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Research Paper

Impact of a wood-based biochar in peat-reduced and peat-free substrates on strawberry cultivation and rhizosphere microbiome dynamics

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ABSTRACT

A disadvantage of soilless cultivation is the use of peat, which contributes to CO_2 emissions. Biochar addition to horticultural substrates has been reported to affect strawberry growth and disease suppression. This study investigated the impact of two substrate types, namely peat-reduced and peat-free substrates, on strawberry growth, health and disease suppression. Additionally, the effect of 10% v/v biochar addition to both substrate types was assessed on these parameters. A strawberry trial was performed in 2021 and 2022. Strawberry yield, aboveground fresh and dry weight, root rot severity caused by *Phytophthora cactorum*, lipid peroxidation, antioxidant capacity and the rhizosphere microbiome were evaluated. Results of the two trials varied partly due to differences in disease severity between years. Overall, the findings showed the potential of replacing peat with local alternatives, as no adverse effects were observed on the growth and health of strawberry plants grown on both substrate types. In 2022, the year with a high disease severity, an increase in root rot severity was found on strawberry plants grown on peat-free substrates compared with peat-reduced substrates, but this adverse effect was not observed in the blend with biochar addition. Results from both years indicate a neutral to positive effect of biochar on the aboveground fresh and dry weight and the stress levels of strawberry plants. The substrate type influenced the rhizosphere microbiome, whereas biochar addition only had little to no effect on the microbial community. This study shows potential for replacing peat in soilless strawberry cultivation with local alternatives, including biochar.

1. Introduction

Modern agriculture faces many environmental problems that could endanger our global food production: climate change, water scarcity, pests, diseases and soil degradation are the most prominent. Estimates indicate that over 3.2 billion people are affected by soil degradation, leading to 800 million people suffering from malnutrition globally (Prăvălie, 2021). This increasing human population demands higher food and biomass production, while protecting the environment at the same time. Exploiting new land for agricultural use is difficult and expensive, but a possible way to increase food production is by optimizing horticultural practices. Horticulture has a potential yield of up to 50 kg per m^2 per year and more, so it can significantly contribute to global food security (Eigenbrod et al., 2015). However, a disadvantage of horticulture, specifically soilless cultivation, is the use of high amounts of peat. Current high prices and stricter protective legislation on wetland destruction for peat excavation are putting its use under pressure (Zulfiqar et al., 2019). Although peatlands cover only 3 % of the earth's surface, they possess one-third of the stored carbon (C), making their conservation a priority in combating climate change (Dunn et al., 2011). In Belgium, >20 % of the total amount of peat in horticulture, or 140 000 m^3, is used for soilless strawberry cultivation (Vandecasteele et al., 2023). Replacing at least part of this share in substrates with local and sustainable alternative materials could contribute to the conservation of peatlands (Fryda et al., 2019; Pot et al., 2022). Local alternatives are necessary to mitigate greenhouse gas emissions associated with

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transportation of materials.

Biochar is one of the promising materials that could partially replace peat (Amery et al., 2021). This carbon-rich material is produced by pyrolysis, a thermochemical conversion process under oxygen-limited conditions of biomass (Cha et al., 2016). Biochar exhibits various properties that makes it a good candidate for peat substitution, such as increased water holding capacity of the substrate, possible increased plant growth and disease suppression, and increased nutrient supply (Amery et al., 2021; Blok et al., 2017; De Tender et al., 2016). Therefore, biochar can serve multiple roles in horticultural substrates. It can be used in small amounts (< 5 %) to reduce disease susceptibility, or replace lime and fertilizers, or in larger amounts (> 5 %) as bulk replacement for peat (Amery et al., 2021; Frenkel et al., 2017). When applied in small amounts, biochar's main mechanisms for increased plant growth and disease resistance is induced via changes in the nutrient dynamics and the rhizosphere microbiome (Amery et al., 2021; Jaiswal et al., 2024). When biochar is used in higher amounts as bulk replacement, i.e., at 10 % v/v or higher, a significant impact on the physicochemical properties of the substrate is expected (Rathnayake et al., 2021). One crucial factor is the acid buffering capacity of the biochar, i.e., the biochar's ability to increase the pH of the substrate, since most biochars are alkaline (Lataf et al., 2022; Margenot et al., 2018). The use of biochar with a high acid buffering capacity could reduce the need for liming but may also result in an alkaline pH combined with electrical conductivity (EC) beyond the optimal range for plant growth when biochar is applied in higher v/v % (Blok et al., 2017; Nobile et al., 2020; Rathnayake et al., 2021). The use of biochars with a low acid buffering capacity is key for successful peat replacement. Wood-based biochars mostly have a low acid buffering capacity (Blok et al., 2017; Lataf et al., 2022). Next to an effect on the pH of the substrate, leachate experiments with biochar produced from different feedstock types indicated that biochars produced from spent growing media could act as a source of nutrients and salts in substrates, in contrast to biochars produced from lignocellulosic biomass (Amery et al., 2021). The biochars in previous studies with plant trials at 10 % v/v biochar application or more, are mainly wood-based biochars (Köster et al., 2021; Rathnayake et al., 2021; Vandecasteele et al., 2023). Studies comparing biochars from different feedstocks conclude that wood-based biochars are more suited for peat replacement than biochars produced from nutrient-rich feedstocks like greenhouse or other agricultural crop residues (Blok et al., 2017; Nobile et al., 2020). These nutrient-rich biochars are too saline and too alkaline to be applied in horticultural substrates since higher biochar pH and EC generally had a detrimental effect on plant biomass.

For peat replacement with biochar, the focus is to obtain at least the same yield as in peat substrates without biochar. Blok et al. (2017) found neutral effects of 10 % wood-based biochar addition on the fresh weight of *Gerbera* sp. plants. Köster et al. (2021) investigated three different concentrations of wood-based biochar (5 %, 10 % and 20 % v/v) and found that the addition of 10 % v/v biochar could increase the shoot dry weight of spruce seedlings, while the other concentrations did not. Additionally, adding 10 % biochar produced from sewage sludge resulted in improved growth of lettuce (Mendez et al., 2017). Hence, the effect of 10 % v/v biochar addition is highly dependent on the biochar type (related to the applied feedstock) and targeted crop species (Nobile et al., 2020). In most trials on peat replacement by biochar, the materials used in the blends are restricted to peat, coir and biochar. To the best of our knowledge, the effect of biochar in a fully peat-free substrate on strawberry growth has not yet been investigated for blends with wood fibre, green compost, plant fibre and bark compost. In addition to biochar, green compost, bark and wood fibres are used in the substrates to reduce the amount of peat or totally replace peat for strawberry cultivation since previous literature already confirmed their success in partly replacing peat (Moelants et al., 2021; Vandecasteele, et al., 2023; Vandecasteele et al., 2024). Therefore, this study aimed to investigate the effect of using horticultural substrates with: (1) a reduced amount of peat compared to peat-free substrates and (2) 10 % v/v biochar amendment in peat-reduced and peat-free substrates on strawberry growth, yield, and disease resistance. The rationale for selecting a 10 % v/v biochar inclusion rate was to test if this rather limited amount of biochar is successful for peat replacement and may even already result in a higher success rate in applying peat-reduced and peat-free blends for strawberry cultivation. The obtained data were linked to the rhizosphere microbiome, nutrient concentrations in the substrates and plants, as well as plant stress parameters as potential explanations for the observed effects.

2. Materials & methods

2.1. Raw materials, substrate composition and physicochemical determination

The replacement of peat was studied by creating four substrates with either a reduced amount of peat or being completely peat-free, with a variant supplemented with a 10 % v/v bulk replacement of wood-based biochar. This resulted in four substrates: peat-reduced, peat-reduced with biochar, peat-free and peat-free with biochar with different compositions (Table 1). Before the substrates were created from the raw materials, the plant fibres were acidified with elemental sulfur (S) to reduce their pH (1 g S/L fibre). In 2021, chopped soft rush was acidified, while in 2022, processed grass clippings from nature conservation were acidified (Vandecasteele et al., 2024). Wood-based biochars were used in both trials and produced from wood chips of landscape management using a rotary kiln reactor at 450 °C, as described by Lataf et al., 2022. The justification for using a wood-based biochar produced at 450 °C is mainly based on the pH and the low acid-buffering capacity of this type of biochar in comparison to biochars produced from other feedstocks and at higher temperatures (Lataf et al., 2022). Biochars with higher acid-buffering capacity may increase the pH of the growing medium blend beyond the optimal pH range. Before mixing into the substrates, biochar was moistened to a moisture content of 50 % fresh weight to allow better mixing and avoid losses through dust formation. The peat-reduced and peat-free substrates of 2021 without biochar were limed with 1.4 g lime/L substrate to reach a pH in the optimal range, while liming the blend was not needed in 2022.

The physicochemical properties of the raw materials and substrates were determined (Table S1 & S2). The sample preparation of the raw materials and substrates for determination of total nutrient content, dry matter content, moisture content and laboratory compacted bulk density was executed according to EN 13.040. The materials were dried at 70 °C and ground. Total N content was determined according to the Dumas method (EN 13,654–2), organic C (OC) and inorganic C were measured using a Skalar Primacs SNC 100 analyser (Skalar, Breda, The Netherlands). Total contents of P, K, Magnesium (Mg) and Calcium (Ca) were determined by 5110 VDV Agilent ICP-OES (Agilent, Santa Clara, CA, USA) in the extract following digestion. For substrates, digestion was executed (120 min at 105 °C) on 0.5 g dried and ground material with 4 mL HNO_3 (p.a. 65 %) and 12 mL HCl (p.a. 37 %) using a DigiPREP MS 200 Block Digestion System (SCP SCIENCE, Québec, Canada). For biochar, digestion of 0.2 g biochar was performed using 8 mL HNO_3 (p.a. 65 %, Chem-Lab NV) and 4 mL H_2O_2 (p.a. 30 %, VWR Chemicals) in a 2:1 ratio using a Milestone ETHOS One high-performance microwave digestion system (in 15 min to 200 °C, hold 15 min at 200 °C, max. 1500 W). Electrical conductivity (EN 13.038) and pH—H_2O (EN 13.037) were measured in a 1:5 solid to water (v/v) suspension.

2.2. Experimental design

Two experimental trials under plastic tunnels with strawberry plants (*Fragaria* x *ananassa* cultivar 'Elsanta') were conducted during the spring of 2021 (April 27th to July 13th) and 2022 (March 23rd to June 28th) at the Research Center for Fruit (50° 46.428′ N, 5° 9.660′ E) (Figure S1).

Table 1
Composition of the raw materials in volume percentages to create the substrate.

Substrate	Peat (% v/v)	Wood fibre (% v/v)	Green compost (% v/v)	Bark compost (% v/v)	Acidified plant fibre (% v/v)	Wood biochar (% v/v)
Peat-reduced	50	25	25	0	0	0
Peat-reduced with biochar	40	25	25	0	0	10
Peat-free	0	25	25	30	20	0
Peat-free with biochar	0	30	25	20	15	10

Cold-stored bare-rooted strawberry transplants were cultivated in 6 litres of substrate using white plastic containers and subsequently placed in two plastic greenhouses. Four replicate plots containing three containers were placed in the plastic tunnels for all four substrate conditions according to a randomized block design (identical design in 2021/2022). A randomized block design was used as experimental design as the trial was run in two separate plastic tunnels with two replicates per tunnel. Each container included five strawberry plants at the start of the experiment. The plants in two of the three containers per plot were artificially inoculated with *Phytophthora cactorum*. Briefly, the zoospore solution was prepared by cultivating 5 different isolates (PCF2338, PCF2339, PCF2344, PCF2345 and PCF 2346) of *P. cactorum* on corn meal agar. When sporangia were formed, the *P. cactorum*-grown agar was mixed with a kitchen blender. A stock solution of 300 ml with a concentration of 1×10^4 sporangia per ml was prepared per object and placed in a cold storage room at 4 °C for two days. Before artificial inoculation, the crown of each plant was wounded by making two wounds with a scalpel. Thereafter, the plants were dipped for 10 min in a 20 L zoospore solution (300 ml of the stock solution in a total of 20 L water) per object and planted in the containers. The remaining dipping volume was equally poured on the surface of the growing medium around the plants of that object. The plants in the third container were allowed to grow without inoculation.

During cultivation, the strawberry plants were drip fertigated using a solution with an EC of 1.5 dS m^{-1} and a pH of 5.5 containing the following elemental concentrations (1 mM NH_4, 3.9 mM K, 4.2 mM Ca, 1.25 mM Mg, 10.6 mM NO_3, 1.6 mM SO_4, 2 mM P, 35 µM Fe, 30 µM Mn, 10 µM Zn, 18 µM B, 1.2 µM Cu and 0.6 µM Mo). After initiation of flowering, irrigation was switched to a solution with an EC of 1.5 dS m^{-1} and a pH of 5.5 containing 5.5 mM K, 3.5 mM Ca, 1.25 mM Mg, 10.5 mM NO_3, 1.25 mM SO_4, 2 mM P, 35 µM Fe, 25 µM Mn, 10 µM Zn, 15 µM B, 1 µM Cu and 0.5 µM Mo. This fertigation regime was used to avoid any nutrient limitation during the trials.

An overview of the experimental timeline for both cultivation years is given in Fig. 1. Root evaluation for *P. cactorum* infection was conducted at three different time points shortly after the start of the experiment. Leaves (mixed sample of three leaves from infected and non-infected plants for all analyses) and strawberries (one strawberry from infected and non-infected plants for all analyses per sample) were sampled for lipid peroxidation analysis (MDA), antioxidant capacity analysis (FRAP) and nutrient analysis precisely 69 days after the start of the experiment for both years. Samples for MDA and FRAP were snap-frozen in liquid nitrogen on-site to stop metabolic activities and placed in a freezer at −80 °C in the lab until further analysis. Samples for nutrient analysis were stored in paper bags and plastic containers for the leaves and strawberries, respectively, and subsequently left to dry in an oven.

2.3. Elemental content of leaves and strawberries during cultivation

After sampling, the leaves and strawberries were placed in an oven at 70 °C until the samples were completely dry. Next, 200 mg dry weight (DW) of the samples was transferred into open heat-resistant tubes (SCHOTT DURAN®), followed by addition of 1 ml 69 % HNO_3 (ARISTAR® for trace analysis) and left overnight at room temperature. The next day, the tubes were placed in a heating block underneath a fume hood for 15 min at 60 °C, followed by a slow temperature increase until they reached 110 °C. The tubes were kept at this temperature until the acids had evaporated entirely. This step of adding 1 ml of 69 % HNO_3 was repeated thrice. The last digestion included adding 1 ml of 37 % HCl (ARISTAR® for trace analysis) and finally, the samples were dissolved in a total volume of 5 mL of 2 % HCl (diluted with milliQ H_2O). The content of K, P, Mg, Ca, S, copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe) was measured through inductively coupled plasma-optical emission spectrometry (ICP-OES 710, Agilent Technologies, Santa Clara, CA, USA).

2.4. Strawberry yield and aboveground biomass

The strawberry yield was determined on all strawberry containers by

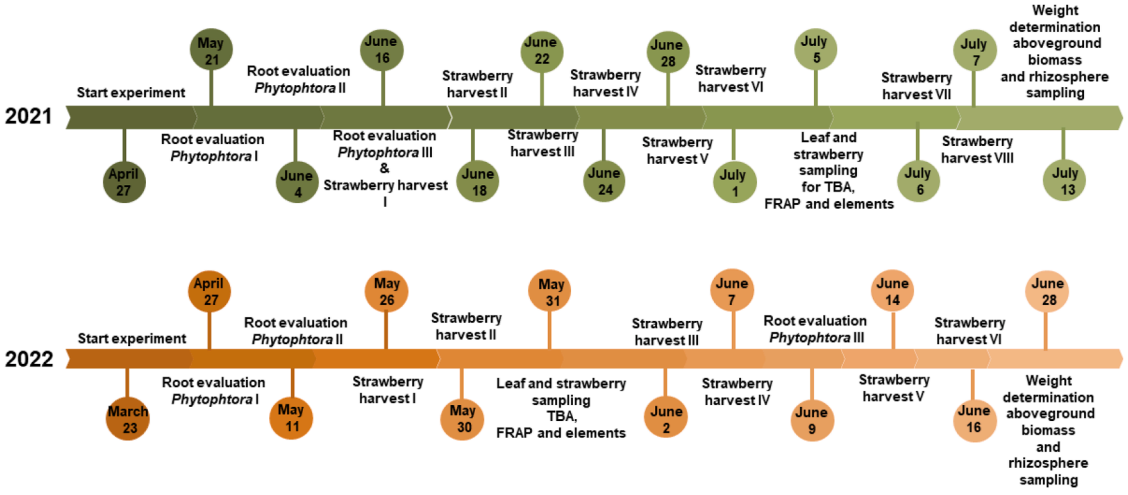

Fig. 1. Experimental timeline for strawberry cultivation in 2021 (green) and 2022 (red). Coloured circles indicated the date at which the corresponding samples were harvested.

picking, sorting and weighing all strawberries at specific moments, as indicated in Fig. 1. Due to the large experimental design, it was for practical reasons not possible to make a differences between with and without inoculation with *P. cactorum*. The sorting process was based on the size and shape of the strawberries, resulting in the strawberry weight being divided over class 4AE (⌀ > 41 mm), 1(⌀ 31–41 mm), 2 (⌀ < 31 mm) and curved.

At the end of the growing season, the strawberry plants in the containers without *P. cactorum* inoculation were harvested to determine the effect of the substrate on the aboveground vegetative biomass of the strawberry plants. This includes the stalks and leaflets of the leaves. All remaining strawberries were removed before harvesting and weighing the vegetative aboveground biomass. Four containers without inoculation per substrate blend were sampled. The roots of these strawberry plants were also processed the same day to determine the rhizosphere microbiome (see 2.8). The biomass of the inoculated strawberry plants was not determined because these plants had growth retardation or died due to the artificial infection. The plant biomass of the infected plant would reflect the effect of *P. cactorum* rather than the potential of the different substrates to grow strawberry plants.

2.5. Element uptake in the aboveground strawberry plant

At the end of the growing season, the aboveground plant material was harvested to determine the level of element uptake. Briefly, the material was dried and grounded and 0.5 g was digested with 4 mL HNO_3 (p.a. 65 %) and 12 mL HCl (p.a. 37 %). Subsequently, the total content of P, K, Mg, Na and Ca was determined with the 5110 VDV agilent ICP-OES (Agilent, Santa Clara, CA, USA) in the extract following digestion (120 min at 105 °C) of 0.5 g dried and ground material with 4 mL HNO_3 (p.a. 65 %) and 12 mL HCl (p.a. 37 %) using a DigiPREP MS 200 Block Digestion System (SCP SCIENCE, Québec, Canada). The total uptake in the aboveground biomass was calculated by multiplying the measured content with the dry biomass of the aboveground plant.

2.6. Disease resistance against Phytophthora cactorum

Phytophthora cactorum disease symptoms were assessed 38, 51 and 80 days after artificial infection in 2021 and 35, 49 and 78 days after artificial infection in 2022. The scoring of symptoms on the strawberry plants was classified as follows: 0 = healthy plant with no symptoms of disease and no growth delay, 1 = growth delay and/or leaf softening, 2 = light wilting of 1 or 2 leaves, 3 = strong wilting of all leaves, 4 = plant is dead. The scoring in 2021 was done on the two infected containers altogether, while in 2022, this was done on the separated infected containers to increase statistical power. The per cent disease index (PDI) per time point was calculated using the following formula

$$\text{PDI} = \frac{\Sigma(\text{Disease class x Number of plants with this class})}{\text{Total number of plants within treatment x Maximum class}}\ x\ 100$$

Subsequently, the Area Under Disease Progress Curve (AUDPC) was calculated using the different PDI scores (Campbell and Madden, 1990; Schandry, 2017).

2.7. Phospholipid fatty acid (PLFA) analysis

After the growing season, the growth substrates without *P. cactorum* infection were collected for PLFA analysis. Total PLFAs were isolated with phosphate buffer, chloroform and methanol at 0.9:1:2 from 0.75 g freeze-dried substrate. Via solid phase extraction phospholipids were separated and saponified to obtain free fatty acids. Fatty acid methyl esters (FAME) were formed by methylating the free fatty acid with 0.2 M methanolic KOH. Fatty acid methyl esters were analysed with a capillary gas chromatograph-flam ionization detector (Perkin Elmer Clarus 600, Perkin Elmer) with a SP-2560 column (100 m length × 0.25 mm ID, 0.20 μm film thickness, Supelco). The temperature program was set as followed: start at 75 °C followed by a heating rate of 10 °C min^{-1} up to 240 °C. Identification of the PLFAs was done via retention time using an external FAME (Restek 35,077 Food Industry FAME mix) and bacterial acid methyl ester (BAME) mix (Supelco, 47,080-U). C values were corrected with a C19:0 internal standard. The following formula was used to calculate the abundance of individual PLFAs in absolute C amounts (PLFA-C, C_x [nmol g^{-1}]) based on the concentration in the liquid extracts:

$$C_x[nmol\ g^{-1}] = \frac{A_x\ \times\ C_i[\mu g] \times\ 1000}{A_i\ \times W[g]\ \times M[\mu g\ \mu mol^{-1}]}$$

C_x represents the concentration of the fatty acids studied, A_x is the peak area of the fatty acid studied, A_i is the peak area of the internal standard, C_i is the absolute amount of internal standard in the vial [μg], W is the amount of substrate [g], M is the molecular weight of the fatty acid [μg μmol^{-1}]. The total microbial biomass was calculated as the sum of 18 PLFAs (i-C15: 0, a-C15: 0, i-C16: 0, i-C17: 0, C16: 1c9, C17: 0cy, C19: 0cy, C14: 0, C15: 0, C16: 0, C17: 0, C18: 0, 10Me-C16: 0, 10Me-C18: 0, C18: 2c9,12, C16: 1c11, C18: 1c9).

2.8. ***Determination*** *of the rhizosphere microbiome*

At the end of the growing season, the rhizospheric substrate was collected from the roots of four strawberry plants per treatment grown in the containers without artificial infection of *P. cactorum* following the protocol of Lundberg et al. (2012). The resulting rhizosphere pellets of 250 mg were stored at −20 °C until DNA extraction. DNA was extracted using the DNeasy 96 PowerSoil Pro kit (Qiagen). Amplicon sequencing was done on the V3-V4 fragment of the 16S rRNA gene and ITS2 gene fragment to determine the bacterial and fungal communities, respectively. The preparation of the libraries, quality control, and pooling of the samples were done according to the protocol of De Tender et al. (2016). The libraries were sequenced by Admera Health (South-Plainfield, NJ, United States) using Illumina MiSeq v3 technology (2 × 300 bp), spiked with 30 % PhiX DNA. Demultiplexing of the raw sequences was done by the sequencing provider. Trimming, filtering, dereplication and merging of the reads, amplicon sequence variant (ASV) calling, chimera removal were done using the DADA2 algorithm (v1.12) and described in detail by Joos et al. (2023). The taxonomy was assigned with assignTaxonomy by the SILVA database (v132) and UNITE (v7) databases for bacterial and fungal taxonomies, respectively (Nilsson et al., 2019; Quast et al., 2012). The metabarcoding data were processed by removing mitochondria and chloroplasts from the bacterial sequences. Low abundant sequences with less than two counts and present in at least three independent samples were removed for both the bacterial and fungal sequences as technical filtering before any analyses. The taxonomic resolution for data clustering was set on family level based on the recommendation of Joos et al. (2020).

2.9. Plant stress parameters: lipid peroxidation and the total antioxidant capacity

Leaves (mixed sample of three leaves per sample with distinction between infected and non-infected plants for all analyses) and strawberries (one strawberry per sample with distinction between infected and non-infected plants for all analyses) from living plants were sampled 69 days after the start of the experiment. Samples were pulverised using a pestle and mortar or two stainless beads in the Retsch® MM 400 Mixer Mill (Retsch), respectively. Afterwards, 80 % ethanol (v/v) was added and samples were centrifuged at 18,407 rcf for 30 min at 4 °C. Subsequently, the supernatant was used for analysis. To determine the malondialdehyde (MDA) concentration as an indicator of lipid peroxidation, the thiobarbituric acid-reactive substances (TBArs) assay was used. Briefly, 500 μL of 0.5 % (w/v) TBA in 20 % (w/v) trichloroacetic

acid solution was added to 250 µL extract. Subsequently, samples were placed at 90 °C for 1 h to activate the reaction. Afterwards, samples were put at 4 °C for 5 min to stop the reaction and centrifuged at 4 °C for 1 min at 2348 rcf. Subsequently, MDA absorbance was measured at 532 nm. In addition, absorbance at 600 nm and 440 nm was measured to correct for unspecific absorbances in a 96-well microplate (Greiner, Bio-One) with a FLUOstar® Omega Microplate reader (BMG Labtech). Malondialdehyde concentration (nmol/ml) was calculated as follows: $(6.45 * (A_{652} - A_{600}) - (0.56 * A_{440}))*3$ (Dhinsa et al., 1981).

The ferric reducing ability of plasma (FRAP) method was used to measure the non-enzymatic total antioxidant capacity (Benzie et al., 1996). 180 µL of reaction buffer (0.3 M acetate (pH 3.6), 10 mM 2,4, 6-Tris(2-pyridyl)-s-triazine and 200 mM $FeCl_3$) was added to 20 µL of leaf extract in 80 % ethanol in a 96-well microplate. Subsequently, samples were placed in the dark and at 4 °C for 20 min. After incubation, absorbance at 600 nm of samples and a 6 hydroxy-2,5,7,8-tetramethylchroman-2 carboxylic acid (Trolox) standard (0–15 nmol) curve was measured with a plate reader as described for MDA concentration.

2.10. Data & statistical analysis

Statistical analyses were performed with R version 4.3.1. Upon conducting a variance component analysis on the complete dataset of both years, a substantial year-related effect was noticed, related to the difference in disease severity after artificial inoculation. Consequently, statistical analyses have been performed on the separated years.

To further assess the disease severity of the *P. cactorum* infection, the Area Under Disease Progress Curve (AUDPC, (Campbell and Madden, 1990; Schandry, 2017)) was calculated for each year separately using the PDI scores of the three different measurement points with the audpc function in the R package agricolae (de Mendiburu et al., 2023).

For the data on the aboveground vegetative biomass, strawberry yield, AUDPC, plant stress, defence, and elemental analysis, the outliers were identified with the Grubbs test and deleted before further statistical analyses. The following models were used to identify significant substrate and bulk biochar effects. The used model for each parameter was selected based on the best model fit.

$$g(\mu) \sim substrate * bulk\ biochar + greenhouse$$

$$g(\mu) \sim substrate * bulk\ biochar$$

In these models, g(.) represents the link function and µ the modelled mean. The identity function was used for the LM or ANOVA analyses, unless specified otherwise. Main and interaction effects are included in the model as indicated with "*". An additive greenhouse effect is indicated with "+". Substrate and bulk biochar were included as a factor term. The normality and homoscedasticity were checked and data was transformed when necessary (logarithmic or inverse).

To account for variation introduced by the different Illumina runs, a block factor (block_run) was added to the metabarcoding analyses. Diversity was calculated using the Shannon-Wiener diversity index using the vegan package (Oksanen et al., 2020) for which the assumption of normality of the residuals was fulfilled. A dissimilarity matrix based on pairwise Bray-Curtis indices was calculated using the ordinate function on the ASV dataset. The homogeneity of variances analysed with a multivariate Levene's test was fulfilled. The bacterial and fungal community composition was analysed with PERMANOVA using the adonis function from the vegan package (Oksanen et al., 2020). A principal coordinate analysis (PCoA) based on the Bray-Curtis dissimilarity matrices was performed. For the differential abundance analyses, data were first aggregated at the family level to assess the main effects of substrates and bulk biochar addition. This aggregation resulted in 278 bacterial families in 2021 and 291 in 2022, as well as 151 fungal families in 2021 and 179 in 2022. The counts on family level were modelled using a nbGLM. The data were normalized using offsets to correct for the effective library size (Robinson et al., 2010). For each comparison of the main factors, the differential abundance of specific contrasts was assessed with a likelihood ratio test. The BH-FDR was used to adjust for multiple testing of each contrasts.

3. Results

3.1. Strawberry yield and aboveground biomass

The effect of substrate type with or without biochar on the strawberry aboveground biomass of non-infected plants exhibited a different response between 2021 and 2022 (Fig. 2A & B). Only in 2022, a higher fresh and dry aboveground biomass was observed in the peat-reduced compared to the peat-free substrates (P-value < 0.05, ANOVA). On the contrary, in 2021, no substrate effect was observed. However, in 2021, biochar addition resulted in an increased fresh aboveground biomass (P-value < 0.05, ANOVA) (Fig. 2A & B). The presence of biochar in the substrate also increased total strawberry yield per plant (measured on both artificially infected plants and non-infected plants; P-value < 0.05, ANOVA, Fig. 2C), thus aligning with the increased aboveground biomass observed in 2021. This was mainly due to an increase in class 2 strawberries (P-value < 0.1, ANOVA; Supplemental Figure 1).

3.2. Disease resistance against Phytophthora cactorum

A distinct difference in disease severity for the artificially infected plants was observed between both years, which is mainly related to differences in climate conditions in 2021 versus 2022. In 2021, the *P. cactorum* infection was less severe compared to 2022 (Fig. 3). Due to the relatively mild root rot development in 2021, no significant differences in root rot severity between the substrate blends were observed. However, in 2022, peat-reduced substrates without biochar had a significantly lower root rot severity than peat-free substrates without biochar (P-value < 0.05, one-way ANOVA). A significant increase in root rot was also observed when biochar was added to the peat-reduced substrates (P-value < 0.05, one-way ANOVA).

For the strawberry plants grown without artificial inoculation with *P. cactorum,* no differences between treatments were found as no clear root rot symptoms were observed.

3.3. Rhizosphere microbiome

Phospholipid fatty acid analysis was used to determine the total microbial biomass. No significant difference was found in the total microbial biomass of the different substrates (Fig. 4). Significant differences in bacterial and fungal community composition based on amplicon sequencing were identified between the substrate types at the end of the trials (P-value < 0.001, PERMANOVA). Furthermore, a difference in the microbiome of substrates of 2021 and 2022 was identified (Fig. 5). In 2022, the peat-reduced substrate exhibited a significant 6% decrease in bacterial diversity and a significant 20% decrease in fungal diversity compared to the peat-free substrate. This effect was not observed in 2021, while a significant decrease in the fungal diversity was found in 2021 in the peat-reduced substrate with biochar compared to the peat-reduced substrate without biochar (Fig. 6) (P-value < 0.05, GLM).

The differences in composition between peat-reduced and peat-free substrates resulted in a different bacterial and fungal community (P-value $= 0.001$, PERMANOVA). In 2021, the addition of biochar to the substrate resulted in a minor shift in the bacterial community (P-value $=$ 0.042, PERMANOVA), and fungal communities (P-value $= 0.001$, PERMANOVA) in the peat-reduced blends (Fig. 5 and Fig. 6).

After conducting differential analyses, it was observed that in total 36 and 37 bacterial families had a significantly different relative abundance between peat-reduced and peat-free substrates in 2021 and 2022, respectively (FDR 5 %).

Only a limited number of families had a significantly different

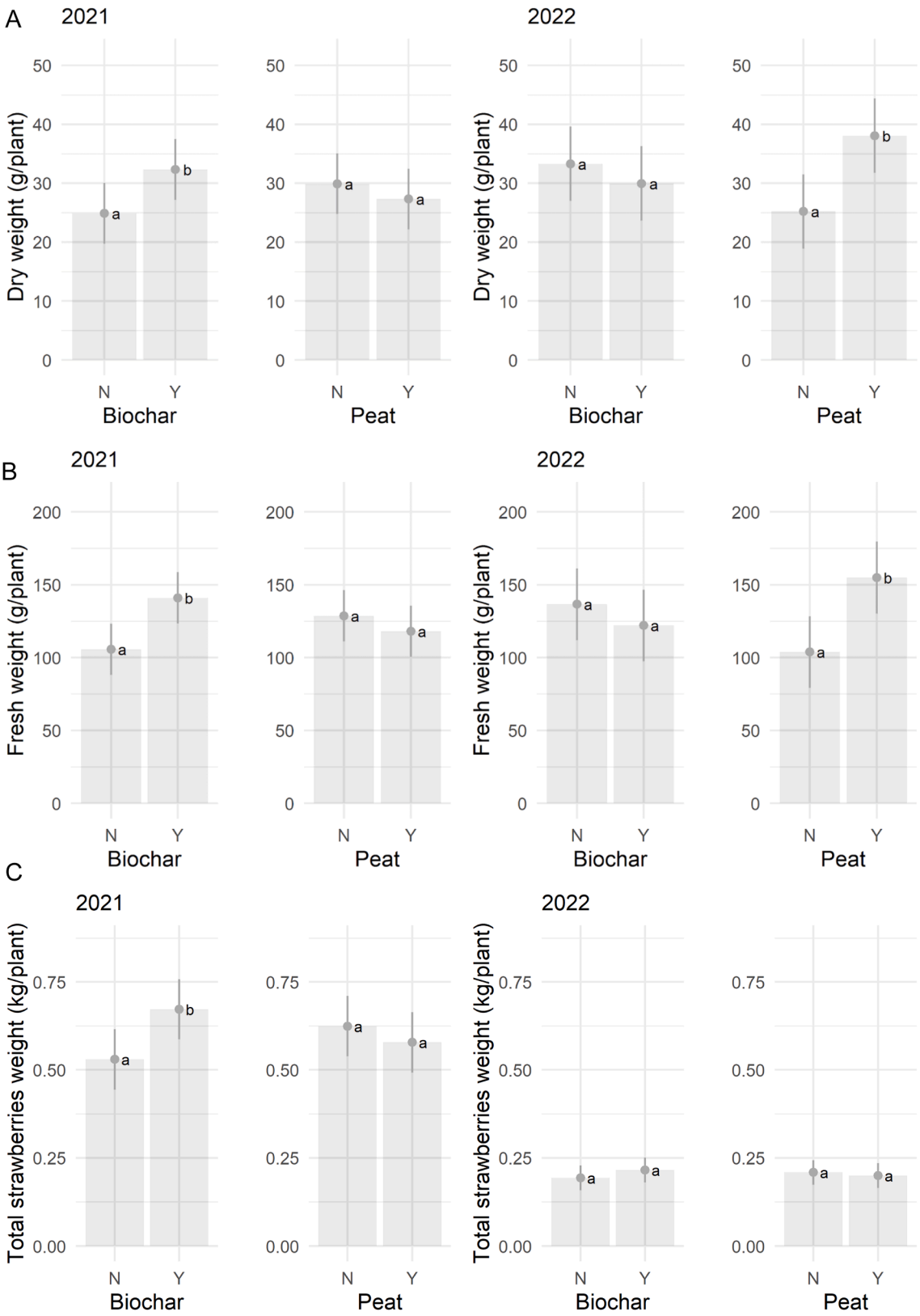

Fig. 2. Effect of the main factors peat and biochar on the fresh and dry aboveground vegetative biomass and strawberry yield in 2021 and 2022. Strawberry plants were grown in a greenhouse trial in four different substrate blends, with differences in peat and biochar addition. The main effect of substrate is shown as peat-reduced (peat Y) vs peat-free (peat N). The effect of bulk biochar is shown as without biochar addition (biochar N) and with biochar addition (biochar Y). Bars represent the estimated marginal mean of at least 3 biological replicates and error bars represent the 95 % confidence interval. Figure A shows the dry weight of the aboveground vegetative biomass (g/plant) of plants without artificial *P. cactorum* infection, figure B shows the fresh weight of the aboveground vegetative biomass (g/plant) without artificial *P. cactorum* infection, figure C show the total strawberry weight (kg/plant) of both plants with and without artificial *P. cactorum* infection. Significant effects are indicated with different letters (P-value < 0.05; two-way ANOVA).

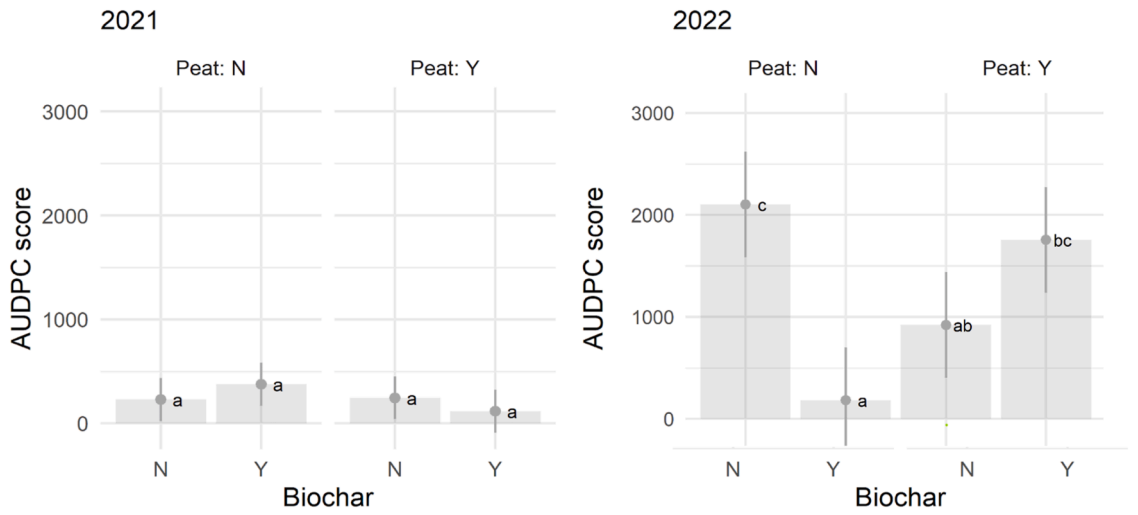

Fig. 3. The mean area under disease progress curve (AUDPC). Strawberry plants were grown on different substrates: peat-reduced (peat Y) vs peat-free (peat N). The effect of bulk biochar is shown as without biochar addition (biochar N) and with biochar addition (biochar Y) and infected with *Phytophthora cactorum* to induce root rot. Root rot was scored at 3 different timepoints after infection and the per cent disease index per time point was used to calculate the AUDPC scores. Bars represent the estimated marginal mean of at least 4 biological replicates and error bars represent the 95 % confidence interval. Significant effects are indicated with different letters (P-value < 0.05; and two-way ANOVA for 2021 and one-way ANOVA 2022).

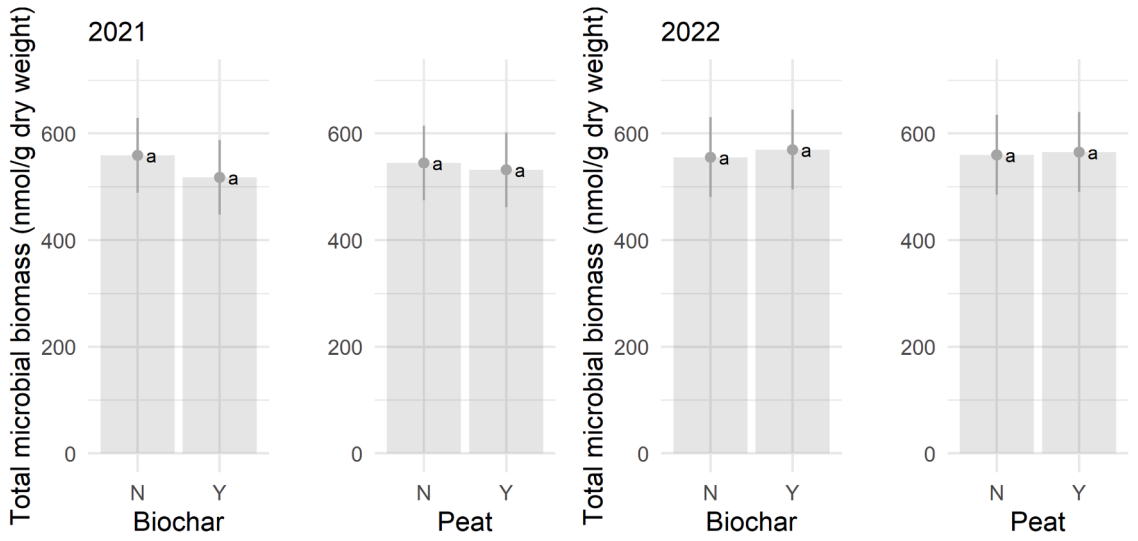

Fig. 4. Effect of the main factors peat and biochar on the total microbial biomass (nmol/g DW) of the substrates in 2021 and 2022. Strawberry plants were grown in a greenhouse trial in four different substrate blends, with differences in peat and biochar addition. After cultivation, substrates were collected for PLFA analysis and total microbial biomass was determined. The main effect of substrate is shown as peat-reduced (peat Y) vs peat-free (peat N). The effect of bulk biochar is shown as without biochar addition (biochar N) and with biochar addition (biochar Y). Bars represent the estimated marginal mean of 4 biological replicates and error bars represent the 95 % confidence interval. Significant effects are indicated with different letters (P-value < 0.05; two-way ANOVA).

abundance within the substrates in both 2021 and 2022, yet there was no consistent pattern of lower or higher abundance observed in those years. When focusing on the families present for at least 1 % in either of the substrates, it was observed that in 2021 the Gemmataceae and Alteromonadaceae had a higher relative abundance in the peat-reduced compared to the peat-free substrates (FDR 5 %) (Table S3). Gemmataceae members are typically found in peat (Dedysh and Ivanova, 2018), whereas the family Alteromonadaceae mostly in marine environments requiring sodium to grow (López-Pérez and Rodríguez-Valera, 2014). A lower relative abundance in the peat-reduced substrates compared to the peat-free substrates was observed for the Thermoactynomycetaceae, Nitrosomonadaceae and Rhizobiales Incertae Sedis (FDR 5 %). Members of these families are often found in compost and can play a role on plant growth and health (e.g., Wei et al., 2024; Nakagawa and Takahashi, 2015; Erlacher et al., 2015). Within 2022, the bacterial families Devosiaceae, Blrii41 and Burkholderiaceae had a higher mean relative abundance in the peat-reduced compared to the peat-free substrates (5 % FDR). Members within these families are often found in the rhizosphere and can be involved in plant growth and health (Nor et al., 2017; Pal et al., 2022.; Petters et al., 2021). The bacterial families Vicinamibacteraceae and an unknown Proteobacterial family had a lower mean relative abundance in the peat-reduced compared to the peat-free substrates (5 % FDR). Member of the relatively new *Vicinamibacteraceae* are not typically known to be involved in plant growth and health (Huber and Overmann, 2018). In general, no significant biochar effect on the bacterial and fungal family abundance was observed.

For 2021, in total 53 fungal families were differentially abundant between peat-reduced and peat-free (5 % FDR), while there were 87 fungal families differentially abundant in 2022. When focusing on those that have a mean abundance above 1 %, the following pattern became

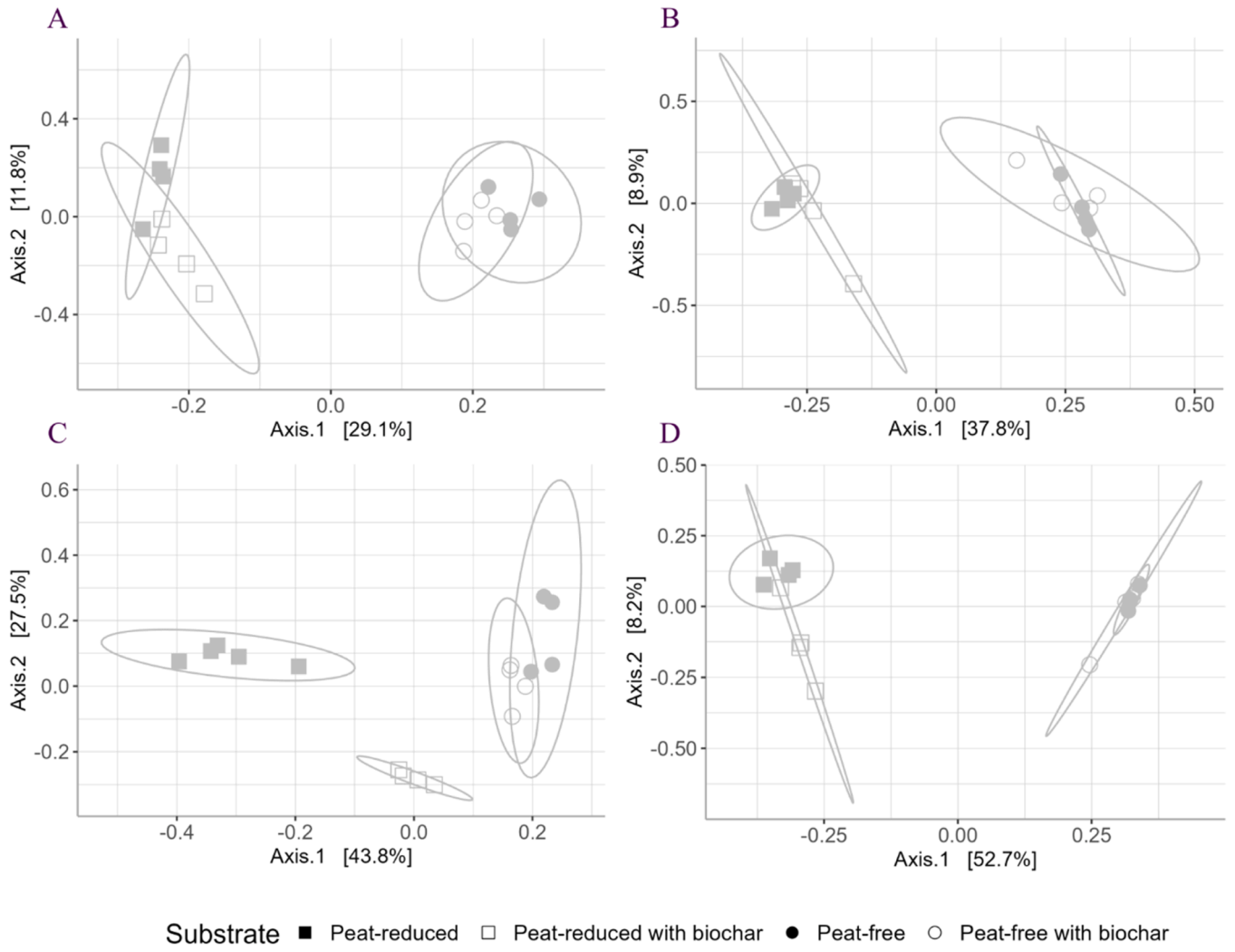

Fig. 5. Bacterial (A and B) and fungal (C and D) community of the strawberry rhizosphere separated for 2021 (A and C) and 2022 (B and D). Figures shows a principal coordinate analysis (PCoA) of the bacterial and fungal amplicon sequence variants (ASV) for the 4 substrates used peat-reduced (filled square), peat-reduced with biochar (empty square), peat-free (filled circle) and peat-free with biochar (empty circle). For each treatment, 4 biological replicates are shown.

clear. In 2021, the peat-reduced substrates had a higher relative abundance of the fungal families Mycosphaerellaceae and Entolomataceae (FDR 5 %) in comparison with the peat-free substrate (Table S4). Members of Mycosphaerellaceae and Entolomataceae are able to colonise diverse niches and vary in lifestyle from pathogens to endophytes, saprobes, epiphytes and fungicolous species (Videira et al., 2017; Peng et al., 2021). Remarkable is the low relative abundance of Lasiosphaeriaceae in the peat-reduced substrates, i.e. 0.6 % [0.3; 0.8] compared to the peat-free substrates, i.e. 14.2 % [10.4; 18]. This difference was also observed in 2022. Members of the Lasiosphaeriaceae occur in diverse environments and are associated with various plant hosts, often as endophytes or pathogens (Marin-Felix et al., 2020). In 2022, also the Sordariales_fam_Incertae_sedis had a lower relative abundance in the peat-reduced substrates compared to the peat-free substrates. This family had an uncertain placement in the Sordariales order with unknown role in plant growth and health. The Microascaceae family had a high relative abundance in the peat-reduced compared to the peat-free substrates (5 % FDR) (Table S4). Few information can be found on the role of members of this family in plant growth or health. After biochar application, only in 2021, an increase in Mortierellaceae was observed, going from 1.1 % [0.5 %; 1.7 %] to 2.3 % [1.4 %; 3.3 %] mean relative abundance (FDR 5 %). Member of these family are known as saprophytes but recently also as beneficial for plant growth and health (De Tender et al., 2024).

3.4. Element analyses of leaves and strawberries during cultivation

Overall, a substrate type or biochar effect was found for P, K, S, Cu, Mn and Fe, while no effect was found for the content of Ca, Mg and Zn in the strawberries or leaves from strawberry plants (Table 2 & 3). Substrate and biochar effects were found for several macronutrients. For instance, a trend towards an increase in the S content was found in strawberry plant leaves grown on peat-free substrate, compared with plants grown on peat-reduced substrate in 2021 (P-value < 0.1, ANOVA). This effect was absent in 2022, but an interaction effect of biochar and substrate was present (P-value < 0.05, ANOVA). Strawberry plants grown on peat-free substrates had an increased K content in their leaves compared to plants grown on peat-reduced substrates in 2022 (P-value < 0.05, ANOVA). However, this effect was absent in 2021. In addition to the substrate effect, a trend towards a biochar effect on the K content was present in the leaves in 2021 (P-value < 0.1, ANOVA) and an interaction effect from biochar and substrate was found in 2022 (P-value < 0.05, ANOVA). Lastly, the P content significantly increased in strawberry leaves grown with biochar, compared to no biochar addition in 2021, whereas no effect was found in 2022.

In the strawberries, a significant S increase was found in 2021 and

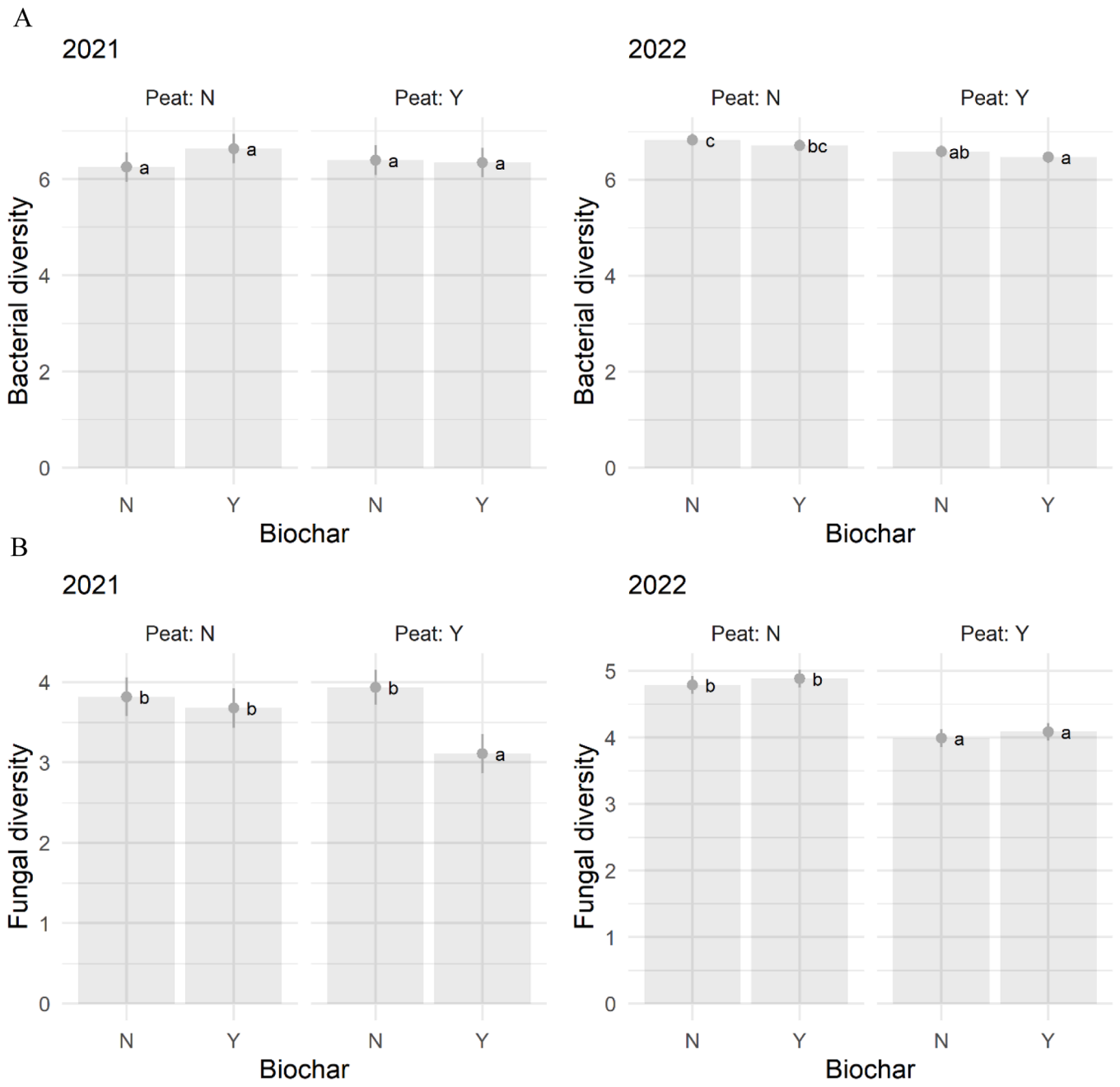

Fig. 6. Bacterial and fungal diversity of the strawberry rhizosphere. Figures display the Shannon diversity of the bacterial (A) and fungal (B) diversity for 2021 and 2022. The different substrate types are peat-reduced (peat Y) vs peat-free (peat N). The addition of biochar is shown by (biochar Y) or without (biochar N). Bars represent the estimated marginal means of four biological replicates and error bars represent the 95 % confidence interval. Significant effects are indicated with different letters (P-value < 0.05; two-way ANOVA).

2022 when plants were grown on peat-free substrates in comparison with plants grown with peat-reduced substrates (P-value < 0.05, ANOVA). Further, the P content increased in strawberries of plants grown with peat-free substrates compared to the peat-reduced in 2021 and 2022 (P-value < 0.05, ANOVA). However, in 2022 an interaction effect was found between biochar addition and substrate type and therefore caution is needed when interpreting the results (P-value < 0.05, ANOVA). Similar to the leaves in comparison with plants grown on peat-reduced substrates, an increase in the K content of strawberries was found when grown with peat-free substrates in 2022 (P-value < 0.05, ANOVA). A significant increase in the P contents was found in the strawberries grown with biochar compared to those without biochar in 2022. Nevertheless, as mentioned above, a significant interaction effect between biochar and substrate was present.

For the micronutrients, a significant decrease in the Mn content of leaves was found in plants grown with peat-free substrate compared to peat-reduced in both 2021 and 2022 (P-value < 0.05, ANOVA). Nevertheless, an interaction effect was present in 2022 for the Mn content of leaves (P-value < 0.05, ANOVA). Additionally, a Cu decrease in the leaves' content was found when grown on peat-free substrates compared with peat-reduced in 2022, as well as an interaction effect from substrate and biochar (P-value < 0.05, ANOVA) and was absent in 2021. In addition to a substrate effect, a biochar effect was found for the Mn contents of strawberry leaves in 2021 (P-value < 0.05, ANOVA) and a trend towards a significant decrease was found in 2022 (P-value < 0.1, ANOVA), as well as an interaction effect of substrate and biochar (P-

Table 2
Element analyses of leaves and strawberries of 2021. Strawberry plants were grown in a greenhouse trial with different growing blends: peat-reduced substrate, peat-free substrate, without biochar addition and with biochar addition and sampled (leaves and strawberries) 69 days after the start. Values are expressed in g/kg DW for the macro-elements and in mg/kg DW for the micro-elements and represent the average of at least 3 replicates ± the standard error. Significantly different effects within the substrate are between biochar treatment are indicated with an asterisk (* P-value < 0.1, ** P-value< 0.05; two-way ANOVA). / means no significant interaction.

2021					
Leaves					
	Substrate		**Biochar**		
	Peat-reduced	**peat-free**	**Biochar**	**no biochar**	**substrate × biochar**
Macro-elements (g/kg)					
Phosphorus (P)	3.75 ± 0.08	3.87 ± 0.08	3.97 ± 0.06	3.65 ± 0.08**	/
Potassium (K)	16.70 ± 0.34	16.82 ± 0.26	17.15 ± 0.31	16.37 ± 0.25*	/
Calcium (Ca)	7.81 ± 0.57	7.43 ± 0.41	7.67 ± 0.60	7.58 ± 0.38	/
Sulfur (S)	1.86 ± 0.03	1.96 ± 0.03*	1.93 ± 0.03	1.89 ± 0.04	/
Magnesium (Mg)	2.69 ± 0.07	2.74 ± 0.07	2.67 ± 0.08	2.75 ± 0.07	/
Micro-elements (mg/kg)					
Iron (Fe)	37.39 ± 3.43	33.71 ± 2.34	35.85 ± 2.69	35.15 ± 3.14	/
Zinc (Zn)	15.61 ± 0.57	16.05 ± 0.78	15.15 ± 0.52	16.51 ± 0.78	/
Copper (Cu)	2.40 ± 0.17	2.73 ± 0.25	2.60 ± 0.22	2.53 ± 0.22	/
Manganese (Mn)	57.07 ± 2.41	35.39 ± 1.66**	49.05 ± 3.63	42.91 ± 3.20**	/
Strawberries					
	Substrate Peat-reduced	**peat-free**	**Biochar biochar**	**no biochar**	**substrate × biochar**
Macro-elements (g/kg)					
Phosphorus (P)	2.62 ± 0.09	2.97 ± 0.10**	2.80 ± 0.08	2.79 ± 0.12	/
Potassium (K)	14.18 ± 0.73	15.26 ± 0.62	15.20 ± 0.50	14.24 ± 0.81	/
Calcium (Ca)	1.38 ± 0.06	1.53 ± 0.07	1.48 ± 0.07	1.44 ± 0.07	/
Sulfur (S)	0.96 ± 0.04	1.12 ± 0.04**	1.02 ± 0.04	1.05 ± 0.05	/
Magnesium (Mg)	1.10 ± 0.04	1.19 ± 0.05	1.16 ± 0.03	1.14 ± 0.06	/
Micro-elements (mg/kg)					
Iron (Fe)	73.81 ± 9.59	65.04 ± 8.34	71.80 ± 9.69	66.93 ± 8.35	**
Zinc (Zn)	7.31 ± 0.39	8.10 ± 0.49	7.49 ± 0.35	7.89 ± 0.54	/
Copper (Cu)	1.10 ± 0.14	0.81 ± 0.10	1.09 ± 0.15	0.83 ± 0.09	/
Manganese (Mn)	40.75 ± 4.70	30.53 ± 2.62**	41.30 ± 4.93	29.99 ± 1.96**	**

Table 3
Element analyses of leaves and strawberries of 2022. Strawberry plants were grown in a greenhouse trial with different growing blends: peat-reduced substrate, peat-free substrate, without biochar addition and with biochar addition and sampled (leaves and strawberries) 69 days after the start. Values are expressed in g/kg DW for the macro-elements and in mg/kg DW for the micro-elements and represent the average of at least 3 replicates ± the standard error. Significantly different effects within the substrate are between biochar treatment are indicated with an asterisk (* P-value < 0.1, ** P-value< 0.05; two-way ANOVA). / means no significant interactions.

2022					
Leaves					
	Substrate		**Biochar**		
	Peat-reduced	**peat-free**	**Biochar**	**no biochar**	**substrate × biochar**
Macro-elements (g/kg)					
Phosphorus (P)	5.25 ± 0.22	5.30 ± 0.19	5.39 ± 0.21	5.15 ± 0.19	/
Potassium (K)	23.56 ± 0.79	26.03 ± 074**	24.37 ± 0.66	25.33 ± 0.97	**
Calcium (Ca)	17.61 ± 1.48	19.35 ± 958.07	17.43 ± 1.11	19.65 ± 1.29	/
Sulfur (S)	2.11 ± 0.06	2.09 ± 0.05	2.06 ± 0.05	2.14 ± 0.06	**
Magnesium (Mg)	5.01 ± 0.34	4.86 ± 0.22	4.72 ± 0.27	5.16 ± 0.29	/
Micro-elements (mg/kg)					
Iron (Fe)	64.68 ± 5.71	61.64 ± 3.07	62.12 ± 4.47	64.14 ± 4.34	/
Zinc (Zn)	15.31 ± 0.42	14.86 ± 0.67	14.50 ± 0.37	15.67 ± 0.73	/
Copper (Cu)	1.28 ± 0.05	1.08 ± 0.06**	1.15 ± 0.07	1.16 ± 0.04	**
Manganese (Mn)	56.95 ± 3.99	31.82 ± 3.32**	39.75 ± 3.94	53.53 ± 8.60*	**
Strawberries					
	Substrate Peat-reduced	**peat-free**	**Biochar biochar**	**no biochar**	**substrate × biochar**
Macro-elements (g/kg)					
Phosphorus (P)	6.75 ± 0.37	8.87 ± 0.39**	8.33 ± 0.33	7.29 ± 0.54**	**
Potassium (K)	21.02 ± 0.79	25.46 ± 0.65**	23.81 ± 0.66	22.67 ± 1.11	**
Calcium (Ca)	1.84 ± 0.14	2.28 ± 0.13	2.13 ± 0.16	2.00 ± 0.13	/
Sulfur (S)	1.37 ± 0.07	1.62 ± 0.08**	1.60 ± 0.07	1.38 ± 0.08	/
Magnesium (Mg)	1.47 ± 0.04	1.59 ± 0.09	1.55 ± 0.05	1.51 ± 0.09	/
Micro-elements (mg/kg)					
Iron (Fe)	24.03 ± 0.75	21.66 ± 1.58	22.82 ± 1.15	22.87 ± 1.41	/
Zinc (Zn)	10.08 ± 0.40	10.97 ± 0.62	10.89 ± 0.47	10.11 ± 0.56	/
Copper (Cu)	1.12 ± 0.07	1.17 ± 0.14	1.17 ± 0.08	1.12 ± 0.12	/
Manganese (Mn)	23.95 ± 1.08	18.73 ± 1.48**	21.04 ± 1.52	21.64 ± 1.41	/

value < 0.05, ANOVA).

As for the leaves, also in strawberries, the content of Mn decreased in plants grown with peat-free substrate in 2021 and 2022 (P-value < 0.05, ANOVA). Nonetheless, like in the leaves, for the content of Mn in the strawberries, an interaction effect of biochar and substrate was present but then in 2021 instead of 2022 (P-value < 0.05, ANOVA). In 2021, an interaction effect from biochar and substrate was found for the Fe content of strawberries (P-value < 0.05, ANOVA).

3.5. Total element uptake in the aboveground strawberry plant

In addition to the element contents of the leaves and strawberries

during the growing season, the total element uptake of P, K, Ca, Na and Mg was determined at the end of the growing season in the aboveground biomass (Table 4). An apparent biochar effect on the P and K uptake was found in 2021, where biochar addition increased the total P and K uptake (P-value < 0.05). On the contrary, in 2022, a trend toward a significant decrease after biochar addition was found for the P and K uptake (P-value < 0.1). In addition, in 2022, a significant effect of the substrate was found for the P, K, Ca and Mg uptake, where the peat-reduced substrate resulted in a higher uptake of P, K, Ca and Mg compared with the peat-free substrate (P-value < 0.05). In 2021, the Na uptake was significantly increased in the plants grown on the peat-free substrate compared to plants grown on peat-reduced substrate (P-value < 0.05). Lastly, a trend toward an increase in the Ca uptake was found with biochar compared to plants grown without biochar (P-value < 0.1).

3.6. Plant stress and defence

To assess stress and damage from reactive oxygen species, the total antioxidant capacity (TAC) was determined using the FRAP assay (Fig. 7). Additionally, lipid peroxidation was measured by determining the concentration of malondialdehyde (MDA) in the leaves and strawberries (Fig. 8). Overall, no significant changes in the TAC or lipid peroxidation of leaves of strawberry plant and strawberries were found in the trial of 2021 and 2022.

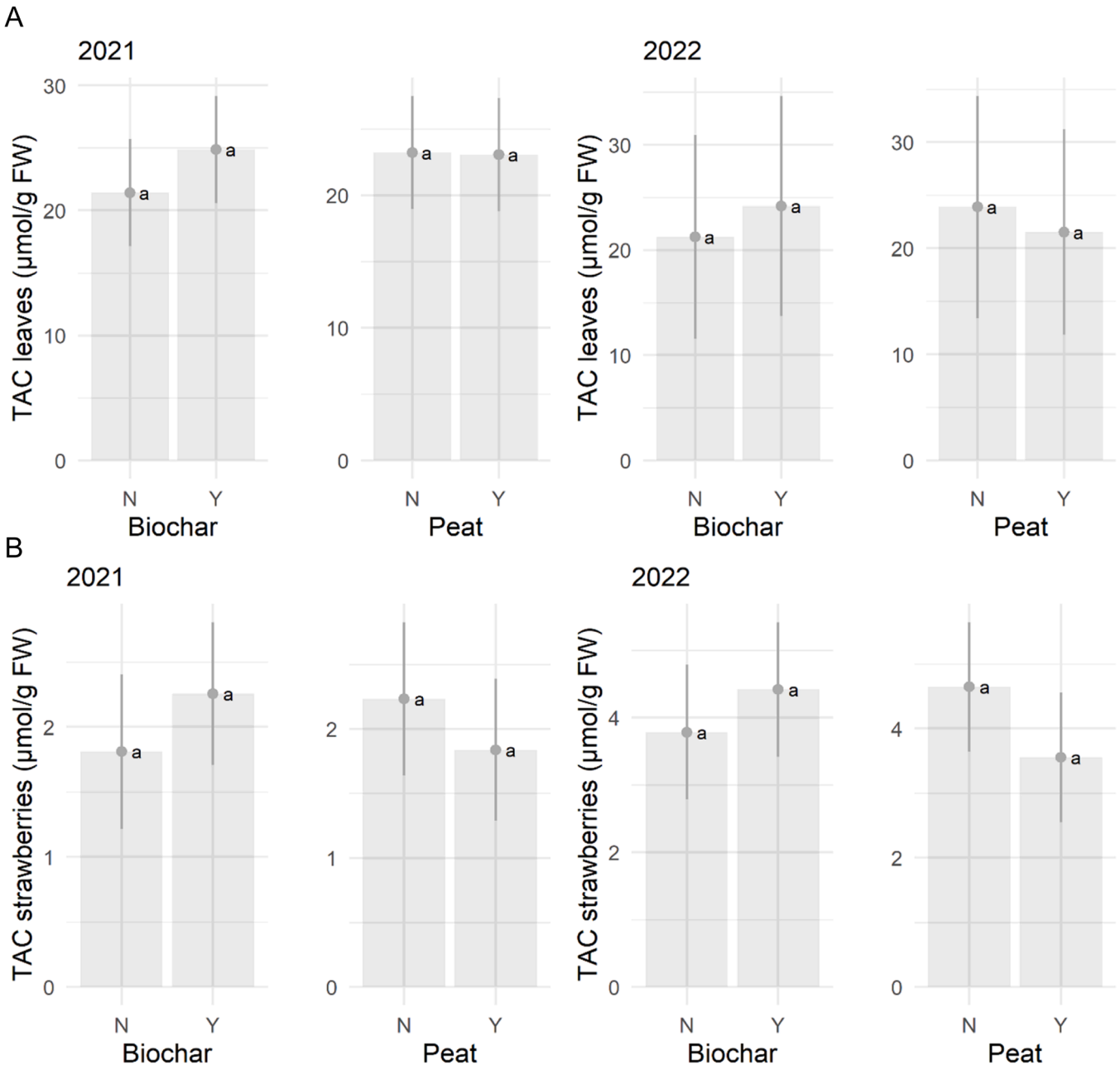

Fig. 7. Main factor effects of the total antioxidant capacity(TAC) in leaves (A) and strawberries (B) of 2021 and 2022. Strawberry plants were grown in a greenhouse trial in four different substrate blends, with differences in peat and biochar addition. The main effect of substrate is shown as peat-reduced (peat Y) vs peat-free (peat N). The effect of bulk biochar is shown as without biochar addition (biochar N) and with biochar addition (biochar Y). Bars represent the estimated marginal mean of at least 3 replicates and error bars represent the 95 % confidence interval. The antioxidant capacity expressed as trolox equivalent (nmol)/ g fresh weight. Significant differences are indicated with different letters P-value < 0.05; two-way ANOVA).

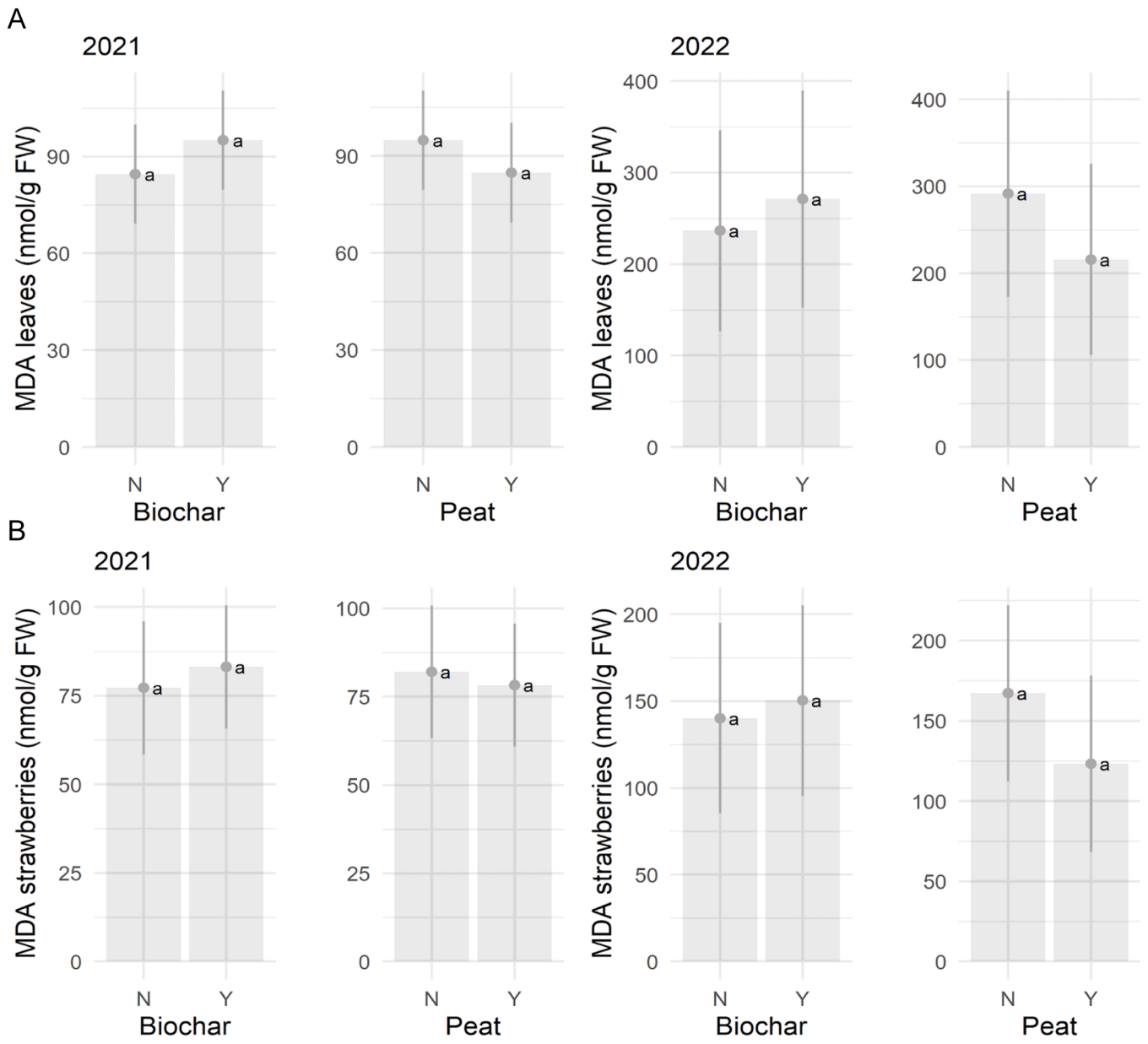

Fig. 8. Main factor effects of the lipid peroxidation in leaves (A) and strawberries (B) of 2021 and 2022. Strawberry plants were grown in a greenhouse trial in four different substrate blends, with differences in peat and biochar addition. The main effect of substrate is shown as peat-reduced (peat Y) vs peat-free (peat N). The effect of bulk biochar is shown as without biochar addition (biochar N) and with biochar addition (biochar Y). Bars represent the estimated marginal mean of at least 3 replicates and error bars represent the 95 % confidence interval. Lipid peroxidation expressed as the concentration of MDA (μmol) per gram fresh weight. Significant differences are indicated with different letters P-value < 0.05; two-way ANOVA).

3.7. Summary of and links between major findings across the two years

In Table 5, we summarize the main findings on the effect of the substrate type (peat-reduced vs. peat-free) and biochar addition on (i) plant growth (above ground biomass and fruit yield), (ii) disease resistance, (iii) the rhizosphere microbiome and (iv) nutrient dynamics.

For the above ground biomass, there was a positive biochar effect in 2021 and a positive substrate type effect in 2022. Similarly, there was also a positive biochar effect in 2021 for the strawberry fruit yield. No differences were found in disease resistance in 2021; whereas in 2022, the highest disease resistance was found in the peat-free substrate with biochar.

As the interaction between horticultural plants and their rhizosphere microbiome is critical to plant growth and health (Pot et al., 2022), we try to link rhizosphere microbiome changes with the parameters for plant growth (above green biomass and yield) and disease resistance. In 2021, the biochar effect on the plant growth promotion can be associated with an increase in the relative abundance of members of the Mortierellaceae family (Table S4). In 2022, there is a link between the rhizosphere microbiome changes (composition and diversity) and the substrate effect on the above green biomass (Table 5). More specifically, lower microbial (bacterial and fungal) rhizosphere diversity and a shift in their composition are associated with a higher above green biomass. Plant growth promoting effect in peat-reduced in 2022 (Fig. 2) may (partially) explained by the increased relative abundance of the 3 bacterial families: Devosiaceae, Blrii41 and Burkholderiaceae and 1 fungal family, Microascaceae. In addition, the lower bacterial rhizosphere diversity in the peat-free substrate with biochar can be linked with the disease resistance.

As changes in nutritious elements are additional drivers for plant

growth and health (Amery et al., 2021; Jaiswal et al., 2024), we also tried to link nutrient dynamics with the plant growth and health parameters (Table 5). In 2021, the increased above green biomass and fruit yield can be linked with changes in macro- and micro-elements and total elements in the above biomass (Table 5). In 2022, the increased above ground biomass in the peat-reduced substrates as compared to the peat-free substrates, can be linked to changes in the total element uptake in the above ground strawberry plant. In addition, the disease resistance in the peat-free substrate with biochar can be linked to changes in nutrient dynamics in both leaves and fruit.

4. Discussion

In general, more effects of substrate type and biochar addition were found during the 2022 trial than in the trial of 2021. The most prominent was the large difference in disease pressure of *P. cactorum* after artificial inoculation. This difference between the two cultivation years can be due to multiple reasons, such as different weather conditions and different planting time (April versus March), which are known to greatly influence Elsanta growth and disease susceptibility (Lieten, 1997; Madden et al., 1993; Murthy and Pramanick, 2014; Verheul et al., 2007). For practical reasons, the specific materials used for the peat-free substrates were changed for the trial of 2022 since chopped soft rush was replaced with processed grass clippings in 2022, which could contribute to differences between the two trials. However, this difference was only induced in the peat-free substrates and the disease severity was higher in all substrates in 2022. Therefore, the difference induced by changing the composition of the peat-free substrates cannot be the main reason for the differences between disease severity. Moreover, different batches of green and bark compost, peat and wood fibre were used in each trial. This induced some small changes between both years, for example, in the Ca concentration and the EC value of the substrates (Table S1). Therefore, differences between the two trials can be partly explained by differences in composition between the substrates used in 2021 vs. 2022. Additionally, also the quality of the strawberry plating material can vary year to year. These 4 factors that may have contributed to the variability between the two years (namely, planting time, climatic conditions, substrate composition, quality in planting material) are intrinsically linked to the fact that the experiments were conducted under on-farm conditions. This is valuable information because it highlights the potential for substantial year-to-year variability in greenhouse trials. Consequently, researchers should exercise caution when drawing conclusions from a single trial conducted under practical conditions, as such results may not be broadly representative or reproducible across different years.

In 2022, a substrate type effect was present, leading to a decreased fresh and dry weight of the plants grown on the peat-free substrate (Fig. 2A & B). This could come from a difference in the biological stability of the plant fibres in the peat-free substrate since this stability was lower in 2022 compared to 2021 (Oxygen uptake rate of value 4.9 in 2021 vs. 12.3 mmol O_2/kg OM/hr in 2022). Furthermore, the total uptake of P, K, Ca and Mg decreased in the aboveground biomass of plants grown on peat-free substrate at the end of the growing season. Despite this decrease, no effect was found on the total strawberry yield (Fig. 2C). This is in line with the results of Vandecasteele et al. (2023), who also reported no substrate effect (peat vs. peat-reduced) on the strawberry yield (Vandecasteele et al., 2023). No biochar effect on plant growth or strawberry yield was found in the 2022 trial. This is in agreement with other studies performed on different crops that found neutral effects on plant growth (Blok et al., 2017; Güereña et al., 2013; Vandecasteele et al., 2023; Vaughn et al., 2013). On the contrary, no substrate effect was present in the trial of 2021, but a positive effect of biochar on the dry and fresh weight of the aboveground part of strawberry plants was demonstrated. This positive effect was also illustrated by the high total strawberry yield (Fig. 2). Positive plant growth effects from using biochar could come from an increase in nutrient availability. Amery et al. (2021) already reported that biochar can be used as a fertiliser, especially for P supply to the substrate. Also, our study found increased P content in the leaves of strawberries grown with biochar compared to without during the growing season and an increased total P and K uptake in the aboveground part of plants grown with biochar at the end of the growing season in the trial of 2021 and an increased P content in the strawberries in 2022, indicating a higher availability during early crop development (Table 2,3 & 4). However, in 2022, this biochar effect was substrate-dependent since there was a significant interaction effect. As P is an essential macronutrient for plant growth, the increased uptake of P could indicate an increased bioavailability of P in the substrates, which could partially explain the 2021 results (Gong et al., 2022). Our results also show a trend towards an increase in the K content in the leaves of strawberry plants grown with biochar compared to plants grown without biochar (Table 2 & 3). Furthermore, the K uptake at the end of the growing season was increased in 2021 in plants grown with biochar compared to plants grown without biochar (Table 4). Overall, no major differences were observed in the element contents and the element uptake, except for the K and P uptake in 2021 after biochar addition. This could be explained by the high fertigation used, as it has been found that the fertilizer replacement value of biochar is more pronounced in suboptimal conditions (Amery et al., 2021).

The relevance of the biochar as source of nutrients in the horticultural substrate is strongly affected by the composition of the blend and the feedstock of the biochar. When biochar is combined with materials low in nutrients like peat, coir or wood fiber, biochar can act as fertilizer, mainly when the biochar is produced from nutrient-rich feedstocks (Amery et al., 2021). The role of biochar as source of nutrients is limited when biochar is combined with materials high in nutrients like green compost, mainly when the biochar is produced from wood being a nutrient-poor feedstock (Nobile et al., 2020; Vandecasteele et al., 2023). The nutrient balance of previous experiments with peat-reduced blends with 15 % v/v green compost and 10 % v/v wood-based biochars

Table 4
Total element uptake of the aboveground strawberry biomass. Strawberry plants were grown in a greenhouse trial with different growing blends: peat-reduced substrate, peat-free substrate, with or without biochar addition.After the growing season, aboveground biomass was sampled for element analysis. Values are expressed in mg/plant and represent the average of at least 3 replicates ± the standard error. Significantly different effects within the substrate between biochar treatment are indicated with an asterisk (* P-value < 0.1, ** P-value< 0.05; two-way ANOVA). No "substrate x biochar" interactions were found.

2021				
	Substrate		**Biochar**	
mg/plant	**Peat-reduced**	**peat-free**	**Biochar**	**no biochar**
Phosphorus (P)	106.3 ± 11.0	112.1 ± 10.2	128.2 ± 7.9	90.2 ± 7.9**
Potassium (K)	625.3 ± 57.4	658.6 ± 58.4	731.3 ± 42.8	552.6 ± 51.7**
Calcium (Ca)	404.7 ± 35.3	446.0 ± 48.0	482.6 ± 35.4	368.1 ± 38.5*
Sodium (Na)	3.8 ± 0.3	6.4 ± 1.1**	5.3 ± 0.6	4.9 ± 1.1
Magnesium (Mg)	106.0 ± 9.4	115.8 ± 12.2	126.7 ± 8.7	95.0 ± 9.8*
2022				
	Substrate		**Biochar**	
mg/plant	**Peat-reduced**	**peat-free**	**Biochar**	**no biochar**
Phosphorus (P)	169.4 ± 17.0	118.1 ± 8.7**	125.1 ± 7.7	156.9 ± 18.9*
Potassium (K)	738.5 ± 58.8	525.6 ± 42.4**	553.5 ± 36.2	687.4 ± 72.4*
Calcium (Ca)	563.1 ± 44.3	399.6 ± 34.1**	446.5 ± 45.2	516.3 ± 51.4
Sodium (Na)	4.0 ± 0.4	3.8 ± 0.4	3.8 ± 0.5	4.0 ± 0.4
Magnesium (Mg)	142.2 ± 10.8	100.9 ± 7.8**	113.6 ± 10.3	129.5 ± 13.3

pointed at green compost being an important source of N, P, K, Ca and Mg in the horticultural substrate. Although only 15 % v/v of green compost was used, it contributed to >70 % of the P, K, Ca and Mg in the peat-reduced blend and >50 % of the N (Vandecasteele et al., 2023). In contrast, the effect of the wood-based biochar amendment on the N, P, Ca and Mg contents in the virgin growing media was limited.

The relevance of the biochar for balancing substrate pH is related to the acid-buffering capacity of the biochar (Blok et al., 2017; Lataf et al., 2022). Biochars with higher acid-buffering capacity may increase the pH of the growing medium blend beyond the optimal pH range.

Next to a link between nutrient dynamics and plant growth (above biomass and yield), a link could also be found between changes in the rhizosphere microbiome (diversity and composition) and plant growth (Table 5). Most remarkably, a higher relative abundance of the Mortierellaceae could be associated with the biochar induced plant growth promotion in the peat-free substrate in 2021. Recently, several members of the Mortierellaceae family are found to be plant growth promoting (De Tender et al., 2024) and therefore this finding merits further investigation.

In addition to plant growth promotion, plant health was assessed via stress indicators by measuring lipid peroxidation and total antioxidant capacity as an indication of oxidative stress in plants. Oxidative stress is a common stress response of plants as a reaction to abiotic stresses (Anzano et al., 2022). It has been demonstrated that biochar can contain potentially toxic elements and induce plant stress (Lataf et al., 2022). In our study, no substrate or biochar effect was found on lipid peroxidation or total antioxidant capacity of leaves and strawberries, indicating that there was no difference in the oxidative stress levels of strawberry plants grown on peat-free or peat-reduced substrates and with or without biochar (Fig. 7 & 8). Similarly, Viger et al. (2015) found no increased stress levels in plants after biochar treatment. Still, they did find an increased susceptibility to abiotic stress indicated by a decrease in the expression of genes that regulate defence responses against abiotic and biotic stresses (Viger et al., 2015). However, Kamran et al. (2019) found that biochar has a positive effect by decreasing oxidative stress in plants exposed to cadmium. Likewise, biochar can reduce oxidative stress in plants during salt stress (Farooq et al., 2020). No differences were found in our study, but as plants were grown without induced abiotic stress, this observation was expected.

Although no differences in oxidative stress were found for both trials, differences in the disease severity after artificial inoculation with *P. cactorum* were found in the 2022 trial. No effect was observed in 2021, as the disease severity in this year was overall very low. In 2022, the disease severity was higher in peat-free conditions without biochar compared to the peat-reduced substrate without biochar (Fig. 3). An apparent biochar effect was present, but the direction of this effect was substrate-dependent. In the peat-free substrates, the addition of biochar decreased the disease severity, whereas in the peat-reduced substrate, no significant effect of biochar addition was found (Fig. 3). De Tender et al. (2021) also reported a decrease in the severity of the aboveground pathogen *B. cinerea* in strawberry fruits after biochar addition in low amounts (< 5 % v/v). Disease suppression is known to be affected by biochar type, pathogen type and concentration, as plants amended with high biochar concentrations (> 5 % v/v) often result in a higher susceptibility to root-borne pathogens such as *Phytophthora* spp. (Frenkel et al., 2017; Jaiswal et al., 2024). However, in our study, differences between trial years cannot be attributed to differences in biochar concentration, as the biochar concentration was the same in both years. Differences in root rot susceptibility in 2022 could be partly explained by the differences in the microbiome of the rhizosphere and nutrient dynamics. More specifically, disease suppression in 2022 in the biochar peat-free substrate was linked to a lower bacterial diversity in the rhizosphere. This is in contrast to previous findings of Jaiswal et al. (2017), Kolton et al. (2017) and De Tender et al. (2021) where biochar mediated suppression was linked to higher bacterial diversity in the rhizosphere. However, these studies were done in less complex substrates (peat or sand), whereas this study incorporated biochar in peat-reduced and peat-free blends (blends with wood fibre, green compost, plant fibre and bark compost) and the interaction with the rhizosphere microbiome may act differently in these blends than in previous less complex substrates.

Our results did clearly show a difference in the bacterial and fungal communities between the substrate types in both years (Fig. 5). Specifically, 37 bacterial and 87 fungal families had a significantly different relative abundance between peat-reduced and peat-free substrates in 2022 (Table S3 & S4). This difference can be explained by the incorporation of bark compost and plant fibre in the peat-free substrates, which can induce changes in the microbiome (Malewski et al., 2023; Van Gerrewey et al., 2020). The peat-free substrate contained plant fibres that were acidified with element S, leading to an increased sulphate (SO_4) content in these substrates due to S oxidation by microorganisms, and also to a higher biological stability of the fibre (Vandecasteele et al., 2024). This increased SO_4 content could attract other microorganisms and thereby change the abundance of several bacterial families (Damo et al., 2023; Pot et al., 2021). The increase in SO_4 content in the peat-free substrate is also visible in the element content of strawberries of plants grown on peat-free substrates as they contained more S compared to plants grown on peat-reduced substrates. Since the nutrient supply through fertigation was set sufficiently high to avoid any nutrient limitation, these differences in nutrient uptake in the crop could be explained by a difference in the elemental contents or the bioavailability of the elements in the substrates.

Table 5
Summary of the main findings on the effect of the substrate type (peat-reduced vs. peat-free) and biochar addition on (i) plant growth (above ground biomass and fruit yield), (ii) disease resistance, (iii) the rhizosphere microbiome and (iv) nutrient dynamics. "x" means that the substrate type (peat), biochar addition (biochar) or both (peat x biochar) had an significant impact on the measured parameter.

		2021			2022		
	Parameter	peat	biochar	peat x biochar	peat	biochar	peat x biochar
i	Green above biomass		x		x		
i	Fruit yield		x				
ii	Disease resistance						x
iii	Bacterial rhizosphere composition	x	x		x		
iii	Fungal rhizosphere composition	x	x		x		
iii	Bacterial rhizosphere diversity				x		x
iii	Fungal rhizosphere diversity			x	x		
iv	Macro-elements in leaves	x	x				x
iv	Micro-elements in leaves	x	x				x
iv	Macro-elements in fruits	x					x
iv	Micro-elements in fruits			x	x		
iv	Total elements above biomass	x	x		x	x	

5. Conclusions

This study shows that peat can be replaced by renewable and regionally available materials like acidified plant fibres, wood fibres and bark or green compost for strawberry substrates without adverse effects on strawberry yield. Additionally, some caution is necessary as it could be that some peat-free substrates are more susceptible to root-rot infection due to differences within the rhizosphere microbiome. However, only the trial with the high infection rate showed an increased disease severity. This increase was also reduced when biochar was added to the peat-free substrate. Therefore, this research showed that at least 10% v/v biochar can be used in peat-reduced and peat-free substrates to partly replace peat. In addition to decreasing the disease severity in peat-free substrates, our study demonstrates that biochar even has the potential to increase the strawberry yield. Further research is necessary to confirm these results. Additionally, it would be useful to repeat the experiments with a lower fertigation regime since it is previously shown that biochar could play a role in fertigation and performs best in suboptimal nutrient conditions.

CRediT authorship contribution statement

I. Pecqueur: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation. **M. Huybrechts:** Writing – review & editing, Supervision, Project administration, Formal analysis, Data curation. **L. Joos:** Writing – review & editing, Supervision, Software, Formal analysis, Data curation. **M. Jozefczak:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **A. Lataf:** Writing – original draft, Methodology, Investigation, Formal analysis. **W. Van Hemelrijck:** Writing – review & editing, Investigation, Conceptualization. **M. Boonen:** Methodology, Investigation. **C. De Tender:** Writing – review & editing, Supervision. **D. Vandamme:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **J. Debode:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **B. Vandecasteele:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **A. Cuypers:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2026.114875.

Data availability

Data will be made available on request.

References

Amery, F., Debode, J., Ommeslag, S., Visser, R., De Tender, C., Vandecasteele, B., 2021. Biochar for circular horticulture: feedstock related effects in soilless cultivation. Agronomy 11 (4), 629. https://doi.org/10.3390/agronomy11040629.

Anzano, A., Bonanomi, G., Mazzoleni, S., Lanzotti, V., 2022. Plant metabolomics in biotic and abiotic stress: a critical overview. Phytochem. Rev. 21 (2), 503–524. https://doi.org/10.1007/s11101-021-09786-w.

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239 (1), 70–76. https://doi.org/10.1006/abio.1996.0292.

Blok, C., Van der Salm, C., Hofland-Zijlstra, J., Streminska, M., Eveleens, B., Regelink, I., Visser, R., 2017. Biochar for horticultural rooting Media improvement: evaluation of Biochar from gasification and slow pyrolysis. Agronomy 7 (1), 6. https://doi.org/10.3390/agronomy7010006.

Campbell, C.L., Madden, L.V., 1990. Introduction to Plant Disease Epidemiology. John Wiley & Sons, New York.

Cha, J.S., Park, S.H., Jung, S.-C., Ryu, C., Jeon, J.-K., Shin, M.-C., Park, Y.-K., 2016. Production and utilization of biochar: a review. J. Ind. Eng. Chem. 40, 1–15. https://doi.org/10.1016/j.jiec.2016.06.002.

Damo, J.L.C., Shimizu, T., Sugiura, H., Yamamoto, S., Agake, S.-i., Anarna, J., Ohkama-Ohtsu, N., 2023. The application of sulfur influences microbiome of soybean rhizosphere and nutrient-mobilizing bacteria in andosol. Microorganisms 11 (5), 1193. https://doi.org/10.3390/microorganisms11051193.

Dedysh, S., Ivanova, A., 2018. Planctomycetes in boreal and subarctic wetlands: diversity patterns and potential ecological functions. FEMS. Microbiol. Ecol. 95, fiy227. https://doi.org/10.1093/femsec/fiy227.

de Mendiburu, F., Yaseen, M., 2023. agricolae: Statistical Procedures for Agricultural Research. R Package Version 1.3-7 2.

De Tender, C., Debode, J., Vandecasteele, B., D'Hose, T., Cremelie, P., Haegeman, A., Maes, M., 2016. Biological, physicochemical and plant health responses in lettuce and strawberry in soil or peat amended with biochar. Appl. Soil Ecol. 107, 1–12. https://doi.org/10.1016/j.apsoil.2016.05.001.

De Tender, C., Vandecasteele, B., Verstraeten, B., Ommeslag, S., Kyndt, T., Debode, J., 2021. Biochar-enhanced resistance to botrytis cinerea in strawberry fruits (But Not Leaves) is associated with changes in the rhizosphere microbiome. Front. Plant Sci. 12, 700479. https://doi.org/10.3389/fpls.2021.700479.

De Tender, C., Vandecasteele, M., Ommeslag, S., De Zutter, N., Vandenbussche, E., Haegeman, A., Debode, J., 2024. Linnemannia elongata: a key species in chitin-based plant growth promotion. Phytobiomes. J. 8 (3), 366–377.

Dhinsa, R.S., Plumb-Dhindsa, P., Thorpe, T.A., 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot. 32 (1), 93–101. https://doi.org/10.1093/jxb/32.1.93.

Dunn, C., Freeman, C., 2011. Peatlands: our greatest source of carbon credits? Carbon. Manage 2 (3), 289–301. https://doi.org/10.4155/cmt.11.23.

Eigenbrod, C., Gruda, N., 2015. Urban vegetable for food security in cities. A review. Agron. Sustain. Dev. 35 (2), 483–498. https://doi.org/10.1007/s13593-014-0273-y.

Erlacher, A., Cernava, T., Cardinale, M., Soh, J., Sensen, C., Grube, M., Berg, G., 2015. Rhizobiales as functional and endosymbiontic members in the lichen symbiosis of Lobaria pulmonaria L. Front. Microbiol. 6, 53. https://doi.org/10.3389/fmicb.2015.00053.

Farooq, M., Rehman, A., Al-Alawi, A.K.M., Al-Busaidi, W.M., Lee, D.-J., 2020. Integrated use of seed priming and biochar improves salt tolerance in cowpea. Sci. Hortic. 272, 109507. https://doi.org/10.1016/j.scienta.2020.109507.

Frenkel, O., Jaiswal, A.K., Elad, Y., Lew, B., Kammann, C., Graber, E.R., 2017. The effect of biochar on plant diseases: what should we learn while designing biochar substrates? J. Environ. Eng. Landsc. Manag. 25 (2), 105–113. https://doi.org/10.3846/16486897.2017.1307202.

Fryda, L., Visser, R., Schmidt, J., 2019. Biochar replaces peat in horticulture: environmental impact assessment of combined biochar & bioenergy production. Detritus 5 (March), 132–149. https://doi.org/10.31025/2611-4135/2019.13778.

Gong, H., Xiang, Y., Wako, B.K., Jiao, X., 2022. Complementary effects of phosphorus supply and planting density on maize growth and phosphorus use efficiency. Front. Plant Sci. 13, 983788. https://doi.org/10.3389/fpls.2022.983788.

Güereña, D., Lehmann, J., Hanley, K., Enders, A., Hyland, C., Riha, S., 2013. Nitrogen dynamics following field application of biochar in a temperate North American maize-based production system. Plant Soil. 365 (1), 239–254. https://doi.org/10.1007/s11104-012-1383-4.

Huber, K.J., Overmann, J., 2018. Vicinamibacteraceae fam. nov., the first described family within the subdivision 6 acidobacteria. Int. J. Syst. Evol. Microbiol. 68 (7), 2331–2334.

Jaiswal, A.K., Elad, Y., Paudel, I., Graber, E.R., Cytryn, E., Frenkel, O., 2017. Linking the belowground microbial composition, diversity and activity to soilborne disease suppression and growth promotion of tomato amended with biochar. Sci. Rep. 7 (1), 44382. https://doi.org/10.1038/srep44382.

Jaiswal, A.K., Frenkel, O., Debode, J., Graber, E.R., 2024. How does biochar influence plant biotic stress? In: Lehmann, J., Joseph, S. (Eds.), Biochar For Environmental Management: Science, Technology and Implementation, 3rd ed Routledge.

Joos, L., Beirinckx, S., Haegeman, A., Debode, J., Vandecasteele, B., Baeyen, S., De Tender, C., 2020. Daring to be differential: metabarcoding analysis of soil and plant-related microbial communities using amplicon sequence variants and operational taxonomical units. BMC. Genomics. 21, 1–17.

Joos, L., De Tender, C., Holderbeke, A., Clement, L., Vandecasteele, B., Debode, J., 2023. Exploring the microbial response as a potential bio-indicator for soil health: insights from a controlled incubator experiment. Agric. Ecosyst. Environ. 356, 108634. https://doi.org/10.1016/j.agee.2023.108634.

Kamran, M., Malik, Z., Parveen, A., Zong, Y., Abbasi, G.H., Rafiq, M.T., Ali, M., 2019. Biochar alleviates Cd phytotoxicity by minimizing bioavailability and oxidative stress in pak choi (Brassica chinensis L.) cultivated in Cd-polluted soil. J. Environ. Manage 250, 109500. https://doi.org/10.1016/j.jenvman.2019.109500.

Kolton, M., Graber, E.R., Tsehansky, L., Elad, Y., Cytryn, E., 2017. Biochar-stimulated plant performance is strongly linked to microbial diversity and metabolic potential in the rhizosphere. New Phytol. 213, 1393–1404. https://doi.org/10.1111/nph.14253.

Köster, E., Pumpanen, J., Palviainen, M., Zhou, X., Köster, K., 2021. Effect of biochar amendment on the properties of growing media and growth of containerized Norway spruce, Scots pine, and silver birch seedlings. Can. J. For. Res. 51 (1), 31–40. https://doi.org/10.1139/cjfr-2019-0399.

Lataf, A., Jozefczak, M., Vandecasteele, B., Viaene, J., Schreurs, S., Carleer, R., Vandamme, D., 2022. The effect of pyrolysis temperature and feedstock on biochar agronomic properties. J. Anal. Appl. Pyrolysis. 168, 105728. https://doi.org/10.1016/j.jaap.2022.105728.

Lieten, P., 1997. The effect of substrate temperature on performance of strawberry on peat bags. Acta Hortic 450, 501–550. https://doi.org/10.17660/ActaHortic.1997.450.63.

López-Pérez, M., Rodríguez-Valera, F., 2014. The Family Alteromonadaceae. In: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), The Prokaryotes: Gammaproteobacteria. Springer, Berlin, Heidelberg, pp. 69–92. https://doi.org/10.1007/978-3-642-38922-1_233.

Lundberg, D.S., Lebeis, S.L., Paredes, S.H., Yourstone, S., Gehring, J., Malfatti, S., Dangl, J.L., 2012. Defining the core Arabidopsis thaliana root microbiome. Nature 488 (7409), 86–90. https://doi.org/10.1038/nature11237.

Madden, L., Wilson, L., Ellis, M., 1993. Field spread of anthracnose fruit rot of strawberry in relation to ground cover and ambient weather conditions. Plant Dis. 77 (9), 861–866.

Malewski, T., Borowik, P., Golińska, P., Okorski, A., Olejarski, I., Oszako, T., 2023. Organic inputs positively alter the bacteriome of post-agricultural soils. Forests 14 (9), 1711. https://doi.org/10.3390/f14091711.

Margenot, A.J., Griffin, D.E., Alves, B.S.Q., Rippner, D.A., Li, C., Parikh, S.J., 2018. Substitution of peat moss with softwood biochar for soil-free marigold growth. Ind. Crops. Prod. 112, 160–169. https://doi.org/10.1016/j.indcrop.2017.10.053.

Marin-Felix, Y., Miller, A.N., Cano-Lira, J.F., Guarro, J., García, D., Stadler, M., Stchigel, A.M., 2020. Re-evaluation of the order Sordariales: delimitation of lasiosphaeriaceae s. str., and introduction of the new families diplogelasinosporaceae, naviculisporaceae, and schizotheciaceae. Microorganisms 8 (9), 1430.

Mendez, A., Cárdenas-Aguiar, E., Paz-Ferreiro, J., Plaza, C., Gasco, G., 2017. The effect of sewage sludge biochar on peat-based growing media. Biol. Agric. Hortic. 33 (1), 40–51. https://doi.org/10.1080/01448765.2016.1185645.

Moelants, J., Similon, L., Bosmans, L., 2021. Sustainable organic growing media in a commercial tomato growing system. Acta Hortic. 1317, 303–312. https://doi.org/10.17660/ActaHortic.2021.1317.35.

Murthy, B.N.S., Pramanick, K.K., 2014. Straberry cultivation in mild-tropics: prospects and challenges from diseases. perspective. Acta Hortic. 1049, 151–159. https://doi.org/10.17660/ActaHortic.2014.1049.13.

Nakagawa, T., Takahashi, R., 2015. Nitrosomonas stercoris sp. nov., a chemoautotrophic ammonia-oxidizing bacterium tolerant of high ammonium isolated from composted cattle manure. Microbes. Environ. 30, 221–227. https://doi.org/10.1264/jsme2.ME15072.

Nilsson, R.H., Larsson, K.-H., Taylor, A.F S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Abarenkov, K., 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic. Acids. Res. 47 (D1), D259–D264. https://doi.org/10.1093/nar/gky1022.

Nobile, C., Denier, J., Houben, D., 2020. Linking biochar properties to biomass of basil, lettuce and pansy cultivated in growing media. Sci. Hortic. 261, 109001. https://doi.org/10.1016/j.scienta.2019.109001.

Nor, M., Sabaratnam, V., Tan, G., 2017. Devosia elaeis sp. nov., isolated from oil palm rhizospheric soil. Int. J. Syst. Evol. Microbiol. 67 (4), 851–855. https://doi.org/10.1099/ijsem.0.001683.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P., O'Hara, B.,… Wagner, H. (2020). Vegan: community Ecology package. R Package Version 2.6-4.

Pal, G., Saxena, S., Kumar, K., Verma, A., Sahu, P., Pandey, A., White, J., Verma, S., 2022. Endophytic Burkholderia: multifunctional roles in plant growth promotion and stress tolerance. Microbiol. Res. 265, 127201. https://doi.org/10.1016/j.micres.2022.127201.

Peng, L., Shan, X., Wang, Y., Martin, F., Vilgalys, R., Yuan, Z., 2021. Hybrid genome assembly and gene repertoire of the root endophyte Clitopilus hobsonii QYL-10 (Entolomataceae, Agaricales, Basidiomycetes). Mol. Plant-Microbe Interact. 34 (6), 711–714. https://doi.org/10.1094/MPMI-11-20-0328-A.

Petters, S., Groß, V., Söllinger, A., et al., 2021. The soil microbial food web revisited: predatory myxobacteria as keystone taxa? ISME J. 15, 2665–2675. https://doi.org/10.1038/s41396-021-00958-2.

Pot, S., De Tender, C., Ommeslag, S., Delcour, I., Ceusters, J., Gorrens, E., Vancampenhout, K., 2021. Understanding the shift in the microbiome of composts that are optimized for a better fit-for-purpose in growing Media. Front. Microbiol. 12, 643679. https://doi.org/10.3389/fmicb.2021.643679.

Pot, S., Tender, C.D., Ommeslag, S., Delcour, I., Ceusters, J., Vandecasteele, B., Vancampenhout, K., 2022. Elucidating the microbiome of the sustainable peat replacers composts and nature management residues. Front. Microbiol. 13, 983855. https://doi.org/10.3389/fmicb.2022.983855.

Prăvălie, R., 2021. Exploring the multiple land degradation pathways across the planet. Earth. Sci. Rev. 220, 103689. https://doi.org/10.1016/j.earscirev.2021.103689.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic. Acids. Res. 41 (D1), D590–D596. https://doi.org/10.1093/nar/gks1219.

Rathnayake, D., Creber, H., Van Poucke, R., Sohi, S., Meers, E., Mašek, O., Ronsse, F., 2021. Biochar from sawmill residues: characterization and evaluation for its potential use in the horticultural growing media. Biochar 3 (2), 201–212. https://doi.org/10.1007/s42773-021-00092-4.

Robinson, M.D., Oshlack, A., 2010. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol. 11 (3), R25. https://doi.org/10.1186/gb-2010-11-3-r25.

Schandry, N., 2017. A practical guide to visualization and statistical analysis of R. solanacearum infection data using R. Front. Plant Sci. 8, 623. https://doi.org/10.3389/fpls.2017.00623.

Van Gerrewey, T., Vandecruys, M., Ameloot, N., Perneel, M., Van Labeke, M.-C., Boon, N., Geelen, D., 2020. Microbe-plant growing Media interactions modulate the effectiveness of bacterial amendments on lettuce performance inside a plant factory with artificial lighting. Agronomy 10 (10), 1456. https://doi.org/10.3390/agronomy10101456.

Vandecasteele, B., Hofkens, M., De Zaeytijd, J., Visser, R., Melis, P., 2023. Towards environmentally sustainable growing media for strawberry cultivation: effect of biochar and fertigation on circular use of nutrients. Agric. Water. Manage 284, 108361. https://doi.org/10.1016/j.agwat.2023.108361.

Vandecasteele, B., Viaene, J., Castejón-del Pino, R., Lataf, A., Cuypers, A., Vandamme, D., 2024. S-enhanced microbial activation of biochars and processed grass fibers for circular horticulture. Sci. Total Environ. 957, 177760. https://doi.org/10.1016/j.scitotenv.2024.177760.

Vaughn, S.F., Kenar, J.A., Thompson, A.R., Peterson, S.C., 2013. Comparison of biochars derived from wood pellets and pelletized wheat straw as replacements for peat in potting substrates. Ind. Crops. Prod. 51, 437–443. https://doi.org/10.1016/j.indcrop.2013.10.010.

Verheul, M.J., Sønsteby, A., Grimstad, S.O., 2007. Influences of day and night temperatures on flowering of Fragaria x ananassa Duch., cvs. Korona and Elsanta, at different photoperiods. Sci. Hortic. 112 (2), 200–206. https://doi.org/10.1016/j.scienta.2006.12.022.

Videira, S., Groenewald, J., Nakashima, C., Braun, U., Barreto, R., Wit, P., Crous, P., 2017. Mycosphaerellaceae – Chaos or clarity? Stud. Mycol. 87, 257–421. https://doi.org/10.1016/j.simyco.2017.09.003.

Viger, M., Hancock, R.D., Miglietta, F., Taylor, G., 2015. More plant growth but less plant defence? First global gene expression data for plants grown in soil amended with biochar. Gcb Bioenergy 7 (4), 658–672. https://doi.org/10.1111/gcbb.12182.

Wei, C., Liang, J., Wang, R., Chi, L., Wang, W., Tan, J., Shi, H., Song, X., Cui, Z., Xie, Q., Cheng, D., Wang, X., 2024. Response of bacterial community metabolites to bacterial wilt caused by Ralstonia solanacearum: a multi-omics analysis. Front. Plant Sci. 14. https://doi.org/10.3389/fpls.2023.1339478.

Zulfiqar, F., Allaire, S.E., Akram, N.A., Méndez, A., Younis, A., Peerzada, A.M., Wright, S. R., 2019. Challenges in organic component selection and biochar as an opportunity in potting substrates: a review. J. Plant Nutr. 42 (11–12), 1386–1401. https://doi.org/10.1080/01904167.2019.1617310.