Biochar and hemp as peat substitutes in organic growing media

– *effects on nutrient availability and nutrient uptake*

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Preface and acknowledgements

‘Rest till Bäst’ is a Vinnova-funded project that was initiated in 2017, with an overall aim to convert different organic residuals into biochar and to develop commercial value for the product (Rest till Bäst 2022). This study is based on a sub-project within the framework of ‘Rest till Bäst’ and is conducted in collaboration with project-partner EcoTopic AB - a consultant company specializing in biochar.

First of all I would like to thank EcoTopic for a rewarding collaboration in this study. I would also like to thank SLU Partnerskap Alnarp for contributing with funding to conduct nutrient analyses, which made it possible to gain deeper insight and understanding of the studied field. Lastly, I want to especially thank my supervisor Helene Larsson Jönsson for her valuable knowledge and support along the way and Jan-Eric Englund for his kind and patient help with the statistical analyses.

Abstract

This study aimed at evaluating the suitability of biochar and hemp as partial or complete replacement of peat in horticultural growing media, with specific emphasis on the effects on nutrient availability and plant nutrient uptake. The study was motivated by the environmental concerns surrounding the use of peat, along with a desire to explore possible uses for biochar and a crop residue from hemp cultivation. In total, 13 growing media treatments were evaluated, comprising of six peat/biochar-treatments, six hemp/biochar treatments (biochar rates 0 – 31.25 % v/v) and one control treatment (a commercial peat-based growing media).

A greenhouse pot experiment was set up to assess the effect on plant growth of lettuce, *Lactuca sativa* L. Prior to cultivation, important physicochemical properties of the media were determined; dry bulk density, water-holding capacity, total porosity, air-filled porosity, pH and electrical conductivity. In order to study the impact on nutrient availability and plant nutrient uptake, growing media samples were analyzed for readily available plant nutrients, both before and after lettuce cultivation. Additionally, the harvested lettuce was analyzed for its nutrient content. Overall, the physicochemical measurements showed more suitable properties in the control treatment and the peat-based growing media, compared to the hemp-based growing media. However, all treatments except the control had a slightly to highly alkaline pH, which was unfavorable for plant growth. Regarding lettuce plant performance, both the peat-based growing media and the hemp-based growing media performed much below a satisfactory level. Severely impaired plant growth could be observed in all treatments except the control but particularly in the hemp-based treatments. The negative plant response was associated with an overall N deficiency and/or reduced N availability in the growing media with increasing biochar rates. This was most likely caused by N volatilization, due to the alkaline pH of the growing media.

Keywords: Growing media · Biochar · Hemp · Peat substitution · Nutrient availability · Nutrient uptake

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Abbreviations

AEC: Anion exchange capacity

BD: Bulk density

CEC: Cation exchange capacity

C/N: Carbon nitrogen ratio

EC: Electrical conductivity

GM: Growing media

H/C: Hydrogen carbon ratio

SSA: Specific surface area

VOC: Volatile organic compounds

WHC: Water-holding capacity

1. Introduction

1.1. Background

Peat (*Sphagnum* peat) is the most widely used component in growing media, accounting for approximately 80 % of all growing media used annually within the horticultural sector in Europe (Prasad et al. 2019). The predominance of peat is attributed to its many beneficial qualities for growing media use. The high water-holding capacity (WHC) and high cation exchange capacity (CEC) of peat is particularly important since container-grown plants rely on a very limited volume of growing media to obtain water and nutrients. Moreover, peat is naturally free from toxins, pathogens, weed seeds and other unwanted elements, which is a fundamental requirement in growing media. From a manufacturing perspective, peat has the benefit of being a fairly homogeneous material that is available in large quantities, to a comparatively low price (Schmilewski, 2008). Based on the excellent growing conditions and the economical aspects of peat, it could be regarded as an ideal material for growing media. However, there are adverse environmental and sustainability aspects associated with peat – aspects that are becoming increasingly important today – which makes the use of peat controversial. Peat is derived from peatlands, also referred to as peatbogs, which are wetlands found in all continents of the world, covering a total of 3 % of the world's land area (Kern et al. 2017). The water-saturated and oxygen-poor conditions created in peatbogs leads to incomplete decomposition of plant material, which is thus slowly accumulated and is capable of storing vast amounts of carbon. Peatbogs have been estimated to account for approximately 30 % of all soil-sequestered carbon globally (Parish et al. 2008). However, with peat extraction, the plant-captured carbon is released back into the atmosphere, contributing to greenhouse gas emissions. In addition, peatbogs represent important ecosystems, recognized for their great biodiversity, which are endangered by an excessive peat extraction. Due to the extremely long regeneration time of peat, it is not considered a renewable resource (Parish et al. 2008).

With rising public awareness, pressure is put on political decision-making and several directives and regulations have been undertaken to conserve and restore peatlands (Joosten et al. 2012). In the UK, one of the major consumers of horticultural peat, a governmental action plan has recently been set to phase out peat by 2030 (HM Government 2018). In Sweden, there are currently no restrictions around the use of peat for horticultural purpose but this may change in a near future (Jordbruksverket 2016; Sveriges Riksdag 2021). Hence, there is a clear incentive to search for sustainable materials that can substitute peat in growing media, either entirely or partly, without compromising on quality.

1.1.1. Growing media qualities

In the search for alternative materials, a good starting point is to define what qualities are desirable in a growing medium. According to Landis et al. (2009), an ideal growing medium should consist of an adequate proportion of macro - and micropores, which is obtained by incorporating a mixture of coarse and fine particles. Fine particles contribute mostly with small pores (micropores), capable of retaining water, while coarse particles create medium to large-sized pores (meso - and macropores) that are vital for the oxygen supply to the plant’s roots as well as the dispersal of carbon dioxide. Additionally, macropores enables drainage of excess water, which is important to reduce the risk of water saturation and the development of anoxic conditions in the bottom of the container (Landis et al. 2009).

Low bulk density is generally a preferable trait in growing media, as it facilitates transportation and handling of the medium, as well as reducing the risk of compaction (Agarwal et al. 2021).

The initial pH of the growing media should be between 5.3 and 6.5, as the majority of plant species show optimal growth within this range (Nobile et al. 2020). pH-values up to 7.0 are still considered acceptable for most crops though (Silber and Bar-Tal, 2008). The electrical conductivity (EC) in growing media is recommended to be ≤ 0.5 dS m^{-1} (Nobile et al. 2020).

The specific surface area (SSA) is a crucial parameter in growing media, as it provides sites for chemical reactions and nutrient retention to occur. A high specific surface area enables high cation exchange capacity (CEC), which is essential for the retention of nutrients. Organic components commonly used in growing media, such as peat and compost, display good cation exchange properties.

Concerning growing media fertility, there has long been a preference within the horticultural sector to use materials with low nutrient content, since it allows for nutrient adjustments with soluble mineral fertilizers, according to the specific crop requirements (Steiner and Harttung 2014). In this context, it is relevant to highlight that there are substantial differences in growing media intended for organic production, compared to conventional growing media; mineral fertilizers are not allowed in organic cultivation and therefore plant nutrients must be provided in an organic form, e.g. from manure or compost. Because organic nutrients are not easily dissolved in water, the possibilities of tailoring plant nutrient levels via soluble fertilizers are limited. For this reason, legislation demands that growing media for organic production must contain the majority of plant nutrients from the start, to assure a sufficient nutrient supply throughout the major part of the growing season (KRAV, 2021).

A wide range of organic materials have been tested as alternatives to peat in growing media. Some of the most commonly evaluated materials in the literature are green waste compost, coir husk fiber, wood fibers and animal manure compost. Limitations that are often encountered with the mentioned materials are high salinity, high pH, insufficient porosity and low stability (Carlile et al. 2015; Vandecasteele et al. 2017). In this study, biochar and hemp will be evaluated for their potential use in growing media.

1.1.2 Biochar

Biochar is a carbon-rich product that is formed under pyrolysis of different organic materials. Pyrolysis refers to the process of thermal degradation of biomass in an oxygen-restricted environment, in which gas and bio-oils are produced in the first hand, and biochar is generated as a solid residue (Lehmann and Joseph, 2009).

The interest in biochar has escalated in recent years, much owing to its well-documented capacity to sequester atmospheric carbon into soil (Lehmann and Joseph, 2009). The carbon sequestration capacity is explained by the exceptionally stabile structure of biochar, which is a result of the pyrolysis process in which aliphatic C is transformed into aromatic C (Tomczyk et al 2020). The aromatic compounds have a recalcitrant structure that makes biochar highly resistant to microbial decay. Unlike plants, which release their carbon content back into the atmosphere once they decompose, the turnover time of biochar has been stated to range all from hundreds to thousands of years, depending on the quality of the biochar and the environmental conditions (Lehmann and Joseph, 2009). Consequently, biochar has been recognized as a carbon sink and as a potential climate mitigation tool (Nemati et al. 2015). It's important to note that this effect requires that the biochar production rate does not exceed the rate at which plants are grown (Lehmann and Joseph, 2009). The longevity of biochar in soils is often exemplified by the highly fertile Amazonian Dark Soils, commonly referred to as '*Terra Preta'*. In these archeological soils that date back more than thousand years, biochar (char) can still be detected and is identified as the reason for the maintained soil fertility (Lehmann and Joseph, 2009).

The observed capacity of biochar to improve soil fertility on a long-term basis has attracted great attention and has been the subject of diverse studies. The nutrient retention capacity is related to the extremely porous structure of biochar, which creates a large surface area that generally comprise a high amount of organic functional groups, such as carboxyl and hydroxyl groups (Tomczyk et al. 2020). These functional groups have different surface charge, which contributes to the adsorption of both cations and anions. For example, studies have demonstrated the ability of biochar to adsorb both NH_4^+ and NO_3^- (Beusch et al. 2019; Yuan et al. 2016). However, the cation exchange capacity (CEC) for biochar is usually more prominent than the anion exchange capacity (AEC). In addition, the porous nature of biochar enables a good water-holding capacity, which is associated with improved nutrient retention (Razzaghi et al. 2020). Thus, biochar has successfully been used to reduce nutrient loss from leaching and gaseous emissions (Taghizadeh-Toosi et al. 2012; Steiner 2010; Mandal et al. 2016).

1.1.3 Biochar in growing media

Up until today, biochar has mainly been used as soil amendment, although other functions have been explored e.g. for soil remediation and water filtration (Lehmann and Joseph, 2009). Only more recently has biochar been evaluated as a component in growing media (Prasad et al. 2019; Nobile et al. 2020). The use of biochar for this purpose is currently limited but has attracted increased research attention. Based on the biochar description provided above, it is evident that biochar and peat share many characteristics and that biochar display qualities that theoretically would function well in growing media. A biochar feature that may be disadvantageous in growing media is the highly alkaline pH (generally ranging from 7.1 to 10.5), which is above the optimal range for plant growth (Chrysargyris et al. 2019). However, in combination with the pH-acidic peat, biochar has been suggested as a potential substitute for liming agents (Steiner and Harttung 2014).

Numerous studies have reported positive plan responses from biochar incorporation in growing media (Messiga et al. 2022; Sabatino et al. 2020; Mendez et al. 2017; Graber et al. 2010). However, other studies have observed negative or neutral plant responses from biochar addition (Huang et al. 2020; Liu et al. 2019). Due to a lack of biochar standardization and often times insufficiently reported research methodology, these results can be difficult to compare (Razzaghi et al. 2020). However, based on the vast body of literature on biochar, it is clear that biochars can have hugely varying properties, which in turn determines their function and potential use.

According to recent literature, biochar characteristics are primarily determined upon the nature of the biochar feedstock. A wide range of organic materials can be used as biochar feedstock, such as wood, manure and compost (Hossain et al. 2020). Wood-derived biochar (characterized by a high lignin content) generally produce a biochar with a more stable structure (i.e. a higher fraction of recalcitrant carbon) and low ash content, whereas biochar produced from mineral-rich feedstock, such as sewage sludge or animal manure, result in a biochar with higher ash content and a higher proportion of labile carbon (Mukome et al. 2013; Singh and Cowie, 2010; Zielinska et al. 2015). High ash content is correlated with elevated levels of P and K. Thus, biochars produced from mineral-rich feedstock could be a potential nutrient source, whereas wood-derived biochars tend to contribute only with modest amounts of nutrients, especially with respect to the N content (Hossain et al. 2020). Important to note is that high ash content can imply the presence of phytotoxic compounds, such as phenols, that may inhibit root development in plants (Rathnayake et al. 2021; Tomczyk et al. 2020). High ash biochars have also been associated with increased hydrophobicity (Mukome et al. 2013). Although some biochars display high nutrient content, a large proportion of the nutrients are often organically bound, and subsequently not directly available to plants. For example, biochars derived from mineral-rich sources can have considerably high N content but have been shown to release only negligible N when applied in soils. Likewise, a large proportion of the S in biochar is usually organically bound while P and K availability show large variations (Chan and Xu, 2009).

Apart from the feedstock material, biochar production conditions have also been shown to greatly influence the final biochar features. This explains why biochars produced from the same feedstock can display largely different properties when produced under different pyrolysis temperatures. Particularly the pyrolysis temperature, but also heating time and rate, have been shown to largely impact chemical and physical features of the biochar. Higher pyrolysis temperatures are correlated with a reduced CEC, due to a loss of functional groups (Silber et al. 2010). Additionally, higher pyrolysis temperatures tend to increase biochar pH and EC (Sabatino et al. 2020; Tomczyk et al. 2020; Nguyen et al. 2017).

Due to the apparent complexity of biochar, characterization of biochar features is recommended prior to growing media application. Although no guidelines have yet been established, some research suggests that wood-derived biochar is more suitable for growing media use because of its highly stabile structure and low ash content (Steiner and Harttung 2014; Prasad et al. 2019).

1.1.4 Biochar and nutrient dynamics in growing media

Biochar is typically not added for direct fertilization but rather as a means of altering the physical and chemical properties of the growing media. Nevertheless, alterations in these properties may have indirect effects on the fertility of the growing medium. Regarding the physical structure, the porous nature of biochar can improve the porosity of the growing medium - an aspect that is crucial for the availability of oxygen and water and thus for the plant's nutrient supply. Biochars have also been shown to provide an important habitat for symbiotic microorganisms, which may promote nutrient cycling (e.g. mineralization, nitrification, immobilization) in the growing medium. Additionally, the large specific surface area of biochar provides sites for chemical reactions to occur, which enables an increased release of available nutrients (Chrysargyris et al. 2020).

Several studies have reported increased nutrient use efficiency (NUE) in growing media as a response to biochar addition, primarily associated with the adsorption-capacity of biochar and its stimulation on microbial activity (Cao et al. 2019; Messiga et al. 2022; Kaudal et al. 2018). However, the specific mechanisms involved have not been fully covered in research (Silber et al. 2010).

Although the adsorption capacity of biochar is generally regarded as a positive trait, when applied in growing media it may temporarily reduce plant nutrient availability with the risk of reducing plant productivity (Modin 2021). Additionally, nitrogen immobilization has been reported as a short-term effect from biochar addition (Lehmann and Joseph, 2009). On this basis, provision of supplemental N is a recommended practice together with biochar application, in order to reduce the risk of N shortage. Pre-charging of biochar with nutrients is also a means of enabling a slow release of nutrients. For this to be efficient, it requires good CEC or AEC properties in the biochar (Modin 2021)

Lastly, biochar addition in growing media is associated with pH alterations, which is well known to influence nutrient mobility and availability (Chrystargyris et al. 2019).

It is hereby evident that biochar can affect nutrient dynamics in several ways, which may imply both advantages and obstacles when applying biochar to growing media. In order to justify the use of biochar in growing media, further knowledge is required on important nutrient aspects, such as the effect on nutrient availability and plant nutrient uptake.

Because of the high variability in biochar properties, an optimal range for biochar incorporation has not been established but should rather be determined based on biochar characteristics and on the specific growing conditions (Sabatino et al. 2020; Graber et al. 2010). However, many studies have reported negative plant responses at higher biochar rates in growing media, suggesting that biochar can only partly substitute peat (Huang et al. 2020; Zulfiqar et al. 2021; Rathnayake et al. 2021). In this study, biochar rates from 6.25 to 31.25 % (v/v) will we evaluated. With the aim of entirely substituting peat, this study will evaluate the combination of biochar and hemp in growing media.

1.1.5 Hemp

The cultivation of hemp (*Cannabis sativa* L) includes different hemp varieties that are used either for fiber production or for food consumption. In the cultivation of oilseed hemp for the food industry, the hemp seeds from the upper parts of the plant are used while the remaining part of the hemp stalk is not harvested. This generates an agricultural residue that currently has no use. One previous initiative has been made to identify uses for this hemp residue, using the fibers for textile production (Kronberg et al. 2021). To my knowledge, there have been no published studies concerning the use of unprocessed hemp in growing media. One study investigated the effect of using synthetically processed hemp fibers as a hydroponic growing medium, in which hemp showed suitable physicochemical properties for growing media and a positive plant response for Daikon radish was reported (Both et al. 2021). Dresbøll and Magid (2006) studied the physical structure and chemical composition of hemp and subsequently its microbial decomposition in compost. The authors concluded that the structural quality of hemp was superior to two other studied straw materials, in term of its stability in compost. Hence, composted hemp was suggested as a suitable material for growing media. In this study, unprocessed hemp that has only been subject to natural retting will be evaluated for its use in growing media. Retting refers to the biological process in which enzymatic degradation of lignin and pectin causes the hemp fibers to release from the woody core of the stem (Thomsen et al. 2005).

2. Aim and objectives

The overall aim of this study is to evaluate the suitability of biochar and hemp as substitutes for peat in horticultural growing media. The main objectives are to examine the effect of biochar and hemp on the physical and chemical properties of growing media and to investigate how these properties affect nutrient availability and ultimately, the plant performance of lettuce.

2.2. Research questions

- How do biochar and hemp affect the physicochemical properties of the growing media?
- How do biochar and hemp affect nutrient availability and plant nutrient uptake in the growing media?
- How do biochar and hemp affect the plant growth of lettuce?

3. Materials and methods

3.1. Greenhouse pot experiment

A greenhouse pot experiment was carried out from 4 of March to 20 of April in Alnarp, Sweden. Greenhouse temperature was maintained at 22± 2°C during the day and 18 ± 0.5 °C at night. The experiment was set up as a randomized complete block design with a total of 13 treatments, each replicated four times. Six of the treatments were peat-based, with biochar rates from 0 to 31.25 %. Six of the treatments were completely peat-free, with hemp as bulk material and biochar rates from 0 to 31.25 %. All the treatments contained equal amounts of composted manure (25 % (v/v), green waste compost (12,5 % (v/v) and clay (6,25 % (v/v). No chemical fertilizers were added to the growing media.

A commercial peat-based growing media ('Solmull växttorv') was used for control treatment, fertilized with dolomite meal and mineral nutrients (1.2 kg NPK and micronutrients (Hasselfors Garden, Sweden) [1]. In total, the experiment comprised of 52 pots. The formulation and composition of the growing media-treatments are presented in Table 1. A description of the individual growing media components will be presented in the next section.

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[1] The control treatment used in this study was included for comparison of a growing media market standard. Treatment PB0 and HB0 function as additional control treatments, as they contain 0% biochar.

Table 1. Formulation and composition (volume) of the different growing media-treatments. PB= Peat Biochar; HB = Hemp Biochar

Treatments	**Peat**	**Biochar**	**Manure**	**Compost**	**Clay**
PB0	56.25 %	0 %	25 %	12.5 %	6.25 %
PB1	50 %	6.25 %	25 %	12.5 %	6.25 %
PB2	43.75%	12.5 %	25 %	12.5 %	6.25 %
PB3	37.5 %	18.75 %	25 %	12.5 %	6.25 %
PB4	31.25 %	25 %	25 %	12.5 %	6.25 %
PB5	25 %	31.25 %	25 %	12.5 %	6.25 %
	Hemp				
HB0	56.25 %	0%	25 %	12.5 %	6.25 %
HB1	50 %	6.25 %	25 %	12.5 %	6.25 %
HB2	43.75 %	12.5 %	25 %	12.5 %	6.25 %
HB3	37.5 %	18.75 %	25 %	12.5 %	6.25 %
HB4	31.25 %	25 %	25 %	12.5 %	6.25 %
HB5	25 %	31.25 %	25 %	12.5 %	6.25 %

Lettuce seeds (*Lactuca sativa* L., cv. 'Cencibel') were sown individually in plug trays using a commercial seed substrate (Hasselfors såjord, Sweden). Two-week-old seedlings were transplanted separately into 1 L pots containing 1 L of growing medium and were placed on saucers. From the date of transplanting, the plants grew for an additional five weeks.

Throughout the experiment, the pots were top-irrigated manually and the moisture content was maintained at 60 % of the WHC of the growing medium. This was obtained by weighing the pots every second day and adjusting the moisture content accordingly.

The lettuce was harvested five weeks after transplanting the plants. The roots were first washed thoroughly to eliminate growing media particles and absorbent paper was used to absorb moisture from the roots, in order to determine the fresh weight of the roots. After measuring the fresh weight, the roots and shoots were dried at 60 °C for 60h to determine the dry weight. Following, the dry shoot samples were analyzed for their nutrient concentration by a commercial laboratory (LMI AB, Helsingborg, Sweden). The Dumas method was used to determine the N content, and ICP-OES (Inductively coupled plasma - optical emission spectrometry) for the other nutrient elements. The nutrient uptake for each nutrient was calculated by multiplying the nutrient concentration by plant shoot dry weight.

3.2. Growing media components

Biochar

A commercial, wood-derived biochar was used (Carbuna CPK, Germany). The biochar had been produced under a maximum pyrolysis temperature of 540 °C and was EBC certified [2] (EBC 2012-2022). Important features and nutritional information of the biochar are listed in Table 2 and 3, according to information provided by the manufacturer. The biochar was first ground and sieved to a particle size less than 0.5 mm. One week prior to preparing the growing media mixtures, the biochar was pretreated with organic blood meal (Nelson Garden®, Sweden) containing 13 % nitrogen (1.9 g blood meal per 1 L biochar, dissolved in 400 ml water).

Table 2. Main properties of the biochar, according to information provided by manufacturer (analysis conducted by 'Eurofins, Bobritzsch-Hilbersdor')

pH *	**VOC**	**H/C**	**Ash content**	**Dry BD** < 3 mm	**SSA**
8.6	9.6 %	0.33	2.1 %	339 kg/m³	417.2 m²/g

* measured in $CaCl_2$

Table 3. Biochar nutrient concentration (*g/kg*) according to information provided by manufacturer (analysis conducted by 'Eurofins, Bobritzsch-Hilbersdor')

N	**P**	**K**	**S**	**Mg**	**Ca**	**B**	**Mn**	**Fe**	**Na**	**Si**
4.7	1.1	3.1	0.5	1.1	6.7	0.012	0.227	0.3	0.1	2.4

Hemp

Locally produced hemp of the oilseed variety 'Finola' was purchased from 'Svensk Hampaindustri'. The hemp material was derived from hemp that had been cultivated in 2019. After harvest, the residual stems were left in the field

[2] EBC stands for European Biochar Certificate – a voluntary industry standard in Europe

for approximately six months, allowing natural retting to occur. Afterward, the stems were cut and stored in bales to later be milled in a hammer mill, with the purpose of entirely separating the hemp fibers from the other components. This could not be fully achieved and hence the hemp material used in this study contained a mix of cellulosic fibers and lignin/hemicellulose-rich parts (see image 1). The hemp material was not subject to any other processing [3]. Nutritional properties of the hemp are presented in Table 4.

Table 4. Hemp nutrient concentration (*mg/kg*), according to analysis conducted by LMI AB, Helsingborg, Sweden. The Dumas method was used to determine the N content and ICP-OES analysis for the remaining nutrient elements.

Total N	P	K	Mg	S	Ca	B	Fe	Mn	Mo	Zn	Si	Cu	Na	Al
6960	704	1260	365	607	3810	6.3	127	94	<0.2	42	42	5.2	321	76

Figure 1. Unprocessed hemp used as bulk material in treatment HB0-HB5 (Photo Jacqueline Hellman)

Peat

A pure sphagnum peat (H2-H4 decomposition degree) with a pH of 4.0 - 5.0 was used as bulk material in the PB-treatments ('Solmull naturtorv', Hasselfors Garden, Sweden). The total N content was 1100 mg/L, according to manufacturer.

[3] Information provided by Clara Norell, CEO Svensk Hampaindustri

Manure

Hygienized horse stable manure was purchased from 'Wiggeby Jordbruk' (Wiggeby Jordbruk AB). Nutritional properties are displayed in Table 5, which include both organically bound N and mineral N, according to the Kjeldahl & Dewardas method (Muñoz-Huerta et al. 2013) Approximately one third of the total N content (1.3 out of 4.27 kg/ton) of the manure was in a readily available ammonium form. The manure had a pH-value of 8.6.

Table 5. Stable manure nutrient concentration (*kg/ton*), according to analysis conducted by Eurofins Agro Testing Sweden AB, Kristianstad Sweden.

Total N	NH_4^+	P	K	Mg	S	Na
4.27	1.3	0.71	5.9	0.82	0.58	0.47

Green waste compost

The green waste compost used in this study was obtained from a local manufacturer (Swerock AB), consisting mainly of park residues. The compost had a pH of 8.5 and EC of 1.9 and was abundant in micronutrients and all macronutrients except N. Table 6 presents its content of available nutrients (Spurway analysis).

Table 6. Green waste compost nutrient concentration (*mg/L*), according to Spurway analysis performed by LMI AB, Helsingborg, Sweden. These values represent available nutrients at the time of the analysis and not the total nutrient content.

Total N	NH_4^+	NO_3-	P	K	Mg	S	Ca	B	Fe	Mn	Na	Al
11	11	1	29	720	160	30	1600	1.3	1.8	3.9	75	1.9

Clay

Clay granules were purchased from 'Bara Mineraler' (Bara Mineraler AB, Malmö, Sweden).

3.3. Growing media characterization

Prior to the pot experiment, main physicochemical properties of each growing medium were determined. All the measurements were performed in duplicates.

3.3.1 Physical properties

To determine the WHC, a cylinder of known volume with an extension ring was filled with growing media up to the edge. The cylinder was then placed in a container and during two days water was gradually added until the growing medium was fully saturated. Following, the cylinder was removed and left to drain for 48 hours, covered with foil. The extension ring was removed and surplus growing media discarded. The wet growing media was put in pre-weighted aluminum pans and weighted before drying in an oven at 105 °C for 24 hours, to determine the dry weight. The WHC was determined using the following formula:

$$WHC\ (\%) = \frac{Wet\ weight\ of\ GM - Dry\ weight\ of\ GM}{Lower\ ring\ cylinder\ volume} \times 100$$

The dry bulk density was measured according to European Standard method. A cylinder of known volume with a spacer ring was filled with growing media to the edge and then compacted with a weight for three minutes. The spacer ring was detached and surplus growing media was removed. To calculate the bulk density, the weight of the remaining growing media in the cylinder was divided by the volume of the cylinder.

The compact density is a measurement of the density of a substrate without its pores. The compact density was measured by filling up approximately half of a pre-weighted 50 mL flask with growing media, then weighing the flask to determine the weight of the substrate. To extract all the air from the pores, 25 mL of 95 % ethanol was added to the flask, which was then sealed with film and put on a shaker for 30 min. Following, the flask was filled with alcohol to the 50 mL mark, noting the volume used. By knowing the total volume of alcohol used, the volume occupied by the substrate could be determined. The compact density is calculated as g/dm^3. By knowing the total density of a substrate (i.e. bulk density) and the density without pores (i.e. compact density), the total porosity of the substrate could be determined. The total porosity of the growing medium was calculated using the following formula:

$$Porosity = (1 - bulk\ density/compact\ density)\ x\ 100$$

The air-filled porosity (macropores) was determined by subtracting the WHC % (micropores) from the total porosity.

3.3.2 Chemical properties

pH and electrical conductivity (EC) was determined according to European Standards (EN 13037:1999 and EN 13038:1999). Growing media was suspended in deionized water in a 1:5 ratio and then placed in an end-over-end shaker for 1 h. After this procedure, the pH and EC were measured using a pHTestr 10 (Eutech Instruments) pH meter and ECTestr 11 (Eutech Instruments) conductivity meter.

Growing media samples from each treatment were collected both before cultivation (18 of March) and after harvest (20 of April). The samples were analyzed for readily available plant nutrients (Spurway analysis, method described by Spurway and Lawton, (2009)) by a commercial laboratory (LMI AB, Helsingborg, Sweden).

The nutrient concentration for each growing medium, according to manufacturer’s information, was calculated in order to determine the theoretical nutrient input in each treatment. The nutrient concentrations are presented in Table 7.

Table 7. Calculated nutrient concentration in each growing medium (*mg/L*), based on the nutrient content of the different components (peat, hemp, biochar, manure and green waste compost). The nutrient input from peat (treatment PB0-PB5) only includes N. The nutrient input from the green waste compost is based on a Spurway analysis (i.e. available nutrients) and not the total nutrient content.

TREATMENT	**N**	**P**	**K**	**S**	**Mg**	**Na**
PB0	1189	90	804	86	119	66
PB1	1211	107	854	94	137	68
PB2	1233	125	903	102	154	69
PB3	1254	142	953	110	172	71
PB4	1276	160	1002	118	190	73
PB5	1298	178	1052	126	207	74
HB0	852	118	855	111	134	79
HB1	911	132	899	116	150	79
HB2	971	147	943	121	166	80
HB3	1030	161	987	126	182	80
HB4	1089	176	1031	132	198	80
HB5	1149	190	1075	137	214	80

3.4 Statistical analysis

All data results were processed in Minitab® 20 and analyzed using General linear model-ANOVA and One-way ANOVA with a confidence level of 95 %. Tukey tests were used for comparison of treatments and Pearson correlation test was used to identity possible correlations between different factors. Statistical tests were conducted for all 13 treatments but also for the individual treatment groups (PB and HB), in order to detect differences related to the biochar rate.

4. Results

4.1. Physiochemical properties

The initial physicochemical properties of the growing media (prior to lettuce cultivation) are listed in Table 8. Statistical differences among all treatments are also indicated. Statistical test results for the individual treatment - groups (PB and HB) are presented in the appendix.

Table 8. Initial physicochemical properties of the growing media-treatments. Treatments that do not share a latter are significantly different at *P* <0.05 significance level among the columns.

Treatment	Dry bulk density (g/cm^3)	Porosity (%)	Air-filled porosity (%)	WHC (%)	pH	EC (µS/cm)
Control	0.46 ^{e}	60,59 ^{b}	-	62 ^{a}	5,4 ^{f}	330 ^{bcd}
PB0	0.59 ^{a}	58,29 ^{b}	-	58 ^{a}	7 ^{e}	330 ^{bcd}
PB1	0.59 ^{a}	59,90 ^{b}	-	57 ^{a}	6.9 ^{e}	290 ^{d}
PB2	0.55 ^{b}	59,91 ^{b}	-	56 ^{a}	7.4 ^{d}	350 ^{abcd}
PB3	0.55 ^{b}	58,36 ^{b}	-	58 ^{a}	7.6 ^{d}	310 ^{cd}
PB4	0.57 ^{ab}	58,11 ^{b}	-	55 ^{a}	8.1 ^{abc}	355 ^{abc}
PB5	0.55 ^{b}	57,38 ^{b}	-	55 ^{a}	8.2 ^{abc}	325 ^{bcd}
HB0	0.41 ^{f}	69,04 ^{a}	34	35 ^{b}	7.95 ^{c}	345 ^{abcd}
HB1	0.48 ^{de}	64,27 ^{ab}	27	37 ^{b}	8 ^{bc}	395 ^{a}
HB2	0.47 ^{de}	62,45 ^{ab}	25	37 ^{b}	8.05 ^{abc}	340 ^{abcd}
HB3	0.49 ^{d}	61,70 ^{ab}	24	38 ^{b}	8.3 ^{ab}	355 ^{abc}
HB4	0.46 ^{e}	63,20 ^{ab}	22	41 ^{b}	8.35 ^{a}	350 ^{abcd}
HB5	0.52 ^{c}	60,48 ^{b}	19	41 ^{b}	8.3 ^{ab}	385 ^{ab}

The dry bulk densities were quite similar among the PB-treatments but were significantly higher compared to all HB-treatments and the control. When testing for statistical differences within the treatments-groups, the bulk density for HB0 was significantly lower than the other HB-treatments. An opposite effect was observed within the PB-treatments, where treatment PB0 was significantly higher than all treatments except PB1. However, this decrease was not further enhanced with increasing biochar rates.

The total porosity was similar among all treatments. The only treatment that deviated was treatment HB0 (0 % biochar), which was significantly higher than the control treatment and all PB-treatments. ANOVA test performed on the individual treatment-groups showed no significant differences within treatment-group PB ($P = 0.927$) whereas within treatment-group HB, treatment HB0 had a significantly higher porosity than the other HB-treatments ($P < 0.001$).

The WHC of the control treatment and all PB-treatments were significantly higher than all HB-treatments but there were no significant differences within the treatment-groups. ANOVA test performed on the individual treatment-groups showed no significant differences either ($P = 0.507$ respectively 0.188).

There were large variations in pH among the treatments. The control treatment had an acidic pH (5.4), treatment PB0 and PB1 had a neutral pH (pH 6.9 and 7) while all other treatments had alkaline pH, ranging from 7.4 to 8.35. As illustrated in Figure 2, there is a clear trend of increasing pH with increasing biochar rates.

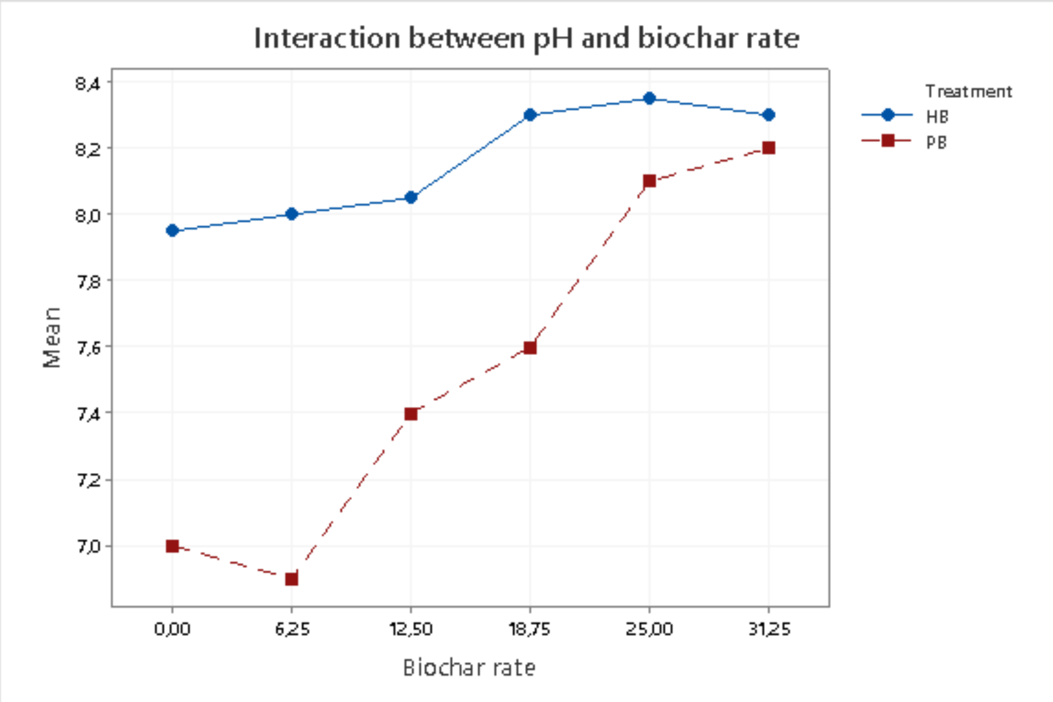

Figure 2. Interaction plot for pH and biochar rate (%) in both treatment-groups (PB and HB). Biochar rates from 0 to 31.25 %.

4.1.2. Growing media nutrient content before cultivation

Results from the initial Spurway analysis are presented in Table 9, displaying the total concentration of readily available nutrients in each growing medium. The most striking difference between the treatments is the N content, where N shortage is observed in all treatments except the control. In the PB-treatments, available N was significantly higher in treatment PB0 (0 % biochar) and a linear decrease in available N was observed with increasing biochar rates. A negative correlation between N content and biochar rates in the PB-group was confirmed with the Pearson correlation test (r-value 0.923 and P = 0.009 (figure 1)). No such correlation could be established in the HB-group. The P content was similar in the control treatment and PB-treatments whereas the HB-treatments with lower biochar rates (0 and 6.25 % particularly but also 12.5 and 18.75 %) showed higher P content. The control treatment had a substantially higher S and Mg content compared to all other treatments whereas it had a lower content of K, Ca and micronutrients (B, Mn and Fe). The HB-treatments were particularly rich in P, Ca and Mn compared to the other treatments. Some of the PB-treatments showed high Al content, compared to the other treatments. The Na content was similar in the PB –

treatments and HB-treatments but higher compared to the control treatment. The 0 % biochar treatments (PB0 and HB0) differed only slightly from the treatments containing biochar. PB0 had a higher S, Mg, B and Mn content, compared to all other PB-treatments. HB0 showed the highest P content of all treatments.

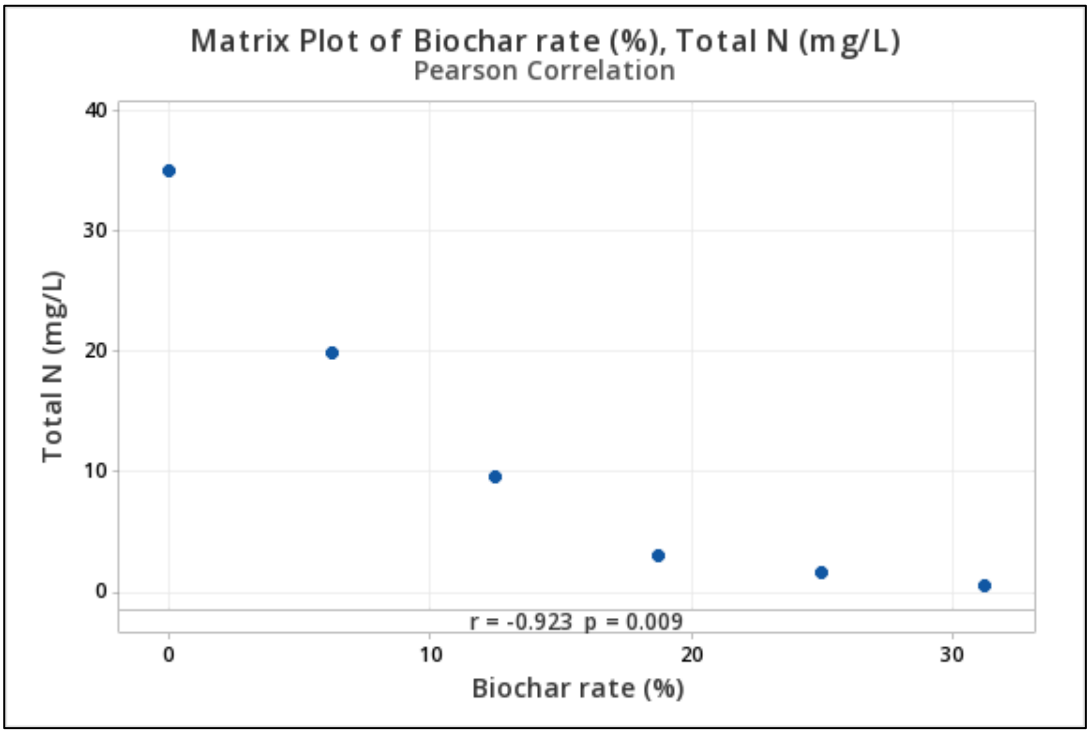

Figure 3. Correlation diagram relating total available N in the growing media – treatments (before lettuce cultivation) to biochar rate (%). Biochar rates from 0 to 31.25 % (treatment PB0 – PB5). A negative correlation was confirmed with the Pearson's correlation test (*P* = 0.009)

Table 9. Nutrient concentration in each growing medium (*mg/L*) before the experiment, based on a Spurway analysis. Analysis conducted by LMI AB, Helsingborg, Sweden

TREATMENT	N	NO_3^-	NH_4^+	P	K	S	Mg	Ca	B	Mn	Fe	Na	Al
Control	300	300	1	64	330	280	240	860	0.36	0.34	0.21	72	<1.0
PB0	35	34	1	56	580	15	110	650	0.48	4.9	1.1	110	1.8
PB1	20	19	1	45	490	10	87	600	0.5	3.5	1.9	98	2.8
PB2	9.6	8	1	62	650	8	96	650	0.64	2.8	1.4	99	2.1
PB3	3.1	1	1	50	590	7	96	680	0.79	1.8	1.2	110	1.7
PB4	1.8	1	1	48	640	6	78	570	0.76	1.7	2.4	100	3.8
PB5	0.63	1	1	42	570	6	77	710	0.78	2.2	2	83	2.8
HB0	0.94	1	1	110	580	5	100	970	0.72	5.7	0.64	96	1
HB1	1	1	1	100	630	5	110	1100	0.77	5.7	0.51	100	<1.0
HB2	1.1	1	1	84	600	6	94	1100	0.82	5.6	0.55	85	<1.0
HB3	1.3	1	1	84	750	6	100	1200	0.86	5.4	0.74	100	<1.0
HB4	0.67	1	1	61	690	6	93	1100	0.85	4.7	1.7	89	2.6
HB5	0.78	1	1	55	630	6	84	990	0.83	4.4	1.3	92	1.8

4.2. Growing media nutrient concentration after cultivation

Readily available nutrients in each growing medium at the end of the experiment are displayed in table 10. From this data it can be observed that the concentration of available K, S, Fe, Na and Al had increased in all treatments (except control) compared to the initial nutrient analysis. Treatment PB0, PB1 and PB2 showed the highest Al content, which were much higher compared to the initial Spurway analysis. Treatment PB0 and PB1 had the highest Fe content, which was not observed in the initial analysis. N content was almost depleted in all treatments, including the control (which showed the greatest decline in N, from 300 mg to 2 mg). A slight decrease in P could be observed in all treatments. The remaining nutrient elements were maintained at a consistent level. The control treatment showed a deviating pattern, with a substantial decrease in all macronutrients.

Table 10. Concentration of available nutrients in each growing medium (*mg/L*) after the experiment, based on a Spurway analysis. Samples for the analysis were collected from all growing media-replicates to obtain the average nutrient content in each treatment. Analysis conducted by LMI AB, Helsingborg, Sweden.

TREATMENT	N	NO_3^-	NH_4^+	P	K	S	Mg	Ca	B	Mn	Fe	Na	Al
Control	2.0	<1	2	28	79	130	160	620	0.30	0.53	0.23	53	<1
PB0	2.3	1	2	40	680	18	100	500	0.44	0.84	6.0	130	8.9
PB1	1.1	1	1	35	710	13	100	590	0.59	0.83	8.1	120	13
PB2	0.19	1	1	37	690	12	89	560	0.67	0.93	1	110	11
PB3	0.37	1	1	44	790	14	96	660	0.76	1.2	3.1	120	4.5
PB4	0.17	1	1	73	740	40	100	660	0.78	1.2	2.6	120	3.5
PB5	0.17	1	1	42	800	19	87	710	0.90	1.3	2.4	120	3.2
HB0	0.46	1	1	72	870	10	100	1100	0.71	3.1	2.3	120	3.2
HB1	0.64	1	1	80	750	11	110	1200	0.82	3.4	0.88	120	1.1
HB2	0.46	1	1	71	720	11	110	1100	0.85	3.1	1.1	110	1.4
HB3	0.65	1	1	66	750	14	95	1000	0.88	3.1	1.3	110	1.7
HB4	0.68	1	1	57	670	14	80	800	0.86	2.5	2.1	110	3.0
HB5	0.21	1	1	55	730	13	87	970	0.91	2.6	2.0	110	2.9

4.3 Plant performance

In the present study, the plant performance of lettuce was assessed by measuring fresh and dry weight of the shoots and roots. As an overall result, the control treatment performed more satisfactory than treatment-group PB and HB, which showed severely impaired plant growth, particularly in the HB-treatments. In all HB-treatments, visual symptoms of nitrogen deficiency (chlorosis in the lower leaves and stunted growth) became apparent at an early stage. The PB-treatments developed the same symptoms but at a later stage and to a lesser degree. In the last week of the experiment, the control treatment also developed chlorosis on some of the lower leaves. Images presented below were taken at the day of harvest.

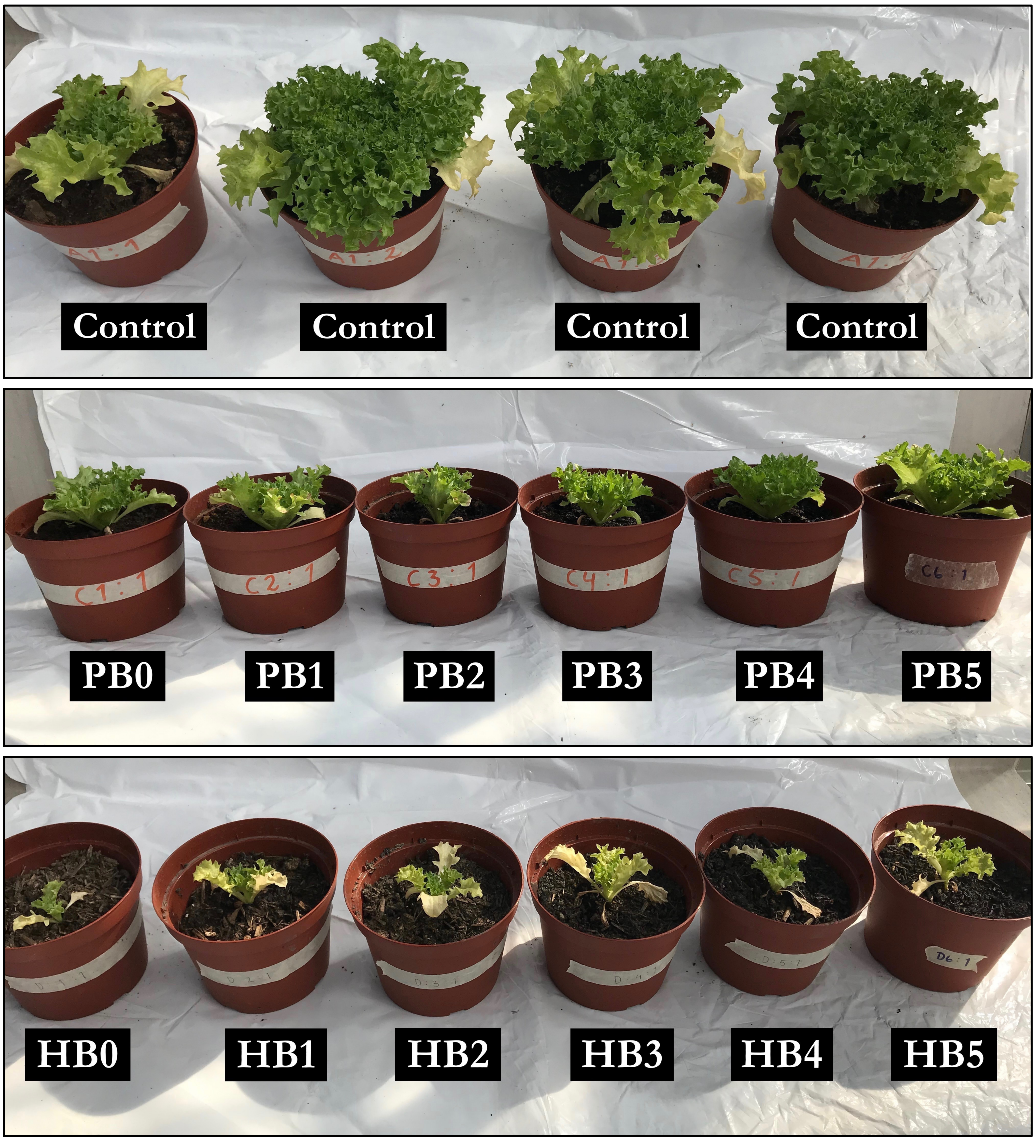

Figure 4: All treatments at the day of harvest (Photo Malin Nilsson). From the top: Control treatment; Treatment PB0-PB5 (Peat-based growing media with biochar rates 0 - 31.25 %) and Treatment HB0-HB5 (Hemp-based growing media with biochar rates 0 - 31.25 %)

Fresh and dry weights of the lettuce shoots are displayed in Table 11. In summary, these results show that the control treatment had by far the highest FW and DW of all treatments. Moreover, The PB-treatments performed superiorly to the HB-treatments. Interestingly, the highest FW and DW within the PB-group were observed for treatment PB0 and PB5, which were the treatments with 0 % respectively 31.25 % biochar. The only statistical difference in FW was observed for the control treatment, which was statistically higher than all other treatments. The control treatment did not have a statistically higher DW than treatment PB5, but was statistically higher than all other treatments. Worth noting is the high standard deviation for the control treatment. Both the lowest FW and DW were observed in treatment HB0 and HB1.

Table 11. Mean values and standard deviations for FW and DW of the shoots. Treatments that do not share a letter are significantly different at *P* <0.05 significance level among the columns

Treatment	N	FW (g)	DW (g)
Control	4	27.95 ± 14.36 [a]	3.86 ± 1.56 [a]
PB0	4	7.41 ± 2.07 [b]	1.40 ± 0.50 [bc]
PB1	4	5.68 ± 0.99 [b]	0.87 ± 0.09 [bc]
PB2	4	3.41 ± 1.11 [b]	0.50 ± 0.12 [bc]
PB3	4	4.92 ± 0.83 [b]	1.24 ± 1.15 [bc]
PB4	4	5.20 ± 0.35 [b]	1.66 ± 1.32 [bc]
PB5	4	8.46 ± 2.05 [b]	2.18 ± 1.34 [ab]
HB0	4	0.44 ± 0.30 [b]	0.21 ± 0.10 [c]
HB1	4	0.93 ± 0.43 [b]	0.24 ± 0.12 [c]
HB2	4	1.47 ± 0.14 [b]	0.37 ± 0.04 [bc]
HB3	4	1.39 ± 0.34 [b]	0.34 ± 0.10 [bc]
HB4	4	1.21 ± 0.42 [b]	0.28 ± 0.10 [bc]
HB5	4	1.03 ± 0.39 [b]	0.24 ± 0.09 [c]

ANOVA-tests without the control treatment revealed that treatment-group PB had statistically higher shoots FW compared to treatment-group HB (*P* <0.001). Within treatment-group PB, a significant difference in FW related to the biochar rate was observed (*P* = 0.001). Treatment PB5 had a statistically higher FW than treatment PB2, PB3 and PB4. No statistical differences in FW were found in treatment-group HB (*P* = 0.117). Treatment-group PB also had statistically higher DW than treatment-group HB (*P* <0.001). However, there were no significant differences in DW within the individual treatment groups (*P* = 0.199 respectively 0.164). As illustrated in figure 2 and 3, no linear relationship could be established between biochar rates and fresh and dry weight.

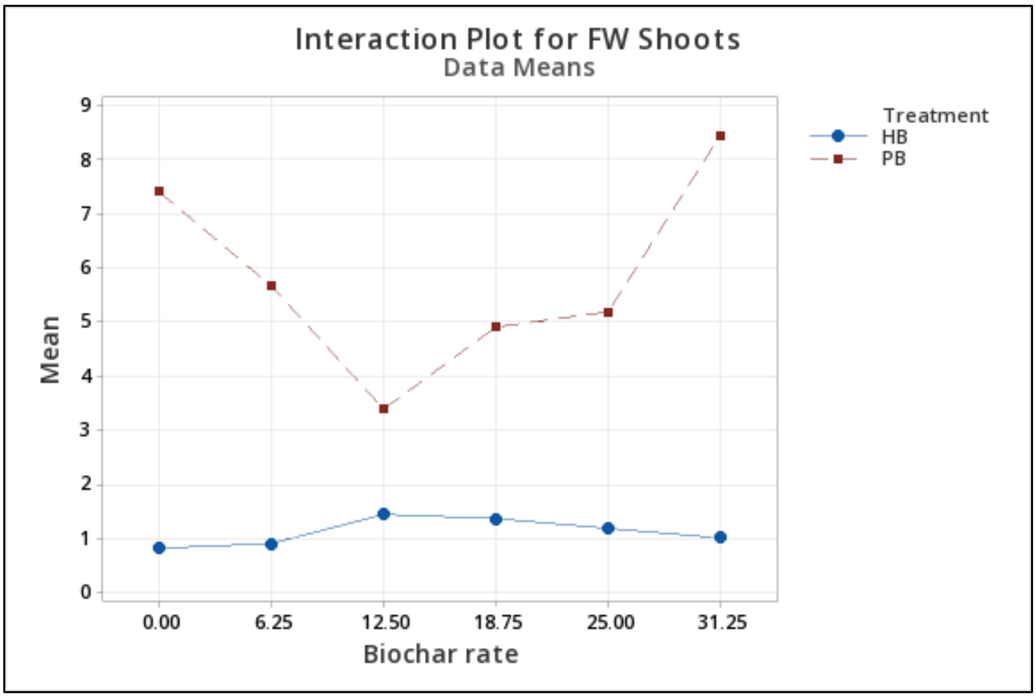

Figure 5. Interaction plot for FW shoots and biochar rate (0 - 31.25 %) in both treatment-groups. A non-linear relationship was observed between the two variables.

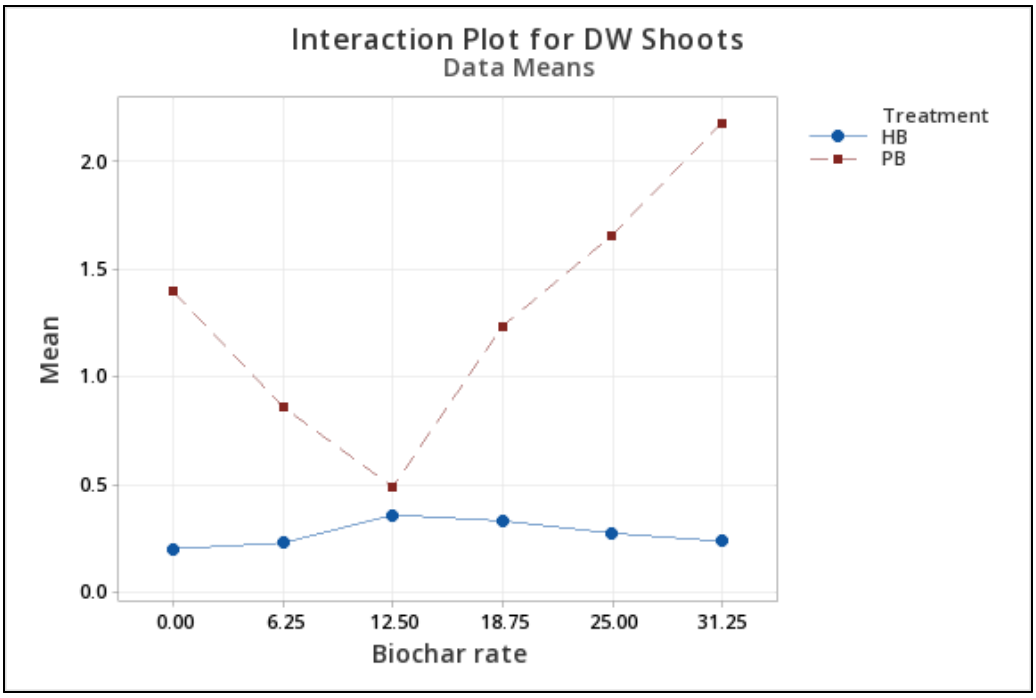

Figure 6. Interaction plot for DW shoots and biochar rate (0 – 31.25 %) in both treatment-groups. A non-linear relationship was observed between the two variables.

Table 12 presents the fresh and dry weights of the roots. The highest FW was observed in treatment PB0, followed by the control treatment. However, the control treatment had the highest DW. As for the shoots, the lowest FW and DW were observed in treatments HB0 and HB1.

Table 12. Mean values and standard deviations for FW and DW of the roots. Treatments that do not share a letter are significantly different at *P* <0.05 significance level among the columns.

Treatment	N	FW ± SD	DW ± SD
Control	4	4.49 ± 1.91 [ab]	0.61 ± 0.25 [a]
PB0	4	5.21 ± 1.56 [a]	0.48 ± 0.20 [ab]
PB1	4	3.41 ± 0.35 [abc]	0.29 ± 0.04 [bc]
PB2	4	2.32 ± 0.64 [cde]	0.22 ± 0.06 [c]
PB3	4	2.71 ± 0.51 [bcd]	0.21 ± 0.05 [c]
PB4	4	3.47 ± 0.57 [abc]	0.25 ± 0.05 [bc]
PB5	4	2.39 ± 1.47 [bcde]	0.28 ± 0.12 [bc]
HB0	4	0.61 ± 0.16 [de]	0.04 ± 0.01 [c]
HB1	4	0.48 ± 0.21 [e]	0.05 ± 0.02 [c]
HB2	4	0.69 ± 0.12 [de]	0.07 ± 0.02 [c]
HB3	4	1.01 ± 0.15 [de]	0.09 ± 0.02 [c]
HB4	4	0.61 ± 0.24 [de]	0.08 ± 0.03 [c]
HB5	4	0.76 ± 0.19 [de]	0.06 ± 0.02 [c]

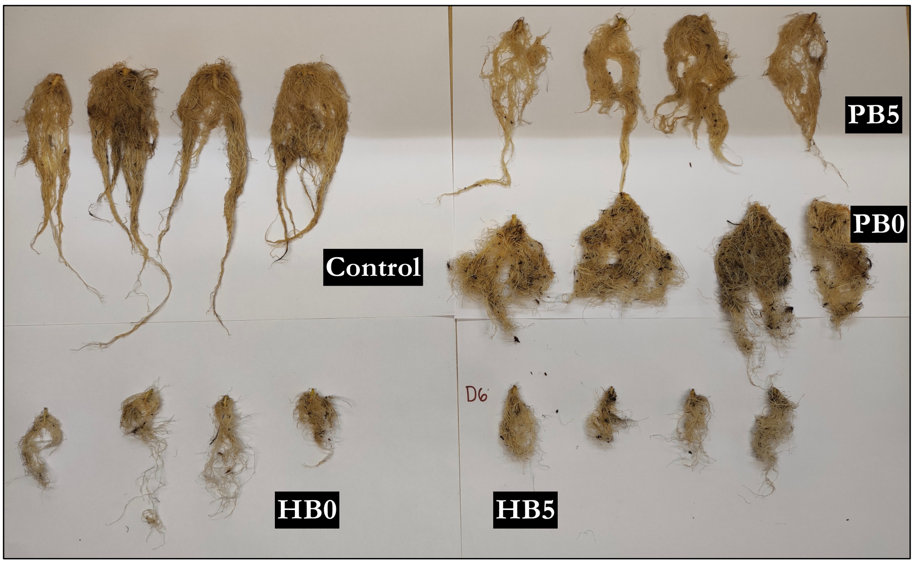

Figure 7: Lettuce roots in the following treatments: Control, PB0, HB0 (0 % biochar treatments), PB5 and HB5 (31.25 % biochar treatments). Photo Malin Nilsson.

4.4. Plant tissue nutrient analysis

Nutrient concentration and total nutrient content of the lettuce shoots are displayed in Table 13, 14 and 15. Statistical analysis could only be performed on the N content, due to insufficient samples in the other nutrient elements. The results revealed that the total N content in the control treatment was only statistically higher than the HB-treatments and treatment PB1 and PB2. In Table 13, it can be seen that treatment PB4 and PB5 have absorbed nearly the same amount of N, P and K as the control treatment. For the other macronutrients (Mg, S and Ca), the nutrient uptake has been notably greater in the control treatment compared to the other treatments, as shown in Table 14. In micronutrient uptake (Fe, B and Mn), what stands out in the results is that PB0 has taken up much more Fe and Mn than all other treatments. Moreover, the uptake of Al has also been notably greater in PB0. In general, micronutrient uptake has been equal in the control treatment as the other PB-treatments. Treatments PB0 and PB5, which were the treatments that obtained the highest shoots dry and fresh weight, showed contrasting nutrient uptake. Treatment PB5 had absorbed much more N, P and K than treatment PB0, whereas treatment PB0 absorbed much more Mg, S, Ca, Fe and Mn than treatment PB5.

Table 13. Plant nutrient values (N, P and K), presented both as concentration (*g/kg*) and as average nutrient uptake per treatment (calculated from the mean DW of the plants with n=4). Based on ICP-OES and Dumas nutrient element analysis, conducted by LMI AB, Helsingborg, Sweden. Treatments that do not share a letter in the N column are significantly different at *P* <0.05 significance level.

Treatment	N		P		K	
	g/kg	*mg/plant*	*g/kg*	*mg/plant*	*g/kg*	*mg/plant*
Control	10.1 ^{c}	39.8 ^{a}	2.8	11.2	32.3	130.7
PB0	12 ^{bc}	16.8 ^{abc}	3.4	4.8	44.3	62.2
PB1	10 ^{c}	8.7 ^{bc}	3.0	2.6	38.0	33.1
PB2	11.8 ^{bc}	5.7 ^{bc}	3.5	1.8	44.0	23.0
PB3	14 ^{ab}	16.5 ^{abc}	4.3	5.3	53.3	65.6
PB4	15.3 ^{a}	25.2 ^{abc}	4.5	7.5	58.5	98.9
PB5	13.8 ^{ab}	30.5 ab	4.1	8.9	54.1	116.8
HB0	5.9 ^{d}	1.19 ^{c}	1.26 *	0.39 *	25.7 *	8.0 *
HB1	5.1 ^{d}	1.19 ^{c}	1.30 *	0.46 *	24.0 *	8.4 *
HB2	4.7 ^{d}	1.72 ^{c}	1.48	0.54	22.8	8.3
HB3	5.3 ^{d}	1.76 ^{c}	1.51	0.57	23.6	8.9
HB4	5.6 ^{d}	1.59 ^{c}	1.51 *	0.65 *	23.1 *	9.5 *
HB5	6.0 ^{d}	1.44 ^{c}	1.59 *	0.57 *	24.5 *	8.8 *

Values are based only on one sample, due to insufficient plant tissue material for the nutrient analysis

Table 14. Plant nutrient values (Mg, S, Ca and Na) presented both as concentration (*g/kg*) and as average nutrient uptake per treatment (calculated from the mean DW of the plants with n = 4). Based on ICP-OES nutrient element analysis, conducted by LMI AB, Helsingborg, Sweden.

Treatment	**Mg**		**S**		**Ca**		**Na**	
	g/kg	*mg/plant*	*g/kg*	*mg/plant*	*g/kg*	*mg/plant*	*g/kg*	*mg/plant*
Control	2.0	8.1	1.1	4.3	7.1	28.4	1.7	6.7
PB0	2.1	7.8	0.9	3.6	8.1	30.4	2.7	10.3
PB1	1.9	1.6	0.8	0.7	6.9	6.0	2.3	2.0
PB2	2.2	1.1	1.0	0.5	7.5	3.9	2.2	1.2
PB3	2.2	2.7	1.3	1.6	8.3	10.1	2.8	3.3
PB4	2.0	3.5	1.5	2.5	7.9	13.5	3.0	5.1
PB5	1.8	4.0	1.3	2.9	7.4	15.8	2.5	5.4
HB0 *	1.4	0.43	0.35	0.11	3.8	1.2	1.7	0.53
HB1 *	1.4	0.50	0.33	0.11	3.8	1.3	1.8	0.64
HB2	1.3	0.48	0.38	0.14	4.0	1.4	1.6	0.57
HB3	1.3	0.50	0.38	0.14	3.7	1.4	1.7	0.66
HB4 *	1.2	0.48	0.40	0.16	3.6	1.5	1.4	0.59
HB5 *	1.4	0.51	0.43	0.15	4.0	1.5	1.6	0.58

- Values are based only on one sample, due to insufficient plant tissue material for the nutrient analysis

Table 15. Plant nutrient values (Al, Fe, B and Mn), presented both as concentration (*mg/kg*) and as average uptake per treatment (calculated from the mean DW of the plants with n = 4). Based on ICP-OES nutrient element analysis, conducted by LMI AB, Helsingborg, Sweden.

Treatment	Al		Fe		B		Mn	
	mg/kg	*mg/plant*	*mg/kg*	*mg/plant*	*mg/kg*	*mg/plant*	*mg/kg*	*mg/plant*
Control	7.5	0.03	27	0.11	15.5	0.06	33.3	0.13
PB0	110.3	0.16	191	0.27	29.5	0.04	248.5	0.34
PB1	63.5	0.06	110.5	0.10	28.3	0.02	120.8	0.11
PB2	67.7	0.04	113.7	0.06	34	0.02	68.7	0.03
PB3	78.5	0.11	93.3	0.09	37.3	0.05	61.8	0.08
PB4	80.5	0.20	55.3	0.10	40.8	0.07	44.3	0.08
PB5	76.3	0.22	38.3	0.09	34.5	0.08	33.8	0.07
HB0 *	25	0.01	51	0.02	15	0.005	23	0.01
HB1 *	54	0.02	93	0.03	16	0.01	23	0.01
HB2	29	0.01	52.8	0.02	15.8	0.01	24.3	0.01
HB3	30.7	0.01	42	0.02	16.3	0.01	23.7	0.01
HB4 *	29	0.01	36	0.01	15	0.01	22	0.01
HB5 *	24	0.01	49	0.02	18	0.01	28	0.01

* Values are based only on one sample, due to insufficient plant tissue material for the nutrient analysis

5. Discussion

In the present study, 13 growing media mixtures were examined for their main physicochemical properties and for their effect on plant performance, with the aim of evaluating the suitability of biochar and hemp as partial or complete replacement of peat in growing media. The physical and chemical properties of the different growing media varied greatly, which will be discussed in detail in the following section.

5. 1 Physicochemical properties

The biochar used in this study had a high bulk density (339 kg/m^3), compared to previously reported biochar densities in growing media (Nieto et al. 2016; Rathnayake et al. 2021). Wood-derived biochar generally display higher bulk densities compared to biochars produced from low-lignin feedstock (Brewer et al. 2014). Nevertheless, the addition of biochar led to a slight decrease in bulk density in the PB-treatments, compared to the 0 % biochar treatment. This indicates that even a high-density biochar may replace peat without negatively affecting the bulk density of the growing medium. The bulk density of the control treatment was significantly lower than all PB-treatments, due to its 100 % peat content. The difference in bulk density is not linked to the addition of biochar but to the incorporation of manure and compost in the PB-treatments, which contributed with higher densities than the low-density peat. The optimal range for bulk density is normally suggested to be 0.2 to 0.4 g/cm^3 in growing media but can stretch up to 0.5 g/cm^3, depending on the crop and growing conditions (Agarwal et al. 2021). Hence, bulk densities in the PB-treatments were at the upper limit, which over time may result in compaction of the growing medium, thereby reducing its aeration properties. Contrary, all the hemp-based growing media showed low bulk densities. The addition of biochar led to a significant increase in bulk density but the values were still within the optimal range.

Biochar is often proclaimed for its great water-holding capacity, which would be an argument for its use in growing media. In this study, the WHC was not enhanced by the addition of biochar but did however remain at a more or less constant level, regardless of increasing biochar proportions in the peat-based growing media. All PB-treatments were within the recommended range for WHC in growing media, which is suggested to be 45-65 % (Zulfiqar et al. 2021). The maintained WHC observed in this study is only partly supported by the existing literature. Méndez et al. (2015) reported an increased WHC when replacing peat with 50 % (v/v) biochar, compared to peat alone. Other studies have observed a notable decrease in WHC when replacing peat with biochar at different ratios (Rathnayake et al. 2021; Nieto et al. 2016). These diverging results are explained by the varying physical properties of biochar, which highlights the importance of biochar characterization prior to its use. In the hemp-based growing media, the addition of biochar led to a slight increase in WHC. Despite this increase, all values were still below the optimal range. In general, the ability of biochar to enhance WHC in growing media has been more prominent when substituting other materials than peat, such as compost and coconut coir (Zhang et al. 2014; Méndez et al. 2015 and Kim et al. 2017). This correlates with the findings of this study.

The total porosity in a growing medium should be 50-80 %, revealing that all treatments had an adequate total porosity (Agarwal et al. 2021). However, the proportion of macro – and micropores is equally important to consider. In the hemp-based growing media, a high proportion of the total porosity was made up of air-filled pores, suggesting that the hemp material contained more coarse particles, which also explains its limited water-holding capacity (provided by micropores). The combination of limited water retention and high aeration is associated with a faster dry-out of the medium, requiring more frequent irrigation (Caron et al. 2004). Due to a likely measurement bias of the total porosity, the air-filled porosity of the control treatment and PB-treatments are not included in the results, since they showed nearly 0 % air-filled porosity, which is unlikely. The total porosity of the control treatment and the PB-treatments is estimated to be higher than the reported values, which would have generated more accurate values for the air-filled porosity. Based on the bulk densities and the well-developed root systems in these treatments, the air-filled porosity has been sufficient.

In terms of the physical properties of the examined growing media-mixtures, the results of this study only support a partial replacement of peat with biochar. A complete replacement of peat with hemp and biochar (HB-treatments) resulted in insufficient water-holding capacity, which would be unfavorable for growing media use.

Although a positive correlation between biochar rate and pH could be established, it should be noted that the other growing media components (i.e. compost and manure) were alkaline in pH and therefor likewise contributed to the pH elevation. This was evident since treatment PB0 (0 % biochar) had a pH of 7, despite the use of peat (with pH 4-5) as bulk material. The lowest biochar rate (6.25 % v/v) did not cause a pH increase in combination with the pH-acidic peat. However, at higher biochar rates the pH elevation was notable and far above the recommended pH range. In the hemp-based growing media, even the 0 % biochar treatment showed a high pH and with increasing biochar rates, the pH rose to unacceptable levels. The pH issue observed in all elaborated growing media-mixtures clearly needs to be addressed, in order for them to be a viable option on the market. Looking into the existing literature on biochar-amended growing media, the effect of biochar on growing media pH has not been consistent. In a study by Chrystargyris et al. (2019), the effect of different biochar types and biochar ratios on growing media properties was evaluated. The authors reported varying pH effects on the growing media (pH 5.32 – 7.06) at a 20 % biochar ratio, which did not correspond to the inherent pH of the biochar. Similar results have been reported in other studies (Sabatino et al. 2020 and Chrystargyris et al. 2020). Moreover, Steiner and Harttung (2014) demonstrated that biochar ratios up to 80 % could be used in combination with peat without raising the pH above 7. A strong liming effect of biochar should therefore not be assumed but needs to be tested for the specific biochar. In the present study, stable manure was used as the primary nutrient source instead of mineral fertilizer, which has otherwise been the standard in research studies of similar kind. Because soluble mineral fertilizers do not cause a similar pH raise, the results from this study are difficult to compare with previous research in this field. However, high pH has frequently been reported as a limitation in organic growing media (Carlile et al. 2015 and Cacini et al. 2021). Reducing growing media pH is usually a challenge in organic growing media, since the use of mineral acids is not allowed (Håkansson 2013). Available options for organic production are citric acid and leonardite (if obtained as a by-product in mining (Livsmedelsverket 2019)). For example, Steiner and Harttung (2014) used leonardite to reduce biochar pH prior to its use in growing media, which reduced the pH from 9 to 5.2. However, after applying the biochar to growing media, the initial pH reduction was lost after six weeks of cultivation. Vaughn et al. (2015) used citric acid to lower biochar pH prior to growing media application. In order to enable the use of biochar in organic growing media, strategies to reduce the pH must be elaborated. Pre-treating the biochar with leonardite or citric acid could be an option but the durability of the pH reduction is uncertain. This is a relevant area to address in future studies.

The use of biochar in growing media has in some cases been associated with inadequately high EC-levels (Nobile et al. 2020). In this study, there was no correlation between biochar and increased EC and the levels were all within the optimal range (<500 µS/cm (Nobile et al. 2020)). The biochar used in this study showed a remarkably low ash content (2.1 %), compared to biochars produced from mineral-rich feedstock, which can display ash content above 50 % (Rathnayake et al. 2021) This suggests that wood-derived, low-ash biochar are safe to use in growing media without causing abnormal EC values.

5.2. Nutrient availability

The Spurway analysis represents a momentary image of the nutrient status in the growing media, indicating the amount of nutrients that will be available for plant uptake in the subsequent days. This information should be regarded with caution, as low nutrient levels may as well indicate a good nutrient uptake by the plants. In this study however, samples for the initial Spurway analysis were taken at the same day of transplanting the plants, and therefore gives reliable information about the amount of available plant nutrients at the start of the experiment. Not surprisingly, the control treatment showed substantially higher N content compared to all other treatments, due to the use of mineral N. In nearly all other treatments, the N content was negligible. Treatment PB0 and PB1 showed slightly higher N content but the levels were still low. According to guidelines elaborated for Swedish plant nurseries, adequate N levels detected in a Spurway-analysis should be 100-150 mg/L (Rudin 1999). Hence, all growing media-treatments except the control were highly deficient in N. Because nitrogen is the most essential nutrient element for plant growth, a limited plant growth could be anticipated, based on the initial Spurway-analysis. However, a Spurway analysis does not provide information about nutrients that are organically bound, which are continuously mineralized and subsequently released as plant available nutrients in the growing medium. Based on the calculated nutrient input in each growing medium, the total amount of N was substantial in all treatments. Approximately two thirds of the total N content in the stable manure (the primary nutrient source used in all treatments) was organically bound, capable of providing a slow and steady nutrient release, under favorable conditions for mineralization to occur. Moreover, the nutritional information provided for the green waste compost was based on a Spurway-analysis and hence the compost component was expected to contribute with more nutrients than what was reported in the calculated nutrient input. A continuous N mineralization in treatments PB0 and PB1, equivalent to the initially measured values, might have been sufficient for lettuce development. However, based on the second Spurway analysis and on the final plant performance, there has not been sufficient (or available) N in neither of the treatments (except for the control). Equally, a large proportion of S is organically bound, which explains the large deviance between the total S input and the measured available S in each growing medium. Therefore, the initially low S content observed in all treatments (except the control) was not very concerning, due to an expected S mineralization throughout the experiment. Contrary, the initially measured nutrient levels in the control treatment should be sufficient for the entire growing season, because of the inherently low nutrient content in peat. Apart from the N and S content, the Spurway analysis showed fairly balanced nutritional properties in all treatments, according to the aforementioned plant nursery-guidelines (Rudin 1999). Compared to the control, the PB-treatments and HB-treatments had either equal nutrient content (P and Ca) or higher nutrient content (K, B, Mn and Fe). This observation is associated with the use of organic amendment, which is known to provide a wide spectrum of nutrients and in particular be a good source of micronutrients (Carlile et al. 2015).

Results from the second Spurway analysis, taken at the day of harvest, showed higher nutrient levels for several nutrient elements (K, S, Ca and Mn) in both the peat-based and hemp-based growing media, compared to the initial analysis. This indicates that a continuous mineralization has occurred, but there has been a lack of subsequent nutrient uptake by the plants. The amount of available N was still negligible and explains the limited plant growth. The treatments with 0 % biochar (PB0 and HB0) had a similar nutrient content than all other elaborated treatments, which suggests that the biochar component did not contribute with nutrients directly. This is in line with previous research, which have shown negligible nutrient supply from wood-derived biochar (Modin 2021; Hossain et al. 2020).

Concerning the growing media pH, it is well known to influence nutrient availability. The treatments showed large variations in pH but few correlations could be drawn to the amount of available nutrients shown in the Spurway analysis. Apart from a reduction in available N, there was a trend of decreasing P at pH-values above 8, which correlates with the reduced bioavailability of P at alkaline pH. The results did however not indicate reduced bioavailability in any of the micronutrients that normally are affected by high pH-values, e.g. B, Mn and Fe (Neina 2019).

Regarding the negative correlation between N content and biochar, there are several plausible mechanisms involved. The lack of readily available N was most severe in the hemp-based growing media. The C/N ratio was not analyzed in this study but other studies have reported high C/N ratio for hemp (Luxhøi et al. 2006), which is known to stimulate microbial degradation and thereby induce immobilization of N (Both et al. 2021). In a study by Vandecasteele et al. (2018), the incorporation of different unprocessed plant fibers in growing media was associated with increased nitrogen immobilization. This supports the theory of nitrogen immobilization induced by a potentially high C/N ratio in the hemp. Contrary, biochar is not expected to have contributed to nitrogen immobilization in the growing media. Even though biochars generally have high C/N ratios, research suggests that biochar do not cause any substantial nitrogen immobilization, due to the high proportion of recalcitrant C in biochar, which is not easily mineralized. This theory has wide scientific support (Fornes et al. 2019; Lehmann and Joseph, 2009). However, despite the recalcitrant structure of biochar, it may contain small fractions of surface labile C that is relatively easily mineralized. Labile C in biochar is a result of incompletely pyrolyzed biomass, which may occur in fast pyrolysis at low temperatures (Bruun et al. 2012). According to the biochar analysis, the H/C ratio of the biochar used in this study was 0.33. The H/C ratio is an important parameter since it reveals the degree of carbonization, which in turn defines the stability of the biochar. According to EBC-guidelines, the H/C ratio should not exceed 0.7, as this would indicate an insufficient pyrolyzation of the organic matter. The amount of Volatile Organic Compounds (VOC) gives additional information about the stability of the biochar, since it represents a labile fraction of the biochar, hereby susceptible to microbial degradation. The biochar used in this study had a VOC content below 10, which is considered low (Rathnayake et al. 2021). Based on this information, it can be concluded that the biochar used in this study is highly stabile. The likelihood of nitrogen immobilization induced by the biochar is therefore low.

Nitrogen adsorption and/or nitrogen volatilization are more plausible explanations for the low N availability. Many studies have reported increased CEC in growing media after biochar application and the ability of biochar to adsorb NH_4+ (Kim et al. 2017; Taghizadeh-Toosi et al. 2012; Steiner 2010). Moreover, studies have demonstrated the ability of biochar to reduce NH_3 volatilization during composting. This effect is attributed to the adsorption of the precursor NH_4^+ (Malińska et al. 2014; Steiner et al. 2010) or to the direct retention of NH_3 by acid functional groups (Mandal et al. 2018). However, there have also been contrasting reports of increased NH_3 volatilization in biochar-amended composts. For example, Febrisiantosa et al. (2018)

investigated the ability of biochar to reduce N volatilization during composting. The results instead showed increased NH_3 volatilization compared to the control. The negative effect was associated with the high pH in the compost (pH 8.69), caused by the addition of alkaline biochar. Similar findings have been reported in other studies (Hestrin et al. 2020). It is well known that ammonia volatilization increases with higher pH-values, due to a shift in the ammonium-ammonia equilibrium at alkaline pH toward the volatile ammonia (Mandal et al. 2010). However, in the mentioned study by Steiner et al. (2010), in which a notable reduction in NH_3 volatilization could be observed, the pH of the compost was above 9 (Steiner et al. 2010). This indicates that the effect is not solely pH-dependent. Mandal et al. (2018) demonstrated that low-pyrolysis biochars had a greater ability of reducing NH_3 emissions, due to an increase in CEC and acid functional groups. However, the mechanisms involved in biochar NH_3 retention have not been fully elucidated (Hestrin et al. 2020). This study did not include analysis of CEC and surface functional groups. However, biochars produced at high pyrolysis temperatures and from lignin-rich feedstock have been reported to have comparatively low CEC (Silber et al. 2010). This suggests that the biochar used in this study would not have any greater capacity to retain NH_4^+ and NH_3. Based on the above discussion, the lack of available N observed in all growing media treatments is most likely a consequence of N volatilization, and not N adsorption. The raise in pH with increasing biochar rates correlates with the observed reduction of N, thereby supporting this theory. Furthermore, due to a research bias in the experiment, the extent of N volatilization has likely been increased. The growing media mixtures were prepared at the same day as sowing the lettuce seeds and were afterwards stored in the heated greenhouse in partly open plastic bags. Due to an uneven development of the lettuce seedlings, the procedure had to be repeated, which delayed the transplanting of the plants. The very alkaline pH-values in many of the growing media-mixtures, combined with the warm greenhouse environment, have therefore likely caused a substantial N loss by NH_3 volatilization.

5.3 Plant performance

The results obtained in the greenhouse pot experiment did not correlate with the positive plant responses frequently reported in biochar-amended growing media (Messiga et al. 2022; Sabatino et al. 2020; Mendez et al. 2017; Graber et al. 2010). In the hemp-based growing media, there were only small differences in the measured plant biomass. In the peat-based growing media there were significant differences between some of the treatments but no correlation could be established between plant biomass and biochar rate. Although the treatment with highest biochar rate (31.25 % in PB5) had the least available N at the start of the experiment, this treatment performed equally to the treatment with no biochar (PB0), which showed the highest amount of available N in the growing medium. These contrasting results are hard to explain. However, based on the measured nutrient uptake of the plants, it is evident that mineralization of organic matter has occurred throughout the experiment. The PB-treatments with highest biochar rates (25 and 31.25 %) have absorbed similar nutrient amounts as the control treatment. Hence, despite of the lack of available N and S at the start of the experiment, these nutrients have become available to the plants sometime during the five weeks. The calculated nutrient input confirmed that all growing media-treatments had substantial amounts of N and S, and that a continuous release of these nutrient elements could be expected. The reason for the stunted plant growth in the PB-treatments is hard to explain in relation to the measured nutrient uptake in these treatments. It is possible that N became available in the growing media at a too late stage, which impaired plant development. Since this study only measured plant performance based on the final biomass, it is not possible to identify differences in plant growth during the five weeks of the experiment. It would have been beneficial to use another model crop in the experiment, such as basil, for which plant development can be measured in height. By measuring plant development during the growing season, information about mineralization in the growing media could have been better understood.

Apart from nutrient release from mineralization, it is possible that the initial pre-treatment of biochar with supplemental N (blood meal) was not plant-available at the start, but has successively been released during the experiment. Regarding the pre-treatment of biochar with additional N, very limited literature could be found regarding practical information on amounts and procedure of this practice. Further research into this area is relevant, such as developing easy methods to determine the amount and time required to nutrient load the biochar.

From this study it is not possible to predict if the biochar addition had a stimulating effect on growing media microbial activity, which might have improved nutrient availability and plant nutrient uptake. Microbial activity and communities were not examined in this study but this would be a relevant aspect to address in future research, in order to gain better understanding on its influence on growing media nutrient dynamics. Moreover, it would be relevant to measure N dynamics in growing media, e.g. N leakage and gaseous emissions, as biochar has been shown to greatly influence these processes.

Conclusions

The greatest limitation encountered in the evaluated growing media-treatments was the alkaline pH, which was far above the optimal pH range for plant growth. This was an issue in all treatments but particularly in the hemp-based growing media. The use of manure and green waste compost contributed to a high pH, which was further elevated with the addition of biochar. This highlights the difficulty of using biochar in organic growing media, due to the challenge in maintaining the pH at an adequate level. The alkaline pH most likely resulted in a substantial N loss through nitrogen volatilization, which is favored at high pH. N shortage was identified as the main reason for the poor plant performance in all treatments. Apart from the N content, the growing media-treatments showed good nutritional properties, which for several nutrient elements were superior to the control treatment. Despite the low plant biomass obtained in all elaborated treatments, two of the peat-based growing media with biochar rates ≥ 25 % had a similar nutrient content to the control treatment. This suggests that the addition of biochar stimulated plant nutrient uptake.

The pH was the only parameter that was notably altered by biochar addition in the peat-based growing media, while biochar addition improved the water-holding capacity in the hemp-based growing media. The pot experiment clearly demonstrated the unsuitability of hemp to be used as growing media material (in an unprocessed form). A potentially high C/N ratio in the hemp may have caused N immobilization, in addition to the N loss by ammonia volatilization. Since the plant performance was equally poor in the 0 % biochar treatments, the negative plant response cannot be solely linked to the addition of biochar. Any potentially positive effects of biochar addition were obscured by the negative effect of the high pH. Investigating pH-reducing strategies in biochar-organic growing media was identified as a future research approach.

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7. Popular science summary

There has been a great interest in biochar in recent years, owing to its observed ability to capture atmospheric carbon and improve soil fertility.
With an excellent capacity to retain water and nutrients when applied to soil, biochar can contribute to long-term soil fertility. As the horticultural industry is facing the challenge of substituting peat – a material that is highly appreciated for its growing properties but is also associated with negative environmental effects – biochar has been suggested as a potential alternative. In this study, the aim was to see if the many positive plant responses previously reported for biochar amendment could be achieved when using biochar in organic growing media. Biochar was used in different proportions and in combination with either peat or hemp for lettuce cultivation in a greenhouse pot experiment. The addition of biochar resulted in such a high pH that the plant growth was severely reduced, which might have obscured any potentially beneficial effect of using biochar. This however highlighted the challenge in using biochar in organic growing media due to the difficulty in maintaining the pH at an adequate level for plant growth.

Appendix 1

Dry bulk density treatment-group PB

Grouping Information Using the Tukey Method and 95% Confidence

Peat treatment	N	Mean	Grouping			
PB0	3	0,59182	A			
PB1	3	0,58765	A	B		
PB4	3	0,57063		B	C	
PB3	3	0,55342			C	D
PB5	3	0,55139			C	D
PB2	3	0,55006				D

Means that do not share a letter are significantly different.

P = <0.001

Dry bulk density treatment-group HB

Grouping Information Using the Tukey Method and 95% Confidence

Hemp treatment	N	Mean	Grouping			
HB5	3	0,51864	A			
HB3	3	0,49113		B		
HB1	3	0,47695		B	C	
HB2	3	0,46713		B	C	
HB4	3	0,46067			C	
HB0	3	0,41244				D

Means that do not share a letter are significantly different.

P = < 0.001

Total porosity in treatment-group PB

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
PB2	2	59,86	A
PB1	2	59,61	A
PB3	2	58,23	A
PB0	2	58,23	A
PB4	2	58,1189	A
PB5	2	57,374	A

Means that do not share a letter are significantly different.

P = 0.927

Total porosity in treatment-group HB

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping			
HB0	2	69,035	A			
HB1	2	64,273		B		
HB4	2	63,200		B	C	
HB2	2	62,450		B	C	D
HB3	2	61,694			C	D
HB5	2	60,488				D

Means that do not share a letter are significantly different.

P = <0.001

WHC in treatment-group PB

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
PB0	2	58,21	A
PB3	2	57,95	A
PB1	2	57,24	A
PB2	2	56,57	A
PB5	2	55,498	A
PB4	2	55,20	A

Means that do not share a letter are significantly different.

P = 0.507

WHC in treatment-group HB

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
HB5	2	41,595	A
HB4	2	41,157	A
HB3	2	37,890	A
HB2	2	37,40	A
HB1	2	37,30	A
HB0	2	34,93	A

Means that do not share a letter are significantly different.

P = 0.188

Shoots FW in treatment-group PB

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping		
PB5	4	8,46	A		
PB0	4	7,41	A	B	
PB1	4	5,678	A	B	C
PB4	4	5,200		B	C
PB3	4	4,922		B	C
PB2	4	3,408			C

Means that do not share a letter are significantly different.

P = 0.001

Shoots FW in treatment-group HB

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping
HB2	4	1,4675	A
HB3	4	1,390	A
HB4	4	1,210	A
HB5	4	1,032	A
HB1	4	0,930	A
HB0	4	0,845	A

Means that do not share a letter are significantly different.

P = 0.117

DW shoots in treatment-group PB

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping
PB5	4	2,183	A
PB4	4	1,663	A
PB0	4	1,400	A
PB3	4	1,240	A
PB1	4	0,8650	A
PB2	4	0,4950	A

Means that do not share a letter are significantly different.

P = 0.199

DW shoots in treatment-group HB

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping
HB2	4	0,3650	A
HB3	4	0,3375	A
HB4	4	0,2800	A
HB5	4	0,2425	A
HB1	4	0,2350	A
HB0	4	0,2075	A

Means that do not share a letter are significantly different.

P = 0.164