Biochar and Hemp as Peat Substituents in Horticultural Substrates and Their Effects on Presence of Microbiota

Louise Hagbard

Master thesis • 30 credits
Swedish University of Agricultural Sciences, SLU
Faculty of Landscape Architecture, Horticulture and Crop Production Science
Department of Plant Breeding
Horticultural Science Programme – Master's Programme
Alnarp 2022

Biochar and Hemp as Peat Substituents in Horticultural Substrates and Their Effects on Presence of Microbiota

Biokol och Hampa som Torv Substituenter I Hortikulturella Substrat och Deras Effekter på Förekomsten av Mikrobiota.

Louise Hagbard

Supervisor:	**Håkan Asp, SLU, Department of Biosystem and Technology**
Assistant supervisor:	Anna Karin Rosberg, SLU, Department of Biosystem and Technology
Examiner:	Sammar Khalil, SLU, Department of Biosystem and Technology

Credits:	30 credits
Level:	Second cycle, A2E, Master's thesis
Course title:	Independent Project in Biology, A2E
Course code:	EX0947
Programme/education:	Horticultural Science Programme
Course coordinating dept:	Department of Plant Breeding
Place of publication:	Alnarp
Year of publication:	2022
Cover picture:	
Copyright:	All featured images are used with permission from the copyright owner.
Keywords:	Biochar, hemp, *Cannabis sativa,* fibers, substrate, growing media, microbiome, *Trichoderma, Pseudomonas, Lactuca sativa,* peat reduction

Swedish University of Agricultural Sciences

Faculty of Landscape Architecture, Horticulture and Crop Production Science

Department of Plant Breeding

Abstract

The extraction of peat has been shown to have a detrimental impact on the environment in several ways, including through emissions of greenhouse gases but also through a negative impact on fragile habitats, ecosystems, and ecosystem services. The horticultural sector is a major user of peat products and thus contributes to both emissions and habitat changes. This work explores the possibilities of reducing the use of peat in cultivation substrates as part of reducing the environmental impact of peat extraction. The aim of the study is to instead use biochar, which has previously been reported to have good effects in the agricultural sector, as well as residual products from seed hemp production, to achieve utilization of residual product streams. The physicochemical properties of the substrates were evaluated as well as the effect on growth in a greenhouse experiment of *Lactuca sativa*. In addition, a screening of microbial communities was conducted to gain an understanding of what properties novel substrates may have on the microbiome. The conclusions from this work are that biochar amendment with 31.25% in a peat substrate had best growth of all treatments. Hemp amendment, however, gave high numbers of colony forming units, poor performance in terms of growth, and development of plants and not satisfactory numbers in terms of water holding capacity and nutrient supply.

Keywords: biochar, hemp, *Cannabis sativa,* peat reduction, peat, microbiome*,* fibers, substrate, growing media, *Trichoderma, Pseudomonas, Lactuca sativa*

Table of contents

List of tables

List of figures

Abbreviations

Biocontrol agents: (BCA), 21
Bulk Density: (BD), 24
Carbon: (C), 20
Carbon dioxide: (CO2), 14
Cation exchange capacity: (CEC), 14
Central Bureau of Statistics: (SCB), 11
Compact density: (CD), 24
Electrical conductivity: (EC), 25
Food and Agricultural Organization of the United Nations: (FAO), 10
King's B Agar: (KB), 25
Life Cycle Analysis: (LCA), 14
Malt extract agar: (MA), 25
Nitrogen: (N), 14
Nutrient Agar: (NA), 26
Phosphorus: (P), 30
Polycyclic aromatic hydrocarbons: (PAH), 14
Pot capacity: (PC), 24
Potassium: (K), 30
Sustainable development goals: (SDG), 10
Trichoderma Selective Medium: (TSM), 26
Tryptic Soy Agar: (TSA), 25
Water holding capacity: (WHC), 19
Δ^9-tetrahydrocannabinol: (THC), 15

1. Introduction

Since the beneficial properties of peat as a substrate for cultivation was discovered, it has been the main component in most commercial substrates intended for pots and containers for both home growers as well as in commercial production. Its physical properties are considered outstanding in terms of substrate component with its low pH and bulk density, high cation exchange capacity, satisfactory aeration, and optimal container capacity (Bohlin & Holmberg, 2004; Landis et al., 1990). However, peat and, above all, its extraction have been shown to have detrimental consequences for the environment. As part of finding more sustainable constituents for cultivation substrates but also to be able to apply more circular flows and hence utilize waste and by-products, other types of substrates must be investigated in terms of suitability for cultivation.

As part of the development of more sustainable food systems and the production of horticultural crops, the out phasing of peat can be seen as a method for improvement. The Food and Agricultural Organization of the United Nations (FAO) emphasizes in the sustainable development goals (SDG) that to keep producing crops and foods there is a need to "nurture healthy ecosystems and support the sustainable management of land, water, and natural resources while ensuring world food security." It is also stated that in order for this transition to be possible it will "require major improvements in the efficiency of resource use, in environmental protection and in systems resilience" (FAO, 2022). Similar reform work is undergoing in Sweden to support the transition to more sustainable and resilient production and consumption systems within the food chain (Swedish Board of Agriculture, 2021). Additionally, the European Commission aims to achieve a bioeconomy with their strategy and action plan *Innovating for sustainable growth: a bioeconomy for Europe* (2012), and the subsequent *Updated Bioeconomy Strategy* (2018). It is emphasized how bioeconomy "encompasses the production of renewable biological resources and the conversion of these resources and waste streams into value-added products, such as food, feed, bio-based products, and bioenergy".

Peat is the common substrate in growing media of horticultural products and around 90% of all growing media used in professional and private horticultural production

in Europe today consists of or is based on peat (Kern, et al. 2017). According to Statistics Sweden (SCB, 2021), an estimated 1.9 million m^3 of peat is used for cultivation purposes within Sweden and the total amount of peat extracted in Sweden in 2016 exceeded 3 million m^3 (SCB, 2017). World wide, 40 million cubic m^3 of peat is estimated to be used annually (Kuisma et al. 2014). In recent years, the large use of peat has been increasingly questioned largely due to the impact that peat extraction has on the climate. It is considered detrimental since greenhouse gas emissions occur when peat is extracted. According to Swedish Environmental Protection Agency (2016), the total extraction, treatment, and transport of peat give rise to relatively large amounts of net emissions of greenhouse gases such as CO_2, CH_4, and N_2O.

The above mentioned motivates to further investigate and evaluate possibilities to provide more sustainable peat substituents for horticultural production which also is equivalent to peats' physical and chemical properties. Hence, this also motivates the survey on these materials' impact on the microbial composition within the substrate and if this affects the growth of plants within such substrate. This work will focus on two products with emerging interest for horticultural production; biochar and hemp fiber. Biochar is not only considered a more sustainable product since it is produced from by-products it also provides a sink of carbon. Fibers from hemp cultivated for food purposes are today residual products without any specific commercial end product. During the past few decades, more attention has been paid to biochar for its potential use in agriculture and environmental purposes (Lehmann & Joseph, 2009). However, not as much attention has been brought to the use of biochar in container substrates. Likewise, assessments of hemp as a substrate in hydroponic growing systems have been conducted (e. g. Dannehl et al., 2015; Both et al., 2021) but studies with hemp as a container substrate are sparse.

A commercial growing media must be possible to produce in homogenous batches with consistent quality (Boudreault, 2010). Further, it is of importance that it is free from weeds and alien invasive species, free from both phytopathogens and human pathogens as well as toxic substances. In addition, it is also of importance that the growing media should be easy to wet and handle for the grower. To be able to produce sustainable substrates, it is an advantage if the components are available nearby to reduce the need for transportation. In general, the biggest advantage of cultivation in substrates compared to in field cultivation is the physical properties of the substrate (Raviv & Lieth, 2008). More specifically, it is its ability to provide adequate levels of both oxygen and water to the rhizosphere and thus to the plant through transpiration. These physical properties of porous substrates are more suitable than soils for the production of most horticultural crops. As the limited volume of the container means weaker matrix forces, it also means that the water is more easily accessible. For this reason, it entails a lower risk of oxygen deficiency

than plants grown in soils close to field capacity. Utilizing these benefits of container cultivation leads to improved yields, both in terms of quantity and quality.

Plants grown in a limited volume of substrate are strongly dependent on the physical structure of the substrate for root growth. Therefore, it is crucial that the physical structure of the substrate has an equilibrium between air and water and that is able to be maintained throughout the whole period of growth (Bilderback et al., 2005). According to Raviv & Lieth. (2008), the limited volume also affects nutrient management compared to plants produced on agricultural land.

In cultures grown in containers with substrates of limited volume entails that the growing medium will have a lower buffer capacity hence, the pH will be more adjustable and the nutrient availability will be limited to the available volume (Raviv & Lieth, 2008). Likewise, the volume will affect the root zone, which leads to a reduced root size but a higher root density. This in turn can lead to increased competition for the space between roots, which in itself affects the activity in the rhizosphere. Most plant production substrates have negative permanent and/or variably charged surfaces. The surface charge properties of a substrate have a great effect on the chemical reactions in the rhizosphere, which in turn affects the availability of applied cations and how effective their uptake is.

1.1 Objective

The aim of this thesis was to investigate the possibilities of utilizing byproducts, such as biochar and hemp fibers, as more sustainable choices of container substrate compared to the traditionally used peat. The thesis was developed on behalf of the company EcoTopic which provided certain predetermined aspects to examine. To incorporate their wishes, the trials were divided into sub-experiments to, on one hand, perform growth assessment and, on the other, look at physicochemical properties and assess the general presence of microbiota and whether there were differences in these factors between the substrates.

1.1.1 Research questions

Can biochar and hemp function as a substitute for peat in growing media and can optimum proportions be determined?

Is it possible to evaluate whether its physicochemical properties are equivalent to peats?

What can a screening of microbial communities tell us about the various substrates and hence the effect on plant growth?

1.2 Materials for Substrate Components

1.2.1 Biochar

Biochar is a material with a large variation that depends on production methods as well as feedstock. According to Lehmann & Joseph (2009) and Vaughn et al. (2013), the joint characterization of biochar is that it is a material of carbonaceous character and produced from the thermochemical decomposition of biomass under temperatures ranging from 350 - 900°C in conditions of quasi absence of oxygen. The high temperature during the production step is one of the advantages as this means that the obtained product is completely free of pests, weed seeds, and pathogenic organisms (Zulfiqar et al., 2019)

In recent years, biochar has been emphasized as a revitalization for soils where it is said to increase the soil's total carbon sequestration as well as increase efficiency and yield of agricultural products. The advantages of biochar in agronomy are several, namely, biochar can inhibit nutrient leaching through sorption and slow desorption. In addition, it tends to reduce the dose of fertilizer (Altland & Locke 2012, 2013; Nemati et al. 2015). However, the claims can be limited to some extent by the biochar's production technology (pyrolysis or gasification conditions) and incoming feedstock, i.e. expectations may not always be met. Research has concluded that biochar is a continuum of black carbon and can have a wide variety of properties given the net result of the production and of the factors in post-processing, that are relevant to the product. Therefore, biochar should not be seen as a uniform product where all forms live up to the same expectations, but rather it is a spectrum of black carbon in all its forms.

The content of different nutrients in biochar is variable due to feedstock and production technology. However, the content of micronutrients as well as K and P are generally prevalent. These nutrients are slowly released and gradually become available to the plant. This inherent nutrient content of biochar reduces the overall need for fertilizer. This property is in itself a reason for reduced greenhouse gas emissions such as carbon dioxide (CO_2) at a general level of a Life Cycle Analysis (LCA). The content of nitrogen (N) in biochar is usually less than 5%, rather around 1% and therefore it should be added before cultivation for proper growth. Like the nutrient content, the particle size varies depending on the production feedstock and technology. However, it can advantageously be atomized in a manner that makes it suitable for container substrates. The distribution of particle sizes can affect several properties of the biochar and potential mixtures. Density, specific surface area, and water retention are all increasing with a decrease in fraction size. In contrast, air content is decreasing with smaller fraction sizes (Quintero et al., 2013). The chemical properties such as pH, cation exchange capacity (CEC), and surface

adsorption also increase with smaller particle size distribution (Jeffery et al., 2011; Mukherjee et al., 2011). The pyrolyzed particles are inherently unaffected by decomposition even during plant production and its impacts (Vaughn et al., 2013). Therefore, the initial physical properties should remain stable over time. This is analogous to other mineral components such as perlite, vermiculite, and sand. The advantage in a production context is that drainage and water retention tend to remain the same throughout the growth period.

Differences in feedstocks can have a great impact on the properties of the obtained biochar. However, the feedstock can consist of almost endless combinations of biomass and hence the properties and suitability will vary. Limiting factors for plant production can be polycyclic aromatic hydrocarbons (PAH) and heavy metal content (Somtrakoon & Chouychai, 2013).

The biochar used in this trial was obtained from Cancun AG (Germany). It is certified according to EBC AgroBio (EBC, N.D) and hence approved as animal feed and for organic production. Analysis obtained from Eurofins (Jena, Germany) determines the biochar as of up to 89% purity. It has a specific area of 417 m^2/g. The pyrolysis was conducted at temperatures of 540º C. According to the analysis, the obtained biochar has a pH of 8,6.

1.2.2 Hemp

Hemp (*Cannabis sativa* L.) is an annual herbaceous crop that has been cultivated in various climates around the world since ancient times (van der Werf, 1994). However, during the 20th century, it remained restricted in many western countries. Sweden allowed cultivation of industrial hemp again in 2003 due to a greater demand for renewable energy sources and increased environmental awareness in society (Bernesson, 2006). It is a debated and controversial crop since it is not only grown for properties such as biomass, seeds, and fibers, but also medicinal substances. There are differences between varieties and the main difference between them is whether they contain the intoxicating compound Δ^9-tetrahydrocannabinol (THC) or not. The varieties that are legally approved for Swedish cultivation must contain less than 0,20% of THC (Swedish Board of Agriculture, 2022). However, despite growing approved varieties, it is still necessary to report to the Swedish Board of Agriculture if hemp should be cultivated.

The industrial varieties of hemp are mainly cultivated on agricultural land and are acclimatized to the same range of climate as wheat. To maintain a stand with proper development a well-prepared seedbed is required. The seedbed advantageously is free from perennial weeds to ensure sufficient growth conditions and capillarity

movement of water (Ranalli, 1999). The density of the stand is affected by whether the crop is grown for fiber or seed production, where a higher density is desired for fiber production and a lower for seed production (van der Werf, 2004). It has been reported that best development and growth are achieved on fertile, medium-heavy soils with sufficient drainage (Ranalli, 1999) According to du Bois (1982) hemp is not only improving soil structure with its root system but also provides a generally high yield of biomass. It has been reported that cellulose production from hemp is more efficient than from e.g., sugar cane and corn (Herer, 1985 see Ranalli, 1999). Another advantage in terms of cultivation is that pesticides can be avoided to a large extent, partly the crop has good competitiveness against weeds and partly it is relatively free from pathogens and pests that cause economically losses (McPartland & Hillig, 2006; Ranalli, 1999)

Hemp production is considered high yielding in terms of biomass. Fibers obtained from plants, such as those from hemp, are commonly referred to as vegetable fibers (Manaia et al., 2019). They have a high content of cellulose which acts as the main structural component. Hemp fibers generally have a cellulose content of 70-74%. Additionally, they contain 15-20% of hemicellulose, 3,5-5,7% of lignin, 1,2-6,2 of wax, 0,8% pectin and 0,8 % ash (Manaia et al., 2019). Fibers from hemp are desirable as a sustainable material in different composites. However, hemp fiber itself can be considered a natural composite because of its complex structure. Cellulose microfibrils and a matrix, mainly composed of pectins and hemicellulose, form this structure which in turn forms different cell wall layers. Fibers and layers are assembled in a way that creates bundles of fibers (Bourmaud et al.,2018; Crônier et al., 2005). To utilize the properties of the fibers, it is an advantage to separate the bundles. A widespread course of action is a process called retting (Paridah et al., 2011). In Europe, this commonly refers to field retting which means that excess plant material is left on the field after harvesting so that natural colonization of microbiota can occur. Once the retting process is initiated, the microbiota contributes to the production of several enzymes which in turn help separate cortex fibers from pectic substances (Henriksson et al., 1997; Mazian et al., 2018).

According to a study done on the microbial composition of hemp during the retting process (Fernando et al., 2019), it has been established that several species of fungi were already present before the process started. The bacterial flora, on the other hand, increased with time of retting. *Pseudomonas* spp. could be observed throughout the retting period. *Pantoea* sp. was instead observed during the latter part of the process as cellulose decay could also be recorded. *Rhizobium soli* and *Methylocapsa aurea* were relatively evenly observed throughout the process. This suggests that the retting process, maturation of stems (early or late harvest), and hence, the chemical composition of the fibers will affect the presence of microbial species. A comparative study conducted by Mazian et al. (2019) supports the idea

of the importance of when and to what extent the retting process is ongoing for the mechanical and structural properties of the fibers.

Prior studies have explored hemp as a substrate in hydroponic crop systems (e.g., Dannehl et al., 2015; Nerlich et al., 2022). However, little literature supports the use of hemp fibers in container substrates. The hemp used in this experiment (Figure 1) was obtained from a local producer (Svensk Hampa Industri, SHI, Smedstorp, Sweden). The hemp in question is from the cultivation year 2019 and is of a low-growing variety, called Finola, which is primarily grown for food. The seeds were threshed in September 2019, with a combine harvester and the stalks were then allowed to remain in the field to undergo retting until March 2020. At that time, the stalks were felled to be able to press into bales. The bales were then stored in an open barn until August 2020. For the purpose of separating the fibers from lignin substances in the trunk, they were processed using a hammer mill.

Figure 1. Hemp fibers used for the purpose of this experiment, a mixture between lignin and cellulosic fibers.

1.2.3 Peat

Peat is an organic soil type extracted from bogs that are formed by the incomplete decomposition of organic substances under wet and oxygen-free conditions. The build up of peat in bogs is a slow process (Kern et al, 2017). Most peatlands in Sweden were formed about 2000 years ago. Bogs and peatlands can be valuable habitats for a wide range of animals such as birds and insects but also mosses and vascular plants. However, they are considered fragile ecosystems but yet they provide important ecosystem services such as conservation of species, water purification, and climate regulation, including carbon sequestration (Joosten et al., 2016). In Sweden, peatlands are not only a characteristic feature of the landscape they also constitute areas for outdoor life and recreation values (Swedish Environmental Protection Agency, N. D.).

Drainage and ditching are part of the process when extracting peat from bogs and this leads to changes in water flows and consequently acidification and changes in aquatic biodiversity (Kern et al, 2017). Furthermore, a healthy intact bog or mire can act as a carbon sink. But when drained, greenhouse gas emissions from peatlands are increasing considerably. Peat used as a substrate is not a renewable source and according to life cycle analysis (LCA) of mushroom production (Robinson et al., 2019) it contributes to 9.71×10^{-2} kg of CO_2 per kg of produce when used as growing media. Management practices regarding peat production are creating both direct emissions, changing ecosystems, and creating biodiversity loss (Kløve et al, 2017). However, emissions can differ depending on methods and practices.

Northern peatlands, which are appreciated to occupy 2.4-4 million square km (Yu, 2011), are distributed across the northern hemisphere of 45◦ N. It is accepted that they have sequestered a large amount of carbon since the Last Glacial Maximum (Treat et al., 2019) thirty percent of the globes soil carbon is estimated to be sequestered in peatlands (Joosten et al., 2016). According to Yu (2011), the average rate of carbon accumulation, which is associated with the increase of peat, is estimated at 18–28 g C m^{-2} yr^{-1}. However, this estimation is rather connected to the depth of peat bogs than to their surface area. Hence, harvesting of peat bogs which leads to shallower stands and thus not the same potential to sequester atmospheric carbon is considered problematic.

Peats' Properties as a Successful Substrate

According to The International Peatland Society, a system of classification factors such as; botanical composition, nutrient status, and degree of decomposition is used to classify peat (Kivinen, 1980, see Raviv et al., 2019) (Table 1). Another commonly used system for classification purposes is the von Post scale which

describes peat's age and decomposition stage divided into three categories; younger and with a larger part of undecomposed material and a low humification (H1-H3); partly decomposed (H4-H6) and older with a large part of highly decomposed material (H7-H10) (von Post 1922, see Raviv et al., 2019). Additionally, in horticultural sectors there is yet another designation for the different stages of peat, namely, H1-H4 are referred to as white peat, H4-H6 as dark peat, and H7-H10 as black peat (Bunt, 1988). However, despite which classification is used, it can tell a lot about the peat's properties since the physical and chemical properties will partly change depending on e. g. the degree of humification, particle size distribution and the composition of the original material (Puustjarvi & Robertson, 1975)

Peat's beneficial properties for plant production are several, but predominantly it has a high porosity. The distribution of pores has a particularly good distribution between micro and macro pores, which means that the plant roots maintain good aeration but at the same time have a high water holding capacity (WHC). Peat also has the benefit of a low pH which is readily adjusted through liming to suit most plants requirements of pH (Raviv et al. 2019). Likewise, the nutrient content of peat is rather low but can easily be adjusted through amendments such as manure or compost, or mineral fertilizers. The many beneficial properties of peat are why it once became the major component in different growing media. Additionally, it is readily available and has a consistent quality and act stable over time when used for cultivation.

Table 1. Overview of classification factors for peat

Botanical composition
Moss peat – mostly *Sphagnum* and other mosses
Sedge peat – sedges, grasses, herbs (e.g., *Carex* and *Phragmites* spp.)
Wood peat – remains of trees and woody shrubs
Degree of humification (H)
Weakly decomposed (H1-H3)
Medium decomposed (H4-H6
Strongly decomposed (H7-H10)
Trophic status
Oligotrophic
Mesotrophic
Eutrophic

1.2.4 Physical Properties of Pot and Container Substrates

As previously mentioned, there are some properties that are desirable and optimal for the purpose of utilizing materials as growing substrates.

The particle distribution of a substrate indicates the size and distribution of particles. Particle size distribution can be directly related to water retention and air porosity. In a study conducted by Graceson et al. (2013), they found an increase of WHC when the proportion of fine particles also increased and thus created more water holding pore spaces. However, WHC is not only dependent to the substrate, WHC and air porosity also depend on the container height. Generally, particle size and air porosity are directly proportional in difference to WHC which is instead inversely proportional (Noguera et al., 2003). Another study (Noguera et al., 2003), could conclude that bulk density (BD) decreased with increasing particle size.

According to a study (Benito et al., 2006), an optimal substrate should have a medium to coarse texture. Particle size distribution should be in the range of 0.25 and 2.5 mm, as this provides enough water availability and oxygen for the plant to absorb, and the total porosity thus increases. In the same study, it could be shown that an increased proportion of larger particles, 0.5-8 mm, led to poorer physical properties and thus a deviation from recommended values. Although there are certain values that seem more or less accepted in an industry context, there seems to be a lack of universal and official standards for the physical properties of a substrate. According to Yeager et al. (1997) there are areas where many of the substrates available and used in commercial production of horticultural crops fail. These include total porosity values from 50% to 85%, container capacity values from 45% to 65%, airspace values from 10% to 30% and bulk densities from 0.19 to 0.70 g cm^{-3}.

1.3 Microbiology in Horticultural Substrates

In cultivation substrates, as well as in soil, both pathogenic and promoting microbes are present (Lugtenberg, 2015). Pathogens and detrimental organisms can adversely affect crop growth through deteriorating growth conditions. Promoting organisms, on the other hand, can instead improve growth conditions through, for example, protection against infestation or in the form of plant growth regulators. Promoting organisms are divided into several classes where (i) reduces or inhibits plant diseases (ii) act as plant growth regulators (iii) inhibits stress responses (iv) promotes growth by neutralizing soil contaminants that may otherwise adversely affect the plant. However, several species of saprophytic fungi and bacteria have no or very little effect on the plant but instead help to metabolize organic material (Postma et al., 2008). Before planting has occurred, the substrate is already colonized and microbial activity can be identified. However, an increase in activity and occurrence of microorganisms can be seen after planting as the plant's rhizosphere has a complex pattern of interaction with the microbiome.

Peat has been seen as favourable in terms of lack of microbial activity. According to Hoitink and Boehm (1999), and Krause et al. (2001), the absence of microbial life is due to the peat's low availability of energy and the relatively high amount of stabilized carbon (C). This means that peat as a substrate does not provide the carbohydrates, chitin and lipids needed for the microorganisms to be able to proliferate.

Hemp has the advantage that it can be provided from local production given its wide range of growth in terms of climate conditions. Therefore, it can be an interesting and sustainable peat replacement material. A possible limitation for the use of hemp in growing media is its interaction with nitrogen (N) (Altland and Locke, 2011; Frangi et al., 2012). N-immobilization occurs when organic materials with high C: N ratios, such as straw and other plant fibers, degrade microbially (Jackson et al., 2009; Vandecasteele et al., 2016), leading to competition with crops for available nitrogen. Hemp is rich in cellulose, which is a source of C for bacteria and fungi (Manaia et al., 2019). According to Sánchez (2009) fungi are considered to be the most efficient degraders. However, the relative proportion of saprophytic fungi can alter in relation to prior treatments of straw or fibers (Debode et al., 2018).

The utilization of circular feedstocks for the production of substrates and growing media does not only provide a more sustainable product it also means a much more diverse microbiome can be expected (Carlile & Coules, 2011). Depending on the structure and communities of the microbiome present, it could potentially suppress phytopathogens and benefit plant growth but likewise, it could be a risk of phytopathogens or other malignant organisms or substances that is detrimental to plant growth. The use of compost as a circular feedstock for alternative substrates is fairly common. It is accepted that three main characteristics of compost are crucial for optimal utilization in growing media: pH, maturation, and organic matter content. According to several studies (e. g. Antoniou et al., 2017; Lutz et al., 2020) composts can stimulate plant growth and suppression of diseases due to their high microbial activity. Biocontrol agents (BCA) such as *Trichoderma*, *Pseudomonas,* and *Bacillus* spp. are genera that commonly are found in composts and are contributors to disease suppression (Lutz et al., 2020).

2. Method and Material

2.1.1 Experimental Design

To evaluate the performance of biochar and hemp as peat alternatives in horticultural growing media an experimental setup was performed in 1 L pots, placed in a block formation on greenhouse tables in a greenhouse (Vegetum Greenhouse, Swedish University of Agricultural Science). The plant assessed was lettuce (*Lactuca sativa* 'Christabel'). In each pot, peat was proportionally exchanged with more sustainable and renewable materials such as biochar and hemp fibers together with composted horse manure, clay, and green compost, and their performances were assessed in comparison to a commercial peat product. The proportions of replacement were based on the company EcoTopics' previous experiences with these materials. Each dose (1-6) was replicated 4 times with a total of 52 pots (n = 52).

Lettuce seeds (*Lactuca sativa* 'Christabel') were individually sown in 80 mL plug trays filled with commercial seed substrate (S-jord Hasselfors, Sweden), kept under controlled greenhouse conditions (temperature 20/16 °C; day/night, ventilation temperature 22/18°C; day/night and 16 h of assimilation light (High-Pressure Sodium, 400 W, Philips)) and watered by adding water when needed. After emergence (2 weeks after sowing) the lettuce seedlings were transplanted respectively into 1L pots with the various substrates assessed (treatments Reference, P, H, and dose 1-6, Table 1 and 2). The setup for the experiment was a randomized block experiment, each block (total 4 blocks) was placed under assimilation light, on a table in Vegetum greenhouse, SLU Alnarp. All pots were irrigated to 60% pot capacity 3 times per week.

2.1.2 Substrate Components

For the purpose of this experiment two types of commercially available peat substrates from Hasselfors (Örebro, Sweden) were used, one with no amendment and without adjusted pH (Solmull Naturtorv) and one with NPK (11:5:18) and dolomite meal added (Solmull Växttorv*)*. The first one was used in the mixed

substrates (treatment P and H, dose 1-6, Table 1 and 2), and the second one as a reference for what is commonly used as horticultural growing media (called Ref.).

Approximately 15 L of biochar (Carbuna AG, Germany) was mixed, using a kitchen blender, to fractions between 0-5 mm. Thereafter, it was activated with blood meal, 13% N (Nelson Garden, Tingsryd, Sweden), to prevent nitrogen immobilization once blended with the other materials. To be able to activate the biochar (15 L), bloodmeal (29 g) was dissolved in water (5 L). The suspension was thereafter added to the biochar and thoroughly mixed. It was set to "rest" for 1 week before adding the other materials to complete the various substrate mixes.

After one week all mixes were prepared in plastic bags in proportions according to tables 1 & 2. Hemp fibers were obtained from SHI (Smedstorp, Sweden). Green compost was obtained from SWEROCK (Malmö, Sweden). Composted horse manure was obtained from Wiggeby Jordbruk (Svartsjö, Sweden). Clay was obtained from Bara Mineraler (Bara, Sweden). All substrate mixes were carefully measured (V/V) for the right proportion and mixed for an even distribution of the different fibers and particle sizes. No adjustments for pH were done on any of the treatments or doses. No further fertilizer was added during the period of growth.

Table 2. Proportions (V/V) of each constituent for peat and biochar mixtures in gradient P

Peat and Biochar gradient (P)						
Treatment	**Dose**	**Peat**	**Biochar**	**Horse manure**	**Green compost**	**Clay**
P	1	56,25%	0,00%	25,00%	12,50%	6,25%
P	2	50,00%	6,25%	25,00%	12,50%	6,25%
P	3	43,75%	12,50%	25,00%	12,50%	6,25%
P	4	37,50%	18,75%	25,00%	12,50%	6,25%
P	5	31,25%	25,00%	25,00%	12,50%	6,25%
P	6	25,00%	31,25%	25,00%	12,50%	6,25%

Table 3. Proportions (V/V) of each constituent for hemp and biochar mixtures in gradient H.

Hemp and Biochar gradient (H)						
Treatment	**Dose**	**Hemp**	**Biochar**	**Horse manure**	**Green compost**	**Clay**
H	1	56,25%	0,00%	25,00%	12,50%	6,25%
H	2	50,00%	6,25%	25,00%	12,50%	6,25%
H	3	43,75%	12,50%	25,00%	12,50%	6,25%
H	4	37,50%	18,75%	25,00%	12,50%	6,25%
H	5	31,25%	25,00%	25,00%	12,50%	6,25%
H	6	25,00%	31,25%	25,00%	12,50%	6,25%

2.1.3 Physical Properties

The physical parameters examined for each treatment were dry bulk density (BD), water holding capacity (WHC) compact density (CD), and porosity.

Bulk Density

Dry bulk density (BD) consists of the total density of the particles and the pores in the substrate. This parameter is primarily important from a practical perspective, for transport and handling. Bulk density was assessed on the different treatments and the different doses according to standard protocols (EN 13040:2007), using an iron cylinder of a known volume. The cylinder was filled with substrate and pressed with a weight for 3 minutes. Thereafter, the extension ring was removed and the excess substrate was carefully removed using a ruler. The remaining content in the cylinder was then weighed and bulk density (g/dm^3) was determined.

Water Holding Capacity

Water holding capacity (also referred to as pot capacity, PC) was assessed in duplicates according to a modified version of the standard procedure. PVC cylinders were filled with substrates and then immersed in water for 48 h. Thereafter they were placed on a rack for drainage at atmospheric pressure for 48 h. All treatments (Ref., P, H) and doses (1-6) were then weighed and thereafter dried in a drying cabinet for 72 h at 105 º C. After drying all samples were weighed again to obtain the dry weight of the substrates. By subtracting the dry weight from the wet weight, the amount of water retained by the substrates could be calculated. The obtained pot capacity was then used to calculate the weight at 60% capacity in 1L pots and further used as irrigation amounts.

Compact Density

Compact density (CD) is the density of the substrate without pores, i.e., the ratio between the mass of the dry substrate and its compact volume. The assessment was carried out in duplicates. A dry 50 ml graduated flask was weighed and filled to about half with dry substrate, respectively. Thereafter flasks with substrate were weighed. To expel air from the substrate, 25 ml of alcohol was added with a burette into each flask. Flasks were then covered with parafilm and shaken for 30 minutes. Thereafter, alcohol was added from a burette to the 50 ml mark on the flask. The total amount of alcohol added to the flask was recorded. The volume not filled by the liquid is the volume of the substrate. The compact density was calculated in g/dm^3.

Porosity

The relationship between the compact density and the dry bulk density was used to calculate the total porosity (micro and macropores) using formula

$$1 - (BD / CD) * 100$$

2.1.4 Chemical Properties

pH and EC

pH and electrical conductivity (EC) were measured in all treatments and doses using European standard EN13038:2011. pH and conductivity were measured in distilled water in a substrate: water ratio of 1: 5 (v / v). 30 ml of substrate was measured with a measuring glass and then transferred to a sample jar; 150 ml of distilled water was added. The samples were then shaken in an "end over end" shaker for 60 minutes. The jars were shaken by hand just before the measurement. The suspension was decanted into the lid of the jar and pH and EC values were determined once values had stabilized. The electrodes were rinsed in distilled water between measurements.

Nutrient Assessment

Nutrient assessment was examined by performing Spurway analyses of available nutrients in all treatments and doses on two different occasions of the experiment, before planting and after harvest. The substrate samples were sent to an external laboratory (LMI, Helsingborg, Sweden) for analysis.

2.1.5 Microbiology

Within the framework of this study, a microbial screening was also performed to evaluate and get an understanding of the differences between the different substrate constituents and the microbiome within. The screening was conducted through the evaluation method of plate counting.

Firstly, samples of substrates were collected before planting, in triplicates from the extremes of each mix (P & H, dose 2 and 6), the control for P (dose 1) and a commercially available peat substrate (Ref.), respectively (Table 4). Samples were put into tubes, which were marked accordingly, and then put in a refrigerator for later assessment.

Five different agar types were prepared for plating samples onto petri dishes.

- Tryptic Soy Agar (TSA) in strength 0,1 was prepared using 4.0 g TSA, 15.0 g of agar, and 1000 ml of distilled H_2O.
- Malt extract agar (MA) was prepared using 10.0 g Malt extract, 20.0 g agar, and 1000 ml of distilled H_2O.
- King's B Agar (KB) was prepared using 20.0 g of proteose peptone, 1.5 g of K_2HPO_4, 1.5 g of MgSO4*7 H_2O, 15.0 ml of glycerol (99%), 15.0 g agar and 1000 ml of distilled H_2O.

- Nutrient Agar (NA) was prepared using 28.0 g of nutrient agar and 1000 ml of distilled H_2O.

All suspensions were thoroughly mixed and then autoclaved for approximately 30 minutes.

Trichoderma Selective Medium (TSM) was prepared with 0.9 g of K_2HPO_4, 0.2 g of $MgSO_4$*7 H_2O, 0.15 g of KCl, 1.05 g of NH_4Cl, 3.0 g of glucose, 0.15 g of Rose Bengal, 20.0 g of agar and 1000 ml of distilled H_2O. Likewise, this suspension was thoroughly mixed and autoclaved. After cooling in temperature, to prevent damage to antibiotics, Streptomycin and Tetracycline were added to the suspension. The antibiotics were prepared in stock solutions using 0.1 g of Streptomycin in 10 ml of distilled H_2O and 0.05g of Tetracycline in 5 ml of distilled water. Both suspensions were carefully mixed and then filtered using a membrane filter.

After plating all agar suspensions on petri dishes, they were put into sealed plastic bags and put into a refrigerator for later use.

From the refrigerated samples, 1 g of each substrate was immersed in a tube with 10 ml of detergent. The tubes were then shaken at appr. 130 rpm for 30 minutes. Thereafter, 1 ml of the solution was diluted in 0,85%, autoclaved, NaCl in tubes. For the purpose of the present experiment, a series of dilutions from 10^{-1} to 10^{-6} was performed for each sample. Conducting the serial dilution, 1 ml of each sample was placed into tubes with 9 ml of 0,85% NaCl, respectively. After vortexing diluted samples, 0,1 ml of chosen serial dilution were placed on prepared agar plates in duplicates (Table 4).

Table 4. Overview of agar types, dilution steps and incubation times used.

Agar type	Purpose	Dilution steps	Incubation time
TSA	a medium that is suitable for non-selective bacterial growth	10^{-4}, 10^{-5}, 10^{-6}	72 h
NA	a non-selective media for growth of e. g. *E.coli, Salmonella, Staphylococcus* etc.	10^{-2}, 10^{-3}, 10^{-4}	24 h
KB	suitable for the detection of several Pseudomonads	10^{-1}, 10^{-2}, 10^{-3}	48 h
MA	a medium for isolation and enumeration of yeasts and molds	10^{-3}, 10^{-4}, 10^{-5}	96 h
TSM	a Trichoderma-selective agar medium	1, 10^{-1}, 10^{-2}	120 h

100 µl of each dilution step, respectively, were plated in duplicates on Tryptic Soy Agar (TSA), Nutrient Agar (NA), King's B Agar (KB), Malt extract Agar (MA) and Trichoderma Selective Agar (TSM). All plates were marked accordingly with substrate sample, dilution, and agar type. Glass beads were added to the plate and

then stirred through motion for 30 s-1 minute for an even spread. Plates were then put in an incubator at 25º C. Each agar type was incubated according to times presented in table 4.

Viable plate count was conducted by counting colonies on plates with 30 up to 300 colonies. Plates with KB agar were put under UV light in order to count fluorescent colonies. CFU/g of substrate sample was calculated using formula;

$$CFU \bullet dilution\ volume / sample\ weight / inoculation\ volume / dilution\ factor\ counted = CFU/g\ substrate\ sample$$

$$Log10(CFU/g/\text{substrate})$$

2.1.6 Assessment of Plant Biomass

To evaluate the different treatments and their impact on growth, an assessment of produced biomass was conducted. After 5 weeks of growth, the plants were assessed as developed enough and lettuce plants were carefully removed from the pots. All growing media was removed from the roots manually. Weights were measured for all treatments, doses and replicates, divided into fresh and dry weights of leaves and roots, respectively. Thereafter, all samples were dried in a drying cabinet at 60º C for 72 h and dry weights of all samples were measured.

2.1.7 Statistical Analysis

Statistical analyses were performed in Minitab version 19. For all statistical analyses, a confidence level of 95% was used. One-way ANOVA, general linear model, and interaction plots were performed. Significant differences were calculated using Tukey's-test at a significance level of $p < 0.05$, where different small letters describe significant differences. Equal variances were assumed for all analyses.

3. Results

3.1 Physical and Chemical Assessment

The results from the physical assessments (Table 5) show that when comparing all doses, treatment P has a significantly higher bulk density (BD) compared to treatment H. Additionally, treatment P has a significantly higher pot capacity (PC) compared to treatment H. However, in treatment P the capacity to hold water is not linear to the increased dose of biochar which seems to be the case in treatment H. In terms of total porosity, the results instead show significantly higher mean values in treatment H.

Regarding the chemical assessments (Table 5), treatment with hemp and biochar (H) had a significantly higher mean value for both pH and EC. However, both treatments show pH that is alkaline compared to the reference value.

Table 5. Results of physical and chemical assessments of substrate mixes of peat, hemp and biochar (see materials and methods for treatments) and reference values for a common commercial substrate based on peat. Data are arithmetic means ± standard deviation, for factors pH, BD, PC and CD which was each repeated in triplicates. The arithmetic means of CD was used to calculate Porosity. EC values are based on substrate samples sent to external laboratory (LMI, Helsinborg, Sweden)

Peat and biochar (P)	pH	EC (mS/cm)	BD (g/dm^3)	PC (g)	CD (g/dm^3)	Porosity (%)
Dose 1	6,5 ± 0,24	2,2	591, 8 ± 7,4	525,6 ± 19,7	1418,9 ± 76,4	58.3
Dose 2	6,8 ± 0,05	1,8	587,7 ± 1,6	516,9 ± 11,6	1465, 6 ± 175,8	59.9
Dose 3	7,2 ± 0,09	2,1	550,1 ± 7,1	510,8 ± 0	1372,4 ± 74,4	59.9
Dose 4	7,6 ± 0,00	1,7	553,4 ± 4,9	523,3 ± 10,6	1329,1 ± 104,2	58.4
Dose 5	8,2 ± 0,05	1,8	570,6 ± 3,5	498,5 ± 9,8	1362,5 ± 1,5	58.1
Dose 6	8,3 ± 0,05	1,6	551,4 ± 4,9	501,6 ± 7,9	1293,9 ± 29,4	57.4
Hemp and biochar (H)						
Dose 1	7,8 ± 0,14	2,5	412,4 ± 9,2	315,5 ± 20,1	1332,6 ± 39,4	69.0
Dose 2	8,1 ± 0,05	2,3	477,0 ± 9,4	336,9 ± 15,8	1335,1 ± 15,7	64.3
Dose 3	8,3 ± 0,12	2,2	467,1 ± 6,8	337,7 ± 27,0	1244,0 ± 5,7	62.5
Dose 4	8,4 ± 0,05	2,3	491,1 ± 3,6	342,2 ± 8,5	1282,6 ± 35,6	61.7
Dose 5	8,9 ± 0,26	2,0	460,7 ± 7,4	371,7 ± 4,2	1251,9 ± 14,8	63.2
Dose 6	9,0 ± 0,33	2,0	518,6 ± 4,3	375,6 ± 1,2	1312,6 ± 6,6	60.5
Reference values (Ref.)	5,3 ± 0,09	4,1	460,5 ± 11,6	563,2 ± 15,6	1168,7 ± 41,5	60.6

The analysis of readily available nutrients (Figure 2) shows a very high content of potassium (K) in all samples, with no significant differences between treatments H & P. However, regarding availability of phosphorus (P) there is a significant difference, where treatment H has the higher mean value. Nitrogen content (N_{tot}), show no significant difference between treatments. However, in comparison to the reference value the means are very low on both treatments and all doses.

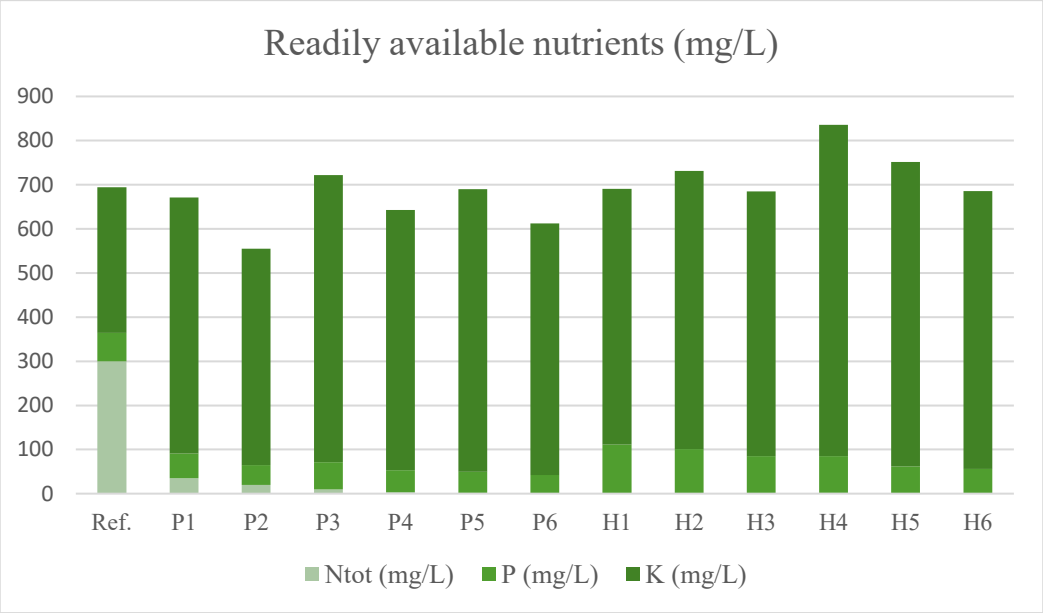

Figure 2. Readily available nutrients based on substrate samples sent to external laboratory (LMI, Helsinborg, Sweden)

3.2 Growth Assessment

According to the mean values of fresh weight (Figure 3), P6 has the significantly highest value of weight of fresh leaves. According to the figure, P6 produced a higher weight of leaves than P1 which acted as a control (0% biochar). However, this treatment (P6) is still separated from the reference value which has a higher leaf weight. For the fresh weight of roots, it is treatment P1 which has the significantly highest mean value. Overall, treatment P has a significantly higher weight than treatment H, for both leaves and roots.

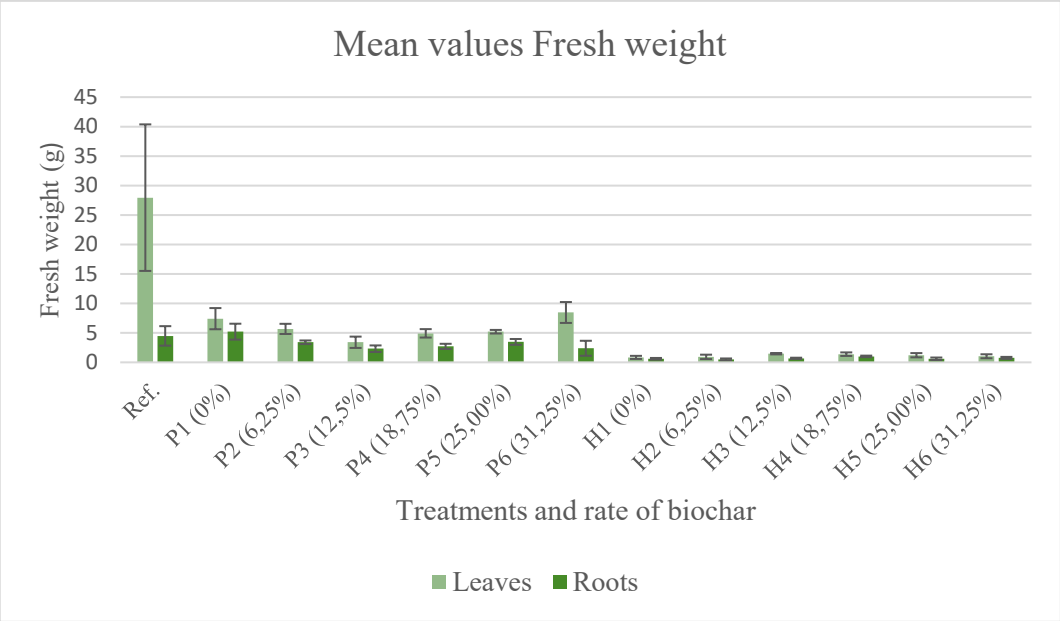

Figure 3. Arithmetic means of fresh weight (g) for leaves and roots, for each treatment and dose with error bars showing standard deviation.

The same pattern is repeated in the values for dry weight (Figure 4), where treatment P has a significantly higher weight but is still different from the reference value. However, when dry weight is measured there is no significant difference between doses of biochar in treatment P (1-6). Also, there is more deviation within treatment P.

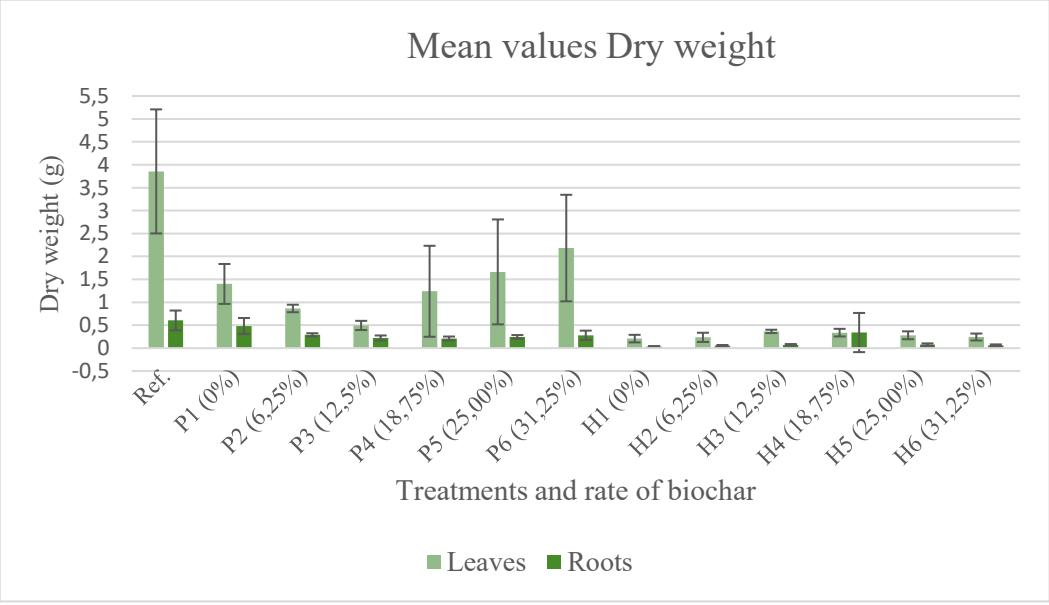

Figure 4. Arithmetic means of dry weight (g) for leaves and roots, for each treatment and dose with error bars showing standard deviation.

When comparing treatment P (with best growth) with treatment H and each dose (1-6), there is a multiplication in growth in grams. Dose 3 in treatment H had the highest mean value of fresh weight of leaves (1,47 g ± 0,12) and dose 3 in treatment P had the lowest mean value of fresh weight of leaves (3,41 g ± 0,96), but still treatment P has an increase of 132% in grams.

Correspondingly to the weights obtained, visual assessment of plant growth indicates a better growth in treatments with peat and biochar (P1-P6) (Figure 5). Here, P6 (with the highest rate of biochar) shows the best growth. All doses of treatment H (Figure 6) show an overall poor performance, lack of growth and deficiency symptoms of nitrogen (N). Interestingly, treatment H though shows a better visual growth with increasing biochar rate.

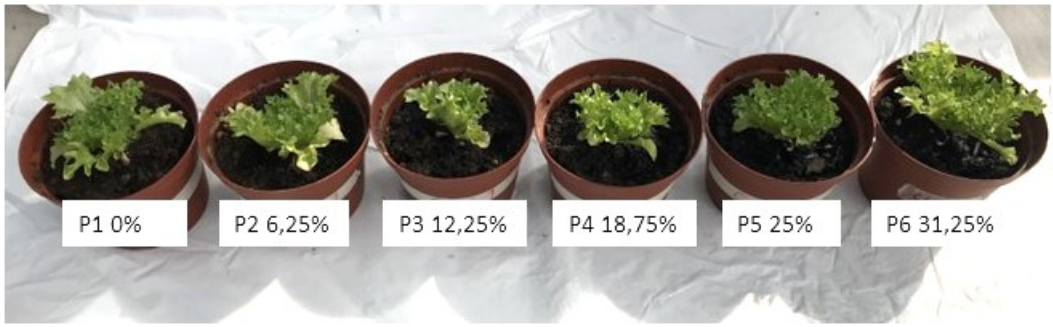

Figure 5. Visual assessment of growth of leaves and overall plant health, treatment P1-P6 with biochar rate in percent. Photo: L. Hagbard

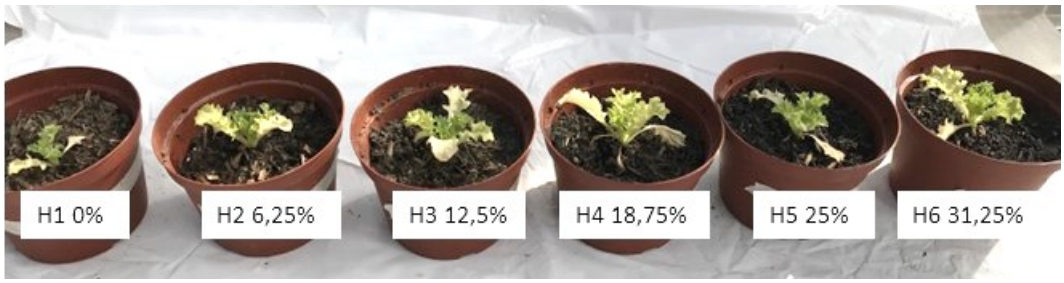

Figure 6. Visual assessment of growth of leaves and overall plant health, treatment H1-H6 with biochar rate in percent. Photo: L. Hagbard

Visual assessment of development of root systems from the extremes in the biochar gradient (Figure 7). H1 & P1 with 0% biochar and H6 & P6 with 31,25% biochar. Treatments with peat and biochar (P1 & P6) have a better developed root system than treatments with hemp and biochar (H1 & H6). The reference (Ref.) shows a root system grown in a commercial peat substrate.

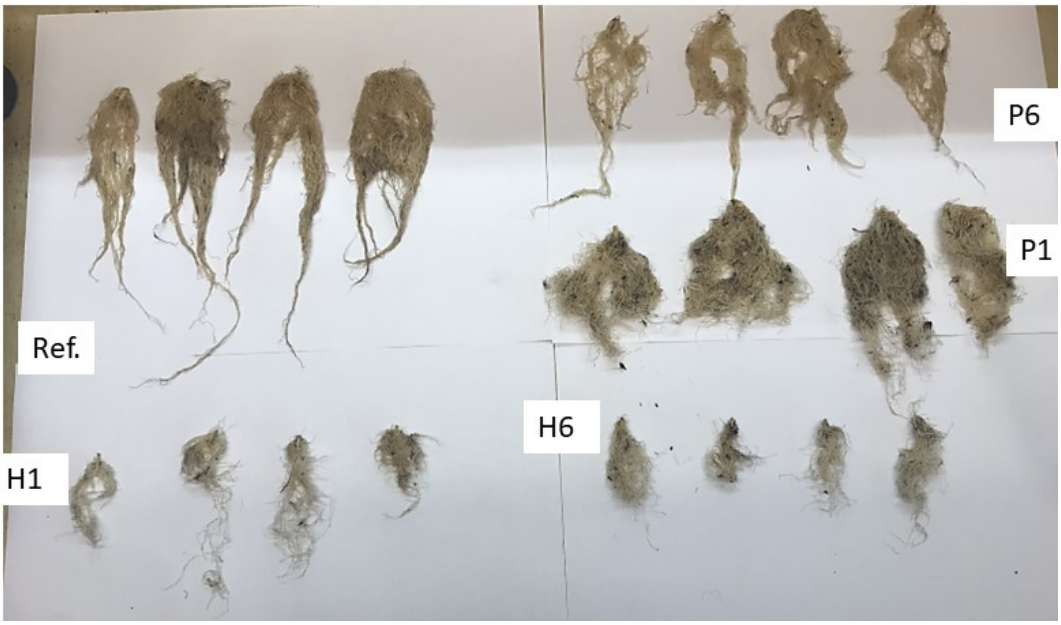

Figure 7. Sample of the extremes of the biochar gradient for each treatment, P1 & P6 and H1 & H6. Commercial peat substrate (Ref.) for reference. Photo: L. Hagbard

3.3 Microbial Assessment

Plates with tryptic soy agar, which is a media for counting of bacteria, show a significantly higher presence of microbiota in treatment H2 (Figure 8). Further, there is a significant difference in presence between treatment H6 and peat with biochar (P1, P2 and P6) which are not significantly separated from each other.

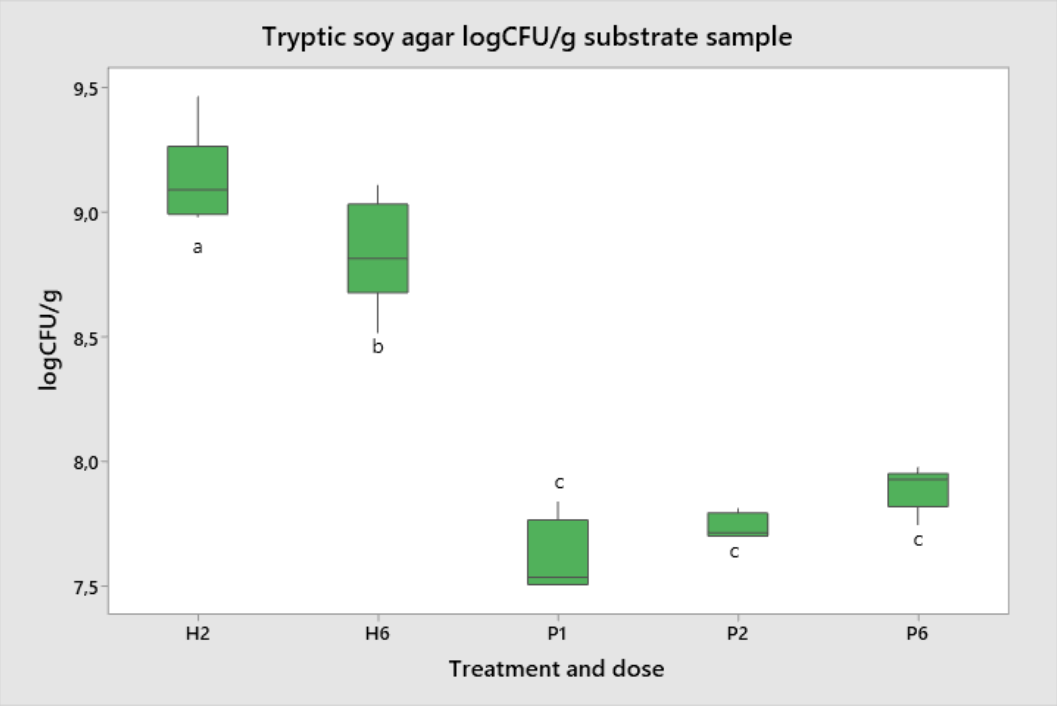

Figure 8. logCFU/g substrate on plates of Tryptic Soy Agar. Treatment H and dose 2 (H2) has the significantly highest amount of CFU/g substrate sample. Data are arithmetic means and whiskers indicating variability outside the upper and lower quartiles.

Plates with nutrient agar, which is a general media for microbial growth, show that treatment H2 has the significant highest presence of microbiota (Figure 9). P6 and P2 are significantly separated from the other treatments but not from each other. In this case, treatment P1(with a biochar rate of 0 %) has the significant lowest amount of microbiota present.

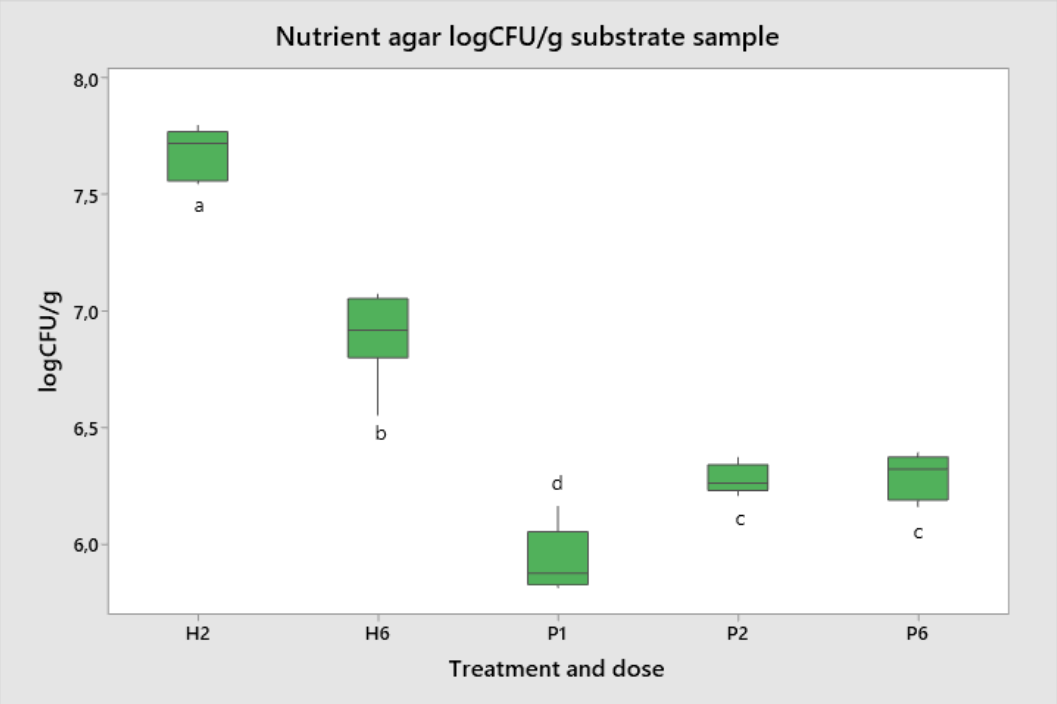

Figure 9. logCFU/g substrate on plates of nutrient agar. Treatment H and dose 2 (H2) has the significantly highest presence of CFU/g substrate sample. P1 shows the least CFU/g substrate sample. Data are arithmetic means and whiskers indicating variability outside the upper and lower quartiles. Means that do not share a letter are significantly different.

Plates with malt extract agar (Figure 10), which promotes growth of molds and yeasts, show a significantly higher presence in treatments with hemp (H2 & H6). None of the treatments with peat and biochar have any significant difference from each other.

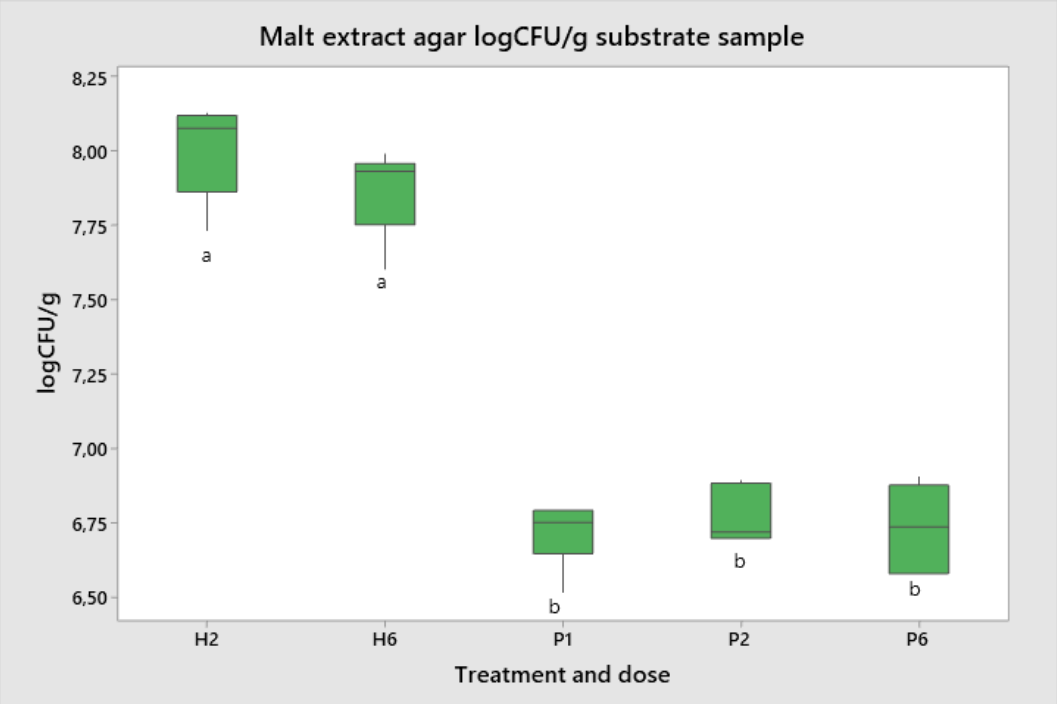

Figure 10. logCFU/g substrate on plates of malt extract agar. Data are arithmetic means and whiskers indicating variability outside the upper and lower quartiles. Means that do not share a letter are significantly different.

Plates with King's B agar, which promotes growth of species of *Pseudomonas* spp., show a significantly higher abundance in treatments with hemp (H2 & H6) (Figure 11).

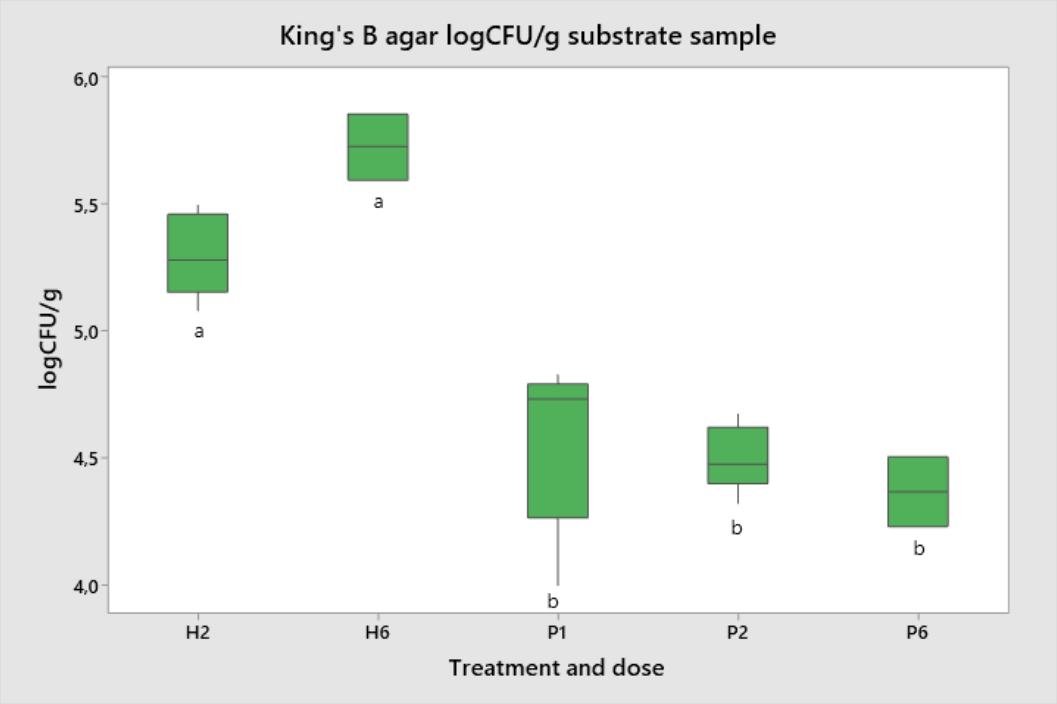

Figure 11. logCFU/g substrate on plates of King's B agar. Data are arithmetic means and whiskers indicating variability outside the upper and lower quartiles. Means that do not share a letter are significantly different.

Plates with TSM, which is a selective media promoting growth of *Trichoderma*, show that treatments P1 and P6 have a significantly higher presence than treatments H2 and H6 (Figure 12). However, when compared with commercial peat, the peat substrate (Ref.) has the significantly highest presence (Figure 13).

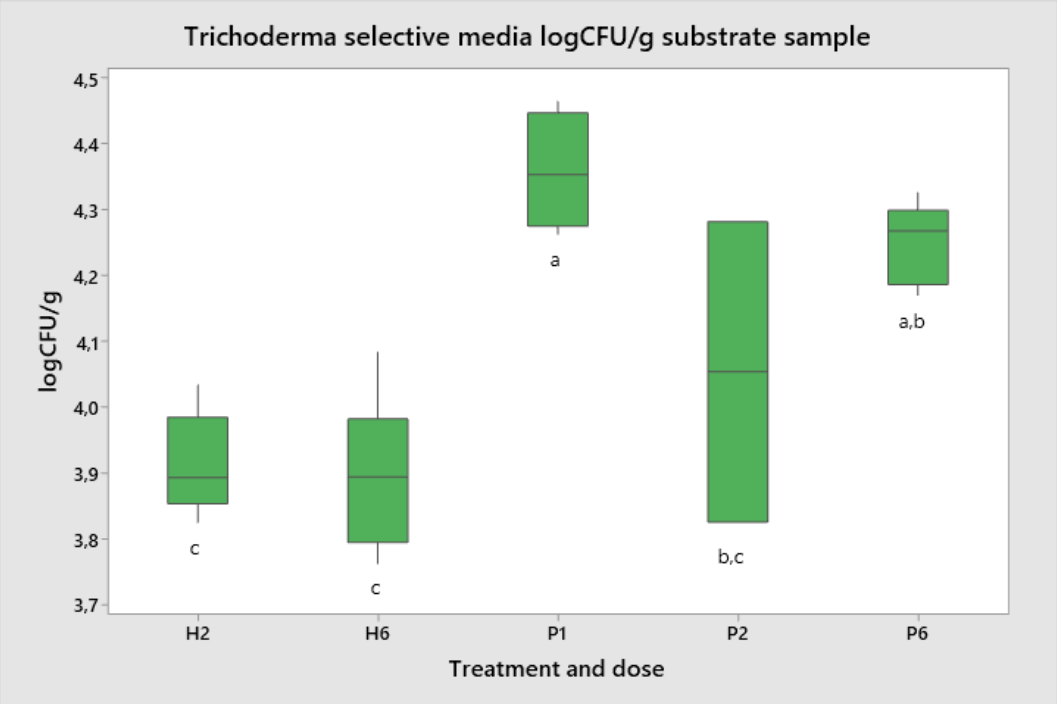

Figure 12. logCFU/g substrate on plates of Trichoderma selective media. Data are arithmetic means and whiskers indicating variability outside the upper and lower quartiles. Means that do not share a letter are significantly different.

Microbial assessments of a general, commercially available peat substrate (Figure 13), where each box correspond respectively to the each of the different figures above. For King's B agar and abundance of *Pseudomonas,* the reference value is similar to the treatment with biochar and peat (P1, P2 & P6). Assessment on malt extract plates show a reference value lower than the both treatments (H & P). Reference value for nutrient agar also indicates a lower value than both treatments. Similarly, the reference value for tryptic soy agar plates is lower than both treatments (H & P). However, on *Trichoderma* selective media the reference value indicates the highest abundance. Of the treatment and doses, P1 shows the highest abundance of *Trichoderma*.

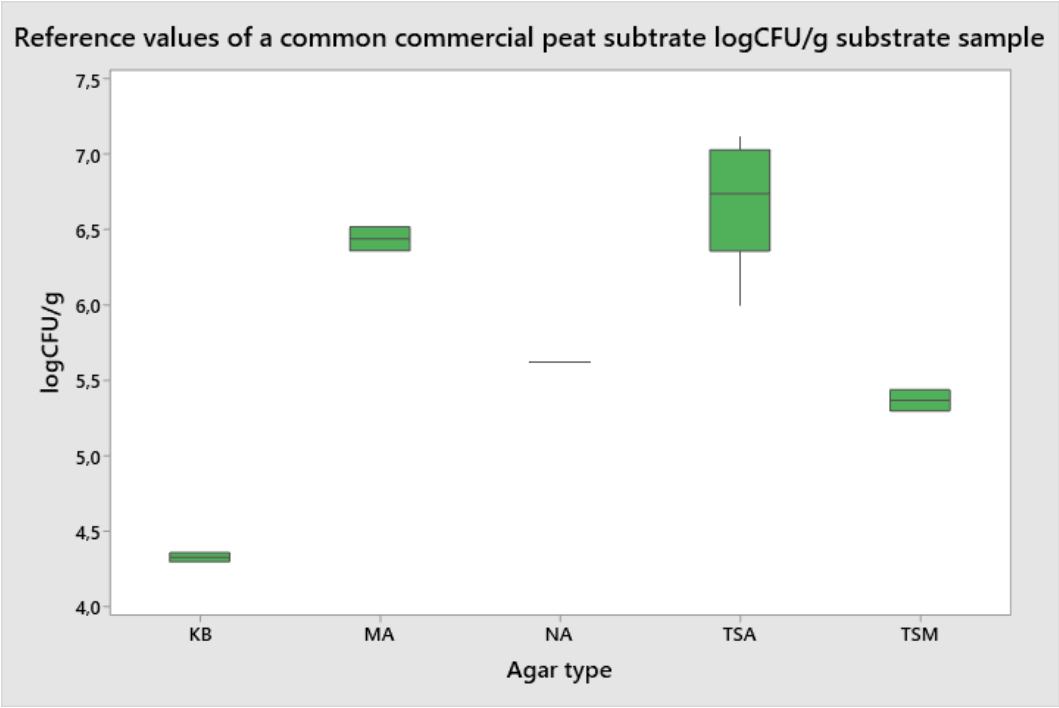

Figure 13. Reference values of logCFU/g for each agar type used in the assessment, in a commercial peat based substrate. Data are arithmetic means and whiskers indicating variability outside the upper and lower quartiles.

4. Discussion

The result from the physical assessments indicated treatment P (peat and biochar) obtained lower bulk density with the highest ratio of biochar added (31,25 %) compared to the control where 0 % biochar was added. In this case, P6 had a 6% higher bulk density than H6. To my knowledge, it seems that standardized values for bulk density aren't available but instead are rather accepted amongst professional growers based on the knowledge of cultivation system and crop. However, e. g. Noguera et al. (2003) recommend a range between 200 -400 g/dm^3 as suitable for pot cultivation. If this should be applied to this study there is none of either treatments or doses, not even the reference, within in this range.

The particle size distribution was not assessed in this work, however, only by looking and working with the different mixtures it became very clear that treatment H contained a larger proportion of bigger particles. This could explain the poor pot capacity in treatment H, since prior studies (Graceson et al., 2013) have found that water holding capacity is related to particle size distribution and primarily fine particles. Benito et al. (2006) emphasized that an optimal substrate should have a particle size distribution in the range of 0.25 and 2.5 mm which indeed seems more applicable to treatment P than treatment H. The same study also showed poorer physical performance when proportion of larger particles, 0.5-8 mm was increased which also could explain the poor performance of treatment H. The particle size distribution is made visible in the context of added rates of biochar to treatment H, primarily. The total porosity for treatment H decreases along the gradient of added biochar which could partly be explained by the fact that components differed in particle size.

The results regarding plant growth from current work indicate that a reduction in peat is possible when substituted with biochar. According to the statistical analyses, the growth of biomass differs significantly between the different treatments (H & P), where treatment H has a poorer performance. This indicates that by maintaining a certain proportion of peat in the substrate and alter with biochar, it can sustain acceptable growth. However, the growth in the treatment containing peat (P), was different from the reference value (Ref.) of a commercial product. The treatment with the highest ratio of biochar, P6, was the one with the best growth (both fresh

weight and dry weight) and also the one with the best developed leaves and root system according to visual growth assessment. This indicates that biochar, after all, positively affected growth and possibly released nutrients over time, as discussed by Wang et al. (2022) in the context of agricultural systems. If this is the case, these factors can be remedied for improved performance, such as higher or more accurate nutrient supply, elaboration with other potential substrates, or a higher proportion of those substrates included in this study, compost, clay or horse manure.

A limitation in utilizing biochar as substrate is its inherent high pH. The high pH values of biochar were already acknowledged in advance prior to the experiment was started. Given that it is difficult to lower the pH in a substrate and in agreement with the client, the decision was made to leave the pH unchanged to still evaluate what growth could be obtained. The results given, demonstrate that all treatments where biochar was added (P2-P6 & H2-H6) have alkaline pH. This is despite the fact that unlimed peat was used in the mixtures to compensate, to some extent, for the high pH of biochar. The pH value correspondingly affects the cation exchange capacity (CEC), as the negative charge of the organic material increases with increasing pH (Eriksson et al., 2005). Which also can explain the immobilization of nutrients in this case. Therefore, to optimize biochar as a substrate for cultivation, some sort of acidic component must be added for accurate pH values. However, as earlier mentioned, to lower pH in a substrate is difficult as well as maintain accurate levels and it could therefore be a limitation in the potential of a general growing media. Different plants have different optimums but a general recommendation is 5.2 – 6.5 (Abad et al., 2001; Noguera et al., 2003).

Regarding the microbial assessment the results show a significant higher level of CFU/g in samples containing hemp (H). Therefore, the results give the impression that the fact that added biochar has not affected the abundance of microorganisms. It is rather a difference between if peat or hemp is present in the mixture. Peat has previously been reported (Hoitink & Boehm 1999; Krause et al. 2001) to have inherent properties such as the absence of microbial life. This is due to low availability of energy and the relatively high amount of stabilized carbon (C). This could explain why treatment P had a lower number of microorganisms apart from *Trichoderma*. On the contrary, the hemp contained high amount of available carbon (C) which led to higher proliferation of microorganisms.

Trichoderma species are naturally abundant in peat, specifically in young peat which has a larger proportion of undecomposed material and a humification level of H1-H3. Between treatments and doses, P1 hade the significant highest CFU/g. The explanation can be assumed to be that there was a certain amount of peat (56,25%) in the mixture. H6 had the lowest presence of *Trichoderma* suggesting that the environment in the treatment was not as favourable as in the other doses.

Probably, the lack of peat in this treatment could be an explanation, in combination with the high pH for this dose (pH 9,0 ± 0,33). A correlation analysis was performed to see if pH and abundance of *Trichoderma* correlated, but the result was not significant and no further conclusions can be drawn between formation of microbiome depending on pH. Further, it has been reported that in general, the presence of bacteria will increase with an increase in pH, whilst fungi will decrease (Rousk et al., 2009). The results suggests that this was accurate in this case where both treatments and all doses were above recommended pH values for potting substrate, as mentioned. Hence, arithmetic means of CFU/g were highest on TSA plates which are used for the purpose of determine proliferation of bacteria. In a study, conducted by Grunert et al. (2016), they evaluated and studied soilless cultivation systems and the groups organic and mineral cultivation media and the microbial communities that inhabits these systems. They identified moisture, potassium content, pH and electrical conductivity (EC) as the main physicochemical properties that drive microbial communities in soilless media. Possibly, this could be utilized to some extent when amendment of biochar is used to be able to provide a suppressive effect in substrates, which is also reviewed by Poveda et al. (2021).

The results from the greenhouse trial were somewhat unexpected given the evidence available, at least in favor of biochar. Based on previous research, these results indicate that something in the experimental set up was unsuccessful. Several possible sources of errors have subsequently been identified, of which the most decisive is believed to be the initial supply of nitrogen (N) to the biochar. The dose has probably been insufficient, which has led to a high C/N ratio and immobilization of nitrogen. Another possible effect on the substrates was the extended time they were left in the greenhouse environment before the actual experiments could start. The delay was due to difficulties in getting the lettuce to germinate and grow evenly in the greenhouse, probably due to too high temperatures. Due to this, the substrates were in an environment (varm and humid) that was beneficial for microbial activity which could also play a role in potential nitrogen release.

It is also important to keep in mind that different species of plants have different growth optimums and therefore they can perform differently even if biochar has properties reported as beneficial. As an example, Vaughn et al. (2015) identified differences in growth between tomato (*Solanum lycopersicum*) and marigold (*Calendula officinalis*) when amendments of biochar were introduced to the substrate. Likewise, Choi et al. (2018) could conclude that mixes with 20% pine bark and 80% biochar (by volume) induced increased fresh and dry weight in chrysanthemum (*Chrysanthemum nakingense*) cultivation studies whilst it decreased the fresh and dry weight of tomato compared to control plants. Further,

lettuce (*Lactuca sativa*) and basil (*Ocimum basilicum*) were left with no effect when grown in 80% biochar mixes. The differences in performance can also be linked to the different properties of biochar depending on which feedstock, as well as pyrolysis method, are used during production.

In present trial, hemp did not perform in accuracy with a desired cultivation substrate. However, if it is possible to find other, or modify the fractions of hemp fibers, or use it in a composted format it may be able to perform with satisfaction. As future outlooks in the field, investigating the effects of biochar in longer cultures to understand the effects on growth and root development of plants in containers but also the microbiome and community structure. As Mattei et al. (2017) reported, there is a research gap in assessing novel materials for substrate utilization and their microbial community structure. Exploring to what extent novel substrates can mitigate soilborne phytopathogens is another potential pathway to better utilizing substrates and thus cultivation in limited containers. Likewise, research on an accumulation of organisms or potentially toxic substances in novel substrates could be of interest if substrates are aimed to be repurposed several times. According to previous studies presented in this thesis, biochar is a material that is persistent over time, but depending on what other material it is mixed with, it may still play a role in the availability of water, nutrients and air i. e. total substrate performance. Nevertheless, research should also be conducted regarding availability, origin and environmental impact. In order to reduce peat use, however, it is important that researchers and users continue to discover renewable materials or residual streams. In order to reach consensus for production and practical purposes regarding novel substrate and their components, standard protocols should be established to a larger extent. In regards of use of biochar in horticultural contexts, protocols on their properties and thus reasonable nutrient demands should also be established for greater usability.

Conclusions

In this work, biochar and hemp were explored as potential reducers of the use of peat in horticultural growing media and how they would affect the presence of microbiota in the media mixtures. The conclusion that can be drawn is that peat with the addition of 31,25 % biochar performed best in growth assessments conducted. Hemp and biochar, however, did not perform with satisfaction regarding growth. Hemp and biochar treatment was also found to have the highest CFU / g in most microbial counts, probably due to high C / N ratio. No decisive conclusion about biochar's effect on the microbiome could be drawn.

It is important to keep in mind that there is no substrate that is optimal for all horticultural plants. Therefore, when introducing a new substrate or substrate component, research and evaluations should be made on its properties and how the effect on plants can turn out. By extensively exploring substrates with several constituents and with knowledge-based applications rewarding their beneficial properties while minimizing their limitations, one can potentially achieve suitable products.

References

Abad, M., Noguera, P. & Burés, S. (2001). National inventory of organic wastes for use as growing media for ornamental potted plant production: case study in Spain. *Bioresour Technol* 77:197–200. https://doi.org/10.1016/S0960-8524(00)00152-8

Altland, J. E., & Locke, J. C. (2011). Use of ground miscanthus straw in container nursery substrates. *Journal of Environmental Horticulture*, *29*(3), 114-118. https://doi.org/10.24266/0738-2898-29.3.114

Altland, J. E. & Locke, J. C. (2012). Biochar Affects Macronutrient Leaching from a Soilless Substrate. *HortScience* 47, nr 8 :1136–40. https://doi.org/10.21273/HORTSCI.47.8.1136.

Altland, J. E. & Locke, J. C. (2013). Effect of Biochar Type on Macronutrient Retention and Release from Soilless Substrate. *HortScience* 48, nr 11: 1397–1402. https://doi.org/10.21273/HORTSCI.48.11.1397.

Antoniou, A., Tsolakidou, M-D., Stringlis, I.A. & Pantelides, I.S. (2017). Rhizosphere Microbiome Recruited from a Suppressive Compost Improves Plant Fitness and Increases Protection against Vascular Wilt Pathogens of Tomato. *Frontiers in Plant Science.* 8. https://www.frontiersin.org/article/10.3389/fpls.2017.02022.

Benito, M., Masaguer, A., Moliner, A. & De Antonio, R. (2006). Chemical and physical properties of pruning waste compost and their seasonal variability. *Bioresour Technol.* 97:2071–2076. https://doi.org/10.1016/j.biortech.2005.09.011

Bernesson, S. 2006. *Hampa till bränsle, fiber och olja - en liten handbok*. SERO, Köping.

Bilderback, T. E., Warren, S. L., Owen, J. S., & Albano, J. P. (2005). Healthy substrates need physicals too!. *HortTechnology*, *15*(4), 747-751. https://doi.org/10.21273/HORTTECH.15.4.0747

Bohlin, C. & Holmberg, P. (2004). Peat: Dominating Growing Medium in Swedish Horticulture. *Acta Horticulturae*, nr 644:177–81.

Both, A. K., Helle, M. A., Madireddy, G. & Cheung, C. L. (2021). Green Chemical Approach to Fabricate Hemp Fiber Composites for Making Sustainable Hydroponic Growth Media. *ACS Agricultural Science & Technology* 1, nr 5:499–506.https://doi.org/10.1021/acsagscitech.1c00118.

Boudreault, S. (2010). *Effects of Physical and Chemical Properties of Substrates on Growth and Development of White Spruce Seedlings in Containers After One Growing Season.* M.Sc. Thesis, Department of Soil and Agri-Food Engineering, Université Laval, Québec, Canada.

Bourmaud, A., Beaugrand, J., Shah, D.U., Placet, V. & Baley, C. (2018). Towards the design of high-performance plant fibre composites: How can we best define the diversity and specificities of plant cell walls? *Prog. Mater. Sci.* 2018, 97, 347–408. https://doi.org/10.17660/ActaHortic.2004.644.22.

Bunt, A.C. (1988). *Media and mixes for container-grown plants: a manual on the preparation and use of growing media for pot plants*. (2. ed.) London: Unwin Hyman.

Carlile, B. & Coules, A. (2011). Towards sustainability in growing media. *International Symposium on Growing Media, Composting and Substrate Analysis*. vol. 1013 (2011), pp. 341-349. 10.17660/ActaHortic.2013.1013.42

Choi, H.S., Zhao, Y., Dou, H., Cai, X., Gu, M. & Yu, F. (2018). Effects of biochar mixtures with pine-bark based substrates on growth and development of horticultural crops. *Hortic. Environ. Biotechnol.* 59, 345–354. https://doi.org/10.1007/s13580-018-0035-x

Crônier, D., Monties, B. & Chabbert, B.R. (2005). Structure and chemical composition of bast fibers isolated from developing hemp stem. *J. Agric. Food Chem*. 53, 8279–8289. https://doi.org/10.1021/jf051253k

Dannehl, D., Suhl, J., Ulrichs, C. & Schmidt, U. (2015). Evaluation of substitutes for rock wool as growing substrate for hydroponic tomato production. *Journal of Applied Botany and Food Quality* 88 (01 mars 2015): 68–77. https://doi.org/10.5073/JABFQ.2015.088.010.

Debode, J., De Tender, C., Cremelie, P., Lee, A. S., Kyndt, T., Muylle, H., De Swaef, T. & Vandecasteele, B. (2018). Trichoderma-Inoculated Miscanthus Straw Can Replace Peat in Strawberry Cultivation, with Beneficial Effects on Disease Control. *Frontiers in Plant Science* 9 (2018). https://www.frontiersin.org/article/10.3389/fpls.2018.00213.

Du Bois, W. F. (1982). *Hemp as a raw material for the paper industry*. *Bedrijfsontwikkeling*.

EBC. (N.D.). *The European Biochar Certificate.* Available: https://www.european-biochar.org/en [2022-04-15]

EC. *Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committe and the Committee of the Regions: A Bioeconomy for Europe*; European Commission: Brussels, Belgium, 2012. [Google Scholar]

EC. *A Sustainable Bioeconomy for Europe: Strengthening the Connection between Economy, Society and the Environment*. Updated Bioeconomy Strategy; Publications Office of the European Union: Luxembourg, 2018; p. 107.[Google Scholar]

Eriksson, J., Nilsson, I. Simonsson, M. & Wiklander, L. *Wiklanders marklära*. Studentlitteratur, 2005.

FAO. (2022). *Ecosystems, biodiversity, genetics | Sustainable Development Goals*. Available:https://www.fao.org/sustainable-development-goals/overview/fao-and-the-2030-agenda-for-sustainable-development/ecosystems-biodiversity-genetics/en/. [2022-05-04]

Fernando, D., Thygesen, A., Meyer, A., & Daniel, G. (2019). Elucidating Field Retting Mechanisms of Hemp Fibres for Biocomposites: Effects of Microbial Actions and Interactions on the Cellular Micro-morphology and Ultrastructure of Hemp Stems and Bast Fibres. *BioResources, 14*(2), 4047-4084. Retrieved from https://ojs.cnr.ncsu.edu/index.php/BioRes/article/view/BioRes_14_2_4047_Fernando_Elucidating_Field_Retting_Mechanisms_Hemp_Fibre

Frangi, P., Amoroso, G. & Piatti, R. (2012). Alternative Growing Media to Peat Obtained From Two Fast Growing Species of *Poaceae. Acta Hortic*. 927, 967-972 https://doi.org/10.17660/ActaHortic.2012.927.119

Graceson, A., Hare, M., Monaghan, J. & Hall, N. (2013). The water retention capabilities of growing media for green roofs. *Ecol Eng* 61:328–334. https://doi.org/10.1016/j.ecoleng.2013.09.030

Grunert, O., Hernandez-Sanabria, E., Vilchez-Vargas, R., Jauregui, R., Pieper, D.H., Perneel, M., Van Labeke, M.-C., Reheul, D. & Boon, N. (2016). Mineral and Organic Growing Media Have Distinct Community Structure, Stability and Functionality in Soilless Culture Systems. *Scientific Reports* 6, nr 1: 18837. https://doi.org/10.1038/srep18837.

Henriksson, G., Akin, D.E., Hanlin, R.T., Rodriguez, C., Archibald, D.D., Rigsby, L.L. & Eriksson, K.L. (1997) Identification and retting efficiencies of fungi isolated from dew-retted flax in the United States and Europe. *Appl. Environ. Microbiol*. 1997, 63, 3950–3956. [Google Scholar]

Hoitink, H. A. J., & Boehm, M. J. (1999). Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. *Annual review of phytopathology*, *37*, 427. https://doi.org/10.1146/annurev.phyto.37.1.427

Jackson, B. E., Wright, R. D. & Alley, M. M. (2009). Comparison of Fertilizer Nitrogen Availability, Nitrogen Immobilization, Substrate Carbon Dioxide Efflux, and Nutrient Leaching in Peat-lite, Pine Bark, and Pine Tree Substrates, *HortScience horts*, *44*(3), 781-790. https://journals.ashs.org/hortsci/view/journals/hortsci/44/3/article-p781.xml

Jeffery, S., Verheijen, F.G.A., van der Velde, M. & Bastos, A.C. (2011). A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agric. Ecosyst. Environ*. 144, 175–187.

Joosten, H., Sirin, A., Couwenberg, J., Laine, J., & Smith, P. (2016). The role of peatlands in climate regulation. In *Peatland restoration and ecosystem services: science, policy and practice* (Vol. 2016, pp. 63-76). Cambridge, UK: Cambridge University Press.

Kern, J., Tammeorg, P., Shanskiy, M., Sakrabani, R., Knicker, H., Kammann, C., Tuhkanen, E-M. et al. (2017). Synergistic use of peat and charred material in growing media – an option to reduce the pressure on peatlands? *Journal of Environmental Engineering and Landscape Management* 25, nr 2,160–74. https://doi.org/10.3846/16486897.2017.1284665.

Kløve, B., Berglund, K., Berglund, Ö., Weldon, S. & Maljanen, M. (2017). Future Options for Cultivated Nordic Peat Soils: Can Land Management and Rewetting Control Greenhouse Gas Emissions? *Environmental Science & Policy* 69: 85–93. https://doi.org/10.1016/j.envsci.2016.12.017.

Krause, M. S., Madden, L. V., & Hoitink, H. A. (2001). Effect of potting mix microbial carrying capacity on biological control of Rhizoctonia damping-off of radish and Rhizoctonia crown and root rot of poinsettia. *Phytopathology*, *91*(11), 1116-1123.

Kuisma, E., Palonen, P. & Yli-Halla, M. (2014). Reed canary grass straw as a substrate in soilless cultivation of strawberry. *Sci Hortic* 178:217–223. https://doi.org/10.1016/j.scienta.2014.09.002

Landis, T. D., Jacobs, D. F., Wilkinson, K. M. & Luna, T. (1990). Growing media. *The container tree nursery manual*, *2*, 41-85

Lehmann, J. & Joseph, S. (2009). *Biochar for Environmental Management.* [e-book]. *Science and Technology.* London: Earthscan.

Lugtenberg, B. (2015). *Principles of Plant-Microbe Interactions.* [e-book]. *Microbes for Sustainable Agriculture*. Cham: Springer International Publishing.

Lutz, S., Thuerig, B., Oberhaensli, T., Mayerhofer, J., Fuchs, J.G., Widmer, F., Freimoser, F.M. & Ahrens, C.H. (2020). Harnessing the Microbiomes of Suppressive Composts for Plant Protection: From Metagenomes to Beneficial Microorganisms and Reliable Diagnostics". *Frontiers in Microbiology* 11 (2020). https://www.frontiersin.org/article/10.3389/fmicb.2020.01810.

Manaia, J.P., Manaia, A.T. & Rodriges, L. (2019). Industrial Hemp Fibers: An Overview. *Fibers*. *7*, 106. https://doi.org/10.3390/fib7120106

Mattei, P., Pastorelli, R., Rami, G., Mocali, S., Giagnoni, L., Gonnelli, C & Renella, G. (2017). Evaluation of dredged sediment co-composted with green waste as plant growing media assessed by eco-toxicological tests, plant growth and microbial community structure. *Journal of Hazardous Materials* 333:144–53. https://doi.org/10.1016/j.jhazmat.2017.03.026

Mazian, B., Bergeret, A., Benezet, J-C. & Malhautier, L. (2018). Influence of field retting duration on the biochemical, microstructural, thermal and mechanical properties of hemp fibres harvested at the beginning of flowering. *Ind. Crops Prod.* 116, 170–181. https://doi.org/10.1016/j.indcrop.2018.02.062

Mazian, B., Bergeret, A., Benezet, J.C., & Malhautier, L. (2019). A Comparative Study of the Effect of Field Retting Time on the Properties of Hemp Fibres Harvested at Different Growth Stages. *Fibers* 7, nr 12:108. https://doi.org/10.3390/fib7120108.

McPartland, J.M. & Hillig, K.W. (2006). Host-Parasite Relationships in Cannabis. *Journal of Industrial Hemp.* 10(2), 85-104. https://doi.org/10.1300/J237v10n02_08

Mukherjee, A., Zimmerman, A.R. & Harris, W. (2011). Surface chemistry variations among a series of laboratory-produced biochars. *Geoderma*, 163, 247–255 https://doi.org/10.1016/j.geoderma.2011.04.021

Nemati, M. R., Simard, F., Fortin, J-P. & Beaudoin, J. (2015). Potential Use of Biochar in Growing Media. *Vadose Zone Journal* 14, nr 6: vzj2014.06.0074. https://doi.org/10.2136/vzj2014.06.0074.

Nerlich, A., Karlowsky, S., Schwarz, D., Förster, N. & Dannehl, D. (2022). Soilless Tomato Production: Effects of Hemp Fiber and Rock Wool Growing Media on Yield, Secondary Metabolites, Substrate Characteristics and Greenhouse Gas Emissions. *Horticulturae*. 8(3):272. https://doi.org/10.3390/horticulturae8030272

Noguera, P., Abad, M., Puchades, R. & Noguera, V. (2003). Influence of particle size on physical and chemical properties of coconut coir dust as container medium. *Commun. Soil Sci. Plant Anal.* 34:593–605. https://doi.org/10.1081/CSS-120017842

Paridah, M.T., Basher, A.B., SaifulAzry, S. & Ahmed, Z. (2011). Retting process of some bast plant fibres and its effect on fibre quality: A review. *BioResources*, 6, 5260–5281. https://ojs.cnr.ncsu.edu/index.php/BioRes/article/view/BioRes_06_4_5260_MdTahir_BSA_Retting_Bast_Fibers_Review

Postma, J., Os, E. V. & Bonants, P. J. M. (2008). Pathogen detection and management strategies in soilless plant growing systems.

Poveda, J., Martínez-Gómez, A., Fenoll, C. & Escobar, C. (2021). The Use of Biochar for Plant Pathogen Control. *Phytopathology®* 111, nr 9: 1490–99. https://doi.org/10.1094/PHYTO-06-20-0248-RVW.

Puustjarvi, V. & Robertson, R. A. (1975). Physical and chemical 12. properties. p 23–38. *Peat in Horticulture. Academic Press, London*.

Quintero, M.F., Ortega, D., Valenzuela, J.L. & Guzman, M. (2013). Variation of hydro-physical properties of burnt rice husk used for carnation crops: Improvement of fertigation criteria. *Sci. Hortic*, 154, 82–87. https://doi.org/10.1016/j.scienta.2013.02.019

Ranalli, P. (1999). Agronomical and Physiological Advances in Hemp Crops. In: Ranalli, P. (Ed.) *Advances in Hemp Research*. pp. 61-84. New York, USA: Food Products Press.

Raviv, M. & Lieth, J.H. (red.) (2008). *Soilless culture theory and practice*. [E-book]. Elsevier.

Raviv, M., Lieth, J.H. & Bar-Tal, A. (red.) (2019). *Soilless culture: theory and practice*. (Second edition.) London: Academic Press, an imprint of Elsevier

Robinson, B., Winans, K., Kendall, J. Dlott, F. & Dlott, J. (2019). A life cycle assessment of ***Agaricus bisporus*** mushroom production in the USA. *Int. J. Life Cycle Assess*., 24 (2019), pp. 456-467. https://doi.org/10.1007/s11367-018-1456-6

Rousk, J., Brookes, P. C. & Bååth, E. (2009). Contrasting Soil pH Effects on Fungal and Bacterial Growth Suggest Functional Redundancy in Carbon Mineralization. *Applied and Environmental Microbiology* 75, nr 6: 1589–96. https://doi.org/10.1128/AEM.02775-08.

Sánchez, C. (2009). Lignocellulosic residues: biodegradation and bioconversion by fungi. *Biotechnology advances*, *27*(2), 185-194. https://doi.org/10.1016/j.biotechadv.2008.11.001

Somtrakoon, K. & Chouychai, W. (2013). Phytotoxicity of single and combined polycyclic aromatic hydrocarbons toward economic crops. *Russ. J. Plant Physiol*. 60, 139–148. [Google Scholar]

Statistics Sweden. (2017). *Skörd av Odlingstorv 1982–2016* [online] Available: https://www.scb.se/hitta-statistik/statistik-efter-amne/miljo/markanvandning/torv-produktion-anvandning-och-miljoeffekter-torv/pong/statistiknyhet/torv-2016--produktion-anvandning-och-miljoeffekter/ [2022-04-05]

Statistics Sweden. (2021). *Peat 2020 - Production, use and environmental impact.* [online] Available: https://www.scb.se/en/finding-statistics/statistics-by-subject-area/environment/land-use/peat-production-use-environmental-impact/ [2022-04-09]

Swedish Board of Agriculture. (2021). *Hållbara livsmedelssystem – Definition, pågående initiativ och förslag på åtgärder.* Rapport 2021:3. Available: https://webbutiken.jordbruksverket.se/sv/artiklar/ra213.html [2022-04-22]

Swedish Board of Agriculture. (2022). *Odla hampa.* [online] Available: https://jordbruksverket.se/stod/lantbruk-skogsbruk-och-tradgard/jordbruksmark/gardsstod-och-stodratter/gardsstod/odla-hampa. [2022-05-04]

Swedish Environmental Protection Agency. (N.D.). *Torvtäkter*. [online] Available: https://www.naturvardsverket.se/vagledning-och-stod/branscher-och-verksamheter/takter/torvtakter/ [2022-04-29]

Swedish Environmental Protection Agency. (2016). *Torvutvinningens och torvanvändningens klimat- och miljöpåverkan* [online] Available: http://www.naturvardsverket.se/upload/miljoarbete-i-samhallet/miljoarbete-i-sverige/regeringsuppdrag/2016/redovisade/ru-torv-skrivelse-slutversion-rattad-2016-06-29.pdf

Treat, C. C., Kleinen, T., Broothaerts, N., Dalton, A. S., Dommain, R., Douglas, T. A., Drexler, J. Z., et al. (2019). Widespread global peatland establishment and persistence over the last 130,000 y. *Proceedings of the National Academy of Sciences* 116, nr 11: 4822–27. https://doi.org/10.1073/pnas.1813305116.

Vandecasteele, B., Boogaerts, C. & Vandaele, E. (2016). Combining woody biomass for combustion with green waste composting: Effect of removal of woody biomass on compost quality. Waste Management, Volume 58. https://doi.org/10.1016/j.wasman.2016.09.012.

van der Werf, H. (1994). Crop physiology of fibre hemp (Cannabis sativa L.). Dissertation. Wacheningen, The Netherlands: Wageningen University.

van der Werf, H. (2004). Life cycle analysis of field production of fibre hemp, the effect of production practices on environmental impacts. *Euphytica* 140(1), 13-23. https://doi.org/10.1007/s10681-004-4750-2

Vaughn, S. F., Kenar, J. A., Thompson, A. R. & Peterson, S. C. (2013). Comparison of Biochars Derived from Wood Pellets and Pelletized Wheat Straw as Replacements for Peat in Potting Substrates. *Industrial Crops and Products* 51: 437–43. https://doi.org/10.1016/j.indcrop.2013.10.010.

Vaughn, S.F., Eller, F.J., Evangelista, R.L., Moser, B.R., Lee, E., Wagner, R.E. & Peterson, S.C. (2015). Evaluation of biochar-anaerobic potato digestate mixtures as renewable components of horticultural potting media. *Ind. Crop. Prod.* 2015, 65, 467–471. https://doi.org/10.1016/j.indcrop.2014.10.040

Wang, C., Luo, D., Zhang, X., Huang, R., Cao, Y., Liu, G., Zhang, Y. & Wang, H. Biochar-Based Slow-Release of Fertilizers for Sustainable Agriculture: A Mini Review" *Environmental Science and Ecotechnology* 10 (01 april 2022): 100167. https://doi.org/10.1016/j.ese.2022.100167.

Zulfiqar, F., Allaire, S. E., Aisha Akram, N., Méndez, A., Younis, A., Masood Peerzada, A., Shaukat, N. & Wright, S. R. (2019). Challenges in organic component selection and biochar as an opportunity in potting substrates: a review. *Journal of Plant Nutrition* 42, nr 11–12: 1386–1401. https://doi.org/10.1080/01904167.2019.1617310

Yu, Z. (2011). Holocene carbon flux histories of the world's peatlands: Global carbon-cycle implications. *The Holocene*. 2011;21(5):761-774. doi:10.1177/0959683610386982

Yeager, T., Gilliam, C., Bilderback, T., Fare, D., Niemiera, A. & Tilt, K. (1997). Best management practices: Guide for producing container-grown plants. S. Nursery Assn., Marietta, Ga

Popular science summary

To secure the supply of food in the coming years, the crop production industry is undergoing improvement work which focuses on the environmentally harmful effects of cultivation. One of these is to reduce the use of the most widely used substrate in pot and container cultivation, peat.

This work aimed to explore the possibilities in using renewable materials in greenhouse pot cultivation. Peat was proportionally exchanged to biochar and hemp and to further reduce the content of peat, compost, horse manure and clay were also added. The experiment was conducted in a greenhouse on plants of lettuce. Additionally, physical and chemical properties were investigated, and a screening of microbial presence was also conducted. All these parameters are to some extent connected and will affect the properties of the substrate and therefore also affect growth of plants.

It was found that hemp and biochar together was not suitable as a substrate in the format used in this experiment. Growth was poor and a high presence of microbiota probably meant that the nitrogen was not available to the plant. Peat and biochar together performed best at a 31,25% ratio of added biochar. However, when compared to a commercially available peat based substrate there were differences in growth and physicochemical properties that can be limiting for growth and thus, do not apply as a substrate for production of food crops where yield is of importance.

According to studies, 3 million m^3 of peat is extracted in Sweden alone annually and used in cultivation but also for other purposes. By allowing parts of peat in pot cultivation to be replaced with more sustainable materials, the hope is to reduce the use of peat in horticulture. Biochar is a material with increasing interest and which has already achieved several benefits in agriculture. Biochar can be created from almost anything and above all you can use things that would not otherwise be used. However, the properties of biochar are affected partly depending on what is used as a feedstock but also what the actual production technology looks like. Hemp has been cultivated for a long time for different purposes. In Sweden, only varieties of industrial hemp are allowed after approval from the agricultural board. The stems

from seed hemp have no specific end product but has been explored as a substrate in hydroponic growing systems. The step to introduce these fibers into container cultivation therefore seemed reasonable.

Continuing to explore new, alternative substrates will remain to be important. In addition, being able to evaluate the properties of the substrates and how these affect the development of different plants can provide answers to how they can best be used. By extensively exploring substrates with several constituents and with knowledge-based applications rewarding their beneficial properties while minimizing their limitations, one can potentially achieve suitable products. In addition to this, there is also a need to explore more of the microbiome within container substrates and how it can be utilized in order to sustain healthy and high yielding plants.

Acknowledgements

I would like to thank my supervisor, Håkan Asp, for guidance and valuable insights throughout this work. Also, I am thankful for the guidance and thoughts from Anna Karin Rosberg regarding the microbiological assessment. Acknowledgement should also be aimed to EcoTopic for the opportunity to be a part of this project.

Finally, I would like to thank my family for supporting me during my studies. And to my biggest supporters, Moa and Malte - thank you for never ceasing to be inquisitive, never stop exploring!

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file. If you are more than one author, the checked box will be applied to all authors. Read about SLU's publishing agreement here:

- https://www.slu.se/en/subweb/library/publish-and-analyse/register-and-publish/agreement-for-publishing/.

☒ YES, I/we hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

☐ NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.