Journal of Cleaner Production 327 (2021) 129375

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

Circular alternatives to peat in growing media: A microbiome perspective

Tanvi Taparia [a,b,c,1,*], Ed Hendrix [a], Els Nijhuis [a], Wietse de Boer [b,c], Jan van der Wolf [a,**]

[a] Biointeractions and Plant Health, Wageningen University and Research, Droevendalsesteeg 1, Wageningen, 6708PB, Netherlands
[b] Department of Soil Biology, Wageningen University and Research, Droevendalsesteeg 3, Wageningen, 6708PB, Netherlands
[c] Department of Microbial Ecology, Netherlands Institute of Ecology, Droevendalsesteeg 10, Wageningen, 6708PB, Netherlands

ARTICLE INFO

Handling editor: M.T. Moreira

Keywords:
Circular agriculture
Peat-alternative
Physico-chemical properties
Microbial composition
Mushroom cultivation
Casing soil

ABSTRACT

Peat use in horticulture is associated with a large ecological footprint. Peat is the predominant growing media in Europe. Modern cropping systems rely heavily on dynamic interactions of the crop with the microorganisms in the growing media and yet, in the search for sustainable peat-alternatives, the microbiome of the growing media has often been ignored. In mushroom cultivation, peat is a prime determinant of productivity, in the form of a casing soil which supplies beneficial microbes. In this study we describe the microbial composition, interactions, and activity of four circular substrates used to proportionally replace peat in mushroom growing media. We also evaluate various physico-chemical characteristics of the peat-alternatives. We characterize the impact of sanitary pre-treatments such as steaming and acidification on the microbiome as well as the agronomical performance of the peat-reduced growing media. We found that grass fibres from agricultural residue streams, peat-moss farmed in degraded peatlands, and spent casing soil recycled from previous cultivation cycles can be used to successfully replace peat in mushroom growing media. Peat moss and spent casing were expectedly similar to peat in physical, chemical, and microbiological properties. However, the grass fibres had unique characteristics, such as high organic matter content, low water holding capacity and a diverse and competitive microbiome. Pre-treatment of the substrates by acidification and steaming significantly affected the microbiome, and reduced the presence of pests, pathogens and competitive fungi in the peat-reduced media. Strong trade-offs existed between the productivity and disease pressure in the circular cropping system, which are also governed by the microbial composition of the growing media. Knowledge on the accessibility, sustainability, and economic viability of these peat-alternatives will further determine the transition away from peat use and towards sustainable growing media.

1. Introduction

Peat has been the primary component of growing media due to its low cost, high availability and unique physico-chemical characteristics (Caron and Rochefort, 2011). Of the total growing media required for horticulture within the European Union, 86% is composed of peat, amounting to 29.3 million m^3 of peat use annually (Altmann, 2008). Wet peatlands are fragile ecosystems with important ecosystem functions such as biodiversity conservation, water purification and climate regulation. They sequester 30% of the global soil carbon despite constituting only 3% of the global terrestrial area (Joosten et al., 2016). Peat excavation is thus associated with a large ecological footprint, and strongly discouraged by EU directives (Owen, 2007). Severe peat supply bottlenecks are expected in the near future due to rapidly declining global deposits and consequent increase in peatland conservation policies (Bos et al., 2011). Increased societal and governmental pressure has fuelled an extensive search for abundant and sustainable alternatives to replace peat in growing media (Alexander et al., 2008).

Black peat, in the form of a casing soil, has been the predominant growing media in mushroom cropping systems since the 1950s due to its suitable physico-chemical properties, consistency, stability, availability, low costs and easy storage (Schmilewski, 2008). This peat-based covering layer is placed on top of mycelium-containing compost, and induces fructification. The casing soil is a prime determinant of productivity in mushroom cultivation because beneficial microorganisms in the peat induce the transformation of vegetative mycelium into fruiting

* Corresponding author. Biointeractions and Plant Health, Wageningen University and Research, Droevendalsesteeg 1, Wageningen, 6708PB, Netherlands.
** Corresponding author.
E-mail addresses: tanvi.taparia@bio.ku.dk (T. Taparia), jan.vanderwolf@wur.nl (J. van der Wolf).
[1] Present address: Section of Microbiology, University of Copenhagen, Universitetsparken 15, Copenhagen 2100, Denmark.

https://doi.org/10.1016/j.jclepro.2021.129375
Received 14 January 2021; Received in revised form 13 October 2021; Accepted 14 October 2021
Available online 19 October 2021

bodies (Baars et al., 2020). However, peat use in casing soils contributes to 9.71 × 10^{-2} kg of CO_2 per kg of produce, which is the highest greenhouse gas (GHG) emissions associated with an individual input or process in mushroom cultivation, according to several life cycle assessments (Robinson et al., 2019). Peat use in the casing soil is shaped by high environmental impacts, as excavation of black peat and its long-distance transport are associated with global warming and biodiversity loss, while the preparation of the casing soil is associated with eutrophication and water eco-toxicity (Robinson et al., 2019; Leiva et al., 2015; Gunady et al., 2012).

In the search for sustainable alternatives, by-products from industrial, forestry or agricultural waste streams have received significant attention as peat-replacement media. Alternatives like coco-peat (Latinoamericana, 2006), fly-ash (Pardo-Giménez et al., 2017), tea waste (Peyvast et al., 2007), paper pulp (Sassine et al., 2005), pine bark (Pardo et al., 2004), green waste compost (Noble and Dobrovin-Pennington, 2005), spent mushroom substrate (Pardo-Giménez et al., 2011) and recycled rock-wool (Noble and Dobrovin-Pennington, 2005) have been tested in commercial scale trials for mushroom cultivation. However, none of these alternatives have resulted in an industrial application due to poor agronomical performance. This is either due to the introduction of pests and pathogens (competitive weed fungi in paper pulp and recycled coco-coir), inappropriate physical characteristics (insufficient water holding capacity of pine bark, lignite and lump chalk), unsuitable chemical composition (soluble salt content in spent mushroom substrate and digestates) or accumulation of toxic residues (pesticide traces in green waste compost and recycled rockwool) (Noble and Dobrovin-Pennington, 2015; Wang et al., 2008; Pardo et al., 2004). But it is also due to political and economic reasons such as unsustainable sourcing (limited supply of coco-coir, tea wastes, coffee wastes and sugarcane bagasse), legislative constraints (EU policy on field disposal of recycled rockwool or spent mushroom compost) or lack of economic viability (prohibitive costs of coir and vermiculite or chemical processing costs of compost) (Noble and Dobrovin-Pennington, 2015; Pardo--Giménez et al., 2011; Sassine et al., 2005; Gülser and Pekşen, 2003).

The microbiomes of alternative growing media have received limited attention until now (Taparia et al., 2021; Carrasco et al., 2020; Van Gerrewey et al., 2020; Vandecasteele et al., 2020). Beneficial and harmful microbes co-inhabit growing media, and they increase in diversity and abundance once the growing media is planted (Postma et al., 2008). Most microbes may be benign, such as saprophytic fungi and bacteria, others may offer positive benefits, such as plant growth promotion, and some may be detrimental to crop health, because they compete with the host for nutrients or cause diseases (Carlile and Schmilewski, 2010). Disease management in horticulture has traditionally aimed at keeping the growing media as 'clean' as possible, by using pathogen-free propagation material and substrates and by using disinfestation and other sanitation techniques (Postma et al., 2008). However, circular alternatives to conventional growing media that are derived from agri-residue streams often contain a diverse and competitive microbiome (Carlile and Coules, 2011). This could support disease suppression and plant growth, but its use may also bear a risk for dissemination of potential human pathogens, antibiotic resistance genes and plant pathogens (De Corato, 2020).

In this research, we explore the possibility of four local, circular and sustainable alternatives to partially replace black peat in mushroom growing media. We attempt to incorporate three principles of circular economy in mushroom cropping systems by utilizing agricultural residues in the casing soil (design out waste), reusing spent casing soil from previous cultivation cycles (keep materials in use), and substituting black peat in the casing soil with peat moss grown in degraded peatlands (regenerate natural systems). We assess the microbiological, physical and chemical characteristics of peat-reduced growing media in connection with the agronomical performance of the circular cropping system. We specifically focus on the role of the diversity, composition and interactions in the microbiome of circular growing media and techniques for its management. We also comment on the accessibility, sustainability and economic viability of these peat-alternatives.

This research aims to elucidate the role of the microbiome in assuring the quality of peat-reduced growing media, together with other physico-chemical properties. It enhances our understanding of the trade-offs between productivity and disease pressure in circular cropping systems that are governed by microbial interactions in the growing media. We believe that this knowledge on alternative growing media is essential to transition away from peat use in horticulture. Finally, our research outcomes also enable a better understanding of the advantages and disadvantages of moving from traditional to more circular farming practices.

2. Methods

2.1. Description of peat alternatives

Conventionally, peat-basing casing soils in Western Europe largely comprise black peat (90%), which is often mixed with additives such as Baltic peat (5%) and garden soil (5%) by volume, to arrive at a suitable consistency and structure. Additionally, 3–7% of sugar beet lime (or ground limestone) is added to the casing soil to maintain a pH between 7.0 and 7.5. In our experiments, ready-to-use casing soil was commercially obtained from KBVB (Netherlands), and used as a negative control. It was proportionally replaced with four types of peat-alternative materials at different ratios to assess their suitability as casing soils, namely peat moss, grass fibres, acifified grass fibres and spent casing.

In this study, peat moss, refers to non-decomposed moss from *Sphagnum* sp. These are grown organically on degraded and drained peatlands that were formerly mined. It has the advantage of being cyclically and renewably harvested every 3–4 years, in comparison to black peat which is conventionally harvested from natural peatlands (Pouliot et al., 2015). As opposed to black peat, which is dug out, peat moss is harvested superficially with minimal damage to below-ground landscape. The moss layer transfer technique allows for *Sphagnum* fibres to be used for ecological restoration of cutover bogs (Graf et al., 2012), thus enabling *Sphagnum* farming on degraded peatlands. It consequently reduces negative environmental effects such as peat oxidation, soil subsidence and CO_2 emissions (Joosten et al., 2012). Peat moss was used to proportionally replace 25% of peat in the casing soil.

Grass fibres used in this study, are produced by a patented circular biorefining process that converts non-woody biomass into lignocellulosic fibres (Vos and Rustenburg, 2015). It is produced from a mild extraction of various agricultural and horticultural residue streams, the energy demands for which are met by producing biogas from the leftover grass-juice, resulting in zero net emission of CO_2. All the water used in this process is cleaned and recycled within the biorefinery (Newfoss, Netherlands). As an additional step, to assess the effect of a reduced microbial community, some of the grass fibres were acidified via an anaerobic fermentation process that gradually reduces the pH to 4.5 over a year. This acidification is chemical-free and performed with the microbiota in the processing fluids from organic feedstocks. Acidified grass fibres were studied as an independent peat alternative. Both grass fibres and acidified grass fibres were used to proportionally replace 50% of peat in the casing soil.

Spent casing soil is mechanically separated from the compost after a cook-out (steaming) of the growing chambers, at the end of the cropping cycle. Thus, spent casing undergoes pasteurization as part of the previous cultivation cycle, with no extra energy or cost associated to it. To be able to re-use spent casing, the use of disinfectants and salts on the mushroom beds need to be avoided during cultivation (Noble and Dobrovin-Pennington, 2015). Separation of the casing soil simultaneously reduces the amount of leftover spent mushroom compost (SMC) for waste disposal, which is associated with high costs. The SMC devoid of casing soils, largely contains compost, with very high N and P content, and increased fertilizer value (Noble and Dobrovin-Pennington, 2015).

Up to 33% of this spent compost can be re-used in phase I compost for mushroom cultivation with no negative effects on yield (Noble and Dobrovin-Pennington, 2015). Spent casing is a local product that was used to proportionally replace 30% of peat in fresh casing soil.

2.2. Setup of cultivation experiments

To evaluate the performance of peat alternatives in casing soils, cultivation experiments were performed at an experimental mushroom growing facility (Unifarm, Wageningen University and Research). Peat was proportionally replaced in the casing soil by 25%, 50%, 50%, and 30% of its volume, respectively with (A) *Sphagnum* moss from degraded peat lands, (B) grass fibres from agricultural residue streams, (C) acidified grass fibres and (D) spent casing soil from previous cultivation cycles. The proportions of peat replacement were recommended by the mushroom growers, based on previous experiences with these materials. Alternative casing soils were prepared from both unsteamed and steamed (at 70 °C for 8 h) raw materials. Finally, before being introduced in the growing room, all the casing soils were mixed with a small quantity of fully colonized phase III compost (100g of compost per m^2 of growing surface), by a process called "CAC-ing", to promote early growth of the crop (MacCanna and Flanagan, 1972). The generic setup for mushroom cultivation and details about the growing conditions are described in Taparia et al. (2020b).

Ginger blotch (*Pseudomonas gingeri*) is a bacterial disease that originates from the casing soil, and is responsible for recent disease outbreaks in European mushroom farms (Taparia et al., 2020a). In order to evaluate the disease pressure in the alternative casings. Five days after filling the growing room, ginger blotch pathogen (strain IPO3777), was added to the mushroom beds at densities of 10^3, 10^4 and 10^5 cfu/g of casing soil. The protocol for pathogen inoculation in the soil is described in Taparia et al. (2020b). Negative controls were mock-inoculated with tap water. Each treatment consisted of three replicates, arranged in a randomized block design. From day 15 onwards mushroom were harvested over three cultivation cycles (flushes) spanning one week each. The entire cultivation experiment was repeated twice with freshly procured raw materials. The overall experimental design and casing soil compositions are described in Tables 1 and 2 respectively.

The agronomical performance of the peat alternatives was described quantitatively by the productivity (total yield) and disease pressure (blotch prevalence), and qualitatively by the post-harvest condition of the mushrooms. The weight of healthy and diseased mushrooms harvested from each treatment were registered daily across the three cultivation cycles. Diseased mushrooms were identified visually by screening for symptoms of ginger blotch. Productivity of the alternative casing soils was measured as the total harvest weight summed across three flushes, per growing surface (kg/m^2). Disease pressure in the alternative casing soils was measured as the proportion of diseased harvest to total harvest (%). Post-harvest quality was observed by visually inspecting the sliced mushroom caps immediately after harvest. The entire cultivation experiment was repeated twice, with freshly procured alternative materials, peat and compost. The productivity and disease pressure was averaged across both repetitions of the experiment.

Table 1
Experimental design of the cultivation experiments, with a summary of the different factors, treatments and measurements.

Table 1. Experimental design

Factors	Description and treatments
Peat-alternative	Type of peat-alternative and its proportion in the casing soil: *i) Peat-moss (25%), ii) Grass fibres (50%), iii) Acidified grass fibres (50%), iv) Spent casing (30%). Control is commercial peat-based casing soil*
Heat Treatment	Heat treatment of raw materials at 70 °C for 8 h before mixing with peat*: i) Steamed and ii) Unsteamed*
Pathogen density	Inoculation density of pathogen: *0, 10^3, 10^4, 10^5 cfu/g of casing soil*
Flush	Progressive harvest cycle: *1*st *flush, 2*nd *flush, 3*rd *flush*
Replicate	Randomized block design within the experiment: *block 1,2,3*
Experiment	Independent replicate experiments, with fresh materials: *exp 1,2*
Quantitative measurements	
Yield	Total harvest weight per m^2 of growing surface
Blotch prevalence	Proportion of diseased harvest to total harvest (by weight)

Table 2
Nine casing soil compositions that are described by three individual factors, type of alternative material used, heat treatment of alternative material and proportion of introduction (by volume) in casing soil to make peat-reduced growing media.

Table 2. Casing soil composition

Casing soil tag	Raw material	% in casing soil	Treatment of raw material
1	Black peat (control)	90%	Un-steamed
2	Grass fibres	50%	Un-steamed
3	Grass fibres	50%	Steamed
4	Acidified grass	50%	Un-steamed
5	Acidified grass	50%	Steamed
6	Black peat (control)	90%	Steamed
8	Peat moss	25%	Un-steamed
9	Peat moss	25%	Steamed
10	Spent casing	30%	Steamed

2.3. Physical and chemical properties of alternatives

Five litres of peat and alternatives were sampled for the assessment of physical and chemical properties, at three time points, from the raw materials itself, from freshly prepared casing soil at the beginning of the cultivation experiments, and at the end of the third cultivation cycle. Before being mixed with peat in the casing soil, the organic matter content (thermogravimetric) (EN13039), dried weight, moisture content (gravimetric) (EN13040) and respiration (EN16087/1) were measured from the raw materials itself, according to European Standards developed by CEN, the European Commission for Standardisation. From the freshly prepared casing soils, physical characteristics such as, moisture content, soil moisture retention capacity and shrinkage were measured according to EN 13041 protocols. Relative increase in moisture content between saturation and drainage was measured according to EN 13041 protocols. Water contents (gravimetric), an archaic characteristic used to describe peaty soils, was also measured (Boelter, 1968). Dry bulk density and wet bulk density were measured according to EN 13040 protocols. Chemical characteristics such as pH (potentiometric) (EN 13037) and electrical conductivity (conductimetric) (EN 13038) were also measured according to European Standards. The pH, electrical conductivity and moisture content were measured again at the end of the third flush, to study temporal variation across the cultivation cycles. Structural properties such as resistance (hardness) and stickiness (adhesiveness) were measured by a CT3 Texture analyser (Ametek Brookfield, Middleboro, MA, USA). All measurements were made in replicates of three. The physico-chemical analyses were jointly performed by BVB Substrates (Maasland, Netherlands) and Groen Agro Control (Delfgauw, Netherlands).

2.4. Sequencing the casing soil microbiome

For microbiome analysis, 1g of casing soil from the growing room was sampled at five timepoints, T0: when freshly prepared casing soil on the mushroom bed was inoculated with pathogen or control (day 5), T1: during the pinhead formation of the first flush (day 12), T2: during pinhead formation of the second flush (day 19), T3: during pinhead formation of the third flush (day 26) and finally, T4: at end of the third flush, before cook-out (day 35). Three replicate samples were collected

per casing soil treatment, from both cultivation experiments. Homogenized soil was used for DNA extraction using a Soil PowerMag DNA Extraction kit (Qiagen, Germany) according to manufacturer's protocol. The soil DNA was quantified fluorometrically using a Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, USA) on the Infinite M200 PRO (Tecan, Switzerland) and then diluted to a concentration of 2 ng/μl.

The soil microbiome was determined from the casing soil DNA by targeted sequencing of the V3–V4 regions of bacterial 16S rRNA gene and the fungal ITS2 gene (as described in Taparia et al., 2021). The libraries for 16S and ITS2 respectively, were sequenced on Illumina MiSeq using MiSeq v2 or v3 reagents (Illumina, USA) to generate 250 or 300bp paired-end reads that overlap. All raw sequences for soil fungi and bacteria were deposited in NCBI under BioProject numbers PRJNA657168 and PRJNA657276 respectively. Downstream processing of the raw data was performed on QIIME2 version qiime2-2020.2 (Bolyen et al., 2019) using the Dada2 workflow (Callahan et al., 2016), resulting in a set of unique sequences and an abundance table of amplicon sequence variants (ASVs) or taxa (as described in Taparia et al., 2021).

2.5. Statistical data analyses

All statistical analysis from cultivation experiments, physico-chemical assessments and microbiome sequencing was performed on RStudio with R version 3.4.0 (Team, 2013). Cultivation data, such as yield and blotch prevalence were transformed using *tidyverse* (Wickham et al., 2019). Generalized linear regression and zero-inflated beta regression were performed using packages, *glm* and *betareg* (Cribari-Neto and Zeileis, 2009) respectively. Widely applicable information criteria (WAIC) and adjusted R squared values were compared to arrive at a final minimally adequate model (Johnson and Omland, 2004). Model assumptions on normality of data and homogeneity of residuals were verified using diagnostic plots and statistical tests (Fox et al., 2012). Data on physico-chemical properties were assessed with analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA).

Analyses of microbiome sequence data was performed with packages *vegan* (Oksanen et al., 2007) and *phyloseq* (McMurdie and Holmes, 2013). Alpha diversity of the microbiome was calculated by the Inverse Simpson index. Beta diversity was measured using the Bray-Curtis distances. The core microbiome was defined as the taxa present above a detection threshold of 0.1% in 90% of the samples from that treatment, and calculated using package *microbiome* (Lahti et al., 2017). Co-occurrence microbial ecological networks were estimated with

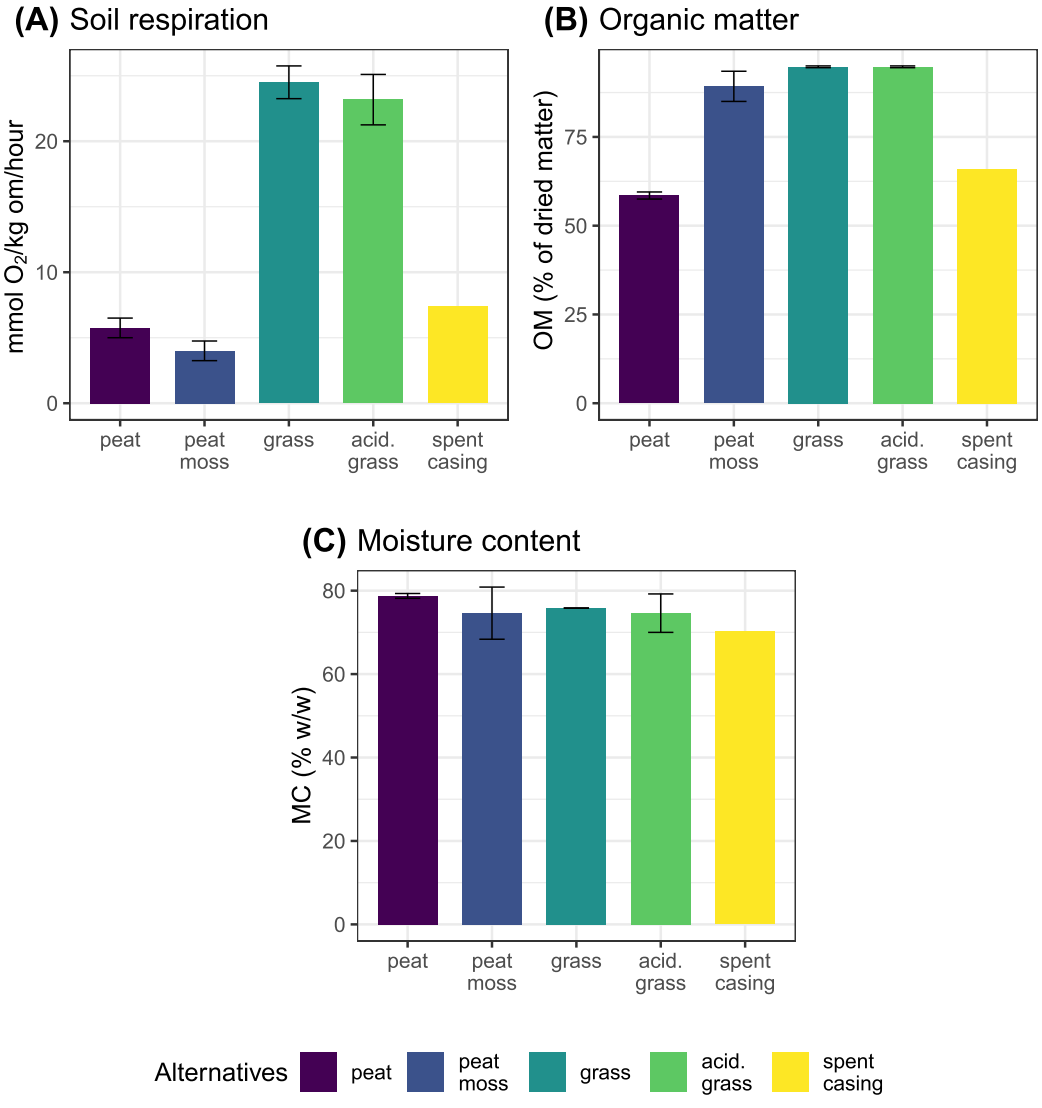

Fig. 1. Physical and chemical properties of alternative raw materials before mixing with peat.

inverse covariance, and performed using packages, *igraph* (Csardi and Nepusz, 2006), *speic-easi* (Kurtz et al., 2015) and *ggnetwork* (Briatte, 2016).

3. Results

3.1. Physical and chemical characteristics

The individual raw materials, before being used to proportionally replace peat in the casing soil, varied significantly in their physico-chemical characteristics such as soil respiration ($P = 0.04$) and organic matter content ($P = 2 \times 10^{-16}$), according to a multivariate ANOVA (Supplementary Table 1). Soil respiration for both grass fibres was higher than the other alternatives. Surprisingly, the respiration of spent casing, which was heat treated, was even higher than that of peat (Fig. 1A). Both grasses and peat moss had a similarly high organic matter content (Fig. 1B). The moisture content of the raw materials was not significantly different from each other (Fig. 1C).

Physical and structural characteristics were evaluated after the raw materials were proportionally mixed with peat to constitute casing soils with varying compositions. The water contents ($P = 0.05$), increase in moisture content ($P = 0.04$), bulk density (wet) ($P = 2 \times 10^{-16}$), bulk density (dry) ($P = 0.05$), resistance ($P = 2 \times 10^{-16}$) and stickiness ($P = 2 \times 10^{-16}$) differed significantly between the alternative casing soils, according to a multivariate ANOVA (Supplementary Table 2). Heat treatment of the alternatives before preparation of the casing soil, had no impact on these parameters ($P < 0.05$). Both grass-based casings had lowest fresh and dry bulk density by volume (Fig. 2A and B). Peat and peat-moss based casing soils had the highest gravimetric water contents (Fig. 2C), whereas both grass-based casing soils had the largest relative increase in moisture content when saturated with water (Fig. 2D). Their physical structure in terms of resistance (hardness) (Fig. 2E) and stickiness (adhesiveness) (Fig. 2F) was also significantly different from other casing soils. Heat treatment of the raw materials did not affect these

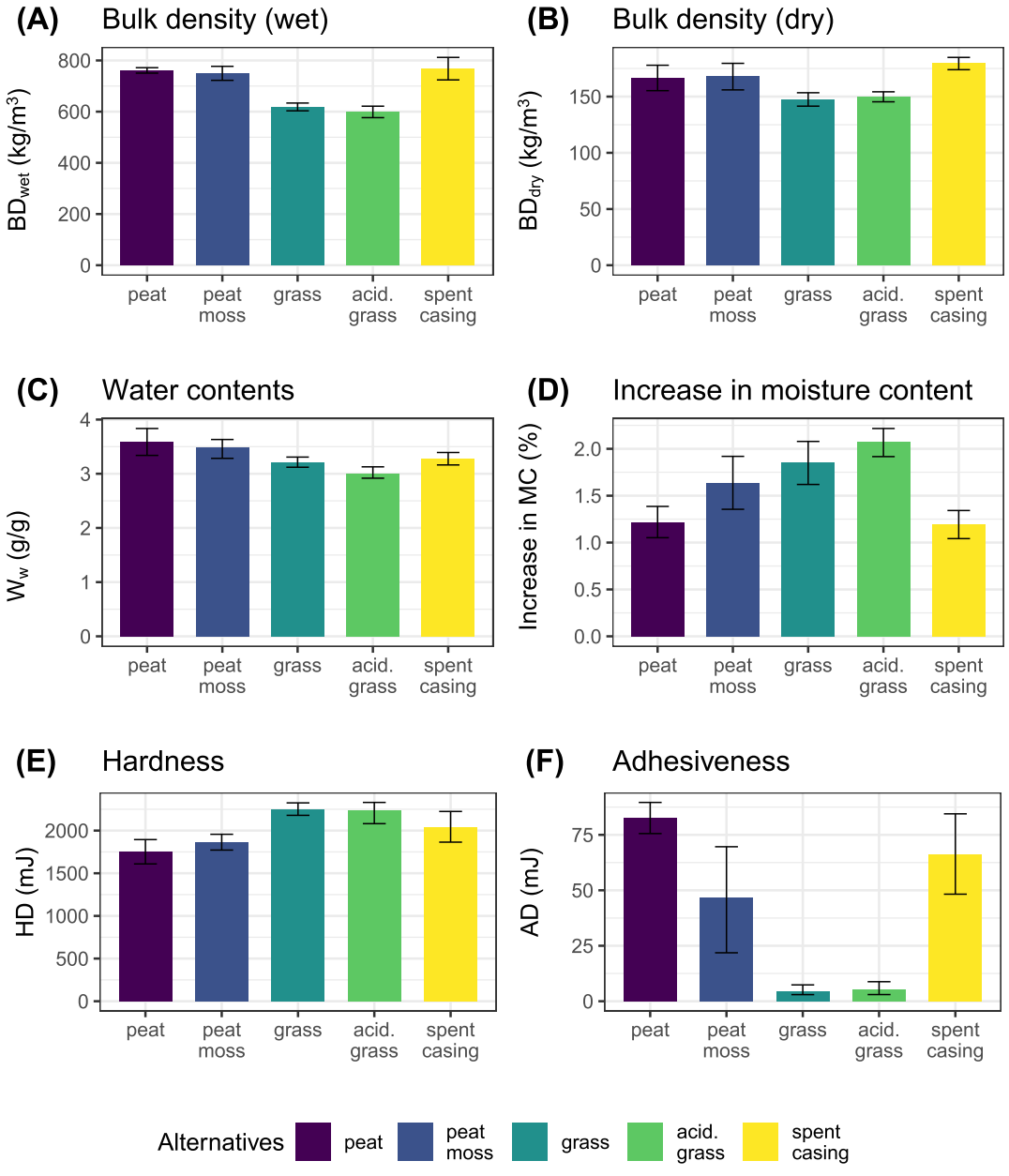

Fig. 2. Soil moisture retention and water relation properties of alternative casing soils.

parameters either (Supplementary Table 2).

The soil moisture retention curves (Fig. 3A) varied significantly between peat alternatives ($P = 2 \times 10^{-16}$). Peat, peat moss and spent casing had similar pF curves. Both the grass-based casings were the fastest in losing soil moisture. Related parameters, such as the moisture content ($P = 0.04$), bulk density ($P = 0.01$) and shrinkage ($P = 2 \times 10^{-16}$) measured during the pF curves also differed significantly among the peat alternatives (Fig. 3B, C and 3D), according to a multivariate ANOVA analysis (Supplementary Table 3). Heat treatment of raw materials before preparation of casing soil, had no impact on soil water retention and related properties. The organic matter content of the casing soils did not differ between the alternatives or due to heat treatment.

The characteristics of the casing soils changed significantly during the cropping cycle (Supplementary Table 4), from being placed onto compost at the start of the experiment (T0) to the end of the third flush (T4). The overall pH differed between peat alternatives ($P = 2 \times 10^{-16}$) although it varied well within the optimal range of 7.0 and 7.5 (Rainey, 1985). The pH reduced slightly from T0 to T4 ($P = 0.01$), more so for the grass-based casing soil (Fig. 4A). The pH of peat and peat moss was similar, as was the pH of acidified grasses and spent casing. Electrical conductivity also differed between peat alternatives ($P = 2 \times 10^{-16}$), and varied in the range of 0.8 and 1.8 between the time points ($P = 2 \times 10^{-16}$). Spent casing had the highest EC compared to all alternatives at T0. However, at the end of the cultivation cycle, at T4, the EC increased for all the casing soils (Fig. 4B). The moisture content of the soils reduced significantly ($P = 2 \times 10^{-16}$) from ~80% at T0 to ~60% at T4 (Fig. 4C). Moisture content also varied due to heat treatment of the alternatives ($P = 2 \times 10^{-16}$). It was particularly low for both of the steamed grasses.

3.2. Microbiological community composition

The bacterial and fungal microbiome of the alternative casing soils were also explored throughout the cultivation cycle. The soil bacterial community comprised of 27,666 amplicon sequence variants (ASVs) or taxa originating from 378 casing soil sample. The soil fungal community was less diverse and comprised of only 2116 taxa. The bacterial alpha diversity of the soils, described by the Inverse Simpson index varied significantly between the peat alternatives ($P = 2 \times 10^{-16}$) according to a univariate ANOVA (Supplementary Table 5). Casing soil composed of only peat was the least diverse, but when it was supplemented with other alternatives, the casing soil had a comparably higher bacterial diversity (Fig. 5A). Grass-based casing soil had the highest alpha diversity. Heat treatment of the raw materials, before being used to proportionally replace peat in the casing soil reduced the alpha diversity ($P = 0.01$) of the casing soil significantly (Fig. 5B), although the magnitude of the effect was different for each alternative. The diversity also

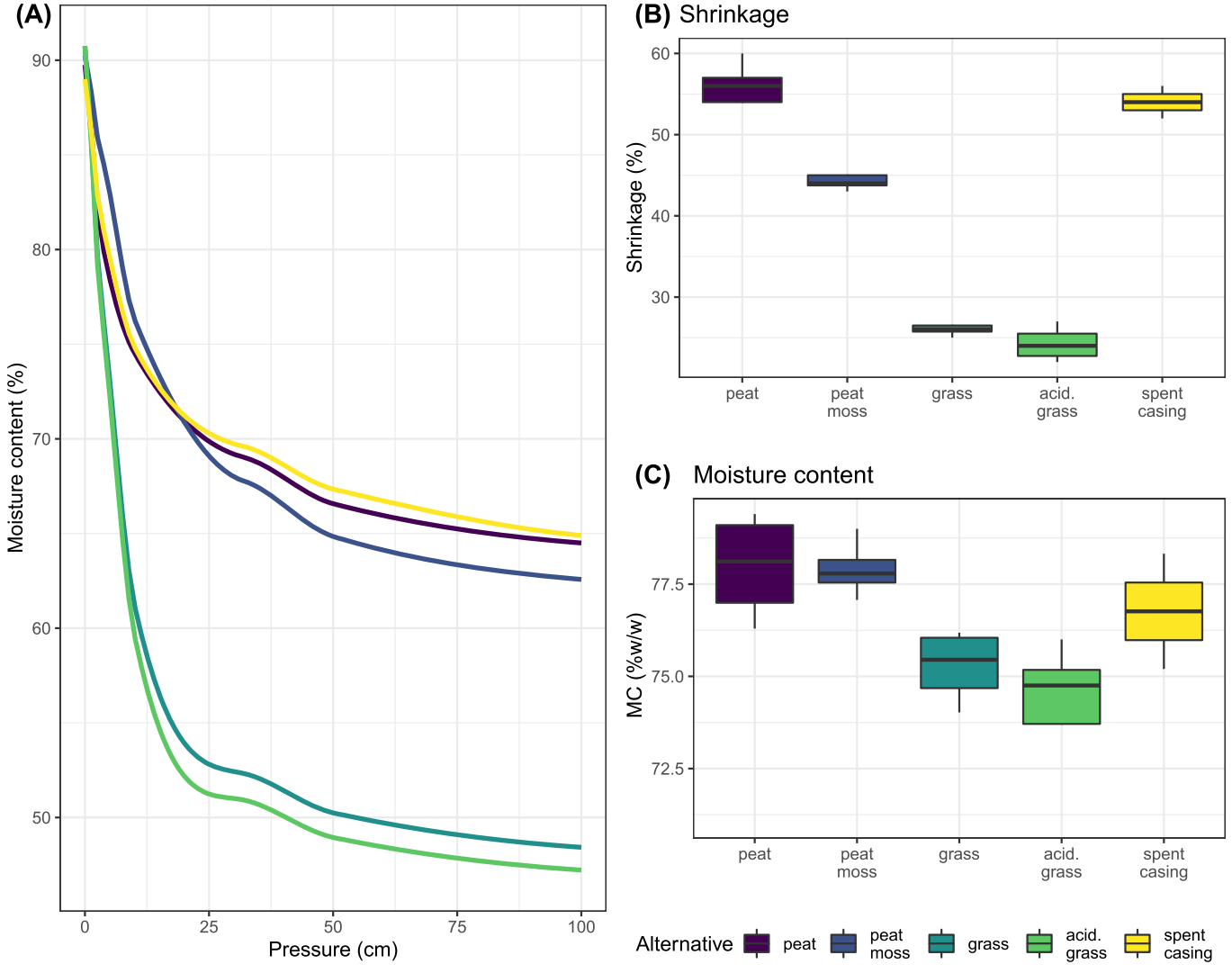

Fig. 3. Physical and chemical properties of alternative casing soils at the beginning of cultivation.

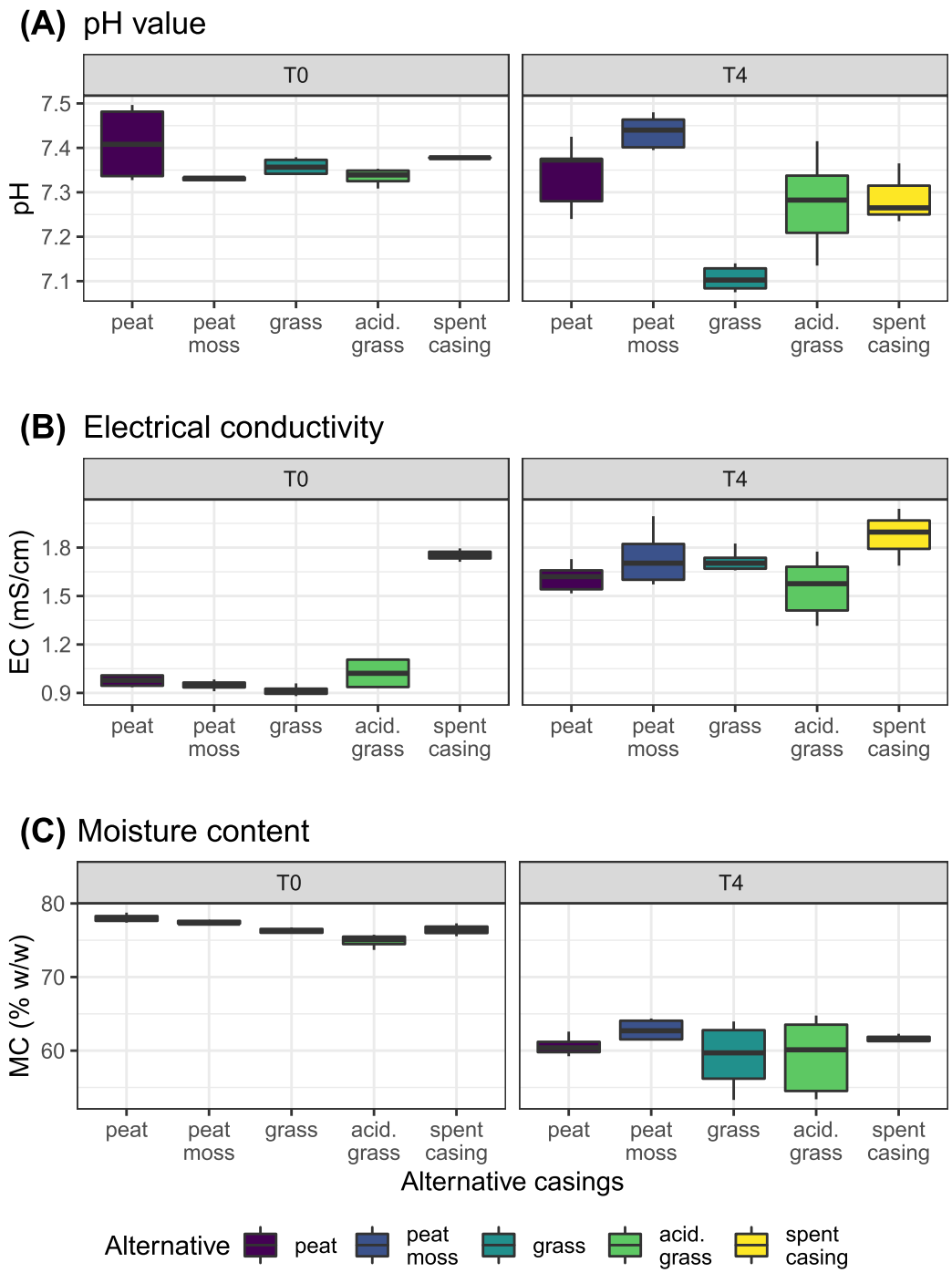

Fig. 4. Moisture content, pH and electrical conductivity at the start of the cultivation experiment (T0) and at the end of the 3rd harvest cycle (T4).

increased consistently across the cultivation cycles ($P = 2 \times 10^{-16}$), for all casing soil compositions (Fig. 5C). The diversity of the alternatives also varied between repetitions of the experiment conducted with freshly procured raw materials ($P = 5.4 \times 10^{-5}$), even though the overall differences between the experiments were only marginal (Fig. 5D).

The bacterial and fungal community composition of the casing soils differed significantly between the peat alternatives ($P = 0.001$), due to heat treatment ($P = 0.001$), across the cultivation cycles ($P = 0.001$) and between the repetitions of the experiments ($P = 0.001$), according to a PERMANOVA (Supplementary Table 6). The core bacterial profile between peat, peat moss and spent casing was very similar on a genus level, but at lower taxonomic levels, peat was the least diverse as it comprised of only 40 bacterial taxa, whereas peat moss and spent casing comprised of 119 and 108 bacterial taxa respectively. Heat treatment of the grass fibres had a relatively smaller effect on the bacterial community compared to acidification of the grass fibres, which reduced the core bacterial microbiome drastically from 119 taxa to 20 taxa. On a genus level, the core microbiomes between the alternatives may look relatively conserved (Fig. 6A), however, most of these changes occur at lower taxonomic ranks, such as the species or strain level.

The core fungal microbiome of peat comprised of only 6 fungal taxa, namely, *Agaricus*, *Apiotrichum, Meliniomyces, Mycothermus, Candida* and *Pseudeurotium* in descending order of abundance (Fig. 6B). Heat treatment of peat reduced the abundance of all other fungi, except *Agaricus*.

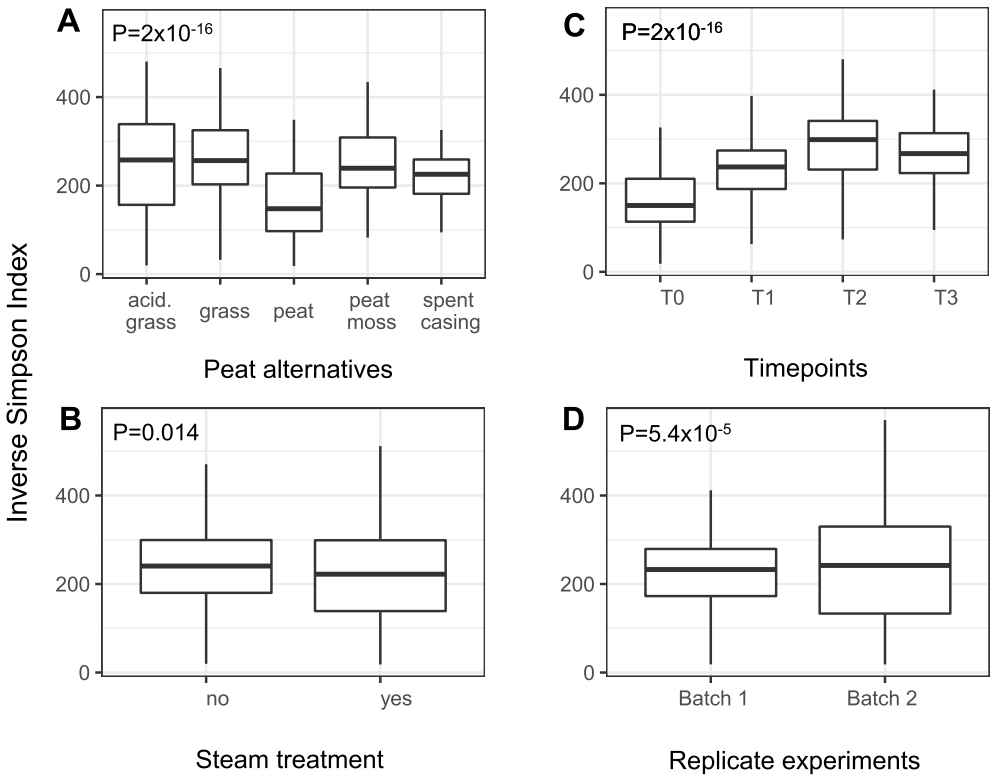

Fig. 5. Differences in bacterial species richness of casing soils between (A) peat alternatives (B) steam treatment (C) timepoints of cultivation cycles and (D) independent repetitions of the experiment.

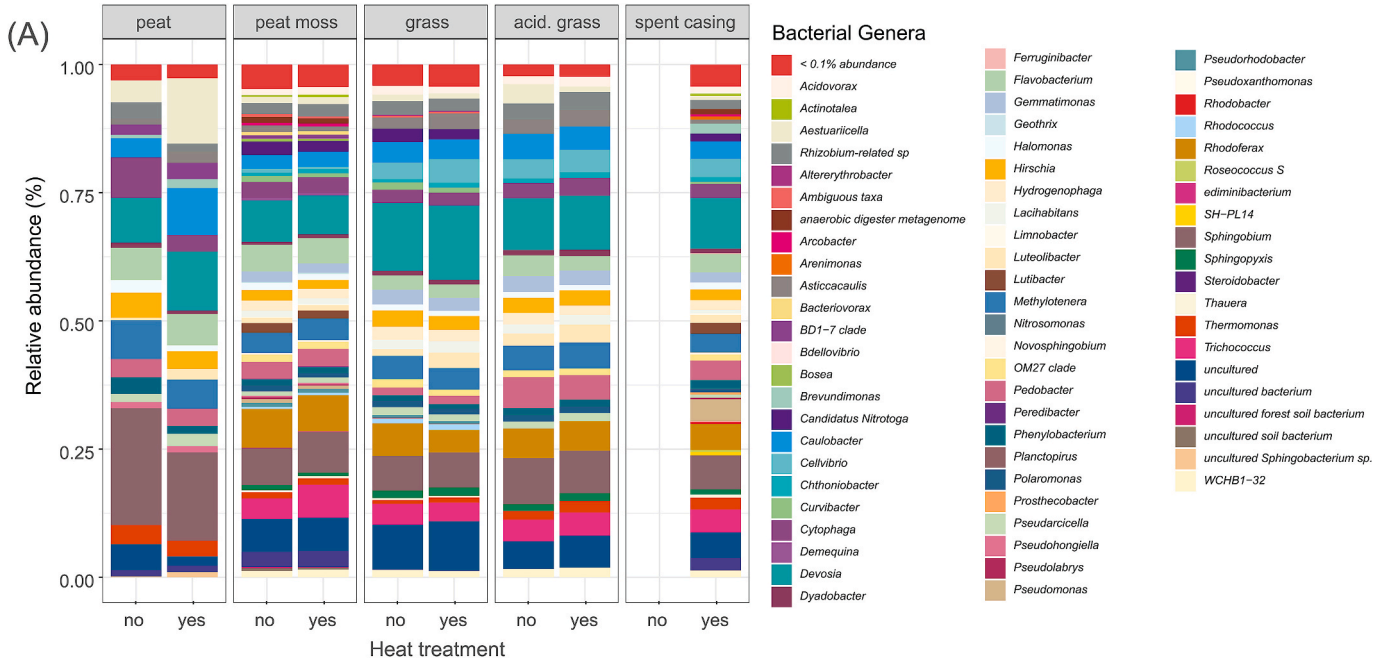

Fig. 6A. Community composition of the core bacterial microbiome of alternative casing soils on a genus level, from combined data across all harvest cycles.

Peat moss additionally comprised of *Pseudallescheria, Pseudeurotium, Saitozyma Solicoccozyma, Trichoderma* and unidentified fungi. Heat treatment of peat moss did not have a significant impact on fungal microbiome of the casing soil. The fungal composition of spent casing, which was steamed, was very similar to that of peat moss. The grass fibres were unique in that, their microbiome was largely dominated by *Pseudeurotium,* most of which was lost from the casing soil after the heat treatment. Acidification of the grass fibres led to reduction of *Pseudeurotium and Dipodascus*, and a relative increase of *Ascobolus* and *Solicoccozyma.*

Interactions within the casing soil microbiome were explored via microbial co-occurrence networks. Significant differences existed in the network topology between the alternative casing soils, indicating that the degree- and type of microbial interactions in the soil community

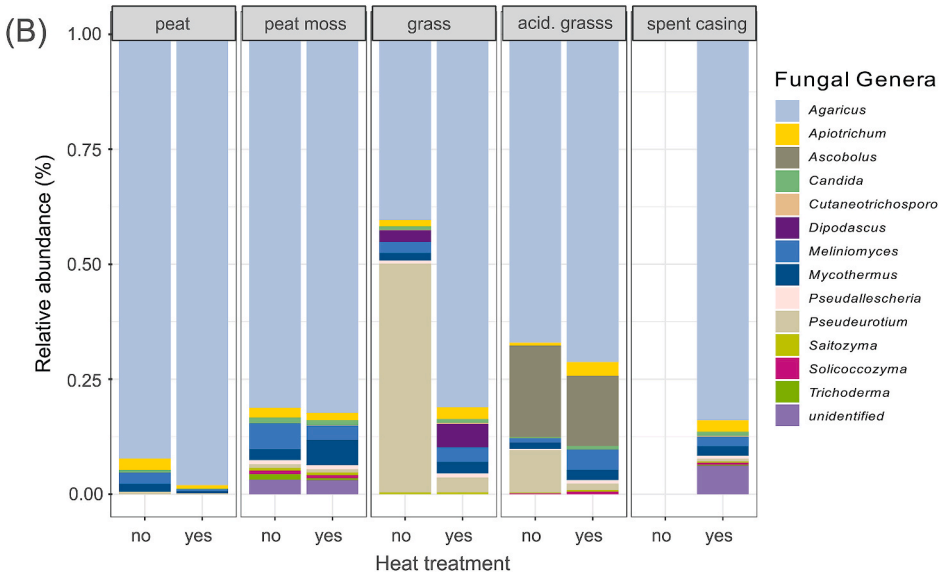

Fig. 6B. Community composition of the core fungal microbiome of alternative casing soils on a genus level, from combined data across all harvest cycles.

were influenced by the use of peat-alternatives (Supplementary Table 7). The bacterial network of peat-based casing soil was compact and dense, as indicated by the low average path length, high graph density and high clustering coefficient. It also had a large number of edges (Fig. 7A). Peat moss-based casings had a similarly network topology. The bacterial network in spent casing was most modular, as indicated by the highest number of vertices, average path length, modularity and node density. Bacterial networks of grass fibres were also modular, but better connected, given their high clustering coefficient. Acidification of grass, simplified the interactions, as evident from the reduced clustering coefficient and graph density of the network. Fungal interactions within the microbiome were significantly reduced compared to that of the bacterial community (Fig. 7B). Peat-based soils lacked a fungal network, whereas peat moss had a minimal but compact fungal network. This was further reduced in heat treated spent casing soil. Grass-based casings had the largest and most complex fungal network, with the highest number of edges, modularity, clustering coefficient, node density and average path length. Acidification of the grass fibres largely reduced the size of the network. High resolution figures with taxonomic labels are available as Supplementary Figs. 1 and 2.

With the limitations of amplicon sequencing, the microbiomes of the alternative casing soils were preliminarily screened for presence of genera that could comprise known human pathogens (*Bacillus cereus, Campylobacter species, Clostridium botulinum, Clostridium perfringens, Cronobacter species, Enterohemorrhagic Escherichia coli, Escherichia coli O157:H7, Listeria monocytogenes, Salmonella species, Shigella species, Staphylococcus aureus* and *Vibrio*). Most of these genera were absent from the alternative casing soils, although a few unidentified species from the genus *Bacillus*, *Clostridium*, *Cronobacter* (ASV223444), *Staphylococcus* (ASV10822), and *Yersinia* (ASV23472 and 23478) were detected. The soil microbiomes were also screened for presence of genera, that could comprise of known plant pathogens (*Pseudomonas fluorescens, Pseudomonas syringae, Agrobacterium tumefaciens, Bacillus caryophylli,*

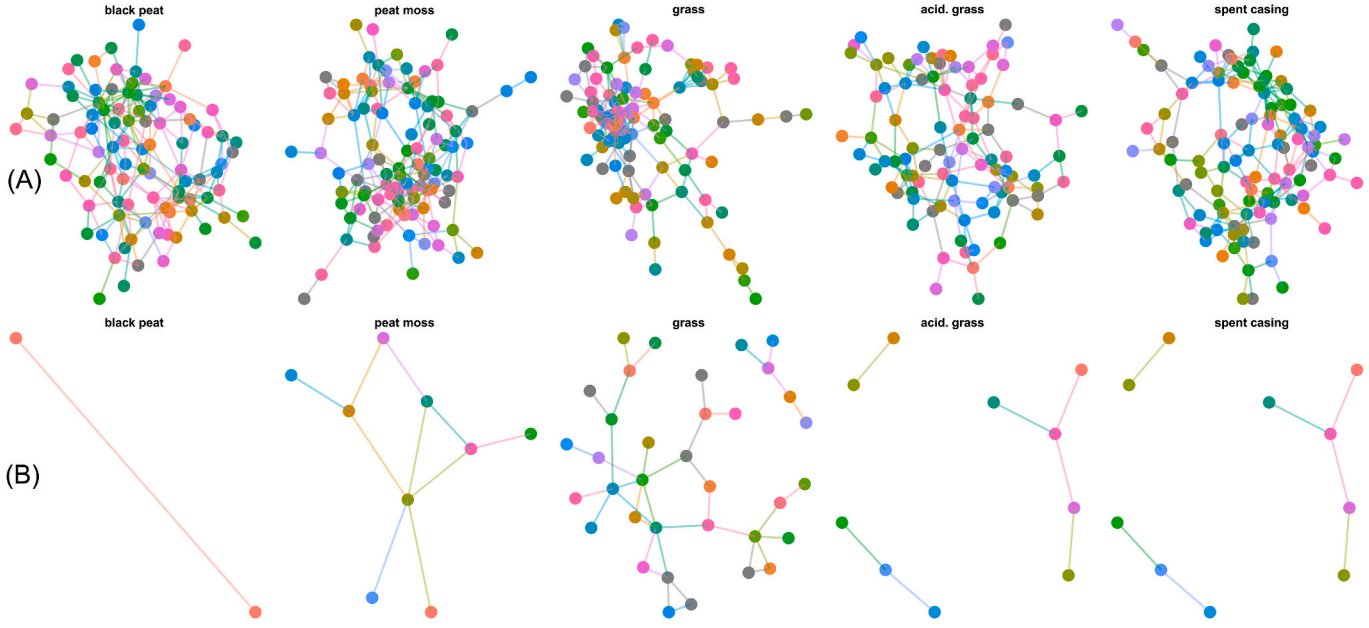

Fig. 7. Co-occurrence networks of the core (A) bacteria and (B) fungi in alternative casing soils, from combined data across both harvest cycles.

Clavibacter michiganensis, Dickeya dadantii, Dickeya dianthicola, Dickeya zeae, Erwinia amylovora, Pantoae ananatis, Pantoae citrea, Pantoae punctata, Pantoae terrea, Pectobacterium atrosepticum, Pectobacterium carotovorum, Pectobacterium wasabiae, Rahnella aquaticus, Ralstonia, Rhodococcus fascians, Serratia plymuthica, Xanthomonas campestris, Xanthomonas euvesicatoria, Xanthomonas fragariae, Xanthomonas gardneri and *Xanthomonas perforans)*. The majority of these species were absent from the casing soil microbiome, however, a few unidentified taxa found in the casing soil microbiome belonged to genera which could potentially include plant pathogens. These include unidentified taxa from the genus *Erwinia* (ASV ASV23428, ASV23426 and ASV23435), *Pantoea* (ASV23422, ASV23423, ASV23424 and ASV23425), *Rahnella* (ASV23450-55), *Ralstonia*, *Rhodococcus, Serratia,* and *Xanthomonas*.

3.3. Agronomical performance of the alternatives

The productivity of the casing soil was measured as total harvest across three consecutive cultivation cycles for each peat alternative, averaged between the two independent repetitions of the experiment conducted with fresh batches of raw materials. The productivity of the casing soils differed significantly between peat alternatives ($P = 2 \times 10^{-16}$), due to heat treatment ($P = 9.2 \times 10^{-5}$) and between repetition experiments ($P = 2 \times 10^{-16}$), according to univariate ANOVA (Supplementary Table 8). Peat-based casing soil produced a mean yield of ~31.1 kg/m^2 of growing surface, and heat treatment significantly increased its yield to 34.5 kg/m^2 (Fig. 8). When peat moss was used to proportionally replace 25% of peat in the casing, the mean yield was 32.6 kg/m^2. Steamed peat moss had a yield of 33.3 kg/m^2, which was not significantly higher. When grass and acidified grass fibres were used to substitute 50% peat in the casing, their yields were similar at 29.3 and 28.5 kg/m^2 respectively. Heat treatment of the grasses did not increase the yield further. When steamed spent casing replaced 30% of peat in the casing soil, yields were maintained similar to peat at 30.9 kg/m^2. Between the repetition experiments, the mean yields reduced by 5.2 kg/m^2. The productivity of all casing soils decreased from the 1st to the 3rd cultivation cycle.

The disease pressure was measured as the bacterial blotch prevalence, across three cultivation cycles, over four pathogen densities and two experiments. In mock-inoculated casing soils, without added pathogen, there were no significant differences in the blotch prevalence between the alternatives ($P = 0.40$), although heat treatment of the peat-alternative significantly ($P = 0.002$) reduced the inherent blotch prevalence of the casing soils. In the pathogen-inoculated casing soils, the mean blotch prevalence varied significantly between peat alternatives ($P = 6.0 \times 10^{-5}$) and their heat treatment ($P = 0.006$), according to univariate ANOVA (Supplementary Table 9). Steamed peat showed the highest susceptibility to blotch with a disease prevalence of 19.94% (Fig. 9). Heat treatment of the raw materials reduced the disease pressure in the alternative casings, with the exception of peat, which had higher blotch prevalence when steamed, although it was statistically insignificant. Casing soil composed of steamed acidified grasses had the lowest susceptibility to blotch, with a disease prevalence of 7.7%, which was significantly less compared to that of peat and peat moss based casing soils. Spent casing and steamed grass fibres shared a similarly lower blotch prevalence of 9.7 and 10.5% respectively.

Beta regression revealed that parameters such as peat alternatives, heat treatment, inoculation density of pathogen and consecutive harvest cycles, significantly affected the disease pressure of the cropping system (Supplementary Fig. 3). The bacterial blotch prevalence increased with inoculated pathogen density in the soil ($P = 2 \times 10^{-16}$), irrespective of the casing soil composition. In the first flush, mock-inoculated soils had 1.5% blotch prevalence, and it increased to 18.5%, 26.9% and 35%, when inoculated with *'P. gingeri'* at 10^3, 10^4 and 10^5 cfu/g of soil respectively. Blotch prevalence declined steeply with consecutive cultivation cycles ($P = 2 \times 10^{-16}$), consistently across all peat alternatives. At the highest inoculation density of 10^6 cfu/g of inoculated pathogen, the mean blotch prevalence decreased steeply from 35% in the first flush to 14.8% and 2.5% in the second and third flush respectively.

There were no differences in the post-harvest quality of the mushrooms grown in peat-based and alternative casing soils, when assessed visually for cap size, broken veil, and other deformities (Supplementary Fig. 4). However, other pests, pathogens and weeds were observed in the circular cropping system (Supplementary Fig. 5). Mushrooms with brown blotch (*Pseudomonas tolaasii*) were found throughout the cultivation cycle, in all casing soil compositions. This was confirmed by detection of the causative agent, *Pseudomonas tolaasii*, on the mushroom caps via diagnostic Taqman™-qPCRs. In the third flush of both repetitions of the experiment, two other competitive fungi from the genus *Peziza* and *Parasola* (formerly *Coprinus*) were also found growing in casing soils composed of unsteamed grasses. Small patches of green mould (*Trichoderma*) were also observed in the third flush, in multiple

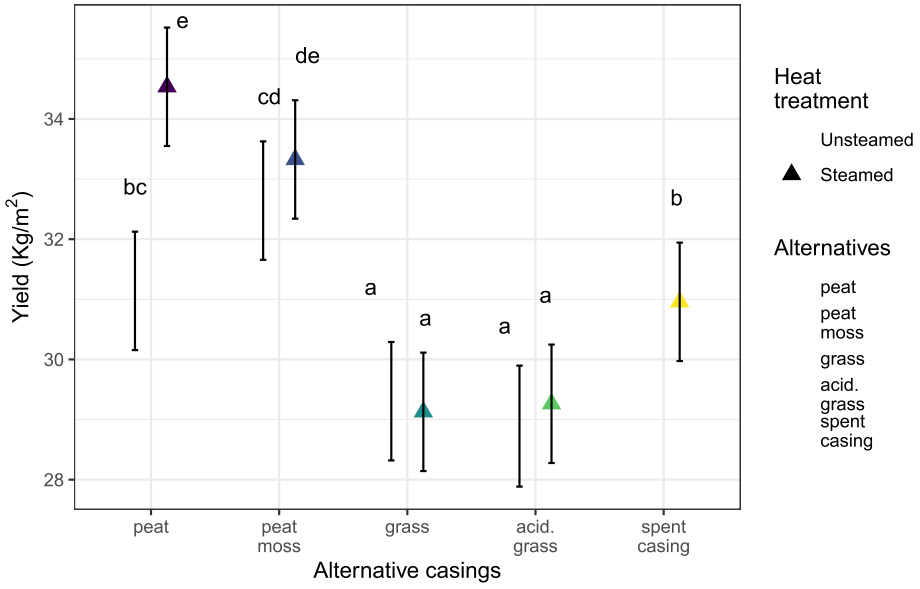

Fig. 8. Linear regression on the productivity (yield) of alternative casing soils and the effect of steaming. Yield is summed across the three cultivation cycles (flushes). Statistically significant comparisons according to Tukey's test are highlighted by letters.

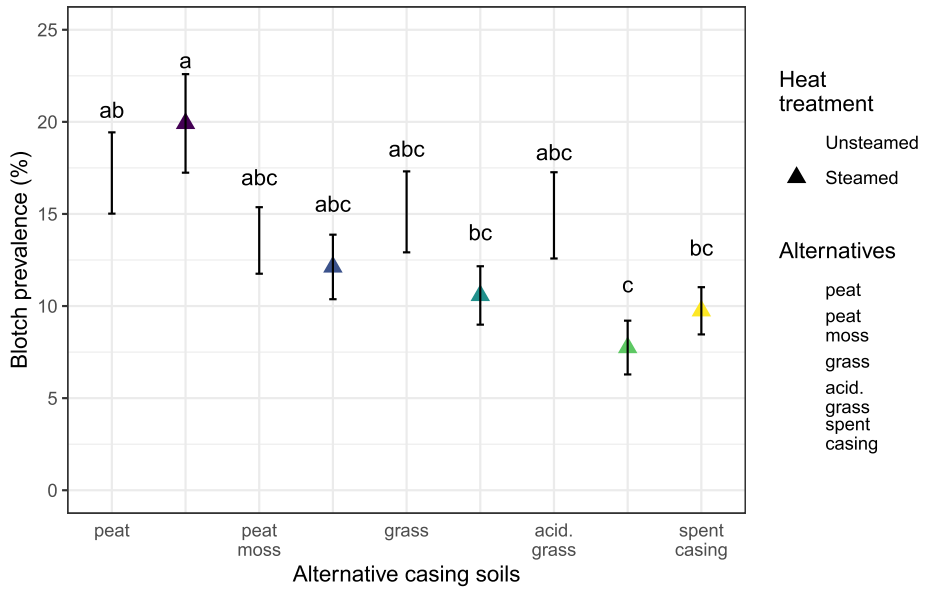

Fig. 9. Linear regression on the disease pressure (ginger blotch prevalence) of alternative casing soils, and the effect of steaming. Statistically significant comparisons according to Tukey's test are highlighted by letters.

casing soil types. In the first experiment, towards the end of the third flush, several pests such as mites, gall midges (*Mycophila speyeri*), and scarid flies (*Lycoriella auripila*) also emerged. They could not be associated to a specific casing soil. These pests were absent from the second repetition of the experiment.

4. Discussion

4.1. Role of the microbiome in mushroom growing media

Mushroom cultivation is different from other horticultural crops in that it is done on a two-component growing media. Firstly, the *A. bisporus* mycelium is grown through a substrate of aerobically fermented compost (Sánchez, 2004). Secondly, a layer of peat-based casing soil is applied on top, which provides physical support to the developing sporophores, acts as a water reservoir for the mycelium and prevents compost desiccation (Pardo-Giménez et al., 2017). Another important microbial function of the casing soil is that it induces fructification of the crop (Baars et al., 2020). Casing soil microbes that have been reported to stimulate mushroom development include, *Pseudomonas putida, Bacillus psilocybe Bacillus megaterium, Arthrobacter terregens, Rhizobium metiloff* and a blue-green alga, *Scenedesmus quadricauda* (Godfrey, 2003). These bacteria are speculated to initiate fructification by removing volatile inhibitors of fruiting body formation (Noble et al., 2009). Pseudomonads have received significant attention for their stimulatory effect on sporocarp initiation and development, however, several related *Pseudomonas* species can also cause bacterial blotch diseases on the mushrooms (Taparia et al., 2020a). Other harmful microorganisms endemic to the casing soil include pathogenic fungi, *Mycogone perniciosa* and various *Trichoderma* species, which cause wet bubble disease and green mould disease on the mushrooms respectively (Fletcher and Gaze, 2007).

The casing soil microbiome of the peat and alternatives, not only supplies beneficial microorganisms to induce fructification of the mushrooms, but it also determines the invasion resistance of the community in the event of a pathogen introduction. This invasion resistance is often determined by the diversity of the resident community and the complexity and stability of its interaction network (Mallon et al., 2015; Latz et al., 2012). Resistant microbial communities are known to show high modularity and complexity instead of a compact interaction network (Mendes et al., 2018). Modularity in the network suggests diversity in species roles and functionality, and consequently efficient consumption of available resources (Poudel et al., 2016). Thus, a modular microbial network implies ecological robustness and an ability to maintain community-level interactions despite fluctuations in the member species or the environment. Lack of modular interactions in the soil microbiome could thus increase the success of a pathogen invasion (Wei et al., 2015; van Elsas et al., 2012).

In mushroom cultivation, the overall species richness, or alpha diversity of the casing soil increased with the ratio of peat substitution. It was expected that addition of organic components like peat moss (25%), and grass fibres (50%), and spent casing (30%) will lead to a higher bacterial diversity in the casing soil, as it increases the nutritional status of the soil with the introduction of N and C rich components such as lignin, chitin, cellulose, and hemicellulose. The alpha diversity of the casing soil also increased across the cultivation cycle, in agreement with previous reports of the casing soil microbiome (Carrasco et al., 2019). It can be suspected that many bacterial taxa were present below the detection limit at the beginning of the cultivation cycle. However, increased colonization of the casing soil by the *A. bisporus* mycelium consequently increases the abundance of mycelial exudates present in the casing soil, which include various sugars and amino acids. Diverse soil bacteria can benefit from these fungal exudates which acts as a nutrient source for their growth (Baars et al., 2020). This is also supported by previous findings that report both increased alpha diversity and soil suppressiveness to bacterial blotch in later cultivation cycles (Taparia et al., 2021; Carrasco et al., 2020). Furthermore, it can also be speculated that the bacterial diversity in the casing soil is affected by increased period of contact with the compost. The casing soil can also acquire bacteria from the compost, which has a unique microbiome compared to that of the casing soil (Carrasco et al., 2019).

The total bacterial microbiome of these casing soils is rich and diverse, and comprise many rare and low abundance organisms, however, the core microbiome of these casing soils is relatively small as most low abundance organisms are not represented. The core fungal microbiome of the casing soil largely comprised of the mushroom of interest, *Agaricus bisporus*, as expected (Carrasco et al., 2019; Pecchia et al., 2014). However, several thermophilic species, such as *Meliniomyces* and *Mycothermus* were also abundant in the core microbiome, and were not lost after steam treatment. In circular horticulture, where growing media originates from other agricultural, forestry or industrial waste streams, sufficient attention also needs to be paid to study the

propagation of human and plant pathogens. In our experiments, we could not detect the presence of known human or plant pathogens in the microbiome of the casing soil prepared from the alternatives, but several unidentified bacterial taxa were detected, belonging to genera that comprise human or plant pathogens. However, most of these genera include only a few pathogenic variants. Unfortunately, amplicon-targeted sequencing does not allow accurate identification on a species or strain level, as the taxonomic resolution is limited due to the short read length.

4.2. Peat as a primary component of casing soil

Peat has been the primary component of casing soils in mushroom cultivation, since the 1950's due to its suitable physico-chemical and microbiological properties (Flegg, 1953). In our experiments, peat-based casing soils had one of the highest yields among the unsteamed soils, which is associated with its high water holding capacity and moisture content (Noble et al., 1999; Rainey, 1985). Peat also had a minimal fungal microbiome, which lacked an interaction network. Peat is generally known to be free of pathogens, and the competitive superiority of *Agaricus* could also be partially attributed to the low densities of other weed fungi in the casing soil (Carrasco et al., 2019). The reduced abundance of- and interactions between-other competing weed fungi, in an otherwise *Agaricus* rich environment, also encourages high productivity. The mean blotch prevalence was highest in casing soils composed of steamed and unsteamed peat. This can be attributed to the composition, interaction and activity of casing soil microorganisms. The core bacterial microbiome was least diverse in peat-based casing soils. Peat had one of the lowest soil respiration rates and organic matter content, which also point towards low microbial activity in the soil. The bacterial network in peat was compact and dense, and lacked modular interactions, implying a microbial community that does not occupy different ecological niches, and is likely not invasion resistant.

Heat treatment of the peat, before preparation of the casing soil, led to a large increase in productivity. This is contrary to the well-documented loss of yield in heat sterilized casing soils (Pizer and Leaver, 1947), which led to the important discovery of the role of the casing soil microflora in fructification of mushroom bodies (Arrold, 1972; Hume and Hayes, 1972; Smith and Hayes, 1972). In our experiments, after the peat in the casing soil was heat treated, the casing soil was mixed with fully colonized phase III compost, by a process called "CAC-ing" (MacCanna and Flanagan, 1972). It potentially allowed beneficial bacteria that are missing from the heat treated casing soil microbiome to be re-supplied via the compost (Reddy and Patrick, 1990) and promoted earlier pinning of the mushroom caps and increased yields (Kertesz and Thai, 2018). Additionally, the *A. bisporus* mycelium was able to colonize the casing soil more easily due to reduced competition from a resident soil microbial community. However, casing soils composed of heat-treated peat also had the highest disease pressure. While the blotch prevalence in steamed peat soils without added pathogen was negligible. Once the pathogen was introduced, casing soil comprising steamed peat was most susceptible to bacterial blotch outbreaks. Heat treatment further reduced the diversity and abundance of the resident microbial community in the peat, which failed to prevent the establishment of rising pathogen populations, in the event of a pathogen invasion.

4.3. Performance of peat alternatives

Sphagnum moss was high in water holding capacity, moisture content and bulk density, similar to black peat, however, it also had high respiration and organic matter content, similar to the grass fibres. In texture, it had intermediate hardness and adhesiveness. Its bacterial and fungal microbiomes were very similar to that of peat, although they were more diverse and relatively abundant, especially at the species level. The co-occurrence network topologies were also alike, indicating similar interactions within the microbial community. When peat moss was used to proportionally replace 25% of peat in the casing soil, it had equivalent productivity and disease pressure to that of peat-based casing soil. These high yields can be attributed to its high soil moisture retention curves and the abundance of endemic *Pseudomonas* sp., both of which are known to encourage fructification (Noble et al., 1999; Rainey, 1989). Heat treatment of peat moss, before preparation of the casing soil, did not have a significant effect on the productivity or disease pressure of the circular cropping system. Steaming of the peat moss significantly reduced the diversity of the bacterial microbiome, although the relative abundance of *Pseudomonas* sp. remained unaffected. Peat moss also had very high bulk density, that was similar to that of peat.

Grass-based casing soils had lower yields compared to other alternatives, but grasses were used to proportionally replace a much higher amount of peat in fresh casing, equivalent to 50%. The lower productivity can be partially ascribed to their inability to act as a water reservoir (Kalberer, 1990). Casing soil composed of grass fibres had the highest relative increase in soil moisture content when they were saturated with water, however, they also had the lowest soil moisture retention capacity, implying that they are quick to both absorb and release water and hence do not provide a good buffering system for soil moisture. Their low water holding capacity may depend on their structure, in which they differ significantly from peat in both hardness and adhesiveness. However, the need for casing soil to be a good buffering system for water, is steadily reducing with automated and frequent watering of mushroom beds in commercial farms (Sánchez, 2004). Genomic and transcriptomic analyses of *A. bisporus* show its adaptation to humic rich and partially decomposed plant material (Morin et al., 2012). Yet the grass fibres were also a competitive environment for *A. bisporus* mycelium to colonize, due to highly diverse bacterial and fungal resident community. This is also evident from their microbial network which was highly connected and modular. Their soil respiration rates and organic matter content, were also indicative of high microbial activity.

Acidification of the grass fibres did not have a clear effect on the agronomical performance of the casing soil or its physical and chemical properties. The pH of the alternative casing soil was not significantly lower at the start of the experiment, because the acidification was neutralized in the preparation of the casing soil by addition of sugar beet lime. Although there was no overall difference in the microbial diversity, the relative composition of the bacterial and fungal microbiome did change after acidification, mostly at the species and strain level for soil bacteria. Changes in soil fungi were observable at the genus level too, where *Pseudeurotium* sp. and *Dipodascus* sp. decreased in relative abundance, and *Ascobolus* sp. and *Solicoccozyma* sp. increased. Contrary to other reports, acidification did not lead to an increase in *Bacteroidetes* and *Firmicutes* (Abendroth et al., 2017). The microbial co-occurrence network reduced in size after acidification, implying fewer interactions within the microbial community. Heat treatment of the grass fibres had no effect on productivity, although steamed acidified grasses, had the lowest disease pressure overall. While, acidification of the grasses increased the relative abundance of *Dyadobacter* sp. in the casing soil, heat treatment increased the relative abundance of *Saitozyma* sp. Both of these have been previously associated with blotch suppressiveness in the casing soil (Taparia et al., 2021). Heat treatment also reduced the abundance of other weed and pathogenic fungi, such as *Coprinus* sp., *Peziza* sp., and *Trichoderma* sp. in the casing soil, which is supported by an earlier finding (Park et al., 1971). However, steamed grasses had one of the lowest moisture levels at the end of the cultivation cycle, and require frequent watering. Acidified grass fibres had the lowest dry and fresh weight by volume.

Spent casing, as expected, had similar physical, chemical and structural properties, to that of black peat, namely, water holding capacity, bulk weight, dried weight, organic matter content, soil respiration and texture. At the beginning of the cultivation experiment, spent casing had the highest electrical conductivity, due to soluble salts that

were deposited during the previous cultivation cycles (Pardo-Giménez et al., 2011). However, at the end of the cultivation experiment the EC was equivalent for all alternatives. If its proportional use in casing soil needs to be increased, the high EC could pose a problem. Recycling of the same casing soil multiple times, can potentially lead to accumulation of soluble salts, which would need to be leached out (Gonani et al., 2011). Despite the heat treatment of spent casing, its microbial community composition was similar to that of peat, although the bacterial diversity was much lower. Its fungal microbiome additionally comprised of *Saitozyma* sp., *Solicoccozyma* sp., *Trichoderma* sp., and an unidentified fungus, which were largely absent from peat-based casing soils. Similar findings were reported from spent compost (Eicker and van Greuning, 1989). When spent casing was used to proportionally replace 30% peat in fresh casing soil, the productivity of spent casing was equivalent to that of peat, although the disease pressure was much lower. Spent casing showed a high suppression of ginger blotch, at higher pathogen densities and also in earlier flushes. This can be attributed to the increased abundance of endemic *Pseudomonas* sp. in spent casing, which have a niche overlap with the pathogen and may compete for nutrients. However, in personal communication, farmers are reluctant to reintroduce spent casing soil in their farms over concerns of pests and pathogen management.

4.4. Accessibility and sustainability

Four circular and sustainable alternatives, *Sphagnum* moss, grass fibres, acidified grass fibres and spent casing can be used to proportionally replace black peat in growing media. Their agronomical performance depends on their related physical, chemical and microbiological characteristics. However, their future use also depends on their accessibility and sustainability. *Sphagnum* moss in growing media has been shown to perform well on a wide variety of crops (Blievernicht et al., 2012; Oberpaur et al., 2010; Emmel, 2005). However, degraded peat-lands on which *Sphagnum* farming is practised, needs to be constantly water-saturated. Even though the land area is widely available, it requires substantial investment in site preparation and irrigation to support a high enough water table (Gaudig et al., 2014). Several studies show the feasibility of large-scale *Sphagnum* farming, which has been practised in Germany, the Netherlands, Latvia, Georgia and Canada (Gaudig et al., 2017). Economic analyses reveal that the use of *Sphagnum* moss in growing media is currently profitable only for niche markets with high revenues, such as orchid cultivation, but it cannot compete with the low cost of black peat. However, it is predicted that at a surcharge of ~10% to the end-consumers, it is economically viable to completely substitute black peat for *Sphagnum* moss for other horticultural crops as well (Wichmann et al., 2020).

Grass fibres used in this study were produced from agricultural residue streams of mixed origins within the Netherlands, which underwent a patented biorefinery process for conversion to lignocellulose fibres. These can include sugar cane bagasse, sorghum, corn cobs, corn stover, rice straw, nut shells and grass clippings. Europe generates about 700 million tonnes of agricultural waste annually (Pawelczyk, 2005), implying that large amounts of lignocellulosic biomass are available for valorisation. It is thus essential to further develop circular bio-economies with integrated processes (Székács, 2017), to simultaneously reduce both resource consumption and waste generation, thereby, mitigating the environmental impact associated with food production (Maina et al., 2017; Commission, 2014). The major limiting factor in availability of these grass fibres is the biorefinery process, which currently only has one full-scale facility, with a production capacity of 1000 kg fibres per hour. A second drawback of the use of these waste streams is the inconsistency in substrate quality, resulting in an unreliable agronomical performance. This can be accounted for by partially mixing with standardized growing media like peat.

Spent casing is an abundant and local resource. The production of 1 kg of mushrooms, generates 5 kg of residual material called spent mushroom substrate (SMS) (Lau et al., 2003). In the Netherlands, more than 800,000 tonnes of SMS (35–40% dry matter content) is produced per year (Oei and Albert, 2012). SMS disposal is a big challenge for mushroom farmers due to regulations from the EU nitrate Vulnerable Zones. An average farm discards about 24 tonnes of SMS per month (Singh et al., 2011), of which 4.8 tonnes comprises spent casing. However, the limiting factor is the separation of the casing soil from the compost. A perforated plastic meshes can be layered between the compost and the casing soil during filling of the room (Farsi et al., 2011; Royse et al., 2008). A MushComb machine can also be used, which allows mechanical recovery of 50–75% of the casing soil (Oei and Albert, 2012). Cost benefit analysis of spent casing soil reveals a significant initial investment, for the casing separator machine and trailer to separate the casing soils, and the hopper and conveyor belt for recycling into fresh casing (Noble and Dobrovin-Pennington, 2015). However, the SMS disposal costs are reduced by 12% and fresh casing soil costs are reduced by 30% (Noble and Dobrovin-Pennington, 2015).

4.5. Future prospects for circular horticulture

Like most agro-ecological systems, the trade-offs between productivity and disease pressure need to be further explored in use of peat alternatives. The management of the microbial community plays an important role in this. The microbiome of the alternative casing soil can be detrimental in that it introduces pathogens to the farm (peat) or competes with the crop for nutrition (grasses). It can also be beneficial, when it supplies microbes for plant growth promoting microbes or disease suppression (spent casing). It can be speculated that with the full replacement of peat, currently known soil-borne diseases that are introduced from peat-based casing soil will decline. However, it is also expected that other pests, pathogens and competitive fungi native to the peat-alternatives, may affect the performance of the alternative casing soils, as was observed for unsteamed grass-based casing soils. Steam treatment of the grass fibres resulted in lack of other competitive fungi. Hence, it is also important to explore other methods to manage the microbiome of the peat alternatives, such as the use of biostimulants, peak-heating, disinfection, fermentation, acidification or via storage.

From an industrial perspective, it is also essential to determine an optimal peat-replacement ratio which balances agronomical performance with environmental impact and economic viability. Trade-offs in circular cropping systems are also driven by economics, energy demands and sustainability of the peat-alternatives, which need to be further investigated. Life cycle assessments are required to quantify the environmental impact of using these peat alternatives. Cost-benefit and supply chain analyses of these peat-reduced growing media are also essential to determine the economic suitability for industrial use. The watering methods, growing conditions and environment currently employed for mushroom cultivation have been optimized for peat-based casing soils over the last 50 years. However, the physical, chemical and structural properties of the alternative casings largely differ from that of peat-based casing soils. Hence, the optimization of the growing conditions can also play an important role in increasing the productivity and reducing the disease pressure in circular cropping systems.

Common concerns about using circular alternatives in food production systems also involve the accumulation of toxic compounds such as heavy metals, pesticide residues and microplastics. The alternative growing media and its produce need to be screened for presence of these elements, for food safety, but also to meet regulatory limits within the EU for disposal of organic wastes. The identity of and risk from human and plant pathogens needs to be established with the help of genomics and cultivation experiments. Finally, sanitisation of the peat alternatives with methods like steaming, dry heat, composting, irradiation, solarisation, dry or cold storage need to be explored to eliminate the propagation of pests and pathogens that can be detrimental to human health and crop health. Heat and chemical treatment of peat-reduced growing media can also lead to the loss of a beneficial microbiome. Hence,

supplementation of the peat-alternatives with known beneficial organisms, either as individual strains or a group of microbes that facilitate growth promotion and disease suppression is also a promising strategy.

5. Conclusions

We demonstrated the successful use of four circular alternatives to proportionally replace peat in mushroom growing media, namely, two grass fibres from agricultural residue streams, peat-moss farmed in degraded peatlands, and spent casing soil recycled from previous cultivation cycles. Specific physical and chemical properties of the casing soils that influence productivity varied between the peat-alternatives. These include, moisture retention capacity, moisture content, pore fraction, soil respiration, electrical conductivity, and organic matter content. Peat moss and spent casing were expectedly similar to peat, but the grass fibres had unique structure and physico-chemical characteristics, such as high organic matter content and low moisture retention capacity. There were no visual differences in the post-harvest quality of the mushrooms grown in peat-based and alternative casing soils.

Mushroom growing media partially composed of peat-alternatives had unique microbial communities, which varied significantly from that of peat. Crop growth and disease suppression were both associated with the microbiome of the growing media, and strong trade-offs existed between the productivity and disease pressure in the circular cropping systems. Grass fibres had the most diverse microbiomes, and their pretreatment by acidification and steaming significantly reduced their microbiome, as well as the presence of pests, pathogens and competitive fungi. Steamed and acidified grass fibres also showed the highest suppression of bacterial blotch, closely followed by steamed spent casing. Peat-alternatives, with the exception of the grasses, had lower disease pressure compared to that of peat, without loss of yield. These insights on the microbiome, management, characteristics and performance of peat-reduced growing media, needs to be supplemented with knowledge on the accessibility, sustainability, and economic viability of these peat-alternatives in order to transition away from peat use and towards circular horticulture.

Funding sources

This project was granted by the Topsector Horticulture and propagation material of the Dutch Ministry of Economic Affairs. It was conducted under Grant No. KV1509-050 in collaboration with members of the Dutch and Belgian mushroom supply chain.

Authors contributions

TT, EH, and JW designed the experiments. TT and EH, performed the bioassay. TT and EN analyzed the data. TT wrote the first draft of the manuscript. JW and WB contributed to subsequent manuscript revision. All authors read and approved the submitted version.

CRediT authorship contribution statement

Tanvi Taparia: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Ed Hendrix:** Investigation, Resources. **Els Nijhuis:** Investigation, Data curation. **Wietse de Boer:** Writing – review & editing, Supervision, Funding acquisition. **Jan van der Wolf:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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.

Acknowledgements

We would like to thank Marjon Krijger, Mark Hendriks, Chula Hok-A-Hin, Jos Amsing, Caroline van der Horst, Johan Baars and Edwin Hamoen for their support in carrying out this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2021.129375.

References

Abendroth, C., Simeonov, C., Peretó, J., Antúnez, O., Gavidia, R., Luschnig, O., Porcar, M., 2017. From grass to gas: microbiome dynamics of grass biomass acidification under mesophilic and thermophilic temperatures. Biotechnol. Biofuels 10, 1–12.

Alexander, P.D., Bragg, N.C., Meade, R., Padelopoulos, G., Watts, O., 2008. Peat in horticulture and conservation: the UK response to a changing world. Mires Peat 3.

Altmann, M., 2008. Socio-economic impact of the peat and growing media industry on horticulture in the EU. Epagma. Brussel.

Arrold, P., 1972. Confirmation of the ability of *Pseudomonas putida* to cause fruiting of the cultivated mushroom. MGA Bull Mushroom Growers Assoc.

Baars, J.J., Scholtmeijer, K., Sonnenberg, A.S., van Peer, A.V., 2020. Critical factors involved in primordia building in *Agaricus bisporus*: a review. Molecules 25, 2984.

Blievernicht, A., Irrgang, S., Zander, M., Ulrichs, C., 2012. The youngest peat-Sustainable production of *Sphagnum* sp. and its use as growing media in professional horticulture. Extended Abstract No. 247. In: Proceedings of the 14th International Peat Congress.

Boelter, D.H., 1968. In: Important Physical Properties of Peat Materials. Proceedings, Third International Peat Congress, Quebec, Canada. Department of Energy, Minds and Resources and National Research Council of, pp. 150–154. Canada.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857.

Bos, M.G., Diemont, W.H., Verhagen, A., 2011. Sustainable Peat Supply Chain: Report of the Ad Hoc Working Group Enhancing the Sustainability of the Peat Supply Chain for the Dutch Horticulture. Alterra.

Briatte, F., 2016. Ggnetwork: geometries to plot networks with'ggplot2. R Package Version 0 5 1.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.

Carlile, B., Coules, A., 2011. Towards sustainability in growing media. In: International Symposium on Growing Media, Composting and Substrate Analysis, vol. 1013, pp. 341–349.

Carlile, W.R., Schmilewski, G., 2010. In: Life in Growing Media: the Good, the Bad and the Ugly. Proceedings of the International Peat Society. Peat in Horticulture–Life in Growing Media, pp. 7–14.

Caron, J., Rochefort, L., 2011. Use of peat in growing media: state of the art on industrial and scientific efforts envisioning sustainability. In: International Symposium on Responsible Peatland Management and Growing Media Production, vol. 982, pp. 15–22.

Carrasco, J., Tello, M.L., de Toro, M., Tkacz, A., Poole, P., Pérez-Clavijo, M., Preston, G., 2019. Casing microbiome dynamics during button mushroom cultivation: implications for dry and wet bubble diseases. Microbiology 165, 611–624.

Carrasco, J., García-Delgado, C., Lavega, R., Tello, M.L., De Toro, M., Barba-Vicente, V., Preston, G., 2020. Holistic assessment of the microbiome dynamics in the substrates used for commercial champignon (Agaricus bisporus) cultivation. Microbial biotechnology 13, 1933–1947.

2014 Commission, E.U, 2014. Towards a Circular Economy: A Zero Waste Programme for Europe. COM, p. 398.

Cribari-Neto, F., Zeileis, A., 2009. Beta Regression in R.

Csardi, G., Nepusz, T., 2006. The igraph software package for complex network research. InterJournal, complex systems 1695, 1–9.

De Corato, U., 2020. Agricultural waste recycling in horticultural intensive farming systems by on-farm composting and compost-based tea application improves soil quality and plant health: a review under the perspective of a circular economy. Sci. Total Environ., 139840

Eicker, A., van Greuning, M., 1989. Economical alternatives for topogenous peat as casing material in the cultivation of *Agaricus bisporus* in South Africa. S. Afr. J. Plant Soil 6, 129–135.

Emmel, M., 2005. Growing ornamental plants in *Sphagnum* biomass. In: International Symposium on Growing Media, vol. 779, pp. 173–178.

Farsi, M., Malekzadeh, K., Jalalzadeh, B., Shahri, M., 2011. Recycling of mushroom peat casing soil through a plastic mesh. In: Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products. ICMBMP7.

Flegg, P.B., 1953. Pore space and related properties of casing materials. Mushroom Sci. 2, 149–161.

Fletcher, J.T., Gaze, R.H., 2007. Mushroom Pest and Disease Control: a Colour Handbook. CRC Press.

Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., Firth, D., Friendly, M., Gorjanc, G., Graves, S., 2012. Package 'car. R Foundation for Statistical Computing, ' Vienna.
Gaudig, G., Fengler, F., Krebs, M., Prager, A., Schulz, J., Wichmann, S., Joosten, H., 2014. *Sphagnum* farming in Germany-a review of progress. Mires Peat 13, 1–11.
Gaudig, G., Krebs, M., Prager, A., Wichmann, S., Barney, M., Caporn, S.J.M., Emmel, M., Fritz, C., Graf, M., Grobe, A., 2017. *Sphagnum* Farming from Species Selection to the Production of Growing Media: a Review. Mires and Peat.
Godfrey, S.A., 2003. Molecular Investigation of Pseudomonads Causative of *Agaricus bisporus* Blotch Disease in New Zealand Mushroom Farms. University of Canterbury, Plant and Microbial Sciences.
Gonani, Z., Riahi, H., Sharifi, K., 2011. Impact of using leached spent mushroom compost as a partial growing media for horticultural plants. J. Plant Nutr. 34, 337–344.
Graf, M.D., Bérubé, V., Rochefort, L., 2012. Restoration of Peatlands after Peat Extraction. Restoration and Reclamation of Boreal Ecosystems. Cambridge University Press, Cambridge, pp. 259–280.
Gülser, C., Pekşen, A., 2003. Using tea waste as a new casing material in mushroom (*Agaricus bisporus* (L.) Sing.) cultivation. Bioresour. Technol. 88, 153–156.
Gunady, M.G.A., Biswas, W., Solah, V.A., James, A.P., 2012. Evaluating the global warming potential of the fresh produce supply chain for strawberries, romaine/cos lettuces (*Lactuca sativa*), and button mushrooms (*Agaricus bisporus*) in Western Australia using life cycle assessment (LCA). J. Clean. Prod. 28, 81–87.
Hume, D.P., Hayes, W.A., 1972. The production of fruit-body primordia in *Agaricus bisporus* (Lange) Sing. on agar media. Mushroom Sci. 7, 527–532.
Johnson, J.B., Omland, K.S., 2004. Model selection in ecology and evolution. Trends Ecol. Evol. 19, 101–108.
Joosten, H., Sirin, A., Couwenberg, J., Laine, J., Smith, P., 2016. The role of peatlands in climate regulation. Peatland Restoration and Ecosystem Services: Science, Policy and Practice 66.
Joosten, H., Tapio-Biström, M.-L., Tol, S., 2012. Peatlands: Guidance for Climate Change Mitigation through Conservation, Rehabilitation and Sustainable Use. Food and Agriculture Organization of the United Nations Rome.
Kalberer, P.P., 1990. Water relations of the mushroom culture *Agaricus bisporus*: study of a single break. Sci. Hortic. 41, 277–283.
Kertesz, M.A., Thai, M., 2018. Compost bacteria and fungi that influence growth and development of *Agaricus bisporus* and other commercial mushrooms. Appl. Microbiol. Biotechnol. 102, 1639–1650.
Kurtz, Z.D., Müller, C.L., Miraldi, E.R., Littman, D.R., Blaser, M.J., Bonneau, R.A., 2015. Sparse and compositionally robust inference of microbial ecological networks. PLoS Comput. Biol. 11, e1004226.
Lahti, L., Shetty, S., Blake, T., Salojarvi, J., 2017. Microbiome R Package. Tools Microbiome Anal R.
Latinoamericana, T., 2006. Fibra de Coco como Material de Cobertura en la Producción de Champiñón. Terra.
Latz, E., Eisenhauer, N., Rall, B.C., Allan, E., Roscher, C., Scheu, S., Jousset, A., 2012. Plant diversity improves protection against soil-borne pathogens by fostering antagonistic bacterial communities. J. Ecol. 100, 597–604.
Lau, K.L., Tsang, Y.Y., Chiu, S.W., 2003. Use of spent mushroom compost to bioremediate PAH-contaminated samples. Chemosphere 52, 1539–1546.
Leiva, F.J., Saenz-Díez, J.C., Martínez, E., Jiménez, E., Blanco, J., 2015. Environmental impact of *Agaricus bisporus* cultivation process. Eur. J. Agron. 71, 141–148.
MacCanna, C., Flanagan, J.B., 1972. Casing types and techniques. Mushroom Sci. 8, 727–731.
Maina, S., Kachrimanidou, V., Koutinas, A., 2017. A roadmap towards a circular and sustainable bioeconomy through waste valorization. Current Opinion in Green and Sustainable Chemistry 8, 18–23.
Mallon, C.A., Van Elsas, J.D., Salles, J.F., 2015. Microbial invasions: the process, patterns, and mechanisms. Trends Microbiol. 23, 719–729.
McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8, e61217.
Mendes, L.W., Raaijmakers, J.M., de Hollander, M., Mendes, R., Tsai, S.M., 2018. Influence of resistance breeding in common bean on rhizosphere microbiome composition and function. ISME J. 12, 212–224.
Morin, E., Kohler, A., Baker, A.R., Foulongne-Oriol, M., Lombard, V., Nagye, L.G., Ohm, R.A., Patyshakuliyeva, A., Brun, A., Aerts, A.L., 2012. Genome sequence of the button mushroom *Agaricus bisporus* reveals mechanisms governing adaptation to a humic-rich ecological niche. Proc. Natl. Acad. Sci. Unit. States Am. 109, 17501–17506.
Noble, R., Dobrovin-Pennington, A., 2015. Developing Alternatives to Peat in Casing Materials for Mushroom Production. Kenilworth, UK.
Noble, R., Dobrovin-Pennington, A., Hobbs, P.J., Pederby, J., Rodger, A., 2009. Volatile C8 compounds and pseudomonads influence primordium formation of *Agaricus bisporus*. Mycologia 101, 583–591.
Noble, R., Dobrovin-Pennington, A., 2005. Partial substitution of peat in mushroom casing with fine particle coal tailings. Sci. Hortic. 104, 351–367.
Noble, R., Dobrovin-Pennington, A., Evered, C.E., Mead, A., 1999. Properties of peat-based casing soils and their influence on the water relations and growth of the mushroom (*Agaricus bisporus*). Plant Soil 207, 1–13.
Oberpaur, C., Puebla, V., Vaccarezza, F., Arévalo, M.E., 2010. Preliminary substrate mixtures including peat moss (*Sphagnum magellanicum*) for vegetable crop nurseries. International Journal of Agriculture and Natural Resources 37, 123–132.
Oei, P., Albert, G., 2012. Recycling casing soil. In: 18th Congress of the International Society for Mushroom Science, pp. 757–765. Beijing, China.
Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M.H.H., Oksanen, M.J., Suggests, M., 2007. The vegan package. Community Ecology Package 10, 719.
Owen, P., 2007. LIFE and Europe's Wetlands: Restoring a Vital Ecosystem. Office for Official Publications of the European Communities.
Pardo-Giménez, A., Pardo-González, J.E., Zied, D.C., 2011. Evaluation of harvested mushrooms and viability of *Agaricus bisporus* growth using casing materials made from spent mushroom substrate. Int. J. Food Sci. Technol. 46, 787–792. https://doi.org/10.1111/j.1365-2621.2011.02551.x.
Pardo-Giménez, A., Pardo, J.E., Zied, D.C., 2017. Casing materials and techniques in Agaricus bisporus cultivation. Edible and medicinal mushrooms: technology and applications 385–413.
Pardo, A., De Juan, A.J., Pardo, J., 2004. Assessment of different casing materials for use as peat alternatives in mushroom cultivation. Evaluation of quantitative and qualitative production parameters. Spanish J. Agric. Res. 2, 267. https://doi.org/10.5424/sjar/2004022-80.
Park, W.M., Kim, D.S., Park, Y.H., Chung, H.S., 1971. Factors affecting growth of *Trichoderma* spp. with special reference to control of green mildew in *Agaricus bisporus*. Growth 9, 2.
Pawelczyk, A., 2005. Eu policy and legislation on recycling of organic wastes to agriculture. ISAH 1, 64–71.
Pecchia, J., Cortese, R., Albert, I., Singh, M., 2014. Investigation into the microbial community changes that occur in the casing layer during cropping of the white button mushroom, Agaricus bisporus. In: Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products, pp. 309–313. New Delhi, India.
Peyvast, G., Shahbodaghi, J., Remezani, P., Olfati, J.A., 2007. Performance of tea waste as a peat alternative in casing materials for bottom mushroom (*Agaricus bisporus* (L.) Sing.) cultivation. Biosciences. Biotechnology Research Asia 4.
Pizer, N.H., Leaver, W.E., 1947. Experiments with soils used for casing beds of the cultivated mushroom, *Psalliota campestris*. Ann. Appl. Biol. 34, 34–44.
Postma, J., Os, E. van, Bonants, P.J.M., 2008. Pathogen Detection and Management Strategies in Soilless Plant Growing Systems.
Poudel, R., Jumpponen, A., Schlatter, D.C., Paulitz, T.C., Gardener, B.B.M., Kinkel, L.L., Garrett, K.A., 2016. Microbiome networks: a systems framework for identifying candidate microbial assemblages for disease management. Phytopathology 106, 1083–1096.
Pouliot, R., Hugron, S., Rochefort, L., 2015. *Sphagnum* farming: a long-term study on producing peat moss biomass sustainably. Ecol. Eng. 74, 135–147.
Rainey, P.B., 1989. The Involvement of *Pseudomonas Putida* in Basidiome Initiation of the Cultivated Mushroom *Agaricus bisporus*.
Rainey, P.B., 1985. A Study of Physical, Chemical and Biological Properties of the Mushroom Casing Layer.
Reddy, M.S., Patrick, Z.A., 1990. Effect of bacteria associated with mushroom compost and casing materials on basidiomata formation in *Agaricus bisporus*. J. Indian Dent. Assoc. 12, 236–242.
Robinson, B., Winans, K., Kendall, A., Dlott, J., Dlott, F., 2019. A life cycle assessment of *Agaricus bisporus* mushroom production in the USA. Int. J. Life Cycle Assess. 24, 456–467.
Royse, D.J., Sanchez, J.E., Beelman, R.B., Davidson, J., 2008. Re-supplementing and re-casing mushroom (*Agaricus bisporus*) compost for a second crop. World J. Microbiol. Biotechnol. 24, 319–325.
Sánchez, C., 2004. Modern aspects of mushroom culture technology. Appl. Microbiol. Biotechnol. 64, 756–762.
Sassine, Y.N., Ghora, Y., Kharrat, M., Bohme, M., Abdel-Mawgoud, A.M.R., 2005. Waste paper as an alternative for casing soil in mushroom (*Agaricus bisporus*) production. J. Appl. Sci. Res. 1, 277–284.
Schmilewski, G., 2008. The role of peat in assuring the quality of growing media. Mires Peat 3.
Singh, A.D., Vikineswary, S., Abdullah, N., Sekaran, M., 2011. Enzymes from spent mushroom substrate of *Pleurotus* sajor-caju for the decolourisation and detoxification of textile dyes. World J. Microbiol. Biotechnol. 27, 535–545.
Smith, J.F., Hayes, W.A., 1972. Use of autoclaved substrates in nutritional investigations on the cultivated mushroom. Mushroom Sci. 8, 355–361.
Székács, A., 2017. Environmental and ecological aspects in the overall assessment of bioeconomy. J. Agric. Environ. Ethics 30, 153–170.
Taparia, T., Krijger, M., Haynes, E., Elphinstone, J.G., Noble, R., van der Wolf, J., 2020a. Molecular characterization of *Pseudomonas* from *Agaricus bisporus* caps reveal novel blotch pathogens in Western Europe. BMC Genom. 21, 1–14.
Taparia, T., Hendriks, E., Hendriks, M., Krijger, M., de Boer, W., van der Wolf, J., 2020b. Comparative studies on the disease prevalence and population dynamics of ginger blotch and brown blotch pathogens of button mushrooms. Plant Dis. https://doi.org/10.1094/PDIS-06-20-1260-RE.
Taparia, T., Hendriks, E., Nijhuis, E., Hendriks, M., de Boer, W., van der Wolf, J., 2021. Soil microbiome mediated suppression of blotch during consecutive cultivation cycles of mushrooms. Soil Biol. Biochem. 155, 108161.
Team, R.C., 2013. R: A Language and Environment for Statistical Computing.
van Elsas, J.D., Chiurazzi, M., Mallon, C.A., Elhottovā, D., Krištůfek, V., Salles, J.F., 2012. Microbial diversity determines the invasion of soil by a bacterial pathogen. Proc. Natl. Acad. Sci. Unit. States Am. 109, 1159–1164.
Van Gerrewey, T., Ameloot, N., Navarrete, O., Vandecruys, M., Perneel, M., Boon, N., Geelen, D., 2020. Microbial activity in peat-reduced plant growing media: identifying influential growing medium constituents and physicochemical properties using fractional factorial design of experiments. J. Clean. Prod. 256, 120323.
Vandecasteele, B., Blindeman, L., Amery, F., Pieters, C., Ommeslag, S., Van Loo, K., De Tender, C., Debode, J., 2020. Grow-Store-Steam-Re-peat: reuse of spent growing media for circular cultivation of *Chrysanthemum*. J. Clean. Prod., 124128
Vos, D.J., Rustenburg, S., 2015. A Process for the Conversion of Biomass of Plant Origin, and a Combustion Process. European Patent Office EP2606140B1.

Wang, J.C., Ho, W.J., Wu, C.Y., 2008. Heavy metal contents of the cultivated culinary-medicinal Royal Sun Agaricus (the himematsutake mushroom) *Agaricus brasiliensis* using different casing materials. Int. J. Med. Mushrooms 10, 2.
Wei, Z., Yang, T., Friman, V.-P., Xu, Y., Shen, Q., Jousset, A., 2015. Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. Nat. Commun. 6, 1–9.
Wichmann, S., Krebs, M., Kumar, S., Gaudig, G., 2020. Paludiculture on former bog grassland: profitability of *Sphagnum* farming in north west Germany. Mires Peat 26.
Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., 2019. Welcome to the tidyverse. Journal of Open Source Software 4, 1686.