit humans. It is not possible to directly influence soil ES, except through the manipulation of the natural stocks or soil processes. For example, the flood mitigation service is underpinned by soil physical properties, such as soil intactness, infiltration characteristics, water holding capacity and porosity (Barbedo et al., 2014). Microbial diversity and abundance and nutrient content can be added to soil physical properties when exploring the stocks and processes underpinning the provision of food (Holt et al., 2016).

The natural capital-ecosystem services concept allows the links between the components of the soil, the condition or quality of the soil, and how any change that occurs under land use management practices impacts on provision of services to humans. This approach makes it possible to explore in more depth the direct and indirect relationships, and to evaluate in simple monetary or wider values the contribution of the stocks or changes in the condition of the stocks to the provision of certain ES (Straton, 2006; Dominati et al., 2014a; Baveye et al., 2016). This enables more informed economic or environmental decisions to be made in the management of land at both fine and wider scales (Ranganathan et al., 2008; Breure et al., 2012; FAO, 2016; Maseyk et al., 2017).

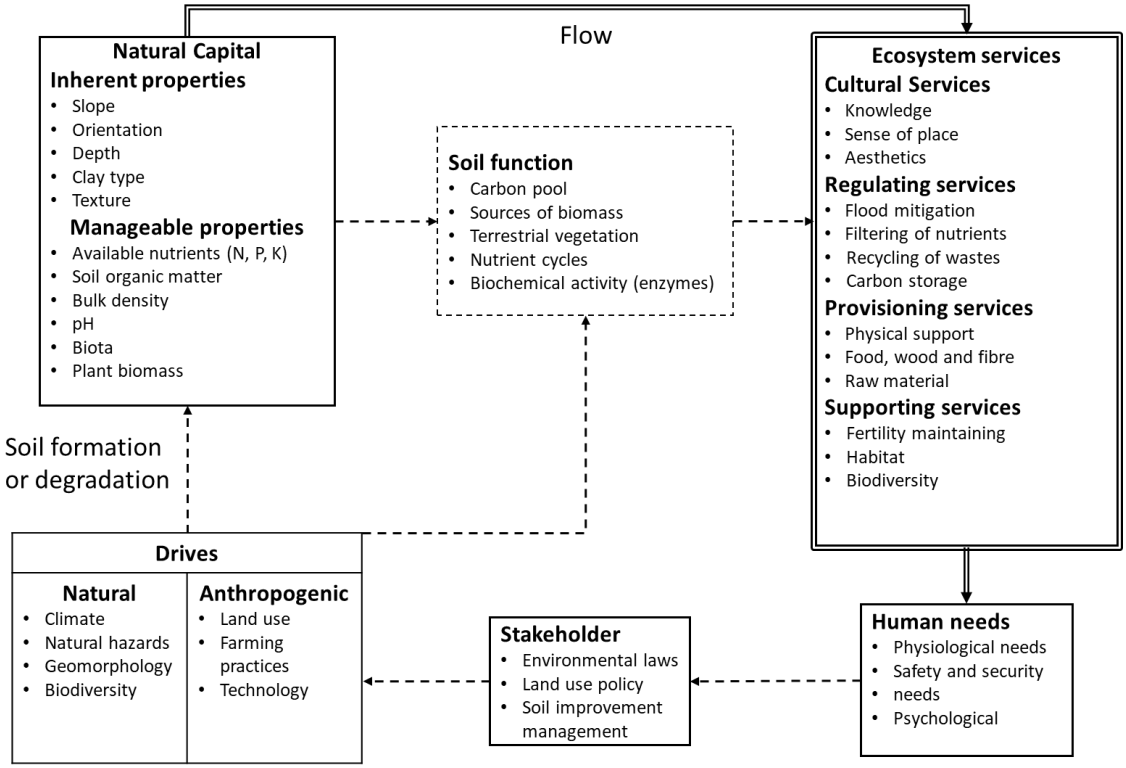

Figure 7.1 Conceptual framework linking soil natural capital, soil processes, and the provision of ecosystem services to meet human needs. Based on Dominati et al. (2010) and Su et al. (2018).

7.2.2 Soil ecosystem services

The Millennium Ecosystem Assessment (MEA, 2005) gave momentum to the concepts of Costanza and Daly (1992) and others from the 1990's who defined "ecosystem services" as "the benefits people obtain from ecosystems" and "natural capital" as the "stocks of natural assets that yield a flow of ecosystem goods or services into the future". The ecosystems approach has its origins in ecological economics, recognising that the economy is a subsystem of the ecological system and that sustainable economic activity needs to operate within the biophysical limits of the natural environment (Rockström et al., 2009). The motivation to call the properties of a soil "natural capital stocks" comes from framing the contribution of natural resources

alongside manufactured capital (factories, buildings, tools), human capital (labour, skills) and social capital (education, culture, knowledge) to the economy (**Fig. 7.1**; Daly and Farley (2011).

Ecosystem services have been previously divided into four categories: (i) provisioning services – e.g. provision of food, water, raw materials and physical support, etc; (ii) regulating services – e.g. flood mitigation, filtering of nutrients, regulating greenhouse gases, carbon storage, etc; (iii) cultural services – e.g. aesthetics, sense of place, recreational, etc; and (iv) supporting services – maintaining fertility, erosion control, pollination, etc. (MEA, 2005; Adhikari & Hartemink, 2015; Baer & Birgé, 2018).

In the Chapter we explore the influence of biochar, as an amendment to the soil, on five ecosystem services: food and fibre provisioning, carbon sequestration, water regulation, disease and pest control, and fertility maintenance. Relationships between ecosystem services and components of soil natural capital (natural capital stocks) in pasture agroecosystems are summarised in **Table 7.1**. The detailed explanation is given below.

Food and raw material provisioning is the main reason for agriculture-based land use. The ability of the soil to provide this service is influenced by many factors, including everything from the soil type and its characteristics through to a wide range of management practices. Root biomass of pastures, as it was explained by Crush and Thom (2011), plays an important role in regulating and maintaining stable growth of aboveground biomass under the constant pressure of defoliation by grazing animals. A developed root system also provides resilience and persistence properties against the treading pressure on the soil from the actions of livestock. Soil microbes and meso/macro fauna (earthworms and arthropods) incorporate, decompose and mobilize nutrients, and increase their bioavailability, which in turn stimulates plant growth (Lavelle et al., 2006; Blouin et al., 2013; Saccá et al., 2017). Soil organic carbon is the main driver of many

soil processes, and on par with limited nitrogen and phosphorus ensures soil provisioning service (Sarmiento et al., 2006; Francaviglia et al., 2018). Reducing soil bulk density (BD) and or increasing soil pH generally has the potential to benefit the processes underpinning the provision of food and raw material. For example, decreasing the BD of a compacted soil (Stirzaker et al., 1996) or reducing soil acidity in acidic soil (Pagani & Mallarino, 2012) has been shown to have a positive impact on crop yields. Soil enzymes, especially those located in or close to the rhizosphere, play an important role in maintaining plant health (Jandera et al., 1989; Egamberdieva et al., 2011) and, as was shown by (Wang et al., 2011), lift plant primary production by maintaining the flow of nutrients and OC.

Carbon sequestration. The balance between atmospheric carbon (CO_2) and carbon (C) stored in the soil is an integral part of global climate regulation. The preservation of soil carbon and its protection from decomposition may be used for climate mitigation and deceleration of global warming (Lal, 2004). The carbon in soil organic matter represents a significant reservoir within the global C cycle. Estimates of C in soil organic matter account for 1200–1550 petagrams (Pg; 1 Pg = 1015 g) and 2370–2450 Pg C to soil depths of 1 and 2 m, respectively (Eswaran et al., 1995). Comparative estimates of organic C contained in living biomass (560 Pg) and atmospheric CO_2-C (760 Pg) indicate that a small shift in the soil organic C pool has the potential to have a significant impact on atmospheric CO_2 concentrations (Lal, 2004). For example, a 5% shift in soil organic C stored in the 0–2 m soil layer could potentially reduce atmospheric CO_2-C by 16% (Baldock & Broos, 2012). This potential mitigation contributes to the equilibria between soil NC stocks via soil C storage and regulation of nitrous oxide and methane emissions (Dominati et al., 2010).

Plant roots protect soil from erosion and carbon loss (Liu et al., 2018a). Soil aggregate stability plays an important role in carbon sequestration (Šimanský & Bajčan, 2014).

(Aislabie & Deslippe, 2013; Liang et al., 2019). Soil aggregate stability is also dependent on microorganisms which bind soil particles by extracellular polymeric substances, and, in case of fungi, by hyphae (Lynch & Bragg, 1985; Tisdall, 1994; Boivin & Kohler-Milleret, 2011; Costa et al., 2018). An increase in the fungi and bacteria in the soil alters the carbon cycling patterns and has been linked to higher C storage potential (Malik et al., 2016). Earthworms may also play a role in regulating the soil carbon sink through their influence on C cycling and sequestration (Zhang et al., 2013). Several studies found links between the activities of soil enzymes and C storage potential (Cenini et al., 2016; Chen et al., 2018); soil enzymes are involved in the processes of transformation of soil C, its integration in biological activities and, therefore, retention of C in the soil system.

Water retention. The ability to retain and regulate water flow is a very important regulating ecosystem service provided by the soil-plant system. The presence and activity of earthworms influences soil porosity at different scales; this has a positive effect on soil water regime, soil erosion reduction (Shuster et al., 2002) and soil water storage (Ehlers, 1975; Clements et al., 1991). Soil bulk density also affects soil water regime, thus in a compacted soil the decrease in BD positively influences water infiltration at the soil surface, as well as water flow through soil horizons. Plant roots have an important influence on soil water regime, by changing the physical properties of soil and through transpiration (Willigen et al., 2012).

Disease and pest regulation. In permanent agricultural systems, such as annual pastures, regulation of pests (plants and animals) and diseases is an essential ecosystem service (Dominati, 2013). Below-ground biotic interactions regulate the structure of soil communities. A balance in the community has the potential to reduce abundance of soil pests and at the same time stimulate plant resistance against diseases (Altieri et al., 2012; Wachira et al., 2014). The diversity in the microbial and faunal communities has the capacity to control contagion agents. It has been shown that earthworms help plants to

tolerate pests and diseases (Bertrand et al., 2015). Soil arthropods (including mites and Collembola) also can control soil-borne harmful organisms, protecting plants from infections (Brussaard, 1997; Bagyaraj et al., 2016).

Soil fertility maintenance. Maintaining soil fertility is a critically important service for plant growth, provided by the soil. Biological activity controls many soil processes responsible for the supply of nutrients. Earthworms and arthropods, through their role in the initial steps in organic matter decomposition and turnover, supply soil with available nutrients and enzymes (Derouard et al., 1997; Barrios, 2007; Bityutskii & Kaidun, 2008). Soil fungi and bacteria are also important in fertility maintenance, and it has been shown that increase of the fungi:bacteria ratio is linked to a reduction in NH_4 losses (de Vries et al., 2006). The composition of soil microbial community is often used as an indicator of soil wellnesses (Li et al., 2016). Available phosphorus and nitrogen can also be used as indicators of soil fertility, as both nitrogen and of phosphorus deficiency are major factors that limit plant productivity throughout the world (Hardie et al., 2014). Soil enzymes, such as phosphatases and ureases, enrich soil with available forms of nutrients, which underpin soil fertility (Yang et al., 2012; Piotrowska, 2014).

Table 7.1 Relationships between ecosystem services and components of soil natural capital (natural capital stocks) in agroecosystem investigated in our study. For this and for next Tables: OC – organic carbon, TN – total nitrogen, BD – soil bulk density, GMea – Geometric Mean of enzyme activities.

Components of natural capital (stocks)	Food and fibre	Carbon sequestration	Water regulation	Disease and pest regulation	Fertility maintenance
Plant Roots	✓	✓	✓		
Fungi	✓	✓		✓	✓
Bacteria	✓	✓		✓	✓
F/B ratio	✓	✓			✓
Earthworms	✓	✓	✓	✓	✓
Arthropods	✓			✓	✓
OC	✓	✓			✓
TN	✓				✓
Olsen P	✓				✓
pH	✓	✓			✓
BD	✓	✓	✓		
GMea	✓	✓			✓

7.2.3 The Natural Capital-Ecosystem Services (NC-ES) approach

The Natural Capital-Ecosystem Services (NC-ES) approach was used to build up a more complete picture of the benefits of biochar as a soil amendment. The opportunity to complete a more holistic assessment of the benefits of biochar is made possible with the data sets from **Chapters 4-6**. We use the framework developed by Dominati et al. (2010) to evaluate the effect of biochar on soil properties (stocks) and processes supporting the provision of services in an agro-ecosystem. The methodology of Hewitt et al. (2015) was used to provide a semi-quantitative assessment of the percent change in adequacies of the soil NC stocks, following addition of biochar or lime, for the provision of a specific service. The soil NC "stocks" are defined by soil properties that can be either directly measured, or estimated within a soil profile (Hewitt et al., 2015).

An inventory of the changes in the properties of soil NC stocks and in soil processes, as influenced by the addition of biochar in two contrasting soils (Andosol and Cambisol) under a pastoral use was assembled first using the framework of Dominati et al. (2010). The list of properties contributing to the NC stocks of the experimental soils, including Geometric Mean of enzyme activities (GMea) are given in **Table 7.2** and **Table 7.3.** These are drawn from **Chapters 4** and **5**.

To quantify the NC stock of the two soils (the Andosol and the Cambisol) used as grazed pastures we applied the methodology proposed by Hewitt et al. (2015). The method of Hewitt et al. (2015) identifies and quantifies NC stocks by estimation based on specific ES provided by the land with a specific land use type. Once the land use type of interest is chosen, the focus shifts to the services of interest from that specific land use type. The next step is to select the soil services that would be expected to be gained from the land use, and to select the NC stocks required for delivering these services. Then, 100% and 0% adequacy for each stock for each soil service are determined. The last step is to aggregate and quantify the stock adequacies for all stocks. Hewitt et al. (2015) used this methodology for non-monetary evaluation and comparison of four processes (nitrate storage, nitrate reduction, phosphorus filtering, and microbial filtering) that contribute to the soil-filtering regulation ecosystem service.

In the present study the land use was four grazed pastures. Our focus was on assessing the effect of biochar, as an amendment. We also included positive control (lime), to isolate the benefits of biochar as a liming agent from the other potential benefits of biochar. Both biochar and lime were compared with the soil receiving neither amendment (negative control). In the present study, we have modified the method of Hewitt et al. (2015) for estimating the effect of biochar on ES resulting from a change in the soil NC stocks by bench marking the change against the NC stocks of the control (untreated) soil, which was assumed to equal 100% adequacy. The links between the soil NC stocks and ES are

listed in **Table 7.1**. The negative or positive influence of biochar on the properties contributing to the soil NC stocks in each of the four pastures, and flow-on effects to the provision of ES, were compared with that of control soil and lime addition. For the soil to which either biochar of lime was added, comparing the sum of the percentage differences for each NC stock to that of the untreated soil, provides an initial indication of the overall effect of biochar, as an amendment, on the provision of the five ES explored in this study. For the soil bulk density (BD), the decrease in BD was assumed as an increase in soil NC (positive percentage difference).

7.3 Results

The NC stocks of the soils of four studied pastures were initially grouped and compared using the framework of Dominati et al. (2010) and the semi-quantitative approach based on the adequacy method of Hewitt et al. (Hewitt et al., 2015) (**Table 7.2**).

The biggest impact of biochar was on the biological properties of the four soils, doubling the biological NC stocks (e.g. doubled fungal biomass carbon, earthworms and arthropods abundance), while the shift in the physicochemical properties of the four soils was of a smaller magnitude (e.g., on average across all four sites soil OC increased by 6.5 g kg^{-1} and Olsen P by 2.9 mg kg^{-1}) (**Table 7.2**). Biochar also had a large significant effect of plant root biomass, increasing root biomass by nearly 50%. This equates to a 12% increase in soil C stocks (**Table 7.2**).

In comparison, the impact of lime on the NC stocks of the four soils was more limited, with a positive effect on soil pH and some soil biological properties (including the doubling of earthworm abundance), no influence on root biomass, TN and Olsen P, and a negative effect on soil OC, which decreased by 1.7 g kg^{-1} (2.9%) on average across all four pastures.

A summary of the links between the NC stocks of the two soils and ES is given in **Table 7.1**. By affecting not only soil biological and chemical properties, but also plant root growth, biochar has the potential to influence all five ES listed in **Table 7.1**.

Nearly all the soil properties (NC stocks) linked to the **provision of food and fibre** measured in the four soils in this study were positively influenced by biochar. Lime did not positively affect BD, OC, TN and Olsen P.

Looking at **C sequestration**, biochar addition had a positive impact on all soil NC stocks linked to this service. Lime, which is important in addressing soil acidity, had a negative effect on OC in all four soils.

Water regulation in our study can be linked with three soil properties – root biomass, earthworm abundance and BD (**Table 7.2**). Biochar addition had a positive effect on all three, while lime only had a positive effect on earthworms.

Disease and pest regulation in our study can be linked to the soil biological properties. Both biochar and lime had positive effect on soil biota, with the impact of the former more pronounced. Nearly all the soil properties (NC stocks) linked to **soil fertility maintenance** were positively influenced by biochar addition. The impact of lime on this ES was less pronounced, again showing that biochar has impact beyond just that of a liming agent.

Using the adequacy methodology of Hewitt et al. (2015), for the four soils in this study we calculated the percent changes in the soil properties contributing to the NC stocks following the addition of biochar or lime (**Table 7.3)**.

Table 7.2 Values of soil natural capital stocks (averaged for 2-9.5 cm and 9.5-17 cm soil depths) for control soil and for soil with biochar or lime (data from **Chapters 4** and **5).** Bold numbers represent the values with the more than 200% increase.

	Andosol NE			Andosol EF			Cambisol LF			Cambisol HF		
SNC	**Control**	**Biochar**	**Lime**	**Control**	**Biochar**	**Lime**	**Control**	**Biochar**	**Lime**	**Control**	**Biochar**	**Lime**
Roots, Mg ha^{-1}	5.8	11.0	5.6	6.9	**16.0**	6.4	26.2	36.3	25.2	15.7	18.8	14.5
Fungi, mg kg^{-1}	372.3	**701**.4	485.1	451.0	**1016.2**	614.1	1121.0	1681.0	1180.2	468.7	**980.7**	616.9
Bacteria, mg kg^{-1}	1105.6	1574.3	1248.9	833.0	1390.4	1141.1	1927.1	2252.9	1954.2	1411.4	1687.7	1508.2
F/B ratio	0.3	0.4	0.4	0.5	0.7	0.5	0.6	0.7	0.6	0.3	0.6	0.4
Earthworms, ind m^{-2}	151.0	**330.3**	**481.2**	273.6	339.7	462.3	325.5	500.1	632.2	188.7	349.1	518.9
Arthropods, 1000 ind m^{-2}	500.0	**1088.5**	908.5	277.2	**592**.3	296.7	678.7	1098.7	1538.7	1212.0	1874.7	1535.7
OC, g kg^{-1}	61.9	69.5	61.9	73.8	82.6	70.1	49.6	55.7	47.9	54.5	57.9	53.0
TN, g kg^{-1}	6.1	7.0	5.6	7.1	7.4	6.9	4.7	5.3	4.8	5.0	5.7	5.2
Olsen P, mg kg^{-1}	34.8	39.5	35.5	19.4	21.6	19.1	4.4	4.9	4.4	18.7	22.9	18.8
pH	5.5	5.9	5.9	5.9	6.2	6.2	5.1	5.2	5.5	5.2	5.4	5.5
BD, g cm^{-3}	0.67	0.64	0.69	0.68	0.63	0.67	0.71	0.65	0.69	0.70	0.66	0.69
GMea	52.7	59.8	58.6	73.3	78.6	76.4	24.5	28.4	29.5	31.4	36.2	34.1

Table 7.3 Percentage increase or decrease in the natural capital stock adequacy for provision of ecosystem services in the four pasture soils treated with either biochar or lime, as calculated using the adequacy method of Hewitt et al. (2015). Values represent increase or decrease compared with the control treatment (assumed to represent 100% adequacy). For the soil BD the decrease in BD was assumed as an increase in percentage difference. Bold numbers represent the most noticeable changes.

	Andosol NE		**Andosol EF**		**Cambisol LF**		**Cambisol HF**	
	Biochar	**Lime**	**Biochar**	**Lime**	**Biochar**	**Lime**	**Biochar**	**Lime**
Roots	**89.5**	-3.0	**131.7**	-6.9	38.6	-3.8	19.7	-7.6
Fungi	**88.4**	30.3	**125.3**	36.1	50.0	5.3	**109.3**	31.6
Bacteria	42.4	13.0	66.9	37.1	16.9	1.4	19.6	6.9
F/B ratio	32.3	16.4	35.9	0.0	28.5	3.1	74.1	21.8
Earthworms	**118.8**	**218.8**	24.1	69.0	53.6	94.2	85.0	175.0
Arthropods	**117.7**	81.6	**113.7**	7.0	61.9	**126.7**	54.7	26.7
OC	12.2	0.0	11.9	-5.1	12.2	-3.6	6.1	-2.8
TN	14.8	-8.5	5.3	-1.9	11.7	-1.9	12.9	3.2
Olsen P	13.5	1.8	11.3	1.1	11.3	-0.1	22.8	0.9
pH	6.7	8.6	3.6	4.8	1.1	6.5	3.7	4.9
BD	5.3	0.6	8.3	2.2	8.4	1.0	5.9	1.3
GMea	13.5	11.3	7.3	4.3	16.1	20.4	15.4	8.8
Sum	**555.1**	**370.8**	**522.1**	**147.8**	**310.7**	**249.3**	**429.2**	**270.6**

The largest shift in the NC stocks in the two Andosols (averaged for two pastures) following the addition of biochar as an amendment were in the biological properties; this included root biomass (110.6%), fungal biomass (106.9%), bacterial biomass C (54.7%), earthworms (71.5%) and arthropods abundance (115.7%) (**Table 7.3**). In comparison, lime addition had less of an effect on biological properties in the Andosols – root biomass (-4.95%), fungal (33.2%) and bacterial biomass C (25.1%), arthropods (44.4%), but a greater impact than biochar on earthworm abundance (143.9%) (**Table 7.3**). The percent shift in the soil physicochemical properties in the Andosol pastures with the addition of either biochar or lime was not as pronounced as the biological properties. Biochar

increased OC, TN and Olsen P by 10-12% and BD by 6.9%, while lime decreased OC and TN, and increased Olsen P and BD by less than 2% (**Table 7.3**).

In the Cambisol pastures addition of biochar as an amendment had a greater effect on biological components of soil NC, including roots biomass (29.2%), fungal (79.7%), and bacterial biomass (18.3%), earthworm (69.3%) and arthropods abundance (58.3%) (**Table 7.3**). Lime addition had less of an effect on roots biomass, fungal and bacterial biomass, but a larger than biochar percent influence on earthworm (134.5%) and arthropods abundance (76.7%). The shift in the physico-chemical properties of the Cambisols with the addition of biochar followed a similar pattern to that seen in the two Andosols.

The sum totals in **Table 7.3** represent the total percent changes in the adequacy of the soil NC stocks to provide ES following the addition of biochar or lime. When summed, the effect of biochar addition on all of the measured soil properties in the Andosol NE and Andosol EF pastures (555.1% and 522.1%, respectively) was much greater than that of a lime addition (184.3 and 374.3 %, respectively). In the two Cambisol soils the overall percent increase from biochar addition was lower (310.7% for the Cambisol LF and 429.2% for the Cambisol HF). The overall percent increase following lime addition for the Cambisol LF and HF soil was 249% and 270.6%, respectively, again less than what was seen with biochar.

The data indicate that in the two Andosol pastures the different management histories appear to have little influence on the effects of the biochar addition. In comparison, lime addition had a more pronounced effect on the pasture soil that had not received effluent (And-NE); this was not the case with biochar addition, despite biochar being a liming agent. Interestingly, in the two Cambisol pastures, either biochar or lime addition had a greater effect in the soil with the high fertility (Cam-HF), except for the effect of lime on arthropods and GMea (**Table 7.3**).

Combining the soil NC increase/decrease values in **Table 7.3** and the links between the soil NC stocks and ES listed in **Table 7.1**, we can now calculate the total percent change in the soil NC stocks that underpin and influence specific ecosystem service (ES).

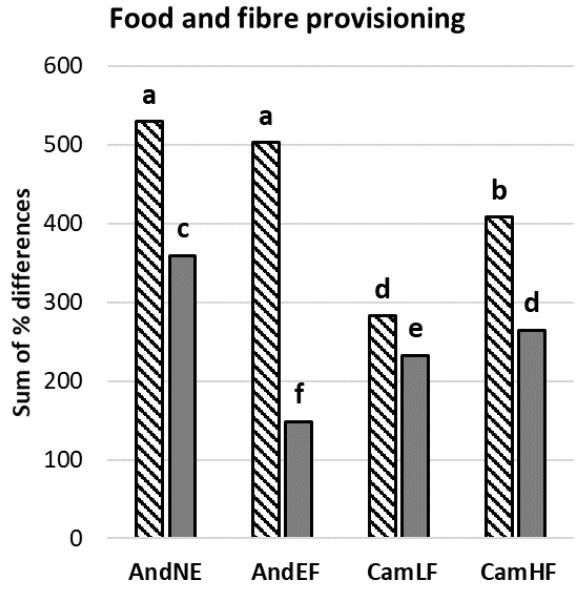

Figure 7.2 Sum of the percentage changes in the soil natural capital stocks that under pin and influence the delivery of the **Provisioning of the food and fibre ES** in the four pasture soils following the addition of either biochar (striped bars) or lime (grey bars). Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between biochar or lime treatments within all four sites.

The **Food and fibre provisioning ES** (contributing NC stocks include roots biomass, fungi, bacteria and their ratio, invertebrates, nutrients, soil BD, pH and biochemical activity) was higher (P<0.005) in soils to which biochar had been added than in the soils with lime (**Fig. 7.2**). This was the case for all four pastures. For the Andosol soils (And-NE and And-EF) biochar addition had similar effect, while lime had more pronounced effect (P<0.005) on the properties of NC stocks in the And-NE soil. In the Cambisol soils the effect of both biochar and lime addition on NC stocks was more pronounced (P<0.005) in the soil with the history of high fertility (Cam-HF).

The sum of the percentage changes in the soil properties (NC stocks) of the four soils contributing to the **C sequestration ES** (contributing NC stocks include biological

properties except arthropods, OC, soil BD, pH and GMea) was higher ($P<0.005$) in the soil to which biochar had been added (**Fig. 7.3 a**). In the both Andosols (And-NE and And-EF), biochar addition had a similar effect, while lime had more pronounced effect ($P<0.005$) on the NC stocks of And-NE than in And-EF soil. In the Cambisols, the effect of both biochar and lime was more pronounced in the high fertility (Cam-HF) than in Cam-LF ($P<0.005$) soil. For both the Cambisol soils the sum of the percent change in the soil NC stocks was higher following the addition of biochar than with lime ($P<0.005$).

The sum percent change in the soil NC stocks that underpin and influence the **Water regulation ES** (contributing NC stocks include roots biomass, earthworms abundance and soil BD) in the Andosol without effluent (And-NE) and the Cambisol with low fertility (Cam-LF) was the same following the addition of either biochar or lime (**Fig. 7.3 b**). For the Andosol pasture receiving effluent (And-EF), the sum of percentage changes in the NC stocks contributing to **Water regulation ES** was higher in soils to which biochar had been added comparing to lime ($P<0.005$), while for the Cambisol with high fertility (Cam-HF) lime addition had a more pronounced effect on **Water regulation ES** than biochar ($P<0.005$).

The sum of the percent changes in the soil NC stocks that underpin and influence the **Disease and pest control ES** (contributing NC stocks include fungal and bacterial biomass C, earthworm and arthropod abundance) for the Andosol sites (And-NE and And-EF) was higher in soils to which biochar had been added ($P<0.005$), compared to the soil with lime (**Fig. 7.3 c**). For the Cambisol soil with low fertility (Cam-LF), the sum percent changes in the NC stocks contributing to this ES was higher for lime amendment than for biochar ($P<0.005$), while for Cam-HF there were no differences between biochar and lime.

The sum of percent changes in the NC stocks that underpin and influence the **Maintaining soil fertility ES** (contributing NC stocks include biological properties,

chemical properties and GMea) for both Andosol soils (And-NE and And-EF) and the Cambisol soil with high fertility (Cam-HF) was higher following biochar addition than lime ($P<0.005$), while in Cam-LF there was no significant difference between biochar and lime (**Fig. 7.4**).

In summary, the biggest impact on the NC stocks that contribute to the five ES was seen in pastures on Andosol soil with biochar addition, and in the Cambisol with a history of high fertility (Cam-HF) (**Table 7.3**).

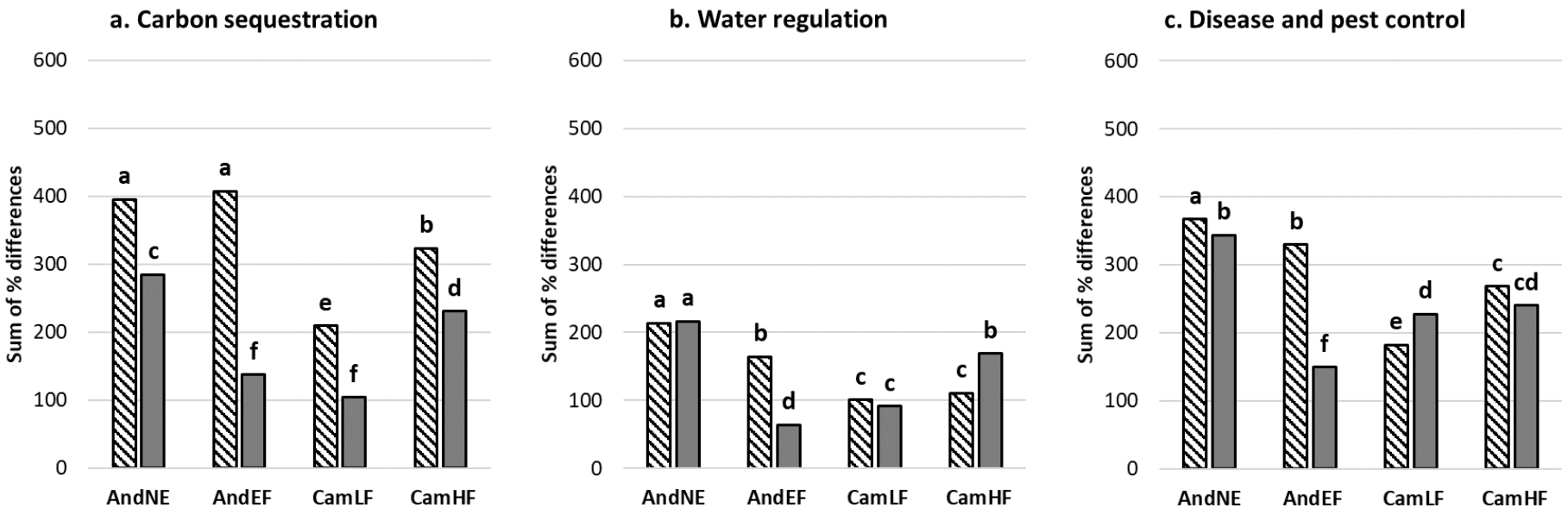

Figure 7.3 Sum of the percentage changes in the natural capital stocks that underpin and influence the delivery of the regulating ecosystem services - **Carbon sequestration ES** (a), **Water regulation ES** (b), **Disease and pest control ES** (c) in the four soils following the addition of either biochar (striped bars) or lime (grey bars). Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between biochar or lime treatments within all four sites.

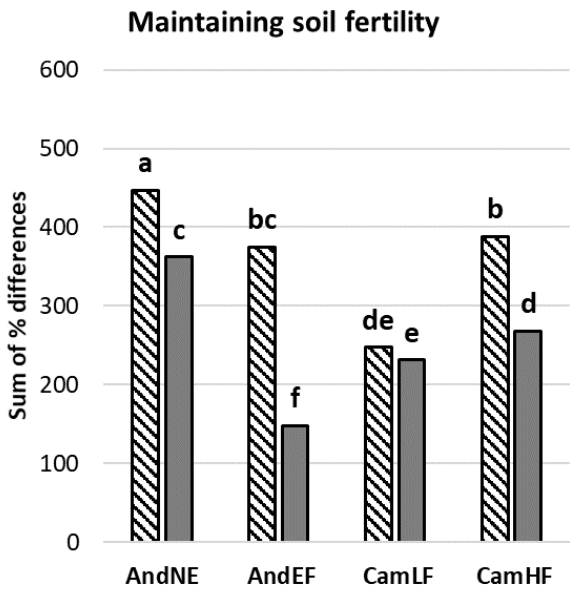

Figure 7.4 Sum of the percentage changes in the soil natural capital stocks that underpin and influence the delivery of the **Maintaining soil fertility ES** in four soils following the addition of either biochar (striped bars) or lime (grey bars). Lowercase letters indicate significant differences (Tukey HSD test, α=0.05) between biochar or lime treatments within all four sites.

7.4 Discussion

Pastoral soil provides a wide range of ecosystem services (ES). Animals grazed on pastures are the source of food (meat and milk), fibre (wool) and by-products (bones, fat, blood) that are an important part of the worldwide economy. Besides, bees use flowers on pastures for honey production. Therefore, sustained plant growth is a factor responsible for the constant delivery of these services by the soil. In addition to the provisioning services, pasture soil delivers regulating and supporting services. Occupying large areas, pastures play a significant role in the ecosystem through carbon storage, flood regulation, nutrient filtering, pest and disease regulation (all regulating services), and fertility maintenance (supporting service). Pastures also are the habitat to a wide range of organisms, supporting their diversity.

The applied NC-ES approach based on Hewitt et al. (2015) used in this study allowed to qualify the contribution of biochar to soil NC stocks, and showed itself useful for exploring the effects and potential benefits of applying amendments application for the delivery of specific soil ES.

Biochar addition had a beneficial effect on the natural capital (NC) stocks of all four soils. Its effect was much greater than that of lime (positive control), which in some cases had a negative effect on some NC stocks. Biochar increased the abundance of bacteria and fungi, as well as earthworms and arthropods. Both groups, microorganisms and fauna, play an important role in maintaining sustainability of soil use (Aislabie & Deslippe, 2013). Biochar also increased root biomass, which is an important component of the NC of the soil-plant system (Crush & Thom, 2011; Bakker et al., 2019), contributing to a wide range of ES including supply of food and raw materials, carbon sequestration, water and erosion regulation, and biological control.

As it stimulates soil microbial diversity and abundance in the rhizosphere (as found in the field experiment in the present study), biochar has been shown to have beneficial effect on plant diseases resistance (Kolton et al., 2011). There is also a link between soil faunal community abundance and diversity and soil fertility (McCormack et al., 2013b). An interesting finding in the present study was the fact that while both biochar and lime enhanced microbial density and abundance of earthworms and arthropods to varying degrees, only biochar increased root biomass. It was suggested (**Chapter 4**) that labile C from biochar (49% of labile C) caused enhanced microbial activity and therefore immobilization of available N. This caused short-term N deficiency for plants, which then allocated more OC into roots.

Besides biota, biochar addition also influences the abiotic part of the soil. Biochar consists of stable and recalcitrant organic carbon which cannot be easily decomposed (Lehmann et al., 2009). Amending soil with biochar and its stable carbon can be used as

an instrument of carbon sequestration (Lorenz & Lal, 2014). Biochar used in the current study has relatively low nutrient content (Fertilizer class - 0), but was still able to provide the soil with other nutrients (nitrogen, phosphorus) that can be used by plants and soil organisms. Lime addition, in comparison, resulted in a decrease in nutrient availability, while biochar mostly increased soil nitrogen and phosphorus. Shen et al. (2016) showed that biochar application increases bioavailability of phosphorus for plants. Both soils used in the present study have low pH (<7) and both biochar and lime reduce soil acidity; lime had a greater effect on soil pH than biochar. In addition to chemical properties, biochar decreased soil bulk density, which can be beneficial to compacted clay soil (Walters & White, 2018).

All soil NC stocks measured in the four soils and linked to the provisioning ES were positively influenced by the addition of biochar. This included the microflora, meso- and macro-faunal abundance, OC, nutrient content, reduced soil acidity and BD, stimulated biochemical activity. Lime addition also had a positive effect on most soil properties, but had no effect on root growth and resulted in a drop in OC and TN content of all soils.

The most noticeable difference in effect of biochar and lime addition was carbon sequestration ES, with biochar increasing, and lime reducing the OC content. Changes in water regulation ES and disease and pest control ES following biochar or lime application were similar, and in some cases, lime had a more pronounced effect. This is can be explained by the high contribution of earthworms to these services, and the positive effect of lime on earthworms (**Chapter 4**). We suggested (**Chapter 4**) that lime had more a pronounced effect on abundance of earthworms, as besides just liming effect (which was also provided by biochar) lime supplied earthworms with available Ca, which is a vital component for their well-being.

7.5 Conclusions

In summary, biochar contributed to the NC stocks and the flow of services in all four soils. Thus, amending soil with biochar has a positive effect on a number of key NC stocks that underpin the provision of a range of ES (provisioning and regulating) which are important in agro-ecosystems (Verheijen et al., 2010; Rodrigues & Horan, 2018). Liming also changed the soil NC stocks and impacted on a range of ES.

As the biochar used in the present study had a low nutrient content (Fertilizer class - 0), but some liming potential (Liming class - 1), the lime application (added at the liming equivalence of the biochar application) was used as a positive control. This study showed very clearly that biochar is more than just a liming agent. The global impact (the total sum of percentage changes) on soil ES provided by biochar was much higher than that provided by lime.

In comparison with the negative control and the lime amendment, biochar increased the components of soil NC responsible for food and fibre production ES. There was also a significant positive impact of biochar on soil properties associated with carbon sequestration ES and fertility maintenance ES. Both biochar and lime had similar positive effect on water regulation and disease and pest control services.

In this Chapter we show that willow biochar made at 350° C and added at an application rate of 12 Mg ha^{-1} had a significant positive impact on the potential of soil to provide regulating, provisioning and supporting services in two contrasting soils (Andosol and Cambisol). Therefore, this specific biochar when added to this specific soil could be considered as a forward-looking soil amendment, which can increase plant productivity, reduce disease and pest risks, improve soil physical properties, while being used as an instrument to mitigate climate change.

DRC 16

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Stanislav Garbuz
Name/title of Primary Supervisor:	Dr Maria Minor
Name of Research Output and full reference:	
Garbuz, S. A., MacKay A. D., Camps Arbestain, M., DeVantier, B., & Minor, M. A. Effect of biochar addition as an amendment on the natural capital stocks and soil processes as they influence the provision of services in two contrasting soils	
In which Chapter is the Manuscript /Published work:	Chapter 7
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	65%
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
The candidate used data obtained from the field-based experiments (described in chapters 4 and 5), analysed the results based on the modified method and discussed findings. The supervisors (A.D. MacKay, M. Minor, M. Camps-Arbestain) provided advice on analysis, comments on the results, and contributed to manuscript writing. Brian DeVantier assisted with the field-based experiment.	
For manuscripts intended for publication please indicate target journal:	
Ecosystem Services	
Candidate's Signature:	Stanislav Garbuz 2020.03.05 14:39:26 +13'00'
Date:	05/03/2020
Primary Supervisor's Signature:	Maria Minor Digitally signed by Maria Minor Date: 2020.03.06 13:19:29 +13'00'
Date:	06/03/2020

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Chapter 7

Chapter 8

Summary and recommendations for future work

8.1 Summary

Soil possesses a range of inherent and dynamic properties, which form its natural capital stocks. The quality and quantity of ecosystem services provided by the soil depend on its natural capital stocks. The purpose of soil management is not only to obtain material profit (e.g. crop, raw material), but to also maintain the ability of the soil to provide all the other services (i.e. regulating and cultural services) which we require from nature.

Intensification of human activity has negative impacts on ecosystems, which require the application of advanced and sustainable technology in all spheres of human activity, including agriculture. Biochar is considered to be an effective soil amendment that has high potential in sustainable agriculture and ecosystem services management. It has been used in agriculture since the beginning of time. Consisting of recalcitrant carbon (C), biochar has a number of characteristics that can be used to improve soil properties. Like all amendments, biochar has an impact on soil biological properties, including micro-, meso- and macro-fauna activity. In addition, depending on the type of biochar, it can offer a wide range of benefits to the provision of ecosystem services, such as carbon sequestration, greenhouse gases regulation, filtering of nutrients and biological control.

Previous works in New Zealand showed either (i) a beneficial effect, or (ii) a no effect of biochar application on pasture growth (Shen et al., 2016; Mahmud et al., 2018). For example, biochar application has been shown to have a high potential for increasing C sequestration in Allophanic and non-Allophanic soils in New Zealand (Calvelo Pereira et al., 2016; McNally et al., 2017). Biochar affects soil physical properties (Herath et al., 2013) and nutrient cycle (Taghizadeh-Toosi et al., 2011; Wisnubroto, 2015), soil

biological properties (Momayezi et al., 2015; Wang et al., 2015a) and plant growth (Free et al., 2010; Biederman & Harpole, 2012; Paz-Ferreiro et al., 2014).

Willow was used as a raw material for the production of biochar in this study, because it is widely distributed in New Zealand, where it is planted for soil and stream bank erosion, as a coastal buffer, and for protective strips (McIvor, 2018; McIvor & Frazer, 2018). The willow was chosen because of its rapid growth, ready availability, low cost of planting and maintenance requirements. During the growth of trees, willow can be trimmed or coppiced, so it is possible to sustainably harvest wood or use old trees to produce biochar. Despite its low nutrient content, biochar produced from willow has certain characteristics that make it promising as a soil amendment (Kwapinski et al., 2010; Hangs et al., 2016).

This thesis tested the hypothesis that adding biochar to the soil as an amendment changes the natural capital stocks of the soil and soil processes, resulting in an increase in the flow of ecosystem services to humans. Biochar, added as an amendment to the soil, adds C and habitat, and behaves in part as a catalyst stimulating biological activity, resulting in a shift in nutrient availability, pore size distribution, rhizosphere environment, and plant root growth. In the thesis, two contrasting soils – the Allophanic (Andosol) and the Brown (Cambisol) – were used to evaluate the dynamic interconnection between biochar addition, soil type, the chemistry and biological properties of each soil, and plant growth. In addition, the influence of management practices (effluent addition or no effluent addition to the Allophanic soil; and low or high fertiliser input to the Cambisol) on the efficiency of the biochar was also examined as part of the study.

8.2 General Discussion and Conclusions

8.2.1 Hypotheses

1. Biochar (produced from willow at low temperature) will have a catalytic effect on the biological and physiochemical properties of an Andosol and a Cambisol under New Zealand pasture
2. There will be synergetic interactions between biochar (produced from willow at low temperature) and different functional group of soil biota as they influence soil processes and plant growth
3. Biochar (produced from willow at low temperature) application will be beneficial for soil ecosystem services within New Zealand pastoral agro-ecosystems.

8.2.2 Biological and physiochemical properties

Both glasshouse and field mesocosm experiments (**Chapters 3** and **4,** respectively) showed that application of willow biochar (12 Mg ha^{-1}) as an amendment (1% w/w) can benefit soil C and N stocks, improve physical properties and neutralise acidity in soils soon after application. Biochar has the potential to increase soil nutrient content, mostly through indirect effects on soil processes.

Biochar can increase soil micro-, meso- and macro-fauna. In **Chapters 4, 5** and **6**, it was shown that biochar increased soil root biomass and stimulated soil trophic levels. Dynamical changes in soil biota caused by biochar were reflected in changes of enzymatic activities.

In the glasshouse experiment (**Chapter 3**), addition of biochar resulted in a significant increase in white clover biomass. This effect was not related to the liming potential of biochar, as clover growth was not stimulated by lime addition. The enhanced clover growth was paralleled by an increase in N_2-fixation and, consequently, an increase in TN content. The experiment design included mesocosms with and without earthworms. This

allowed the interaction between biochar and earthworms to be explored as part of the study. The interaction between biochar and earthworms was found to be soil-type specific. For example, on their own, biochar and earthworms both increased clover growth in the Cambisol, but there was no additive effect. In the Andosol a positive synergistic effect of biochar and earthworms on soil biochemical processes and clover growth was evident. The synergistic effects of biochar and earthworms were reflected in increased abundance of Collembola and soil fungal biomass.

In the field-based mesocosm experiment (**Chapters 4-6**) we investigated the effect of biochar addition as an amendment to soil on the biological, biochemical and physico-chemical properties in grazed pastures on two contrasting soils – a sil-andic Andosol and a dystric Cambisol. With each soil there were two sites managed under different agricultural practices. On the Andosol the management of site practices included with or without the application of dairy shed effluent. In the Cambisol the two sites had either a low or high P fertilizer history. The field study lasted one year and included two sampling times – after six months (autumn) and at the end of experiment (12 months, spring).

Total N, OC and Olsen P contents and bacterial (Cb) and fungal (Cf) C biomass, and Collembola abundance were all higher ($P<0.005$) in all four soils to which biochar had been added compared with initial values and controls. Biochar showed a strong beneficial effect on root growth, with up to a two-fold increase in standing root biomass. The overall increase in root C in the biochar-amended soils (compared with the negative control) represented 1.2 to 4.0 Mg C ha^{-1}, in addition to the OC added with the biochar itself. The increase in root biomass of the pastures in the presence of biochar in the field study could be related to the short-term N immobilization (Nguyen et al., 2017; Gao et al., 2019) caused by biochar labile OC addition, which forced plants to allocate more OC into roots (Hill et al., 2006). We suggested that biochar stimulates OC accumulation by enhancing the root growth of the pasture (which includes legumes) and biological activity. The

increase in TN is potentially related to enhanced N_2 fixation from increased activity of the legume component of the pasture (Rondon et al., 2007; Mia et al., 2014; de Assumpção, 2017). Biochar added to the soil, besides contributing some P itself, can increase available soil P through enhanced P mineralization (Makoto et al., 2011; Gao et al., 2019).

Biochar addition significantly reduced soil BD, by 7% across all paddocks, while estimated decrease (made based on BD values of the biochar and soil, and their ratios in the mixture) should be 1.9%. We suggested that biochar has additional influence on factors contributing to the soil structural stability and pore structure through its interaction with soil biota and by stimulating root growth.

Biochar application significantly increased microbial (bacteria and fungi) biomass, abundance of soil arthropods (Collembola, Oribatida and Gamasina) and earthworms. The micro-intra-particle structure of biochar can provide a physical growth matrix for arbuscular mycorrhizal fungi (Hammer et al., 2014). Biochar contains labile C and also can also provide soil microorganisms with nutrients present in the ash fraction (Anderson et al., 2011; Camps-Arbestain et al., 2017; Gao et al., 2017). Increase in soil arthropods and earthworms could be related to the positive effect of biochar on root detritus and microbial biomass, which would increase the food supply available for the soil food web (Hale et al., 2013; Conti et al., 2015). The results provide evidence that biochar is behaving a like a catalyst, in part by providing habitat, which has a positive effect on the soil food web, soil structure and plant growth.

Biochar addition had a pronounced effect on the activity of a number of enzymes. The observed increase in cellulase activity in the soil treated with biochar could be the result of the enhanced root biomass providing a source of cellulose. Dehydrogenase activity, which is closely related to microbial activity, increased with biochar addition, reflecting the increased size of fungal and bacterial communities in all four soils treated with

biochar. Biochar application also increased urease activity, but the mechanism of action is not clear. Nitrate reductase activity was higher in the soil mixed with biochar than in control, however, lime addition had an even more pronounced positive effect on nitrate reductase activity, due, as we suggested, to its strong positive link to earthworm activity which was greater in the soils treated with lime. Geometrical mean of enzyme activities (GMea) showed a significant positive effect of biochar application on total biochemical activity of the soil, and this was evident throughout the 12 months of the field study **Chapter 6**.

8.2.3 Synergetic interactions between biochar, functional groups of soil biota, and plants

As was mentioned above, biochar had a positive effect on plant growth (**Chapter 3**) and plant root biomass (**Chapter 4**). This additional plant detritus, along with the labile C, nutrients and habitat provided by the biochar, stimulated microbial populations, which in turn created additional food sources and stimulated other trophic levels – arthropods and earthworms. The lower BD and reduced acidity of the soil resulting from the addition of biochar would also have had a positive effect on microbial activity. The net effect of the interactions between the soil biota and plants following biochar application was the changes in metabolic functions, which were reflected in activities of certain enzymes. Increased biological activity and enzymatic activity stimulated by biochar led to additional long-lasting changes in soil chemical properties. Our results support what was shown elsewhere – that, in general, biochar application has a positive effect on soil biological conditions and biochemical processes (Khadem & Raiesi, 2017; Mierzwa-Hersztek et al., 2019).

8.2.4 Effect of biochar on soil ecosystem services

The results of the present study showed that the addition of willow biochar produced at low temperature had a beneficial effect on the soil natural capital stocks and

the flow of ecosystem services in pasture-based agro-ecosystems (**Fig. 8.1**). Biochar addition increased root biomass through changes to a number of the NC stocks and processes contributing to plant growth (Crush & Thom, 2011; Bakker et al., 2019). Biochar increased the abundance of bacteria and fungi, as well as earthworms and arthropods. All these members of the soils biological community play an important role in sustaining the stocks and processes for the ongoing functions of a soil (Aislabie & Deslippe, 2013).

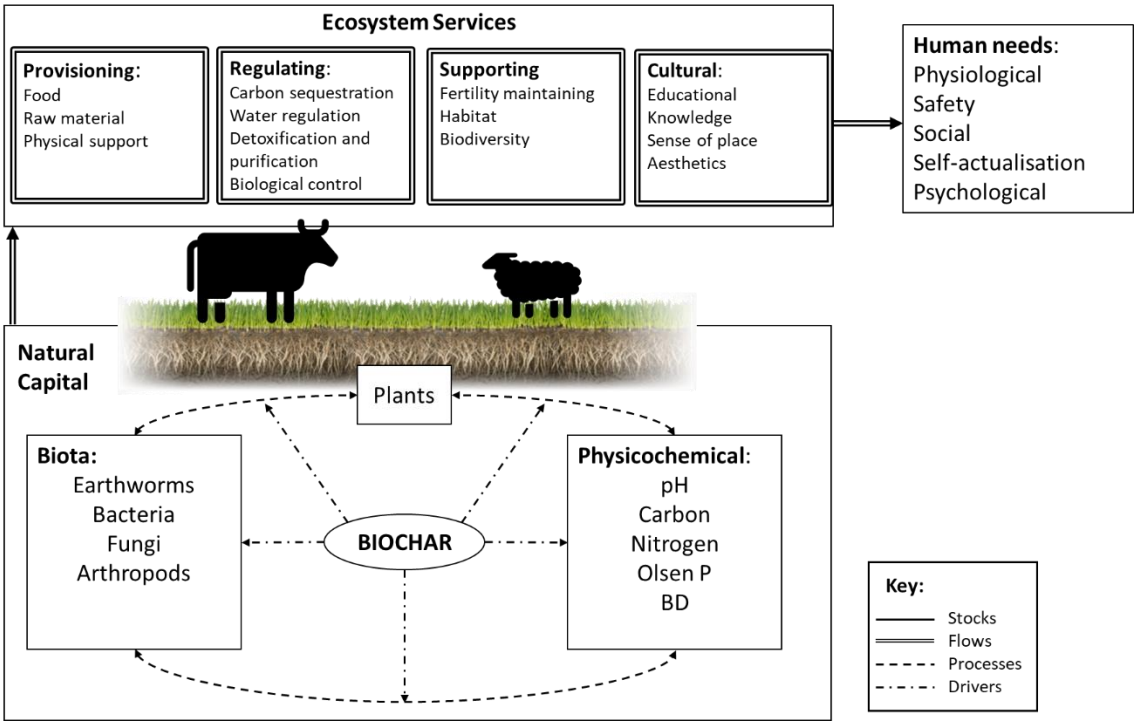

Figure 8.1 Graphic highlights: the effects of biochar on the Natural Capital stocks and processes that underpin the ecosystem Services and Human needs, according to the existing frameworks (Dominati et al., 2010; Su et al., 2018).

This willow biochar could be considered as a forward-looking amendment in the studied systems, as it can increase plant productivity, reduce disease and pest risks, improve soil physical properties, and at the same time might be used as an instrument to mitigate climate change. In our example, biochar made from willow contributed to the natural capital stocks and flows in two distinctly different soil orders, and under different

effluent and fertiliser practices. Elsewhere, it was shown that biochar can interact with standard fertilisers and enhance their effect (Oladele et al., 2019; Zhang et al., 2019). Amending soil with biochar contributes to the existing nutrient status of the soil, and stimulates the provision of ecosystem services (provisioning and regulating) by the soil (Verheijen et al., 2010; Rodrigues & Horan, 2018).

It should be considered that applying biochar as a part of sustainable agricultural practice can contribute to social services. Using agricultural techniques which improve crop production and its quality, and at the same time do not harm (or improve) soil health, promotes the fulfilment of humankind "I am looking after the land" attitude and the sense of place. Charcoal as a soil amendment is an old practice, going back to the first agricultural revolution (slash-and-burn agriculture). Returning and reflecting on an age-old experience can increase our knowledge about our place on Earth.

Also, importantly, agriculture has an important place in Māori social and economic life. By treating the soil with respect, Māori place soil health (*Mauri*) and human health on the same level. Māori farming practices use *mātauranga Māori* (Māori knowledge) to maintain soil ecosystem services. Besides the obvious provisioning and regulating services, *mātauranga Māori* included cultural services in their system long before the modern frameworks for ES were developed. Biochar application could be considered as a promising technique in sustainable agriculture, which meets the view of Māori on land use.

8.3 Recommendations for future work

The glasshouse experiment (**Chapter 3**) showed a very interesting interaction between biochar and legume plants (white clover) in relation to soil biological and chemical properties. This interaction made it difficult to explore the influence of biochar on N availability from the soil versus N availability from the additional N_2 fixation. For future studies, it would be interesting to conduct a similar study with ryegrass (one of the main

components of NZ pasture) as a mesocosm plant, rather than a legume. The absence of active plant N_2 fixation would enable an investigation of the impact of biochar on existing N and C cycle.

The design of the field-based mesocosm study is shown to be a promising model to undertake other experiments in field conditions. Future studies should include additional soil orders and management practices (land use types and fertiliser application) for more complete understanding of biochar impact on soil processes and biota. Measurements would need to be extended to include a measure of pasture growth, pasture species composition, and pasture quality, to be able to link the influence of the biochar addition through its effect on soil and processes through to plant roots, plant growth, and animal performance.

In the present study, due to the presence of positive control (lime), it was shown that the effect of biochar mainly was not related to its liming potential. In the future studies we advise to include biochar that has more available nutrients (higher Fertiliser Class) and use the equivalent amount of the mineral fertilisers as positive control. This will allow to identify in more detail the mechanisms of biochar influence on soil processes.

Study of enzymatic activity of soils treated with biochar provides insights into biochemical interactions between functional group of soil biota, plants, and biochar. For future work, it would be useful to analyse other enzymes, such as invertase – to evaluate the effect of biochar on transformation of simple sugars, chitinase – to evaluate the effect of biochar on decomposition of chitin, component of cell walls of fungi and the exoskeleton of invertebrates, etc.

Molecular biology methods, such as Quantitative PCR (or Real-Time PCR) will be useful for more detailed analysis of effects of biochar on components of soil biota and soil processes. For example, the method based on measuring presence and abundance of universal bacterial (e.g. Eub338 and Eub518) and fungal (e.g. ITS1F and ITS4) genes

will be useful to characterize soil microbial community (Fierer et al., 2005; Manter & Vivanco, 2007). The specific bacterial genes nirS (Cytochrome-cd1 nitrite reductase genes), nirK (Copper containing nitrite reductase genes), and nosZ (nitrous oxide reductase genes) can be measured to quantify denitrification rate and nitrous oxide emission. Presence and quantity of these genes in the soil are indicative of denitrification rate and N_2O emission, and their quantitative determination can be used for evaluating the effect of soil management practises on denitrification process in general, and N_2O emission, in particular. The ability to manipulate the C cycle, be N emissions

The present study was limited to 12 months. Future work needs to establish the longevity of the changes found in soil properties and pasture root mass following the addition of willow biochar made on 350°. Do the differences persist for longer than 12 months? One year is also insufficient to separate out seasonal dynamics from linear time dynamics.

Further work is also warranted in trying to better understand the mechanism(s) contributing to the increased root growth, because C in roots is the main source of labile C for C sequestration into soil organic matter. Little of the above-ground litter or dung in a pasture system is sequestered into soil organic matter. A practice that could increase the amount of root C has the potential to also change the C stocks in the soil. This, along with the impacts of biochar on the N cycle, might offer a tool for manipulating the C cycle.

In the present study the willow biochar was mixed throughout the upper 15 cm of soils. It would be interesting to investigate the impact of biochar addition to the surface of the soil, similar to lime application, to see if the same changes would result. It would also be interesting to explore the impact of multiple applications.

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