EFFECT OF BIOCHAR ON SELECTED SOIL PHYSICAL PROPERTIES OF SANDY SOIL WITH LOW AGRICULTURAL SUITABILITY

by

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ABSTRACT

Biochar has been labelled to be a key factor in the global carbon mitigation act and has been described as the modern day equivalent (*terra nova*) to the *terra preta* dark earth soils of the Brazilian Amazon. Globally biochar has been evaluated as a means to improve soil fertility and to mitigate greenhouse gases (GHGs). Little research has however been published on the effects of biochar incorporation on soil physical properties.

The objective of this study was to evaluate the effect of pine sawmill waste derived biochar (locally-produced *via* slow pyrolysis – 450℃) on selected soil physical properties, soil-water dynamics and crop production and- performance, when amended to a Kroonstad (Kd 1000 – Morgendal) soil form. This soil form is commonly found in the Western Cape area (South Africa) and can be classified as having low agricultural suitability for perennial- and annual crop species.

Two pot trials were carried out in an atmospheric controlled greenhouse, where winter wheat and green beans respectively were planted, with five different application levels of biochar (0t/ha, 1t/ha, 10t/ha, 50t/ha and 200t/ha). Soil physical properties namely, water-stable aggregates, bulk density and water-retention capacity along with physiochemical characterisation of the sandy soil and biochar was determined. The water-use was monitored throughout the trials (evapotranspiration, volumetric water content and biomass water use efficiency, BWUE). The above- and below ground (specific leaf traits for the green bean and the root structural development for the winter wheat) biomass was collected and analysed at harvest.

There was significantly higher volumetric water content measured for the 50t/ha and 200t/ha biochar treatments. This effect can be ascribed due to a change in the soil's tortuosity and porosity where more meso- and micro-pores were present as the biochar rate increased. The same results were evident when a water-retention curve was established *in vitro* by means of the sandbox method. The bulk densities were only significantly lower for the 200t/ha biochar treatments.

The wheat root systems differed greatly among the fertilised biochar treatments: the 50t/ha and 200t/ha treatments had a more complex fibrous root system (more extensive branching and thinner roots) than 0t/ha, 1t/ha and 10t/ha application levels. This is attributed to the increased water-holding capacity along with a reduction of N- and P availability with increasing addition of biochar. Several leaf traits were measured for the green bean crops; however the leaf nitrogen- and carbon content, chlorophyll content index (CCI) and carbon isotope fractionation yielded the most interesting findings. Concerning the fertilised biochar treatments, there was established that the 10t/ha treatments had the highest leaf nitrogen- and carbon content. The leaf chlorophyll content did not differ significantly between the fertilised biochar treatments; however a very interesting observation was evident regarding the measured leaf CCI for the unfertilised treatments. A decreasing trend and lower leaf CCI was measured as the biochar application levels increased. This effect was ascribed to be due to a decrease in N uptake by the plants as the biochar application increased, the C/N ratio also increased, and this leading to N immobilisation. The lowest leaf carbon isotope fractionation was measured for the 10t/ha fertilised treatments and is inversely correlated with BWUE and therefore endorses the conclusion that the 10t/ha biochar application had a positive effect on the long term water use efficiency for the green bean plants.

Biochar promoted aggregation in the sand-rhizosphere interface for winter wheat, increased water-holding capacity and enhanced crop performance for green beans. The findings reported here provide new information on the effect of biochar on the structural development of sandy soil, combined with biochar- and root growth effects for winter wheat; along with detailed interpretations of specific leaf traits associated with crop production for commercial green beans. The addition of biochar at low application levels (approximately 1-10t/ha to 15 cm depth) increased the biomass yield and water use efficiency of the crop species. Besides long term carbon storage, biochar can have immediate positive effects on the physical properties of sand and plant growth.

OPSOMMING

Biokoolstof word beskou as ‘n sleutel komponent rakende die wet op globale koolstofvermindering en is al beskryf as die moderne ekwivalent (*terra nova*) van die *terra pret*a donker-aardgronde wat aangetref word in die Brasiliaanse Amasone. Wêreldwyd word biokoolstof tans geëvalueer met die doel om grondvrugbaarheid te verbeter asook kweekhuisgasse (KHG) se nadelige gevolge te verlig. Min navorsing was tot dus ver gedoen rakende die uitwerking met toediening van biokoolstof op grondfisiese-eienskappe.

Die doel van hierdie studie was om die effek van biokoolstof, wat afkomstig is van denne-saagmeul-afval (plaaslik geproduseer is en d.m.v. stadige perolise - 450℃) te evalueer aangaande die volgende faktore: geselekteerde grondfisiese-eienskappe, grond-waterdinamika interaksie en die uitwerking op gewasproduksie; met toediening aan 'n Kroonstad (Kd 1000 - Morgendal) grondvorm. Hierdie grondvorm word as algemeen in die Wes-Kaap (Suid-Afrika) bestempel en kan geklassifiseer word as ‘n lae-geskiktheid landbougrond vir meerjarige- en eenjarige gewasse.

Twee potproewe is uitgevoer onder beheerde atmosfeer in ‘n kweekhuis, waar winter koring en groenbone geplant is, met vyf verskillende behandelings van biokoolstof (0t/ha, 1t/ha, 10t/ha, 50t/ha en 200t/ha). Die volgende grondfisiese-eienskappe is ondersoek, naamlik water-stabiele aggregaat formasie, bulkdigtheid en waterhouvermoë, asook die fisiochemiese karakterisering van die sanderige grond en biokoolstof wat gebuik is. Waterverbruik is gedurende die proewe gekontroleer (evapotranspirasie, volumetriese waterinhoud en die biomassa se water verbruiksdoeltreffendheid, BWVD). Die bo- en ondergrondse biomassa, spesifiek die blaareienskappe van die groenboontjie en die strukturele ontwikkeling van die winter koring se wortels, is tydens die oes ondersoek en ontleed.

Die volumetriese waterinhoud was betekenisvol, asook hoër vir die 50t/ha en 200t/ha behandelings. Hierdie effek word toegeskryf as gevolg van 'n verandering in die grond se kronkeligheid en porositeit; waar meer meso- en mikroporieë teenwoordig was soos die biokoolstof inhoud toegeneem het.

Dieselfde resultate was verkry met die opstelling van ‘n water-retensie kurwe *in vitro* d.m.v. die Sandboks metode. Bulkdigtheid was slegs betekenisvol verskilled asook aansienlik laer vir die 200t/ha biokoolstof behandelings. Die koring se wortelstelsel het drasties verskil tussen die verskillende bemeste biokoolstof behandelings: die 50t/ha en 200t/ha behandelings het 'n meer komplekse en veselagtige wortelstelsel gevorm (hoër graad van vertakking en dunner wortels was aanwesig) as die 0t/ha, 1t/ha en 10t/ha behandelings. Die effek word toegeskryf aan die toenemende waterhouvermoë, tesame met 'n tekort aan N- en P-beskikbaarheid soos die biokoolstof toedieningshoeveelhede verhoog het. Verskeie blaareienskappe is gemeet vir die groenboon gewasse, maar die blaar stikstof- en koolstof-inhoud, chlorofil inhoud indeks (CII) en koolstof-isotoop fraksionering het die mees interessante bevindinge opgelewer. Die hoogste blaar stikstof-en koolstof-inhoud is gemeet vir die 10t/ha bemeste biokoolstof behandelings. Die blaar chlorofil inhoud het nie beduidend verskil tussen die bemeste biokoolstof behandelings nie, maar daar was egter 'n baie interessante waarneming vir die onbemeste biokoolstof behandelings.

‘n Tendens was aanwesig waar die CII afgeneem het soos die biokoolstof toedieningshoeveelheid ook afgeneem het vir die onbemeste behandelings. Die effek word toegeskryf as gevolg van 'n afname in N-opname deur die plant soos die biokoolstof toedieningshoeveelheid verhoog is en tot gevolg gehad het dat die C/N-verhouding ook toegeneem het, wat gelei het tot N-immobilisasie. Die laagste blaar koolstof-isotoop fraksionering was geassioseer met die 10t/ha bemeste biokoolstof behandelings en is omgekeerd gekorreleerd met BWVD en onderskryf dus die gevolgtrekking dat die 10t/ha biokoolstof behandeling 'n positiewe uitwerking het op die langtermyn waterverbruiksdoeltreffendheid vir groenboontjie plante.

Biokoolstof het aggregasie bevorder binne die wortelsone, asook deurgans die waterhouvermoë verhoog en gewasproduksie verbeter. Hierdie bevindinge lewer nuwe inligting oor die effek van biokoolstof op die strukturele ontwikkeling van sanderige grond en die gekombineerde interaksie met biokoolstof toediening en hoe dit wortegroei beïnvloed van winter koring; asook 'n gedetailleerde interpretasie van spesifieke blaareienskappe wat verband hou met die produksie van gewasse vir kommersiële verbouing soos die groenboontjie. Die toediening van biokoolstof by die lae hoeveelhede (ongeveer 1-10t/ha tot op 15 cm diepte) het die opbrengs en waterverbruiksdoeltreffendheid van die gewasse verbeter.

Behalwe vir die langtermyn koolstofvaslegging, kan biokoolstof toediening onmiddellike positiewe resultate teweeg bring aangaande die fisiese eienskappe van sandgronde en plantegroei.

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INTRODUCTION

Awakening interest, especially in the modern- and sustainable agricultural community, has came forth were an age old concept, regarding ancient agricultural practices of the Amazonian people are being rediscovered namely biochar. Biochar is believed to be one of the key solutions in nullifying our previous anthropogenic wrongdoings to our environment.

Worldwide population pressures are on the rise and not only are we, the human-race, aiming for dramatic yield increases regarding agricultural commodities; we have all together shift our focus to being more environmental friendly and overall striving to adapt sustainable practices, regarding energy- and food production systems. This is where biochar has gained its sophistication in the last few years, as bio-oil production increased by means of the demand for more sustainable energy technologies, so has this by-product surfaced in today's modern agricultural sciences.
Low potential sandy soils are commonly found in the Western Cape area (South Africa). The role of biochar in the modification and possible stabilisation of soil structure, especially in sandy soils, is therefore a desirable field of study, particularly when events such as root logging, erosion and drought of annual agricultural crop species could be minimised. However, there has been no attempt currently to critically evaluate the effects of biochar amendment on South African soils regarding physical properties, moreover agricultural productivity.

The project has two principal objectives:

Firstly it aims to provide an assessment of the physical properties for the selected soil used throughout the experimental procedure, and the characterising of the biochar. The physical properties that will be focused on, includes the following: particle- and bulk density, particle-size distribution, specific surface area and aggregate stability formation (as structural formation increase for sandy soils, erosion will decrease).

The second main objective is to determine the influence of biochar, at different application levels, and how this effect selected soil physical properties and crop productivity. Crop productivity will further be assessed, by studying in detail selected plant responses (chlorophyll content and all traits that will be indicative towards plant-water dynamic interaction), thereby evaluating the possible benefits of biochar application to specific agronomy crops.

There are several uncertainties coupled with biochar as a soil amendment, and therefore this dissertation will aim to help understand the crop, soil (physical properties) and biochar specific interactions under controlled conditions for a South African sandy soil, with low agricultural suitability.

The first Chapter is a literature review on biochar production and why this subject has reached global demand. It also includes previous studies and research findings with their exclusive results regarding biochar addition with detailed summarised agricultural scenarios.

The second Chapter is dedicated to material and methods and all related equations used throughout the experiment.

The third Chapter focuses on the structural development of a sandy soil after biochar addition and how it influences a commercially grown agricultural crop's root structural development and plant water status.

The fourth Chapter investigates and focuses on crop specific responses and the plant nutrients status (focusing on N), along with a section specifically researching the water-retention capacity.

A general conclusion as well as future research recommendations in soil physical science and biochar agricultural research has been included.

CHAPTER 1: LITERATURE REVIEW

1.1 Bioenergy production from biomass resources and biochar formation: An introduction

Bioenergy sources contribute around one-tenth of the global primary energy usage, where 10% is produced from modern bioenergy technology. Bioenergy conversion can be applied to various processes, such as: power generating (electricity), heat- and fuel production (transport). Biofuel production has especially increased within the last decade and according to Blaschek *et al*. (2010), the total sustainable technical potential of bioenergy is estimated to be around a quarter of current global energy use.

In a broader sense, worldwide we are rethinking the usage of fossil fuels. The era of bioenergy production has step forward for several reasons:

- increased greenhouse gas emissions in the atmosphere - seeking to promote environmental benefits and- health;
- by making biofuel employment is created;
- international investment in science has lead to new alternatives – displacing coal derived energy;
- countries dependent on oil import can benefit economically and politically, by local production of bioenergy;
- lastly, we have a limited supply of fossil fuels (Pandey, 2009).

Our heightened interest in bioenergy can mainly be attributed to the depletion of oil resources and the negative environmental impact associated with the use of unsustainable energy resources (Sims, 2002; Brownsort, 2009; Pandey, 2009).

The global demand for renewable- and non-polluting fuels are on the rise, as is the global energy demand for a rapid growing population of 7 billon people. This scenario can be seen as highly problematic.

According to Pandey (2009), renewable energy is the energy derived from resources that are regenerative or, for all practical purposes, cannot be depleted. Renewable sources like, wind, solar, geothermal, hydrogen, and biomass play a combined and critical role in future energy consumption and- needs. All of the above sources have great potential as reliable and sustainable energy resources, but they lack the array of energy-related products produced from biofuels. Biomass combustion yields the following products, namely: bio-oil, gas and char. The other renewable energy sources can only support generative electrical- and/ or mechanical energy.

Biofuels are the only alternate energy resource for the foreseeable future and can still form the basis of sustainable development in terms of socioeconomic and environmental concerns (Pandey, 2009). Bioenergy is the word used for energy associated with biomass, and biofuel is the bioenergy carrier, transporting solar energy stored as chemical energy (Van Loo and Koppejan, 2008). Biomass resources include wood from sustainably grown plantation forests, residues from agricultural or forest production and organic waste by-products from food and fibre industries, domesticated animals and human activities (Sims, 2002).

Looking at the bigger picture it is safe to say that agriculture is the basis of any- and every successful nation. One of our most fundamental needs is our basic physiological dependence on food and water.

Biochar has been labelled a key factor in the global carbon mitigation act and has been described as the modern day equivalent to the Terra Preta-dark earth-soils of the Amazon (Sohi *et al.*, 2009). Char production has three main purposes in the economy as standing: activated char used in the metallurgical industry, charcoal-briquette production for cooking and as a future soil amending agent to help mitigate carbon dioxide (CO_2), namely biochar (Sims, 2002).

The following question arises: how feasible is biochar application in agricultural practices and what is the long term impact on the environment?

Promoting bioenergy and sustainability, we must think and make use of biochar as a by-product when converting chemical energy contained in the biomass. Biomass has stored chemical potential energy and this energy is derived from photosynthesis. According to Sims (2002), biomass can be defined as: recent organic matter originally derived from plants as a result of the photosynthetic conversion process, or from animals, and which is destined to be utilised as a store of chemical energy to provide heat, electricity, or transport fuels.

There are many forms of biomass, as mentioned before, and to simplify the concept one can see biomass as a potential fuel. The value of this fuel is revealed when the stored chemical energy is released *via* combustion, gasification, pyrolysis and biochemical processes, such as fermentation (Sims, 2002). We can regard biomass and its use to fuel primary energy conversion technology, to be bound by the laws of thermodynamics (Sims, 2002).

The first law of thermodynamics is that the total energy input in a system must always equal that same total energy output (Sims, 2002). Therefore we can not create nor destroy energy, even when changed from one form to another. The second law of thermodynamics basically rest on the concept that as energy flows through successive transformation processes, the total energy to complete useful work at the end will be less (Sims, 2002). Meaning that with the transformation of the energy, from one form to another, work has been done to complete the systematic flow.

The logic behind the laws of thermodynamics (energy) cannot be ignored: our greatest asset to treasure as man is the soil we use for food production and the natural fresh water reservoirs. Biochar is still a relative new field of study and before we start playing the ‘carbon-stock-market’ we need to be clear on the science behind this stable carbon source. The application level, life-cycle and environmental effects of biochar need to be studied in depth, with case specific experiments.

1.2 Main biomass conversion processes

Various processes can be used to convert the useful energy that is stored in biomass. The choice of conversion process depends on the type and quantity of the biomass feedstock, the desired form of the environmental standards, economic conditions, and specific factors for the project (Manual *et al*., 2002).

According to Saxena *et al*., 2008, the two main processes for the conversion of biomass are thermochemical processes and biochemical/ biological processes. We can go further and classify the technology into primary -and secondary conversion processes. Primary technologies referrers to, when raw biomass fuel is converted directly into heat or into more convenient fuels or energy carriers, such as gases (CH_4 and H), liquid fuels (CH_3OH and C_2H_5OH), or char (C).

Secondary technology referrers to the process where these energy carriers that was formed in the primary process part, is transformed to the final and desired energy form. The secondary conversion technology includes energy forms such as: boilers, gas turbines and internal combustion engines. However, in the present context, only the primary thermochemical conversion technologies will be briefly named: combustion, gasification, pyrolysis, liquefaction and hydrogenation.

1.2.1 Combustion

Combustion is one of the oldest methods of obtaining energy (heat). Basically it involves burning biomass in the presence of air, where the stored chemical energy is converted to heat. Combustion can also be used for generating electricity and mechanical power. We can apply combustion on a small domestic scale and on a large scale such as in industrial production.

Combustion can supply hot gases at temperatures that range from 800℃ to 1000℃. The combustion process has many drawbacks, because the biomass used for burning is not always in an acceptable form. Straw, wood, and some other types of biomass require primary treatment such as compressing, chopping, and grinding for better combustion, which can be expensive (McKendry, 2002).

Domestic use can be labelled as being very inefficient, because with heat transfer, loss of 30% to 90% energy can occur.

1.2.2 Gasification

Biomass gasification is based on the partial combustion of the feedstock in restricted oxygen or air supply, therefore yielding gas mixtures. The gas consists mainly out of CO_2, H and CH_4. Producer gas, when converted by secondary energy conversion technology, can fuel the following: gas engines, gas turbines, heat production and electricity.

Producer gas can be used as synthetic gas and this gas allows to produce ammonia and/ or methanol; both used in synthetic petrol production or as a hydrogen source.

1.2.3 Pyrolysis

Pyrolysis can be defined as: the process where biomass is heated in the absence of oxygen in an inert atmosphere where useful products, such as low molecular gases (CO and CO_2), solid products (carbonaceous char) and liquids are yielded. A detailed description of the pyrolysis system will follow in section 1.3 of this chapter, being that this is the main thermochemical process system through which biochar is produced.

1.2.4 Liquefaction

Liquefaction is a low-temperature, high pressure, thermochemical process using a catalyst with the addition of hydrogen and producing a marketable liquid product (Pandey, 2009). Interest in liquefaction is low because the reactors and fuel feeding systems are more complex and more expensive than for the pyrolysis and gasification processes (Demirbas, 2001).

1.2.5 Hydrogenation

Hydrogenation leads to methane production. After the formation of synthesis gas, the gas reacts with hydrogen to produce methane. This is only one route of methane production *via* hydrogenation.

1.3 Pyrolysis of biomass to biochar

In section 1.2.3, the term pyrolysis was defined, and the following definition was given by Pandey (2009) and is worth mentioning: pyrolysis is the thermal decomposition of an organic material in the absence of oxygen, leading to the formation of liquid, gases, and a highly reactive carbonaceous char.

Pyrolysis systems has various parameters, which can be controlled and therefore manipulate the quantity and quality of the products yielded. These parameters include the following: reaction temperature, pressure, heating rate, reaction time and the biomass composition (feedstock). The pyrolysis system can be simplified as such:

1. A heat source generates heat and leads to an increase in the biomass feedstock (fuel) temperature.
2. As the temperature increases the pyrolysis reaction is initiated, leading to the formation of char and the release of volatiles.
3. The volatiles start to flow out as the heat is transferred between the hot volatiles and the cooler unpyrolysed fuel.
4. Tar is produced as the volatiles condensate in the cooler parts of the fuel.
5. The pyrolysis process continues *via* autocatalytic reactions, initiating a secondary pyrolysis process.

The pyrolysis process can be both exothermic and endothermic, all depending on the reaction temperature. The process is endothermic up to 280℃ but exothermic above this, and so requires variable amounts of energy (Antal and Gronli, 2003).

The process steps can be broken down as follow: drying of biomass feed, grinding the feedstock in small particles - suited for rapid reaction, pyrolysis reaction, and the separation of the products.

1.3.1 Types of pyrolysis

Pyrolysis can be grouped in three different categories according to the heating rate and the heat duration, which the feedstock is exposed to. This section will only give a brief introduction to these processes and not an in-depth discussion; also the types of pyrolysis reactors will not be discussed in this context (Figure 1.1).

1.3.1.1 Slow pyrolysis

This is the conventional process whereby the heating rate is kept slow (approximately 5-7℃/min) (Ozbay *et al.*, 2001).This slow pyrolysis process where lower temperature (typically 400℃) and longer vapour residence times is maintained, leads to higher char yields than liquid- and gaseous products. The target product is the char and little liquid- and gas products are usually recovered.

1.3.1.2 Fast pyrolysis

This process is maintained by a high heating rate (approximately 500℃) and a short vapour residence time and favours the production of liquid yields. A very fine feedstock is usually used for this process and in the bio-oil production industry; fluidised-bed reactors are best suited to maintain high heating rates and to help rapidly remove the hot vapour from the solid feedstock.

1.3.1.3 Flash pyrolysis

This is an improved version of fast pyrolysis, whereby high reaction temperature is obtained within a few seconds (Pandey, 2009). This process has an extremely high heating rate (about 1000℃/min) and reaction time lapsed of a few seconds.

The process is usually carried out at atmospheric pressure and various categories are outlined and briefly discussed in the *Handbook of Plant-Based Biofuels* by Pandey (2009).

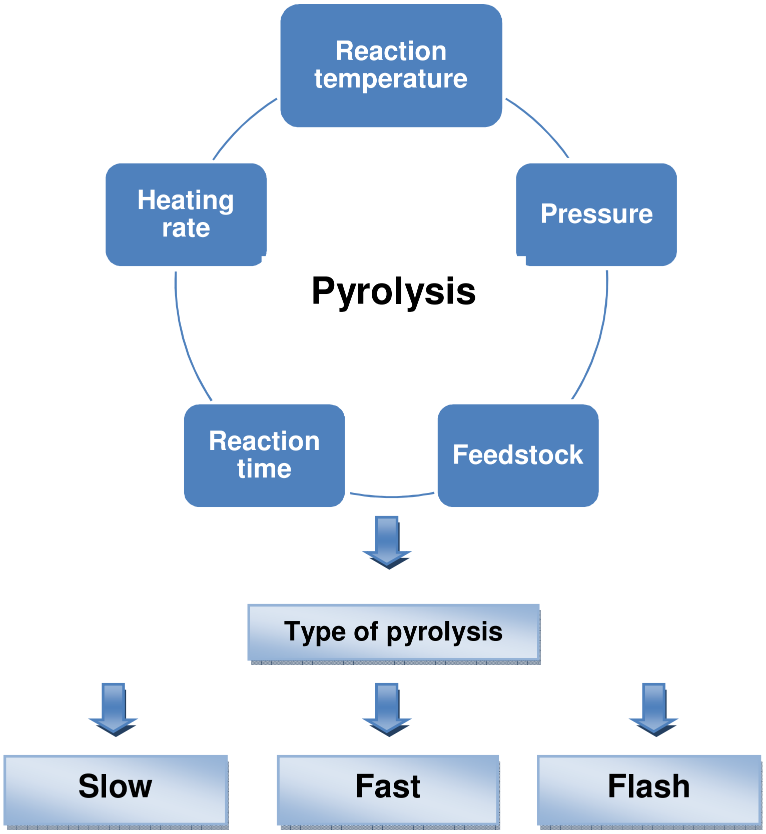

Figure 1.1 Various pyrolysis types

1.3.2 Biochar properties according to feedstock supply

Feedstock materials can be divided into three comprehensive categories: residue plant material, waste derived materials, and energy crops specifically grown as a source of feedstock, for energy supply purposes. Residues from crop production and/ or forestry have been labelled as producing "cleaner" biochars, when measured against waste derived biochar producing systems.

Biochar made from residue feedstock do not lead to land use changes such as in the case of energy crops grown on purpose. When crop residues are not used as a feedstock in pyrolysis, it can simply be worked back into the soil and also contribute to the organic content of the specific soil.

Waste derived feedstock are cheap, in abundance and readily available sources, therefore on paper it sounds ideal. The risk of using waste derived biochar on agricultural soils is due to the fact that contaminants can be present. After overcoming the safety barrier for waste biochars, there's still the term 'waste' connected to the product and therefore less appealing for commercial usage on agricultural soils. Waste chars can rather be used to fuel energy production systems *via* combustion than applying to soil.

Various annual- and perennial crop species have been identified as having high efficiency properties when converting solar energy into stored biomass, which can then be converted into heat, electricity or transport fuels with zero or very low net carbon emissions (Sims, 2002). Concern can be raised if biochar would reach commercial practicality and mass production, because this could lead to the sudden increase of unsustainable energy crops and/ or plantations, especially if non-woody traditional food crops would be used, namely: wheat, sugar cane and sorghum.

1.3.2.1 Feedstock composition

Biomass is generally composed of three main groups of natural polymeric material: cellulose, hemicelluloses and lignin (Brownsort, 2009). As biomass feedstock differs, so does the proportion of the three main groups, as recalled in the above passage, therefore with pyrolysis the product distribution will also be different. Broadly classified, the primary products that form with pyrolysis of hemicelluloses and celluloses are liquid- and gas products. Lignin decomposes to liquid, gas and solid char products (Brownsort, 2009). The primary decomposition of biomass containing lignin and cellulose components, contribute to char yields.

Minerals in biomass, particularly the alkali metals, can have a catalytic effect on pyrolysis reactions leading to increased char yields in some circumstances, in addition to the effect of ash contributing directly to char yield (Brownsort, 2009). Minerals also affect the reactivity and ignition properties of chars (Antal and Gronli, 2003).

1.3.2.2 Feedstock resources

Biomass can be broadly categorised as woody- and non-woody feedstock (Figure 1.2). The following will only serve as an introduction to potential feedstock resources.

Woody biomass consists of plant materials comprising mainly cellulose, hemicelluloses and lignin, and it therefore differs from other biomass materials such as sludges, municipal waste and some agricultural/ horticultural crops (Sims, 2002). An average tree contains about 20% to 30% lignin, and lignin has almost twice the heat value of cellulose. Resources of woody biomass are sources such as: forest residues, residues from wood processing activities (sawmills), energy forest plantations (purposeful grown), and green municipal waste.

Non-woody biomass has the potential to provide both rural and urban areas with renewable energy, especially since there's an array of biomass resources, including the following: energy crops (both annual- and perennial varieties), agricultural crop residues, sewage sludge (both anthropogenic- and/ or animal-derived wastes), and municipal wastes and landfill gas.

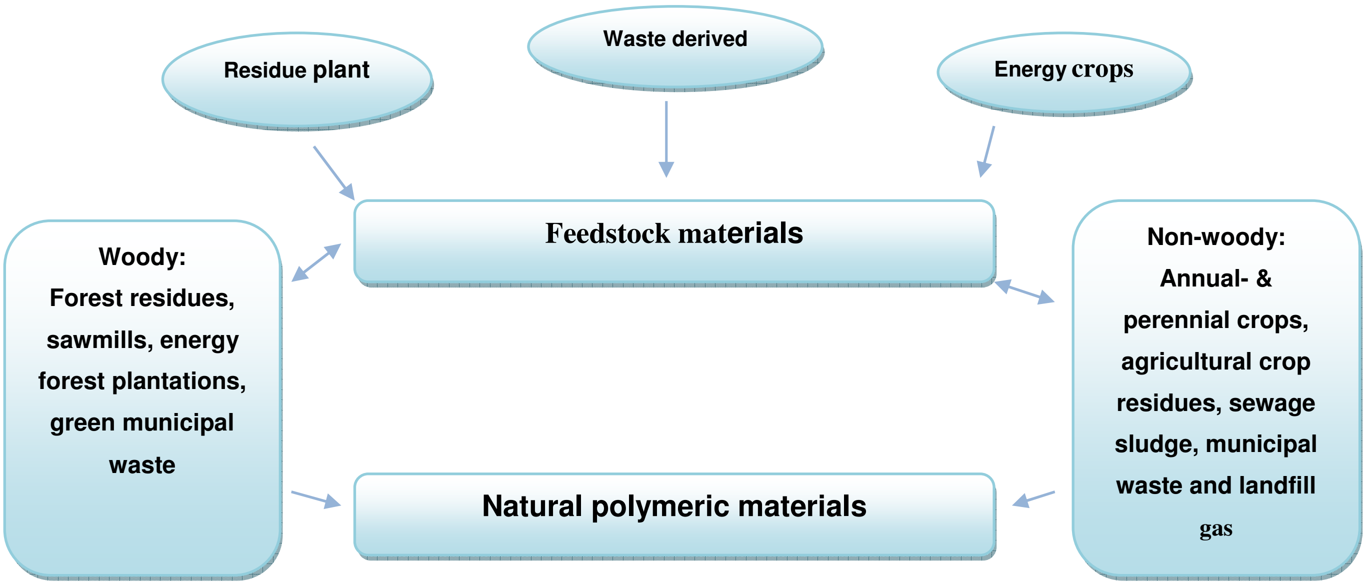

Figure 1.2 Categorised feedstock materials

1.3.2.3 Physical- and chemical properties

Defining the physical and chemical properties of a biochar are essential to help agricultural and pyrolysis engineers understanding the way in which a specific biochar functions within a specific soil type. Atkinson *et al*. (2010) pointed out that a biochar's key characteristics are vital in developing an understanding of its agricultural impacts. Amending soils with biochar had the following modifications reported: structure, texture, porosity, particle size distribution and density (see Amonette and Joseph, 2009), thereby altering the air oxygen content, water storage capacity and microbial and nutritional status within the rhizophere.

It is also apparent that the soil water regime can itself modify biochar stability depending on the initial properties of the feedstock used as biochars produced at lower temperatures and from more labile feedstock are more easily altered (Nguyen and Lehmann, 2009). Biochar is a source of stable carbon fixed as polycyclic aromatic hydrocarbons and therefore hosts an array of functional groups.

The high resistance potential against chemical and/ or microbial decay is derived from the chemical stability, conjugated in the aromatic structure as a six-carbon-carbon ring structure (simples form, benzene) with alternating single- and double bonds.

The heterogeneous composition of biochar means that their surfaces can exhibit hydrophilic, hydrophobic, acidic and basic properties, all of which contribute to their ability to react with soil solution substances (Atkinson *et al*., 2009). Biochar's physical and chemical properties depend primarily on the feedstock material used, the availability of oxygen and the temperature intensity during pyrolysis. It is therefore critical to report in experiments, when amending soils with biochar, the following: feedstock material, duration- and maximum temperature exposure of the feedstock, during pyrolysis. Temperature is primarily responsible for the level of carbon lost during pyrolysis and the physical and structural changes apparent (Downie *et al*., 2009).

Downie *et al*. (2009) remarked that biochar porosity, which determines its surface area, shows pore-size distribution that is highly variable and encompasses nano- (< 0.9 nm), micro- (< 2 nm) to macro-pores (> 50 nm).

Macro-pores will primarily contribute to soils *via* its ability to promote aeration and hydrology and even provide refuge for microbes (mycorrhizae and bacteria). Smaller pores are involved with molecules adsorption and transport (Atkinson *et al*., 2009).

Each soil type varies according to soil structure and all structural properties are normally linked to the particle-size distribution within a soil. Sandy soils can only store a limited and small quantity of water and nutrients, because of the low specific surface area it has (0.01 - 0.1 $m^2 \cdot g^{-1}$), where compared to the great specific area of clay soils (5 - 750 $m^2 \cdot g^{-1}$) (values adapted from Troeh and Thompson, 2005). Biochar can thereby help to improve the specific surface area of sandy soils when amended. The physical- and chemical properties of biochar feedstock, alongside the conditions during pyrolysis, all have an obvious effect on the properties of the biochar being produced.

1.4 Agricultural effects from biochar application

Hammond (2009) stated the following: different types of biochar affect different types of soil in different climates in different ways, and the effects vary for different crops. We can however make some generalisations according to how biochar will affect the soil's chemical-, physical-, and biological properties. Plant productivity, after biochar amendment, on the other hand has not been studied in depth, meaning that in most literature, only aboveground yield were interpreted.

Table 1.1 provides an overview of the basic information according to the following criteria: feedstock material, pyrolysis system, results obtained, plant test species used, and the soil type. It should be stressed that Table 1.1 is only a simplified overview of previous published articles; the focus is based on the main results obtained with the application of biochar during the individual research trials.

1.4.1 Soil properties

Biochar soil additions cause numerous soil changes, ranging from chemical, physical and biological effects (soil biota). The following text will function as a summary of reported positive effects with biochar application.

1.4.1.1 Chemical

Dramatic chemical soil improvements have been reported with biochar applications to agronomy soils and included the following:

1. Increased soil pH (Chan *et al*., 2007; Novak *et al*., 2009; Laird *et al*., 2010; Van Zwieten *et al*., 2010; Peng *et al*., 2011), thus reducing lime requirements.
2. Increased cation exchange capacity (CEC) (Chan *et al*., 2007; Laird *et al*., 2010; Van Zwieten *et al*., 2010; Peng *et al*., 2011).
3. Reduced N leaching thereby can possibly reduce fertiliser requirements (Chan *et al*., 2007; Van Zwieten *et al*., 2010).
4. Acts as a bioremediation source, by reducing the mobility of heavy metals and organic soil contaminants such as insecticides (Hilber *et al*., 2009).

Biochar is generally of alkaline pH and may alter soil pH in a favourable direction for most crops (Chan and Xu, 2009). The ash content of biochar is primary responsible for the modification of the soil's pH. Elevated CECs are due to increases in charge density per unit surface of organic matter which equates with a greater degree of oxidation, or increases in surface charge area for cation adsorption, or a combination of both (Atkinson *et al*., 2010). Steiner *et al*. (2008) confirmed that biochar can act as an absorber reducing N leaching and increasing N use efficiency. Nitrogen use efficiency is of great importance, especially to sustain future population growth.

Table 1.1 Case specific studies for biochar applications (summarised articles)

Feedstock	Process Type	Selected results	Plant test species	Soil	Reference	Remarks
Papermill waste (wood pulped with sodium hydroxide &-sulphate)	Slow pyrolysis	Increase in pH, CEC, soil carbon, microbial activity (soybeans);reduced exchangeable Al; combination of biochar with fertiliser increased N uptake	Radish (*Raphanus sativus*), Wheat (*Triticum aestivum*) & Soybean (*Sorghum bicolour*)	Ferrosol	Van Zwieten *et al*. (2010)	Two separate soil- and biochar types where used; only ferrosol effects have been summarised
Wood residues and rosewood (produced from local lumbermill)	Processed into biochar mound method (FAO, 1983)	Improved saturated hydraulic conductivity (SHC), xylem sap flow (XSF), water permeability, water holding capacity; leaf chlorophyll decreased with biochar application	Rice cultivars (*Oryza sativa L.*)	Only location specified	Asai *et al.* (2009)	The soil type was not specified, but a good summary can be found in Table 1 of the article
Pecan shells	Multiple-step pyrolysis temperature program	Increased SOC, pH, soil minerals – Ca, K & P	Bare soil and vegetative growth is not specified	Fine loamy sand	Novak *et al.* (2009)	An in depth discussion of the pyrolysis process and biochar analysis is given
Poultry litter	Slow pyrolysis (HTT=450 ℃)	Increase in dry matter yields; reduced soil strength; changes in the plant elemental composition	Radish (*Raphanus sativus*)	Alfisol	Chan *et al.* (2008)	Two biochar treatments where present: non activated- and activated biochar
Green waste (grass clippings, cotton trash and plant pruning's)	Slow pyrolysis (HTT=450℃)	Increase in yields (biochar and fertiliser had a combined effect) – improved N use efficiency; increases in pH, CEC, organic C & reduction in soil strength	Radish (*Raphanus sativus var*. Long Scarlet)	Alfisol	Chan *et al.* (2007)	

Variety	Assume fast pyrolysis (HTT=550°C)	No significant data reported, namely: germination effects	Maize (*Zea mays*)	Fine sandy loam and sand.	Free *et al.* (2010)	Five different feedstock where used
By-products of birch charcoal production	Slow pyrolysis (HTT=400°C)	Increased CH_4 uptake (immediately after addition), soil water capacity (11%)	Wheat (*Triticum aestivum*)	Silt loam	Karhu *et al.* (2011)	Interesting remark by the authors that the increased CH_4 uptake could be due to better soil aeration
Wood	Commercially made from cooking using a traditional mound kiln technique	The single biochar application to the soil, improved crop yields up to 4 years after application	Maize (*Zea mays*)	Oxisol: Infertile acidic, kaolinitic sandy soil	Major *et al.* (2010)	Details on feedstock used to make the biochar and production conditions are not available
Mixed hardwood (Oak and hickory trees)	Slow pyrolysis (traditional kiln)	Reduced bulk density; increased water holding capacity, CEC, specific surface area, pH and the retention of P and several other plant nutrients	Bare soil	Fine-loamy soil	Laird *et al.* (2010)	
Rice straw	Slow pyrolysis (HTT=450°C)	Increased pH, CEC & maize biomass. No effect on aggregate stability	Maize (*Zea mays*)	Ultisol	Peng *et al.* (2011)	This article focused on the spectral properties of biochar and how it's influenced by the charring temperatures and duration

1.4.1.2 Physical

Little research has been published on the effects of biochar incorporation on soil physical properties, and as main focus of this work is on the physical soil-biochar interaction; a more profound discussion will follow for this specific section and the possible mechanisms behind previous findings. The physical soil properties studied is as follow:

1. Enhanced soil water-holding capacity (Asai *et al*., 2009; Laird *et al*., 2010; Karhu *et al*., 2011).
2. Improved soil water permeability (Asai *et al*., 2009).
3. Improved saturated hydraulic conductivity (SHC) (Asai *et al*., 2009).
4. Reduced soil strength (Chan *et al*., 2007, 2008; Busscher *et al*., 2010).
5. Modification in soil bulk density (ρ_b) (Laird *et al*., 2010).
6. Modified aggregate stability (Busscher *et al*., 2010; Peng *et al*., 2011).

Laird *et al*. (2010) reported that biochar amended soils retained more water at gravity drained equilibrium (up to 15% for 20 g·kg^{-1} treatment), had greater water retention at -1 and -5 bars soil water matric potential, (13% and 10% greater, respectively for 20 g·kg^{-1}), and no effect was detected regarding saturated hydraulic conductivity. Soil columns were used and treatments consisted of 0, 5, 10, and 20 g-biochar·kg^{-1}, with- and without manure (laboratory incubated studies).

Similar soil-water parameters were studied by Asai *et al*. (2009): they found that applying biochar to upland rice paddies, improved soil water permeability and water holding capacity, thereby the plant's water availability (field studies). They also found that biochar amendment improved the saturated hydraulic conductivity. Chan *et al*. (2008), reported that the field capacity of the biochar-amended soil only increased with increased levels of biochar application but significant increases where detected only at the higher treatment levels of 50 t·ha^{-1} and 100 t·ha^{-1} of biochar.

Biochar enhances the water retention of soils, thus improving dry or sandy soils and reducing irrigation requirements (Liang *et al*., 2006). By increasing the water retention capacity of a soil, one increases the potential for crops to retain more plant available water and thereby increasing crop yields and reducing water stress during critical periods of water restriction. When soils are saturated, the highest hydraulic conduction will be noted for the soil with the larger- and more continuous pore system, while the opposite will be noticed for soils with a more predominant micro-pore system.

According to Kemper and Rosenau (1986), large pores in the soil are generally associated with high infiltration rates, good tilt, and adequate aeration for plant growth. The main mechanism behind the increased water holding capacity and improved saturated hydraulic conductivity can be attributed to the modification of the soil's pore system.

Sandy soils have low water holding capacities, due to a dominant macro-and meso-pore systems present, with little to no organic material and/ or clay at hand (Hillel, 1980). Therefore water molecules can only be held by capillary forces and not by adsorption such as in clayey soils (Hillel, 1980). It can be hypothesised that when amending a sandy soil with biochar one modifies the pore-system and thereby helps to increase the water content, by adsorbing more water molecules, as the biochar is highly porous and exhibits a variety of binding sites. Soils which are compacted and therefore have a low infiltration potential, can become waterlogged (Hillel, 1980) and thereby restrict root growth and lead to reduced crop yields. Hypothetically one can expect when amending a soil where the pore-system consist of mainly micro-pores (clay or silt), the hydraulic conductivity will increase, as biochar will help to shift the pore system to more macro- and/ or meso-pore sizes.

Busscher *et al*. (2010) concluded that biochar showed the tendency to reduce soil strength, but he did not report significant results regarding to soil aggregation. Guant and Cowie (2009) pointed out that strong clay soils require more energy for field operations (such as ploughing), and biochar may lessen this by reducing soil strength, but this effect is still unproven.

Soil strength is an indication of how loose or to what degree a soil is compacted (Hillel, 1980). Bulk density is therefore a key parameter to measure, because it is directly affected by the soil structure (Hillel, 1980). The effect is possibly due to the modification in bulk density, where before biochar amendment it was more compacted and therefore a higher bulk density present, and with biochar amendment the soil strength decreased as the bulk density decreased. Biochar has a very low bulk density (0.30 – 0.43 $g{\cdot}cm^{-3}$) (values adapted from Pastor-Villegas *et al.*, 2006) and particle density (1.47 $g{\cdot}cm^{-3}$; pine wood) (value adapted from Brown *et al.*, 2006); hence for the volume it occupies within the soil and its low mass due to its porous nature, soil strength will be reduced with application. Generalising, one can expect that the soil strength (including ρ_b) will decrease as the content of biochar increases.

Important factors to mention and which influence aggregate formation and stabilisation are the following:

- soil fauna, specifically earthworms and termites;
- microorganisms such as bacteria and fungi;
- plant roots;
- inorganic binding agents, namely oxides and calcium; and
- environmental properties such as freeze-thaw cycles, dry-wet cycles and fire (for further reading, refer to the review paper by Six *et al.*, 2004).

Peng *et al*. (2011) reported no effect on aggregate stability. Their experimental design was not scientifically sound and their evidence can be seen as weak. Firstly the study was only concluded over an 11-day period, where 50 g of soil was incubated with 1% biochar application level (by dry weight).

The sample size along with the short experimental period and without any plant test species is very unrealistic to test the effect of biochar on soil aggregation, which takes time in soils and is dependant on biotic factors.

The formation of soil structure in sands will be briefly reviewed so that confusion with respect to the forces, which stimulate aggregation of particles and those that stabilise- or degrade aggregation, can be avoided. It should be noted that aggregation of sandy soil's will be the primary focus in the text to follow.

According to Oades (1993), sand has structure because it has a pore-size distribution created by the size and the packing of sand grains and that this structure can be changed by altering the packing of the sand grains by tillage or compaction or rearrangement by soil animals. The structure is not altered significantly by drying and wetting cycles because the shrink-swell capacity is virtually zero (Oades, 1993), because of the absence or lack of clay- and organic matter content. Therefore biological factors such as the root-microbial interaction in the rhizophere need to play a major role in structural development for sands.

Plants influence the rate, extent and the spatial development of the drying phase (Oades, 1993), modified by their root interactions and need for water. After aggregate formation, aggregate stabilisation follows. Stabilisation of aggregates of sand particles involves the growth of higher plants, fungi and bacteria in the pore system between grains (Oades, 1993). The sand grains are then held together by (a) colonies of organisms and their mucilage's (microbial aggregates), (b) roots and hyphae (root microbial aggregates) and (c) metabolic products from the decomposition of fragments of higher plants (Forster, 1979, 1990) (Figure 1.3). As illustrated in Figure 1.3, there is a distinctive difference between the three types of aggregates.

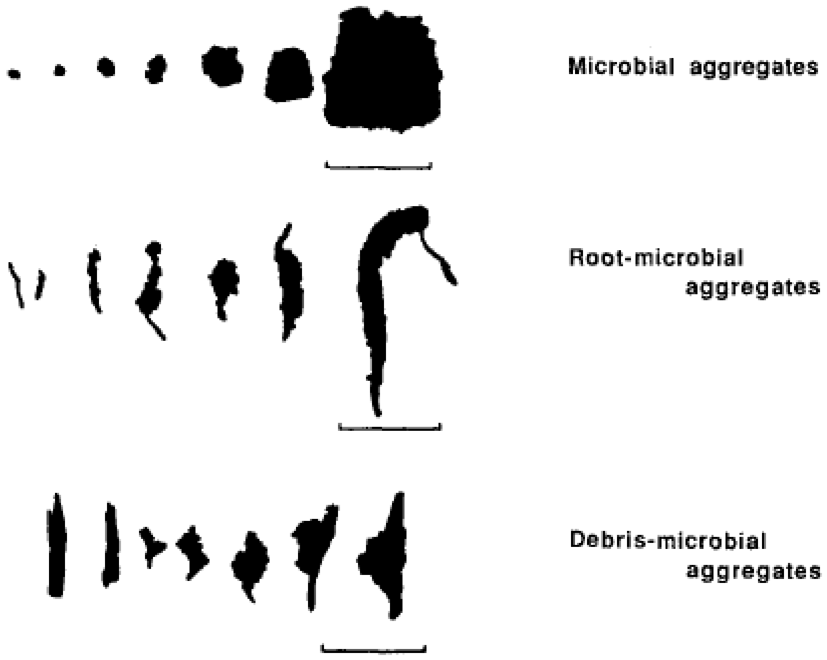

Figure 1.3 Aggregate stabilisation in sands (after Forster, 1990). Bar = 10 mm.

Aggregate formation in sandy soil will help to combat soil loss due to overland flow and wind erosion. The mechanisms by which biochar can stabilise aggregates is poorly understood and no attempt has been made by soil physicists to identify the physical-, chemical, and biological factors, which could help in the formation and stabilisation of aggregation. The following are all possible factors and mechanisms that could contribute to aggregate stabilisation in sandy soils with biochar application (Hillel, 1980):

- Biochar can improve root growth and thereby stimulate aggregation (roots can grow into biochar pores as more water and plant nutrients are adsorbed).
- Enhanced microbial activity (especially rhizopheric bacteria and mycorrhizal fungi, which in direct association with roots form a more extensive rooting system *via* filaments know as mycelia and hyphae).
- Calcium carbonate, more so calcium (Ca).

Czimczik and Masiello (2007) reported that Ca was shown to increase biochar stability, most likely by enhancing interactions with mineral surfaces.

1.4.1.3 Biological

The functioning of different biological communities within soils is a complex field of study. The following positive effects have been reported:

1. Enhanced biological N fixation (rhizobia) (Rondon *et al*., 2007).
2. Improved colonisation of mycorrhizal fungi.
3. Earthworms showed preference for biochar amended soils (Van Zwieten *et al*., 2010).
4. Increased CH_4 uptake (Karhu *et al*., 2011).
5. Potential catalyst in reducing N_2O to N_2 (Van Zwieten *et al*., 2009).

Rondon *et al*. (2007) found the following: evidence exists to show that increasing biochar amendments to soil can increase the proportion of N derived from fixation by *Phaseolus vulgaris* (common green bean) and this increased yields. When preparing acidic soils, the increased alkalinity effect of applied biochar, could help to increase rhizobia numbers, especially when they function optimum in neutral pHs.

Van Zwieten *et al*. (2010) found that earthworms showed a very distinct preference for biochar amended ferrosol soils, when compared to the control. Karhu *et al*. (2011) found that increased CH_4 uptake was beneficial and available immediately after fresh biochar application to soil. The reason for the increased CH_4 uptake is unclear. It has been suggested by Van Zwieten *et al*. (2009), that biochar improves soil aeration, and thus decrease CH_4 production and increase CH_4 oxidation.

There have been hypothesised that biochar may have the potential to catalyse the reduction of N_2O to N_2 (Sohi *et al*., 2009), but Van Zwieten *et al*. (2009) did not find supporting evidence to this claim. This could be due to the fact that we are dealing with case specific scenarios and that each soil type will be affected differently according to the biochar (feedstock and pyrolysis needs to be defined) used and the amount applied under specific climatic conditions.

1.4.2 Biochar application and crop production effects

The response of agricultural crops to different biochars and various application levels is essential for devising suitable and applicable strategies for long term carbon sequestration in sustainable farming. Atkinson *et al*. (2010) highlighted that the importance attached to the extent with which biochar application might increase agricultural production is an important driver in any attempt to develop systems that economically incorporate pyrolysis products within the soil.

At the moment there is restricted research that has been conducted on plant specific responses. Most experiments were conducted thus far on annual crops and in the majority, only yield responses were given. The response of different crops to various biochar applications levels is summarised in Table 1.2.

Asia *et al.* (2009) studied the effects of biochar application on rice yields (*Oryza sativa* L.) and selected plant traits (Table 1.1). They found the following: biochar application lead to higher grain yields; improved the response to N fertiliser treatments; reduced leaf chlorophyll concentration; improved xylem sap flow; and concluded that biochar application is highly dependent on soil fertility and fertiliser management.

Van Zwieten *et al*. (2010) found for wheat in the ferrosol soils, there was no significant difference in the absence of fertiliser, however with fertiliser, significant increases in biomass production were recorded, indicating a strong fertiliser by biochar interaction (see Table 1.2 for additional information regarding results).

A pot trial was carried out by Chan *et al*. (2008) and they found in the absence of N fertiliser, biochar significantly increased total dry matter (TDM) of radish even at the lowest level of application (10 $t \cdot ha^{-1}$), and the yield increased with increased levels of biochar application to 50 $t \cdot ha^{-1}$ (more information available in Tables 1.1 and 1.2).

Rondon *et al*. (2007) had contradicting data, were their pot trial experiments obtained the following results: bean yield increased by 46%; biomass production increased by 39% over the control at 60 $g \cdot kg^{-1}$ and 90 $g \cdot kg^{-1}$ biochar application; total N uptake decreased when biochar application were increased to 90 $g \cdot kg^{-1}$; and soil N uptake by N-fixing beans decreased by 14%, 17% and 50% when 30, 60, and 90 $g \cdot kg^{-1}$ biochar were added to soil. C/N ratios increased from 16 to 23.7, 28, and 35, respectively (Rondon *et al*., 2007). At the moment there are no long term field studies for biochar grown crops and therefore we do not know whether increased plant yields will be sustainable over the long term.

No studies have been published where shoot-to-root ratios increased while plant growth decreased, which would indicate a direct toxic effect of biochar on plant roots through the presence of organic and inorganic (heavy metals) compounds (Lehmann *et al*., 2011). Thus far studies have not shown any severe negative results from biochar amendments to agricultural soils.

Table 1.2 Effect of biochar application on crop yield (case specific scenarios)

Crops	Soil type	Biochar treatment ($t{\cdot}ha^{-1}$)	Fertiliser treatment ($kg{\cdot}ha^{-1}$)	Yield increase over control [a] (%)	Additional information	Reference
Wheat	Ferrosol	0 & 10	1.25g Nutricote® per 250g soil [b]	+ 250	Yield increase was only for wheat on ferrosol soil and the results was given in total dry weight (g). There was a similar response for soybean and radish observed. Calcarolsol soils, amended with fertiliser and biochar gave varied crop responses, where soybean biomass increased, but wheat and radish yield decreased. All trials conducted without fertiliser for wheat and soybean had no significant results, while radish biomass showed increased responses.	Van Zwieten *et al*. (2010)
Radish	Alfisol	0, 10, 25 & 50	N (100)	+ 320	Biochar treatment trials without fertiliser had significantly increased the total dry matter (TDM) of radish, even at the lowest level of biochar application. The highest TDM observed was for the non-activated poultry litter derived biochar (50 $t{\cdot}ha^{-1}$ and fertiliser).	Chan *et al*. (2008)
Radish	Alfisol	0, 10, 50 & 100	N (100)	+ 95 to + 266	Application of biochar to soils without fertiliser had no significant increase on the TDM production. The magnitude of yield response increased as the biochar application increased with nitrogen addition.	Chan *et al*. (2007)
Maize	Fine sand loam & sand	0, 2.5, 5.0 & 10	Nil	No effect	Free *et al*. (2010) reported the following: biochar feedstock at any applied level did not affect the dry weight of coleoptiles, roots, remaining seed or coleoptiles length.	Free *et al*. (2010)

Wheat	Silt loam	0 & 9	Nil	No effect	The yield, number and weight of wheat seeds was not affected by the biochar. The main objective of the study was to study gas emissions, hence biomass was not measured.	Karhu *et al*. (2011)
Maize	Oxisol	0, 8 & 20	Lime (dolomite) (2.2); N (156 – 170; P (30 – 43); K (84 – 138)	+ 28 (2004) + 30 (2005) + 140 (2006)	Nitrogen was applied as urea, K as KCl and P as acidified rock phosphate.	Major *et al*. (2010)
Maize	Ultisol	0 & 2.4	N (150); P_2O_5 (100); K_2O (150)	+ 146	Pot trials were used and maize biomass was increased by 64% (without fertiliser) to 146% (with fertiliser) after biochar amendment.	Peng *et al*. (2011)
Beans	Oxisol	0, 30, 60 & 90[c]	Lime (300); N (20); P (20)	+ 39	Biochar had significantly increased biomass, but when > 60 $g{\cdot}kg^{-1}$ was applied, production declined.	Rondon *et al*. (2007)

[a] Control indicates that no biochar was used and + and – sings indicates if yields increased or decreased.

[b] Nutricote® contains 15.2% N, 4.7% P, 8.9% K, 3.3% Ca, 1.1% S, and micronutrients.

[c] Fertiliser applied was in $g{\cdot}kg^{-1}$ (pot trial).

1.5 Social and environmental issues

The global trend is slowly but surely, moving towards sustainable production systems, waste minimisation, reduced fossil fuel transport, alternative energy generating projects, conservation of native vegetation and mitigation of greenhouse gas emissions (Sims, 2002; Brownsort, 2009; Pandey, 2009, Blaschek *et al*., 2010). When considering pyrolysis technology and biochar production, the following may arise, concerning the different pyrolysis methods employed in processing plants: is fast or slow pyrolysis systems more cost sufficient?

The difference in production costs and products generated may be vital to the economic feasibility of biochar (Pratt and Moran, 2010).

From an economic point of view, producing biochar for agricultural amendment will only be profitable if the income generated is greater than fast pyrolysis (bio-oil) production systems. We do know that applying biochar to agricultural soils, produced dramatic yield improvements, reduced soil acidity (reduced lime requirements) and increased the water holding capacity (less irrigation needed). These are all agricultural benefits that will lead to greater economic income, but these results were proven by short term trials and for selected crop species.

Primary motivations to produce biochar and applying commercially are as follow:

- mitigating GHG (especially CO_2);
- selected soil chemical, physical and biological benefits;
- increased agricultural crop yields;
- economical growth (creating employment and contributing to the carbon-stock-market); and
- alternative ‘cleaner’ fuel option (substitute for fossil fuels; bio-oil- and gas production favoured).

Biochar is incompletely combusted (lack of oxygen during pyrolysis); therefore in the event of a fire, the applied biochar will complete the process of combustion and release extra carbon amounts in the atmosphere. This hypothetical scenario can be devastating especially since this will counter the exact event it was relieving.

There is also evidence where charcoal was applied to undisturbed forest soils (carbon rich soils) in northern Sweden and studied over a 10 year period. As a result, there was mineralisation (decomposition) of native soil organic matter with accelerated emissions of CO_2 (Wardle *et al.*, 2008). Again, applying biochar defeated the original purpose of mitigating greenhouse gases. Biochar application may be limited in the future to only degraded agricultural soils. Biochar use as an alternative mitigation technology needs to be evaluated and measured intensively according to: effectiveness (relieving atmospheric CO_2), cost efficiency (compare to bio-oil production) and sustainability (long term agricultural effects).

1.6 Gaps in knowledge

Based on the literature reviewed we have to focus on the following for future biochar research:

The mechanistic understanding of how biochar affects the soil's physical properties, needs to be established and hence also crop interaction and- yields, because very little to no research has been provided on this combined topic. The physical properties that mainly have to be focused on will include porosity, particle-size distribution, bulk density, aggregate formation and- stabilisation. Ultimately the soil structural effect after biochar application needs to be defined, as soil productivity and favourable yield responses will be the aim. Roots are not only controlled on a genetic level, but also on an environmental level, hence we need to know how biochar will affect root growth and yields of agronomy crop species.

Specific risks and gaps in knowledge that needs to be solved before applying biochar commercially are as follow:

- unknown soil processes, after biochar application, needs to be identified (longevity in soils);
- can supplying feedstock on a commercial level be sustainable in the long run;
- changing the publics perception (activism groups and 'waste' terminology);
- funding long term projects; and
- detailed studies to form a catalogue of different biochar types for site and crop specific interactions.

To date, no research has specifically focused on aggregate formation and other related measurements within the soil-root interface, such as plant-soil-water dynamics.

Coordinated research is needed at the moment so that biochar can be assessed for future use as a commercial agricultural soil amendment; and to date only short term studies has been conducted and none of these has been done for South African soils and- conditions.

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CHAPTER 2: EFFECT OF BIOCHAR ON SELECTED SOIL PHYSICAL PROPERTIES AND CROP PERFORMANCE OF WINTER WHEAT POT PLANTS

2.1 Introduction

The role of biochar in the modification and possible stabilisation of soil structure, especially in sandy soils, is a desirable field of study, particularly when events such as root logging, erosion (*via* water runoff or wind) and drought of annual agricultural crop species could be minimised. In the Western Cape area (South Africa), sandy soils are commonly found and can be classified as having low agricultural suitability for perennial- and annual crops. This is due to the following: very little organic matter present, low- to acidic pH, massive soil structure and low to very low water- and nutrient holding capacity. Bronick and Lal (2005) stated that soil structure is a key factor in the functioning of soil, its ability to support plant and animal life, emphasis on soil carbon (C) sequestration and water quality.

The use of biochar in soils can lead to changes in soil structure, texture, porosity, particle-size distribution and bulk density (Atkinson *et al*., 2010). Hence, if biochar can contribute to soil structure formation in sandy soils, while also increasing the C-storage and plant available water (PAW), this product could be ideal to increase the yield capacity for agronomic plant species using the same amount of applied irrigation water and- fertiliser, as well as facilitating and enhancing the rooting system. However a soil structural formation and a plant-soil interface study have not been attempted at present.

Improving water use efficiency (WUE) is one of the main targets of crop research for Mediterranean environments (see Hamdy *et al*., 2003 for a more in-depth review) and if biochar can help to alleviate the current rising water demand and- scarcity as well addressing food security, it should be rated higher than currant, as this alternative soil amendment could contribute to better management of water.

Biological factors such as root-microbial interaction in the rhizosphere play a major role in structural development for sandy soils therefore as biochar is added to the soil the rhizosphere needs to be studied and how this stable carbon agent will affect the root formation and- growth of plants. The physical properties of biochar may have a direct impact on root growth itself, because plant root growth is mostly determined by penetration resistance, porosity and nutrient- and water availability.

Theoretically, different cohering interactions would be expected, because each soil type formed a unique relationship with the specific applied biochar. Therefore the following needed to be defined: feedstock material, pyrolysis conditions, biochar application level, the soil type used within the study, and the specific crop planted along with yield responses and vegetative traits (preferably above- and below the soil surface). Kuwagaki (1990) proposed that the following properties should be measured for a quality assessment for agronomy-used char: pH, volatile compound- and ash content, water holding capacity, bulk density, pore volume, and specific surface area.

Conceptually the main physical mechanism of importance with biochar application for the long term, according to crop production improvement, is believed to be due to the effect it will have on root growth; as more water is retained in especially sandy soils as well as plant nutrient retention due to the higher specific surface area, after biochar application, but this statement has not been proven scientifically and will be addressed in this Chapter.

The objectives of this study were: (a) to establish the biomass water use efficiency, field capacity and plant available water content, for each application level, (b) to determine the above- and below ground biomass yields for each treatment combination, (c) to exclusively study the plant response below the soil surface, focusing on the root formation and- morphology, along with aggregate formation within the rhizosphere, and (d) to determine the optimum biochar application level for winter wheat and to establish what possible negative effects biochar amendment may have on these agronomy crops.

2.2 Material and methods

2.2.1 Soil and biochar

The soil was collected near Brackenfell in the Western Cape region of South Africa (33° 53’ 43.08” South, 18° 43’ 24.24” East). The region has Mediterranean climate and the soil had no history of agricultural practices. Plant cover consisted of, *Pennisetum clandestinum*; common name is kikuyu grass and weeds. The soil was classified as a Kroonstad (Kd 1000 - Morgendal) soil form (Soil Classification Working Group, 1991). Before sampling, the thin A-horizon was removed (0–100 mm) from the surface and only soil from the E-horizon was collected (100–1000 mm). There was no restrictive layer present up to the sampling depth of 1000 mm.

The feedstock material used for the biochar production system, was pine sawmill waste. The producer is a small-scale commercial charcoal-briquette company in the Eastern Cape area. The feedstock material was exposed to slow pyrolysis at 450℃ (highest heat treatment temperature, HTT) and crushed afterwards. The physical appearance of the biochar was predominantly granular, however powdery residue prevailed especially whilst sieving, making this a messy product to work with.

Particle-size analysis for the soil was determined with the pipette method (Gee and Bauder, 1986) and particle density was determined using volumetric flasks of known volume (Blake and Hartge, 1986b), for both soil and biochar analysis. Particle size range for biochar was done by sieving dry samples with sieves ranging from 53–2000 µm. All laboratory studies were replicated four times.

2.2.2 Plant growth trial

Soil and biochar samples were air dried and then passed through a 2 mm sieve before filling plastic pots (total volume per pot, 7.10 L). The experiment was carried out in an climate controlled greenhouse. The experimental design was setup as a randomised two-factorial block experiment, with four replicates per treatment. The treatments consisted out of five biochar treatments (0%, 0.05%, 0.5%, 2.5% and 10%; weight-to-weight basis, w/w), with fertilised- and unfertilised replicates (see Table 2.1).

The soil and biochar of all treatment combinations were tumbled in a rotary cement mixer for approximately 10 minutes. During the tumbling process for each treatment, the predetermined amount of biochar was slowly added to the soil, thereby ensuring that the biochar and sand mixtures were well mixed (homogenous). A broad spectrum fertiliser was used, namely Chemicult Hydroponic Powder (CHP) and the plant test species, *Triticum aestivum* (common name, winter wheat) where planted and fertigated three times throughout the 12 week pot trial. The hydroponic powder contained macronutrients (N: 0.065 g/L, P: 0.027 g/L, K: 0.013 g/L, Ca: 0.07 g/L, Mg: 0.022 g/L, S: 0.075 g/L) and micronutrients (Fe: 0.0015 g/L, Mo: 0.00001 g/L, Cu: 0.00002 g/L, B: 0.00024 g/L, Mn: 0.00024 g/L, Zn: 0.00005 g/L).

The fertigation schedule was as follow (percentage is according to the concentration volume applied of an 1.769g CHP per 100mL distilled water dilution): 70% at sowing period, 30% four weeks after germination, and again 30% after four weeks had passed. A total of ten seeds were sown per pot and thinned to the best four, following germination. With harvest, all above- and below ground biomass was collected separately and then oven dried at 60°C to a constant mass and weighed.

The biomass water use efficiency (BWUE) was calculated as follow (equation 2.1): the total above ground biomass (dry weight yield in grams) for each treatment and their specific surface areas (pot's area in cm^2) were determined and divided by the total amount of water applied (mm) throughout the experimental period. BWUE was then converted to $kg \cdot ha^{-1} \cdot mm^{-1}$ (equation 2.2).

$BWUE = Yield \,/\, TWA$.. (2.1)

Where:

$BWUE$ = biomass water use efficiency ($kg{\cdot}ha^{-1}{\cdot}mm^{-1}$)

$Yield$ = total vegetative biomass yield ($kg{\cdot}ha^{-1}$)

TWA = total amount of water applied (mm)

$\text{Area}_{\text{pot surface}} = \pi \times \text{radius}^2$.. (2.2)

$\text{Area}_{\text{pot surface}} = (3.141...) \times (12 \text{ cm})^2$

$\text{Area}_{\text{pot surface}} = 452.3893 \text{ cm}^2$

Yield was measured as follow: dry weight of above ground biomass was measured in grams, thus to convert grams to kilograms multiply the yield value (g) with 10^{-3} to convert to yield in kilograms. To convert cm^2 to hectare, you need to multiply with 10^{-8}.

Calculation example: average above ground yield ($g{\cdot}cm^{-2}$) for fertilised wheat which received 1 ton of biochar per hectare.

$\text{Yield}_{\text{F1BC1}} = 23.10 \text{ g} \div 452.38 \text{ cm}^2 = 0.0510058 \text{ g}{\cdot}\text{cm}^{-2}$

$0.0510058 \text{ g}{\cdot}\text{cm}^{-2} \times (10^{-3} \text{ kg} \div 10^{-8} \text{ ha}) \times (1\text{cm}^2 \div 1\text{g}) = 5101 \text{ kg}{\cdot}\text{ha}^{-1}$

Representative root sample for each of the different biochar treatments (only fertilised treatment *via* core sampler) were collected and stored, so that the root structure could be evaluated qualitatively. After aggregates where harvested photo's where taken using a Celestron® handheld digital microscope camera (Photo 2.6 to 2.10).

2.2.3 Selected soil physical properties

2.2.3.1 Soil water

Field capacity (FC) was determined as described by Cassel and Nielsen (1986), according to the container capacity method (equation 2.3). The pots where weighed and irrigated on a weekly basis to FC and after seven weeks into the experiment the irrigation frequency was modified to twice a week. The permanent wilting point (PWP) was calculated *via* the SPAW – Soil Water Characteristics software program (Version 6.02.74), which is based on the work done by Saxton and Rawls (2006). Plant available water (PAW) for each treatment combination was calculated and based on the values measured for FC, PWP, bulk density (ρ_b) and the soil depth (d) (equation 2.4).

$$FC = \theta_v \times \rho_b \times d \quad (2.3)$$

Where:

FC = field capacity (mm)

θ_v = volumetric water content ($m^3 \cdot m^{-3}$)

ρ_b = bulk density ($g \cdot cm^{-3}$)

d = depth of soil in pot (mm)

$$PAW = (FC - PWP) \times \rho_b \times d \quad (2.4)$$

Where FC and PWP is in $m^3 \cdot m^{-3}$, ρ_b is in $g \cdot cm^{-3}$, d and PAW is in mm.

The volumetric water content ($\theta_{v,}$ $m^3 \cdot m^{-3}$; equation 2.5) and evapotranspiration (ET, mm; equation 2.6) were also calculated throughout the 12 weeks for each treatment combination and their replicates. It was derived from the soil water balance (equation 2.7) as the total amount of water that evapotranspirated was monitored throughout the experiment by weighing each pot just prior to watering to FC.

$$\theta_v = [(M_{wet} - M_{dry}) / M_{dry}] \times \rho_b \times \rho_w \text{ ....(2.5)}$$

Where:

M_{wet} = mass of wet soil

M_{dry} = mass of dry soil

ρ_b = bulk density ($g \cdot cm^{-3}$)

ρ_w = density of water ($g \cdot cm^{-3}$)

$$ET = I - \Delta W \text{ .... (2.6)}$$

Where:

ET = evapotranspiration (mm)

I = total amount of water applied *via* irrigation (mm)

ΔW = water balance (mm)

$\Delta W = R + I - A - P - E - T$.. (2.7)

Where:

ΔW = change in the soil water content (mm)

P = precipitation (mm)

I = irrigation (mm)

R = run-off from the soil surface (mm)

D = deep percolation or drainage from the root zone (mm)

E = evaporation from the soil surface (mm)

T = transpiration from the plant cover (mm).

It was assumed throughout the experimental period that P, R and D were zero.

2.2.3.2 Bulk density (ρ_b)

At harvest bulk density samples (*via* standard core sampler with a sampling volume of 71.27 cm^3) where collected for each treatment and all of its replicates, making use of the core method (Blake and Hartge, 1986a). The samples were collected at 0–5cm and >5–10cm depth and oven dried for approximately 24 hours at 60℃ to a constant mass.

The temperature was set lower than what is indicated in this method (105℃) when drying in the oven, because of the highly flammable and combustible nature of biochar. Biochar bulk density was determined (equation 2.8) by dividing the known mass of biochar, by the biochar volume it occupied:

ρ_b = biochar mass / volume occupied by soil sample ....(2.8)

where biochar was in grams and the volume was measured in cm^3.

2.2.3.3 Aggregate stability and pH measurement

The wet sieving method is used to determine the aggregate stability of a soil, which gives a good indication on how resistant a soil's structure is against mechanical- or chemical destructive forces. The fraction of water-stable aggregates (WSA) per treatment was determined by making use of the method based on the work done by Kemper and Rosenau (1986). The sand-biochar samples were collected in the root zone (rhizophere). As the wet-sieving method relies on the use of a dispersion agent soil pH (White, 1997) was determined in both distilled water and 1.00 M KCl.

Four grams of air dried aggregates (1-2 mm) where weighed and placed on a 250 μm sieve (apparatus can hold eight sieves). Samples were pre-moistened with distilled water. Two set of cans (weighed and numbered beforehand) need to be present and pre-prepped per sample. The first set of cans (non-WSA) needs to be filled with distilled water and the second set of cans (WSA) has to be filled with a dispersion agent. The dispersion agent/ solution used are as follow, according to the pH measured in water for the soil:

- pH > 7 will need a 0.05 M sodium hexametaphosphate solution (Galgon); and
- pH < 7 will need a 0.05 M NaOH solution.

When the cans are ready and the samples are wetted, submerged sieves with samples in the cans containing distilled water, start the motor by putting the main switch into "3 min" position. The stroke length is set at 1.3 cm and the cycle is about 34 times per minute. At the end of this first sample run, the motor will stop automatically.

Raise the sieve holder out of the water and when no more water is leaking, replace the cans with the non-WSA (n-WSA) fraction with the second set of cans filled with the dispersion agent. Place the sieve holder in the working position and start the motor by switching into "continue". Continue the sieving until only loose soil particles are left on the sieve (about 5-8 minutes).

When finished raise sieve holder and wait until no more leakage is present. Place the first and second set of cans in an oven and leave to dry for 24 hours at 105℃. The fraction of water-stable aggregates was calculated as follow (equation 2.10):

$$fraction = WSA / (WSA + n\text{-}WSA) \quad \text{...} (2.10)$$

Where:

WSA = water-stable aggregates

$n - WSA$ = non-water-stable aggregates.

2.2.4 Statistical analysis

The overall experimental design included four biochar mixing ratios, one control and four replicates per treatment combination for both fertilised- and unfertilised treatments; hence the experimental design is of a randomised block design with two factors. The factors to take into account were first the biochar treatment and the second factor was the presence- or absence of fertiliser (Table 2.1).

One- and two-way analysis of variance (ANOVA) were used to evaluate biochar treatment effects on plant biomass yield (above-and below ground) and soil physical properties by means of STATISTICA 10.0 (StatSoft) software. The significant difference between the biochar treatment means were calculated *via* a *post hoc* test comparison namely, Tukey's t-test. Column charts were drawn for most of the measured parameters and error bars were used and illustrated according to standard error values, while only the average values for the different treatment combinations were given in the graphs. All statistical analysis were compared and treated as being significantly different, if the *P*-value was less than 0.05.

Table 2.1 Treatment combination for two-factorial experimental design

Fertiliser	F0			F1	
Biochar treatment	BC0	BC1	BC2	BC3	BC4
(t/ha)	0	1	10	50	200
Two-factorial treatment combination	F0BC0	F0BC1	F0BC2	F0BC3	F0BC4
(four replicates per treatment)	F1BC0	F1BC1	F1BC2	F1BC3	F1BC4

The biochar treatment, as indicated in t/ha, is based on a depth increment of 15 cm from the soil surface

2.3 Results

2.3.1 Soil and biochar characterisation

The sandy soil consists mainly of medium- to fine sand particles (Table 2.3). Very little clay was present and less than 3% silt was at hand. The soil had a low pH and very low electrical conductivity (EC) value and it could be concluded that quarts material was the main mineral present, as the particle density is 2.63 $g{\cdot}cm^{-3}$ and therefore a very low ability to adsorb essential plant macro- and micro minerals (K^+, Ca^{2+}, Mg^{2+}, NH^{4+}, P, Al^{3+}, and Fe^{2+}) and nutrients. The effective cation exchange capacity (ECEC) for the sandy soil was determined at $1.96cmol_c.kg^{-1}$ (Sika, 2011). Coarse- and fine sands have a very low specific surface area of respectively, 0.01 $m^2{\cdot}g^{-1}$ and 0.1 $m^2{\cdot}g^{-1}$ (values adapted form Troeh and Thompson, 2005). Due to the dominant macro-pore system for sandy soils in combination with small specific surface area, sands exhibit limited capacity to store water (due to the fact that capillary forces are very low) and plant nutrients.

The pine sawmill waste derived biochar consisted mainly of particles in the coarse size fraction (Table 2.3). Particles ranged mainly from 500-2000 µm. The bulk density was extremely low, 0.33 $g{\cdot}cm^{-3}$, and the reported value correlated well with 0.30 – 0.43 $g{\cdot}cm^{3}$ reported by Pastor-Villegas *et al*. (2006). Particle density was also very low 0.85 $g{\cdot}cm^{-3}$. According to previous studies where pine wood was used to make biochar, the value differed and Brown *et al*. (2006), reported a value of 1.47 $g{\cdot}cm^{-3}$ for pine wood derived biochar.

This difference could be due to the fact that the pyrolysis temperature was different (higher HTT) from our study, and that the feedstock pre-treatment was not the same, for example more coarse or moist plant material was used than the fine sawmill waste used in this specific case study. The high pH of the biochar (Table 2.3) was also of great importance and can therefore be used as a liming agent and help increase the pH of the sandy soil. Soil pH played an essential role in plant nutrient uptake, especially in more acidic soils.

The biochar had a relatively high specific surface area (Table 2.3) and corresponds with previously published values for pine woody plant material derived biochar (Brown *et al*., 2006; Cetin *et al*., 2004). The micro-porosity (Table 2.3) also correlates with the results of Laine *et al.* (1991) which showed that the average micro-porosity values of different biochars ranged between 0.2 $cm^{3}{\cdot}g^{-1}$and 0.5 $cm^{3}{\cdot}g^{-1}$.

When ameliorating a sandy soil as such with biochar, it is expected that the total soil-specific surface will be increased significantly. The following plant available macro- and micro nutrient values are worth mentioning, namely: K^{+}, Na^{+}, Ca^{2+}, Mg^{2+}, P, Al^{3+} and Fe^{3+} and their respective values were 1720.85 $mg{\cdot}kg^{-1}$, 126.39 $mg{\cdot}kg^{-1}$, 1432.49 $mg{\cdot}kg^{-1}$, 301.37 $mg{\cdot}kg^{-1}$, 89.59 $mg{\cdot}kg^{-1}$, 19.37 $mg{\cdot}kg^{-1}$ and 42.88 $mg{\cdot}kg^{-1}$, all of the heavy metals were below the norm (Sika, 2011). Therefore toxicity is not believed to be a concerning factor regarding plant health.

It should be noted that the reported results mainly focused on the physical interactions and- mechanisms and that chemical analysis, based on the work done by Sika, (2011), was reported for a more thorough overview.

Table 2.2 Branauer-Emmett-Teller (BET; Brunauer *et al*., 1938) surface area- and micro-porosity for pine sawmill waste derived biochar and additional chemical analysis (adapted from Sika, 2011)

	Ash content (%)	Volatiles (7 min at 900℃; %)	Specific surface area ($m^2 \cdot g^{-1}$)	Micro-porosity ($cm^3 \cdot g^{-1}$)	Average micro-pore diameter (nm)
Biochar	˜3.04	˜19.90	387.15	0.190	1.97

Table 2.3 Mean values of basic chemical- and physical properties of the soil and biochar used in the pot trial

EC	pH		C	N	Particle-size distribution (%)[a]					ρ_d	ρ_b	f[b]
(ds/m)	H_2O	KCl	(%)		CSa	MSa	FSa	Silt	Clay	(g·cm^{-3})		
0.06˜	5.40˜	4.30˜	0.16˜	0.03˜	0.87	71.43	27.62	2.80	0.31	2.63	1.57	0.40
EC	pH		C	N	Particle-size distribution (%)[c]					ρ_d	ρ_b	f
(ds/m)	H_2O	KCl	(%)		Cf	Mf	Ff	< 53µm		(g·cm^{-3})		
0.75˜	9.39˜	8.57˜	82.71˜	0.53˜	68.56	7.53	6.85	5.09		0.85	0.33	0.61

[a] CSa: coarse sand (250-500 µm), MSa: Medium sand (106-250 µm), FSa: fine sand (53-106 µm)

[b] f: porosity

[c] Cf: coarse fraction (500-250 µm), Mf: medium fraction (250-106 µm), Ff: fine fraction (106-53 µm)

˜Adapted from Sika (2011)

2.3.2 Plant growth responses

2.3.2.1 Above-ground vegetative growth

In the case of the fertilised treatments, only the 1t/ha and 10t/ha biochar applications increased the above-ground biomass, i.e. by 22.0% and 26.9% respectively, compared to the control (Figure 2.1). The highest above-ground biomass was obtained where 10t/ha and fertilisers were applied. In contrast, the fertilised 200t/ha biochar application reduced the vegetative growth drastically, i.e. by 74%, compared to the control. In the case of the unfertilised treatments, only 10t/ha and 50t/ha biochar applications increased the above-ground biomass by 31.8% and 39.5%, respectively, compared to the control (Figure 2.1). Similar to the fertilised treatments, 200t/ha biochar without fertilisers reduced the vegetative growth by 47.3% compared to the control. Above-ground biomass of the fertilised wheat was substantially higher compared to the unfertilised plants ($P < 0.001$). The biochar application level also resulted in differences in the above-ground biomass ($P < 0.001$). There were also differences in the above-ground biomass when all treatments were compared ($P < 0.001$).

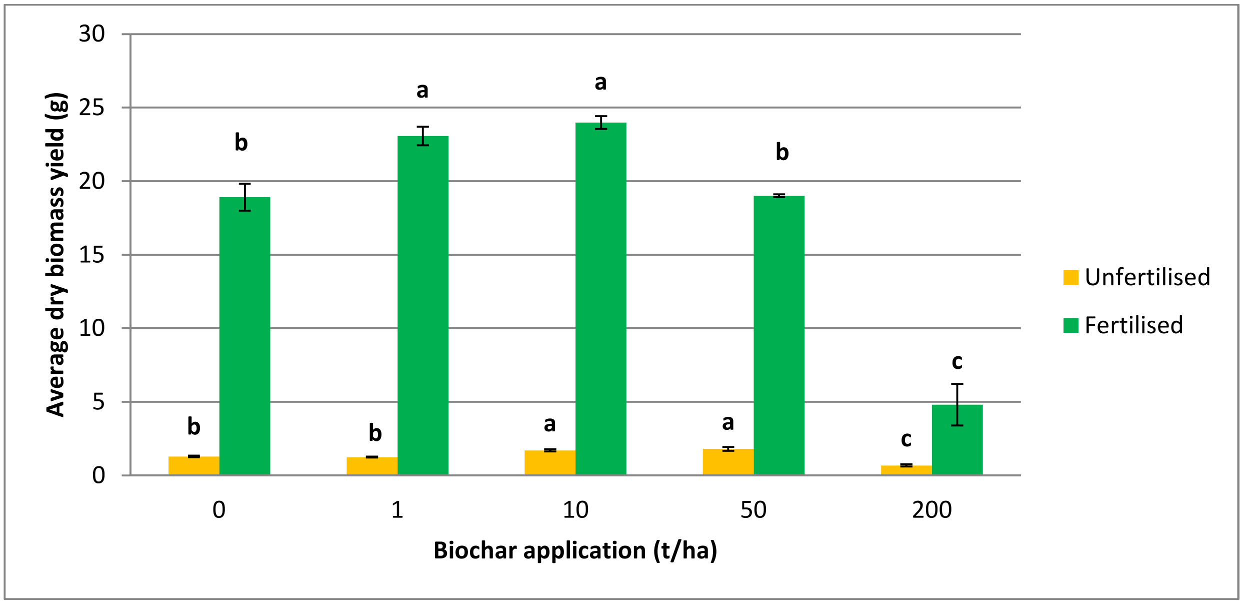

Figure 2.1 Effect of biochar amelioration on dry vegetative biomass of unfertilised and fertilised winter wheat

2.3.2.2 Root biomass and root structure

In the case of the fertilised treatments, the 1t/ha, 10t/ha and 50t/ha biochar applications increased the root biomass, i.e. by 61.7%, 44.3% and 28.9% respectively, compared to the control (Figure 2.2). The highest dry root yield was obtained where the 10t/ha and fertilisers were applied. In contrast, the fertilised 200t/ha biochar application reduced the root growth drastically, i.e. by 75.1%, compared to the control. In case of the unfertilised treatments, only 10t/ha and 50t/ha biochar applications increased the dry root biomass by 24.0%, respectively, compared to the control (Figure 2.2).The unfertilised treatments, 1t/ha and 200t/ha biochar treatments, reduced root growth by 0.8% and 64% compared to the control. Root biomass of the fertilised wheat was substantially higher compared to the unfertilised plants ($P < 0.001$). The biochar application level also resulted in differences in the root biomass ($P < 0.001$). There were also differences in the root biomass when all treatments were compared ($P < 0.001$).

It should be noted that the root structural interpretation, was only a qualitative illustration and therefore no statistical data was interpreted and discussed, but only the visual effects that was observed at harvesting (Photo 2.1 to 2.10). The structure of the root systems differed greatly among the fertilised biochar treatments. As the level of biochar application increased, the monocot plant species developed more nodal/ branched roots. Over the 12 week pot trial the 50t/ha and 200t/ha treatments had a more complex fibrous root system than when measured against the control and the 1t/ha and 10t/ha, lower biochar applications. More extensive branching and thinner roots can be seen and noticeably higher proportions of root-aggregates that were still bound to the rooting systems are present (Photo 2.4 and 2.5).

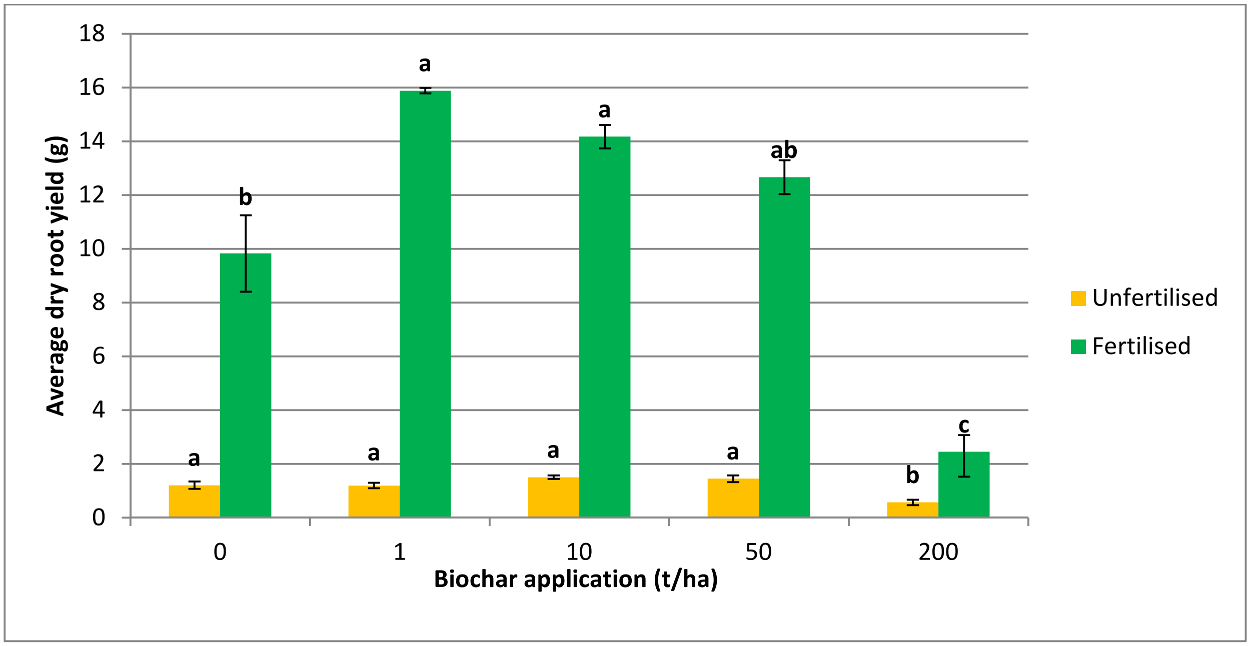

Figure 2.2 Effect of biochar amelioration on dry root biomass of unfertilised and fertilised winter wheat

Photo 2.1 An example of the root structural development of winter wheat in a sandy soil

Photo 2.2 An example of the root structural development of fertilised winter wheat in a sandy soil ameliorated with 1t/ha biochar

Photo 2.3 An example of the root structural development of fertilised winter wheat in a sandy soil ameliorated with 10t/ha biochar

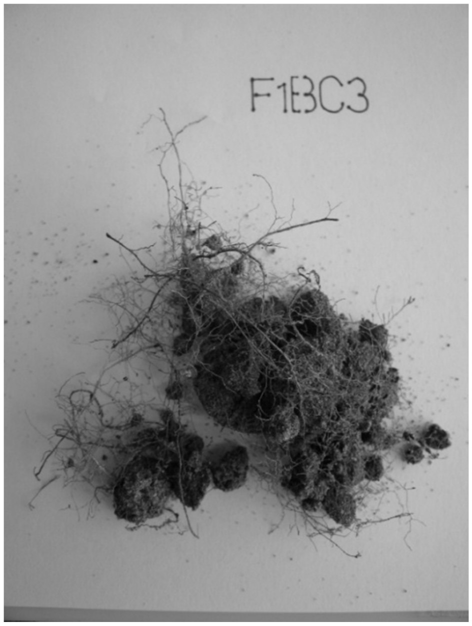

Photo 2.4 An example of the root structural development of fertilised winter wheat in a sandy soil ameliorated with 50t/ha biochar

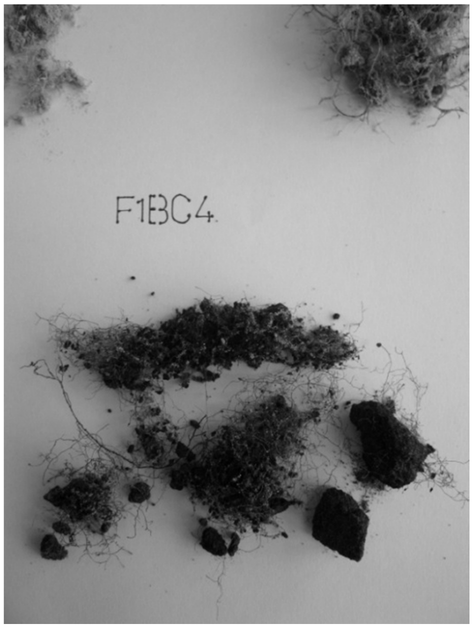

Photo 2.5 An example of the root structural development of fertilised winter wheat ameliorated with 200t/ha biochar

The root hairs were more prominent for the control, 1t/ha and 10t/ha biochar treatment applications. It was also very difficult to remove the sand particles from the roots, without breaking or damaging the roots itself, especially for the fertilised 10t/ha biochar treatments (Photo 2.8), hence the only visual present, without root hairs, but it can be concluded that the sand and biochar particles are strongly bound by the root hairs. The fertilised 50t/ha and 200t/ha biochar applications had less root hair formation (Photo 2.10). It should be mentioned that the 200t/ha biochar treatments performed poorly regarding yield response (above- and below the soil surface), due to the fact that they became diseased within 7 to 8 weeks after planting. It was suspected to have been some form of a soil born pathogen (alleged to be *Fusarium*- or *Phythium* spp.). Symptoms included the following: brown irregular lesions on the lower part of the stem and within the tenth- and eleventh week, the affected plants one-sidedly collapsed where the lesions where concentrated.

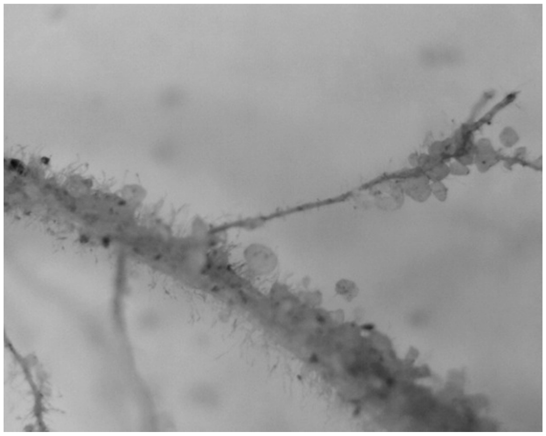

Photo 2.6 An example of root hairs of fertilised winter wheat in a sandy soil

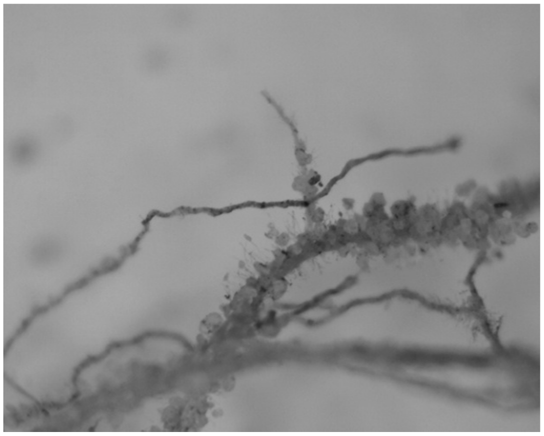

Photo 2.7 An example of root hairs of fertilised winter wheat in a sandy soil ameliorated with 1t/ha biochar

Photo 2.8 An example of root hairs of fertilised winter wheat in a sandy soil ameliorated with 10t/ha biochar

Photo 2.9 An example of root hairs of fertilised winter wheat in a sandy soil ameliorated with 50t/ha biochar

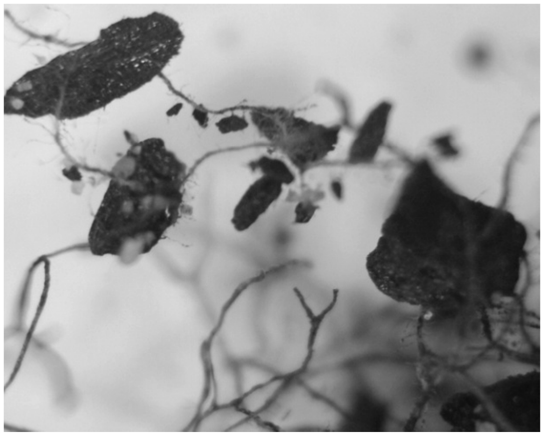

Photo 2.10 An example of root hairs of fertilised winter wheat in a sandy soil ameliorated with 200t/ha biochar

2.3.2.3 Above- and below ground biomass ratio

In the case of the unfertilised treatments, only the 10t/ha and 200t/ha biochar applications increased the shoot-to-root biomass ratio, i.e. by 31.6% and 44.0%, compared to the control (Figure 2.3). In the fertilised treatments the same effect was evident where 10t/ha and 200t/ha biochar application increased the shoot-to-root biomass ratio by 57.3% and 120.9%, respectively, compared to the control (Figure 2.3). Shoot-to-root biomass ratio of the fertilised wheat was substantially higher compared to the unfertilised plants ($P < 0.001$). The biochar application level also resulted in differences in the shoot-to-root biomass ratios ($P = 0.005$). There were however no differences in the shoot-to-root biomass ratios when all treatments were compared ($P = 0.163$) (this showed that a similar response was proportionally the same and present for the unfertilised- and fertilised-biochar treatments).

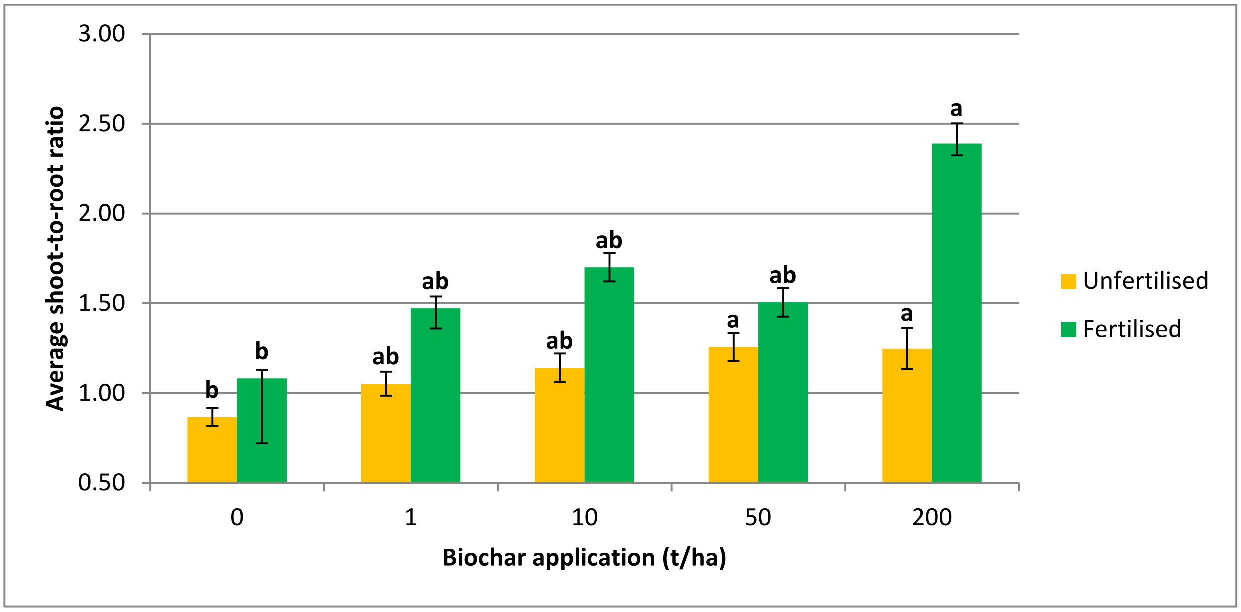

Figure 2.3 Effect of biochar amelioration on shoot-to-root ratio of unfertilised and fertilised winter wheat

2.3.2.4 Evapotranspiration

The average daily ET was calculated and summarised in Table 2.4. Daily ET of the fertilised wheat was substantially higher compared to the unfertilised plants ($P < 0.001$). The biochar application levels did however not differ from each other ($P = 0.356$). There were however no differences in daily ET when all treatments were compared ($P = 0.996$).

See Figures 2.4 and 2.5 for the average ET measurements for both treatment combinations and their individual biochar treatment applications, as measured throughout the pot experiment over the 13 week period.

Table 2.4 Effect of fertilisation and biochar amelioration on evapotranspiration (ET) of winter wheat in a sandy soil. Data are mean of unfertilised- (F0) and fertilised (F1) biochar (BC) treatment combinations where the significant interaction between the F0BC and F1BC combinations were calculated independently from each other

Biochar application	ET ($mm \cdot d^{-1}$)	
t/ha	Unfertilised	Fertilised
0	6.15^a	8.03^a
1	6.72^a	8.32^a
10	7.24^a	9.24^a
50	6.50^a	8.38^a
200	6.15^a	7.64^a

Data are means of 12 observations over the growing season. Within each column and each treatment combination, values with different superscripted letters are significantly different according to Tukey's t-test ($P < 0.05$)

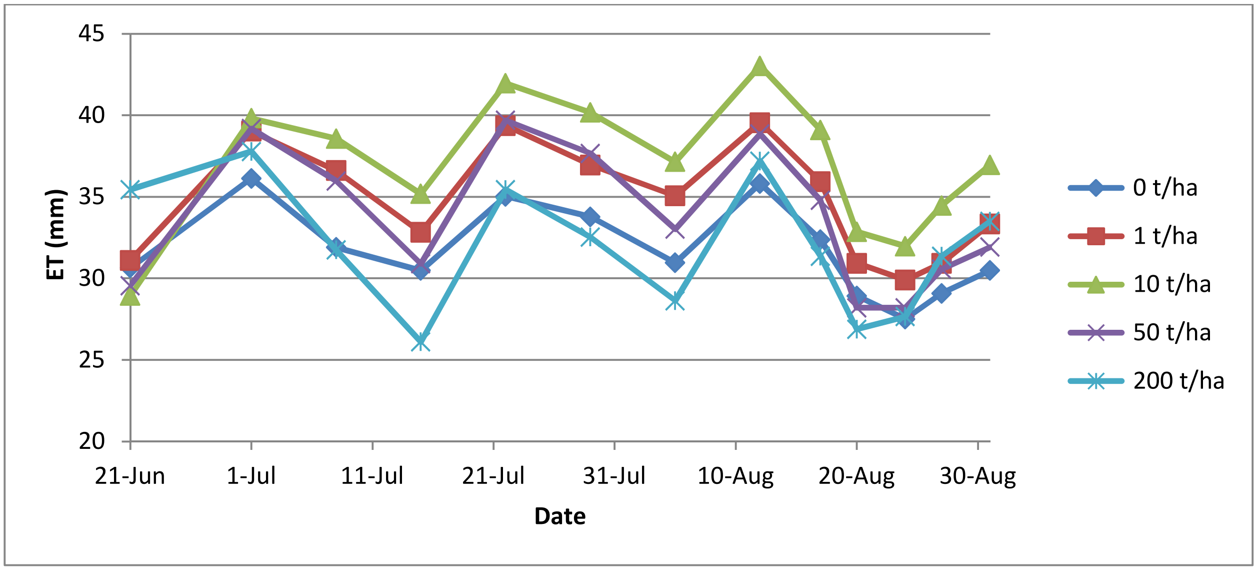

Figure 2.4 Effect of biochar amelioration on evapotranspiration (ET) measured for unfertilised pots

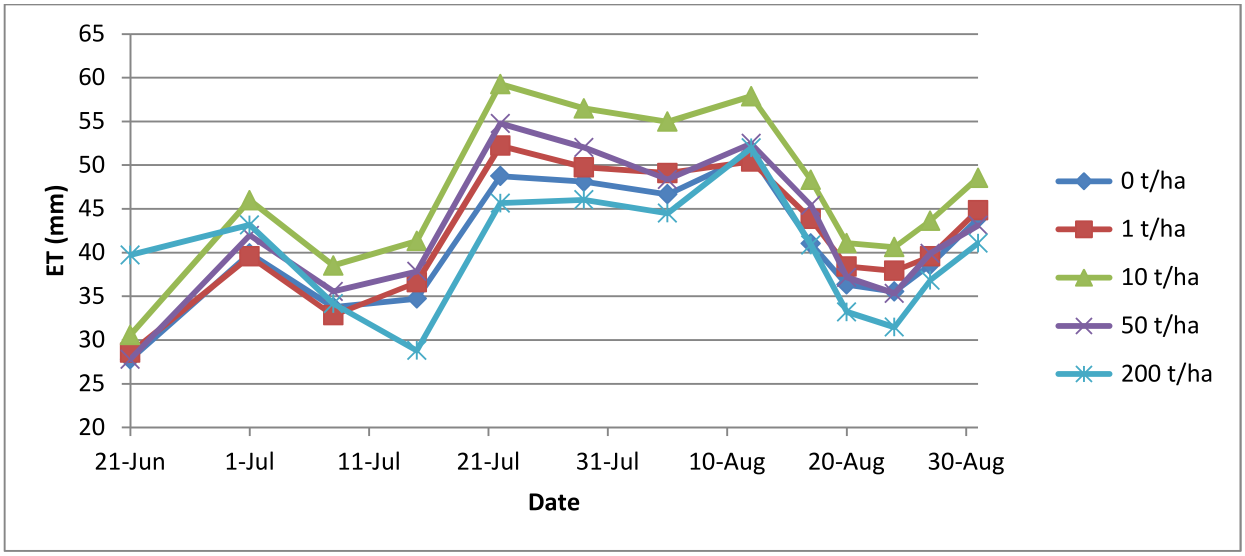

Figure 2.5 Effect of biochar amelioration on evapotranspiration (ET) measured for fertilised pots

The total amount of water applied (mm) for each treatment combination was calculated at the end of the pot trial (Table 2.5) by basically taking the total amount of water over the experimental period. It should be mentioned that the plants were irrigated according to the amount deficit from the FC weight of the pot and therefore water use efficiency could be determined at the end with harvest.

As the above ground biomass yield (dry mass in g) results were already presented in section 2.3.2.1, the biomass yield in kg·ha^{-1} will not be discussed again in this section. The yield data in this section is merely a converted value to calculate BWUE, as this is a more practical manner and unit value to interpret.

The unfertilised-biochar treatments responded as follow regarding BWUE: 1t/ha and 200t/ha biochar treatments showed to decrease with 9.34% and 68.13% respectively, and the 10t/ha and 200t/ha biochar applications increased with 1.10% and 7.14%. The fertilised-biochar treatments performed as follow regarding BWUE: 1t/ha, 10t/ha, 50t/ha and 200t/ha led to a +8.44%, -3.62%, -1.75% and -79.26% yield effect, all measured against the control, respectively.

Table 2.5 Means of unfertilised- (F0) and fertilised (F1) biochar (BC) treatment combinations where the significant interaction between the F0BC and F1BC combinations were calculated independently where irrigation (mm), yield (kg·ha^{-1}) and the total biomass water use efficiency (BWUE, kg·ha^{-}1·mm^{-1}) was determined

Treatment	Biochar (ton·ha^{-1})	Number of irrigations	Total amount ^{x} of water applied (mm)	Above ground ^{y} Biomass Yield (kg·ha^{-1})	BWUE ^{z} (kg·ha^{-1}·mm^{-1})
Unfertilised	0	13	156^{c}	284^{b}	1.82^{a}
	1	13	166^{bc}	273^{b}	1.65^{a}
	10	13	205^{b}	376^{a}	1.84^{a}
	50	13	206^{b}	397^{a}	1.95^{a}
	200	13	255^{a}	149^{c}	0.58^{b}
Fertilised	0	13	251^{b}	4179^{b}	16.59^{ab}
	1	13	283^{ab}	5100^{a}	17.99^{a}
	10	13	331^{ab}	5302^{a}	15.99^{ab}
	50	13	287^{ab}	4199^{b}	14.64^{b}
	200	13	302^{a}	1062^{c}	3.44^{c}

Data are the mean of 4^{X}, 4^{y} and 4^{z}. Within each column and each treatment combination, values with different superscripted letters are significantly different according to Tukey's t-test ($P < 0.05$).

2.3.3 Effect of biochar on selected soil properties

2.3.3.1 Plant available water and soil water content

Biochar addition had a profound effect on the water-holding capacity (also referred to as field capacity) indicated in Table 2.6 as mean values for the control and four different biochar applications. The 1t/ha, 10t/ha, 50t/ha and 200t/ha biochar applications had a respectable increase in the total amount of water held at FC, where they increased with 3.63%, 4.03%, 8.06% and 26.21%, respectively.

The permanent wilting point (PWP) was calculated individually for each treatment, as to observe how the total amount of plant available water content (mm) with each biochar treatment application was affected. After amending the sandy soil with the different biochar application levels and thoroughly mixing of the content; biochar had the following effect on plant available water (according to the SPAW software model), for each treatment combination, measured against the control: 1t/ha, 10t/ha, 50t/ha and 200t/ha resulted in an increase of 3.29, 10.82, 8.47 and 21.19 %, respectively. It should be mentioned that the SPAW results should be treated as arbitrary values rather then exact values, because the organic matter content in % on a weight basis, was substituted with the biochar treatment concentrations and is believed that the water-holding capacity will differ. Hence, the PAW content differed from the values generated by the SPAW system since the biochar had a profound effect on the particle-size distribution with higher amounts of biochar added to each pot. Chapter 3 will focus on the water holding capacity as a water retention curve was calculated for each treatment.

Table 2.6 Mean for field capacity (FC), permanent wilting point (PWP), total plant available water (PAW) and available water per pot for the different biochar treatments

Biochar application (ton·ha^{-1})	FC (θ_v, m^3·m^{-3})	PWP [x] (θ_v, m^3·m^{-3})	PAW (θ_v, m^3·m^{-3})	Soil depth [y] (mm)	Water available per pot (mm)
0	0.248	0.005	0.243	140	34.02
1	0.257	0.006	0.251	140	35.14
10	0.267	0.007	0.260	145	37.70
50	0.268	0.022	0.246	150	36.90
200	0.313	0.096	0.217	190	41.23

[x] PWP was determined (SPAW software) by the mean values for each treatment combinations regarding the following properties: bulk density, salinity and organic matter content (including biochar application %).

[y] Soil depth differed, as the sand-biochar applications were mixed, according to a weight-to-weight basis.

The volumetric water content was measured throughout the experiment by weighing the pots prior to irrigation (Figures 2.6 and 2.7). The mean values (see Table 2.7) were determined for each treatment combination and there was a difference in the volumetric water content when the biochar applications levels were compared ($P < 0.001$).

The unfertilised biochar treatments had the following outcome: 1t/ha, 10t/ha, 50t/ha and 200t/ha caused a -2.27%, nil, +36.36% and +152.27% effect, respectively. The fertilised biochar applications had the following effect: 1t/ha, 10t/ha, 50t/ha and 200t/ha increased respectively with 10.71%, 17.14%, 23.57%, and 75%.

Table 2.7 Effect of fertilisation and biochar amelioration on volumetric water content (θ_V, $m^3 \cdot m^{-3}$) of winter wheat in a sandy soil. Data are mean of unfertilised- (F0) and fertilised (F1) biochar (BC) treatment combinations where the significant interaction between the F0BC and F1BC combinations where calculated independently from each other

Biochar application ($ton \cdot ha^{-1}$)	Θ_V ^{X} ($m^3 \cdot m^{-3}$)	
	Unfertilised	Fertilised
0	0.140^c	0.088^b
1	0.155^{bc}	0.086^b
10	0.164^{bc}	0.088^b
50	0.173^b	0.120^b
200	0.245^a	0.222^a

Data are the mean of 13^X. Within each column and each treatment combination, values with different superscripted letters are significantly different according to Tukey's t-test ($P < 0.05$).

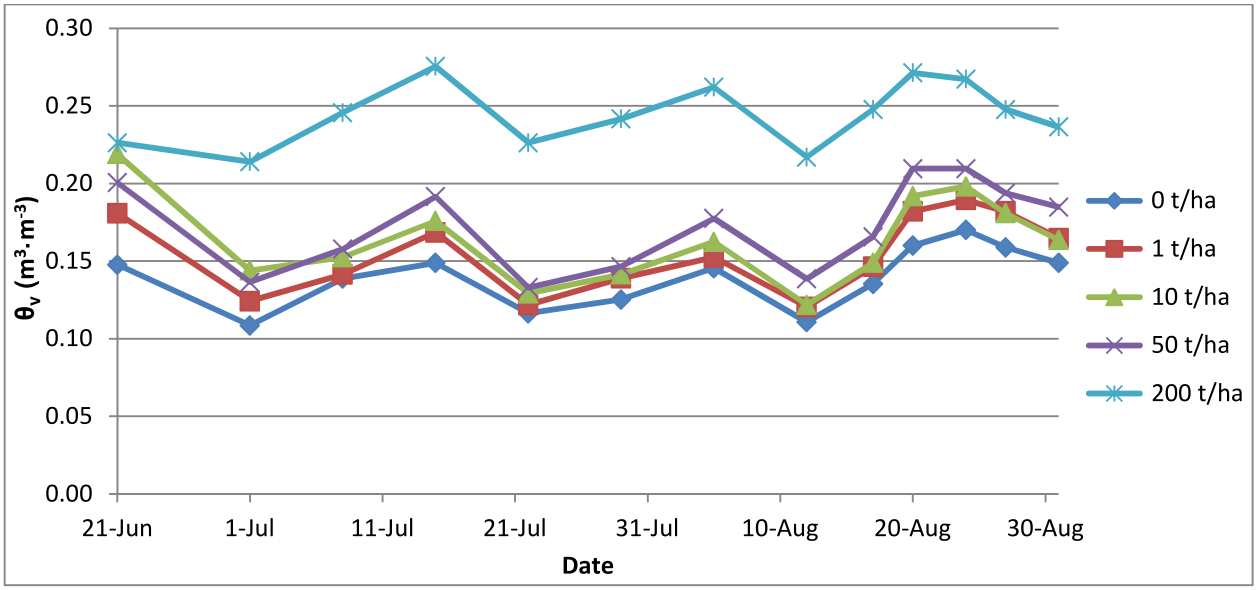

Figure 2.6 Effect of biochar amelioration on volumetric water content (θ_v) measured for unfertilised pots

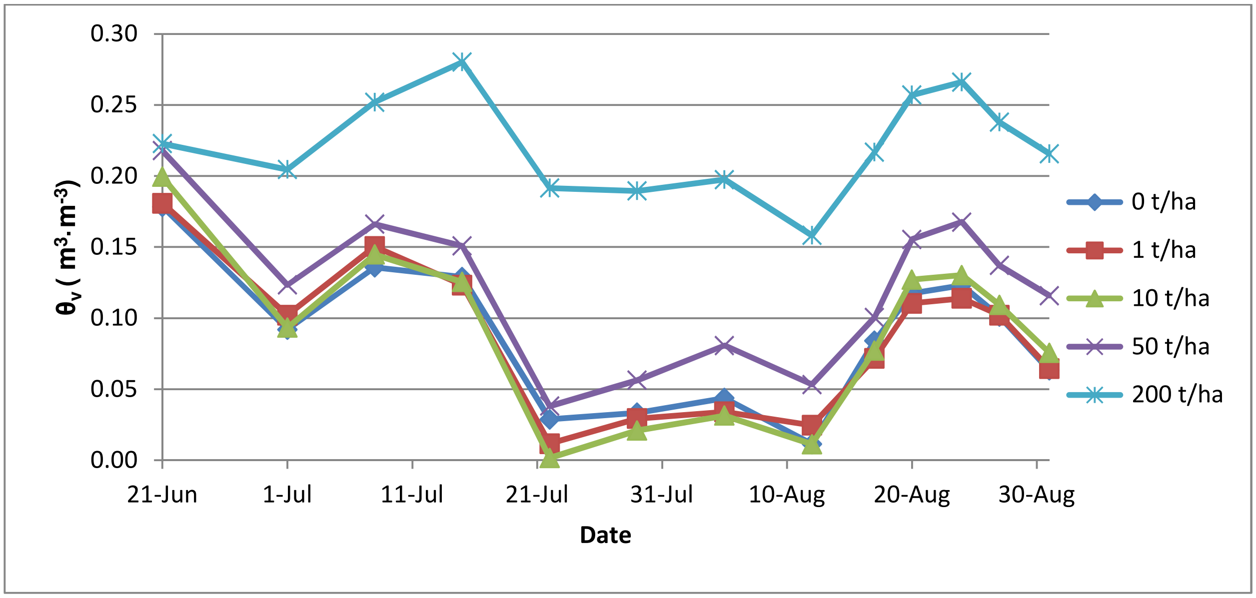

Figure 2.7 Effect of biochar amelioration on volumetric water content (θ_v) measured for fertilised pots

2.3.3.2 Bulk density (ρ_b)

Bulk density did not differ when compared between fertilised and unfertilised treatments ($P = 0.575$) (Figure 2.8). However the biochar application level resulted in differences in the bulk density ($P < 0.001$). There was no difference in the bulk density when all treatments were compared ($P = 0.496$). Only the 200t/ha biochar treatments for both fertilised- and unfertilised treatments where significantly lower, correspondingly 12.8% and 12.4%, when compared to their individual controls.

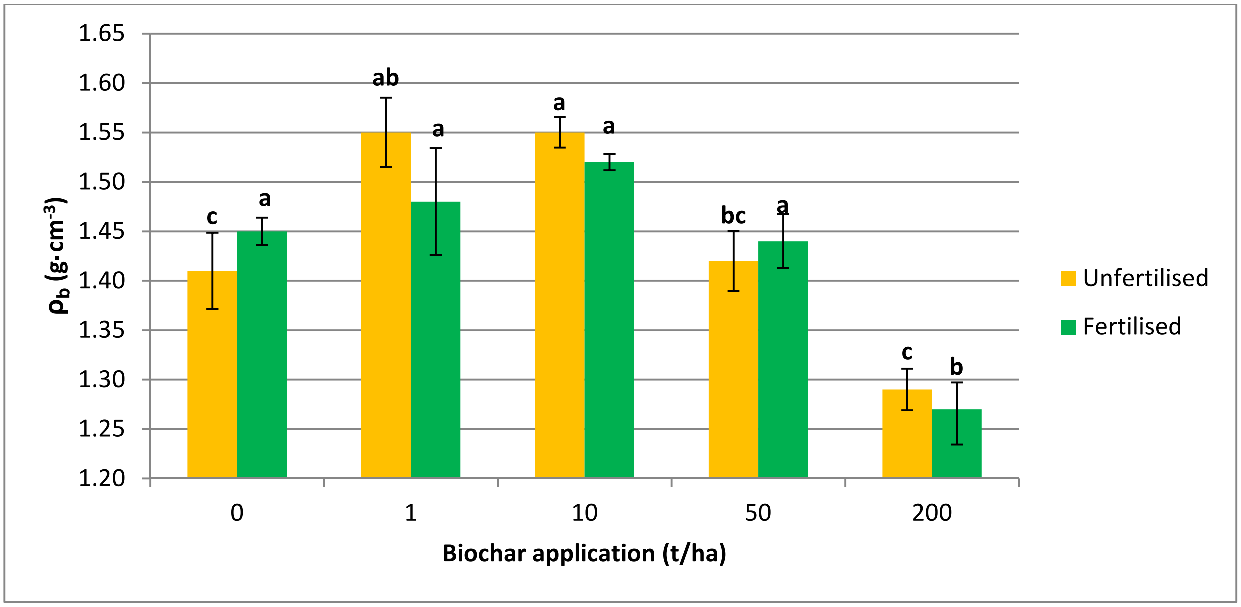

Figure 2.8 Effect of biochar amelioration on bulk densities (ρ_b) of unfertilised and fertilised winter wheat, measured 0-10 cm from soil surface

2.3.3.3 Rhizophere pH measurement

Rhizophere pH (H_2O) of the unfertilised wheat was substantially higher compared to the fertilised pH (H_2O) measurements ($P < 0.001$). The biochar application level also resulted in differences in the pH (H_2O) ($P < 0.001$) (Figure 2.9). There were no differences in the pH (H_2O) when all the treatments were compared ($P = 0.090$), this only indicates that the same trend was observed for the unfertilised- and fertilised treatments with the biochar application.

As the biochar application levels increased, so did the pH (H_2O) for both fertilised- and unfertilised treatments. There was a significant increase in the pH measured against the control for the following unfertilised treatments: 10t/ha, 50t/ha and 200t/ha, which increased correspondingly with 0.2, 0.37 and 1 unit. The fertilised treatments that increased significantly against the control were as follow: 1t/ha, 50t/ha and 200t/ha and increased correspondingly with 0.17, 0.27 and 1.22 unit.

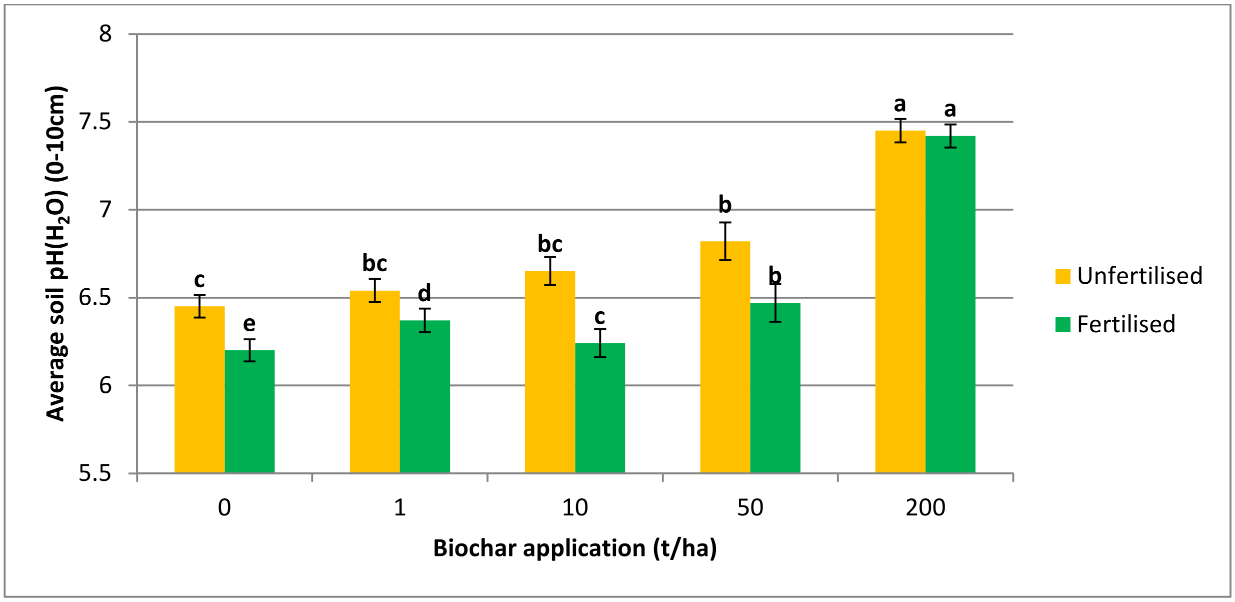

Figure 2.9 Effect of biochar amelioration on pH_{H2O} measured within the rhizosphere (after harvest) of unfertilised and fertilised winter wheat, measured 0-10 cm from soil surface

Rhizophere pH (KCl) of the unfertilised wheat was substantially higher compared to the fertilised pH (KCl) measurements ($P < 0.001$). The biochar application level also resulted in differences in the pH (KCl) ($P < 0.001$) (Figure 2.10). There were also differences in the pH (KCl) when all treatments were compared ($P < 0.001$).The pH increased with more than 1 unit for the unfertilised 200t/ha and the fertilised 50t/ha and 200t/ha treatments, respectively 1.69, 1.12 and 2.3.

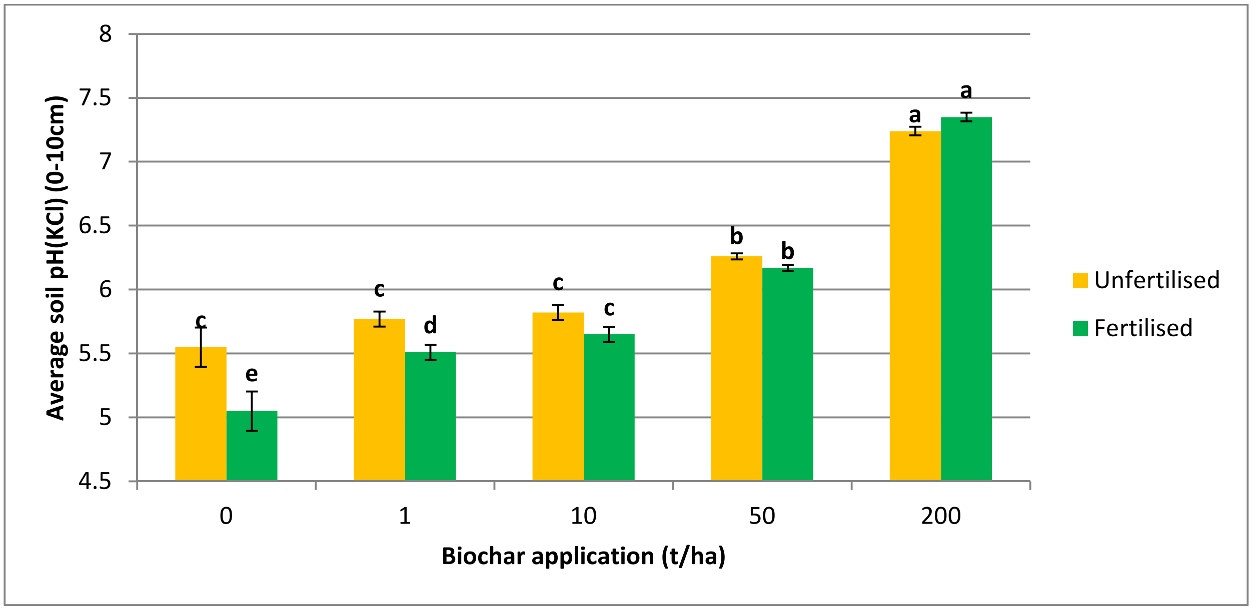

Figure 2.10 Effect of biochar amelioration on pH_{KCL} measured within the rhizosphere (after harvest) of unfertilised and fertilised winter wheat, measured 0-10 cm from soil surface

2.3.3.4 Water-stable aggregates

Water-stable aggregates of the fertilised wheat were substantially higher compared to the unfertilised plants (*P* < 0.001) (Figure 2.11). The biochar application level also resulted in differences in the water-stable aggregates (*P* < 0.001). There were no differences in the water-stable aggregates when all treatments were compared (*P* = 0.183). However the 10t/ha, 50t/ha and 200t/ha fertilised-biochar treatments were significantly higher when compared to the control and increased respectively by 13.33%, 36% and 33.33%. The unfertilised WSA only increased significantly against the control for the 200t/ha biochar treatment and increased with 70.37%. The only decrease in WSA formation was for the 1t/ha biochar-unfertilised treatment, but it did not differ significantly nor deviated from the control.

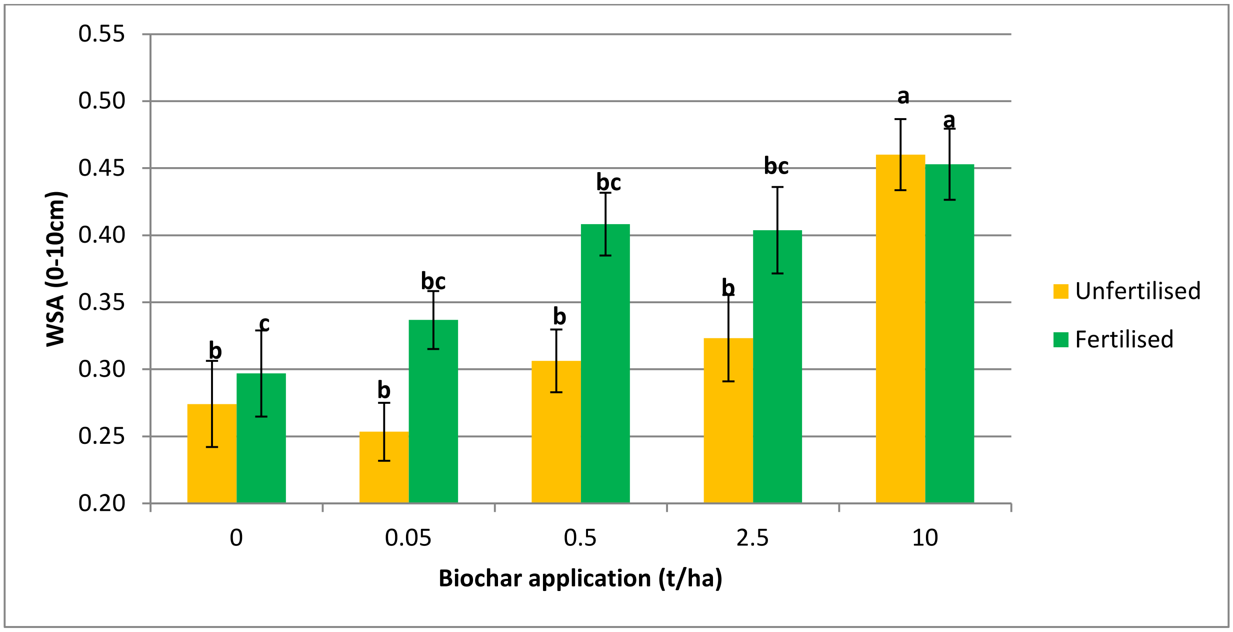

Figure 2.11 Effect of biochar amelioration on water-stable aggregates (WSA) measured within the rhizosphere (after harvest) of unfertilised and fertilised winter wheat, measured 0-10 cm from soil surface

2.4 Discussion

Results reported here offer new information on the impact of biochar on root growth and the structural development of sandy soils, as well as overall soil-plant water status. The main results that will be elaborated on were: (a) high association between fine root systems and their morphology, with increased biochar applications regarding aggregate formation (defining the possible mechanisms), (b) the highest efficiency concerning yield response was for the 10t/ha biochar-fertilised treatment combinations (model plant response concerning biochar application), (c) the BWUE was found to be the most efficient for the 1t/ha biochar-fertiliser combination, and (d) there was a negative wheat yield response when the 50t/ha biochar application was exceeded (threshold value) and we need to define a sound explanation for this effect to facilitate and assist future research.

These results confirmed that biochar application had a positive and to a lesser extent a negative effect on wheat yields (above- and below the soil surface) as well as a profound positive effect on soil structural development (water-stable aggregate formation) within the rhizosphere.

There is up to date no information regarding the possible interaction between sandy soils and structural development with biochar application and therefore also no published work on likely mechanisms regarding root associated aggregate formation for any agricultural crop species, thereby evidently no literature referencing winter wheat crops specifically.

Strong evidence indicated that as the root mass increased, the vegetative yield also increased for the fertilised 10t/ha biochar treatment, which not only produced the highest yield, but also had the second highest dry root biomass, shoot-to-root ratio and water-stable aggregate fraction within the rhizophere (41 % of sampled aggregates were WSA). An increase in yield response as well as shoot-to-root ratio, is a good indication of favourable soil conditions. Wilson (1988) described this effect as an indication of improved resource supply that requires fewer roots to sustain the same above-ground biomass production.

A resent review by Lehmann *et al*. (2011) stated that: “No studies have been published where shoot-to-root ratios increased while plant growth decreased, which would indicate a direct toxic effect of biochars on plant roots through the presence of organic or inorganic (heavy metals) compounds”. This effect was evident and present as results indicated for the higher biochar applications, but this is not believed to be due to a toxic effect, but rather nutrient deficiency and hence these plants were more susceptible to pathogenic microbial infections (soil born diseases). Fageria and Moreira (2011) reported that roots with adequate nutrient supplies may also have more root hairs than nutrient deficient soils. This phenomenon was observed for the fertilised-biochar treatments (Photo’s 2.6-2.10) and supports the previous statement that the decrease in yield was resulting from nutrient deficiency.

Although deficiencies of many mineral elements influence plant growth and root-shoot relationships, constant water and N deficiency limit shoot growth the most (Fageria and Moreira, 2011). Therefore the high shoot-to-root ratio for the 200t/ha biochar treatments can be assigned due to nitrogen deficiency as drought was not a factor. The structural development changed for N deficient roots, because more photosynthates are being used by the roots as they develop greater length to obtain more N and this could also help to explain the higher grade of branching and finer roots that developed as the biochar level increased. Wardlaw (1990) also stated that partitioning of photosynthates and their effects on dry matter distribution were influenced by several environmental factors such as low temperature, drought, and mineral nutrient deficiency.

Phosphate availability affects shoot- and root growth and has been associated with fine root hair formation in the past (Forde and Lorenze, 2001). Gahoonia *et al*. (1997) estimated that the presence of root hairs increased the total root surface area by 95-341% for winter wheat and Hoad *et al*. (2001) remarked the special importance of less mobile nutrient uptake, such as phosphorus (P) by root hairs. The decline in yield for the 200t/ha biochar treatment can be ascribed to its enhanced ability to retain more water and the evident effect it had on the C/N ratio, since there were two possible hypothesis or a combination of the two regarding nutrient absorption.

Firstly, the plant macro- and micro nutrients could have been absorbed by the biochar and be less readily available due to a huge difference brought about regarding the C/N ratio (specifically N-adsorption) and the increase in pH. Secondly the nutrients could have leached from the soil column, as the higher application levels of biochar enhanced water retention and thereby also water mass movement. As the biochar consisted mostly of coarse fragments it's believed to have helped in facilitating percolation through modifying the soil particle-size distribution and overall tortuosity.

The leaching effect could be more applicable to nitrogen, as this mineral is more mobile than phosphorus. Phosphorous is primarily dependent on the soil pH, regarding availability for root uptake (Hopkins and Hüner, 2004), as of its capacity to be polyprotic and therefore changes as the pH changes.

A polyprotic acid contains more than one proton, each with a different dissociation constant (Hopkins and Hüner, 2004). The soil pH plays a major role in the availability of phosphorus, and therefore it is believed that the decrease in yield for the biochar 200t/ha treatment was due to P immobilisation.

Phosphorus is most readily available for root uptake at a pH less than 6.8 where the complex is known as a monovalent orthophosphate anion ($H_2PO_4^-$) and between pH 6.8 and pH 7.2; the predominant form is HPO_4^{2-}, which is even less readily available. As the pH exceeds 7.2 (alkaline soil conditions), the trivalent PO_4^{3-} complex forms and is not available for root uptake (values adopted from Hopkins and Hüner, 2004). The reported pH for the 200t/ha biochar-fertilised treatment was 7.42, and therefore it is most likely that the negative yield response could be described to P deficiency as almost no root hairs could be noticed and that the pH exceeded the threshold value for readily available P. No visual P deficiencies were noticed for the aboveground growth. However the wheat plants of the 200t/ha treatments became diseased during the experimental period and it is most likely due to nutrient deficiency and believed to have made the plants more susceptible to secondary infections such as soil pathogens.

The highest yield response, namely the 10t/ha biochar-fertiliser treatment can be ascribed to the following factors: as sand has a very low specific surface area, its natural capacity is weak regarding nutrient adsorption, therefore with biochar application, which had a high specific area it could be explained as having a complimentary effect with fertiliser application, as it helps to adsorb more plant nutrients and also contributed to the total nutrient availability due to its ash content, along with an optimum pH of 6.24 in the rhizosphere, plant nutrients was readily available for root uptake. Regarding the total amount of water irrigated the 10t/ha fertilised treatments had the highest values and can be simply explained due to a greater need for water as more water was lost due to more transpiration by bigger plants. Another interesting effect to report was that although the 200t/ha treatments performed poorly regarding plant growth and yield (lowest responses), they still had relatively high evapotranspiration throughout the duration of the pot experiment.

The following is only a hypothetical justification, but the main effect regarding water loss for the 50t/ha and 200t/ha biochar-fertilised (which had the lowest yields), could be due to higher evaporation caused by a lowered albedo value, since a larger soil surface area was exposed due to less vegetation present to intercept radiation. As albedo decreases (darker colour) more short wavelengths are adsorbed by the soil body and leads to an increase in soil temperature, hence increased water loss due to evaporation.

The most interesting findings were for the water stable aggregates. The reported aggregate size class (< 250 μm) indicated the micro-aggregate stability fraction; and as micro-aggregates formed, they tend to participate in the formation of macro-aggregates and therefore micro-aggregation can be described as the foundation for favourable aggregation in sandy soils. The complex dynamics of aggregation are the results of the interaction of many factors including the environmental, soil management factors, plant influences and soil properties such as mineral composition, texture, soil organic carbon concentration, pedogenic processes, microbial activities, exchangeable ions, nutrient reserves, and moisture availability (Kay, 1998).

There were several mechanisms regarding aggregate formation, but different mechanisms will be operating in different soil types. According to Bronick and Lal (2005) the rate and stability of aggregation generally increased with soil organic carbon (SOC) and clay surface area and CEC, and in soils with low SOC or clay concentrations, aggregation may be dominated by cations. As the sand had very little to almost no organic matter content along with low specific surface area and- CEC, it's believed that the main mechanisms regarding biochar amendment was related to a higher concentration of cations, increased carbon content, precipitation of P, microbial- and root exudates.

Aggregate stability was the highest for the 10t/ha and 200t/ha biochar-fertilised treatments, but it is not believed to be due to the same mechanism, as the root morphology and plant yields differed greatly. The dry root mass was the highest for the 1t/ha and 10t/ha biochar-fertilised treatments and these plant species, had less branching with more pronounced root hair formation, probably due to more luxurious consumption of plant available nutrients and favourable pH. The active mechanism is possibly owed to greater association with microbial organisms and root mucilage and-exudates. Plant roots in sand tended to form more readily microbial-root aggregates, as the absence of clay and low SOC with low CECs and surface areas, limit root associated aggregation with the previously stated factors.

Chemically, roots enhance aggregation by releasing a variety of compounds, which have a cementing effect on soil particles (Bronick and Lal, 2005). Roots affect the soil pH (due to active nutrient uptake and the release of H^+) and the 10t/ha had lower rhizosphere pH measurements than all the other biochar amended treatments. This indicates that greater root uptake and root activity was present. Therefore the higher WSA was believed to be due to root- and microbial interaction as the biochar retained more nutrients and created a so-called "hot spot" for biotic factors.

The highest WSA formation was delivered by the 200t/ha biochar-fertilised treatments. Here we found the opposite as discussed in the above passage. The highest fractions of WSA are now associated with the lowest yield responses.

The mechanisms can surely not be the same. Therefore it's believed to be due to the alkaline nature of the applied biochar (liming effect and P precipitation), along with higher concentrations of bivalent cations such as Mg^{2+} and Ca^{2+} (woody plant material derived biochar), which can also precipitate as bicarbonates, and the heterogeneous chemical external- and internal surface area. Generally, Ca^{2+} is more effective than Mg^{2+} in improving soil structure (Zhang and Horn, 2001). It's a well known fact that bivalent cations, such as Ca^{2+}, improve structural formation *via* cation bridging.

Chan and Heenan (1999) suggested that the increase in aggregate stability in limed soils were due to the effect of strong bonding involved with Ca^{2+} bridge formation. Application of P as fertiliser and phosphoric acid can lead to the formation of Al^{3+} or Ca^{2+} phosphates, which act as aggregate bonding agents (Haynes and Naidu, 1998). Bronich and Lal (2005) reported in their review that increased pH, cations, bicarbonate (HCO_3^-), dissolved carbonates and CO_2 can react with available cations to form secondary carbonate coatings on primary soil particles. The main mechanisms regarding the WSA formation and biochar application, is believed to be due to the increase in pH with biochar amelioration, along with the additional presence of Ca^{2+} plant available mineral fraction, as the biochar had relatively high amounts of K^+ and Ca^{2+}, and lastly the fertilised derived P that reacted with calcium and precipitated through the increase in pH.

2.5 Conclusion

Application of biochar to sandy soil had significantly increased field capacity and PWP.

The highest aboveground vegetative wheat biomass yield was obtained at 10t/ha, which is attributable to the following factors: optimum pH, along with additional plant minerals (ash content) and the biochar's high specific surface area (better nutrient retention), therefore facilitated the wheat crops to grow under optimum soil conditions - this can be studied in more depth as this could be a possible future solution to decrease fertiliser loss *via* leaching for sandy soils.

The higher application level of biochar is suspected to have limited adequate supplies of macro- and micro nutrients and hence created elemental imbalances and this was believed to be the main effect regarding the finer root system formation and decrease in yield for the 200t/ha biochar-fertilised treatments.

BWUE increased significantly for the 1t/ha fertilised plants (second highest yield response) and this can be seen as a significant finding regarding biochar research related to agricultural crop production. Water use efficiency was enhanced and could help to address the water constraints for more arid agricultural regions, as there is a dire need to help conserve- and apply water correctly and efficiently.

Increased knowledge of crop specific scenarios regarding root architecture and root development dynamics with biochar application should be studied in depth and for case specific studies. It was evident that biochar at low applications levels, such as 1-10t/ha could have a synergistic effect when applied with fertiliser on overall plant nutrient availability and can lead to more effective agricultural management practices for agronomy crops.

Water-stable aggregation was promoted in the sand-rhizosphere interface, but more research needs to be done so that a comprehensive understanding of how and what exactly occurs can be formulated and trusted, but thus far biochar significantly improved sandy soil’s aggregation and therefore can help to combat water- and wind erosion.

Biochar proved to be most efficient when applied to a sandy soil at the 1t/ha and 10t/ha fertilised applications and significantly increased biomass yield, aggregate stability and BWUE; all of these factors are challenging for sandy and Mediterranean climatic areas and therefore it is believed that biochar can play a key role in addressing future agricultural research regarding these problematic soil- and climatic conditions, for South African and international agriculture.

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CHAPTER 3: BIOCHAR AMENDMENT AND ITS INFLUENCE ON SELECTED PLANT GROWTH TRAITS AND- SOIL PHYSICAL PROPERTIES FOR THE COMMON GREEN BEAN (*PHASEOLUS VULGARIS* L.)

3.1 Introduction

Mitigation of greenhouse gases (GHGs), facilitating nutrient retention and increasing the potential to absorb or store more soil water, were some of the previous observations and focuses of international studies regarding biochar applications to agricultural soils (to recap see Table 1.1 in Chapter 1). However, as these attempts yielded predominantly positive results, currently lacking is full scientific explanations on the modification with biochar amendments on physical soil properties and how this will influence the overall wellbeing of agricultural crop species (focusing on plant-water status).

Understanding the factors influencing water- and plant nutrient availability with application of biochar to agro-systems, should be critically studied and evaluated for future and successful management of irrigation- and fertilisation programs, if biochar should become a commercial soil amendment. No published work to date incorporated comprehensive studies, were the plant-soil interface was considered as one entity and defined regarding biomass water use efficiency (BWUE), water balance of the soil, yield responses (above- and below the soil surface) and plant nutrient status in combination with plant specific traits. Previous work regarding biochar have focused mostly on climatic regions were tropic- and sub-tropic weather patterns are found, and to a lesser extent for Mediterranean climatic zones, where water scarcity and nitrogen availability are the main limitations concerning yield, especially for sandy soils.

The main aims of the study were: (a) to assess the combined effect of imposing different biochar treatments regarding plant productivity, focusing on yield response and biomass water use efficiency; (b) to study the relationship between the different biochar treatments and how plant response were influenced; and (c) determining the effects of biochar amendment on the sandy soil's water-holding capacity.

3.2 Material and methods

3.2.1 Soil and biochar

Seeing that the same soil and biochar was used throughout the study and standard methods, when it is applicable, reference to Chapter's 2 sections will be given and any additional details will be specified alongside the referred section. See section 2.2.1 for detailed information regarding the sampling for the soil.

The pH was determined as described by White (1997) in a 1:2.5, soil to distilled water solution, and the total C- and N content was determined by means of the dry combustion method (Nelson and Sommers, 1996) *via* the EuroVector Elemental Analyzer. The cation exchange (CEC) capacity of the soil was determined by making use of 1 M NH_4OAc solution (Summer and Miller, 1996).

See section 2.2.1 for detailed information regarding the biochar used.

The pH for the biochar was determined in a 1:20, biochar to distilled water solution, and is based on the work done by Cheng and Lehmann (2009). Total C- and N content was also determined by making use of the dry combustion method and the EuroVector Elemental Analyzer.

Soil chemical analysis regarding the different soil-to-biochar treatments were conducted for the following chemical parameters: pH (distilled water, 1:20); electrical conductivity (EC; 1:5, soil to solution), total C- and N content using the dry combustion method described by Nelson and Sommers (1996) (using a EuroVector Elemental Analyser), exchangeable cations by making use of ammonium acetate method (1 M NH_4OAc for

60 min at a pH of 7; analysed by CAF lab, Department of Soil Science, Stellenbosch University, using a Varian atomic absorption spectrometer), and total macro- and micro-elements were determined (Soltanpour and Schwab, 1977) by using $NH_4HCO_3^-$ - DTPA extraction solution (method standard for alkaline soil samples). The macro- and micro-elemental extracts were respectively analysed at the Department of Geology, Stellenbosch University, using inductively coupled plasma atomic emission spectrometry (ICP-AES) and- mass spectrometer (ICP-MS) analysers.

3.2.2 Plant growth trial and sampling

See section 2.2.2 for details of experimental design and preparation. The bean pot trial only had three replicates per treatment combination.

A broad spectrum fertiliser was used, namely Chemicult Hydroponic Powder (CHP) and the plant test species, *Phaseolus vulgaris* (common name, green bean) where planted on the 18 of April 2011 as one week old seedlings and fertigated on a weekly basis throughout the 39 day pot trial (harvest was determined according to bloom initiation).

It should be mentioned that all pots were pre-treated with Xanbac D (which is a fungicide - active ingredient is dichlorophen), 16 days prior to planting of the seedlings. Each pot received 300 mL of the fungicide solution and the reason for the Xanbac D application was because with the first attempt of this bean trial, post emergence damping-off occurred and *Pythium aphanidermatum* was isolated from the tested plant material and diagnosed. The analysis was completed by the Department of Plant Pathology, Stellenbosch University.

The fertigation solution was arranged and schedule as follow: a stock solution was prepared before each fertigation application, where 1 g of CHP was diluted for every litre of tap water used; each pot received on the date of planting, and thereafter on a weekly basis, 250 mL of the CHP stock solution. One seedling per pot were planted and irrigated to field capacity (FC) on a weekly basis.

At harvest the leaf area was determined by making use of the LI-3100 Area Meter for three pre-selected leafs per plant test species. Specific leaf area (SLA) was also determined as follow: the one-sided area of a fresh leaf divided by its oven-dry mass (Cornelissen *et al*., 2003).

The relative chlorophyll concentration was measure for all the dicot test species on the day prior to harvesting, by only measuring the relative chlorophyll content index (CCI) for their youngest completely expanded leaves, where a CCM-200 plus (manufactured by Opti-Science, Inc., Hudson, New Hampshire, USA) handheld apparatus was used.

Maximum photosynthesis (A_{max}), transpiration (E) and water use efficiency (A/E) were recorded for all fertilised treatment combinations over a short period, starting late-morning (11:43am) on the 12 of June 2011 *via* an infrared gas analyser (IRGA; Licor, Li-6400 Portable photosynthesis system, Lincoln, Nebraska, USA). WUE was derived by dividing A_{max} (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) with the measured E value (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) and all the measurements were taken on the first fully expanded leaves in full sunlight. Before any measurements were recorded, a response curve of assimilation against light intensity was constructed and was found to be 1500 μmol photons$\cdot m^{-2} \cdot s^{-1}$.

Above- and below ground plant biomass was separated with harvesting and the fresh- and dry weight was determined. The biomass was oven dried at 80 °C to a constant mass and weighed (after two days).

Note should be taken that all the oven-dried plant material, that were send for analysis, were milled before hand using a rotor Retsch Ultra Centrifugal Mill ZM 200 model, set at 1200 rpm, where the plant material was milled to < 40 μm size.

The three leaves that were measured regarding leaf area and SLA, where kept separated and was analysed for leaf carbon and nitrogen ratios, $\delta^{13}C$ (O'Leary et al., 1992) and $\delta^{15}N$ (Evans, 2001) as a means to determine if any of the plant test species responded differently regarding the biochar treatment and water stress relations. The C- and N isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) were calculated as follow:

$$\delta = 1000\% \text{º} \, [R_{sample}/R_{standard}] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.1)$$

where R is the molar ratio of the heavier to the lighter isotope of the specific sample and the standard was defined according to the work done by Farquhar *et al*. (1989).

The sample size was between 2.100 mg and 2.200 mg and weighed into 8 mm by 5 mm tin capsules (Elemental Microanalysis Ltd., Devon, U.K.) on a Sartorius microbalance (Goettingen, Germany). Samples were then combusted in a Fisons NA 1500 (Series 2) CHN analyser (Fisons Instruments SpA, Milan, Italy). The carbon and nitrogen gas release were determined on a Finnigan Matt 252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany), which was connected to a CHN analyser by Finnigan MAT Conflo control unit, and the values represented the measured $\delta^{13}C$ and $\delta^{15}N$, respectively. Three standards were used to correct the samples for machine drift and was as follow: two in-house standards (Merck Gel and Nasturtium) and one IAEA (International Atomic Energy Agency) standard-$(NH_4)_2SO_4$.

The rest of the oven-dried plant material were send to a commercial laboratory (BemLab, De Beers Rd, Somerset West, South Africa), for plant macro- and micro-elemental analysis (root and shoot material was milled together for each treatment) by using ICP-MS and a LECO-nitrogen analyser with Spectrascan standards (Norway).

3.2.3 Selected soil properties

3.2.3.1 Soil water

See section 2.2.4.1 regarding field capacity (FC), permanent wilting point (PWP) and volumetric water content (θ_v) calculations.

3.2.3.2 Soil water retention and bulk density

The water retention curve (WRC) for the different biochar applications and control were determined using a sandbox apparatus (08.01 Sandbox, Eijkelkamp, Giesbeek, The Nederlands) according to manufacturer's instructions (Eijkelkamp, 2007). Undisturbed soil samples were collected *via* a standard core sampler for each biochar treatment and the control, respectively. The samples were collected at 0–5 cm and > 5–10 cm depth. The sampling rings with the respective samples were covered at their bottom end, by sealing it with a piece of ultra-thin capillary material (filtration paper) and adhesive, as recommended by the manufacturer, aiming to prevent sample loss as the rings needed to be removed for weighing. Each of the four biochar applications and the control was replicated five times. Initially, the samples where saturated with distilled water by placing them in 1 cm of water in the sandbox, waiting for 3 h and then slowly raising the water level to the top of the ring (half a cm from the rim) for a period of 24 h. Following saturation, the samples were subjected to a range of water suction's of up to 10 kPa.

The water content of the samples were gradually decreased by exposing them to increased suction regimes, initially from 0.5, 1, 2, and up to 10 kPa, with 1 kPa unit difference between each sampling point. To ensure establishment of steady moisture conditions in the bulk of the samples at each suction level, the time intervals between each step of suction increase were at least 3 days or until the difference between two successive weighings were at least more than 0.2 grams. After establishment of moisture equilibrium at each suction level, ranging from 0.5 – 10 kPa, the samples were removed from the sandbox, weighed, and returned immediately back to their original position.

All the samples were oven-dried for approximately 24 hours at 60 ℃ to a constant mass and weighed. The volumetric water content was thus estimated by deducing the dry weight from the actual weight of the sample at the specific suction regime and multiplying it by die bulk density.

The bulk density (ρ_b) was determined as sample dry weight to volume ratio ($g \cdot cm^{-3}$). The WRC of the four biochar treatments and the control were graphically illustrated by presenting the mean of the five measured values of volumetric water content at each suction level.

3.2.4 Statistical analysis

Refer to section 2.2.4 and Table 2.1 for statistical analysis.

3.3 Results

3.3.1 Soil- and biochar characterisation

Seeing that the same soil and biochar was used throughout the study, refer to section 2.3.1 and Tables 2.2 and- 2.3, regarding basic physiochemical analyses. This section will focus on the results obtained regarding the different biochar applications and interpreting what the effects were on the soil macro- and micro-nutrient status, specifically focusing on nutrients which affect photosynthesis and correlating these results with specific vegetative plant traits in the discussion section.

As the biochar application levels increased, so did the pH measurements for both fertilised- and unfertilised treatments (only pH measured in distilled water will be interpreted) (Table 3.1). There was an increase in the pH_{H2O} measured against the control for the following unfertilised treatments: 1t/ha, 10t/ha, 50t/ha and 200t/ha, which increased correspondingly with 0.67, 0.89, 2.08 and 3.43 units.

The fertilised treatments increased as follow, when measured against their individual control: 1t/ha, 10t/ha, 50t/ha and 200t/ha increased correspondingly with 0.64, 0.75, 1.7 and 2.99 units. The electrical conductivity (EC) for the unfertilised treatments performed as follow, when measured against the control: 1t/ha, 10t/ha, 50t/ha and 200t/ha, led to a +15.90%, -12.27%, +1.31% and +89.26% effect, respectively.

The N- and C content (%) and the carbon-to-nitrogen ration (C/N) were exclusively determined per biochar treatment, before planting of the crop species (Table 3.2). The biochar application level resulted in differences in the N content (%), C content (%) ($P < 0.001$) and C/N ratios ($P = 0.002$).

Table 3.1 Average of unfertilised- (F0) and fertilised (F1) biochar (BC) treatments combinations where the interaction between the F0BC and F1BC combinations where determined for pH (H_2O and KCl) and EC, after harvest initiation of the green beans

Treatment	Biochar (t/ha)	pH (H_2O)	pH (KCl)	EC (ds/m)
Unfertilised	0	6.17	5.58	0.612
	1	6.84	6.30	0.463
	10	7.06	6.35	0.400
	50	8.25	6.99	0.672
	200	9.60	8.56	1.833
Fertilised	0	6.00	5.29	0.591
	1	6.64	6.21	1.264
	10	6.75	6.24	0.971
	50	7.70	8.25	1.247
	200	8.99	8.47	2.152

Note should be taken that the EC measured, was determined with planting of the crop species as this value was used in correlation with the calculation of the PWP *via* the SPAW software program.

Table 3.2 Mean for N- and C content (%) and the carbon-tot-nitrogen ratio (C/N) per biochar treatment combination

Biochar (t/ha)	N ^{x} (%)	C ^{x} (%)	C:N ^{x}
0	0.012^b	0.305^c	27^b
1	0.011^b	0.245^c	23^b
10	0.010^b	0.400^c	40^b
50	0.020^b	1.285^b	64^b
200	0.070^a	7.660^a	111^a

^{x} Data are the mean of 2 samples. Within each column and each treatment combination, values with different superscripted letters are significantly different according to Tukey's t-test ($P < 0.05$).

The total macro- and micro element concentrations for the different sand-biochar treatment applications are summarised (Table 3.3). The following micro elements, also known as heavy metals were not included in Table 3.3, as they were below the norm, regarding toxicity levels, namely: Co, Cd and Pd. Only the elements which are known to have a specific metabolic function, regarding the plant's photosynthesis capacity, have and will be interpreted in this section, to be exact: N (results from Table 3.2), P, Mg, Fe and Mn.

The different biochar treatments (refer to Table 2.1 for conversion to t/ha) had the following effect on Mg concentration, when compared to the control: 1t/ha, 10t/ha, 50t/ha and 200t/ha, increased with 27.50%, 18.91%, 64.64% and 154.19%, respectively. P decreased as follow for the 1t/ha, 10t/ha, 50t/ha and 200t/ha biochar treatments, compared to the control: 87.45, 88.32, 83.01 and 66.97 %, respectively. Fe performed as follow when to the control for 1t/ha, 10t/ha, 50t/ha and 200t/ha application levels: +4.82, +1.49, -15.51 and -18.93 %, respectively. Mn concentration increased as follow for the 1t/ha, 10t/ha, 50t/ha and 200t/ha biochar treatments, correspondingly: 36.33%, 14.88%, 22.84% and 111.76%.

It should be noted that the Ca^{2+}, K^{+}, Mg^{2+} and Na^{+} levels increased correspondingly as the biochar treatments increased. This mineral fraction was believed to be the main rationale as to the increase in pH along with the increase in EC, as salinity increases with addition of these salts (ash content of biochar contains these mineral fractions). The high mono- and divalent cations also correlates with the fact that the biomass feedstock was derived from woody plant biomass, as especially calcium plays an essential role in plant structural support and is relatively high in perennial tree species and therefore with biochar addition as seen in Table 3.3.

Table 3.3 Total macro- and micro element concentrations for the different sand-biochar treatment applications

Biochar (ton·ha^{-1})	Macro elements (mg·kg^{-1})				
	Ca	K	Mg	P	Na*
0	149.360	24.103	13.073	43.022	12.431
1	125.560	38.255	16.668	5.398	19.909
10	111.080	29.600	15.545	5.026	11.055
50	109.300	69.670	21.524	7.311	21.201
200	155.830	196.410	33.230	14.211	36.940
	Micro elements (mg·kg^{-1})				
	Al	B	Cr	Mn	Fe
0	10.58	0.334	0.020	0.289	11.41
1	9.45	0.209	0.022	0.394	11.96
10	10.25	0.265	0.023	0.332	11.58
50	5.43	0.111	0.024	0.355	9.64
200	4.83	0.181	0.043	0.612	9.25
	Micro elements (mg·kg^{-1})				
	Ni	Cu	Zn	As	Mo
0	0.020	0.959	0.886	0.023	0.006
1	0.018	0.561	0.953	0.030	0.009
10	0.020	0.253	0.821	0.030	0.008
50	0.012	0.831	0.789	0.095	0.013
200	0.007	0.558	1.021	0.332	0.011

* non-essential element

3.3.2 Plant growth response

3.3.2.1 Above- and below ground biomass yield

Above-ground biomass of the fertilised beans was substantially higher compared to the unfertilised plants ($P < 0.001$). The biochar application level however did not differ in the above-ground biomass ($P = 0.122$). There were also no differences in the above-ground biomass when all treatments were compared ($P = 0.283$). In the case of the fertilised treatments, the highest yield was obtained for the 10t/ha biochar applications, i.e. by 66% (Table 3.4). In contrast, the fertilised 200t/ha biochar application reduced the vegetative growth, i.e. by 22.60%, compared to the control. In the case of the unfertilised treatments, the same trend was evident with 10t/ha biochar application leading to the highest above-ground biomass, i.e. by 73.33% (Table 3.4). Similar to the fertilised treatments, 200t/ha biochar without fertilisers reduced the vegetative growth by 6.63% compared to the control.

Dry root biomass of the fertilised beans was substantially higher compared to the unfertilised plants ($P < 0.001$). The biochar application level also resulted in differences in the dry root biomass ($P = 0.001$). There was however no differences in the dry root biomass when all treatments were compared ($P = 0.137$). In the case of the fertilised treatments, the highest root yield was obtained for the 10t/ha biochar applications, i.e. by 56.73%, compared to the control (Table 3.4). In contrast, the fertilised 200t/ha biochar application reduced the root growth, i.e. by 52.88%, compared to the control (Table 3.4). In the case of the unfertilised treatments, the same trend was evident with 10t/ha biochar application leading to the highest dry root biomass, i.e. by 81.48%, compared to the control (Table 3.4). The lowest dry root biomass was associated with the 200t/ha biochar treatment without fertilisers.

The shoot-to-root biomass ratio was different for the unfertilised-biochar (P = 0.046) applications, when compared to the control. In the case of the fertilised treatments no difference was found (P = 0.811), compared to the control (Table 3.4). Shoot-to-root biomass ratio of the fertilised beans was substantially higher compared to the unfertilised plants (P < 0.001). The biochar application level did not bring any differences to the shoot-to-root biomass ratios (P = 0.908). There were also no differences in the shoot-to-root biomass ratios when all treatments were compared (P = 0.440).

Table 3.4 Dry vegetative biomass bean yield for unfertilised- and fertilised-biochar application

Treatment	Biochar (t/ha)	Shoots [x] (g)	Roots [x] (g)	Shoot:root [x] ratio
Unfetilised	0	0.30^{a}	0.27^{a}	1.13^{ab}
	1	0.40^{a}	0.34^{a}	1.26^{a}
	10	0.52^{a}	0.49^{a}	1.04^{ab}
	50	0.33^{a}	0.41^{a}	0.82^{b}
	200	0.28^{a}	0.29^{a}	0.99^{ab}
Fertilised	0	1.86^{a}	1.04^{a}	1.72^{a}
	1	2.43^{a}	1.60^{a}	1.52^{a}
	10	2.52^{a}	1.63^{a}	1.51^{a}
	50	1.94^{a}	1.16^{a}	1.82^{a}
	200	0.85^{a}	0.49^{a}	1.97^{a}

[x] Data are the mean of 3 replicates. Within each column and each treatment combination, values with different superscripted letters are significantly different according to Tukey's t-test (P < 0.05).

3.3.2.2 Evapotranspiration and biomass water use efficiency

The total amount of water applied/ irrigated (mm) for the fertilised bean treatments did not differ from the unfertilised treatments (P = 0.190) (Table 3.5). The biochar application level however resulted in differences in the water used for the bean plant (*P* < 0.001). No differences were also evident when all treatments were compared (*P* = 0.123). As the above ground biomass yield (dry mass in g) results were already presented in section 3.3.2.1, the biomass yield in $kg{\cdot}ha^{-1}$ will not be discussed in this section. The yield data in this section is merely a conversed value to help calculate BWUE, as this is a more practical manner and unit value to interpret.

No differences were measure for the green beans regarding BWUE between the different biochar applications for both unfertilised- (*P* = 0.104) and fertilised (*P* = 0.089) treatments (Table 3.5). BWUE of the fertilised green beans was substantially higher compared to the unfertilised plants (*P* < 0.001). The biochar application level however did not differ for BWUE (*P* = 0.112). There was also no difference in the BWUE when all treatments were compared (*P* = 0.280). In the case of the unfertilised treatments for BWUE no difference were found when compared to the control, however the highest BWUE was associated with the 1t/ha treatments, i.e.69.11%, compared to the control. Similar to the unfertilised treatments no difference were found, however the highest BWUE was associated with the 10t/ha treatments, leading to 44.94% increase, compared to the control.

There were no significant differences measured for the unfertilised-biochar treatments regarding ET (*P* = 0.170) (Table 3.5). There was on the other hand a highly substantial difference between the biochar applications for the fertilised treatments (*P* < 0.001). There was a highly significant difference between the fertilised treatments when compared to the unfertilised treatments (*P* < 0.001) as well as a highly substantial difference between ET for the biochar applications (*P* < 0.001) and when all treatments were compared also a substantial difference (*P* < 0.001). The unfertilised-biochar treatments, when measured against the control, responded as follow: 1t/ha, 10t/ha, 50t/ha and 200t/ha increased with 5.95%, nil, 8.33% and 9.52 %, respectively.

The fertilised-biochar treatments responded as follow, measured against their individual control: 1t/ha, 10t/ha, 50t/ha and 200t/ha, led to a -3.23%, +12.90%, +10.75% and +20.43%, effect respectively. See Figures 3.1 and 3.2 for the average ET measurements for both treatment combinations and their individual biochar applications, as measured throughout the pot experiment over the 6 week period.

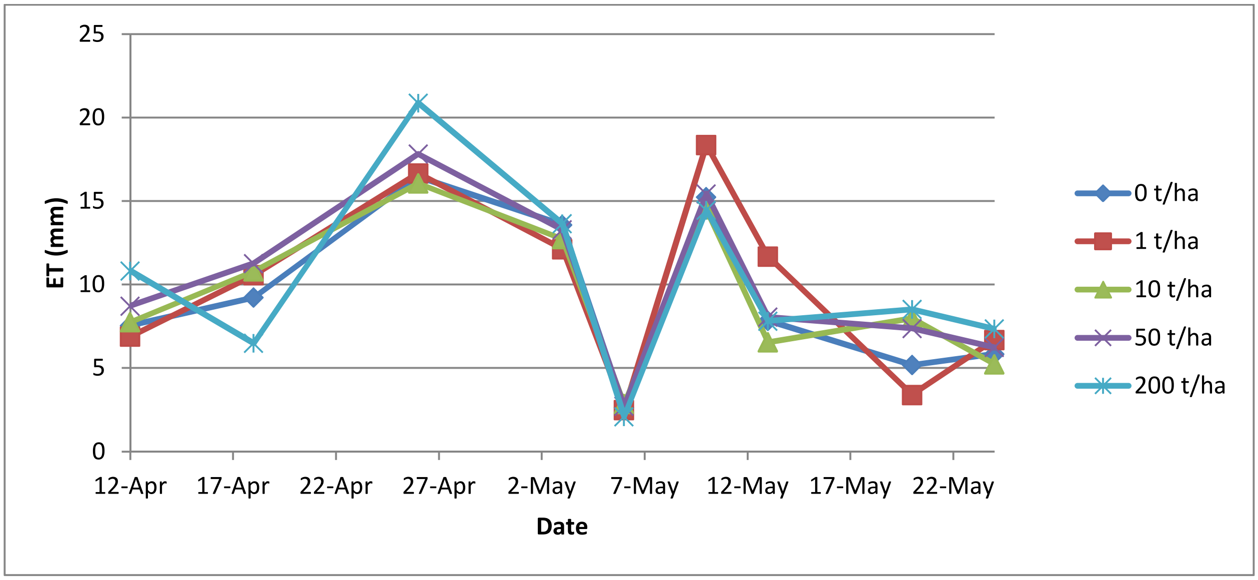

Figure 3.1 Effect of biochar amelioration on evapotranspiration (ET) measured for unfertilised pots

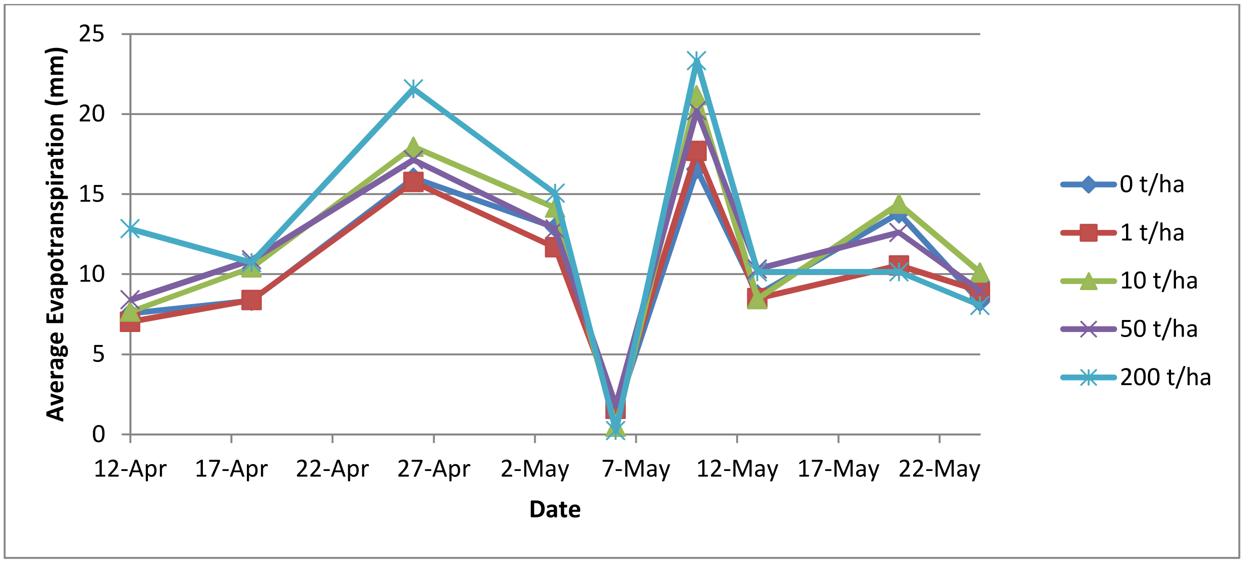

Figure 3.2 Effect of biochar amelioration on evapotranspiration (ET) measured for fertilised pots

Table 3.5 Means of unfertilised- (F0) and fertilised (F1) biochar (BC) treatment combinations where the significant interaction between the F0BC and F1BC combinations were calculated independently where irrigation (mm), yield (kg·ha^{-1}) and the total biomass water use efficiency (BWUE, kg·ha^{-}1·mm^{-1}) was determined

Treatment	Biochar (t/ha)	Total amount of water applied ^{x} (mm)	Above ground Biomass yield ^{x} (t/ha)	BWUE ^{x} (kg·ha^{-1}·mm^{-1})	ET ^{x} (mm)
Unfertilised	0	200^{b}	66^{a}	0.327^{a}	84^{a}
	1	164^{b}	88^{a}	0.553^{a}	89^{a}
	10	213^{b}	114^{a}	0.536^{a}	84^{a}
	50	229^{b}	74^{a}	0.329^{a}	91^{a}
	200	311^{a}	62^{a}	0.205^{a}	92^{a}
Fertilised	0	191^{b}	411^{a}	2.194^{a}	93^{b}
	1	178^{b}	536^{a}	3.033^{a}	90^{c}
	10	176^{b}	557^{a}	3.180^{a}	105^{a}
	50	197^{b}	428^{a}	2.170^{a}	103^{a}
	200	325^{a}	189^{a}	0.582^{a}	112^{a}

^{X} Data are the mean of 3 replicates. Within each column and each treatment combination, values with different superscripted letters are significantly different according to Tukey's t-test ($P < 0.05$).

There were no significant differences between the fertilised-biochar treatments, regarding maximum photosynthesis, transpiration and water use efficiencies (Table 3.6). There was however correlating results regarding the WUE measured *via* the IRGA and the BWUE (calculated after harvesting of the bean plants), were the 10t/ha biochar application had the highest WUE measurements. The maximum photosynthesis was also the highest for the 10t/ha biochar application, along with the lowest transpiration value.

It should be noted that A_{max} and *E* only were measured in the fertilised-biochar plant leaves, as the unfertilised plants had very small leaves that were not fully developed at the time of the leaf measurements.

Table 3.6 Mean for maximum photosynthesis (A_{max}), transpiration (*E*) and water use efficiency (A_{max}/E = WUE $_{instantaneous}$) measured with the IRGA for all the fertilised-biochar treatment combinations

Biochar (t/ha)	A_{max} ^{x} (µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	E ^{x} (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)	WUE $_{instantaneous}$ ^{x}
0	24.74^{a}	3.18^{a}	8.14^{a}
1	28.29^{a}	2.99^{a}	9.49^{a}
10	35.49^{a}	2.21^{a}	17.40^{a}
50	30.80^{a}	2.76^{a}	11.30^{a}
200	28.72^{a}	2.54^{a}	11.98^{a}

^{x} Data are the mean of 3 replicates. Within each column and each treatment combination, values with different superscripted letters are significantly different according to Tukey's t-test ($P < 0.05$).

3.3.3 Leaf characteristics and plant nutrient status

3.3.3.1 Actual leaf area

A highly substantial difference was observed between the fertilised treatments when compared to the unfertilised treatments regarding leaf area ($P < 0.001$) (Figure 3.3). The biochar applications also differed ($P = 0.005$) for both fertilised- and unfertilised treatments and as the biochar increased, leaf area increased correspondently. There were also differences in the actual leaf area when all treatments were compared ($P = 0.010$). The unfertilised-biochar applications did not differ ($P = 0.522$) between each other, but the following effect was evident: 1t/ha, 10t/ha, 50t/ha and 200t/ha, led to a +77.13%, +82.57%, +34.92% and +67.68%, yield response when measured against the control, respectively. The fertilised treatments were significantly different ($P = 0.011$) and performed as follow when measured against the control: 1t/ha, 10t/ha, 50t/ha and 200t/ha, led to a +32.79%, +38.44%, +22.44% and -42.25%, yield response, correspondingly.

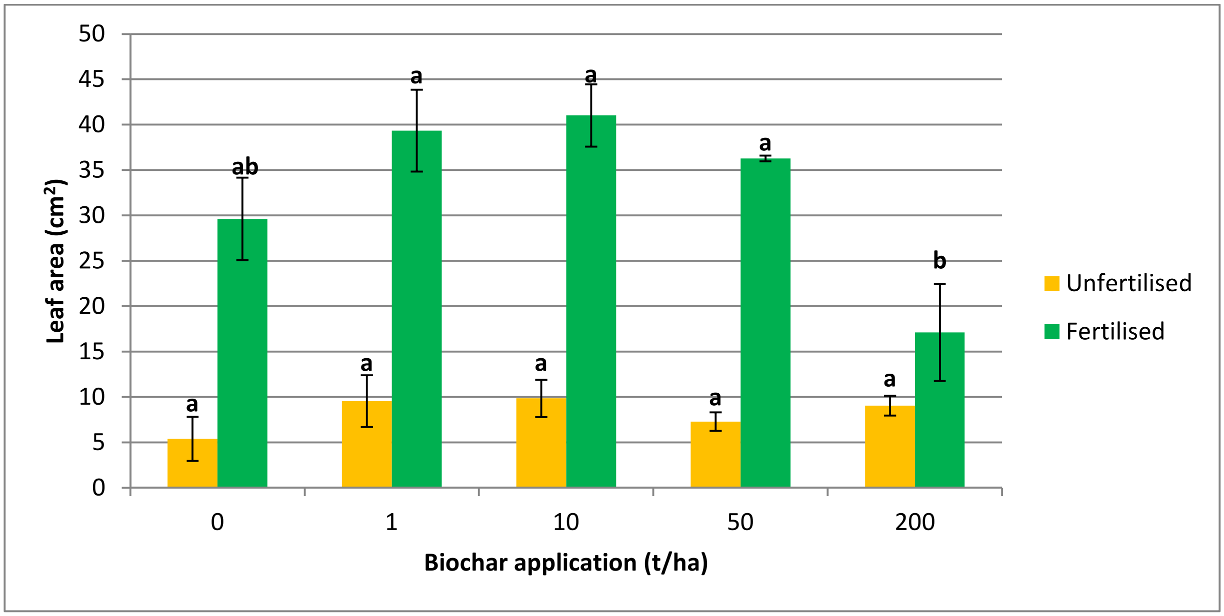

Figure 3.3 Effect of biochar amelioration on leaf area (cm^2) of unfertilised and fertilised green bean

3.3.3.2 Specific leaf area (SLA)

SLA of the fertilised wheat was not substantially higher compared to the unfertilised plants (*P* = 0.312). The biochar application level also did not resulted in any differences in the SLA (*P* = 0.792). There were also no differences in the SLA when all treatments were compared (*P* = 0.792) (Figure 3.4). There was however an increase, as follow in SLA for the unfertilised treatments: 1t/ha, 10t/ha, 50t/ha and 200t/ha biochar applications, increased the SLA by 17.53%, 22.45%, 12.98% and 39.63%, respectively, as measured against their control. The fertilised treatments performed as follow, when measured against their control: 1t/ha, 10t/ha, 50t/ha and 200t/ha, decreased with 5.2%, 14.48%, 7.8% and 10.67%, correspondingly.

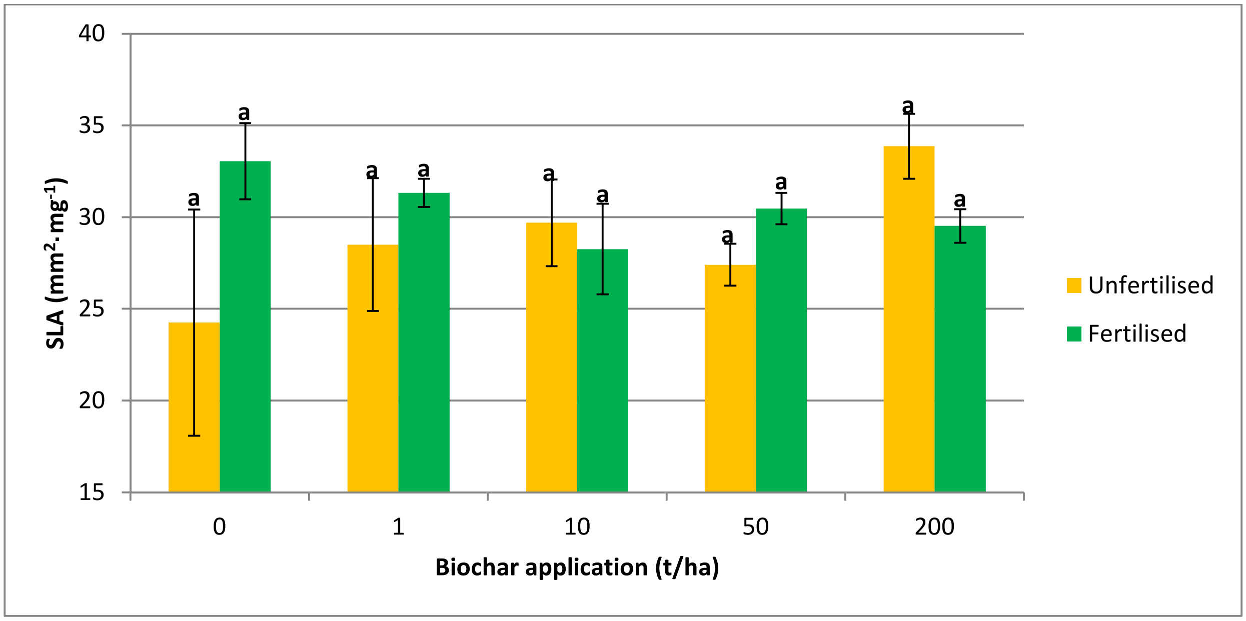

Figure 3.4 Effect of biochar amelioration on specific leaf area (SLA) of unfertilised and fertilised green bean

3.3.3.3 Chlorophyll content index (CCI) and plant nutrient status

There was a highly significant difference between the unfertilised- and fertilised treatments ($P < 0.001$) and also a significant difference between the different biochar applications ($P = 0.006$), but overall there was no significant interaction found for the different treatment combinations, regarding the CCI measurements ($P = 0.236$) (Figure 3.5). There was no significant difference between the biochar applications for the fertilised treatments ($P = 0.206$), and when measured against their control performed as follow: 1t/ha, 10t/ha, 50t/ha and 200t/ha biochar applications let to a +0.25%, -18.86%, +8.44% and -44.42% yield effect, respectively. There was a significant difference ($P = 0.002$) between the unfertilised treatments and when, measured against their individual control, performed as such: 1t/ha, 10t/ha, 50t/ha and 200t/ha decreased correspondingly with 3.67%, 30.79%, 52.20% and 61.58%.

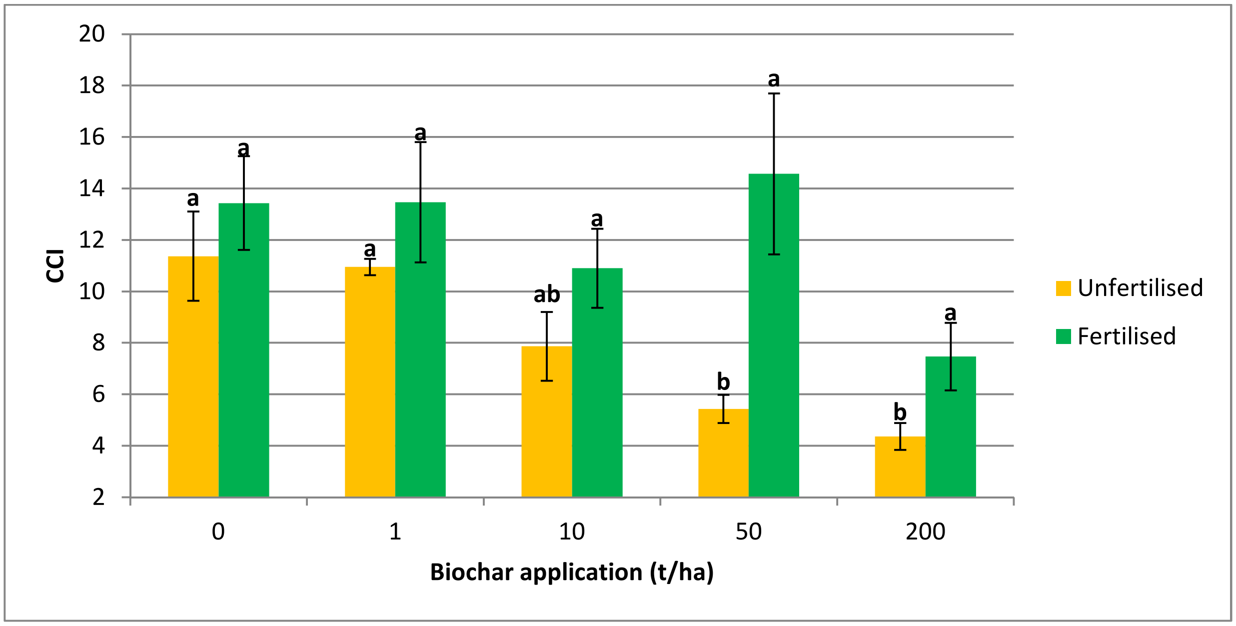

Figure 3.5 Effect of biochar amelioration on leaf chlorophyll content index (CCI) of unfertilised and fertilised green bean

The plant element composition (whole plant was dried and milled, including the roots) is given in Table 3.7 for both the unfertilised- and fertilised-biochar treatment combinations. It should be stressed that only certain elements will be interpreted and discussed in section 3.4, as in with conjunction with the CCI results (Figure 3.5), the main aim is to give sound explanations regarding the effect of biochar application, at different levels, for the selected plant trade, namely, the plant's photosynthetic wellbeing as indicated by the chlorophyll content index (CCI), in this case scenario.

In Figure 3.6, it was evident that as the biochar application increased the CCI index for the unfertilised treatments decreased and one can almost referrer to this response as being antagonistic from each other. We will predominantly focus on the following elements, namely: N, P, Mg, Mn and Fe, as these elements play an essential- and functional role in ensuring that the biochemistry- and photosynthetic activity are optimised, to ensure optimum yield. The mono- and divalent cations (K^+, Na^+, Ca^{2+} and Mg^{2+}) will also be interpreted in section 3.4, as there was an interesting effect noticed: as the biochar applications increased, the monovalent cation concentrations also increased correspondingly and the divalent cation concentrations decreased.

Only the *P*-values will be specified (2-ANOVA), for the results obtained in Table 3.7, and any significant results between the specific biochar treatments for the unfertilised- and fertilised combinations were indicated in Table 3.7 (ANOVA). There was a highly significant ($P < 0.001$) difference measured for nitrogen between the different biochar applications as well as a significant difference between the fertilised- and unfertilised treatments ($P = 0.002$), but overall no significant difference was measured between the treatment combinations ($P = 0.619$) (this shows that a equal trend/ response was proportionally the same and present for the unfertilised- and fertilised-biochar treatments). Phosphate differed highly significantly between the different biochar applications ($P < 0.001$), however there was no significant difference between the fertilised- and unfertilised treatments ($P = 0.286$) and overall no significant interaction between the treatment combinations ($P = 0.066$). There were highly significant differences measured between the biochar applications for potassium, fertilised- and unfertilised treatments and the overall interaction between treatment combinations were highly significantly different (*P* < 0.001). There were no significant difference measured between the biochar applications for calcium (*P* = 0.247), however there was a highly significant difference between the fertilised- and unfertilised treatments (*P* < 0.001), and overall no significant interaction regarding the treatment combinations (*P* = 0.495). No significant differences were measured for magnesium: between different biochar applications (*P* = 0.457), fertilised- and unfertilised measurements (*P* = 0.063) and the overall interaction regarding the different treatment combinations effect (*P* = 0.937). There were highly significant differences measured between the fertilised- and unfertilised treatments (*P* < 0.001) and also significant differences between the biochar treatments (*P* = 0.002), regarding sodium, moreover the overall interaction between the different treatment combinations were as well significantly different (*P* = 0.038).

There was a highly significant difference (*P* < 0.001) between the fertilised- and unfertilised treatment combinations for manganese, as well as a highly significant difference between the biochar applications (*P* < 0.001), but the overall interaction between the treatment combinations was not significantly different (*P* = 0.055). There was a significant difference for iron (*P* = 0.013) between the fertilised- and unfertilised treatment combinations, as well as a significant difference between the biochar applications (*P* = 0.005), but the overall interaction between the treatment combinations was not significantly different (*P* = 0.153).

Copper, zinc and boron were highly significantly different between the fertilised- and unfertilised treatments (*P* < 0.001) and between the different biochar applications was Cu not significantly different (*P* = 0.321), but Zn (*P* = 0.015) and B (*P* = 0.024) was however significantly different. The overall interactions for these trace elements were all insignificant between the treatment combinations: Cu (*P* = 0.509), Zn (*P* = 0.269), and B (*P* = 0.142).

Table 3.7 Plant analysis for green bean plants

Treatment	Biochar (t/ha)	N [x] (%)	P [x] (%)	K [x] (%)	Ca [x] (%)	Mg [x] (%)	Na [x] (mg/kg)	Mn [x] (mg/kg)	Fe [x] (mg/kg)	Cu [x] (mg/kg)	Z [x] (mg/kg)	B [x] (mg/kg)
Unfertilised	0	2.34^{a}	0.23^{a}	1.74^{c}	0.65^{e}	0.48^{a}	2446^{b}	38.33^{a}	459^{a}	11.67^{a}	231.00^{a}	30.33^{a}
	1	2.15^{ab}	0.19^{a}	1.56^{c}	1.10^{b}	0.49^{a}	3381^{b}	33.50^{ab}	445^{a}	13.50^{a}	224.50^{a}	27.00^{a}
	10	2.02^{bc}	0.17^{a}	2.78^{c}	1.22^{a}	0.48^{a}	1562^{b}	36.00^{ab}	355^{a}	11.00^{a}	178.67^{a}	29.67^{a}
	50	1.76^{cd}	0.22^{a}	4.38^{b}	0.88^{c}	0.40^{a}	2445^{ab}	20.33^{b}	393^{a}	15.00^{a}	219.00^{a}	27.67^{a}
	200	1.67^{d}	0.30^{a}	5.99^{a}	0.67^{d}	0.38^{a}	4286^{a}	14.00^{b}	306^{a}	13.33^{a}	188.33^{a}	27.33^{a}
Fertilised	0	2.45^{a}	0.50^{a}	4.21^{a}	0.81^{d}	0.49^{a}	1303^{a}	85.00^{a}	300^{b}	6.33^{a}	165.67^{a}	40.67^{a}
	1	2.29^{ab}	0.45^{a}	4.67^{a}	1.35^{b}	0.51^{a}	1562^{a}	52.00^{c}	301^{b}	6.33^{a}	133.67^{a}	39.00^{ab}
	10	2.20^{ab}	0.48^{a}	4.91^{a}	1.19^{a}	0.49^{a}	1648^{a}	68.33^{b}	384^{a}	7.00^{a}	93.33^{ab}	38.00^{ab}
	50	1.93^{b}	0.54^{a}	5.32^{a}	0.90^{c}	0.47^{a}	1642^{a}	30.33^{d}	265^{c}	7.33^{a}	62.33^{bc}	32.33^{ab}
	200	2.03^{ab}	0.43^{a}	5.01^{a}	0.63^{e}	0.38^{a}	2131^{a}	16.33^{e}	178^{d}	6.67^{a}	55.33^{c}	26.33^{b}

[x] Data are the mean of 3 samples. Within each column and each treatment combination, values with different superscripted letters are significantly different according to Tukey's t-test ($P < 0.05$).

3.3.3.4 Leaf nitrogen- and carbon contents

The leaf N contents differed significantly between the unfertilised- ($P = 0.010$) and fertilised-biochar applications ($P = 0.030$) (Table 3.8). There were also highly significant differences between the fertilised- and unfertilised treatments and between the different biochar applications ($P < 0.001$), moreover a significant difference was evident regarding the overall interaction between the different treatment combinations ($P = 0.029$). The unfertilised-biochar treatments performed as follow, when measured against their control: 1t/ha, 10t/ha, 50t/ha and 200t/ha, decreased with 19.70%, 39.90%, 45.67% and 49.52%, respectively. The fertilised-biochar treatments responded as follow, measured against the control: 1t/ha, 10t/ha, 50t/ha and 200t/ha, led to a -12.39%, +9.40%, -18.12%, and -20.41%, respectively.

There were no significant differences measured between the unfertilised- ($P = 0.305$) and fertilised-biochar applications ($P = 0.250$) for the leaf C content, however there were small differences measured between the treatment combinations (Table 3.8). No significant differences were found between the different biochar applications ($P = 0.171$), unfertilised- and fertilised treatments ($P = 0.124$), and overall the interaction for the different treatment combinations were also insignificant ($P = 0.409$). The unfertilised-biochar applications performed as follow when measured against their individual control: 1t/ha, 10t/ha, 50t/ha and 200t/ha, decreased with 3.63%, 4.74%, 7.59% and 6.67%, correspondingly. The fertilised-biochar treatments did as follow: 1t/ha, 10t/ha, 50t/ha and 200t/ha, led to a -1.31%, +1.14%, -1.92% and -0.48%, response measured against the control, respectively.

The leaf carbon-to-nitrogen ratio were determined to be highly significantly different between the biochar applications for the unfertilised treatments ($P < 0.001$), but no significant difference were found between the fertilised treatments ($P = 0.115$) (Table 3.8). There were highly significant differences between the different biochar applications, unfertilised- and fertilised treatments ($P < 0.001$), moreover an overall significant difference was evident between the different treatment combinations ($P = 0.004$).

The unfertilised- and fertilised treatments yielded the following results, when measured against their individual controls, regarding the C/N ratio effect at the 1t/ha, 10t/ha, 50t/ha and 200t/ha biochar applications: +18.77%, +53.72%, +63.85%, and +78.85% effect for unfertilised treatments and +13.19%, -7.69%, +21.10% and +30.11% effect for the fertilised treatments, respectively.

The leaf $\delta^{15}N$ did not differ significantly between the biochar applications for the unfertilised treatment (*P* = 0.472), but there was well a significant difference measured between the fertilised-biochar treatments (*P* = 0.042) (Table 3.8). No significant interactions were found between the fertilised- and unfertilised treatments (*P* = 0.504), biochar applications (*P* = 0.249), and the overall interaction between the different treatment combinations was not significantly different (*P* = 0.368). The unfertilised-biochar treatments performed as follow, measured against the control: 1t/ha, 10t/ha, 50t/ha and 200t/ha, led to a -21.99%, +13.04%, -56.27%, and -36.57% responses, respectively. The fertilised-biochar treatments had the following result, when measured against the control: 1t/ha, 10t/ha, 50t/ha and 200t/ha, led to a +36.09%, +12.84%, +14.07% and -31.50%, response, respectively.

The leaf $\delta^{13}C$ determination yielded no significant differences between the unfertilised- (*P* = 0.545) and fertilised treatment effects (*P* = 0.374) (Table 3.8). Also there was no significant differences measured between the unfertilised- and fertilised treatments (*P* = 0.087), biochar applications (*P* = 0.563) and the overall interaction between the different treatment combinations were in addition insignificant (*P* = 0.465). The unfertilised-biochar treatments performed as follow: 1t/ha, 10t/ha, 50t/ha and 200t/ha led to a -0.60%, +1.34%, +3.63% and +0.88%, response measured against the control, correspondingly. The fertilised treatments yielded the following results, respectively: 1t/ha, 10t/ha, 50t/ha and 200t/ha, led to a +0.79%, -0.83%, +0.38% and +0.17%, effect measured against the control.

Table 3.8 Mean of unfertilised- (F0) and fertilised (F1) biochar (BC) treatment combinations where the significant interaction between the F0BC and F1BC combinations where calculated independently from each other for total leaf N- and C content (%), C/N ratio, and leaf N- (δ 15N/ 14N) and C isotope ratios (δ 13C/ 12 C)

Treatment	Biochar (t/ha)	N ^{x} (%)	C ^{x} (%)	C/N ^{x}	N ^{x} (- δ)	C ^{x} (- δ)
Unfertilised	0	4.16^b	40.47^a	10.07^b	3.91^a	28.36^a
	1	3.34^{ab}	39.00^a	11.96^{bc}	3.05^a	28.19^a
	10	2.50^a	38.55^a	15.48^{ac}	4.42^a	28.74^a
	50	2.26^a	37.40^a	16.50^a	1.71^a	29.39^a
	200	2.10^a	37.77^a	18.01^a	2.48^a	28.61^a
Fertilised	0	4.36^{ab}	39.62^a	9.10^a	3.27^{ab}	29.06^a
	1	3.82^{ab}	39.10^a	10.30^a	4.45^b	29.29^a
	10	4.77^b	40.07^a	8.40^a	3.69^{ab}	28.82^a
	50	3.57^{ab}	38.86^a	11.02^a	3.73^{ab}	29.17^a
	200	3.47^a	39.43^a	11.84^a	2.24^a	29.11^a

[x] Data are the mean of 3 leaf samples. Within each column and each treatment combination, values with different superscripted letters are significantly different according to Tukey's t-test (*P* < 0.05).

3.3.4 Effect of biochar on selected soil physical properties

3.3.4.1 Soil water dynamics

Field capacity (see Figure 3.6 and Table 3.9) was calculated for each biochar application and the control and the values given below are the mean of 8 replicates. There was a highly significant difference between the different biochar applications for FC ($P < 0.001$) and the response was as follow: 1t/ha, 10t/ha, 50t/ha and 200t/ha, led to a +2.27%, -4.07%, +4.48%, and -22.38%, measured effect against the control, respectively.

The permanent wilting point (Table 3.9) was calculated by means of the SPAW software program and the following effect was evident regarding PAW when measured against the control: 1t/ha, 10t/ha, 50t/ha and 200t/ha, led to a -4.20%, +5.94%, -0.22% and +9.81%, response, respectively.

Table 3.9 Mean for field capacity (FC), permanent wilting point (PWP), total plant available water (PAW) and available water per pot for the different biochar treatments

Biochar application (t/ha)	FC (θ_v, $m^3 \cdot m^{-3}$)	PWP ^{x} (θ_v, $m^3 \cdot m^{-3}$)	PAW (θ_v, $m^3 \cdot m^{-3}$)	Soil depth ^{y} (mm)	Water available per pot (mm)
0	0.266	0.004	0.262	140	36.68
1	0.257	0.006	0.251	140	35.14
10	0.275	0.007	0.268	145	38.86
50	0.278	0.034	0.244	150	36.60
200	0.309	0.097	0.212	190	40.28

^{X} PWP was determined (SPAW software) by the mean values for each treatment combinations regarding the following properties: bulk density, salinity and organic matter content (including biochar application %).

^{Y} Soil depth differed, as the sand-biochar applications were mixed, according to a weight-to-weight basis.

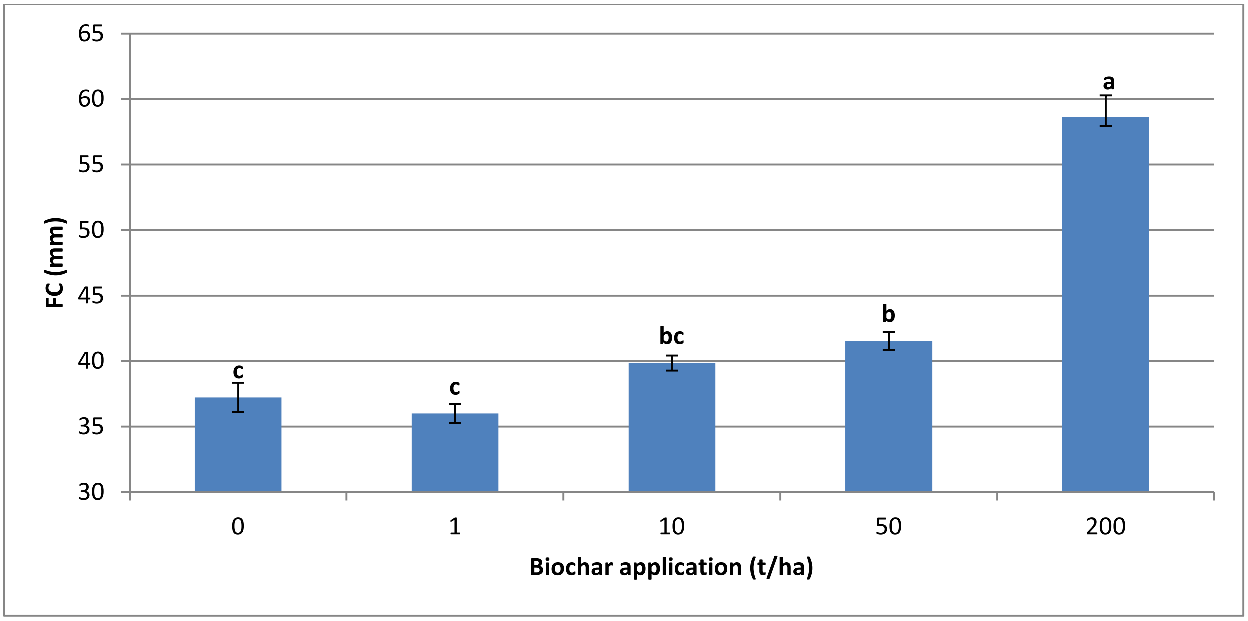

Figure 3.6 Field capacity (FC) measured for each sand-biochar applications

The volumetric water content was measured throughout the experiment by weighing the pots prior to the deficit irrigation regime (see Figures 3.7 and- 3.8). The mean values (see Table 3.10) were determined for each treatment combination and the unfertilised- and fertilised treatments were significantly different (P = 0.038) from each other, concerning the biochar applications, there was a highly significant difference (P < 0.001), however the overall interaction was not significant (P = 0.241).

The unfertilised-biochar treatments had the following outcome (no significant difference, (P = 0.060): 1t/ha, 10t/ha, 50t/ha and 200t/ha caused a -28.89%, +5.19%, +9.63% and +31.11% effect respectively. The fertilised-biochar treatments had the following effect (highly significant, P < 0.001): 1t/ha, 10t/ha, 50t/ha and 200t/ha, led to a -8.70%, -23.48%, -5.22% and +53.91%, response when measured against the control.

Table 3.10 Mean of unfertilised- (F0) and fertilised (F1) biochar (BC) treatment combinations where the significant interaction between the F0BC and F1BC combinations where calculated independently from each other for volumetric water content (θ_V, $m^{3\cdot}m^{-3}$)

Biochar application (ton·ha^{-1})	Θ_V ^{X} (m^3·m^{-3})	
	Unfertilised	Fertilised
0	0.135^a	0.115^b
1	0.096^a	0.105^b
10	0.142^a	0.088^b
50	0.148^a	0.109^b
200	0.177^a	0.177^a

Data are the mean of 8^X measurements. Within each column and each treatment combination, values with different superscripted letters are significantly different according to Tukey's t-test ($P < 0.05$).

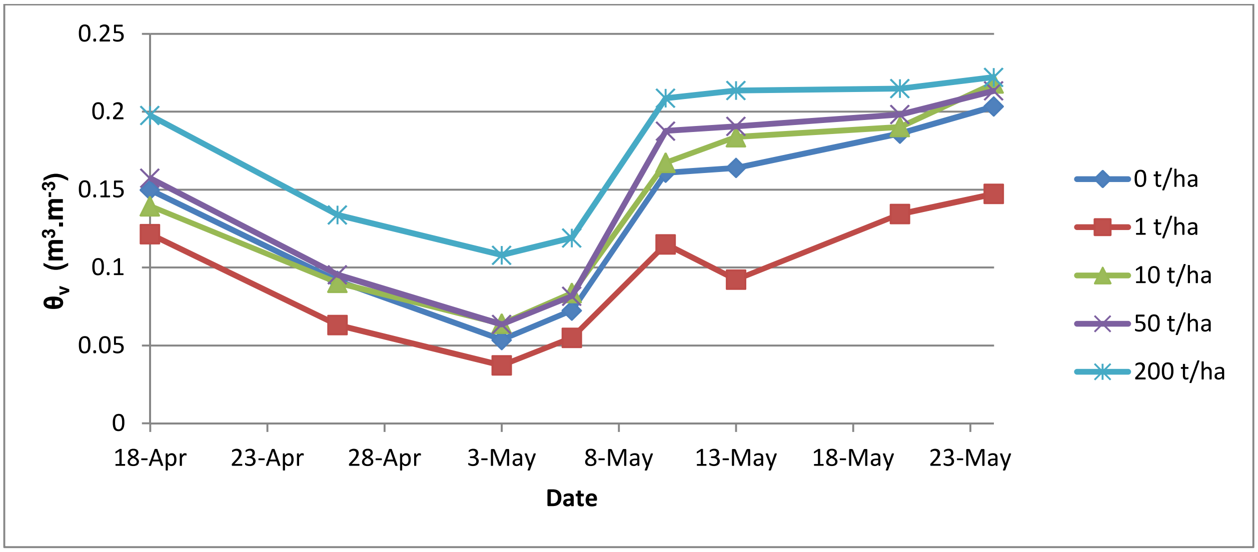

Figure 3.7 Effect of biochar amelioration on volumetric water content (θ_v) measured for unfertilised pots

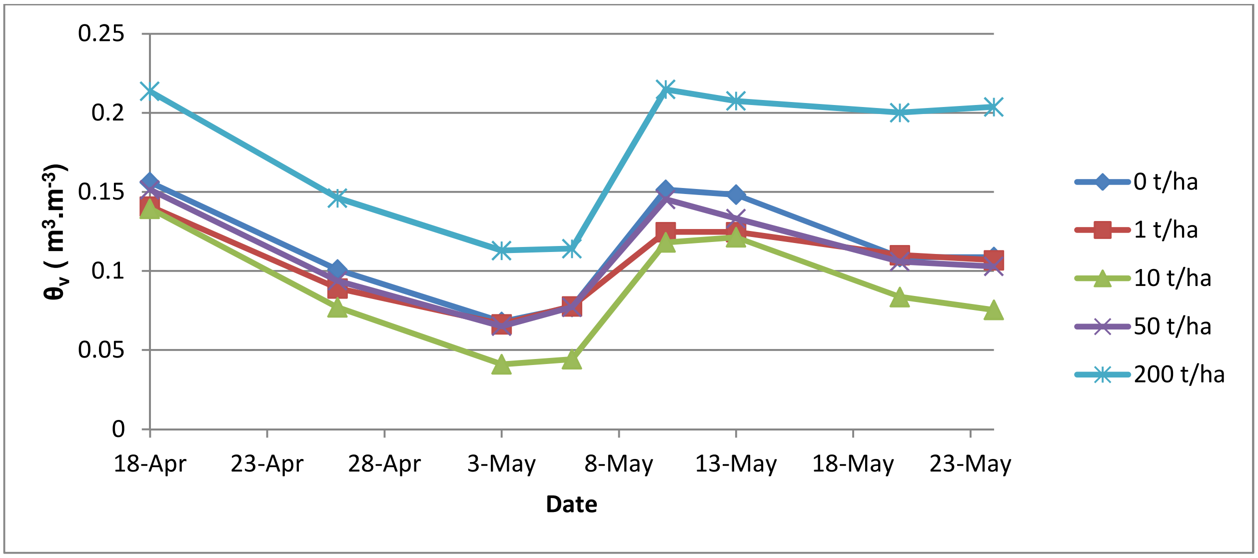

Figure 3.8 Effect of biochar amelioration on volumetric water content (θ_v) measured for unfertilised pots

3.3.4.2 Water-retention curve

The water-retention curve (Figure 3.9) shows the mean values of 5 replicates for each biochar applications and the control, at different matric suction levels. There was a highly significant difference ($P < 0.001$) regarding the average volumetric water content, between the different treatments and the control. To simplify the results the average volumetric water content was determined throughout the experimental procedure and the significant difference between the treatments were determined as such, when measured against the control: 1t/ha, 10t/ha, 50t/ha and 200t/ha increased with 1.15^{a}, 5.06^{a} %, 21.20^{b} %, and 32.07^{c} % (where the values with different superscripted letters are significantly different according to Tukey's t-test ($P < 0.05$); take note, the control was also donated an "a" superscript letter).

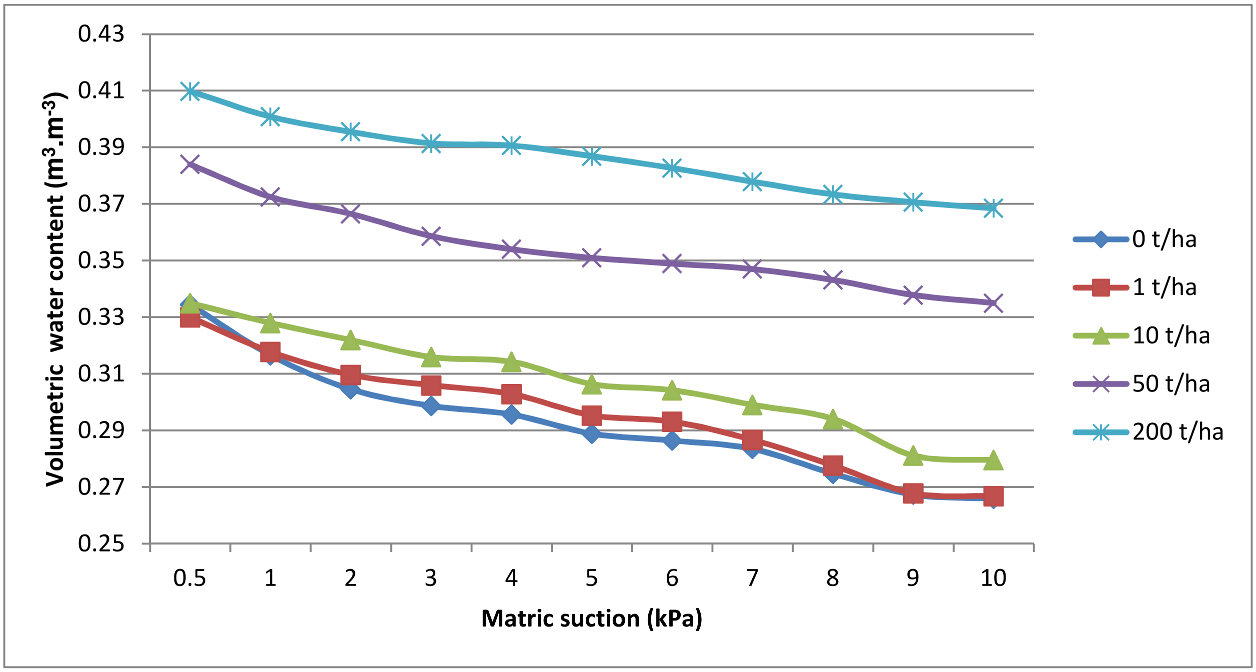

Figure 3.9 Soil water-retention curve for the different biochar applications

3.3.4.2 Bulk density (ρ_b)

There was a highly significant difference ($P < 0.001$) between each biochar treatment (Figure 3.10). The biochar applications performed as follow regarding bulk density: 1t/ha, 10t/ha, 50t/ha and 200t/ha led to a +2.29%, +4.07%, -4.48% and -22.38% effect, all measured against the control, respectively.

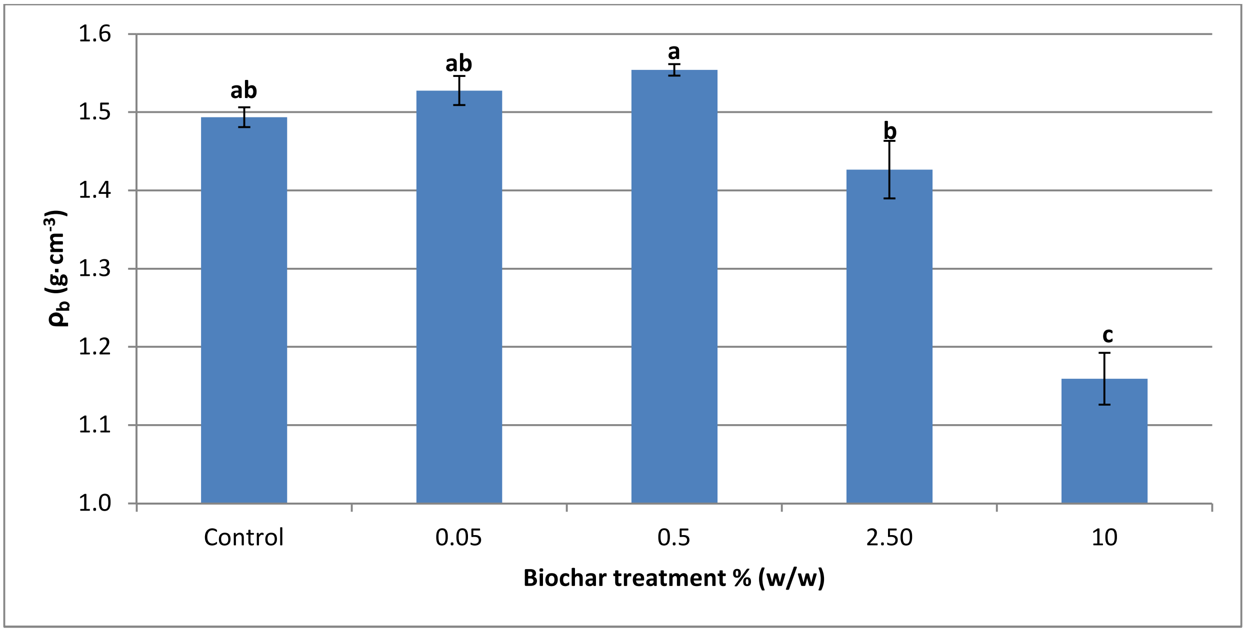

Figure 3.10 Average bulk densities (ρ_b) for different biochar treatment combinations

3.4 Discussion

Overall, the 10t/ha fertilised-biochar application displayed numerous positive plant responsive effects, regarding particularly the following factors, in addition to the measured performances of the alternative biochar treatments and the specific control: overall yield response, leaf C/N ratio, BWUE, leaf area, photosynthesis (A_{max}), WUE $_{\text{instantaneous}}$ and leaf N- and C content (%). Not all of these parameters were significant, however definite positive trends were apparent throughout the pot trial and the results clearly showed favourable enhancements for the green bean, with the 10t/ha fertilised-biochar applications (unfertilised- and fertilised treatments).

Baronti *et al*. (2010) also reported that the most favourable biochar application was at 10t/ha and increased durum wheat yield, which was cultivated in Italy, with 10%.

A threshold value was evident throughout the whole experimental procedure regarding the measured plant growth- and specific leaf trait responses, in particular leaf N content (%). The positive results deteriorated when the biochar application level exceeded the 50 t/ha and 200 t/ha biochar applications, were the yield, plant physiological responses and plant-water regimes were affected negatively or yielded less favourable effects (observed responses were mostly under the norm/ control).

The focus of this discussion will predominantly revolve around the previously stated observations in the passage above and try to explain the results relating to the different biochar applications and to assist in deriving sound explanations for the positive- and negative interactions with biochar amendment, regarding the cultivated plant's response.

There is up currently no literature where crop yield, BWUE, specific leaf traits (not as detailed as have been attempted in this chapter), isotope fractionation and water-retention as a whole, have been attempted and interpreted regarding biochar research; moreover there's no literature to consult taken the whole picture into account and therefore an entirely new venture in agricultural sciences, especially soil physical science was established.

Firstly the 10t/ha biochar-fertilised treatments and their related effects and- analysis will be addressed and interpreted, as the highest yield was obtained for this treatment and could further be referred to as a model plant response.

Table 3.1, the 10t/ha fertilised-biochar treatment had an optimum pH_{H2O} measurement of 6.75 and therefore it's believed to facilitate the plant roots in nutrient uptake, however in Table 3.2 the C/N ratio was determined to be less favourable.

It's a well known fact in soil science that a C/N ratio > 30, leads to N immobilisation and the ideal C/N ratio is linked to extend between a 20-30 ratio (were mineralisation nor immobilisation will dominate) as a ratio below 20 is normally associated with readily N mineralisation *via* soil micro-organisms.

The measured C/N ratio for the 10t/ha biochar treatment was determined to be 40. On the other hand the C/N ratio was determent before planting of the seedlings and it's believed to have shifted (<40) as the experiment progressed over time. The leaf C/N ratio was on the other hand, found to be ideal (see Table 3.8), were the 10t/ha fertilised treatments had an average leaf C/N ratio of 8.40, the lowest when compared between the other measured treatments. Regarding the soil macro- and micro element contents for the different biochar treatments, it was evident that as the biochar increased so did most of the elements, except the following: aluminium, boron, iron and copper, and it's believed to be due to the increase in pH (Table 3.1).

In Table 3.4 were the above- and below ground yields and shoot/root ratios were reported, it was clear that biochar increased both the shoot- and root growth at the 10t/ha biochar applications for both fertilised- and unfertilised treatments, however the shoot/root ratio were the only significant results yielded. The fertilised 10t/ha treatments had the lowest ratio and the 200t/ha fertilised treatments had the highest ratio, furthermore a lower shoot/root ratio is normally associated with improved resource supply to roots, as was previously also reported and discussed in Chapter 2 regarding the wheat plants.

Concerning the plant-water regimes, the 10t/ha biochar-fertilised and- unfertilised treatments had the most efficient water usage and therefore also the highest measured BWUE (Table 3.5).

The 200t/ha biochar applications performed poorly regarding the following factors: total amount of irrigation water applied (highest application needed to sustain FC conditions), BWUE (lowest) and ET (highest water loss).

In relation to the fact that the highest evapotranspiration was measured for the 50t/ha and 200t/ha biochar treatments throughout the experimental period; it's obviously associated with the increase in FC, because as the biochar level increased (Tables 3.9 and 3.10, and Figure 3.6) so did the water content and therefore also the evapotranspiration potential.

This corresponds with the measured volumetric water content (θ_v) (Table 3.10 and Figurers 3.7 and 3.8), were the θ_v was determined to still be the highest for the 200t/ha applications, throughout the experiment, even though the highest ET was also assigned to this specific biochar treatments.

The water-retention curve (Figure 3.9) exhibits in relation to the above findings an equivalent effect, were the 50t/ha and 200t/ha biochar applications significantly retains more water. In combination with the measured bulk density (Figure 3.10) were it only significantly decreased at the 50t/ha and 200t/ha biochar applications, it is believed that the biochar increased the water-holding capacity of the sandy soil by means of its own porous nature and also a corresponding modification was brought about regarding the pore-size distribution, when applied to the sandy soil. Based on this, it can be assumed that a shift in the pore-ratio system has occurred, were micro- and meso-pores have increased and macro-porosity decreased, leading to a higher association/ interaction of water molecules with the sand- and/ or biochar particles *via* adhesion- and cohesion attraction forces within the soil-water interface; and therefore promoting capillary forces and overall the water-holding capacity in the sandy soil.

The most interesting findings were believed to be related to the plant specific traits. Although no significant results were obtained regarding A_{max}, E and WUE $_{\text{instantaneous}}$ (IRGA measurements) between the different fertilised-biochar treatments, there was however a definite trend regarding the 10t/ha biochar treatments and the effect it had on the plants photosynthetic capacity. The 10t/ha treatment had the highest A_{max}, lowest E and highest WUE, all positively related with the additional plant yield results (Table 3.6).

The highest leaf area was also found to be for the 10t/ha biochar applications, both fertilised- and unfertilised treatments (Figure 3.3). The SLA was found to be the lowest for the 10t/ha fertilised-biochar treatment and the unfertilised control treatments (Figure 3.4). SLA of a species is in many cases a good positive correlate of its potential relative growth rate or mass-based maximum photosynthetic rate (Cornelissen *et al*., 2003). According to Cornelissen *et al*. (2003) lower SLA values tend to correspond with relatively high investments in leaf structural components and a longer leaf lifespan.

As C, H and O are the main “building blocks” of plant structural components; it is believed to be the main reason for the corresponding decrease in leaf C content (Table 3.8), with the increase in biochar applications for both unfertilised- and fertilised treatments.

The leaf chlorophyll content (Figure 3.8) did not differ significantly between the fertilised-biochar applications; however a very interesting observation was evident regarding the unfertilised-biochar treatments and the measured leaf CCI. A decreasing trend and lower leaf CCI was observed and measured as the biochar applications increased. This corresponds with previous work conducted by Asai *et al*. (2009). However, no statistical and significant differences were found, but Asai *et al*. (2009) did report that there was a prominent decrease in leaf chlorophyll content for their unfertilised-biochar treatments and that the leaf chlorophyll measurements were reported to be lowest with the highest biochar applications (in their study the biochar treatments consisted out of 4-, 8- and 16t/ha, including a control plot). They described this effect to be due to a decrease in N uptake by the plants and that the lower N uptake was attributed to the high C/N ratio brought about by biochar addition to the soil and leading to N immobilisation. Rondon *et al*. (2007) also reported that foliar N concentrations decreased with all applications of biochar. Regarding the unfertilised-biochar treatments, in relation to the plant’s N content, a corresponding decrease in N as the biochar applications increased, were evidently reported in both the plant analysis (Table 3.7) and leaf analysis (Table 3.8), respectively.

The same trend was related to the fertilised-biochar treatments, were only the 50t/ha biochar treatment was found to have the lowest N uptake and not the 200t/ha level, other than that the N content decreased as the biochar application increased (take note that it’s based on the plant N content regarding the whole plant analysis in Table 3.7).

Concerning the fertilised-biochar treatments, there was also establish that the 10t/ha treatments had the highest leaf N- and C content (Table 3.8) and this strengthens the results measured with the IRGA, were it had the highest maximum photosynthetic rate. Chlorophyll concentrations, photosynthesis rate and plant growth have been observed to decrease with N deficiency (Evans and Terashima, 1987; Marschner, 1995).

It should be stressed that not only N has an essential role regarding the photosynthetic functioning in plants, but also P, Mg, Fe, and Mn. Regarding these minerals, named in the latter it was also found that all of these plant nutrients decreased with an increase of biochar addition to the sand, except P. Only Mn and Fe (merely for unfertilised treatments) significantly decreased with the increased biochar applications.

The carbon isotope fractionation ($^{12}C/^{13}C$) of the leaf indicated no significant differences between the different biochar applications for both fertilised- and unfertilised treatments (Table 3.8). Farquhar *et al*. (1982) reported that WUE is inversely correlated with $\delta^{13}C$, and therefore no differences in the leaf $\delta^{13}C$ would mean that there would be no differences in the long-term WUE between the treatments. However, it should be pointed out that the lowest leaf $\delta^{13}C$ was evidently measured for the 10t/ha fertilised-biochar treatment and correlated inversely regarding the measured $WUE_{\text{instantaneous}}$ and BWUE and therefore unmistakably endorses the conclusion that the 10t/ha biochar application had a positive effect not only on the overall yield response but also the water use efficiency for the green bean plants at this specific application.

The nitrogen isotope fractionation ($^{14}N/^{15}N$) of the leaf indicated only significant differences between the fertilised-treatments (Table 3.8). Where the 200t/ha biochar treatment was associated with the lowest leaf $\delta^{15}N$ value and the highest values were obtained for the 1t/ha, 10t/ha, 50t/ha biochar treatments.

The lower leaf $\delta^{15}N$ is believed to the due to the fact that less available soil derived N was at hand due to the high C/N ratio and the increase in pH along with the fact that with the higher water-holding capacity as the biochar level increased the N was predominantly in the NH_4^+ (ammonium) form (plant root's absorb more readily NO_3^-). Raven *et al*. (2004) and Guo *et al*. (2007) pointed out the fact that plants which are supplied with NH_4^+ is generally associated with lower WUE than those supplied with NO_3^-, were Cramer and Lewis (1993) stated that toxicity symptoms of NH_4^+ included growth inhibition, wilting and other water stresses.

3.5 Conclusion

The highest yield- and water use efficiency was found to be for the 0.5 % biochar application (10 ton·ha^{-1}) and (with fertiliser application) plant nutrient status, especially N uptake was significantly improved. A threshold value was evident throughout the experiment regarding plant production, were at the moment the recommended biochar application would be associated to be between 1-10 ton·ha^{-1} without any detrimental plant-soil side effects, when applied to a Kroonstad soil form or for any other sand dominated soil type, with a low- to acidic pH.

It was clearly illustrated that as biochar application increased N availability was reduced and led to a decrease in leaf traits such as leaf chlorophyll content, specific leaf area, and leaf area. This was most readily encountered for the unfertilised treatments. The leaf C- and N isotope analysis yielded additional information, were the leaf δ^{13}C was positively correlated to the measured BWUE and the δ^{15}N was associated with the fact that as biochar increased less N was taken-up by the bean plants and this effect was assigned due to the high C/N ratio and were NH_4^+- above NO_3^- formation was favoured.

Lastly it was illustrated that biochar had a profound effect on the water-holding capacity when amended with a medium- to fine textured sandy soil. Biochar application greater than 1 ton·ha^{-1}, significantly increased the field capacity and water-retention was significantly enhanced at 50-200 ton·ha^{-1} biochar applications.

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GENERAL CONCLUSIONS AND FUTURE RESEARCH RECOMMENDATIONS

General conclusions drawn from this study are presented below, followed by future research recommendations, but first the main objectives will be recapped:

There were two principal objectives broadly defined at the end of the introduction:

1. Provide an assessment of the physical properties for the sandy soil used throughout this experimental procedure, and the characterising of the specific biochar used.

This was necessary in facilitating future research, where we could help in contributing to international biochar research by means of evaluating the effect of the specific biochar applied to an agricultural soil regarding the crop-, soil- and water dynamics/ interaction as a whole, and for a case specific study, under South African climatic conditions and soil type. This was also necessary in guiding one to a better understanding and giving sound explanations for the specific soil physical- and plant results measured.

2. Determine the influence of biochar, at different application levels on selected soil physical properties and crop productivity (this being the core of the dissertation).

Chapter's two and three focused on different properties and each Chapter addressed different objectives, specifically defined in building towards and attending to the uncertainties coupled with biochar amendment to agricultural soil's and how the specific applied biochar influence soil physical properties and overall crop production, under South African soil and climatic conditions.

General conclusions drawn in hindsight of this study:

- Application of biochar to the sandy soil had significantly increased field capacity and this is believed to be due to its porous nature and it is speculated to have shifted the pore-size from more macro- to meso- and micro-pores.
- The highest biomass yield was obtained at the 10 t/ha biochar applications.
- The higher application levels of biochar (50- and 200 t/ha) limited adequate supplies of macro- and micro nutrients, especially nitrogen uptake, and was believed to be due to alkaline soil conditions.
- Biomass water use efficiency (BWUE) increased significantly for wheat crops and a strong trend was observed and also applicable to green bean crops at the same treatment level namely, 10 t/ha biochar application.
- It was evident that biochar had a synergistic effect when applied in conjunction with a standard fertiliser on the agricultural crops and overall plant nutrient availability – this was only true for the 1 t/ha and 10 t/ha biochar applications throughout the study and was believed to be due to a more favourable pH owing to the biochar's alkaline nature and improved nutrient retention.
- Water stable aggregation was promoted in the sand-rhizosphere interface and the exact mechanism is still undefined however strong evidence indicates that two possible mechanisms may be at hand.
- A threshold value was evident throughout the study regarding crop production, where yields decreased when biochar was applied at 50 t/ha or greater to the sandy soil.

Future research is necessary to increase the level of certainty regarding the effects of biochar application on soil physical properties, crop production and- performance along with the effect on the plant- and soil water regime.

Measuring the soil surface albedo after biochar application, is an additional and imperative physical property to attend to in future research, because as biochar is added the soil the albedo will decrease - meaning the surface temperature will increase and this could have the reversed effect in mitigating greenhouse gases due to modification in the radioactive balance at earth’s soil interface.

The long term sustainability of biochar application should be determined for different soil types and different climatic conditions.

Application of biochar to agricultural soils and thorough mixing of this organic product to soil bodies should be studied, especially on a commercial scale, hence to determine if this product will be economically viable when used in sustainable farming practices. The latter is important since it would be expensive and labour intensive to obtain thorough mixing by means of heavy equipment.

The effect of biochar on hydrophyllic behaviour of sandy soil should be investigated.