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Microbial activity in peat-reduced plant growing media: Identifying influential growing medium constituents and physicochemical properties using fractional factorial design of experiments

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A B S T R A C T

The development of peat-reduced plant growing media for horticulture is needed to tackle sustainability concerns related to the peat production process. Alternative growing media constituents with unique physicochemical properties, like coir pith, wood fiber, compost, and inorganic materials are currently used. However, little is known about the impact of these materials on microbial activity. Because microbial communities play an important role in plant growth and development, the impact of various growing media constituents on microbial activity was analyzed using two-level fractional factorial statistical design of experiments. The results showed that growing media composition greatly affected microbial activity. White peat (+2.4% CO_2) and composted bark (+1.5% CO_2) significantly promoted microbial activity over black peat and green waste compost respectively. In contrast, the organic materials coir pith and wood fiber, as well as the inorganic materials perlite and sand, had little effect on microbial activity. Physicochemical analysis of the different growing media mixtures revealed that the water-filled porosity (WFP) was the most important parameter driving microbial growth, increasing microbial activity (+2.1% CO_2) at 62.95% WFP compared to 82.95% WFP. Our results are instrumental for developing novel organic growing media with distinct microbial features to move towards a low peat sustainable horticulture.

1. Introduction

Peat has been the most reliable growing medium (a.k.a. substratum) in horticulture for decades because of its performance and low economic cost (Barrett et al., 2016). The high cation exchange capacity (CEC) of peat favors plant nutrient uptake (Carlile et al., 2015). Peatlands are easily available in the northern hemisphere making peat a relatively cheap resource (Barrett et al., 2016). Although peatlands only cover 3% of the global land area, they contain 30% of the global soil carbon (Parish et al., 2008). However, unsustainable peat extraction damages the unique habitat of many species (Kern et al., 2017). Also, drainage and excavation of disturbed peatlands releases high amounts of CO_2 and CH_4 into the atmosphere (Gorham, 1991). Drainage-related peatland greenhouse gas emissions, including drainage for cultivation, peat extraction, and other economic uses, total close to 1 billion ton CO_2eq annually (Tubiello et al., 2016). Because of these concerns involving peat production, there is increasing governmental and societal pressure to decrease or phase out peat as a growing media constituent (Carlile and Coules, 2013). To reduce the environmental pressure of peat production, industry and NGO initiatives like ‘Responsibly Produced Peat’ (RPP) were set-up to responsibly select, manage and restore peatland (RPP Foundation, 2018). Simultaneously, industry and researchers are looking for plant growing media alternatives to minimize the use of peat while maintaining optimal plant growth properties.

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Abbreviations

GHG	Greenhouse gas
CEC	Cation exchange capacity
RPP	Responsibly Produced Peat
EC	electrical conductivity
WFP	water-filled porosity
PGPR	plant growth-promoting rhizobacteria
CFU	colony-forming unit
DOE	Design of Experiments
GC	gas chromatography
M1-16	Growing media mixture 1-16
C1-2	control 1-2
PT	peat
OO	other organics
CM	composted materials
IM	inorganic materials
AG	Arabic gum
C	C-source
DM	dry matter content
OM	organic matter content
ρ_b	bulk density
WC	water capacity
V_a	air volume at water saturation point
V_w	water volume at water saturation point
TPV	total pore volume

Principal plant growing media alternatives for peat are coir pith, wood fiber, compost and inorganic materials (Carlile et al., 2015). As a peat alternative, coir pith is often used either as a pure substratum or as a constituent in horticulture growing media mixtures. Because of its high salt content coir pith needs to be washed and buffered before it is ready for horticultural use (Verhagen and Zevenhoven, 2017). A second alternative for peat is wood fiber. The application of wood fiber in growing media mixtures helps improve air content. However, wood fiber can cause unwanted microbial nitrogen fixation during cultivation (Gruda, 2012a; Vandecasteele et al., 2018). As a third alternative, compost is considered. Due to its variability in physicochemical and microbial properties, composts are mainly used as mixture components (Schmilewski, 2008). Sand and perlite are inorganic materials commonly used in growing media mixtures. The use of sand can help improve peat plug stability (Verhagen and Zevenhoven, 2017). Application of inert perlite in peat media improves air content and water uptake (Carlile et al., 2015).

Peat plugs, which are mainly composed of black peat, are commonly used for plant propagation and in hydroponic cultivation systems. Peat plugs have adhesive properties allowing for easy handling and transplantation. In the search for peat growing media alternatives, biological adhesives need to be found that can introduce these adhesive properties in peat-reduced or peat-free substrates. Arabic gum is a biological adhesive derived from *Acacia* trees (Dror et al., 2006). It is commonly used as a food additive and has the potential to be used as a growing media binding agent.

Growing media quality depends on several physicochemical properties, which are unique to each type of growing media constituent, and can be steered to assure optimal plant growth. Electrical conductivity (EC), pH and nutritional elements (N, P, K, Ca, Mg and P) are important chemical characteristics (Carlile et al., 2015). Having a low pH, peat growing media are easily adjusted to desirable pH levels through the addition of lime, which also is a source of Ca and Mg. Peat alternatives have typically higher pH levels, with consequent less margin to steer pH and adjust Ca and Mg contents. Soil moisture strongly affects microbial activity. Use of water-filled porosity (WFP; a.k.a. soil water saturation) as an expression for soil moisture content allows for accurate prediction of microbial activity across different soil types (Linn and Doran, 1984; Moyano et al., 2012). Organic growing media have a distinctive and stable microbial community providing functional diversity, temporal stability, and resilience to a heterogeneous and fluctuating hydroponics environment (Grunert et al., 2016). Steering the microbial community of organic growing media in hydroponics could have an important impact on the productivity and quality of the final crop. However, the impact of alternative growing media constituents on microbial activity has not been investigated.

This study aimed to determine which growing media constituents contribute to increased microbial activity. Peat-reduced growing media mixtures composed of 4 groups of growing media constituents were tested. These 4 groups were: peat (black peat and white peat), other organics (coir pith and wood fiber), composted materials (composted bark and green waste compost) and inorganic materials (perlite and sand). Because of the high number of variables involved in organic growing media and microbial community interactions, optimizing growing media composition by testing every possible combination of variables is laborious and inefficient. To this end, parameters affecting microbial activity were investigated using two-level fractional factorial statistical design of experiments (2_{IV}^{8-4}). The control factors investigated were: growing media constituents (peat, other organics, composted materials, and inorganic materials), Arabic gum, WFP, carbon source, and a plant growth-promoting rhizobacteria (PGPR) inoculum. The effect of growing media composition on microbial activity was linked to the physicochemical properties of the tested growing media constituents.

2. Materials and methods

2.1. Design of experiments

Statistical fractional factorial design of experiments (DOE) was used to investigate microbial activity in organic growing media allowing us to study multiple variables simultaneously. The number of experiments is limited while maintaining sufficient data points for qualitative comparisons. By systematically changing control factors, the effects of these control factors and their interactions on microbial activity are determined. The production of CO_2, O_2, CH_4, and H_2S was measured in relation to maximal microbial activity. Microbial activity was evaluated using 1/16 fractional factorial statistical design of experiments (2_{IV}^{8-4}).

2.1.1. Selection of control factors and factor levels

The microbial activity fractional factorial design included 8 major control factors at 2 factor levels (low −1 and high +1 level) (Table 1). To properly detect the effect of changing each control factor from the low to high level, the differences between both factor levels are preferred to be as far apart as possible. Therefore, for each group of growing media alternatives (control factors A, B, C, and D) two raw materials were chosen that have dissimilar physicochemical properties to properly assess differences in microbial activity. Similarly, for Arabic gum (control factor E), both a low dose (1 kg m^{-3}) and a high dose (5 kg m^{-3}) were chosen. The selection of the factor levels for control factors F, G, and H are shown in sections 2.3. and 2.4.

Table 1
Control factors and factor level settings for growing media optimization.

	Control factor	Low (−1)	High (+1)
A	Peat	Black peat	White peat
B	Other organics	Coir pith	Wood fiber
C	Composted materials	Composted bark	Green waste compost
D	Inorganic materials	Perlite	Sand
E	Arabic gum ($kg.m^{-3}$)	1	5
F	WFP (% v/v)	62.95	82.95
G	C-source (g glucose.L^{-1} substratum)	0	5
H	PGPR ($CFU.g^{-1}$ substratum)	0	10^7

Testing all combinations (= full factorial design) would require $2^8 = 256$ experiments. Using a 1/16 fraction factorial design (resolution IV) requires 16 combinations to be tested. The 2_{IV}^{8-4} fractional factorial design presented in Table 2 was established using Minitab 17 (Minitab Inc., State College, Pennsylvania, United States).

No center points were included in the 2_{IV}^{8-4} fractional factorial design. Control factors A, B, C, and D are categorical, so issues of nonlinearity do not occur. Control factors E, F, G, and H were assumed to be linear.

Fractional factorial design can result in confounding interaction effects (a.k.a. aliasing). It was assumed that three-factor and higher-order interactions are extremely rare and can be neglected. In the 2_{IV}^{8-4} fractional factorial design, aliasing occurs between two-factor interactions (AB + CG + DH + EF; AC + BG + DF + EH; AD + BH + CF + EG; AE + BF + CH + DG; AF + BE + CD + GH; AG + BC + DE + FH; AH + BD + CE + FG). Using the heredity principle it was assumed that an interaction effect is likely significant when the main effects involved are also significant (Bingham and Sitter, 2003).

2.1.2. Statistical analysis of fractional factorial design

The factorial designs were analyzed statistically in Minitab 17 (Minitab Inc., State College, Pennsylvania, United States) using probability effects plots and analysis of variance (ANOVA) (Daniel, 1959).

2.2. Growing media composition

The growing media constituents were divided into 4 raw material groups containing 2 raw materials each (see control factors A, B, C and D in Table 1). Based on the 2_{IV}^{8-4} fractional factorial design (Table 2) 16 different growing media mixtures were composed. The bulk density of the raw material was determined following the standard procedure EN 12580. Volumetric composition of growing media mixtures was as follows: 60% v/v peat, 20% v/v other organics, 10% v/v composted materials and 10% v/v inorganic materials. Using the raw material bulk densities and the volumetric composition of the growing media mixtures, the necessary weight to be added was determined for each constituent. Growing media mixtures (25 L) were made by mixing the desired amount of raw materials in a cement mixer. Afterward Arabic gum was added at a dose of 1 kg m^{-3} or 5 kg m^{-3} depending on the factor level (see control factor E in Table 2). The pH was adjusted to 5.8 by liming, following Regeling Handelspotgronden (RHP) guidelines (Verhagen and Zevenhoven, 2017).

Table 2
The 2_{IV}^{8-4} fractional factorial design and the 2^4 full factorial design highlighted in gray.

Standard order	Run order and growing media mixture	Factors A	B	C	D	E	F	G	H
5	1	-1	-1	1	-1	1	1	1	-1
2	2	1	-1	-1	-1	-1	1	1	1
4	3	1	1	-1	-1	1	1	-1	-1
9	4	-1	-1	-1	1	1	1	-1	1
1	5	-1	-1	-1	-1	-1	-1	-1	-1
6	6	1	-1	1	-1	1	-1	-1	1
12	7	1	1	-1	1	-1	-1	-1	1
10	8	1	-1	-1	1	1	-1	1	-1
3	9	-1	1	-1	-1	1	-1	1	1
13	10	-1	-1	1	1	-1	-1	1	1
8	11	1	1	1	-1	-1	-1	1	-1
14	12	1	-1	1	1	-1	1	-1	-1
11	13	-1	1	-1	1	-1	1	1	-1
16	14	1	1	1	1	1	1	1	1
15	15	-1	1	1	1	1	-1	-1	-1
7	16	-1	1	1	-1	-1	1	-1	1

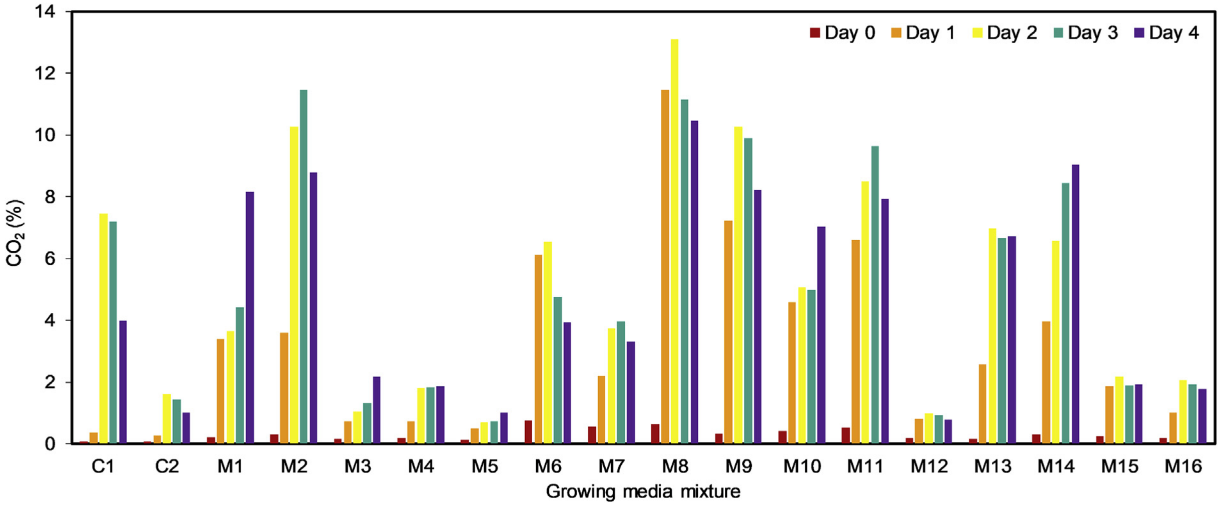

Fig. 1. Dynamics of microbial activity CO_2 (%) of 16 growing media mixtures and 2 control treatments over time.

2.3. Water-filled porosity

To determine the WFP factor level suitable for analysis of microbial activity, growing media mixtures were placed in a vertical hydroponics indoor farm. Specifically, 6 cups (cup volume 0.2 L) were filled with the different growing media mixtures. The cups were placed inside a crate (dimensions: 80 cm × 60 cm fitted with a water overflow of 0.7 cm). Each crate fitted 24 cups. The crates were irrigated with excess nutrient solution. The growing media mixtures were left inside the hydroponics system for 24 h. Next, the substrata were removed from the cups, mixed and WFP (% v/v) was determined in triplicate (Linn and Doran, 1984). The average WFP (= 72.95 ± 2.5% v/v) of all 16 growing media mixtures was used as a baseline. For the microbial activity experiment factor levels −10% WFP_{avg} (= 62.95%; low factor level) and +10% WFP_{avg} (= 82.95%; high factor level) were used (Table 1).

2.4. PGPR inoculum and C-source

A mix of *Bacillus* sp. with plant growth-promoting properties was used as inoculum (Vitact R, De Ceuster Meststoffen NV, Grobbendonk, Belgium). The PGPR inoculum factor levels were set at a dose of 0 CFU g^{-1} substratum for the low factor level (no addition of PGPR inoculum) and 10^7 CFU g^{-1} substratum for the high factor level (Table 1). Glucose was used as a positive control, providing a C-source for microbial growth. Factor levels were set at 0 g glucose.L^{-1} substratum (low factor level) and 5 g glucose.L^{-1} substratum (high factor level).

2.5. Analytical methods

2.5.1. Gas-phase analysis

Microbial activity was determined using the respiration method described by Anderson and Domsch (1978). Each growing media mixture (200 mL) was placed inside a 1 L high-density polyethylene container (20% substratum, 80% headspace). The containers were sterilized with 70% ethanol before use. A volume of buffered nutrient solution (10 mM phosphate buffer, pH 5.8), depending on the WFP factor level, was added. Glucose and/or the PGPR inoculum were added to the growing media mixture depending on the factor level (Table 2). Next, the substratum was thoroughly mixed. The container was closed airtight with a lid that was fitted with a septum to sample the headspace. Besides the 16 growing media mixtures, two controls without substratum were included: control 1 (C1): glucose (5 g.L^{-1} substratum), PGPR-mix (10^7 CFU g^{-1} substratum) in 200 mL buffered nutrient solution, and control 2 (C2): no glucose (0 g.L^{-1} substratum), PGPR-mix (10^7 CFU g^{-1} substratum) in 200 mL buffered nutrient solution. The samples were incubated at room temperature. The experiment took place over 5 days, headspace gas samples were taken once a day. The gas-phase composition was analyzed with a Compact GC (Global Analyser Solutions, Breda, The Netherlands), equipped with a Molsieve 5A pre-column and Porabond column (CH_4, O_2) and an Rt-Q-bond pre-column and column (CO_2 and H_2S) at 50 °C using He (3 bar) as carrier gas. Concentrations of gases were determined using a thermal conductivity detector. At the end of the experiment, the pH of each mixture was determined in a 1:5 soil:water extract.

2.5.2. Physicochemical analysis

The correlations between growing media composition, microbial activity and the physicochemical properties of the constituents were determined. The analytical tests were performed before liming and before the addition of Arabic gum to the growing media mixtures. Using full factorial statistical design of experiments (2^4) on the control factors peat (A), other organics (B), composted materials (C) and inorganic materials (D), the physicochemical properties of the growing media mixtures were related to its constituents (Table 2). The chemical analysis was carried out according to Gabriels et al. (1998). The physical analysis of the growing media was performed as described by Verdonck and Gabriels (1992).

3. Results and discussion

3.1. Microbial activity

3.1.1. Dynamics of microbial activity

The CO_2 production (%) of 16 growing media mixtures was measured over time as a proxy for general microbial activity. The concentration of CH_4 and H_2S remained below the GC detection limit throughout the experiment, suggesting that anaerobic metabolism did not take place. Variation in O_2 concentration over time showed similar, yet inverse, trends in comparison to CO_2 concentration (Appendix A). Changes in headspace CO_2 concentration over time are shown in Fig. 1. Growing media mixtures with (M1; M2; M8; M9; M10; M11; M13; M14) or without (M3; M4; M5; M6; M7;

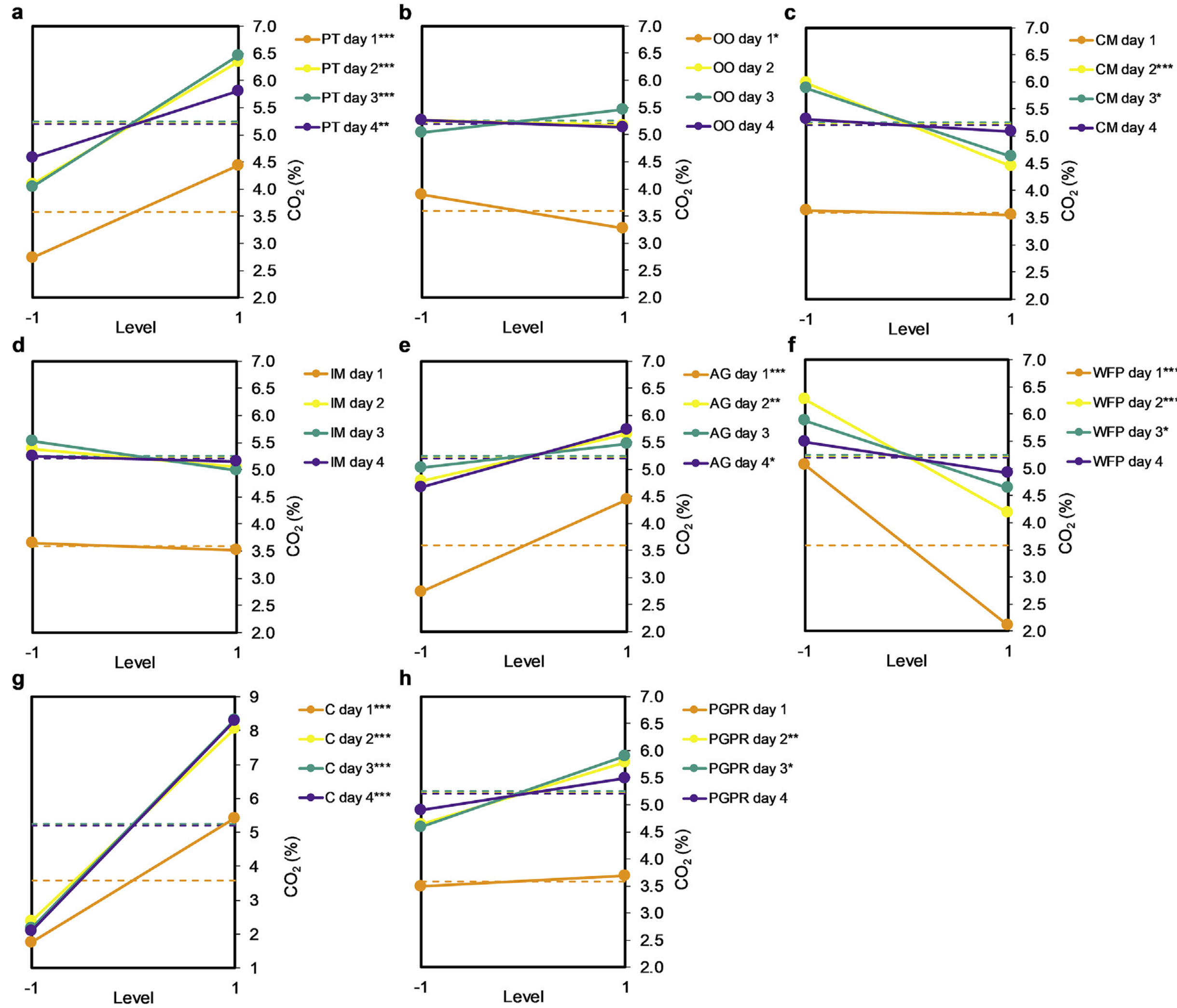

Fig. 2. Main effects of growing media constituents on CO_2 concentration (%) over time. (a) peat (PT; −1 = black peat and 1 = white peat), (b) other organics (OO; −1 = coir pith and 1 = wood fiber), (c) composted materials (CM; −1 = composted bark and 1 = green waste compost), (d) inorganic materials (IM; −1 = perlite and 1 = sand), (e) Arabic gum (AG; −1 = 1 kg m^{-3} and 1 = 5 kg m^{-3}), (f) water-filled porosity (WFP; −1 = 62.95% v/v and 1 = 82.95% v/v), (g) C-source (C; −1 = 0 g glucose.L^{-1} substratum and 1 = 5 g glucose.L^{-1} substratum) and (h) bacterial inoculant (PGPR; −1 = 0 CFU g^{-1} substratum and 1 = 107 CFU g^{-1} substratum). Day 1 (●), day 2 (●), day 3 (●) and day 4 (●). Dashed lines indicate mean levels of CO_2 concentration for each day. Asterisks indicate level of significance: $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

M12; M15; M16) addition of glucose were classified as different groups. As expected, the addition of glucose causes a large increase in CO_2 concentration in comparison to growing media mixtures where no C-source was provided. The respiration method used for the estimation of microbial activity is based on the initial respiratory response of microbial communities to the addition of an excess carbon energy source (Anderson and Domsch, 1978). Growing media mixture 8 (M8) reached the highest overall CO_2 concentration (13.1%) on day 2. While on the same day M5 only reached a CO_2 concentration of 0.7%. Different trends in microbial activity are observed. For example, M8, M9, and M13 show a strong increase in CO_2 concentration during the first 48 h, leveling out or even decreasing during the final 48 h. M14 CO_2 concentration increases gradually throughout the 4-day experiment. After a sharp increase during the first 24 h, M1 and M10 CO_2 concentration is stable for the next 2 days, increasing strongly again on the final day. The CO_2 concentration for all growing media mixtures measured on average 3.6% on day 1, increasing to 5.2% on day 2, stabilizing on day 3 (5.3%) and day 4 (5.2%). The fractional factorial DOE analysis described in sections 3.1.2., 3.1.3. and 3.1.4. elucidates the trends visible in Fig. 1.

3.1.2. Microbial activity of control factors

The effect of each control factor on microbial activity is illustrated by comparing CO_2 production at high and low factor levels (Fig. 2).

3.1.2.1. Peat. The presence of white peat over black peat significantly increased CO_2 production, up to 2.4% on day 3 ($P < 0.001$) (Fig. 2 a), suggesting that white peat stimulates microbial growth and or respiration. Peatlands are formed over thousands of years by accumulation of partially degraded plant material under anaerobic conditions. The peat profile varies in age: young, undecomposed white peat is located in the upper layers of the peat profile while highly decomposed black peat can be found at the bottom of the peat profile (Carlile et al., 2015; Moore, 1989). The older, more decomposed subsurface peat is less favorable as microbial energy source resulting in reduced CO_2 production in the lower parts of the profile and higher CO_2 production at the top of the peat profile (Fisk et al., 2003; Glatzel et al., 2004).

3.1.2.2. Other organics. Changing the type of organic material, coir pith and wood fiber had little to no effect on CO_2 concentration (Fig. 2 b). Only on day 1 CO_2 concentration (0.6%) significantly increased when using coir pith instead of wood fiber ($P = 0.013$). The higher organic matter content of coir pith and wood fiber would imply that these would also stimulate microbial respiration and growth, however, little difference in microbial activity when using coir pith or wood fiber was observed. The moderately decomposed materials had a minor effect on microbial activity after day 2 and 3 of incubation, suggesting that the earlier composting allowed further degradation.

3.1.2.3. Composted materials. The type of composted material influenced the CO_2 levels on day 2 and day 3 (Fig. 2 c). On day 2, composted bark significantly increased CO_2 concentration with 1.5% in comparison to green waste compost ($P < 0.001$). Compost in growing media mixtures is known to improve disease suppression against soil-borne plant pathogens, indicating that the moderate support of microbial activity by compost is sufficient (Raviv, 2013). Composts lack uniformity, even at batch level, resulting in high variability in physicochemical and biological properties (Raviv, 2013). This variability between batches is caused by the type and ratios of starting materials, and composting parameters (temperature, moisture) used (Carlile et al., 2015). Because of this, the effect of compost on the microbial community may vary.

3.1.2.4. Inorganic materials. The type of inorganic material did not significantly affect CO_2 concentration (Fig. 2 d). The lack of impact of inorganic materials on microbial growth agrees with the absence of a C-source. Moreover, perlite is produced at high temperature (±1000 °C) killing all microbial life and is therefore sterile or poorly recolonized (Verhagen and Zevenhoven, 2017).

3.1.2.5. Water-filled porosity (WFP). Next to growing media constituents, the effect of WFP, the addition of a C-source, Arabic gum and of a PGPR inoculum on CO_2 production was determined. The level of WFP influenced CO_2 concentration substantially (Fig. 2 f). At the low WFP factor level, the CO_2 concentration was 2.1% higher compared to the high WFP factor level on day 2 ($P < 0.001$). Soil moisture strongly affects microbial activity. WFP is used as an expression for soil moisture as it is known to accurately predict microbial activity across different soil types (Linn and Doran, 1984; Moyano et al., 2012). Our results show that relatively low WFP (62.95% v/v) is beneficial for microbial activity compared to high WFP (82.95% v/v). At low soil water content, the supply of solutes to the microbial community is reduced because water inside the soil pores becomes physically disconnected. Reaching soil water saturation, soil pores filled with water reduces the oxygen availability and thus the metabolic activity of aerobic microorganisms. The optimal moisture content supporting respiratory metabolic activity is ±60% v/v, where microbial activity is maximally reducing towards both moisture extremes (Moyano et al., 2013).

3.1.2.6. C-source. Adding glucose as a C-source significantly stimulated CO_2 concentration throughout the experiment, increasing CO_2 levels up to 6.2% on day 3 and day 4 in comparison to the control without added glucose ($P < 0.001$) (Fig. 2 g). The addition of glucose as a C-source to growing media mixtures provided a positive control for microbial growth. In line with the dependence on a C-source, the microbial growth was strongly enhanced after the addition of glucose. Our experiments did not include plant growth tests, but plant root exudates could be sufficient to provide a source of energy to support microbial growth, since they are known to constitute a significant source of carbon in soil that steers rhizosphere microbial community structure (Hirsch et al., 2013; van Dam and Bouwmeester, 2016).

3.1.2.7. Arabic gum. The application dose of Arabic gum substantially affected CO_2 levels during the first day, losing significance over time (Fig. 2 e). A high dose of Arabic gum instead of a low dose significantly increased the CO_2 concentration by 1.7% on day 1 ($P < 0.001$). Because Arabic gum is not semi sterile and is a relatively chemically inert material, the microbial communities present in the growing media mixtures are suspected to use Arabic gum as an energy source for growth.

3.1.2.8. Plant growth-promoting rhizobacteria (PGPR). Inoculating the growing media mixtures with PGPRs increased CO_2 concentration on days 2 and 3 (Fig. 2 h). The CO_2 concentration significantly increased with 1.1% on day 2 when growing media mixtures were inoculated ($P = 0.001$). Application of PGPRs can stimulate plant growth, increase crop yield and quality, provide disease resistance, decrease biotic and abiotic stress and increase nutrient availability and uptake (Berg, 2009; Compant et al., 2010; Lee and Lee, 2015). The addition of a PGPR mix of *Bacillus* sp. had a positive effect on microbial activity during the middle of the incubation period. This indicates the PGPRs can establish, but that the growing media mixtures have an active native microbial community that might prove difficult for the inoculant to persist.

3.1.3. Control factor interaction effects

To illustrate interactions between control factors, day 2 growing media mixture CO_2 concentrations on were compared because they reached the highest levels (M8; 13.1%) compared to other days. In the absence of glucose, the type of composted material had little effect on mean CO_2 concentration. However, growing media mixtures containing composted bark showed much higher average CO_2 levels than mixtures containing green waste compost when 5 g glucose.L^{-1} substratum was added as a positive control (Fig. 3 a). A similar dependence of CO_2 production on additional glucose was observed with white and black peat. In the absence of additional glucose, the mean CO_2 concentrations were not different. The addition of glucose to media containing white peat led to substantially higher mean CO_2 levels compared to black peat (Fig. 3 c). The addition of glucose activates the higher respiration potential of both bark compost and white peat by supplying an excess source of carbon.

At high WFP, no significant difference in mean CO_2 concentration between growing media mixtures containing black or white peat was observed. At low WFP, however, growing media mixtures containing white peat showed a much higher mean CO_2 concentration in comparison to mixtures containing black peat (Fig. 3 b). The WFP property of a growing medium strongly influences the effect of peat on the microbial activity. At high WFP, there is no difference in microbial activity between black peat and white peat. High WFP strongly inhibited the activity of white peat microbial community which is known to be more active than black peat (Glatzel et al., 2004). Only at low WFP did white peat mixtures show higher microbial activity than black peat mixtures. High WFP inhibits the microbial activity potential of white peat because of a lack of oxygen availability.

Because of the occurrence of aliasing effects when using fractional factorial designs, significant interaction effects were selected based on the heredity principle (Bingham and Sitter, 2003). The changes in main effect significance over time (Fig. 2) resulted in shifts in the selected aliased two-factor interaction that had a significant effect on CO_2 concentration on a certain day. For the aliased interactions AB + CG + DH + EF, the selected significant interaction

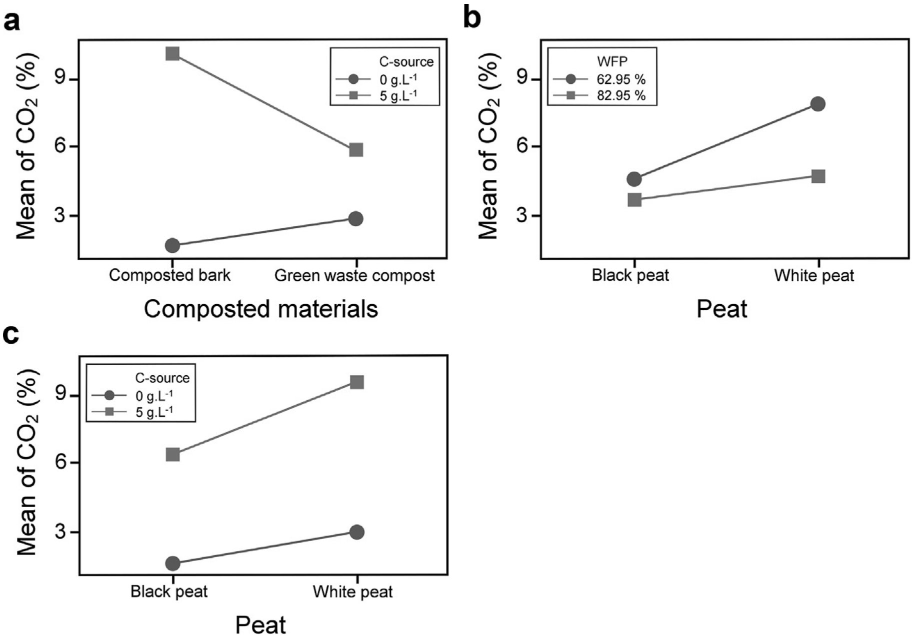

Fig. 3. Significant interactions between plant growth media constituents on day 2 CO_2 concentration. (a) Composted materials and C-source (g glucose.L^{-1} substratum; $P < 0.001$), (b) peat and water-filled porosity (WFP; % v/v; $P = 0.001$) and (c) peat and C-source ($P = 0.006$).

effect shifted from EF on day 1 to CG on days 2 and 3. Overall, the interactions effects CG, AF, and AG contributed the most to the headspace CO_2 concentration throughout the experiment.

3.1.4. Control factor contribution to total microbial activity

The Pareto chart in Fig. 4 (a) shows how much each control factor and their interactions contribute to total CO_2 concentration in the headspace on day 2. Only significant factors and interactions are shown. The higher the absolute standardized effect of a control factor, the more it affected overall CO_2 concentration. The normal probability plot (Fig. 4 b) reveals in which direction the significant control factors and interactions affect day 2 CO_2 concentration when changing from low to high factor level. The control factor C-source had the largest effect on CO_2, increasing in concentration when applying glucose to the growing media mixtures. The standardized effect of control factor C-source (G) was double the second-largest effect, which was caused by the interaction between composted materials and C-source decreasing CO_2 concentration when using green waste compost instead of bark compost (CG). This confirms the need for a C-source to activate the microbial activity potential of growing media mixtures and bark compost in particular. The following control factors in order of importance are peat and WFP. White peat (A, high factor level) had a positive effect while high WFP (F) decreased CO_2 levels. Both composted materials (C) and the interaction between peat and WFP (AF) negatively affected CO_2 concentration at the high factor level. The addition of PGPR inoculant had a positive effect on CO_2 production (H), although its contribution was lower compared to peat and compost. This shows that the inoculant was able to establish, but the native microbial communities present in peat and compost contribute more to total microbial activity making it difficult for the inoculant to persist. Finally, Arabic gum (E) and the interaction between peat and C-source (AG) both contributed equally to CO_2 increasing its concentration at the high factor level.

Harvested peat has a low colony-forming unit (CFU) count (10^4 CFU g^{-1} DW) because of the low oxygen conditions under which it develops. Coir pith has on average 10^6 CFU g^{-1} DW and wood fiber is known to show more microbial activity than peat substrata. Composted materials can have as high as 10^8 CFU g^{-1} DW (Carlile et al., 2015; Gruda, 2012b). Although peat has low microbial counts, the Pareto chart (Fig. 4 a) shows it contributed most to microbial activity compared to organic materials and composted materials. Of course, ratios in which the growing media constituents were added also played a role (60% v/v peat, 20% v/v other organics, 10% v/v composted materials).

3.2. Physicochemical properties

3.2.1. Chemical properties

The influence of peat, other organics, composted materials and inorganic materials on the physicochemical properties of growing media are presented in Table 3. Focusing on the chemical properties, the type of peat had a strong significant effect ($P < 0.001$) on pH, Ca^{2+}, Mg^{2+}, SO_4^{2-} and Fe^{2+}-concentration. The mean pH of growing media mixtures containing black peat (pH 5.3) was higher than mixtures containing white peat (4.7). Using black peat as a growing media constituent resulted in a 137% increase in Ca^{2+}, a 61% increase in Mg^{2+} and a 102% increase in SO_4^{2-}-concentration in comparison to white peat mixtures. Fe^{2+}-concentration doubled when using white peat instead of black peat as a growing media constituent.

Mean Na^+-concentration increased significantly in mixtures containing coir pith (41.4 mg.L^{-1} substratum) as opposed to wood fiber (28.7 mg.L^{-1} substratum) ($P = 0.013$). The growing media constituents peat, composted materials, and inorganic materials did not alter the Na^+-concentration when changing their factor levels. Non-processed coir pith is highly contaminated with seawater and adds Na^+ to the growing media, which is toxic for most crops (Munns et al., 2016). Because of its high salt content, coir pith is buffered repeatedly with a nutrient solution containing Ca and Mg salts before it is suitable for horticultural use (Carlile et al., 2015; Verhagen and Zevenhoven, 2017). Despite buffered coir was used in our experiments, it still contained high enough Na^+ levels to contaminate the growing media mixtures, a problem that is

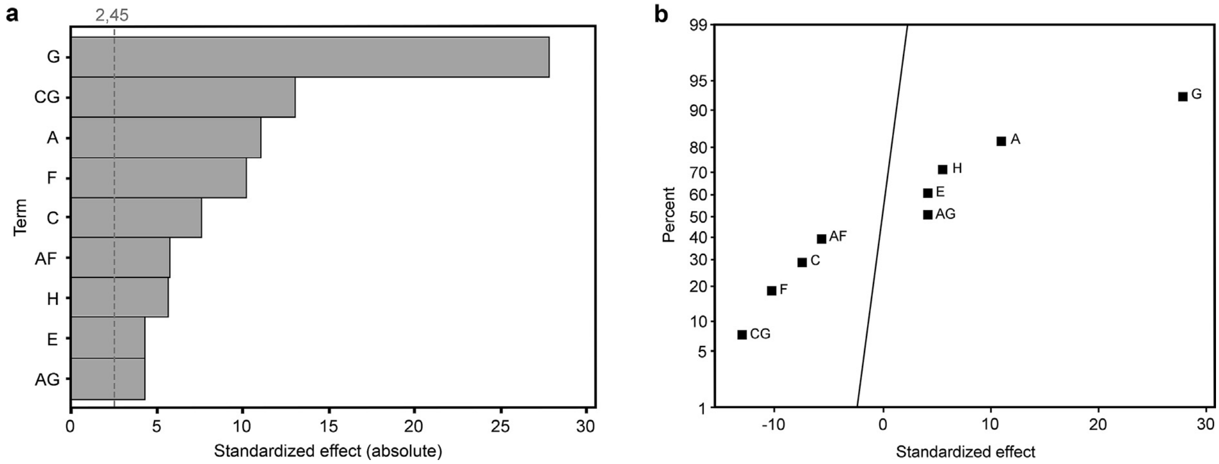

Fig. 4. (a) Pareto chart of standardized effect (absolute) of the significant terms on day 2 CO_2 concentration. Terms are ordered from the largest to the smallest effect. The dashed reference line indicates the statistical significance of effects. (b) Normal probability plot of standardized effect of the significant terms on day 2 CO_2 concentration. Peat (A), composted materials (C), Arabic gum (E), WFP (F), C-source (G), PGPR (H) and significant interactions AF, AG, and CG. Significance at $P < 0.05$.

avoided by using wood fiber.

The type of composted material used had a highly significant effect ($P < 0.001$) on all other chemical properties tested. Growing media mixtures containing green waste compost were high in pH, EC and mineral content. The application of composted bark doubled the amount Mn^{2+} (12.0 mg.L^{-1} substratum) in comparison to green waste compost (6.0 mg.L^{-1} substratum). Improper composting of bark can cause accumulation of Mn^{2+}, resulting in manganese toxicity in plants when used as a growing media constituent (Solbraa, 1985). The growing media mixtures tested did not reach toxic Mn^{2+} levels.

3.2.2. Physical properties

The type of inorganic material used did not affect the chemical properties of the final growing media mixture. Sand and perlite are chemically inert and contain little to no nutrients, and change primarily the physical properties of growing media mixtures (Verhagen and Zevenhoven, 2017). All physical properties tested, except for growing medium shrinkage, were strongly altered by the type of inorganic material. Sand significantly increased dry matter (DM) content (54.9% fw) in comparison to use of perlite (38.0% fw) ($P < 0.001$). Dry matter of growing media mixtures with sand contained more ash (64.8% dw) than organic matter (OM; 35.2%

Table 3
Main effects of plant growth media constituents on physicochemical properties. Peat (PT; −1 = black peat and 1 = white peat), other organics (OO; −1 = coir pith and 1 = wood fiber), composted materials (CM; −1 = composted bark and 1 = green waste compost), inorganic materials (IM; −1 = perlite and 1 = sand) and mean of all 16 mixtures. Chemical properties: pH, EC (μS.cm^{-1}) and NO_3^-, NH_4^+, P_2O_5, K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, Na^+, Cl^-, Fe^{2+} and Mn^{2+} concentrations (mg.L^{-1} substratum). Physical properties: dry matter content (DM; % fresh weight), organic matter content (OM; % dry weight), ash content (% dry weight), bulk density (ρ_b; g.L^{-1}), shrinkage (% v/v), water capacity (WC; g.(100 g dry matter)$^{-1}$), air volume at water saturation point (V_a; % v/v), water volume at water saturation point (V_w; % v/v), total pore volume (TPV; % v/v), and water-filled porosity at water saturation point (WFP; % v/v). Asterisks indicate level of significance: not significant (n.s.), $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

Property	PT			OO			CM			IM			Mean
Chemical	−1	1		−1	1		−1	1		−1	1		
pH	5.3	4.7	***	5.0	5.0	n.s.	4.7	5.4	***	5.0	5.0	n.s.	5.0
EC	111	97	*	109	100	n.s.	60	149	***	106	102	n.s.	104
NO_3^-	5.4	1.5	**	3.2	3.7	n.s.	0.0	6.9	***	3.6	3.3	n.s.	3.5
NH_4^+	3.7	3.4	n.s.	4.3	2.8	**	1.2	5.9	***	4.1	3.1	*	3.6
P_2O_5	29.2	39.4	**	31.9	36.7	n.s.	10.7	57.9	***	33.2	35.5	n.s.	34.3
K^+	150	148	n.s.	161	137	*	70	228	***	152	146	n.s.	149
Ca^{2+}	1233	520	***	889	864	n.s.	688	1065	***	867	886	n.s.	877
Mg^{2+}	134	83	***	110	106	n.s.	87	129	***	108	109	n.s.	108
SO_4^{2-}	107	53	***	91	69	**	63	97	***	87	73	n.s.	80
Na^+	31.8	38.4	n.s.	41.4	28.7	*	31.1	39.0	n.s.	37.0	33.1	n.s.	35.1
Cl^-	55.3	53.0	n.s.	57.3	51.0	*	22.2	86.0	***	56.1	52.1	n.s.	54.1
Fe^{2+}	0.6	1.1	***	0.8	0.9	**	0.6	1.1	***	0.8	0.9	**	0.9
Mn^{2+}	9.3	8.7	*	8.7	9.2	*	12.0	6.0	***	8.9	9.1	n.s.	9.0
Physical													
DM	43.2	49.7	***	43.8	49.1	***	45.0	47.8	**	38.0	54.9	***	46.4
OM	58.7	51.7	***	55.4	55.0	n.s.	59.8	50.6	***	75.2	35.2	***	55.2
Ash	41.3	48.3	***	44.6	45.0	n.s.	40.2	49.4	***	24.8	64.8	***	44.8
ρ_b	234	157	***	193	198	n.s.	189	202	*	125	265	***	195
Shrinkage	31.8	27.5	*	32.8	26.5	**	29.9	29.4	n.s.	29.9	29.4	n.s.	29.6
WC	351	533	***	450	435	n.s.	470	414	**	615	269	***	442
V_a	15.1	23.2	***	18.8	19.5	n.s.	19.1	19.2	n.s.	20.5	17.8	***	19.1
V_w	72.6	68.8	***	71.1	70.3	**	71.0	70.5	**	72.0	69.4	***	70.7
TPV	87.7	92.2	***	90.1	89.8	n.s.	90.2	89.7	n.s.	92.6	87.3	***	89.9
WFP	82.8	74.7	***	79.1	78.4	n.s.	78.8	78.7	n.s.	77.8	79.7	**	78.7

dw), while mixtures with perlite contained more organic matter (75.2% dw) than ash (24.8% dw). Water volume at water saturation point (V_w; 72.0% v/v) and total pore volume (TPV; 92.6% v/v) of mixtures containing perlite was significantly higher than sand mixtures (V_w = 69.4% v/v and TPV = 87.3% v/v) ($P < 0.001$). This resulted in a significantly lower WFP, the ratio between V_w and TPV, at water saturation point for perlite mixtures (77.8% v/v) compared to sand mixtures (79.7% v/v) ($P = 0.002$). Perlite is used in growing media mixtures to improve pore volume, while sand has low pore content (Verhagen and Zevenhoven, 2017). Indeed, the DOE results clearly show TPV of growing media mixtures containing perlite was highest, while the use of sand decreased TPV. Another reason why perlite is commonly used as a growing media constituent is its ability to improve water uptake (Verhagen and Zevenhoven, 2017). Our results show significantly higher water capacity in mixtures containing perlite.

The type of peat used significantly affected all physical properties tested. White peat increased dry matter content (black peat: 43.2% fw; white peat: 49.7% fw) and amount of ash (black peat: 41.3% dw; white peat 48.3% dw). V_w (black peat: 72.6% v/v; white peat: 68.8% v/v) was significantly lower for white peat mixtures ($P < 0.001$) while TPV (black peat: 87.7% v/v; white peat: 92.2% v/v) significantly increased ($P < 0.001$), causing WFP to decrease with 8.1% v/v when using white peat as growing media constituent (black peat: 82.8% v/v; white peat: 74.7% v/v). White peat and perlite significantly decreased WFP at water saturation of the growing media mixtures. As WFP correlates with microbial activity and is considered to be healthier for the plant, the use of white peat and perlite as growing media constituents can promote microbial activity in a wet hydroponics environment. White peat decreased shrinkage (27.5% v/v) in comparison to black peat (31.8% v/v).

Likewise, the type of other organic constituent affected mixture shrinkage. Wood fiber decreased shrinkage (26.5% v/v) in comparison to the use of coir pith (32.8% v/v). Shrinkage, which is a measure for the loss in substratum volume when dried, is unfavorable because it affects plant performance and the aesthetic value of a flower pot (Raviv, 2011; Verhagen and Zevenhoven, 2017). Other organics had little effect on the other physical properties measured.

Mainly DM, OM, and ash were influenced by the type of composted material. DM content increased to 47.8% fw when using green waste compost in comparison to composted bark (45.0% fw). Dry matter of mixtures with green waste compost contained equal amounts of ash (49.4% dw) and OM (50.6% dw), while mixtures with composted bark contained more OM (59.8% dw) than ash (40.2% dw).

3.2.3. Physicochemical interaction effects

Substantial interaction effects occurred between different growing media constituents for multiple physicochemical properties. Interaction of other organics and inorganic materials caused significant changes in SO_4^{2-}-concentration ($P = 0.017$). When using wood fiber as an organic constituent the type inorganic materials used did not affect SO_4^{2-}-concentration. Contrary, when combining coir pith with perlite SO_4^{2-}-concentration (107 $mg.L^{-1}$ substratum) increased compared to the combination of coir pith and sand (75 $mg.L^{-1}$ substratum). While the type of other organic did not affect NH_4^+-concentration when combined with composted bark as composted material constituent, NH_4^+-concentration was 1.7 times higher when green waste compost was combined with coir pith (7.5 $mg.L^{-1}$ substratum) compared to wood fiber (4.3 $mg.L^{-1}$ substratum). Growing media shrinkage was lowest when composted bark was combined with perlite (composted bark + perlite: 28.1% v/v; composted bark + sand: 31.8% v/v) and when green waste compost was combined with sand (green waste compost + perlite: 31.8% v/v; green waste compost + sand: 27.0% v/v). When using sand as inorganic material the type peat used did not affect water capacity. Contrary, when combining perlite with white peat, water capacity (759 g.(100 g dry matter)$^{-1}$) was 1.6 times better compared to the combination of perlite and black peat (472 g.(100 g dry matter)$^{-1}$). The type of other organic constituent did not affect water volume when combined with black peat, water volume was significantly higher when white peat was combined with coir pith (69.8% v/v) instead of wood fiber (67.8% v/v).

4. Conclusions

In conclusion, fractional factorial DOE is presented here as a tool for developing microbially active peat-reduced growing media mixtures for horticulture applications. The analysis revealed that white peat (+2.4% CO_2) and composted bark (+1.5% CO_2), in contrast to black peat and green waste compost respectively, are strongly promoting microbial activity. Moreover, white peat has an active native microbial community that might prove difficult for microbial biostimulants to persist after establishment. Our results, also, confirm that relatively low WFP values favor microbial growth. A WFP value of 62.95% increased CO_2 concentration with 2.1% compared to 82.95% WFP. Further research should focus on determining the effect of growing media constituents on plant growth and quality, and how this is associated with microbial community structure.

Author contributions

Thijs Van Gerrewey: Investigation, Formal analysis, Data curation, Visualization, Writing the Original Draft.

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Maaike Perneel: Conceptualization and Project administration.

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Danny Geelen: Conceptualization, Funding acquisition, Writing – Review and Editing, Supervision.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

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