Final Report

Developing a database of bio-markers for compost quality control to maximise mushroom production yield

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Public summary

The quality and yield of mushroom crops depends critically on the quality of the compost used, but the methods used to measure compost quality have not changed much in the last forty years. Recent advances in our understanding of the microbiology of mushroom composting have suggested that this plays a crucial role in determining compost quality. The aim of this project was to develop a robust, largescale database of microbial biomarkers at different stages of mushroom composting, and use this database to link the microbial diversity in the compost with the yield and quality of the resulting mushroom crop. A preliminary survey of three successive compost crops from Australian composting facilities around Australia confirmed the feasibility of the study, and a major sampling program was then undertaken to determine bacterial and fungal dynamics during mushroom composting at compost yards in four Australian states over a 12-month period, taking samples from four timepoints at every second compost crop. The microbes in 425 samples from 113 compost crops were identified by next generation DNA sequencing, and their populations correlated with the yield and quality profile of the mushroom crops harvested from those composts. The resulting database comprises over 60 million DNA sequences, which will provide a detailed resource for further data mining in coming years. Biomarker analysis revealed that the bacterial taxa present at the end of Phase I show the most significant correlation with crop yield and quality. The most promising candidate as a biomarker is *Thermus*, but many of the other bacteria identified are poorly characterized or previously unknown, making it difficult to use them as specific biomarkers for crop management. This aim is also frustrated by variations in composting process between composters and the effect of market requirements (which influence harvested yield), and no consistent set of biomarkers for yield/quality could be identified that will be valid across the entire Australian mushroom industry.

Technical summary

The quality and yield of mushroom crops depends critically on the quality of the compost used, but the methods used to measure compost quality have not changed much in the last forty years. Recent advances in our understanding of the microbiology of mushroom composting have suggested that this plays a crucial role in determining compost quality. The aim of this project was to develop a robust, largescale database of microbial biomarkers at different stages of mushroom composting, and to use this database to link the microbial diversity in the compost with the yield and quality of the resulting mushroom crop. A preliminary survey of the microbial diversity of successive compost crops from Australian composting facilities around Australia was carried out using 16S V3-V4 amplicon sequencing (Illumina Miseq), and confirmed that the bacterial succession during composting was consistent at individual compost yards, and that the microbial dynamics reflected traditional measures of compost quality such as C/N ratio, humification, microbial biomass, and nitrification ratio. A major sampling program was then undertaken to determine bacterial and fungal dynamics during mushroom composting at compost yards in four Australian states over a 12-month period, taking samples from four timepoints at every second compost crop. The microbes in 425 samples from 113 compost crops were identified by next generation DNA sequencing (V3-V4 16S for bacteria; ITS1 for fungi), and their populations correlated with the yield and quality profile of the mushroom crops harvested from those composts. After quality filtering, chimera removal and taxonomic assignment (dada2 pipeline on the R platform), the resulting ASVs were assembled into a database comprising over 60 million DNA sequences, including 87,000 bacteria ASV's and almost 3,000 fungal ASV's. This database will provide a detailed resource for further data mining in the coming years. Biomarker analysis revealed that the bacterial taxa present at the end of Phase I show the most significant correlation with crop yield and quality ($p < 0.05$; filtered for taxa present at > 0.1% in > 80% of samples of each composting phase). The most promising candidate as a biomarker is *Thermus*, but many of the other bacteria identified are poorly characterized or previously unknown, making it difficult to use them as specific biomarkers for crop management. This aim is also frustrated by differences in composting process between composters and the effect of market requirements (which have a major influence on harvested yield), and no consistent set of biomarkers could be identified that will be valid across the entire Australian mushroom industry.

Keywords

Compost, mushrooms, microbial diversity, *Mycothermus*, *Agaricus,* mushroom yield, mushroom quality, biomarker; compost quality

Introduction

Current techniques for measuring mushroom compost quality have changed very little in 40 years. They depend largely on a limited number of chemical assays and on the empirical personal expertise of composters, acquired over years of experience. These measures are invaluable, but effective compost management requires them to be more strongly integrated with the outcomes of modern compost research. In particular, since composting is a microbial process characterized by a complex succession of microorganisms, there is a need to understand the optimum microbe concentrations (both the total population, and the populations of key microbial groups) in order to design a compost quality indicator that correlates microbial load with crop yield and quality.

Indicators for compost maturity/stability are used in many composting processes, including municipal composting and composting of various manures. Typical variables measured include water-soluble carbon, C/N ratio, nitrification ratio (NH_4/NO_3), content of humic material, and microbial respiration (measured as CO_2 release). For composts used in the horticultural industry, plant related variables such as seed germination and seedling survival are sometimes also included. Usually a combined "compost maturity index" (CMI) is generated, which is composed of several factors (e.g. the Solvita compost test, which combines values for NH_3 and CO_2 production). Since the aim of composting is to stabilize nutrients from e.g. manure in a form that does not damage the environment, there are often legal requirements for "compost stability" that must be met before compost is used, and these form a minimum list for a "compost quality index" for these composts (Bernal et al. 2017).

For mushroom composting, by contrast, the most important use of a compost quality indicator is to determine that the compost will afford high yield and quality of mushrooms. Methods that correlate physicochemical compost parameters with mushroom yield have been reported periodically, but have not found great take-up in the industry. Sharma and Kilpatrick (2000), for example, identified a combination of pH, dry matter, ammonia, carbon, ash, Cu, Fe, and Na as explaining >90% of yield variation. Similar results were reported more recently by Spanish researchers (Zied et al. 2011), who measured properties of casing and compost separately, though with less accurate predictions of yield. It should be noted that the number of parameters required for yield prediction in these studies would make them impractical for composters to use routinely, and the number of crops evaluated in the reports was relatively low.

The last ten years have seen a rapid expansion in our knowledge and understanding of the microbial dynamics of mushroom composting. From a starting point in 2003 where fewer than 100 different bacterial and fungal taxa were known in mushroom compost (Ryckeboer et al. 2003), we can now identify and track tens of thousands of different species, all of which are active at different stages of mushroom composting, spawn run and cropping (Cao et al. 2019; Carrasco et al. 2020; Kertesz et al. 2016; Thai et al. 2022; Vieira and Pecchia 2021). The aim of the current project was to generate a largescale database of compost microbes, covering a 12-month timespan of composting at multiple sites within Australia, and to use this database to identify bacterial taxa whose populations at different stages of composting are diagnostic of increased yield or quality in the subsequent mushroom crop, and which can act as biomarkers for yield management during mushroom production.

Methodology

Experimental design

The project consisted of two major experimental components: a proof-of-concept program, sampling from three successive compost crops at individual composters, followed by a full-scale compost sampling program, conducted over 12 months. In the original program design, each of these sampling programs envisaged sampling from 6-8 composting facilities with a geographical spread over all four mainland Eastern Australian states.

The "proof-of-concept" stage of the program consisted of compost sampling from three successive compost crops (four timepoints per crop) at four different compost facilities in different states. The aim of this initial study was to determine the reproducibility of microbial diversity at each compost yard (successive crops were expected to be very similar in quality and microbial composition), and to evaluate how well the changes in microbial diversity during composting reflected physicochemical parameters of compost that are traditionally used to measure composting efficiency and compost quality.

The major sampling program commenced in October 2022, after the "proof-of-concept" stage had been completed. In this program, composters sampled compost at four time points from every second compost crop over a period of 12 months (a total of 26 crops per composter). Four composters (in Queensland, Victoria and two from South Australia) provided a complete 12-month set of samples, while one facility was only able to provide a subset of the expected number of samples. The analysis below focusses on the data obtained from the four participants, in three Australian States, who completed the full 12-month sampling program.

Compost sampling from commercial composting facilities

Compost samples used in the project were obtained from five commercial mushroom composting facilities located in New South Wales, Queensland, Victoria, and South Australia. For confidentiality reasons, the composters are not named in this report. Compost samples taken at specific timepoints during the composting process (start phase I (bunker fill); mid Phase 1 (on turning); end phase 1; end phase 2). Each of these samples were pooled samples, produced by combining ten independent samples taken from across the face of the compost pile at each timepoint. Sampling was done on site by industry personnel, who were provided with appropriate training by the research team using a training video made by the team (https://www.youtube.com/watch?v=TZG_QKWlOyA), and additional training where needed. Compost samples were frozen on site (freezers were provided on request by the project team) and the collected, frozen samples were shipped to the University of Sydney for analysis every 2-3 months. The samples were sent by express courier and received within 24 hours, and they were stored at -20 °C when received.

Before analysis, compost samples (0.5 - 1 kg) were thawed to room temperature and thoroughly homogenized in a rotating 25 litre plastic drum (containing three internal baffles) for 1 minute. Samples were then emptied onto a sterilized stainless steel worktop (thoroughly cleaned with 80% ethanol) and randomized subsamples collected for chemical analysis (ca. 500 g) and DNA analysis (10 g). Where possible, approximately 100 g of each compost was retained (stored at -20 °C) for future analysis of other parameters.

Compost physicochemical measurements

Water content of the compost was measured gravimetrically by weighing subsamples of compost before and after oven drying. Moisture content was expressed as a percentage of the fresh weight. Ash content was determined gravimetrically after heating the dried sample for 2 h in a muffle furnace at 550 °C. pH and electrical conductivity of casing and compost extracts were determined using a pH meter and digital conductivity meter in 1:10 water extracts. Total carbon (C), nitrogen (N), and sulfur (S) content of dried and finely ground samples of feedstocks and compost substrates were determined by combustion. Total water-extractable C and N was measured using a TOC analyser. Microbial biomass in compost was estimated using the fluorescein diacetate (FDA) hydrolase assay (Shaw and Burns 2006). The humification index (HIX) in compost was measured by fluorescence spectroscopy of compost extracts (Ohno 2002). Nitrate and ammonium levels in composts were measured in water extracts using anion chromatography and cation chromatography,

respectively. Total free amino acids in compost were measured using a ninhydrin-based method (Zhang et al. 2013).

Molecular characterization of microbial diversity in compost

Microbial diversity in compost samples was analyzed by high-throughput amplicon sequencing of total compost DNA. Total DNA was extracted from freeze-dried, ground compost samples using a beadbeating method and purified using DNA binding magnetic beads. Bacterial communities were investigated using the V3-V4 region of the 16S rRNA gene, while fungal communities were studied using the ITS1 locus. Amplicon sequencing (Illumina MiSeq) was done by the Australian Genome research Facility, and the resulting sequence files were processed using the error-correcting DADA2 pipeline, implemented on the R platform. Taxonomy was assigned by comparisons with the SILVA database for bacteria and the UNITE database for fungi.

Measurements of mushroom crop yield and quality, and correlation with microbial diversity.

Records of the mushroom yield and quality obtained from individual compost crops were provided by growers on a confidential basis. Participating growers were closely associated with the respective compost producer, to ensure that each compost was used alone and was not blended with composts from other sources. Records kept by individual growers were not entirely consistent, since different producers record quality differently, using different scales. The data received were therefore normalized to a consistent scheme. Yield was recorded as (a) yield per flush (kg/m^2); (b) yield over first two flushes (kg/m^2); (c) total yield per crop (kg/m^2); and (d) biological efficiency (kg/kg compost dry wt). Quality was recorded as (a) % Premiums (white, round, no blemishes); (b) % A grade (off-white, slightly off shape, some blemished); (c) % B grade (off-shape, blemished, very dirty); and (d)% C grade (not edible, diseased, dead).

Crop yield and quality were correlated with microbial diversity using a linear mixed modelling approach in the R platform. The yield and quality parameters listed above were incorporated as meta-data in the microbial database, and microbial taxa that were significantly correlated ($p<0.05$) with changes in yield/quality were identified. These were further filtered, to only include taxa that were present in >80% of compost samples, and made up >0.1 % of the microbial population concerned.

Results and discussion

Impact of the Covid-19 pandemic on the Biomarkers project

The project commenced shortly before the outbreak of Covid-19 in 2020, and this led to considerable difficulties in the sampling programs and disruption of the selection of composters that were able to participate. Several collaborating compost facilities went out of business over the period of the project, and changed supply/demand/labour conditions in the industry meant that others were forced to withdraw from the sampling program (mainly due to a lack of available staff and financial constraints imposed by the changed market conditions).

The most pronounced effect of the Covid-19 pandemic was a severe delay in implementation of the project, mainly because of the need to sample from a defined sequence of crops (3 successive crops for the “proof of concept” stage; 26 crops, sampling every second crop over 12 months for the main stage). The “proof-of-concept” stage of the project finally commenced in May 2021, but had to be halted again in June 2021 because of Covid lockdowns during the second half of 2021. Sampling for this part of the project was finally completed in January – April 2022. There was no effect on data integrity for the “proof of concept” stage, because intact sampling series were obtained by restarting sampling after lockdowns.

For the main phase of the sampling program, two of the original participants had to abandon the program shortly after commencement, due to the market and management problems outlined above. Replacement composters were recruited, but these necessarily had a delayed start to their sampling program. Although each of the analysed datasets contained a full 12-months’ worth of samples, the overall sampling period lasted from October 2022 – February 2024 and the exact 12-month sampling period undertaken by participating composters was therefore not identical for all participant (e.g. one yard sampled from October 2022 - November 2023, while another sampled from January 2023 – February 2024). For the analysis below, each set of results have been re-aligned to match a calendar year, so that seasonality can be taken into account.

Consistency of microbial markers at Australian compost yards.

The major aim of this project was to establish a database of microbial biomarkers in mushroom compost, and to link these to changes in mushroom crop yield and crop quality. In order to guarantee the value of this database, two pre-conditions must be met:

- Measured microbial diversity in mushroom composts must be consistent, with similar microbial communities observed reproducibly at specific stages of the composting process at different compost yards;
- Measured microbial diversity must correlate with known measures of compost quality at different stages of the composting process (e.g. pH, C/N content, humification, moisture content, ammonia content)

These two preconditions were investigated in an initial "proof-of-concept" sampling program. Samples were taken from four timepoints (bunker fill, mid Phase I, end Phase I and end Phase II) during three successive compost crops at each of four composting yards. Bacterial diversity was measured in these samples, and compared with measurements of a range of physicochemical parameters: moisture content, C/N ratio, pH, Humification Index, total C, total N, soluble C, total N, microbial biomass, ammonium content, nitrification ratio (ammonium:nitrate).

Successive compost crops showed a high level of consistency both in physicochemical parameters and in bacterial diversity (Fig. 1). Composts sampled at the start of Phase 1 (wetted compost immediately before bunker fill, after addition of brew) showed a relatively diverse range of chemical markers (Fig. 1A) and bacterial diversity (Fig. 1B) from different composters. Compost samples with similar microbial communities appear in close proximity to each other on the plot (Fig. 1), so close clustering indicates a close microbial relatedness between compost samples. For each compost yard, the three successive crop samples were found to be closely aligned at each stage of the composting process, confirming the consistency at each yard. At bunker fill there was a broader divergence of microbes in composts from different yards, which was expected because yards in different States source their raw materials from different suppliers and different parts of the country. However, the compost microbial communities become more similar later in the composting process, and the Phase 2 microbial communities from all yards are very similar. This demonstrated that successive compost crops from an individual composter are highly reproducible in the succession of bacterial diversity, and that a major sampling program is unlikely to suffer from high levels of random variation between crops.

Correlation of bacterial biomarkers to physicochemical measurements of compost quality

This initial sampling program also addressed the question of whether the microbial composition of compost varies in a similar manner to physicochemical measures of compost quality. A range of currently used physicochemical parameters were monitored in the samples taken. These included measures of compost maturity (C/N ratio, pH); conversion of plant material to humic acids (humification index); release of soluble nutrients during breakdown of plant material (soluble C, N); microbial biomass (FDA hydrolase activity); nitrogen metabolism (ammonium content and nitrification ratio). The combined data are presented in Fig. 1A. Once again, compost samples with similar sets of physicochemical parameters appear close to each other on the plot, so chemically similar composts appear close together. The results are very similar to those obtained for total microbial diversity (Fig. 1B), with good reproducibility between successive crops at each individual yard, and broader variation between yards. The bunker fill samples showed highest variation, but across all yards the end Phase 1 samples were less consistent with each other, and the end Phase 2 samples more closely related still. Traditional physicochemical compost quality measures are thought to be related to the resulting mushroom crop yields and quality, though developing a reliable predictive tool has proved elusive despite many attempts in the last forty years. The similarity in the progression between phases observed for physicochemical indicators (Fig. 1A) and biological indicators (Fig. 1B) confirms that microbial biomarkers are closely correlated with known markers of compost quality, and may therefore feasibly be related to mushroom crop yield and quality. However, the resolution of the microbial data is much higher (up to 500 different bacterial taxa in each sample, versus eleven physicochemical indicators), so the probability of being able to discover and optimize a reliable prediction of yield/quality is much higher.

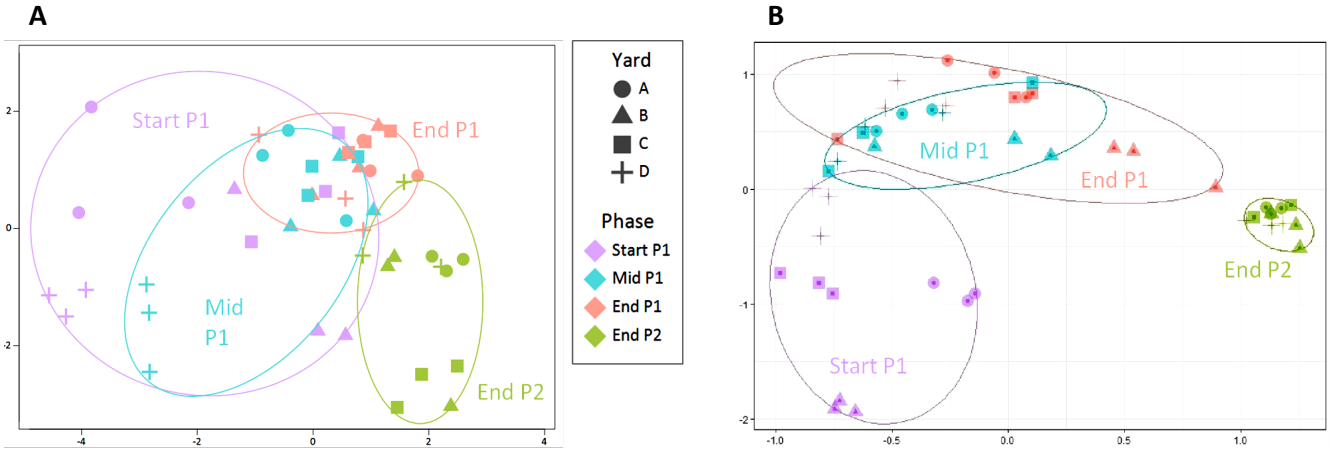

Figure 1. Chemical and biological markers of compost quality. **A**. Chemical indicators of compost quality, including C/N ratio, pH, Humification Index, soluble C, N, microbial biomass, ammonium content, nitrification ratio. **B.** Biological indicators**:** bacterial diversity measured by DNA analysis (next generation DNA sequencing). Plots represent principal component analysis (PCA) of combined data sets. The data were obtained from three successive crops at four composting yards, and four timepoints in composting. Ellipses represent 95% confidence intervals.

Constructing a large-scale compost microbial diversity database – sampling and data collection.

The observed microbial community in compost at different stages of composting is roughly consistent at different composting facilities (Fig. 1B), but there are many factors that may affect the specific microbes present at each stage and the quality of the final product. Several of these are related to the geographic location of the facility. Compost yards in different regions of Australia obtain their wheat straw from different locations (e.g. NSW yards largely from Western NSW and from the Riverina, South Australian yards largely from the Eyre peninsula), though this varies according to seasonal availability, especially in drought years. Different wheat-growing areas use different wheat cultivars, which are optimized for the respective soil and climate conditions, and this can affect the microbial content/diversity of the straw. Storage conditions for straw after harvest also vary across regions, modifying the effect and duration of adding "new season-straw" on the composting process. Compost microbial diversity is also strongly affected by differences in compost management processes, and it was important to include a range of different composting methods in order to achieve good oversight of methods used by Australian composters. Phase II pasteurization/curing methods are very similar at most Australian composters, and Phase II is commonly done in a closed tunnel over about 5-7 days (though one of the participating composters uses a tray pasteurization system instead of a tunnel). By contrast, Phase I composting varies greatly between different composters. Differences include the duration of wetting and of thermophilic composting, the number of turns (or rate of aeration), the use of an indoor tunnel, outdoor bunker, or outdoor windrow, the rate of application of water or goody water, the timing of poultry manure addition and the addition of supplementary feedstocks (e.g. stable bedding, feather-meal, oilseeds, or inorganic components such as urea).

In order to investigate annual variation in composting (e.g. the effect of adding new season straw), samples were taken were taken from every second crop (i.e. fortnightly) over a 12-month period. Randomized 10-fold samples were taken across the bulk of the compost (taken during turning in order to sample top, middle and bottom of the compost), and thoroughly blended before analysis. The participating composters included two producers using entirely indoor processes, and others producing Phase I compost in an outdoor bunker or on an aerated slab. The use of goody water varied, with some composters using only goody water for straw wetting, while others used either a lesser proportion of goody water or used goody water initially and then switched to fresh water. The pattern of goody water use is particularly relevant, since it contains a large population of microbes and acts as an inoculum for the straw (Safianowicz et al. 2018). In addition, the mixture and proportion of feedstocks varied considerably between composters. All these factors are expected to have a considerable influence on microbial content and diversity. Unfortunately, it was not possible to include this compost management information in the database, because of the need to ensure industrial confidentiality.

The composters included in the sample set have been anonymized (see below), but inclusion of method data would have too readily allowed their identification, especially as it is linked to yield and quality data.

The compost samples taken during the project, and the bacterial/fungal sequences obtained, are summarized in Table 1. A full year's samples (26 crops) were obtained from four yards, (W, X, Y and Z). A fifth yard (V) provided a smaller set of samples. In all, 113 compost crops were sampled at multiple timepoints, providing 425 samples. After DNA sequencing and quality control of the sequences, this provided a total of 58 million bacterial DNA sequences and 11.9 million fungal DNA sequences, representing over 87,000 different kinds of bacteria, and nearly 3,000 different kinds of fungi. The distribution of these microbes in different composts is presented in the database (Appendix 2), and an initial analysis is provided below and in the attached technical Appendix 1.

Table 1. Compost samples analyzed in the 12-month farm sample sequence, and the bacterial/fungal data obtained from these composts.

Composter	V	W	X	Y	Z	Total
Start of sampling	Sep-22	Aug-22	Dec-22	Oct-22	Oct-22	
Crops sampled[a]	9	26	26	26	26	113
Total sample number	35	102	96	93	99	425
Missed samples[b]	12.50%	1.90%	4.00%	10%	3.90%	5.10%
Bacterial DNA sequences[c]	4,795,434	14,025,730	13,365,172	11,494,847	14,432,191	58,113,374
Fungal DNA sequences (end Phase I)[d]	1,085,698	4,534,853	2,350,012	3,070,520	2,714,184	11,923,976
Total bacterial genotypes	13,642	21,013	29,443	25,819	24,962	87,353
Total fungal genotypes (end Phase I)	428	1,044	734	1,021	915	2,950
Crops with associated yield/quality data	0[e]	25	25	23	26	99

a) Samples were taken from every second compost crop, so 26 crops represent one calendar year of sampling.
b) Missed samples within the crops sampled, (operator error or sample loss through freezer breakdown).
c) The bacterial and fungal sequence numbers are for high quality sequences used in analysis (after removing noise, low quality sequences and chimeric sequences). Raw sequence counts were approximately 2-fold higher.
d) Bacterial diversity was determined at four timepoints for each crop, while fungal diversity was only determined for Phase I composts.
e) Composter V was only able to provide a subset of a year's samples (9 crops), so it was not possible to include comparable yield/quality data from Yard V in the overall dataset.

Microbial diversity in the 12-month farm sample sequence.

The bacterial communities in composts from the 12-month farm sample sequence revealed a clear succession of the types of bacteria present during composting (Fig. 2), as expected from the "proof-of concept" trial (Fig. 1B). Samples from bunker-fill (purple symbols) are biologically different from end-Phase I (red symbols), with Mid-Phase I samples intermediate between the two. End-Phase II samples (green symbols) are biologically very different from the others, confirming the importance of proper conditioning in the biological production of a high-quality compost.

The original project design envisaged bacterial analysis of compost from 6 yards, rather than the four used in the final database. To compensate for this shortfall, fungal community analysis was added to the program, and was carried out for all end-Phase I samples (Fig. 2). Phase II composts were not analyzed, since they are generally dominated (>95%) by *Mycothermus (Scytalidium)* (Thai et al. 2022), and diversity analysis is therefore less informative. The fungal diversity of Phase I composts was very similar for three of the yards studied, but slightly different for Yard Z (Fig. 2). This difference was not reflected in the bacterial communities, where all four yards appeared quite similar.

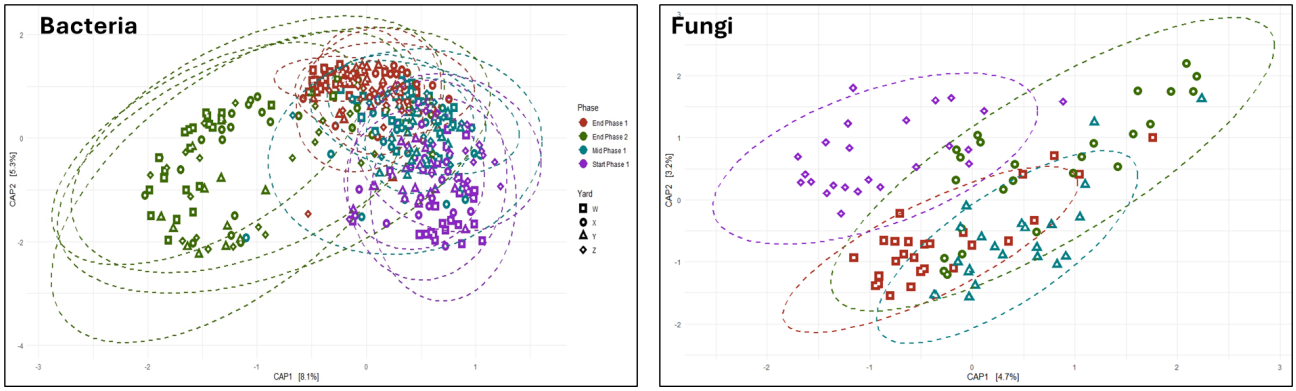

Figure 2. Bacterial and fungal communities in mushroom compost. Bacteria: Communities are grouped according to the phase of composting. Fungi: All samples are from end Phase I compost, communities are grouped according to composter. Compost samples with similar bacterial or fungal communities are close together on the plot. Ellipses represent 95% confidence intervals.

As discussed above, annual changes in feedstock quality were anticipated to cause cyclical changes in the microbial diversity of the compost. Diversity parameters were compared across the annual cycle (making allowance for the fact that different composters commenced sampling at different times (Table 1)), and considerable variation was indeed observed. Unexpectedly, this variation did not show a cyclical pattern, and was also not correlated with the presence of particular microbial taxa in the compost. New season straw is considerably different from weathered straw in its microbial load and diversity, and breaks down much more slowly during composting, so composters are careful to blend new season straw very gradually into their compost feedstocks when it becomes available. This care appears to be effective, since it does not lead to an annual cycle in the bacterial fingerprint of the compost. Seasonal variations in weather conditions might also be predicted to cause annual variation, especially for composters using outdoor composting (e.g. for Phase I bunkers or windrows), but this was also not observed.

Mushroom crop yield and quality in the 12-month farm sample sequence.

Data on the yield and quality of mushrooms produced from 378 of the composts studied were obtained from the production records of the enterprises concerned. No attempt was made to validate the data provided by growers, or to benchmark these data through independent growth trials using the same composts (this had been proposed in the initial project but was removed on the advice of the Project Reference Group).

Measurements of quality may take many different forms, but this study relied on a market-oriented approach, reporting % Premiums, and A-, B- and C-grade mushrooms. Unexpectedly, this led to complexity, since different growers recorded different scales, depending in part on the requirements of the wholesaler, so the values used for each composter have been standardized to the descriptors listed under methodology above. Although samples of each compost have been retained for possible future analysis of other parameters, it was unfortunately not possible to obtain samples of the mushrooms produced. Future projects could potentially also examine nutrient composition of the mushrooms (minerals, protein, vitamins, etc) to correlate with cultivation parameters and compost microbial diversity.

The annual cycle of yield/quality for Yard W (Fig. 3) shows typical variation at an individual composter. There was some variation in yields of first and second flush during the year, and third flush was only harvested where required. No annual cycle was apparent. The picked crop was >95% Premiums and A-grade mushrooms, but it is not clear from the data whether this is an accurate representation of the entire crop, or whether lower grade mushrooms were not accurately recorded because of their lesser market value. It is noteworthy that the average quality was lower in yards where a third flush was regularly harvested, due to the lower quality normally obtained for third flush.

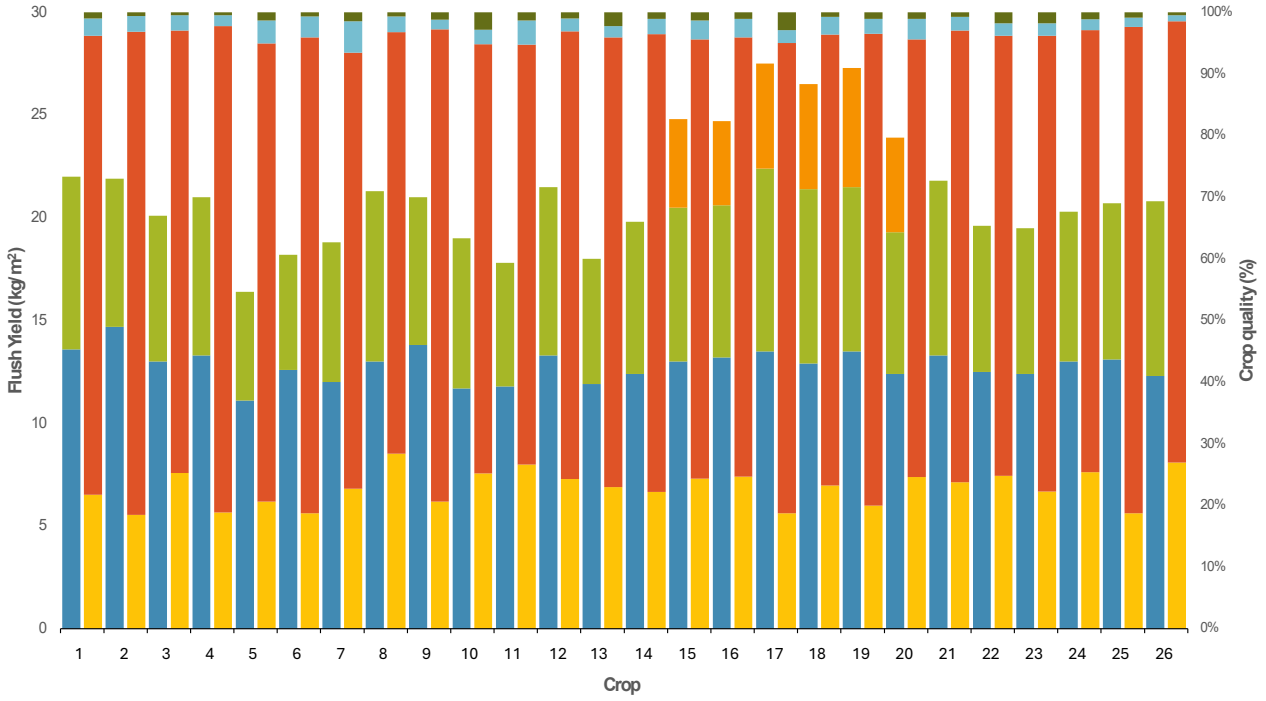

Figure 3. Variation in mushroom crop yield and quality over 12-months. Representative data are shown for Yard W – other yards are shown in the Technical Appendix. Yield (kg/m^2) for each flush is shown on the left-hand vertical axis (flush 1 – dark blue, flush 2 – green, flush 3 – orange). Crop quality (%) is shown on the right-hand vertical axis (Yellow – Premium grade, red – A grade, light blue – B grade, dark green – C grade).

Correlation of microbial diversity with mushroom crop parameters – compost biomarkers for mushroom crop yield and quality.

The most important aim for the project was to correlate microbial biomarkers at each of the composting phases with observed increases (or decreases) in crop yield and/or quality. Identification of key diagnostic biomarkers that are linked to enhanced yield could provide an important tool for better compost management. For example, the presence of a particular "increased-yield" biomarker in Phase I compost might allow the grower to negotiate better market contracts prior to harvesting, while the presence of a "reduced-yield" biomarker might prompt remedial action in composting, or even recycling of the compost to avoid the costs associated with a low-yielding mushroom crop.

No individual bacterial genotypes were identified that were statistically correlated with crop yield or quality, so further bacterial biomarker analysis focused on the level of bacterial genus. Several genera (i.e groups of closely related species) were identified that were significantly correlated with increased or decreased yield/quality in the overall database (Table 2). Significant effects (both positive and negative) were found most frequently for Phase I compost, and no diagnostic biomarkers were found in Phase II compost. The most interesting correlation was the correlation of *Thermus* in Phase I with an increase in yield. *Thermus* is one of the most abundant bacterial taxa observed at the end of Phase I and makes

up a large proportion of the bacterial population of goody water (Safianowicz et al. 2018), so this may be a useful diagnostic result.

Additional detail relating to the individual participating composters is provided in the Technical Appendix (Appendix 1). This revealed a larger number of positive and negative yield correlations, which were statistically significant at individual yards, but were not apparent in the combined database because of variation in microbial diversity between composters.

Table 2. Bacterial genera correlated with increased or decreased total mushroom yield and quality ($p<0.05$) in the overall database, combining all the composters tested. The genera with the strongest effect are shown for each Phase (blank fields indicate no significant correlations). Genera in the final column are statistically associated with the designated effect whenever they appear. Positive and negative correlations are indicated in green and red, respectively.

	Effect	Bunker-fill	mid Phase I	end Phase I	end Phase II	All phases
Yield	**positive**			*Thermus*		
	negative	*Chelativorans*	*Thermobispora*	*Caldicoprobacter*		*Thermobacillus*
Quality	**% Premiums**			*Blastocatella*		
	% Premiums			*Catenococcus, Devosia, Bacillus*		
	% A-grade			*Xiphinematobacter, Pelomonas*		*Flavobacterium*
	% A-grade					
	% B-grade					
	% B-grade					*Thermobacillus, Sphaerimonospora, Chelatococcus*

Because bacterial diversity was the main aim of the project, the fungal diversity was only measured at the end of Phase I. This choice reflected the observation that most bacterial biomarker correlations with crop yield and quality were observed at the end of Phase I. In addition, since the fungal community at the end of Phase II is generally composed of >95% *Mycothermus*, it was thought unlikely that small changes in this fungal composition would have a significant effect on crop yield (but see additional comments in the Recommendations section below). However, no fungal genera in Phase I compost were found to be significantly correlated with crop yield or quality, either at individual composters or in the combined dataset.

Key conclusions for the observed correlations between compost biomarkers and yield/quality of the mushroom crop.

1. **Statistically significant correlations (>95% certainty) of individual bacterial genera with mushroom yield/quality were more frequent for end Phase I compost than for compost at other composting timepoints.** The Phase I process is the most variable stage of mushroom composting. Across the yards studied, there was considerable variability in how Phase I was implemented, including (a) use of outdoor bunkers, outdoor windrows or indoor bunkers, (b) varied length of Phase I composting, (c) varied frequency of compost turning, (d) nitrogen (poultry manure and/or inorganic N) and other components added to the straw in different forms and at differing timepoints, (e) water and goody water added in differing proportions and at different times. All of these variables

directly affect the microbial succession observed during composting and contribute to the considerable variability in the Phase I microbial community observed between composters (Figure 2).

In addition to differences between the standard processes used by each composter, there are also slight variations that are applied by each composter in the course of their daily work. These may be partly experimentation (e.g. adding part of the manure at a later timepoint), or may be simple changes caused by e.g. shortage of staff, changed quality of feedstocks, reduced demand, etc. In this project, composters were asked to record major changes to the composting and growing process that might have affected yield/quality (e.g. loss of one flush, disease problems, delayed ammonia clearance in Phase II). These were controlled for during the modelling stage, but even when the affected crops were eliminated from the model, there was no significant change in the biomarkers observed.

The project aimed to develop biomarkers that would be applicable to the whole industry, and so no attempt was made to identify differences caused by the overall composting process used at each composter (e.g. details of temperatures, times, turning rates, aeration rates etc). This was partly because the details were regarded as commercial-in-confidence, and could therefore not be recorded in the database, and partly for statistical reasons. With only four yards sampled, effects due to e.g. the use of an outdoor bunker vs an indoor bunker could not have been distinguished with statistical confidence from other composter-dependent effects. It was also not possible to include all the minor, day-to-day changes in composting process, as these are not usually recorded and their inclusion would have provided a very heavy additional burden for the participating composters.

The compost microbe community at the end of Phase I needs to be appropriate to ensure effective conditioning of the compost, mediating the complete removal of ammonia and the growth of *Mycothermus* and its associated microbes by the end of Phase II. The results obtained here reveal that slight changes in this bacterial community have a significant effect on mushroom yield, presumably because of their influence on the microbial succession during Phase II. Interestingly, these effects on yield are more significant than changes in the bacterial community at the end of Phase II – this may be because of effects of the Phase I bacterial community on development of the end-Phase II fungal community, which is dominated by *Mycothermus* (see the Recommendations below).

Effect of feedstock variability - Variability in microbial diversity in the feedstocks (i.e. start Phase I) was even larger than at end Phase I (Figure 2), but most of this diversity was in mesophilic taxa (i.e. those that grow optimally at temperatures of 20 – 45 °C). These taxa are sensitive to the high temperatures experienced in Phase I (>70 °C), and are killed during high-heat composting (including potential human pathogens in the poultry manure). This provides a good explanation of the lack of correlation of these taxa with final yield/quality.

Effect of Phase II variability - Microbial variability between composters at the end of Phase II was considerably lower than at the end of Phase I, because the Phase II process of pasteurization and conditioning in a closed tunnel was quite uniform at the different yards studied. It was nonetheless expected that the bacteria present at the end of Phase II might be strongly correlated with mushroom yield/quality, because the Phase II microbial community is essential for strong mycelium proliferation during spawn run. For individual compost yards this was indeed the case, with some of the strongest correlations observed at end Phase II (see Technical Appendix). However, the bacterial biomarkers observed were different at different composters, and so they did not provide uniform biomarkers that could be used to predict crop yield across the industry.

2. **Most of the statistically significant correlations (>95% certainty) showed a negative correlation with yield or quality, not a positive effect. The magnitude of these observed effects was generally quite small**. This is a phenomenon that is not uncommon when working with large datasets. Most frequently, the negative correlations are observed because the two correlated phenomena (in this case the population of a particular bacterial genus and the crop yield) are not directly linked, but are correlated through linkage to a third variable, for example, a physical parameter such as temperature, or an association with another organism. The magnitude of the effect is an indication of the utility of these taxa as biomarkers, but it must always be borne in mind that the respective genus does not cause the observed effect on yield.

   The frequency of negative correlations can also increase when there is selectivity of sampling, i.e. where certain groups of samples are either not tested, or not included in the analysis. In the current project, the four composters

that provided a complete set of data were operating at high efficiency, so there was very little variation in the observed yield or quality of the resulting crops. This means that even very small changes in conditions might affect particular bacterial populations and also be associated with an apparent marginal decrease in yield, hence yielding negative correlations. In principle, this could indeed be described as "selective sampling", since we consistently used composts which were high-yielding, and were not able to include data from low-yield composts. This was not done intentionally (no samples were omitted from analysis), but due to the lack of availability of low-yield composts for testing. There were low-yield composts available at the time, but the respective composters were (for excellent reasons) more concerned with their own compost management, and they declined to participate in the study.

3. **Almost all of the significant correlations were for genera (groups of bacterial species) that have been poorly characterized, if at all**. This is an important limitation on practical application of the results obtained. The understanding of microbial diversity in the environment has made enormous strides forward in the last ten years, and from DNA sequencing studies it is now clear that there are many thousands-fold more bacterial species in environments like compost than were previously known. However, these species are identified by characterization of a single gene that is present in all bacteria – for most of these species there is no further information (yet) available on how they grow, and what their function is in the environment. This makes it almost impossible to speculate on why an increase in their population might be correlated with higher (or lower) crop yields, and whether this effect is merely a correlation (i.e. the increased crop yield and the increased population are both due to a secondary factor) or a causative effect (i.e. the increased population directly stimulates cropping efficiency). If the latter is true, then such strains could potentially be used as a "prebiotic" inoculum for compost – but many of these taxa have never been isolated or cultivated in the laboratory (they are known only from the environmental DNA sequences), so this is not yet possible.

4. **There was significant variation between individual composters, and the biomarkers identified were generally not consistent across different compost yards.** The initial "proof-of-concept" phase of the project demonstrated that the compost microbial populations from each composter were consistent over successive crops, and that the bacterial succession during composting was consistent with changes in physicochemical compost maturity measures, which have been used in the past to measure compost quality. This eliminated the factor of random variation between crops, i.e. that successive compost crops might look microbially quite different from each other, regardless of compost quality. Nonetheless, the most significant statistical factor differentiating crops in the 12-month study was the composter. As described above, this was probably due to routine differences in the composting process between composters, e.g. effects of type and length of Phase I, time of addition of nitrogen, frequency of turning etc, all of which will affect the microbial population. These factors were not recorded and were not included in the analysis, partly these "tweaks" to the composting process are commercially sensitive, but mainly because the level of replication (only four composters included) would make it statistically impossible to evaluate the effects of individual changes. It was anticipated that biomarkers would be identified that are correlated with increased yield despite slight differences in the composting process – in the event, the only genus that was linked to increased crop yield was *Thermus* in end Phase I compost (see Recommendations section).

Outputs

Table 4. Output summary

Output	Description	Detail
Database correlating microbial signature against yield and quality data	Database correlating microbial signature against yield and quality data	Appendix 2
Publication	Article in Australian Mushroom journal (2019)	Appendix 3
Webinar	Webinar on mushroom compost biomarkers (AHR videos, 14th October, 2021)	https://www.youtube.com/watch?v=unj5yTnPEI4
Conference presentation	Presentation of the Compost biomarkers database at the 44th AMGA conference, Adelaide, 28th October 2022.	https://youtu.be/1cY80XVap7I
Conference presentation	Presentation of the Compost biomarkers database at the 45th AMGA conference, Auckland, 24th October 2024.	https://www.youtube.com/watch?v=CasEI1tzKuY
Conference presentation	Presentation of the Compost biomarkers data at the ISMS/NAMC conference, Las Vegas, 28th February, 2024.	Invited presentation of the O'Neil Lecture, North American Mushroom Conference.

Outcomes

Table 5. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Improved awareness of microbial characteristics of compost at various stages of the production cycle and the impact on production outcomes.	Aligned to SIP 2017-2021: Outcome - *Mushroom growers are profitable and sustainable through increased yields, reduced costs and effective risk management;* Strategy - *Improve production by increasing yield and quality;* *KPI - identification of yield productivity increases*	The database developed by the project is the largest source of microbial information about mushroom compost ever compiled. It provides key information about the influence on mushroom yield and quality of bacterial and fungal biomarkers in compost at four stages of the composting process.	Feedback from composters and growers at AMGA conferences, Feedback from composters and growers via the Marsh Lawson Steering Committee. Direct feedback from composters/growers participating in the project
Mushroom growers equipped to increase profitability by managing compost microbes at optimum levels throughout the production cycle.	Aligned to SIP 2017-2021: Outcome - *Mushroom growers are profitable and sustainable through increased yields, reduced costs and effective risk management;* Strategy - *Improve production by increasing yield and quality;* *KPI - identification of yield productivity increases*	Production of a "biomarker calculator" and best practice sheet were projected outcomes for the project. However, detailed statistical analysis of the database did not reveal consistent biomarkers of yield/quality that can be implemented across the industry at this stage. Individual participants have been provided with data from their own company, which will guide their own production strategies.	See Results and Discussion above. Discussion and advice from the Project Reference Group. Feedback from composters and growers at AMGA conferences
Australian Mushroom grower access to microbial biomarker database for compost quality control.	Aligned to SIP 2017-2021: Outcome - *Mushroom growers are profitable and sustainable through increased yields, reduced costs and effective risk management;* Strategy - *Improve production by increasing yield and quality;* *KPI - identification of yield productivity increases*	Mushroom growers provided with an anonymized spreadsheet version of the database for further analysis. Direct use for compost quality control at all composters across the industry is not supported at this point, since no consistent biomarkers of yield/quality (either negative or positive) were found.	See Results and Discussion above. Database introduced to growers at AMGA conference 2024, and feedback provided by delegates (composters and growers). Individual participants provided with

Monitoring and evaluation

Table 6. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
To what extent has the project achieved its expected outcomes?	The project has achieved its major outcome of generating an extremely large database of microbial biomarkers in compost, and linking these to mushroom yield and quality. Because of changes in the number of industry participants, the database was expanded from the original aim of only measuring bacterial biomarkers, to also providing Phase I fungal biomarkers. A broad range of compost biomarkers correlated with mushroom yield and quality were identified at individual composters, but there were not consistent across the industry.	Delays in project realization because of the Covid-19 pandemic and because the project required an uninterrupted 12-month sampling period has meant that the database is at present only available in spreadsheet form. The very large amount of data provided provides extensive opportunities for further data mining, which will provide further insights into the influence of the compost microbial community on mushroom crop parameters.
To what extent has the project met the needs of industry levy payers?	The project has met the industry need for more knowledge about the impact of compost bacteria and fungi on mushroom crop yield and quality, with a massive increase in the data available for mushroom production on Australian farms. Unfortunately it has also demonstrated that variability in compost management (driven by market factors) plays a key role in determining both microbial characteristics and crop yield, and measured biomarkers of crop yield/quality are not consistent across the industry.	Further insights into the impact of compost bacteria and fungi on mushroom crop yield and quality will be available through further data mining of the database provided.
To what extent were mushroom composters and growers engaged with the biomarker database, the biomarker calculator, and the optimized user guide?	A broad range of compost biomarkers correlated with mushroom yield and quality were identified at individual composters, but there were not consistent across the industry. The biomarker database has been generated and made available for further data mining, but it was not appropriate to produce a calculator or user guide at this point in time.	Ongoing engagement during the project was hampered by the fact that the database was only available as a final output of the project. With completion of the initial data analysis, publication of the data in scientific journals is not planned, which will broaden the impact of the project considerably.
Did the project engage with industry levy payers through their preferred learning styles (factsheets, User	The project engaged with levy payers through industry presentations (webinars and conference	A factsheet is planned, which will explain the impact of compost microbes on compost productivity

guide, webinars, demonstration trials)?	presentations) and through articles in the industry journal (one published article, one article planned for early 2025). As described above, engagement through factsheets and an optimized user guide was not possible, due to the nature of the results obtained (no consistent compost biomarkers of yield/quality identified that can be used across the industry).	and its influence on mushroom crop yield and quality, but it will not have the predictive impact that was anticipated (and will therefore not amount to a “user guide for better crop yield”).

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Recommendations

- The bacterial genus *Thermus* was identified in end-phase I compost as a positive bacterial biomarker of crop yield. This genus is abundant in end-Phase I compost, and more work is required to validate this finding over a range of both high yielding and lower-yielding composts, to determine the minimum level of *Thermus* in compost that is predictive of high crop yield. If appropriate, a rapid quantitative test for *Thermus* in end Phase I compost could then be developed (either PCR-based or antigen based).
- *Thermus* strains have previously been found at high levels in goody water, so when high-quality goody water is used for straw wetting it effectively acts as a positive inoculum. However, this has only been studied at one compost yard (Safianowicz et al. 2018). This needs to be evaluated at other composters. If the finding is confirmed, then goody water management may be the most important aspect of maximizing crop yield. More detailed studies of microbial dynamics in goody water at a range of composters are urgently required.
- The fungus *Mycothermus* is always present at high levels in end-Phase II compost (usually >95% of the fungal population). The current project has only analyzed fungal biomarkers at end-Phase I, but it is possible that the exact levels of *Mycothermus* at end-Phase II may be diagnostic of yield. Further analysis of the end-Phase II fungal population should be carried out immediately, using the samples from the current study, to determine whether a minimum level of *Mycothermus* in Phase II compost is predictive of high crop yield.
- Although the participating composters in the database have been de-identified in the database, individual participants have been provided with their personal dataset of biomarkers. This can now be combined with additional confidential process data for each crop that they may have recorded, which will provide considerable additional predictive power for yield and quality.
- The bacterial biomarker database generated in this project provides a detailed resource for further data mining. At present, analyses have focused on identification of individual bacterial genotypes or genera that are statistically correlated with crop yield and quality. Further data mining is required to determine whether combinations of microbial taxa may be more effective than single genera or species in predicting crop yield and quality.
- Most of the bacterial biomarkers in the database that are linked to increased yield and quality are uncharacterized genera, most of which have never been isolated or studied in the laboratory. More research is required to isolate and characterize these organisms, and understand their function in compost. A good example of this is the recent characterization of a novel bacterial species that is dominant in Phase II, *Mycovorax composti*, which attacks the fungus *Mycothermus* (Thai et al. 2024). Some functional characterization is possible using inference programs based on 16S bacterial diversity, such as PICRUST (Douglas et al. 2020) or Tax4Fun2 (Wemheuer et al. 2020), which have been used in the past to predict tentative functional profiles in anaerobic digestion or composting (Ijoma et al. 2021; Jimenez et al. 2014; Wang et al. 2018) However, their performance is unreliable when used for inference outside human-based datasets (Sun et al. 2020), and the results must be interpreted with care.
- For future studies of this nature, benchmarking of cropping is essential. This was proposed in the original tender, but was abandoned on the insistence of the Project Reference Group. The use of grower data introduced numerous confounding variables, since measured crop yield and quality were dependent not just on compost quality, but on a multitude of other factors (e.g. market factors, labour availability, customer requirements for e.g. size, weight, whiteness, shelf-life). In addition, statistical analysis of yield/quality required a uniform set of records kept by all participating growers -a minimum set of cropping details was requested for the project, but this was not always adhered to (especially for growers selling to customers with less rigorous requirements, who did not need to keep exact records of crop quality etc). Meaningful statistical analysis of the total dataset is restricted to parameters that are recorded for all crops, and this is most easily done by conducting standard benchmark trials, e.g. in the Marsh Lawson Mushroom Research Unit.

Scientific publications

Journal article

Kertesz, M. (2019) Compost biomarkers. A crystal ball for yield and quality? *Australian Mushrooms Journal* Spring 2019: 36-37.

References

Bernal MP, Sommer SG, Chadwick D, Qing C, Li GX, Michel FC (2017) Current approaches and future trends in compost quality criteria for agronomic, environmental, and human health benefits. Adv Agron 144:143-233 doi:10.1016/bs.agron.2017.03.002

Cao GT, Song TT, Shen YY, Jin QL, Feng WL, Fan LJ, Cai WM (2019) Diversity of bacterial and fungal communities in wheat straw compost for *Agaricus bisporus* cultivation. Hortscience 54:100-109 doi:10.21273/Hortsci13598-18

Carrasco J, Garcia-Delgado C, Lavega R, Tello ML, De Toro M, Barba-Vicente V, Rodriguez-Cruz MS, Sanchez-Martin MJ, Perez M, Preston GM (2020) Holistic assessment of the microbiome dynamics in the substrates used for commercial champignon (*Agaricus bisporus*) cultivation. Microb Biotechnol 13:1933-1947 doi:10.1111/1751-7915.13639

Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI (2020) PICRUSt2 for prediction of metagenome functions. Nat Biotechnol 38:685-688 doi:10.1038/s41587-020-0548-6

Ijoma GN, Nkuna R, Mutungwazi A, Rashama C, Matambo TS (2021) Applying PICRUSt and 16S rRNA functional characterisation to predicting co-digestion strategies of various animal manures for biogas production. Scientific Reports 11:13 doi:10.1038/s41598-021-99389-4

Jimenez DJ, Dini-Andreote F, van Elsas JD (2014) Metataxonomic profiling and prediction of functional behaviour of wheat straw degrading microbial consortia. Biotechnology for Biofuels 7:17 doi:10.1186/1754-6834-7-92

Kertesz M, Safianowicz K, Bell T (2016) New insights into the microbial communities and biological activities that define mushroom compost. Science and cultivation of edible fungi 19:161-165

Ohno T (2002) Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. Environ Sci Technol 36:742-746 doi:10.1021/es0155276

Ryckeboer J, Mergaert J, Vaes K, Klammer S, De Clercq D, Coosemans J, Insam H, Swings J (2003) A survey of bacteria and fungi occurring during composting and self-heating processes. Ann Microbiol 53:349-410

Safianowicz K, Bell TL, Kertesz MA (2018) Bacterial population dynamics in recycled mushroom compost leachate. Appl Microbiol Biotechnol 102:5335-5342 doi:10.1007/s00253-018-9007-4

Sharma HS, Kilpatrick M (2000) Mushroom (*Agaricus bisporus*) compost quality factors for predicting potential yield of fruiting bodies. Can J Microbiol 46:515-519 doi:10.1139/cjm-46-6-515

Shaw LJ, Burns RG (2006) Enzyme activity profiles and soil quality. In: Bloem J, Hopkins DW, Benedetti A (eds) Microbial methods for assessing soil quality. CABI Publishing, Wallingford, pp 158-182

Sun S, Jones RB, Fodor AA (2020) Inference-based accuracy of metagenome prediction tools varies across sample types and functional categories. Microbiome 8 doi:ARTN 46

10.1186/s40168-020-00815-y

Thai M, Bell TL, Kertesz MA (2024) *Mycovorax composti* gen. nov. sp. nov., a member of the family *Chitinophagaceae* isolated from button mushroom compost. Int J System Evol Microbiol 74:Article 6496

Thai M, Safianowicz K, Bell TL, Kertesz MA (2022) Dynamics of microbial community and enzyme activities during preparation of *Agaricus bisporus* compost substrate. ISME Commun 2:88 doi:10.1038/s43705-022-00174-9

Vieira FR, Pecchia JA (2021) Bacterial community patterns in the *Agaricus bisporus* cultivation system, from compost raw materials to mushroom caps. Microb Ecol 84:20-32 doi:10.1007/s00248-021-01833-5

Wang K, Chu C, Li XK, Wang W, Ren NQ (2018) Succession of bacterial community function in cow manure composting. Bioresour Technol 267:63-70 doi:10.1016/j.biortech.2018.06.028

Wemheuer F, Taylor JA, Daniel R, Johnston E, Meinicke P, Thomas T, Wemheuer B (2020) Tax4Fun2: prediction of habitat-specific functional profiles and functional redundancy based on 16S rRNA gene sequences. Environ Microbiome 15:12 doi:10.1186/s40793-020-00358-7

Zhang YF, Fu Y, Zhou SF, Kang LX, Li CZ (2013) A straightforward ninhydrin-based method for collagenase activity and inhibitor screening of collagenase using spectrophotometry. Anal Biochem 437:46-48 doi:10.1016/j.ab.2013.02.030

Zied DC, Pardo-González JE, Minhoni MTA, Pardo-Giménez A (2011) A reliable quality index for mushroom cultivation. J Agric Sci 3:50-61

Intellectual property

No project IP or commercialisation to report.

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Appendices

Appendix 1. Detailed technical report, methods and results.

Appendix 2. Database of microbial biomarkers in mushroom compost crops, linked with yield and quality data.

Appendix 3. Kertesz, M. (2019) *Australian Mushrooms Journal*

Appendix 1. Detailed Technical report

Table of Contents

1 Background- Microbial diversity in Australian mushroom composts

2 Methods

2.1.1 Compost sampling

Compost samples used in the project were obtained from five commercial mushroom composting facilities located in New South Wales, Queensland, Victoria and South Australia. For confidentiality reasons, the composters are not named here. Compost samples taken at specific timepoints during the composting process were pooled samples of approximately 1-2 kg, produced by combining ten independent samples taken from across the face of the compost pile during turning. Sampling was done on site by industry personnel, who were provided with appropriate training by the research team (https://www.youtube.com/watch?v=TZG_QKWlOyA), and the samples were shipped to the University of Sydney for analysis.

2.1.2 DNA extraction, amplification and sequencing

Total compost DNA was extracted according to Lever et al. (2015) with some modifications. Ground compost samples (200 mg) were suspended in 200 mM sodium hexametaphosphate (100 µL), lysis buffer 1 was added (30 mM Tris/HCl, 30 mM EDTA, 800 mM guanidinium chloride, 0.5% (v/v) Triton X-100, pH 10.0) (500 mL), and the samples were lysed using a homogenizer (MoBio Laboratories Inc.,) at 2000 rpm for 5 min. Lysis buffer 2 (2.5 M sodium chloride, 2% (w/v) cetyltrimethylammonium bromide, 0.1% (w/v) polyvinylpolypyrrolidone) (500 µL) was added, followed by incubation at 65 °C with agitation (500 rpm) for 30 min and centrifugation, Supernatants were extracted once with an equal volume of chloroform:isoamyl alcohol (24:1) and DNA was recovered from the aqueous phase using DNA binding magnetic beads (GE Life Sciences, Australia) in SPRI solution, following the manufacturer's

instructions. Bacterial diversity was analysed using primers 341F and 806R (Caporaso et al. 2011; Muyzer et al. 1993) to amplify the V3-V4 16S rRNA gene hypervariable region, with the Illumina MiSeq platform (paired 300 bp read lengths) at the Australian Genome Research Facility (AGRF) (Melbourne, Australia).

2.1.3 Bioinformatics

Raw FASTQ files were processed in R v3.6.1.(R Core Team 2019) Raw read quality was determined using FastQC. Trimming and filtering was determined using the DADA2 function 'filterAndTrim', (Callahan et al. 2016) discarding forward and reverse reads with an expected error score higher than 3 and 4, respectively. Low quality reads were removed during trimming and filtering by setting 'truncLen' parameters to 285 and 240 for the forward and reverse reads, respectively. Forward and reverse primers were trimmed from the 5' end by setting the 'trimLeft' function to 17 and 20, respectively. The sequences were denoised and dereplicated using the 'dada' and 'derep' functions, unique sequences were merged with a minimum overlap of 20 base pairs and a sequence table was constructed with the resulting sequence variants.

Taxonomy was assigned using a pre-trained SILVA Naïve Bayes classifier clustered at 99% identity (SILVA release v132) (Quast et al. 2012). Species assignment was done in a separate step using the SILVA release v132 for species assignment. 16S gene sequences that were affiliated with chloroplasts and mitochondria were removed prior to downstream analysis. Sequence variants which occurred in fewer than three samples and with fewer than three reads in each of these samples were also removed (singletons and doubletons). A phylogenetic tree was constructed using the packages "phangorn" (Schliep 2010) and "DECIPHER", (Wright 2016) using the neighbour-joining method.

Statistical analysis was done using the packages "phyloseq" (McMurdie and Holmes 2013) and "vegan".(Dixon 2003) All graphs and plots were visualized using "ggplot2" (Wickham 2016). Shannon and Simpson alpha-diversity analyses were performed using the 'plot_richness' function from the phyloseq package before singletons and doubletons were removed from the dataset. Differences in the bacterial community (beta-diversity) were analysed in R (R Core Team 2019) using a canonical analysis of principal coordinates with unweighted UniFrac as the distance metric.

DNA sequencing data are available at NCBI Short Read Archive under BioProject PRJNA867030.

3 Consistency of microbial diversity in Australian mushroom composts

Table 3.1. Number of bacterial genotypes found in composts from different composting yards at different stages of the composting process

Yard	Crop	Sample			
		Start-Phase 1	Mid-Phase 1	End-Phase 1	End-Phase 2
A	1	1479	1573	1759	1712
	2	1553	1321	1456	1060
	3	1189	885	2610	2130
B	1	1925	1553	1933	2052
	2	2199	1544	2023	1983
	3	2409	2131	3593	1174
C	1	2199	2034	1764	1388
	2	1625	968	1341	872
	3	2115	348	2183	1400
D	1	2004	1563	2014	1529
	2	1380	1619	1113	1540
	3	1304	1656	2618	1904

Figure 3.1. Key bacterial taxa identified in composts from different yards. Only genera constituting >3% of the total population are shown.

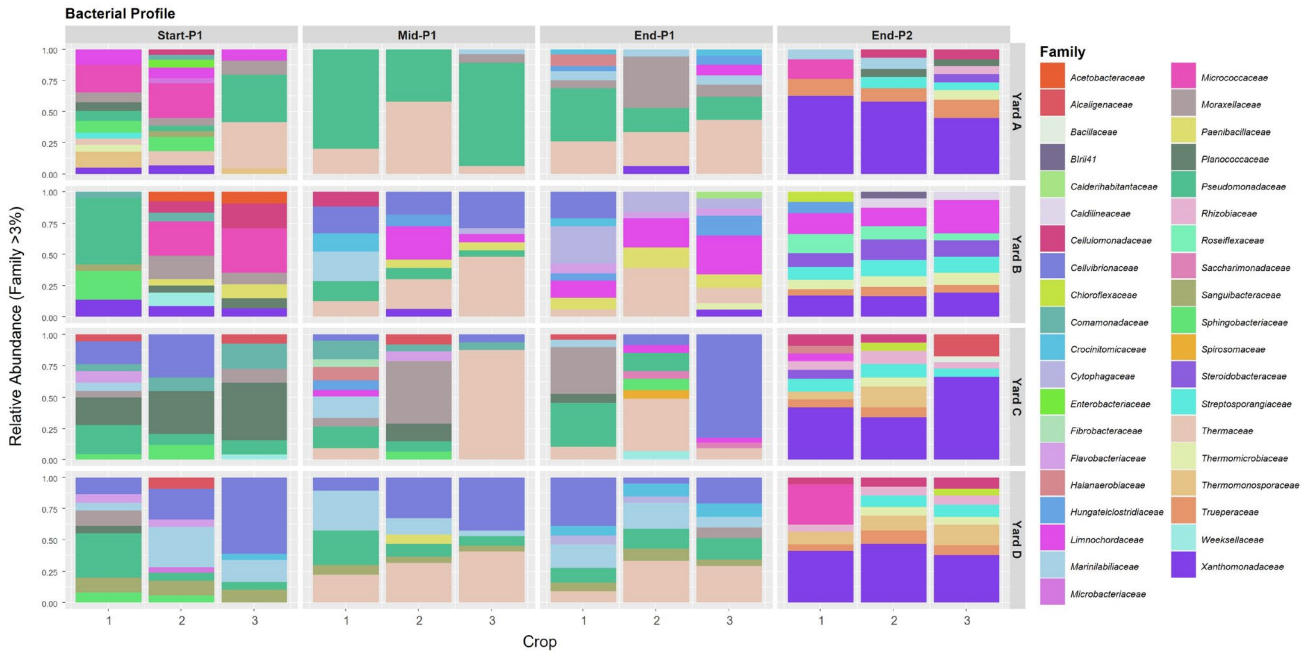
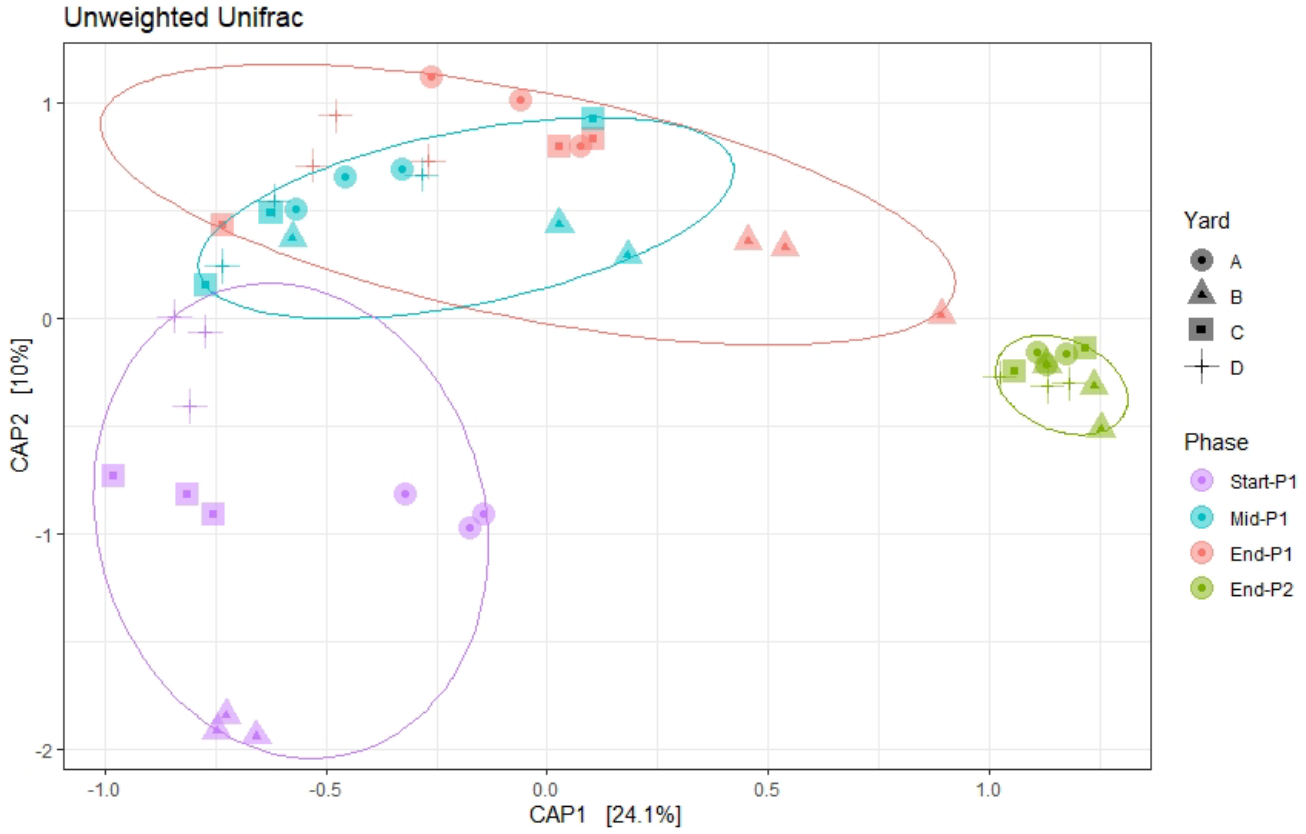


Figure 3.2. Distribution of bacterial communities in different phases of composting at four different Australian compost yards. Points that cluster close to each other contain similar bacterial communities. Ellipses represent 95% confidence intervals.



4 Physicochemical parameters of mushroom compost quality and correlation with bacterial diversity

Table 4.1. Physicochemical parameters measured in three successive compost crops at four compost yards (designated A, B, C,D) in eastern Australia.

Compost Yard	Crop	Phase	Moisture Content %	pH	Total Carbon (g/kg)	Total Nitrogen (g/kg)	Carbon/ Nitrogen Ratio	Total Extractable Carbon (g/kg)	Total Extractable Nitrogen (g/kg)	Humification index	Biomass (FDA activity; µg/min/ gram compost)
A	1	Start Phase 1	69.68	8.01	410.86	12.20	36.02	18.15	1.87	7.27	0.14
A	1	Mid Phase 1	73.78	8.1	369.88	16.10	23.02	8.22	1.45	8.64	17.68
A	1	End Phase 1	71.99	7.8	353.22	19.32	18.29	6.15	1.05	10.07	7.13
A	1	End Phase 2	65.20	6.77	354.15	24.96	14.19	6.01	0.95	10.49	72.69
A	2	Start Phase 1	68.83	7.92	399.03	11.92	33.59	10.36	1.18	6.99	25.63
A	2	Mid Phase 1	72.69	7.91	371.97	16.34	22.80	7.43	1.35	10.23	6.89
A	2	End Phase 1	71.41	7.67	360.49	19.09	18.89	5.17	0.91	8.76	20.05
A	2	End Phase 2	65.50	7.04	351.93	25.54	13.79	6.37	1.01	9.55	77.08
A	3	Start Phase 1	69.01	8.01	429.70	7.93	55.50	10.89	1.02	10.39	4.88
A	3	Mid Phase 1	72.44	8.2	331.79	20.99	15.87	6.68	1.33	6.50	47.32
A	3	End Phase 1	69.65	7.02	337.42	20.13	16.84	6.45	1.17	10.36	4.05
A	3	End Phase 2	64.98	7.25	347.93	24.05	14.50	6.12	1.03	10.00	81.11
B	1	Start Phase 1	72.84	7.87	363.67	12.22	29.81	6.66	1.75	8.30	64.47
B	1	Mid Phase 1	70.79	8.2	340.47	18.21	18.77	5.44	1.17	8.42	9.87
B	1	End Phase 1	71.89	7.89	348.31	16.16	21.56	4.89	1.51	7.46	40.87
B	1	End Phase 2	63.82	7.74	350.29	26.59	13.18	7.18	1.20	7.23	11.17
B	2	Start Phase 1	70.04	7.85	327.41	16.24	20.22	6.51	1.61	6.25	188.47
B	2	Mid Phase 1	72.29	7.93	355.30	18.63	19.08	6.68	1.84	6.81	45.87
B	2	End Phase 1	71.17	7.55	355.10	19.52	18.25	5.45	1.17	11.48	1.47
B	2	End Phase 2	63.93	6.66	344.01	27.04	12.73	7.50	1.22	5.64	136.87

B	3	Start Phase 1	68.94	7.46	348.93	14.91	23.44	4.38	1.13	6.99	215.37
B	3	Mid Phase 1	72.75	7.59	356.53	18.49	19.29	4.14	0.83	5.54	17.67
B	3	End Phase 1	71.38	7.91	343.28	20.58	16.68	6.07	1.30	8.68	4.27
B	3	End Phase 2	63.90	7.42	357.53	25.11	14.24	7.93	1.24	7.47	16.67
C	1	Start Phase 1	70.19	7.99	374.90	19.21	19.52	8.76	1.89	5.84	18.35
C	1	Mid Phase 1	73.48	8.22	367.78	20.59	17.97	7.31	1.33	9.75	49.59
C	1	End Phase 1	70.26	7.76	370.62	21.52	17.23	6.37	1.08	11.44	15.16
C	1	End Phase 2	63.24	7.8	355.34	28.70	12.39	7.01	1.06	3.76	144.70
C	2	Start Phase 1	74.25	7.61	363.39	17.30	21.11	6.08	1.20	10.25	12.78
C	2	Mid Phase 1	72.70	8.08	359.40	20.31	17.73	5.14	0.94	8.63	18.75
C	2	End Phase 1	71.15	7.75	356.77	19.55	18.28	4.62	0.82	11.13	23.22
C	2	End Phase 2	63.95	7.5	343.53	28.22	12.18	4.72	0.76	4.94	123.60
C	3	Start Phase 1	72.76	7.74	365.33	19.26	18.97	7.03	1.29	7.59	14.87
C	3	Mid Phase 1	72.73	7.82	371.56	19.33	19.27	8.28	1.40	9.49	60.63
C	3	End Phase 1	71.61	7.95	363.40	20.30	18.04	6.44	1.09	10.28	27.70
C	3	End Phase 2	64.40	7.69	352.24	27.08	13.01	5.24	0.81	3.86	123.21
D	1	Start Phase 1	63.16	8.16	430.19	13.46	32.29	17.47	1.64	6.66	63.01
D	1	Mid Phase 1	64.86	8.37	411.88	18.74	22.53	15.93	1.76	5.90	34.46
D	1	End Phase 1	61.28	8.12	399.49	12.75	31.71	5.71	0.68	10.65	2.54
D	1	End Phase 2	59.91	7.79	347.37	21.00	16.56	6.04	0.70	10.71	3.87
D	2	Start Phase 1	62.44	8.04	434.56	8.87	50.38	14.63	1.28	5.77	96.07
D	2	Mid Phase 1	63.41	7.89	419.42	15.57	27.08	15.75	1.51	5.55	58.00
D	2	End Phase 1	61.16	7.37	376.21	19.19	19.62	6.79	0.86	9.33	29.24
D	2	End Phase 2	56.92	7.74	360.98	19.64	18.54	7.11	0.81	9.06	48.28
D	3	Start Phase 1	62.32	8	428.59	10.52	41.13	16.67	1.57	6.94	121.14
D	3	Mid Phase 1	60.92	7.81	416.25	15.50	27.22	15.95	1.58	5.23	115.41
D	3	End Phase 1	57.86	7.86	357.10	16.98	21.51	6.70	0.81	10.24	20.55
D	3	End Phase 2	55.96	7.19	311.70	20.78	15.01	7.60	0.93	9.05	21.88

5 Variation in compost microbial diversity, yield and quality over a 12-month period

It was anticipated that an annual cycle in bacterial diversity during composting, and also in the yield and quality of the mushroom crops might be observed, driven by changes in annual mean temperature (especially in the southern States, and especially if phase I composting is done in outdoor bunkers or windrows).and by annual variation in the quality of the wheat straw feedstocks. These vary from new season straw, available immediately after harvest in ca. November, through to weathered straw, available during the late winter months. Because the new season straw is much more shiny and hard, it decomposes much less readily, and composters generally plan to introduce the new straw to their compost mix progressively over several months rather than switching abruptly.
Bacterial diversity in composts at different composting phases was determined from the database, and divergence from the mean is shown in Figure 5.1. The data reveal that although compost bacterial diversity changes during the year, there is no cyclical pattern, i.e. the diversity in January is not reflected by the diversity in the following January. There is also considerable variation between crops and between composters.

Mushroom yield and quality is also very dependent on individual composters (Figure 5.2). This reflects different market pressures for different farms (e.g. demand from supermarkets, labour availability). The data reveal variations in harvesting of third flush, and also differences between yards in the quality of mushrooms retained. The data are presented as yield per area of compost (kg/m^2) and not as total yield, but they nonetheless also partly reflect the experiences of mushroom farmers – annual cycles in demand are reflected in e.g. picking of more third flush mushrooms in winter, and a slightly lower yield in summer. However, these do not necessarily reflect the influence of compost parameters on crop yield and quality, but rather the market strategy of the individual farmers.

The data also reflect some individual problems that were experienced in gathering the data. A number of samples were not received (23 samples of a total of 416 from the 4 main yards; 5%), including a period of nearly two months from Yard Y, due to a freezer breakdown on site (visible in Figure 5.2). These omissions were largely due to unintentional oversights at the participating composters, but are well within tolerance levels for the entire dataset.

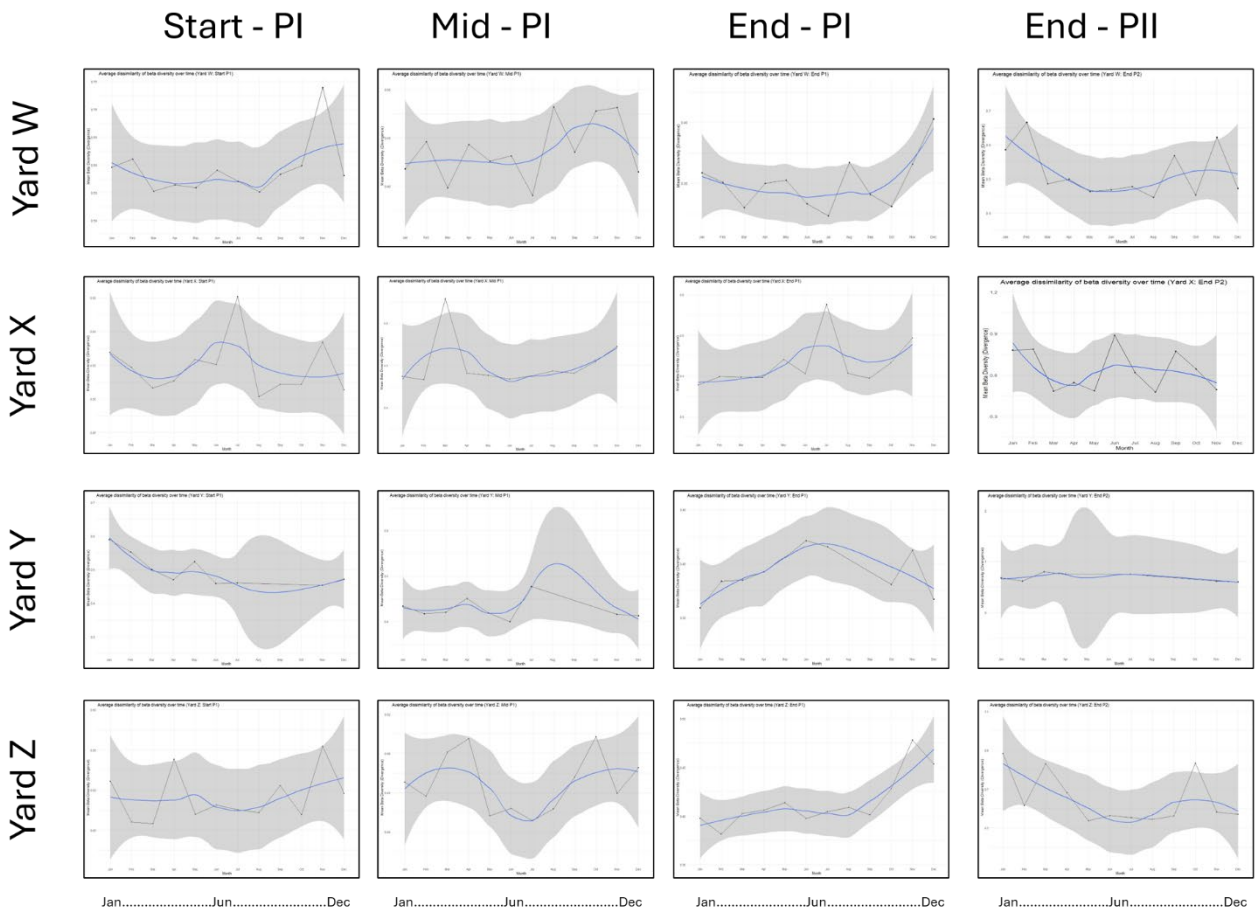


Figure 5.1. Bacterial diversity changes at different composting yards over a twelve-month period. The mean beta diversity divergence values for crops at different stages (start-Phase I, mid-Phase I, end-Phase I, end-Phase II) are shown in black. Because every second crop was sampled (fortnightly intervals between samples), each data point represents the mean of two successive crops. The smoothed progression of beta diversity through the year is shown in blue, with 95% confidence interval (grey shading). Sampling was only conducted for one 12-month period, so the data do not represent a multiple year average.

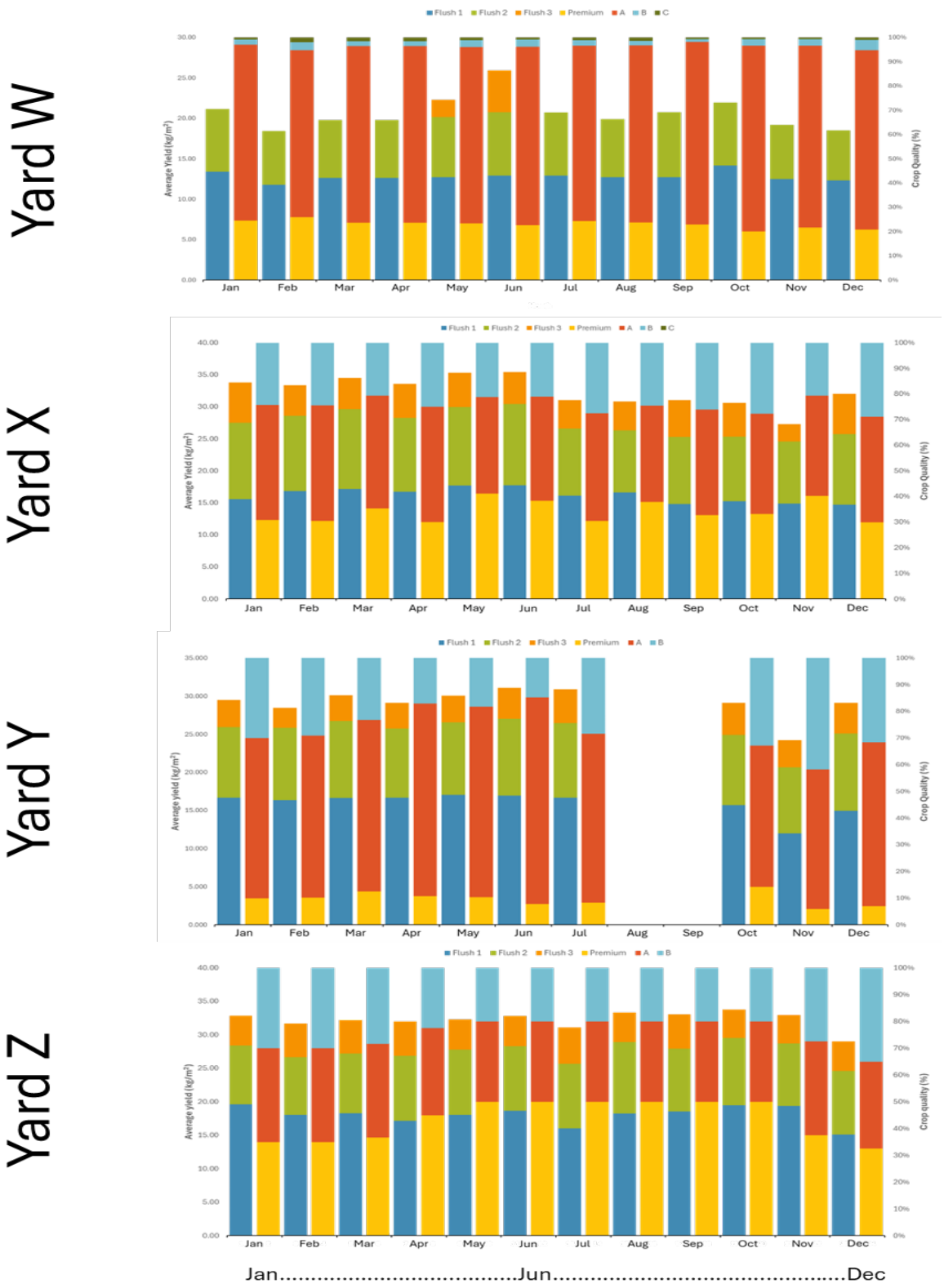


Figure 5.2. Yield and quality parameters at different composting yards over a twelve-month period. Average yield for each flush is shown on the left-hand vertical axis (dark blue - flush 1, light green – flush 2, orange– flush 3). Crop quality is shown on the right-hand vertical axis (yellow - % premiums, red - % A grade, light blue - % B grade, dark green - % C grade). Each data point represents the average of two successive crops. Sampling was only conducted for one 12-month period, so the data do not represent a multiple year average.

6 Correlation of bacterial and fungal biomarkers with mushroom crop yield and quality

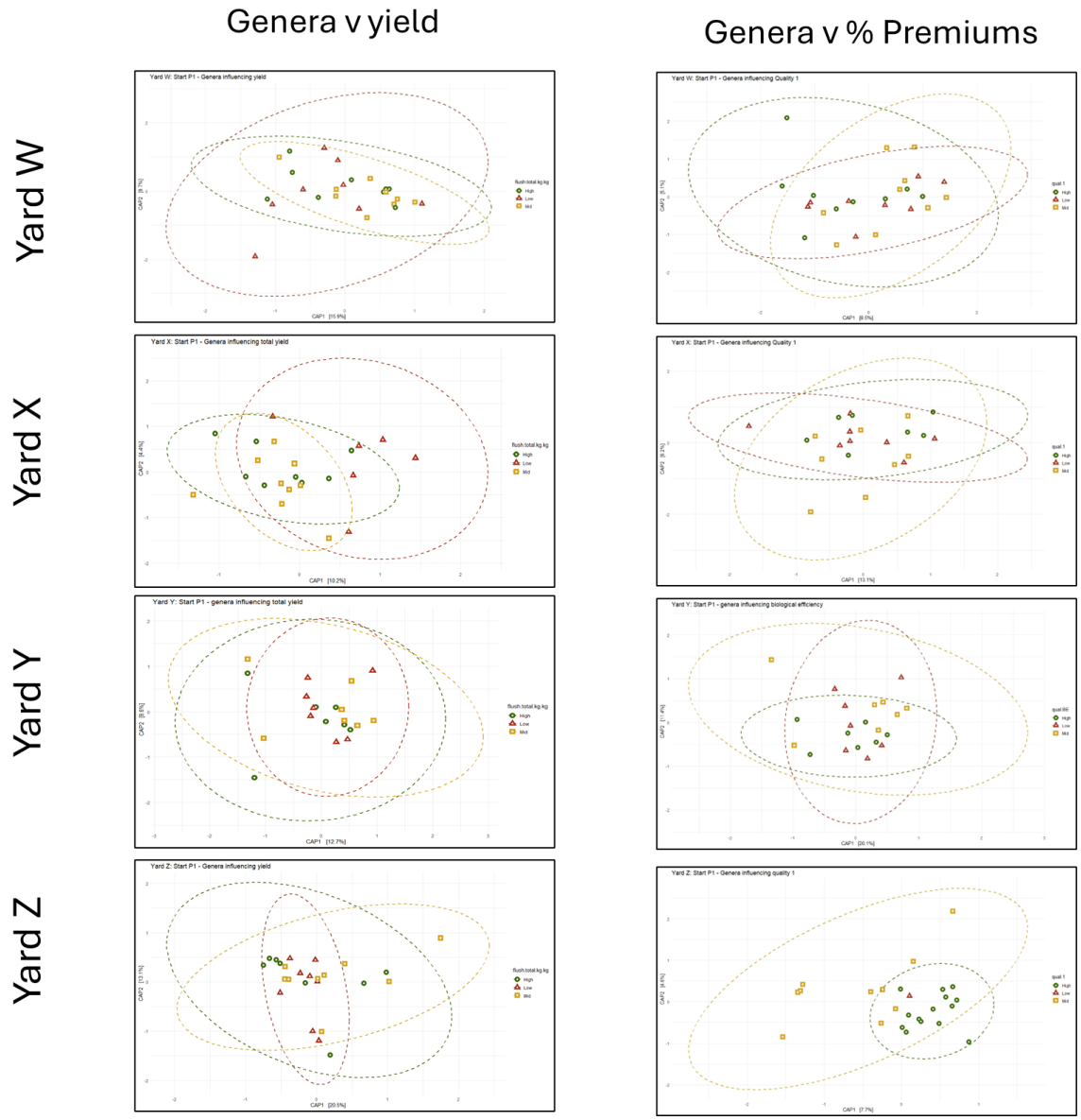


Figure 6.1. Correlation of Start phase I bacterial diversity with mushroom crop yield and quality (% premiums). Bacterial diversity is shown as genera. Crops with high yield or quality are shown in green, medium high yield/quality in yellow and low yield/quality in red.

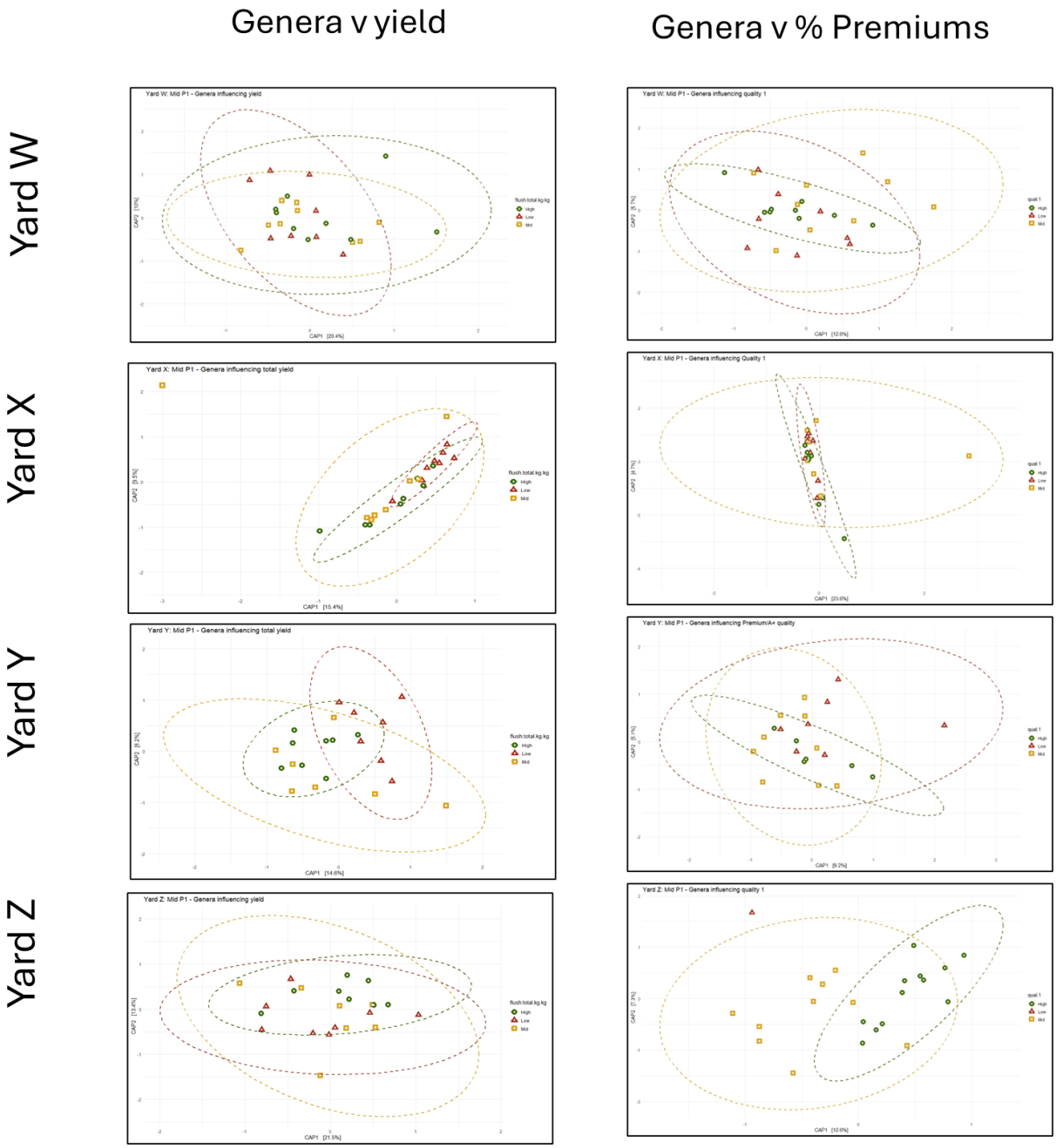


Figure 6.2. Correlation of Mid phase I bacterial diversity with mushroom crop yield and quality (% premiums). Bacterial diversity is shown as genera. Crops with high yield or quality are shown in green, medium high yield/quality in yellow and low yield/quality in red.

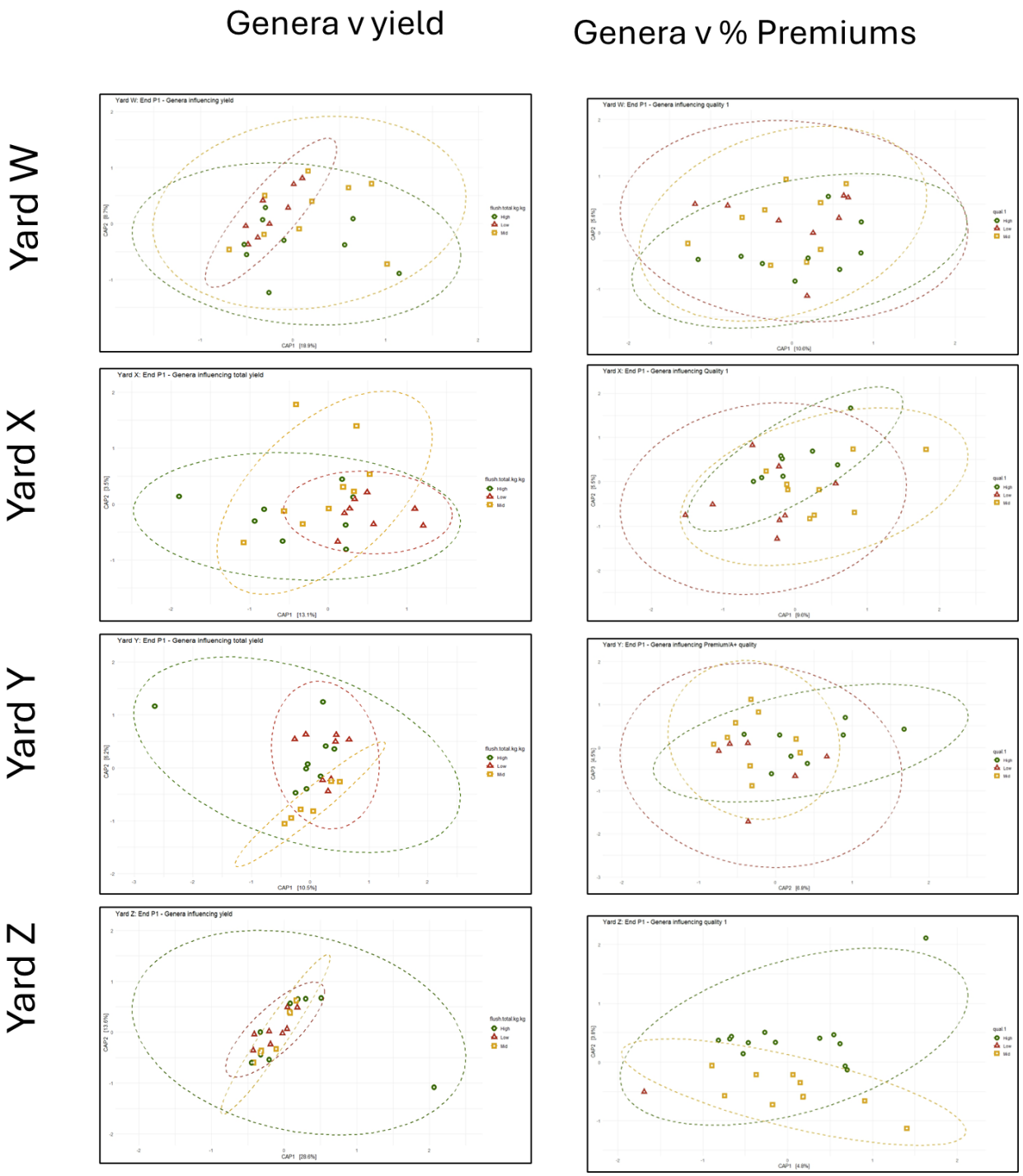


Figure 6.3. Correlation of end phase I bacterial diversity with mushroom crop yield and quality (% premiums). Bacterial diversity is shown as genera. Crops with high yield or quality are shown in green, medium high yield/quality in yellow and low yield/quality in red.

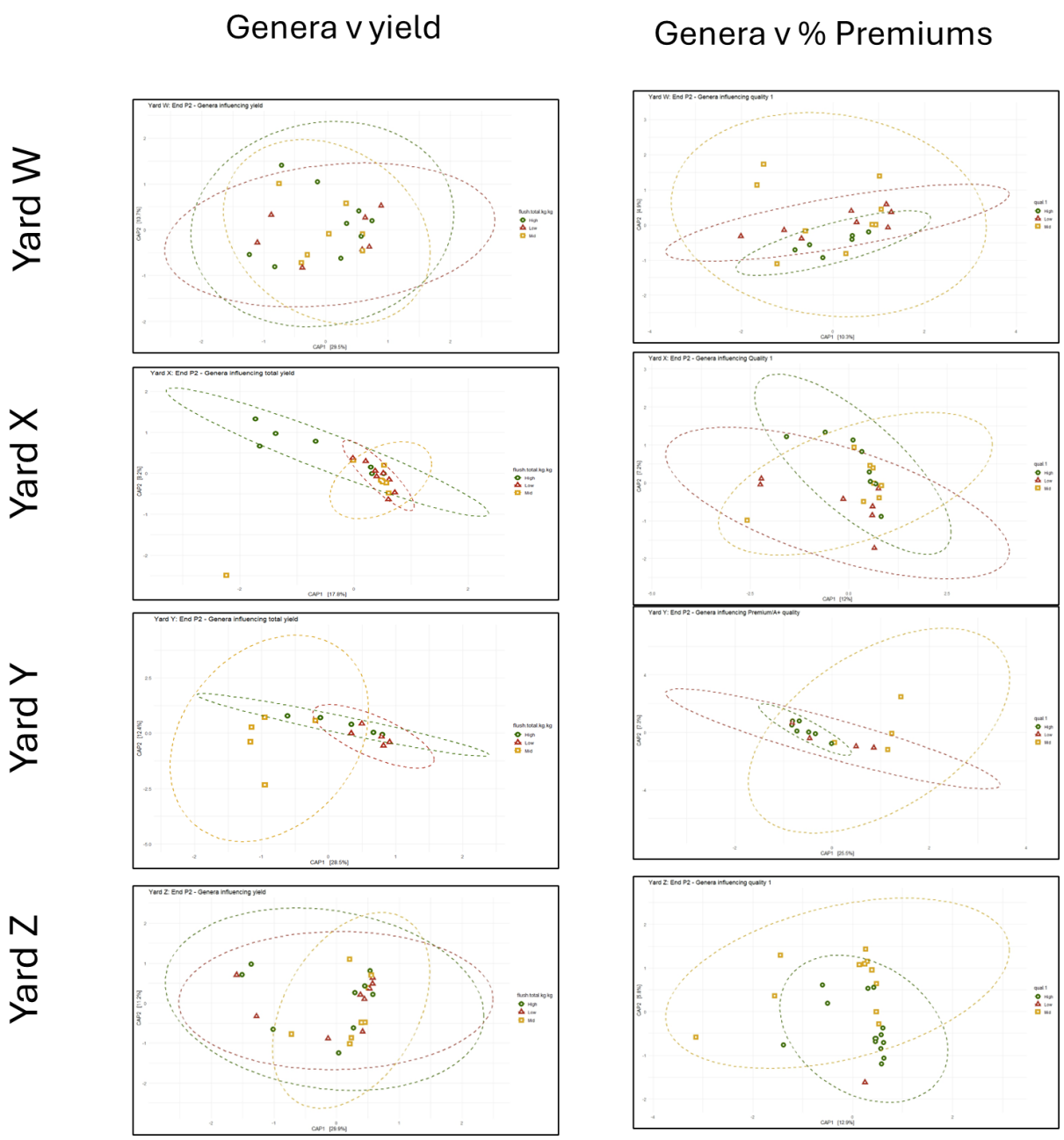


Figure 6.4. Correlation of end phase II bacterial diversity with mushroom crop yield and quality (% premiums). Bacterial diversity is shown as genera. Crops with high yield or quality are shown in green, medium high yield/quality in yellow and low yield/quality in red.

Table 6.1. Bacterial genera correlated with increased or decreased total mushroom yield ($p<0.05$) at individual composters. The three genera with the strongest effect are shown for each Phase and Composter (blank fields indicate no significant correlations). Genera in the "Overall" column are statistically associated with the designated effect whenever they appear.

Composter	Effect	Bunker-fill	mid Phase I	end Phase I	end Phase II	Overall
W	**increase**		*Garciella* *Desulfohalotomaculum* *Nitrolancea*		*UCG-012* *Mycobacterium* *Cerasibacillus*	*Garciella*
X	**increase**			*Solibacillus* *Pseudaminobacter* *Ochrobactrum*		
Y	**increase**		*Chryseomicrobium* *Anoxybacillus* *Pusillimonas*	*Trichococcus*		
Z	**increase**			*Thermoactinomyces* *Pedobacter* *Agrococcus*		*Propioniciclava*
W	**decrease**		*Treponema* *Ornithinimicrobium*	*Chthoniobacter*	*Sphingobacterium* *Taibaiella*	
X	**decrease**			*Corynebacterium* *Clostridium* *Aquamicrobium*		
Y	**decrease**	*Thermobifida* *Thermopoly-spora*		*Tomitella* *Streptococcus* *Fermentimonas*		
Z	**decrease**			*Caryophanon* *Caldanaerobacter* *Camelimonas*		*Caryophanon* *Anaerosalibacter* *Gulosibacter*

7 References

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: High-resolution sample inference from Illumina aplicon data. Nat Meth 13:581-583 doi:http://doi.org/10.1038/nmeth.3869

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci USA 108:4516-4522 doi:10.1073/pnas.1000080107

Dixon P (2003) VEGAN, a package of R functions for community ecology. J Veget Sci 14:927-930 doi:10.1111/j.1654-1103.2003.tb02228.x

Lever MA, Torti A, Eickenbusch P, Michaud AB, Santl-Temkiv T, Jorgensen BB (2015) A modular method for the extraction of DNA and RNA, and the separation of DNA pools from diverse environmental sample types. Front Microbiol 6:Article 476 doi:10.3389/fmicb.2015.00476

McMurdie PJ, Holmes S (2013) phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8:e61217 doi:10.1371/journal.pone.0061217

Muyzer G, Waal ECd, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695-700 doi:doi:10.1128/aem.59.3.695-700.1993

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

R Core Team (2019) R: A language and environment for statistical computing. R Foundation For Statistical Computing, Vienna, Austria

Schliep KP (2010) phangorn: phylogenetic analysis in R. Bioinformatics 27:592-593 doi:10.1093/bioinformatics/btq706

Wickham H (2016) ggplot2: Elegant graphics for data analysis. Springer, New York

Wright ES (2016) Using DECIPHER v2.0 to analyze big biological sequence data in R. R Journal 8:352-359

COMPOST BIOMARKERS
A CRYSTAL BALL FOR YIELD & QUALITY?

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Button mushrooms (*Agaricus bisporus*) are grown commercially in Australia on a specialised compost that is usually prepared from wheat straw and poultry manure. The quality and yield of the mushroom crop depends critically on the quality of the compost, but the way compost quality is measured has changed very little in the last forty years. It depends largely on a limited number of chemical assays and on the empirical personal expertise developed by composters over years of experience.

These measures are invaluable, but for effective compost management they need to be more strongly integrated with the outcomes of modern compost research.

In particular, recent research has provided a much better understanding of the complex succession of microorganisms that catalyse the composting process (Kertesz and Thai, 2018), and there is now an opportunity to design compost quality indicators that correlate crop yield and quality with the levels of active bacteria and fungi in the compost (both the total populations, and the populations of key microbial groups).

MEASURING COMPOST QUALITY

Indicators for compost maturity/stability are used in many composting processes, including municipal composting and composting of various manures. Typical variables measured include water-soluble carbon, C/N ratio, nitrification ratio (ammonium:nitrate), content of humic material, and microbial respiration (measured as CO^2 release). For composts used in the horticultural industry, plant related variables such as seed germination and seedling survival are sometimes also included.

Usually a combined "compost maturity index" (CMI) is generated, which is composed of several factors (e.g. the Solvita compost test, which combines values for NH^3 and CO^2 production (https://solvita.com/compost/)).

Since the aim of municipal or manure composting is to stabilise nutrients in a form that does not damage the environment, there are often legal requirements for "compost stability" that must be met before compost is used, and these form a minimum list for a "compost quality index" for these composts (Bernal *et al.*, 2017).

For mushroom composting, the criteria are rather different. The most important purpose of a mushroom compost quality indicator is to determine whether the compost will afford high yield and quality of mushrooms, rather than to measure stability of the compost. Methods to correlate physicochemical compost and casing parameters with mushroom yield have been reported in the past, but have generally been too complex to find great take-up in the industry. One published method, for example, can predict yields with >90% accuracy, but requires measurements of compost pH, dry matter, ammonia, carbon, ash, copper, iron, and sodium in order to do so (Sharma and Kilpatrick, 2000). A second model is slightly less demanding (it only requires values for compost moisture, C/N ratio, pH and total nitrogen, and casing water holding capacity, porosity, pH and electrical conductivity), but it also provides less accurate predictions of yield (Zied *et al.*, 2011). Clearly, the number of parameters required for yield prediction in these studies make them impractical for composters to use routinely, and the number of crops evaluated in these studies was relatively low.

Infrared spectroscopy of Phase 2 compost has also been used as a rapid method to predict yield. Compost absorbs UV or near- or mid-infrared (NIR/MIR) light in a way that is related to its chemical and microbial composition, and this has been reported to explain up to 84% of yield variation (Sharma *et al.*, 2005). In the past, these methods have required expensive and complex instrumentation, but the recent development of hand-held spectrometers may make this technique useful in the future, and the newest instruments cost only a few hundred dollars. Compost NIR spectra change in a predictable manner during composting, but correlation of the measurements with e.g. cellulose or lignin content varies greatly between yards, and this is likely also to apply to yield predictions. Notably, a recent comprehensive review of mushroom composting and cultivation methods (Zied and Pardo-Giménez, 2017) does not mention mushroom compost quality indicators, confirming that more work is needed in this field.

WHY ARE COMPOST BIOMARKERS NEEDED?

Achieving consistency of compost is an ongoing issue for the industry. All growers have experienced fluctuations in availability and quality of wheat straw and chicken manure that influenced crop yield and quality. Compost made with new season straw, for example, often takes longer to reach maturity, and gives reduced crop yields. More frustratingly, occasional compost batches give lower mushroom yields which growers are at a loss

to understand or remedy, despite years of experience. Low profit margins in the industry mean that even slight yield reductions are economically painful. The ability to predict potential yields earlier in the production process will allow growers to manage composting conditions, inputs and the growing environment more effectively, and take timely remedial action.

Targeted biomarkers could provide:

- a stop/go signal for compost crops, i.e. a signal that yield from a particular batch of compost is likely to be poor, allowing compost crops to be blended or halted if necessary;
- a physical management signal, e.g. that a particular compost crop may need a different level of treatment (e.g. temperature, time, water);
- a biological management signal, e.g. presence of a reduced population of an essential microbe, or elevated population of a potential mushroom pathogen, signalling that effective composting may require bioaugmentation or other treatment.

These signals are only useful, however, if the methods used are easy for the grower to carry out routinely and if they provide feedback in a useful timeframe for composting (see Box below).

THE AUSTRALIAN COMPOST BIOMARKERS PROJECT

The Biomarkers project is supported by Hort Innovation through the levy project *Developing a database of bio-markers for compost quality control to maximise mushroom production yield* (MU17006).

The project is measuring the variation in compost microbe populations throughout composting at a range of Australian mushroom composting facilities and correlating this with the mushroom yield and quality obtained with each compost. The data will be used to develop an Australian Mushroom Industry Database of compost bio-markers, that can be used to assist growers to maximise mushroom yield and productivity. In particular, the project will:

- Conduct a global scan for novel and innovative options using biomarkers to maximise mushroom yield via mushroom compost quality control.
- Compare microbial diversity and dynamics at a range of composting facilities across the Australian mushroom industry, studying every second crop over a continuous period of one year to develop a database of unprecedented size.
- Establish a correlation between microbial populations at these composting facilities and the yield/quality of the resultant mushroom crops.
- Develop fact sheets and calculators to help Australian mushroom growers to use the microbial load database to improve yield and manage composting.

Many composters and growers have experimented with different ways to predict yields over the years. Many of these attempts are documented in industry magazines and reports, but others have only been passed on anecdotally and the project work will look to tap into this wealth of empirical experience. The project has already been discussed with several composting facilities, and the project team would be delighted to receive information outlining any experiences or suggestions.

Growers' needs in a potential compost biomarker of crop yield and quality

- **Sampling method** should be practical and reproducible for the composter or grower.
- **Cost** of sampling and analysis should be low.
- **Speed** - results should be available as soon as possible. Can the analysis be done on-site, or must it be outsourced to an external laboratory?
- **Reliability** and reproducibility of analysis method - how well is the biomarker linked to yield/quality of mushroom crops?
- **International acceptance** - integration of method with the international mushroom industry and other related industries.

REFERENCES

Bernal, M.P., Sommer, S.G., Chadwick, D., Qing, C., Li, G.X., and Michel, F.C. (2017) Current approaches and future trends in compost quality criteria for agronomic, environmental, and human health benefits. *Adv Agron* 144: 143-233.

Kertesz, M.A., and 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.

Sharma, H.S., and Kilpatrick, M. (2000) Mushroom (*Agaricus Bisporus*) compost quality factors for predicting potential yield of fruiting bodies. *Can J Microbiol* 46: 515-519.

Sharma, H.S.S., Kilpatrick, M., and Lyons, G. (2005) Prediction of potential mushroom yield by visible and near-infrared spectroscopy using fresh phase II compost. *Appl Spectrosc* 59: 1054-1059.

Zied, D.C., and Pardo-Giménez, A. (2017) *Edible and Medicinal mushrooms. Technology and applications.* New Jersey: John Wiley & Sons.

Zied, D.C., Pardo-González, J.E., Minhoni, M.T.A., and Pardo-Giménez, A. (2011) A reliable quality index for mushroom cultivation. *J Agric Sci* 3: 50-61.

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This project has been funded by Hort Innovation using the mushroom research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au