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Article

Biochar for Horticultural Rooting Media Improvement: Evaluation of Biochar from Gasification and Slow Pyrolysis

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Abstract: Peat is used as rooting medium in greenhouse horticulture. Biochar is a sustainable alternative for the use of peat, which will reduce peat derived carbon dioxide emissions. Biochar in potting soil mixtures allegedly increases water storage, nutrient supply, microbial life and disease suppression but this depends on feedstock and the production process. The aim of this paper is to find combinations of feedstock and production circumstances which will deliver biochars with value for the horticultural end user. Low-temperature (600 °C–750 °C) gasification was used for combined energy and biochar generation. Biochars produced were screened in laboratory tests and selected biochars were used in plant experiments. Tests included dry bulk density, total pore space, specific surface area, phytotoxicity, pH, EC, moisture characteristics and microbial stability. We conclude that biochars from nutrient-rich feedstocks are too saline and too alkaline to be applied in horticultural rooting media. Biochars from less nutrient-rich feedstocks can be conveniently neutralized by mixing with acid peat. The influence of production parameters on specific surface area, pH, total pore space and toxicity is discussed. Biochar mildly improved the survival of beneficial micro-organisms in a mix with peat. Overall, wood biochar can replace at least 20% v/v of peat in potting soils without affecting plant growth.

Keywords: alkalinity; biochar; gasification; pH; phytotoxicity; pyrolysis; salinity; stability; degradability

1. Introduction

Biochar is the carbon rich co-product of pyrolysis or gasification of biomass. Biochar application to soils is of public and agricultural interest [1]. The public interest in biochar application to soil is focused on the potential to decrease global net carbon dioxide emission by an increased soil storage of carbon [2]. The agricultural interest is focused on a number of positive properties [3], the most striking being plant growth stimulation by increased water storage [4], increased nutrient supply [5,6], increased beneficial microbial life [7,8] and disease suppression [7,9]. Just as for agriculture, biochar application to horticultural rooting media (soiless substrates) is of public and agricultural interest. (1) The public interest is to use biochars from renewable organic residual streams to substitute part of the peat used in rooting media in greenhouse horticulture [10,11]. Peat bogs are important carbon (C)

stocks and regulate the local water quality and water regime [12]. In the light of environmental concerns, peat substitution by biochar will preserve peat bogs and lower global carbon dioxide emissions linked with the use of peat extraction and use [13]; (2) The horticultural interest in biochar apart from peat substitution is the use and manipulation of bacterial communities for the protection of plants against diseases, either by direct protection or by induced plant resilience [14–16]. In certain plant growth media, biochar amendment results in chemical responses in the plant as well as shifts in the rhizosphere microbiome [17]. In greenhouse horticulture, the use of high input fertigation systems makes biochar related increases in water storage and nutrient supply of less economic consequence than for agricultural applications. An advantage of greenhouse testing is the improved control over climate effects including rain related water content and nutrient concentration fluctuations.

Several researchers have looked into the potential of biochar as a rooting medium for horticulture [14,18,19], including the substitution of peat. The study presented in [17] demonstrates the effect of oak wood pyrolysis biochar on strawberry grown in white peat and lettuce grown in field soil. In the strawberry bioassay, addition of 3% w/w biochar to peat resulted in (1) a higher fresh and dry plant weight; (2) a lower susceptibility for the fungal pathogen *Botrytis cinerea* on both leaves and fruits; and (3) changes in the rhizosphere microbiology such as an increase of bacterial diversity and a shift in composition of the rhizosphere microbiota. Extra inorganic plant nutrition and lime added to the peat reduced these effects of biochar on the strawberry plants. In [11] the authors reported that the hydrophysical properties of peat based growing media changed with the addition of various biochars up to 70% v/v. Lettuce (*Lactuca sativa*) grown in the peat/biochar mixtures showed substantially higher yields than with peat alone. In addition, this study confirmed the importance of biochar production conditions on the product properties. In [20] Dumroese et al. found that peat moss, amended with various ratios of pellets comprised of equal proportions of biochar and wood flour, generally had chemical and physical properties suitable for service as a rooting medium during nursery production of plants. A mixture of 75% v/v peat and 25% v/v pellets enhanced hydraulic conductivity and water availability at low (< -10 kPa) matric potentials. In [21] it was demonstrated in rooting media used for container production of greenhouse crops that biochars from wood gasification were able to buffer peat acidity, eliminating the need for liming agents. Gasification biochars reduced shrinkage of peat acting as a stable skeleton, and reduced the ammonium/nitrate ratio in the peat after a fertilization event. Lastly, biochars added stable and high levels of potassium to rooting media. In [22], the performance of tomato crop green-waste pyrolysis biochar as a rooting medium for hydroponic tomato production was compared with an existing, commercially acceptable rooting medium, pine sawdust. No peat was used in these rooting media. The EC of rooting media containing, or consisting entirely of biochar was reduced by rinsing with water before use, believing there is potential to capture and recycle nutrients flushed during this process. In terms of growth, yield, or fruit quality no differences were found among biochar and pine sawdust rooting media. In another attempt to completely replace peat [23] as well as vermiculite, a dried anaerobic digestate remaining after the fermentation of potato processing wastes, was mixed with three biochars produced from either wood pellets, pelletized wheat straw or field pennycress press cake. All three biochars were acidified and combined in 50%/50% v/v ratios with the digestate before comparing with a 50%/50% v/v sphagnum peat moss/vermiculite control medium containing slow-release chemical fertilizers. A growth increase of tomato was observed for the mix containing the wood pellet biochar.

Horticultural interest in biochar also seems justified in the light of prior results with biochar-like materials such as charred rice husks [24,25] and torrefied Gramineae [26,27]. Torrefaction or carbonization is a process that happens at temperatures (250–400 °C) with the objective of creating an energy-dense, solid biofuel with gas and liquids as by-products. The term biochar usually describes the by-product of pyrolysis and gasification. Pyrolysis is carried out at temperatures of 400 °C to 650 °C, without oxygen, while gasification occurs at temperatures above 600 °C. Both pyrolysis and gasification have as main objective the production of syngas, liquid fuels and chemicals. Charred rice

husks (synonymous with “burnt” rice husks) are used in mixes and on their own on a large scale in horticulture in South East Asia, notably Indonesia, as well as in South America [28].

In [24] the study of gasified rice hull biochar (GRHB) on available nutrients in a rooting medium for containers revealed that GRHB provides sufficient P and K to support a production cycle of geranium, but lacked either the correct concentration or balance of micronutrients for healthy growth. In [25] a number of crops were grown on standard commercial rooting medium composed of sphagnum peat moss/perlite (85%/15% v/v) and mixed with 0%, 5%, or 10% v/v GRHB. GRHB provided a source of readily available phosphate and potassium when incorporated at 5% or 10% v/v. The amount of available phosphate and potassium became depleted after a period of up to 6 weeks. Torrefied reed (*Phragmites australis*) was successfully used in an experiment with up to 50% v/v as compared to peat [27]. Torrefaction was used as low oxygen gasification of organic materials at temperatures of 150–400 °C [26]. Both charred rice husks and torrefied reed indicated potential for charred products at dosages far beyond 25% by volume.

A large and growing body of literature has reported no beneficial, adverse or contradicting effects on plant growth when using biochar [29–31]. The negative and neutral effects of biochar application have resulted in increased attention on methods to characterize biochars in general and for specific applications [32–34]. A wide variety of growing media are already being used in horticulture and a set of specific quality parameters and measuring methods has been developed [35]. Evaluating biochar for horticultural applications requires both, a material characterization with rooting media tests and a quantified link with results in field or container experiments. Such data will then fill the gap between biochar engineering and horticultural application results. Evidence of the importance of production factors has been reported for the nature of feedstocks [36,37]; the temperature of production [34,37,38]; the supply of oxygen [34,39]; and the cooling procedure to prevent condensation of toxic substances [14]. Gray et al., 2014, have reported a decreased hydrophobicity and related greater water entry at higher production temperatures [40].

The objective of our paper is a follow up on an earlier study by Fryda and Visser [41], who related the feedstock materials and thermal processes (pyrolysis and gasification) to the properties of the produced biochar. They concluded that biochars of widely different properties can be produced using the same feedstock under different production conditions. The authors further concluded that phytotoxic properties caused by condensation of tar loaded gas on the biochar particles can be avoided by using higher temperatures and early separation of gas from solids. They also reported increased internal particle porosity with gasification temperatures. In the present paper, we aim to first show the influence of feedstock and production parameters on a set of growth influencing properties, and second, to investigate the impact of two ways of disease suppression on rooting media. Our hypotheses are: (1) biochar is a potting soil constituent which can be used in growing medium blends in quantities of 20% v/v without negative growth effects; (2) biochar can induce disease suppression.

Our approach is to use well-defined materials for testing on horticultural properties, like water retention, water uptake rate, phytotoxicity, pH buffer values, nutrient and EC levels, cation exchange capacity (CEC), microbial stability and nitrate immobilization [35]. In addition we use biochars in plant tests to find potentially positive effects of biochar addition on the suppression of powdery mildew and *Fusarium*. Powdery mildew is selected based on possible biochar induced stimulation of plant hormones, which are related to induced plant resistance against biotrophic pathogens [9]. *Fusarium* is selected based on possible biochar enhanced *Fusarium* suppression by beneficial microorganisms [7].

2. Materials and Methods

2.1. Biochars Produced

Eight biochars were used in this study (Table 1). All biochars were produced by ECN (Petten, The Netherlands) in a lab-scale gasifier, at 670 °C gasification temperature, except for one sample (number 5) which was produced at 750 °C (Table 1). The facilities and test procedures are described

elsewhere in detail [41]. All biochars were continuously collected from the fluidized gasification bed under stable and continuous conditions. Initial laboratory testing [41] was supplemented with horticultural tests [35]. In close cooperation between biochar producers and horticultural specialists, it was decided to make biochar grains of 3–4 mm (with high internal particle porosity) because of the foreseen mixing with milled peat and aimed at optimal texture and drainage of the biochar/peat mixture.

Table 1. Overview of the biochars produced by ECN and pyrolysis conditions.

Code	Biomass	T	Moisture	Ash	Volatiles	C	HHV
		°C	%, a.r.	%, d.m.	%, d.m.	%, d.m.	MJ/kg
1. Beech/Tomato	80% beech wood + 20% tomato leaves	670	5.4	17.5	12.3	70.2	27.5
2. Wood/Tomato	80% wood chips-1 * + 20% tomato leaves	670	3.3	22.5	13.3	64.2	24.4
3. Wood chips-1	Batch spring 2015 *	670	-	-	-	-	-
4. Sweet pepper waste	Vegetable residues (Spain)	670	4.5	33.6	14.7	51.7	21.3
5. Sweet pepper waste	Vegetable residues (Spain)	750	4.5	26.5	15.1	58.4	21.2
6. Wood chips-2 **	Batch July 2015 *	670	3.2	10.7	10.2	79.1	29.7
7. Wood chips-3	Batch August 2015 *	670	-	-	-	-	-
8. Wood chips ***	Beech wood chips	670	2.7	23.8	6.0	70.2	27.1

T = Temperature; C = organic matter calculated as remaining mass without ash and volatiles; HHV = Higher Heating Value as measure of combustion value; a.r. = as received; d.m. = dry matter; * Wood cuttings from forestry Purmerend; ** Used in greenhouse experiment; *** Used in climate chamber experiment.

2.2. Rooting Medium Testing

Rooting media are tested for about twenty properties to evaluate their suitability for use as a rooting medium in horticulture [35,42]. Not all tests are relevant for all rooting media materials used. For biochar as constituent of peat based potting soil mixes, we limited tests to dry bulk density, organic matter content, ash content, specific surface area (SSA), salt content by electro conductivity (EC), acidity (pH), nutrient availability, total nutrient content, cation exchange capacity (CEC), base saturation, water retention and available air curve, toxicity and degradability. Reports by production sciences and agriculturists often use % w/w and air filled space (a ratio of two unknowns) to describe water content, air content, biochar addition and organic matter content. The consequence is that such reporting often cannot be interpreted for rooting media with a dry bulk density different from soil as those common in horticulture. To evaluate the material's suitability in horticulture, results should be reported in % v/v and air filled space (a ratio of the fixed total sample volume). The reason is that plants and bacteria sense and react to the environment on a per volume basis. We believe the observations above merit a place in the biochar assessment procedures already available [33].

2.2.1. Physical Characterization of Biochars

Dry weight and bulk density were determined after drying at 110 °C [43]. The organic matter content was determined by loss on ignition at 500 °C [44]. The specific surface area (SSA), elemental contents and levels of organic compounds were analyzed according to a biochar protocol by a commercial laboratory (Eurofins, Obitzsch-Hilbersdorf, Saxony, Germany). The proximate analysis of the biochar samples was carried out at ECN under EN ISO/IEC 17025 accreditation (Table 1). The proximate analysis includes moisture content of the sample as received and the remaining dry matter divided into the ash (mineral) content, the volatile content and the organic matter content

(also referred to as free carbon). Finally, the high heating value (HHV) based on complete combustion of the sample to carbon dioxide and liquid water is given. The actual carbon content as total C (% in dry sample) was determined later (Table 2).

Table 2. Properties of biochars produced from different feedstocks or with different temperatures.

Parameter	Unit	1	2 *	3	4	5	6 *	7	8 *
		Tomato		Wood	Pepper	Pepper	Residual wood		
		Beech	Wood	Beech	650 °C	750 °C	Batch 1 **	Batch 2	Batch 3 ***
pH	-	11	12	11	12	12	9.4	9.9	10
EC	dS·m ⁻¹	6.8	13	0.68	9.6	11	0.53	0.61	0.71
NH ₄	mmol·L ⁻¹	<0.1	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
K	mmol·L ⁻¹	49.1	94.3	3.2	61	84.3	3.6	3.6	4.5
Na	mmol·L ⁻¹	0.5	1	0.1	5.1	7.4	0.2	0.2	0.3
Ca	mmol·L ⁻¹	0.3	3	0.3	0.3	0.5	0.2	0.3	0.2
Mg	mmol·L ⁻¹	0.1	<0.1	<0.1	0.2	0.1	0.2	0.2	0.1
Si	mmol·L ⁻¹	0.3	0.3	<0.1	0.1	0.2	<0.1	<0.1	<0.1
NO ₃	mmol·L ⁻¹	<0.1	0.2	<0.1	0.1	0.1	<0.1	0.1	<0.1
Cl	mmol·L ⁻¹	23.6	48.5	0.1	47.8	65.2	0.2	0.4	0.6
SO ₄	mmol·L ⁻¹	10.8	21.4	0.3	4.1	4.3	0.4	0.2	0.2
HCO ₃	mmol·L ⁻¹	3.3	7.2	3.6	18.9	19.5	3.2	3.7	4.3
P	mmol·L ⁻¹	<0.05	<0.05	<0.05	<0.05	<0.05	0.1	<0.05	<0.05
Fe	μmol·L ⁻¹	0.4	0.9	<0.4	<0.4	<0.4	0.5	0.6	0.5
Mn	μmol·L ⁻¹	0.2	<0.1	<0.1	<0.1	<0.1	2.2	0.7	0.2
Zn	μmol·L ⁻¹	0.1	0.1	<0.1	<0.1	<0.1	0.2	0.1	0.1
B	μmol·L ⁻¹	13	9	10	28	35	8	7	6
Cu	μmol·L ⁻¹	0.1	5	<0.1	0.2	0.1	<0.1	<0.1	<0.1
Mo	μmol·L ⁻¹	0.5	0.9	<0.1	<0.1	<0.1	<0.1	0.10	<0.1
Dry weight	%	96.7	97.1		95.5	95.5	97.8		
Bulk density	kg·m ⁻³	131	113		104	129	102		
SSA	m ² ·g ⁻¹ d.m.	59	81		39	29	119		
Ash	% d.m.	19	28		35	34	13		
total C	% d.m.	77	68		59	59	82		
total H	% d.m.	1.3	1.4		1.2	1.3	1.5		
Total N	% d.m.	0.7	1.0		0.8	0.9	0.8		
C/N ratio	mol·mol ⁻¹	128	79		86	76	119		

Nutrient concentrations and pH were determined in a 1:1.5 v/v water-extract. d.m. stands for dry matter. * Wood cuttings from forestry Purmerend; ** Used in greenhouse experiment; *** Used in climate chamber experiment.

2.2.2. Water Holding Capacity

The water retention at different water potentials (expressed as suction forces) was determined with the sand box method, using a suction device which can be set to a series of standard suction forces [43]. The method allows prediction of the water content in field circumstances as well as comparing various rooting materials. First, the samples were nearly saturated (−3 cm). Thereafter, the water potential was decreased to −31.5 and −50 cm water column and the water content was measured when equilibrium was established. The drying steps were followed by a rewetting step in which the sample was again brought to near-saturation in order to determine the effect of the drying on water uptake.

2.2.3. Chemical Characterization of Biochars

Water-extractable nutrients were determined in a water extract with a sample-solution ratio of 1:1.5 v/v [45]. Extraction after dilution is the accepted method to find plant available elements when extraction by suction or pressure is not applicable. The exchangeable cations were extracted with concentrated BaCl_2 [46,47]. Water-extractable nutrients and exchangeable cations were analyzed with ICP by a commercial laboratory (Groen Agro Consult, Delft, The Netherlands).

2.2.4. Acid-Neutralizing Capacity

The acid-neutralizing capacity of the biochar was determined by a method using titration with acid with time-stepped acid addition to allow for dissolution and reaction kinetics [48]. The method uses an automated titration unit (Metrohm, Schiedam, The Netherlands). Concentrated acid (HCl) was dosed for five minutes followed by an equilibration period of 45 min without acid dosing. During dosing, acid was added until a pH of five was reached. During equilibration, the pH slowly increased due to buffering of the medium. This procedure was repeated ten times. The titration took therefore nine hours and was performed in duplicate. The total amount of acid dosed during the titration was thought equal to the acid-neutralizing capacity of the rooting medium expressed in $\text{mol}\cdot\text{kg}^{-1}$. The same procedure was used to determine the base neutralizing capacity of peat by using concentrated base (KOH) instead of acid.

2.2.5. Phytotoxicity Test

Phytotoxicity was tested using water extracts (1:2 volume ratio) from the biochars as received (without any pre-washing), using an established plant response method for rooting material quality [49,50]. Water-extracts were filtered (8 μm paper, Merck, Schiphol-Rijk, The Netherlands). Thereafter, the pH values of the extracts were adjusted to pH 5.5 using concentrated nitric acid, the EC values were adjusted to $2\text{ dS}\cdot\text{m}^{-1}$ by dilution with demi water, and nutrients (NPK) were added to reach the required standard concentrations [50]. The bioassays were carried out using: *Sorghum saccharatum* (L.) Moench (sorghum), *Lepidium sativum* L. (garden cress) and *Sinapis alba* L. (mustard). For each treatment, 4 plates per plant species with 10 seedlings per plate were prepared, i.e., 120 seedlings per treatment. After incubation for 3 days at $25\text{ }^\circ\text{C}$, in darkness, photos of the plates were taken. The number of germinated seedlings was counted and root- and shoot length of seedlings were measured. Statistical analyses on the data were performed in GenStat (ANOVA).

2.2.6. Oxygen Uptake Rate

The oxygen uptake rate (OUR) method determines the maximum oxygen uptake rate measured under conditions ideal for microbial degradation of organic matter [51]. This method results in a value for the maximum possible breakdown rate of the rooting material tested and allows ranking of materials in classes for stability over time. Media were mixed with a nutrient solution ($4.3\text{ g}\cdot\text{L}^{-1}\text{ NH}_4\text{Cl}$, $5.4\text{ g}\cdot\text{L}^{-1}\text{ CaCl}_2\cdot 2\text{H}_2\text{O}$, $4.3\text{ g}\cdot\text{L}^{-1}\text{ MgSO}_4\cdot 7\text{H}_2\text{O}$, $0.03\text{ g}\cdot\text{L}^{-1}\text{ FeCl}_3\cdot 6\text{H}_2\text{O}$, $5.0\text{ g}\cdot\text{L}^{-1}\text{ EDDHA}$ 6% iron chelate, $1.4\text{ g}\cdot\text{L}^{-1}\text{ MnSO}_4$, $1.1\text{ g}\cdot\text{L}^{-1}\text{ ZnSO}_4$, $4.2\text{ g}\cdot\text{L}^{-1}\text{ Na}_2\text{B}_4\text{O}_7$, $0.2\text{ g}\cdot\text{L}^{-1}\text{ CuSO}_4$, $0.13\text{ g}\cdot\text{L}^{-1}\text{ Na}_2\text{MoO}_4$, $1\text{ mL}\cdot\text{L}^{-1}\text{ HCl}$ (36%)) to ensure that microbial activity was not limited by nutrients or moisture. The pH of the medium-nutrient mixture was adjusted to pH 5.5 and a nitrification inhibitor, allylthiourea (ATU), was added. The rooting media were put in a closed vessel and placed on a horizontal shaker (150 rpm) for five days at a temperature of $30\text{ }^\circ\text{C}$. The pressure in the vessels was continuously measured over the course of the incubation period. CO_2 was scrubbed from the gas phase. Therefore, the decrease in pressure was completely attributed to consumption of O_2 . The O_2 concentration was plotted as a function of time and the maximum O_2 consumption rate (dO_2/dt) was derived from this graph. The results are expressed as oxygen consumption per unit of time per mass of dry organic matter ($\text{mmol}\cdot\text{h}^{-1}\cdot\text{kg}^{-1}\text{ DOM}$).

2.3. Climate Chamber Experiment with *Gerbera* and Powdery Mildew

In a pot experiment in a climate chamber, the direct (inherent) effect of biochar addition on the induction of plant resistance in young *Gerbera jamesonii* plants was tested. After three weeks challenging time with biochar, the plants were artificially infected with powdery mildew (*Erysipheles*) and monitored on disease development. Powdery mildew is a bio-trophic fungus that feeds on living plants. The biochar used was beech wood derived (Table 1, code 8).

2.3.1. Treatments

Four treatments, with 20 plants each were included in the climate chamber experiment:

- A. Standard rooting medium (milled white peat)
- B. Standard rooting medium with 20% v/v biochar
- C. Standard rooting medium + fungicide (0.1% triflumizool)
- D. Standard rooting medium + SAR elicitor (chemically induced disease resistance)

In treatment B, the rooting medium was produced by mixing 3 L of biochar with 2 L of acid peat in order to neutralize the high pH of the biochar. This neutral peat/biochar blend was then mixed with standard peat medium to achieve the proper treatment ratio. The volume of biochar in the final rooting medium was 20% v/v. In treatment C, the chemical fungicide applied was Rocket which is triflumazool at 0.1% w/w (Certis Europe, Utrecht, The Netherlands). In treatment D the chemical elicitor used was INA which is 2,6-dichloroisonicotinic acid (Sigma-Aldrich, St. Louis, MO, USA). INA is a Systemic Acquired Resistance (SAR) elicitor that induces disease resistance against powdery mildew through stimulation of natural defense processes in the plant (i.e., activation of the salicylic acid route).

The experiment was performed in a climate chamber set at temperature 20 °C; relative humidity 85%; light duration 16 h; light level 240 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR light. After three weeks, plants were infected with a spore suspension of powdery mildew spores derived from infested *Gerbera* plants (2×10^4 spores per mL).

2.3.2. Assessment and Harvest

Two weeks after the first symptoms of mildew infection appeared the mildew was scored per plant with an index made by Spencer [52]. After five weeks, a plant health assessment was performed and the leaf chlorophyll content was measured with a SPAD 502 Plus meter (Konica Minolta Business Solutions Europe, Langenhagen, Lower Saxony, Germany). After six weeks, plants were harvested and fresh weights were analyzed.

2.4. Greenhouse Experiment with Added Microorganisms and *Chrysanthemum*

A potting soil mix with biochar, behaving equally well as a pure peat based medium, would already establish biochar as a peat alternative. However, an additional claim on benefits for plant health would be even more convincing. Such claims could be based on either direct effects of biochar itself, suppressing a particular pathogen, or on indirect effects of biochar, such as the promotion of beneficial microorganisms which then suppress pathogen activity. A greenhouse experiment was performed to assess whether the addition of biochar to peat affects plant growth and plant health. More specific, it was tested whether (1) biochar addition enhances the ability of *Chrysanthemum* \times *morifolium* cv Euro to suppress diseases caused by the wilting pathogen, *Fusarium oxysporum*; and whether (2) addition of rhizobacteria spp to the rooting medium enhances the colonization of biochar with bacteria followed by suppression of *Fusarium* incidence.

2.4.1. Treatments

Four treatments, with 30 plants per repetition were included in the greenhouse experiment:

- A. Peat
- B. Peat + plant-growth promoting rhizobacteria
- C. Peat + biochar
- D. Peat + plant-growth promoting rhizobacteria

Treatments A, B and D received Baltic white peat (Jiffy Substrates, Dordrecht, The Netherlands), limed to pH 5.0 with $4 \text{ kg} \cdot \text{m}^{-3}$ calcium magnesium carbonate.

Treatment C received a mix of the same but unlimed peat and biochar-6 in an 85%:15% v/v ratio limed to pH 5.0 with $2 \text{ kg} \cdot \text{m}^{-3}$ of calcium-magnesium carbonate. The biochar-6 used was delivered in two batches, which were mixed in a 1:1 ratio (Table 1; ECN, Petten, The Netherlands). The mixing ratios above had been established in a preliminary mixing experiment. NPK 12-14-24 fertilizer including trace elements was added to all treatments at $1 \text{ kg} \cdot \text{m}^{-3}$ (PG-mix, Yara, Vlaardingen, ZH, The Netherlands). The rooting media were prepared two weeks before the start of the experiment. The pH and nutrient content were analyzed shortly before the start of the experiment.

All treatments were infected with the plant-pathogen *Fusarium oxysporum* isolated from chrysanthemums. *Fusarium* was added on day 14 of the experiment in a concentration of 10^4 cfu per pot (cfu = colony forming units per g dry matter). Because there were only minor indications of infection, *Fusarium* was added again in a concentration 10^4 cfu per pot on day 49.

Treatment B and D were treated with a commercial product, Compete Plus which is a mix of organisms that promote plant-growth and/or increase disease suppression (Plant Health Cure B.V, Oisterwijk, The Netherlands). Compete Plus claims to contain $>5 \times 10^7$ cfu of each of 6 *Bacillus* strains (*licheniformis*, *megaterium*, *polymyxa*, *pumilus*, *subtilis*, *azotofixans*) as well as 1×10^7 cfu of *Trichoderma harzianum* and 1×10^6 cfu *Streptomyces griseoviridis* as well as various organic feed supplements. Rhizobacteria were added every two weeks, totaling four applications per pot and starting one day before planting the chrysanthemums. For the first addition, each pot received 100 mL of solution prepared with $1 \text{ g} \cdot \text{L}^{-1}$ Compete Plus. For the latter additions, each pot received 50 mL of solution prepared with $0.4 \text{ g} \cdot \text{L}^{-1}$ Compete Plus. The rhizobacteria were poured on top of the rooting medium in order to ensure they spread throughout the whole pot volume.

Each treatment was repeated 6 times in fields of 30 plants, i.e., 180 plants per treatment, 720 in total. The fields were distributed over 6 blocks, each block having all four treatments. Within a block there were 2 sub irrigation tables with each two treatments. Treatments without rhizobacteria (A and C) and with rhizobacteria (B and D) were kept on separate sub irrigation tables and received water from separated storage tanks to prevent spreading of rhizobacteria. For each block, 14 plants were guard plants and observations were restricted to the 16 remaining plants.

2.4.2. Cultivation

The pot-experiment was performed with chrysanthemums which were reproduced by stem cuttings. Ten days after planting the unrooted cuttings on a standard peat medium, the plants had formed a sufficient rooting system and were transplanted into the final pots. Plants were grown in containers of 0.7 L. For the first two weeks, plants were irrigated manually on top of the containers to ensure mixing of the rhizobacteria throughout the containers. From day 14 onwards, plants were irrigated from below by 15 min flooding cycles every one or two days, depending on weather conditions. After 70 days, plants were harvested and dry weight, fresh weight and nutrient content were determined.

2.4.3. Bacterial Analysis

Abundance of bacteria and fungi in the media was checked with semi quantitative PCR technique (qPCR) with primers sets, and 338F and 518R and 5.8s and ITSrev for bacteria and fungi respectively. Additional qPCR was performed to establish the numbers of *Firmicutes* (bacterial phylum to which *Bacillus* species belong). At the end of the greenhouse experiment, three mixed rooting media samples of each treatment were collected by mixing rooting medium from 5 pots and manually removing plant roots. After manual homogenization of the sample by sieving them through 2 mm sieve, 3 subsamples were collected for DNA isolation. DNA was isolated using the commercially available PowerSoil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA, USA) according to manufacturer's protocol. qPCR was performed using SYBR Green chemistry (Promega Inc., Leiden, The Netherlands).

3. Results

3.1. Suitability of Biochars as a Rooting Medium

This section will show the results on the physical, chemical and biological characterization of the various biochars and will interpret these results in terms of suitability of biochar as a rooting medium.

3.1.1. Physical Properties

The biochars produced had low dry bulk densities 100–130 kg·m⁻³ (Table 2). The low dry bulk densities were related to very high total pore space 92%–94% v/v. Low dry bulk density and high total pore space are hallmarks for established potting soil constituents like peat and coir which also combine high water contents with air contents >15% v/v. The ash content of tomato or sweet pepper derived vegetable waste biochar was 34%–35% w/w, reflecting the high content of mineral nutrients of horticultural crops. The wood biochar sample showed only 10% w/w of ash and the wood/vegetable biochar mixes were in between. The volatile matter of the pepper based biochars in the proximate analysis was 14.7%–15.1% w/w which is higher than the amount of volatiles found in the wood biochars (6.0%–10.2% w/w) with the wood/tomato mixes in between (Table 1). The biochars produced showed high organic carbon contents, 59%–82% w/w (Table 2). The low hydrogen and nitrogen contents of <1.5% w/w reflect the low oxygen level in the gasification. Another apparent difference between biochar produced from wood and vegetable waste is the specific surface area, which is 2 to 3 times higher for biochar produced from wood compared to vegetable waste. A high specific surface area facilitates water uptake and the establishment of microbial life on biochar particles. Two possible explanations of the reduced specific surface area found in sweet pepper based biochar are: (1) Clogging of internal pores by volatile tars [34]; (2) Low temperature melting of ash compounds with high levels of calcium and potassium, which is in line with slagging reported for such samples [41].

3.1.2. Water Holding Capacity

The water holding capacity was measured at different suction values to represent the wetting and drying cycles that occur in rooting media during plant growth. Even under saturated conditions (i.e., after watering), rooting media need to contain a sufficient volume of air (>15%) to prevent anaerobic conditions [53]. Peat has a water holding capacity ranging from 74% to 35% v/v at suction forces of –10 cm respectively –50 cm (Figure 1a). The water holding capacity of the biochar (produced from wood chips) is much lower and ranges from 56% to 25% v/v (Figure 1b). Biochar consists of relatively coarse particles of 3–4 mm. The pores in between these particles are too large to retain moisture. Therefore, the 100% v/v biochar sample has a poor water holding capacity. However, once the biochar is mixed with peat, such pores are filled and the water holding capacity is similar to the water holding capacity of the peat alone (Figures 1 and 2), just as has been reported on perlite-peat mixes [54]. Under nearly saturated conditions, the peat and peat-biochar mixture contain at least 20% v/v air meaning that the rooting media contain a sufficient amount of oxygen. For both biochar and peat, less than 10% v/v of the material volume is occupied by solid particles showing the porous

nature of both materials. To conclude, a peat/biochar mixture containing 15% v/v biochar has an almost similar water holding capacity as peat and is in that aspect suitable for application as a rooting medium in horticulture.

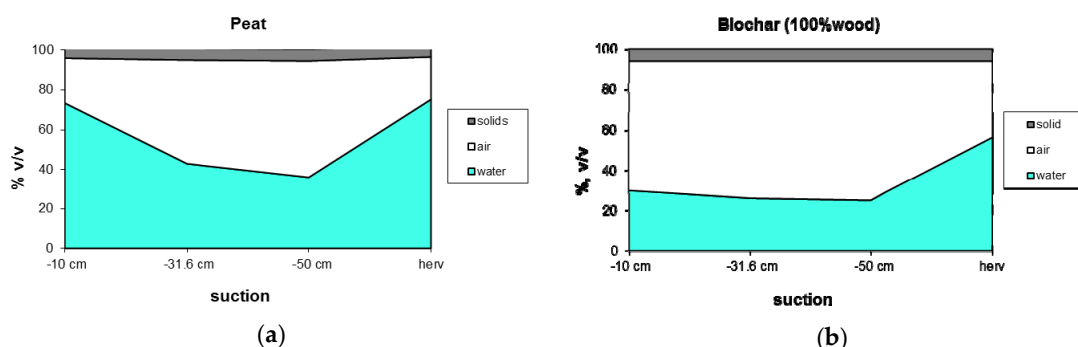


Figure 1. Water content of peat (a) and 100% v/v biochar from wood chips (b) in % v/v as a function of suction strength in cm water column. The water holding capacity of 100% v/v wood biochar of 3–4 mm grains is low compared to peat and too low to ensure sufficient water supply to plants. The higher water-content after re-saturation compared to the initial situation is partly explained by re-arrangement of the biochar particles leading to an increase in the presence of small pores.

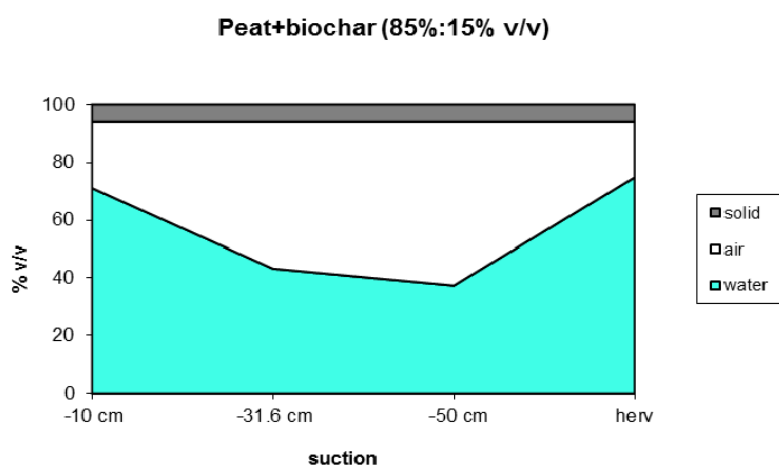


Figure 2. Water content in % v/v of a peat/biochar mixture (85%:15% v/v) as a function of suction strength in cm water column. The biochar particles are 3–4 mm and are produced from wood chips. The water holding capacity of the peat/biochar mixture is similar to the water holding capacity of peat because the large pores in between the biochar particles are now filled with peat. Based on the water holding capacity, the peat-biochar mixture is suitable for application in horticulture.

3.1.3. Chemical Composition and Nutrient Values of Biochar

The high pH values (9.4–12) and high ECs (6.8–13 dS·m⁻¹) of the biochars based on vegetable waste, are problematic for application in horticulture since a pH of about 5.5 and an EC value below 1 dS·m⁻¹ are required for growing pot plants (Table 2). The results also show that the EC of the biochar is about 20 times higher when using vegetable waste as feedstock compared to wood materials. Two of the tested biochars were produced from feedstock that contained 80% v/v wood and only 20% v/v tomato leaves. The EC of these biochars is still considerably higher than the biochars produced from 100% wood. The addition of small amounts of nutrient-rich waste materials to the feedstock thus has a strong negative effect on biochar quality. Biochar produced from wood (beech or residual wood) has a low EC value (<0.6 dS·m⁻¹), low Na and SO₄ concentrations (<0.5 mmol·L⁻¹) and somewhat lower pH values (9.4–11). The EC value of the wood based biochar remains below the maximum EC value

for rooting media. However, the pH value is still much higher than the desired pH range (pH 5.0–6.0) meaning that the biochar should be mixed with other acidic media or additives to produce a mixture with the desired pH.

Biochars do not deliver substantial amounts of plant nutrients as measured with the 1:1.5 extraction, except for potassium. The potassium levels however, especially in the vegetable residues, are up to ten times higher than the desired $3\text{--}5\text{ mmol}\cdot\text{L}^{-1}$ and may induce shortages of other elements by oversupply. Biochars based on vegetable residues also contain unwanted elements such as Na, Cl, SO_4 and HCO_3 in quantities high enough to reduce growth at dosages of over 10% v/v.

The concentrations of exchangeable cations are about $10\text{ meq}\cdot\text{L}^{-1}$ (about $100\text{ meq}\cdot\text{kg}^{-1}$) which is of limited practical consequence even though the cation exchange complex is low in Na and rich in K (33%), Ca (33%) and Mg (28%), which are all plant nutrients. The potassium will be readily exchanged for calcium, which is routinely handled by potting soil producers preparing mixes, i.e., of no consequence for the grower.

Because of the very high C/N ratio of biochar (65–110), mineralization of biochar will at first lead to N immobilization rather than N mineralization. The high C/N ratio also indicates a material as stable as peat.

3.1.4. Acid-Neutralizing Capacity

In order to produce a biochar/peat mixture with a neutral pH (pH 5), common for horticultural cultivation, the acid-neutralizing capacity of the biochar and the base-neutralizing capacity of the peat were determined. For this test, biochar made from wood chips was used. The results of the acid titration of biochar (Figure 3) show that biochar has a high acid-neutralizing capacity and that 258 mmol H^+ per kg dry matter are needed to bring the pH of biochar to pH 5.0 (Table 3). The titration curve shows that multiple acid dosages were needed to reduce the pH to the desired level due to the rather slow buffering of the biochar (Figure 3). Similar slow buffering processes occurred when biochar was mixed with acid peat and the final pH of the mixture could only be established after an equilibration period of at least 7 days.

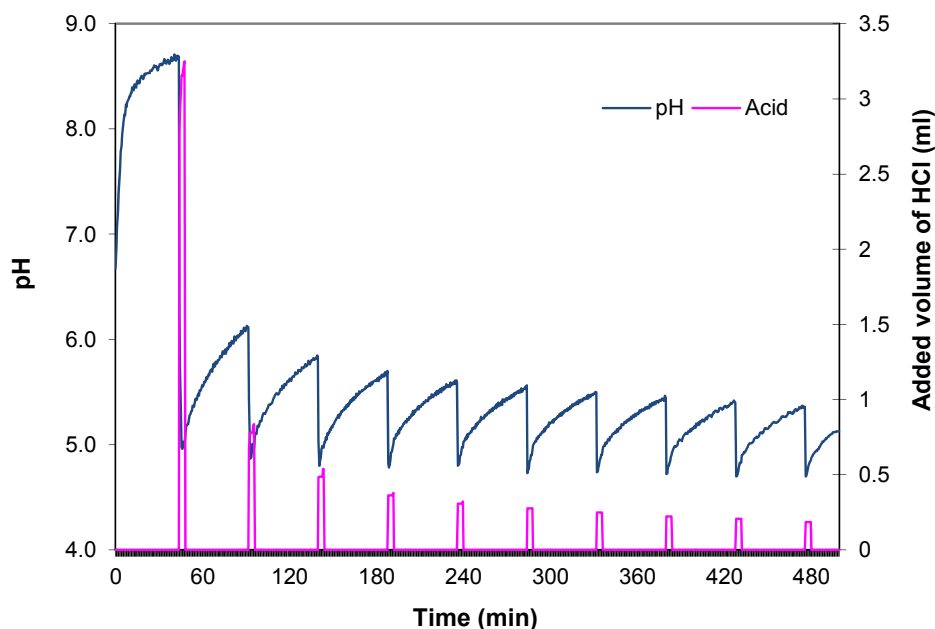


Figure 3. Acid neutralizing capacity of biochar from wood chips as determined by titration. The blue line is the pH; the pink spikes are the acid dosage.

Table 3. Acid buffering capacity of biochar and base buffering capacity of peat, determined by titration¹, expressed per unit weight of dry matter (d.m.) and per unit of volume.

Medium	Acid Buffering Capacity		Base Buffering Capacity	
	mmol·kg ⁻¹ d.m.	mol·m ⁻³	mmol·kg ⁻¹ d.m.	mol·m ⁻³
Biochar (wood chips)	258	26	-	-
Peat ²	-	-	90	9

¹ Titration with a titrino using concentrated HCl or KOH; ² Acid milled white peat, no fertilizer or calcium-carbonate added.

The acid neutralizing capacity of biochar (258 mmol·kg⁻¹ dry matter) is 2.9 times higher compared to the base neutralizing capacity of peat (90 mmol·kg⁻¹ dry matter) (Table 3). For horticultural purposes mixing ratios are based on a volume basis using the bulk density of both materials, biochar (102 kg·m⁻³) and peat (91 kg·m⁻³), which changes the ratio very slightly to 3.2. This means that mixing 24 kg of biochar and 76 kg of acid peat will give a mixture with a neutral pH (Table 4).

Table 4. Bulk density and volumetric contribution of solid particles and pores in peat, biochar and peat-biochar mixture¹.

Substrate	Bulk Density	Solid Fraction	Total Pore Space
	kg·m ⁻³	% v/v	% v/v
Biochar (wood chips)	100 ± 2.6	6.2 ± 0.17	93.8 ± 0.17
Peat ²	91 ± 0.87	5.8 ± 0.06	94.2 ± 0.06
Peat/biochar mixture (85%:15%)	91 ± 1.7	5.8 ± 0.11	94.2 ± 0.11

¹ Data collected for determination of pF curves; ² Acid milled white peat, no fertilizer or calcium-carbonate added.

It is usual in this stage to compensate for interstitial filling of the mix, a density increase of the mix caused by small particles filling up larger pores. Interstitial filling up to 20% v/v is common in rooting media and increases production costs. In this case, the dry bulk density and total pore space of the separate peat and biochar and the 85%/15% v/v mixed material remained similar (Table 4). It was therefore concluded that interstitial filling was negligible. This was somewhat unexpected as the high water content of a peat/biochar mix 85%/15% v/v in Figure 2 seemed to indicate some degree of interstitial filling.

3.1.5. Phytotoxicity

The results of the phytotoxicity test (Figure 4) provide insight into the presence of toxic compounds that affect germination and early growth of seedlings. The tested biochar materials were produced from three different feedstocks; sweet pepper waste, wood chips and a mix of tomato leaves and wood chips. The biochar produced from sweet pepper waste performed rather well in the phytotoxicity test (Figure 4) as there was no significant difference in germination and early growth of seedlings as expressed by the observed root and shoot length compared with the control treatment. In contrast, the biochars produced from wood and tomato leaves showed significant (above 20%) adverse effects on root and shoot development indicating that this biochar contains water-soluble phytotoxic compounds. The biochar produced from 100% wood chips is not as phytotoxic as the wood/tomato leaf biochar, but still a significant reduction in root length of *Sorghum* was found, indicating that even the biochar from 100% wood chips is not completely free of toxic compounds. The presence of phytotoxic compounds is a point of concern. Prior research showed that toxicity is caused by condensation of volatile tar components in the cooler parts of the product [41]. Early separation of solid product and fumes is supposed to prevent this type of problem so further production optimization is called for.

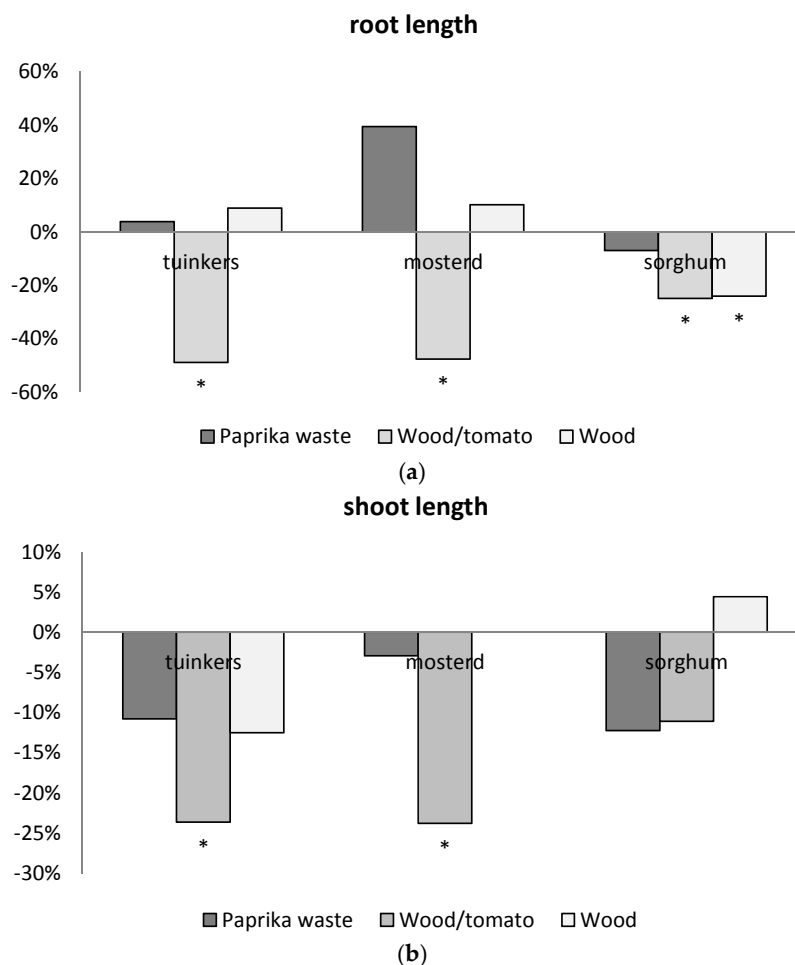


Figure 4. Average difference in root (a) and shoot length (b) between treatments and the control in the phytotoxicity test for three biochars produced from sweet pepper waste, wood and tomato leaves, and wood chips only. The asterisks denote a statistically significant reduction in root or shoot length at $p = 0.05$.

3.1.6. Stability

The oxygen uptake rate (OUR) is a measure for bacterial degradation under ideal conditions (i.e., ideal pH, EC and sufficient amounts of nutrients present). The result is reported as mmol oxygen used by bacteria per hour per kilogram of dry organic matter (DOM). The OUR of biochar is very low ($2.5 \text{ mmol} \cdot \text{h}^{-1} \cdot \text{kg}^{-1} \text{ DOM}$) and almost as low as the OUR of peat (Table 5). This is an indication the biochar is very stable. A low OUR is beneficial for application in horticulture because it means that degradation of the material, and consequently shrinkage of the medium, will not occur. A consequence of the low OUR is that biochar is a poor source of carbon for micro-organisms. Thus, if one aims to stimulate microbial activity in potting soil, an additional carbon source like fresh wood fibre may be needed.

Table 5. Oxygen uptake rate (OUR) of biochar and peat in $\text{mmol} \cdot \text{h}^{-1} \cdot \text{kg}^{-1}$ dry organic matter.

Material	OUR Value
Biochar Woodchips	2.5
Peat	1.9
Compost (for reference)	5–10

3.2. Effect of Biochar on Plant Growth and Powdery Mildew Infection of *Gerbera*

A pot experiment was performed with *Gerbera* plants cultivated on rooting media with and without beech wood chip biochar in a climate controlled room (20 °C, RH 80%, light:dark period, 16 h:8 h). Plants were infected with powdery mildew to assess the effect of biochar addition on the ability to induce plant resistance and thereby suppress powdery mildew infection.

3.2.1. Effect of Biochar Addition on Rooting Medium Quality

The pH in the rooting medium with 20% v/v biochar was higher than the pH in the 100% v/v peat medium (Table 6), which shows that the amount of acidic peat used to neutralize biochar was not yet sufficient. This is consistent with the result of the neutralization test, which showed the test's duration is not long enough to cover the reaction time of biochar.

Table 6. Nutrient concentrations at the end of the cultivation period, including pH and EC of peat and peat with 20% v/v biochar ¹.

Parameter	Unit	Medium	Medium
		Peat	Peat + Biochar
pH	-	5.3	6.4
EC	dS·m ⁻¹	0.65	0.54
NH ₄	mmol·L ⁻¹	<0.1	<0.1
K	mmol·L ⁻¹	2.3	2.4
Na	mmol·L ⁻¹	0.3	0.4
Ca	mmol·L ⁻¹	0.7	0.4
Mg	mmol·L ⁻¹	0.5	0.3
Si	mmol·L ⁻¹	<0.1	<0.1
NO ₃	mmol·L ⁻¹	2.7	0.6
Cl	mmol·L ⁻¹	0.1	0.1
SO ₄	mmol·L ⁻¹	0.8	1.1
HCO ₃	mmol·L ⁻¹	<0.1	<0.1
P	mmol·L ⁻¹	0.65	0.65
Fe	μmol·L ⁻¹	4.7	2.8
Mn	μmol·L ⁻¹	0.4	0.2
Zn	μmol·L ⁻¹	0.8	0.8
B	μmol·L ⁻¹	6	8
Cu	μmol·L ⁻¹	0.2	0.4
Mo	μmol·L ⁻¹	0.1	0.2

¹ Measured in a 1:1.5 v/v soil/water extract. Biochar was produced from beech wood.

At the end of the pot experiment, the nitrate concentration was much lower in the rooting medium with biochar compared to the standard peat medium. This indicates nitrogen immobilization by microbial life involved in the degradation of biochar. Degradation of biochar was expected because of the high C:N ratio of biochar (>65, Table 2) which is much higher than the C:N ratio needed for bacterial growth. The amount of nitrogen immobilization is acceptable for practical purposes as it can easily be compensated by addition of extra nitrogen in the form of calcium-nitrate. Even though plant yield and leaf chlorophyll content were not visibly affected, the amount of nitrogen immobilization must be known to potting soil producers in order to allow for exact compensation.

3.2.2. Effect of Biochar Addition on Plant Growth and Disease Suppression

The fresh weight of *Gerbera* plants was similar for plants grown on 100% v/v peat than on the peat-biochar 80%–20% v/v after six weeks of cultivation (Figure 5). Thus, biochar addition did not lead to any phytotoxic or other growth-reducing effects. Fresh weight of the plants receiving SAR elicitor, a product to chemically induce disease resistance, was lower compared to other treatments.

This product is known to cause a reduction in yield in *Gerbera* plants but is used as a positive control on induction of plant hormones.

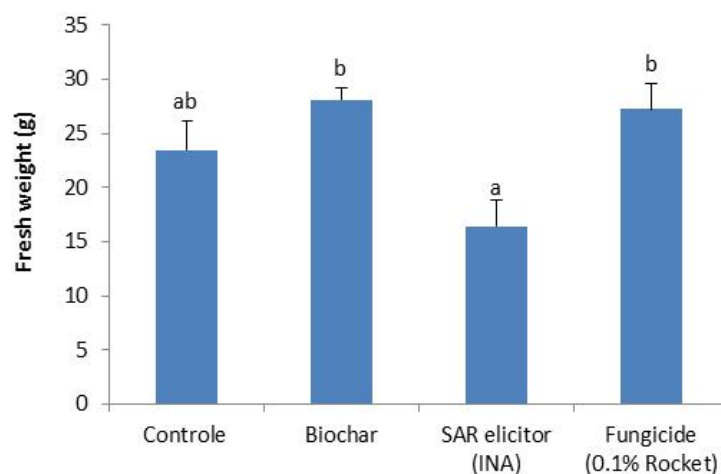


Figure 5. Average fresh weight of *Gerbera* after six weeks. No significant effects of biochar addition were found compared to the untreated plants (Tukey's test, $p < 0.05$). Treatments which do not share the same letters a or b differ significantly (Tukey's test, $p < 0.05$).

Nineteen days after inoculation with mildew, plants were assessed for symptoms of powdery mildew infection (Figure 6). *Gerberas* cultivated on peat and peat/biochar showed symptoms of powdery mildew infection and the area of leaf spots was similar for both treatments. Thus, biochar addition does not affect the level of severity of powdery mildew infection. Generally, the severity of infection with powdery mildew was rather low since less than 5% of the plant surface was affected. Other tests with gerbera plants showed that infection is more efficient at lower relative humidity (<85%). As mildew symptoms were not reduced, the plant material was not further analyzed on increased levels of plant hormones related with systemic acquired resistance. Therefore a systemic effect on disease resistance against biotrophic pathogens, e.g., powdery mildew, was neither proven nor disproven for the wood biochar tested.

Overall, it can be concluded that cultivation of *Gerbera* on media containing 20% v/v of biochar was successful and gave similar yields as compared to the standard peat medium.

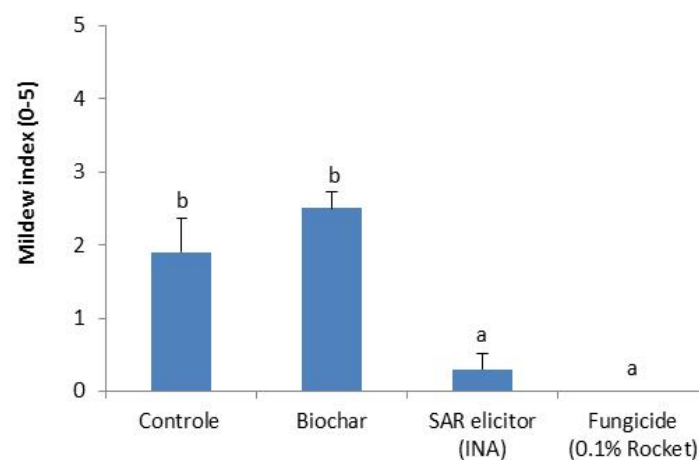


Figure 6. Symptoms of powdery mildew infection (mildew index) on *Gerbera* plants, 19 days after inoculation. Symptoms were quantified on a scale between 0 and 5 (0 = 0, 1 = 0.1%–2%, 2 = 2%–5%, 3 = 5%–20%, 4 = 20%–40%, 5 = >40%). Treatments which do not share the same letters a or b differ significantly (Tukey's test, $p < 0.05$).

3.3. Effect of Biochar and Added Microorganisms on Plant Growth of *Chrysanthemum*

A pot experiment was performed with *Chrysanthemum* plants cultivated on rooting media with and without biochar in a greenhouse setting. Plants were infected with *Fusarium* and the rooting media were pre-treated with a mix of beneficial microorganisms to assess the effect of biochar addition on the ability of beneficial microorganisms to suppress *Fusarium* infection.

3.3.1. Effect of Biochar Addition on Rooting Medium Quality

EC, pH and elemental composition including nitrate of the mix after cultivation resembled that of the reference, peat only, with the exception of potassium, which is 25% higher in the biochar mixtures because of the high level in the biochar itself (Table 2). This means the addition of 20% v/v of biochar did not require adjustments in the fertilization scheme other than the prior neutralization of alkalinity.

At the start of the experiment, the dissolved organic carbon (DOC) concentration was measured in peat (1392 mg·kg⁻¹) and in the peat/biochar 80%/20% v/v mix (723 mg·kg⁻¹). The level of DOC in peat is caused by naturally occurring organic acids. Addition of the biochar leads to a marked decrease in the DOC concentration, indicating adsorption of organic acids on biochar. This mechanism might be used to load biochar with digestible compounds to support microorganisms.

3.3.2. Effect of Biochar Addition on Plant Growth and Added Microorganisms

No effect of treatment on the plant fresh- and dry weight was observed and no differences in nutrient content of the plants were observed. This is consistent with the observation that rooting medium solution pH, EC and nutrient availability are similar in treatments with and without biochar. Thus, for the tested biochar, biochar addition had no consequences for nutrient uptake or plant growth. No effect of *Fusarium* incidence was found meaning that the effect of biochar addition on disease suppression could not be tested.

Numbers of bacteria, based on the copy numbers of 16S gene per gram of rooting medium, did not differ significantly between treatments (Figure 7).

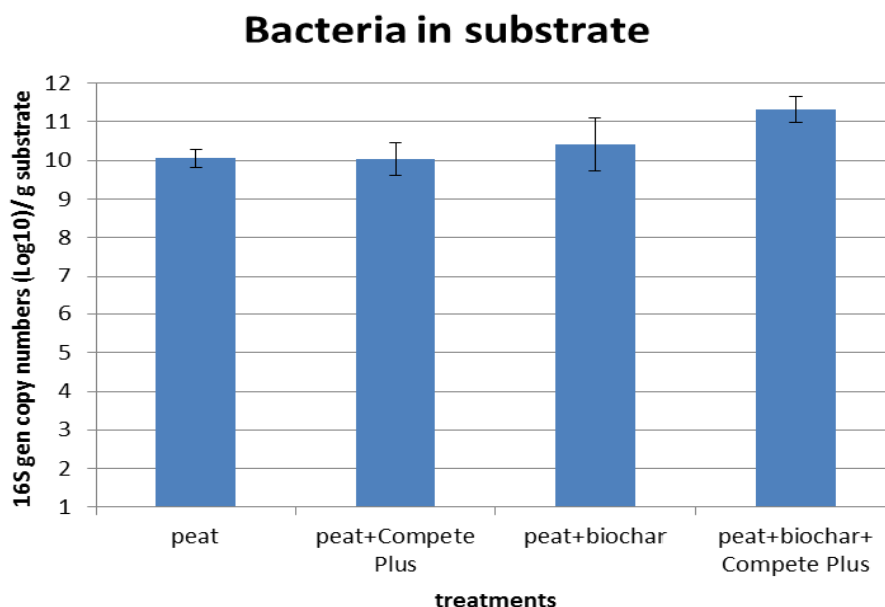


Figure 7. Total Bacteria numbers (on basis of log10 of copy numbers of 16S gene per g dry medium).

Hypothetically, addition of Compete Plus should result in higher number of *Firmicutes* in the studied rooting media with Compete Plus, as *Firmicutes*, i.e., *Bacillus* species, are abundant in Compete Plus. However, the numbers of *Firmicutes* found are not particularly high (Figure 8). We added in total

160 mg of Compete Plus per 700 mL container. With a specified level of about 3×10^8 *Bacilli* per gram Compete Plus, this is about 7×10^5 cfu with a medium of $100 \text{ kg} \cdot \text{m}^{-3}$. The actual numbers were over 100 times lower for all treatments (Figure 8). It is difficult to explain why the numbers of this phylum are so low and presumably have decreased after addition.

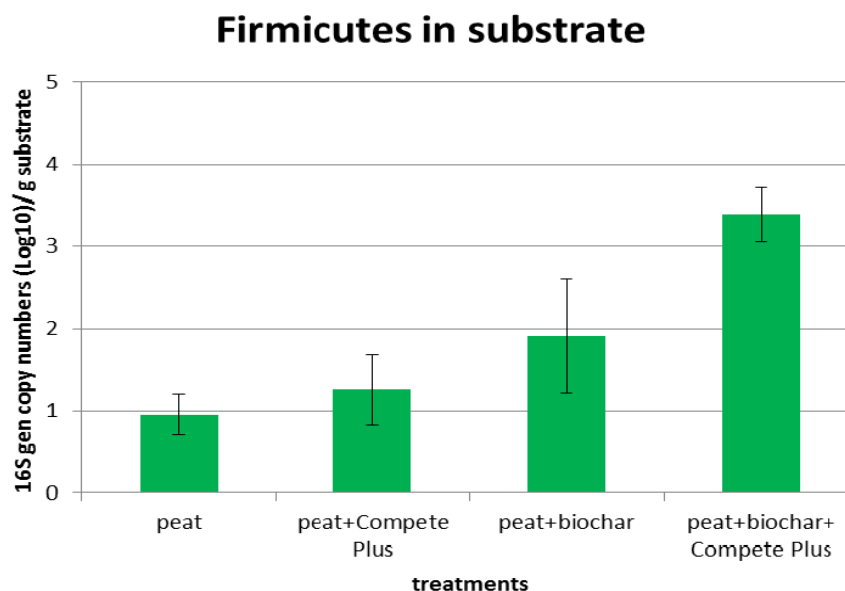


Figure 8. *Firmicutes* numbers (on basis of log10 of copy numbers of 16S gene per g dry medium).

Even so, in the peat+biochar+Compete Plus treatment, the numbers of *Firmicutes* showed a statistically significant higher level compared with the other treatments. This could, arguably, indicate that biochar slows the decline of *Firmicutes* numbers, which remains open for further research.

3.4. Suitability of Feedstocks for Application as Rooting Medium

The biochars based on vegetable waste had to be rejected for further experimentation based on both, a too high level of nutrient elements and a too high alkalinity. Both nutrient level (EC) and alkalinity (pH and buffering capacity) restrict the amounts of vegetable biochar which can be mixed with unlimed acid peat to levels below 10% v/v, which are too low for commercial applications. EC and pH restrictions are valid for rooting media mixes used for protected horticulture but not so much for open field horticulture or agriculture. Open field applications usually use a much smaller volume ratio of biochar to mix with soil, which reduces the effects of salinity and alkalinity. Furthermore, soil can buffer salinity and pH effects much more efficiently than common rooting media. Finally, the nutrient input is a direct substitute for nutrients which open field farmers otherwise have to buy and which represent a large operational cost factor. For protected horticulture growers, nutritional costs are a relatively small factor which plays no role in choosing a rooting medium.

4. Discussion

4.1. Feedstock Related Effects of Biochar

Biochars produced from beech wood or mixed residual wood have a higher specific surface area compared to biochars produced from tomato or sweet pepper waste. This is expected to increase its water retention and to increase the cation exchange capacity (CEC). Increased water content is a positive property if mixtures of >35% v/v biochar are going to be used as these might become too dry on their own. A higher CEC is not valued in professional horticulture as interference of the CEC with the nutrient solutions used, changes the ratios of the elements in the nutrient solutions to undesirable

values. The values for CEC measured here are low enough to allow them to be disregarded in mixtures with <50% v/v of biochar. CEC is thought to be proportional to surface area of the biochar but also the result of negatively charged carboxyl (COO^-) groups. The amount of carboxyl groups depends initially on the feedstock, in which composted leaves are known to have more of these groups per unit weight than woody products.

Biochar produced from source materials containing nutrient-rich biomass (i.e., tomato leaves, sweet pepper waste) appears unsuitable for use as potting soil because of its high salt content and high pH. Even biochar produced from feedstock with only 20% v/v tomato leaves and 80% v/v wood chips is too saline to be used as an ingredient for potting soil. We interpret this as evidence that the cations in the feedstock oxidize into oxides (Na_2O , K_2O , CaO and MgO) during biochar production. As these oxides are readily soluble in water and highly alkaline this explains why feedstock influences EC and pH. It also explains how feedstock can be quantitatively tested prior to use on salinity and alkalinity, notably by measuring the content of alkali metals Na, K, Ca and Mg. Even so there are reports where high EC feedstocks are used for biochar for horticultural purposes [23] in which case the EC was lowered by either prior washing with water or dilution with low EC biochar.

4.2. Production Setting Related Effects of Biochar

The temperature of the production process increases salinity in accordance with the mass loss i.e., the salt ions concentrate in the remaining solids. Conversely, to produce higher amounts of biochar from a mass unit of dry feedstock, the process temperature and oxygen levels should be reduced. Higher process temperatures (>500 °C) and higher process oxygen levels, including the oxygen in the process water vapor, will increase the degree of oxidation of feedstock cations which will increase the pH and pH buffer capacity of the material. The influence of temperature and oxidation level on alkalinity was observed before [34], as was a smaller but still relevant relation of ash content with pH [41]. Generally if nutrient poor feedstock is used the biochar qualities are closer to those of peat.

The amount of carboxyl groups in the end product is influenced by the process parameters' temperature and oxygen level. Higher temperatures and lower oxygen levels reduce the amount of carboxylic groups left in the biochar and therefore presumably reduce the cation exchange capacity.

The surface area of the feedstock is reduced by the level of clogging of smaller pores. Such surface area reduction supposedly by clogging is reported for condensation of volatile tar [34] and may also have been caused by slagging [41]. Slagging arguably is worse in materials with higher amounts of potassium and calcium salts which act as viscosity reductants for the slag.

Condensation of plant toxic volatile tar products is possible over the total temperature range of 200–900 °C [36,55,56], as long as the volatiles are not immediately transported away from the biochar mass, especially during the cooling of the mass.

The particle size of the biochar is a production variable which affects physical and chemical properties of the materials as well as the properties of rooting media which are mixed with a biochar. (1) Physical properties affected are density, specific surface area (SSA) and water retention, which all increase with smaller particle size; and air content, which decreases with smaller particle size [28,56,57]; (2) Chemical properties affected are pH, cation exchange capacity (CEC) and surface adsorption, which all increase with smaller particle size [4,34,58]; (3) Rooting media properties require that the biochar particle size is tuned to an intended application such as coarse particles >4 mm to replace peat fractions or bark; 3–4 mm particles to replace milled peat; or powdered <1 mm particles to replace lime [58]. This demonstrates that even a biochar from one specific feedstock, produced at one set of specific production parameters still can produce disappointing results if the particle size does not match the application. On the other hand, particle size manipulation before the heating process is one of the easier ways to diversify biochar product applications.

4.3. The Survival Rate of Beneficial Microorganisms as Affected by Biochar

In two pot experiments, plants were grown on mixtures of peat and biochar (beech wood respectively residual wood). In both experiments, nutrient availability and plant growth were similar in the biochar treatment compared to the control treatment without biochar with the exemption of a slight decrease in nitrate availability. There were no signs of phytotoxic or growth reducing effects of biochar. Thus, the pot experiments confirm that peat/biochar media, with substantial biochar content (20% v/v), can be used in horticulture without yield reduction and without adjustment of the fertilization scheme, other than a preventive increase in nitrate to counteract nitrogen immobilization.

Results of the pot experiment with *Gerbera* show that the severity of powdery mildew infection is similar for *Gerberas* cultivated on rooting medium with 20% v/v biochar and the control treatment without biochar. Thus, biochar addition does neither decrease nor increase severity of powdery mildew infection in *Gerberas* and a systemic mode of action on disease resistance is not proven for the biochar used. In the experiments with chrysanthemums, no *Fusarium* incidence was found meaning that the effect of biochar addition on disease suppression could not be tested. More research is needed in order to elucidate if, and if so under which conditions, biochar additions can enhance disease suppression of diseases.

The addition of biochar did not increase the overall numbers of micro-organisms in the potting soil. A reason the level of micro-organisms did not respond to biochar addition could be the low level of degradation ($OUR < 3$), showing biochar particles to be a poor environment for micro-organisms needing carbon for growth. Possibly, colonization of biochar can be enhanced by loading the biochar with biodegradable organic acids, which would make biochar a more attractive environment for microbes. This is made more plausible by the observation that 20% v/v biochar can halve the amount of free organic carbon in a peat sample, presumably by absorbing the organic acids. Thus, it may be possible to pre-treat biochar with small organic molecules which can then feed a population of micro-organisms establishing themselves on the biochar particles. This will be the topic for further work.

Our results showed that biochar offered some temporary or more permanent protection to *Firmicutes*, the bacteria which include the plant health supporting *Bacilli*. Whether the level of *Firmicutes* is high enough to influence disease suppression remains to be seen and merits further research.

4.4. Horticultural Perspectives of Biochar

Biochar dry bulk density and total pore space ($100 \text{ kg} \cdot \text{m}^{-3}$ and 93% v/v) are well within the range required for horticultural rooting media.

Peat/biochar mixtures, containing 20% v/v biochar, still have properties (EC, pH, water holding capacity, nutrient availability) similar to standard peat and therefore can be qualified as a proper peat alternative. The water retention of biochars (45% v/v at -10 cm) is about 20% v/v lower than for peat but by no means too dry for optimum growth. The water holding capacity can be improved by using relatively wet rooting materials like peat or coir pith but the water holding capacity of biochar can also be improved by mixing with a finer grade of biochar. The wettability of biochar can be increased by increasing the specific surface area by choosing a feedstock low in nutrients and/or increasing process temperature [34]. It may be of practical interest to note that biochars with higher specific surface areas will reduce the effectivity of some crop protection agents by adsorption [30].

Examples in literature show it is possible to grow plants just as successfully as on standard peat mixtures with 50%–100% v/v rice husk and reed based biochar [24,27]. It is, therefore, expected that the wood based biochars used in this work may be used in ratios up to 50% v/v if the pH is properly neutralized.

Because the pH of most biochars is too high, those biochars have to be acidified to obtain an optimal potting mixture. Most rooting media in their natural i.e., unlimed and unfertilized state are neutral to slightly alkaline (pH 6–7.5). The only exception is peat which can be harvested at pHs as low as pH 3.0–4.0. This leads to this paradox: in order to use biochar as a peat alternative one needs

unlimed and acid peat. The mixing ratio differs depending on the biochar as well as on the peat used but expected maximum biochar levels range from 20%–50% v/v. The pH buffer method described is effective but the method needs to be adapted by an increase of the 16 hours measuring period to allow for the slow reaction of biochar with added acids.

The very low oxygen uptake rate (OUR) of biochar is a positive property for horticultural media since it indicates that biochar is poorly degradable, reducing the need for additional nitrate and reducing the risks of shrinkage of the rooting medium due to microbial degradation of biochar.

However, the presence of some phytotoxic compounds in biochar from wood chips in these experiments, despite that the problem was reported as technically solvable [41], remains a point of concern that needs further attention in process design.

5. Conclusions

The first hypothesis was: (1) biochar is a potting soil constituent which can be used in growing medium mixes in quantities of 20% v/v without negative growth effects. This hypothesis is proven for mixtures up to 20% v/v of wood based biochar and may hold true for mixtures up to 50% v/v, provided pH effects can be compensated. Biochar based on vegetable waste had a high EC and a high alkalinity. These characteristics limit the amount of biochar that can be mixed with unlimited peat, to levels below 10%, which are too low for commercial applications; The second hypothesis was: (2) biochar can induce disease suppression. This hypothesis was not proven. In a climate chamber experiment we found no difference in the powdery mildew infection between peat and peat/biochar mixtures. A greenhouse experiment with *Chrysanthemum* and addition of the pathogen *Fusarium* showed no effects of *Fusarium* on both the peat and the peat/biochar mixtures. It was however found that some added and potentially beneficial microorganisms remained more numerous in a mix of peat with 20% v/v biochar than in 100% v/v of peat.

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References

1. Lehmann, J.; Joseph, S. *Biochar for Environmental Management: Science and Technology*, 2nd ed.; Earthscan: London, UK, 2015.
2. Laird, D.A. The charcoal vision: A win-win-win scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water quality. *Agron. J.* **2008**, *100*, 178–181. [[CrossRef](#)]
3. Atkinson, C.; Fitzgerald, J.; Hipps, N. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: A review. *Plant Soil* **2010**, *337*, 1–18. [[CrossRef](#)]
4. Singh, B.; Singh, B.P.; Cowie, A.L. Characterisation and evaluation of biochars for their application as a soil amendment. *Soil Res.* **2010**, *48*, 516–525. [[CrossRef](#)]
5. Glaser, B.; Wiedner, K.; Seelig, S.; Schmidt, H.-P.; Gerber, H. Biochar organic fertilizers from natural resources as substitute for mineral fertilizers. *Agron. Sustain. Dev.* **2014**, *35*, 667–678. [[CrossRef](#)]
6. Olmo, M.; Villar, R.; Salazar, P.; Alburquerque, J. Changes in soil nutrient availability explain biochar's impact on wheat root development. *Plant Soil* **2016**, *399*, 333–343. [[CrossRef](#)]
7. Akhter, A.; Hage-Ahmed, K.; Soja, G.; Steinkellner, S. Compost and biochar alter mycorrhization, tomato root exudation, and development of *Fusarium oxysporum* f. Sp. *lycopersici*. *Front. Plant Sci.* **2015**, *6*. [[CrossRef](#)] [[PubMed](#)]

8. Nielsen, S.; Minchin, T.; Kimber, S.; van Zwieten, L.; Gilbert, J.; Munroe, P.; Joseph, S.; Thomas, T. Comparative analysis of the microbial communities in agricultural soil amended with enhanced biochars or traditional fertilisers. *Agric. Ecosyst. Environ.* **2014**, *191*, 73–82. [[CrossRef](#)]
9. Elad, Y.; David, D.R.; Harel, Y.M.; Borenshtein, M.; Kalifa, H.B.; Silber, A.; Graber, E.R. Induction of systemic resistance in plants by biochar, a soil-applied carbon sequestering agent. *Phytopathology* **2010**, *100*, 913–921. [[CrossRef](#)] [[PubMed](#)]
10. Nemati, M.R.; Simard, F.; Fortin, J.-P.; Beaudoin, J. Potential use of biochar in growing media. *Vadose Zone J.* **2015**, *14*. [[CrossRef](#)]
11. Nieto, A.; Gascó, G.; Paz-Ferreiro, J.; Fernández, J.M.; Plaza, C.; Méndez, A. The effect of pruning waste and biochar addition on brown peat based growing media properties. *Sci. Hortic.* **2016**, *199*, 142–148. [[CrossRef](#)]
12. Steiner, C.; Harttung, T. Biochar as growing media additive and peat substitute. *Solid Earth Discuss.* **2014**, *6*, 1023–1035. [[CrossRef](#)]
13. Verhagen, J.; van den Akker, J.; Blok, C.; Diemont, H.; Joosten, H.; Schouten, M.; Schrijver, R.; Verweij, P.; Wösten, H. *Climate Change. Scientific Assessment and Policy Analysis: Peatlands and Carbon Flows: Outlook and Importance for the Netherlands*; Wab 500102 027; Netherlands Environmental Assessment Agency PBL: Bilthoven, The Netherlands, 2009.
14. Graber, E.R.; Harel, Y.M.; Kolton, M.; Cytryn, E.; Silber, A.; David, D.R.; Tsechansky, L.; Borenshtein, M.; Elad, Y. Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. *Plant Soil* **2010**, *337*, 481–496. [[CrossRef](#)]
15. Jaiswal, A.; Frenkel, O.; Elad, Y.; Lew, B.; Graber, E. Non-monotonic influence of biochar dose on bean seedling growth and susceptibility to rhizoctonia solani: The “shifted rmax-effect”. *Plant Soil* **2015**, *395*, 125–140. [[CrossRef](#)]
16. Elad, Y.; Cytryn, E.; Meller Harel, Y.; Lew, B.; Graber, E.R. The biochar effect: Plant resistance to biotic stresses. *Phytopathol. Mediterr.* **2012**, *50*, 335–349.
17. De Tender, C.A.; Debode, J.; Vandecasteele, B.; D’Hose, T.; Cremelie, P.; Haegeman, A.; Ruttink, T.; Dawyndt, P.; Maes, M. Biological, physicochemical and plant health responses in lettuce and strawberry in soil or peat amended with biochar. *Appl. Soil Ecol.* **2016**, *107*, 1–12. [[CrossRef](#)]
18. Altland, J.E.; Locke, J.C. Biochar affects macronutrient leaching from a soilless substrate. *HortScience* **2012**, *47*, 1136–1140.
19. Kloss, S.; Zehetner, F.; Wimmer, B.; Buecker, J.; Rempt, F.; Soja, G. Biochar application to temperate soils: Effects on soil fertility and crop growth under greenhouse conditions. *J. Plant Nutr. Soil Sci.* **2014**, *177*, 3–15. [[CrossRef](#)]
20. Dumroese, R.K.; Heiskanen, J.; Englund, K.; Tervahauta, A. Pelleted biochar: Chemical and physical properties show potential use as a substrate in container nurseries. *Biomass Bioenergy* **2011**, *35*, 2018–2027. [[CrossRef](#)]
21. Bedussi, F.; Zaccheo, P.; Crippa, L. Pattern of pore water nutrients in planted and non-planted soilless substrates as affected by the addition of biochars from wood gasification. *Biol. Fertil. Soils* **2015**, *51*, 625–635. [[CrossRef](#)]
22. Dunlop, S.J.; Arbestain, M.C.; Bishop, P.A.; Wargent, J.J. Closing the loop: Use of biochar produced from tomato crop green waste as a substrate for soilless, hydroponic tomato production. *Hortscience* **2015**, *50*, 1572–1581.
23. Vaughn, S.F.; Eller, F.J.; Evangelista, R.L.; Moser, B.R.; Lee, E.; Wagner, R.E.; Peterson, S.C. Evaluation of biochar-anaerobic potato digestate mixtures as renewable components of horticultural potting media. *Ind. Crops Prod.* **2015**, *65*, 467–471. [[CrossRef](#)]
24. Altland, J.E.; Locke, J.C. Gasified rice hull biochar is a source of phosphorus and potassium for container-grown plants. *J. Environ. Hortic.* **2013**, *31*, 138–144.
25. Locke, J.C.; Altland, J.E.; Ford, C.W. Gasified rice hull biochar affects nutrition and growth of horticultural crops in container substrates. *J. Environ. Hortic.* **2013**, *31*, 195–202.
26. Trifonova, R.; Postma, J.; Ketelaars, J.J.M.H.; van Elsas, J.D. Torrefied grass fibres as a substitute for peat in potting soil. Presented at ORBIT Conference, Wageningen, The Netherlands, 13–15 October 2008.
27. Blok, C.; Rijpsma, E.; Ketelaars, J.J.M.H. New growing media and value added organic waste processing. *ISHS Acta Hortic.* **2016**, *1112*, 269–280. [[CrossRef](#)]

28. Quintero, M.F.; Ortega, D.; Valenzuela, J.L.; Guzman, M. Variation of hydro-physical properties of burnt rice husk used for carnation crops: Improvement of fertigation criteria. *Sci. Hortic.* **2013**, *154*, 82–87. [[CrossRef](#)]
29. Singh, B.; Macdonald, L.M.; Kookana, R.S.; van Zwieten, L.; Butler, G.; Joseph, S.; Weatherley, A.; Kaudal, B.B.; Regan, A.; Cattle, J.; et al. Opportunities and constraints for biochar technology in Australian agriculture: Looking beyond carbon sequestration. *Soil Res.* **2014**, *52*, 739–750. [[CrossRef](#)]
30. Graber, E.; Tsechansky, L.; Gerstl, Z.; Lew, B. High surface area biochar negatively impacts herbicide efficacy. *Plant Soil* **2012**, *353*, 95–106. [[CrossRef](#)]
31. Abiven, S.; Schmidt, M.W.I.; Lehmann, J. Biochar by design. *Nat. Geosci.* **2014**, *7*, 326–327. [[CrossRef](#)]
32. Schimmelpfennig, S.; Glaser, B. One step forward toward characterization: Some important material properties to distinguish biochars. *J. Environ. Qual.* **2012**, *41*, 1001–1013. [[CrossRef](#)] [[PubMed](#)]
33. International Biochar Initiative. *Standardized Product Definition and Product Testing Guidelines for Biochar That Is Used in Soil*; International Biochar Initiative: Westerville, OH, USA, 2012.
34. Mukherjee, A.; Zimmerman, A.R.; Harris, W. Surface chemistry variations among a series of laboratory-produced biochars. *Geoderma* **2011**, *163*, 247–255. [[CrossRef](#)]
35. Blok, C.; Kreijl, C.D.; Baas, R.; Wever, G. Analytical Methods Used in Soilless Cultivation. In *Soilless Culture: Theory and Practice*; Elsevier: Amsterdam, The Netherlands, 2008.
36. Kloss, S.; Zehetner, F.; Dellantonio, A.; Hamid, R.; Ottner, F.; Liedtke, V.; Schwanninger, M.; Gerzabek, M.H.; Soja, G. Characterization of slow pyrolysis biochars: Effects of feedstocks and pyrolysis temperature on biochar properties. *J. Environ. Qual.* **2012**, *41*, 990–1000. [[CrossRef](#)] [[PubMed](#)]
37. Morales, V.L.; Pérez-Reche, F.J.; Hapca, S.M.; Hanley, K.L.; Lehmann, J.; Zhang, W. Reverse engineering of biochar. *Bioresour. Technol.* **2015**, *183*, 163–174. [[CrossRef](#)] [[PubMed](#)]
38. Mimmo, T.; Panzacchi, P.; Baratieri, M.; Davies, C.A.; Tonon, G. Effect of pyrolysis temperature on miscanthus (*Miscanthus × giganteus*) biochar physical, chemical and functional properties. *Biomass Bioenergy* **2014**, *62*, 149–157. [[CrossRef](#)]
39. Keiluweit, M.; Nico, P.S.; Johnson, M.G.; Kleber, M. Dynamic molecular structure of plant biomass-derived black carbon (biochar). *Environ. Sci. Technol.* **2010**, *44*, 1247–1253. [[CrossRef](#)] [[PubMed](#)]
40. Gray, M.; Johnson, M.; Dragila, M.I.; Kleber, M. Water uptake in biochars: The roles of porosity and hydrophobicity. *Biomass Bioenergy* **2014**, *61*, 196–205. [[CrossRef](#)]
41. Fryda, L.; Visser, R. Biochar for soil improvement: Evaluation of biochar from gasification and slow pyrolysis. *Agriculture* **2015**, *5*, 1076–1115. [[CrossRef](#)]
42. Urrestarazu Gavilán, M. *Tratado de Cultivo Sin Suelo*; Mundi-Prensa: Madrid, Spain, 2004.
43. CEN, European Committee for Standardization. *Soil Improvers and Growing Media—Determination of Physical Properties—Dry Bulk Density, Air Volume, Water Volume, Shrinkage Value and Total Pore Space*; prEN 13041; Technical Committee 223: Berlin, Germany, 2006.
44. CEN, European Committee for Standardization. *Soil Improvers and Growing Media—Determination of Organic Content and Ash*; prEN 13039; Technical Committee 223: Berlin, Germany, 2007.
45. CEN, European Committee for Standardization. *Soil Improvers and Growing Media—Determination of Electrical Conductivity*; prEN 13038; Technical Committee 223: Berlin, Germany, 2008.
46. Verhagen, J.B.G.M. Cec and the saturation of the adsorption complex of coir dust. *Acta Hortic.* **1999**, *481*, 151–155. [[CrossRef](#)]
47. ISO/DIS 11260. *Soil Quality—Determination of Effective Cation Exchange Capacity and Base Saturation Level Using Barium Chloride Solution*; Technical Committee 190: Berlin, Germany, 2011.
48. Blok, C.; Kaarsemaker, R. Ph in rockwool propagation blocks: A method to measure the pH buffer capacity of rockwool and other mineral wool media. *Acta Hortic.* **2013**, *1013*, 65–72. [[CrossRef](#)]
49. Blok, C.; Wever, G.; Persoone, G. A practical and low cost microbiotest to assess the phytotoxic potential of growing media and soil. *Acta Hortic.* **2008**, *779*, 367–374. [[CrossRef](#)]
50. CEN, European Committee for Standardization. *Soil Improvers and Growing Media—Determination of Plant Response—Part 2: Petri Dish Test*; EN 16086; Technical Committee 223: Berlin, Germany, 2008.
51. CEN, European Committee for Standardization. *Pren_00223089 2009 Determination of Aerobic Biological activity—Part 1: Oxygen Uptake Rate (Our)*; EN 16087; Technical Committee 223: Berlin, Germany, 2009.
52. Spencer, D.M. Standardized methods for the evaluation of fungicides to control cucumber powdery mildew. In *Crop Protection Agents—Their Biological Evaluation*; McFarlane, N.R., Ed.; Academic Press: London, UK, 1977; pp. 455–464.

53. Verhagen, J.B.G.M. Oxygen diffusion in relation to physical characteristics of growing media. *Acta Hortic.* **2013**, *1013*, 313–318. [[CrossRef](#)]
54. Londra, P.A. Simultaneous determination of water retention curve and unsaturated hydraulic conductivity of substrates using a steady-state laboratory method. *HortScience* **2010**, *45*, 1106–1112.
55. Trifonova, R.; Postma, J.; Verstappen, F.W.A.; Bouwmeester, H.J.; Ketelaars, J.J.; Van Elsas, J.-D. Removal of phytotoxic compounds from torrefied grass fibres by plant-beneficial microorganisms. *FEMS Microbiol. Ecol.* **2008**, *66*, 158–166. [[CrossRef](#)] [[PubMed](#)]
56. Giuffrida, F.; Consoli, S. Reusing perlite substrates in soilless cultivation: Analysis of particle size, hydraulic properties, and solarization effects. *J. Irrig. Drain. Eng.* **2016**, *142*. [[CrossRef](#)]
57. Dede, O.H.; Dede, G.; Ozdemir, S.; Abad, M. Physicochemical characterization of hazelnut husk residues with different decomposition degrees for soilless growing media preparation. *J. Plant Nutr.* **2011**, *34*, 1973–1984. [[CrossRef](#)]
58. Jeffery, S.; Verheijen, F.G.A.; van der Velde, M.; Bastos, A.C. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agric. Ecosyst. Environ.* **2011**, *144*, 175–187. [[CrossRef](#)]



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