MINI-REVIEW

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Microbial ecology of the *Agaricus bisporus* mushroom cropping process

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Abstract

Agaricus bisporus is the most widely cultivated mushroom species in the world. Cultivation is commenced by inoculating beds of semi-pasteurised composted organic substrate with a pure spawn of *A. bisporus*. The *A. bisporus* mycelium subsequently colonises the composted substrate by degrading the organic material to release nutrients. A layer of peat, often called "casing soil", is laid upon the surface of the composted substrate to induce the development of the mushroom crop and maintain compost environmental conditions. Extensive research has been conducted investigating the biochemistry and genetics of *A. bisporus* throughout the cultivation process; however, little is currently known about the wider microbial ecology that co-inhabits the composted substrate and casing layers. The compost and casing microbial communities are known to play important roles in the mushroom production process. Microbial species present in the compost and casing are known for (1) being an important source of nitrogen for the *A. bisporus* mycelium, (2) releasing sugar residues through the degradation of the wheat straw in the composted substrate, (3) playing a critical role in inducing development of the *A. bisporus* fruiting bodies and (4) acting as pathogens by parasitising the mushroom mycelium/crop. Despite a long history of research into the mushroom cropping process, an extensive review of the microbial communities present in the compost and casing has not as of yet been undertaken. The aim of this review is to provide a comprehensive summary of the literature investigating the compost and casing microbial communities throughout cultivation of the *A. bisporus* mushroom crop.

Keywords *Agaricus bisporus* · Compost · Casing · Microbial ecology · Bacteria · Fungi

Introduction

Agaricus bisporus is the most widely cultivated mushroom crop in the world, with an estimated annual worth of up to US$ 4.7 billion as of 2009 (Sonnenberg et al. 2011). Commercial cultivation of *A. bisporus* crops is performed worldwide, with production mostly concentrated in North America, Europe, India and China (Kabel et al. 2017). Currently, cultivation of *A. bisporus* is dominated by a single hybrid strain produced in the Netherlands in the 1980s by cross-breeding the Horst-U1 and U3 strains (Savoie et al. 2013). This lack of genetic diversity in *A. bisporus* cultivars has resulted in worldwide *A. bisporus* production facing similar threats from pathogenic species, which appear to originate from the mushroom growing medium (Largeteau and Savoie

2010). Despite this, in-depth investigations of the microbial communities co-habiting the growing medium have, until recently, been relatively unexplored. However, in the past few years studies using advanced techniques for characterising microbial communities, such as molecular fingerprinting techniques, next generation sequencing (NGS) and phospholipid fatty acid analysis (PLFA), have revealed new insights into the microbial dynamics present in the mushroom growing medium (McGee et al. 2017a; McGee et al. 2017b; Siyoum et al. 2016; Szekely et al. 2009; Pecchia et al. 2014; Vos et al. 2017a; Zhang et al. 2014). The aim of this review is to summarise the current understanding of the activities and dynamics of the microbial communities inhabiting the mushroom growing medium throughout the mushroom cropping process.

Preparation of the mushroom growing medium and the mushroom cropping process

Large-scale commercial cultivation of *A. bisporus* using pure inoculants was first performed by the Pasteur Institute in 1894 using substrates of partially fermented horse manure and straw

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from stables (Van Griensven and Van Roestel 2004). Modern cultivation of *A. bisporus* is predominantly performed using a substrate of semi-pasteurised composted organic material, the main components of which are generally gypsum, manure (horse or chicken) and wheat straw (Straatsma et al. 1994). The growing medium substrate is a mixture that is optimal for growth and production of the *A. bisporus* crop. Typically, the composted substrate is covered by a layer of "casing" soil, which is usually peat-based, prior to inducing production of the mushroom crop (Berendsen et al. 2012). The mushroom composted substrate undergoes three distinct phases during its production and preparation prior to being used for producing the mushroom crop (Kabel et al. 2017). Phase 1 of mushroom compost production is a short composting process of the raw ingredients usually performed outdoors in windrow stacks (Derikx et al. 1990). In phase 2 of production, the mushroom compost is conditioned by heat treatment to enable the development of thermophilic microbial communities and to remove insects prior to *A. bisporus* inoculation (Mouthier et al. 2017; Zhang et al. 2014). Phase 3, sometimes known as the "spawn run", involves the inoculation of phase 2 compost, with an inoculation/spawn of *A. bisporus*, which subsequently colonises the substrate (Van Griensven and Van Roestel 2004). Upon completion of phase 3, the colonised compost is generally transferred to a mushroom production house/unit where the compost surface is covered with a peat-based casing layer (Sharma and Kilpatrick 2000). The initial mushroom crop or "flush" is induced by controlling environmental conditions such as temperature, CO_2 and moisture inside the cropping house (Sharma et al. 2005). A graphical representation of the

colonisation of the mushroom growing medium is presented in Fig. 1. The mushroom cropping process consists of harvesting a series of two to three flushes from the compost bed before the substrate is considered spent (spent mushroom compost: SMC), and the process is terminated (Royse et al. 2008). Successive mushroom crop flushes produce diminishing crop yields, despite the substantial levels of nutrients remaining in the compost substrate (Pecchia et al. 2014). A substantial body of research has been conducted into investigating the capacity of the *A. bisporus* mycelium to release carbohydrate nutrients from the straw fibres present in the compost substrate. The genetics and biochemistry relating to carbohydrate degradation of *A. bisporus* has been extensively reviewed in Kabel et al. (2017). This review will focus on the activity and composition of the wider microbial community present in the mushroom growing medium.

Scope of review

Mushroom compost and casing is a very heterogeneous environment which is inhabited by a broad range of microbial species including archaea (Derikx et al. 1989), bacteria (McGee et al. 2017b), fungi (McGee et al. 2017a) and viruses (Eastwood et al. 2015). Regardless of this, investigations of microbial communities in the mushroom cropping process have generally been limited to bacteria and fungi. Therefore, the scope of this review is limited to focusing on the bacterial and fungal communities of the compost and casing substrates, as the larger body of work conducted in these areas allows for determination
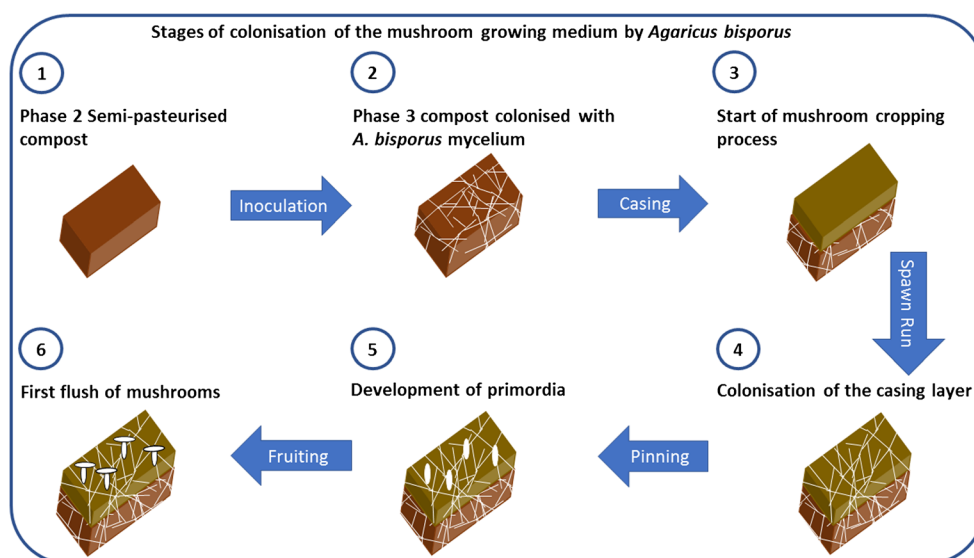

Fig. 1 Stages of colonisation of the mushroom growing medium by *Agaricus bisporus*. Stage 1: Phase 2 semi-pasteurised compost is inoculated with a pure spawn of *A. bisporus*. Stage 2: Colonised phase 3 compost is transferred to a mushroom growing facility where the casing layer is applied to the surface commencing the initiation of the mushroom cropping process. Stage 3: The compost *A. bisporus* mycelium colonises the casing layer i.e. Spawn run. Stage 4: *A. bisporus* mycelium in casing layer condenses into "pins" which develop into primordia. Stage 5: Primordial pins develop into full mushroom fruiting bodies for harvesting in stage 6

of certain distinguishing trends. This review also considers studies which report compost and casing enzyme activities that reflect activity of the whole microbial community present. The review aims to address the microbial commutes present during the mushroom cropping process only, and does not cover the earlier stages of mushroom growing medium production. A list of the relevant mushroom cropping process microbial ecology literature is presented in Table 1. A substantial body of literature has been published on microbial pathogens of the *A. bisporus* crop; these have been well reviewed in Largeteau and Savoie (2010). This review only refers to known microbial pathogens when it is pertinent, and reported in surveys of microbial communities in mushroom compost and casing.

Enzymatic activity of the mushroom compost microbial community

Microbial degradation of straw fibres in the mushroom compost is critical in releasing carbohydrate nutrition that feeds the *A. bisporus* mycelium (Savoie 1998). However, large amounts of lignin and hemicellulose have been found to remain in the spent mushroom compost after the mushroom cropping

process has been completed (Jurak et al. 2015; Vos et al. 2017b). As a result of this, a large body of work has been conducted investigating the microbial community enzymatic activities involved in degrading the complex carbohydrates in the mushroom compost substrate (Bonnen et al. 1994; Jurak et al. 2015; Savoie 1998; Vos et al. 2017b), though little or no work has reported enzyme activities in the casing substrate. The carbohydrate-degrading enzymes which have been most commonly studied are (1) the lignin-degrading enzymes laccase and manganese peroxidise (MnP), and (2) the hemicellulose-degrading enzymes hemicellulase, xylanase and β-xylanase (Bonnen et al. 1994; Jurak et al. 2015; Kabel et al. 2017; Savoie 1998; Vos et al. 2017a).

Investigations of enzyme activities present in the compost layer indicate that activity of the *A. bisporus* mycelium dominates in the degradation of lignin straw residues. The study of Savoie (1998) indicated that laccase and MnP activities in the compost fraction were associated with *A. bisporus* inoculation, as activities of these enzymes were below limits of detection (LOD) in the microbial community present in uninoculated mushroom compost. The absence of laccase activity in uninoculated mushroom compost was supported by the study of Vos et al. (2017a). There are indications that the

Table 1 Summary of current literature associated with the microbial ecology of the *A. bisporus* mushroom cropping process

Reference	Substrate	Techniques	Target
Bonnen et al. (1994)	Compost	MnP activity	Enzymatic activity
Savoie (1998)	Compost	Endo-cellulase, xylanase, β-glucosidase, β-xylosidase, β-N-acetyl-glucosaminidase, laccase, MnP and protease activity	Enzymatic activity
Cai et al. (2009)	Casing	Cultivation of isolates; PLFA	Bacteria; fungi
Choudhary et al. (2011)	Casing	Cultivation of isolates	Bacteria
Szekely et al. (2009)	Compost	PLFA; T-RFLP	Bacteria; fungi
Sharma et al. (2013)	Compost	ARDRA	Bacteria
Zhang et al. (2014)	Compost	NGS (DNA)	Bacteria; fungi
Pecchia et al. (2014)	Casing	NGS (DNA)	Bacteria
Jurak et al. (2015)	Compost	α-Arabinofuranosidase, cellobiohydrolase, endo-xylanase, endo-glucanase, β-mannosidase, β-xylosidase and β-glucanase activity	Enzymatic activity
Siyoum et al. (2016)	Casing; compost	Cultivation of isolates; PLFA	Bacteria; fungi
McGee et al. (2017a)	Compost	NGS (cDNA and DNA)	Fungi
McGee et al. (2017b)	Compost	NGS (cDNA and DNA)	Bacteria
Vos et al. (2017a, b)	Compost	Chitin quantification; laccase activity; PLFA	Bacteria; fungi; enzymatic activity

degradation of mushroom compost lignin by MnP activity is limited by low levels of the co-factor H_2O_2 in the mushroom compost (Vos et al. 2017b). The results from these studies indicate that the wider microbial community plays little or no part in the degradation of lignin in mushroom cropping compost. Fungi are known to be more active decomposers of lignin than bacteria (Brown and Chang 2014). It is probable that the phase 1 composting process and phase 2 semi-pasteurisation effectively suppress fungi capable of degrading lignin in the mushroom compost substrate which may compete with A. bisporus in phase 3 and the mushroom cropping process.

In distinct contrast to lignin degradation, production of xylanase enzymes for degrading hemicellulose in mushroom compost appears to be highly associated with the wider mushroom compost microbial community in addition to A. bisporus. The study of Savoie (1998) found xylanase and β-xylanase activities to be higher in uninoculated mushroom compost, compared to mushroom compost inoculated with a spawn of A. bisporus. Their study found that after the application of a casing layer on the 14th day of the spawn run period, xylanase and β-xylanase activities markedly decreased in the A. bisporus-inoculated mushroom compost compared to the uninoculated mushroom compost. Xylanase activity of the A. bisporus mycelium appears to decrease throughout the mushroom cropping process, with lower xylanase activity observed in successive mushrooms flushes (Patyshakuliyeva et al. 2013, 2015). This may be as a result of the A. bisporus mycelium lacking the ability to degrade the highly substituted xylan residues that build up in the mushroom compost as the cropping process progresses (Jurak et al. 2015).

Distinguishing the difference between enzyme activity of the A. bisporus mycelium and the wider microbial community is difficult. Few studies have investigated comparisons between inoculated and uninoculated mushroom compost. The study of Savoie (1998) indicated that in addition to xylanase activity, the wider microbial community in uninoculated mushroom compost had higher potential to produce endocellulases and laminarases for degrading cellulose fibres in the mushroom compost. The wider microbial community therefore clearly contributes substantially to the release of carbohydrate nutrients through the degradation of the straw fibres in the mushroom compost.

Microbial ecology of the compost substrate

Compost fungal community

The mushroom compost substrate has been shown to be dominated by the A. bisporus mycelium throughout the cropping process (Vos et al. 2017a). PLFA conducted on mushroom compost found the A. bisporus mycelium to make up 6.8%

w/w of the mushroom compost after complete colonisation, though less than half of the mycelium was found to be active (Vos et al. 2017a). This work was supported in the study of McGee et al. (2017a) which investigated fungal activity based on cDNA internal transcribed spacer (ITS) transcripts. Activity of the A. bisporus mycelium was found to dominate the fungal compost community up to the first mushroom crop flush (> 90%), before decreasing substantially thereafter to generally < 30%, with activity peaking slightly during the second and third mushroom crop flushes (McGee et al. 2017a). The decrease in activity of the A. bisporus mycelium resulted in the fungal community becoming dominated by other fungal species, particularly the pathogen Lecanicillium fungicola and an unidentified fungal species (McGee et al. 2017a). Fungal pathogens affecting the A. bisporus mycelium, such as L. fungicola, Trichoderma aggressivum, Mycogone perniciosa and Cladobotryum dendroides are typically associated with the later flushes of the mushroom cropping process (Largeteau and Savoie 2010). The decreased activity of the A. bisporus mycelium after the first flush may be a critical factor allowing fungal pathogens to gain an increased niche in the compost environment and an opportunity to attack the mushroom fruiting crop and mycelium.

In-depth studies investigating the composition of the fungal mushroom compost community have been limited to date. The study of Zhang et al. (2014) used NGS to target the DNA-derived compost fungal community at one point during the mushroom cropping process, in a survey aimed at phase 1, 2 and 3 and mushroom cropping. Their study detected only a single unclassifiable fungal species in addition to A. bisporus, in the mushroom compost substrate at the budding stage of the first flush of the mushroom crop. Isolation of fungal species using culturing techniques in the study of Siyoum et al. (2016) identified a greater diversity of fungi in mushroom compost, particularly yeast species. The study of Siyoum et al. (2016) isolated two species of Penicillium (meleafrinum and brevicompactum) from the compost along with five yeasts: Cystofilobasidium infirmominiatum, Rhodotorula mucilaginosa, Candida glaebosa, Trichosporon moniliforme and Trichosporon cutaneum from two sampling points (pinning and first flush). However, the isolation of cultivatable fungal species does not indicate whether the species are active or dormant. The study of McGee et al. (2017a) identified a broad diversity of fungal species (211) in the mushroom cropping compost over 11 sampling points using NGS to target the DNA-derived community. However, analysis of the cDNA-derived community identified only 51 active species over the same period, indicating that the majority of species detected in the DNA-derived community were dormant and possibly legacy species from phases 1, 2 and 3. Determining microbial communities using culturing and DNA-derived methodologies has the limitation of not distinguishing between dormant species and species that are active when the

substrate is sampled. Interestingly, the DNA-derived community was found to be dominated by Ascomycota species, with only one other Basidiomycete species detected in addition to *A. bisporus*. In contrast to this, the active cDNA-derived community was dominated by a mixture of *Ascomycota* and *Basidomycota* (McGee et al. 2017a). Some similarities were observed between the active fungal species identified from the cDNA-derived community and the fungal species isolated in the study of Siyoum et al. (2016) such as the presence of yeast species such as *Trichosporon cutaneum*, *Candida* and *Rhodotorula*. The mushroom cropping compost has a high level of extractable simple sugars (Jurak et al. 2015) and tends to be quite moist at > 60% (McGee et al. 2017b), both factors which are highly associated with yeast-rich environments (Nagy et al. 2017). Yeast species are known to be highly active in thermophilic composting processes; however, little is currently known about their influence in the mushroom compost cropping process.

Certain fungal pathogens which attack the *A. bisporus* mycelium, such as *Trichoderma aggressivum* which causes the green mould disease, are known to typically originate from the mushroom compost (Samuels et al. 2002). However, reports on the compost fungal community have rarely detected the presence of this pathogen. It is possible that published studies on the microbial community have tended to use pathogen-free or relatively free cropping cycles for the basis of their studies.

Compost bacterial community

While growth of the *A. bisporus* mycelium may dominate the mushroom growing medium, the bacterial community present in the mushroom cropping compost is known to be highly diverse (McGee et al. 2017a; Siyoum et al. 2016; Szekely et al. 2009; Vos et al. 2017a; Zhang et al. 2014). Several studies have indicated that bacterial diversity is higher than fungal diversity in the compost substrate throughout the mushroom cropping process (Siyoum et al. 2016; Zhang et al. 2014). Colonisation of the mushroom compost by the *A. bisporus* mycelium has been shown to affect the bacterial community structure throughout the cropping process (Cai et al. 2009). A comparison between *A. bisporus*-inoculated and uninoculated mushroom compost found abundance of the bacterial community to be significantly reduced by the presence of the *A. bisporus* mycelium (Vos et al. 2017a). Studies have shown that the *A. bisporus* mycelium feeds on bacteria present in the mushroom compost utilising them as a source of nitrogen (Fermor and Wood 1981; Fermor et al. 1991). There is some indication that gram-negative bacteria may be more susceptible to the *A. bisporus* mycelium than gram-positive bacteria. Gram-positive compost bacterial relative abundance has been shown to increase in the compost substrate compared to gram-positive bacteria (Vos et al. 2017a). However, an NGS characterisation of the DNA-derived bacterial community at budding of the first flush of mushrooms found the bacterial community to be dominated by gram-negative Actinobacteria such as *Streptomyces* (60%), *Ilumatobacter* (8%), *Microbispora* (2%), *Saccharopolyspora* (2%) and an unknown gram-negative bacterial species (19%) (Zhang et al. 2014). NGS characterisation of the DNA and cDNA-derived bacterial communities after sampling 11 points throughout the mushroom cropping process has indicated that there is a highly dynamic community present in the compost substrate (McGee et al. 2017b). Characterisation of the bacterial community using NGS indicated that the mushroom compost is dominated by *Actinobacteria*, *Firmicutes*, *Proteobacteria*, *Planctomycetes* and *Deinococcus-Thermus* (McGee et al. 2017b). In total, 273 and 274 species exceeding 0.1% relative abundance were identified in the DNA and cDNA-derived bacterial communities, respectively. A substantial change in the composition of the bacterial compost community structures was found to occur after the first flush of mushrooms (McGee et al. 2017b). As the mushroom cropping process progressed, abundance of the *Deinococcus-Thermus* and *Proteobacteria* was found to decrease in relative abundance as the *Actinobacteria* and *Firmicutes* inversely increased (McGee et al. 2017b).

Culturing techniques have shown thermophilic bacterial species to play prominent roles in phase 1 composting, but were found to be below LOD or become dormant at the mesophilic temperatures of phase 3 (Szekely et al. 2009). Inoculation of phase 1 mushroom compost with thermophilic bacteria capable of degrading lignocellulosic material has been shown to improve the efficacy of the composting process (Ahlawat and Vijay 2010). Thermophilic bacteria of the phyla *Deinococcus-Thermus* and *Thermodesulfobacteria* were shown to be highly active in the mushroom cropping compost until the first flush of the mushroom crop, based on cDNA transcripts (McGee et al. 2017b). However, little is known about the possible role thermophilic bacteria may play in the mushroom compost throughout the cropping process.

The study of McGee et al. (2017b) found bacteria with nitrifying properties, such as *Rhizobium* and *Stenotrophomonas*, to become more active in the mushroom compost after the first flush. The increase in activity of nitrifying bacteria was found to correlate with increased levels of extractable nitrate in the mushroom compost, which steadily increased until the termination of the cropping process. Interestingly, the addition of cyanobacteria with nitrifying properties, such as *Nostoc*, to mushroom compost during the cropping process has been shown to increase crop yield (Riahi et al. 2011). Spent mushroom compost (SMC) has been shown to be a good source of nitrogen for plants when added to soil-based systems (Hackett 2015; Stewart et al. 1998). This may possibly be a result of the increasing levels of extractable nitrate production in compost media throughout the cropping process; however, more research is needed to establish

whether nitrification is a consistent property related to the mushroom compost substrate during the cropping process.

The study of Siyoum et al. (2016) found cultivatable compost bacterial isolates to be dominated by three species of *Proteobacteria*—*Citrobacter kosen*, *Serratia marcescens*, *Pseudomonas putida*—and one *Actinobacteria*, *Arthrobacter arilaitensis*, though sampling was limited to two points in the mushroom cropping process. In contrast to this, the bacterial community they characterised using denaturing gradient gel electrophoresis (DGGE) targeting extracted DNA identified a much more diverse community. In the Siyoum et al. (2016) study DGGE identified *Bacteroidetes* (*Flavisolibacter* sp., *Rhodothermus obamensis*) and *Firmicutes* (*Bacillus badius*, *Cellulomonas* sp., *Microbispora* sp. and *Blastococcus* sp.), in addition to *Proteobacteria* (*Bordetella* sp., *Luteimonas* sp. and *Pseudomonas* sp.). The study of McGee et al. (2017b) found *Pseudomonas* species to be present throughout the mushroom cropping process, with activity appearing to peak during the first flush of mushrooms. The presence of certain *Pseudomonas* species in the peat casing layer have been shown to aid in inducing the development of the mushroom crop; however, other species of *Pseudomonas* such as *P. gingeri*, *P. aeruginosa*, *P. fluorescens* and *P. tolaasii* are known to be pathogens of the fruiting mushroom cap (Largeteau and Savoie 2010).

Microbial ecology of the casing layer

Casing fungal community

The fungal casing community has rarely been studied in detail. The majority of studies investigating fungal species associated with the mushroom casing layer generally relate to pathogenic species attacking the *A. bisporus* mycelium and fruiting bodies. Fungal pathogens which have been observed to colonise the mushroom casing layer and attack fruiting mushroom bodies include *C. dendroides* (Carrasco et al. 2017), *L. fungicola* (Berendsen et al. 2010), *M. perniciosa* (Fu et al. 2016) and *Trichoderma* spp. (Samuels et al. 2002) all of which belong to the phylum *Ascomycota*. The occurrence and epidemiology of these pathogens been extensively reviewed in Largeteau and Savoie (2010).

The study of Cai et al. (2009) characterised the abundance of microbial casing communities through the cropping process until the second flush of a mushroom crop. Their study found the fungal casing community to have a higher abundance than the bacterial casing community throughout the cropping process. The fungal casing community abundance was found to increase almost exponentially until the inter-flush period between the first and second mushroom flushes, and was most likely dominated by *A. bisporus*. However, upon completion of the first mushroom flush, the ratio of fungal to bacterial biomass was found to decrease during the second flush primordia formation period and into the second mushroom flush.

To date, the fungal community present in the mushroom casing layer has been best characterised in the study of Siyoum et al. (2016). Their study characterised fungal isolates cultivated from the mushroom casing layer during the cropping process. Similar to their findings in the mushroom compost layer, they found a broad selection of fungal yeast species belonging to the phylum *Ascomycota*; three *Candida* spp. and one *Pichia* sp. In addition to *A. bisporus*, several filamentous fungal species were identified in the casing layer, which also belonged to the phylum *Ascomycota*: *Bionectria* sp., *Chaetomium* sp., *Chromelosporium* sp., *Pseudallescheria* sp., *Trichoderma* sp. and three *Penicillium* spp.

Similar to the compost substrate, the majority of fungal species in the casing layer appear to belong to the phylum *Ascomycota*. This is a somewhat perplexing property, given the optimisation of the growing medium to favour a *basidiomycete* species. Knowledge of the casing community is severely limited at present due to a lack in studies and sufficient sampling throughout the mushroom cropping process. In order to more fully understand how and why these pathogens or potential competitors for nutritional resources come to establish themselves in the casing layer, a more complete knowledge of the casing fungal community dynamics is required in future studies.

Casing bacterial community

Characterisation of bacterial casing community structures has received substantially more attention than the fungal community. The bacterial casing community abundance has been shown to be lower than fungal casing community abundance throughout the cropping process (Cai et al. 2009). However, bacterial abundance, as measured in PLFAs, has been shown to increase in the casing layer during primordia formation of the first and second mushroom flushes, decreasing during other stages of mushroom growth and development (Cai et al. 2009). The study of Cai et al. (2009) indicated that gram-negative bacteria were in higher abundance than gram-positive bacteria throughout a mushroom cropping process (Cai et al. 2009).

The study of Pecchia et al. (2014) investigated the bacterial casing community throughout the cropping process using NGS and determined that bacteria were dominated by species from four phyla: *Actinobacteria*, *Bacteriodetes*, *Firmicutes* and *Proteobacteria*. Their study indicated that as the mushroom cropping process progressed, relative abundance of the *Actinobacteria* (*Actinomycetales* and *Coriobacteriales*) and *Firmicutes* (*Bacillales* and *Clostridiales*) increased

successively after both the first and second mushroom flushes. Relative abundance of the *Proteobacteria* (*Rhizobiales*, *Caulobacteriales*, *Sphingomonadeles*; *Burkholderiales*; *Xanthomonadales* and *Pseudomonadales*) decreased after the first mushroom flush, while relative abundance of the *Bacteriodetes* (*Flavobacteriales* and *Sphingobacteriales*) increased between the first and second flush, but decreased between the second flush and third flush. The study of Siyoum et al. (2016) identified cultivatable bacterial isolates from the same four phyla detected in the study of Pecchia et al. (2014): *Actinobacteria* (*Rhodococcus* sp. and *Microbacterium* sp.), *Bacteriodetes* (*Terrimonas* sp. and *Sphingobacterium* sp.), *Firmicutes* (*Trichococcus collinsii*) and *Protobacteria* (*Acidovorax temperans*, *Aminobacter* sp., *Bosea thiooxidans*, *Devosia hwasunensis*, *Mesorhizobium* sp., *Paracoccus koreensis*, *Pseudaminobacter salicylatoxidans*, *Rhizobium* sp. and *Shinella kummerowiae*) in the casing layer throughout a mushroom cropping process. However, when the bacterial casing community was characterised using DGGE, only *Proteobacteria* were identified such as *Alpha-Proteobacteria* (*Chelativorans multitrophicus*, *Rhizobium* sp., *Shinella* sp. and *Sphingomonas* sp.), *Beta-Proteobacteria* (*Curvibacter* sp.) and four *Pseudomonas* of the *Gamma-Proteobacteria* (*P. nitroreducens*, *P. plecoglossicida*, *P. putida* and *P. reactans*). The study of Choudhary (2011) also found cultivable species of bacteria in casing to be dominated by strains of *Acinetobacter* and *Pseudomonas* throughout the mushroom cropping process.

The *Pseudomonadales* were observed to decrease throughout the mushroom cropping process monitored in the Pecchia et al. (2014) study. The *Pseudomonadales* contain the genera *Pseudomonas*, which play influential roles in the development of mushroom fruiting bodies cropping process (Noble et al. 2003). It has been indicated that *Pseudomonas* species in the casing layer can aid in the development of the mushroom crop by metabolising volatile compounds that are inhibitory to the formation of *A. bisporus* primordia (Noble et al. 2009). *Pseudomonas* species are also known to cause numerous cavity diseases on mushroom fruiting bodies (Jolivet et al. 1998).

Conclusions

The fungal community within the mushroom growing medium is highly dynamic and responsive to the stages of the mushroom cropping process. The *A. bisporus* mycelium dominates the mushroom cropping substrate (Vos et al. 2017a), but also appears to have significantly reduced activity after the first flush of mushrooms, based on compost laccase activity (Savoie 1998; Vos et al. 2017a) and cDNA synthesis (McGee et al. 2017a). The reduced *A. bisporus* activity after the first flush of

mushrooms results in increased activity of other fungal species within the compost substrate, and possibly facilitates the development of some fungal pathogens (McGee et al. 2017a). Apart from the *basidiomycete A. bisporus*, most fungal species found in the mushroom growing medium tend to belong to the phylum *Ascomycota* (McGee et al. 2017a; Siyoum et al. 2016), particularly the pathogenic species associated with the mushroom cropping process (Largeteau and Savoie 2010). A considerable amount of yeast species have been identified in the mushroom substrate, though as of yet their influence on the cropping process is unknown.

The abundance (Vos et al. 2017a) and structure (McGee et al. 2017b) of the compost bacterial community appear to be very responsive to growth of the *A. bisporus* mycelium throughout the mushroom cropping process. The compost and casing bacterial communities are very diverse and predominantly composed with members of the phyla *Actinobacteria*, *Bacteriodetes*, *Firmicutes* and *Proteobacteria* (Pecchia et al. 2014; McGee et al. 2017b; Siyoum et al. 2016). Members of the Pseudomonadales tend to be frequently detected in both compost and casing (Choudhary 2011; McGee et al. 2017b; Siyoum et al. 2016; Pecchia et al. 2014) and are highly associated with development of *A. bisporus* primordia, fruiting bodies (Noble et al. 2003, 2009) and fruiting body diseases (Jolivet et al. 1998; Largeteau and Savoie 2010).

The understanding of microbial communities in the mushroom cropping process is still far from being fully understood. Though most fungal pathogens of the *A. bisporus* crop appear to colonise and inhabit the casing layer, no attempt has yet been reported that characterises the fungal casing community using advanced molecular techniques. The archaeal community has still as of yet received no characterisation. In order to more fully understand the influence of microbial community dynamics on the mushroom cropping process, more advanced studies are required, particularly those sampling the cropping process at higher frequencies. The use of advanced molecular techniques will also help facilitate the complexities of the microbial communities influencing the *A. bisporus* mushroom cropping process.

Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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