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Biochars improve tomato and sweet pepper performance and shift bacterial composition in a peat-based growing medium

Vicky Lévesque[a,*], Thomas Jeanne[b], Martine Dorais[c], Noura Ziadi[a], Richard Hogue[b], Hani Antoun[c]

[a] Quebec Research and Development Centre, Agriculture and Agri-Food Canada, 2560 Hochelaga Blvd., Quebec City, QC G1V 2J3, Canada
[b] Institut de Recherche et de Développement en Agroenvironnement, 2700 Einstein St., Quebec City, QC G1P 3W8, Canada
[c] Centre de Recherche et d'Innovation sur les Végétaux, Université Laval, Pavillon de l'Envirotron, 2480 Hochelaga Blvd., Quebec City, QC G1V 0A6, Canada.

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ABSTRACT

We previously observed that the biochars used in this study can replace 15% (v/v) of perlite in a peat-based growing medium (PBGM) without causing any harm to microbial biomass. However, little is known about their impacts on the plant-microbe-soil interactions and crop productivity. Thus, the aim of the present work was to evaluate in greenhouse trials, the effect of substituting perlite in PBGM by three types of biochar on tomato cv. Micro-Tom and sweet pepper cv. Redskin, growth and yield, on their water and fertilizer use efficiency and on the bacterial diversity found in PBGM. Biochars were derived from maple bark pyrolysed at 550 °C (M550) and 700 °C (M700) and from pine chips pyrolysed at 700 °C (P700). Tomato and sweet pepper plants were grown in a greenhouse and fertigated with mineral fertilizer at full or half of the recommended level. Compared to the control without biochar, adding 5, 10 or 15% (v/v) biochar improved plant water use efficiency and increased tomato and sweet pepper fruit dry weight yield respectively, by up to 32% and 54%. Biochar significantly increased N and P uptake efficiency, while reducing loss of NO_3^- and PO_4^{3-} in leachates. In PBGM used to grow sweet pepper under full fertigation, the presence of 15% (v/v) M700 or P700 biochars significantly increased bacterial richness probably through the improvement of some ecological micro-niches such as C accessibility. This also allowed the establishment of potential plant growth promoting bacteria particularly *Agrobacterium*, *Cellvibrio* and *Streptomyces*, which showed a positive correlation with plant productivity and the chemical properties of PBGM. Our results suggest that the higher PBGM pH reached by adding 15% (v/v) biochar improved plant growth by increasing carbon, nitrogen and phosphorus availability and by favoring the establishment of plant beneficial bacteria. Therefore, substituting 15% (v/v) of perlite in PBGM with the three tested biochars appears to be a beneficial practice towards sustainable greenhouse production of tomato and sweet pepper, and this practice merit further investigation with other crops such as indeterminate cultivars.

1. Introduction

The most common substrate used as a growing medium in horticulture is peat, because of its favorable physicochemical properties for plant growth and its freedom from weeds and pathogens (Barrett et al., 2016). However, peat is a non-renewable resource due to its very long regeneration time (Kern et al., 2017). The physicochemical properties of a peat-based growing medium (PBGM) can be improved by adding aggregates such as perlite, but they are expensive (Allaire and Lange, 2017; Nemati et al., 2015; Zulfiqar et al., 2019) and not sustainable (Zulfiqar et al., 2019). To produce cheaper PBGM, alternative materials to substitute part of the peat and aggregates have been investigated (Evans, 2011; Nemati et al., 2015; Zulfiqar et al., 2019). Biochar was found to be an interesting sustainable component for PBGMs (Huang and Gu, 2019; Kern et al., 2017). A report indicated that 30% by volume of biochar made from hardwood logs, softwood bark, or hardwood waste by-products can replace perlite (Nemati et al., 2015). Biochar is a cheap component and it can have many additional benefits when used as a supplement to growing media (Nemati et al., 2015; Zulfiqar et al., 2019). For example, in comparison to the control, addition of 3% (w/w) holm oak biochar produced at 650 °C to a white peat used to grow strawberries for 13 weeks, increased plant dry and fresh weights and the rhizosphere bacterial diversity (De Tender et al., 2016). A meta-analysis using data representing normal field crop

* Corresponding author.
E-mail address: vicky.levesque@canada.ca (V. Lévesque).

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production conditions indicated that addition of biochar to soil increased crop yield, and soil carbon (C), phosphorus (P), potassium (K) and total nitrogen (N) in comparison to the control, suggesting a reduction of nutrient leaching losses (Biederman and Harpole, 2013). Similarly, addition of 20 or 60% (v/v) of an urban biochar to a commercial composted pine bark significantly reduced cumulative N and P leaching in comparison to the control containing 20% sphagnum peat (Kaudal et al., 2018). However, no effect on silverbeet biomass was observed after 11 weeks of growth in the greenhouse. Biochar may also have a synergistic effect with peat, as a pH-controlling agent for acidic PBGMs (Kern et al., 2017). By increasing the pH and the labile organic C and N compounds of soil, biochar may stimulate microbial activity (Anderson et al., 2011; Kolton et al., 2011; Zheng et al., 2016), promote biological immobilization of N and limit N leaching (Vaccari et al., 2015).

Biochar amendments can favor the development of specific microorganisms beneficial to plant growth and crop productivity. For example, increased yields of pepper in sandy soil (Kolton et al., 2011) and of tomato in growing medium containing coconut fiber-tuff amended with biochar (Graber et al., 2010) were partially attributed to the increase of plant-growth-beneficial microorganisms. Systemic resistance against foliar fungal pathogens was also observed in pepper and tomato after the addition of citrus wood biochar to soil or coconut-fiber-tuff potting medium (Elad et al., 2010). Biochar can improve the ability of arbuscular mycorrhizal fungi to protect host plant roots against infections transmitted by pathogens (Matsubara et al., 2002). In addition, biochar pores can serve as a refuge for bacteria and fungi in soil, protecting them from predatory soil microarthropods (Gul et al., 2015; Warnock et al., 2007).

Although biochar amendment has shown positive impact on soil microbial communities and on plant growth and productivity, its effect varies and depends on the physicochemical properties of biochar, its application rate and according to the biomass species used as feedstock (Huang and Gu, 2019).

In a previous incubation study using five biochars as alternative amendments to PBGM, we found that biochars differently influenced greenhouse gas emissions, C and N mineralization and microbial activity (Lévesque et al., 2018). Since the incorporation of 15% biochar by volume in PBGM did not show any detrimental effect on microbial biomass, we suggested that biochar can be advantageously integrated into PBGM used for the production of greenhouse horticultural crops to improve their properties and plant performance (Lévesque et al., 2018). To investigate how biochars influence plant growth and PBGM microbial communities, we evaluated how replacing perlite with different biochars in three proportions affects biomass production, nutrient uptake and water use by greenhouse tomato and sweet pepper cultivated under two levels of fertigation. Our hypotheses were a) replacing perlite with biochar in PBGM improves water and fertilizer-use efficiency, thus increasing crop biomass and yield; b) addition of biochar to PBGM increases microbial diversity and alters the composition of bacterial communities, promoting the establishment of plant growth-promoting bacteria that can be selected by the crop.

2. Material and methods

2.1. Biochar types and PBGM formulations

The production methods and physicochemical characteristics of the three biochars used in this study were previously described by Lévesque et al. (2018). Two biochars were derived from maple bark (*Acer saccharum*) pyrolysed at 550 °C (M550) and 700 °C (M700) and one was a commercial biochar (Biochar Engineering, Golden, CO USA) derived from pine chips (*Pinus strobus*) produced at 700 °C (P700). Biochars were ground and sieved to 2 mm before their use.

Ten PBGMs were formulated from a mix of sphagnum peat moss, perlite and biochar (M550, M700, or P700) at varying volumetric rates: (i) 73% peat + 27% perlite (control PBGM without biochar); (ii) 73% peat + 22% perlite + 5% biochar; (iii) 73% peat + 17% perlite + 10% biochar and (iv) 73% peat + 12% perlite + 15% biochar. As performed industrially (Premier Tech, Rivière-du-Loup, QC, Canada), initial PBGMs pH was adjusted with dolomitic ($1\,g\,L^{-1}$) and calcitic ($0–4.3\,g\,L^{-1}$) limestone to reach a pH of 5.5, and a starter granular fertilizer ($100\,mg\,N\,L^{-1}$, $23\,mg\,P\,L^{-1}$, and $90\,mg\,K\,L^{-1}$ of PBGM), a commercial wetting agent (AquaGro 2000 L; Aquatrols, Paulsboro, NJ, USA) and a spore suspension of *Rhizoglomus irregulare* DAOM 197,198 ($135\,spores\,L^{-1}$ of PBGM) were added. Once the pH of the mixtures was stabilized (approximately 2 weeks after the PBGM formulations), the initial chemical characteristics of the PBGMs were determined on a dry-matter basis according to the saturated media extract (SME) method (Warncke and Krauskopf, 1983) and are presented Table A.1.

The control PBGM had a bulk density of $0.12\,g\,cm^{-3}$ and a total porosity of $0.94\,cm\,cm^{-3}$ (Table A.1). Even though the used biochars had different bulk density (Lévesque et al., 2018), the addition of 5, 10 and 15% (v/v) of biochar had little effect on the final PBGMs bulk density (ranging from 0.12 to $0.14\,g\,cm^{-3}$) and the porosity (ranging from 0.92 to $0.94\,cm\,cm^{-3}$). In mass equivalent (w/w), 5, 10 and 15% (v/v) rates of biochar applications are respectively equivalent to 16, 28 and 38% for M550; 15, 27 and 36% for M700; and 7, 14 and 20% for P700.

2.2. Plant growth conditions

Two independent experiments were carried out in a Venlo greenhouse ($150\,m^2$) located at the Université Laval (Quebec City, QC, Canada; 46° 46′N, 71° 16′W) in 2014. The first experiment was conducted from 29 July to 30 September with tomato, *Solanum lycopersicum* cv. Micro-Tom (Tomato Growers Supply Company, Fort Myers, FL, USA), and the second took place from 29 October to 30 December with sweet pepper, *Capsicum annuum* cv. Redskin, (Norseco, Laval, QC, Canada). A climate-management system (Priva, De Lier, Netherlands) was used to adjust greenhouse relative humidity to 50%, corresponding to 1.24/0.97 kPa day/night vapor pressure deficit. Plants were grown under 21 °C/17 °C day/night temperature with a 16 h photoperiod of supplemental lighting (photosynthetic photon flux density of $150\,\mu mol\,m^{-2}\,s^{-1}$ at 1 m above the ground; HPS 600 W; P.L. Light Systems Inc., Beamsville, ON, Canada).

Seeds were sown and germinated in $64\,cm^3$ humid rockwool cubes (Delta; Grodan, Roermond, Netherlands) using tap water, then $100\,mg\,N\,L^{-1}$ was added when the first pair of true leaves unfolded. Seedlings (10 and 15 cm height for tomato and sweet pepper, respectively) were transplanted into plastic pots chosen to allow adequate root development (Ø 15.2 cm × 30.5 cm height; Stuewe and Sons, Inc., Tangent, OR, USA). The bottom of each pot was filled with 0.5 L of gravel separated with a geotextile membrane from the top 5 L of PBGM (Fig. 1). To measure the gas concentration in PBGMs air, 7 cm of a 10-cm long tube (Ø 3 mm; product code EW-06490-12; Cole Parmer, Montreal, QC, Canada) with a female connector (product code RK-45512-04; Cole Parmer) and a male Luer-lock stopper with an injectable ABS and polyisoprene membrane (product code 80891.00B; Vygon, Lansdale, PA, USA) was permanently inserted in each pot at a depth of 15 cm (Fig. 1). To assess bacterial diversity, a Nitex screen (50 µm mesh opening size; Dynamic Aqua-Supply Ltd., Surrey, BC, Canada) rectangular bag (7 cm long × 4 cm wide) was inserted vertically into each pot at a depth of 10 to 17 cm (Fig. 1) to prevent root penetration and to optimize the interaction between microorganisms and medium under the influence of roots, and to facilitate sampling (Wallander et al., 2001). Each bag was filled with the same mixture ($\sim 28\,cm^3$) added to each pot and was sealed with a thermal impulse sealer. The bags were removed 63 d after transplanting and kept at −80 °C in sterile Whirl-Pak bags before DNA extraction.

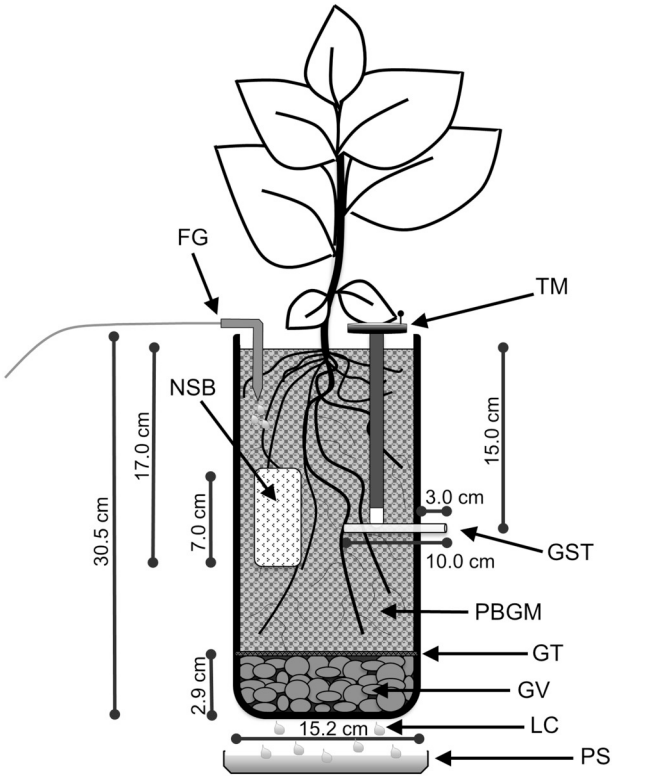

Fig. 1. Schematic illustration of the pots and measurement devices used for the greenhouse experiments.
FG, Fertigation; NSB, Nitex screen bag; TM, Tensiometer; GST, Gas sampling tube; PBGM, Peat-based growing medium; GT, Geotextile; GV, Gravel; LC, Leachates; PS, Plastic saucer.

For each crop, nine biochar treatments (3 biochar types × 3 biochar application rates) plus a control without biochar and two levels of fertigation, 100% (F100) or 50% (F50) of the recommended level (MAFF, 1996), were established in a greenhouse in a randomized complete block design with six replicates (1 pot per experimental unit), for a total of 120 experimental units. The pots were spaced 40 cm apart to prevent leaf overlapping. The mineral concentration in the F100 solution was equivalent to 200 mg N L^{-1} (1150 mg of 6-11-31 NPK and 850 mg of 15.5-0-0 NPK), and in the F50 solution to 100 mg N L^{-1}. The pH of the nutrient solution was adjusted to 5.5 with phosphoric acid 75% (Plant Products, Laval, QC, Canada; MAFF, 1996). The plants were fertigated with drippers, and the irrigation was controlled by tensiometers (0 to −80 kPa) connected to a pressure sensor (model ptd25-10-vh; Mécatronique DL Inc., Lévis, QC, Canada) and installed at a depth of 15 cm below the PBGM surface (Fig. 1). The matric potential was monitored with a data logger (LabVIEW; Mécatronique DL Inc.), and tension averages were sent at 15-min intervals to a water management system (Priva). Because of water retention by biochar, a preliminary experiment allowed us to determine irrigation thresholds for each biochar rates in the PBGMs, in order to maintain a similar water content (data not shown).

Accordingly, diurnal irrigation thresholds were set in PBGM at −3.0 kPa in the absence of biochar; at −3.5 kPa with 5% and −4.0 kPa with 10% and 15% biochar. At night irrigation was set at −7.5 kPa for all treatments. The average daily volume of irrigation water used during fertigation varied according to the biochar rate used (Table A.2). The leachates, corresponding to 10% to 30% of the daily volume of irrigation water, were collected in plastic saucers placed under each pot (Fig. 1). Means of daily leached water for each treatment of each crop during the 63-d growth are presented in Table A.3.

2.3. Plant measurements

Plants were harvested 63 d after transplanting, and shoot (leaf and stem), fruit and root biomass were measured. Roots were placed on a sieve (0.053 mm) to limit loss and were gently washed with tap water to remove attached peat. Root subsamples were stained with Trypan blue (Dalphe and Hamel, 2008) and the percentage of root segments colonized with *R. irregulare* was determined using the grid intercept method (Giovannetti and Mosse, 1980). Throughout the experiment, mature fruits were picked and dried, and yield was expressed as the total dry weight produced per plant. Shoots, fruits and roots were dried at 55 °C in a forced-draft oven for 14 d. The dried tissues were ground to 1 mm (Wiley Mill ED-5; Thomas Scientific, Swedesboro, NJ, USA), mineralized in a mixture of sulfuric and selenious acids (Isaac and Johnson, 1976), and the contents of N and P were measured (Bélanger et al., 2011) by automated continuous-flow injection (QuikChem 8000 system; Lachat Instruments, Loveland, CO, USA).

Efficiency of N or P uptake was calculated as the percentage of N or P present in the dry matter of the whole plant (shoot, root and fruit) taken up from the total amount of N or P retained in PBGM (g of nutrient applied – g of nutrient loss by leaching) during the 63 d of plant growth.

Water meters recorded the daily volume of irrigation water for each experimental unit throughout the experiment. The water-use efficiency (WUE) was calculated as follows:

$$WUE = TP/Wc$$

where *TP* is the total plant biomass (g dry matter $plant^{-1}$), and *Wc* is the total water consumption (L of water applied – L water loss by leaching) by the plant during the 63 d of growth.

2.4. Chemical analysis of PBGMs and leachates

After 63 d of plant growth, the concentrations of NO_3^-, PO_4^{3-} and dissolved organic carbon (DOC) as well as the pH were measured in the PBGMs by mixing 75 mL of deionized water with 150 mL of PBGM (Lévesque et al., 2018; Warncke and Krauskopf, 1983). A 12-g subsample was used to determine gravimetric water content by drying at 105 °C for 24 h. The leachates collected on days 35, 42, 49 and 56 after transplanting, and PBGM extracts were filtered using a Nynaflo disk filter (0.45-µm pore openings) and analyzed within 24 h. Concentrations of DOC in PBGMs, NO_3^- and PO_4^{3-} in leachates and in PBGMs were measured as previously described (Lévesque et al., 2018). For leachates, the total loss of nutrients during the 63 d of plant growth was calculated as the mean concentration of nutrients in leachate (mg L^{-1}) multiplied by the total volume of water collected (L).

2.5. Characterization of PBGMs microbiome

2.5.1. Microbial biomass in PBGMs

Microbial biomass C (MBC) was measured 63 d after seedling transplantation in the PBGMs and was determined by using the chloroform fumigation-extraction method (Voroney et al., 2008) as previously described (Lévesque et al., 2018). The MBC concentration was calculated as proposed by Voroney et al. (2008) using extraction efficiency coefficient of 0.35 (K_{EC}).

2.5.2. Gas measurement in the PBGM air

Air samples (20 mL) from the PBGMs were collected weekly on days 29, 36, 43, 50, and 57 after plant transplantation to measure respiration (CO_2 production). The samples were collected before the first irrigation of the day to avoid any disruption of gas diffusion by the fertigation event. The samples were transferred into pre-evacuated 12-mL vials (Exetainer vials; Labco, High Wycombe, UK). Concentrations of CO_2 were determined within 7 d with a gas chromatograph equipped with a flame ionization detector (Model 3800; Varian Inc., Walnut Creek, CA,

USA) and a headspace auto-injector (Combi Pal; CTC Analytics, Zurich, Switzerland). The CO_2 concentration was used as an indicator of PBGM respiration, including root respiration and soil microbial activity. An average daily concentration of CO_2 was calculated from air samples for each treatment collected during the growth of each crop.

2.5.3. DNA extraction and high-throughput sequencing of 16S rRNA gene amplicons

Bacterial diversity was assessed in the 15% biochar–F100 treatments and compared to the control without biochar. This rate of biochar proved to have no adverse effect on microbial biomass (Lévesque et al., 2018) and as practiced by the producers the recommended complete fertilization level was used to reflect bacterial communities present in PBGM under commercial production conditions. For this purpose, DNA was extracted from 0.255 g of PBGM sampled in each Nitex screen bag using a PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA). The concentration and quality of DNA were determined by spectrophotometry at 230, 260 and 280 nm (NanoVue Plus spectrophotometer; GE Healthcare, Amersham, UK).

Quantification of total bacteria was performed using a CFX96 detection system (Bio-Rad, Hercules, CA, USA). Briefly, total bacteria were quantified with the amplification systems established by Fierer et al. (2005), using SYBR Green qPCR Mastermix (Qiagen, Toronto, ON, Canada) as the reagent. The qPCR assay was performed in a 96-well plate, using EUB338 and EUB518 primers (Fierer et al., 2005) at a concentration of 0.3 µM in a final volume of 10 µL. Standard curves ($n = 3$) were generated from a known quantity of amplified DNA fragments, and then 10-fold serial dilutions over a 4-log range were carried out in order to determine the number of amplification units in each sample. The qPCR program consisted of an initial denaturation step at 95 °C for 15 min followed by 39 cycles of denaturation at 95 °C for 20 s, annealing at 53 °C for 30 s, and elongation at 72 °C for 30 s. The amplification efficiency was 89.5% with an R^2 of 0.992. The DNA extracts were tested for the presence of PCR inhibitors using the qPCR detection system program described above with an M13-assay that amplifies a cloned M13 sequence made with the TOPO PCR cloning kit (Thermo Fisher Scientific). No inhibition of the amplification process during the qPCR M13-assay was detected.

Bacterial diversity in the PBGMs was determined by Illumina MiSeq sequencing. The V6–V8 region of the bacterial 16S rRNA gene was amplified using primers B969F and BA1406R (Comeau et al., 2017), and a dual-indexed PCR approach specifically designed for sequencing with Illumina MiSeq technology was performed using the Genomic Analysis Platform (IBIS, Université Laval, Quebec City, QC, Canada). Briefly, sequences of specific genes were fused to the Illumina TruSeq primers, and PCR amplifications were conducted in a 50-µL volume containing 1 × Q5 buffer (New England Biolabs, Whitby, ON, Canada), 0.25 µM of each primer, 200 µM of each nucleotide (dNTPs), 1 unit of Q5 High-Fidelity DNA Polymerase (New England Biolabs), and 1 µL of reference cDNA. The qPCR program consisted of an initial denaturation step at 98 °C for 30 s followed by 35 denaturation cycles at 98 °C for 10 s, annealing at 55 °C for 10 s, and elongation at 72 °C for 30 s, and a final elongation at 72 °C for 2 min. The PCR products were purified with a clean-up solution (Axygen PCR clean-up kit; Fisher Scientific, Ottawa, ON, Canada), and their quality was verified on a 1% (w/v) agarose gel. The purified PCR products were diluted 50- to 100-fold and used as templates for a second PCR assay in order to attach dual indices and sequencing adaptors to all fragments. The second PCR assay was carried out as described above, except that only 12 cycles were performed after the denaturation step. Purification of the second PCR products was carried out as in the first assay, and the quality and quantity of the purified products were verified on a DNA 7500 Bioanalyzer chip (Agilent, Waldbronn, Germany). The purified PCR-amplified DNA fragments were pooled in equimolar amounts and then paired-end sequenced on the Illumina MiSeq platform using a 2 × 300 bp strategy (Comeau et al., 2017).

2.5.4. Downstream data analysis

The quality of Illumina MiSeq sequencing was verified, and the sequences were demultiplexed on the basis of the tags used, by determining the density of the clusters and the distribution of the Q-score (mostly above 30). Forward and reverse fragments were paired using the QIIME open-source bioinformatics pipeline, version 1.9.1 (Caporaso et al., 2010) and the Fastq-Join tool with a minimum overlap of 50 bp. In total, 587,140 fragments were obtained for all treatments in the two greenhouse crops, with an average of 12,232 fragments per sample. The quality of the reconstructed fragments was verified by FastQC, and the average reconstructed length was 436 ± 7 bp. Paired fragments were then clustered and filtered using the multiple_split_libraries_fastq.py command in QIIME. The fragments were clustered into operational taxonomic units (OTUs) with a similarity level of 97% using an open-reference approach with the Greengenes reference database, version 13.8 (DeSantis et al., 2006), to generate a bacterial OTU table. Singletons were removed from the bacterial OTU table by means of filtering using a minimum threshold of 10 sequences per OTU. The OTU table of 16S amplicon sequencing was normalized at 4000 sequences per sample on the basis of the rarefaction curves. The number of bacterial OTUs was determined and three alpha-diversity indices, Faith's PD, Shannon, and Chao1, were calculated from the bacterial OTU table derived from the QIIME (http://qiime.org/) command alpha rarefraction to estimate the bacterial richness within each sample. The indices Shannon and Chao1 also estimate the evenness within sample diversity (importance of rare OTUs) and Faith's PD takes into consideration the phylogeny of microbes to estimate diversity across a tree. The beta-diversity was also calculated using an unweighted UniFrac distance matrix from the bacterial OTU table in order to estimate the bacterial composition differences between samples (Lozupone and Knight, 2005), which was then visualized using the "capscale" function with unconstrained multidimensional scaling (MDS).

2.6. Statistical analyses

Statistical analyses were conducted by using the Mixed procedure of SAS 9.3, version 2012 (SAS Institute Inc., Cary, NC, USA). Data from the tomato and sweet pepper experiments were analyzed separately according to a randomized complete block design in order to compare the control PBGM with the nine biochar treatments. The chemical and biological analyses of PBGMs and leachates were performed on same four blocks randomly selected (Lévesque et al., 2018), whereas all six blocks were used to measure plant parameters because of their potential higher variability. A two-way analysis of variance (ANOVA) was performed to determine the effects of biochar, fertilization, and their interactions on plant biomass, total plant N and P contents, WUE, and N or P uptake efficiency, and on chemical biological properties in PBGM (pH, concentrations of DOC, NO_3^-, PO_4^{3-}, MBC, PBGM air CO_2), and nutrient leaching from PBGMs. The normality of data was examined using the Shapiro-Wilk test, and no transformation was required. Dunnett's test was used to compare the significant differences ($p < 0.05$) between means of the control and of each biochar PBGM, and polynomial contrasts were performed between the three biochar application rates (5, 10, and 15%).

A one-way ANOVA was applied to study differences among the mean alpha-diversity indices of bacteria according to a randomized complete block design with four replicates (same blocks used for chemical and biological analyses). Significant differences between means were established by Tukey's test at $p < 0.05$. A pairwise comparison of beta-diversity between bacterial communities in samples was tested using the "adonis" function, a nonparametric statistical analysis, on the basis of the unweighted UniFrac comparison matrix in QIIME. An R similarity test ("vegan" package in R) was calculated with the level of statistical significance at $p < 0.05$. The "envfit" function from the "vegan" library of the R package was used to correlate environmental variables with bacterial communities, and vectors were fitted onto the

Table 1
Effect of biochar types (M550, M700 and P700) and application rates (5, 10 and 15% v/v) on plant dry matter biomass, total plant N and P contents, and water use efficiency after 63 d of tomato (cv. Micro-Tom) and sweet pepper (cv. Redskin) growth.

Treatments	Plant biomass (g plant^{-1})			Total content[†] (mg plant^{-1})		WUE[‡] (g L^{-1})
	Shoot	Fruit	Root	N	P	
Tomato	Biochar effect (n = 12)					
Control	15.8	26.2	4.5	817	267	3.5
M550 (5%)	21.0*	32.2**	5.0	1019**	326**	5.1**
M550 (10%)	19.7	29.5	5.0	949	281	4.8**
M550 (15%)	20.7	29.2	4.9	996*	233	4.7**
M700 (5%)	19.1	29.4	5.1	924	318*	5.4**
M700 (10%)	21.2*	30.9*	5.1	1043**	321*	5.0**
M700 (15%)	21.6*	33.8**	5.1	1049**	272	4.8**
P700 (5%)	22.0**	34.7**	4.9	1003*	331**	5.3**
P700 (10%)	23.3**	31.8**	4.8	1056**	359**	5.2**
P700 (15%)	22.5**	32.9**	5.2	1044**	366**	4.8**
	Fertilization effect (n = 60)					
F50	20.7	31.4	5.1	977	300	5.0
F100	20.7	30.7	4.9	1003	315	4.8
Pepper	Biochar effect (n = 12)					
Control	38.8	51.5	7.8	2421	507	2.7
M550 (5%)	59.3**	66.6	9.0	3301**	637	4.5**
M550 (10%)	60.3**	79.1**	9.9**	3561**	690*	5.7**
M550 (15%)	53.2**	68.6	8.7	3073	492	5.1**
M700 (5%)	69.7**	78.1**	9.9**	4069**	803**	5.2**
M700 (10%)	56.2**	76.4**	9.4	3360**	673	5.9**
M700 (15%)	54.3**	72.2*	9.8*	3126*	576	5.2**
P700 (5%)	53.2**	70.7*	8.6	3193*	689*	4.5**
P700 (10%)	49.9	71.2*	8.9	3116*	673	5.6**
P700 (15%)	46.7	64.8	8.5	2805	625	4.9**
	Fertilization effect (n = 60)					
F50	44.8 b	65.4 b	8.1 b	2615 b	479 b	5.4 a
F100	63.6 a	74.6 a	10.0 a	3790 a	794 a	4.6 b

In each column, means followed by asterisks (biochar effect) are significantly different (*$p < 0.05$; **$p < 0.01$) from the control (Dunnett's test); means followed by different letters (fertilization effect) are significantly ($p < 0.05$) different (Tukey's test). Control, without biochar; biochar treatments: maple bark produced at 550 °C, M550 and 700 °C, M700, or pine chips produced at 700 °C, P700. Treatments were fertilized with 50% (F50) or 100% (F100) of the recommended level. All units are expressed on dry matter basis.

[†] Total mineral content present in shoot, fruit and root.

[‡] WUE, water use efficiency [g plant dry matter L^{-1} of total water consumption by plant (L of water applied – L water loss by leaching during the 63 d of plant growth; Table A.3)].

MDS ordination plots. Variation in the composition of bacterial communities between two treatments was estimated with 999 permutations. Pearson's product-moment correlation was also performed to assess the linear relationship between two normally distributed interval variables.

3. Results

3.1. Effect of biochar and fertilization on plant biomass, yield, total plant N and P contents and mycorrhizal colonization

Addition of biochar to PBGM significantly affected shoot and fruit biomass of tomato and all measured biomass in sweet pepper (Table A.4). None of the biochars had an adverse effect on shoot, fruit and root dry biomass for both crops. Significant increases in tomato shoot biomass ranging from 33% (5% M550) to 47% (10% P700) and in fruit dry matter yields ranging from 18% (10% M700) to 32% (5% P700) were observed (Table 1). With a few exceptions, significant increases in pepper shoot biomass and fruit dry matter yields ranging from 37% to 54% were observed with all biochar treatments (Table 1). The root biomass of pepper was on average 26% higher than in the control, in PBGM receiving 10% M550, and 5% or 15% M700. Fertilization levels had no effect on tomato root, shoot and fruit dry matter biomass, while pepper plants receiving the complete fertilization F100 produced significantly more shoot, fruit and root dry matter biomass (Tables 1; A.4).

In general, tomato and pepper plants cultivated in the presence of biochar contained more N and P than plants grown without biochar (Tables 1; A.4). Indeed, increases in total N content in tomato ranged from 22% (15% M550) to 29% (10% P700), and in pepper from 29% (10% P700) to 68% (5% M700) (Table 1). Similarly, in plants receiving biochar, total P increased from 19% with 5% M700 to 37% with 15% P700 in tomato and from 36% to 58% in pepper with the lowest rates (5%) of P700 and M700, and with 10% M550. The significant linear polynomial contrast analysis (Table A.4), however, indicated that total N and P contents in the whole pepper plants tend to decrease with increasing rates of biochar applications. In fact, with M700 and P700, pepper N and P content decreased when > 5% biochar was added to PBGM (Table 1). The level of fertilization did not influence the total N and P in tomato (Table A.4), but pepper plants receiving the complete F100 fertilization contained 45% more N and 66% more P than plants grown under F50 (Table 1).

In the control and all biochar and fertilization treatments, no mycorrhizal colonization was observed in tomato and pepper roots (data not shown).

3.2. Effect of biochar on uptake efficiency of N and P and on WUE

The percentage of N and P taken up by both crops from nutrients supplied by fertigation F50 generally increased in the presence of biochar (Table 2). However, with the complete fertilization F100, biochar had no effect on tomato N uptake efficiency and the use of 10% or more

Table 2
Effect of biochar types (M550, M700 and P700) and application rates (5, 10 and 15% v/v) on the percentage of N and P uptake efficiency after 63 d of tomato (cv. Micro-Tom) and sweet pepper (cv. Redskin) growth.

Treatments	Tomato[†]				Pepper	
	N (%)		P (%)		N (%)	P (%)
	F50	F100	F50	F100	F	
	Biochar effect (n = 6)				Biochar effect (n = 12)	
Control	51.2	36.6	34.9	40.2	40.2	20.1
M550 (5%)	82.4**	44.1	55.7**	42.2	62.8**	29.5
M550 (10%)	66.3	38.7	42.7	28.5**	73.3**	36.3**
M550 (15%)	69.5*	40.5	35.7	22.9**	69.0**	26.1
M700 (5%)	73.2**	38.6	54.7**	37.7	72.4**	34.9**
M700 (10%)	69.0	46.6	47.3	35.9	75.9**	36.8**
M700 (15%)	78.5**	38.4	43.1	25.9**	69.8**	30.3*
P700 (5%)	83.8**	44.7	59.9**	41.8	60.1**	31.5**
P700 (10%)	79.5**	48.0	60.7**	43.1	69.6**	38.9**
P700 (15%)	73.7**	40.3	54.4**	41.1	68.4**	37.8**
					Fertilization effect (n = 60)	
F50	–	–	–	–	74.8 a	32.0
F100	–	–	–	–	57.6 b	32.5

Uptake efficiency is the percentage of N or P, present in the dry matter of the whole plant (shoot, root and fruit) taken up from the total amount of N or P retained in PBGM (g of nutrient applied – g of nutrient loss in leaching; Table A.3) during the 63 d of plant growth.

In each column, means followed by asterisks (biochar effect) are significantly different (*$p < 0.05$; **$p < 0.01$) from the control (Dunnett's test); means followed by different letters (fertilization effect) are significantly ($p < 0.05$) different (Tukey's test). Control, without biochar; biochar treatments: maple bark produced at 550 °C, M550 and 700 °C, M700, or pine chips produced at 700 °C, P700. Treatments were fertilized with 50% (F50) or 100% (F100) of the recommended level. All units are expressed on dry matter basis.

[†] A significant interaction ($p < 0.05$) was observed for the efficiency of N and P uptake (Table A.4), therefore simple effects are presented.

M550 or 15% M700 reduced P uptake efficiency (Table 2), thus explaining the significant biochar × fertilization interaction found with tomato (Table A.4). For tomato crops under F50, biochar additions increased N and P uptake efficiency by 36% to 64% and by 56% to 74%, respectively, in comparison to the control (Table 2). For pepper, added biochars increased N uptake efficiency by 50% to 89%, and uptake was more effective with the low fertigation F50 (Table 2). With few exceptions, P uptake by pepper was 51% to 94% higher in biochar PBGMs than in the control and fertilization levels had no effect.

Biochar addition significantly improved WUE by tomato and pepper (Table 1). Fertilization levels had no effect on WUE of tomato, but pepper plants cultivated with F50 had a higher WUE than plants receiving F100 (Table 1).

3.3. Chemical properties of PBGMs

A significant biochar × fertilization interaction for the chemical properties of the PBGMs used to grow tomato and pepper for 63 days was observed (Table A.4). As indicated by polynomial contrast analyses for both crops fertilized with F50 and F100, the pH increased linearly with increasing maple biochar application rates and it was higher than in PBGM without biochar (Table 3). With a few exceptions, the rate of application of P700 did not change the pH of PBGMs of the two tested crops.

PBGMs of tomato had a similar DOC content regardless of the biochar or fertilization treatments, except for M700 (15%) under F100, where DOC was 54% higher than in the control (Table 3). For pepper under F100, higher DOC content was only observed in PBGM in the presence of 10% and 15% M550 or M700 (Table 3). Under both fertigation regimes, in M700 amended PBGMs used to grow pepper, DOC increased linearly with increasing biochar application rates. Under F100 fertilization, NO_3^- concentration in tomato PBGMs supplemented with all rates of maple biochar was 21% to 41% lower than in the control, whereas no significant effect was observed among all treatments under F50 fertigation (Table 3). The NO_3^- concentration was similar between the P700 PBGM and the control for both fertigation regimes in tomato. For pepper, NO_3^- concentration was 4- to 11-fold lower than the control, in 10% and 15% M550 and M700 under F50, but it was 2-fold higher in P700 (15%) under F100. For both crops under F50 and F100, the concentration of PO_4^{3-} decreased linearly in PBGM when more maple biochars were used (Table A.4), and it was lower than in the control without biochar (Table 3). Concentration of PO_4^{3-} was similar among P700 and the control PBGMs, except for P700 (15%) under F50, where it was higher than in the control of the two crops.

3.4. Nutrient leaching from PBGMs

The concentrations of NO_3^- and PO_4^{3-} in leachates collected from PBGM used to grow tomato and pepper were significantly influenced by the addition of some biochars. However, the biochar effect varied according to the level of fertigation as indicated by the observed significant biochar × fertilization interaction (Table A.4). For the two crops fertigated with F100, the addition of any biochar tested at a rate of 15% significantly reduced the concentration of NO_3^- and PO_4^{3-} in leachates, while under F50, their concentrations were lower in the PBGM amended with 15% M500 or M700 biochar than in the control (Table 4). The significant linear polynomial contrast analyses (Table A.4) indicate that the concentration of these two anions in leachates tends to decrease with the increasing rate of biochar applications for the two crops (Table 4).

3.5. Microbial biomass in PBGMs and respiration

The MBC in PBGM of tomato fertigated with F50 was similar in biochar treatments and in the control without biochar (Tables 3; A.4).

Table 3
Effect of biochar types (M550, M700 and P700) and application rates (5, 10 and 15% v/v) on chemical and biological properties of peat-based growing medium at the end of the 63 d of tomato (cv. Micro-Tom) and sweet pepper (cv. Redskin) growth.

Treatments	Chemical analysis								Biological analysis	
	pH		DOC (g C kg dry peat^{-1})		NO_3^- (g N kg dry peat^{-1})		PO_4^{3-} (g P kg dry peat^{-1})		MBC (mg C kg dry peat^{-1})	
	F50	F100	F50	F100	F50	F100	F50	F100	F50	F100
Tomato										
Control	6.64	5.93	1.15	0.61	0.41	5.77	0.50	1.24	1759	1687
M550 (5%)	7.03**	6.35**	1.12	0.67	0.26	4.20**	0.28**	0.67**	2242	1558
M550 (10%)	7.40**	6.91**	0.81	0.77	0.58	4.41**	0.27**	0.44**	1916	2064
M550 (15%)	8.12**	7.63**	1.07	0.70	0.17	3.38**	0.15**	0.21**	1788	960**
M700 (5%)	6.97**	6.36**	0.89	0.47	0.25	3.82**	0.38	0.72**	1769	1719
M700 (10%)	7.26**	6.77**	0.85	0.83	0.60	4.57**	0.32**	0.42**	2454	1839
M700 (15%)	7.89**	7.37**	1.31	0.94**	0.05	3.85**	0.16**	0.25**	1386	1295
P700 (5%)	6.67	5.99	0.74	0.61	0.36	5.08	0.40	1.25	1597	1113**
P700 (10%)	6.73	6.00	1.02	0.63	0.52	4.83	0.45	1.29	1291	1239*
P700 (15%)	6.51	6.00	0.89	0.63	0.85	4.79	0.80**	1.30	1765	1165**
Pepper										
Control	6.55	6.05	0.52	0.35	0.64	2.54	0.80	1.10	1362	1230
M550 (5%)	6.95**	6.32**	0.47	0.56	0.28	3.90	0.64*	0.93	2528**	1491
M550 (10%)	7.27**	7.39**	0.61	0.97**	0.11**	2.13	0.42**	0.26**	2350**	1555
M550 (15%)	7.91**	7.69**	0.61	0.69**	0.06**	3.32	0.23**	0.29**	2010**	1551
M700 (5%)	6.92**	6.44**	0.47	0.43	0.35	2.78	0.57**	0.82*	1881*	1326
M700 (10%)	7.14**	7.07**	0.74*	1.03**	0.16**	3.23	0.43**	0.36**	2340**	1419
M700 (15%)	7.69**	7.69**	0.82**	1.34**	0.07**	2.12	0.31**	0.23**	2204**	2600**
P700 (5%)	6.78**	6.24	0.48	0.58	0.72	3.96	0.79	1.32	2008**	1542
P700 (10%)	6.73**	6.16	0.56	0.47	0.33	4.06	0.76	1.37	1938*	1339
P700 (15%)	6.64	6.15	0.74*	0.56	0.27	4.72**	1.04**	1.31	2008**	1422

A significant interaction ($p < 0.05$) was observed for the chemical and biological analyses in peat-based growing medium (Table A.4), therefore simple effects are presented. Means ($n = 4$) in each column followed with an asterisk are significantly different of the control according to Dunnett's test (*$p < 0.05$; **$p < 0.01$). Control, without biochar; biochar treatments: maple bark produced at 550 °C, M550 and 700 °C, M700, or pine chips produced at 700 °C, P700. DOC, dissolved organic carbon; MBC, microbial biomass carbon.

Table 4
Effect of biochar types (M550, M700 and P700) and application rates (5, 10 and 15% v/v) on the total nutrient loss by leaching during the 63 d of tomato (cv. Micro-Tom) and sweet pepper (cv. Redskin) growth.

Treatments	Tomato		Pepper	
	Quantity in leachate (mg)[a]		Quantity in leachate (mg)[a]	
	NO_3^--N	PO_4^{3-}-P	NO_3^--N	PO_4^{3-}-P
F50				
Control	108.4	73.9	262.0	221.3
M550 (5%)	66.9	69.0	134.7	288.5
M550 (10%)	65.9	64.1	47.3*	248.6
M550 (15%)	25.5**	16.2**	14.3*	34.8**
M700 (5%)	161.9	121.1*	106.6	235.8
M700 (10%)	92.7	70.1	75.8	250.5
M700 (15%)	9.6**	11.7**	32.7*	52.2**
P700 (5%)	70.6	86.4	52.4*	317.2**
P700 (10%)	71.4	87.4	266.0	517.4**
P700 (15%)	70.3	74.4	32.4*	253.9
F100				
Control	648.9	371.5	1169.8	444.1
M550 (5%)	533.8*	195.3**	1143.1	351.0**
M550 (10%)	613.6	102.3**	261.6**	81.8**
M550 (15%)	530.5*	60.3**	399.9**	55.6**
M700 (5%)	578.1	222.6**	942.5	271.2**
M700 (10%)	339.2**	61.4**	926.3	175.8**
M700 (15%)	306.7**	62.2**	251.7**	15.2**
P700 (5%)	629.2	243.7**	1436.4	485.6
P700 (10%)	622.1	177.1**	916.5	318.3**
P700 (15%)	260.4**	110.7**	828.3**	313.6**

A significant interaction ($p < 0.05$) was observed for the total N and P loss by leaching (Table A.4), therefore simple effects are presented. In each column, means ($n = 4$) followed with an asterisk are significantly different from the control according to Dunnett's test (*$p < 0.05$; **$p < 0.01$). Control, without biochar; biochar treatments: maple bark produced at 550 °C, M550 and 700 °C, M700, or pine chips produced at 700 °C, P700.

[a] Quantity in leachates (mg) is the total amount of NO_3-N or PO_4-P loss by leaching during the 63 d of plant growth [Concentration in leachate (mg L^{-1}) × Volume of water loss by leaching (L); Table A.3].

However, under F100, MBC was significantly lower in PBGM receiving all rates of P700 or 15% M550 (Table 3). For pepper under F50, MBC in biochar treated PBGM was 38% to 86% higher than in the control. Under F100, MBC was similar in all treatments except with 15% M700, where it was 2-fold higher than in the control (Table 3).

For the two tested crops, PBGM CO_2 concentrations, produced by root respiration and soil microbial activity, were influenced by biochar addition, but not by the level of fertigation (Table A.4). The average CO_2 concentration in the PBGMs air of tomato crop increased with increasing biochar application rates as shown by the significant linear polynomial contrast analyses (Table A.4), and it was significantly higher than the control in the presence of 15% M550 or M700 (Fig. 2a). Concentration of CO_2 was 46% to 61% higher than the control in PBGM air when pepper was grown in the presence of any rate of maple biochars or with 5% P700 (Fig. 2b).

3.6. Bacterial diversity in PBGMs

Addition of 15% (v/v) biochar had a significant effect on OTUs and alpha-diversity indices in PBGM after 63 d of pepper growth under F100 fertigation, but no significant effect was observed with tomato (Table 5). In fact, pepper PBGM receiving M700 or P700 contained more OTUs than the control without biochar. The Faith's PD index in PBGM increased with the addition of biochar, particularly M700, and Chao1 and Shannon indices were, respectively, 15% and 6% higher than the control with M700 and P700. For both crops, significant differences in the beta-diversity of bacteria were also observed between the biochar treated PBGMs and the control, as assessed by the "adonis" statistical test (Table A.5). Significant dissimilarities in bacterial composition were noted between the two maple PBGMs, the control and P700 (Fig. 3).

The composition of bacterial communities was significantly linked to the chemical and biological properties of the PBGMs amended with biochar (Fig. 3). For both crops, positive correlations were observed between the bacterial communities of M700 and DOC; communities of M550 and M700 and the pH; and communities of the control and P700 and the PO_4^{3-}. For tomato, NO_3^- was correlated with bacterial communities from P700 and the control (Fig. 3a). For pepper, CO_2 was positively correlated with the bacterial communities of M550 and M700, whereas MBC was positively correlated with those of M700 (Fig. 3b).

Several heterotrophic bacteria were found in the PBGMs of both crops and were significantly influenced by biochar amendments, particularly maple biochars (Table 6). Indeed, the relative abundance of bacteria related to the families *Oxalobacteraceae*, *Methylophilaceae*, and *Cytophagaceae* and the genera *Devosia*, *Hyphomicrobium*, and *Cellvibrio*, was generally higher with maple biochars than with P700 and the control, whereas bacteria related to the family *Sphingobacteriaceae* and the genera *Bradyrhizobium* and *Flavobacterium* were less abundant. For both crops, the relative abundance of bacteria related to the genera *Streptomyces* and *Rhodanobacter* were higher in P700 than in the other PBGMs. Bacteria related to the genus *Rhodoplanes* were more abundant with M700 for pepper, and those of the genus *Mucilaginibacter* were higher in P700 for tomato. Except for *Sphingobacteriaceae*, *Methylophilaceae*, *Cytophagaceae*, *Hyphomicrobium* and *Rhodoplanes*, bacteria found in PBGMs have previously been reported to play a beneficial role in plant growth (Table 6).

According to the Pearson product-moment correlation coefficient, the relative abundance of the genera *Agrobacterium*, *Cellvibrio* and *Streptomyces* showed significant positive correlations with total plant biomass, total plant N and P contents, N uptake efficiency and total P in PBGMs (Table A.6). *Agrobacterium* and *Cellvibrio* were also positively correlated with DOC, while *Agrobacterium* and *Streptomyces* were positively correlated with total N in PBGMs.

4. Discussion

4.1. Plant productivity in biochar amended PBGM under two fertigation regimes

In the absence of biochar, the growth of tomato cultivar Micro-Tom was not affected by the fertilization level, indicating that the nutrients in F100 solution exceed plant requirements. On the contrary, sweet pepper dry matter biomass and N and P contents were significantly reduced under F50 fertilization (Table 1) confirming the high nutritional requirement of this plant (Olsen et al., 1993).

Addition of biochar improved tomato and sweet pepper shoot biomass under both fertigation regimes, this can be partly explained by a better nutrient uptake efficiency and a higher WUE (Table 1). In fact, PBGMs receiving 15% (v/v) biochar, especially maple biochars, lost significantly less N and P by leaching than the control without biochar (Table 4), suggesting a better availability of nutrients for plant growth. This is due to the more alkaline nature of the maple biochar, which also had a higher concentration of calcium carbonate than the P700 biochar (Lévesque et al., 2018), thus favoring PO_4^{3-} precipitation on its surface (Alburquerque et al., 2013; Major et al., 2009). The three biochars used in this study also previously showed significant potential for adsorbing nitrogen (NH_4^+ and NO_3^- forms) and retaining water on their surfaces (Lévesque et al., 2018). These results are also confirmed by the significant and positive correlations observed between tomato and pepper plant biomass, total plant N and P contents, and WUE (Table A.7). Our results agree with a previous report indicating that biochar can improve crop productivity by reducing nutrient leaching, thus increasing nutrient uptake efficiency (Hussain et al., 2017). As previously reported

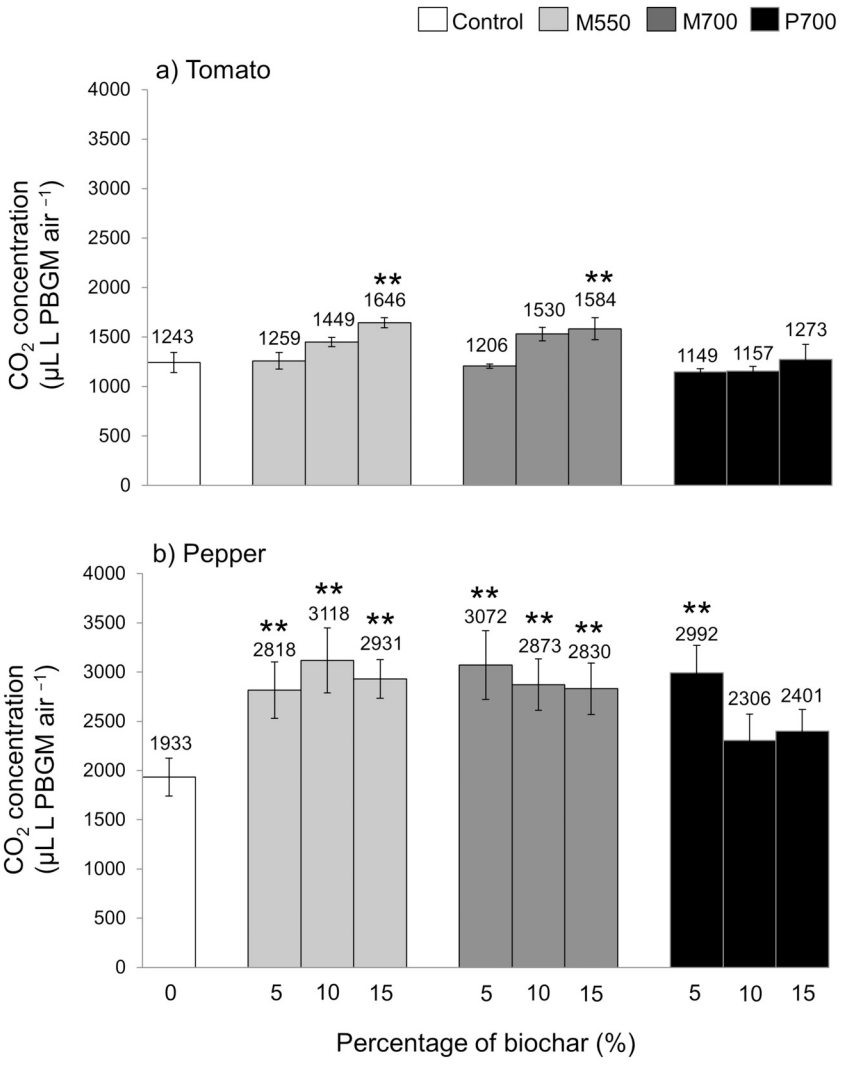

Fig. 2. Effect of biochar types (M550, M700 or P700) and application rates (5, 10 or 15% v/v) on average daily CO_2 concentration detected in the PBGM air calculated from samples collected 29, 36, 43, 50 and 57-d after transplantation of tomato (cv. Micro-Tom) and sweet pepper (cv. Redskin).
Means (n = 40) of treatments marked with an asterisk are significantly different of the control according to Dunnett's test (**$p < 0.01$). Bars show standard errors (± SE) of means. PBGM, peat-based growing medium. Control, without biochar; biochar treatments: maple bark produced at 550 °C, M550 and 700 °C, M700, or pine chips produced at 700 °C, P700.

(Jaiswal et al., 2017; Kaudal et al., 2018; Vaughn et al., 2013), the increase of the biochar application rate increased PBGM pH and was not deleterious to plant growth. Our results are also congruent with those of Dorais et al. (2016), indicating that up to 30% (v/v) of a biochar, produced by pyrolysis at 750 °C of a mixture of balsam fir and white and black spruce, can replace peat in PBGM without causing any harmful effect on sweet pepper growth.

4.2. Respiration and bacterial diversity in PBGM as affected by biochars

Soil microbial activity is strongly influenced by the metabolizable C compounds in biochar (Gul et al., 2015). The labile C fraction in biochar can provide more available C for the microbial community, leading to increase microbial respiration (Lévesque et al., 2018). Additionally, high mineral ash biochars can be oxidized faster than those

Table 5
Effect of biochar types (M550, M700 and P700) on the number of observed OTUs and on alpha-diversity indices of bacteria in peat-based growing medium after 63 d of tomato (cv. Micro-Tom) and sweet pepper (cv. Redskin) growth and fertilized with the complete recommended level.

Crop	Treatments	OTUs observed	Alpha-diversity indices		
			Faith's PD	Chao1	Shannon
Tomato	Control	920 ± 63	62 ± 3	1752 ± 140	8.1 ± 0.2
	M550	932 ± 82	68 ± 4	1795 ± 143	7.7 ± 0.6
	M700	1028 ± 14	73 ± 1	1927 ± 32	8.3 ± 0.1
	P700	1000 ± 47	69 ± 3	1964 ± 92	8.2 ± 0.4
Pepper	Control	983 ± 10 c	67 ± 1 c	1771 ± 15 b	8.1 ± 0.1 b
	M550	1031 ± 23 bc	73 ± 1 b	1936 ± 48 ab	8.4 ± 0.1 ab
	M700	1108 ± 12 a	77 ± 1 a	2081 ± 38 a	8.7 ± 0.1 a
	P700	1059 ± 20 ab	73 ± 1 b	2006 ± 64 a	8.5 ± 0.1 a

For pepper, means ± standard errors (n = 4) in each column followed by different letters are significantly different at $p < 0.05$ according to Tukey's test. For tomato, biochar addition had no significant effect. Control, without biochar; biochar treatments received 15% (v/v) of: maple bark produced at 550 °C, M550 and 700 °C, M700, or pine chips produced at 700 °C, P700.
OTUs, operational taxonomic units; Faith's PD, Faith's phylogenetic diversity.

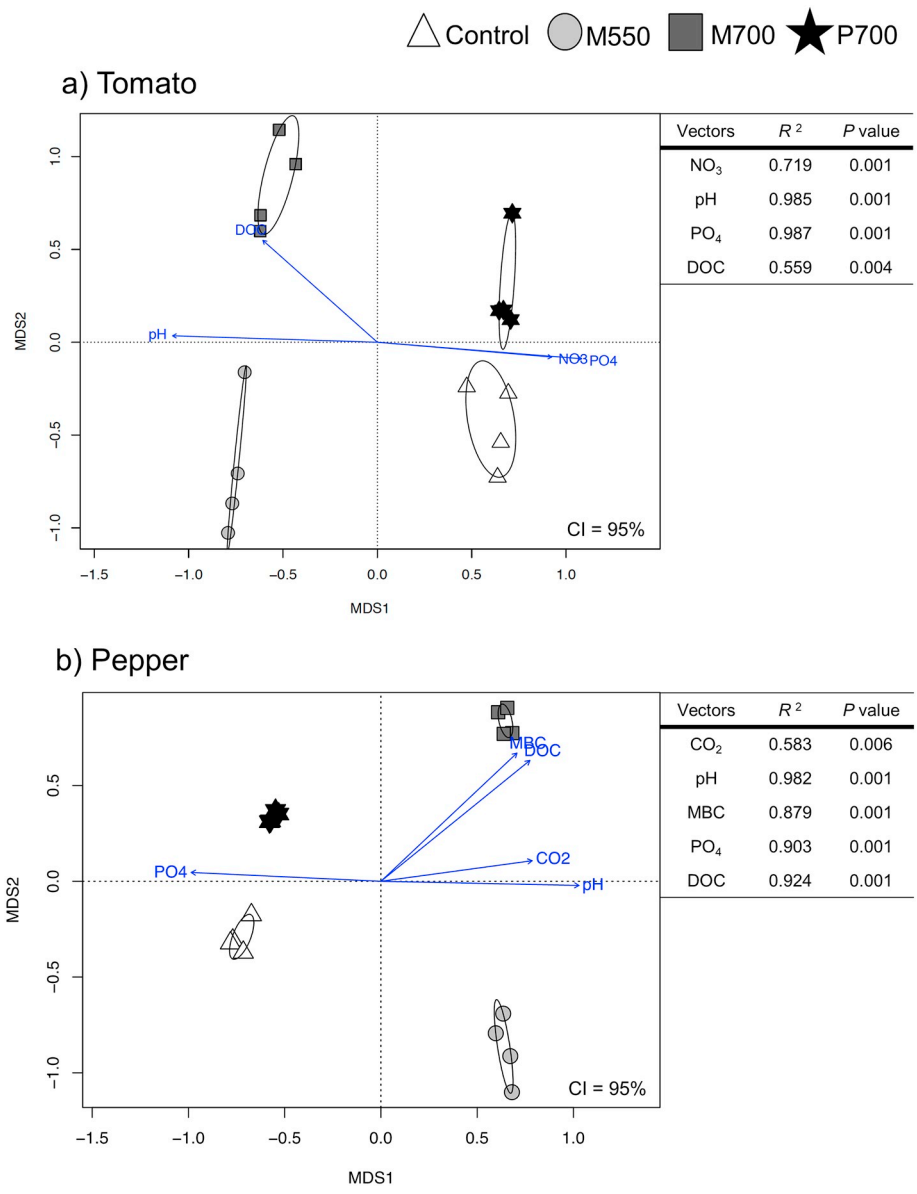

Fig. 3. Metric multidimensional scaling (MDS) ordination patterns based on unweighted UniFrac distance matrix of the beta-diversity of bacterial communities present in the peat-based growing media of tomato (cv. Micro-Tom) and sweet pepper (cv. Redskin) grown for 63 d in the presence or absence of biochar and fertilized with the complete recommended level.

The correlations between vectors and bacterial communities of tomato and pepper crops were tested using the function of 'envfit' from 'vegan' library of the R package (based on 999 permutations), and significant vectors (R^2-values and *P*-values were shown on the right panels) were fitted onto the MDS ordination plots.

MBC, microbial biomass of carbon; DOC, dissolved organic carbon; CI, confidential interval to separate clustering of bacterial groups. Control, without biochar; biochar treatments received 15% (v/v) of: maple bark produced at 550 °C, M550 and 700 °C, M700, or pine chips produced at 700 °C, P700.

with low mineral ash, and they possibly improve nutrient availability for growth and activity of soil microorganisms (Joseph et al., 2010). Méndez et al. (2017) also reported that stimulation of microbial activity can, in part, be attributed to an increase of PBGMs pH and by the mineralization of a fraction of biochar C and other nutrients used as an energy source. Biochar derived from maple has a higher mineral ash content than biochar derived from pine (Lévesque et al., 2018), which can explain, in part, the greater production of CO_2 in the PBGMs amended with 15% maple biochars, especially for tomato. According to polynomial contrasts, the linear relation between CO_2 production and the biochar application rates in PBGM of tomato (Table A.4) corroborate with the increase of pH by biochar.

On the contrary, no positive correlation was observed between CO_2 production and biochar application rates in the PBGM of pepper (Fig. 2b; Table A.4). Joseph et al. (2010) reported that a complex set of reactions can occur between biochar, plant roots and microorganisms in the rhizosphere, affecting microbial activity. Concentration of CO_2 in the air of the substrate can also be affected by plant root density and physical properties of the substrate, such as porosity and water retention. In general, a greater plant biomass releases more root exudates and root detritus in soil, thus improving microbial activity through a better availability of organic C in soil (Jaiswal et al., 2017; Liu et al., 2016). Our results obtained with sweet pepper corroborate these observations since the high pepper plant biomass released substantially more CO_2 than the lower biomass produced by the dwarf tomato plant. As reported by Dijkstra et al. (2006), we observed positive correlations between CO_2 production and leaf biomass ($R^2 = 0.841$; $p < 0.0001$) and between CO_2 and aboveground biomass ($R^2 = 0.853$; $p < 0.001$) (Fig. A.1). Moreover, the positive correlation obtained between the CO_2 production and root biomass ($R^2 = 0.785$; $p < 0.0001$) confirms that a high root biomass and higher root respiration support a more important microbial respiration caused by a better C availability in PBGM.

The results of the MDS analysis showed that in PBGM amended with 15% (v/v) maple biochars used to grow tomato and sweet pepper, the pH, and to a lesser extent the DOC, strongly correlated with the composition of the bacterial community (Fig. 3). This agrees with previous reports indicating that the pH of soil or growing media used with different crops and their content in available C are the main factors mediating microbial diversity (Gul et al., 2015; Hussain et al., 2017; Jaiswal et al., 2017; Kolton et al., 2017). Therefore, our results suggest that the pH of PBGM amended with biochar had the highest impact on plant growth by improving the availability of nutrients such as C, N and

Table 6
Effect of biochar types (M550, M700 and P700) on the relative abundances of families and genera of heterotrophic bacteria identified in a peat-based growing medium after 63 d of tomato (cv. Micro-Tom) or sweet pepper (cv. Redskin) growth and fertilized with the complete (F100) recommended level. Putative beneficial roles of bacteria on plant growth and references are also included.

	Tomato				Pepper				Beneficial roles for plants	References
	Control	M550	M700	P700	Control	M550	M700	P700		
Family (%)										
Cytophagaceae	1.38 b	32.17 a	18.18 a	0.57 b	0.39 D	18.57 A	6.93 B	1.38 C	–	McBride et al., 2014
Methylophilaceae	0.18 bc	4.48 a	1.46 b	0.06 c	0.04 C	6.09 A	1.50 B	0.02 C	–	Doronina et al., 2014
Oxalobacteraceae	0.74 b	0.98 ab	2.00 a	0.54 b	0.19 B	1.36 A	1.71 A	0.18 B	PGP, PS	Green et al., 2007; Sylva et al., 2017
Sphingobacteriaceae	7.30 a	0.32 b	0.68 b	5.84 a	5.18 A	0.48 C	1.29 B	8.06 A	–	Lambiase, 2014
Genus (%)										
Agrobacterium	0.17 b	0.40 ab	0.59 a	0.47 ab	0.30C	0.90 B	1.55 A	0.77 B	PS	Rodríguez and Fraga, 1999
Bradyrhizobium	0.75 a	0.05 c	0.15 b	0.23 ab	0.54 A	0.02 B	0.03 B	0.64 A	PS, N_2 fixation, BCA, PGP,	Antoun et al., 1998; Jaiswal et al., 2017
Cellvibrio	0.01 b	0.28 a	0.17 a	0.01 b	0.09 B	1.26 A	1.35 A	0.01 B	N_2 fixation, PGP, PSRI	Fonte et al., 2000; Jaiswal et al., 2017; Wu and He, 2015
Devosia	0.77 b	1.52 a	0.76 b	0.57 b	0.15 D	1.19 A	0.60 B	0.38 C	N_2 fixation, PGP, PSRI	Jaiswal et al., 2017; Verastegui et al., 2014
Flavobacterium	4.37 a	0.04 b	0.13 b	2.72 ab	13.47 A	0.52 B	0.17 C	1.41 AB	BCA	Jaiswal et al., 2017; Kolton et al., 2011
Hyphomicrobium	0.09 b	0.10 b	0.34 a	0.11 b	0.07 B	0.13 B	0.21 A	0.09 B	–	Oren and Xu, 2014
Mucilaginibacter	0.03 b	0.01 b	0.02 b	0.19 a	0.03 AB	0.02 AB	0.01 B	0.04 A	PGP	Jaiswal et al., 2017
Pseudomonas	0.01 b	0.01 b	0.05 a	0.01 b	0.05 B	0.11 AB	0.12 AB	0.24 A	PGP, PS	Rodríguez and Fraga, 1999
Rhodanobacter	1.89 b	0.02 c	0.33 bc	4.91 a	0.17 B	0.02 B	0.09 B	0.47 A	BCA,	Jaiswal et al., 2017
Rhodoplanes	5.03 a	4.76 a	5.40 a	5.43 a	4.34 B	5.49 B	12.05 A	4.04 B	–	Oren and Xu, 2014; Ye et al., 2016
Streptomyces	0.17 b	0.75 b	1.85 a	1.85 a	0.72 B	3.47 B	3.5 B	8.21 A	BCA, PGP, PS	Jog et al., 2014; Kämpfer et al., 2014; Viaene et al., 2016

Means ($n = 4$) in each row followed by different letters are significantly different at $p < 0.05$ (Tukey's test). Control, without biochar; biochar treatments received 15% (v/v) of: maple bark produced at 550 °C, M550 and 700 °C, M700, or pine chips produced at 700 °C, P700.
BCA, biocontrol agent; PGP, plant growth promotion; PS, phosphate solubilization; and PSRI, plant systemic resistance inducers.

P and by shifting bacterial community structures in the PBGM. Our study, performed with three different biochars and two crop species, further showed that bacterial diversity response (richness and composition) depends on the interaction between the biochar type and the crop species used.

The bacterial phyla such as *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Gemmatimonadetes Proteobacteria* and *Verrucomicrobia* were identified in the rhizosphere of healthy tomato plants cultivated in different media (Nassal et al., 2018; Tian et al., 2015, 2017). These phyla were also dominant in the PBGM without biochar of tomato (Fig. A.2). Among bacterial endophytes isolated from healthy tomato root microbiome, Tian et al. (2017) observed several genera, including *Bradyrhizobium*, *Devosia*, *Flavobacterium*, *Pseudomonas*, *Rhodanobacter* and *Streptomyces*, which were also found in all PBGMs after 63 d of tomato and sweet pepper growth.

Several heterotrophic bacteria found in the PBGM amended with biochar could potentially be beneficial for plant growth, such as those involved as biocontrol agents, in plant growth promotion, in the solubilization of phosphates and in N_2 fixation (Table 6). A previous study observed a positive effect of biochar-amended sand on canopy biomass of tomato and plant resistance towards the foliar fungal pathogen *Botrytis cinerea* (Kolton et al., 2017). The authors hypothesized that the biochar stimulation of these plant-defense mechanisms is linked to different factors, such as shifts in root-associated microbial communities and biochar-associated chemical constituents or changes in plant physiological parameters. The authors also revealed that root-associated bacteria belonging to the phyla *Bacteriodetes* and *Proteobacteria* increased with biochar amendment, as observed in our study (Table A.8), and these were linked to the stimulation of plant-defense mechanisms. In addition, some species from the genera *Agrobacterium* (Rodríguez and Fraga, 1999), *Cellvibrio* (Fonte et al., 2000; Jaiswal et al., 2017; Wu and He, 2015), *Devosia* (Jaiswal et al., 2017; Verastegui et al., 2014), *Rhodanobacter* (Jaiswal et al., 2017) and *Streptomyces* (Jog et al., 2014; Kämpfer et al., 2014; Viaene et al., 2016) are known to be beneficial to plant growth. These bacteria were also detected in this study, and their abundance was higher in the PBGMs amended with biochar (Table 6). In addition, *Agrobacterium*, *Cellvibrio* and *Streptomyces* were positively correlated with parameters measured in the biomass and in the PBGMs (Table A.6). However, we cannot exclude the proliferation of detrimental bacteria in the PBGM amended with biochar, as the genera *Agrobacterium* and *Pseudomonas* contain several plant pathogens strains. In a previous study, biochar added in organic potted plants of sweet pepper did not suppress of root colonization by *Pythium ultimum*, but no negative effect on plant growth was observed (Dorais et al., 2016).

The absence of mycorrhizae in the treatments of both crops is probably caused by the high P concentration added in PBGMs (Warnock et al., 2010).

5. Conclusions

This study showed that the replacement of perlite by three different types of biochar at up to 15% by volume (v/v) in PBGMs improved their properties, resulting in an increased plant biomass and fruit yield of dwarf tomato and sweet pepper. The improved nitrogen and phosphorus retention in PBGM amended with 15% biochar increased the availability of nutrient for plant growth and reduced their loss by leaching. In addition, enhancement of the microbial abundance, activity and diversity in the PBGM amended with biochars was likely due to the improvement of the ecological micro-niche through a better nutrient accessibility, especially in PBGM amended with maple bark pyrolyzed at 700 °C. The increase of pH and dissolved organic carbon in PBGM amended with this biochar strongly influenced the bacterial composition in the PBGM. Our results support the premise that the positive effects of biochar on crop yield are attributed, in part, to the increase in bacterial richness and to shifts in bacterial composition

towards beneficial heterotrophic bacteria such as *Agrobacterium*, *Cellvibrio* and *Streptomyces*. As hypothesized, the addition of biochars to the PBGM significantly improved plant water-use efficiency and nutrient uptake efficiency, and, as observed with the dwarf cultivar of tomato, it may also allow a reduction of fertilizer inputs without reducing fruit yield. However, further work is required to determine how biochar can reduce fertilizer input without reducing fruit yields when different horticultural crops are considered. Nevertheless, the present study confirms the suitability of the three tested biochars for replacing perlite at up to 15% (v/v) in PBGM and use as amendments to greenhouse substrates without affecting negatively plant performance and bacterial diversity in PBGM.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2020.103579.

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