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**Investigation on the effect of biochar
addition and the use of pasture species
with different rooting systems on soil
fertility and carbon storage**

**A thesis presented in partial fulfilment of the
requirements for the degree of**

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Abstract

There is a potential to increase soil carbon (C) sequestration in New Zealand pastoral soils, especially in the subsoils where the soil C stocks have been reported to have a greater C saturation deficit than the topsoils. Selecting pasture species with deeper root systems will enhance soil formation at depth and mineral weathering, thus enhancing the potential for soils to stabilize organic matter (OM). Moreover, the addition of biochar may increase the stable C pool of soils and provide other additional benefits. Up to the present time few studies have investigated the potential of biochar to promote root growth and allocation of plant C to the subsoil. A glasshouse study was carried out to examine the effect of adding a nutrient-rich biochar at various dose (0, 1.5, 5 and 10 Mg ha⁻¹ without nitrogen (N) fertiliser; 0, 1.5, 10 and 20 Mg ha⁻¹ with N fertiliser at a dose of 113 kg N ha⁻¹) to a sandy soil on plant growth (above- and below-ground). The results indicated that, in the absence of N limiting conditions, biosolids-derived biochar could improve plant biomass yield as a result of the addition of available P and K. This amendment also caused an increase in plant root length. Subsequently, a 2-year lysimeter trial was set up to compare changes in C stocks of soils under deep- or shallow-growing pastures as well as to investigate whether biochar addition below the top 10 cm could promote root growth at depth. For this: i) soil ploughing at cultivation for pasture establishment was simulated in two different soils (a silt loam soil and a sandy soil) by inverting the 0–10 and 10–20 cm depth soil layers, and biochar was mixed at a rate of 10 Mg ha⁻¹ in the buried soil layer, where appropriate; and ii) three pasture types with contrasting root systems were grown. Distinctive biochars were selected for these two soils so that soil-specific plant growth limitations could be overcome. In the silt loam, soil inversion resulted in a net loss of native organic C in the buried horizon under shallow-rooted species, but not under deep-rooted species. The addition of a C-rich pine biochar (equivalent to 7.6 Mg C ha⁻¹) to this soil resulted in a net C gain (6–16% over the non-biochar treatment, calculated up to 30 cm; $P < 0.05$) in the buried soil layer under all pasture treatments; this overcame the net loss of native organic C in this horizon under shallow-rooted pastures. In the sandy soil all pasture species were able to maintain soil C stocks at 10–20 cm depth over time. In this soil, the exposure of a skeletal and nutrient-depleted soil layer at the surface may have fostered root growth at depth. The addition of a nutrient-rich biosolids biochar (equivalent to 3.6 Mg C ha⁻¹) to this soil had no apparent effect on total C stocks. In this 2-year study, none of the biochar amendments affected either pasture yield or root growth. More research is needed to understand the mechanisms through which soil C stocks at depth are preserved.

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1.1. Overall importance of the research

The 2014 New Zealand greenhouse gasses (GHGs) inventory report indicated the existence of a significant increase in total GHGs during the previous decade, from 60,641.4 Gg carbon dioxide equivalents (CO₂-e) in 1990 to 76,048.0 Gg CO₂-e in 2012 (MfE, 2014). The agricultural sector in New Zealand has contributed to a substantial amount of these emissions (e.g., 46.5% of total emission in 2012; MfE, 2014), which are to a great extent related to the existing intensive pastoral agriculture system that contributes through the enteric fermentation from ruminants and the nitrous oxide (N₂O) emissions from soil. Pastoral farming is the dominant land use in New Zealand (MacLeod and Moller, 2006; Mudge *et al.*, 2011), with almost 54.6% of land use being grassland (MfE, 2014). In the last few decades, the intensification of pastoral farming in New Zealand with an increase in stocking rates has led to an increased use of N fertilizer, better pasture management, and the greater use of supplementary feeding (Mudge *et al.*, 2011; Clark *et al.*, 2007).

The large GHGs emissions from the New Zealand agricultural sector could be offset under the Kyoto Protocol with an increase in soil organic carbon (SOC) stocks (Parsons *et al.*, 2009), which is generally denoted as soil carbon (C) sequestration. Soil C sequestration is considered as the removal of atmospheric C using plants to absorb CO₂ through photosynthesis and their storage in stable organic C form in soil (Lal, 2004; Powlson *et al.*, 2008). The enhancement of these stocks through land management practices can be carried out by increasing SOC inputs and/or slowing its losses, which mainly occur through decomposition (Powlson *et al.*, 2011; Smith, 2008). The C sequestration of New Zealand pastoral soils may thus play an important role as GHGs mitigation option.

Pasture/grassland systems generally have a high inherent soil C content compared with agricultural soils (Conant *et al.*, 2001). The conversion of pasture into cropland leads to a loss of soil C (Davidson and Ackerman, 1993; Schipper *et al.*, 2001), whereas the conversion of arable land to pasture has the opposite effect (Schipper *et al.*, 2001;

Guo and Gillford, 2002; Goulding and Poulton, 2005). The higher soil C contents of grasslands compared with arable soils are attributed to the enhancement of the following processes: (1) the physical protection of organic matter (OM) from plant roots and litter as particulate OM; (2) the chemical stabilisation of decomposition products originated from particulate OM; and (3) the formation of soil aggregates to protect native soil OM (Balesdent *et al.*, 2000; Soussana *et al.*, 2004). Recent studies have proposed the existence of a SOC saturation limit for a specific soil under a specific land use and management practices (Baldock and Skjemstad, 2000; Hassink, 1997, Six *et al.*, 2002). If the soil is unsaturated with respect to soil C, there are several GHGs mitigation options that could be carried out to improve soil C under a pasture/grassland system, namely: (i) a better grazing management (Conant *et al.*, 2001; Conant and Paustian 2002); (ii) an increasing pasture productivity followed by the associated plant litter return and C storage in soil (Conant *et al.*, 2001; Schnabel *et al.*, 2001; Beukes *et al.*, 2010); (iii) introducing pasture species with higher C allocation by deeper roots in subsoil (Carter and Gregorich, 2010; Fisher *et al.*, 1994); and (iv) applying organic matter amendment material that could increase pasture productivity and subsequent C input to grassland soils (Ryals *et al.*, 2014; Schimmelpfennig *et al.*, 2014; Slavich *et al.*, 2013).

The reported trends in SOC stocks in New Zealand pastures are unclear, with a decline in soil C as described by Schipper *et al.* (2007; 2010), an increase in soil C stocks as indicated by Mudge *et al.* (2011), and no soil C change as illustrated by Tate *et al.* (2003, 2005) and Schipper and Sparling (2011). In a recent study, Dodd *et al.* (2011) stated that the organic C content in the top 10 cm of most New Zealand pastoral soils has reached either a steady state or its maximum capacity. If soils are close to their C saturation limit under their specific management practices, a further increase in SOC in New Zealand pastoral soils becomes a challenge, unless increases in SOC stocks occur deeper in the soil horizon, where their SOC contents are below their saturation limit (Stewart *et al.*, 2008; Dodd *et al.*, 2011).

There is growing interest in the study of SOC contents and characteristics in the subsurface horizons (Batjes, 1996; Jobbagy and Jackson, 2000; Rasse *et al.*, 2005; Rumpel and Kogel-Knabner, 2011). The subsoil SOC stock not only represents an

important fraction of total SOC (e.g., 61% of soil C stock was stored below 30 cm in permafrost region; Tarnocai *et al.*, 2009), but is generally in a more stable form than in surface horizons (Paul *et al.*, 1997; Rumpel and Kogel-Knabner, 2011). The stabilisation of SOC in subsoil horizon is mostly related to the sorption of organic compounds to the soil mineral surfaces, also known as organo-mineral complexes (Kogel-Knabner *et al.*, 2008; Schoning and Kogel-Knabner, 2006). All this has led to theories about the potential of subsurface horizons to sequester C through an increase in C inputs in the forms of roots biomass, rhizodeposition, and the protection of organic matter in the form of organo-mineral complexes (Lorenz and Lal, 2005; Kogel-Knabner *et al.*, 2008; Powlson *et al.*, 2011; Dodd *et al.*, 2011).

The increase of SOC at depth through soil resource manipulation and the use of multi-species pasture could represent an attractive tool to mitigate GHGs (Dodd and Mackay, 2011). Recently, Carter and Gregorich (2010) reported that the increase of SOC in the subsoil of a perennial grass is associated with the higher root biomass in the deeper soil horizon. However, in most cases the increase in SOC stocks in the subsurface horizons in pastoral systems through root proliferation is challenged by the fact that roots of common pasture species are concentrated in the top soil layer (0–10 cm). Reid and Crush (2013) observed that ryegrass species in typical New Zealand pastoral farming systems had 80% of their root biomass in the topsoil layer. Root distribution is strongly influenced by nutrient concentration in soil (Russell, 1977), and this tends to be greatest in the topsoil. A modification in soil nutrient distribution could thus influence that of plant roots distribution (Dodd *et al.*, 2011).

Another approach that has acquired high interest in the past years is the addition of organic amendment with highly recalcitrant C to soil (e.g., biochar), thus modifying the C saturation limit of a specific soil. Biochar is charcoal to be used as soil amendment to enhance soil functions and to mitigate GHGs emissions that would otherwise occur if the original biomass from which it was produced decomposed (Lehmann, 2007; Sohi *et al.*, 2009). Biochar has a condensed aromatic C structure that makes it resistant to decomposition and therefore it will remain in soil for a longer period of time compared with the original feedstock (Antal and Gronli, 2003; McHenry 2009; Joseph *et al.*, 2010). The potential beneficial effect of biochar on crop growth could also increase the

capture of C through photosynthesis and root growth (Pendergrast-Miller *et al.*, 2013). Moreover, there is evidence that biochar application may protect native soil OM against decomposition – the so-called negative priming – by providing additional reactive surfaces through which organic compounds are stabilised (Liang *et al.*, 2010; Keith *et al.*, 2011; Zimmerman *et al.*, 2011). Joseph *et al.* (2010) provided a detailed review on soil-biochar interactions, including the soil mineral-biochar-OM reaction and biochar-roots interactions, which further address the potential of biochar as C sequestration tools.

Biochar, with its high nutrient value, is expected to enhance the proliferation of roots when applied to a poorly fertile soil. However, there is still limited information available on soil mineral–plant roots–biochar interactions that lead to an additional soil C sequestration to that attained with the addition of stable biochar C (Prendergast-Miller *et al.*, 2014; Ventura *et al.*, 2014), either by enhancing root biomass production in deeper soil horizons or through the stabilisation of native SOC in biochar surfaces. Based on the above considerations, this thesis proposed the study of the effect of biochar application on the development of deeper rooting pasture plant species in two contrasting soils.

1.2. Objectives of the research

The main objective of this Masters thesis was to study the effect of biochar application to typical New Zealand pasture soils on the SOC sequestration through (i) the increase of stable C (originated from the biochar C) in soil, (ii) the increase in biomass and root carbon input following biochar application, and (iii) the protection of native OM by biochar.

The specific objectives were:

- To study the suitability of a biochar derived from (i) biosolids only, and (ii) from biosolids and greenwaste on ryegrass growth when added in the top horizon of a low fertility sandy soil.
- Based on the results of the sub-objective 1, where an increase in the fertiliser value of the two biochars studied was proven and an increase in root growth was

detected in the presence of biochar (even under limited nutrient conditions), a second experiment was carried out to investigate the effect of a depth application of biochar (< 10 cm depth) in two contrasting soils (a loamy soil and a sandy soil) on the production of above-ground and root biomass of various pasture types to build root biomass for soil C sequestration. The biochar added to each soil was tailor-made to fulfil the specific needs of each soil: a biosolids-green waste biochar was applied to the sandy soil, whereas a pine biochar was applied to the loamy soil.

The hypotheses behind this Masters thesis are:

- A high nutrient value biochar added below the top 10 cm of a low fertility sandy soil and balanced for other nutrients will enhance pasture root growth and increase the soil C sequestration associated with root density in the soil;
- A high nutrient value biochar added to 10–20 cm depth of a low fertility sandy soil and balanced by other nutrients will help stimulate pasture growth at depth and stabilise root-derived organic matter;
- A low ash with high C value biochar added below the 10–20 cm depth of a poorly drained loamy soil will enhance pasture root growth at depth and help stabilise root-derived organic matter.

1.3. Outline of the thesis with the hypotheses and objectives

This Master Thesis is divided into 6 chapters. The first two chapters are a general introduction and a literature review, respectively. The following chapters (3–5) are written as “stand alone” chapters that have their own introduction, specific objectives, material and methods, results, discussion, and conclusions. As indicated above, the second experiment (referred to as the lysimeter experiment) was designed based on the results of the study described in chapter 3 and is described in chapters 4 and 5. The last chapter (chapter 6) consists of general discussion, conclusions, and recommendations for future works.

- Chapter 1 A general introduction, explaining the background for this research, followed by an outline of the research objectives and the structure overview of this Master thesis.
- Chapter 2 A review of literature, providing information and insight about: (1) soil C sequestration in pasture soils; (2) the concept of soil C protection and possible C saturation in soil; (3) biochar properties and the application of biochar to sequester C; and (4) the soil-biochar interaction.
- Chapter 3 A study of biochar application to low fertility sandy soil. This chapter will explain the properties of biochar derived from biosolids and biosolid-green waste material, and the effect of the application of these biochars to the topsoil (0–10 cm) on plant biomass production, nutrient uptake, and root proliferation.
- Chapter 4 A report of a lysimeter experiment on the effect of tailor-made biochars on different pasture species mixtures growth in two contrasting soils: 1.) Description of the setting of lysimeter trial and above- and below-ground plant biomass production over the 2-year experiment.
- Chapter 5 A report of a lysimeter experiment on the effect of tailor-made biochars on different pasture species mixtures growth in two contrasting soils: 2.) Changes in soil C and N stocks over the 2-year experiment.
- Chapter 6 This final chapter contains a summary of the overall research, conclusions, and recommendations for future work.

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2.1. Soil Organic Matter

Despite the relatively small contribution of soil organic matter (SOM) to total soil mass of a typical mineral soil (e.g., 0.1 to 10% of total soil mass), SOM is essential to soil functions. SOM plays a key role in (I) nutrient fertility, (II) retention of available nutrients at cation exchange sites, (III) improving soil physical properties (e.g., aggregation, water retention), and (IV) influencing soil ecological dynamics (Brady and Weil, 2002). In recent years, there has been growing interest in managing SOM not only to improve the overall soil fertility so that sustainable food production is attained, but also in enhancing soil C pools as a mitigation option to reduce greenhouse gases (GHGs) emissions from soil (Lal, 2004; Smith, 2004).

The turnover of SOM is an important aspect of the global carbon (C) cycle, since C is the major constituent of SOM. Any process that changes the turnover of SOM will affect the balance between the C stored in soil and atmospheric CO₂ concentration (von Lutzow *et al.*, 2006). Activities that accelerate the turnover of SOM result in an increase of the atmospheric CO₂, and can thus contribute to global warming (Lal, 2004; Smith, 2004). Understanding processes that control SOM dynamics, and especially the mechanisms through which C is stabilised in soil, is important if CO₂ emissions from soil are to be reduced (Marschner *et al.*, 2008; von Lutzow *et al.*, 2006).

2.1.1. Soil OM stabilisation in soil

The stabilisation of SOM refers to the processes that decrease the loss of SOM that would otherwise occur through mineralization, erosion, and leaching (Sollins *et al.*, 1996). Soil OM stabilisation includes the following mechanisms: (1) the spatial inaccessibility of SOM to enzymes and microorganisms through physical protection; (2) the chemical interaction between SOM and soil minerals or/and metals, which increases the energy needed by microbes to decomposed associated-SOM; and (3) the selective preservation of recalcitrant compounds (Christensen, 2001; Marschner *et al.*, 2008; Six *et al.*, 2002; Sollins *et al.*, 1996; von Lutzow *et al.* 2006; von Lutzow *et al.*, 2008). The spatial inaccessibility of SOM to biodegradation agents (enzymes and

microorganisms) includes the occlusion of SOM in the form of micro-aggregates (Six *et al.*, 2002), intercalation of SOM between phyllosilicates, and encapsulation in organic macro-molecules (von Lutzow *et al.*, 2006). The interaction between soil OM and minerals and, to some extent metals ions, reduces OM solubility and provides protection against OM decomposition (Marschner *et al.*, 2008; Sollins *et al.*, 1996; von Lutzow *et al.* 2006). Inherent bio-chemical properties of specific organic compounds may cause their selective preservation although it is now known that this mechanism has only a significant relevance to carbonised material rich in condensed aromatic C (Sollins *et al.*, 1996; von Lutzow *et al.*, 2006).

The traditional view of the molecular structure of SOM considered it a mixture of plants litter and secondary products such as rhizodeposits, black carbon materials, microbial substrates, and humic substances (Kogel-Knabner, 2002), each with different resistance to decomposition. Molecular properties, such as molecule size, polarity, ether bridges, quaternary C-atoms, molecular organic unit, type of C functions groups, and long chain (hydrophobic) hydrocarbons, were related to the inherent recalcitrance of organic compounds, with alkyl C and aromatic C being considered as recalcitrant (Sollins *et al.*, 1996). Recent studies by Marschner *et al.* (2008) and Schmidt *et al.* (2011) stressed that (I) selective preservation of SOM is not a major process in the protections of SOM against biodegradation, and (II) that lignin – traditionally considered as a recalcitrant compound – is degraded during the early stages of OM decomposition. This was corroborated by ^{13}C NMR analyses that showed the relatively fast chemical alteration of the lignin molecule, which is not being stabilised in soil in the long term (Baldock and Nelson, 2000; Kogel-Knabner, 2000; Kiem and Kogel-Knabner, 2003). Estimates of turnover times for different organic compounds are shown in Figure 2.1; these provide evidence of the low stability of lignin.

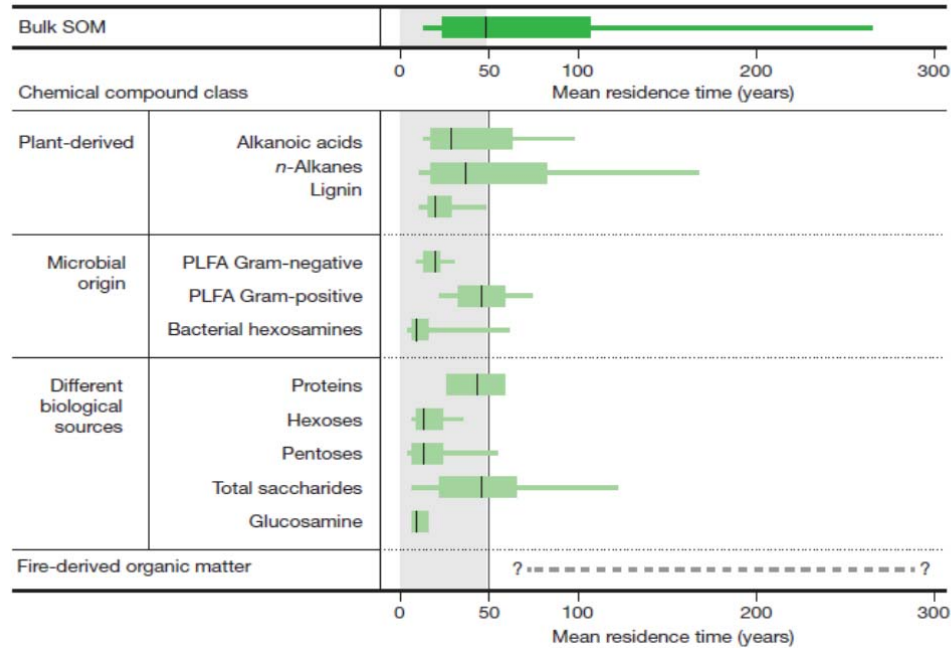


Figure 2.1. Mean Residence Time of Molecular Organic Structure
(Source: Schmidt *et al.* 2011)

2.1.1.1. The spatial Inaccessibility

Spatial inaccessibility of SOM can be achieved by: (1) occlusion of SOM into soil aggregates; (2) intercalation of SOM within phyllosilicates; and (3) hydrophobicity-related properties of SOM (von Lutzow *et al.*, 2006). Soil aggregates formation occurs through abiotic and biotic mechanisms. Even though biotic mechanisms are the major processes influencing soil aggregation (Six *et al.*, 2002), abiotic mechanisms are also considered important, especially in soils with high Al- and Fe-oxy-hydroxides, and short-range ordered aluminosilicates, such as allophane, content (Mayer *et al.*, 2004; Sollins *et al.*, 1996).

Plant litter and root growth enhance the formation of macroaggregates from microaggregates (von Lutzow *et al.*, 2006), whereas root exudates, microbial cells, and faunal mucus mostly contribute to the formation of microaggregates as binding agents. The microaggregation process is also influenced by clay content and type. Different clay types provide different binding potential because of their differences in surface area and cation exchange capacity (CEC) (Six *et al.*, 2002). Clay types with high

CEC and specific surface area, such as montmorillonite, have a higher SOM binding potential compared with clay minerals with low CEC and low specific surface area.

Macroaggregates (> 250 μm) are formed by biogenic aggregation and enmeshment by roots and fungal hyphae, with particulate OM being the stabilizing agent (von Lutzow *et al.*, 2006). Organic matter stabilisation within macroaggregates includes a wide range of macromolecules rich in lignin and O-alkyl or long-chain straight hydrocarbons. The occlusion of SOM within microaggregates (< 250 μm) is more stable compared with that within macroaggregates (Six *et al.*, 2002), as the OM compounds in microaggregates have a lower C/N ratio compared with those in macroaggregates (von Lutzow *et al.*, 2006). The stabilisation of SOM by aggregation occurs due to (1) reduced access for the microorganisms and their enzymes to SOM; (2) reduced enzymes diffusion into the intra-aggregates space; and (3) reduced diffusion of oxygen, thus restricting the aerobic SOM decomposition (von Lutzow *et al.*, 2006).

The other mechanism through which access to SOM is restricted is the intercalation of SOM within phyllosilicates. Organic compounds, specifically ligands from enzymes, protein, fatty acids or organic acids have been found to be occluded within the interlayer spaces of expandable phyllosilicates in acid soils (Violante and Gianfreda, 2000; Kennedy *et al.*, 2002), with van der Waals forces also contributing to it (Theng, 2012). The concept of the encapsulation of labile OM into more recalcitrant polymers or humic pseudo-macromolecules has been suggested by several researchers (Knicker *et al.*, 1996; Zang *et al.*, 2000; Piccolo, 2002). Labile OM is thought to be protected against decomposition by the hydrophobicity of humic pseudo-macromolecules (Piccolo *et al.*, 1999; Spaccini *et al.*, 2002). A literature review carried out by von Lutzow *et al.* (2006) considered these mechanisms to be important for stabilisation of OM but acknowledged the limitations on proving these concepts.

The accessibility of microorganisms to SOM is also restricted to the availability and distribution of water within the soil pores (von Lutzow *et al.*, 2006). In the absence of water in soil pores, less SOM decomposition was observed (Goebel *et al.*, 2004; Jandl *et al.*, 2004). The excess of water also causes a decline in SOM decomposition due to the O₂ limitation of soil microbial activity (Franzluebber, 1999; Skopp *et al.*, 1990). The

hydrophobic characteristics of SOM compounds are also considered to have an important role in reducing the microbial accessibility to SOM. Organic matter coverage is influenced by the surface charge of the soil organo-mineral complexes, which in turn, controls formation of micro-aggregates, the wettability of the surfaces, and hence, the physical accessibility of the microorganisms and their enzymes to the SOM (Bachmann *et al.*, 2008).

As expected, the surface wettability of soil OM is reduced as hydrophobicity increases. Bachmann *et al.* (2008) explained that a reduction in soil wettability is associated with the creation of water-repellent properties that may cause the formation of discontinuous pathways for enzymes and nutrient transfer in unsaturated soils. Under these conditions, SOM becomes non accessible to microorganisms and is thus stabilised against degradation. The coating of SOM on soil mineral surfaces alters the wettability of soil pores; hence this process may provide protection against microbial decomposition, since it will inhibit the mobility of microorganisms to the SOM (von Lutzow *et al.*, 2006; Bachmann *et al.*, 2008). Water repellency of soils was found to be positively correlated with SOM content (Chenu *et al.*, 2000; Doerr *et al.*, 2000) with the formation of large contact angles on the surface of the SOM-mineral coating (Bachmann *et al.*, 2008). Land use and management practices are also considered to influence soil wettability (Woche *et al.*, 2005). Arable soils generally have a low contact angle (indicating high wettability) compared with forest soils (Bachmann *et al.*, 2008). The effect of a reduced wettability is considered a key factor in SOM decomposition as it controls the microbial accessibility of water, nutrients, and oxygen and is crucial for the diffusion of enzymes in non-saturated soils.

2.1.1.2. *Organo-mineral Complexes*

The interaction between SOM and soil mineral particles also provides protection of SOM compounds against degradation. The binding of SOM with soil mineral particles in subsoil horizons has been shown to reduce the mineralisation of SOM up to 30% compared to its mineralisation in soil solution (Kalbitz *et al.*, 2005). Soil OM present in silt and clay fractions is considered to be more stable compared with other SOM in any other soil fractions (Hassink, 1997; Chenu and Stotzky, 2002). Why SOM associated

with soil minerals is stabilized against degradation is not clearly understood, although many, varied experimental approaches have been undertaken (von Lutzow *et al.*, 2006). The sorption of small organic molecules on mineral surfaces reduces the solubility of organic molecules (Chenu and Stotzky, 2002). However, microbial secretion could contribute to the desorption of small organic molecules (Chenu and Stotzky, 2002). Large molecules are considered to be more strongly attached to soil minerals than are small ones (Chenu and Stotzky, 2002).

In general, the sorption of organic compounds onto soil mineral surfaces is determined by the mineralogy of soil minerals, and especially their surface reactivity and specific surface area (Kleber *et al.*, 2004; von Lutzow *et al.*, 2006). Minerals with a higher specific surface area provide a larger sorption area for SOM, e.g., layer silicates (with a particle size <2 µm), sesquioxides (5–100 nm), Fe-oxides (3–10 nm), and amorphous Al-oxides (<3 nm). Of the different aluminosilicates, the 2:1 clay types (e.g., montmorillonite) provide higher surface area for SOM sorption than the 1:1 clay types (e.g., kaolinite) (Six *et al.*, 2002). Kaiser and Guggenberger (2003) suggested a preferential sorption of SOM occurs on reactive surface such as rough edges and micropores (generally at the edges of these; Kaiser and Guggenberger, 2003).

Metal ions in soil can also interact with SOM, creating bindings that contribute to SOM stabilisation. The chemistry of SOM-metals binding is well understood (Baldock and Skjemstad, 2000; Tipping, 2002) but there is less information on how these bindings relate to the stability of SOM. Common metals in soil, such as Ca^{2+} , Al^{3+} , and Fe^{3+} , are considered as potential ions that stabilise OM in soils (Baldock and Skjemstad, 2000). In calcareous soils, Ca^{2+} is a dominant cation contributing to SOM stability, whereas Al^{3+} is dominant in acid soils (Lundstrom *et al.*, 2000; Zysset and Berggren, 2001; Nierop *et al.*, 2002). Van Hees *et al.* (2003) reported that in the subsoil horizons, Al and Fe oxy-hydroxides minerals are the primary sorbents of soluble organic compounds. The specific Al ions interaction with SOM is common in acid forest soils under coniferous trees in temperate-cold climate regions, as a result of the acidification and mobilisation of Al by shoot-derived complexing agents (Rasse *et al.*, 2005). Moreover, the stabilisation of root-derived C compounds with the Al ions contributes to the selective preservation of C against biodegradation (Parfitt, 2009).

Various conceptual models have been proposed to explain SOM stabilisation mechanisms (Wershaw *et al.*, 1986; Kleber *et al.*, 2007). A recent review by Kleber *et al.* (2007) proposed a concept of zonal structure of organo-mineral associations (Figure 2.2) based on the amphiphilicity (dual properties of hydrophilicity and hydrophobicity) of SOM. This concept suggests that SOM attached to a mineral surface is segregated into more than one layer of molecules, indicating that not all the stabilised SOM is attached to the mineral surface. The zonal structure model (Figure 2.2) divides the interactions of SOM and mineral particles into several zones (contact zone, hydrophobic interactions zone, and kinetic zone). In the contact zone, the amphiphilic SOM fragments are bound to mineral surfaces through electrostatic interactions and direct the hydrophobic fragments outwards to the aqueous solution. The hydrophobic fragments interact with other amphiphilic SOM compounds in the aqueous solution, binding to each other. In the kinetic zone, the accumulation of OM is possible through the multivalent cations present in the soil solution.

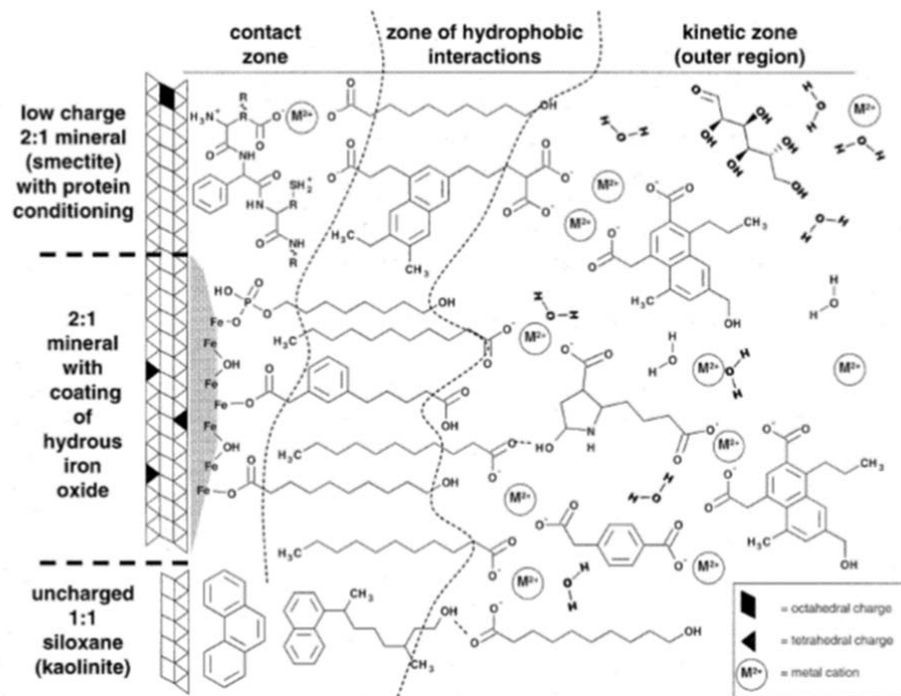


Figure 2.2. The zonal model of organo-mineral interactions
(Source: Kleber *et al.*, 2007)

2.1.2. Soil C saturation

The C storage capacity of a soil depends on the C input from fresh OM to the soil and the C output through process of decomposition, erosion, and leaching. Many of the earlier SOC decomposition models used the first-order kinetic approach and, as a result, models assumed the linearity between the C input and the SOC storage capacity at steady state (Stewart *et al.*, 2007). This linear relationship infers the lack of an upper boundary in the capacity of soils to store C at increasing C inputs (Figure 2.3.a). However, long-term experiments have shown that this is not always the case (Six *et al.*, 2002; Stewart *et al.*, 2007). In a comprehensive review, Six *et al.* (2002) determined the upper limit of soil C storage based on C input information and soil C data from 11 sites. These authors indicated that the data best fitted to an asymptotic model (Figure 2.3.b) rather than to the classic linear model. Using soil C data from 14 sites, these results were corroborated by Stewart *et al.* (2007), whose findings suggest that soils have an upper limit to store C. Hence, once a soil reaches its C saturation level, an additional input of C (under a specific land use and management) would be unlikely to contribute to increase soil C concentration (unless this stability of C in this new input does not depend on its interaction with soil, e.g., charcoal).

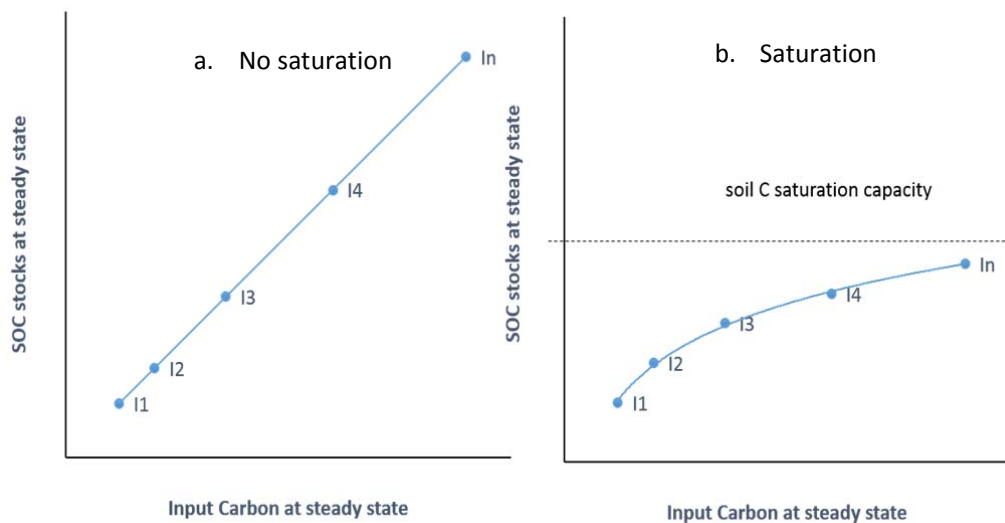


Figure 2.3. Relationship between input level of C and SOC at steady state

The limitation of soil to store additional C was reported earlier by Hassink (1997). This was attributed to the fact that, with the increase in the sorption of SOM to soil mineral surface, there is less surface area available for additional SOM to become adsorbed. Six *et al.* (2002) proposed a concept of soil C saturation in which this limit was not only related to the chemically-protected SOC as Hassink (1997) suggested, but to four different C pools, namely the physically, chemically, biochemically protected and the unprotected soil C pools, the chemically and biochemically protected C pools being considered to be the most stable C fractions (Stewart *et al.*, 2008). The deficit between theoretical SOC saturation level and the actual concentration of the SOC associated with soil fine fraction is considered as the saturation deficit (Angers *et al.*, 2011; Beare *et al.*, 2014; Stewart *et al.*, 2007; Six *et al.*, 2002). The soil saturation deficit provides information on the potential of a soil to sequester additional C.

Studies on soil C in New Zealand pastoral systems have reported either loss in soil C (Schipper *et al.*, 2007; Schipper *et al.*, 2014), increases (Mudge *et al.*, 2011; Parfitt *et al.*, 2014; Schipper and Sparling, 2011) or no change (Schipper *et al.*, 2007; Tate *et al.*, 2005) in soil C stocks. Recently, Beare *et al.* (2014) used the New Zealand National Soil Database (NSD) to evaluate the methodological concept of soil C saturation limit by Hassink (1997) and Feng *et al.* (2013). For this, they investigated the upper limit of soil C stabilisation based on the fine mineral fraction and specific surface area on two different soil depths (topsoil of 0–15 cm and subsoil of 15–30 cm). The results from multi-regression analysis showed that almost all soils in New Zealand have a positive saturation deficit, meaning there is potential to increase the soil C stocks in New Zealand pastoral soils, this unsaturation being greater in the subsoil, thus providing a greater potential for SOM stabilisation at depth (Six *et al.*, 2002; von Lutzow *et al.*, 2006; Chenu and Plante, 2006).

2.2. Deep Soil Organic Matter: Potential for Storing C in the Subsoil

In recent years, the study of both the dynamics of subsoil OC and the C stabilisation mechanisms in the deep soil horizon has increased (Rasse *et al.*, 2005; Rumpel and Kogel-Knabner, 2011; Salome *et al.*, 2010; and Schrumpf *et al.*, 2013). Salome *et al.* (2010) suggested that the dynamics of C in the topsoil and subsoil are controlled by

different mechanisms. Hence, understanding the C dynamics in the subsoil will help manage soils so that soil C sequestration in the deeper soil layer is increased. The following sub-sections will address the subsoil SOM sources, the proposed specific stabilisation mechanisms of root-derived C, and potential soil management strategies to increase subsoil C.

2.2.1. *Dynamic of subsoil OM*

In the subsoil, there are four main input of C, namely root detritus, root exudates, Dissolved Organic Carbon (DOC), and SOM incorporated through bioturbation by soil organisms and plant roots (Rumpel and Kogel-Knabner, 2011). The relative importance of these SOM inputs to the SOC in subsoil layers depends on climate (Kaiser and Guggenberger, 2000), soil properties (Schrumpf *et al.*, 2013), and land management (Rumpel and Kogel-Knabner, 2011). Of the above four OM inputs, plant roots are the most important C source in the subsoil layer, not only because of their high C allocation, but also because of the types of compounds originating from them (e.g., more prone to selective preservation in the short- and mid-term). Plant rooting patterns contribute to the variability of vertical SOC profile (Jobbagy and Jackson, 2000). Rasse *et al.* (2005) estimated that the average relative contribution factors of plant root vs shoot to SOC is 2.4 from in-situ experiments, and 1.3 from an incubation trial. This means plant roots always contribute more to SOC compared with shoot litter. These authors also found that root-derived SOM had longer mean residence time than that shoot-derived SOM.

The different dynamics of SOC in topsoil and subsoil layers have been mainly attributed to differences in the quality of OM substrate (Fierer *et al.*, 2003; Fontaine *et al.*, 2007; Salome *et al.*, 2010; Xiang *et al.*, 2008). Salome *et al.* (2010) found that the mineralisation of C per gram of soil was significantly greater in the topsoil compared with the subsoil. This was attributed to a greater protection of soil C within soil aggregates in the subsoil compared with the topsoil. In the same study, these authors also found a more dominant positive priming effect of the SOM in the topsoil than in the subsoil samples.

2.2.2. Influence of root chemical composition on SOM stabilisation of root-derived C

The importance of plant roots as the source of C to SOC in subsoil layers has been reviewed quite intensively during the last decade (Lorenz and Lal, 2005; Rasse *et al.*, 2005; Rumpel and Kogel-Knabner, 2011). Rasse *et al.* (2005) suggested a conceptual model (Figure 2.4) to explain the mechanisms of root-derived C stabilisation occurring in the subsoil, which are 1) chemical recalcitrance of root-derived OM compounds (in the short- and mid-term); 2) micrometer physical protection of root litter by soil aggregation; and 3) physico-chemical interactions between root and soil mineral particles (Figure 2.4.). Fundamentally, these three mechanisms are the same C stabilisation mechanisms that have been reviewed in sub-chapter 2.1.1; however, plant roots have specific traits and characteristics in response to the C stabilisation processes, which will be described below.

The chemical recalcitrance of plant roots is attributed to the high content of recalcitrant macro-molecules compounds such as tannins, suberins, and cutins, which are considered to be relatively resistant to degradation by soil enzymes and microorganisms in the short- to mid-term (Lorenz and Lal, 2005; Lorenz *et al.*, 2007; Rasse *et al.*, 2005). However, as discussed in a previous section, the existence of a true chemical recalcitrant has been widely debated (Rumpel and Kogel-knabner, 2011; Schmidt *et al.*, 2011). The protection of root-derived C in the subsoil layer then seems to be more related to the physico-chemical interaction between root-derived SOM and soil mineral surfaces and to the occlusion of root-derived SOM within soil aggregates (Schoning and Kogel-Knabner, 2006).

Root-derived material contributes to the formation of soil aggregates and the aggregates, in turn, provide a physical stabilisation for root-derived SOM (Rasse *et al.*, 2005). Some root-derived SOM is reported to be part of particulate SOM (POM) that has been physically stabilised as occluded POM (Rumpel and Kogel-Knabner, 2011). In terms of soil aggregation processes, root hairs hold soil particles together and promote microbial biomass growth, and this produces organic polymers that act as binding agents (Jastrow *et al.*, 1998; Tisdall and Oades, 1979).

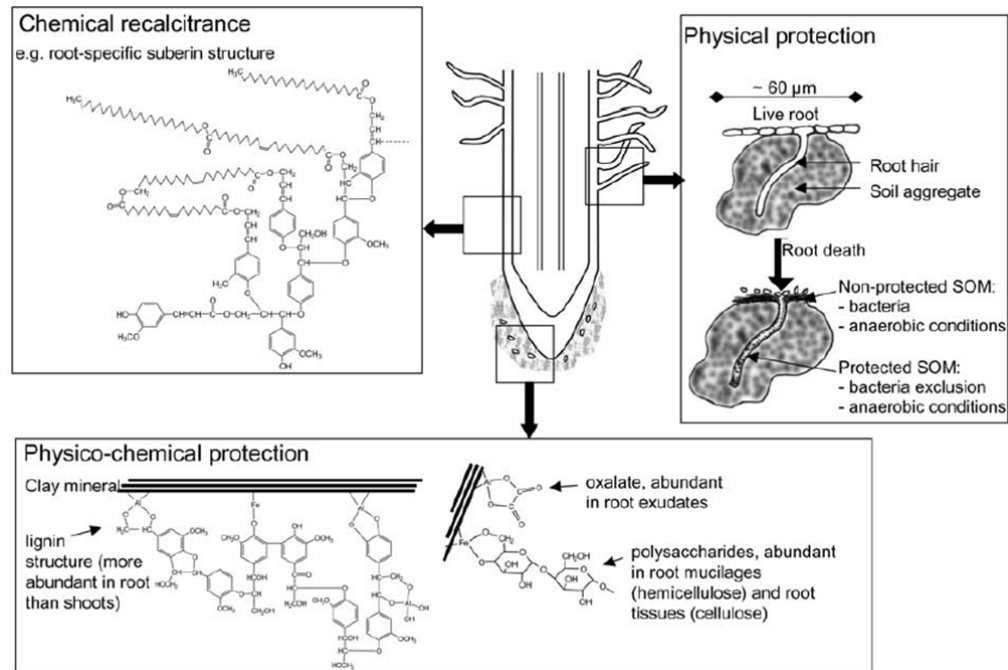


Figure 2.4. Various stabilisation mechanisms of C from roots
(Source: Rasse *et al.*, 2005)

Roots hairs are also an input of SOM and can penetrate into soil microaggregates, and their detritus can become protected within (Figure 2.4). Six *et al.* (2002) argued that the physical protection of root-derived SOM within soil aggregates included two main mechanisms: (1) the inaccessibility of decomposer agents (soil enzymes and microorganisms) to SOM entrapped in the fine pores of microaggregates; and (2) the anoxic conditions common in microaggregates that hampers the activity of aerobic microorganisms.

There are two main types of organic compounds derived from roots: (1) water-soluble organic compounds, such as sugar, amino acids and organic acids; and (2) water-insoluble materials, such as cell walls and other root debris and mucilage (Brimecombe *et al.*, 2001). Both soluble and insoluble root organic acids are reported to vary in chain length, and considered to be labile compounds in the soil matrix that can be mineralized within hours of released from roots (Chabbi *et al.*, 2001; Rasse *et al.*, 2005). Despite these root exudates being relatively prone to biodegradation, they can also be retained at soil mineral surfaces (Figure 2.4.).

It has been suggested that the sorption of root-derived SOM to the soil mineral surfaces is more effective in the subsoil horizons, given that, in many instances, the general lower contents of SOM at depth prevent reaching a C saturation state (Rasse *et al.*, 2005; Rumpel and Kogel-Knabner, 2011). Previous research by Jobbagy and Jackson (2000) and Rumpel *et al.* (2004) reported a positive correlation between the vertical SOC distribution and clay content in deep soil horizon, suggesting the clay surface is a key stabilising mechanism for the subsoil root-derived C. However, in New Zealand soils under grassland (and probably under other land uses) the stable SOC concentrations do not relate to the clay fraction but to reactive Al (Percival *et al.*, 2000; Beare *et al.*, 2014).

2.2.3. Soil management practices to increase subsoil C

Increasing SOC stocks in the subsoil by enhancing root growth at depth can be a promising strategy to contribute to GHG mitigation (Lal, 2004; Smith, 2004; Six *et al.*, 2002). At the ecosystem level, land-management strategies intended to increase subsoil C stocks have intensively been discussed by Lal and Lorenz (2005). These authors support (1) the idea of promoting root growth at depth, and (2) the enhancement of the formation of stable, organo-mineral complexes. One of the strategies they propose is the selection of plants and cultivars rich in more recalcitrant compounds (e.g., tannins and suberin) in the below-ground biomass (Lorenz and Lal, 2005). Despite the fact that the biochemical recalcitrance of root-derived organic compounds is being debated (e.g., Schmidt *et al.*, 2011), the role of root exudates to promote mineral weathering in acid soils, and enhance the formation of organo-mineral complexes should not be disregarded (Rasse *et al.*, 2005).

Grasslands ecosystems generally provide a higher below-ground biomass production than croplands (Jobbagy and Jackson, 2000); in some instances, higher than forest ecosystems (80% and 69% respectively) (Kuzyakov and Domanski, 2000). Moreover, there is growing interest in enhancing deep soil C content with the selection of deep-rooted pasture species (Dodd and Mackay, 2011). Recently, Carter and Gregorich (2010) reported that 7 years after conversion from barley to tall fescue perennial

grassland soil C content in the subsoil (40–60 cm) increased from 6.9 Mg ha⁻¹ to 12.4 Mg ha⁻¹.

Another management option to increase soil C in the deeper soil layer is by the allocation at depth of a soil amendment rich in C resistant against biodegradation. This is the case of biochar, a product of biomass thermo-chemical conversion in zero or low oxygen condition (also known as pyrolysis), which has a condensed aromatic C structure and thus is highly resistant to decomposition (Lehmann and Joseph, 2009). This product has also been reported to improve soil functions (Lehmann and Joseph, 2009; Shackley *et al.*, 2010; Verheijen *et al.*, 2009).

2.3. Biochar

Carbonised material – commonly referred to as black C (BC) – is a product of incomplete combustion of biomass (Schmidt *et al.*, 2001). Black C can be formed during the natural vegetation fire events (Knicker, 2007) and as a consequence of slash and char agricultural practice (Glaser *et al.*, 2002). Black C also includes charcoal produced by thermoconversion under limiting oxygen condition as pyrolysis (Lehmann *et al.*, 2006). Biochar is defined as charcoal to be used as soil amendment to improve soil functions while reducing the GHG emissions that would otherwise be produced from the original uncharred biomass (Lehmann and Joseph, 2009). In recent years, research publications have provided more insight on the role of biochar in restoring soil quality and increase soil C sequestration (Atkinson *et al.*, 2010; Herath *et al.*, 2013; Jeffery *et al.*, 2011, 2015; Oram *et al.*, 2014; Wang *et al.*, 2012a, b). The following section reviews the role of biochar as a sequestration strategy and how this is influenced by different environmental conditions.

2.3.1. Biochar production system

Biochar is produced from the thermal degradation of biomass in an oxygen-limited condition, which is known as pyrolysis. Three products are generated from the pyrolysis of biomass: (1) non-condensable gases, (2) a flammable bio-oil representing the condensable liquid-tar, and (3) solid co-product in the form of charcoal (Spokas *et al.*, 2012). In an environment where oxygen is limited, the degree of oxidation during

pyrolysis of biomass is smaller, compared with normal combustion (Verheijen *et al.*, 2010). As a result, the proportion of C converted to CO₂ is lower than the C retained in the pyrolysis products, either as bio-oil or as the solid product of biochar. The characteristics and properties of biochar produced from pyrolysis depend on several factors: the pyrolysis method, the type of biomass feedstock, and the final heating rate (Manya, 2012; Verheijen *et al.*, 2010). The pyrolysis is always carried out above 300°C, but the highest heating temperature and the heating rate will vary.

There are two common types of pyrolysis through which biochar are produced: slow pyrolysis and fast pyrolysis. Slow pyrolysis is characterized by slow heating (minutes to hours) of the biomass feedstock, and a relatively long holding temperature once the peak temperature has been reached (Mohan *et al.*, 2006; Verheijen *et al.*, 2010). Biomass heated with fast pyrolysis technique, however, is exposed to a heat transfer for a few milliseconds to seconds (Bridgewater *et al.*, 1999; Laird *et al.*, 2009). The difference between those two pyrolysis methods is summarised in Table 2.1. The solid, liquid, and gas distribution of the original mass strongly depends on whether slow or fast pyrolysis is carried out (Spokas *et al.*, 2012). Biochar properties can still vary considerably within a specific pyrolysis type, given these will also depend on the specific heating rate, the highest heating temperature, the type of feedstock, and its particle size (Brewer *et al.*, 2009; Brunn *et al.*, 2012; Brown, 2009).

Table 2.1. Pyrolysis methods and characteristic of product generated from pyrolysis

Conversion type	Temp. range	Residency time	Heating rate	Product production (% of original feedstock mass)			Solid Proximate analysis (%)			
				Solid	Liquid	Gas	Moisture	VM	Ash	Fixed C
Slow Pyrolysis	350–700	Hours	1–100	15–40	20–55	20–60	0–5	5–20	2–10	40–90
Fast Pyrolysis	450–550	< 1 min	> 1000	10–30	50–70	5–15	0–5	40	30	40–60

(source: Spokas *et al.*, 2011)

2.3.2. Biochar C Stability

The stability of biochar in soil is important because it will determine (1) how long biochar will remain in the soil and contribute to the soil C sequestration, and (2) for how long it will provide additional benefit to the soil (Lehmann *et al.*, 2009). The intrinsic properties of biochar are the main factor influence the C stability (Calvelo-

Pereira *et al.*, 2011). However, the pedoclimatic conditions have proved to be an important factor in determining the fate of biochar after its application to the soil (Herath *et al.*, 2015). The following sub-section provides a more in-depth discussion on the characteristics of stable C in biochar and the effect of biochar application to the native SOM (priming effect).

2.3.2.1. Long term stability of biochar

The recalcitrance of organic C changes significantly after the biomass conversion into biochar by pyrolysis (Lehmann and Joseph, 2009). The changes in chemical composition occur by a complete destruction of cellulose, transformation of lignin, and the forming of polycyclic aromatic hydrocarbons structures (Almendros *et al.*, 2003; Krull *et al.*, 2009; Schmidt and Noack, 2000). The condensed aromatic C structure consists of six C atom rings with double bonds in which the delocalized pi electrons overlap rendering delocalized electrons – known as aromatic compounds – linked together without oxygen or hydrogen (Schmidt *et al.*, 2001; Lehmann and Joseph, 2009). This aromatic structure of biochar-C has a greater chemical and microbial stability against degradation than other organic C compounds (Cheng *et al.*, 2008; von Lutzow *et al.*, 2006). Hence, the C-aromaticity of biochar becomes one of its key properties in increasing the stable organic C pools in soil.

The degree of biochar stability against degradation can be estimated using several approaches. Zimmerman *et al.* (2011) used the volatile content of biochar as an indicator to determine the degree of biochar stability, with high volatile content suggesting a low stability. The elemental composition of biochar also can be used to determine the degree of biochar stability (Calvelo-Pereira *et al.*, 2011; Schimmelpfennig and Glaser, 2012; Spokas, 2010). The International Biochar Initiative (IBI) (2012) has established a relationship between atomic H/C_{organic} ratios and the stability of biochar. Carbonaceous materials with H/C_{organic} ratios higher than 0.7 are not considered biochar (IBI, 2012). Moreover, the elemental composition (C, H and O) of a biochar – and more specifically the atomic H/C_{organic} ratio – can be used to predict its C-aromaticity (Wang *et al.*, 2013). These authors tested and modified several existing mathematical model to estimate the C-aromaticity of biochars.

It has to be noted that although biochar is rich in stable C, it also contains a labile C fraction that can be subject to abiotic and biotic degradation (Cross and Sohi, 2010; Zimmerman, 2010). Studies have shown a flush in soil respiration rate immediately after the addition of biochar to soil (Ameloot *et al.*, 2013; Brunn *et al.*, 2008; Zimmerman, 2010), suggesting the increasing activity of soil microorganisms following the application of biochar, although desorption of CO₂ and dissolution carbonates and the corresponding release of CO₂ from acid environments cannot be disregarded. The initial fast mineralization rate of biochar-C is reported to be variable, approximately 2–20% of C mineralized between 2 (Smith *et al.*, 2010) and 60 days (Kuzyakov *et al.*, 2009; Steinbeiss *et al.*, 2009).

2.3.2.2. *Priming effect after biochar application to soil*

The application of biochar as soil amendments is intended to increase soil C sequestration and provide additional benefit to soil properties. However, biochar in the soil ecosystem may accelerate the decomposition of native OM (NOM) or protect it against decomposition, depending on the interaction between biochar, soil microbes, and NOM in the soil (Kuzyakov *et al.*, 2009; Lehmann *et al.*, 2011; Zimmerman *et al.*, 2011). The addition of new substrates to soil may change the mineralisation of NOM in the soil, also known as priming effect (Kuzyakov *et al.*, 2000).

The positive priming effect of biochar on the decomposition of NOM (e.g., acceleration of decomposition) has been widely reported by several researchers (Kuzyakov *et al.*, 2009; Luo *et al.*, 2011; Steinbeiss *et al.*, 2009; Wardle *et al.*, 2008). There are several mechanisms that explain this positive priming effect: (1) biochar provides a habitat for microorganisms, and in turn increases the soil microbial activity (Thies and Rillig, 2009); (2) the easily mineralisable C fraction from biochar triggers the co-metabolism of NOM (Wardle *et al.*, 2008; Luo *et al.*, 2011); and (3) the addition of nutrients with biochar that were deficient in that specific soil. It is not only positive priming of biochar on NOM that can occur, but also the reverse reaction, i.e. NOM priming effect on biochar, as has been reported by Hamer *et al.* (2004), Luo *et al.* (2011), and Kuzyakov *et al.* (2009).

On the other hand, other studies have found that the application of biochar did not result in changes in NOM mineralisation rates, and even reported negative priming effects (Cross and Sohi, 2011; Jones *et al.*, 2011; Zimmerman *et al.*, 2011). The negative priming effect of biochars on NOM can be the result of: (1) the sorption of NOM on to biochar surface and/or encapsulation of NOM within biochar pores (Zimmerman *et al.*, 2011); (2) the possible deactivation of microbial enzymes on biochar surfaces (Zimmerman *et al.*, 2011); (3) the promotion of soil aggregation by biochar (Herath *et al.*, 2013) and the occlusion of NOM within the aggregates (Hilscher and Knicker, 2011); (4) the presence of toxic compounds to micro-organisms in the biochar (Liu *et al.*, 2009); and (5) the short-term “dilution” effects of biochar labile C fraction on to total SOC pools (Whitman *et al.*, 2014). In the recent years, more studies have provided more information on the prevalence of the negative priming effect caused by biochar application (Herath *et al.*, 2015; Whitman *et al.*, 2014; Zimmerman *et al.*, 2011).

Therefore, despite biochar enhancing the mineralisation of SOM at the earlier stage of its application to soil (positive priming effect), this effect decreases over time, and can even turn into a negative priming effect, thus enhancing SOM stabilisation against decomposition (Whitman *et al.*, 2014; Zimmerman *et al.*, 2011). Recently, Herath *et al.* (2015) reported the lower soil respiration rates (1.5 to 0.5 mg CO₂-C kg⁻¹ soil h⁻¹ within a few days) of soil amended with corn-stover biochar compared with those from the same soil amended with fresh corn-stover (4–1 mg CO₂-C kg⁻¹ soil h⁻¹ within 1–2 months). These authors also reported that the total CO₂ evolution from a biochar-treated Alfisol soil (not considering that from biochar) was lower (4.2 g kg⁻¹) compared with that from the nil biochar treatment (5.3 g kg⁻¹), indicating the existence of a negative priming effect of biochar on soil NOM decomposition. Based on the stable C isotopic measurement, Zimmerman *et al.* (2011) suggested that in a soil-biochar system, both the NOM and the biochar can act as a priming agent, where NOM positively primed biochar in the early stage of incubation, while biochar might be responsible for retarding SOC decomposition in a later stage.

2.3.3. *The availability of nutrients from biochar*

The nutrient concentration in biochar is largely dependent on the type of feedstock and pyrolysis condition (Singh *et al.*, 2010), while the availability of these nutrients is associated with the nature of chemical compounds present in the biochar (Wang *et al.*, 2012b). In general, wood-derived feedstock produced biochar with higher total C content, but low N and P. Contrary to this, animal/human-based waste feedstock produced biochar with relatively higher N and P compared with the plant biomass feedstock (Table 2.2). It has to be noted that the same type of feedstock can produce different nutrient concentration of biochar (Chan and Xu, 2009). The difference in quality of feedstock, as well as the pyrolysis condition is attributed to such variability.

Table 2.2. Nutrient properties of selected biochar from various type feedstocks

Feedstock Type	Temperature of pyrolysis	Total Corg (g kg ⁻¹)	Total N (g kg ⁻¹)	Available N (g kg ⁻¹) ^a	Total P ₂ O ₅ (g kg ⁻¹)	Available P (g kg ⁻¹)	Source
Mix Manure-Eucalyptus	450	481.3	15.5	1.05	18.25	7.09 ^b	Wang <i>et al.</i> , 2012a, b
Mix Manure-Eucalyptus	550	547.1	15.9	1.48	19.03	6.55 ^b	
Mix Biosolid-Eucalyptus	450	367.5	18.5	3.07	109.39	20.14 ^b	
Mix Biosolid-Eucalyptus	550	357.4	16.6	0.76	115.98	18.37 ^b	
Cattle Feedlot Manure	550	760	6.10	-	15.83	5.10 ^c	Slavich <i>et al.</i> , 2013
Greenwaste	550	440	2.20	-	0.44	0.06 ^c	

^a measured using 6M HCl

^b extracted using 2% Formic-acid

^c extracted using 1.1 M neutral Ammonium Citrate

The total N content in the biochar does not reflect its availability to plant uptake. Almendros *et al.* (2003) reported that charring organic material will result in the formation of heterocyclic N, which is considered to be resistant to microbial degradation and limit the N availability to plant uptake (Chan and Xu, 2009; Yao *et al.*, 2010). However, recent studies have shown the form of heterocyclic N is less recalcitrant than it was assumed, since part of this when present in grass charcoals was reported to be subject to N mineralisation and become available to soil microorganisms and plant uptake (De La Rosa and Knicker, 2011; Hilscher and Knicker 2011). Recently, Wang *et al.* (2012b) found that increasing pyrolysis temperature results in a reduction in the fraction of hydrolysable N of biochar produced from a

mixture of eucalyptus wood-manure and eucalyptus wood-biosolid. The total hydrolysable N fraction of biochar is represented by the labile fraction of N and considered to be bioavailable in the short term (Wang *et al.*, 2012b).

Despite the existence of a labile N fraction in biochar, it is very low compared with that in other organic amendments and fertilisers. The release of N from biochar is most likely to co-mineralise with the C mineralisation from a biochar, and is reported to be less than 2% (Fang *et al.*, 2014; Singh *et al.*, 2012; Zimmerman, 2010). Using data from Table 2.2, the mixture manure-eucalyptus biochar produced at 450°C is likely to contain 1.05 g N kg⁻¹ biochar, therefore the application of 1 Mg ha⁻¹ biochar only provides 1.05 kg ha⁻¹ of N to soil. It is not therefore plausible to use biochar to provide additional N to soil.

Phosphorus in biochar is most likely to be concentrated in the ash fraction of biochar, and the amount can be considerable if produced from animal-based waste such as manure, poultry litter, or sewage sludge/biosolids (Gaskin *et al.*, 2008; Hossain *et al.*, 2011; Wang *et al.*, 2012a). Increasing pyrolysis temperature was reported to increase total P concentration in the biochar, with 100% recovery of P is expected in the biochar (Bridle and Pritchard, 2004; Hossain *et al.*, 2011; Wang *et al.*, 2012a). Phosphorus in biochar could be in the form of calcium (Ca) or magnesium (Mg) complexes (Bridle and Pritchard, 2004; Wang *et al.*, 2012a) or aluminium-bound P compounds (Wang *et al.*, 2012a) which are not available for plant uptake. Using a 2% formic acid extraction, Wang *et al.* (2012a) found that biochar made from a mixture of manure and eucalyptus wood contained 70–80% of total P in an extractable form, which indicates the high availability of P in this particular biochar. This was further confirmed by the greater P recovery in the plant (39–93%) grown in soils amended with corresponding biochar. These authors also reported that the maximum dry matter yield from biochar-treated soils was similar to those from P fertilised treatments, indicating the potential of manure-derived biochar as an alternative to P source in the soil.

Biochar also contain a high amount of potassium (K) in the form of K-containing salts. Potassium in biochar has been reported to be highly available to plant uptake (Yao *et al.*, 2010). During the thermal-degradation of biomass, ester bonded sulphur (S) is

reported to undergo changes into sulphate-S (Churka Blum *et al.*, 2013). Yao *et al.* (2010) found that biochar produced from biosolid at 550°C contained the sulphate-S in non-crystalline form. Other cations such as Ca and Mg are found to be concentrated in the biochar if it is produced at temperature < 500°C, but are elutriated otherwise. Xu and Sheng (2011) reported that biochar made from cornstalk had a higher Ca and Mg availability for plant uptake (98% of the total Ca and Mg is soluble in soil solution).

2.4. Biochar and root interaction

The application of biochar as a soil amendment is reported to influence the proliferation of plant roots (Lehmann *et al.*, 2011). Earlier research by Makoto *et al.* (2010) and Noguera *et al.* (2010) showed that root growth was increased by the application of biochar to soil, although the mechanistics explaining root proliferation in soil-biochar systems are unclear (Lehmann *et al.*, 2011) and reports are not always consistent. The addition of biochar to soil can directly provide nutrients to plants (mainly P and K) but also indirectly alter soil nutrient availability through the retention of nutrient cations, which in turn will affect plant root growth (Prendergast-Miller *et al.*, 2014). Biochar can also improve soil physical properties that favour root growth, such as soil water content or the improvement on soil aeration (Lehmann *et al.*, 2011). Recently, Brunn *et al.* (2014), investigating the effect of biochar application on soil mechanical constraint to root growth, found that the application of 1% straw-biochar reduced the soil mechanical resistance and improved root penetration to subsoil. Therefore, the improved soil physical properties under biochar treatment increase the total average root density (54% root coverage) compared with the control (33% root coverage).

2.5. Conclusion and research gap identified in the literature review

The summary of literature review and current research gaps are summarized below:

- Studies on the potential increase of soil C storage in the subsoil of grassland ecosystems with the use of species with deep rooting systems are scarce.
- The potential effect of biochar application at depth on stimulating root growth in deeper horizons has not yet been explored.

- More information is needed on the potential priming effect of biochar on NOM.

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Chapter 3. The Application of Biochar Made from Biosolids to Increase The Fertility of A Sandy Soil: A Glasshouse Study

Raw sandy soils have low nutrient fertility and low water storage capacity, which make their management a challenge for farmers. The aim of this experiment was to test the ability of two ash-rich biochars to be used as amendment to support ryegrass growth in a low fertility sandy soil under glasshouse conditions. Two biochars were produced at 550°C from (i) biosolids (BS biochar) and (ii) a mixture (50:50; wt:wt) of biosolids and green waste (BG biochar). Biochars were applied to a typical Udipsamment soil at different rates: 0, 1.5, 5 and 10 Mg ha⁻¹ without nitrogen (N) fertiliser; 0, 1.5, 10 and 20 Mg ha⁻¹ with N fertiliser at a dose of 113 kg N ha⁻¹. In the absence of N fertiliser, biochar amendments significantly reduced ($P < 0.05$) the above ground-biomass yield (mean: 1.2–1.8 g) compared with the control (mean: 3.0 g). When fertilised with N, biochar application significantly increased ($P < 0.05$) the above-biomass yield from 3.3 g to up to 7.5 g. The addition of biochar, with and without the presence of N fertiliser, significantly increased ($P < 0.05$) plant's root length (0.68 – 1.28 cm cm⁻³) compared with the controls (0.32 – 0.50 cm cm⁻³). The results obtained indicated the two biochars produced in this study can (i) induce short-term N immobilisation, if this nutrient is deficient in the soil, and (ii) be a good source of P, K and Ca to plants. Adequate information should thus be provided to farmers to ensure the added value of biochar is maximised.

Chapter 3. The Application of Biochar Made from Biosolids to Increase The Fertility of A Sandy Soil: A Glasshouse Study

3.1. Introduction

Sandy soils are characterised by having more than 68% sand and less than 18% clay in the first 100 cm of the soil profile (WRB, 2006). The coarse texture of these soils accounts for their high water infiltration and low water storage capacity. A lack of reactive surfaces gives these soils a low cation exchange capacity (CEC) and poor structure, which partly explains their low capacity to protect organic matter (OM) from microbial decomposition (Baldock and Skjemstad, 2000).

Biochar has been suggested as a means to amend the physical properties of sandy soils – and particularly, to increase the soil water retention capacity because of its general high porosity (Novak *et al.*, 2009). In addition, the high porosity of biochar is expected to have a direct positive influence on the water retention capacity of soils (Laird *et al.*, 2010) although not all the water retained in the biochar pores may be available for plants (Verheijen *et al.*, 2009). In addition, biochar develops surface activity as it weathers, which enables it to retain cationic nutrients (Hina *et al.*, 2010; Uchimiya *et al.*, 2012) and interact with soil particles (Joseph *et al.*, 2010). Some biochars may be rich in nutrients (Yao *et al.*, 2010; Wang *et al.*, 2012ab, Albuquerque *et al.*, 2013). The initial nutrient content of biochar largely depends on the type of feedstock and pyrolysis conditions (Singh *et al.*, 2010; Calvelo Pereira *et al.*, 2011), whereas nutrient availability depends on the element considered (DeLuca *et al.*, 2009; Yao *et al.*, 2010), feedstock type and pyrolysis conditions (Wang *et al.*, 2012ab).

Biosolids, as a major part of human waste, are rich in plant micro- and macro-nutrients, as well as in organic C. If of good quality (e.g., low in heavy metals) and blended with cellulosic material to facilitate carbonisation (Hossain *et al.*, 2009), biosolids could be an ideal feedstock for biochar production (Strezov and Evans, 2009; Hossain *et al.*, 2010). With the exception of Nitrogen (N), most of the nutrients in biochars produced from biosolids are found in the ash fraction, either as soluble salts (e.g., sulphates), less soluble salts (e.g., phosphates) (Wang *et al.*, 2012a), or highly insoluble silicate minerals (e.g., feldspars, mica) (Yao *et al.*, 2010; Hossain *et al.*, 2011);

the latter are mostly derived from local soils and sediments entering wastewater systems and their interactions with water treatment chemicals such as aluminium. Recycling nutrients through the carbonisation of biosolids might thus be a suitable waste management option, by simultaneously eliminating pathogens, reducing waste volume and odour problems, and decreasing the availability of pollutants (Karayildirim *et al.*, 2006; Hossain *et al.*, 2011). The resulting biochar from biosolids also offers additional value as soil conditioner for acidic soils as it has been shown to have liming properties (Wang *et al.*, 2012a).

The application of biochar produced from biosolids to sandy soils could therefore have several benefits for the functions of these soils through: (i) an increase of the nutrient fertility, either directly by the input of nutrients, or indirectly, by increasing the CEC; (ii) minor additions of clay and silt fractions, (iii) promotion of soil aggregation; and (iv) increase soil water retention. Yet given the low caloric value of these wastes, mixing the biosolids with cellulosic material prior to pyrolysis is recommended in order to ensure exothermic reactions are produced (Hossain *et al.*, 2009).

The research objectives of this study are: (i) to report the characteristics of biochars from biosolids and a mixture of biosolids and green wastes (both produced at a highest heating temperature of 550 °C); and (ii) test the hypothesis that biochar from biosolids and mixtures of biosolids and greenwaste can be used as amendments so sandy soil to increase plant yield and root growth via improved plant nutrition.

3.2. Material and Methods

3.2.1. Production of biochars

The biosolids, provided by Palmerston North City Council (Manawatu, New Zealand), underwent a primary sedimentation and dewatering process at the wastewater treatment plant. The green waste was collected from the compost unit of Massey University at Palmerston North (New Zealand). This consisted of a mixture of leaves, hedge prunings, and chipped wood materials. The chemical characteristics of the two wastes are reported in Table 3.1. Both feedstocks were air-dried and stored until used. Prior to pyrolysis, the feedstock were oven-dried at 60 °C for overnight. The biochar produced from biosolids was referred to as BS biochar and the one from a

mixture of biosolids and greenwaste (50:50, wt:wt) as BG biochar. Two hundred grams of each feedstock were pyrolysed in a gas-fired rotating drum kiln made of stainless steel with inner volume of 5 l, as described by Calvelo Pereira *et al.* (2011). The feedstock was heated to 550 °C at an averaged heating rate of ~ 16 °C min⁻¹. The 550°C temperature was selected based on the study of Yao *et al.* (2010), where the nutrient availability of the same biosolids biochar (pyrolysed at the same temperature but in a different batch) was investigated through an enhanced biochar weathering using a Soxhlet reactor.

Table 3.1. Chemical characteristics of the materials used to produce the feedstocks.

	Feedstock	
	Biosolids	Green waste
Total C (g kg ⁻¹)	336.9	490.9
Total N (g kg ⁻¹)	36.2	5.2
C/N ratio	9.3	94.5
Total P (g kg ⁻¹)	153.3	7.3
Total Fe (g kg ⁻¹)	10.33	0.06
Total Al (g kg ⁻¹)	19.0	0.1
Total K (g kg ⁻¹)	1.9	3.0
Total Ca (g kg ⁻¹)	21.0	5.0
Total Na (g kg ⁻¹)	0.44	0.27
Total Mg (g kg ⁻¹)	2.73	0.73

3.2.2. Characterisation of biochars

The pH of the biochar was measured according to Ahmedna *et al.* (2000). Basically, a suspension of 1% (wt:wt) ground biochar in deionised water was heated until the temperature reached 90 °C, and stirred for 20 minutes to allow dissolution of the biochar component. After cooling at room temperature, the pH was measured. Sub-samples of both biochars were analysed for total C, H and N, using the LECO CHN-932 Microanalyser (Leco Corp., St Joseph, MI). The inorganic C-CO₃ content of the biochars (IC) was determined using a modification of the static chamber method (Bundy and Bremner, 1972; Tiessen *et al.*, 1983). Organic C was calculated as the difference of total C and IC.

Fourier-transformed infrared (FTIR) spectra of the carbonised materials were measured in triplicate with a Nicolet 5700 FTIR with an ATR attachment (Omni Sample

Nexus). Spectra were obtained using KBr as a beam splitter, with a resolution of 4 cm^{-1} and the spectral range was $4000\text{--}700\text{ cm}^{-1}$, with an aperture size of 34 cm. The reflectance was measured and analysed using omnic v7.1 with Happ-Genzel apodisation and Mertz phase correction. The obtained reflectance of each sample was then compared with the literature (Smith, 1999; Reig *et al.*, 2002; Smidt and Meissl, 2007) to correlate the relevant bands to possible functional groups.

Thermogravimetric (TG) analyses were carried out by using a SDTQ600 instrument (SDT Q600, TA Instrument, Melbourne, Australia). For this, the biochar samples were placed in an Al_2O_3 crucible and heated from room temperature to $900\text{ }^\circ\text{C}$ (at a rate of $5\text{ }^\circ\text{C min}^{-1}$) under a N_2 atmosphere; weight loss (in weight %) was recorded continuously; thereafter, an air current was provided and the ash was determined when there was no further weight change. The TG signals were exported to the TA universal analysis software for further analysis. The weight loss between 110 and $900\text{ }^\circ\text{C}$ in a N_2 atmosphere was considered as the volatile matter content of biochar, whereas the loss of weight at $900\text{ }^\circ\text{C}$ after the introduction of air current was considered as stable, thermo-resistant fraction or fixed C (Calvelo Pereira *et al.*, 2011, after Sevilla and Fuertes, 2010).

Total N and P in biochar were determined after Kjeldhal digestion (Blackmore *et al.*, 1987). Total K, Ca, Mg and Na in biochar were determined using an atomic absorption spectrophotometer (GBCAvanta; Braeside, Australia) after digestion with concentrated $\text{HNO}_3\text{-HClO}_4$. Acid hydrolysis of the two biochars was conducted using 6 M HCl according to the method of Pansu and Gautheyrou (2006) as modified by Wang *et al.* (2012b). Carbon and N concentrations from the non-hydrolysate residues were determined by CNS analyser (LECO fp-2000 CNS analyser; LECO corp., St Joseph, MI). Available P in biochar was estimated using the 2% formic acid extractable P (FA-P) following the methodology of Rajan *et al.* (1992) and AOAC (2005) as modified by Wang *et al.* (2012a). The standard vanado-molybdate colorimetric method (AOAC, 2005) was used to determine the P content in the biochar, by reading the absorbance at 420 nm on a spectrophotometer.

3.2.3. Soil collection

The soil used for this experiment was collected from the surroundings of Foxton beach, under a pine plantation, in the Horowhenua county, New Zealand ($40\pm 33^{\circ}\text{s}$, $175\pm 12^{\circ}\text{e}$); it is known as Waitarere soil (Molloy, 1993) and classified as Typic Udipsamment (Soil Survey Staff, 2006). The sample was taken from the Ap horizon (to a depth of ~ 15 cm) and air dried. The air-dried soil was passed through a 2-mm diameter sieve. The soil had an 88% sand and 5.7% clay, and a bulk density of 1.17 g cm^{-3} . The chemical composition of the soil is reported in Table 3.2.

Table 3.2. Chemical characteristic of the soil used in the experiment

Material	pH	CEC ^a	Total C (g kg^{-1})	Total N (g kg^{-1})	C/N ratio	Olsen P (mg L^{-1})	Exchangeable cations (cmol kg^{-1})			
							K	Ca	Na	Mg
Soil	5.8	2.04	5	0.5	9.4	7	0.13	1.1	0.15	0.66

^aCEC; cation exchange capacity.

3.2.4. Glasshouse experiment

The experiment was carried out in a glasshouse by using open PVC columns (15 cm diameter, 30 cm height) as pots. The following factorial combination of treatments was considered: (i) biochar type (BS and BG); (ii) N application (0 and 113 kg N ha^{-1} as urea; N0 and N1, respectively); and (iii) biochar dose (0, 1.5, 5.0, and 10.0 Mg ha^{-1} for the N0 treatment, and 0, 1.5, 5.0 and 20.0 Mg ha^{-1} for the N1 treatment). Overall, there were 16 treatment combinations. The experimental design was completely randomized with 3 replicates.

When packing the soil columns, the bottom 15 cm was filled with soil alone (equivalent to 4.1 kg soil) as the lower layer. Above this layer, a mixture of soil (equivalent to 2.1 kg soil) and biochar was added to all the pots, except for the controls, to fill the top 12 cm of the soil column. The pots were then watered with deionised water until they reached field capacity (14% w/w for BS and 18% w/w for BG treatment). Eighty seeds of Moata's tetraploid Italian ryegrass (*Lolium multiflorum* lam.) were germinated in each pot, and thinned to 60 plants after 2 weeks. The soil water content was returned to field capacity at twice weekly intervals throughout the experiment (90 days). Before

harvesting, all pots were leached with 1.2–1.4 pore volumes of deionised water. Leachates were collected and analysed for mineralised N. Plant harvest was carried out by cutting the above-ground biomass to 1 mm height. The grass was oven dried at 60 °C to constant weight (~ 2 days) and then weighed.

3.2.5. *Root length measurements*

At harvest, measurements of root length were carried out for the roots present on the top 12 cm depth following the method described by Tennant (1975). This consisted of wet-sieving $\frac{1}{8}$ of the 0–12 cm soil volume so the roots could be separated from the soil. The root length was estimated by counting the number of root intercepts in a 0.5 cm × 0.5 cm grid area. The root length was calculated by multiplying the number of intercepts × grid unit × conversion factor ($\frac{11}{14}$). The total root length in the top 12 cm of soil was then calculated in cm by multiplying the estimated root length by 8.

3.2.6. *Plant and leachate analysis*

Before plant analysis, ryegrass samples were ground. Total N and P in above-ground biomass were determined after Kjeldhal digestion (Blackmore *et al.*, 1987). Total K, Ca, Mg and Na in above-ground biomass were determined using an atomic absorption spectrophotometer (GBCAvanta; Braeside, Australia) after digestion with concentrated HNO₃-HClO₄, according to the US EPA 200.2 method (Martin *et al.*, 1994). Nutrient uptake (mg pot⁻¹) was calculated from the plant elemental analyses and the above-ground dry matter weights. Leachates collected from the trial were analysed for NO₃⁻ and NH₄⁺ concentrations using a technicon autoanalyser (Technicon, Dublin).

3.2.7. *Statistical analysis*

A General Linear Model (GLM) was conducted using a Minitab 17 software (MINITAB Inc. 2014). The GLM was used to see the effect of treatments on the differences variables measured. There were 2 factors used, i) biochar application rate and, ii) the addition of N fertilizer. A Least Significant Difference (LSD) posthoc tests were conducted to see the significant difference (at P < 0.05) between variables measured.

3.3. Results

3.3.1. Materials used for feedstocks and biochar characteristics

The biosolids were characterised by a lower C content than the green waste (Table 3.1) but contained much higher concentrations of N, P, K and Ca. These chemical attributes were carried forward to properties of the biochars. The yield (ratio of mass recovered after pyrolysis and initial mass of feedstock) of the BS biochar was considerably higher than that of the BG biochar (56 vs 41%) (Table 3.3), as expected, given the higher ash content of the former. Conversely, total C content was considerably smaller in the BS than in the BG biochar (26.1 and 45.2%, respectively).

Table 3.3. Characteristics of biochar made from biosolids (BS) and from a 1:1 (dry weight basis) mixture of biosolids and green waste (BG)

	BS	BG
Yield (%)	56	41
Total C (g kg ⁻¹)	261	452
Total N (g kg ⁻¹)	26.6	19.6
Total H (g kg ⁻¹)	16.2	11.3
Inorganic C (g kg ⁻¹)	0.63	0.39
Organic C (g kg ⁻¹)	260	452
C _{org} /N ratio	9.7	23.1
Atm H/C _{org} ratio	0.7	0.7
N Kjeldahl (g kg ⁻¹)	26	19
Hydrolysable N (g kg ⁻¹)	0.21	0.18
C _{labile} [*] /Hydrolysable N	395	755
Total P (g kg ⁻¹)	27.35	17.57
FA-Extractable [#] P/Total P (%)	34.54	31.42
Total K (g kg ⁻¹)	1.91	2.43
Total Ca (g kg ⁻¹)	13.48	14.71
Total Na (g kg ⁻¹)	0.97	0.99
Total Mg (g kg ⁻¹)	3.27	2.75
Fixed C (%)	17.7	31.6
Volatile content (%)	14.1	17.1
Ash content (%)	64.8	46.4

[#] extraction using 2% of Formic Acid

^{*} C_{labile} obtain by: Total C – fixed C (in g kg⁻¹)

The atomic H/C_{org} ratio of both biochars was 0.7. Total N of BG biochar was lower (1.9%) than that of BS biochar (2.6%), which was expected, given that the N content of

the biosolids became diluted after mixing with a relatively N-poor green waste. This resulted in a C:N ratio of the BS biochar almost 3 times smaller than that of the BG biochar (9.7 and 23.1, respectively) (Table 3.3). Nitrogen extractable by acid hydrolysis was a small fraction of total N in both biochars (0.78 % N for biochar BS and 0.80 % N for biochar BG), evidencing the low N availability in the charred material. Phosphorus extractable with formic acid accounted for approximately one third of the total P present, being slightly greater for BS biochar than BG biochar (34.5% and 31.4% respectively). The BS and BG biochars had pH values measured in water of 7.9 and 8.6, respectively.

The FT-IR spectra of the two biochars showed similar reflectance spectra, but with different intensity (especially in the region of 1600–1000 cm^{-1}) (Figure 3.1). The main bands in both spectra were at wave numbers ~ 1600 , 1420, 1020 and 750 cm^{-1} . The band at 1600 cm^{-1} was assigned to molecular vibration of ring stretching in C=C; 1420 cm^{-1} to C-H bending; 1020 cm^{-1} to Si-O bonds of silicates; and 750 cm^{-1} to N-H secondary amine groups, respectively (Smith, 1999; Smidt and Meissl, 2007). Mixing biosolids with green waste increased the intensity of the reflectance in the 1500–1600 cm^{-1} and 750–875 cm^{-1} regions (Figure 3.1), associated with C=C bonds moieties (Chen *et al.*, 1996; Smith, 1999) and the aromatic C-H out-of-plane bending at 875 cm^{-1} (Reig *et al.*, 2002).

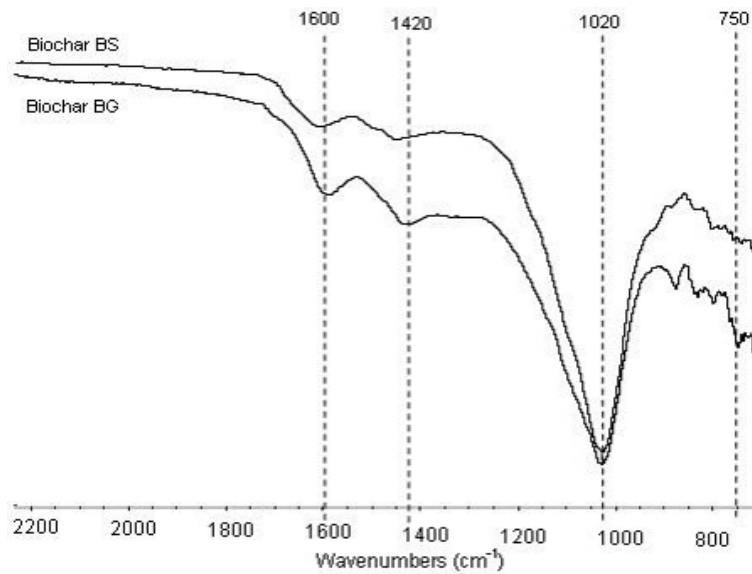


Figure 3.1 FTIR spectra of the biochar made from biosolids (BS), and the biochar made from a 1:1 (mass basis) mixture of biosolids and greenwaste.

3.3.2. Above-ground biomass.

The biochar application rate and both the addition and the absence of N fertiliser significantly ($P < 0.05$) influenced the above-ground biomass production (Figure 3.2). In the absence of N fertiliser, the application of biochar at all rates significantly ($P < 0.05$) decreased plant yield (3.0 g pot^{-1} in the control to $1.2\text{--}1.8 \text{ g pot}^{-1}$ in the biochar-treated pots) (Figure 3.2). The addition of N fertiliser to the soil without biochar did not significantly increase the above-ground biomass. However, the simultaneous addition of N fertiliser and biochar increased above-ground biomass ($112\text{--}229 \%$ compared with the N fertilised control), this effect being more pronounced at the highest biochar doses (Figure 3.2). The type of biochar used in this experiment had a significant effect ($P < 0.05$) on the plant yield in the absence of N fertilizer, with greater biomass recorded on the BG treatment.

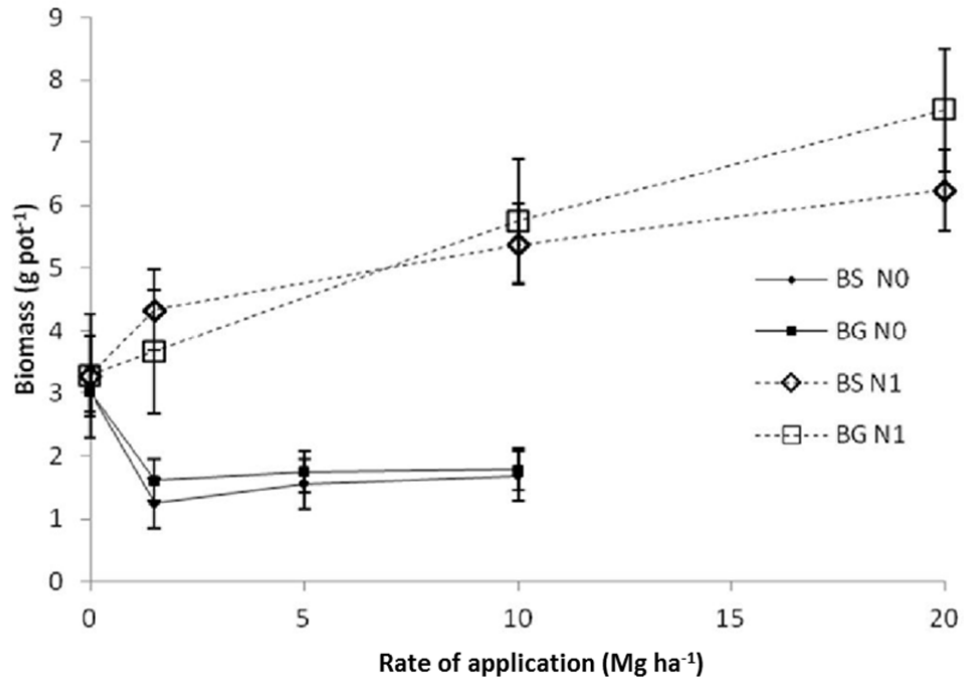


Figure 3.2. Above-ground biomass yield (g DM pot⁻¹) of ryegrass grown on pots of Waitarere soil fertilized with 0 and 113 kg N ha⁻¹ (N1 and N0, respectively) and different rates and types of biochar (biosolids biochar, BS; biosolids-green waste biochar, BG). Data represent the average and the standard error of the mean (SEM) (n = 3)

3.3.3. Root length measurement.

There was a significant ($P < 0.05$) effect of (i) the addition of N fertiliser, and (ii) the rates of biochar addition (either to fertilised or non-fertilised pots) on the root length of plants (Figure 3.3). The BG biochar treatment showed higher root length value than BS biochar in the absence of N fertiliser ($P < 0.05$), but the opposite result occurred when N fertiliser was added to the soil.

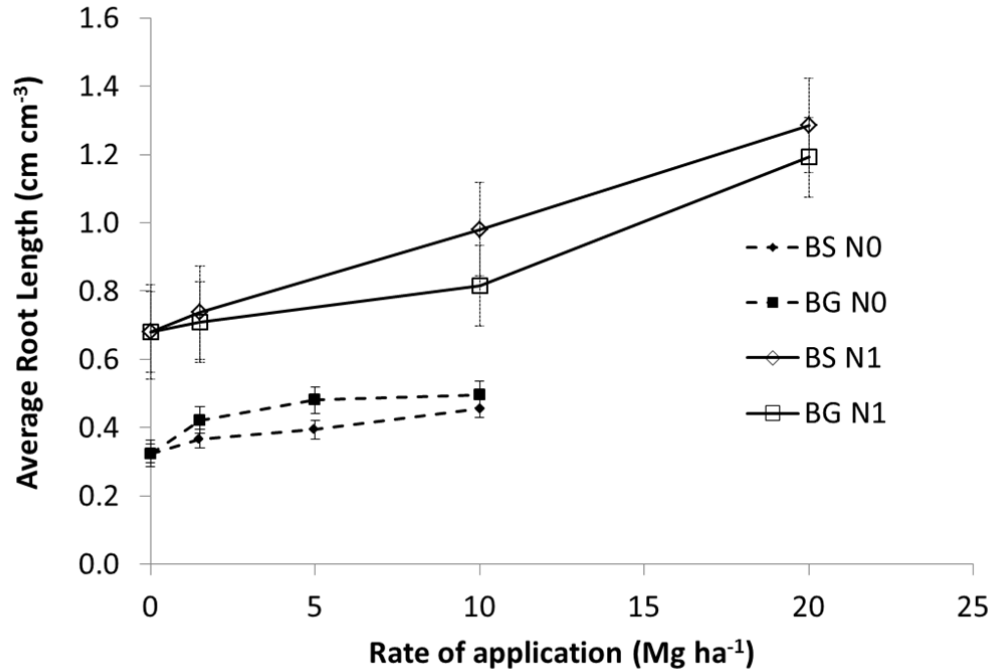


Figure 3.3. Root length (cm cm⁻³; in the top 12 cm) of ryegrass grown on pots of Waitarere soil fertilized with 0 and 113 kg N ha⁻¹ (N1 and N0, respectively) and different rates and types of biochar (biosolids biochar, BS; biosolids-greenwaste biochar, BG). Data represent the average and the standard error of the mean (SEM) (n = 3)

3.3.4. Nutrients in above-ground biomass

3.3.4.1. Nitrogen

Concentrations of N in the shoot biomass ranged from 12 to 39 mg g⁻¹ (Figure 3.4a), which indicates that N was a limiting nutrient for ryegrass growth (Cornforth, 1997). The addition of biochar caused a significant decline ($P < 0.05$) in the plant-N concentration of all treatments compared with the control. In the zero-N pots, plant-N concentration diminished from 20 to < 14 mg g⁻¹ and, in the N-treated pots, from 39 to < 19 mg g⁻¹ along with biochar application (Figure 3.4a). In contrast, the total N taken up by plant in N-treated soils significantly ($P < 0.05$) increased with increasing biochar doses (Figure 3.4b). This resulted in an enhanced recovery of applied N-urea in plant biomass from 40% in the fertilised control to >70% at the highest dose of BG biochar. In the zero-N pots, total N taken up by plants was always below that of the corresponding control (Figure 3.4b).

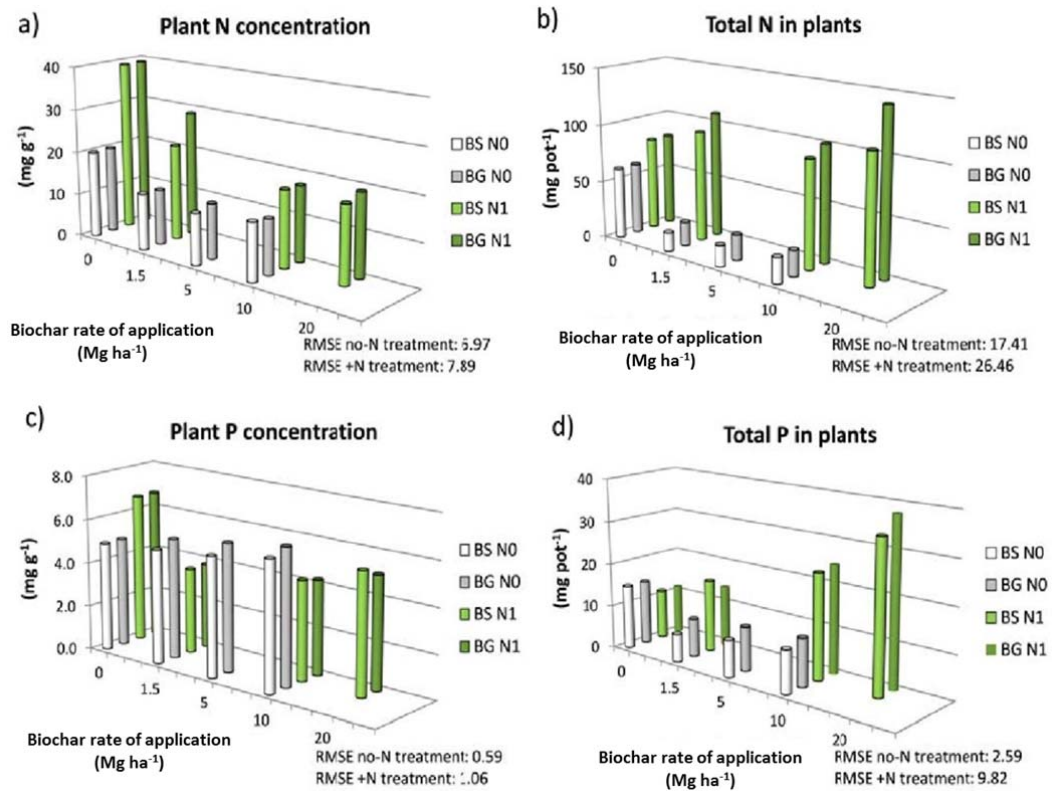


Figure 3.4. Nitrogen and P concentrations and total amounts in above-ground biomass of ryegrass grown on pots of Waitarere soil fertilised with 0 and 113 kg N ha⁻¹ (N1 and N0, respectively) and different rates and types of biochar (biosolids biochar, BS; biosolids-green waste biochar, BG). Data represent average and the residual mean square error (RMSE) (n = 3)

3.3.4.2. Phosphorus

Phosphorus concentration in the above-ground biomass ranged from 3.8 to 6.7 mg g⁻¹ and was not in the range limiting ryegrass growth (Cornforth, 1997). Plant-P concentration significantly increased ($P < 0.05$) with increasing biochar doses, although the N fertilised control showed the highest P concentrations of all treatments (Figure 3.4c). Biochar addition had a more evident effect on total P uptake than in plant P concentration, with a significant increase ($P < 0.05$) in the N-fertilised plants at increasing biochar doses (from 11.3 to 37.9 mg P pot⁻¹). The total P taken up by plants in the zero-N pots was always below that of the corresponding control (Figure 3.4d). The type of biochar only affected plant-P uptake significantly ($P < 0.05$) in the absence of N fertilizer, but differences were minor (Figure 3.4d).

3.3.4.3. Potassium

Potassium (K) concentration in the above-ground biomass ranged from 30 to 40 mg g⁻¹ (Figure 3.5a) and just fell in the range limiting ryegrass growth (Cornforth, 1997). There was no significant effect of (i) type of biochar, (ii) biochar dose, and (iii) presence/absence of N fertiliser on K concentrations in ryegrass (Figure 3.5a). Potassium concentration in the above-ground biomass remained relatively constant, ranging from 36 to 39 mg g⁻¹ in the zero-N treatments, and from 30 to 41 mg g⁻¹ in the N-fertilised treatments. Total K taken up by the N-fertilised plants increased significantly (P < 0.05) at increasing biochar doses (Figure 3.5b), from a value of 80 mg pot⁻¹ in the control to >200 mg pot⁻¹ at the highest dose. In the zero-N treatments, adding biochar to soils significantly (P < 0.05) decreased K uptake (Figure 3.5b).

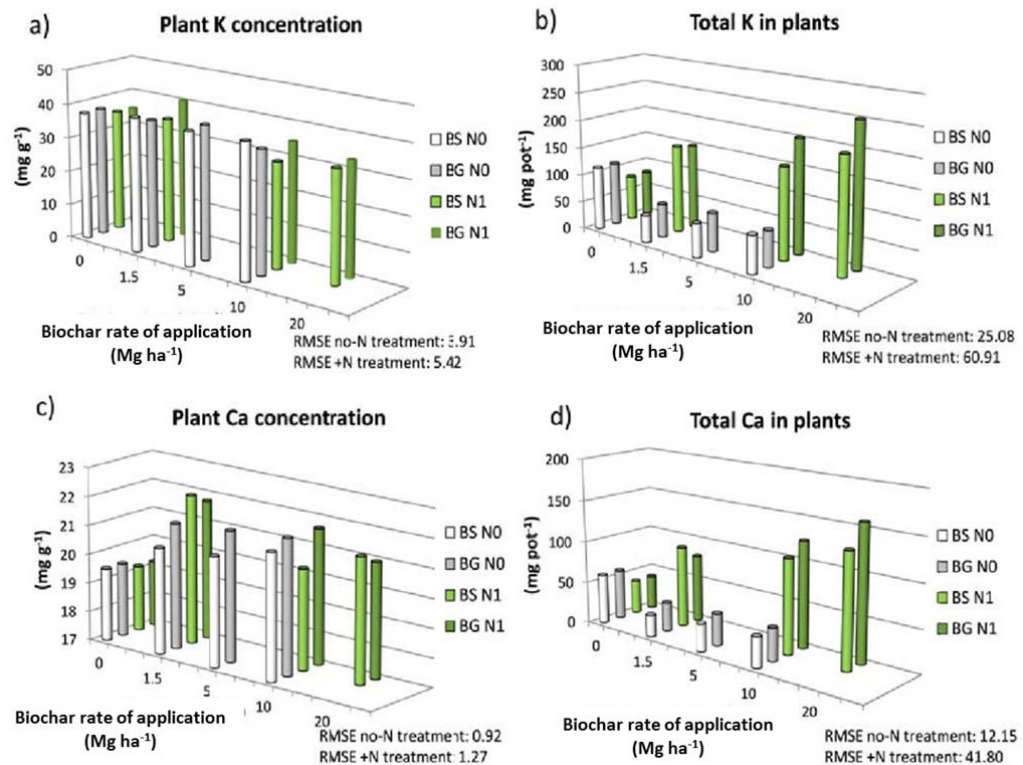


Figure 3.5. Potassium and Ca concentrations and total amounts in above-ground biomass of ryegrass grown on pots of Waitarere soil fertilised with 0 and 113 kg N ha⁻¹ (N1 and N0, respectively) and different rates and types of biochar (biosolids biochar, BS; biosolids-green waste biochar, BG) Data represent average and the residual mean square error (RMSE) (n = 3)

3.3.4.4. Calcium

The Calcium (Ca) concentration in the above-ground biomass was significantly increase ($P < 0.05$) with the addition of biochar compared with the control (Figure 3.5c). The plant Ca concentrations ($19 - 22 \text{ mg g}^{-1}$) of all treatments showed higher values than those reported to limit ryegrass growth (7.8 mg g^{-1}) (Cornforth, 1997). Total Ca taken up by the N-fertilised plants was increase significantly ($P < 0.05$) at increasing biochar doses, from 40 to $>130 \text{ mg pot}^{-1}$ (Figure 3.5d). There was no significant difference between biochar BS and BG on the Ca uptake by plants. Similar to plant-Ca concentration, the plant-Mg concentration ($2.7 - 4.2 \text{ mg g}^{-1}$) (data not shown) was above the limiting value of ryegrass growth (2.2 mg g^{-1}) (Cornforth, 1997).

3.3.5. Nitrogen in leachates

Concentration of inorganic N in leachates (either as NO_3^- or NH_4^+) at the end of the experiment was always below $0.2 \text{ } \mu\text{g ml}^{-1}$ (Table 3.4). Mean NO_3^- concentration in leachates collected from soils fertilised with urea was significantly higher ($P < 0.10$) than those without addition of urea. Mean NH_4^+ concentration of plants on fertilised pots was not significantly different to that of unfertilised treatments, although values tended to be lower without N fertiliser (Table 3.4). In the absence of N fertiliser, the addition of biochar to soil decreased the NO_3^- concentration in leachates compared to the control. In the N-treated soil, the increasing rates of biochar application had opposite effects on NO_3^- concentration in leachates, depending on the type of biochar used. When the BS biochar was applied to the N-treated soil, the concentration of NO_3^- significantly ($P < 0.05$) increased along with increasing rate of the biochar, whereas the inverse pattern was observed for the BG biochar (Table 3.4). In unfertilised soil, increasing biochar application rates significantly ($P < 0.05$) decreased the NH_4^+ concentration, but this was not observed in the N-treated soils.

Table 3.4. Nitrate-N and ammonium-N concentrations in leachates from plant studies

Fertiliser	Biochar Types	Rate of application Mg ha ⁻¹	Leached NO ₃ ⁻ (µg N mL ⁻¹)	Leached NH ₄ ⁺ (µg N mL ⁻¹)	
0 mg N	-	0	0.131 c*	0.171c	
	BS	1.5	0.069 a	0.043 b	
		5	0.064 a	<d.l. ** a	
		10	0.076 a	<d.l. a	
		Average	0.089	0.032	
	BG	1.5	0.105 b	0.014 a	
		5	0.090 b	<d.l. a	
		10	0.087 b	<d.l. a	
		Average	0.111	0.033	
		200 mg N	-	0	0.164 c
BS			1.5	0.046 a	0.006 a
	10		0.084 b	0.045 b	
	20		0.136 c	0.035 a	
	Average		0.111	0.033	
BG	1.5		0.199 c	0.021 a	
	10		0.082 b	0.036 a	
	20		0.066 a	0.030 a	
	Average		0.111	0.033	

* Rows followed by different letters indicate significant differences ($P < 0.05$) between control BS and BG treatments with the same N application.

** <d.l.: below detection limit.

3.4. Discussion

3.4.1. Biochar properties

Both biochars had a high mass recovery (> 40%) compared to biochars produced from woody feedstocks at the same final temperature of 550 °C of pyrolysis and using the same kiln (< 30%; Calvelo-Pereira *et al.*, 2011). This is due to the high fraction of mineral matter in the biosolids, which becomes concentrated as the organic material thermally degrades during the pyrolysis. The BS biochar had a smaller organic C content (260 g C kg⁻¹) than the BG biochar (452 g C kg⁻¹), as expected. Reported organic C contents of biochars from biosolids range widely from 20% (Hossain *et al.*, 2011) to 47% (Bridle and Pitchard, 2004). Variability in these values not only depends on process conditions but, to a large extent, on the characteristics of the biosolids. The ratio of $\frac{\text{volatiles matter}}{(\text{volatiles matter} + \text{fixed C})}$ in biochar decreased when biosolids were mixed with green waste (44% vs 34%), suggesting the presence of a greater stable C fraction in the carbonised mixture of BG biochar. The atomic H/C_{org} ratio of both biochars was 0.7 (Table 3.3). Both biochars are thus within the limit of ≤ 0.7 established by the IBI Guidelines for Specifications of Biochars (IBI, 2012) based on the correlation of this index with the degree of thermal degradation and the presence of

condensed aromatic structures. The nature of C=C and C-H bonds contributing to the FT-IR spectra corroborates the high degree of aromaticity of these biochars. Yao *et al.* (2010), using the same BS biochar as in the present study, but from a different batch, reported a CP/MAS¹³CNMR spectrum dominated by aryl-C, with substantially lower intensity of alkyl C.

The addition of an organic waste with a low N content (5 g kg⁻¹) to the biosolids before pyrolysis had a dilution effect on the final N content of the biochar (26 g kg⁻¹ for the BS biochar and 16 g kg⁻¹ for the BG biochar), as expected. Nitrogen in the biochars was poorly available, as reflected by the low hydrolysable-N values, which were less than 1% of total N in biochar. This was in agreement with the results of Yao *et al.* (2010), where N released during an accelerated geochemical weathering using the same biochar was also < 1 %. Nitrogen in charred material has been reported to be mostly found locked as heterocyclic form (Knicker, 2007) although some recent publications suggest it may be available to some extent (De la Rosa and Knicker, 2011; Hilscher and Knicker, 2011); this fraction increases as the temperature of pyrolysis increases (Chan and Xu, 2009; Wang *et al.*, 2012b), with a dominance of pyridine-like compounds at temperatures >600 °C (Bagreev *et al.*, 2001), thus explaining the low N availability of both biochar.

3.4.2. Above-ground biomass production

The application of biochar had a significant negative effect ($P < 0.05$) on ryegrass yield when no N fertiliser was added to the pots, and this was observed at all biochar doses (Figure 3.2). The results are consistent with the low plant availability of N in biochar, as estimated with acid hydrolysis, where both biochars only added 0.18 to 0.21 mg of hydrolysable N per g of added biochar. The results from the present pot trial also supports the view that soil N becomes less available to plants when a biochar produced at 550°C is added to a soil poor in N (Figure 3.4b). The N concentration of above-plant biomass in the non-fertilised soils amended with biochar was less than the N concentration in the corresponding control (Figure 3.4a), whereas the opposite trend was observed for the rest of nutrients analysed (Figure 3.4a and 3.5a), thus indicating that N was the growth limiting nutrient and this was exacerbated by the addition of biochar (Figure 3.4b). This could be attributed to either (i) microbial N

immobilisation – as reported by Steiner *et al.* (2008) –, and/or (ii) the adsorption of N forms (e.g., NH_4^+) on the surface of the biochar (Steiner *et al.*, 2008; Hina *et al.*, 2010).

Wang *et al.* (2012b) suggested the use of $C_{\text{labile}}/N_{\text{labile}}$ ratio of biochar to assess the N mineralisation or immobilisation that may occur in soil; C_{labile} being estimated as total C_{org} – fixed C, and the N_{labile} being considered the hydrolysable N from biochar acid extraction. The $C_{\text{labile}}/N_{\text{labile}}$ ratio was 395 for BS biochar and 755 for BG biochar (Table 3.3) and therefore well above the value of 25, which is considered to indicate the probability of net N immobilisation. The addition of biochar to this soil thus triggered microbial N immobilisation and reduced N availability. When N fertiliser was applied to the soil, the N concentration in above-ground biomass of the control + urea almost doubled that of the control treatment without urea (Figure 3.4a). Nonetheless, no increase in the growth of above-ground biomass was observed in the control + urea compared with the control (Figure 3.2), revealing that other nutrients were also deficient in this soil, in addition to N. Based on the plant nutrient concentrations in the N-fertilised control, it is apparent that P was not the main nutrient limiting plant growth (Cornforth, 1997) in the N-fertilised control despite the low Olsen P value of the soil (Table 3.2).

The application of biochar to the Waitarere soil along with N fertiliser greatly increased the above-ground biomass (Figure 3.2) and the uptake of major nutrients by plants (Figures 3.4 and 3.5). This increase was more evident at high doses of biochar, suggesting that biochar was providing nutrients to plants other than N, mainly P, K, Ca and Mg. The absence of biochars showed a significant decrease ($P < 0.05$) in P, K, Ca and Mg concentrations in plant biomass with increased yield (Figures 3.4 and 3.5) indicated that the supply of these nutrients from biochars met plant demand. This is in agreement with the work of Yao *et al.* (2010), where noticeable amounts of K, Ca and P were released through an accelerated weathering of the BS biochar under high leaching conditions (specifically 10, 21 and 15 %, respectively).

The results of the present pot trial indicated that recovery of P added with the biochar in above-ground biomass decreased at increasing biochar doses (from ~ 12% to < 4%). Based on the results of Yao *et al.* (2010) – where available P in BS biochar was

estimated to be ~ 15 % – it is apparent that not all available P in biochar was used by the growing plants, especially at high biochar doses, suggesting that other nutrients – probably N – were limiting growth. Wang *et al.* (2012a) also calculated the percentage of P recovery from biosolid-biochar application under no nutrient-limiting conditions, and they reported that 16-35% of P was recovered in the biomass. These authors proposed the use of 2% formic acid extraction to predict the availability of P in ash-rich biochars, such as biochars produced from biosolids. Based on the P-formic acid extraction results (Table 3.4), the BS and BG biochars were able to supply 9.7 and 5.9 g of available P per kg of biochar, respectively. The amount of P in above-ground biomass represented 7–21% of formic acid-P added to soil with biochar as amendment.

The response of plant yield to the two different types of biochar produced in this study was only significantly different ($P < 0.05$) at the highest biochar dose (20 Mg ha^{-1}), where the BS biochar yielded a lower biomass than the BG biochar. This was accompanied by a lower N, P, K, Ca and Mg uptake by the former. Based on the elemental composition of the feedstocks, the BS biochar had a higher Ca and Mg content than that of the BG biochar, whereas the inverse was expected for K. The lower K content of the BS biochar compared to the BG biochar could, at least partially, explain the differences in above-ground biomass at high biochar doses. Nonetheless, the results obtained denote an overall similar response of ryegrass yield to both biochars. Based on these findings, it would seem convenient to dilute the biosolids with greenwaste to ensure exothermic reactions are reached during pyrolysis, as this would make the whole process more energetically favourable (Hossain *et al.*, 2009) and would not negatively affect ryegrass growth.

An increase in crop yield due to biochar application along with N fertiliser has also been reported by Chan *et al.* (2007), working with radish grown on an Alfisol amended with biochar from greenwaste (produced at $450 \text{ }^{\circ}\text{C}$), and by Chan *et al.* (2008), working the same crop and soil, but amended with biochar produced from poultry litter (produced at $450\text{--}550 \text{ }^{\circ}\text{C}$). In the former work (Chan *et al.*, 2007), no beneficial effect of biochar was observed on plant yield in the absence of N fertiliser. However, in the latter study, a positive effect of biochar on plant yield was observed in the absence of

N fertiliser. An increase in tomato production was also reported by Hossain *et al.* (2011) working with biochar from wastewater sludge (produced at 550 °C) added at a dose of 10 Mg ha⁻¹ along with N fertilizer. Ozuma *et al.* (2011) added cow manure biochar (produced at 500°C) at 15 Mg ha⁻¹ in NPK-fertilized sandy soils and observed an increase in maize growth by 64.6% compared with the control. These results emphasize the need to know the amount of available nutrients in biochar before adding it to soil. Moreover, it is evident that the characteristics of soil together with crop needs must be known prior to the addition of this amendment.

3.4.3. Biochar and C sinks

The results from measuring root length in the top 12 cm of soils (depth to which the biochar was applied) indicate that all biochar treatments promoted root growth compared to the control, even in the absence of N fertiliser. The increased root length under low nutrient availability is attributed to the adaptation response of roots under nutrient deficiency to increase the root spatial development (Neumann and Romheld, 2002); this could be promoted by BS and BG biochars, although the reason behind this pattern is unknown and more work is needed to understand this. Conversely, the increase in root length under the N fertiliser treatment, at increasing biochar doses, was mainly caused by the enhanced plant growth induced by the increasing presence of nutrients. The results obtained thus indicate that biochar not only increases the organic C pool of this soil through the addition of a highly recalcitrant C source, but through promoting plant growth and thus photosynthesis, this amendment also increases the C pool in above- and below-ground biomass.

Within grassland systems, the pattern of soil organic C sequestration in soils has been reported to correlate well with plant root density and turnover times (Rees *et al.*, 2005). The long-term stabilisation of the enhanced root biomass will largely depend on the C saturation level of this soil under this new scenario (e.g., biochar-amended soil) (Stewart *et al.*, 2007). The maximum C sequestration capacity of sandy soils is generally very limited, given (i) the low presence of the reactive surfaces of these soils able either directly interact with organic matter (e.g., silt + clay protected) or physically protect organic matter through aggregation (e.g., micro-aggregate protected) (Six *et al.*, 2002), and (ii) the poor plant growth in soils with low water and nutrient storage

capacity. Biochar produced from biosolids could increase the reactive surface of these soils through (i) the addition of a considerable amount of silt and clay mineral particles, and (ii) the presence of acidic functional groups at the charcoal surface, which would further increase as charcoal weathers (Cheng *et al.*, 2006). Moreover, biochar surface can also interact with soil organic matter through hydrophobic interactions following the conceptual model of Kleber *et al.* (2007). This, together with the added nutrient fertility value of this high-ash biochar, may increase the C sequestration potential of these soils. Nonetheless, long-term studies are needed to determine the persistence of this additional C input in this specific soil, as well as the potential role of biochar as reactive surface to stabilise soil C.

3.5. Conclusions

Biochars, produced from biosolids and municipal green waste to reduce GHG emissions, also captured plant-available P, K, Ca and Mg. Nitrogen was also captured in the biochar but was essentially unavailable to plants in this short-term study. When added to nutrient-poor sandy soil fertilized with urea biochar stimulated ryegrass shoots and root growth, thereby increasing the potential to sequester more atmospheric CO₂. Biochar application caused immobilization of existing soil and fertilizer N and reduced shoot growth in the absence of urea. Biochar addition stimulated root growth whether urea was applied or not, suggesting that an important agronomic property of biochar may be root growth stimulation. Root growth stimulation from biochar addition has considerable potential to increase further soil carbon stocks and requires study in a range of soil-plant-climate conditions.

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Chapter 4. Biochar Lysimeter Trial: 1. Description of The Setting of Lysimeter Trial and Above- and Below- Ground Plant Biomass Production Over The 2-Years Experiment

The selection of deep-rooting pasture species has been proposed as one of the options to increase the soil carbon (C) storage in the deeper soil depth. A lysimeter trial was conducted to study the changes in plant C allocation to soil under different pasture species on two contrasting coarse and loamy soil and investigate the effect of biochar application to the subsoil (10 cm below topsoil) to pasture root proliferation at depth. The lysimeter trial was arranged by i) simulating soil ploughing at cultivation for pasture establishment by inverting the topsoil (0–10 cm) and subsoil (10–20 cm) from the field; ii) the application of biochar at 10 Mg ha⁻¹ in the buried soil layer; and iii) the selection of pasture species with different rooting systems. The biochars used in this trial was specifically produced to overcome the soil-specific plant growth limitations. A high-ash biochar produced from mixture of biosolid and greenwaste (BG-550) was used in the low fertility coarse soil, while the low ash pine biochar (PI-350) was selected to ameliorate the physical soil constrain in the loamy soil. The results from 2 years of this trial suggested that biochars did not significantly improve the pasture dry matter production and below-ground biomass on both coarse and loamy soil. Pasture species was significantly influenced ($P < 0.05$) by the production of above- and below-ground biomass, with the highest biomass recorded on the mixture of red-clover and cocksfoot.

A paper from this study has been submitted for publication:

Calvelo Pereira R, Hedley M, Camps Arbestain M, Wisnubroto E, Green S, Saggari S, Kusumo BH, Mahmud AF (2015) Net changes of soil C stocks in two grassland soils 26 months after simulated pasture renovation including biochar addition. GCB Bioenergy.

Chapter 4. Biochar Lysimeter Trial: 1. Description of The Setting of Lysimeter Trial and Above- and Below Ground Biomass Production over The 2-Years Experiment

4.1. Introduction

Agricultural soils can act either as a source or sink of C (Lal, 2004; Smith, 2008). In New Zealand, pastoral farming is the main agricultural land use, with almost 54.6% of New Zealand land use being dairy farming and grazed pasture (MfE, 2014). The soil C under grassland is considered to be a considerable soil C sink compared with annual or conventional crop farming (Jones *et al.*, 2006; Soussana *et al.*, 2004), with the relative translocation of plant C into soil in pasture being 1.5 to 2 times higher than the cereal crops (Kuzyakov and Domanski, 2000). In a survey of 29 farms in Alabama, Georgia, South Carolina, and Virginia, Franzleubbers *et al.* (2012) have shown that SOC stocks under pasture down to 20 cm depth were significantly greater than under conventional tillage cropping. In France, Soussana *et al.* (2004) estimated the average soil C stocks (0–30 cm) under permanent grassland to be around 70 Mg C ha⁻¹, which is 25 Mg C ha⁻¹ higher than cropland system.

Although the SOC stocks of grassland soils are greater than those of other agricultural systems, GHGs emissions from these systems are one of the major contributions to the total agricultural GHGs profile in New Zealand (MfE, 2014), mostly associated with emissions of N₂O and CH₄ from urine patches and N fertilisation, and ruminant fermentation, respectively. Pastoral farming thus faces a big challenge to reduce and mitigate its GHGs emissions. There are various management practices to directly reduce these N₂O and CH₄ emissions, including grazing management (Lal, 2004), the reduction in stocking rate (Clark, 2009), and the application of nitrifier inhibitors (Di *et al.*, 2007). Alternatively, the indirect mitigation of GHGs emissions from grasslands could be achieved by increasing the soil C stocks, also known as soil C sequestration (Parsons *et al.*, 2009).

Improving the efficiency of pastoral production may help increase the soil C stock under pastoral farming (Beukes *et al.*, 2010). With a proper pasture management, biomass production (above ground and below ground) could be increased and the plant litter and animal excreta returned to soil, hence contributing to an increase in

the soil C input (Beukes *et al.*, 2010; Conant *et al.*, 2001; Schnabel *et al.*, 2001). Carter and Gregorich (2010) found an increase of 44% in the total subsoil (40–60 cm) C following the conversion from barley to tall fescue perennial grasslands. These findings highlight the importance of deeper allocation of C in the subsoil horizon of grasslands. The presence of soil C at depth is closely related to the root architecture of plants (Conant and Paustian, 2002; Rasse *et al.*, 2005). Furthermore, it has been suggested that decomposing root biomass is more likely to be stabilised within soil aggregates in the subsoil, resulting in an increased C residence time (Denef and Six, 2006). Based on the above considerations, it is clear that the selection of plants with deeper root systems in grasslands is crucial for building up a higher C stock in the subsoil horizon.

Biochar is a carbonaceous material produced from incomplete combustion (in the absence or low amounts of oxygen) of various organic feedstocks and intended to be used as soil amendment to improve soil functions and sequester C (Joseph *et al.*, 2010; Lehmann and Joseph, 2009). Biochar has been proposed as one of the techniques to improve soil properties under grasslands (Ohsowski *et al.*, 2012). The application of biochar as soil amendment has been shown to improve in soil functions, such as soil hydraulic conductivity (Herath *et al.*, 2013), soil pH (Novak *et al.*, 2009; van Zwieten *et al.*, 2010a), soil cation exchange capacity, and nutrient retention (Laird *et al.*, 2010; Lehmann *et al.*, 2003; Liang *et al.*, 2006). Biochar is also considered to have additional nutrient value when it is applied to poor fertility soil (Hossain *et al.*, 2010; Wang *et al.*, 2012). While biochar is considerably low in Nitrogen (Yao *et al.*, 2010), high-ash biochars typically contain other nutrients (such as Phosphorus and Potassium) that were reported to be available for plant uptake (Wang *et al.*, 2012; Yao *et al.*, 2010).

Although some studies have shown that the application of biochar has no effect on crop yield (Deenik *et al.*, 2010; Gaskin *et al.*, 2010; Slavich *et al.*, 2013), others, especially those studying its application to poor quality soils, have reported a significant increase in crop yield (Lehmann *et al.*, 2003; Major *et al.*, 2010; Rondon *et al.*, 2007; van Zwieten *et al.*, 2010a). The difference in plant response to biochar application is due to differences in properties among biochars (mainly dependent on production process and feedstocks type) and their ability to match the soil constraints that limit plants growth (van Zwieten *et al.*, 2010b). Therefore, it is important to understand

clearly the properties of biochar that will be used as soil amendment if soil constraints are to be matched.

Plant responses to biochar application are not limited to above-ground biomass growth, but also to below-ground biomass/root. In recent years, awareness of root responses to soil-biochar application has increased (Brunn *et al.*, 2014; Noguera *et al.*, 2010; Prendergast-Miller *et al.*, 2014; Ventura *et al.*, 2014). However, little is known about the pasture root response to biochar application. The aim of this study was to investigate the effect of biochar application in two contrasting soils on the production of above-ground and root biomass of various pasture types (with different rooting patterns), with particular attention paid to the effect on soil C stocks. For this a lysimeter trial was established. This chapter reports on the set up of lysimeter experiments and the results of a 2-year lysimeter trial on plant above- and below-ground biomass production. The results of soil C and N will be discussed in chapter 5.

4.2. Materials and Methods

4.2.1. Feedstock and Biochar Production

Two types of feedstock were used to produce two different biochars: (1) pine sawdust, and (2) a mixture of biosolids and green waste. Pine sawdust was acquired from a local saw mill in Palmerston North, New Zealand. The biosolids were collected from the Palmerston North City Council waste-water treatment, where there is no contribution from industrial wastes. The biosolids were collected after primary sedimentation and dewatering processes. The green waste consisted of a mixture of leaves, hedge-prunings, trees branches, and chipped wood materials that were collected from the compost unit of Massey University, Palmerston North, New Zealand. Both biosolids and green waste were oven-dried at 70°C for overnight before biochar production (pyrolysis).

Biosolids and green waste were used to produce a high-ash biochar with a final temperature of 550°C. This high ash biochar (BG-550) is expected to add the nutrients and clay mineral particles present in the ash fraction, thus increasing soil nutrient fertility and contributing to the promotion of soil aggregation. A mixture of biosolids

and green waste (at 1:1 ratio and 200 g of feedstock per batches) was pyrolysed using a gas-fired rotating drum-kiln with an inner volume of 5 L, as described by Calvelo Pereira *et al.* (2011). The heating rate was set to $16^{\circ}\text{C min}^{-1}$, and after reaching a final temperature of 550°C , the heating source was turned off and the biochar was left to cool to room temperature.

The pine sawdust was pyrolysed to produce a low-ash biochar intended to be used to ameliorate the soil physical constrain in the Pallic soils and promote root growth at depth. The production of biochar from pine sawdust (PI-350) was carried out in a gas-fired, rotating drum kiln with an inner volume of 25 L using a heating rate of approximately $16^{\circ}\text{C min}^{-1}$ to reach a final temperature of 350°C .

4.2.2. Biochar Characterisation

The pH of the biochar was measured according to Ahmedna *et al.* (2000). Basically, a suspension of 1% (wt:wt) ground biochar in deionised water was heated until the temperature reached 90°C , and stirred for 20 minutes to allow dissolution of the biochar component. The pH of the suspension was then determined using a combined pH electrode (PHM83, Radiometer, Copenhagen, Denmark).

The degree of alkalinity of the biochar was determined by measuring the lime equivalence. Briefly, the lime equivalence was determined by using 1 g of ground biochar dissolved in 25 mL of 1 M HCl; the solution was stirred frequently for 1 h and then subsequently neutralized to pH 7 by using standardized NaOH 1 M. The pH change was monitored continuously by using an automated titrator (pH/EP/IP Titration Workstation TIM865, Radiometer, Copenhagen). Samples of 1 g of pure CaCO_3 were used as control.

Thermogravimetry analysis were carried out using a SDTQ600 instrument (SDT Q600, TA Instrument, Melbourne, Australia) to analyse the ash content, volatile matter content (dry basis) and the stable thermo-resistant fraction or fixed C (dry basis) according to the methods describe by Calvelo Pereira *et al.* (2011). Total C, H, and N contents were determined using a TruSpec CHNS analyzer (LECO Corp. St. Joseph, MI).

The oxygen content was estimated as follows: $O = 100 - (C + H + N + \text{ash})$ (all in % of weight).

The Cation Exchange Capacity or CEC of biochars used in this experiment was analysed using a modified version of the methodology developed by Matsue and Wada (1985) and Blakemore *et al.* (1987), as described by Calvelo Pereira *et al.* (2015a). Briefly, the CEC of biochars (0.2 g of a fine ground biochars samples) were measured by replacing the exchangeable cations with Sr^{2+} using 50 mL of 0.01 M SrCl_2 . A semi-micro leaching system employing a peristaltic pump with a gentle suction rate (1 ml min^{-1}) was used to promote exchange. Commercial acidified sand was used as a packing element in leaching tubes (ratio biochar:sand 0.1:2 g/g) to avoid formation of channels and air-locks in the column during leaching. A macerated filter paper plug was placed at the bottom of the column to avoid the loss of material from leaching the tubes. The equilibrium pH was measured in the SrCl_2 equilibrium solution. The excess counter-ion solution was eliminated by a highly diluted solution of SrCl_2 ($\sim 0.0001 \text{ M}$), avoiding dispersion or excessive hydrolysis. The retained Sr was finally replaced with 50 mL of 0.5 M HCl, determining the Sr by atomic absorption spectrophotometry (GBCAvanta; Braeside, Australia); the amount of Sr displaced by the acid solution was termed CEC.

The estimation of C-aromaticity (fa) of biochars used in this experiment was predicted using the modified densimetric approach to estimate the fa of biochar by Wang *et al.* (2013), based on Mazumdar (1999). Based on the equation by Mazumdar (1998), the carbon aromaticity can be predicted using equation:

$$fa = (1 - \frac{H'}{C'}) + \alpha (\frac{Mc}{d} - 5.34) \quad (1)$$

where H'/C' is the atomic ratio of H and C, α is a modification coefficient {Wang *et al.*, (2013) proposed value of $\alpha = 0.110$ for biochars sample}, and 5.34 is the average molar volume of graphite-C atom. The value of Mc/d is defined as the average molar volume of C-atom, and can be estimated (Mazumdar, 1999) by using the equation:

$$\frac{Mc}{d} = 5.34 + 9.15(\frac{H'}{C'}) - 2.9(\frac{H'}{C'})^2 \quad (2)$$

The equations above were originally used to determine the degree of carbon aromaticity of polyaromatic hydrocarbons (PAH), which only consisted of C and H. Biochars, in other hand, does not contain only C and H, but also O, N, S, and other elements. To correct the H'/C' value of biochars to be used in those equations, Wang *et al.* (2013) calculated the H'/C' value as bellow:

$$H'/C' = \left(\frac{H\%}{1} + 2\theta * \frac{O\%}{16} \right) / \left(\frac{C\%}{12} + \frac{N\%}{14} \right) \quad (3)$$

with θ being the value of the molar ratio of C=O bond to CO bonds. Wang *et al.* (2013) found that the θ value can be constant (0.290) in biochars with atomic ratio H/C < 0.7. The calculation of *fa* was based on the dry-ash-free basis. This was done to minimise the overestimation of C-aromaticity of the corresponding biochar measured (Wang *et al.*, 2013).

4.2.3. The Establishment of Lysimeter Trial with biochar application

Two experiments were conducted using lysimeter studies and run for 2 years. The first experiment was done to study the effect of low-ash biochar (PI-350) for improvement of soil physical constraints in the Tokomaru (TK) soil and the enhancement of plant root growth. The second experiment was done to study the effect of high-ash nutrient-rich biochar (BG-550) addition to the low fertility Motuiti brown sandy (MS) soil. A control treatment (nil biochar addition) was included to evaluate the effect of biochar application to the variables measured (plant biomass production, below-gournd biomass, soil C and N concentration)

The lysimeter experiments were conducted in the experimental field of Plant and Food Research, Palmerston North, New Zealand. The setting of the lysimeter experiment was carried out in December 2010/January 2011. The lysimeter experiment set up used PVC columns with 40 cm depth and 20 cm diameter attached to a 1.3 m PVC drainage collection columns or Flux meters, as described by Deurer *et al.* (2008) (Fig. 4.1). The soils were collected for this trial in October 2010. The two soils selected were a Tokomaru silt loam soil (USDA: Typic Fragiudalf; coded TK), and a Motuiti brown sand soil (USDA: Typic Udipsamment, coded MS).

After the sampling process, the soil samples were reconstructed within the lysimeter, by inverting the original surface soil to become the subsoil in the lysimeter. The soil properties for the silt loam Tokomaru soil and sandy Motuiti soil after the reconstructed process are described in Table 4.1. As expected, the subsoil (10–20 cm) of both soils was higher in C content (35.6 and 27.6 g C kg⁻¹ for experiments 1 and 2, respectively) compared with the topsoil layer (1.17 and 1.54 g C kg⁻¹). When the two soils used in this trial were compared, the silt loam TK soil had higher total C compared with the loess sandy MS soil (1.8, 1.3, and 3.8 times higher total C in the 0–10, 10–20, and 10–30 cm respectively). The C/N ratio of the TK soil is relatively low compared with the sandy soil, given the higher total N content of TK soil than the MS soil.

Table 4.1. Soil Chemical Properties used in This Experiment

Property	Soil Depth, cm ^a	Experiment 1, silt loam soil			Experiment 2, sandy soil		
		0–10	10–20	20–30	0–10	10–20	20–30
Bulk density	Mg m ⁻³	1.17	0.92	1.38	1.54	1.3	1.8
C	g kg ⁻¹	20	35.6	12.5	11.3	27.6	3.3
N	g kg ⁻¹	2.3	3.6	1.3	1.1	2.4	0.4
C/N	-	9.4	9.8	9.4	10.3	11.7	8.9
Olsen P	mg kg ⁻¹	20.8	78.1	6.2	13.7	16.6	7.6
SO ₄	mg kg ⁻¹	12.5	18.3	9	5	12	5
K	cmol kg ⁻¹	0.79	1.55	0.45	0.18	0.35	0.17
Ca	cmol kg ⁻¹	5.1	6.9	5.5	2.4	4.4	1.2
Mg	cmol kg ⁻¹	0.84	1.46	0.84	0.44	1.03	0.19
Na	cmol kg ⁻¹	0.09	0.14	0.2	0.04	0.12	0.03
CEC	cmol kg ⁻¹	17	19	15	6	10	3

^a reconstructed soil in the lysimeter at the beginning at the experiment (time zero)

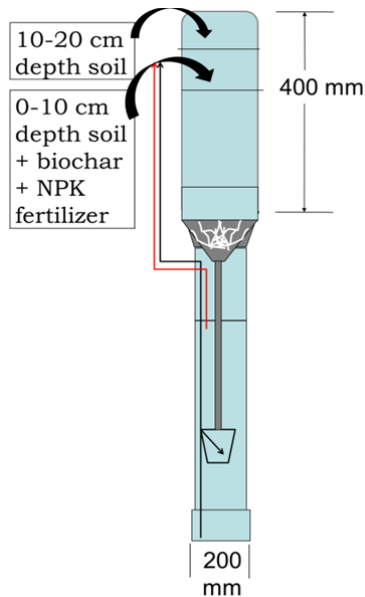


Figure 4.1. Drainage collection column used in the experiment

In the TK soil (experiment 1) the intact soil cores were taken from the field site at 20–40 cm depth (Picture 4.1). The topsoil samples (0–10 cm and 10–20 cm) were taken separately. The PI-350 biochar was applied to the top soil layer of half of the soil cores at an application rate of 10 Mg ha^{-1} . A basic fertilizer (NPK; urea, 80 kg ha^{-1} ; 100 kg K ha^{-1} as KCl and 10 kg P ha^{-1} as single super phosphate/SSP) was added to the 0–10 cm soil layer of all cores. In the process of repacking the soil column for the lysimeter trial, the top soil layer from the field (0–10 cm) was added to the 10–20 cm depth in the column. This was done to simulate ploughing during the common pasture preparation (by inverting the topsoil into subsoil layer). The 10–20 cm soil layer from the field then was added to the top soil (0–10 cm) in the column without any amendments.

The procedure for soil sampling for the MS soil (experiment 2) differed slightly. Since the soil had a poor structure, it was not possible to take an intact core of soil at 20–40 cm depth. Soil sampling was therefore carried out by separately collecting the soil at 0–10 cm, 10–20 cm, and 20–40 cm depth. Samples at each different sampling depth were repacked individually in the soil column, again inverting the 10–20 and 0–10 cm layers. Before this inversion, the biochar made from biosolid-green waste biochar (BG-550) was added to the 0–10 cm depth layer at an application rate of 10 Mg ha^{-1} to half

of the soil cores. The 10–20 cm layer received the same basic fertilizer dose of urea (80 kg ha^{-1}), KCl (100 kg K ha^{-1}), and SSP (10 kg P ha^{-1}).



Picture 4.1. Sampling of Tokomaru silt loam soil

Soil temperature was measured using thermocouples that were installed in one of the 4 replicates of each treatment. Time-domain Reflector (TDR) sensors and water flux-tipping buckets were also installed to the same replicates to monitor the soil moisture fluctuation during the trial and volume of drainage, respectively (Picture 4.2). The experimental field was also equipped with a mini weather station to monitor the rain intensity and humidity throughout the experiment.



Picture 4.2. Soil column with TDR installed

4.2.4. Plant Development and Monitoring

The pasture species chosen to be used in this experiment were as follows: (1) perennial ryegrass (*Lolium perenne*); (2) mixture of cocksfoot (*Dactylis glomerata*) and red clover (*Trifolium pretense*); (3) chicory (*Cichorium intybus*) (for experiment 1 only); and (4) lucerne (*Medicago sativa*) (for experiment 2 only). Those plant species were chosen because of their deeper rooting profile. Both the ryegrass and the red clover-cocksfoot mixture were planted in TK soil and MS soil. The trial was set up with a randomized block design, with a total of 24 soil columns for each experiment (4 replicates per treatment). The experiment was started on 1 December 2010, and the seedling was done on the second week of December 2010, with 25 seeds of ryegrass per pot, 60 cocksfoot seed per pot, and 15 seeds per pot for each of the red clover, cocksfoot, and chicory treatments. The thinning was done on the first week of January 2011 so that the ryegrass treatment consisted of 17 healthy plants, 50 for cocksfoot, and 11 for red clover, Lucerne, and chicory.

In the event of a heavy rain, leaching of water from the soil column was collected from the tube that connects to the bottom of the lysimeter. The tipping bucket in the lysimeter (tipping-bucket water flux-meter – TWFM; Tranzflo NZ Ltd, Palmerston North) was used to monitor the volume of any drainage event (Deurer *et al.*, 2008). A manually operated lateral drip irrigator was used to maintain the soil moisture content above the wilting point during periods of low rain (Picture 4.3).

The trial lasted 777 days after the pasture establishment. The first fertilization event was conducted during the pasture seedling (13 December 2010), with a surface application of NPKS fertilizer (20 kg N ha⁻¹) to help the germination of the pasture species. During the 2001 autumn season another fertilizer application was done only on the MS soil, with surface application of 50 kg N ha⁻¹ as Urea, 30 kg P ha⁻¹ as Single Super Phosphate (SSP), and 50 kg K ha⁻¹ as Potassium Chloride (KCl). The third fertilization event was applied during the mid-spring season of 2011 (October–November) for both soils: 50 kg N ha⁻¹ (as ammonium sulphate), 21 kg P ha⁻¹ (as SSP), and 50 kg K ha⁻¹ (as KCl). The last fertilization event was done during the mid-spring season of 2012 (October–November), before the trial ended. Both soils received 50 kg

N ha⁻¹ (as Urea), and 30 kg P ha⁻¹ (as SSP). The monthly global radiation, average soil temperature, and cumulative monthly rainfall are presented in the Figure 4.2.

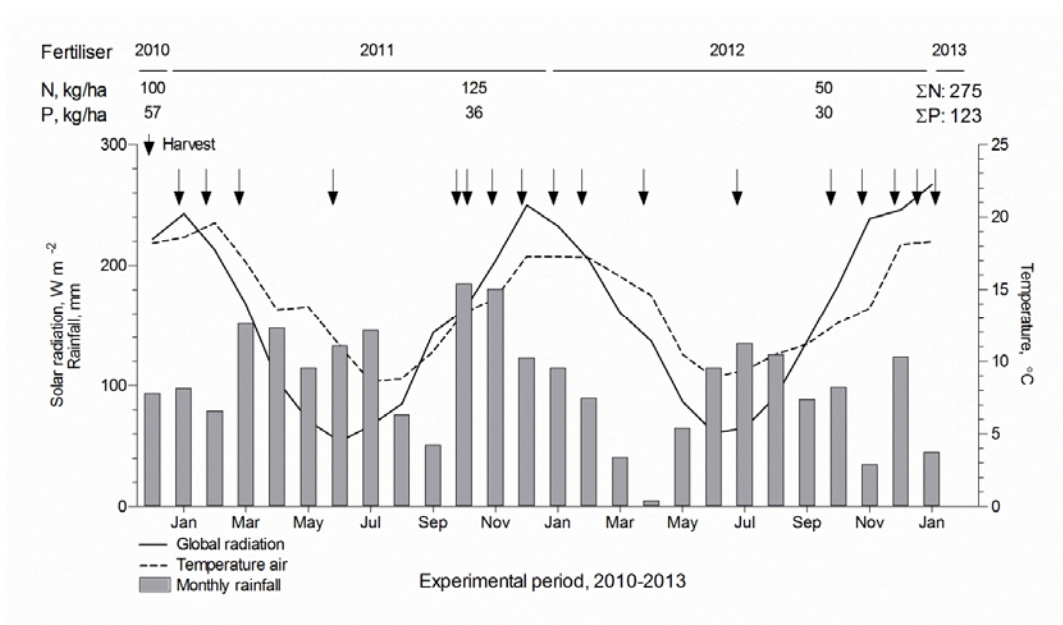


Figure 4.2 Monthly global radiation, average soil temperature, and cumulative monthly rainfall during the 2 years of the trial. The events of fertilizer addition to soil and plant “harvest” are also indicated.



Picture 4.3. Lateral drip irrigation machine used in the experiment

4.2.5. Above Ground and Bellow Ground Biomass Sampling

During the germination and establishment phase, the pastures were slightly trimmed to promote vegetation growth. After the establishment phase, the cutting of above ground biomass was done by cutting the biomass 5 cm height from soil surface. The cutting was intended to simulate a grazing event, and there were 9 grazing events during the first year of the trial (2010–2011) and 8 grazing events during the second year of the trial (2011–2012). Fresh above-ground biomass was oven dry at 60°C for 3 days, and then calculated as plant dry matter (DM).

Following grazing, manure and synthetic urine were applied to replace the C and N removed from the harvest, as in a normal grazing event in the field. Dry cow dung was re-applied to replace 34% of the removed C from biomass. Synthetic urine was made according to Holland and During (1977), equivalent to 500 kg K ha⁻¹ and 400 kg N ha⁻¹. The synthetic urine was applied to replace 47% of the removed N from the harvest.

The below-ground biomass samples were collected at the end of the trial (29 January 2013). Each soil column was dismantled from the lysimeter, and 20-mm cores were taken at five different soil depths: 0–20 mm, 20–40 mm, 60–80 mm, 140–160 mm, and 200–220 mm (Figure 4.3). The first three sample depths were taken to represent the 10 cm topsoil, while the fourth sampling depth (140–160 mm) was done as a representative sample for a particular layer that incorporated with biochar, and the final sampling depth of 200–220 mm was the original soil layer from the field (in the case of TK soil, this particular depth is the intact core that was collected directly from the field). From these, soil samples for bulk density and root samples were separately taken using two ring samples (inner diameter: 47 mm; height: 20 mm). Fresh weight and air dry weight of the soil + root samples were measured. Root separation from the soil was done using a wet sieving method. The soil samples for root biomass were washed under a running tap water through a sieve stack starting from 2000 µm, 1500 µm, 1000 µm, 500 µm, and 350 µm. The roots mass retained on every sieve then were oven dried at 50°C for 4 days.

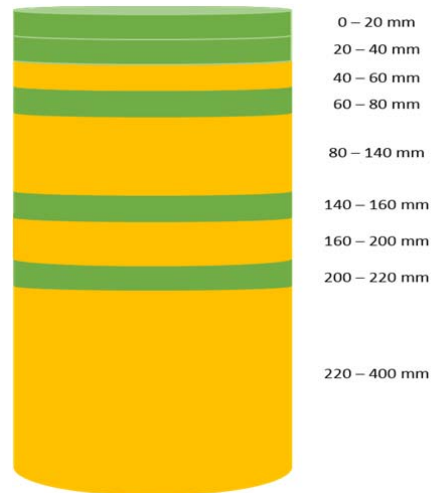


Figure 4.3 Schematic figures of soil sampling.

4.2.6. Statistical Analysis

A General Linear Model (GLM) was conducted using a Minitab 17 software (MINITAB Inc. 2014). The GLM was used to see the effect of treatments on the differences of soil C and N in each soil. There were 2 factors used in each soil: i) pasture species, and ii) biochar amendment. A Least Significant Difference (LSD) posthoc tests were conducted to see the significant difference (at $P < 0.05$) between variables measured.

4.3. Results

4.3.1. Biochar Properties

The properties of biochars used in this lysimeter experiment are presented in Table 4.2. The pH of BG-550 biochar was higher than PI-350 biochar, and the former also had higher lime equivalence (195.4 and $7.4 \text{ kg CaCO}_3 \text{ t}^{-1}$, respectively). As expected, wood-derived biochar (PI-350 biochar) had a higher total C (759 g C kg^{-1}) than the waste-derived biochar (BG-550 biochar, 356 g C kg^{-1}). On the other hand, the total N of PI-350 biochar was lower than the BG-550 biochar (2.7 g kg^{-1} for PI-350 and 14 g kg^{-1} for BG-550). The fixed-C of PI-350 was higher than that of BG-550 (65.8% and 32.1% , respectively), while BG-550 had a higher ash content (53.8%) than PI-350 had (2.7%). The C-aromaticity of both biochars based on estimation model by Wang *et al.* (2013) were relatively the same (0.80 – 0.83), despite their having been produced at different highest heating temperature. The atomic $\text{H}/\text{C}_{\text{org}}$ ratio for the PI-350 biochar was lower

(0.63) than that of the BG-550 biochar (0.76), which suggests that the method used to estimate aromaticity might not be suitable. Calvelo Pereira *et al.* (2015b) reported the actual C-aromaticity of PI-350 and BG-550 biochars based on NMR spectra were 0.73 and 0.87, respectively. The cation exchange capacity (CEC) of the BG-550 biochar was higher (11.3 cmol kg⁻¹) than the PI-350 biochar (0.88 cmol kg⁻¹).

Table 4.2. Biochar properties used in the experiment

Variables	Unit	PI-350	BG-550
pH	-	7.2	8.2
Lime equivalence	kg CaCO ₃ t ⁻¹	7.4	195.4
Total C	g kg ⁻¹	759	356
Total N	g kg ⁻¹	2.7	14
Total H	g kg ⁻¹	39	22
Total O	g kg ⁻¹	173	70
H/Corg	At. Ratio	0.63	0.76
O/Corg	At. Ratio	0.17	0.11
Ash	%	2.7	53.8
Volatile Matter	%	31.5	14.1
Fixed C	%	65.8	32.1
CEC	cmol kg ⁻¹	0.88	11.3
C-Aromaticity (<i>fa</i>) ^a	%	83	80

^a fraction of biochar-C that is aromatic, based on the estimation Mazumdar (1999) modified by Wang *et al.* (2013)

4.3.2. Above-ground Biomass Production

4.3.2.1. Pasture yield on silt-loam Tokomaru Soil (experiment 1)

As expected, the pasture growth mostly occurred during spring and summer, while that occurring during the late autumn–winter period was smaller. The application of PI-350 biochar did not significantly alter the daily DM production of the pasture treatments in this experiment. The daily DM yield was highest in the mixed pasture type, ranging from 7.6 kg ha⁻¹ d⁻¹ (biochar, November 2012) to 314.8 kg ha⁻¹ d⁻¹ (control, January 2013); and the lowest daily herbage production was observed in the chicory, which ranged between 4.7 kg ha⁻¹ d⁻¹ (biochar, October 2012) and 151.6 kg ha⁻¹ d⁻¹ (biochar, February 2011). The average growth rate of all pastures was lower in 2012 compared with that in 2011, especially during the autumn–spring period (April–October) (Fig. 4.3). Of three pastures used in the TK soil, chicory suffered the highest reduction of average growth rate with 61.1% reduction in the biochar-treated pots and 55.7% reduction in nil-biochar pots (Table 4.3).

Table 4.3 Average growth rate per year of pasture in Tokomaru soil

Soil Type and Treatment	Average growth rate (kg DM ha ⁻¹ day ⁻¹)		
	Ryegrass		
	2011	2012	P-value
Tokomaru Control	50.0	41.0	0.029*
Tokomaru + PI350 Biochar	52.8	41.9	0.103 ^{ns}
Mixture			
Tokomaru Control	93.0	71.9	0.082 ^{ns}
Tokomaru + PI350 Biochar	90.6	68.8	0.003*
Chicory			
Tokomaru Control	44.5	19.7	0.001*
Tokomaru + PI350 Biochar	42.4	16.5	0.000*

Note: * significant at 5% ($P < 0.05$); ^{ns} not significant ($P > 0.05$)

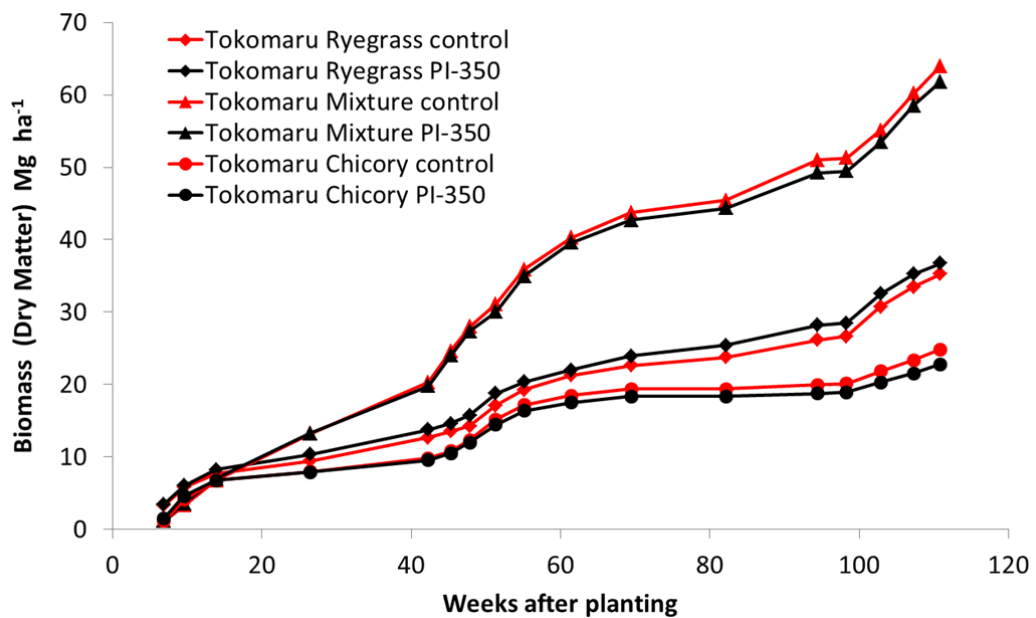


Figure 4.4 Cumulative above-ground biomass productions of different pasture plants on Tokomaru soil.

When comparing the total DM production of the different pastures, significant differences ($P < 0.05$) were found between them (Fig. 4.4, and Table 4.4). The red clover and cocksfoot mixture showed a significantly higher total DM production ($P < 0.05$) compared with the other pasture species, regardless of the application of biochar (Fig. 4.4). The application of PI-350 biochar to the TK soil did not significantly affect the total DM production at any time (Table 4.4). General differences in DM production were found when comparing the 2 different growth periods (Table 4.4). Above-ground biomass production on TK soil decreased from period 1 (late 2010–2011) to period 2 (2012–early 2013) (Table 4.4). All pastures grown in the TK soil showed lower total

herbage yield in 2012 (6.4 to 28.1 Mg ha⁻¹) compared with that in 2011 (16.4 Mg DM ha⁻¹ to 35.9 Mg DM ha⁻¹). The highest decrease in herbage yield over time was observed with chicory, regardless of the presence or absence of biochar in the soil (Table 4.4).

Table 4.4 Average total DM of pasture in Tokomaru soil

Soil/ Pasture	Treatment	Year 1 (386 days) Mg ha ⁻¹	Year 2 (390 days) Mg ha ⁻¹	Δ change to year 1	Cumulative (777 days) Mg ha ⁻¹	Δ change to control
Ryegrass	Control	19.3	16.0	-17% ^{ns}	35.3	3.2%
	PI-350 Biochar	20.4	16.3	-20% ^{ns}		
Mixture	Control	35.9	28.1	-22% ^{ns}	63.9	-3.3%
	PI-350 Biochar	35.0	26.8	-23% ^{ns}		
Chichory	Control	17.2	7.7	-55%*	24.9	-9.2%
	PI-350 Biochar	16.4	6.4	-61%*		
P value						
Factor	Biochar	0.818 ^{ns}	0.627 ^{ns}	0.504 ^{ns}	0.638 ^{ns}	
	Pasture	0.000*	0.000*	0.000*	0.000*	
	Interaction	0.598 ^{ns}	0.867 ^{ns}	0.939 ^{ns}	0.684 ^{ns}	

Note: * significant at 5% ($P < 0.05$); ^{ns} not significant ($P > 0.05$)

4.3.2.2. Pasture yield on coarse sandy Motuiti Soil (Experiment 2)

The daily DM production on the MS soil followed a similar trend to that on the TK soil, with the growth in the dry season (November–February) being higher than that in the wet season (June–August). The application of biochar to the MS soil did not always affect the daily herbage production, with the only significant ($P < 0.05$) increase observed within the cocksfoot and red clover mixture in January 2013 compared with the control (307.2 kg ha⁻¹ day⁻¹ and 218.1 kg ha⁻¹ day⁻¹, respectively). Similar to the result of experiment 1, the highest daily DM yield was also obtained with the mixture pasture, between 5.1 kg ha⁻¹ day⁻¹ (biochar; January 2011) and 307.2 kg ha⁻¹ day⁻¹ (biochar; January 2013); while the lower herbage production was observed under lucerne, with values ranging from 4 kg ha⁻¹ day⁻¹ (biochar; January 2011) to 188.5 kg ha⁻¹ day⁻¹ (biochar; January 2013). In this experiment, the average growth rate per year on MS soil was higher in 2012 compared with 2011, although this effect was only

significant ($P < 0.05$) for lucerne, irrespective of the presence or absence of biochar (Table 4.5).

Table 4.5. Average growth rate per year of pasture in Motuiti soil

Soil Type and Treatment	Average growth rate (kg DM ha ⁻¹ day ⁻¹)		
	Ryegrass		
	2011	2012	P-value
Motuiti Control	37.0	39.4	0.250 ^{ns}
Motuiti + BG-550 Biochar	34.9	35.7	0.750 ^{ns}
Mixture			
Motuiti Control	51.1	50.5	0.732 ^{ns}
Motuiti + BG-550 Biochar	48.9	54.1	0.067 ^{ns}
Lucerne			
Motuiti Control	35.5	40.8	0.020*
Motuiti + BG-550 Biochar	34.6	41.5	0.008*

Note: * significant at 5% ($P < 0.05$); ^{ns} not significant ($P > 0.05$)

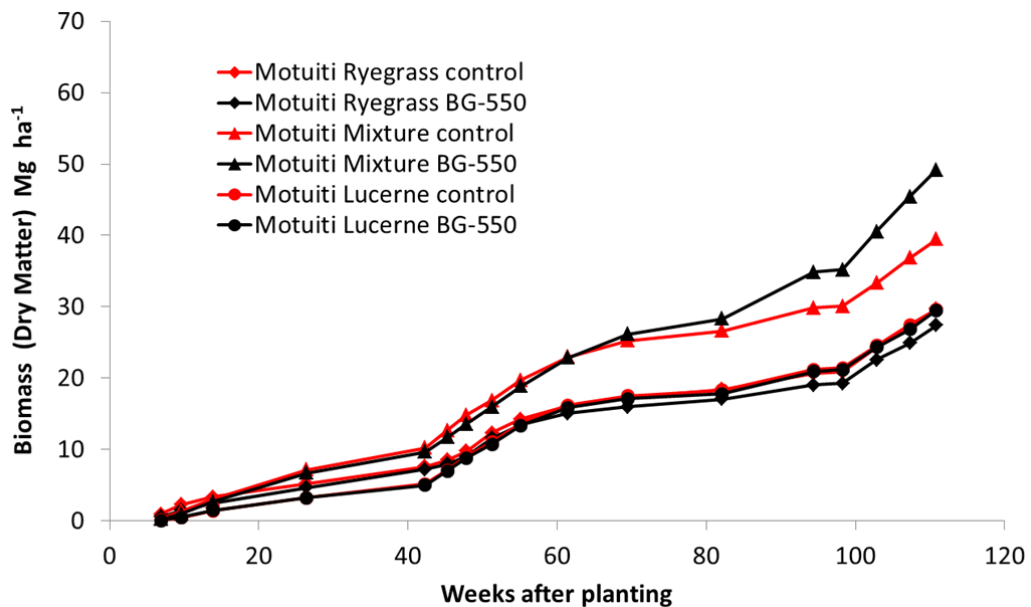


Figure 4.5 Cumulative above-ground biomass productions of different pasture plants on Motuiti soil.

Cumulative DM yields of pasture species grown in the MS soil are shown in Figure 4.5. Similar to the cumulative DM production in the TK soil, the mixture of red clover and cocksfoot produced the highest DM yield. In the first 80 weeks after the establishment of the trial (January 2011 to July 2013), the application of BG-550 biochar did not show any significant difference on DM yield. The high-ash biochar application had an effect

on pasture dry matter yield in the red clover and cocksfoot treatments starting from July 2012 to the end of the trial (Fig. 4.5). The total DM production of most pastures growing on MS soil tended to be higher in 2012 than in 2011 (Table 4.6), which contrasted with what was observed for the TK soil. At the end of the trial, the BG-550 biochar application resulted in a 25% increase of total average DM production in the cocksfoot and red clover mixture compared with the nil-biochar mixture. However, this was not significant, because only 1 of 4 replicates was observed to have a very large increase in DM (replicate 4: 174% DM increase, while the 3 other replicates averagely only 10% DM increase) compared with the nil biochar treatment.

Table 4.6 Total average DM of pasture in Motuiti soil

Soil/ Pasture	Treatment	Year 1 (386 days)	Year 2 (390 days)	Δ change to year 1	Cumulative (777 days) Mg ha ⁻¹	Δ change to control
		Mg ha ⁻¹	Mg ha ⁻¹			
Ryegrass	Control	14.3	15.4	8%	29.7	
	BG-550 Biochar	13.5	13.9	3%	27.4	-8.3%
Mixture	Control	19.7	19.7	0%	39.4	
	BG-550 Biochar	18.9	30.3	60%	49.2	25%
Lucerne	Control	13.7	15.9	16%	29.6	
	BG-550 Biochar	13.4	16.2	21%	29.6	-0.3%
P value						
Factor	Biochar	0.217 ^{ns}	0.326 ^{ns}	0.164 ^{ns}	0.480 ^{ns}	
	Pasture	0.000*	0.031*	0.419 ^{ns}	0.003*	
	Interaction	0.902 ^{ns}	0.256 ^{ns}	0.168 ^{ns}	0.334 ^{ns}	

Note : * significant at 5% ($P < 0.05$); ^{ns} not significant ($P > 0.05$)

4.3.3. Live and dead root biomass

4.3.3.1. Live and dead root biomass in Experiment 1

The measuring of below-ground biomass in this trial could not distinguish the difference between plants live or dead roots, hence the recorded biomass was determined as live and dead root biomass. In the TK soil, pasture types had a significant effect ($P < 0.05$) on live and dead root biomass (Fig. 4.6 a,b,c). Ryegrass grown in the TK soil showed a significantly lower ($P < 0.05$) below-ground biomass compared with the other plants species, while the highest biomass was observed in the mixture pasture type. Significant differences ($P < 0.05$) in below-ground biomass

were also detected when comparing different soil depths, with majority of biomass recovered in the top 10 cm soil depth. The below-ground biomass measured in the layer incorporated with biochar (10–20 cm) was lower compared with the one in the topsoil (0–5 cm). The application of PI-350 biochar did not significantly increase the below-ground biomass in TK soil, as expected. There was no clear effect of this biochar on the total amount of below-ground biomass after 2 years of trial; while a notable increase of live and dead root biomass was recorded on the 0–10 cm of cocksfoot and red clover mixture and the 10–20 cm of chicory (Figure 4.6 b,c; Table 4.7). Both ryegrass and the mixture pasture showed a lower amount of biomass covered in soil layer incorporated with biochar (28–55% lower than the corresponding control), while an increase of total below ground biomass was observed in the chicory (78% higher than the nil biochar treatment).

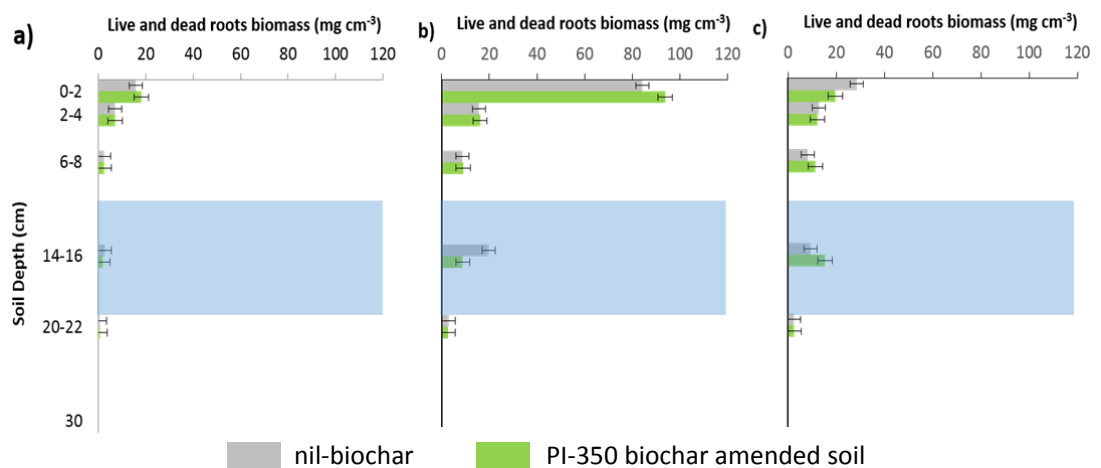


Figure 4.6 Live and dead roots biomass distribution (mg cm⁻³) with depth after biochar addition and growth of different pasture species in lysimeter containing Tokomaru silt loam soil (experiment 1): a) ryegrass; b) red-clover and cocksfoot mixture; c) chicory. Data represent the average and the standard error of the means (SEM) (n=4) (For each depth, bars with different letters indicate significant differences at P < 0.05 between Control and biochar amended soil; shaded areas represent the depth of biochar application)

Table 4.7 Estimation of live and dead roots amount in corresponding TK soil layer

Treatment		Below ground biomass (g) soil depth ⁻¹			Δ to control		
		0–10 cm	10–20 cm	20–30 cm	0–10	10–20	20–30
Ryegrass	Control	186.8	88.5	27.0	4%	-28%	36%
	PI-350 biochar	193.5	63.8	36.7			
Mixture	Control	814.6	684.6	158.1	16%	-55%	-13%

	PI-350 biochar	943.0	310.6	137.4			
Chicory	Control	399.0	297.4	102.9			
	PI-350 biochar	392.8	528.4	106.1	-2%	78%	3%
P Value							
Factor	Pasture	0.000*	0.009*	0.000*			
	Biochar	0.551 ^{ns}	0.595 ^{ns}	0.875 ^{ns}			
	Interaction	0.698 ^{ns}	0.082 ^{ns}	0.721 ^{ns}			

Note: * significant at 5% ($P < 0.05$); ^{ns} not significant ($P > 0.05$)

4.3.3.2. Live and dead root biomass in Experiment 2

The different pasture species in MS soil showed significant ($P < 0.10$) difference in the below-ground biomass along 30 cm soil profile (Fig. 4.7 a,b,c). As with experiment 1, the ryegrass also showed the lowest live and dead root biomass compared with the other pasture plants, and the highest below-ground biomass was observed on the mixture of cocksfoot and red clover (Fig. 4.7). The below-ground biomass measured in the subsoil (10–20 cm) was significantly ($P < 0.05$) lower than that measured in the topsoil (0–5 cm soil depth). However, when estimating the total below ground biomass, the subsoil (10–20 cm) was calculated to have a higher total below-ground biomass compared with the topsoil, regardless of pasture types and biochar application (Table 4.8). The application of BG-550 biochar to the subsoil of MS soil did not result in a significantly higher total below-ground biomass in the corresponding soil depth (Table 4.8).

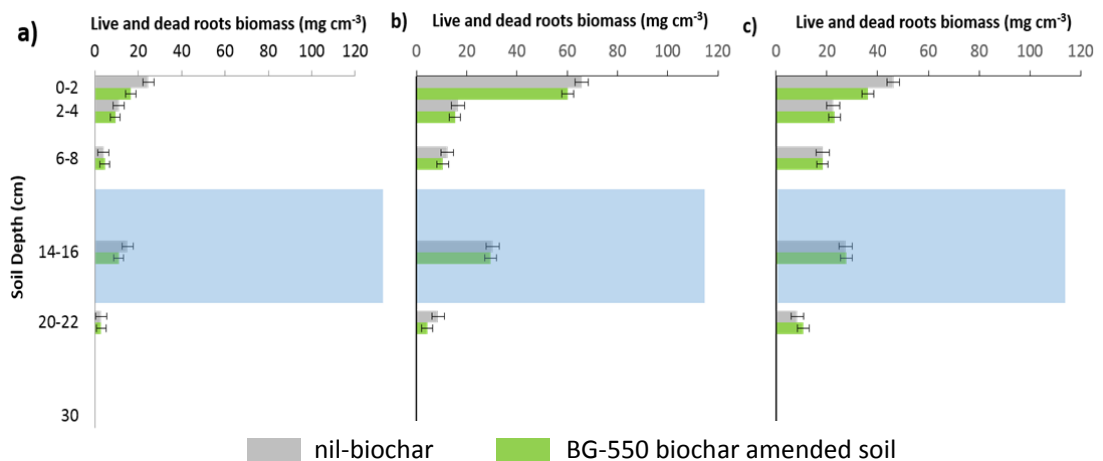


Figure 4.7. Live and dead roots biomass distribution (mg cm⁻³) with depth after biochar addition and growth of different pasture species in lysimeter containing Motuiti sandy soil (experiment 2): a) ryegrass; b) red-clover and cocksfoot mixture; c) lucerne. Data represent the average and the standard error of the means (SEM) ($n=4$) (For each depth, bars with

different letters indicate significant differences at $P < 0.05$ between Control and biochar amended soil; shaded area represent the depth of biochar application)

Table 4.8 Estimation of live and dead roots amount in corresponding MS soil layer

Treatment		Below ground biomass (g) soil depth ⁻¹			Δ to control		
		0–10 cm	10–20 cm	20–30 cm	0–10	10–20	20–30
Ryegrass	Control	291.0	497.4	155.1	-17%	-26%	-13%
	BG-550 biochar	241.7	365.6	134.2			
Mixture	Control	752.9	997.9	392.1	-21%	3%	-52%
	BG-550 biochar	598.1	1031.3	187.9			
Lucerne	Control	660.0	1004.0	364.7	4%	-12%	22%
	BG-550 biochar	689.6	886.1	443.8			
		P Value					
Factor	Pasture	0.000*	0.001*	0.003*			
	Biochar	0.448 ^{ns}	0.551 ^{ns}	0.359 ^{ns}			
	Interaction	0.610 ^{ns}	0.821 ^{ns}	0.104 ^{ns}			

Note: * significant at 5% ($P < 0.05$); ^{ns} not significant ($P > 0.05$)

4.4. Discussion

4.4.1. Pasture DM production

The two soils used in this trial differed in their constraints to support plant growth. The TK soil is a silt loam soil (Typic Fragiudalf) that is characterized by an increase in soil bulk density with depth associated with a high clay content in the soil B horizon down to 750 mm (Scotter *et al.*, 1979). Some of the TK topsoil (0–10 cm) properties are reported in Table 4.1, with soil pH 5.07; bulk density 0.94 Mg m⁻³; total C content 35.6 g kg⁻¹; C/N ratio of 9.8, and Olsen P 78.1 g kg⁻¹. Physical constraints in the TK soil are associated with compaction, which is the main condition that limits plant growth (Scotter *et al.*, 1979). Contrasting with the TK soil, the MS soil is a brown sandy soil (Typic Udipsamment) with a sandy soil texture in the whole soil profile (Cowie and Rijkse, 1977). The topsoil (0–10 cm) properties of the MS soil are as follows: soil pH

5.94, bulk density 1.23 Mg m^{-3} , total C content 27.6 g kg^{-1} , C/N ratio 11.7, and Olsen P 16.6 g kg^{-1} . The major factors limiting plant growth in the sandy soil are low water availability (Smith and Stiefel, 1977) and low nutrient supply (Cowie and Rijkse, 1977).

Soil texture had a strong influence on the fertility of the soils used in this experiment, where the MS soil with a coarse soil texture had lower water and nutrient retention compared with the TK soil. Consequently, pasture production on the TK soil was greater compared with the MS soil, as shown by the overall biomass production. This was observed in the ryegrass and the mixture pasture (without biochar) as they were common vegetation in both soil types. Of those two pasture types, the greatest yield in both soils was observed in the mixture of cocksfoot and red clover (Tables 4.4 and 4.6). The use of multi-pasture, including cocksfoot and clover species, has been reported to increase pasture productivity compared with common perennial ryegrass pasture (Goh and Bruce, 2005; Nie *et al.*, 2004; Skinner *et al.*, 2006). In the environment that limited ryegrass growth, such as low water availability, multi-species pasture plants offer an alternative approach to overcome those limitations (Nie *et al.*, 2004).

In this lysimeter experiment, the selection of cocksfoot and red clover mixture on both TK and MS soils was based on the ability of cocksfoot to growth at depth, and thus on the greater tolerance of this plant under dry conditions (Volarie and Thomas, 1995). On the other hand, the legume red-clover offers advantages such as the biological N_2 fixation (Goh and Bruce, 2005) and the dominant growth in summer period (Nie *et al.*, 2004). The red-clover-based mixed pasture has been reported to have higher biological N_2 ($135.9 \text{ kg N ha}^{-1} \text{ year}^{-1}$) than the New Zealand standard pasture of ryegrass and white clover mixture ($52 \text{ kg N ha}^{-1} \text{ year}^{-1}$) (Goh and Bruce, 2005). Red clover is susceptible to water stress in dryer conditions (Sallinger *et al.*, 2003); however, mixing red clover with more resilient cocksfoot will help maintain sward production in the summer. During the summer season of periods 1 and 2, this pasture mixture produced higher DM (up to 80%) compared with other pasture types, regardless of the difference in soil types.

Chicory is one pasture plant commonly used in New Zealand because of its high DM yields and its ability to produce high growth rates in lamb, deer, and cattle (Li *et al.*,

1997; Li and Kemp, 2005). In this 2-year experiment, the herbage daily growth of chicory was limited during winter period (June–August), with an almost nil growth of chicory during the 2012 winter. Chicory is an actively growing plant during the warm spring–summer period, but dormant during the cold winter season (Li and Kemp, 2005). In the first year of this experiment, the chicory produced a relatively high DM – about 42.4 – 44.5 kg ha⁻¹ day⁻¹ – but this was still lower than the maximum value of 150 kg ha⁻¹ day⁻¹ reported by other researchers (Hare *et al.*, 1987; Matthews *et al.*, 1990). During the second year there was a sharp decline in the daily herbage production of chicory of almost 60% decrease in DM yield (Table 4.3.4). One of the reasons for this decline could be the commonly observed decline in chicory yield with time. In fact, Li *et al.* (1997) found that, after 4 years, the chicory yield declined to only half of the harvest in the first year.

The total annual DM production of lucerne in the MS soil (13–15 Mg DM ha⁻¹ year⁻¹) was in the range of the common lucerne production in New Zealand (6.5 – 28 Mg DM ha⁻¹ year⁻¹) (Moot, 2012). In the MS soil, the growth of lucerne was also limited during the winter period, with the lowest daily herbage production being recorded of 7.8 – 9.2 kg ha⁻¹ day⁻¹ in July 2012. As with chicory in the TK soil, lucerne has a lower seasonal growth in winter because of its inability to adapt to cold temperatures (Goh and Bruce, 2005). The higher recovery of lucerne DM in the period 2 could be explained by its deeper root allocation in the coarse MS soil (Fig. 4.6). Plants with deeper root distribution and high root density are likely to reduce nutrient leaching in the sandy soil (Moot, 2012). With lower nutrient leaching, more nutrients are available for plant uptake, and in turn increase the biomass production of lucerne in the MS soil.

4.4.2. Biochar influence on the total DM production

The application of PI-350 biochar in the TK soil did not result in a significant increase in pasture DM production compared with the nil biochar treatment (Table 4.4), which was to some extent expected, as the PI-350 biochar lacks nutrient fertility value (Table 4.2) and is only intended to increase the allocation of root biomass at depth by improving soil aeration. Nonetheless, some minor effects were observed in the ryegrass DM yield, as this was greater in the biochar-amended soil compared with the

non-biochar treatment in both years (6% DM improvement in 2011 and 2% in 2012). However, when PI-350 biochar was applied to the mixture pasture and chicory, the DM yield was lower compared with the control (Table 4.4). It must be noted that the decline in DM production following PI-350 biochar application in these pasture types was not significant. Similarly, Slavich *et al.* (2013) found that there was no significant effect of green waste-derived biochar amendment on the total pasture production of an acidic Ferralsol. In contrast to these results, Jones *et al.* (2012) reported that using a somewhat similar wood-derived biochar produced at 450°C, resulted in biochar amendment that had a positive effect on the DM yield of cocksfoot grown in a sandy clay loam soil.

The decline in DM production following biochar application in this experiment can be explained, at least in part, to the immobilisation of N by biochar. The decline in DM production following biochar application to a poor fertility soil referred to in Chapter 3 might also confirm the immobilisation of soil N, and numerous studies also reported the immobilisation of N following biochar application (Bridle and Pritchard, 2004; Kloss *et al.*, 2014). In the second season of the experiment, chicory suffered the most DM decline – up to 16% DM reduction compared with the control. Chicory is a non-legume pasture plant; hence the application of N-fertiliser is essential in a pure chicory stand to produce higher DM (Li and Kemp, 2005). The low content and availability of N from PI-350 biochar and the possible N immobilisation in the TK soil might explain the decline of DM yield in chicory. Other possible explanations of this decline in DM production might be related to the presence of aromatic compounds that are potentially toxic to soil microflora, such as terpenoids and their derivatives (Calvelo-Pereira *et al.*, 2011).

The application of BG-550 biochar to MS soil also did not result in a significantly higher DM production, although the mixture of cocksfoot and red clover did show a relatively high DM yield on the second year of the experiment (Table 4.6). An increase in DM yield following the application of high-ash biochars has also been reported by Chan *et al.* (2008), Hossain *et al.* (2010), Slavich *et al.* (2013), van Zwieten *et al.* (2010a), and Wang *et al.* (2012). The high-ash biochar had a considerable liming equivalence and also a considerable amount of nutrients (e.g., P and K; Wang *et al.*, 2012), and thus

may have contributed to the amelioration of this poor-fertility sandy soil. Wang *et al.* (2012) produced a high-ash biochar using biosolids from the same wastewater treatment plant as the ones used for the BG-550 biochar, although in this case the biosolids were mixed with eucalyptus wood instead of green waste to increase the calorific value of the biosolids during pyrolysis.

The P bioavailability test using 2% formic acid extraction following the methodology described by Wang *et al.* (2012) showed that about 16.71 g kg⁻¹ of P in the biosolids-eucalyptus biochar was available for plant uptake. Hossain *et al.* (2010) also noted that the high availability of P from the wastewater sludge biochar contributed to a better yield of tomato plant in a Chromosol. The increase in pasture DM production following the application of BG550 biochar could be partially explained by the increasing P availability as a result of the dissolution of adsorbed P in this slightly acidic MS soil, as has been reported by Slavich *et al.* (2013). More detailed observation on the plant nutrient uptake is needed to confirm this result.

The application of PI-350 and BG-550 biochar in this trial did not significantly influence the cumulative pasture DM in either the first (2010–2011) or second period (2011–2012) of the trial. The biochar used in both experiments did not succeed in providing additional nutrient to the loamy and coarse soil. It seems that the periodic application of fertilizer and the return of animal waste (cow manure and synthetic urine) following the “grazing/harvest” events supplied sufficient nutrients for pasture growth. Thus, it is important to understand the limitation of each biochar in supplying the available nutrients for plant uptake before applying it to the soil.

4.4.3. *Live and dead root biomass*

The wet sieving method in this study was not adequate to differentiate live plant roots and dead root litter. Hence, the below-ground biomass recovered in this study was determined as live and dead root biomass, and the results could not be used as an indicator of plants root proliferation. As expected, the difference in pasture species significantly influenced ($P < 0.05$) the total below-ground biomass in both experiments. The mixture of cocksfoot and red clover was consistently recorded to have the highest below-ground biomass in both coarse MS soil and silt loam TK soil (Figures 4.6 and

4.7). The higher below-ground biomass production of a mixture pasture species has also been reported by Skinner *et al.* (2006), who found that 11-pasture mixtures had a greater root biomass (30–62%) compared with the less complex pasture. In this trial, although red clover has a shallow root pattern, the cocksfoot provides a greater vertical root growth, regardless of the contrasting soil condition of coarse and silt loam soil. When comparing the below-ground biomass pattern (Tables 4.7 and 4.8), pasture in experiment 2 (coarse sandy MS soil) showed a higher below-ground biomass recovered in the subsoil (10–20 cm) than in the topsoil layer (0–10 cm). Originally, the subsoil in the lysimeter pot was the topsoil layer in the field and had a low nutrient content and low soil bulk density (Table 4.1). As a result of the simulated mouldboard ploughing, the subsoil in the lysimeter pots was favourable for vertical root growth, as the plant tried to enhance its nutrient and water uptake in the deeper soil layer.

In the silt loam soil (experiment 1), the high bulk density is the main constraint to pasture root growth. The incorporation of PI-350 biochar in the subsoil of silt loam soil resulted in a lower soil bulk density, regardless of the difference in pasture species (Appendix 2). However, the addition of biochar was not clearly stimulated the below-ground biomass production (Table 4.7), where only chicory amended with biochar showed a higher biomass (78% higher) compared with the nil biochar treatment. Recent studies have reported a positive influence of biochar on plant root growth, associated with the increasing water retention by biochar (Brunn *et al.*, 2014) and the soil nutrient transformation (Prendergast Miller *et al.*, 2014). The PI-350 biochar has low CEC (Table 4.2), hence it did not improve nutrient retention to enhance root growth on the deeper soil layer.

Similar to experiment 1, the addition of BG-550 biochar did not significantly influence the below-ground biomass production on the sandy MS soil (Table 4.8). It was expected that applying high-ash biochar (BG-550) would provide additional nutrient value to the poor fertility sandy soil; however, this was not the case in experiment 2. During the 2 years of this trial, several fertilization events (N and P) were carried out when pasture was not performing well (Fig. 4.2). As a result, the topsoil layer might have already acquired a substantial amount of P from the fertilization events, and the

available P from BG-550 biochar in the subsoil might not have enhanced the vertical root proliferation.

4.5. Conclusion

The production of above- and below-ground biomass of different pasture species in this study was highly influenced by the type of pasture rather than by the biochar-amended treatment, regardless of the difference in soil types. In general, the pasture in the relatively fertile silt loam soil showed a higher DM production compared with the coarse sandy soil. The mixture of cocksfoot and red clover showed a higher annually DM production ($18.9 - 35.9 \text{ Mg ha}^{-1} \text{ year}^{-1}$) compared with other pasture species in both soil types. In the silt loam soil, the application of PI-350 biochar did not stimulate higher DM production compared with the nil biochar treatment. This was expected, since the PI-350 biochar was made from wood material that did not contain a substantial amount of nutrient to improve soil fertility. The addition of nutrient rich biochar BG-550 to the coarse sandy soil did not result in a significantly higher DM production as expected. The lack of biochar application effect on DM production in the sandy soil could be explained by the adequate nutrient availability to plant uptake resulting from the fertilizer event during the experiment and also from the return of animal excreta following the “harvest/grazing” management.

In this study, the application of low-ash PI350 to a loamy soil did not enhance the belowground biomass production of different pasture species, despite the lower bulk density, compared with the nil biochar treatment. By lowering the soil bulk density, the PI-350 biochar succeeded in providing better soil aeration for root growth. However, the localised available nutrient in the topsoil following the fertilisation event may result in a lower vertical root growth. In the coarse sandy soil, the BG-550 biochar did not stimulate root growth as expected. This may be due to the lack of additional nutrients especially P from the BG-550 biochar, that could have enhanced root growth at deeper soil depth. Further study is needed to understand the mechanisms of biochar types and the effect of biochar in altering soil properties that favour root growth at deeper soil depth.

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Chapter 5. Biochar Lysimeter Trial: 2. Changes in Soil C and N Stocks over the 2-Year Experiment

Increasing soil Carbon (C) storage at greater depth could be achieved by enhancing plant C allocation to deeper soil depth. Using organic amendment, such as biochar, provides additional advantages of storing C with greater longevity; especially when it is applied to the subsoil layer. A 2-year lysimeter trial was set-up to investigate the changes in C and Nitrogen (N) stocks of two different soils following the addition of biochar at subsoil layer (below 10 cm). Distinctive biochars were selected for these two soils so that soil-specific plant growth limitations could be overcome. In the Tokomaru silt loam, the “cultivation” by inverting topsoil and subsoil of original sample resulted in a net loss of native organic C in the buried horizon under shallow-rooted species, but not under deep-rooted species. The addition of C-rich pine biochar at 10 Mg ha⁻¹ (equivalent to 7.6 Mg C ha⁻¹) to this soil resulted in a net C gain (6–16% over the non-biochar treatment, $P < 0.10$) in the buried soil layer under all pasture treatments. The additional C from this biochar helped overcome the net loss of native organic C under shallow-rooted pastures. In the Motuiti sandy soil, all pasture species were able to maintain soil C stocks at 10–20 cm depth over time. In this soil, the exposure of a skeletal and nutrient-depleted soil layer at the surface may have fostered root growth at depth. The addition of a nutrient-rich biosolids biochar at 10 Mg ha⁻¹ (equivalent to 3.6 Mg C ha⁻¹) to this soil had no apparent effect on total C stocks. More research is needed to understand the mechanisms of biochar could influence the C storage at greater soil depth.

A paper from this study has been submitted for publication:

Calvelo Pereira R, Hedley M, Camps Arbestain M, Wisnubroto E, Green S, Saggar S, Kusumo BH, Mahmud AF (2015) Net changes of soil C stocks in two grassland soils 26 months after simulated pasture renovation including biochar addition. GCB Bioenergy.

5.1. Introduction

In New Zealand, the dominant agricultural land use is pastoral farming, with almost 54.6% of the agricultural land use being grassland (MacLeod and Moller, 2006; Mudge *et al.*, 2011; MfE, 2014). Pasture/grassland ecosystems generally have a high inherent soil carbon (C) content compared with cropland soil (Conant *et al.*, 2001). This has been attributed to the allocation of considerable amounts of C to the below-ground biomass by pasture species (Kuzyakov and Domanski 2000; Rasse *et al.*, 2005; Soussana *et al.*, 2004).

The reported trends in total soil C stocks in New Zealand pastures are unclear, with a decline in soil C as described by Schipper *et al.* (2007, 2010), an increase in soil C stocks as indicated by Mudge *et al.* (2011), and no soil C change as illustrated by Tate *et al.* (2003, 2005) and Schipper and Sparling (2011). Such contrasting trends could be explained by vertical, lateral, and temporal variability in soil properties, and differences in the methodological approach used. There have been recent discussions on the existence of an upper limit above which a specific soil may not be able to store more C – the so-called “C saturation limit” – (Baldock and Skjemstad, 2000; Hassink, 1997). Dodd *et al.* (2011) suggested that the top 10 cm of most New Zealand pastoral soil might have reached either a steady state or its maximum capacity to store additional C. An opportunity then arises to increase the soil C stock in the subsoil, where the soil C content might be below the so called saturation level (Stewart *et al.*, 2008; Dodd *et al.*, 2011). Recently, Beare *et al.* (2014) estimated the upper C stabilisation limit of New Zealand pastoral soils based on data from measurable properties of the fine mineral fraction and found that most soils have a C deficit, this being greater in subsoil (median value 15 mg C g⁻¹) than in top soils (median value 12 mg C g⁻¹).

Several management practices can result in an increase of soil C stocks under pastoral agricultural system: (i) improvement of grazing management by increasing pasture

productivity and adequate grazing regimes that allow an increase in the return of C into soil (Conant *et al.*, 2001; Conant and Paustian, 2000; Schnabel *et al.*, 2001; Beukes *et al.*, 2010); (ii) introduction of deep-rooted pasture species to increase plant C allocation directly into the subsoil (Carter and Gregorich, 2010; Fisher *et al.*, 1994); and (iii) application of organic C-rich amendments (such as compost, manure or biochar) (Ryals *et al.*, 2014; Schimmelpfennig *et al.*, 2014; Slavich *et al.*, 2013) , which in the case of biochar will be able to increase soil C stocks independently of the original C saturation status of a specific soil.

Biochar is a C-rich material that results from the carbonization of organic material under no/low oxygen conditions and intended to be used as soil amendment to improve soil functions and increase the soil C stocks. By transforming biomass C (otherwise rapidly degradable) into a more stable form (Lehmann and Joseph, 2009), biochar can contribute to mitigate anthropogenic CO₂-C emissions (Singh *et al.*, 2012; Woolf *et al.*, 2010). Biochar application as soil amendment can have no effect on plant biomass production (Biederman and Harpole, 2013; Kloss *et al.*, 2014), as discussed in Chapter 4. However, when fulfilling the specific needs of a soil/crop, biochar amendment can result in an increase in plant biomass production [Chapter 3 of this thesis; Steiner *et al.* (2007)].

Biochars made from woody plant material usually have a high C content and have an impact on soil physical properties as bulk density or water discharge (Herath *et al.*, 2013; Jeffery *et al.*, 2015), whereas those biochars made from ash-rich feedstock (such as animal waste) have a greater impact on soil chemistry, including nutrient cycling (Wang *et al.*, 2012ab). The results from Chapter 3 showed that the addition of an ash-rich biochar to a sandy soil provided nutrients that were limiting plant growth (e.g., P, K) and had a positive effect on roots growth. However, results from Chapter 4 of this thesis suggested that, when crop nutrient needs are balanced by the addition of other amendments (e.g., fertilisers), the effect of a nutrient-rich biochar on both above- and below-ground biomass productions is negligible.

The objective of this study was to investigate changes in soil C 2 years after the application of biochar to two contrasted soil types under intensive grazing. For this,

the same lysimeter experimental set up as in chapter 4 – where the effect of biochar on plant biomass production was investigated – was used. This chapter focuses on the relationship between the below-ground biomass production and the changes in soil C and N stocks.

5.2. Material and Methods

5.2.1. Lysimeter Trial

Details on the biochars (PI-350 and BG-550) and soils used in this study (the Tokomaru silt loam and the Motuiti sand), and the experimental set-up of the lysimeter trial, management and monitoring are described in Chapter 4. Basically, two experiments were carried out: (i) experiment 1 on the Tokomaru soil, which included the use of either ryegrass, or cocksfoot and red clover mixture, or chicory; (ii) experiment 2 on the Motuiti soil, which included the use of either ryegrass, or cocksfoot and red clover mixture, or lucerne.

5.2.2. Soil Sampling and C-N Analysis

Soils were sampled at the end of the trial (29 January 2013). Each soil column was dismantled from the lysimeter and soil samples were taken at different depths: 0–20 mm, 20–40 mm, 60–80 mm (represent 60–100 mm), 140–160 mm (represent 100–200 mm), and 200–220 mm (represent 200–300 mm). For this a ring sample (inner diameter: 47 mm; height: 20 mm), as described in Chapter 4 was used. An air-dried, ground (< 250 µm) soil subsample per slice was analysed for total C (TC) and total N (TN) determinations using a vario MACRO cube CHNS elemental analyser (Elementar 237 Analysensysteme GmbH, Hanau, Germany).

5.2.3. Calculation on the cumulative soil C and N stock

Soil TC (and TN) stocks (Mg TC ha⁻¹ soil) under each pasture and treatment were calculated at a fixed depth using C concentration, sampling depth and soil bulk density (Calvelo Pereira *et al.*, 2015). The commonly used fixed depth (FD) method is considered by the Intergovernmental Panel on Climate Change (IPCC) as suitable to estimate the TC stocks of soils down to 30 cm depth (Gifford and Roderick, 2003). The FD method does not consider changes on soil bulk density (BD), and hence might be

inaccurate, either overestimating or underestimating the actual soil TC stock (Norris, 2014). Several authors have proposed alternative methods to overcome the inaccuracy of the FD method, such as the equivalent soil mass method (Ellert and Bettany, 1995; Wendt and Hauser, 2013). In this chapter, two methods of calculating soil TC and TN were used in addition to the FD method: (i) the fixed mass (FM) method by Ellert et al. (2008), and (ii) the equivalent soil mass (ESM) method by Wendt and Hauser (2013), both of which intend to overcome the inaccuracies caused when comparing C stocks of different mass of soil, as with the FD method. The specific calculations of soil TC stocks by the methods considered here (FD, FM, and ESM) are fully detailed in the Appendix 4 of this thesis.

5.2.4. *Statistical Analysis*

A General Linear Model (GLM) was conducted using Minitab 17 software (MINITAB Inc. 2014). The GLM assessed the effect of treatments on the differences in soil C and N of each soil and sampling depth. For a specific soil, 2 factors were used: i) pasture species, and ii) biochar amendment. Least Significant Difference (LSD) post hoc tests were conducted to see the significant difference (at $P < 0.05$) between variables measured.

5.3. **Results**

5.3.1. *Soil C and N content*

5.3.1.1. *Soil C and N concentrations in the Tokomaru (TK) soil under different pasture types with and without PI-350 biochar (experiment 1) after 2 years of pasture establishment*

For all treatments and after 2 years of pasture establishment (T2), the top 0–10 cm of TK soil had a higher TC concentration (ranging from 22 to 49 g C kg⁻¹) than that of the same soil layer at T0 (Fig. 5.1), although TC values were pasture type-dependent. At T2, values of TC for the 0–2-cm depth layer ranged between 24 and 49 g C kg⁻¹ soil; the highest values for this layer corresponded to the TK soil under the mixture of cocksfoot and red clover (on average, 39–49 g TC kg⁻¹) (Fig. 5.1b). At the 10–20 cm depth, the addition of PI-350 biochar significantly increased the soil TC compared with the control treatment (nil biochar) for all the pastures considered (Fig. 5.1). At this particular soil depth and in the absence of biochar, soil TC of the TK soil under ryegrass and chicory

swards at the end of the experiment was lower (between 24.5 and 28.2 g C kg⁻¹) than the corresponding one at T0 (Fig. 5.1). No C loss was detected under the mixture. Soil TC concentration in the 20–30 cm depth (10.2 – 12.9 g TC kg⁻¹) was similar to that at T0 (Fig. 5.1), with no effect of either pasture type or amendment.

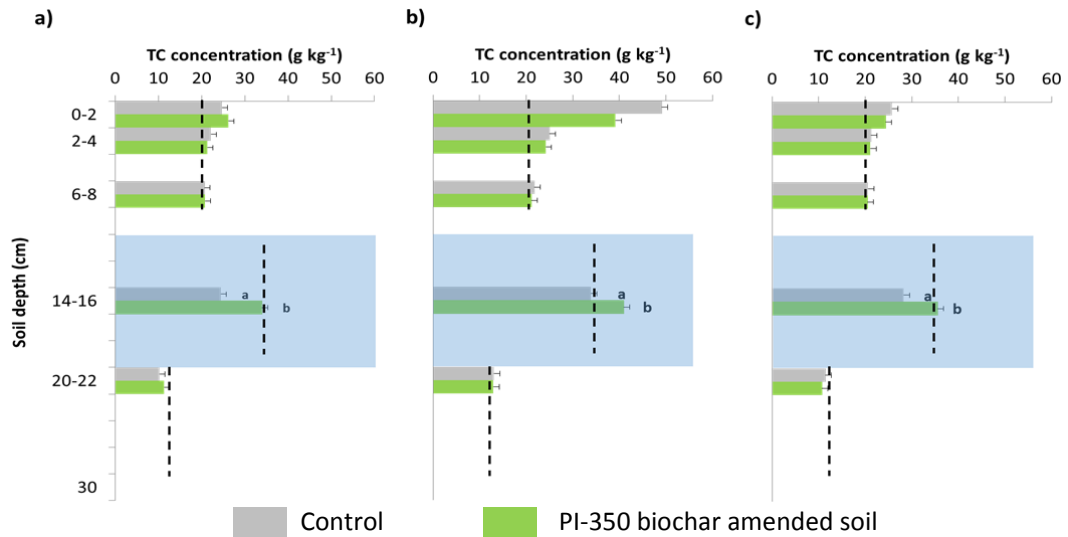


Figure 5.1 Changes in soil TC concentration (g kg⁻¹) with depth after biochar addition and growth of different pasture species in lysimeters containing Tokomaru silt loam soil (experiment 1): a) ryegrass; b) red clover-cocksfoot mixture; and c) chicory. Data represent the average and the standard error the means (SEM) ($n = 4$) (For each depth, bars with different letters indicate significant differences at $P < 0.05$ between control and biochar amended soil; shaded area represent the depth of biochar application) vertical dashed line represent the TC concentration at T0.

After 2 years, TN concentration in the top 10 cm of soil (2.2 – 2.8 g TN kg⁻¹; Fig. 5.2) was similar to that at T0 (Fig. 5.2). In the 10–20-cm soil layer, all pasture types had a lower TN concentration than that at T0 (Fig. 5.2). The addition of PI-350 biochar did not influence soil TN concentration in this particular soil layer (Fig. 5.2).

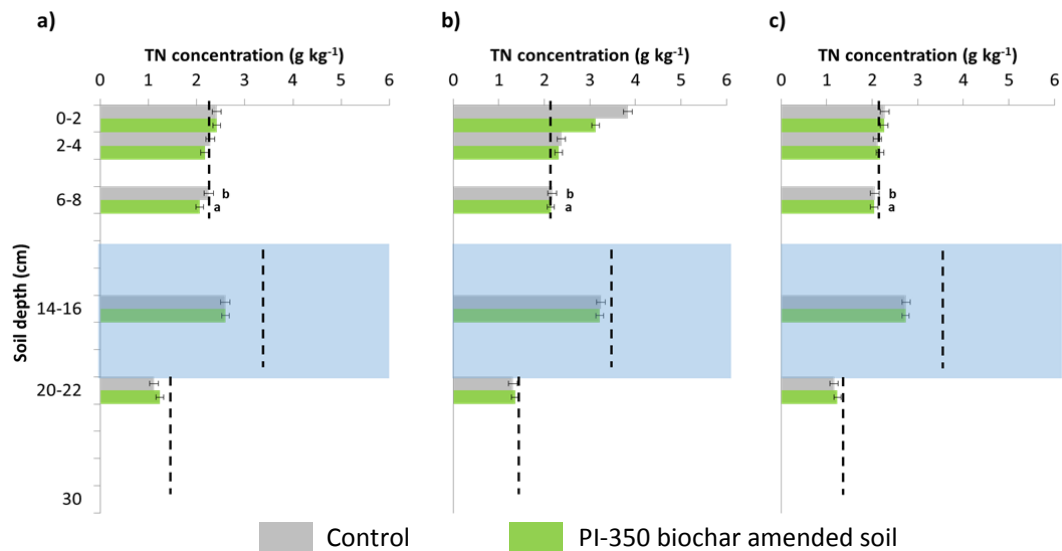


Figure 5.2 Changes in soil TN concentration (g kg^{-1}) with depth after biochar addition and growth of different pasture species in lysimeters containing Tokomaru silt loam soil (experiment 1): a) ryegrass; b) red clover-cocksfoot mixture; and c) chicory. Data represent the average and the standard error the means (SEM) ($n = 4$) (For each depth, bars with different letters indicate significant differences at $P < 0.05$ between control and biochar amended soil; shaded area represent the depth of biochar application) vertical dashed line represent the TN concentration at T₀.

5.3.1.2. Soil C and N concentrations in the Motuiti soil (MS) soil under different pasture types with and without the addition of BG-550 biochar (experiment 2) after 2 years of pasture establishment

After 2 years of pasture establishment (T₂), the top 0–10 cm the MS soil showed a higher soil TC concentration ($12.4 - 15.4 \text{ g TC kg}^{-1}$) than that at T₀ (Fig. 5.3). At 0–2 cm soil depth and in the absence of biochar, TC concentration under the mixture pasture (18 g TC kg^{-1}) ($P < 0.05$) was significantly higher than those found under ryegrass ($15.9 \text{ g TC kg}^{-1}$) and lucerne ($14.1 \text{ g TC kg}^{-1}$) (Fig. 5.3). The soil TC concentration in the 10–20 cm soil depth after 2 years of pasture establishment ($24.3 - 29 \text{ g C kg}^{-1}$) was relatively similar to that at T₀ (Fig. 5.3). However, the TC concentration in the MS soil, independently of pasture type, did not significantly increase in the presence of BG-550 biochar (Fig. 5.3). After 2 years and for all pasture treatments, the TN concentration in the top 10 cm of the MS soil ($1.1 - 1.3 \text{ g TN kg}^{-1}$) was similar to that at T₀ (Fig. 5.4). The application of this ash-rich BG-550 biochar did not change the TN content in the 10–20 soil depth over time (Fig. 5.4).

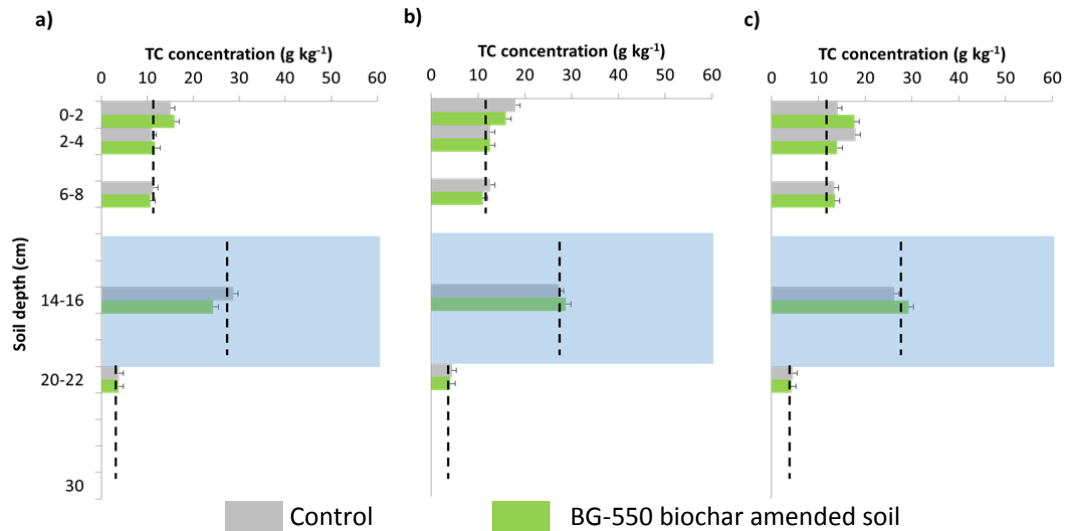


Figure 5.3 Changes in soil TC concentration (g kg^{-1}) with depth after biochar addition and growth of different pasture species in lysimeters containing Motuiti sandy soil (experiment 2): a) ryegrass; b) cocksfoot and red clover mixture; and c) lucerne. Data represent the average and the Standard error the means (SEM) ($n = 4$) (For each depth, bars with different letters indicate significant differences at $P < 0.05$ between control and biochar amended soil; shaded area represent the depth of biochar application) vertical dashed line represent the TC concentration at T_0 .

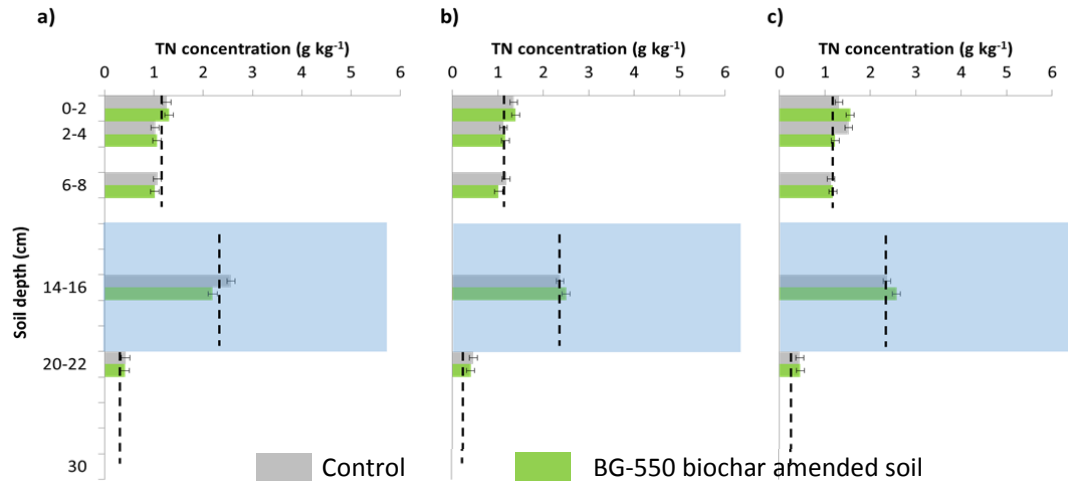


Figure 5.4 Changes in soil TN concentration (g kg^{-1}) with depth after biochar addition and growth of different pasture species in lysimeters containing Motuiti sandy soil (experiment 1): a) ryegrass; b) cocksfoot and red clover mixture; and c) lucerne. Data represent the average and the standard error the means (SEM) ($n = 4$) (For each depth, bars with different letters indicate significant differences at $P < 0.05$ between control and biochar amended soil; shaded area represent the depth of biochar application) vertical dashed line represent the TN concentration at T_0 .

5.3.2. Assessment of soil C and N stocks

5.3.2.1. Comparison between the different methodologies to calculate soil C (and N) stocks

The soil TC (and TN) stocks were first computed by using the more common calculation at a fixed depth (FD). In order to avoid any inaccuracy resulting from differences in soil BD caused by the presence of biochar and plant roots, calculations on soil TC (and TN) then were made using an alternative methods of a fixed mass (FM) and an equivalent soil mass approach (ESM). The soil mass and the respective soil TC and TN stocks are presented in the Table A4.01 and Table A4.02 in Appendix 4. Both the FM and the ESM methods showed a strong positive correlation ($r = 0.967$ and $r = 0.958$, respectively) with the net difference in soil TC stocks (i.e., $TC_{\text{Control}} - TC_{\text{biochar-amended}}$) calculated for each nominal depth using the FD method. The strong correlation value indicates that both the FM and ESM methods have a similar trend in calculating the differences in soil TC stocks between biochar treatments. The application of ESM calculations is often used when there are no soil BD data and only the TC concentration and the soil mass data are needed. On the other hand, the FM method amends the soil C stocks calculated at a fixed depth by subtracting the additional C due to differences in BD at each nominal depth. As data on soil BD (Appendix 3 and 4; Calvelo Pereira *et al.*, 2015) were available for all the soil samples, it was considered convenient here to use the FM approach for a detailed assessment of TC changes with depth in both pastures with different root systems and biochar as soil amendment.

5.3.2.2. Change in soil C and N stocks in experiment 1

Soil TC and TN stocks for the TK soil down to a nominal depth of 30 cm were calculated at T0 and T2 using the FM method; and values are shown in Table A4.01 and Table A4.02 in Appendix 4. At T0, the soil TC stock was $64.1 \text{ Mg C ha}^{-1}$, with 29, 48, and 23% of it distributed at 0–10, 10–20, and 20–30 cm soil layers, respectively. At the end of the experiment and in the absence of biochar, the TK soil under ryegrass and chicory always had a lower soil TC stocks (53.2 and $58.0 \text{ Mg C ha}^{-1}$, respectively) compared with the corresponding TC stock at T0 ($64.1 \text{ Mg TC ha}^{-1}$). The mixture of cocksfoot and red clover at T2, however, showed a higher soil TC stock (73 Mg TC ha^{-1}) compared with the TC stock at T0. When comparing between pasture species, the mixture of

cocksfoot and red clover always showed the highest soil TC ($P < 0.05$), regardless the addition of PI-350 biochar (Table A4.01, in Appendix 4). At T2, the application of PI-350 biochar to the TK soil significantly increased ($P < 0.05$) the soil TC of all pasture compared with the control treatment (Table A4.01 in Appendix 4). The highest increase of soil TC was recorded in the ryegrass with PI-350 biochar (an increase of 9.9 Mg C ha⁻¹) compared with the other pasture types (mixture pasture: 4.3 Mg C ha⁻¹; chicory: 5.3 Mg C ha⁻¹).

The soil TN stocks showed a similar pattern to the soil TC stocks described above (Table A4.02 in Appendix 4). At T2, the soil TN stocks showed lower values than those at T0, with N losses ranging from 1.2 to 2.1 Mg N ha⁻¹. The application of PI-350 biochar did not affect TN stocks.

5.3.2.3. Changes in soil C and N stocks in experiment 2

Soil TC and TN stocks for the Motuiti soil were calculated at T0 and T2 using the FM method down to a nominal depth of 30 cm, and are shown in Table A4.01 and Table A4.02 in Appendix 4. In the absence of biochar, the soil TC stock at T0 was 50.6 Mg C ha⁻¹, with 30, 61, and 9% of it being distributed in the 0–10, 10–20 and 20–30 cm depth. After 2 years (T2) of pasture establishment and the absence of biochar addition, all pasture was recorded as having have soil TC stocks similar to those at T0 (T0 = 50.6 Mg C ha⁻¹; T2 = 52.5 – 54.0 Mg C ha⁻¹). The addition of the ash-rich BG-550 biochar to this sandy MS soil did not influence the soil TC stocks under any pasture treatment (Table A4.01 in Appendix 4). Interestingly, the Motuiti soil amended with BG-550 biochar under ryegrass showed a slightly lower TC stock ($P > 0.05$) compared with the corresponding control treatment (47.4, and 52.5 Mg C ha⁻¹, respectively). Similar trends to those described for TC were also observed in the TN stocks (Table A4.02 in the appendix 4), with losses of N being also observed in the ryegrass, especially at the 20-30 cm soil depth.

5.3.3. Effect of biochar on net changes in TC and TN stocks compared to non-biochar treatments at T2

The net effect of biochar addition to soil TC and TN stocks was tested by assessing the net difference in soil TC (and TN) stocks [i.e., TC (or TN)_{Control} – TC (or TN)_{biochar-amended}]

at T2 for each nominal depth considered (Table A4.01 and Table A4.02 in the appendix 4). In experiment 1 on the TK soil, the application of PI-350 biochar (equivalent to 7.6 Mg C ha⁻¹) resulted in a net increase (ranging from 6 to 16%) in the TC stocks for the soil profile down to nominal depth of 30 cm (Fig. 5.5). Changes in the soil TC stocks were always more apparent for the 10–20 cm depth these being always significant (Fig. 5.6). The treatment of TK soil amended with PI-350 biochar under ryegrass showed a higher net C gain compared with the other pasture species (Fig. 5.5). The addition of PI-350 biochar (equivalent to 0.03 Mg TN ha⁻¹) did not influence the net difference in TN stock (Fig. 5.5), as expected.

In the experiment 2 on the Motuiti soil, the addition of BG-550 biochar (equivalent to 3.6 Mg C ha⁻¹ and 0.14 Mg N ha⁻¹) did not change the net soil TC and TN stock difference at T2 for the entire soil profile to a nominal depth of 30 cm (Fig. 5.6).

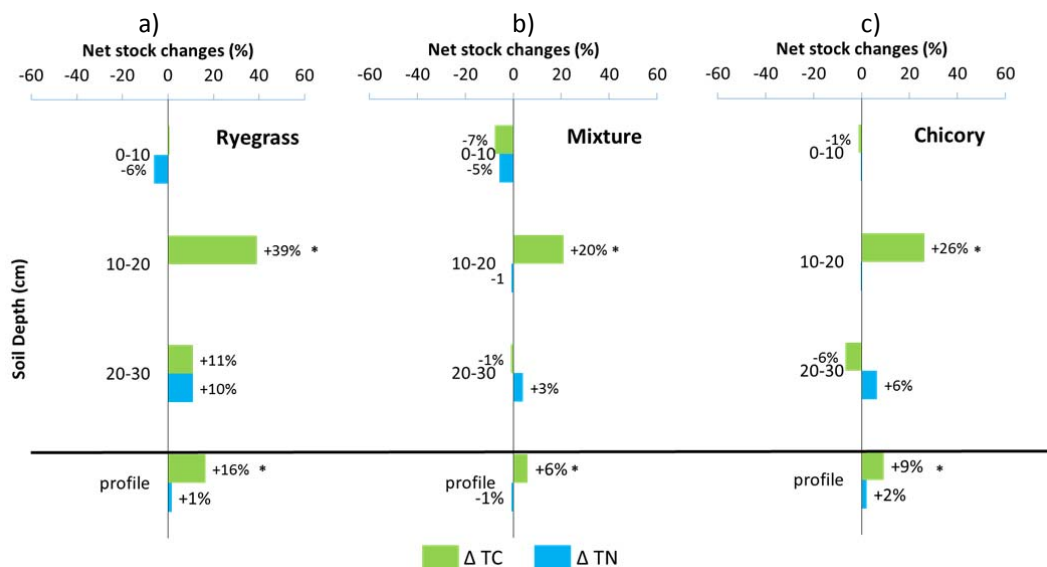


Figure 5.5 Net gain and losses of TC and TN stocks in the biochar-amended treatments compared to the non-amended ones [Δ TC and Δ TN: (stocks_{control} – stocks_{biochar-amended}), in percentage] after 2 years of pasture establishment in the TK soil: a) ryegrass, b) cocksfoot and red clover mixture, c) chicory. Value followed by * showed a significant difference at $P < 0.05$

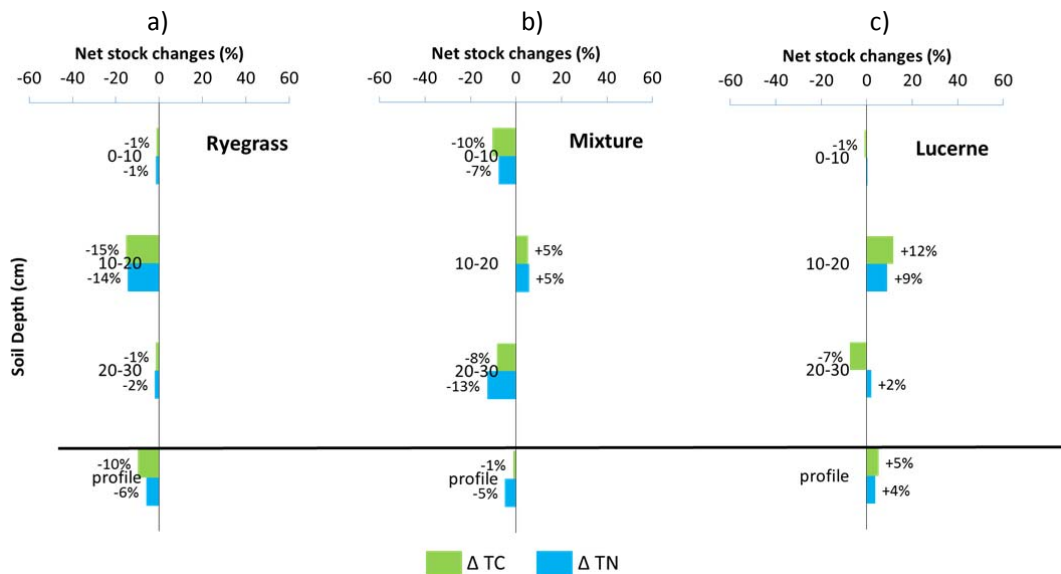


Figure 5.6 Net gain and losses of TC and TN stocks in the biochar-amended treatments compared to the unamended ones [Δ TC and Δ TN: (stocks_{control} – stocks_{biochar-amended}), in percentage] after 2 years of pasture establishment in the MS soil: a) ryegrass, b) cocksfoot and red clover mixture, c) lucerne . Value followed by * showed a significant difference at $P < 0.05$

5.4. Discussion

5.4.1. TK soil under different pastures with and without PI-350 biochar amendment (experiment 1)

After 2 years, TK soils under ryegrass and chicory showed losses of soil C, especially at the 10–20 soil depth, which was the original topsoil layer before to soil inversion. It is hypothesised that a considerable fraction of the organic C in this soil layer had a fast turnover rate (Calvelo Pereira *et al.*, 2015) implying that, when in the topsoil, decomposing C was adequately replenished by the C input from growing pasture roots. However, once the original topsoil layer was inverted, i.e., placed at 10–20 cm depth, the input of C from roots from shallow-rooting species was not able to maintain the original C level of the buried layer (as seen in Chapter 4; Fig. 4.6). Similar results have been reported under field conditions (Linsler *et al.*, 2010; Rutledge *et al.*, 2014; Velinga *et al.*, 2004).

Ryegrass and chicory have shallow root systems. Moreover, the fact that during the 2 years of this lysimeter experiment, substantial amounts of nutrient were applied at the

surface as inorganic fertilizer and also as cow dung and urine (Chapter 4; Fig. 4.2) may have contributed to the concentration of roots in the 0–10 cm soil depth (Chapter 4; Fig. 4.6), resulting in lower plant C allocation to roots in the 10–20 cm depth soil layer.

The Tokomaru soil under the cocksfoot and red clover mixture was able to maintain and even increase the soil TC stocks over time, despite the soil inversion (Table A4.01 in Appendix 4). In fact, under this pasture, a slight increase of soil TC stocks (22–32%) compared with the T0 TC stocks was observed in the top 0–10 cm depth. Assuming that root biomass contains approximately 40% of biomass C for all pastures, the mixture of cocksfoot and red-clover added 28–32 g C kg⁻¹ (calculated from the total live and dead root biomass data; Table 4.7 in Chapter 4) into the soil at 0–10 cm depth over the 2 years of study, which was more than what added ryegrass and chicory (both adding 22 g C kg⁻¹). In the subsoil, the cocksfoot and red clover mixture was able to total 34–41 g C kg⁻¹, which compensated for the loss of C that resulted from the decomposition of native OM. Previous studies have demonstrated that diverse pasture species were associated with a greater plant root biomass (Mueller *et al.*, 2013; Tilman *et al.*, 1996), and had concomitant effect on soil TC stocks.

The application of wood derived PI-350 biochar to the Tokomaru soil at the 10–20 cm depth increased the net TC stocks, especially in the 10–20 cm soil depth (Fig. 5.5). This was expected, since the C-rich PI-350 biochar was added to this particular soil depth. An increase of soil TC in the same Tokomaru soil amended with a C-rich biochar from corn stover was found by Herath *et al.* (2015). Since no increase in below-ground biomass production was detected following biochar application (Table 4.7, in Chapter 4), it is suggested that the C content from the PI-350 biochar (equivalent to 7.6 Mg C ha⁻¹) might alone be responsible for the increase in the soil TC stock, as shown by Calvelo Pereira *et al.* (2015), inferring the C originated from biochar as an increase in the non-oxidisable fraction (the fraction that does not react with potassium dichromate).

The results from experiment 1 also suggest that the PI-350 biochar is not actively contributing to the protection of soil OM against decomposition. Herath *et al.* (2015) showed that the addition of corn stover biochar produced at two different

temperatures (350 and 550°C) into the topsoil of the same Tokomaru soil contributed to the protection of the native soil OM after 510 d of incubation in the laboratory. More studies are needed to understand the circumstances favouring the protection of native soil OM against decomposition in the presence of wood biochar.

5.4.2. *MS soil under different pastures with and without BG-550 biochar amendment (experiment 2)*

After 2 years, soil TC and TN stocks of the Motuiti soil under all pasture treatments were similar to the initial (T0) values, with the only exception showed by the ryegrass amended with BG-550 biochar. In contrast to the results from experiment 1, there was no apparent loss of soil TC in the 10–20 cm soil layer. As this layer – originally constituting the Motuiti topsoil as sampled in the field – was inverted (i.e., placed at 10–20 cm depth), the buried OM-rich Motuiti topsoil may have contributed to soil water and nutrient retention at depth. The fact that the soil inversion exposed an OM-depleted soil with a very poor structure and low water retention capacity may have led roots deeper in the profile, as inferred from the estimates of live and root biomass in Table 4.8 (Chapter 4), and subsequently help maintaining the levels of soil C at depth.

After 2 years of pasture establishment in Motuiti soil, both the mixture and the lucerne swards had the highest TC stocks (Table A4.01 in Appendix 4). Similarly, Skinner *et al.* (2006) found that a mixture of pasture species that included clover with deep-rooted cocksfoot had a higher soil TC content compared with the more common ryegrass species. Pastures with higher root biomass production might provide higher allocation of plant C to soil at depth, and increase the TC stocks of soils under a grazing management that includes a high N and P input via commercial fertilizer and cow dung and urine.

The presence of BG-550 biochar (added at a rate of 10 Mg ha⁻¹, equivalent to only 3.6 Mg C ha⁻¹) had no apparent effect on the changes on TC and TN stocks (Fig. 5.6). However, it should be noted that the variability of the TC and TN stocks recorded in this particular depth (standard deviation ranging from 1.8 to 5.1 Mg C ha⁻¹) may infer the difficulty to detect differences between control and the BG-550 biochar treatment. Contrary to the results obtained in Chapter 3 of this thesis, the addition of a biochar

produced from a mixture of biosolids and greenwaste (BG-550 biochar) did not influenced biomass production or allocation (Chapter 4). As opposed to that experiment, where low P and K content in the soil was balanced with the addition of biochar rich in P and K, in the present study a general application of fertilizer was carried out, thus overshadowing the nutrient fertiliser benefit of this biochar. Interestingly, the Motuiti soil under ryegrass amended with BG-550 biochar showed an apparent net loss of soil C (Fig. 5.6a). To some extent, this could be related to the liming effect of the biochar, which may have buffered the acidification process that follows fertilizer application and legume growth. The absence of legumes in this ryegrass treatment may have contributed to a lesser drop of pH, which, along with the presence of a biochar with liming properties, may have promoted conditions more adequate for microbial growth and decomposition of native soil OM (Whitman *et al.*, 2014). More studies are needed to understand the overall effect of amending soils with ash-rich biochars.

5.5. Conclusion

Changes in soil TC stocks in this study were highly influenced by the difference in pasture species grown in the Tokomaru silt loam soil and the Motuiti sandy soil. Of the three pastures growing in the Tokomaru soil, only the mixture of cocksfoot and red-clover was able to maintain (even enhance) the soil TC stocks, and the use of mixed pastures therefore deserves future attention. Both ryegrass and chicory in the TK soil suffered the loss of C associated with the “cultivation”, as simulated by the soil mixing and inversion. The application of the C-rich PI-350 biochar at a dose of 10 Mg ha^{-1} resulted in a net gain of C in this soil. In the Motuiti sandy soil, all pastures were able to maintain the soil TC stocks at depth and no apparent loss of soil C was recorded, independently of the presence and absence of the BG-550 biochar. In the sandy soil, therefore, the inversion of soil horizons could be considered an attractive alternative to enhance pasture soil C stocks in conditions of intense grazing such as those in New Zealand. However, under field conditions, the inversion of horizons in sandy soils will probably be hampered by their poor structure and the risk of wind erosion during the cultivation and establishment phase. Injection of this biochar to a nutrient-depleted subsurface horizon might then be the only viable option. In such a case, biochar would

be added to a low organic matter and low-nutrient subsurface horizon, more prone to respond positively to biochar addition. More research, especially in the field, is needed to understand the mechanisms of which biochar application can enhance the preservation of soil C at depth.

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6.1. Overall Summary and general conclusions

Recent reports on soil carbon (C) stocks in New Zealand pastoral soils suggest the potential for increasing soil C sequestration in subsoils, where C saturation deficits are likely to be greater. The selection of deep-rooted pasture species, as well as the application of a stable C-rich organic amendment, such as biochar, offers the opportunity to increase soil C stocks in subsoils. Converting organic materials into biochar increases the longevity of C. The difference in the nature of feedstocks and pyrolysis conditions could result in a wide range of biochar properties. Because of the variability of biochar properties, the application of specific biochar to a given soil type must be evaluated carefully to avoid negative effects on soil functions. This thesis reported on the effect of biochar addition and the use of pasture species with different rooting systems on soil fertility and carbon storage.

6.1.1. Ryegrass response to the addition of high ash-nutrient rich biochar in the top 10 cm of soil depth

The conversion of organic waste (biosolids and municipal green waste) into biochar not only increases the proportion of stable C, but also captures plant available phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) (Chapter 3). Soil, fertiliser, and biochar-derived nitrogen (N) were found to be less available for uptake by ryegrass during a short-term glasshouse study, when biochar was added to a Waiterere sandy soil (USDA classification: *typic Udipsamment*) at different rates: 0, 1.5, 5 and 10 Mg ha⁻¹ without N fertiliser and at 0, 1.5, 10, and 20 Mg ha⁻¹ with N fertiliser at a dose of 113 kg N ha⁻¹ to the top 10 cm depth. The results indicated that the application of this biochar to a low fertility sandy soil without the addition of N fertilizer might result in soil N immobilisation. This soil N immobilisation was suggested as the main reason for the reduced growth of ryegrass with increased biochar application. The simultaneous application of this high-ash biochar along with N fertilizer was found to increase the ryegrass DM production from 3.3 g pot⁻¹ (control + N fertilizer) to 7.5 g pot⁻¹ (20 Mg ha⁻¹ biochar + N fertilizer). This increase in ryegrass DM indicated that in a soil where N

is not the limiting nutrient factor, the biosolids-derived biochar could be an excellent source of P, K, and Ca to plants.

Another important result from the short-term glasshouse study was that the application of biosolids-derived biochar increased the ryegrass root length, regardless of whether N fertilizer was applied or not. The results obtained from the glasshouse study thus indicate that, under the experimental conditions used, biochar not only increased the soil C stocks as a result of the addition of a highly stable C fraction, but also promoted plant growth and increasing the allocation of C into below-ground biomass.

6.1.2. Pasture response and soil C change following the addition of C-rich biochar in the subsoil of a silt loam soil (lysimeter experiment 1)

Results from this experiment indicated that pasture dry matter (DM) production was more influenced by the pasture species selected than by the application of PI-350 biochar to this silt loam Tokomaru soil at a dose of 10 Mg ha⁻¹ (equivalent to the addition of 7.6 Mg C ha⁻¹) below the top 10 cm depth after simulating cultivation (Chapter 4). The mixture of cocksfoot and red-clover showed the highest total above-ground biomass compared with the other pasture species studied (rye grass and chicory). The application to this soil of woody C-rich biochar produced at 350°C did not result in an increase in either pasture DM production or below-ground root production, which was expected, given the low nutrient fertility value of this biochar and the positive effect of biochar on root growth in the earlier glasshouse study.

The simulated “cultivation” by inverting topsoil into subsoil layer of the Tokomaru soil in experiment 1 resulted in a loss of soil TC observed in the ryegrass and chicory treatments. This apparent loss of soil TC was not detected in the mixture of cocksfoot and red clover. The application of biochar at a dose of 7.6 Mg C ha⁻¹ to the silt loam Tokomaru soil resulted in a net C gain (6–16% over the nil-biochar treatment, $P < 0.05$). This increase in soil total C (TC) stocks mainly occurred in the subsoil (10–20 cm soil depth) layer where the biochar was applied. In the case of ryegrass and chicory, the addition of C from the PI-350 biochar overcame the net loss of native organic C.

Further studies on the impact of biochar on pasture root growth are needed to clarify under what conditions biochar can increase pasture root proliferation.

6.1.3. Pasture response and soil C change following the addition of high ash nutrient-rich biochar in the subsoil of a coarse sandy soil (lysimeter experiment 2)

The application of a nutrient-rich biochar derived from biosolids (BG-550 biochar) to the Motuiti sandy soil at a dose of 10 Mg ha⁻¹ (equivalent to the addition of 3.6 Mg C ha⁻¹) below the top 10 cm depth after simulating cultivation did not significantly ($P > 0.05$) increase the pasture DM production (Chapter 4). The BG-550 biochar used in this experiment had a considerable liming value and nutrient fertiliser value, especially for P and K. However, the periodic application of maintenance fertilizer and the return of animal waste during the 2 years of the experiment might have overshadowed the nutrient value of BG-550 biochar.

The inversion of topsoil into subsoil horizon did not result in the loss of soil TC as observed in the experiment 1. The application of high-ash BG-550 biochar at a dose of 3.6 Mg C ha⁻¹ to this sandy soil did not result in changes of soil TC stocks ($P > 0.05$), but part of this was attributed to the low amount of C added and the high variability of the data (Chapter 5). It was expected that the addition BG-550 biochar to the subsoil (10–20 cm soil depth) could increase the nutrient content at a greater soil depth and subsequently enhance plant root proliferation, but the fact that the soil received periodic surface applications of maintenance fertilizer and animal waste (contrary to what done in experiment 1) may explain the results obtained.

6.2. Recommendation for future research

The experimental work conducted in this thesis was able to deepen our understanding of soil-plant-biochar systems in pasture/grasslands using different swards in New Zealand soils. However, it raises new questions as listed below:

- Changes in the quantity and quality of native organic matter after soil inversion deserve further attention, more specifically the role of different swards in maintaining the C input into the buried layer. For this we propose that the

current soil samples, at present stored in a cold room, are further fractionated and the organic matter is characterised using pyrolysis-GC/MS.

- The native organic matter-biochar interactions and changes over time deserve further research.
- Field studies are required to prove the feasibility of the most favourable options resulting from the current study (e.g., soil inversion + use of mixture sward in the Tokomaru soil), as failure of seedling establishment may compromise its success.
- In the sandy soil, the inversion of topsoil into subsoil horizon could be an alternative soil management to increase soil C stocks under intensive pasture land use. However, this was not feasible in the field conditions of this research since the risk of wind erosion to this sandy soil is high. Hence, the introduction of C-rich biochar into nutrient-depleted subsoil of this sandy soil might provide a better option to increase soil C stocks at depth and deserves future study.

Appendices

Appendix 1. Raw data of the preliminary glasshouse experiment (Chapter 3)

Table A1.01. Average biomass (dry matter) data of ryegrass

Biochar Type	Rate of application	Added N fertilizer	Biomass (g)
BS biochar	1.5 Mg ha ⁻¹	0 mg N	1.24
	1.5 Mg ha ⁻¹	200 mg N pot ⁻¹	4.34
	5 Mg ha ⁻¹	0 mg N	1.56
	10 Mg ha ⁻¹	0 mg N	1.68
	10 Mg ha ⁻¹	200 mg N pot ⁻¹	5.38
	20 Mg ha ⁻¹	200 mg N pot ⁻¹	6.25
BG biochar	1.5 Mg ha ⁻¹	0 mg N	1.61
	1.5 Mg ha ⁻¹	200 mg N pot ⁻¹	3.67
	5 Mg ha ⁻¹	0 mg N	1.75
	10 Mg ha ⁻¹	0 mg N	1.78
	10 Mg ha ⁻¹	200 mg N pot ⁻¹	5.75
	20 Mg ha ⁻¹	200 mg N pot ⁻¹	7.52
control	control	0 mg N	3.03
control	control	200 mg N pot ⁻¹	3.28

BS biochar : biosolid derived biochar

BG biochar : 1:1 biosoild and greenwaste derived biochar

Table A1.02. Root length (cm cm⁻³) measurement and raw data

$$\text{Root length (cm)} = \frac{11}{14} \times \text{grid unit} \times \text{number of intercept}$$

$$\text{Total root length (cm cm}^{-3}\text{)} = \frac{\text{root length} \times 8}{\text{area}}$$

$$\text{Area} = \pi \times 7.5^2 \times 12$$

Biochar	Rate (Mg ha ⁻¹)	Added N (mg)	Number of Intercept	Grid unit	Root length (cm)	Average Root length (cm cm ⁻³)
BS	1.5	0	281	0.5	110.39	0.37
BS	5	0	298	0.5	117.07	0.39
BS	10	0	287	0.5	112.75	0.46
BG	1.5	0	293	0.5	115.11	0.42
BG	5	0	367	0.5	144.18	0.48
BG	10	0	285	0.5	111.96	0.5
BS	1.5	200	548	0.5	215.29	0.74
BS	10	200	631	0.5	247.89	0.98
BS	20	200	858	0.5	337.07	1.28
BG	1.5	200	413	0.5	162.25	0.71
BG	10	200	429	0.5	168.54	0.82
BG	20	200	798	0.5	313.5	1.19
control	control	control	193	0.5	75.82	0.32
control	control	200	412	0.5	161.86	0.68

Table A1.03. Average plant nutrient (Nitrogen and Phosphorus) uptake

Biochar	Added N (mg)	Rate (Mg ha ⁻¹)	Total N biomass (%)	Plant N Uptake (mg pot ⁻¹)	Total P Biomass (%)	Plant P uptake (mg pot ⁻¹)
BS	0	1.5	1.31	16.21	0.51	6.39
BS	0	5	1.18	18.36	0.54	8.45
BS	0	10	1.32	22.07	0.59	9.82
BG	0	1.5	1.29	20.51	0.54	8.69
BG	0	5	1.28	22.18	0.58	10.09
BG	0	10	1.26	22.41	0.62	10.94
BS	200	1.5	2.20	95.85	0.39	16.57
BS	200	10	1.78	92.87	0.45	23.96
BS	200	20	1.77	109.02	0.55	34.14
BG	200	1.5	2.88	109.06	0.38	14.01
BG	200	10	1.75	100.79	0.43	24.61
BG	200	20	1.91	140.89	0.51	37.90

Appendix 2. Data of lysimeter trial experiment 1 (Pastures on loamy Tokomaru soil with and without the application of PI-350 biochar) (Chapter 4 and Chapter 5)

Table A2.01. Average biomass data

Starting date:	Days after sowing	48	67	97	185	296	317	335	359	386
12/13/2010	Sampling date	1/31/2011	2/19/2011	3/21/2011	6/17/2011	10/06/2011	10/27/2011	11/14/2011	12/08/2011	1/04/2012
Pasture	Treatment	g	g	g	g	g	g	g	g	g
ryegrass	control	9.43	7.05	5.28	5.13	9.13	2.40	2.13	8.08	6.20
ryegrass	PI-350 biochar	9.88	7.28	6.28	6.03	9.63	2.43	3.18	8.53	4.63
mixture	control	3.25	6.08	9.83	18.28	20.13	12.30	9.38	8.95	13.53
mixture	PI-350 biochar	3.45	6.68	9.78	17.70	18.50	12.03	9.53	7.50	14.05
chicory	control	3.30	8.40	7.68	3.18	5.43	2.73	4.25	8.13	5.65
chicory	PI-350 biochar	4.45	8.60	6.08	3.35	4.65	2.68	4.18	6.98	5.43

Starting date:	Days after sowing	430	486	575	661	688	720	751	776
12/13/2010	Sampling date	2/17/2012	4/13/2012	7/11/2012	10/05/2012	11/02/2012	12/04/2012	1/04/2013	1/29/2013
Pasture	Treatment	g	g	g	g	g	g	g	g
ryegrass	control	5.63	3.85	3.25	6.75	1.48	11.53	7.73	5.10
ryegrass	PI-350 biochar	4.65	5.43	4.33	7.80	0.78	11.53	7.75	4.15
mixture	Control	12.40	9.75	4.90	15.78	0.83	10.78	14.40	10.70
mixture	PI-350 biochar	13.00	8.85	4.80	13.83	0.60	11.28	14.33	9.43
chicory	control	3.65	2.65	0.00	1.50	0.55	4.88	4.33	4.23
chicory	PI-350 biochar	3.28	2.35	0.00	1.13	0.53	3.83	3.65	3.45

Table A2.02. Average live and dead root biomass data

Pasture	Treatment	average live and dead root biomass (mg cm ⁻³)				
		sampling depth (cm)				
		0-2	2-4	6-8	14-16	20-22
ryegrass	control	15.91	7.04	2.48	2.75	0.69
ryegrass	PI-350 biochar	18.07	7.06	2.40	1.97	0.82
mixture	control	84.24	15.82	8.71	19.85	3.06
mixture	PI-350 biochar	93.66	16.15	9.17	8.91	2.68
chicory	control	28.55	12.69	8.14	9.35	2.47
chicory	PI-350 biochar	19.65	12.19	11.35	15.31	2.35

Table A2.03. Average soil Carbon and Nitrogen concentration data

Pasture	Treatment	average soil C concentration (g kg ⁻¹)				
		sampling depth (cm)				
		0-2	2-4	6-8	14-16	20-22
ryegrass	control	24.71	22.17	20.73	24.48	10.25
ryegrass	PI-350 biochar	26.24	21.37	20.84	34.01	11.37
mixture	control	49.15	25.08	21.77	33.91	13.05
mixture	PI-350 biochar	39.18	24.21	21.17	41.00	12.93
chicory	control	25.74	21.31	20.63	28.24	11.53
chicory	PI-350 biochar	24.41	21.13	20.55	35.61	10.75

Pasture	Treatment	average soil N concentration (g kg ⁻¹)				
		sampling depth (cm)				
		0-2	2-4	6-8	14-16	20-22
ryegrass	control	2.41	2.28	2.25	2.60	1.11
ryegrass	PI-350 biochar	2.41	2.17	2.07	2.59	1.23
mixture	control	3.84	2.38	2.18	3.24	1.31
mixture	PI-350 biochar	3.12	2.32	2.14	3.22	1.36
chicory	control	2.28	2.12	2.06	2.74	1.17
chicory	PI-350 biochar	2.26	2.17	2.04	2.74	1.24

Table A2.04. Average soil bulk density data

Pasture	Treatment	Soil Bulk Density (g cm ⁻³)				
		0-2 cm	2-4 cm	6-8 cm	14-16 cm	20-22 cm
ryegrass	control	1.21	1.24	1.22	1.16	1.45
ryegrass	PI-350 biochar	1.20	1.26	1.25	1.06	1.43
mixture	control	0.86	1.07	1.11	0.94	1.36
mixture	PI-350 biochar	0.98	1.09	1.05	0.90	1.27
chicory	control	1.14	1.18	1.19	1.04	1.42
chicory	PI-350 biochar	1.16	1.21	1.19	0.99	1.41

Appendix 3. Data of lysimeter trial experiment 2 (Pastures on sandy Motuiti soil with and without the application of BG-550 biochar) (Chapter 4 and Chapter 5)

Table A3.01. Average biomass data

Starting date:	Days after sowing	48	67	97	185	296	317	335	359	386
12/13/2010	Sampling date	1/31/2011	2/19/2011	3/21/2011	6/17/2011	10/06/2011	10/27/2011	11/14/2011	12/08/2011	1/04/2012
Pasture	Treatment	g	g	g	g	g	g	g	g	g
ryegrass	control	2.83	3.65	3.05	5.25	6.88	2.58	3.48	7.28	5.45
ryegrass	BG-550 biochar	1.73	2.58	2.85	6.00	7.25	2.18	3.05	7.25	5.23
mixture	control	0.85	3.00	4.63	11.78	8.80	6.95	6.00	5.90	8.05
mixture	BG-550 biochar	0.73	2.08	4.88	11.33	8.38	5.88	5.38	6.78	8.13
lucerne	control	0.38	0.85	2.83	5.20	5.40	5.48	5.63	5.45	7.58
lucerne	BG-550 biochar	0.13	1.25	2.75	5.05	5.03	5.68	5.15	5.48	7.35

Starting date:	Days after sowing	430	486	575	661	688	720	751	776
12/13/2010	Sampling date	2/17/2012	4/13/2012	7/11/2012	10/05/2012	11/02/2012	12/04/2012	1/04/2013	1/29/2013
Pasture	Treatment	g	g	g	g	g	g	g	g
ryegrass	control	5.10	3.33	3.33	6.25	0.70	9.88	7.40	7.68
ryegrass	BG-550 biochar	4.50	2.70	3.10	5.55	0.70	9.33	6.70	7.03
mixture	control	9.15	6.53	3.78	9.23	0.63	9.23	10.05	7.40
mixture	BG-550 biochar	11.25	9.38	6.13	18.55	0.95	15.23	13.88	10.60
lucerne	control	7.05	3.78	2.33	8.08	0.78	8.78	8.25	6.10
lucerne	BG-550 biochar	7.13	3.50	1.95	8.90	0.80	8.88	7.10	7.65

Table A3.02. Average live and dead root biomass data

Pasture	Treatment	average live and dead root biomass (mg cm^{-3})				
		sampling depth (cm)				
		0-2	2-4	6-8	14-16	20-22
ryegrass	control	24.71	11.02	4.11	15.13	2.88
ryegrass	BG-550 biochar	16.64	9.44	4.61	11.02	2.95
mixture	control	65.75	16.51	12.36	30.35	8.67
mixture	BG-550 biochar	60.22	15.34	10.41	29.58	4.29
chicory	control	46.21	22.58	18.44	27.47	8.36

Table A3.03. Average soil Carbon and Nitrogen concentration data

Pasture	Treatment	average soil C concentration (g kg^{-1})				
		sampling depth (cm)				
		0-2	2-4	6-8	14-16	20-22
ryegrass	control	14.99	10.94	11.31	28.71	3.80
ryegrass	BG-550 biochar	15.93	11.68	10.62	24.34	3.75
mixture	control	17.99	12.61	12.63	27.33	4.39
mixture	BG-550 biochar	15.98	12.59	10.97	28.77	4.03
chicory	control	14.09	17.93	13.37	26.19	4.51
chicory	BG-550 biochar	17.71	14.07	13.50	29.26	4.18

Pasture	Treatment	average soil N concentration (g kg^{-1})				
		sampling depth (cm)				
		0-2	2-4	6-8	14-16	20-22
ryegrass	control	1.26	1.02	1.07	2.56	0.42
ryegrass	BG-550 biochar	1.31	1.06	1.02	2.19	0.42
mixture	control	1.35	1.12	1.18	2.37	0.46
mixture	BG-550 biochar	1.39	1.17	1.01	2.51	0.40
chicory	control	1.31	1.52	1.14	2.37	0.45
chicory	BG-550 biochar	1.56	1.23	1.17	2.58	0.46

Table A3.04. Average soil bulk density data

Pasture	Treatment	Soil Bulk Density (g cm ⁻³)				
		0-2 cm	2-4 cm	6-8 cm	14-16 cm	20-22 cm
ryegrass	control	1.28	1.43	1.52	1.25	1.62
ryegrass	BG-550 biochar	1.28	1.48	1.53	1.28	1.63
mixture	control	1.33	1.53	1.59	1.28	1.71
mixture	BG-550 biochar	1.32	1.46	1.59	1.25	1.76
lucerne	control	1.39	1.39	1.52	1.40	1.73
lucerne	BG-550 biochar	1.34	1.49	1.45	1.32	1.70

Appendix 4. Methods to calculate soil Total Carbon (TC) and Total Nitrogen (TN) stocks (Chapter 5)

A. Fixed depth (FD) method

The most basic method for calculating soil C is by the fixed depth approach, which is the product of soil bulk density, depth and C concentration. The fixed depth method has been proposed by the Intergovernmental Panel on Climate Change (IPCC, 2003) as the common method to assess soil TC stock. The soil TC stock in a certain soil depth (in Mg C ha⁻¹) is calculated by:

$$\text{Soil TC}_{\text{FD}} (\text{Mg C ha}^{-1}) = \text{BD} \times \text{soil C concentration} \times L \times 0.1 \quad (1)$$

where BD is the soil bulk density of the corresponding soil layer (g cm⁻³), soil C concentration is in mg C g⁻¹ soil, L is the length/depth of the soil layer considered (cm) and 0.1 is the correction factor from mg C cm⁻² to Mg C ha⁻¹. The total/cumulative soil TC stock up to 30 cm soil depth results from summing up soil C stock of each layer:

$$\text{Soil TC}_{\text{FD}} (\text{Mg C ha}^{-1}) = \text{TC}_{0-2\text{cm}} + \text{TC}_{2-4\text{cm}} + \text{TC}_{4-10\text{cm}} + \text{TC}_{10-20\text{cm}} + \text{TC}_{20-30\text{cm}} \quad (2)$$

B. Fixed mass (FM) method

The fixed mass (FM) method is a correction to the FD method, as proposed by Ellert and Bettany (1995) and Ellert *et al.* (2006). The FD method has been argued to be either over- or under- estimate TC stocks when soil bulk density differs between the treatments being compared. The FM method fixes this mismatch by correcting the excess soil mass in the corresponding soil layer. For all samples in the same soil, the soil mass to a certain soil depth (in Mg ha⁻¹) is calculated by:

$$M_{\text{soil}} (\text{Mg ha}^{-1}) = \text{soil BD} \times L \times 100 \quad (3)$$

Where soil BD is the corresponding soil bulk density in g cm⁻³, L is the thickness of the soil layer (cm), and 100 is the correction factor from g cm⁻² to Mg ha⁻¹. The soil mass reference (M_{ref}) for each soil is the smallest soil mass to a prescribed depth from all treatments. The soil mass excess (M_{ex}) in a certain depth is calculated subtracting the corresponding soil mass (M_{soil}) with the soil mass reference of the same depth:

$$M_{\text{ex}} (\text{Mg ha}^{-1}) = M_{\text{soil}} - M_{\text{ref}} \quad (4)$$

The corrected soil TC stock then calculated as:

$$\text{TC}_{\text{FM}} (\text{Mg C ha}^{-1}) = \text{TC}_{\text{FD}} - \left(M_{\text{ex}} \times \frac{c_{\text{layer}}}{1000} \right) \quad (5)$$

the soil TC_{FM} is the corrected soil TC stocks for a fixed mass of M_{ref} (Mg dry soil); the C_{layer} is the soil C concentration in the same layer (kg C Mg⁻¹ dry soil). The soil TC stock to 30 cm soil depth results from summing up the TC_{FM} of each sampling layer.

$$\text{Soil TC}_{\text{FM}} (\text{Mg C ha}^{-1}) = \text{TC}_{0-2\text{cm}} + \text{TC}_{2-4\text{cm}} + \text{TC}_{4-10\text{cm}} + \text{TC}_{10-20\text{cm}} + \text{TC}_{20-30\text{cm}} \quad (6)$$

C. *Equivalent soil mass (ESM) method*

This method was proposed by Wendt and Hauser (2013), to eliminate the error caused by the variation in soil bulk density caused by different management techniques on the same soil by using a given reference soil mass. The equivalent mass of a soil of a specific soil layer (soil mass per unit area/ M_{Lsoil} , Mg ha^{-1}) provides a consistent basis for comparing soil TC differences between treatments using the same soil. The soil mass layer of sampled area with inside diameter (D , cm) and number of cores sampled (N) is calculated by:

$$M_{\text{Lsoil}} (\text{Mg ha}^{-1}) = \frac{\text{mass}}{\text{area}} = \frac{M_{\text{Sample}}}{\pi \left(\frac{D}{2}\right)^2 \times N} \times 10000 \quad (7)$$

The M_{Lsoil} reference (as the equivalent soil mass) for a specific layer (e.g., 0-10 cm) is that of the heaviest soil mass among the different treatments of a soil (Wendt and Hauser, 2013). The TC stock in the corresponding depth layer (TC_{ESM} , Mg ha^{-1}) is the product of its soil mass reference and the C concentration:

$$\text{TC}_{\text{ESM}} (\text{Mg ha}^{-1}) = M_{\text{Lsoil}} (\text{Mg ha}^{-1}) \times \text{soil C concentration (g kg}^{-1}) / 1000 \quad (8)$$

The cumulative M_{Lsoil} and TC_{ESM} are then calculated by summing up the respective depth, hence:

$$\text{Soil TC}_{\text{ESM}} (\text{Mg C ha}^{-1}) (0-30 \text{ cm}) = \text{TC}_{\text{ESM } 0-10} + \text{TC}_{\text{ESM } 10-20} + \text{TC}_{\text{ESM } 20-30} \quad (9)$$

Reference.

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- Wendt JW, Hauser S (2013) An equivalent soil mass procedure for monitoring soil organic carbon in multiple soil layers. *European Journal of Soil Science* 64(1), 58-65.

Table A4.01 Soil mass and soil TC stocks calculated at a Fixed Depth, a Fixed Mass and an Equivalent Soil Mass

Soil	Pasture	Treatment	Calculated at a Fixed depth		Calculated at a Fixed Mass ^a		Calculated at an Equivalent Soil Mass ^b	
			Soil Mass Mg ha ⁻¹	soil TC Mg C ha ⁻¹	Soil Mass Mg ha ⁻¹	soil TC Mg C ha ⁻¹	Soil Mass Mg ha ⁻¹	soil TC Mg C ha ⁻¹
Tokomaru	ryegrass	control	3838.0	69.9	2950.8	53.3	3134.6	58.3
	ryegrass	PI-350 biochar	3740.3	79.9	2950.8	63.1	3134.6	69.2
	mixture	control	3376.8	78	2950.8	69.3	3134.6	80.2
	mixture	PI-350 biochar	3214.2	79.4	2950.8	73.5	3134.6	83.2
	chicory	control	3651.1	71.6	2950.8	58.0	3134.6	63.5
	chicory	PI-350 biochar	3605.4	76.3	2950.8	63.3	3134.6	69.3
	T0	T0	3467.6	73.7	2950.8	64.1	3134.6	69.2
Motuiti	ryegrass	control	4314.5	61.5	3773.5	52.5	3773.6	52.8
	ryegrass	BG-550 biochar	4384.6	54.2	3773.5	47.4	3773.6	48.2
	mixture	control	4509.6	62.9	3773.5	53.9	3773.6	54.5
	mixture	BG-550 biochar	4511.0	61.2	3773.5	53.3	3773.6	54.1
	lucerne	control	4592.7	65.1	3773.5	54.0	3773.6	54.2
	lucerne	BG-550 biochar	4461.5	66.3	3773.5	56.9	3773.6	57.1
	T0	T0	4607.4	58.5	3773.5	50.7	3773.6	49.8

^a Fixed mass method by Ellert *et al.* (2008)

^b Equivalent soil mass method by Wendt and Hauser (2013)

Table A4.02 Soil mass and soil TN stocks calculated at a Fixed Depth, a Fixed Mass and an Equivalent Soil Mass

Soil	Pasture	Treatment	Calculated at a Fixed depth		Calculated at a Fixed Mass ^a		Calculated at an Equivalent Soil Mass ^b	
			Soil Mass Mg ha ⁻¹	soil TN Mg C ha ⁻¹	Soil Mass Mg ha ⁻¹	soil TN Mg C ha ⁻¹	Soil Mass Mg ha ⁻¹	soil TN Mg C ha ⁻¹
Tokomaru	ryegrass	control	3838.0	7.4	2950.8	5.7	3134.6	6.1
	ryegrass	PI-350 biochar	3740.3	7.2	2950.8	5.7	3134.6	6.2
	mixture	control	3376.8	7.5	2950.8	6.6	3134.6	7.5
	mixture	PI-350 biochar	3214.2	7.1	2950.8	6.5	3134.6	7.2
	chicory	control	3651.1	7.0	2950.8	5.7	3134.6	6.2
	chicory	PI-350 biochar	3605.4	7.0	2950.8	5.8	3134.6	6.3
	T0	T0	3467.6	7.8	2950.8	6.7	3134.6	7.4
Motuiti	ryegrass	control	4314.5	4.8	3773.5	5.1	3773.6	4.8
	ryegrass	BG-550 biochar	4384.6	5.1	3773.5	4.4	3773.6	4.4
	mixture	control	4509.6	5.6	3773.5	4.8	3773.6	4.8
	mixture	BG-550 biochar	4511.0	5.5	3773.5	4.8	3773.6	4.8
	lucerne	control	4592.7	5.9	3773.5	4.9	3773.6	4.9
	lucerne	BG-550 biochar	4461.5	6.0	3773.5	5.1	3773.6	5.2
	T0	T0	4607.4	5.4	3773.5	4.6	3773.6	4.6

^a Fixed mass method by Ellert *et al.* (2008)

^b Equivalent soil mass method by Wendt and Hauser (2013)