scientific reports

OPEN

Spent casing, *Sphagnum* moss, grass fibers, and green compost as peat alternatives in casing soils for *Agaricus bisporus* cultivation

Florien A. Gorter[1]✉, Roxanne van Rooijen[2], Ed Hendrix[1], Marc Hendriks[1], Odette Mendes[1], Carin Lombaers[1], Marjon Krijger[1], Tanvi Taparia[1,3] & Jan M. van der Wolf[1]

***Agaricus bisporus*, the button mushroom, is commonly grown in compost, which is overlaid with casing soil to induce fructification of the crop. Casing currently consists mainly of peat, but peat use is under pressure due to environmental concerns. We previously identified multiple materials that can be used to partially replace peat in casing soil without having a substantial negative effect on mushroom yield or disease suppressiveness. Here, we investigated what percentage of peat can be replaced by each of these materials (spent casing, *Sphagnum* moss and a processed grass component) as well as green compost, and if combinations of materials perform better than individual constituents. We found that peat can be replaced by up to 75% of alternative casing materials without a significant effect on yield or ginger blotch incidence, with the exception of an unsteamed grass component. Across all casing soils, moisture content at low suction pressure was the best predictor of yield. Physico-chemical properties did not predict blotch incidence, which was instead associated with sourcing of the material and could be mitigated by steaming. Several alternative materials contained increased levels of heavy metals and fungicides, but in all cases, these were still well below the legal limits.**

Keywords *Agaricus*, Casing, Peat, Yield, Bacterial blotch, *Pseudomonas*

Peat has been defined as sedentarily accumulated material that consists of at least 30% dead organic material[1]. It is formed by the slow decomposition of plant material in wet, acidic ecosystems, where anaerobic conditions dominate[2]. Peat is used extensively in horticulture in growth substrates due to its excellent physical, chemical and microbiological properties, as well as its wide availability and cheap sourcing[3]. However, its use is under pressure. Peat is extracted from peatlands, which provide many important ecosystem services, such as water regulation, biodiversity conservation and carbon sequestration and storage[4,5]. In fact, peatlands store the equivalent of as much as 75% of atmospheric carbon, more than all other vegetation types combined[6]. 50% of all peatlands have been degraded over the past century[7] and each year, around 20 million tons of peat are extracted in the EU alone[8]. While to date no overarching EU provision for the extraction of peat has been defined[9], on a national level several strategies have been developed for phasing out peat[8]. Within the Netherlands, a broad coalition of parties has recently expressed its ambition to exclusively use CO_2-neutral substrates by 2050[10]. Alternatives for peat in horticulture are thus urgently needed.

Peat also plays an important role in mushroom cultivation. Mycelium of *Agaricus bisporus*, the button mushroom, is commonly grown in compost consisting of manure, straw and gypsum, which is overlaid with peat mixed with lime to induce fructification of the crop[11]. This transition from the vegetative to the reproductive phase of the mushroom is driven by a combination of cues, which reflect the natural conditions to which *Agaricus* is adapted: reduction in temperature, nutrients, CO_2 and self-inhibitory volatile organic compounds (VOCs)[12,13]. Therefore, a good casing material should be nutrient poor and sufficiently porous, so that gases can escape easily. It should also be conducive to the growth of *Pseudomonas* bacteria, which play an important role in the degradation of VOCs[14], but at the same time be free of pests and pathogens. Finally, it needs to provide sufficient mechanical support, structural integrity, and water holding capacity, a low salt content and high cationic exchange capacity[15]. Based on these criteria, peat is an excellent casing material.

[1]Biointeractions and Plant Health, Wageningen Plant Research, Wageningen, Netherlands. [2]Innovation Department, Kekkilä-BVB, De Lier, Netherlands. [3]Department of Biology, University of Copenhagen, Copenhagen, Denmark. ✉email: florien.gorter@wur.nl

It has proven difficult to find a good replacement for peat. Starting from the 1950s, a large number of alternative materials has been assessed for its effect on mushroom yield and disease suppressiveness[16]. These materials can broadly be categorized as materials of mineral origin (such as clay, gravel and vermiculite), materials of vegetal origin (such as different peat types, bark and cellulosic material from the paper industry), synthetic materials (such as polyacrylamide and silica gels) and other materials (such as spent mushroom substrate, manure and various composts). Results have been mixed, and most materials are associated with one or more drawbacks that currently preclude their application on an industrial scale[17]. For example, wood fiber has insufficient water holding capacity[18], spent mushroom substrate contains excessive soluble nutrients, and paper wastes are conducive to *Trichoderma* and other molds. Other materials are associated with prohibitive costs or insufficient supplies in mushroom producing countries.

One potential way to overcome the drawbacks associated with the individual materials, is to combine them. In a previous study, we used three promising materials to proportionally replace peat[19]. The use of these materials reflects different principles of circular economy, that is, design out waste (fermented grass fibers), keep materials in use (spent mushroom casing) and regenerate natural systems (*Sphagnum* moss). Materials were used at 50, 30 and 25%, respectively. Incorporation of grass slightly reduced yield, whereas incorporation of spent casing and *Sphagnum* moss did not. This raises the possibility that reduction in yield is dependent on the percentage at which peat is replaced rather than the material that is used per se. Disease suppressiveness against the bacterial blotch pathogen *Pseudomonas gingeri*[20]—which was added at different inoculum densities—was not compromised by the incorporation of alternative materials. In fact, spent casing decreased blotch prevalence, in line with previous findings[21]. Steaming of the materials prior to use generally reduced blotch prevalence, although individual contrasts (i.e. contrasts between the steamed and unsteamed version of the same material) were not significant following post-hoc testing. This is a promising result that invites further investigation.

Here, we asked what percentage of peat in mushroom casing can be replaced with fermented grass fibers, spent mushroom casing or *Sphagnum* moss, without having a negative effect on mushroom yield, harvest properties or intrinsic ginger blotch incidence. We asked the same question for green compost, which is a promising material as well based on its physicochemical and microbiological characteristics[22,23]. We also assessed the effect of combining these materials. Moreover, we investigated how physicochemical properties differed between casing soils, and which of these differences drove yield and blotch incidence. Finally, we measured contaminant levels in each material in order to determine whether it can safely be used for mushroom cultivation.

Results

Components interact to affect total mushroom yield

The average total mushroom yield over the course of two flushes when peat was used as a casing was 33.4 kg/m^2 (Fig. 1). Replacing part of or all peat with other components generally slightly reduced yield (average yield for all other casings: 31.9 kg/m^2) and this reduction in yield was better explained by a model with separate factor levels for each casing than by—simplified—models with factors for the presence/absence or volumetric percentage of each component ($\chi^2 = 143.60$, d.f. = 29, $P < 0.0001$ and $\chi^2 = 70.59$, d.f. = 28, $P < 0.0001$, respectively). This indicates

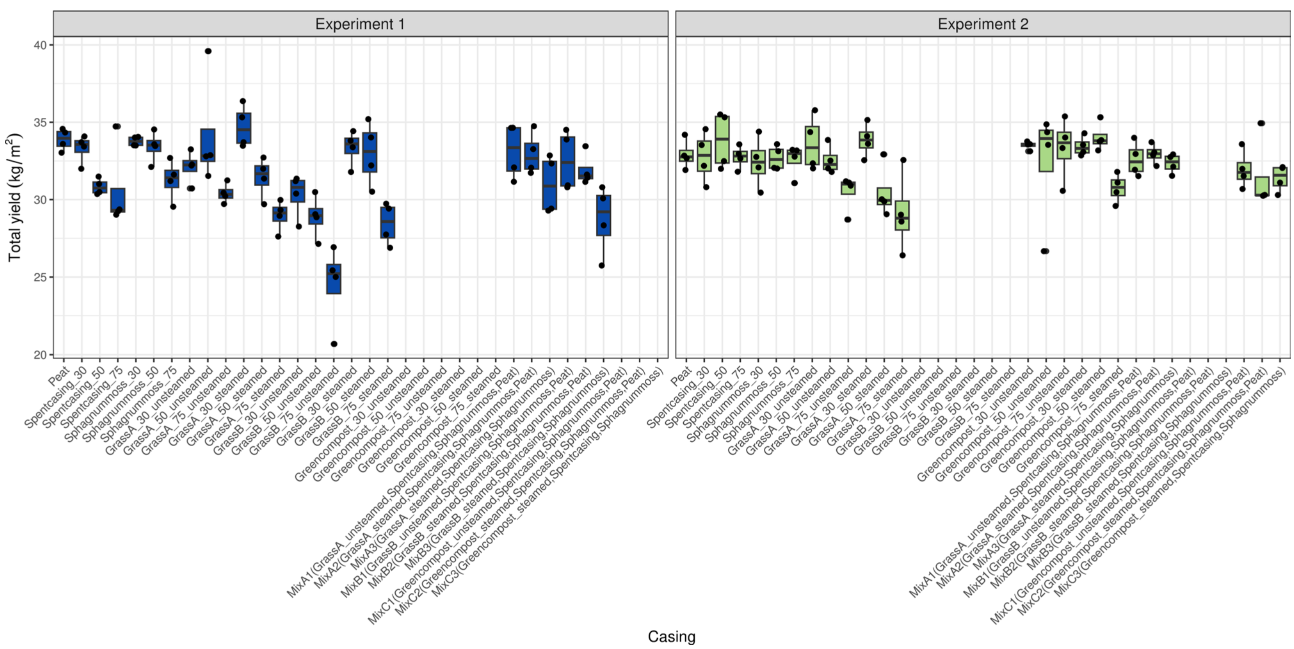

Fig. 1. Effect of casing on total mushroom yield (kg/m^2) over the course of two flushes. Numbers behind individual constituents indicate the volumetric percentage of peat that was replaced by that constituent. Black dots indicate the yield from individual replicate growing boxes, whereas boxplots provide the five point summary for each casing per experiment. See Table 3 for details about the composition of each casing.

that there were significant non-linear effects and/or interactions between the components and their treatment with respect to their effect on yield.

Overall, higher percentages of peat replacement led to higher reductions in yield. However, for both *Sphagnum* moss and unsteamed green compost, replacements as high as 75% did not have a significant effect on yield. Spent casing and unsteamed grass A reduced yield only at 75% replacement, whereas unsteamed grass B reduced yield already at 30% replacement. Steaming had a different effect depending on the component to which it was applied: for green compost and grass A, it increased the reductive effect (steamed green compost reductive at 75%, steamed grass A reductive at 50% replacement), while for grass B, it decreased the reductive effect (reductive only at 75% replacement). Casing mixtures that contained peat at 25% did not significantly reduce yield compared with peat. However, when peat was taken out altogether, yield was consistently reduced, with the largest reductive effect in MixB3 (predicted reduction: −1.74, −4.67, and −2.01 kg/m^2 in mixA3, mixB3, and mixC3, respectively. $P < 0.05$ in all cases).

Complex effects of casing on harvest dynamics

An important criterium for mushroom growers is how equally distributed the mushroom yield is over the different days within a flush, as well as between flushes. To assess the effect of casing on these properties, we calculated the coefficient of variation (CV) in yield per box both within each flush and between flushes (see Figure S1 for overall dynamics of yield over time). The CV within flush (Figure S2) was significantly affected by the presence/absence of the individual grass and compost components (i.e. in a different way by unsteamed and steamed grass A, unsteamed and steamed grass B and unsteamed and steamed green compost), flush, as well as the interactions between these components and flush. Flush had by far the largest effect, with much higher variation in yield in the second flush ($F_{1,385} = 140.36$, $P < 0.0001$; predicted CV for peat 0.71 *vs.* 1.18 for flush 1 and flush 2, respectively). There were significant interactions between grass type, steaming and flush. In the first flush, grass B increased the CV relative to peat while green compost and steamed grass A decreased the CV. In the second flush, both grass A and grass B decreased the CV relative to peat. Experiment had an important effect again, the difference in CV between flushes being much larger in experiment 2.

The CV between flushes (Figure S3) was affected by the volumetric percentage of all components, including peat, where a higher proportion of a component increased the CV, with the exception of unsteamed grass B, which did not have a significant effect ($P < 0.004$ in all other cases). This implies that mixed casing soils have a more similar yield across flushes than casing soils consisting of mostly one component.

Blotched yield strongly increased for casings with unsteamed grass B

The mean blotched yield per box was low (1.03 kg/m^2) (Fig. 2). However, there were large differences in blotch incidence between casings. Like for yield, a model with separate levels of a single factor better explained the data than simplified models with factors for the presence/absence or volumetric percentage of each component ($\chi^2 = 60.61$, d.f. = 29, $P = 0.0005$ and $\chi^2 = 182.67$, d.f. = 29, $P < 0.0001$, respectively). All casings with an unsteamed grass B component had a very high blotch incidence (8.90, 11.33, 11.23 and 8.89 kg/m^2 increase relative to peat

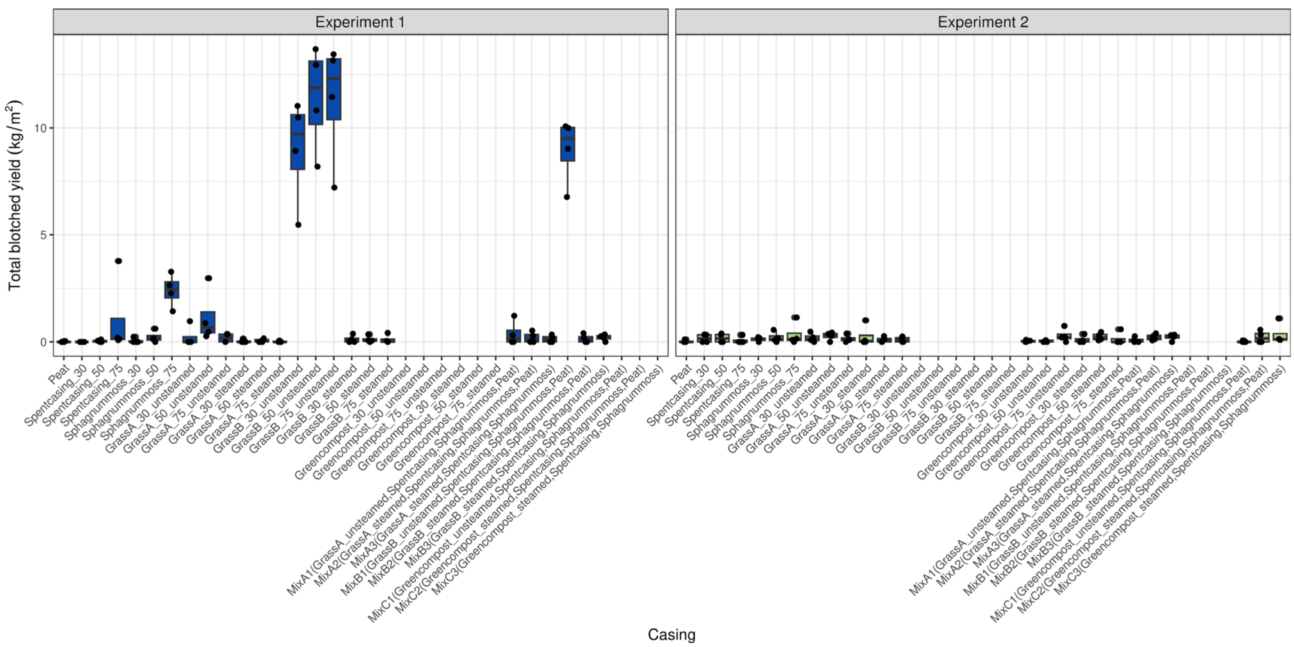

Fig. 2. Effect of casing on total blotched mushroom yield (kg/m^2) over the course of two flushes. Numbers behind individual constituents indicate the volumetric percentage of peat that was replaced by that constituent. Black dots indicate the yield from individual replicate growing boxes, whereas boxplots provide the five point summary for each casing per experiment. See Table 3 for details about the composition of each casing.

for 30, 50 and 75% peat replacement and mixB1, respectively; $P < 0.0001$ in all cases). However, casing with 75% *Sphagnum* moss also had a significantly increased blotch incidence (1.35 kg/m^2 increase, $P = 0.0008$), which appears to be largely driven by experiment 1.

Components affect casing physico-chemical properties in a consistent manner

Individual components affected the physico-chemical properties of the compound casing in a consistent manner, i.e. casings with a more similar composition clustered together more closely in non-parametric multi-dimensional space, and a higher percentage replacement of peat was associated with a larger distance from peat (Fig. 3; compare different colors *versus* squares/circles/triangles of one color). Casings containing spent casing or green compost were most similar to peat and were generally associated with a higher moisture content at low suction pressure (−10 cm water column) and adhesiveness, whereas casings containing grass or *Sphagnum* moss were associated with higher readily available water and hardness.

We used PERMANOVA to assess whether individual components had a significant effect on the physico-chemical profile of the compound casing, and compared the performance of—simplified—models with presence/absence *vs.* volumetric percentage of each component as explanatory variables. The minimal adequate model contained terms for the presence/absence of spent casing, *Sphagnum* moss, green compost and grass in general (spent casing: $F_{1,30} = 8.90$, $P = 0.004$; *Sphagnum* moss: $F_{1,30} = 8.20$, $P = 0.004$; green compost: $F_{1,30} = 6.96$, $P = 0.014$; grass: $F_{1,30} = 55.02$, $P = 0.001$). No significant effects of grass type or steaming were found. Absence or presence of grass explained the majority of all variance ($R^2 = 0.51$), whereas presence or absence of the three other components explained a more limited part of all variance ($R^2 = 0.06–0.07$). Note, however, that this latter observation may partially be driven by the fact that fewer casings contained these components (e.g., 18 casings contained grass, whereas only 12 casings contained *Sphagnum* moss).

Moisture content at low suction pressure correlates with yield

To evaluate the effect of physico-chemical properties on mean total yield, we used multiple regression. Because moisture content at −10 cm water column and mean water fraction, as well as hardness and adhesiveness, were strongly correlated ($r = 0.95$ and −0.83 for each pair, respectively), we included only moisture content at −10

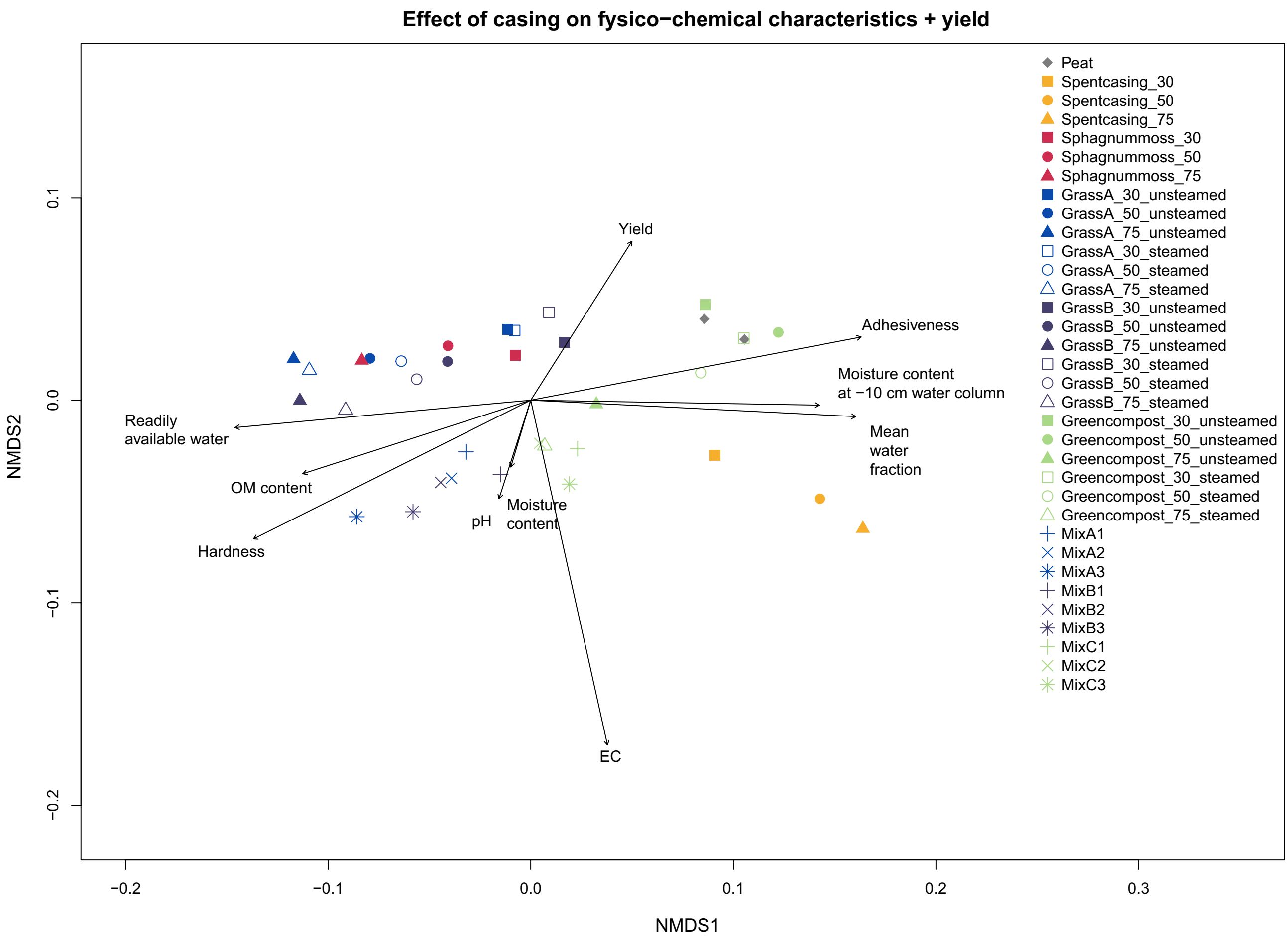

Fig. 3. Effect of casing on physico-chemical characteristics and mean mushroom yield (kg/m2) over the course of two flushes. The presence or absence of grass (blue and purple symbols) in the casing explains the physico-chemical properties for > 50% (k = 3, stress: 0.048).

Constituent	WHC starting material	WHC saturated material
Peat	3.81	5.17
Spent casing	2.98	5.23
Sphagnum moss	6.20	14.86
Grass A	2.02	11.47
Grass B	1.95	9.97
Green compost	1.41	3.12

Table 1. Mean water holding capacity of individual constituents (g water/g dry matter).

Constituent	Zn	Pb	Ni	Hg	Cu	Cr	Cd	As	Pesticides
Peat	45.6 ± 39%	2.5 ± 32.2%	1.7 ± 32.6%	0.017 ± 54.6%	13.2 ± 44.4%	4.5 ± 36.6%	0.45 ± 16%	2.2 ± 25.4%	-
Spent casing	55.2	**18.2**	**2.6**	**0.028**	15.8	4.8	*0.33*	1.70	-
Sphagnum moss	32.5	**7.5**	1.7	**0.055**	*4.0*	1.4	*0.23*	*0.87*	-
Grass A	30.4	*0.83*	1.6	0.013	*5.5*	1.6	*0.06*	*0.15*	0.022 mg/kg fluxapyroxad
Grass B	26.3	*0.83*	**5.4**	0.030	7.3	**7.2**	*0.11*	*0.33*	0.023 mg/kg fluopicolide
Mix B3	53.6	**16.8**	2.0	**0.027**	13.6	4.7	0.41	1.60	0.12 mg/kg metrafenone

Table 2. Heavy metals (mg/kg DS) and pesticide residues in individual constituents from experiment 1 and mix B3 (steamed grass B, spent casing and *Sphagnum* moss). Concentrations in bold are higher than the concentration of the same metal in peat plus percentage measurement uncertainty. Concentrations in italics are lower than the concentration of the same metal in peat minus percentage measurement uncertainty.

cm water column and adhesiveness in our models. We chose to use −10 cm water column because it is less strongly correlated with readily available water than mean water fraction (which is expected, because mean water fraction is an aggregate metric that contains information on both moisture content at low suction pressure, as well as the decrease in moisture content when suction pressure is increased). In turn, adhesiveness was less strongly correlated with moisture content at −10 cm water column than hardness, suggesting it might have more independent explanatory power. Model simplification yielded a minimal adequate model with only moisture content at −10 cm water column as an explanatory variable ($F_{1,32}=11.65$, $P=0.002$). However, readily available water, adhesiveness and EC were all (borderline) significant in combination with moisture content at −10 cm water column and each other, suggesting that these variables may have some additional explanatory power.

We measured bulk density of casings only in the first experiment. As a result, we were unable to include this parameter in the above analyses. However, bulk density is thought to be an important driver of mushroom yield, so we explored its relationship with the other physico-chemical properties, as well as mushroom yield, by an NMDS plot of the data from experiment 1 (Figure S5). This plot confirmed that bulk density is positively correlated with yield. However, this correlation was weak ($r=0.37$), whereas the correlation between bulk density and moisture content at −10 cm water column (the main explanatory variable from the above model) was relatively strong ($r=0.71$). The correlation between moisture content at −10 cm water column and yield was moderate ($r=0.45$), suggesting that this property may be a better predictor of yield than bulk density.

Mean blotch incidence was negatively correlated with yield ($r=0.60$; Figure S4b). However, blotch incidence did not correlate strongly with any of the explanatory variables ($r<0.30$ in all cases) and was mainly associated with the presence of unsteamed grass B in the casing material. Given that grass B is no different from grass A, and unsteamed grass is no different from steamed grass, in terms of physico-chemical properties (see previous sections and Fig. 3), we did not formally test for the effect of these properties on blotch, because such modelling is likely to detect spurious associations. Instead, we conclude that the measured physico-chemical properties are not a good predictor of blotch incidence, at least within the context of our experiments.

Physico-chemical characteristics of individual constituents

We determined the mean water holding capacity (WHC) of the individual constituents. WHC of all constituents was significantly different from peat for both the starting material and the saturated material, with the exception of spent casing in case of the saturated material (Table 1; general linear mixed effect models with experiment as a random effect: $F_{5,38}=196.97$, $P<0.0001$ and $F_{5,38}=725.76$, $P<0.0001$, respectively). For the saturated material, the same divide exists as for the overall physico-chemical analyses: values for peat, spent casing and green compost were relatively similar, and values for *Sphagnum* moss and the grasses were relatively similar. The latter components have a high water holding capacity but a low moisture content already at −10 cm water column, suggesting they can take up a high amount of water but do not effectively retain it.

Individual constituents and mix B3 (steamed grass B, spent casing and *Sphagnum* moss) differed in their heavy metal and pesticide content (Table 2). Spent casing, *Sphagnum* moss and mix B3 contained elevated levels of lead and mercury compared with peat, whereas grass B contained elevated levels of nickel and chromium. The most striking differences were those for lead in spent casing and mix B3: the levels of this element were more than six times higher in these casing materials than in peat. Both grasses and mix B3 contained low levels of fungicides. However, the fungicide that was found in grass B was not detected in mix B3, and vice versa. This is likely due to the fact that the measured concentrations are close to the detection threshold, and this type of measurement is known to vary substantially between technical replicates.

Discussion

We previously identified several materials that can be used to partially replace peat in casing soil without having a substantial negative effect on mushroom yield or disease suppressiveness against inoculated bacterial blotch[19]. Here, we asked what percentage of peat can be replaced by these materials (spent casing, *Sphagnum* moss, and a processed grass component), as well as green compost, without having a negative effect on yield, harvest properties and intrinsic blotch incidence. We also asked what the effect of combining these materials is. Moreover, we investigated how physicochemical properties differed between casing soils, and which of these differences were correlated with yield and blotch incidence. Finally, we measured contaminant levels in each material in order to determine whether it can safely be used for mushroom cultivation.

We found that *Sphagnum* moss and green compost can be used to replace up to 75% of peat without having a significant negative effect on total mushroom yield over the course of two harvest cycles. Spent casing and grass A (sourced from nature areas) could be used to replace up to 50% of peat, whereas grass B (sourced from airports) had a significant negative effect on yield already at 30% replacement. These effects were modified by steaming of the component prior to use, which had a positive effect in case of grass B and a negative effect in case of green compost and grass A. 1:1:1:1 mixtures of either grass A, grass B or green compost with spent casing, *Sphagnum* moss and peat performed no worse than peat, whereas 1:1:1 mixtures of either grass A, grass B or green compost with spent casing and *Sphagnum* moss resulted in a significantly reduced yield. This indicates that casing materials can also be combined to replace up to 75% of fresh peat, but are—in the current combinations—unable to entirely emulate peat.

Blotch incidence was low for all casings, except for those that contained unsteamed grass B. This suggests that this material contained high endogenous levels of *P. gingeri*, the causative agent of ginger blotch, which were eradicated by the steam treatment. The yield was also higher after the steam treatment, but in our previous study we did not find a direct association between blotch and yield[21]. One explanation for this discrepancy may be, that blotch infection was more severe in the current study and yield is affected only above a certain pathogen threshold. Nonetheless, the opposite effect of steaming of the different grass types (which had a very similar physicochemical profile) on yield suggests that (partial) eradication of the endogenous microbiome can have a positive or negative effect on yield. However, it may be hard to predict a priori the effect of steaming.

The variation in yield within flush was consistently larger in the second flush than in the first flush. This is probably due to the fact that in the second flush production is already diminishing, due to a limitation of specific nutrients in the compost. There were also several complex effects of individual components in interaction with flush on within flush variation in yield. However, these effects were more limited than the effect of flush per se. The same was true for the variation in yield between flushes. Our data suggest that more heterogeneous casing mixtures reduce this metric, possibly because such mixtures result in a more balanced physicochemical profile over time and thus in a prolonged period of fructification. Repeating experiments with increased sample sizes and repetitions would be required to assess the generality of the observed patterns.

Overall physicochemical properties of peat, spent casing, and green compost were similar to each other, as were those of grass and *Sphagnum* moss. The first category of materials was characterised by a high moisture content at low suction pressure but low readily available water and water holding capacity after saturation, whereas the second category was characterised by the opposite properties. This combination of properties is somewhat puzzling at first sight, but may reflect different shapes of the water retention curve, analogous to those commonly observed for clay vs. sand[24]: whereas the first category of materials has a relatively low water holding capacity at saturation, it is much better at retaining the water at increased suction pressures, which presumably shows already at the relatively low pressure of −10 cm water column (−10 hPa). Nonetheless, the negative relationships between readily available water (i.e. decrease in moisture content between −10 cm and −50 cm water column) and moisture content at −10 cm water column, as well as yield, are highly unexpected. Possibly, suction force of the mycelium is so high, that readily available water is consumed too easily, such that materials that score high on this property are quickly desiccated when used as casing.

Multiple regression indicated that mushroom yield is associated with moisture content at low suction pressure, although readily available water, EC and adhesiveness also appear to be factors of influence. The decreased yield of casings that contained spent casing relative to those that contained only fresh peat may best be explained by their relatively high EC. EC has previously been identified as one the main limiting factors in the search for alternative casing materials and attempts have been undertaken to remedy this problem in affected materials[16]. By contrast, grass and *Sphagnum* moss appear to have nearly identical physico-chemical properties, but are nonetheless associated with different reductions in yield when used at high proportions. Possibly, *Sphagnum* moss contains a more beneficial microbiome for mushroom cultivation. Interestingly, we previously found that peat and *Sphagnum* microbiomes are highly similar at the genus level, whereas grass contains a unique microbiome that is dominated by the fungus *Pseudeurotium*[19].

Several alternative constituents contained increased levels of non-essential heavy metals. Spent casing and *Sphagnum* moss contained increased levels of lead and mercury, whereas grass B—which was sourced from airports—contained increased levels of nickel and chromium. This latter observation is in line with a recent study that used *Sphagnum* moss bags for biomonitoring of aircraft emissions: both nickel and chromium were in the top five of metals that were significantly enriched (up to eight times higher concentrations after exposure) at sampling sites near the airport[25]. Still, for all three materials, the measured values were well below the maximum values permitted for compost by the Dutch government[26]. Nonetheless, it should be taken into account that mushrooms have a very high ability to bioconcentrate metals[27,28]. For example, *Agaricus* grown on Fe-enriched substrate contained an up to 100 × higher concentration of Fe than the substrate itself. Fortunately, lead and mercury do not appear to accumulate to the same extent in *Agaricus*[29], and the concentrations that we found are unlikely to result in concentrations in the mushroom that exceed the European norms[30–34]. Nickel and chromium are actively excluded from at least wild mushrooms[35,36], and should thus not pose additional risks either. More definitive statements about food safety would require measurements of the metal content of the mushrooms themselves, rather than the casing soil.

For the fungicides, it is not immediately obvious whether the levels that we detected are safe. The allowed daily intake for fluxapyroxad, fluopicolide and metrafenone is 0.02, 0.08 and 0.25 mg/kg body weight, respectively[37–39]. Maximum residue levels in cultivated mushrooms have not been established for fluxapyroxad and fluopicolide (but range from 0.01 to 20 mg/kg for other foodstuffs, and are set at 0.01 mg/kg for unrecognised pesticides) and have been set at 0.4 mg/kg for metrafenone[40]. Assuming a bioconcentration factor of < 60 × for all three chemicals, consumption of up to 1 kg of mushrooms a day should be within the aforementioned limits for a 70 kg person. This suggests that the measured concentrations should not pose a problem. Metal and fungicide concentrations were not determined for green compost. For a fair and complete comparison of the components, these analyses should also have been performed.

In summary, we have shown that peat can be replaced by up to 75% of alternative casing materials without a significant effect on yield or ginger blotch incidence in small-scale growing experiments (with the exception of an unsteamed grass component, which was associated with a high blotch incidence). *Sphagnum* moss and green compost could replace peat up to 75% without supply of other constituents, and thus appear to be the most promising materials. All tested 1:1:1:1 mixtures—containing either grass or green compost in combination with spent casing, *Sphagnum* moss and peat—had a comparable effect. The final choice for a peat-alternative in casings will depend on a combination of economic and sustainability aspects. For grass fibers, sphagnum moss, and spent casing, life cycle assessments are underway to estimate the environmental impact of these alternatives (Goglio et al., in preparation). The use of spent casing may be less suitable for (partial) replacement of fresh peat, because use of this material strictly requires pasteurisation, and this process is associated with prohibitive costs. Leaving out peat from the mixtures altogether did significantly reduce yield. This might be due to a difference in crucial physicochemical properties such as water retention properties and EC, or due to a difference in microbiological composition. The first hypothesis could be tested by for example leaching out spent casing to reduce the EC of this component[16], while the second hypothesis could be tested by adding an extract from fresh peat to the casing.

Our work underlines the importance of not only physicochemical, but also microbiological properties of casing soils. Grass components with a similar physicochemical profile had a very different effect on mushroom growth and yield, and these differences were cancelled out by a steaming treatment. Because we do not know a priori whether a component contains a beneficial or an antagonistic microbiome, steaming seems the wiser course of action, because it prevents disease, even if it may also cause a—limited—reduction in yield. Follow-up work should assess in more detail what the contribution of different microbes is to the parameters that we studied and how the different casing materials perform in the presence of pathogens.

Methods

Casing soils

The casing soil mixtures used in this study are composed of different raw materials, which were previously found to have no or only limited negative effect on mushroom yield and blotch incidence, when used to partially replace peat. The materials are described in detail in the previous study[19]. Briefly, spent casing is casing from the last cycle of an earlier mushroom cropping, and is mechanically separated from the spent mushroom compost following cook-out (steaming) of the growing chambers. *Sphagnum* moss (*Sphagnum spp.*) is cultivated on degraded peatlands, can be sustainably harvested every 20—30 years[41] and is not steamed prior to use. Grass A and grass B are produced by NewFoss using a proprietary biorefining procedure that converts non-woody biomass into lignocellulosic fibres. The raw material for grass A comes from nature areas, whereas the raw material for grass B comes from airports. Green compost is sourced by Kekkilä BVB. The grasses and compost were used in our study in both unsteamed and steamed form. Steaming was performed at 70 °C for 12–13 h.

In the previous study, 30, 25 and 50% of peat was replaced with spent casing, *Sphagnum* moss and grass A, respectively. Here, we varied the proportion of replacement from 30–75% for all individual components and used different combinations of components to replace peat (Table 3). Because grass B was not available at the time of the second experiment, green compost was used instead.

Cultivation experiments

Mushrooms were grown in an experimental mushroom cultivation facility (Unifarm, Wageningen University and Research) as previously described[42]. In short, plastic boxes with an area of 0.1 m^2 were filled with 8.5 kg of compost that was fully colonized by *A. bisporus* strain A15 (Sylvan). 5 L of casing soil mixed with 100 g/kg of phase III compost was applied on top, the temperature was gradually lowered to 18 °C until pinhead formation started, and mushroom yield and blotch incidence were recorded over the course of two flushes. Both yield and blotch incidence were expressed as mushroom weight in kg/m^2. Two experiments were performed, in both of which 25 different casings were tested, with four replicate boxes per casing. Casing soils containing grass B (13–19 and 29–31) were used only in experiment 1, casing soils containing green compost (20–25 and 32–34) were used only in experiment 2. All other casings were used in both experiments.

Physico-chemical analyses

In the first experiment physico-chemical properties of all casing soils were determined. In the second experiment, these properties were determined only for casing soils containing green compost and again for 100% peat. More

Casing		Peat	Spent casing	*Sphagnum* moss	Grass A		Grass B		Green compost	
					-	+	-	+	-	+
1	**Peat**	**100**								
2	Spent casing_30	70	30							
3	Spent casing_50	50	50							
4	Spent casing_75	25	75							
5	*Sphagnum* moss 30	70		30						
6	*Sphagnum* moss 50	50		50						
7	*Sphagnum* moss 75	25		75						
8	Grass A_30_unsteamed	70			30					
9	Grass A_50_unsteamed	50			50					
10	Grass A_75_unsteamed	25			75					
11	Grass A_30_steamed	70				30				
12	Grass A_50_steamed	50				50				
13	Grass A_75_steamed	25				75				
14	Grass B_30_unsteamed	70					30			
15	Grass B_50_unsteamed	50					50			
16	Grass B_75_unsteamed	25					75			
17	Grass B_30_steamed	70						30		
18	Grass B_50_steamed	50						50		
19	Grass B_75_steamed	25						75		
20	Green compost_30_unsteamed	70							30	
21	Green compost_50_unsteamed	50							50	
22	Green compost_75_unsteamed	25							75	
23	Green compost_30_steamed	70								30
24	Green compost_50_steamed	50								50
25	Green compost_75_steamed	25								75
26	Mix A1 (Grass A unsteamed, Spent casing, *Sphagnum* moss, Peat)	25	25	25	25					
27	Mix A2(Grass A steamed, Spent casing, *Sphagnum* moss, Peat)	25	25	25		25				
28	Mix A3(Grass A steamed, Spent casing, *Sphagnum* moss)		33	33		33				
29	Mix B1(Grass B unsteamed, Spent casing, *Sphagnum* moss, Peat)	25	25	25			25			
30	Mix B2(Grass B steamed, Spent casing, *Sphagnum* moss, Peat)	25	25	25				25		
31	Mix B3(Grass B steamed, Spent casing, *Sphagnum* moss)		33	33				33		
32	Mix C1(Green compost unsteamed, Spent casing, *Sphagnum* moss, Peat)	25	25	25					25	
33	Mix C2(Green compost steamed,Spent casing, *Sphagnum* moss, Peat)	25	25	25						25
34	Mix C3(Green compost steamed, Spent casing, *Sphagnum* moss)		33	33						33

Table 3. Composition of casing soils used in this study (% v/v). Each casing soil contains an additional 3% (v/v) of sugar lime. Bold casing numbers indicate casings that were only used in the first experiment, italic casing numbers indicate casings that were only used in the second experiment.

specifically, we determined moisture content in the supplied material (% wet weight), pH, electrical conductivity (EC) (µS/cm), hardness (g), adhesiveness (mJ), and organic matter (OM) content (% dry matter). Bulk density (kg/m^3) was determined only in the first experiment. Measurements were performed in 1 (bulk density), 2 (pH, EC, OM content), or 6 (moisture content, hardness, adhesiveness) technical replicates. Water retention characteristics of each material were determined by measuring moisture content at −3.2, −10, −32, −50 and −100 cm water column, which corresponds with suction pressures of −3.2 to −100 hPa and pF values of 0.5 to 2[43]. Mean water fraction was calculated across all suction pressures. Moisture content at −10 cm was taken as a measure of water fraction in the saturated material, whereas readily available water was calculated as the difference in water fraction between −10 and −50 cm water column. Water buffer was calculated as the difference in water fraction between −50 and −100 cm water column. However, because these values were low (1, 2 or 3%) and not measured with high accuracy, we decided to exclude this measure from further analyses because it would only introduce noise.

For each raw material, water holding capacity (WHC) of both the start material and the saturated material was determined (g water/g dry matter). WHC was determined only in the first (peat and grass B), only in the second (green compost), or in both experiments (other constituents). In all cases, WHC of the starting material was determined in 1 technical replicate, and WHC of the saturated material was determined in 5 technical replicates. For WHC determination on the saturated material, the exact same sample was used as for determination on the start material. Material was saturated with water for 2 h and then left to drain for 2 h prior to measurement. In the first experiment, heavy metal – Zn, Pb, Ni, Hg, Cu, Cr, Cd and As – content (mg/kg DM) of the raw materials was determined by ICP-MS in 1 technical replicate. Pesticide (fluxapyroxad, fluopicolide, and metrafenone) content was determined for peat, spent casing, *Sphagnum* moss, grass A, grass B and mix B3 by Normec Groen Agro Control[44].

Statistical analyses

Data from experiment 1 and 2 were analysed together. General linear mixed effect models (with experiment as a random effect) were used to assess the effect of casing on total yield. Three different modelling approaches were compared. In the first approach, a model was fitted with a single factor with separate levels for each casing. In the second approach, a model was fitted with separate factor levels for the presence *vs.* absence of each individual constituent. In the third approach, a model was fitted with separate continuous factor levels for the volumetric percentage of each individual constituent. For the latter two approaches, model simplification was then used to find the minimal adequate model, and these models were compared with the model from the first approach using the Aikaike Information Criterion (AIC). Statistics are reported from the most parsimonious model.

A similar approach was used for analysing total blotched yield. Variation in yield was captured by two different metrics. Coefficient of variation (CV) in yield within flush was calculated as the standard deviation in yield (per day) within flush, divided by the mean yield (per day) within flush, for each replicate box separately. Similarly, coefficient of variation (CV) in yield between flushes was calculated as the standard deviation in (total) yield of the two different flushes, divided by the mean (total) yield of the two different flushes, for each replicate box separately. CVs were analysed using the same approach as above.

We used permutational analysis of variance (PERMANOVA) to test for the effect of individual components on the overall physico-chemical profile of the casings. To enable joint analysis of all physico-chemical data, we used the mean value from the technical replicates where applicable. Model simplification was done using Akaike information criterion (AIC), and we compared a minimal model with presence/absence of each component with a minimal model with volumetric percentage of each component. To assess the effect of physico-chemical properties on yield, we used multiple regression. We first checked for collinearity between explanatory variables and used this information to select a sensible subset of variables for further modelling. We then fitted separate models with quadratic terms and different subsets of all two-way interaction terms and performed model simplification. These two approaches yielded the same minimal model, which only contained main effects.

In all cases, model assumptions were checked. For post-hoc testing, we evaluated only a limited number (k−1) of meaningful contrasts such that we did not have to correct for multiple testing. Statistical analyses were performed in R version 4.1.0[45].

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Received: 28 February 2025; Accepted: 15 September 2025
Published online: 23 October 2025

References

1. Joosten, H. & Clarke, D. *Wise Use of Mires and Peatlands - Background and Principles including a Framework for Decision-making.* (NHBS Ltd., 2002).
2. Maher, M., Prasad, M. & Raviv, M. Organic Soilless Media Components. In *Soilless Culture* 459–504 (2008).
3. Altmann, M. Socio-economic impact of the peat and growing media industry on horticulture in the EU. (2008).
4. Silva, J. P., Phillips, L., Jones, W., Eldridge, J. & O'Hara, E. LIFE and Europe's wetlands: Restoring a vital ecosystem. (European Commission, 2007).
5. Joosten, H., Tapio-Biström, M.-L. & Tol, S. Peatlands - guidance for climate change mitigation through conservation, rehabilitation and sustainable use. (Food and Agriculture Organization of the United Nations Wetlands International, 2012).
6. IUCN National Committee UK, P. P. UK Peatland Strategy 2018–2040. (2018).
7. Greifswald Mire Centre & Wetlands International. Higher ambition for Peatlands in the EU Nature Restoration Law Proposal. (2022).
8. Hirschler, O. & Osterburg, B. Peat extraction, trade and use in Europe: A material flow analysis. *Mires Peat* https://doi.org/10.19189/MaP.2021.SNPG.StA.2315 (2022).
9. Peters, J. & Von Unger, M. Peatlands in the EU regulatory environment: survey with case studies on Poland and Estonia. (2017).
10. Glastuinbouw Nederland. *Veenconvenant moet leiden tot snellere reductie milieu-impact potgrond en substraten.* https://www.glastuinbouwnederland.nl/nieuws/veenconvenant-moet-leiden-tot-snellere-reductie-milieu-impact-potgrond-en-substraten/ (2022).
11. McGee, C. F. Microbial ecology of the Agaricus bisporus mushroom cropping process. *Appl. Microbiol. Biotechnol.* **102**, 1075–1083. https://doi.org/10.1007/s00253-017-8683-9 (2018).
12. Eastwood, D. C. et al. Environmental regulation of reproductive phase change in Agaricus bisporus by 1-octen-3-ol, temperature and CO(2). *Fungal Genet. Biol.* **55**, 54–66. https://doi.org/10.1016/j.fgb.2013.01.001 (2013).
13. Baars, J. J. P., Scholtmeijer, K., Sonnenberg, A. S. M. & van Peer, A. V. Critical Factors Involved in Primordia Building in Agaricus bisporus: A Review. *Molecules*. **25**. https://doi.org/10.3390/molecules25132984 (2020).
14. Rainey, P. B. *The involvement of Pseudomonas putida in basidiome initiation of the cultivated mushroom Agaricus bisporus* PhD thesis, University of Canterbury (1989).
15. Pardo-Gimenez, A., Zied, D. C. & Pardo-Gonzalez, J. E. The use of peat as casing material in mushroom cultivation. In *Peat: Formation, Uses and Biological Effects* (eds C. Draguhn & N. Ciarimboli) (2012).
16. Pardo-Gimenez, A., Pardo-Gonzalez, J. E. & Cunha-Zied, D. Casing Materials and Techniques in Agaricus bisporus Cultivation. In *Edible and medicinal mushrooms: technology and applications* (eds D. Cunha-Zied & A. Pardo-Gimenez) (John Wiley & Sons Ltd., 2017).
17. Noble, R. & Dobrovin-Pennington, A. The use of peat as casing material in mushroom cultivation. (AHDB Horticulture, 2015).
18. Dittrich, C. et al. Extrusion of different plants into fibre for peat replacement in growing media: Adjustment of parameters to achieve satisfactory physical fibre-properties. *Agronomy* **11**, 1185 (2021).

19. Taparia, T., Hendrix, E., Nijhuis, E., de Boer, W. & van der Wolf, J. Circular alternatives to peat in growing media: A microbiome perspective. *J. Clean. Prod.* **327**. https://doi.org/10.1016/j.jclepro.2021.129375 (2021).
20. Wells, J. M. et al. Postharvest discoloration of the cultivated mushroom *Agaricus bisporus* Caused by *Pseudomonas tolaasii*, *P. 'reactans'*, and *P. 'gingeri'*. *Phytopathology* **86**, 1098–1104 (1996).
21. Taparia, T. et al. Comparative studies on the disease prevalence and population dynamics of ginger blotch and brown blotch pathogens of button mushrooms. *Plant Dis.* **105**, 542–547. https://doi.org/10.1094/PDIS-06-20-1260-RE (2021).
22. Benito, M., Masaguer, A., Moliner, A. & De Antonio, R. Chemical and physical properties of pruning waste compost and their seasonal variability. *Bioresour. Technol.* **97**, 2071–2076. https://doi.org/10.1016/j.biortech.2005.09.011 (2006).
23. Zhang, J.-H. et al. Evaluation of organic solid wastes composts as peat substitutes for seedling production. *J. Plant Nutr.* **36**, 1780–1794. https://doi.org/10.1080/01904167.2013.805222 (2013).
24. Quang Chon, N. Soil Water Retention - pF curve. (2021).
25. Turgut, E. T. et al. Elemental characterization of general aviation aircraft emissions using moss bags. *Environ. Sci. Pollut. Res. Int.* **26**, 26925–26938. https://doi.org/10.1007/s11356-019-05910-8 (2019).
26. Nederlandse Overheid.
27. Orywal, K. et al. Health risk assessment of exposure to toxic elements resulting from consumption of dried wild-grown mushrooms available for sale. *PLoS ONE* **16**, e0252834. https://doi.org/10.1371/journal.pone.0252834 (2021).
28. Falandysz, J. et al. Survey on composition and bioconcentration potential of 12 metallic elements in King Bolete (Boletus edulis) mushroom that emerged at 11 spatially distant sites. *J. Environ. Sci. Health B* **46**, 231–246. https://doi.org/10.1080/03601234.2011.540528 (2011).
29. Tüzen, M., Özdemir, M. & Demirbas, A. Heavy metal bioaccumulation by cultivated Agaricus bisporus from artificially enriched substrates. *Z. Lebensm. Unters. Forsch. A* **206**, 417–419 (1998).
30. European Commission. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance) (2006).
31. European Commission. Commission Regulation (EU) No 488/2014 of 12 May 2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of cadmium in foodstuffs Text with EEA relevance. pp. 75–79 (2014).
32. European Commission. Commission Regulation (EU) 2015/1005 of 25 June 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of lead in certain foodstuffs (Text with EEA relevance). pp. 9–13 (2015).
33. European Commission. Commission Regulation (EU) 2015/1006 of 25 June 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs (Text with EEA relevance). pp. 14–16 (2015).
34. European Commission. Commission Regulation (EU) 2018/73 of 16 January 2018 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for mercury compounds in or on certain products (Text with EEA relevance.) (2018).
35. Siric, I. et al. Heavy metal bioaccumulation by wild edible saprophytic and ectomycorrhizal mushrooms. *Environ. Sci. Pollut. Res. Int.* **23**, 18239–18252. https://doi.org/10.1007/s11356-016-7027-0 (2016).
36. Garcia, M. A., Alonso, J. & Melgar, M. J. Bioconcentration of chromium in edible mushrooms: Influence of environmental and genetic factors. *Food Chem. Toxicol.* **58**, 249–254. https://doi.org/10.1016/j.fct.2013.04.049 (2013).
37. European Food Safety Authority. Conclusion on pesticide peer review regarding the risk assessment of the active substance fluopicolide. *EFSA J.* **7**, 299r. https://doi.org/10.2903/j.efsa.2009.299r (2009).
38. European Food Safety Authority. Reasoned opinion on the modification of the existing MRLs for metrafenone in various crops. *EFSA J.* **11**, 3075. https://doi.org/10.2903/j.efsa.2013.3075 (2013).
39. European Food Safety Authority *et al.* Review of the existing maximum residue levels for fluxapyroxad according to Article 12 of Regulation (EC) No 396/2005. *EFSA J.* **18** , e05984 (2020). https://doi.org: https://doi.org/10.2903/j.efsa.2020.5984
40. European Parliament. Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. (2009).
41. Silvan, N., Silvan, K., Näkkilä, J., Tahvonen, R. & Reinikainen, O. Renewability, use and properties of Sphagnum biomass for growing media purposes. In *Book of abstracts. The 14th International Peat Congress, Peatlands in Balance.* 3–8 (2012).
42. Taparia, T. et al. Casing soil microbiome mediates suppression of bacterial blotch of mushrooms during consecutive cultivation cycles. *Soil Biol. Biochem.* **155**. https://doi.org/10.1016/j.soilbio.2021.108161 (2021).
43. NEN. NEN-EN 13041:2011 en. In *Soil improvers and growing media - Determination of physical properties - Dry bulk density, air volume, water volume, shrinkage value and total pore space* (2011).
44. Normec Groen Agro Control. *Analyselijst grond en substraten versie 5*. https://normecgroenagrocontrol.com/analyselijsten/ (2024).
45. R Core Team. R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria, 2022).

Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 101000651. The project was also financially supported by the Dutch Ministry of Economic Affairs via the 'Topsector program' (Grant KB-50-006-008-WPR). We would like to thank Patricia van der Zouwen for technical support and Wim van den Berg for help with statistical analyses. NewFoss and Kekkilä BVB are acknowledged for their technical support and providing casing materials.

Author contributions

TT and JMvdW designed the study. RvR, EH, MH, OM, CL and TT performed the experiments. FAG and JMvdW analysed the data and drafted the manuscript. All authors commented on the manuscript and gave approval for/are accountable for the final version of the publication.

Funding

Horizon 2020 Framework Programme, 101000651, 101000651, 101000651, 101000651, 101000651, 101000651, 101000651, 101000651, 101000651, Ministerie van Economische Zaken, KB-50-006-008-WPR, KB-50-006-008-WPR, KB-50-006-008-WPR, KB-50-006-008-WPR, KB-50-006-008-WPR, KB-50-006-008-WPR, KB-50-006-008-WPR, KB-50-006-008-WPR, KB-50-006-008-WPR.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-20453-4.

Correspondence and requests for materials should be addressed to F.A.G.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.