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The bacterial and fungal strawberry root-associated microbiome in reused peat-based substrate

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Abstract

Background Reuse of plant growing substrate can contribute to lowering the carbon footprint of horticulture production systems. Here, we assessed the impact of substrate reusing on the root-associated microbiome of strawberries. The cultivars Elsanta and Malling Centenary were grown in a substrate-based hydroponic system using either fresh peat-based substrate or substrate reused up to three times, with comparisons made between not steamed and steam-treated substrate. The root-associated microbiome was analyzed using 16S rRNA gene and ITS1 DNA sequencing to determine bacterial and fungal communities.

Results Substrate reusing without steaming increased the bacterial and fungal community diversity whereas steaming reduced the bacterial diversity and increased fungal diversity in the root-associated microbiome. The root-associated bacterial communities recruited by the two cultivars were diverse, even more so than the diversity recorded for the different times of reused substrate.

Conclusion These observations demonstrate the ability of strawberry to establish a genotype-specific root-associated microbiome when plants are cultured on reused substrate. The bacterial microbiome showed a higher consistency over the times substrate was reused, while the fungal community composition showed stronger adaptation to the substrate reusing. Pathogenic fungi accumulated over the reusing times, underscoring the necessity of substrate sanitation through steaming to minimize the risk of pathogen infections.

Clinical trial number Not applicable.

Keywords Strawberry cultivation, Root-associated microbiome, Circular horticulture, Bacterial and fungal community

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Introduction

Peat is an excellent growth medium for a variety of crop systems because of its ability to retain moisture and provide good aeration to plant roots [1]. However, the use of peat raises environmental concerns due to peatland mining, which threatens important carbon sinks and habitats for many species. Additionally, peat is considered a non-renewable resource [2]. To reduce the use of peat in horticulture, several studies have investigated alternative materials. For example, biochar, coir, wood fibers, green compost and vermicompost have been compared as peat alternatives in soilless cultivation [3, 4]. Despite these available alternatives, replicating the excellent physical properties of peat, which are keeping an air-water balance and physical support, is challenging.

Although the reuse of peat does not address its limited availability, it is an approach to expand its lifespan, reduce peat consumption, and minimizes the cost of disposing of growing media at the end of the growing season [5]. Used peat substrate is often applied as soil improver [6], or used for biochar production, allowing heavy metal absorption [7]. Peat largely maintains its structural properties and multiple reuses do not significantly alter the plant availability of critical minerals like P, K, Ca and Mg [8]. Nitrogen is typically decreased after the first time use and needs to be replenished [8].

A study in which perlite was reused in a hydroponic cultivation system showed a slow degradation of its physical properties [9]. Degradation of substrate, with increase in water holding capacity and decrease in air capacity, was reported for a mix of peat, perlite, and rice hull upon reusing [10]. Despite these structural changes, the reuse of these substrate did not alter the quality or yield of the cultured crops [9]. Successful experiences have also been reported where coir, peat, and wood fiber were reused three times in strawberry cultivation (Malling Centenary, *Fragaria* x *ananassa*) [11]. However, the reuse of perlite led to an increase in pathogen populations, indicating a significant risk associated with reusing substrate [8].

It has been suggested that the degradation in substrate quality is related to the enrichment of host-specific pathogens [12].

Common methods for substrate disinfection include steaming, solarization, and chemical treatments. Steaming has limited effects on the substrate's physicochemical characteristics, and efficiently reduces the risk for pathogens and weeds [8]. To address the feasibility of reusing growth substrate, we conducted a trial in which peat-based plant growth media were reused from strawberries grown in a commercial production setting in Belgium. Repeated use of the substrate previously showed no alteration in nutrient retention or physical properties, and reused substrate, including the steamed ones, resulted in similar yields as the virgin blends [13].

In this study, we investigated the bacterial and fungal communities of strawberries grown on steamed and not steamed reused substrate. The impact of peat reuse on the structure and dynamics of the strawberry root-associated microbiome of the cultivars Elsanta and Malling Centenary was determined by investigating (1) the root-associated microbiome of strawberry grown on reused substrate; (2) how the bacterial and fungal microbial community shifted upon reuse; (3) the impact of the strawberry cultivar on the microbiome.

Material and method

Experimental setup

The strawberry cultures were initiated using tray plants. The strawberry cultivar (hybrid *Fragaria* × *ananassa)* Elsanta was cultured in a greenhouse at the research station Inagro (Ieperseweg 87, 8800 Rumbeke-Beitem, Belgium) and Malling Centenary was purchased from de Kemp BV (Kempweg 15, 5964 ND Horst-Meterik, Netherlands). The tray plants were cultivated and fertilized in Inagro (Belgium) as described [13]. During the vegetative stage, all plants were irrigated with nutrient solution: 3.37 mmol/L K^+, 1.05 mmol/L Mg^{2+}, 3.79 mmol/L Ca^{2+}, 1.00 mmol/L NH_4^+, 10.65 mmol/L NO_3^-, 0.85 mmol/L P, 1.28 mmol/L SO_4^{2-}, 40.00 µmol/L Fe^{3+}, 25.00 µmol/L Mn, 1.00 µmol/L Cu, 10.00 µmol/L Zn, 20.00 µmol/L B, 0.75 µmol/L Mo. During harvesting stage, the nutrient solution contained: 6.50 mmol/L K^+, 1.08 mmol/L Mg^{2+}, 2.69 mmol/L Ca^{2+}, 11.01 mmol/L NO_3^-, 0.87 mmol/L P, 1.08 mmol/L SO_4^{2-}, 30.00 µmol/L Fe^{3+} and 10.00 µmol/L B. Each pot (Bato, 4.7 L, Bato Plastics B.V.,129 Zevenbergen, The Netherlands, 24 cm in diameter, and 20 cm in height) was filled with 4.2 L substrate and 4 tray plants (rooted in 250 ml of substrate) were placed and grown in the corners of the pot. "Excellent mix" substrate was purchased from Kekkilä-BVB (Vantaa, Finland). It consists 20% v/v coarse peat, 20% v/v medium coarse peat, 40% v/v coir grit and 20% v/v perlite, mixed with lime and mineral fertilizer (400 g/m³). 6 drip-irrigated pots were placed in one gutter, which is part of a closed-loop gutter system. To steam the spent substrate from previous growing cycles, substrate was placed in a barrel, injected with 100 °C steam for 1 to 2 min, and then continuously steamed for 1 h to maintain a temperature of around 70 °C. The physicochemical properties of the substrate are described [13]. The growing season started from August to December, 2022. Plants were sampled at the end of the harvesting stage on December 5th, 2022, by carefully removing the plants from the pots and shaking of loose substrate to preserve root system integrity. Plants were transferred in ice box and handling was done using disinfected bags and gloves.

Root-associated microbiome extraction

4 individual strawberry plants per treatment from different pots were collected. The strawberry plants, together with the surrounding substrate was transported to the lab. After arriving, larger clumps of substrate were firstly removed, and the substrate around the main root system was carefully removed by gentle shaking. The root-associated microbiome of strawberry plants was extracted by performing two washing steps, which enable us to collect both the rhizosphere and the rhizoplane microbiome. The root system of a single plant was firstly soaked in 100 ml 0.9% NaCl solution at 4 °C for 90 min on a rotating platform at 125 rpm for rhizosphere extraction. Then it was placed in 100 ml of 0.9% NaCl + 0.01% Tween 80 and mixed again at 125 rpm for rhizoplane extraction. The solutions obtained from both washing steps were combined to 50 mL plastic tubes and centrifuged at 5000 rpm for 10 min to pellet the substrates. The pellets were washed in 2 mL of 0.9% NaCl and resuspended in 1 ml of TSB + 15% glycerol for storage at -80 °C until DNA extraction.

DNA extraction and microbiome sequencing

Microbial pellets were processed using DNeasy® PowerSoil® Pro Kit (QIAGEN, Hilden, Germany), following the protocol, 30 µl of microbial genomic DNA was extracted in the end. The concentration of DNA was measured and the sample was stored at -20°C for downstream analyses. The quality of the extracted microbial DNA was assessed by PCR amplification of the 16S rRNA and ITS1 regions, confirming the presence of bacterial and fungal DNA and they're suitable for sequencing. The primers used were 27F (5'-GAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGYTACCTTGTTACGACTT-3'), which specifically amplify the 16S rRNA gene; ITS4 (5'-CTCCGCTTATTGATATGC-3') and ITS7 (5'-GARTCATCGAATCTTTG-3') for detecting the fungal ITS1 region. The PCR reaction mix contained 15.8 µl H_2O, 2 µl DreamTaq buffer, 0.5 µl dNTP, 0.2 µl forward primers, 0.2 µl reverse primers, 0.2 µl DreamTaq DNA Polymerases (Thermo Fisher Scientific) and approximately 100 ng DNA. The thermal cycling was 1 min at 95 °C, 30 s at 95 °C, 35 cycles of 55 °C for 30 s, 72 °C for 45 s and 10 min at 72 °C. PCR amplification products were visualized by 2% agarose gel electrophoresis. 15 µl of the original genomic DNA extract was sent to LGC Genomics GmbH (Berlin, Germany) for library preparation and sequencing on the Illumina MiSeq platform using the 27F and 1492R primers for bacteria, and ITS4 and ITS7 primers for fungi. Raw sequence data was processed using the R package DADA2. Primer sequences were removed, and reads were truncated at a quality score cut-off (truncQ = 2). Additional filtering removed reads with ambiguous base calls or high expected errors (maxEE = 2,2). After dereplication, unique reads were processed using the Divisive Amplicon Denoising Algorithm (DADA) error estimation algorithm under self-Consist mode for sample inference [14]. Error rates were inspected to ensure accuracy, and denoised reads were subsequently merged. After chimera removal, the resulting Amplicon Sequence Variant (ASV) table was used for taxonomy assignment. Taxonomy was assigned using the Naive Bayesian Classifier with the DADA2-formatted Silva v138.1 database for bacterial sequence reads and the UNITE v8.3 database for fungal sequence reads [15, 16].

Statistical analysis

ASV tables were constructed from the bacterial and fungal sequences and the normalized dataset was analyzed using R. The ASV tables were imported by using phyloseq and vegan packages. Microbial alpha diversity was determined by using breakaway package (richness) and the DivNet package for determining the Shannon diversity. Statistical analysis of the diversity was achieved by using the estimates' variance in a mixed model approach. For beta diversity analyses, Bray-Curtis dissimilarity index was used as input for the visualization in a PCoA plot. A PerMANOVA (with a significance level of $\alpha = 0.05$) on the Bray-Curtis distances evaluated the homogeneity of variances and indicated the differences in microbiome communities. Sourcetracker was used in R (v3.2.4) to estimate the percentage of microbiome transferred within different cycles. Stacked plots (with connecting lines) were made with SRplot [17], the asterisks indicate genera which were significantly different among different time of reuse as determined by two-way ANOVA ($p < 0.05$).

Results

Relative abundance of microbes in the root-associated microbiome of strawberry in reused substrate

A total of 2,370,173 and 1,640,764 sequence reads were obtained for Elsanta and Malling Centenary combined, identifying 9,648 bacterial and 2,252 fungal ASVs. The bacterial sequences covered 31 phyla, 83 classes, 380 families, and 736 genera and the 2,252 fungal ASVs covered 11 phyla, 37 classes, 224 families, and 393 genera.

To gain an overview of the microbial variation in the root-associated microbiome of strawberry plant grown in reused substrate, beta diversity in bacterial and fungal microbiome derived from different time of substrate using was clustered using Principal Coordinate Analysis (Fig. 1). The distance between the dots indicates the similarity and divergence in microbial composition. The bacterial communities were more similar across the different reused substrate than the fungal communities. Overall, there was a trend of increasing differences between samples with the number of times the substrate was reused

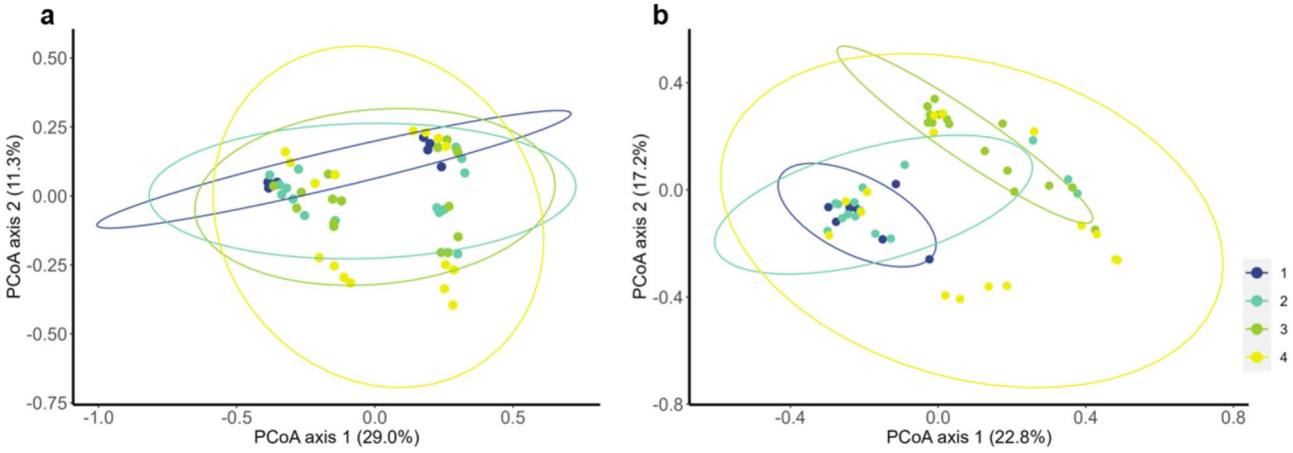

Fig. 1 PCoA plots showing the structure of the root-associated bacterial (**a**) and fungal (**b**) communities as a function of the times the substrate was used. Each dot indicates the root-associated microbiome isolated from each replicant and data ellipses were drawn based on treatments. The times of substrate being used are indicated in different colors, $n = 8$ (fresh substrate) and $n = 48$ (reused substrate). (a. $R^2 = 0.09803$, $p < 0.01$; b. $R^2 = 0.16878$, $p < 0.001$)

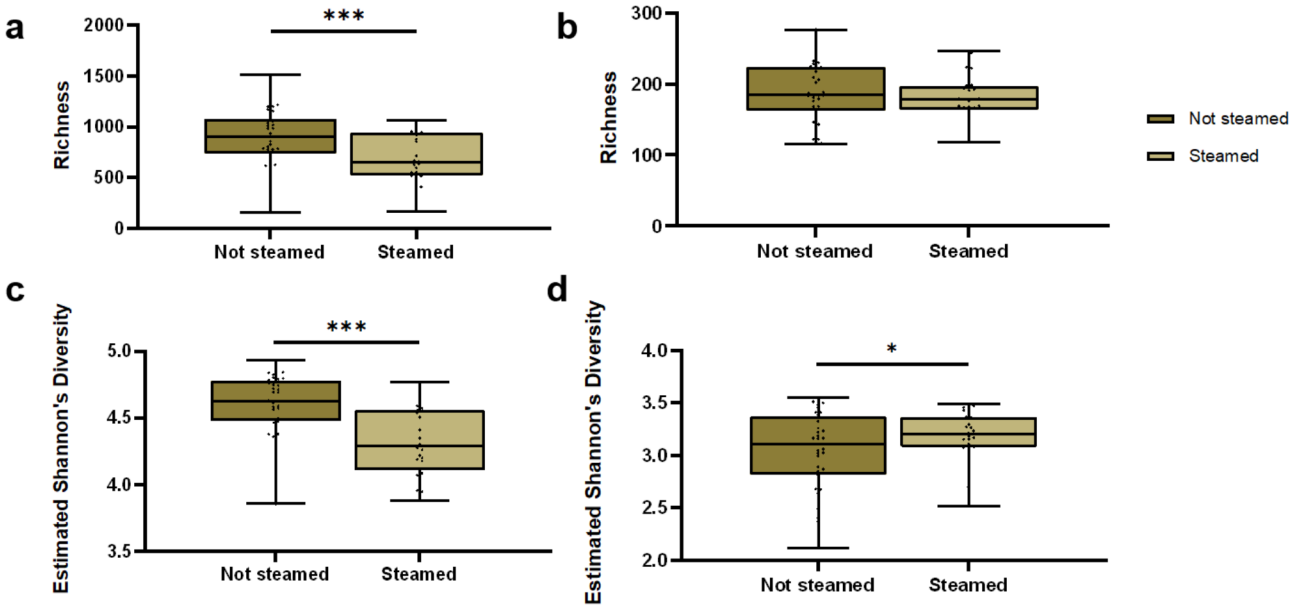

Fig. 2 Boxplot showing the richness (**a** and **b**) and Shannon's diversity index (**c** and **d**) of root-associated bacteria (**a** and **c**) and fungi (**b** and **d**) comparing steamed or not steamed substrate. Asterisks indicate level of significance: $p < 0.001$(***) and $p < 0.05$(*), $n = 32$ (not steamed) and $n = 24$ (steamed)

(Fig. 1). These results suggest that reusing of substrate influences the microbial composition of the root-associated microbiome, with a stronger shift in the fungal community compared to the bacterial community.

Comparison of the root-associated microbiome of plants grown on steamed and not steamed substrate

To further investigate the impact of steaming on the root-associated microbiome, we compared the microbial richness and diversity of bacteria and fungi (Fig. 2). The analysis revealed that steaming significantly reduced bacterial richness (number of species) and Shannon diversity (the abundance and distribution of individual species). The fungal richness was not significantly affected by steaming, and a small but significant impact was observed for the Shannon's diversity index (Fig. 2).

We further examined the structure of both the bacterial and fungal community as affected by steaming treatment. Principal coordinates analysis revealed that bacterial communities extracted from plants cultured on steamed substrate exhibit greater similarity (Fig. 3). This result aligns with the reduced richness and Shannon's diversity of the root-associated microbiome from plants grown on steamed substrate (Fig. 2). In contrast, the fungal communities derived from steamed and not steamed substrate were more conserved (Figs. 2 and 3). These findings suggest that residual bacteria contribute to a big

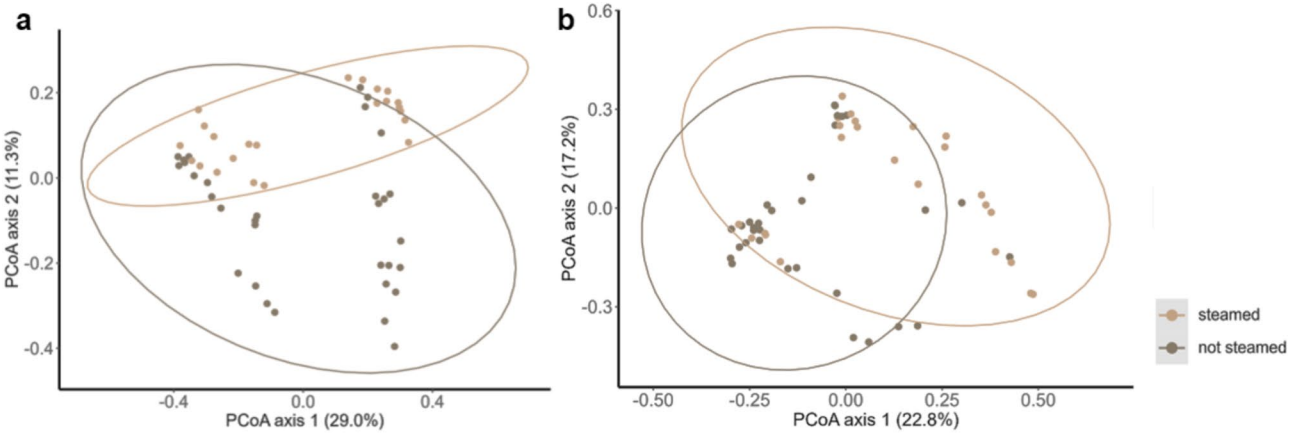

Fig. 3 PCoA plots showing the structure of the root-associated bacterial (**a**) and fungal (**b**) communities as a function of whether the substrate was steamed or not. Each dot indicates the root-associated microbiome isolated from each replicant and data ellipses were drawn based on treatments. Steamed (light brown) or not steamed (dark brown) substrate is indicated in different colors, $n = 32$ (not steamed) and $n = 24$ (steamed). (a. $R^2 = 0.07096$, $p < 0.001$; b. $R^2 = 0.06237$, $p < 0.001$)

extent to bacterial community establishment, more than that for fungi.

Transition of the root-associated microbiome across the reused substrate

To assess the impact of substrate reuse, the root-associated microbial community was compared across the times of substrate reuse. The level of microbial transition, i.e. the proportion of microbial community "retained" from the previous substrate use, was calculated by using the SourceTracker R package, a Bayesian mixed model-based tool for microbial community data analysis. It uses microbial data from known source samples to estimate the contribution of each source in the tested samples [18, 19]. This calculation results in a transition percentage which reflects the proportion of microbial data in samples that can be found in samples from plants grown on substrate from a previous cycle (Fig. 4).

The transition percentage calculated for the root-associated microbiome of 2 subsequent cultures was generally higher than those that was separated by 1 or 2 cycles (Fig. 4), supporting the view that microbes in the substrate contribute to the establishment of the root-associated microbial community. There was, however, a clear difference between the microbiomes derived from plants grown on steamed and not steamed substrate. Steaming resulted in a higher overlap between subsequent cultures as well as cultures separated by 1 or 2 cycles, suggesting that the sterilized substrate provides a relatively consistent condition for the establishment of the microbiome. In contrast, the root-associated microbiome from subsequent cultures using not steamed substrate shifted, resulting in a smaller overlap of the microbiomes derived from plants grown on reused substrate. We also observed that the bacterial communities were more similar than the fungal communities in both the steamed and not steamed substrates. This suggests that, not so much the substrate, but rather the plant determines the identity of bacteria establishing at the root-associated microbiome. The transition of the fungal identities was more dependent on whether the substrate was steamed or not. Steaming the substrate resulted in high similarity of the fungal root-associated microbiomes. It increased the fungal transition across substrate reusing, with the Malling Centenary sample derived from strawberries grown on the 4th time use of substrate (Fig. 4). Collectively, these results show that the root-associated microbial community is largely transferred upon the reuse of substrate and that steaming has stronger impact on the establishment of bacterial community compared to the fungal community.

Dynamics of the relative abundance of root-associated microbial genera

To assess the impact of substrate reusing on the microbial succession in strawberry root systems, the relative abundance of both bacteria and fungi were compared at the genus level. More than 60% of bacteria was classified as "others" (Fig. 5). The 12 most abundant genera remained relatively stable across the reused substrate. One exception was the strong decrease in abundance of the genus *Burkholderia-Caballeronia-Paraburkholderia* in bacterial community of Malling Centenary, dropping from about 10% to around 1% after the first reuse. Compared to Malling Centenary, Elsanta contained fewer of the *Proteobacteria SWB02* but more *Streptomyces*.

The fungal genera varied in relative abundance more than the bacteria (Figs. 5 and 6). When grown on fresh substrate, both cultivars Elsanta and Malling Centenary exhibited a relatively even distribution of the top 12 most

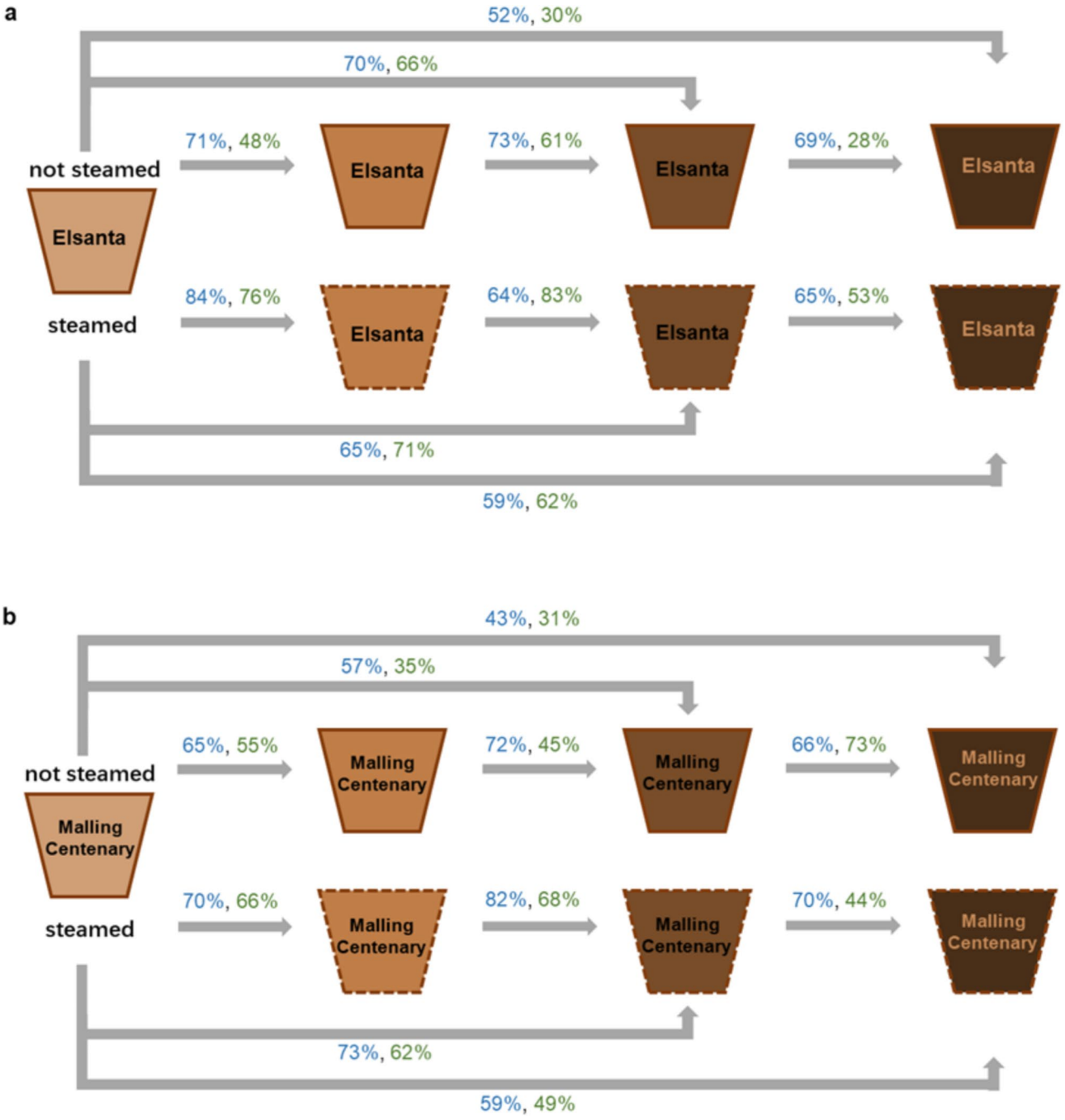

Fig. 4 Microbiome transfer between different times of substrate reuse. Two Cultivars Elsanta (**a**) and Malling Centenary (**b**) are shown separately. The trapezoids represent fresh, 2-time-used, 3-time-used and 4-time-used substrate (as the color become deeper). The dashed line represents the steaming treatment. The transition percentage of bacteria is shown in blue and for fungi in green

abundant fungal genera (Fig. 6). The fungal microbiome of Elsanta showed a strong accumulation of *Mortierella* (3-time-used substate). In both Elsanta and Malling Centenary, unidentified *Chaetothyriaceae* strongly accumulated at the 3-time-used substrate (29% and 24%, respectively). The root-associated fungal community of Malling Centenary also exhibited a significant accumulation of *Thelonectria*, increasing after the first reusing (Figs. 3 and 10–39% at the 2-time-used substrate). Additionally, the relative abundance of *Oidiodendron* and *Humicola* decreased sharply upon reusing. *Olpidium* and *Plectosphaerella* reached peak abundance by the 3rd cycle, while a similar trend was observed in the root-associated microbiome of Elsanta with *Mortierella*.

Next, we compared the bacterial and fungal microbiome of plants grown on reused substrate that was

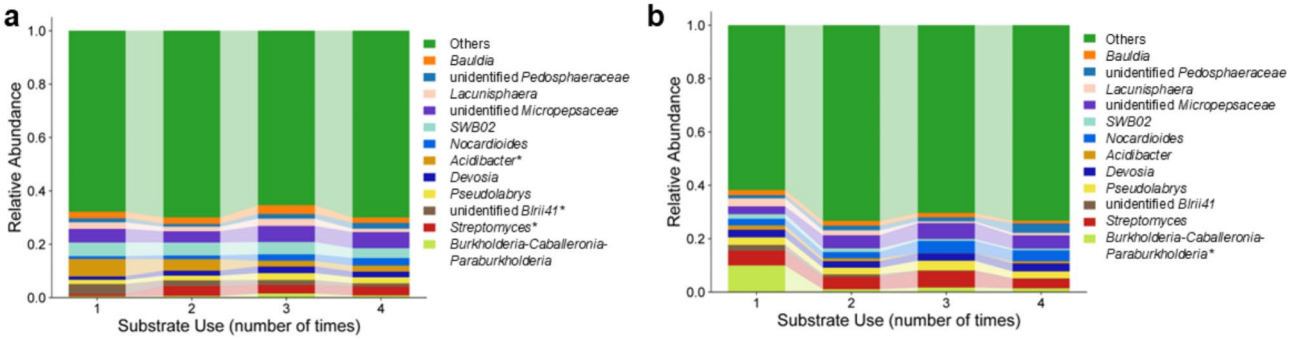

Fig. 5 Stacked bar plot of relative abundance of the top 12 root-associated bacterial genera of Elsanta (**a**) and Malling Centenary (**b**) in not steamed reused substrate. Less abundant bacterial genera are categorized as "Others". Identities of top 12 genera are color coded as in legend on the right

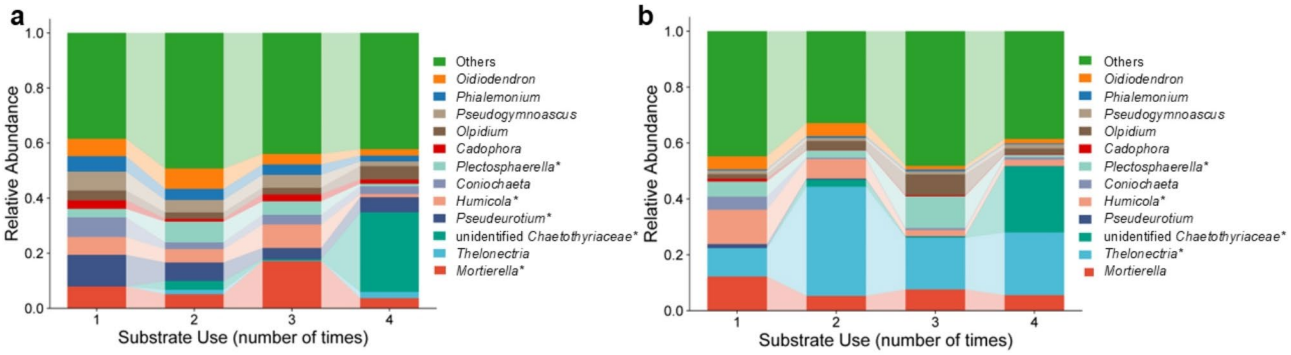

Fig. 6 Stacked bar plot of relative abundance of the top 12 root-associated fungal genera of Elsanta (**a**) and Malling Centenary (**b**) in reused not steamed substrate. Less abundant fungal genera are categorized as "Others". Identities of top 12 genera are color coded as in legend on the right

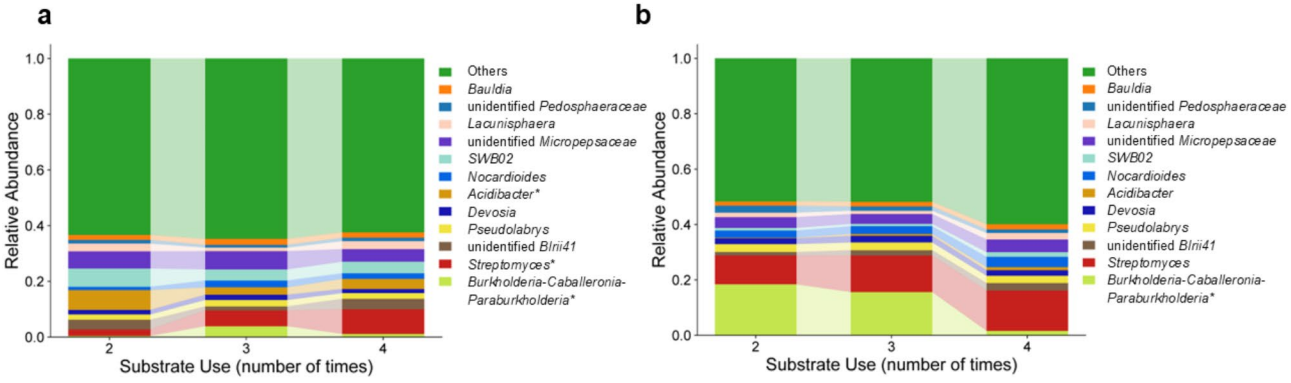

Fig. 7 Stacked bar plot of relative abundance of the top 12 root-associated bacterial genera from Elsanta (**a**) and Malling Centenary (**b**) along substrate reusing (steaming treatment applied). Less abundant bacterial genera are categorized as "Others". Identities of top 12 genera are color coded as in legend on the right

steamed (Fig. 7). In both Elsanta and Malling Centenary, *Streptomyces* became more dominant with the times the substrate was reused. Several genera accumulated to similar abundance indicating that the root systems of Elsanta and Malling Centenary were overall able to recruit similar microbes in each experiment. The *Burkholderia-Caballeronia-Paraburkholderia* showed a strong abundance in Malling Centenary in fresh substrate accounting for 20.0% (Fig. 7). Its abundance was, however, much lower when the substrate was 3-time-used reaching 1.6%. Although there was some variation in *Burkholderia-Caballeronia-Paraburkholderia* abundance in the Elsanta root-associated bacterial community, Malling Centenary accumulated much more microbes of this genus.

Compared to the fungal relative abundance (not steamed, Fig. 3), the root-associated microbiota exhibits greater stability after steaming, including *Oidiodendron*, *Phialemonium*, *Plectosphaerella* and *Coniochaeta* (Fig. 8). Though there were some fluctuations, no significant enrichment of any genus was observed, with minimal

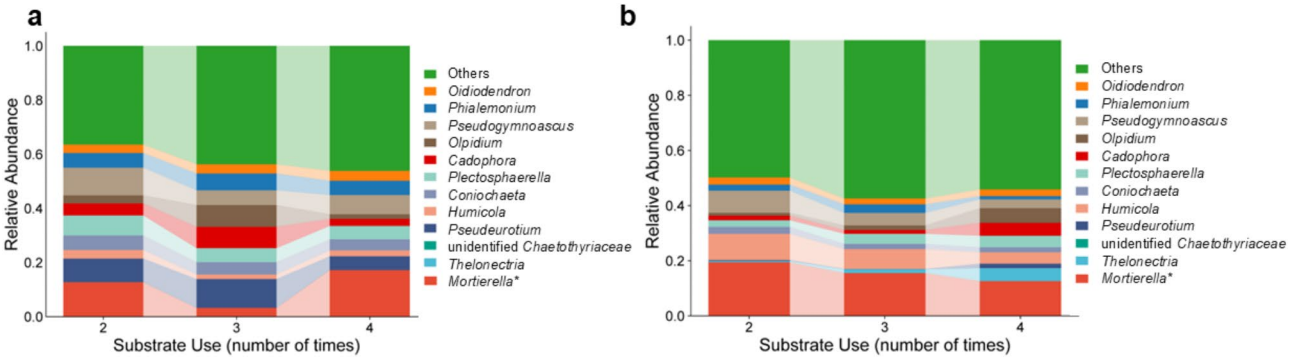

Fig. 8 Stacked bar plot of relative abundance of the top 12 root-associated fungal genera from Elsanta (**a**) and Malling Centenary (**b**) along substrate reusing (steaming treatment applied). Lower abundant bacterial genera are categorized as "Others". Identities of top 12 genera are color coded as in legend on the right

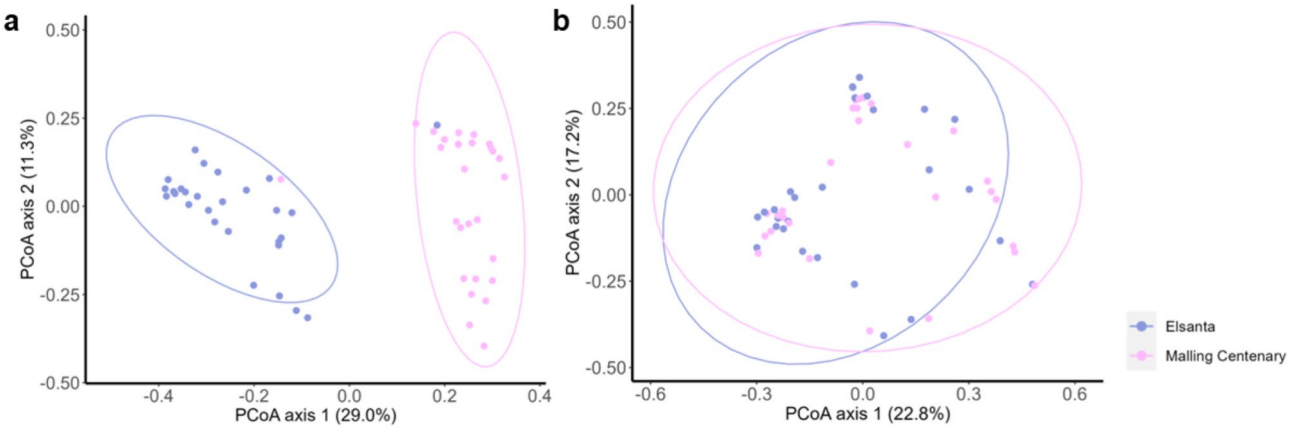

Fig. 9 PCoA plots showing the structure of the root-associated bacterial (**a**) and fungal (**b**) communities as a function of cultivars. Each dot indicates the root-associated microbiome isolated from each replicant and data ellipses were drawn based on cultivars. Elsanta and Malling Centenary are indicated in blue and pink, respectively, $n = 28$. (a. $R^2 = 0.24475$, $p < 0.001$; b. $R^2 = 0.01548$, $p = 0.581$)

occurrences of unidentified *Chaetothyriaceae* and *Thelonectria*. Additionally, *Pseudeurotium* was detected in the root-associated fungal community of Elsanta, while *Humicol*a was detected in Malling Centenary, with both not enriched in the other cultivar, indicating *Pseudeurotium* and *Humicola* are cultivar-specific fungal genera. A list of fungi including those that potentially cause disease are listed in the supplementary Table 1.

Comparison of the root-associated microbiome from Elsanta and Malling Centenary

The extent of variation in microbial community structure and composition was compared for the 2 cultivars. PCoA of the Bray-Curtis dissimilar matrix revealed a strong difference in the root-associated bacterial communities between the cultivars whereas the fungal communities were largely similar (Fig. 9). It indicates that genotype is the main factor driving the differences in the bacterial community. Fungal community, on the other hand, is more strongly influenced by the substrate (Fig. 9).

Discussion

With the aim to improve the sustainability of strawberry soilless culture production, the impact of substrate reuse on the bacterial and fungal root-associated microbiome of strawberry was analysed. The analysis of two strawberry cultivars root-associated microbiome shows that the reuse of substrate has a more pronounced effect on the fungal than the bacterial microbiome. Overall, however, the impact is relatively modest when comparing the bacterial communities of different cultivars. Substrate reuse and steaming both affect the composition and diversity of the microbial community, but in a subordinate manner.

Substrate as a determining factor of the bacterial and fungal root-associated microbiome

The presence of root-associated microbes can confer advantages to the plant host by stimulating growth, nutrient uptake, and resistance to pathogens [20]. Plants recruit protective microorganisms to suppress pathogens in the rhizosphere [21], further demonstrating that

controlling the root-associated microbiome may be a valuable strategy to improve crop protection, as an alternative to chemical pesticides. The success in optimizing plant health using such a method depends on how well the grower can control the establishment and maintenance of the desired microbiome. It will be a major challenge to manage the microbiome in field conditions, because of strong variations in soil composition, structure and edaphon [22]. Crop production systems that use growing media or horticultural substrate, however, provide a context that is much easier to control and will therefore be more suitable for creating the conditions of a profitable application involving the control of the root-associated microbiome.

The presented data revealed that the plant root-associated bacterial communities are less influenced by steaming and the reuse of substrate than the fungal communities, suggesting these treatments are ineffective in altering the bacterial communities. This implies that the root-associated bacterial community is more strongly influenced by the plant and surrounding microbes, which is in line with previous studies [23, 24]. The impact of reusing substrate on the microbial communities found in plants at the end of the growing cycle was limited with a similarity that was around 70% (± 13%) for the bacteria and around 55% (± 27%) for the fungal community. These results indicate that substrate used for plant cultivation is altered in structure or chemical composition such that it influences the establishment of the root-associated fungal community. The establishment of the bacterial community appears not to be as strongly affected by substrate reuse. In line with the similarity of the root-associated bacterial communities in reused substrate, a study in cucumber showed strong similarity of the root-associated bacterial communities in plants cultured on different commercial substrates, suggesting that the substrate characteristics are not the main determinant for establishing the root-associated bacterial community [25]. This contrasts with the behavior of the fungal community. The underlying mechanism by which the substrate influences the fungal community is not clear, but since peat and coir consist of carbohydrate polymers that are a potential energy source and adhesion surface for fungal growth, we presume that impartial decomposition did occur and could have affected its capacity to support fungal growth.

The microbial community is in part established by the selection of microbes from the original soil microbial community structure and then shaped continually by the plant and the environment [26]. Seedlings interact with microbes living in and around the seed from which it originated, pointing to the importance of the starting seed and soil microbiome [27, 28]. In addition, the rhizosphere microbiome can be vertically transmitted across generations, influencing the plants rhizosphere in the next generation, leading to what is called soil succession, also referred to as "soil memory" [29]. In our experimental setup, the microbes recruited to the rhizosphere or the rhizoplane either derived from strawberry tray plants, from the (reused) substrate, or from the nutrient solution and the hydroponic culturing system. The comparison of the root-associated microbial communities of steamed and not steamed substrate allowed assessing the importance of succession of the microbial community structure and composition. In contrast to the fungal microbial communities, steaming substantially reduced the bacterial diversity. These results indicate that bacteria in the reused (not steamed) substrate play an important role in establishing the bacterial root-associated microbiome. Furthermore, the PCoA analyses show a strong clustering of the root-associated microbiomes derived from plants grown on substrate of the same reuse time. This indicates that substrate decay and changes in its physiochemical properties due to prior use also contributes to determining the root-associated microbiome. These findings corroborate with reports showing that bacterial substrate preferences contribute to the microbiome assembly in the rhizosphere [30].

Impact of the cultivar on the root-associated microbiome

Root-associated microbiotas are to a large extent driven by root exudates whereby younger plants secret considerably higher amounts of metabolites than older ones, suggesting that the root-associated microbiome assembly is primarily controlled during the early phase of crop culturing [31, 32]. The various primary and secondary metabolites it produces influences the root-associated microbial community, recruiting and promoting beneficial microorganisms, as well as resisting harmful microorganisms [33]. Several reports provide evidence for a substantial contribution of the crop cultivar to the bacterial and fungal rhizosphere community under different soil conditions [34–36]. Soil does not stay the same over time and continued culturing of a crop like tomato was shown to modulate the root-associated microbiome over each cycle [37]. These reports are consistent with our study showing a gradual shift in root-associated microbiome over the consecutive cycles. Comparison of two strawberry cultivars showed a strong difference in bacterial community and high similarity of the fungal community. This compelling finding is in line with other studies, e.g., in *Chrysanthemum* where five varieties exerted strong selection of the bacterial community, but not the fungal one [38]. Besides, substrate and cultivation conditions also have an important impact. For example, a study of five tomato cultivars showed stronger microbial variation when cultured hydroponically compared to cultivation in soil [39]. In our study we analyzed

the root-associated microbiome at the end of the strawberry culture, but do not have information on the initial growth phase when microbes were being recruited from the original tray plant. It is therefore possible that Elsanta and Malling Centenary microbiomes at the early growth phase were much more similar. The root-associated plant microbe interactions are highly complex and influenced by multiple factors involving soil type, environment, and plant genotype. The shift in the influence of the plant genotype and selection factors is illustrated in a study of *Arabidopsis* using an artificial ecosystem (mixture of substrate and field soil) following the microbiome over 8 generations [40]. They found that over time, the genotype gained more influence, suggesting that the plants exert a selection pressure on the bacteria driving the community to a smaller and more favorable composition [40].

Limitations of substrate reusing in strawberry cultivation

In horticultural production context, the potential risk of pathogen accumulation and economic consequences is a primary concern. Several pathogenic microbes were found in the reused substrate, with a particular stronger increase after the 3rd cycle. *Thelonectria*, a reported pathogens of hardwood trees and shrubs [41], was detected in the root-associated microbiome of Malling Centenary as a subdominant genus. *Chaetothyriaceae* also accumulated. They are epiphytic fungi generally considered as secondary pathogens, which do not infect the plants directly, but rather grow on leaf surface and ultimately negatively affect plant growth by covering leaf surfaces and reducing photosynthesis [1, 42]. Steaming strongly limited the relative abundance of both *Chaetothyriaceae* and *Thelonectria*, demonstrating that sanitizing the substrate was effective.

In addition to the top 12 species with higher relative abundance, we also observed a limited accumulation of other fungal pathogens, for instance *Fusarium* and *Verticillium* which both cause wilt in strawberries, leading to symptoms such as blackening and rotting of the roots [43, 44], and *Cylindrocladium* that causes black rot in the roots as well, and in severe cases, results in stunted growth and reduced fruit quality [45]. These 3 genera are all more abundant in Malling Centenary than in Elsanta. Moreover, they appeared more frequently in the substrate that were reused without steam treatment in between. Among them, *Verticillium* was prominent in the root-associated microbiome after 3 cycles, regardless of whether steam treatment was applied.

Substrate reuse also led to the accumulation of beneficial microorganisms like *Streptomyces* which was found in the root-associated bacterial community of Malling Centenary. *Streptomyces* species, with *Streptomyces lydicus* as represent, often serve as biocontrol and plant growth-promoting agents [46]. As for Elsanta, there was rare presence of *Streptomyces* in the fresh substrate, but it accumulated after reusing, under both steamed and not steamed condition, suggesting that Elsanta specifically recruited this species. *Burkholderia-Caballeronia-Paraburkholderia* is also viewed as beneficial to plants and shown to suppress fungal pathogens [47]. In previous studies members of the *Paraburkholderia* were identified as a plant growth-promoting bacteria [48]. *Burkholderia-Caballeronia-Paraburkholderia* exhibits high relative abundance in the root-associated bacterial community of Malling Centenary specifically after steaming of the substrate, suggesting that it may have been recruited from the tray plant. A sudden decline in relative abundance was observed at the 4th cycle, suggesting a possible substantial accumulation and dominance of fungi competing with and ultimately exerting pressure on the growth of the beneficial bacteria.

Conclusion

The analysis of the root-associated microbiome of strawberry hydroponic cultures revealed that plant genotype and the inherited microbiome of tray plants strongly influence bacterial composition, while substrate reuse primarily affects the fungal community. Overall, the impact of reusing substrate was limited and stronger differences in composition were found between Elsanta and Malling Centenary as cultivars. The accumulation of fungal pathogens showed that there is a risk in reusing the substrate. Steaming of the substrate may help limiting the accumulation of pathogens, yet it alters the establishment of the root-associated microbiome.

Abbreviations

DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
16S rRNA	16S ribosomal ribonucleic acid
ITS	Internal transcribed spacer
TSB	Tryptic soy broth
ASV	Amplicon sequence variant
PCoA	Principal coordinates analysis
PerMANOVA	Permutational multivariate analysis of variance

Supplementary Information

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Supplementary Material 1

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Author contributions
XH and JC performed sampling and experiments, JC, BV, SC and DG developed the research plan and DG designed the study. XH and DG wrote the manuscript. All authors reviewed the manuscript.

Data availability
Sequence data that support the findings of this study was deposited in the National Center for Biotechnology Information with the primary Bioproject code PRJNA1179400.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Barrett GE, Alexander PD, Robinson JS, Bragg NC. Achieving environmentally sustainable growing media for soilless plant cultivation systems– A review. Sci Hort. 2016;212:220–34.
2. Kern J, Tammeorg P, Shanskiy M, Sakrabani R, Knicker H, Kammann C, et al. Synergistic use of peat and charred material in growing media–an option to reduce the pressure on peatlands? J Environ Eng Landsc Manage. 2017;25:160–74.
3. Atzori G, Pane C, Zaccardelli M, Cacini S, Massa D. The role of peat-free organic substrates in the sustainable management of soilless cultivations. Agronomy. 2021;11.
4. Azizi Yeganeh M, Shahabi AA, Ebadi A, Abdoosi V. Vermicompost as an alternative substrate to peat moss for strawberry (Fragaria ananassa) in soilless culture. BMC Plant Biol. 2024;24.
5. Diara C, Incrocci L, Pardossi A, Minuto A. Reusing greenhouse growing media. Acta Horticulturae. 2012;927:793–800. https://doi.org/10.17660/ActaHortic.2012.927.98.
6. Vandecasteele B, Similon L, Moelants J, Hofkens M, Visser R, Melis P. End-of-life stage of renewable growing media with biochar versus spent peat or mineral wool. Nutr Cycl Agroecosyst. 2023. https://doi.org/10.1007/s10705-023-10315-8.
7. Lataf A, Carleer R, Yperman J, Schreurs S, D'Haen J, Cuypers A, et al. The screening of various biochars for Cd2 + removal at relevant soil pH. Waste Manag. 2023;168:376–85.
8. Vandecasteele B, Blindeman L, Amery F, Pieters C, Ommeslag S, Van Loo K et al. Grow - Store - Steam - Re-peat: reuse of spent growing media for circular cultivation of Chrysanthemum. J Clean Prod. 2020;276.
9. Giuffrida F, Leonardi C, Marfà O. Substrate Reuse in Tomato Soilless Cultivation. In: Acta Horticulturae. 2008. pp. 1577–82.
10. Yoon HS, Hwang YH, An CG, Hwang HJ, Shon GM. Effects of Reuse of Organic Substrate on Growth and Yield of Strawberry in Soilless Culture. In: Acta Horticulturae. 2007. pp. 521–6.
11. Woznicki T, Kusnierek K, Vandecasteele B, Sønsteby A. Reuse of coir, peat, and wood fiber in strawberry production. Front Plant Sci. 2023;14.
12. Zucchi P, Longa CMO, Bertoldi D, Martinatti P, Pantezzi T. Effects of organic substrate reuse on growth and yield of everbearing Capri Strawberry. Acta Horticulturae. International Society for Horticultural Science; 2017;579–86.
13. Vandecasteele B, Claerbout J, Denaeghel H, Craeye S. The repeatability of reusing peat as horticultural substrate and the role of fertigation for optimal reuse. Waste Manag. 2024;190:296–305.
14. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13.
15. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41.
16. Abarenkov K, Nilsson RH, Larsson KH, Alexander IJ, Eberhardt U, Erland S et al. The UNITE database for molecular identification of fungi - recent updates and future perspectives. New Phytol. 2010;186.
17. Tang D, Chen M, Huang X, Zhang G, Zeng L, Zhang G et al. SRplot: A free online platform for data visualization and graphing. PLoS One. 2023;18 11 October.
18. Knights D, Kuczynski J, Charlson ES, Zaneveld J, Mozer MC, Collman RG, et al. Bayesian community-wide culture-independent microbial source tracking. Nat Methods. 2011;8:761–5.
19. Olimi E, Kusstatscher P, Wicaksono WA, Abdelfattah A, Cernava T, Berg G. Insights into the microbiome assembly during different growth stages and storage of strawberry plants. Environ Microbiome. 2022;17:21.
20. Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. Plant–microbiome interactions: from community assembly to plant health. Nat Rev Microbiol. 2020;18.
21. Berendsen RL, Pieterse CMJ, Bakker PAHM. The rhizosphere microbiome and plant health. Trends Plant Sci. 2012;17.
22. Snyder AB, Martin N, Wiedmann M. Microbial food spoilage: impact, causative agents and control strategies. Nat Rev Microbiol. 2024;22:528–42.
23. Shao QY, Dong CB, Han YF, Liang ZQ. Research progress in the rhizosphere microbiome of plants. J Plant Nutr Fertilizers. 2021;27.
24. Bai B, Liu W, Qiu X, Zhang J, Zhang J, Bai Y. The root microbiome: community assembly and its contributions to plant fitness. J Integr Plant Biol. 2022;64(2):230–243. https://doi.org/10.1111/jipb.13226.
25. Zhou F, Wu X, Gao Y, Fan S, Zhou H, Zhang X. Diversity shifts in the Root Microbiome of Cucumber under different plant cultivation substrates. Front Microbiol. 2022;13.
26. Bakker MG, Chaparro JM, Manter DK, Vivanco JM. Impacts of bulk soil microbial community structure on rhizosphere microbiomes of Zea mays. Plant Soil. 2015;392.
27. Morella NM, Weng FCH, Joubert PM, Jessica C, Lindow S, Koskella B. Successive passaging of a plant-associated microbiome reveals robust habitat and host genotype-dependent selection. Proc Natl Acad Sci U S A. 2020;117(2):1148–59. https://doi.org/10.1073/pnas.1908600116.
28. Wei Z, Gu Y, Friman VP, Kowalchuk GA, Xu Y, Shen Q et al. Initial soil microbiome composition and functioning predetermine future plant health. Sci Adv. 2019;5.
29. Kong HG, Song GC, Ryu CM. Inheritance of seed and rhizosphere microbial communities through plant–soil feedback and soil memory. Environ Microbiol Rep. 2019;11.
30. Mawarda PC, Le Roux X, van Dirk J, Salles JF. Deliberate introduction of invisible invaders: a critical appraisal of the impact of microbial inoculants on soil microbial communities. Soil Biol Biochem. 2020;148.
31. Chen L, Liu Y. The function of Root exudates in the Root colonization by Beneficial Soil Rhizobacteria. Biology. 2024;13.
32. Zhalnina K, Louie KB, Hao Z, Mansoori N, Da Rocha UN, Shi S et al. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nat Microbiol. 2018;3.
33. Venturi V, Keel C. Signaling in the Rhizosphere. Trends Plant Sci. 2016;21.
34. Marasco A, Buonincontri P, van Niekerk M, Orlowski M, Okumus F. Exploring the role of next-generation virtual technologies in destination marketing. J Destination Mark Manage. 2018;9.
35. Schlemper TR, Leite MFA, Lucheta AR, Shimels M, Bouwmeester HJ, van Veen JA, et al. Rhizobacterial community structure differences among sorghum cultivars in different growth stages and soils. FEMS Microbiol Ecol. 2017;93(8). https://doi.org/10.1093/femsec/fix096.
36. Zhong Z, Han X, Xu Y, Zhang W, Fu S, Liu W et al. Effects of land use change on organic carbon dynamics associated with soil aggregate fractions on the Loess Plateau, China. Land Degrad Dev. 2019;30.
37. Cordovez V, Rotoni C, Dini-Andreote F, Oyserman BO, Carrión VJ, Raaijmakers JM. Successive plant growth amplifies genotype-specific assembly of the tomato rhizosphere microbiome. Sci Total Environ. 2021;772:144825. https://doi.org/10.1016/j.scitotenv.2020.144825.
38. Rotoni C, Leite MFA, Pijl A, Kuramae EE. Rhizosphere microbiome response to host genetic variability: a trade-off between bacterial and fungal community assembly. FEMS Microbiol Ecol. 2022;98.
39. Escobar Rodríguez C, Novak J, Buchholz F, Uetz P, Bragagna L, Gumze M et al. The bacterial microbiome of the Tomato Fruit is highly dependent on the Cultivation Approach and correlates with Flavor Chemistry. Front Plant Sci. 2021;12.

40. Shankar N, Shetty P, Melo TC, Kesseli R. Multi-generation Ecosystem Selection of Rhizosphere Microbial Communities Associated with Plant genotype and Biomass in Arabidopsis thaliana. Microorganisms. 2023;11.
41. Salgado-Salazar C, Rossman AY, Chaverri P. The genus Thelonectria (Nectriaceae, Hypocreales, Ascomycota) and closely related species with cylindrocarpon-like asexual states. Fungal Divers. 2016;80:411–55.
42. Ma M, Jiang X, Wang Q, Ongena M, Wei D, Ding J et al. Responses of fungal community composition to long-term chemical and organic fertilization strategies in Chinese mollisols. Microbiologyopen. 2018;7.
43. Fang X, You MP, Barbetti MJ. Reduced severity and impact of Fusarium wilt on strawberry by manipulation of soil pH, soil organic amendments and crop rotation. Eur J Plant Pathol. 2012;134(3):619–629. https://doi.org/10.1007/s10658-012-0042-1
44. Bhat RG, Subbarao KV. Host range specificity in Verticillium Dahliae. Phytopathology. 1999;89.
45. Schoch CL, Crous PW. First report of Cylindrocladium root and petiole rot of Spathiphyllum in South Africa. South Afr J Bot. 1999;65:208–11.
46. Al-Quwaie DA. The role of Streptomyces species in controlling plant diseases: a comprehensive review. Australas Plant Pathol. 2023;53:1–14. https://doi.org/10.1007/s13313-022-00959-z.
47. Inderbitzin P, Ward J, Barbella A, Solares N, Izyumin D, Burman P, et al. Soil microbiomes associated with verticillium wilt-suppressive broccoli and chitin amendments are enriched with potential biocontrol agents. Phytopathology. 2018;108:31–43.
48. Rahman M, Rahman M, Sabir AA, Mukta JA, Khan MMA, Mohi-Ud-Din M et al. Plant probiotic bacteria bacillus and Paraburkholderia improve growth, yield and content of antioxidants in strawberry fruit. Sci Rep. 2018;8.

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