Microbial dynamics in Australian mushroom compost

By

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Originality statement

This thesis is submitted to The University of Sydney in fulfilment of the requirement for the Doctor of Philosophy.

The work presented in the thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

Meghann Thai

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Presentations

Part of the research in this thesis has been presented at the following events:

- Presentation at the Australian Mushroom Growers Association Conference, Sydney, October 2018
- Presentation at the SOLES HDR Showcase, The University of Sydney, November 2019
- Presentation at the eCongress of the International Society for Mushroom Science, Online, September-November 2021
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Abstract

Button mushrooms (*Agaricus bisporus*) are highly nutritious, containing key minerals, vitamins and high levels of protein (~25% of dry weight). They are grown on a composted substrate made from wheat straw, poultry manure and gypsum. The composting process is a microbially driven and highly aerobic process. It is a high heat composting process with a final curing stage. A previous study using a single compost yard showed that during the pasteurisation/curing phase, there is a stable bacterial co-existence of *Pseudoxanthomonas*, *Steroidobacter* and a novel chitin degrading bacterium from the *Chitinophagaceae* family, and the dominant fungus is *Mycothermus thermophilus*.

In this study, DNA sequencing revealed compost microbes during the curing stage were dominated by the ascomycete *Mycothermus thermophilus*, and the bacterium *Pseudoxanthomonas*, in all compost yards studied. *Pseudoxanthomonas* has been known to produce nitrous oxide (N_2O) in aerobic and microaerobic environments. However, under these conditions the N_2O released was very low, suggesting that other compost microbes may be using the N_2O as an energy source. *In vitro* interactions of *M. thermophilus*, *Pseudoxanthomonas* spp. and a novel chitin degrading bacterium (Family *Chitinophagaceae*) showed that *M. thermophilus* boosted the growth of all bacteria. Understanding the interactions between compost microbes will aid in determining where losses in nitrogen occur and how to optimise nutrient inputs and maximise outputs.

General abbreviations

ASV – Amplicon Sequence Variant

CTAB – Hexadecyltrimethylammonium bromide

df – Degrees of freedom

EDTA – Ethylenediaminetetraacetic acid

N_2O – Nitrous oxide

NH_3 – Ammonia

NO_2^- – Nitrite

NO_3^- – Nitrate

PCR – Polymerase Chain Reaction

PERMANOVA – Permutational multivariate analysis of variance

P1 – Phase I

P2 – Phase II

RFLP – Restriction fragment length polymorphism

YpSs – Yeast, Phosphorus, Soluble starch

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General Introduction

1.1 Background

In Australia, mushroom farmers produced over 64,000 tonnes of edible mushrooms a year and production is worth more than AUD$350 million (Horticulture Innovation Australia, 2017). This makes mushrooms the sixth most valuable horticultural crop in Australia (Australian Mushrooms, 2021).

Button mushrooms are considered high in protein compared to other vegetables (Chang & Miles, 2004, Ferreira *et al.*, 2017) and are often compared to chickpeas, rice, eggs and milk for their nutritive value (Chang & Miles, 2004, Pesti, 2014). Button mushrooms contain approximately 14-27% of crude protein (% DW) (Chang & Miles, 2004, Kalač, 2016, Ferreira *et al.*, 2017). Furthermore, button mushrooms have all nine essential amino acids, the most abundant being lysine (Miles & Chang, 2004). There are many factors that influence the nutritive content of mushrooms, for button mushrooms, the main factors are the substrate it is grown on and the degree of maturity when harvested (Pesti, 2014). For example, protein content in mushrooms, is mostly dependent on the compost composition (Braaksma & Schaap, 1996). Mushrooms obtain a considerable portion of their protein content from the microbial biomass in the substrate (Wood & Fermor, 1982, Vos *et al.*, 2017) which makes the microbiology of compost a key area of interest for producing mushrooms of high quality and yield.

There have been several studies that have described bacterial succession in mushroom compost using DNA fingerprinting techniques (Székely *et al.*, 2009, Vajna *et al.*, 2012, Siyoum *et al.*, 2016), and more recently with Next-generation sequencing (Kertesz *et al.*, 2016, Cao *et al.*, 2019, Carrasco *et al.*, 2020, Vieira & Pecchia, 2021). However, these studies focussed on single compost yards and, usually, only on single crops. In some studies, *Pseudoxanthomonas taiwanensis* was one of the most

abundant bacterial taxa found in Phase II composting (Székely *et al.*, 2009, Vajna *et al.*, 2012, Kertesz *et al.*, 2016). *Pseudoxanthomonas taiwanensis* is a known heterotrophic nitrifier that can produce nitrous oxide (N_2O) in thermophilic, aerobic environments (Chen *et al.*, 2002).

1.2 Mushroom compost process

The white button mushroom is grown on a specific mushroom compost that is a selective medium made from composted wheat straw, poultry manure and gypsum (Royse & Beelman, 2007, Pudełko, 2014, Siyoum *et al.*, 2016). In some countries, e.g., USA and Netherlands, stable bedding is used alongside poultry manure as the primary nitrogen source (Straatsma *et al.*, 1994, Vos *et al.*, 2017). In China, a mixture of organic and chemical nitrogen sources are used for mushroom composting (Cao *et al.*, 2019). Mushroom compost made from stable bedding is often called "traditional" or "conventional" compost, while mushroom compost produced without stable bedding is referred to as "synthetic" compost.

The type of straw used for composting is also very variable. In Australia, wheat straw is the primary carbon source for mushroom compost, however in other countries, rice straw is commonly used (Song *et al.*, 2014, Wang *et al.*, 2016), and more recently, reed straw has also been used (Wang *et al.*, 2021). In an attempt to find alternative and more readily available carbon sources, Pangola grass has been used in place of wheat straw in Mexico (Sanchez *et al.*, 2008) and corn stover, rape straw, bean straw and linseed straw have also been trialled (Noble *et al.*, 2002).

Nitrogen (N) content in raw materials vary greatly and many composters "tweak" the proportion of carbon (C) and N inputs to achieve the desired C/N ratio (25-35) (Hayes, 1972, Miles & Chang, 2004, van der Wurff *et al.*, 2016). A C/N ratio that is

too low will result the raw materials degrading too quickly and producing too much heat (van der Wurff *et al.*, 2016). On the other hand, a C/N ratio of above 35 will result in slow degradation of organic matter (van der Wurff *et al.*, 2016). Additional nitrogen sources are becoming more commonly used due to decreasing nitrogen content in poultry manure from increased bedding and less protein in chicken feed (Beyer *et al.*, 2000, Nahm, 2007). Additional nitrogen sources can be organic, such as seed meal and brewers' grain (Beyer, 2003, Carrasco *et al.*, 2018), or inorganic, including ammonium sulphate and urea (Noble *et al.*, 2002, Demirer *et al.*, 2005).

Mushroom composting is done in three main phases. Firstly, straw is wetted and softened with water and compost leachate before mixing with poultry manure and gypsum (Noble & Gaze, 1996, Safianowicz *et al.*, 2018). The materials are piled into windrows or loaded into bunkers and left to self-heat with periodic mixing to aerate the pile and homogenise the compost pile (Phase I). Phase I is a partial fermentation step that is initially mesophilic but becomes thermophilic within the first 48 hours due to microbial activity, with peak temperatures reaching up to 80-82 °C (Noble *et al.*, 2002). To maintain a moisture content of 70% (w/w) at thermophilic temperatures, compost leachate is repeatedly added during Phase I whenever the compost piles are turned (Safianowicz *et al.*, 2018). Modern compost yards utilise aerated floors to maintain high oxygen levels in the compost pile (Royse & Beelman, 2007, Pudełko, 2014). Phase I can be completed within 6 days (Weil *et al.*, 2013) or can take up to 14 days (Noble & Gaze, 1996).

Phase I compost is subjected to two stages in Phase II, which are carried out in temperature-controlled tunnels designed to provide uniform temperatures and aeration (Noble & Gaze, 1996, Straatsma *et al.*, 2000). Phase I compost is first pasteurised by keeping the compost at 58-60 °C for 4-6 h, which kills pathogenic organisms,

nematodes, and insects in the compost (Noble & Gaze, 1996). This is followed by conditioning or curing, where compost temperatures (55-45 °C) is held for 4-5 days. The total period of Phase II is six days and is complete when ammonia concentration in the process air is below 5-10 ppm (Noble & Gaze, 1996).

Phase II compost is then inoculated with *Agaricus bisporus* mycelium grown on grain (usually sorghum or millet) and proliferates throughout the compost for 15 days (Phase III). Phase III, also known as spawn run, is also highly aerobic and temperatures are maintained at 25 °C for *A. bisporus* (Noble & Gaze, 1996, Royse & Beelman, 2007).

Microbes, particularly bacteria and fungi, are the main contributors in composting. They are responsible for the rapid heat generation and release of ammonia during Phase I and the degradation of the raw materials during conditioning in Phase II. More on how they do this is covered in the next section.

1.3 Bacterial diversity during mushroom composting

The majority of the microbes involved in mushroom composting come from poultry manure (Bolan *et al.*, 2010). More established compost yards use compost leachate, which is enriched with compost organisms (Safianowicz *et al.*, 2018). Throughout Phase I, there is a rapid succession of dominant bacterial and fungal taxa (Kertesz *et al.*, 2016). Microbial growth in Phase I is mainly supported by readily accessible nutrients in the feedstocks (protein, lipid, low molecular weight carbohydrates), and the amount of complex carbohydrates in the compost does not decrease significantly (Jurak *et al.*, 2015). However, during Phase II, the microbial community changes twice, during pasteurisation, pathogenic and non-pathogenic microbes are killed. During conditioning, a stable bacterial community including *Pseudoxanthomonas*,

Steroidobacter and an unclassified genus from the *Chitinophagaceae* family has been found, while the fungal community is dominated by *Mycothermus thermophilus* (syn. *Scytalidium thermophilum*) (Kertesz *et al.*, 2016). Microbial activity is the most intriguing during conditioning, where 50-60% of the cellulose from the wheat straw can be degraded by the end of Phase II (Jurak *et al.*, 2015). Excess ammonia produced during the thermophilic stages is assimilated by the microbes during the conditioning process (Noble & Gaze, 1996). The microbial community at the end of Phase II becomes the "food" that supports *Agaricus* mycelium growth (Wood & Fermor, 1982, Vos *et al.*, 2017).

M. thermophilus has been extensively studied during Phase II composting and is the primary cellulose degrader during conditioning (Straatsma *et al.*, 1989, Wiegant *et al.*, 1992, Basotra *et al.*, 2016). *Pseudoxanthomonas* and *Chitinophagaceae* are two of the most abundant bacterial taxa during Phase II (Kertesz *et al.*, 2016). *Pseudoxanthomonas* has been found in mushroom compost and in cellulose degrading consortia (Kato *et al.*, 2005, Székely *et al.*, 2009, Vajna *et al.*, 2010, Du *et al.*, 2015). Some species of *Pseudoxanthomonas* produce cellulose degrading enzymes, while other species degrade inhibitory compounds from cellulose degradation (Chen *et al.*, 2002, Kumar *et al.*, 2015). *Chitinophagaceae* is a family of known chitin degrading bacteria, and its abundance is most likely correlated to the dominance of *M. thermophilus* during Phase II. More detail on these three taxa are covered in the next sections.

1.3.1 Mycothermus thermophilus (Syn. Scytalidium thermophilum)

Mycothermus thermophilus was first isolated from mushroom compost in 1964 by Cooney and Emerson and was named *Torula thermophila* (Ross & Harris, 1983,

Natvig *et al.*, 2015). It was renamed to *Scytalidium* by Austwick in 1976 based on physiological and morphological features (Natvig *et al.*, 2015), and reclassified from *Scytalidium thermophilum* to *M. thermophilus* by Natvig *et al.* (2015) based on the internal transcribed spacer (ITS) sequences of the rRNA gene region.

In addition to taxonomic investigations, *M. thermophilus* has been shown to promote the growth of *Agaricus bisporus* (Wiegant *et al.*, 1992, Straatsma *et al.*, 1994, Ahlawat & Vijay, 2004). *M. thermophilus* is one of the main microorganisms in mushroom compost that aids in assimilating ammonia from the compost and immobilizing nutrients (Wiegant *et al.*, 1992, Straatsma *et al.*, 1994), but it does not remove ammonia efficiently on its own (Wiegant *et al.*, 1992). It is also able to produce cellulases and xylanases (Basotra *et al.*, 2016). *In vitro*, *M. thermophilus* can double the extension rate of *A. bisporus* mycelium when inoculated on sterile compost pre-grown with *M. thermophilus* (Wiegant *et al.*, 1992). The presence of *M. thermophilus* has been positively correlated with mushroom yield (Straatsma *et al.*, 1994). However, the *in vitro* growth promoting effect was removed when *M. thermophilus* was killed before inoculation of *A. bisporus* (Wiegant *et al.*, 1992). This may be due to the fact that respiration of CO_2 by *M. thermophilus* provides a stimulatory role in *Agaricus* mycelial extension (Straatsma *et al.*, 1994).

M. thermophilus is dominant during Phase II, but has also been detected during Phase I (Sharma *et al.*, 2000). It is most likely introduced into the compost through wheat straw, manure and other substrates (Straatsma *et al.*, 1994), but remains inactive until Phase II when temperatures are stabilised at 45 °C (Straatsma *et al.*, 1994). *M. thermophilus* is capable of surviving temperatures greater than 60 °C (Natvig *et al.*, 2015, Vos *et al.*, 2017), however, the maximum temperature for growth is at 55 °C (Straatsma *et al.*, 1994).

M. thermophilus is almost exclusively the only thermophilic fungus researched in *Agaricus* mushroom compost. Other thermophilic fungi that exhibit similar thermophilic and growth promoting effects to *M. thermophilus* are *Myriococcum thermophilum* and *Chaetomium thermophilum* (Straatsma *et al.*, 1994). *Myriococcum* can increase the density of compost (Straatsma *et al.*, 1994), which can affect the filling rate of compost in farms, as density is used to predict the yield of mushrooms (Straatsma *et al.*, 1994). *C. thermophilum* has a number of genes associated with cellulose degradation (Li *et al.*, 2009) and also produces extracellular xylanase (Katapodis *et al.*, 2007). However, many fungal growth promoting studies emphasise the success of *M. thermophilus* and focus less on other thermophilic fungal species (Wiegant *et al.*, 1992, Straatsma *et al.*, 1994, Straatsma *et al.*, 1994). This could be due to the fact that *M. thermophilus* makes up approximately 60-95% of fungal population during Phase II composting, and 100% of the fungal population by the end of Phase II (Souza *et al.*, 2014, Kertesz *et al.*, 2016). Moreover, there is no literature regarding *Chaetomium* and *Myriococcum* strains that can remove ammonia. Hence, the importance of *M. thermophilus* is more extensively researched in mushroom compost compared to others.

1.3.2 Pseudoxanthomonas

The *Pseudoxanthomonas* genus was first described by Finkmann *et al.* (2000) as aerobic, Gram-negative rods. This description was amended in 2004 to non-spore forming rods, which are strictly aerobic and can reduce nitrite but not nitrate (Thierry *et al.*, 2004). Prior to 2000, there was no genus characterised for *Pseudoxanthomonas* based on its 16S rRNA gene sequence. The closest phylogenetic relatives are *Xanthomonas, Stenotrophomonas* and *Xyella. Pseudoxanthomonas* is distinguished

from these genera because it lacks the 3-hydroxyl-iso-tridecaconic fatty acid and has branched fatty acids (Finkmann *et al.*, 2000, Garrity *et al.*, 2007). *Pseudoxanthomonas* colonies are yellow, and the type strain isolated by Finkmann *et al.* (2000), *Pseudoxanthomonas broegbernensis*, can only reduce nitrite to nitrous oxide instead of reducing nitrate.

Other species of *Pseudoxanthomonas,* along with *P. broegbernensis*, can reduce nitrite to nitrous oxide (Table 1.1), however, there are two species, *P. kaohsiungensis* and *P. suwonensis* that are able to reduce nitrate to nitrite (Chang *et al.*, 2005, Wang *et al.*, 2011). There are also two species of *Pseudoxanthomonas* that are incapable of reducing either nitrite or nitrate (Yang *et al.*, 2005) (Table 1.1).

Pseudoxanthomonas spp. have been found in a range of environments, for example, strains have been isolated from hot springs (Chen *et al.*, 2002), oil-polluted wastewater (Chang *et al.*, 2005), polluted soils (Harada *et al.*, 2006, Wang *et al.*, 2011), unpolluted soils (Yang *et al.*, 2005), ammonia-supplied biofilters (Finkmann *et al.*, 2000), waste composts (Weon *et al.*, 2006) and the digestive tract of insects (Rani *et al.*, 2010) (Table 1.1). Many species assigned to this genus are only able to grow under mesophilic temperatures, but several species are thermotolerant/thermophilic (Table 1.1).

Table 1.1. Characteristics of different species of *Pseudoxanthomonas*.
Strains: 1, *Pseudoxanthomonas broegbernensis* B1616/1^T (Finkmann *et al.*, 2000); 2, *Pseudoxanthomonas taiwanensis* CB-226 (Chen *et al.*, 2002); 3, *Pseudoxanthomonas mexicana* AMX 26B (Thierry *et al.*, 2004); 4, *Pseudoxanthomonas japonensis* 12-3 (Thierry *et al.*, 2004); 5, *Pseudoxanthomonas kaohsiungensis* J36 (Chang *et al.*, 2005); 6, *Pseudoxanthomonas koreensis* T7-09 (Yang *et al.*, 2005); 7, *Pseudoxanthomonas daejoneonensis* TR6-08 (Yang *et al.*, 2005); 8, *Pseudoxanthomonas kalamensis* JA40 (Harada *et al.*, 2006); 9, *Pseudoxanthomonas suwonensis* 4M1^T (Weon *et al.*, 2006); 10, *Pseudoxanthomonas icgebensis* ICGEB-L15^T (Rani *et al.*, 2010); 11, *Pseudoxanthomonas jiangsuensis* wax^T (Wang *et al.*, 2011); 12, *Pseudoxanthomonas spadix* IMMIB AFH-5^T (Young *et al.*, 2007)

Type Strain	1	2	3	4	5	6	7	8	9	10	11	12
Source of isolation	Biofilters	Hot springs	Anaerobic sludge	Polluted urban soil	Oil-polluted site	Ginseng field soil	Ginseng field soil	PAH-contaminated soil	Cotton waste compost	Larval mid-gut	DDT-polluted soil	Oil-contaminated soil
Growth range (°C)	20-40	25-65	10-37	10-37	10-40	10-37	10-37	30-37	10-45	30-50	10-45	22-37
Aerobic	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	-	-	-	-	+	-	-	-	+	-	+	-
Nitrite reduction	+	+	+	+	ND	-	-	+	-	ND	-	ND
Motile	+	-	+	+	+	-	+	-	+	-	+	+
Oxidase			ND	ND	+	+	+	+	+	-	-	+
Catalase	+	+	+	-	-	+	+	+	+	+	-	+
β-glucosidase	-	+	+	+	+	-	+	+	+	ND	-	+
Cellulase activity	ND	-	+	ND	ND	ND	ND	ND	+	ND	ND	ND

Pseudoxanthomonas taiwanensis was identified in *Agaricus bisporus* mushroom compost by Székely *et al.* (2009) using two molecular techniques in comparison with standard *Pseudoxanthomonas* cultures: denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP). In a mixed culture, this species can accelerate cellulose degradation (Haruta *et al.*, 2002, Kato *et al.*, 2005, Du *et al.*, 2015). Furthermore, this species of *Pseudoxanthomonas* has been found in many lignocellulolytic consortia (Wang *et al.*, 2011). *P. taiwanensis* is capable of degrading cellobiose into glucose with β-glucosidase (Chen *et al.*, 2002) although it cannot utilise cellulose. It has been hypothesised that *P. taiwanensis* accelerates cellulose degradation by degrading rate-limiting compounds and buffering pH (Kato *et al.*, 2004, Du *et al.*, 2015). However, this could not be confirmed when *P. taiwanensis* was grown in pure culture (Kato *et al.*, 2005), or when β-glucosidase was added to the medium (Du *et al.*, 2015).

P. taiwanensis can produce N_2O from NO_2^- under thermophilic, aerobic and microaerobic conditions, but lacks the ability to reduce nitrate (Chen *et al.*, 2002). This partial denitrification pathway is similar to *Nitrosomonas europaea*, which reduces NO_2^- to N_2O under anoxic conditions (Chen *et al.*, 2002).

It is currently unknown if *P. taiwanensis* produces N_2O under Phase II composting conditions. Despite being found in bacterial consortia, it is also unknown how *P. taiwanensis* interacts with other compost microbes.

1.3.3 Chitinophagaceae

Members of the *Chitinophagaceae* family have been detected in various compost systems (de Gannes *et al.*, 2013, Eichorst *et al.*, 2013, Kertesz *et al.*, 2016, Siyoum *et al.*, 2016, Pandin *et al.*, 2018). In mushroom compost, they are abundant during Phase

II (Kertesz *et al.*, 2016).The family, *Chitinophagaceae*, was first proposed in 2011 by Kämpfer *et al.* (2011) and was defined according to 16S rRNA gene sequence similarities. Members of the *Chitinophagaceae* family are mesophilic, the cells can be thin, rod- or filamentous- shaped, Gram negative, usually non-motile and aerobic or facultatively anaerobic (Kämpfer *et al.*, 2011, Rosenberg, 2014). The major menaquinone is MK-7 and the typical fatty acid profile of members in the *Chitinophagaceae* family includes iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and iso-$C_{15:1}$ G. Several genera are included in this family, with the type genus being *Chitinophaga* (Rosenberg, 2014). *Chitinophaga* was first proposed in 1981 by Sangkhobol & Skerman (1981) based on colony development and DNA melting temperature.

The type species of the *Chitinophaga* genus, *Chitinophaga pinensis*, is known to degrade chitin (Rosenberg, 2014), which is of concern because fungal cell walls are comprised of chitin. Additionally, there are some species that are capable of hydrolysing cellulose and chitin, such as *Chitinophaga oryziterrae* (Chung *et al.*, 2012, Rosenberg, 2014). This functional capacity is of importance because cellulose degradation is necessary for composting. Another species, *Chitinophaga arvensicola*, is a xylanolytic organism that was isolated from Sphagnum peat bogs (Pankratov *et al.*, 2006), which is one of the most common types of peat used for casing. This species would be beneficial in mushroom production because it can degrade xylan, cellobiose and can also reduce nitrate (Pankratov *et al.*, 2006), all of which are characteristics that are ideal for mushroom compost production. However, it has not been detected in other composts and has an optimum growing temperature 30 °C (Pankratov *et al.*, 2006).

A recently described genus, *Compostibacter*, also belongs to this family (Siddiqi *et al.*, 2016). This bacterial strain was isolated from mushroom compost originating

from Korea, and its colonies are described as milky-white in colour instead of yellow or orange like the majority of the genera in the family (Siddiqi *et al.*, 2016). However, *Compostibacter* is phylogenetically affiliated with the family *Chitinophagaceae* because it shares similar characteristics with other genera in this family (e.g., major respiratory quinone is MK-7 and phosphatidylethanolamine (PE) as the major polar lipid) (Siddiqi *et al.*, 2016). Currently, there is only one type strain associated with this genus. With some members of the *Chitinophagaceae* family expressing chitinolytic characteristics, it is unlikely that members of the *Chitinophagaceae* family will negatively affect the growth of *M. thermophilus* because most species are unable to grow between 45-55 °C. Furthermore, the abundance of *Chitinophagaceae* declines during mushroom proliferation (Kertesz *et al.*, 2016). However, more research on the interaction between members of the *Chitinophagaceae* family, *A. bisporus* and *M. thermophilus* is needed.

1.4 Nitrogen transformation in mushroom compost

Nitrogen is an important element in mushroom production and composting, particularly for the degradation of raw materials (Ryckeboer *et al.*, 2003). If nitrogen is limited in a composting system, then degradation of lignocellulosic materials will be slow (Ryckeboer *et al.*, 2003). On the other hand, excess nitrogen will lead to leaching as nitrate or loss of nitrogen through ammonia gas (Savoie *et al.*, 1996, Ryckeboer *et al.*, 2003).

Sources of organic nitrogen in mushroom composting include poultry manure, cotton seed meal, brewery waste and whey powder (Noble *et al.*, 2002, Beyer, 2003, Carrasco *et al.*, 2018). Inorganic sources of nitrogen, such as ammonium nitrate, ammonium sulphate and urea, are also used in the mushroom industry (Noble *et al.*,

2002, Beyer, 2003). The conventional sources of nitrogen in mushroom composting are horse and poultry manure (Noble *et al.*, 2002, Demirer *et al.*, 2005). However, poultry farms are now adding ammonia suppressants into feed to reduce ammonia release from the litter (González-Matute & Rinker, 2006), which has led to an increase in nitrogen content in poultry manure (Choi & Moore, 2008). One ammonia suppressant was positively correlated to increasing mushroom yields (González-Matute & Rinker, 2006). Knowledge on the effect of ammonia suppressants on mushroom compost is limited, and more research in this field is needed (González-Matute & Rinker, 2006).

In poultry manure, urea and uric acid are the main forms of nitrogen, and are readily converted to ammonia and CO_2 by urease and uricase (Mahimairaja *et al.*, 1994). During composting, thermophilic microorganisms convert nitrogen from the raw materials into microbial protein through fermentation (Hayes, 1972, Romaine & Raid, 1991, Jurak *et al.*, 2015), and protein is usually released as either ammonia gas or water soluble ammonium (Savoie *et al.*, 1996). Ammonium is the most important form of nitrogen in mushroom composting, but ammonia is detrimental to *Agaricus* mycelium (Wood & Fermor, 1982, González-Matute & Rinker, 2006).

Ammonia assimilation by fungi is usually catalysed by two pathways, NADP-glutamate dehydrogenase/glutamine synthetase (NADP-GHS/GS) and glutamine synthetase/glutamate synthetase (GS/GOGAT) (Baars *et al.*, 1994). In high ammonia environments, the NADP-GHS/GS pathway is favoured more than the GS/GOGAT pathway (Hernández *et al.*, 1983). Although ammonia assimilation by *M. thermophilus* has not been investigated, the NADP/GS pathway is most likely being used by *M. thermophilus* for ammonia assimilation.

Heterotrophic nitrifiers can respire with oxygen, as an electron acceptor, or nitrogen, as electron donors in the form of organic nitrogen or nitrite and are large contributors of N_2O (Baggs, 2011, Chen & Ni, 2011, Stange *et al.*, 2013). There are three pools where N_2O can be generated; N_2O from oxidised organic N sources (heterotrophic nitrification), N_2O from NO_3^- oxidation and N_2O from ammonium (NH_4^+) oxidation (Baggs, 2011, Zhang *et al.*, 2015). Production of N_2O by heterotrophic nitrification is dependent on C/N ratio, oxygen concentration and pH (de Boer & Kowalchuk, 2001, Jiang *et al.*, 2011, Li *et al.*, 2017). Nitrogen transformations in mushroom composting have been investigated reasonably thoroughly but, to date, understanding microbial nitrogen transformation during composting has not been attempted.

1.5 Thesis outline

The overall aim of the study was to determine if bacterial diversity is consistent in multiple compost yards in Australia and to investigate the interactions between compost bacteria and the dominant fungus, *Mycothermus thermophilus*. Furthermore, given the abundance of *P. taiwanensis* during Phase II and, with its ability to produce N_2O, it was hypothesised that majority of the nitrogen lost during Phase II was in the form of N_2O gas.

There is limited research on the microbial diversity across multiple compost yards and this chapter (**Chapter 1**) describes the background and aim of the research conducted in this PhD thesis. It summarises the current knowledge on the bacterial communities found in button mushroom compost and microbial nitrogen transformations during composting. A better understanding of how compost microbes interact is needed, in order to predict nitrogen losses during Phase II composting and design methods to improve the reproducibility of composting.

Chapter 2 describes the microbial communities found at three key composting time-points, end-Phase I, mid-Phase II and end-Phase II. Bacterial communities were compared among five compost yards from around Australia, which follow different Phase I composting procedures but relatively similar Phase II processes. Compost bacteria and *Mycothermus thermophilus* were isolated from the compost from these yards to study the interaction among them for Chapter 3.

In **Chapter 3**, the biological interactions were tested between the dominant fungus, *Mycothermus thermophilus,* and two dominant compost bacterial taxa, *Pseudoxanthomonas* and a novel chitin degrading bacterium, *Mycovorax composti*. Compost microbes were grown in co-culture to evaluate if *Pseudoxanthomonas* species or *M. composti*, affected the growth of *M. thermophilus in vitro*.

In **Chapter 4**, total nitrogen was measured in end-Phase I and end-Phase II mushroom compost from nine compost yards in Australia to calculate the proportion of nitrogen lost during Phase II. To determine the fraction of gaseous nitrogen lost, gas from a conventional Phase II composting tunnel at one compost yard was sampled in the return air ducting and a mass balance equation was used to calculate N_2O flux in mushroom compost.

Chapter 5 describes the classification of a novel chitin degrading bacterium, *Mycovorax composti* C216, isolated from Phase II mushroom compost.

In **Chapter 6**, the results obtained in this thesis are summarised and discussed. In order to gain a better understanding of the compost dynamics, microbial interactions and nitrogen transformations during Phase II composting are discussed, with regards to the bacterial community found in Phase II compost samples from multiple compost yards.

Microbial diversity in button mushroom compost

Abstract

Button mushrooms are one of the most widely cultivated, edible mushrooms, globally. In Australia, mushrooms are grown on a selective substrate that is made exclusively from wheat straw, poultry manure and gypsum. Bacterial diversity and succession have previously only been studied in single compost crops and at single compost yards. This study describes the variability in the bacterial composition of mushroom compost sampled at three key timepoints across five different compost yards, using next-gen Illumina sequencing. Bacterial composition in Phase I compost varied with yard size, processing conditions and climate. Meanwhile Phase II compost showed more consistent genera in the bacterial profile. The overall bacterial diversity followed the same trend in bacterial communities, suggesting that a particular group of bacteria were selected during the Phase II process. As such, next-gen sequencing has provided insight into the "flow" of bacterial taxa in multiple compost yards in Australia.

2.1 Introduction

Button mushrooms, *Agaricus bisporus*, are one of the most widely cultivated edible mushrooms. They are grown on a highly selective substrate usually made from wheat straw, stable bedding, poultry manure and gypsum, although there are variations in feedstocks used in different countries. In Europe and the USA, for instance, there is a heavy dependence on stable bedding as the primary carbon and nitrogen source (Beyer, 2003, Jurak *et al.*, 2015, Vos *et al.*, 2017) and is traditionally called "conventional" compost. In Australia, mushroom compost is produced almost exclusively from wheat straw, poultry manure and gypsum, and in China, rice straw is often used in place of wheat straw (Wang *et al.*, 2016). Compost that does not include stable bedding is known as "synthetic" compost (Stoller, 1943). Smaller amounts of other agricultural by-products such as canola meal, soybean meal and cotton seed meal are also often added as supplements to stimulate microbial activity at the start of composting, depending on seasonal availability (Carrasco *et al.*, 2018).

Composting is a microbial process in which lignocellulosic waste materials are converted into a nutrient-rich humus-containing medium (Adams & Frostick, 2008, Liu *et al.*, 2018, Reyes-Torres *et al.*, 2018). Bacteria and fungi are the main contributing microorganisms in composting (Ryckeboer *et al.*, 2003, Kertesz & Thai, 2018). Mushroom composting occurs in two phases that are defined by changes in compost temperature, with an initial thermophilic phase (Phase I, 70-80 °C) followed by a pasteurization phase (Phase II, 60 °C) and subsequent conditioning at mesophilic temperatures (45 °C) (Figure 2.1. Representation of the mushroom composting process.) (Fermor *et al.*, 1985, Jurak *et al.*, 2015, Gómez, 2017).

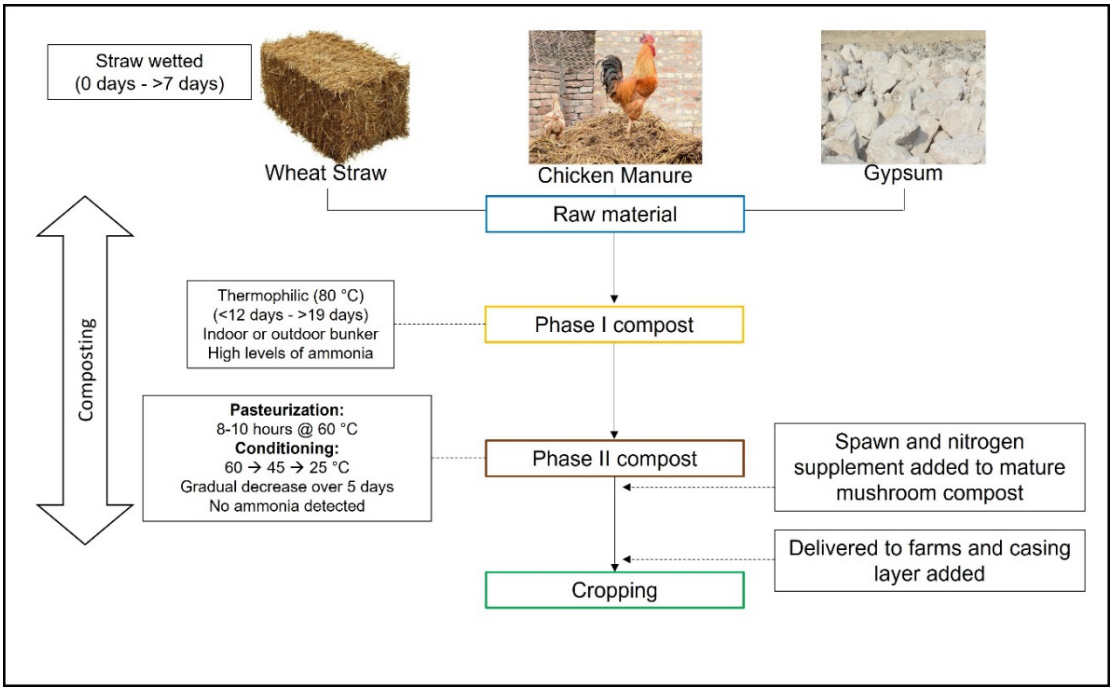

Figure 2.1. Representation of the mushroom composting process.
Outlining the parameters that need to be met in Phase I and Phase II compost before it is ready to be inoculated with mushroom spawn. Cropping includes adding a low nutrient layer, usually consisting of peat moss, to induce fruiting of the mushrooms.

Phase I, the thermophilic phase, is a partially controlled process that takes place in an enclosed bunker, with air supplied through an aerated floor to maintain at least 5% oxygen concentration in the compost (von Minnigerode, 1981, Fermor *et al.*, 1985, Gómez, 2017). Compost temperatures are initially mesophilic (25-45 °C) and rise to thermophilic conditions (45-80 °C) (Fermor *et al.*, 1985, Zhou *et al.*, 2015) as microbial activity increases. The temperature of the compost is controlled by mechanical turning of the compost or by altering airflow through the aerated floor, both of which influence the succession of microorganisms that degrade increasingly complex organic matter from the raw material (Székely *et al.*, 2009, Kertesz *et al.*, 2016, Vieira & Pecchia, 2021). In Australian compost yards, the Phase I process was found to be highly variable (Table 2.1). Phase I is complete when the ammonia

concentration reaches levels of 150-800 ppm due to proteolysis and ammonification (Miller *et al.*, 1991, Jurak *et al.*, 2015).

Phase II composting is done in enclosed tunnels over 6 days, and the process is similar across all compost yards in Australia (Table 2.1). Temperature and oxygen are more closely controlled by ventilation than during Phase I (Miller *et al.*, 1990). Composting in enclosed tunnels ensures that the compost is kept axenic after pasteurization and conditioning. In contrast to Phase I composting, Phase II is initially thermophilic (60 °C) during pasteurisation and mesophilic (decreasing from 55-25 °C) during conditioning. Following pasteurization, conditioning occurs with a slow decrease in temperature from 55 to 45 °C (then rapidly cooled to 25 °C for colonisation by *A. bisporus*), which is the ideal temperature range for bacteria, Actinobacteria and fungi to reassimilate free ammonia back into the compost (Souza *et al.*, 2014, Jurak *et al.*, 2015, Carrasco & Preston, 2020).

Thermophilic fungi are essential in Phase II composting because they convert nutrients from the raw material into microbial biomass and, in doing so, contribute to the selectivity of mushroom composting. One particularly important thermophilic fungus, *Mycothermus thermophilus* (syn. *Scytalidium thermophilum/Humicola insolens*) aids the reassimilation of ammonia into the compost (Fermor *et al.*, 1985, Wiegant, 1992, Vos *et al.*, 2017). In the presence of *M. thermophilus*, hyphal elongation of *A. bisporus* is doubled *in vitro* (sterile compost: 3.2 mm day^{-1}; non-sterile compost: 8.6 mm day^{-1} (Wiegant *et al.*, 1992, Straatsma *et al.*, 1994)). *M. thermophilus* also suppresses other fungal competitors of the mushroom, such as *Chaetomium globosum* (Ross & Harris, 1983, Vos *et al.*, 2017). *M. thermophilus* is the dominant fungal taxon in Phase II compost and makes up most of the microbial biomass in the total microbial community (Straatsma *et al.*, 1994, Coello-Castillo *et*

al., 2009, Székely *et al.*, 2009, Kertesz *et al.*, 2016, Vieira & Pecchia, 2018). However, it is one player in a multifaceted microbial community.

Bacteria, particularly thermophiles, are also key participants in mushroom composting. During Phase I composting there is a rapid succession of bacterial taxa in the compost as easily accessible nutrients are rapidly assimilated (Ryckeboer *et al.*, 2003, Kertesz *et al.*, 2016, Kertesz & Thai, 2018). By mid- to end-Phase I, bacterial diversity is at its peak (Kertesz *et al.*, 2016, Cao *et al.*, 2019). During Phase II, bacterial dynamics change from rapid succession to a more stable community (Kertesz & Thai, 2018). Thermophilic bacteria and the fungus *M. thermophilus* assimilate ammonia into microbial biomass that will provide nutrition for the mushrooms (Buth, 2017, Vos *et al.*, 2017, Vieira & Pecchia, 2018). Thermophilic bacteria also have a significant role in breaking down complex polysaccharides such as cellulose and hemicellulose (Székely *et al.*, 2009, Jurak *et al.*, 2015, Carrasco & Preston, 2020).

Earlier reports on bacterial and fungal succession in compost during mushroom composting have each focussed on a single compost yard, while hypothesising significant variability in microbial diversity among different compost yards (Székely *et al.*, 2009, Kertesz *et al.*, 2016, Vos *et al.*, 2017). In this study, this putative variability was investigated directly by determining the bacterial community diversity in compost from five geographically separated composting yards in south-eastern Australia. The study focusses on three important timepoints in the composting process to determine if the bacterial community structure in synthetic mushroom compost is constant among facilities, or if variation in the bacterial communities can be explained by functional redundancy in species composition.

2.2 Materials and methods

2.2.1 Compost sampling

Samples of mushroom compost were obtained from five commercial composting yards located along the east and southeast of Australia in July and August 2017. The compost yards are referred to in this study as Yards A–E to preserve commercial confidentiality. Compost was prepared according to the typical schedule for each yard (Table 2.1), with the general composting process described here. Briefly, wetted raw materials were subject to an initial uncontrolled self-heating step, with temperatures increasing to approximately 80 °C. This step typically lasted for 14-21 days with two to three turns (Phase I). Pasteurization of the compost was at 60 °C for 6-10 hours and conditioning was initiated by gradually cooling the compost to 45 °C over four to five days (Phase II), before rapidly cooling to 25 °C for *Agaricus* inoculation.

A single sample (approximately 500 g) of compost was sampled at the end of Phase I (samples P1), during Phase II before conditioning (samples P1.5), and at the end of Phase II (samples P2). Some compost yards were able to provide samples for successive crops. One crop is a complete cycle of the composting process from prewetting to the end of Phase II. The crop number is indicated as 1, 2 or 3. Furthermore, due to sampling difficulties during Phase II, not all compost yards were able to provide a sample during Phase II. Single samples were taken by hand around the edge of the compost pile, stored in Ziplock bags and transported to the laboratory without cooling within 3 days. Each bulk sample was mixed thoroughly, and five subsamples were taken and stored at -20 °C.

Table 2.1. Variations in the composting process at various yards around Australia. Including Average rainfall, minimum and maximum temperatures.

Compost yard	Average rainfall (mm)	Average temperatures (°C)	Yard size* (80-1600 t per crop)	Raw materials	Prewet▲ (48 h-14 days)	Phase I• (9-21 days)**	Phase II♦ (6-10 h)
A	702.4	Min: 11.5 Max: 25.4	Large	Wheat straw Poultry manure Gypsum Added nitrogen supplements 100% compost leachate	Medium duration Aerated Indoor	Medium duration Aerated indoor bunker Crop turned three times	Medium duration pasteurization Bulk
B	401.8	Min: 8.7 Max: 22.1	Medium	Wheat straw Poultry Manure Gypsum 100% compost leachate	Medium duration Aerated Outdoor	Short duration Aerated outdoor bunker Crop turned two times	Long duration pasteurization Bulk
C	390.9	Min: 11.5 Max: 23.5	Small	Wheat straw Poultry manure Gypsum Added nitrogen supplements 100% compost leachate	Medium duration Outdoor	Medium duration Aerated outdoor bunker Crop turned five times	Medium duration pasteurization Bulk
D	482.0	Min: 10.1 Max: 21.3	Large	Wheat straw Poultry manure Gypsum 50% compost leachate 50% fresh water	Short duration Outdoor	Long duration Outdoor concrete slab in windrows Crop turned 10 times	Short duration pasteurization Trays
E	826.5	Min: 7.7 Max: 16.9	Small	Wheat straw Poultry manure Gypsum Added nitrogen supplements 100% compost leachate	Long duration Outdoor	Medium duration Outdoor concrete slab in windrows Crop turned six times	Short duration pasteurization Trays

*Yard size – Small: 80-200 tonnes (t) of PI, Medium: 200-800 T of PI, Large: >800 T of PI

**No O_2 data was recorded during Phase I

▲Timing of prewet – Short: <3 days, Medium: 3-6 days, Long: >7 days

•Timing of Phase I – Short: <12 days, Medium: 12-18 days, Long: >19 days

♦Timing of pasteurization period for Phase II – Short: <7 h, Medium: 8-9 h, Long: > 10 h

2.2.2 DNA extraction

Total DNA was extracted from mushroom compost samples according to Lever *et al.* (2015), with some modifications. Compost samples (approximately 1 g) were ground into a fine powder in liquid nitrogen using a sterile mortar and pestle. Subsamples of ground compost (200 mg) were weighed aseptically into 2 mL bead beating tubes containing 0.1 mm zirconium-silicate beads and two 3 mm diameter glass beads. The samples were suspended in 200 mM sodium hexametaphosphate (100 µL), lysis buffer 1 was added (30 mM Tris/HCl, 30 mM ethylenediaminetetraacetic acid (EDTA), 800 mM guanidinium chloride (GuHCl), 0.5% (v/v) Triton X-100, pH 10.0, final concentration of sodium hexametaphosphate in tubes; 120 mM) (500 µL), and the samples were lysed using a homogenizer (MoBio Laboratories, USA) at 2000 rpm for 5 min. The samples were then incubated at 50 °C with agitation at 500 rpm for 1 h and subjected to two cycles of freezing (-80 °C) and thawing (50 °C).

Lysis buffer 2 (2.5 M sodium chloride, 2% (w/v) cetyltrimethylammonium bromide (CTAB), 0.1% (w/v) polyvinylpolypyrrolidone (PVPP)) (500 µL) was added, followed by incubation at 65 °C with agitation (500 rpm) for 30 min. Following incubation, the samples were centrifuged, and the supernatant (600 µL) was transferred to a fresh tube. Samples were extracted once with an equal volume of chloroform:isoamyl alcohol (24:1) and centrifuged at 14,000 × g for 10 min to separate the two phases. The top aqueous phase was transferred to a fresh tube (300 µL).

DNA binding magnetic beads (GE Life Sciences, Australia) in SPRI solution (10 mM Tris-HCl, 1 mM EDTA, 2.5 M NaCl, 20% (w/v) PEG 8000, 0.05% (v/v) Tween 20, 0.02% (w/v) sodium azide, pH 8.0) (120 µL) were added and samples were incubated at room temperature for 5 min to allow DNA binding to occur. The beads were

washed twice with 80% (w/v) ethanol and the DNA was eluted with Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The DNA samples were stored at -20 °C until further analysis.

2.2.3 Bioinformatics

DNA sequencing was carried out by the Australian Genome Research Facility (AGRF) (Melbourne, Australia). The V3-V4 16S rRNA gene hypervariable region was sequenced using universal primers 341F and 806R (Muyzer *et al.*, 1993, Caporaso *et al.*, 2011), using the Illumina MiSeq platform (paired 300 bp read lengths). Barcoded primer design, sequencing and initial quality filtering were performed by the AGRF using their standard protocols.

Raw FASTQ files were returned and the data analysis was 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 & 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.

2.3 Results

The bacterial community composition of mushroom compost was determined in 20 mushroom compost samples provided by composting enterprises in Victoria, New South Wales, South Australia and Tasmania. The samples analysed included seven samples from Yard A, four samples from Yard B, and three samples each from Yards C, D and E and were taken from end-Phase I, mid-Phase II and end-Phase II composts. The bacterial profiles obtained were compared across all compost yards, to test for possible enrichment of particular taxa or groups of taxa in yards with different sizes, using different processes, or in different climate and geographical zones.

2.3.1 Variability in compost yards

All composting yards examined used the same fundamental composting process, but there were substantial differences among them in scale and process details (Table 2.1). Phase I compost production by large-, medium- and small-scale facilities varied from 80 to 1600 t per crop, but all used the same raw materials (poultry manure, wheat straw and gypsum). Two of the small yards and the largest yard provided additional nitrogen either as inorganic supplements (urea or ammonium sulphate) or organic materials (e.g., cottonseed meal or soybean meal). Additional nitrogen supplements are used to meet C/N ratio requirements for optimal composting (25-35) (van der Wurff *et al.*, 2016).

All compost yards had a period of prewetting their raw materials with water and/or compost leachate, which ranged from 48 hours to 14 days. The Phase I process was similar for compost yards A, B and C. However, Yards B and C both did their prewet outdoors, while yard A did an indoor prewet. In the case of yards D and E, both yards

did their prewet outdoors and the piles of raw materials were not aerated. Yard D had a short prewet while yard E had a long prewet.

The Phase I process was the most variable step among compost yards. All compost yards turned the compost piles to homogenize temperatures within the compost pile. However, compost yards A and B had additional aeration with air being pushed through the compost from below ensuring that compost piles did not need to be turned frequently. Compost yards D and E needed to turn their compost more frequently as they relied solely on turning to aerate the compost. Yard C also had an aerated Phase I process, however they turned the compost more frequently than yards A and B. Yards A, B and C used bunkers for Phase I, whereas yards D and E ricked compost into windrows. The timing of Phase I was also variable ranging from 9 to 21 days. Compost yards A, C and E all had a similar timing for Phase I (12-18 days), while Phase I at yard B was shorter and longer at yard D. Yard A had an indoor facility for their Phase I process, all other yards did their Phase I process outdoors.

The variability in process in Phase II was much less than Phase I. For all compost yards, Phase II was done in enclosed tunnels, usually in bulk. However, for yards D and E, the compost end-Phase I compost is preloaded onto trays before being sealed in Phase II tunnels. Pasteurization times varied among yards. For a short pasteurization, the air temperature in the tunnel was held at 60 °C for 6 h, for a longer pasteurization the hold time was 10 h. Compost yards D and E had short pasteurization times, Yards A and C had mid length pasteurization time (8-9 h) and Yard B had the longest pasteurization time. Conditioning time was the same for all compost yards.

Although varying in size, yards A, B and C were most similar in processing. However, yard A did most of their processing indoors. For Yards D and E, the main differences were in prewet and Phase I, the Phase II process was the same.

Weather/climate conditions were variable among compost yards due to their geographical locations. The five-year average for maximum and minimum temperature ranged from 16-25 °C and 7.5-11.5 °C, respectively (Table 2.1). Average rainfall in the last five years prior to sampling was also highly variable with the lowest rain averaging 390 mm, to the highest rainfall averaging 826 mm (Table 2.1).

2.3.2 Bacterial diversity in mushroom compost

A total of 2,619,127 raw paired-end reads were obtained for the twenty samples analysed, with an average of 130,956 reads per sample (Table 2.2). Filtering and trimming yielded 1,852,543 high-quality 16S rRNA reads (>80% of reads passed). These high-quality reads were denoised, dereplicated and paired-end reads were merged, and 9,486 amplicon sequence variants (ASVs) were then inferred using the DADA2 package. Approximately 55% of the ASVs were removed as chimeras, but these variants only made up 4% of total reads. The non-chimeric sequence variants were classified into 4,305 ASVs, which were affiliated with 22 phyla and 182 families using the SILVA database. Less than 5% of the ASVs could be assigned to species level using the SILVA database.

Shannon-Weiner and Simpson diversity indices both showed that the bacterial diversity was the highest in end-Phase I compost for most of the samples (Table 2.3). This could be due to the Phase I process selecting against mesophilic taxa, resulting in low abundances of many taxa.

Table 2.2. Analysis of sequencing reads from compost samples.
Sample names are denoted by yard (A, B, C, D or E), Phase (end-Phase I; P1, Mid-Phase II; P1.5 and end-Phase II; P2) and crop (1, 2 or 3). Overall, approximately 80% of reads in all samples passed quality filtering.

Sample	Yard	Phase	Crop	Reads in	Filtered	Chimeras removed	ASVs inferred
AP11	A	End-Phase I	1	112973	92817	10422	1585
AP1.51	A	Mid-Phase II	1	118038	99346	1118	270
AP21	A	End-Phase II	1	129202	109269	2848	546
AP12	A	End-Phase I	2	135794	107832	1661	1173
AP1.52	A	Mid-Phase II	2	136135	110560	2775	1052
AP22	A	End-Phase II	2	94434	79154	8585	846
AP23	A	End-Phase II	3	62483	51821	1523	218
BP11	B	End-Phase I	1	166011	140408	1004	1202
BP21	B	End-Phase II	1	197271	161988	2056	883
BP12	B	End-Phase I	2	182118	146461	6162	1507
CP11	C	End-Phase I	1	135271	110694	1991	1137
CP21	C	End-Phase II	1	224814	183598	3562	1074
CP12	C	End-Phase I	2	116037	93351	4535	1255
CP22	C	End-Phase II	2	114612	95936	2604	1134
DP11	D	End-Phase I	1	110449	90835	4826	1218
DP1.51	D	Mid-Phase II	1	102632	84383	2448	779
DP21	D	End-Phase II	1	115720	94090	2926	864
EP11	E	End-Phase I	1	111882	92334	3800	1558
EP1.51	E	Mid-Phase II	1	142064	115642	2051	915
EP21	E	End-Phase II	1	111187	89427	4997	1000

Table 2.3. Species diversity and richness of bacterial communities in five compost yards.
Sample names are denoted by yard (A, B, C, D or E), Phase (end-Phase I; P1, Mid-Phase II; P1.5 and end-Phase II; P2) and crop (1, 2 or 3). For most of the compost yard samples, end-Phase I had the highest species diversity and species richness and all end-Phase II samples had the lowest species diversity and richness.

Sample	Compost Yard	Phase	Shannon (H)	Simpson (D)
AP11	A	End-Phase I	4.647	0.9645
AP1.51	A	Mid-Phase II	4.951	0.9790
AP21	A	End-Phase II	3.798	0.8949
AP12	A	End-Phase I	4.534	0.9668
AP1.52	A	Mid-Phase II	3.795	0.9058
AP22	A	End-Phase II	3.505	0.8847
AP23	A	End-Phase II	3.872	0.9286
BP11	B	End-Phase I	5.686	0.9918
BP21	B	End-Phase II	4.985	0.9812
BP12	B	End-Phase I	5.274	0.9844
CP11	C	End-Phase I	5.394	0.9870

CP21	C	End-Phase II	5.225	0.9851
CP12	C	End-Phase I	5.234	0.9857
CP22	C	End-Phase II	4.851	0.9768
DP11	D	End-Phase I	5.001	0.9781
DP1.51	D	Mid-Phase II	4.822	0.9811
DP21	D	End-Phase II	4.802	0.9768
EP11	E	End-Phase I	5.750	0.9925
EP1.51	E	Mid-Phase II	5.269	0.9892
EP21	E	End-Phase II	4.929	0.9842

2.3.3 Bacterial diversity in end-Phase I compost is highly variable

The bacterial profiles of end-Phase I compost are shown in Figure 2.2.2. Compost yards A and B revealed similar bacterial taxa, while yards C and E were more similar to each other. The longer Phase I process in yard D may be the cause of differences in the bacterial profile. However, there are some shared taxa between yard A, B and D.

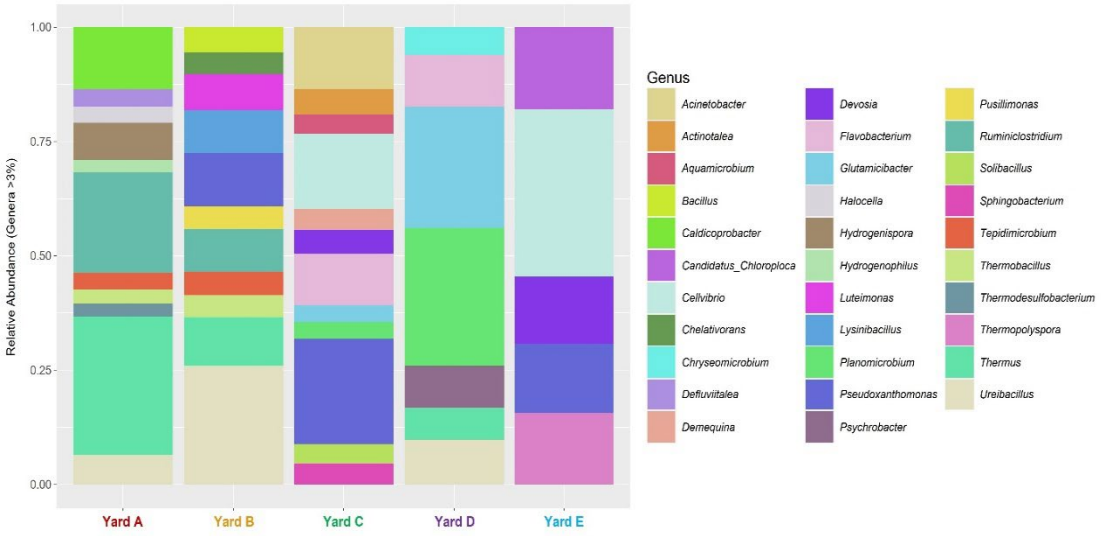

Figure 2.2. Bacterial profile of end-Phase I compost from all compost yards at genus level.
Rare taxa that had a relative abundance of less than 3% and ASVs not classified to genus level are not shown.

For yards A and B, the most common genera found in end-Phase I compost were *Ruminiclostridium*, *Thermus*, *Thermobacillus* and *Ureibacillus* (15-30%). The bacterial profile for yard D had a higher proportion of mesophilic taxa such as

Glutamicibacter and *Planomicrobium* (approximately 12%). In yards C and E, common genera were *Cellvibrio*, *Pseudoxanthomonas* and *Thermopolyspora* (8-15%). Compost yard D shared some bacterial taxa with yards A and B, but there was a larger proportion of mesophilic bacteria in compost from yard D (e.g., *Glutamicibacter*, *Planomicrobium* and *Psychrobacter*) compared to yards A and B, which had much higher proportions of thermophilic bacteria (e.g., *Thermus* and *Ureibacillus*). Compost yard A also had a much higher proportion of *Thermus* than yard B. Compost from yard A is processed at a much larger scale than yard B and therefore a consistently high temperature can be maintained throughout the compost pile (Table 2.1).

2.3.4 Bacterial profiles are similar in Phase II for all compost yards

Variation in pasteurization time provides the greatest source of process variability among compost yards in Phase II (Table 2.1).

The common taxa across all yards were *Chelativorans* (1-7%), *Pseudoxanthomonas* (2-31%) and *Thermopolyspora* (1-2.5%). In yards B, C and E, a smaller proportion of *Pseudoxanthomonas* (2-10%) in the bacterial profile corresponded with a larger proportion of other thermophilic taxa such as *Thermobacillus* (1%), *Thermopolyspora* (2-4%) and *Truepera* (2-4%) (Table 2.3).

At the end of Phase II, the bacterial profiles for yards A, B and C were similar, with additional common genera for these yards being *Actinotalea* and *Thermobacillus* (Figure 2.3). However, in yard C there was a considerably higher proportion of "*Candidatus* Chloropoloca". Much like Phase I, the similarities in bacterial taxa among these yards are likely to be related to process, where these three compost yards

run their Phase II in bulk, compared to compost yards D and E with Phase II done in trays.

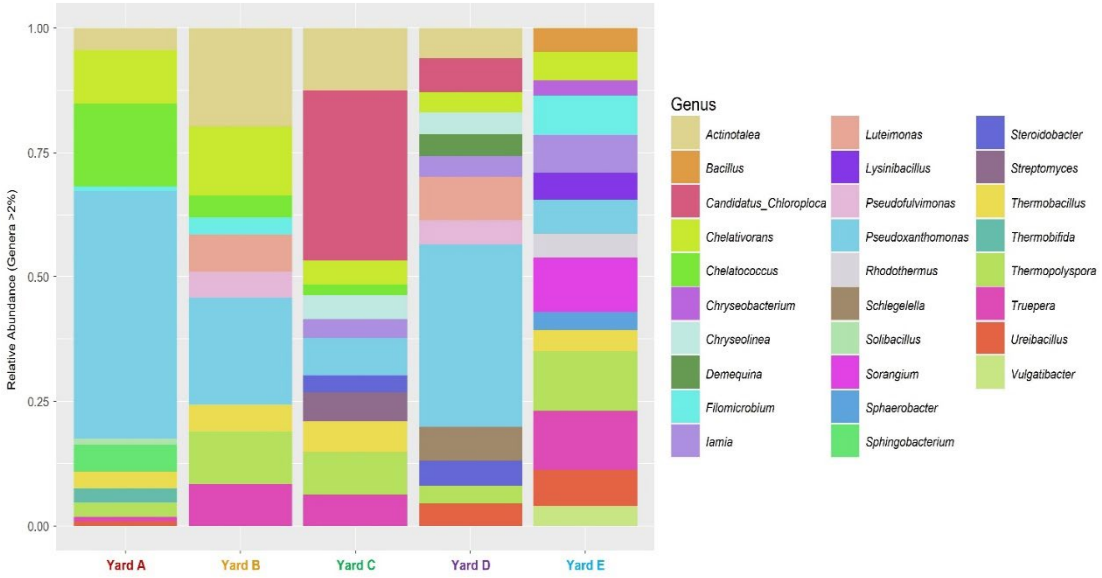

Figure 2.3. Bacterial profile at end-Phase II for all compost yards at genus level. Rare taxa that had a relative abundance of less than 2% and ASVs that could not be classified to genus level are not shown.

2.3.5 The bacterial community in end-Phase II is established by mid-Phase II

Three compost yards, two small-scale yards and one large-scale yard, were able to provide mid-Phase II samples. Entering a Phase II tunnel is a health risk and was not possible for other compost yards. One taxon, *Ureibacillus,* was persistent throughout all three sampling points for all three compost yards. However, for all mid-Phase II samples, almost all the taxa are also present in the end-Phase II samples (Figure 2.4). For example, *Chelativorans*, *Pseudoxanthomonas* and *Thermopolyspora* were consistently present at all three yards for mid-Phase II and end-Phase II. Yards D and E had a smaller proportion of *Pseudoxanthomonas*, and this genus was replaced in these yards by other thermophilic taxa, such as *Lysinibacillus* and *Sphaerobacter*.

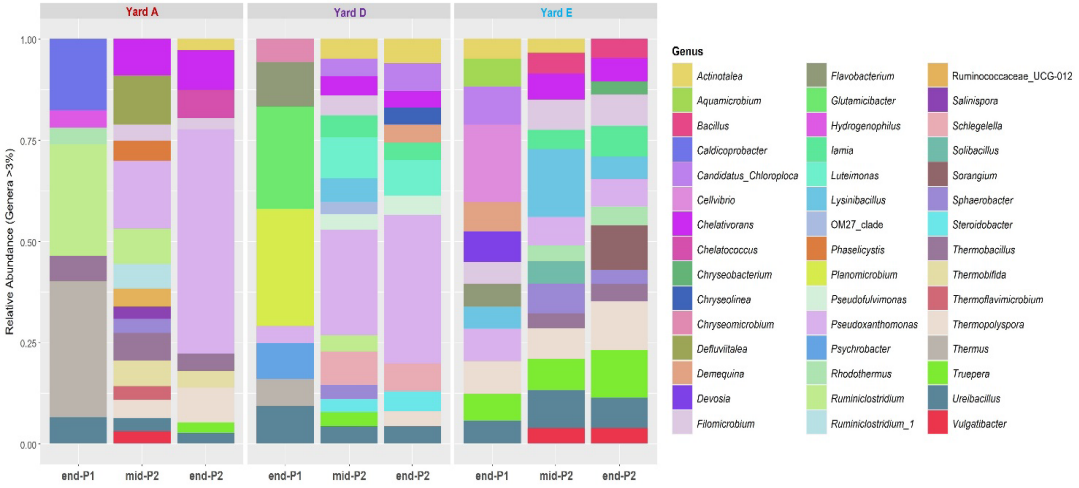

Figure 2.4. Bacterial profiles at key timepoints during Phase II.
Rare taxa that had a relative abundance less than 3% and ASVs that could not be assigned to genus level are not shown. Mid-Phase II was sampled after pasteurisation, before the conditioning step. Due to the health risk of entering a Phase II tunnel, only these three yards were able to provide mid-Phase II compost samples.

2.3.6 Successive crops have similar bacterial communities by the end of Phase II

The bacterial profile of two successive crops from one compost yard was also included to determine the effect of sampling on reproducibility. Successive crops were chosen to ensure that the feedstocks remained the same and therefore, the most significant variable would be sampling technique.

The bacterial profiles of two full successive crops from compost yard A, including mid-Phase II samples that were taken just after the pasteurization process are shown in Figure 2.5. There was more variability in the bacterial profile in Phase I between the two crops. However, the dominant taxon for both crops was *Thermus*. Comparing the two mid-Phase II samples, the bacterial profile for the first crop was more similar to the Phase I profile, while the profile for the second crop was more similar to an end-Phase II profile with a larger proportion of *Sphingobacterium* (Figure 2.5). The differences between the two samples is most likely due to inconsistent sampling

technique where samples were most likely taken from the edge of the pile and were not entirely representative.

By the end of Phase II, the bacterial profiles of the two crops were very similar, with common taxa being *Chelativorans*, *Chelatococcus*, *Pseudoxanthomonas*, *Thermobacillus* and *Thermobifida*. Therefore, despite the variability found at the end of Phase I, the process involved during Phase II composting selects for a particular profile of bacteria.

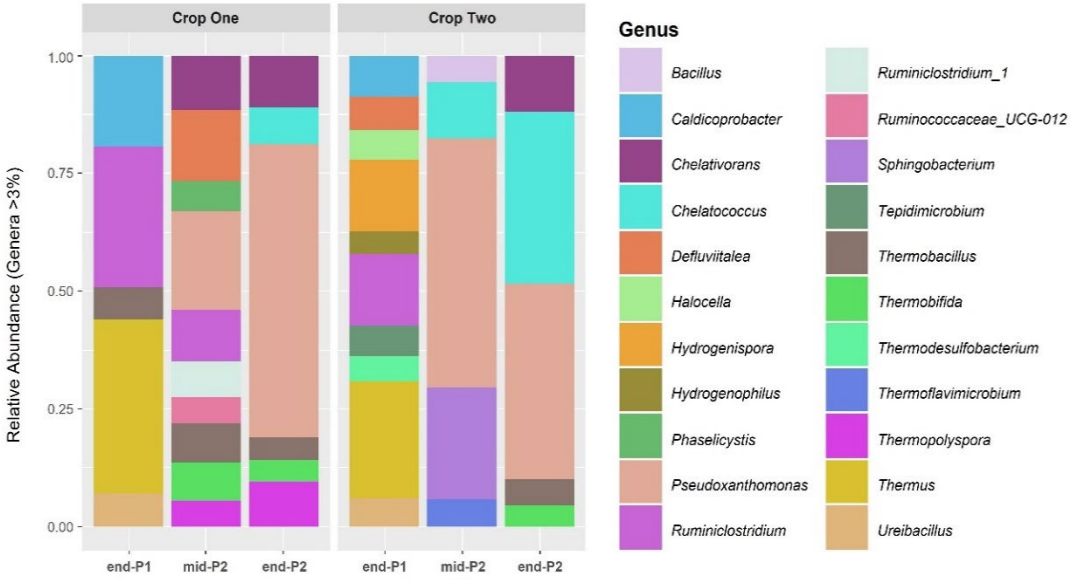

Figure 2.5. Bacterial profiles of two successive compost crops.
Rare taxa that had a relative abundance of less than 3% and ASVs that could not be classified to genus level are not shown.

2.3.7 Bacterial diversity changes in a similar manner during composting at all yards

An unweighted Unifrac distance metric was used to compare bacterial communities among compost yards and phases (Figure 2.6). The bacterial communities in yard A clustered separately from other yards in the ordination plot (Figure 2.6). The combination of operational scale and indoor Phase I processing (Table 2.1) may be the reason for such distinct differences in the bacterial communities.

The bacterial community associated with Yard B, particularly for end-Phase I, was more closely related to Yard A than any of the other yards. This could be seen earlier in the bacterial profiles in Figure 2.2.

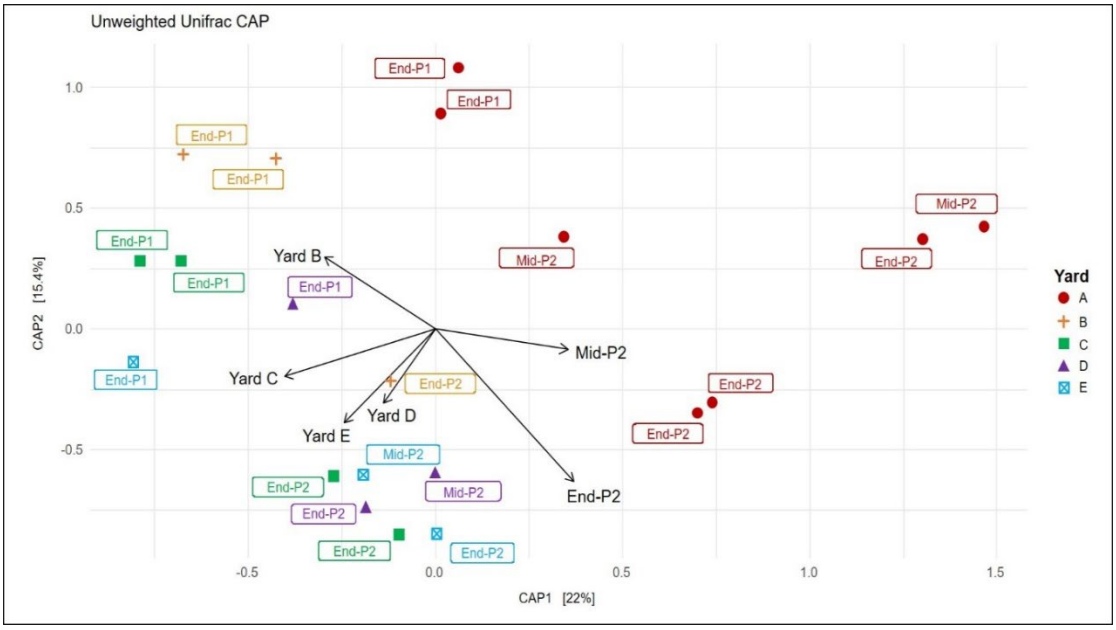

Figure 2.6. Unweighted Unifrac Canononical Analysis of Principal Components exploring the similarity in bacterial profiles among compost yards.
ANOVA: P <0.05, df = 6. Arrows represent the differences in bacterial communities between compost yards and phases. Compost yard A clustered very differently on the ordination plot from other yards.

The bacterial communities in yards D and E were relatively similar, particularly in Phase II. This is most likely due to both yards having the same Phase II procedure (Table 2.1). In fact, most of the Phase II samples clustered closely together on the ordination plot, suggesting that the bacterial communities in Phase II were similar (Figure 2.6).

The differences between these two compost yards are reflected in the Phase I samples where compost yard E has a much longer prewetting process and but a shorter Phase I compared to yard D. Compost yard D had the longest Phase I compared to other yards and, as a result, the bacterial profile indicated progression towards a Phase II community (Figure 2.6).

The succession of different bacterial communities throughout the composting process followed the same general pattern in all compost yards. This was indicated with end-Phase I samples at the top left of the plot, progressing to end-Phase II at the bottom right (Figure 2.6). The bacterial community was significantly different from that in the mid- and end-Phase II (PERMANOVA (Phase): $F = 2.4399$, $R^2 = 0.1764$, $P < 0.05$, $df = 3$).

2.4 Discussion

In this study, bacterial diversity was compared in mushroom compost from five compost yards that are located in widely different locations across southeast Australia. The bacterial profiles in two of the compost yards sampled were significantly different from the other three in both Phase I and Phase II. However, beta diversity analysis showed that the bacterial diversity of Phase I and Phase II followed the same trend, suggesting that there is functional redundancy in the bacteria required for the composting process.

Several taxa were consistently present in Phase II compost from all compost yards studied, particularly *Chelativorans*, *Pseudoxanthomonas* and *Thermopolyspora*. *Pseudoxanthomonas* was the dominant bacterial taxon in end-Phase II compost in three out of five compost yards. *Pseudoxanthomonas taiwanensis* has also been identified as a key species in other mushroom composts (Székely *et al.*, 2009, Cao *et al.*, 2019), including oyster mushroom compost (Vajna *et al.*, 2012), and in other cellulose degrading consortia (Du *et al.*, 2015). In this study, these microbes were identified directly by amplicon sequencing. Whereas earlier studies used fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) to identify these microbes (Székely *et al.*, 2009, Vajna *et al.*, 2012).

Where *Pseudoxanthomonas* (2-4%) was not the dominant organism in end-Phase II samples, there was a higher proportion of Actinobacteria (8-12%) and Bacilli (1-1.5%) in the bacterial profile (Figure 2.3). In yards C and E, *Pseudoxanthomonas* appeared in end-Phase I compost and the proportion was much higher than in their respective end-Phase II samples (Figure 2.2 andFigure 2.3). The dominant Actinomycete in Yards C and E was *Thermopolyspora* (Figure 2.3). This pattern has

been seen in composts that use chemical nitrogen or other grasses (e.g., alfalfa) as their main nitrogen source (Vajna *et al.*, 2010, Cao *et al.*, 2019). In mushroom compost produced in China, high throughput sequencing comparing the top 10 operational taxonomic units (OTU) showed that *P. taiwanensis* was the dominant organism in their mid- to end-Phase I samples and *Thermobispora*, an Actinomycete, was the dominant organism in end-Phase II samples (Cao *et al.*, 2019). However, the proportion of Bacilli was significantly smaller compared to the Actinomycete population (Cao *et al.*, 2019). Phase I compost for this study was done in windrows and details of the temperatures attained during the study were not provided (Cao *et al.*, 2019), but it would seem likely that temperatures were substantially lower than 80 °C and had more temperature variability in windrows than in bunkers seen in Australian compost yards.

In compost preparation for oyster mushrooms, *Pseudoxanthomonas taiwanensis* is dominant in the thermophilic stage, equivalent to Phase I (Vajna *et al.*, 2012). Actinobacteria, such as *Thermopolyspora*, and various Bacilli, such as *Thermobacillus* and *Ureibacillus*, dominate mature compost, the Phase II equivalent (Vajna *et al.*, 2012).

Although the microbial composition and proportion in the five compost yards sampled varied, the bacterial community for each Phase clustered relatively close together, suggesting that the bacterial communities were similar (Figure 2.6). Unweighted Unifrac statistical analysis compared the bacterial diversity of 20 samples and showed clear separation in the bacterial diversity in end-Phase I and end-Phase II. Furthermore, all Phase I and Phase II samples followed the same trend on the PCA plot (Figure 2.6). This suggests that despite the variability in Phase I composting, the Phase II composting process selects for bacteria that fulfill the role or transforming

nutrients from the raw materials into a selective growth substrate for *Agaricus* (i.e., cellulose degradation).

The majority of the cellulose breakdown occurs during Phase II (Jurak *et al.*, 2015, Carrasco *et al.*, 2020) and it seems likely that *Pseudoxanthomonas* plays a significant role in mushroom composting by boosting cellulose degradation. *Pseudoxanthomonas* was first classified in 2000 by Finkmann *et al.* (2000) after isolation from biofilters and *P. taiwanensis* was first classified in 2002 by Chen *et al.* (2002) after isolation from hot-springs. *Pseudoxanthomonas taiwanensis* promotes cellulose degradation in consortia used in biofuel production, and it has been suggested that this is due to its production of β-glucosidase (Haruta *et al.*, 2002, Kato *et al.*, 2005, Du *et al.*, 2015). However, this has been difficult to prove, and previous authors concluded that although *P. taiwanensis* shared a complex relationship with other members of the microbial consortium, β-glucosidase production was not the sole reason for enhanced cellulose degradation (Du *et al.*, 2015). Similarly, *Pseudoxanthomonas* has been shown to aid cellulose breakdown by a consortium by consuming acetate and buffering the pH of the liquid medium (Kato *et al.*, 2005), but this could not be confirmed when growing *Pseudoxanthomonas* as a pure isolate (Kato *et al.*, 2005). Therefore, the role in which *Pseudoxanthomonas* plays in cellulose breakdown must be intrinsically involved with the other microbes and the relationship among them is complex.

It is also likely that the scale and peak temperatures achieved in Phase I are important for establishing the bacterial profile in Phase II, despite the controlled process of Phase II. When the abundance of *Pseudoxanthomonas* was low, the abundances of Actinobacteria and Bacilli were greater (Figure 2.3). This was most likely due to the temperatures achieved and the process used in Phase I (Table 2.1). Phase I was done

outdoors in a bunker for yards B, C, D and E and the temperatures profiles were most likely more variable than the indoor process of yard A (Table 2.1). However, due to the larger scale of yard D compared to yards B, C and E, larger compost piles may have been able to reach higher temperatures (Table 2.1). For a range of composts (mushroom and partial green waste compost) that did not reach a peak of 80 °C, the bacterial community favoured more actinomycetes, whereas when temperatures were greater than 80 °C, the bacterial profile had more Bacilli and *P. taiwanensis* (Székely *et al.*, 2009, Karadag *et al.*, 2013).

Thermobifida, Thermomonospora and *Thermopolyspora* were among the most abundant of thermophilic Actinobacteria found in this study as well as other studies (Zhang *et al.*, 2014, Cao *et al.*, 2019, Song *et al.*, 2021). There is also a succession of dominant actinomycetes at different stages of mushroom composting (Zhang *et al.*, 2014). These genera are known for their cellulose degrading enzymes, and *Thermopolyspora* and *Thermomonospora* also produce hemicellulases (Lin & Stutzenberger, 1995, Goodfellow *et al.*, 2005, Zhang *et al.*, 2014). *Thermobifida cellulolytica* is able to completely degrade cellulose (Kukolya *et al.*, 2002). *Thermopolyspora* dominates end-Phase II compost and is able to produce thermostable hemicellulose degrading enzymes (Zhang *et al.*, 2014). Similar succession in actinomycetes were found in end-Phase I and end-Phase II compost derived from different straw types (Song *et al.*, 2021). *Geobacillus* and *Ureibacillus* have both been isolated from compost samples in this study (data not shown) as well as other composts (Weon *et al.*, 2007, Poli *et al.*, 2011). For oyster mushroom compost, a combination of Actinobacteria and Bacilli dominate in mature compost (Vajna *et al.*, 2012). Bacilli are often found in cellulose degrading systems (Gavande *et al.*, 2021), particularly composts, due to their heat resistant, spore-forming nature.

Bacillus, *Geobacillus* and *Ureibacillus* have highly active lignocellulolytic enzymes (Xu *et al.*, 2019), and therefore it is not surprising that these genera are found in mushroom compost as well as other composting systems (Vajna *et al.*, 2012, Karadag *et al.*, 2013, Rathinam *et al.*, 2020).

Another important genus in mushroom compost is the α-Proteobacterium *Chelatococcus*. In this study, *Chelatococcus daeguensis* was the most common species of this genus and was found in all yards sampled. *Chelatococcus* was first classified by Auling *et al.* (1993), and five species have been classified since then. *Chelatococcus daeguensis* was classified in 2008 by Yoon *et al.* (2008), and is able to grow on several carbon sources, including cellobiose (Yoon *et al.*, 2008). The whole genome has been sequenced and a hypothetical protein that has 99.2% amino acid similarity to a cellulase gene from GH5 (Yang *et al.*, 2017). It has been proposed that *C. daeguensis* aids lignocellulose degradation by activating lignin breakdown (Gavande *et al.*, 2021). However, this has not been fully confirmed.

Variability in successive crop cycles of compost is not well captured in this study as the microbial community was only examined for two crops from a single yard. Despite this, bacterial profiles of two successive crop cycles showed similar compositions, with the dominant organisms remaining the same between both cycles. Microbial succession is likely to vary with season, corresponding to annual variation in substrate quality and composition. However, the varying proportions of the less abundant taxa was most likely due to sampling technique. To give more resolution to the rapid changes in the bacterial diversity, sampling many times throughout the composting process is necessary. This has been previously done with a single compost yard (Kertesz *et al.*, 2016). However, sampling consistently at multiple timepoints across multiple compost yards can be a difficult task, with care required to ensure

reproducible and representative sampling of highly variable compost piles. Ongoing studies are investigating the best practice for sampling multiple compost yards and successive compost cycles, with the aim of identifying microbial indicators of higher producing mushroom compost.

2.5 Conclusion

Changes in microbial diversity during Phase I are related to differences in composting process factors such as time, production scale and temperature achieved. This resulted in diverse bacterial profiles across five compost yards. However, the compost bacterial diversity followed the same progression trend in all compost yards. This suggests that, despite differences in the composition of the bacterial profile in Phase I and Phase II, the Phase II composting process selects for a particular profile of bacteria that are actively involved with cellulose degradation.

In vitro study of mushroom compost bacteria and their interaction with *Mycothermus thermophilus*

Abstract

Composting is a microbially intense process that involves specific interactions between bacteria and fungi to break down polymeric compounds such as cellulose, hemicellulose and lignin. Rapid succession in dominant bacterial and fungal taxa is seen in Phase I mushroom composting. However, during Phase II, a stable community of *Pseudoxanthomonas*, a novel genus from the *Chitinophagaceae* family and the thermophilic ascomycete, *Mycothermus thermophilus* has been previously noted. Understanding the interactions between organisms in this stable community will provide more insight into the dynamics of composting. Three species of *Pseudoxanthomonas* and a novel chitin degrading bacterium, *Mycovorax composti*, were grown in co-culture with *M. thermophilus* to determine the effect these bacteria had with the fungus. *Pseudoxanthomonas taiwanensis* had a neutral interaction with *M. thermophilus* in co-culture, while *P. suwonensis* and *P. koreensis* had a negative influence on the growth of the fungus. Meanwhile, *M. composti* stopped fungal growth and showed chitinolytic activity. Therefore, *M. thermophilus* most likely benefits in the presence of *P. taiwanensis*, while *M. composti* is potentially releasing

nutrients bound in the fungus to support the growth of other bacteria and ultimately *Agaricus bisporus*.

3.1 Introduction

Composting is the process of breaking down polymeric compounds such as cellulose or chitin into lower molecular weight substrates. In the mushroom industry, composting is a three-phase process (Chapter 2) resulting in a substrate that is highly selective for button mushrooms (*Agaricus bisporus*). Composting is primarily driven by bacteria and fungi that break down lignocellulosic complexes in wheat straw. An important bacterial-fungal interaction is chemotaxis where bacteria actively migrate towards fungi or fungal-derived metabolites and adhere to fungal surfaces (Frey-Klett *et al.*, 2011, Deveau *et al.*, 2018). The direct association of bacteria with fungi can be both beneficial and detrimental to fungi (Frey-Klett *et al.*, 2011). One of the beneficial interactions between bacteria and fungi is cooperative metabolism, where bacterial and fungal partners provide nutrients to one another through the degradation of polymeric or recalcitrant materials (Frey-Klett *et al.*, 2011). It is a synergistic process that mostly occurs during the conditioning stage in Phase II and is the critical point at which the compost becomes selective for *Agaricus bisporus*. Here, microbial partnerships are established to breakdown cellulose and hemicellulose before inoculation of the substrate with *A. bisporus* (Straatsma *et al.*, 2000, Jurak *et al.*, 2015, Vieira & Pecchia, 2018).

A detrimental example of chemotaxis is mycophagy, where chitinolytic bacteria colonise fungal hyphae and cause fungal lysis by degrading chitin in the fungal cell walls (de Boer *et al.*, 1998). Chitin is the main structural polymer in fungal cell walls. Chitin is a linear polymer that consists of β-1,4-N-acetylglucosamine and is broken down into N-acetylglucosamine by a series of chitinases (Hamid *et al.*, 2013).

The presence of *Mycothermus thermophilus* in mushroom compost has been known since the early days of mushroom cultivation and *M. thermophilus* has been recognised as a mushroom growth promoting fungus since 1992 (Straatsma *et al.*, 1989, Wiegant, 1992, Straatsma *et al.*, 1994, Straatsma *et al.*, 1994, Coello-Castillo *et al.*, 2009). *Mycothermus* has also been found in a range of other composts made from various starting materials (Langarica-Fuentes *et al.*, 2014, Meng *et al.*, 2018, Wang *et al.*, 2018, Wang *et al.*, 2020). In mushroom compost, the conditioning stage of Phase II is almost exclusively dominated by *M. thermophilus* (Kertesz *et al.*, 2016, Vieira & Pecchia, 2021). *Mycothermus thermophilus* is known to produce a range of carbohydrate-active enzymes (CAZymes), such as endoglucanase, β-glucosidase and cellobiohydrolase, and these useful, thermostable enzymes can be exploited by other industries (Basotra *et al.*, 2016). The by-products of these enzymes can become inhibitory for further breakdown of cellulose (Du *et al.*, 2015), therefore other heterotrophic microbes are needed to aid *M. thermophilus* in the degradation of these inhibitory compounds.

Pseudoxanthomonas taiwanensis, a Gamma proteobacterium (described in the previous chapter), is the dominant bacterial taxon in Phase II mushroom compost (Chapter 2). *Pseudoxanthomonas* was identified in mushroom compost in 2009 using culture independent techniques such as molecular fingerprinting (Székely *et al.*, 2009). Prior to its discovery in mushroom compost, it was found in lignocellulose degrading consortia and is known to produce β-glucosidase but does not express cellulase activity (Haruta *et al.*, 2002, Kato *et al.*, 2005, Wang *et al.*, 2011, Du *et al.*, 2015). The production of β-glucosidase by *Pseudoxanthomonas* has been hypothesized to increase cellulose degradation, however this has not been confirmed (Du *et al.*, 2015).

A novel chitin degrading bacterium, *Mycovorax composti*, from the *Chitinophagaceae* family was recently isolated in mushroom compost in this study, the classification of which is described in Chapter 5. A previous study that identified a bacterial taxon belonging to the *Chitinophagaceae* family in relatively high abundance during the conditioning stage of Phase II composting (Kertesz *et al.*, 2016), this was identified as the novel genus-species *Mycovorax composti*, that was isolated from Phase II compost and is described in Chapter 5. *M. composti* grows in the same temperature range (40-50 °C) as *M. thermophilus*, and the bacterium is capable of hydrolysing chitin (Chapter 5).

Interactions between *M. thermophilus* and other compost microbes have not yet been extensively studied, *in vitro* or *in situ*. A better understanding of microbial interactions may provide valuable insight on how to improve the reproducibility of mushroom composting, particularly for nutrient transformation throughout the composting process. One way to make progress in this area is to identify which microbes work synergistically to produce a substrate specific for *A. bisporus*. The study presented here reports the isolation and identification of key compost microorganisms as the first step in determining how compost microbes, especially the dominant fungus and bacterium, interact *in vitro*.

3.2 Materials and methods

3.2.1 Collection of mushroom compost samples

Compost from 10 compost yards were sampled across eastern and southern Australia. Samples of finished uninoculated mushroom compost (end-Phase II) were collected by composters as described in the previous chapter, and transported unrefrigerated to The University of Sydney, Camperdown, within 48 h of sampling.

3.2.2 Isolation and identification of compost bacteria

Isolates of compost bacteria were obtained from Phase II mushroom compost as follows. Unhomogenised, roughly chopped mushroom compost (1 g) was suspended in 9 mL of liquid nutrient medium (R2A, Hi-Media) (Reasoner & Geldreich, 1985) and serial dilutions of the extract were incubated either at 30 °C and 50 °C for 48 h. Extracts were plated onto a solid nutrient medium (R2A agar (1.5%), BactoDifco, Australia) and incubated at 50 °C for 48 h. For bacterial isolates that were unable to grow at 50 °C after 48 h, the plates were incubated at 30 °C for a further 48 h.

The bacterial strains obtained were classified by partial sequencing of the 16S rRNA gene after PCR amplification with primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Lane, 1991) in 25 µL reactions using MyTaq^{TM} DNA Polymerase (Bioline, Australia). PCR amplification was done with a thermocycler (BioRad, S1000) using the following program: 5 min initial denaturation at 95 °C, 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 55 °C, 1 min extension at 72 °C and a final extension for 5 min at 72 °C.

The amplicons were purified (ISOLATE II PCR and Gel Kit, Bioline, Australia) following the manufacturers protocol and Sanger sequencing of the PCR product was

done by Macrogen Inc., South Korea. The taxonomic identity of the isolates was determined by comparison of the resulting sequence with the GenBank database using BLAST (Altschul *et al.*, 1990). All isolated strains were preserved in 25% (v/v) glycerol and kept at -80 °C.

3.2.3 Isolation and identification of Mycothermus thermophilus

Several strands of composted wheat straw from Phase II compost (2 cm long) were placed onto YpSs agar (per L: 15 g soluble starch, 4 g yeast extract, 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 20 g agar, pH 6.5)(Atlas, 2010) containing chloramphenicol (35 µg mL^{-1}) and incubated at 50 °C for 4 days. Dark green to black fungal colonies which had chained conidia in the aerial mycelia viewed under light microscopy were isolated and purified from plates inoculated with composts from yards A-J and maintained on solid YpSs agar with 35 µg mL^{-1} of chloramphenicol added to supress bacterial growth. The strains obtained were classified by amplification and sequencing of the ITS regions using primers ITS1F (5'-CTT GGT CAT TTA GAG GAA GTA A-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White *et al.*, 1990, Op De Beeck *et al.*, 2014) in 25 µL reactions using MyTaq^{TM} DNA Polymerase (Bioline, Australia). The following PCR protocol was used: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 60 s. A final extension step at 72 °C for 5 min completed the PCR reaction. Amplicons were purified and sent for Sanger sequencing using the same method as the bacterial isolates.

3.2.4 Extraction of DNA

DNA from the isolated bacteria was extracted using the miniprep method described by Wilson (2001), with some modifications. Briefly, bacterial cells were harvested from late exponential phase (R2A, HiMedia) and resuspended in Tris-EDTA buffer (10 mM Tris-HCl, 1mM ethylenediaminetetraacetic acid (EDTA)) containing 1 mg mL^{-1} lysozyme (Sigma-Aldrich, Australia). The cell suspensions were incubated in a water bath at 37 °C for 30 min. Pre-warmed CTAB buffer (500 µL; 2% hexadecyltrimethylammonium bromide (CTAB), 100 mM Tris-HCl; pH 8.0, 20 mM EDTA, 1.4 M NaCl) was added and incubated in a water bath at 50 °C for 60 min. Chloroform:isoamyl alcohol (24:1) (600 µL) was added and the solutions were emulsified before centrifuging at 14,000 × *g* for 10 min. The top aqueous supernatant (600 µL) was transferred to a fresh tube, 480 µL of isopropanol was added, and the precipitated DNA was collected by centrifugation at 12,000 × *g* for 20 min. The pellet was washed three times with 80% (w/v) ethanol, dried resuspended in 10 mM Tris/HCl, 1mM EDTA, pH 8.0.

For extraction of fungal DNA, fungal material was scraped from a 10 × 10 mm area of a freshly grown plate culture (YpSs) and homogenised in CTAB buffer with zirconium-silicate beads (0.1 mm, Daintree Scientific, Australia) with two soda lime glass beads (3 mm, John Morris Group, Australia). DNA extraction for the fungal isolates was continued following the same chloroform:isoamyl alcohol step that was used for bacterial DNA extraction.

3.2.5 Restriction fragment length polymorphism

Restriction fragment length polymorphism analysis of the 16S rRNA gene was done for all bacterial isolates, in order to classify them into similarity groups. After 16S

PCR amplification of bacterial DNA, described as above, the amplicons were digested with *HhaI* and *HinfI* (New England Biolabs, Australia) at 37 °C for 2 h. The fragments were visualised on a 2% (w/v) agarose gel in TAE (40 mM Tris base, 20 mM acetate and 1mM EDTA, pH 8.6) buffer at 50 V. The fragment sizes were analysed using gel electrophoresis software package GelAnalyzer v2010a (Lazar & Lazar, 2010).

3.2.6 Analysis of Mycothermus thermophilus clones using BOX PCR

BOX regions in *M. thermophilus* strains were amplified using the BOXA1R primer (5'-CTA CGG CAA GGC GAC GCT GAC G-3') (Versalovic *et al.*, 1994), in 25 µL reactions with *M. thermophilus* DNA (5× MyTaq^{TM} Red Reaction buffer (Bioline, Australia), 5 µL; 10 µM BOXA1R primer, 2 µL; bovine serum albumin (BSA, 10 mg/mL), 0.25 µL; MyTaq^{TM} polymerase (Bioline, Australia), 0.25 µL; deionised water, 16.5 µL) were amplified using the following protocol; 5 min denaturation at 95 °C, 30 cycles of 1 min denaturation at 95 °C, 1 min annealing at 50 °C and 8 min extension at 65 °C, followed by a final extension for 10 min at 65 °C. Amplicons were visualised on a 1.5 % TAE agarose gel at 90 V.

Ophiostoma ips and *Agaricus bisporus* were used as fungal controls and *E. coli* was used as a bacterial control. *O. ips* and *A. bisporus* were commonly used fungal isolates in the lab and these fungal species were used due to their phylogeny. *O. ips* is an Ascomycete and *A. bisporus* is a Basidiomycete. These controls were used to ensure the specificity of the BOXA1R primer.

3.2.7 Interaction between M. thermophilus and compost bacteria

The interactions between *M. thermophilus* and bacterial isolates from compost were tested using both solid and liquid medium. On solid medium (YpSs 1.5% agar), two parallel streaks approximately 10 cm apart were grown to stationary phase at 50 °C or 30 °C in a moist box to prevent the plates from drying. *M. thermophilus* was subsequently inoculated, with an agar plug (5 × 5 mm) from a freshly grown plate, between the bacterial streaks and incubated for four days at 50 °C in a moist box to prevent evaporation. Simultaneous growth of fungi and bacteria with liquid medium was done using a method adapted from van Schöll *et al.* (2006). Briefly, soda-lime glass beads (5 mm diameter; John Morris Group, Australia) were placed in a single layer in a Petri dish and overlaid with a 45 µm nylon mesh (8.5 cm diameter disc) (SEFAR, Australia) overlaying the glass beads in 15 mL of liquid YpSs medium. An agar plug (5 × 5 mm) of freshly grown *M. thermophilus* was placed in the middle of the nylon overlay and incubated for four days at 50 °C in a closed moist box to minimise evaporation. Bacterial cultures were grown overnight with shaking (200 rpm) until mid-exponential phase (OD_{600}: 0.4-0.5). An aliquot (4 ml) of the liquid medium was removed and replaced with the same volume of the bacterial cultures to be tested. The plates were incubated statically at 50 °C in a moist box. Growth of the bacteria in the co-culture was monitored by measuring OD_{600}, until the bacterial culture reached stationary phase. Daily growth of the fungus was monitored by measuring radial growth until the fungus fully colonised the nylon membrane or hyphal growth had ceased.

3.2.8 Visualisation of Mycothermus thermophilus under microscopy

Mycothermus thermophilus hyphae were live-mounted onto glass slides and viewed using light microscopy (1000 times magnification) by using a compound microscope mounted with a camera (Olympus BX51; Olympus, Australia) with the imaging software cellSens (Olympus, Australia). Hyphae were stained with 1% (w/v) Congo Red, which binds strongly to chitin, to determine if *M. composti* had degraded the cell walls of *M. thermophilus* (Matsuoka *et al.*, 1995). Staining and visualisation were done after hyphal growth had ceased and the bacterial growth had reached stationary phase.

3.3 Results

A total of 146 bacterial colonies were isolated from mushroom compost sampled from five yards (Supplementary Table 2). The majority of bacterial colonies were isolated at an elevated temperature (50 °C) to mimic Phase II compost temperatures, but some bacteria were also isolated from mesophilic conditions (30 °C). The selection of an elevated temperature was mainly due to the interest in *Pseudoxanthomonas* as the dominant taxon during the conditioning stage of Phase II composting (Chapter 2).

The most common bacterial colonies observed on plates inoculated with Phase II mushroom compost were circular colonies with entire margins, varying in colour from straw to bright yellow. *Pseudoxanthomonas* strains were anticipated to be present due to their known dominance in the compost sample, and based on the description by Chen *et al.* (2002). Approximately 30% of the bacterial isolates had these characteristics, while the remaining 70% were a mixture of bacterial isolates that were chosen due to their unique morphologies. The aim of isolating as many compost bacteria as possible was to develop a strain collection of compost bacteria that can be used for interaction analyses and potentially to develop bioaugmentation strategies.

In order to distinguish between bacterial isolates, restriction enzyme digests of the 16S rRNA gene region identified that there were at least 32 RFLP groups. At least one, and if possible, two bacterial isolates from each RFLP group were sent for 16S rRNA Sanger sequencing for identification. RFLP analysis determined three different bacterial groups for the *Pseudoxanthomonas* isolates (Supplementary Table 2). A total of 42 bacterial isolates were identified as *Pseudoxanthomonas* from comparison of the RFLP patterns and 16S rRNA gene sequencing. Three strains were chosen as representative strains based on the RFLP pattern and given the following strain designations: EP2452, SP22492 and MP2294. DNA sequencing confirmed that the

three strains were different species of *Pseudoxanthomonas:* EP2452 was *P. taiwanensis* (100% sequence similarity), SP22492 was *P. suwonensis* (100% sequence similarity) and MP2294 was *P. koreensis* (99% sequence similarity). Strains EP2452 and SP22492 both grew at elevated temperatures as these two species are thermophilic, however MP2294 was strictly mesophilic and only grew at 30 °C.

Amongst many cream and white bacterial colonies, two other bacterial colonies which were distinct had orange pigmentation. These isolates were given the strain designations C216 and C2112. Comparative analysis of the 16S rRNA gene sequence showed that the strains were most closely related to *Niabella terrae,* with 93% sequence similarity. These isolates were identified as novel chitin degrading bacteria from the *Chitinophagaceae* family and the classification of strain C216 will be described in Chapter 5.

3.3.1 Fungal diversity of Mycothermus thermophilus in Australia were undifferentiated

All 15 fungal isolates showed similar morphological features to those described by Straatsma & Samson (1993), with white margins on the fungal colony that became pigmented due to sporulation (Figure 3.1A). All 15 fungal isolates showed identical band patterns when amplified with BOXA1R primer, suggesting that there was little to no genetic diversity in the DNA of the fungal isolates. Furthermore, DNA fingerprinting patterns of the fungal isolates were differentiated from *Ophiostoma ips* and *Agaricus bisporus*, which were used as fungal controls (Figure 3.2). The vegetative hyphae of all putative strains of *M. thermophilus* were transparent and chains of conidia were pigmented and round to ellipsoidal in shape (Figure 3.1B). All

fungal isolates were confirmed as being *M. thermophilus* by comparison of the ITS region.

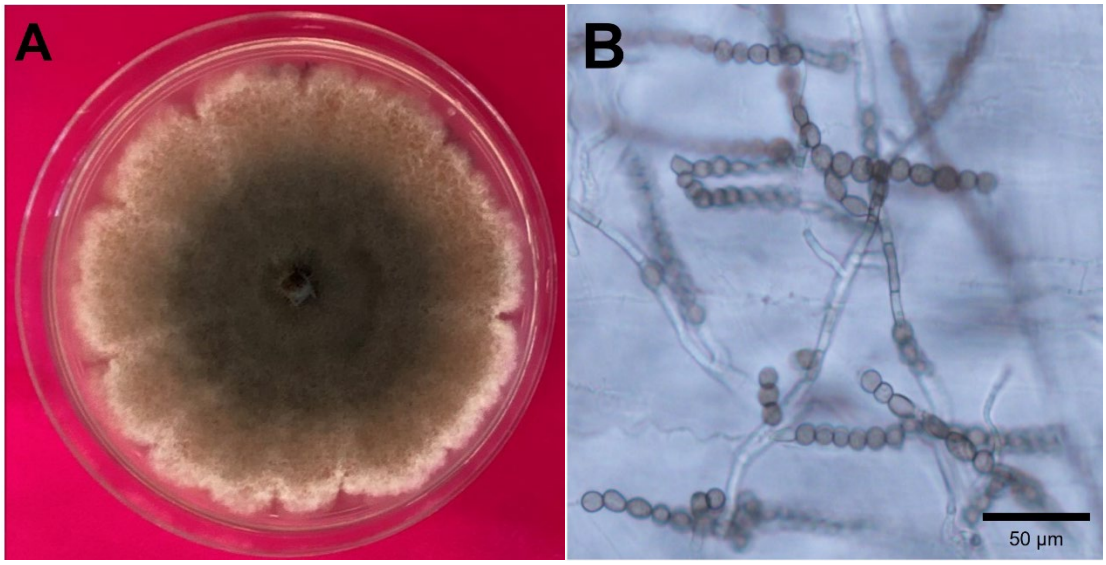

Figure 3.1. Macroscopic and microscopic features of *Mycothermus thermophilus*. (A) *M. thermophilus* grown on YpSs, featuring dark green pigmentation and lobed margins. (B) Light microscopy image of aerial hyphae featuring pigmented conidia in chains.

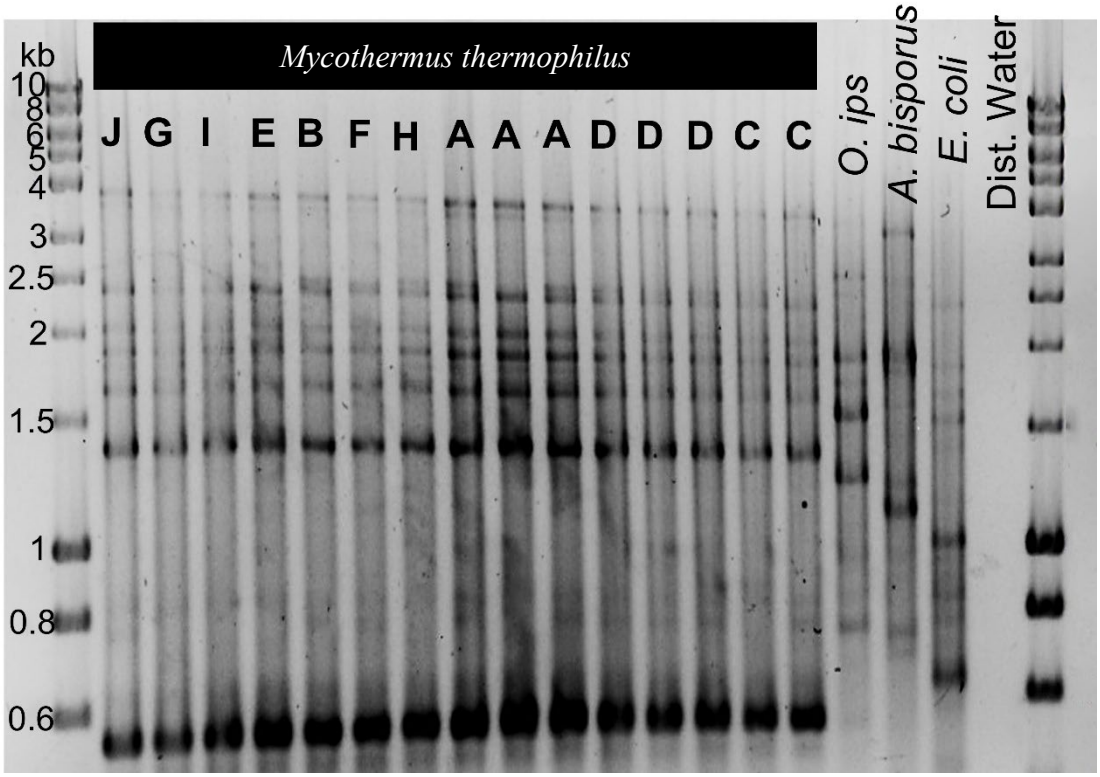

Figure 3.2. Gel image of BOX PCR.
Molecular ladder: 1 kb ladder (Bioline, Australia); *Mycothermus thermophilus* strains isolated from compost yards A - J; *Ophiostoma ips* (*O. ips*), *Agaricus bisporus* (*A. bisporus*) and *Escherichia coli* (*E. coli)* DH5α (fungal and bacterial controls); distilled water (No template control). *O. ips*, *A. bisporus* and *E. coli* were used as controls to ensure specificity of BOXA1R primers to the repetitive BOX sequences in the DNA.

3.3.2 Pseudoxanthomonas spp. impacted the growth of Mycothermus thermophilus

On solid medium, *Mycothermus thermophilus* fully colonised the plate within four days and showed strong sporulation with no curled margins (Figure 3.3A). However, two of three species of *Pseudoxanthomonas*, *P. taiwanensis and P. koreensis*, had relatively little effect on the hyphal growth of *Mycothermus thermophilus* (Figure 3.3B and C), whereas *Pseudoxanthomonas suwonensis* had a greater impact on growth of *M. thermophilus* (Figure 3.3D).

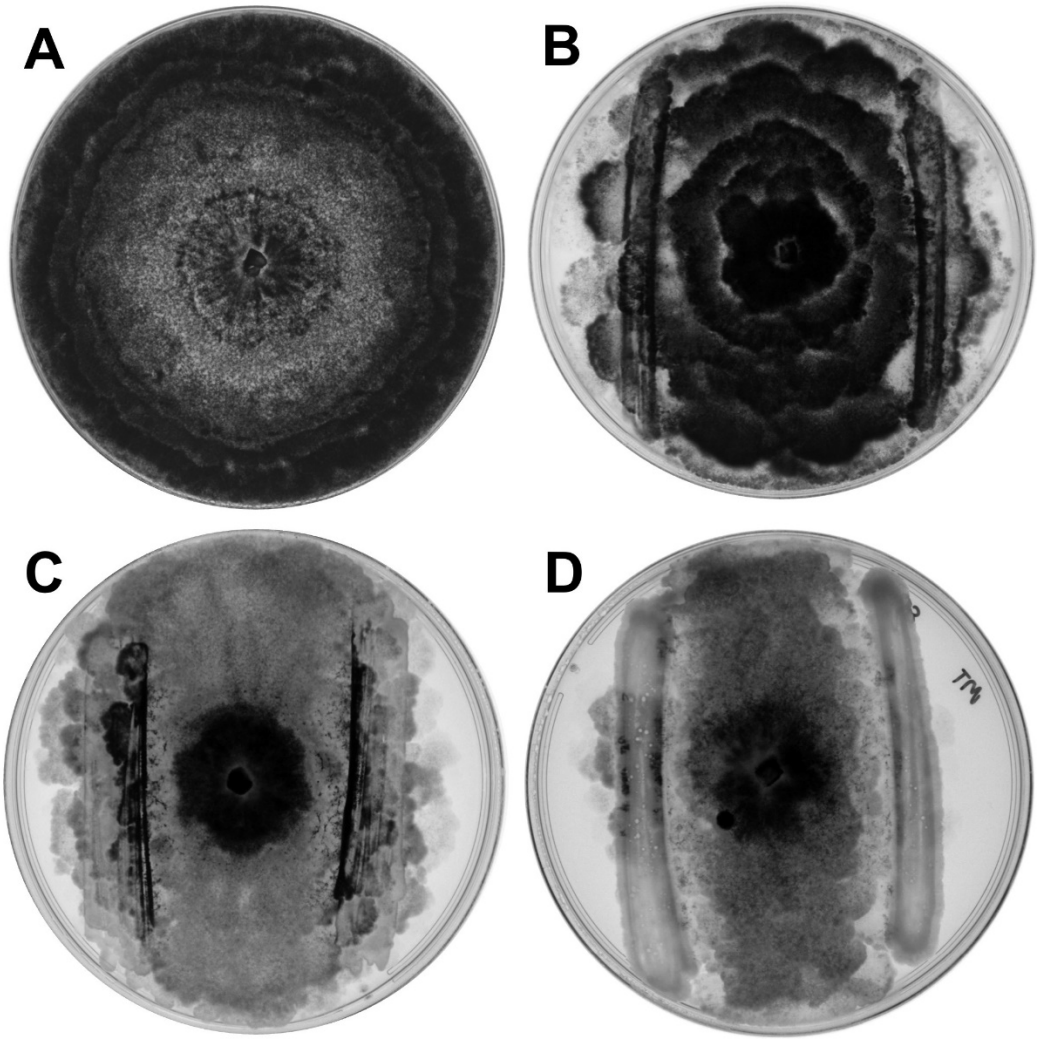

Figure 3.3. Interaction of *Mycothermus thermophilus* with *Pseudoxanthomonas* spp.
(A) *Mycothermus thermophilus* control, (B) *M. thermophilus* with *Pseudoxanthomonas taiwanensis*, (C) *M. thermophilus* with *P. koreensis*, and (D) *M. thermophilus* with *P. suwonensis*. Bacteria (vertical streaks) were grown to stationary phase before inoculation with *M. thermophilus*. The fungus was inoculated equidistant between the bacterial streaks.

Pseudoxanthomonas taiwanensis had the smallest impact on the growth of *M. thermophilus*. In the presence of *P. taiwanensis*, *M. thermophilus* showed a curled margin (Figure 3.3B), however the fungus was able to sporulate and the growth characteristics of *M. thermophilus* were similar to the control (Figure 3.3A). In the presence of *P. koreensis, M. thermophilus* did not have the same curled margin, and instead formed an undulate or lobate margin (Figure 3.3C). Furthermore, sporulation

only occurred around the initial inoculation point (~2 cm diameter) of *M. thermophilus,* and hyphal growth was more restricted in the presence of *P. koreensis* but was still able to grow over the bacteria (Figure 3.3C). In the presence of *P. suwonesis*, *M thermophilus* showed similar margin and sporulation morphology as with *P. koreensis* (Figure 3.3D). However, *M. thermophilus* hyphae were not able to grow over the bacteria and there was a clear inhibition zone between the hyphae and the bacterial streaks (Figure 3.3D).

3.3.3 Mycothermus thermophilus stimulates the growth of Pseudoxanthomonas spp.

Glass bead plates were used to establish the interactions of *Pseudoxanthomonas* spp. with *M. thermophilus*, *in vitro*. This method allowed the bacterial to display optimal growth in the liquid medium, and the fungus to display optimal growth on a surface while still allowing direct interactions between the two during the growth phase of both organisms. After inoculation of the liquid medium with bacteria, *Pseudoxanthomonas taiwanensis* had the smallest impact on the radial growth of the fungus (Figure 3.4). *M. thermophilus* continued growing after the liquid medium was inoculated with *P. taiwanensis*, however radial growth started to slow after 4 days. In comparison, radial growth of the *M. thermophilus* slowed soon after the liquid medium was inoculated with *P. suwonensis* or *P. koreensis* (Figure 3.4).

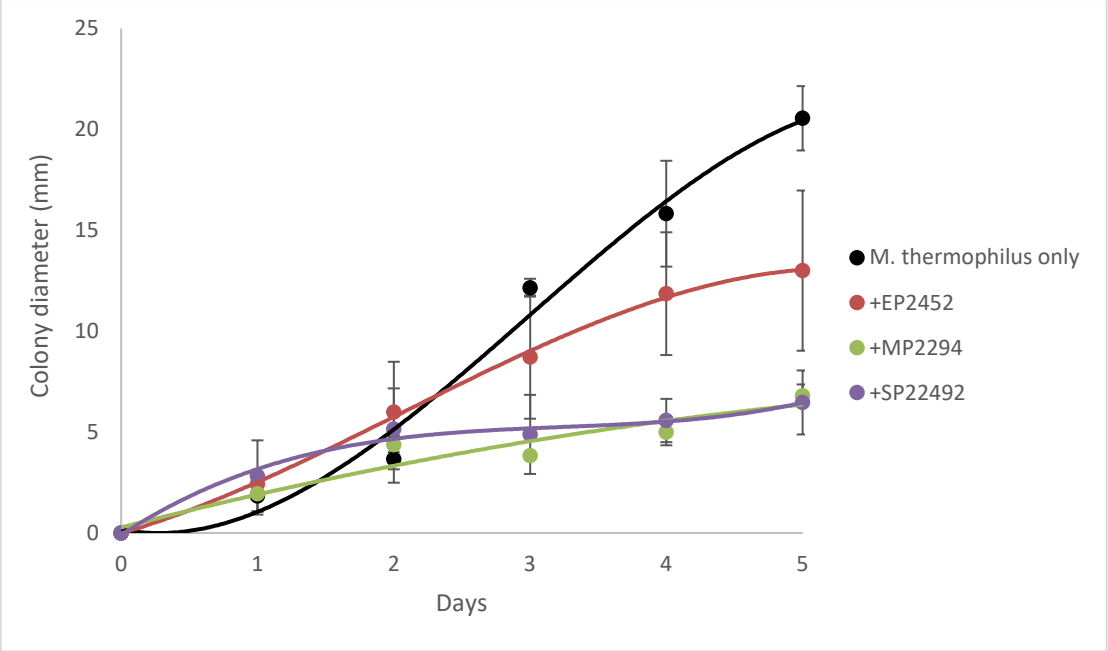

Figure 3.4. Colony diameter of *Mycothermus thermophilus* after inoculation with *Pseudoxanthomonas* spp.
Error bars represent standard error of the mean (n = 3). '*M. thermophilus* only' was used as a control where radial growth was not influenced by bacterial cells. EP2452 – *P. taiwanensis*, MP2294 – *P. koreensis*, SP22492 – *P. suwonensis*.

M. thermophilus promoted the growth of bacteria when grown in co-culture with the fungus (Figure 3.5). Optical density was used as a proxy to measure the bacterial growth. In the absence of *M. thermophilus*, bacterial growth was almost negligible, the greatest optical density reached by *P. suwonensis* strain SP22492 was 0.156 after 5 d of growth and the slowest growth was from *P. taiwanensis*, with the maximum optical density reaching 0.045 after 5 d growth. The growth of these bacteria without the influence of *M. thermophilus* was much slower than anticipated, considering that there are no other competing organisms in the medium (Figure 3.5A). In the presence of *M. thermophilus*, *P. suwonensis* and *P. taiwanensis* reached a maximum optical density of 0.468 and 0.406, respectively (Figure 3.5B).

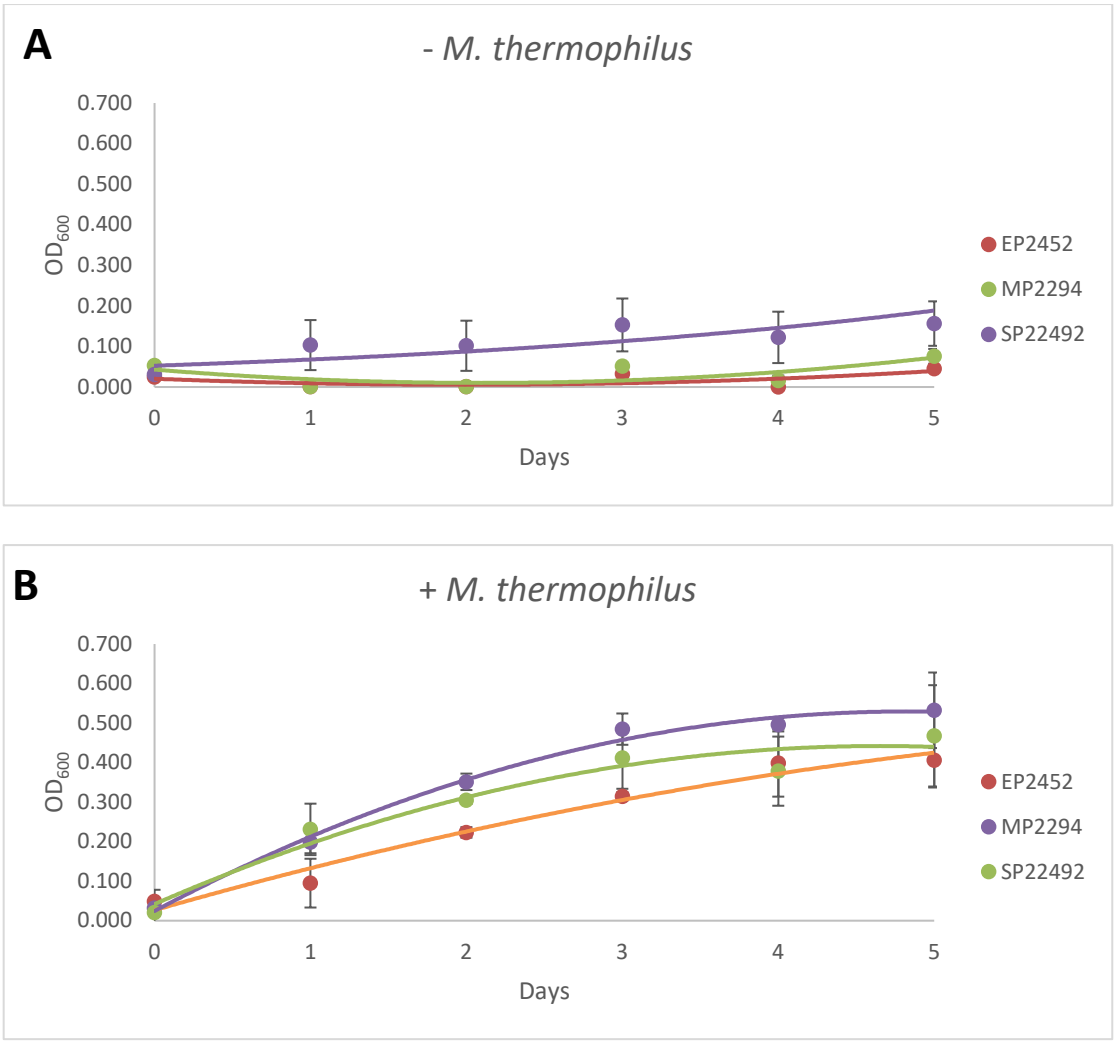

Figure 3.5. Growth of *Pseudoxanthomonas* spp. in co-culture with or without *Mycothermus thermophilus*.
Error bars represent standard error of the mean (n = 3). (A) Growth of *Pseudoxanthomonas* spp. without *M. thermophilus*. (B) Growth of *Pseudoxanthomonas* spp. with *M. thermophilus*. EP2452 – *P. taiwanensis*, MP2294 – *P. koreensis* and SP22492 – *P. suwonensis*.

3.3.4 Mycovorax composti *degrades* Mycothermus thermophilus

The interaction of *M. thermophilus* and the novel chitin degrading bacterium described in Chapter 5, *Mycovorax composti* C216, was tested to determine how the strain's measured chitin-degrading phenotype would affect growth of the fungus. On solid medium, *M. thermophilus* had the curled margin that was found previously with *P. taiwanensis*, however it was much more pronounced in the presence of *M.*

composti. *M. composti* showed strong inhibition of hyphal growth and sporulation of *M. thermophilus* (Figure 3.6).

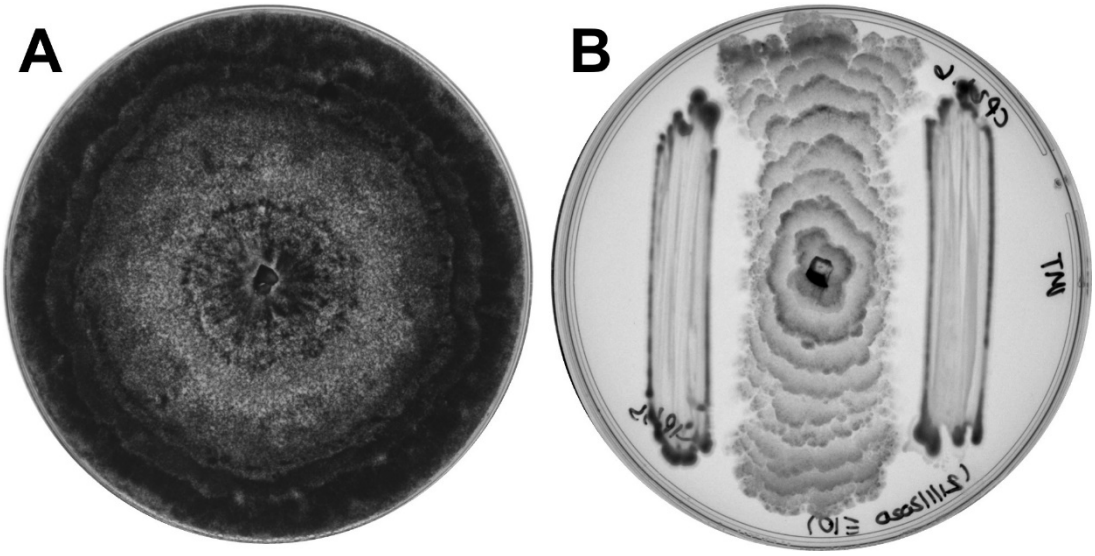

Figure 3.6. Interaction of *Mycothermus thermophilus* with *Mycovorax composti*. (A) M. thermophilus control, (B) M. thermophilus in the presence of Mycovorax composti.

In liquid medium, hyphal growth of the fungus continued for 24 hours after inoculation with the bacterial strain, but then stopped (Figure 3.7). In comparison, in the absence of *M. composti*, *M. thermophilus* continued to grow over the 5-day period (Figure 3.7).

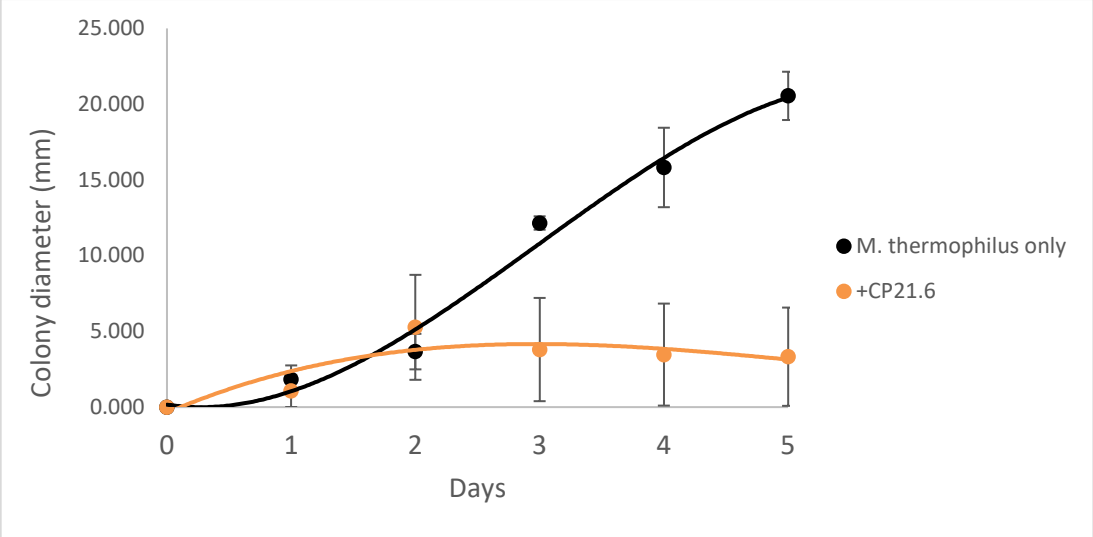

Figure 3.7. Colony diameter of *Mycothermus thermophilus* with (black) and without (orange) *Mycovorax composti*.
Error bars represent standard error of the mean (n = 3).

In the presence of *M. thermophilus*, growth of *M. composti* was faster, and reached a maximum OD_{600} of 0.177 after 5 days (Figure 3.8). The growth of *M. composti* without the influence of *M. thermophilus* was still relatively fast (OD_{600} = 0.119). Overall, growth of *M. composti* was slower than *Pseudoxanthomonas*. The increase in growth of the bacterium in the presence of *M. thermophilus*, and the degradation of *M. thermophilus* in the presence of *M. composti* suggests that the bacterium was exploiting *M. thermophilus* for additional nutrients.

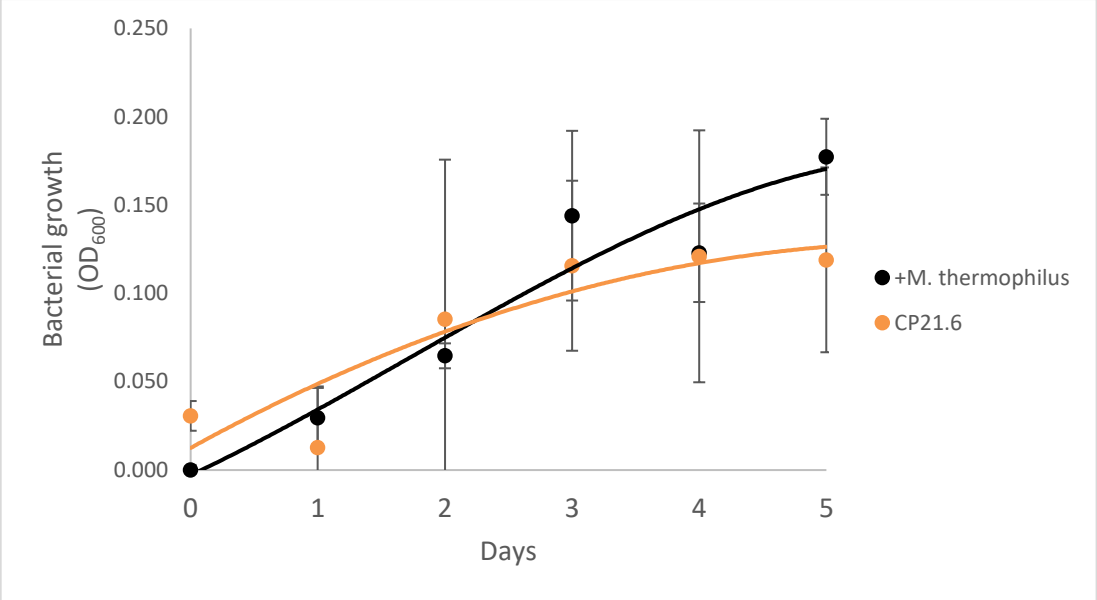

Figure 3.8. Growth of *Mycovorax composti* in the presence of *Mycothermus thermophilus* (black) and without *M. thermophilus* (orange).
Error bars represent standard error of the mean (n= 3).

After the 5-day growth period, hyphae from *M. thermophilus* were stained with Congo Red to determine if *M. composti* had any effect on fungal cells. In the absence of *M. composti*, hyphal cell walls remained intact indicated by the stain strongly binding to the cell walls (Figure 3.9A). In the presence of *M. composti*, particularly where hyphae were in direct contact with the bacteria, the fungal cell walls appeared to be deteriorated, and the stain did not bind as strongly to the hyphal fragments (Figure 3.9B). Degraded hyphae were only observed when the bacterium was directly associated with *M. thermophilus* below the nylon membrane in the liquid medium. Mature hyphae above the nylon overlay had the same macroscopic features as the uninoculated control.

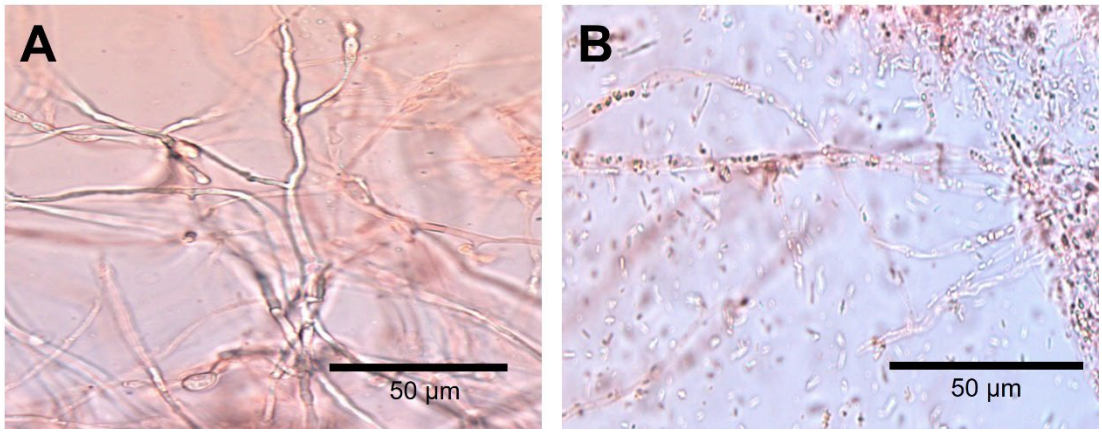

Figure 3.9. Light microscopy images of *Mycothermus thermophilus*.
(A) Healthy *Mycothermus thermophilus* hyphae. (B) Mycothermus thermophilus hyphae after incubation with *Mycovorax composti*. Stained with 1% (w/v) CongoRed.

3.4 Discussion

In this study, 15 strains of *Mycothermus thermophilus* were isolated from 10 compost yards across Australia. DNA fingerprinting showed that all strains were clonal. The interaction of *Pseudoxanthomonas taiwanensis* (identified as the dominant bacterial taxon during Phase II in Chapter 2) with *M. thermophilus* showed that the bacterium did not inhibit the growth of the fungus when grown in co-culture. In comparison, *M. thermophilus* stimulated the growth of *P. taiwanensis* and other *Pseudoxanthomonas* species (Figure 3.5). When *Mycovorax composti*, a novel chitin degrading bacterium, was grown in co-culture with *M. thermophilus*, growth of the bacteria was stimulated and fungal hyphae was degraded over time (Figure 3.7 and Figure 3.8).

Strains of *M. thermophilus* isolated from mushroom compost have been shown to have different patterns of growth and development (Straatsma & Samson, 1993). However, cultures of *M. thermophilus* are commonly described as white, quickly turning dark green or grey to black (Straatsma & Samson, 1993, Natvig *et al.*, 2015). Young cultures of *M. thermophilus* have lobed margins which became even when matured and continuous subculturing caused the pigmentation to become lighter (Straatsma & Samson, 1993). Microscopically, there are two types of *M. thermophilus* growth identified by Straatsma & Samson (1993). Type 1 was characterised as having single darkly pigmented spores on short lateral hyphal branches, and Type 2 had intercalary chained spores that were lightly pigmented (Straatsma & Samson, 1993). However, in this study, only type 2 *M. thermophilus* spores were isolated from all compost yards. *Agaricus bisporus* has been known to colonise compost with both types of *M. thermophilus*, but shows higher affinity for compost with spore type 2 (Lyons *et al.*, 2000).

DNA fingerprinting of isolated strains of *M. thermophilus* by BOX-PCR showed the same fingerprinting pattern for all strains (Figure 3.2), which suggests that, despite the geographical distances between yards, all *M. thermophilus* strains were clonal. A similar pattern was found in mushroom composts produced in Iran, where there was no genetic diversity in 39 strains *of M. thermophilus* isolated from compost yards in nine different provinces (Mehrparvar *et al.*, 2017), however, the authors used random amplified polymorphic DNA (RAPD) fingerprinting technique to distinguish differences among strains (Mehrparvar *et al.*, 2017), a method which is similar and comparable to BOX-PCR (De León *et al.*, 2009, Jarocki *et al.*, 2016). It would be interesting to determine whether there are clonal differences between the *M. thermophilus* strains isolated from Australian mushroom compost and *M. thermophilus* strains isolated in Iran , USA, and Europe.

One logistical reason for the lack of genetic variation in strains of *M. thermophilus* in Australian mushroom compost may be that feedstocks come from similar or the same suppliers, as most of the compost yards sampled were along the eastern and south-eastern coast of Australia. Regardless, the lack of a teleomorph for *M. thermophilus* means there is potentially no genetic variation from sexual reproduction (Straatsma & Samson, 1993).

M. thermophilus had growth stimulating effects on all *Pseudoxanthomonas* species examined in this study (Figure 3.5B). In comparison, growth of *M. thermophilus* was mostly indifferent to *P. taiwanensis* but showed restricted growth in the presence of *P. suwonensis* and *P. koreensis* (Figure 3.3 and Figure 3.5A). *P. taiwanensis* has been known to aid other bacteria when grown in co-culture, through degrading inhibitory compounds or buffering pH (Kato *et al.*, 2004, Kato *et al.*, 2005, Wang *et al.*, 2011, Du *et al.*, 2015). In bio-ethanol production, optimising the population of *P.*

taiwanensis in proportion to the other bacteria proved to be the most efficient solution for efficient lignocellulose degradation (Du *et al.*, 2015). In another consortium, *P. taiwanensis* enhanced degradation of lignocellulose by stabilising pH and utilising substrates derived from breakdown of cellulose (Kato *et al.*, 2005). However, these studies focused on anoxic environments where cellulose degradation was done by *Chlostridium* spp (Kato *et al.*, 2005, Du *et al.*, 2015). Thus, if *Pseudoxanthomonas* plays a similar role in composting, which is aerobic, the mechanisms are likely to be different. Slowing of growth of *M. thermophilus* in the presence of *P. taiwanensis* is most likely due to nutrients becoming limiting during the growth period (Figure 3.4). Likewise, with *P. koreensis* and *P. suwonensis*, both species of bacteria grew faster than *P. taiwanensis* in co-culture (Figure 3.5) and were most likely more efficient at nutrient uptake than *M. thermophilus*. Growth of *P. koreensis* in the presence of *M. thermophilus* at 50 °C was unexpected as this species of bacteria is strictly mesophilic when grown in pure culture (Yang *et al.*, 2005), suggesting that this *P. koreensis* isolate may not be the one described by Yang *et al.* (2005).

In compost, *M. thermophilus* can breakdown cellulose from the wheat straw into more accessible compounds with the wide range of CAZymes it can express (Basotra *et al.*, 2016). The interaction between *P. taiwanensis* and *M. thermophilus* is most likely similar to that of the consortia described above. For this partnership, *P. taiwanensis* utilises substrates derived from cellulose breakdown by *M. thermophilus* and removes inhibitory compounds that would otherwise accumulate, such as acetate and cellobiose (Kato *et al.*, 2005, Du *et al.*, 2015). Inhibitory compounds such as acetate may not be an issue during mushroom composting as it is a result of anaerobic fermentation. With regards to *P. suwonensis*, the most probable reason for its antagonistic behaviour towards *M. thermophilus* is competition. *Pseudoxanthomonas*

suwonensis is also capable of degrading cellulose (Kumar *et al.*, 2015) and does have cellulose degrading genes (Hou *et al.*, 2015), however is not as effective as *M. thermophilus* (Basotra *et al.*, 2016). In this study, none of the *P. suwonensis* isolates showed cellulase activity (Supplementary Table 2). In the case of *P. koreensis* and *M. thermophilus*, inhibitory compounds that affect the growth of the fungus may have accumulated as *P. koreensis* cannot produce β-glucosidase (Yang *et al.*, 2005). Furthermore, this species is a strict mesophile (Yang *et al.*, 2005) and therefore growth at the elevated temperature was unexpected.

Growth of *M. thermophilus* in the presence of the novel chitin degrading bacterium, *Mycovorax composti*, was stunted and fungal hyphae was degraded over time (Figure 3.7). This suggests that *M. composti* is breaking down the chitin in the fungal cell wall. Whole genome sequencing and *in silico* annotation of the novel bacterial isolate indicated that *M. composti* did have chitinase genes and is proposed that this bacterium can express chitinase activity (Supplementary Table 2 and Chapter 5). Genome annotation predicted that *M. composti* has putative *nagA* and *nagZ* genes, which are responsible for breaking down chitobiose or larger chitin oligosaccharides into N-acetyl-D-glucosamine. However, there were no chitinase genes predicted from the genome (Chapter 5).

Bacterial-fungal associations were identified using a complex liquid medium. When nutrients became limiting, *M. composti* showed signs of anti-fungal activity, however, degradation of the fungal hyphae was only evident where the bacterium was directly in contact with the fungus. Chitinolytic bacteria often express anti-fungal activity when nutrients are limited and have been known to colonise living hyphae as the substrate, rather than just chitin (de Boer *et al.*, 1998). However, anti-fungal effects have not been associated with increased chitinase activity (de Boer *et al.*, 1998).

Furthermore, colonisation of the fungal hyphal tips is most likely due to young hyphae being more susceptible than mature hyphae, as no or little anti-fungal effect has been found with mature hyphae (Ordentlich *et al.*, 1988). A similar result was found in this study, where hyphal degradation was only observed where the bacterium had direct contact with the fungus (Figure 3.9). Hyphae of *M. thermophilus* that were above the nylon overlay and out of contact with the bacterium showed the same macroscopic features when there was no bacterial influence. This suggests that the *M. composti* was not able to use *M. thermophilus* as a fungal "highway" to colonise the hyphae above the nylon overlay.

The degradation of *M. thermophilus* hyphae by *M. composti* potentially provides nutrients for *A. bisporus* by hydrolysing the chitin in cell walls of *M. thermophilus*. Chitin, an essential element for the production of mushrooms, contains approximately 6-7% nitrogen (Hamid *et al.*, 2013). In Phase III mushroom compost that was not inoculated with *A. bisporus*, the living fungal biomass decreased by 50% and chitin content in compost did not significantly increase (Vos *et al.*, 2017), suggesting that some mycophagy of *M. thermophilus* most likely occurred. The increase in chitin content may also be due to the fungi in the compost dying (Vos *et al.*, 2017).

Further investigation of microbial interactions among *Pseudoxanthomonas* spp., *M. composti* and *M. thermophilus* is highly recommended and should also be extended to include *A. bisporus*. These interactions need to be (i) tested *in vitro* in minimal media with cellulose as the sole carbon source, and (ii) in a compost matrix to determine if there is a similar response within the compost biome. Investigation of other potential bacterial associations with *M. thermophilus* is also recommended, particularly with compost bacteria that are fungiphiles of *M. thermophilus*. Alternatively, *A. bisporus* is known to consume the microbiota during colonization of the selective substrate

(Fermor *et al.*, 1991, Vos *et al.*, 2017). Therefore, in the presence of *A. bisporus*, *P. taiwanensis* and *M. composti* could potentially be consumed by *A. bisporus* itself.

3.5 Conclusion

Genetic diversity in *M. thermophilus* strains isolated from mushroom compost from several spatially dispersed compost yards in Australia were clonal. *In vitro* studies of the interaction between *M. thermophilus* and compost bacteria showed that the fungus promoted the growth of all bacteria tested. *P. taiwanensis* was the only bacterium that did not have an adverse effect on the mycelial growth of *M. thermophilus*. The novel chitin degrading bacterium, *M. composti*, had an extreme adverse effect on the growth of *M. thermophilus*, where colony diameter of *M. thermophilus* decreased over time. This interaction may be providing nutrients for growth of *A. bisporus* by degrading the chitin in the cell walls of *M. thermophilus* and accelerating hyphal lysis, but further investigations are needed to confirm this.

The ins and outs of nitrogen in mushroom composting

Abstract

Button mushrooms are rich in protein content and are often promoted for consumption using this characteristic. Protein content in mushrooms is correlated to the nitrogen content in the substrate that it is grown on. Much of the nitrogen in mushroom compost comes from poultry manure, however, additional feedstocks are often needed and are an additional expense for composters. *Pseudoxanthomonas taiwanensis* is a heterotrophic nitrifier that produces N_2O in thermophilic, aerobic environments. During Phase II, *P. taiwanensis* is the dominant bacterial species in mushroom compost and nitrogen losses during Phase II are most likely as N_2O. Mapping the nitrogen losses during mushroom compost may be the key to minimising nitrogen losses. In this study, N_2O and NH_3 gas was sampled in a conventional Phase II tunnel at a commercial compost yard. It was found that N_2O and NH_3 concentrations in the tunnel airspace decreased during Phase II but, overall, the total loss of nitrogen as N_2O and NH_3 gas was very small. As such, despite the dominance of *P. taiwanensis* during Phase II, this species is most likely not producing N_2O during Phase II.

4.1 Introduction

Button mushrooms are highly nutritious and have been called the 'meat' of vegetarians due to their protein content. The protein content of white button mushrooms is approximately 2.89 g per 100 g (fresh weight) (U.S. Department of Agriculture, 2021) or 14-30% (dry weight) crude protein (Chang & Miles, 2004, Reis *et al.*, 2012). Button mushrooms also have levels of amino acids which are comparable to milk, with glutamic acid, aspartic acid and tyrosine being the most abundant (Mattila *et al.*, 2002, Chang & Miles, 2004). The nutritional content of the mushroom is strongly related to the substrate on which it is grown (Wood & Fermor, 1982).

Button mushrooms are grown on a composted substrate made from wheat straw, poultry manure and gypsum. The composting process has been outlined in Chapter 2. Nitrogen inputs and losses to the composting process are outlined in Figure 4.1.

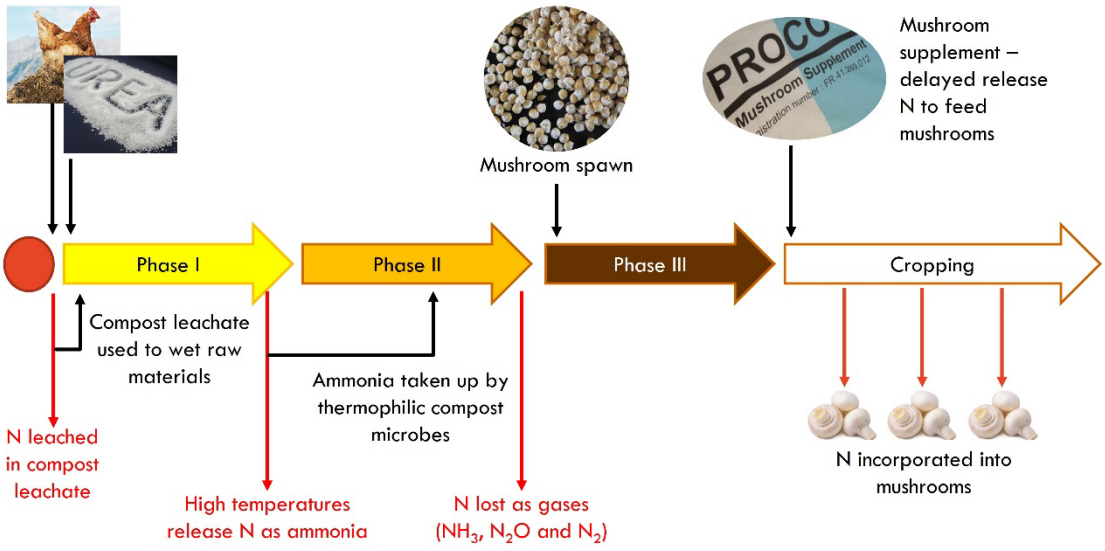

Figure 4.1. Inputs and outputs of nitrogen during mushroom composting. Inputs of nitrogen (black arrows) come from poultry manure, additional nitrogen supplements, compost leachate and ammonia assimilation. Compost leachate, ammonia gas, nitrous oxide gas and harvested mushrooms are known outputs of N losses (red arrows). A significant proportion of added nitrogen is also found in spent mushroom compost and casing (not shown).

Poultry manure is the primary nitrogen source in Australian mushroom compost due to its accessibility and relatively cheap cost (Stoller, 1943, Beyer, 2003). Other countries use stable bedding or synthetic nitrogen as their primary nitrogen source (Noble & Gaze, 1996, Cao *et al.*, 2019). Some composters also use additional feedstocks that are high in nitrogen at the start of their composting process to reach C/N ratio requirements (25-35) (Beyer, 2003, van der Wurff *et al.*, 2016). Additional nitrogen can be provided using organic sources such as cottonseed meal or soybean meal (Beyer, 2003, Carrasco *et al.*, 2018), or inorganic nitrogen sources such as ammonium nitrate, ammonium sulphate or urea (Noble *et al.*, 2002, Demirer *et al.*, 2005). Commercial supplements are also used during cropping and are designed to delay the release of nitrogen to maximise nitrogen availability for the mushrooms (Carrasco *et al.*, 2018).

The composting process is a microbially intense process in which nutrients from the raw materials are broken down and transformed into biomass (Derikx *et al.*, 1990, Vos *et al.*, 2017). Phase I compost is typically characterised by a strong ammonia (NH_3) odour due to proteolysis (Miller *et al.*, 1991, Jurak *et al.*, 2015). Ammonia is toxic to *Agaricus* mycelium and must be removed before spawning. During the conditioning step in Phase II, the microbial community assimilates free NH_3 into biomass (Straatsma & Samson, 1993, Vos *et al.*, 2017, Vieira & Pecchia, 2018). A key bacterial taxon, *Pseudoxanthomonas taiwanensis*, has been identified as the dominant bacterium in Phase II compost in this study (Chapter 2) and in other compost studies (Székely *et al.*, 2009, Pandin *et al.*, 2018, Cao *et al.*, 2019). This species is a heterotrophic nitrifier (Chen *et al.*, 2002) and, in mushroom compost, it is dominant during the conditioning step of Phase II and throughout Phase III (Kertesz *et al.*, 2015).

Nitrous oxide (N_2O) is produced via three metabolic pathways, nitrification, denitrification, and heterotrophic nitrification, and are outlined in Figure 4.2. Heterotrophic nitrifiers can transform organic or inorganic forms of nitrogen into nitrate (NO_3^-) and N_2O (de Boer & Kowalchuk, 2001, Zhang *et al.*, 2015). They can use oxygen as an electron acceptor, or nitrate/organic nitrogen as an electron donor (Zhang *et al.*, 2015). Here, N_2O is produced from the oxidation of hydroxylamine by heterotrophic nitrification (Figure 4.2).

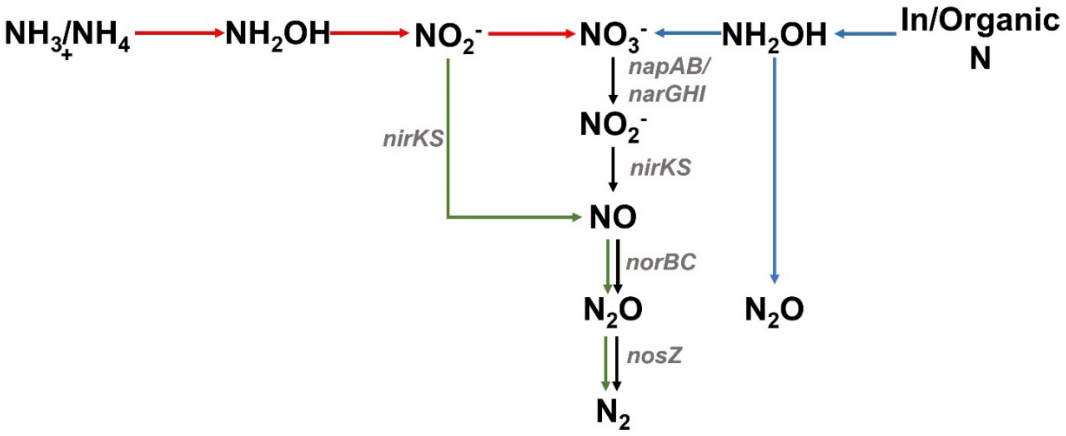

Figure 4.2. Nitrogen transformations adapted from Zhang et al. (2015). Arrows indicate the transformation of nitrogen: autotrophic nitrification (red), heterotrophic nitrification pathway (blue), nitrifier denitrification pathway (green), the denitrification pathway (black) and nitrogen cycling functional genes involved in denitrification (grey).

The more conventional pathway for N_2O production is the denitrification pathway, which has been well characterised and is known to use succession of enzymes that are encoded by a series of nitrogen transforming functional genes (Baggs, 2011, Zhang *et al.*, 2015) (Figure 4.2). In this pathway, N_2O is reduced from nitrite (NO_2^-) or NO_3^- by ammonia-oxidising bacteria (nitrifier-denitrification) or other heterotrophic organisms (denitrification) and is conventionally done under anaerobic environments (Baggs, 2011). Under aerobic conditions, it is possible for nitrite/nitrate reduction to occur, but this is likely when bacteria are exposed to oxygen after an anaerobic phase (Baggs,

2011). Under these conditions, all denitrification enzymes are active except N_2O reductase, which may result in an accumulation of N_2O in aerobic systems (Morley *et al.*, 2008, Baggs, 2011).

In this study total nitrogen was measured in Phase I and Phase II compost samples from several commercial compost yards in Australia to develop a mass balance equation for modelling nitrogen inputs and outputs. Furthermore, N_2O and NH_3 gas from Phase II was monitored daily at an industrial scale compost yard to determine nitrogen loss as gas during Phase II.

4.2 Materials and methods

4.2.1 Compost sampling

Samples were obtained from one compost yard in Queensland, two compost yards in New South Wales, three compost yards in Victoria and two compost yards in South Australia for end-Phase I and end-Phase II compost. For each yard, a single, unpooled sample was taken from the face of the bulk compost pile (approximately 500 g) and transported to The University of Sydney, Camperdown, unrefrigerated. Compost samples were stored at -80 °C until needed for further analysis.

Compost was randomly subsampled at 10 different points before freeze drying (Christ Alpha 1-2 LDplus freeze dryer). Freeze dried compost was ground in a mortar and pestle and stored at -80 °C for downstream analysis.

4.2.2 Total nitrogen

Freeze-dried compost (50 µg) taken from Phase I and Phase II was used for total nitrogen analysis. Total nitrogen was determined by combustion in an Elementar Vario MACRO cube CHNS analyser. The standard used was phenylalanine (30 mg) (SigmaAldrich, Australia) and no sample was used for the blanks. All standard, blanks and samples were run in triplicate.

4.2.3 N_2O/NH_3 sampling

For analysis of N_2O, air was sampled from the return air ducting of a conventional Phase II tunnel at a commercial compost yard near Windsor, NSW. Approximately 800 mL of gas was pulled from sampling vent in the return air ducting using a battery-operated handheld vacuum pump at a rate of one L min^{-1}. The gas was cooled using a 500 mL Dreschel flask in an ice bath and stored in a dual-valve one litre aluminium/polyethylene lined gas sampling bag (HedeTech, China) (Figure 4.3). The

whole system was flushed with Phase II tunnel gas for 5 min before sampling (i.e., about 5 L). Samples were taken in triplicate with 15-min intervals between each sample.

Ammonia was quantified using the Dräger Accuro® handheld pump fitted with Dräger tubes capable of detecting levels of NH_3 between 2.5-1500 ppm (Dräger, Germany). Ammonia gas was sampled from the same sampling point as N_2O. Approximately 500 mL (5 full pumps) of air was sampled per day. If NH_3 was present at the time of sampling, the resulting colour change in the Dräger tube was estimated using the calibrated concentration marked on the tube.

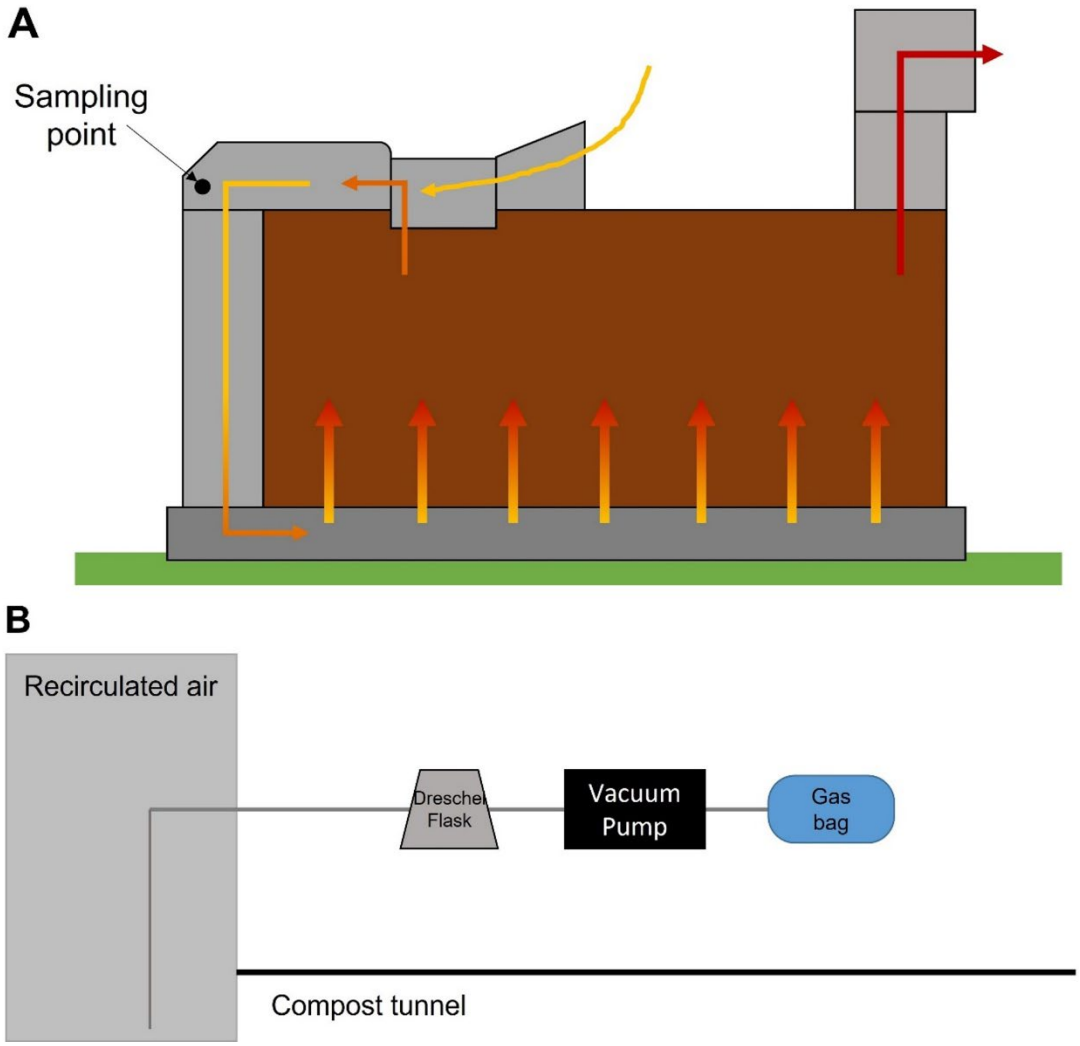

Figure 4.3. Tunnel and gas sampling schematic of Phase II.
A) Schematic of air recirculation in a conventional Phase II tunnel. Yellow arrows indicate fresh air being introduced into the tunnel, red arrows indicate air leaving the tunnel and orange arrows indicate recirculated air. B) Schematic of how gas was sampled from the return air ducting in a Phase II tunnel. Gas was pulled from the tunnel with a vacuum pump, cooled in a 500 mL Dreschel flask in an ice bath before being stored in a 1 L gas bag.

4.2.4 N_2O analysis

Quantification of N_2O in the gas taken from the tunnel was done by gas chromatography. Gas samples (250 µL) were injected with a 1:1 injection split and 30 °C inlet temperature, onto a 100% divinylbenzene Rt-Q-Bond capillary gas chromatography (GC) column (30 m × 0.53 mm, 20 µm film thickness; Restek

Corporation, Bellefonte, PA, USA) coupled to an electron capture detector (ECD; Model 7890A GC; Agilent). Ultra-high purity helium (BOC Ltd, Sydney, NSW) was used as the carrier gas at a flow rate of 3.2 mL min^{-1} and ultra-high purity nitrogen used as the make-up gas for the ECD. The GC oven was held isothermally at 30 °C for 7 min. The GC-ECD system was calibrated using high purity N_2O diluted with ultra-high purity N_2 (BOC Ltd).

4.2.5 N_2O mass balance modelling

A mass balance model for compost tunnel N_2O concentration was adapted from a general mass balance equation developed by Yalçın *et al.* (2015), using their general mass balance equation:

$$\underset{(t+\Delta t)}{mass\ at\ time} - \underset{(t)}{mass\ at\ time} = \underset{from\ t\ to\ \Delta t}{entered\ mass} + \underset{between\ t\ and\ \Delta t}{generated\ mass} - \underset{from\ t\ to\ \Delta t}{exited\ mass}$$

To do this, the following equations were used:

For a single tunnel, the pollutant mass equation was:

$$V_i \frac{dC_i}{dt} = S_i - L_i C_i \qquad (1)$$

Where C_i was the concentration of N_2O in the tunnel i (mg m^{-3}), t is time (day, d) V_i is the volume of the tunnel (m^3), S_i is the summed generation rate of N_2O from the compost in the tunnel (mg d^{-1} m^{-3}) and L_i is the summed rate of N_2O losses from the system (d^{-1}). S_i was calculated from the following equation:

$$S_i = \frac{1}{V_i}(C_{OA} P Q_{OA} + C_{RA} Q_{RA} + G_i) \qquad (2)$$

Where C_{OA} is the concentration of ambient outdoor N_2O at the site (mg m^{-3}), P is the proportion of N_2O that entered the tunnel from outside the system, Q_{OA} is the volumetric air flow rate that from outside to inside (m^3 d^{-1}), C_{RA} is the concentration

of N_2O in the recirculated air (mg m^{-3}), Q_{RA} is the volumetric air flow rate of the recirculated air (m^3 d^{-1}) and G_i is the generation rate of N_2O in the tunnel.

$$Q_T = Q_{OA} + Q_{RA} \quad (3)$$

The total volumetric flow rate (Q_T, m^3 d^{-1}) is the sum of Q_{OA} and Q_{RA}, which is determined from the proportion of outside air that entered the system and varied daily. L_i is calculated from the following equation:

$$L_i = \frac{1}{V_i}(Q_{OA} + Q_{RA}) \quad (4)$$

Finally, equations (2) and (4) were substituted into equation (1) and the following equation was used to calculate summed generation rate of N_2O (S_i) from the compost:

$$C_i = C_O e^{-L_i t} + \frac{S_1}{L_i}(1 - e^{-L_i t}) \quad (5)$$

C_O is the initial concentration of N_2O in the tunnel (mg m^{-3}). In order to calculate S_i, G_i was calculated using the Solver function in Microsoft Excel by changing G_i until the solution of Equation (5) (C_i) was equal to the measured concentration of N_2O in the tunnel.

4.3 Results

Total nitrogen of end-Phase I and end-Phase II compost was measured across multiple compost yards to calculate N losses during Phase II. In a previous chapter (Chapter 2), *P.taiwanensis* was found to be the dominant taxon in end-Phase II compost. N_2O and NH_3 gas were measured over the duration of Phase II to determine whether the high abundance of *P. taiwanensis* leads to an increase in N_2O production during Phase II. The Phase II process was mostly consistent in Australian compost yards (Chapter 2, Table 2.1).

4.3.1 Nitrogen inputs and nitrogen losses are variable in compost yards

All compost yards used poultry manure as their primary nitrogen source (75-85% N). Additional nitrogen-containing feedstocks and timing of nitrogen addition was extremely variable among yards. Compost yards B, D and F did not add any additional nitrogen into their initial mixture (Table 4.1). Compost yards A, H, I and J used a combination of synthetic and organic nitrogen in addition to poultry manure to reach C/N requirements (Table 4.1). Compost yard C added synthetic nitrogen in addition to poultry manure and Compost yard G used organic nitrogen in addition to poultry manure (Table 4.1). Timing of nitrogen addition also varied across multiple compost yards, with some yards adding all of their nitrogen components at bale break, before Phase I commenced (Yards B, C, F and I) and some yards adding nitrogen feedstocks throughout the Phase I process (Yards A, D and G). Only one yard added nitrogen feedstocks during straw wetting (Yard J) (Table 4.1). Compost yards C, I and J added more nitrogen at the end of Phase I, where Yards C and I used sources of inorganic nitrogen and Yard J added an organic nitrogen source (Table 4.1).

Table 4.1. Total nitrogen and percentage of nitrogen lost during Phase I. Type of nitrogen and timing of addition. Organic and inorganic nitrogen feedstocks are in addition to poultry manure. Numbers have been normalised to end-Phase I by correcting for mass loss during composting.

	Additional N inputs		Timing of N input addition ***	Summed N in feedstocks▲ (kg N/T P1)	N content at end-P1 (kg N/T P1)	Loss of N during P1 (%)
	Organic *	Inorganic **				
Yard A	CS, FM	U	3	5.331	4.806	10
Yard B	-	-	2	6.991	5.580	20
Yard C	-	U, AS	2,4	7.591	5.727	25
Yard D	-	-	3	8.790	5.697	35
Yard F	-	-	2	7.379	5.457	26
Yard G	SB	-	3	6.790	4.120	39
Yard H	CS, CT	U	2	7.071	5.469	23
Yard I	RS	AS	2,4	7.416	5.540	25
Yard J	C, CT	U	1,4	6.645	4.741	29

***C, CS, CT, FM, RS, SB – Canola, Cottonseed meal, Cotton trash, Feather meal, Rice straw, Soybean meal**
****AS, U – Ammonium sulphate, Urea**
*****1, 2, 3, 4 – N addition during prewet, N addition at bale break, N addition during P1, N addition after P1**
▲– calculated from reported feedstocks

Compost yards G and D had the highest loss of nitrogen during their Phase I process while yard A had the lowest. The minimal losses of nitrogen found for yard A are most likely reflective of the Phase I process (Chapter 2). There are substantial differences in the loss of nitrogen during Phase I, but because of the many changing variables, it was not possible to deduce a single cause of this.

During Phase II, all compost yards had a net loss of total nitrogen, except for Yard J (Table 4.2).

Table 4.2. Total nitrogen (N) and proportion of nitrogen lost/gained (%) during Phase II (P2) composting.
End-Phase II values have been normalised to end-Phase I (P1) by accounting for mass loss during composting.

Compost Yard	Total N in end-P1 (%DW*)	Total N in end-P2 (%DW*)	N in end-P1 (kg N/T P1)	N in end-P2 (kg N/T P1)	N loss/gain during P2 (%)
Yard A	1.60	2.20	4.806	4.358	-9
Yard B	1.86	2.00	5.580	4.022	-28
Yard C	1.91	1.86	5.727	4.013	-30
Yard D	1.90	2.22	5.697	4.997	-12
Yard F	1.82	1.90	5.457	3.967	-27
Yard G	1.37	2.03	4.120	3.044	-26
Yard H	2.21	2.60	5.469	5.468	-18
Yard I	1.85	1.82	5.540	3.936	-29
Yard J	1.58	2.06	4.741	4.823	2

*** – dry weight**

Compost yards C and I had the greatest loss of nitrogen during Phase II. This was most likely due to these yards adding inorganic nitrogen at the end of the Phase I process. Yard J also had an additional nitrogen input at the end of Phase I (Table 4.1), however, this yard used organic nitrogen, which is not as accessible to compost microbes as inorganic nitrogen sources. Compost yards B and F also had relatively high losses of nitrogen during Phase II, their losses are most likely due to only using poultry manure in their feedstocks and the timing of their feedstock addition (Table 4.1). Yard G had a very low nitrogen content at the end of Phase I. This may be due to sampling inconsistencies where it is difficult to obtain a representative sample from single sampling point in a highly variable system. As with Phase I, yard A had the least amount of nitrogen loss during Phase II, again, most likely reflective of their composting process (Chapter 2, Table 2.1).

4.3.2 N_2O and NH_3 levels decrease during Phase II

With the sizable amount of nitrogen lost during Phase II for some compost yards, it was important to discover where nitrogen losses occurred. To determine how much nitrogen was lost as N_2O and NH_3 gas during Phase II, gas from the return air ducting in a conventional mushroom compost tunnel, from one compost yard, was sampled daily for three crops. The concentrations of N_2O and NH_3 in the recirculating air were analysed using gas chromatography (GC) and Dräger tubes, respectively. Using the mass balance equation developed by Yalçın *et al.* (2015), the net release of nitrogen as N_2O and NH_3 was modelled over the duration of Phase II.

The average amount of nitrogen lost from the bulk compost pile (from approximately 200 T) in the studied tunnels, calculated by subtracting total N at the end of Phase II from total N at the start of Phase II, was approximately 5-9%. Over three Phase II crops, the average generation of N_2O or NH_3 from the compost pile was 25.7 (± 2.74) g and 3.48 kg, respectively. The average amount of N_2O and NH_3 that was released from the tunnel in the exhaust gas was 70.7 (± 16.4) g and 4.2 (± 0.6) kg, respectively. Therefore, the total nitrogen losses during Phase II as N_2O or NH_3 gas were 22.5 (± 5.2) g and 3.5 (± 0.5) kg, respectively. The combined loss of N, as N_2O and NH_3 gas, lost to the atmosphere only accounted for about a tenth of the overall N loss during Phase II.

Over the duration of Phase II, all three crops showed a decline in N_2O concentration in the tunnel airspace (Figure 4.4).

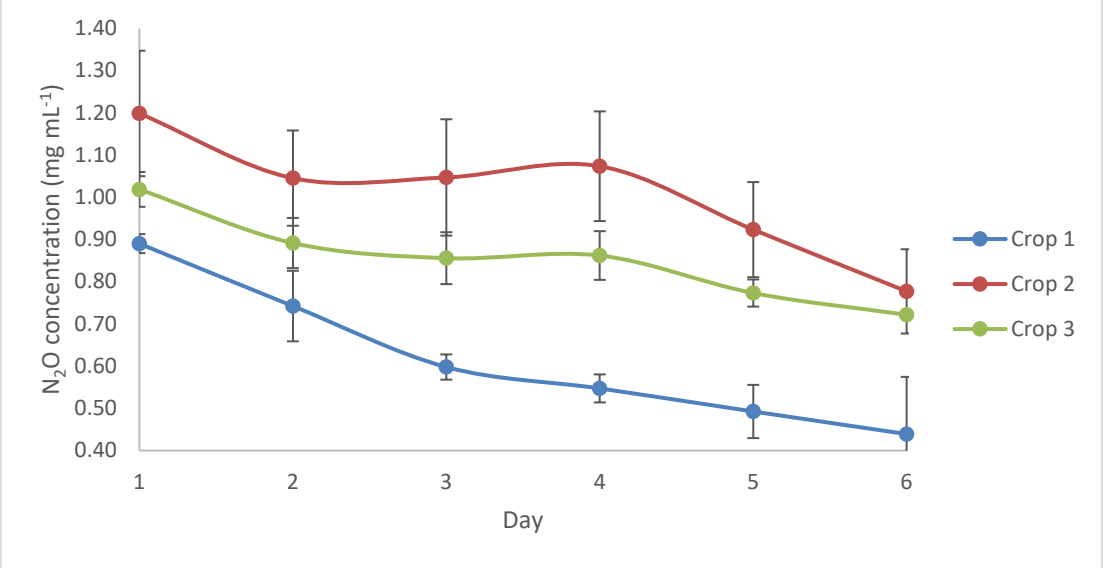

Figure 4.4. Nitrous oxide (N_2O) concentration in the airspace of a conventional compost tunnel for three successive crops.
Error bars indicate standard deviation of three technical replicates.

The rate of N_2O production by the compost decayed exponentially over time and showed the same pattern of decline over the three crops (Figure 4.5). On day four for the second and third crops, N_2O generation increased slightly or did not decrease at the same rate as the previous days.

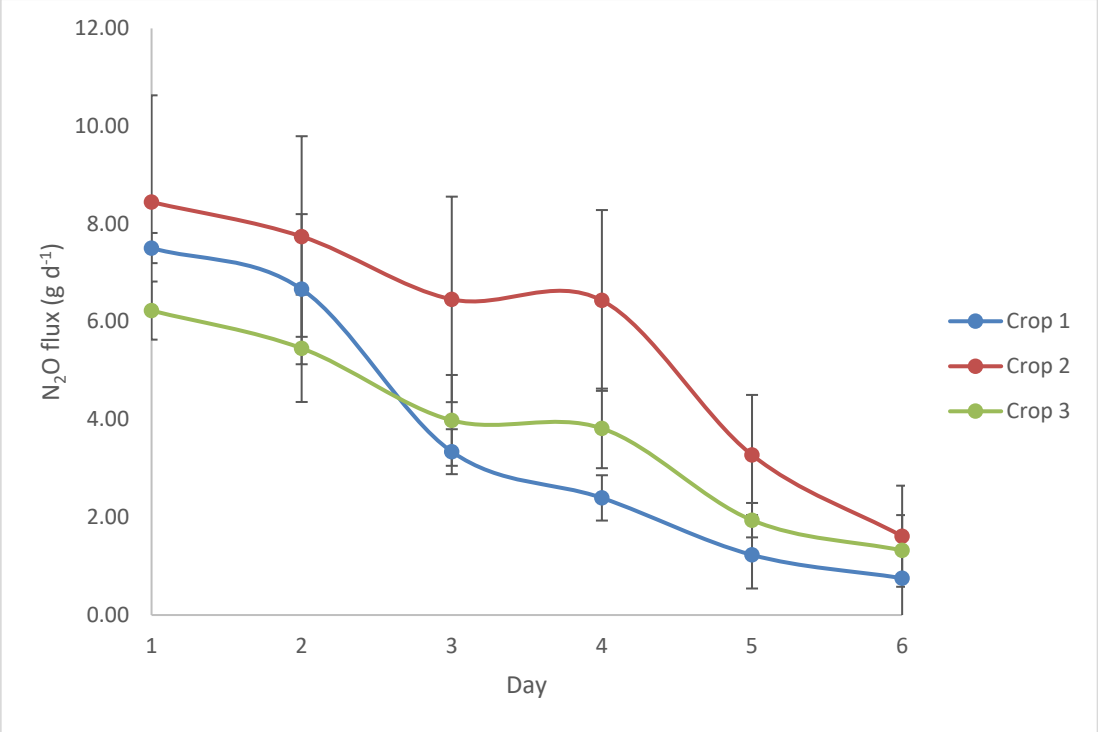

Figure 4.5. Calculated emission rate of nitrous oxide (N_2O) for the compost in one tunnel (approximately 200 T) over the duration of Phase II.
Error bars indicated the standard deviation of three technical replicates.

As expected, NH_3 concentration in the tunnel airspace dropped rapidly within the first 24-48 h for all three Phase II crops (Figure 4.6). Although, there was a small variation in the rate of disappearance of NH_3 among the three crops, the differences were small.

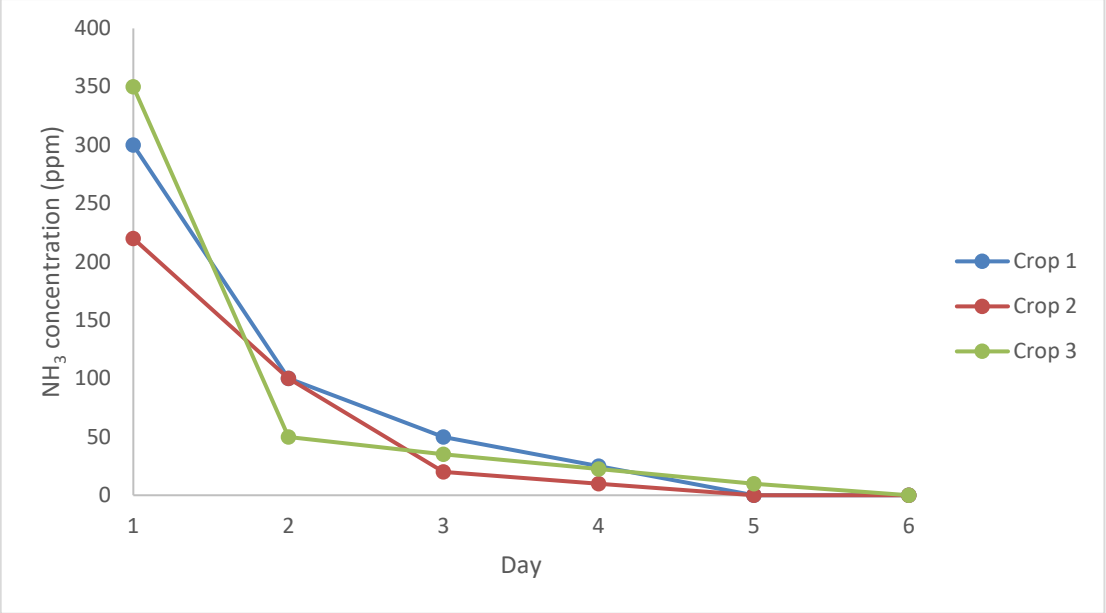

Figure 4.6. Ammonia (NH_3) concentration in a conventional compost tunnel measured quantified by Dräger tubes.
Concentration of NH_3 dropped rapidly within the first 24-48 hours. For two crops, NH_3 was not detected after day 5 of Phase II composting.

4.4 Discussion

Nitrogen inputs are a substantial cost to composters, but much of this is lost during composting. Improving nitrogen management could be a major financial benefit, but not enough is known about where and how nitrogen leaves the composting system.

In Phase I, total nitrogen lost at nine commercial compost yards ranged between 10-40% (Table 4.1) and variability in nitrogen losses during Phase I composting were due to timing, proportion and types of nitrogen used (Table 4.1). Much of the N loss that occurs during Phase I is most likely due to ammonia volatilization from proteolysis (Jurak *et al.*, 2015) as compost with high levels of organic nitrogen can reach higher peak heat temperatures than those with inorganic nitrogen sources (Noble *et al.*, 2002). However, as Phase I is an outdoor process at most compost yards it is difficult to accurately quantify where and how nitrogen is lost during Phase I. Furthermore, nitrogen content for all feedstocks reported were not directly measured, therefore the values in this study are approximate.

The Phase II composting process is similar in commercial compost yards around Australia. Total nitrogen lost during Phase II from the nine commercial compost yards sampled was 9-30% (Table 4.2). From the microbial profiling in Chapter 2, *Pseudoxanthomonas taiwanensis* was the dominant bacterial species found in end-Phase II compost (Chapter 2, Supplementary Table 1). This bacterial species is a heterotrophic nitrifier that can transform NO_2^- to N_2O in aerobic, thermophilic environments (Chen *et al.*, 2002). It was hypothesised that majority of the nitrogen lost during Phase II was as nitrogen gas (NH_3, N_2O, N_2).

To gain a better understanding of how nitrogen is lost during Phase II, this study measured N_2O and NH_3 gas during Phase II composting at one commercial compost yard. Nitrous oxide emissions from the Phase II process were very small, contributing

approximately 70 g of nitrogen lost as N_2O gas and approximately 4 kg of nitrogen lost as NH_3 gas from approximately 200 T of compost over six days. Furthermore, the rate of N_2O production by the compost pile also decreased over the duration of Phase II for all three crops (Figure 4.5). During this time, the population of *P. taiwanensis* actively increases, thus the N_2O production during Phase II and *P. taiwanensis* population are not well-related. Overall, the amount of nitrogen lost as N_2O and NH_3 gas in Phase II was approximately 4.31 kg.

P. taiwanensis may not be producing N_2O during Phase II because of highly aerated processing during Phase II. A similar outcome has been found in other compost systems where concentrations of O_2 greater than 15% resulted in low N_2O concentration (Shen *et al.*, 2011, Li *et al.*, 2017). Production of N_2O by heterotrophic nitrification is also strongly correlated to low pH (Zhang *et al.*, 2015) and mushroom compost pH is usually within the neutral to alkaline range due to the high concentration of NH_3. The hypothesis that the high abundance of *Pseudoxanthomonas* during Phase II composting would lead to considerable production of N_2O was therefore not confirmed. Given the greenhouse gas warming potential of N_2O (300 times more potent than CO_2), this is a highly welcomed result.

Lower concentrations of N_2O during the later duration of Phase II (Figure 4.4) may likely be from other heterotrophic microbes in the compost converting N_2O to N_2. In composts that used dairy manure and rice straw as their starting materials and when aeration was more frequent, nitric oxide reductase (*norB*) and nitrous oxide reductase (*nosZ*) genes had higher copy numbers and lower concentrations of N_2O (Li *et al.*, 2017). This indicates that microbes were further transforming N_2O into N_2 gas when aeration was more frequent (Li *et al.*, 2017). It is unknown whether nitrogen

transformation functional genes would be similar to Phase II mushroom composting, where constant aeration is essential.

The gaseous losses of nitrogen during Phase II were smaller than expected. However, there are several factors that may be the reason for this. During the first 24-48 hours of Phase II, the ammonia in the tunnel airspace dropped rapidly for all three crops (Figure 4.6). The rapid drop in free ammonia is most likely due to *M. thermophilus* assimilating NH_3 as glutamate, catalysed by glutamine synthetase or glutamate synthetase (Ross & Harris, 1983, Wiegant *et al.*, 1992, Baars *et al.*, 1994). It is also the dominant fungus during the conditioning stage of Phase II (Kertesz *et al.*, 2016, Vieira & Pecchia, 2018). Ammonia is also quickly removed by ammonia oxidising bacteria (Jurak *et al.*, 2015). However, there are not many known thermophilic bacterial ammonia oxidisers, and the more commonly encountered ammonia oxidising bacteria (*Nitrosomonas* and *Nitrosospira*) (Baggs, 2010) were not abundant in any compost samples used in this study (Chapter 2, Supplementary Table 1). Thus, NH_3 assimilation was assumed to be done predominantly by *M. thermophilus*.

Another source of nitrogen that could be stimulating bacterial growth during Phase II could be from chitin degradation. As outlined in the previous chapter (Chapter 3), chitin is the main structural polymer of fungi and contains approximately 7% nitrogen (Hamid *et al.*, 2013). The novel chitin degrading bacterium, *Mycovorax composti*, can degrade chitin and potentially release the nitrogen that is bound in the hyphae of *M. thermophilus* (Chapter 5). However, chitin content may not be as much as a contributing factor to nitrogen content during Phase II. In unspawned mature mushroom compost, live fungal biomass, measured as fungal-specific phospholipid fatty acid (PLFA), and chitin content, measured as N-acetylglucosamine (GlcNAc), were measured over 26 days at spawn run temperature (25 °C) (Vos *et al.*, 2017).

While the authors did not measure chitinase activity, over the 26-day period, fungal PLFA significantly decreased and chitin content increased slightly (Vos *et al.*, 2017), thus, the dead fungal biomass in the compost may be an important nitrogen source for bacteria in Phase II. N-acetylglucosamine is also found in the peptidoglycan of bacterial cell walls and therefore, measuring GlcNAc maybe overestimating the chitin content. There is also a small amount (7.53 g N L^{-1}) of soluble nitrogen found in compost leachate (Safianowicz *et al.*, 2018). Another study detected even lower concentrations of soluble nitrogen in compost leachate (3.2-6.4 mg N L^{-1}), mostly in the form of urea, ammonia-N, P-serine, and other amino acids (Noble *et al.*, 2006). Therefore, the amount of nitrogen potentially lost as compost leachate during Phase II is minimal. However, this is not easily quantified because most compost yards do not collect the leachate from Phase II, therefore, making it difficult to quantify N loss as leachate.

Further analysis of N_2O fluxes in Phase II composting is highly recommended. Taking multiple daily samples throughout the Phase II process could help with reducing assumptions that were made in this study. It would also be interesting to compare the composition of Phase II gas from different composting facilities. Quantifying nitrogen cycling functional genes during Phase II could also shed light on how nitrogen is transformed during Phase II and if majority of the nitrogen lose during Phase II is N_2 gas.

4.5 Conclusion

In this study, total nitrogen was measured in end-Phase I and end-Phase II compost collected from several yards around Australia. The total nitrogen lost over the duration of Phase II varied between 9-30%. Monitoring N_2O concentration for the

duration of Phase II had found that emissions of N_2O was very small and the amount exiting the tunnel was minor. Ammonia concentration in the tunnel airspace dropped rapidly within the first 48 hours of Phase II, due to the ammonia assimilating compost microbes that are present during Phase II. Furthermore, the amount of nitrogen lost as N_2O and NH_3 gas only contributed approximately 0.49% of the total nitrogen lost during Phase II. Further analyses are needed to determine the other losses of nitrogen during Phase II, which is most likely from other heterotrophic compost microbes converting N_2O to N_2.

Mycovorax composti gen. nov. sp. nov., a member of the family *Chitinophagaceae* isolated from button mushroom compost

Abstract

A Gram-negative, aerobic, rod-shaped, orange coloured bacterium, designated strain C216, was isolated from mature mushroom compost from Victoria, Australia. Optimal growth was found to occur at 50 °C, at pH 7.25 and in the absence of NaCl on Emerson's 350 YpSs medium at 40 °C. The draft genome sequence is 3,122,820 bp in length with a G + C content of 40.5%. Phylogenetic analyses based on the full 16S rRNA gene sequence showed that strain C216 forms a distinct lineage in the family Chitinophagaceae and the closest identified relative is Niabella aquatica (93.5% 16S rRNA gene sequence similarity). These data demonstrate that strain C216 represents a novel genus and novel species within the family Chitinophagaceae, for which we propose the name Mycovorax composti. The type strain is C216^{T} (= DSMZxxxx)*

*** - Submission to DSMZ delayed due to COVID-19 pandemic**

The following chapter is in the format for submission to the International Journal of Systematic and Evolutionary Microbiology

5.1 Introduction

Mushroom compost is a highly selective substrate that is used to support the growth of button mushrooms (*Agaricus bisporus*). Mushroom compost is produced from wheat straw, poultry manure and gypsum, and nutrients in the raw material are transformed by the bacteria and fungi that colonise the feedstocks. Composting starts with wetting the wheat straw for a period of 3-10 days before the addition of poultry manure and gypsum (Noble & Gaze, 1996). The mixture is then piled and turned periodically to homogenise and aerate the compost over 6-14 days (Phase I) (Noble & Gaze, 1996, Weil *et al.*, 2013). During Phase I, heat is rapidly generated due to microbial activity and peak compost temperatures are as high as 80 °C (Straatsma *et al.*, 2000). Following Phase I, the partially fermented compost is subjected to two stages in temperature-controlled tunnels (Phase II). Phase II tunnels are designed to provide uniform temperature and aeration to the compost (Noble & Gaze, 1996). To pasteurise the compost, the compost pile is heated to 58-60 °C for 6-8 h (Noble & Gaze, 1996), followed by conditioning or curing, where compost temperatures decreased to 48 °C and held for 4 days (Vieira & Pecchia, 2018). The microbial dynamics during conditioning are of particular interest as 50-60% of the carbohydrates are degraded during this period (Jurak *et al.*, 2015). The microbial community also represents a climax community, and A. bisporus utilises the bacterial and fungal biomass of this community as a key source of nutrition during mycelial proliferation. (Vos *et al.*, 2017). *Chitinophagaceae* has been found in various compost systems (de Gannes *et al.*, 2013, Eichorst *et al.*, 2013, Kertesz *et al.*, 2016, Siyoum *et al.*, 2016) and in mushroom compost, it is one of the most abundant families found during the conditioning stage in Phase II (Kertesz *et al.*, 2016).

The family *Chitinophagaceae* is part of the phylum Bacteroidetes and was first described by Kämpfer *et al.* (2011). More than 40 genera belong to this family, the type genus being *Chitinophaga* which was first described by Sangkhobol & Skerman (1981) as a chitinolytic myxobacterium but was later transferred to the *Chitinophagaceae* family by Kämpfer *et al.* (2011). Other genera in the *Chitinophagaceae* family include *Compostibacter* (Siddiqi *et al.*, 2016), *Filimonas* (Shiratori *et al.*, 2009), and *Pseudocnuella* (Maeng *et al.*, 2021). Cells from the genera in the *Chitinophagaceae* family contain menaquinone MK-7 as the major quinone and the major fatty acids are iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, and iso-$C_{15:1}$G (Kämpfer *et al.*, 2011). Cells are usually thin, rod-shaped, and non-motile; however, swarming motility may be observed. They are usually mesophilic, aerobic or facultatively anaerobic, and some members of this family have limited fermentative capabilities.

A bacterial strain, designated C216, was isolated from end-Phase II button mushroom compost. This strain demonstrated chitinolytic activity in vitro and was antagonistic to *M. thermophilus* during growth in laboratory media. The most closely related taxon to this strain is *Niabella aquatica* RP-2 in the *Chitinophagaceae* family (Siddiqi & Im, 2016), which has a 16S rRNA gene sequence similarity to strain C216 of 93.54%.

5.2 Isolation and Ecology

End-Phase II mushroom compost, made from wheat straw, poultry manure and gypsum, was collected from a commercial mushroom compost yard in Victoria, Australia. One gram of the compost sample was suspended in nine millilitres of R2A broth (HiMedia Laboratories, Pennsylvania USA) and incubated at 40 °C for 48 hours, with shaking at 200 rpm. A single orange colony that was round, convex with an entire margin was found and purified by routine subculturing at 40 °C for 48 hours on R2A agar plates (Oxoid, Australia). The bacterial strain was designated C216 and

routinely cultured on 350 Emerson's YpSs agar at 40 °C and preserved at -80 °C in glycerol suspension (25% v/v).

Prior to isolation, 16S diversity profiling with Illumina sequencing had identified this bacterial taxon in a comprehensive study that focused on the microbial succession during mushroom composting at a commercial compost yard located in Windsor, NSW Australia (Kertesz *et al.*, 2016). The organism was part of a stable bacterial community that developed in Phase II mushroom compost, composed of *Pseudoxanthomonas*, *Steroidobacter* and the novel genus from the *Chitinophagaceae* family reported here. The fungal community in the same compost was dominated by the thermophilic, cellulolytic ascomycete *Mycothermus thermophilus* (Kertesz *et al.*, 2016), which is dominant in Phase II mushroom composts (Kertesz *et al.*, 2016, Vieira & Pecchia, 2021) and has been extensively studied for its role in promoting growth of *A. bisporus* (Wiegant *et al.*, 1992). Interactions between *M. thermophilus* and compost bacteria have not yet been investigated. When strain C216 was grown in co-culture with *M. thermophilus*, the bacterium exhibited chitinolytic activity on the hyphal tips of the fungus.

5.3 16S RNA phylogeny

Genomic DNA was extracted using the CTAB method, according to the method of Wilson (2001). Briefly, late exponential phase bacterial cells were harvested and resuspended in Tris-ethylenediaminetetraacetic acid (TE) buffer (10mM Tris-HCl, 1 mM EDTA; 100 µL, pH 8.0) with 1 mg/mL lysozyme (Sigma-Aldrich) and incubated at 37 °C for 30 minutes without agitation. Hexadecyltrimethylammonium bromide (CTAB) extraction buffer (2% CTAB (w/v), 100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl; 500 µL) was added and incubated at 50 °C for one hour without agitation. Chloroform:isoamyl alcohol (24:1) was added in equal volume and the solution was

emulsified before centrifuging at 14,000 × g for 10 minutes to separate the two phases. The top aqueous phase (400 µL) was transferred to a fresh tube and cold isopropanol (320 µL) was added. The precipitated DNA was collected by centrifugation (20,000 × g for 20 minutes) and washing twice with 80% (v/v) ambient ethanol. The DNA pellet was resuspended in TE buffer (pH 8.0) and stored at -20 °C.

Whole genome sequencing of the C216 genomic DNA was done by the Microbial Genome Sequencing Center (Pittsburgh, USA), using their standard Illumina protocols (NextSeq 2000; 2 × 150 bp). The resulting FastQ files were analysed using the Galaxy software package (Afgan *et al.*, 2018). Genome annotation was done in Galaxy using the Prokka tool (Seemann, 2014). DNA G + C contents of strain C216 was determined from whole genome sequencing. 16S rRNA sequences of closely related genera were obtained from the List of Prokaryotic names with Standing in Nomenclature (LPSN) (Parte *et al.*, 2020). The 16S rRNA sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994). A phylogenetic tree was constructed using the software package MEGA-X (version 10.2.2) (Kumar *et al.*, 2018). Distances were determined using the Kimura two-parameter model and clustering was done with the neighbour joining (NJ) method and maximum likelihood (ML) method. The reliability of the trees obtained was confirmed using bootstrap values based on 1000 replicates. The NJ tree (Figure 5.1) revealed that strain C216 was closely related to members of the *Chitinophagaceae* family within the phylum Bacteroidetes, with strain C216 forming a branch between *Niabella aquatica* and *Terrimonas ferruginea*. The ML tree showed similar topology. Comparison of the full 16S rRNA gene sequence of strain C216 (1526 bp), was related to *Niabella aquatica* and *Terrimonas ferruginea*, with 92-93.5% 16S rRNA gene sequence similarity to the type strains of these species.

5.4 Genome Features

The total predicted genome size of strain C216 was 3,332,569 bp. DNA G + C content of strain C216 was 40.5 mol%. The draft genome was scaffolded into 23 contigs which contained 2,883 predicted genes, including 2,841 coding DNA sequences, three rRNA regions, 38 tRNA regions and one tmRNA region. In addition, two repeat regions found in the genome. There were no antibiotic resistance genes identified in the genome. 46.6% of the protein-coding genes could be assigned putative functions by Prokka, while the remaining genes were annotated as hypothetical proteins.

Pairwise analysis of the whole genome was done using the type strain genome server (TYGS) (Meier-Kolthoff & Göker, 2019). Pairwise analysis of the whole genome of strain C216 had found that *Myroides aquimaris* strain CGMCC 1.10825 was the closest related species, with 34.6% similarity in digital DNA-DNA hybridisation (dDDH) (formula d_4). *Niabella aurantiaca* DSM 17617^T had only 19.4% pairwise similarity with strain C216 when comparing dDDH (d_4).

The main characteristic feature of strain C216 is its interaction with *Mycothermus thermophilus.* Chitin degrading enzymes were suspected to be present due to strain C216 breaking down *M. thermophilus* hyphae (Figure 5.3). Chitin is the main structural polymer in fungal cell walls, it is degraded by chitinases (EC 3.2.1.14). No predicted chitinase genes were found in the genome of strain C216, but putative genes for exo-N-aceytlglucosaminidases (EC 3.2.1.52) were present. Exo-N-aceytlglucosaminidases cleave chitin oligosaccharides such as chitobiose and chitotriose (Walter *et al.*, 2021).

Because the strain was isolated from wheat straw compost, the genes that are responsible for xylan degradation were also of interest. Xylan is a type of hemicellulose commonly found in wheat straw, and is constructed of a β-1,4-xylose

backbone with α-L-arabinofuranosyl, acetyl and 4-O-methyl glucuronyl branched units (Dodd & Cann, 2009, Jurak *et al.*, 2015). Analysis of the C216 genome revealed putative genes encoding endo-1,4-β-xylanase (EC 3.2.1.8), β-D-xylosidase (EC 3.2.1.37), α-L-arabinofuranosidase (EC 3.2.1.55) and acetyl xylan esterase (EC 3.1.1.72), suggesting that strain C216 is able to almost completely degrade xylan.

5.5 Physiology and Chemotaxonomy

Cell morphology was determined by growing cells in Emerson's 350 YpSs broth for 24 hours at 45 °C and examined under scanning electron microscopy (Sigma VP HD, Zeiss, Germany). Growth at different temperatures was tested at 30, 37, 40, 45, 50, 55 and 60 °C and at pH values of 4.0 – 9.0 (in increments of 0.25 pH units). Salt tolerance was tested in R2A liquid medium supplemented with 0, 1, 2, 3, 4, 5, 6 and 9% (w/v) NaCl. Motility was assessed using the hanging drop method under light microscopy at 400 times magnification. Gram staining was done according to Smibert *et al.* (1994). Catalase activity was determined by observing bubble production in 3% (v/v) H_2O_2. Oxidase activity was determined by swatching a single bacterial colony, grown on R2A agar for 48 hours at 45 °C onto paper saturated with 1% (w/v) tetramethyl-para-phenylenediamine.HCl. The presence of flexirubin-type pigments was tested for by flooding the plate with 20% (w/v) KOH and observing colour change (Bernardet *et al.*, 2002). Biochemical characterisation was done using API20E (bioMérieux) kit, following the manufacturer's instructions.

The cellular fatty acid profile was determined using cells grown in Emerson's 350 YpSs broth for 24 hours at 45 °C. Analysis of cellular fatty acids was carried out by the Identification Service, Leibniz- Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkuturen GmbH, Braunschweig, Germany. The cellular fatty acids were analysed after conversion into fatty acid methyl esters by saponification,

methylation, and extraction according to the protocol of Sherlock Microbial Identification System (MIDI, Microbial ID, Newark, DE USA).

Cells of strain C216 were Gram-negative, short rod shaped, aerobic, non-motile and non-spore forming. Colonies of strain C216 grown on R2A and Emerson's 350 YpSs agar at 45 °C for 48 h were circular, convex, opaque and orange in colour. Cells were approximately 0.8 – 1.0 μm in length and 0.4 – 0.5 μm in width (Figure 5.2). Growth on YpSs and R2A occurred at 30 – 55 °C and at pH 6.5 – 8.0. NaCl tolerance was 0 – 1% (w/v). Dark red to brown flexirubin-type pigments were observed when the plate was flooded with 20% (w/v) KOH. Phenotypic and chemotaxonomic characteristics that differentiate this strain from closely related taxa are listed in Table 5.1.

The DNA G + C contents of strain C216 was 40.5%, which was below those of *Niabella* species (45.0%) and *Terrimonas* species (48.9%) (Table 5.1). The major respiratory quinone was MK-7. The major fatty acids identified in C216 were iso-$C_{15:0}$ (56.7%), iso-$C_{17:0}$ 3-OH (15.6%) and iso-$C_{15:1}$G (6.1%) The total fatty acid profile of strain C216 in comparison to other closely related genera are summarized in Table 5.2. The much higher levels of fatty acid iso-$C_{15:0}$ (56.7%) clearly distinguished strain C216 from *Niabella* and *Terrimonas* species and also contributes to its thermotolerant/thermophilic nature.

In conclusion, from the data and observations described, strain C216 represents a member of a novel genus within the phylum *Bacteroidetes*, for which the name *Mycovorax* gen. nov. is proposed, and a novel species within this genus which we propose as *Mycovorax composti* sp. nov.

5.6 Description of *Mycovorax* gen. nov.

Mycovorax is named for the strain's interaction with *Mycothermus thermophilus*, an ascomycete fungus, where the bacterium degraded the fungal mycelium of *M. thermophilus*. *Myco*- relating to fungi, *vorax*- eating/gluttonous.

Gram-negative, aerobic and non-motile. The major fatty acids are iso-C15:0, iso-C17:0 3-OH and iso-C15:1G. The major respiratory quinone is MK-7. Positioned phylogenetically within the phylum *Bacteroidetes*. The type species is *Mycovorax composti*.

5.6.1 Description of Mycovorax composti sp. nov.

Mycovorax composti (com.pos'ti. N.L. gen. n *composti* of compost). Isolated from button mushroom compost.

Colonies are orange, two millimetres in size, circular, opaque and convex with entire margins when grown on 350 Emerson YPSs agar at 45 °C for two days. Positive for flexirubin-type pigments. Cells are aerobic, Gram-stain negative, non-motile and non-spore forming short rods that are 0.8 – 1.0 μm in length and 0.4 – 0.5 μm in width. Grows at 37-55 °C (optimum, 45-50 °C), at pH 6.5-8 (optimum, pH 7.0) and in the presence of 0-2% (w/v) NaCl (optimum, 0-1% NaCl). Grows on 350 Emerson YpSs, R2A and LB. Weakly positive for catalase activity and positive for oxidase activity. Indole and H_2S are not produced. Does not hydrolyse gelatine or urea. Does not ferment glucose, mannose, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin or arabinose. Positive for chitinase, xylanase, α-N-acetylgalactosaminidase, β-galactosidase and amylase. The dominant isoprenoid is MK-7. The major fatty acids are iso-$C_{15:0}$ and iso-$C_{17:0}$ 3OH. When grown in co-culture with the thermophilic

ascomycete, *M. thermophilus*, *Mycovorax composti* strain C216 degrades the fungal mycelium and exhibits anti-fungal activity by inhibiting mycelial growth.

The type strain is C216 which was isolated from end-Phase II mushroom compost in Victoria, Australia. The DNA G+C content of the type strain is 40.5 mol%.

5.7 Figures and Tables

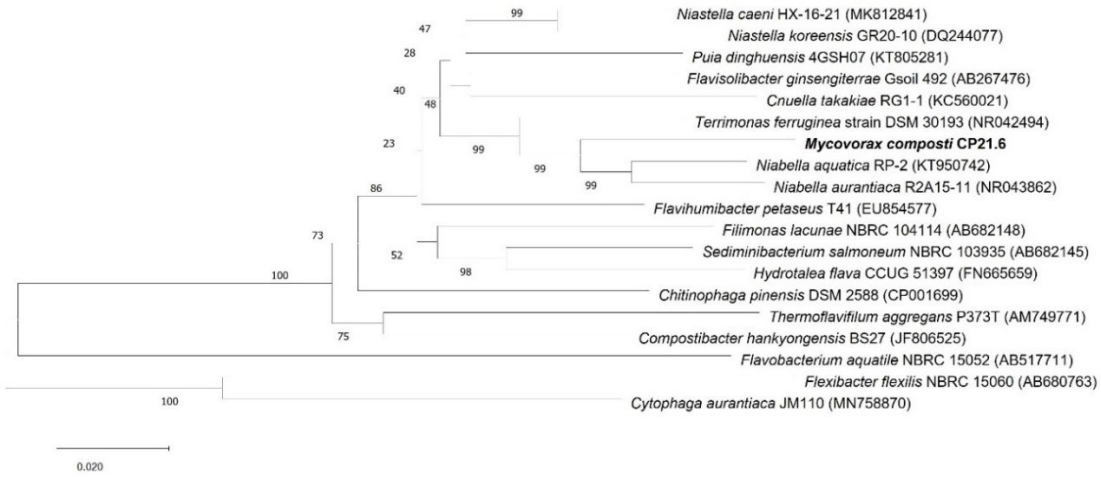

Figure 5.1. Neighbour-joining phylogenetic tree for strain C216 and related strains based on partial 16S rRNA gene sequences.
Numbers at nodes indicates the percentages of bootstrap support based on 1000 replications. Bar, 0.02 substitutions per nucleotide position. *Flavobacterium aquatica* NBRC 15052, *Flexibacter flexilis* NBRC 15060 and *Cytophaga aurantiaca* JM110 were used as outgroups.

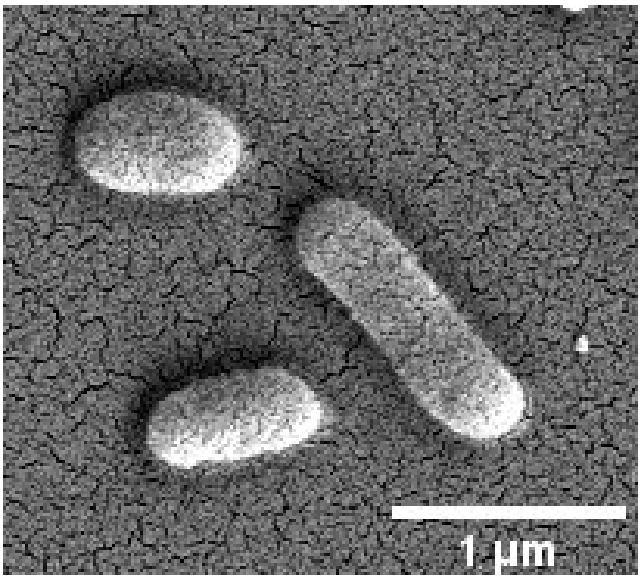

Figure 5.2. Scanning electron micrograph of C216 cell morphology.
The longer cell on the right is an elongated cell that is about to divide. No flagella or other external structures can be seen on the cell.

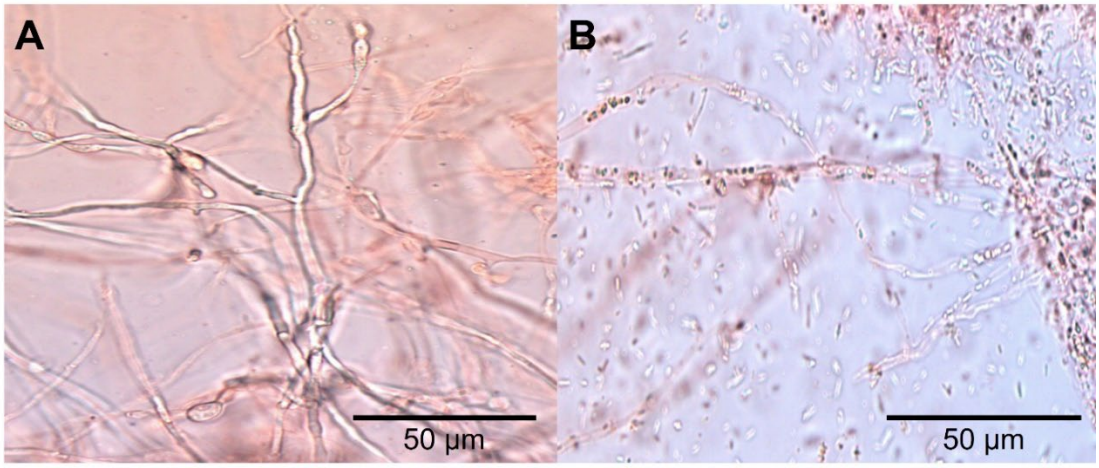

Figure 5.3. Interaction of strain C216 with *Mycothermus thermophilus* in YpSs liquid medium.
A) Healthy *M. thermophilus* hyphae in the absence of strain C216, B) *M. thermophilus* hyphae incubated with strain C216. Stained with 1% (w/v) CongoRed

Table 5.1. Phenotypic and chemotaxonomic characteristics of strain C216 and type strains of closely related genera in the family Chitinophagaceae.

Taxa: 1, C216; 2, Niabella aurantiaca R2A15-11^T; 3, Hydrotalea flava CGUG 51397^T; 4, Compostibacter hankyongensis BS27^T; 5, Terrimonas ferruginea ATCC 13524^T; 6, Niastella koreensis GR20-10^T; 7, Chitinophaca pinensis UQM 2034.

Data in columns 2 – 7 are taken from (Kim *et al.*, 2007), (Kämpfer *et al.*, 2011), (Siddiqi *et al.*, 2016), (Xie & Yokota, 2006), (Weon *et al.*, 2006) and (Sangkhobol & Skerman, 1981). +, Positive; -, negative; w, weak; ND, no data available

	1	2	3	4	5	6	7
Colony colour	Orange	Orange	Orange-Yellow	Milky white	Salmon red	Light Yellow	Yellow
Cell Morphology	Short Rod	Rods	Thin Rods	Short Rod	Rods	Filamentous	Rod
Gram Stain	-	-	-	-	-	-	-
Relationship to O2	Aerobic	Aerobic	Aerobic	Aerobic	Strictly Aerobic	Aerobic	Aerobic
Gliding motility	-	-	-	-	-	+	+
Growth temperature range (°C)	37-55	10-30	20-37	15-37	10-37	10-37	12-37
Optimum temperature (°C)	40-50	25-30	ND	30	25-32	ND	24
pH Range	6.5-8.0	5.0-8.0	ND	6.0-9.0	ND	5.0-8.0	4-10
Salinity range for growth (%)	0-2.0	0-3.0	0-4.0	0-1.5	0-1.0	ND	0-1.5
Flexirubin production	+	+	-	-	ND	-	ND
Catalase	w	+	+	+	w	-	+
Oxidase	+	-	+	-	+	-	-
Indole	-	+	ND	-	-	-	-
H_2S	-	ND	-	ND	-	ND	-
Hydrolysis of;							
Starch	*	-	ND	-	+	-	-
Chitin	+	-	ND	ND	-	+	+
CM-Cellulose	*	-	ND	-	ND	+	-
Xylan	*	-	ND	-	ND	ND	ND
Casein	*	+	ND	-	ND	+	+
Gelatine	-	-	ND	-	+	+	+
Urea	-	-	ND	-	-	-	+
VP	*	ND	ND	ND	+	ND	ND
Nitrate reduction	*	-	-	+	+	-	-
DNA G+C content (mol%)	40.5	45.0	42.0	53.0	48.9	45.8	44.6

Fermentation of sugars							
Amygdalin	-	ND	-	ND	ND	ND	ND
Arabinose	-	ND	-	-	ND	ND	ND
Cellobiose	*	ND	-	ND	+	ND	ND
Dulcitol	*	ND	-	ND	-	ND	ND
Fructose	*	ND	ND	ND	-	ND	ND
Galactose	*	ND	ND	ND	-	ND	ND
Glucose	+	-	-	+	+	-	+
Inositol	-	ND	-	-	-	ND	ND
Lactose	*	ND	ND	ND	ND	ND	+
Maltose	*	ND	-		+	ND	ND
Mannose	-	ND	-	+	+	ND	ND
Melibiose	-	ND	-	+	+	ND	ND
Raffinose	*	ND	-		-	ND	ND
Rhamnose	-	ND	-	ND	+	ND	ND
Sorbitol	-	ND	-	+	-	ND	ND
Sucrose	-	ND	-	+	-	ND	+
Xylose	*	ND	ND	ND	+	ND	ND

*** - Could not be tested due to COVID-19 pandemic**

Table 5.2. Fatty acid profile of strain C216 and related type strains of genera in the family *Chitinophagaceae*.

Taxa: 1, C216; 2, *Niabella aurantiaca* R2A15-11^T; 3, *Hydrotalea flava* CGUG 51397^T; 4, *Compostibacter hankyongensis* BS27^T; 5, *Terrimonas ferruginea* ATCC 13524^T; 6, *Niastella koreensis* GR20-10^T; 7, *Chitinophaga pinensis* UQM 2034.

Data for taxa 2 – 7 was collected from (Kim *et al.*, 2007), (Kämpfer *et al.*, 2011), (Siddiqi *et al.*, 2016), (Xie & Yokota, 2006), (Weon *et al.*, 2006) and (Sangkhobol & Skerman, 1981). Summed feature 1 is $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$H, summed feature 2 is $C_{12:0}$ aldehyde, $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$I, summed feature 3 is $C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH and summed feature 4 is iso-$C_{17:1}$I and/or anteiso-$C_{17:1}$B. -, not detected; TR, trace amounts.

	1	2	3	4	5	6	7
Saturated							
$C_{14:0}$	0.8	-	-	1.0	0.5	-	1.0
$C_{15:0}$	0.7	-	0.6	-	2.7	-	-
$C_{16:0}$	3.5	3.5	0.9	1.9	1.7	2.6	1.9
Branched							
iso-$C_{13:0}$	0.1	-	4.2	-	0.5	-	1.1
iso-$C_{13:0}$ 3-OH	-	-	-	-	-	-	-
iso-$C_{14:0}$	-	-	-	-	1.1	-	TR
iso-$C_{14:0}$ 3-OH	0.1	-	-	-	-	-	-
$C_{14:0}$ 2-OH	0.1	-	-	-	-	-	-
iso-$C_{15:0}$	56.7	33.7	34.8	29.7	28.4	26.8	29.0
iso-$C_{15:0}$ 3-OH	2.0	2.9	4.0	4.4	2.2	1.3	3.2
anteiso-$C_{15:0}$	0.4	1.6	1.7	3.7	0.6	4.9	0.5
$C_{15:0}$ 2-OH	0.3	-	0.4	-	0.8	-	-
iso-$C_{16:0}$	0.1	-	0.7	-	1.4	1	0.8
iso-$C_{16:0}$ 3-OH	0.4	-	1.2	-	0.7	-	-
$C_{16:0}$ 2-OH	1.4	-	-	2.6	-	-	-
$C_{16:0}$ 3-OH	1.6	2.4	0.9	2.4	1.3	-	2
iso-$C_{17:0}$	0.6	-	-	7.4	-	1.6	TR
anteiso-$C_{17:0}$	-	-	-	-	-	-	-
iso-$C_{17:0}$ 3-OH	15.6	-	16.9	22.7	-	29.4	11.3
$C_{17:0}$ 2-OH	0.1	-	0.5	-	-	3.5	-
$C_{17:0}$ 3-OH	-	15.5	-	-	15.3	1.2	-
Unsaturated					-	-	
$C_{15:1}$ω6c	-	-	-	-	-	-	-

iso-$C_{15:1}$G	6.1	22.3	8.2	-	26.2	15.6	15.6
anteiso-$C_{15:1}$A	-	-	-	-	-	-	TR
$C_{16:1}\omega5c$	-	-	-	1.7	-	-	6.0
$C_{17:1}\omega6c$	0.1	-	-	-	-	-	-
iso-$C_{17:1}\omega9c$	-	-	5.9	-	-	-	-
iso-$C_{17:1}\omega10c$	-	-	-	-	-	-	-
Summed Features							
1	-	-	-	-	-	-	-
2	0.1	-	-	-	-	-	-
3	7.3	10.6	4.7	13.1	11.2	4.3	23
4	-	-	-	9.4	-	-	-
Unknown							
11.543	0.1	-	0.5	-	-	-	-
13.565	1.0	-	8.4	-	1.3	-	-
16.582	1.1	1.2	1.7	-	1.3	1.4	-

General Discussion

Phase II composting is one of the most microbially intense steps in mushroom composting and is the critical point at which the compost is selective for *Agaricus bisporus* (Kertesz & Thai, 2018, Vieira & Pecchia, 2021). The transformation of wheat straw, gypsum, and poultry manure into a cocktail of microbial biomass and humus-lignin complexes leads to the selectivity of the compost for *A. bisporus* (Straatsma *et al.*, 1994, Vos *et al.*, 2017).

Phase II is done in temperature-controlled tunnels to provide uniform temperature and aeration throughout the compost pile. There are two stages in Phase II, pasteurisation and curing/conditioning. During pasteurisation, compost temperatures are held at 58-60 °C for 6-8 h (Straatsma *et al.*, 2000), and insects, nematodes, pathogens, and other fungal competitors are killed. Holding the pasteurization temperature at higher than 60 °C will favour more bacilli, subsequently altering the microbial community during conditioning (Vieira & Pecchia, 2018).

During conditioning, compost temperatures are cooled to 48 °C and held for four days, before rapidly cooling to 25 °C for mushroom colonisation (Straatsma *et al.*, 2000). At this point, 50-60% of the cellulose and hemicellulose has been degraded (Jurak *et al.*, 2015). The free ammonia released during Phase I is assimilated back into biomass by compost microbes during curing (Straatsma *et al.*, 1989, Wiegant *et al.*, 1992). Ammonia assimilation during conditioning can be reduced at higher pasteurisation temperatures which lowers the total nitrogen content (Vieira & Pecchia, 2018).

In this study, five compost yards with variable Phase I processes but similar Phase II processes were compared to investigate changes in the bacterial community during Phase II (Chapter 2). The interactions between compost bacteria and *Mycothermus thermophilus* were examined *in vitro*, to determine their biological compatibility and

further understand compost ecology (Chapter 3). Total nitrogen and Phase II gaseous emissions were sampled daily at a commercial compost yard to calculate how much nitrogen and in what form is lost during Phase II (Chapter 4). Comparing nitrogen losses with the bacterial community profile associated with Phase II composting allowed the identification of heterotrophic nitrifying bacteria that could be responsible for transformation of nitrogen during Phase II. Compost bacteria were isolated (Chapter 3) to better understand the ecology of Phase II microbes and their potential use for bioaugmentation. As part of this collection, a novel chitin degrading bacterium with an interesting interaction with *M. thermophilus* was discovered (Chapters 3 and 5).

6.1 Microbial diversity in Australian mushroom compost

Microbial diversity at end-Phase I, mid-Phase II and end-Phase II were compared among five compost yards in locations with different climates and temperatures. Microbial diversity in Phase I compost was highly variable, and the variability was related to differences in composting process factors such as time, scale and temperatures achieved (Chapter 2, Figure 2.2 and Figure 2.6). Some compost yards had a relatively high abundance of *Pseudoxanthomonas* by the end of Phase I (Chapter 2, Figure 2.2). This was unexpected as it was previously only found to be abundant or present in Phase II (Székely *et al.*, 2009, Kertesz *et al.*, 2016). The high abundance of *Pseudoxanthomonas* has been found in other composting systems that do not use animal manures as their primary nitrogen source (Vajna *et al.*, 2012, Cao *et al.*, 2019).

The bacterial profile in end-Phase II compost was more consistent across all compost yards sampled. *Pseudoxanthomonas* was the dominant genus for most of the compost yards (Chapter 2, Figure 2.3) and *Pseudoxanthomonas taiwanensis* was the dominant

species (Chapter 2, Supplementary Table 1). It is well established that *Pseudoxanthomonas* is found in cellulose-degrading consortia, and the relationships between *Pseudoxanthomonas* and cellulose-degrading bacteria are complex. It is likely that *Pseudoxanthomonas taiwanensis* can boost cellulose degradation during Phase II composting. There are several hypotheses, for the way in which *Pseudoxanthomonas* is involved with cellulose breakdown *in vitro*, including β-glucosidase production (Du *et al.*, 2015), buffering pH, and consuming acetate (Kato *et al.*, 2005). *Pseudoxanthomonas taiwanensis* is not known to produce cellulase. However, other species of *Pseudoxanthomonas* can produce cellulose degrading enzymes, they are *P. mexicana* and *P. suwonensis* (Chapter 1).

Pseudoxanthomonas suwonensis was identified in the bacterial profile of Phase II mushroom compost in this study but was much less abundant than *P. taiwanensis* (Chapter 2, Supplementary Table 1). While both species of *Pseudoxanthomonas* can produce β-glucosidase (Chapter 1, Table 1.1), *P. taiwanensis* is the only species of *Pseudoxanthomonas* reported in mushroom compost and cellulose degrading consortia. Perhaps a drop in the abundance of *P. taiwanensis* and an increase in the abundance of *P. suwonensis* in Phase II compost could affect the quality of the compost and have a negative effect on the yield. The cellulolytic *P. suwonensis* would most likely be competing with *M. thermophilus* for the same nutrients, thus competition would occur between *P. suwonensis* and *M. thermophilus*.

In the compost yards where *Pseudoxanthomonas* was dominant in end-Phase I compost, actinomycetes and bacilli were more abundant in end-Phase II compost than *Pseudoxanthomonas* (Chapter 2, Figure 2.3). The lower abundance of *Pseudoxanthomonas* in Phase II could be attributed to two factors, the most obvious may be due to sampling inconsistencies. A consistent and reproducible compost

sample from a bunker, windrow or tunnel is a difficult task that needs further optimisation. Most species of *Pseudoxanthomonas* do not grow above 45 °C (Chapter 1, Table 1.1) and most likely are not *P. taiwanensis*, which is consistently found in end-Phase II compost. However, it should be noted that the summed proportion of actinomycetes and bacilli was higher than *Pseudoxanthomonas*, but the relative abundance of *Pseudoxanthomonas* in compost still placed it amongst the top 20 genera (Chapter 2, Supplementary Table 1).

Overall, the bacterial diversity in end-Phase I, mid-Phase II and end-Phase II composts followed the same pattern as fungal diversity, where there was rapid succession of different bacterial taxa from end-Phase I to end-Phase II (Chapter 2, Figure 2.6). While the abundances of individual taxa in bacterial communities in Phase I and Phase II compost varied among compost yards, there were no observable differences in the compost output, and similar productivity and selectivity for *Agaricus* were achieved. Bacterial communities in mushroom compost produced in the United States tended to show the same pattern, despite the carbon and nitrogen coming primarily from straw bedded horse manure, rather than from straw and chicken manure as is normal in Australia (Vieira & Pecchia, 2021).

The end-Phase I bacterial diversities were relatively similar, except for one compost yard where Phase I processing was vastly different to the other yards (Chapter 2, Figure 2.6). The bacterial diversity in successive crops from the same compost yard was very similar (Chapter 2, Figure 2.6). However, bacterial diversity at end-Phase I was quite distinct among compost yards most likely due to Phase I processing, the scale of the compost yard and the prevailing weather conditions influencing each yard.

On the other hand, mid- and end-Phase II bacterial diversities for most compost yards

were similar (Chapter 2, Figure 2.6). Distinct bacterial diversity during Phase II for one compost yard was most likely due to their Phase I process (unfortunately these differences are confidential, and cannot be discussed) (Chapter 2, Table 2.1). Focusing on the interactions between Phase II compost microbes could provide more insight into the selection of bacterial taxa during Phase II. Furthermore, profiling the bacterial community in mid- and end-Phase II compost would provide valuable insight into which taxa are likely to be responsible for nitrogen transformations during composting.

6.2 Interactions of compost microbes

To gain a better understanding of the microbial ecology of mushroom compost, this study investigated the biological interactions among the most abundant bacterial and fungal taxa in Phase II. From the data shown in Chapter 2, *Pseudoxanthomonas* was the dominant bacterial genus and *Mycothermus thermophilus* was the dominant fungal species. The latter species has been identified as the dominant fungus in Phase II elsewhere (Straatsma *et al.*, 1989, Kertesz *et al.*, 2016, Vieira & Pecchia, 2021) and is known to have a significant impact on the selectivity of mushroom compost (Straatsma *et al.*, 1989, Wiegant *et al.*, 1992, Straatsma *et al.*, 1994, Sánchez & Royse, 2009).

Three species of *Pseudoxanthomonas* was isolated from end-Phase II compost, *P. taiwanensis*, *P. suwonensis* and *P. koreensis*, along with a novel chitin degrading bacterium, *Mycovorax composti* (Chapter 3, Supplementary Table 2). On solid and in liquid culture, *P. taiwanensis* did not inhibit the growth of *M. thermophilus* (Chapter 3, Figure 3.3 and Figure 3.4). *P. taiwanensis* may have a neutral or stimulatory effect on *M. thermophilus* in compost as this species has been involved in other

lignocellulolytic consortia and can aid *M. thermophilus* with the degradation of inhibitory compounds such as cellobiose or acetate, and consequently buffering the pH of the compost (Haruta *et al.*, 2002, Kato *et al.*, 2005, Wang *et al.*, 2011, Du *et al.*, 2015). The *in vitro* interaction between *M. thermophilus* and *P. suwonensis* or *P. koreensis* was most likely due to competition for nutrient resources (Chapter 3, Figure 3.3 and Figure 3.4) and, if this interference also occurs in mushroom compost, could result in a decrease in lignocellulose degradation (de Boer *et al.*, 2005). High proportions of *P. suwonensis* or *P. koreensis* in Phase II compost may impact colonisation of *M. thermophilus* during conditioning and potentially lower compost quality. The likeliness of this to occur with *P. suwonensis* is higher than *P. koreensis*. *P. suwonensis* is a thermotolerant species, whereas *P. koreensis* is strictly mesophilic.

Hyphal growth of *M. thermophilus* in the presence of *M. composti* was severely inhibited, with evidence that hyphae were degraded as the bacterium grew (Chapter 3, Figure 3.7). Putative genes for hemicellulose degradation and exo-chitinase were identified in the genome sequence for *M. composti* C216 (Chapter 5). Non-motile and non-filamentous bacteria produce chitin degrading enzyme to protect cellulosic or hemicellulosic resources from competing actinomycetes and fungi (de Boer *et al.*, 2005). *M. composti* is non-motile and non-filamentous bacterium (Chapter 5) and this antagonistic interaction may be occurring in mushroom compost between *M. composti* and *M. thermophilus*. In end-Phase II mushroom compost, living fungal biomass, quantified by measuring fungi-specific phospholipid fatty acid, declines and chitin content increases over the incubation period (Vos *et al.*, 2017). Increases in chitin content during the later days of sampling were the same and may be overestimated as chitin was measured as N-acetylglucosamine (Vos *et al.*, 2017), which is also

abundant in the cell membranes of bacteria. The authors did not measure chitinase activity, thus the quantification of chitin may be overestimated.

6.3 Nitrogen in Phase II

Nitrogen transformation during Phase II is not well characterised. Diversity profiling of the bacterial community during composting was done to identify taxa potentially responsible for nitrogen transformation during Phase II. The most interesting taxa in this respect were *Chelatococcus daeguensis* and *Pseudoxanthomonas taiwanensis* (Chapter 2, Supplementary Table 1) as they are known to display unusual nitrogen metabolism (Chen *et al.*, 2002, Yang *et al.*, 2014). Total nitrogen content in end-Phase II compost decreased by 5% during Phase II (Chapter 4) and it was hypothesized that most of the nitrogen in compost was released as gaseous N_2O and NH_3. However, N_2O and NH_3 production during Phase II only made up approximately 0.5% of the total nitrogen lost (Chapter 4).

Chelatococcus daeguensis was found in the top 20 genera for most compost yards (Chapter 2, Supplementary Table 1). This species is a heterotrophic nitrifier/aerobic denitrifier that can reduce nitrate under thermophilic conditions (Liang & Huang, 2016). *Chelatococcus daeguensis* TAD1 contains the gene for nitrous oxide reductase (*nosZ*), however it is not known if this gene is expressed under aerobic conditions (Yang *et al.*, 2014). *Pseudoxanthomonas taiwanensis*, the most abundant species (up to 20% of the total bacterial community) in Phase II compost, has also been identified as a thermophilic, aerobic heterotrophic nitrifier capable of producing N_2O (Chen *et al.*, 2002).

Heterotrophic nitrification and aerobic denitrification both increase with increasing C/N ratios, however denitrification efficiency can start at a C/N ratio as low as 2, to as high as 15 (Jiang *et al.*, 2011, Song *et al.*, 2021). Furthermore, N_2O production can

increase under aerobic conditions (Jiang *et al.*, 2011). However, despite the relatively high C/N ratio and aerobic nature of Phase II composting, the rate of N_2O being generated by microbes in the compost decreased over the duration of Phase II (Chapter 4, Figure 4.5). Therefore, *P. taiwanensis* does not produce N_2O under these conditions, or the produced N_2O is being reduced or assimilated by other heterotrophic organisms. Given the high abundance of *Chelatococcus daeguensis* in end-Phase II compost and the decreasing levels of N_2O during Phase II, nitrogen may also be lost as N_2 gas. Other losses of nitrogen could be as compost leachate, however, the soluble nitrogen content in compost leachate is small (7.5 g N L^{-1}) (Safianowicz *et al.*, 2018), potentially insignificant in other countries (3-6 mg N L^{-1}) (Noble *et al.*, 2006), and the volume of leachate from Phase II is often not measured (Safianowicz *et al.*, 2018).

For the compost yard sampled, the nitrogen lost as gas and difference in nitrogen content from their end-Phase I to end-Phase II compost samples were smaller than expected. This is most likely due to their composting process (Chapter 2, Table #). Further analysis and comparisons with other compost yards are needed.

6.4 Recommendations

With the rise in plant-based meat alternatives, mushrooms provide an important source of protein. Increasing the nitrogen content, consequently increasing protein content, of mushrooms would increase the market value of mushrooms. Mushrooms acquire their nitrogen from the biomass in the compost (Wood & Fermor, 1982, Vos *et al.*, 2017), thus minimising nitrogen losses during composting would lead to more nitrogen available for the mushrooms and if mushrooms can incorporate more nitrogen. To maximise the available nitrogen in mushroom compost, several key

research points need to be investigated and several outputs could be produced. For example, changes in bacterial communities at critical time points (i.e., end-Phase I or end-Phase II) that are associated with lower mushroom yields could be augmented by re-establishing the bacterial community by adding the necessary organisms. A similar approach could be used in predicting whether a particular crop may be predisposed to pathogens during cropping. To make these more accessible at field level, a detection assay for individual microorganisms or enzymes could be developed. Such a tool requires more research into the biological interactions that occur with compost microbes and their effect on *A. bisporus*. To accomplish this output, monitoring changes in the microbial ecology of end-Phase I and end-Phase II and relating these changes to mushroom yield would provide powerful knowledge for the mushroom industry. This would have to be done with successive crops from multiple compost yards and their collaborating mushroom farms. This will be no small task. Ensuring consistent, reproducible, and representative samples of highly variable composts from multiple compost yards will be challenging and will need to be optimised.

Lowering the cost or need for additional nitrogen inputs for composters and growers is also a recommended output. To do this, minimising nitrogen losses during Phase I and Phase II could also reduce supplement addition during cropping and delay the timing of addition of nitrogen supplements at cropping. This may involve manipulating the microbial community to suppress other heterotrophic organisms in the compost. Quantifying functional genes for nitrogen transformation and associating gas fluxes during Phase I and Phase II could provide better insight for how nitrogen is lost during composting. Furthermore, due to the nature of Phase I composting, gas sampling during Phase I will need to be optimised for outdoor Phase I composting yards.

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Supplementary Materials

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
Kingdom	Archaea		0	0	0	0	0	0	0	0	0	0	0	12	0	0	21	0	28	0	0	0
	Bacteria		99426	95838	58842	65225	49612	97278	103910	134023	156389	119444	169806	99986	76692	81916	72592	73145	81325	65067	71649	105389
Phylum	Acidobacteria		50	1386	1752	23	0	850	1168	1774	6707	203	5131	384	2223	292	351	2130	2675	1422	2584	3116
	Actinobacteria		4563	12883	7981	2748	2652	8636	13933	12915	34597	10266	36018	23599	18224	13497	13663	12755	13993	10629	14331	21194
	Aquificae		48	0	0	4	0	0	0	0	0	13	0	0	0	0	22	0	0	0	0	0
	Bacteroidetes		2927	1628	1950	337	8003	1701	12814	20301	8335	2040	10040	13974	7083	14899	8293	2173	5316	10945	2674	2784
	BRC1		12	1041	327	4	194	68	496	702	569	113	742	284	363	131	123	239	166	227	325	518
	Chlamydiae		0	0	0	0	0	0	0	0	0	0	3	6	0	0	0	0	0	0	0	0
	Chloroflexi		1218	3871	4594	541	340	2263	6107	3148	13338	3930	38062	1539	19489	1849	2916	11502	12732	6704	16698	21204
	Cyanobacteria		155	338	57	83	9	324	318	1051	1126	358	903	212	293	163	197	298	454	350	231	578
	Deferribacteres		0	2	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0
	Deinococcus-Thermus		15148	1420	824	8262	0	289	798	3103	4951	7660	5049	1267	1666	1613	2364	1119	971	1016	2047	2571
	Dependentiae		0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
	Epsilonbacteraeota		0	0	0	0	0	0	0	188	0	551	0	5	0	10	0	0	0	71	0	0
	Euryarchaeota		0	0	0	0	0	0	0	0	0	0	0	12	0	0	21	0	28	0	0	0
	FBP		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
	Fibrobacteres		0	0	0	0	0	0	0	124	0	7	0	43	0	0	0	0	0	415	0	0
	Firmicutes		65002	39064	4912	44271	14575	16920	10013	28124	21445	73902	24512	14370	6349	17053	30913	12499	9221	7205	8619	25488
	Gemmatimonadetes		620	1388	751	326	51	1150	372	2185	2224	1894	1632	353	698	229	1079	1314	1034	849	2155	3250
	Halanaerobiaeota		151	355	48	2170	0	0	35	61	36	386	143	55	47	54	9	4	0	13	4	70
	Hydrogenedentes		0	0	0	0	0	0	9	0	41	0	149	0	44	5	0	14	54	9	17	18
	Patescibacteria		71	441	167	23	22	28	281	1581	974	289	396	1993	199	314	117	354	368	774	121	372
	Planctomycetes		17	316	220	15	5	17	342	249	582	94	1272	134	555	29	65	371	391	149	483	482
	Proteobacteria		8767	30963	35126	6030	23305	64445	56958	55606	60550	15942	42747	41079	18086	31563	12232	27981	33434	23419	20866	23327
	Spirochaetes		11	6	4	5	0	0	0	64	18	196	11	0	15	15	0	9	42	114	0	16
	Synergistetes		7	0	0	0	0	0	0	7	0	0	3	0	0	0	0	0	5	2	0	0
	Tenericutes		277	535	16	301	98	7	121	186	166	1236	126	98	44	28	81	62	32	34	34	31
	Thermotogae		344	7	0	77	0	211	0	43	0	66	0	3	0	10	6	0	5	0	0	0
	Verrucomicrobia		31	157	88	0	258	299	122	2580	663	253	2698	577	1276	136	161	298	418	642	435	370
	WPS-2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	0	0
	WS4		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0
Class	ABY1		0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0
	Acidimicrobiia		222	4278	1827	186	240	2079	2829	2674	9768	2132	12938	1388	5661	1187	3133	8363	8057	4245	9086	11852
	Acidobacteriia		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
	Actinobacteria		4180	7893	5871	2458	2380	5956	10855	9792	23165	7437	22071	21748	12089	12116	10243	3732	5371	6052	4653	8439
	Alphaproteobacteria		3245	8494	7435	1355	4151	31904	14590	13518	18889	5683	15576	13514	5902	8718	2968	4725	4761	6406	4111	7059
	Anaerolineae		73	1462	1736	80	105	630	1367	1339	6260	1632	9826	321	3513	440	1059	4582	4199	2256	9805	13259
	Aquificae		48	0	0	4	0	0	0	0	0	13	0	0	0	0	22	0	0	0	0	0
	Babeliae		0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
	Bacilli		20663	12093	3361	11079	6936	11483	8239	18589	12557	37878	17908	12717	4343	12480	20614	6663	5061	4253	5523	17377

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
Class	Bacteroidia		1148	732	1287	197	8003	1173	12386	19749	7193	1248	6378	13731	5601	14059	7933	1677	4854	10464	1354	967
	BD2-11_terrestrial_group		0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0
	BD7-11		0	0	15	0	0	0	18	0	22	0	35	0	19	0	0	18	0	0	7	9
	Blastocatellia_(Subgroup_4)		4	0	0	0	0	0	0	0	0	0	709	0	423	34	0	0	45	464	109	93
	Campylobacteria		0	0	0	0	0	0	0	188	0	551	0	5	0	10	0	0	0	71	0	0
	Chlamydiae		0	0	0	0	0	0	0	0	0	0	3	6	0	0	0	0	0	0	0	0
	Chloroflexia		1102	2145	2745	441	235	1633	4566	1527	6772	2086	27977	1190	15877	1390	1789	6766	8387	4389	6817	7648
	Clostridia		32892	21769	543	29133	7037	4194	1233	7475	4608	20451	4673	1158	1386	2964	5303	4454	3237	1630	954	2845
	Coriobacteriia		4	0	0	0	0	0	0	7	12	0	0	0	0	0	0	0	0	0	0	0
	Deferribacteres		0	2	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0
	Dehalococcoidia		0	3	5	0	0	0	0	0	0	0	36	0	30	0	0	8	21	2	0	16
	Deinococci		15148	1420	824	8262	0	289	798	3103	4951	7660	5049	1267	1666	1613	2364	1119	971	1016	2047	2571
	Deltaproteobacteria		1814	7520	4238	2295	998	3187	2596	4890	7720	2918	9432	1739	4380	1034	1627	4473	5967	2904	7942	6734
	Erysipelotrichia		0	0	0	0	0	0	0	43	5	297	0	0	0	3	50	0	0	31	0	0
	Fibrobacteria		0	0	0	0	0	0	0	124	0	7	0	43	0	0	0	0	0	415	0	0
	Gammaproteobacteria		3698	14941	23430	2377	18156	29354	39739	37189	33841	7341	17671	25824	7767	21806	7631	18761	22671	14107	8775	9494
	Gemmatimonadetes		0	49	27	0	0	0	13	0	4	0	36	32	0	0	0	14	33	18	0	0
	Gracilibacteria		0	0	0	0	0	0	0	83	0	0	0	41	0	7	0	0	0	128	0	0
	Halanaerobiia		151	355	48	2170	0	0	35	61	36	386	143	55	47	54	9	4	0	13	4	70
	Hydrogenedentia		0	0	0	0	0	0	9	0	41	0	149	0	44	5	0	14	54	9	17	18
	Ignavibacteria		13	117	0	9	0	0	0	25	10	12	0	0	3	9	26	30	19	0	0	0
	JG30-KF-CM66		0	0	0	0	0	0	0	0	0	0	28	6	13	0	0	0	0	21	0	0
	Leptospirae		11	6	4	5	0	0	0	24	18	196	11	0	15	15	0	9	42	5	0	16
	Limnochordia		11386	4908	1000	3805	602	1243	541	1995	4222	14895	1894	495	612	1603	4908	1352	880	1291	2132	5256
	Longimicrobia		0	82	38	0	0	27	37	179	182	25	240	43	132	3	10	98	87	45	123	126
	Melainabacteria		49	23	0	6	0	0	138	178	461	42	78	0	22	39	22	13	27	0	0	23
	Methanobacteria		0	0	0	0	0	0	0	0	0	0	0	12	0	0	21	0	20	0	0	0
	Methanomicrobia		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0
	Mollicutes		277	535	16	301	98	7	121	186	166	1236	126	98	44	28	81	62	32	34	34	31
	Negativicutes		4	0	0	24	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0
	Nitriliruptoria		5	71	35	0	0	353	53	27	191	41	332	21	144	37	57	329	240	88	253	361
	OLB14		43	229	98	20	0	0	174	282	292	204	167	22	36	19	68	134	102	36	47	218
	OM190		0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0
	Oxyphotobacteria		0	0	0	0	9	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0
	Phycisphaerae		13	213	159	5	5	17	258	69	325	31	1000	48	387	10	30	252	268	51	386	258
	Planctomycetacia		4	103	46	10	0	0	66	180	235	63	231	86	149	19	35	101	123	98	90	215
	Rhodothermia		1766	777	663	131	0	528	428	527	1132	780	3662	243	1479	831	334	466	443	481	1320	1817
	S0134_terrestrial_group		620	1257	686	326	51	1123	322	2006	2038	1869	1356	278	561	226	1069	1202	914	786	2032	3124
	Saccharimonadia		71	441	167	23	22	28	281	1487	974	289	396	1952	199	307	117	354	368	646	121	372
	Sericytochromatia		106	315	57	77	0	324	180	873	665	306	825	212	271	124	175	285	427	350	231	555

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
	Spirochaetia		0	0	0	0	0	0	0	40	0	0	0	0	0	0	0	0	0	109	0	0
	Subgroup_21		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0
	Subgroup_6		46	1386	1752	23	0	850	1168	1774	6707	203	4422	384	1800	258	351	2130	2630	956	2465	3023
Class	Synergistia		7	0	0	0	0	0	0	7	0	0	3	0	0	0	0	0	5	2	0	0
	Thermoleophilia		152	641	248	104	32	248	196	415	1461	652	677	442	322	157	230	331	320	244	325	542
	Thermotogae		344	7	0	77	0	211	0	43	0	66	0	3	0	10	6	0	5	0	0	0
	Verrucomicrobiae		31	157	88	0	258	299	122	2580	663	253	2698	577	1276	136	161	298	418	642	435	370
	211ds20		0	0	0	0	0	0	0	13	10	0	22	0	0	0	0	0	7	0	0	0
	Absconditabacteriales_(SR1)		0	0	0	0	0	0	0	17	0	0	0	3	0	0	0	0	0	0	0	0
	Acetobacterales		10	39	45	0	0	0	39	27	13	30	181	11	40	16	14	63	83	22	48	58
	Acholeplasmatales		0	138	12	2	0	0	76	65	63	204	26	59	25	6	8	25	13	11	0	0
	Acidiferrobacterales		0	3	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	5
	Actinomarinales		97	2075	886	106	175	728	818	1075	5832	1203	7467	333	3317	594	1675	4871	5306	2515	6176	7251
	Actinomycetales		38	0	0	8	0	0	0	0	22	22	0	0	0	0	0	0	0	0	0	0
	Aeromonadales		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0
	Alteromonadales		0	0	0	0	0	0	0	82	0	65	0	44	0	52	34	0	0	196	0	0
	Anaerolineales		0	30	26	0	0	0	0	0	162	27	443	9	78	22	13	86	115	101	724	496
	Aquificales		48	0	0	4	0	0	0	0	0	13	0	0	0	0	22	0	0	0	0	0
	Ardenticatenales		6	0	7	0	0	0	6	0	26	26	74	15	38	0	10	29	37	40	137	129
	Azospirillales		0	0	0	0	0	0	0	79	39	0	27	18	24	0	12	0	57	11	0	0
	B108		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	B55-F-B-G02		0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Babeliales		0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
Order	Bacillales		18755	11893	3318	10278	6845	11165	8213	13015	11709	33009	17426	11746	4261	10509	19409	6607	5007	3978	5474	17241
	Bacteroidales		147	12	27	39	0	0	84	3178	1463	410	762	1817	1044	327	46	60	1003	1212	12	20
	Bacteroidetes_VC2.1_Bac22		15	0	0	0	0	0	0	688	8	27	0	604	0	36	70	0	0	248	0	0
	Balneolales		0	0	0	0	0	0	0	36	0	0	0	7	0	0	0	0	0	5	0	0
	Bdellovibrionales		87	600	596	48	0	0	777	1227	1345	562	1112	465	512	114	161	1105	924	347	365	500
	Beggiatoales		0	11	7	2	0	0	9	4	0	96	8	0	0	0	0	0	0	0	8	4
	Betaproteobacteriales		2245	1069	1383	1683	1527	2049	2506	5121	2450	929	1898	2571	938	1109	1084	2844	2536	1372	489	949
	Bifidobacteriales		0	0	0	4	0	0	0	7	0	17	0	8	0	11	0	0	0	0	0	0
	Blastocatellales		0	0	0	0	0	0	0	0	0	0	709	0	423	34	0	0	45	464	109	93
	Bradymonadales		0	0	0	0	0	0	0	187	52	5	117	10	15	11	0	0	0	168	0	0
	Caldilineales		17	287	291	5	105	305	377	521	1314	172	2097	146	857	86	175	824	864	288	916	1102
	Campylobacterales		0	0	0	0	0	0	0	188	0	551	0	5	0	10	0	0	0	71	0	0
	Candidatus_Peregrinibacteria		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0
	Caulobacterales		42	12	0	0	0	0	200	90	183	13	132	85	15	73	5	16	114	75	0	35
	CCD24		51	740	525	60	49	80	395	282	1873	207	1476	28	542	61	150	772	613	360	2451	1689
	Cellvibrionales		7	32	0	15	0	0	0	8024	183	1070	73	5736	38	7215	454	9	13	7042	7	16
	Chitinophagales		68	340	639	12	923	626	1833	3465	2271	68	1590	1198	1397	867	537	520	966	1885	8	25

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
Order	Chlamydiales		0	0	0	0	0	0	0	0	0	0	3	6	0	0	0	0	0	0	0	0
	Chloroflexales		60	167	2103	0	235	1381	4038	476	4372	23	25453	139	14716	351	523	4725	6746	2928	4302	2639
	Chloroplast		0	0	0	0	9	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0
	Chthoniobacterales		0	84	5	0	173	29	41	122	81	5	1848	91	789	42	36	84	149	216	18	38
	Clostridia_Incertae_Sedis		5	9	0	0	0	0	0	13	39	0	6	0	0	4	11	46	46	0	0	0
	Clostridiales		30348	20753	520	24911	7037	4194	1194	7106	4235	17280	4304	1019	1287	2701	4257	4095	2981	1545	826	2466
	Coriobacteriales		4	0	0	0	0	0	0	7	12	0	0	0	0	0	0	0	0	0	0	0
	Corynebacteriales		859	167	37	421	0	316	25	448	97	502	164	197	57	221	102	112	40	72	34	100
	Cytophagales		47	25	33	27	0	101	91	4090	1469	69	3327	3446	2353	1054	199	690	1398	3155	205	373
	D8A-2		151	62	0	810	0	0	0	0	0	239	0	0	0	0	36	3	2	0	0	0
	Deferribacterales		0	2	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0
	Deinococcales		167	702	799	48	0	95	773	2446	4809	1751	4804	1040	1599	308	401	1005	818	909	1951	2350
	Desulfarculales		0	0	0	0	0	0	0	0	0	0	118	0	50	4	0	0	5	10	50	15
	Desulfobacterales		0	0	0	0	0	0	0	24	0	0	0	0	0	0	0	0	0	16	0	0
	Desulfovibrionales		4	16	0	13	0	0	0	5	60	50	15	3	19	0	17	51	61	26	5	0
	Desulfuromonadales		0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0
	DTU014		12	0	0	103	0	0	0	0	0	47	0	0	0	0	6	0	3	3	0	4
	Ectothiorhodospirales		0	57	25	0	0	0	46	44	72	0	86	22	27	0	22	78	113	17	25	24
	Elsterales		0	9	32	5	0	0	20	16	65	14	111	0	39	7	5	24	11	6	65	55
	Enterobacteriales		5	3	0	0	0	0	0	15	328	0	0	0	0	4	0	0	0	0	0	9
	EPR3968-O8a-Bc78		0	199	198	15	0	0	159	252	1302	39	687	0	223	18	85	347	348	92	458	297
	Erysipelotrichales		0	0	0	0	0	0	0	43	5	297	0	0	0	3	50	0	0	31	0	0
	Euzebyales		5	71	35	0	0	353	53	27	191	41	332	21	144	37	57	329	240	88	253	361
	Fibrobacterales		0	0	0	0	0	0	0	124	0	7	0	43	0	0	0	0	0	415	0	0
	Flavobacteriales		134	12	16	79	0	0	32	6317	364	590	125	4030	261	7923	5771	61	1071	3206	990	423
	Frankiales		0	0	0	0	0	0	0	0	0	0	0	5	0	9	0	0	0	0	0	0
	Gaiellales		0	14	8	4	0	0	0	10	7	14	15	71	5	11	0	9	7	64	15	41
	Gammaproteobacteria_Incertae_Sedis		0	13	36	0	221	99	65	0	43	0	987	241	277	0	0	22	25	20	19	0
	Gemmatales		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	Gemmatimonadales		0	49	27	0	0	0	13	0	4	0	36	32	0	0	0	14	33	18	0	0
	Glycomycetales		59	25	10	13	0	0	0	2	0	9	8	0	0	0	9	0	0	2	0	6
	Halanaerobiales		151	355	48	2170	0	0	35	61	36	386	143	55	47	54	9	4	0	13	4	70
	Haloplasmatales		262	236	2	276	29	0	23	35	25	842	63	3	9	22	61	15	6	14	24	31
	Halothiobacillales		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0
	Hydrogenedentiales		0	0	0	0	0	0	9	0	41	0	149	0	44	5	0	14	54	9	17	18
	IMCC26256		0	0	0	0	0	0	0	0	26	0	13	0	20	3	0	0	20	0	31	40
	Izimaplasmatales		15	151	2	14	69	0	16	51	62	190	28	24	10	0	5	17	13	7	10	0
	JGI_0000069-P22		0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	65	0	0
	Kallotenuales		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0
	KI89A_clade		0	0	0	0	0	0	0	0	3	0	24	0	14	0	0	8	7	0	7	8

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
Order	Lactobacillales		712	78	8	511	91	201	18	5322	322	3838	15	744	0	1774	1028	0	0	219	0	2
	Legionellales		0	0	0	0	0	0	0	0	0	0	12	43	25	5	0	0	0	7	4	0
	Leptospirales		11	6	4	5	0	0	0	24	18	196	11	0	15	15	0	9	42	5	0	16
	Limnochordales		11386	4908	1000	3805	602	1243	541	1995	4222	14895	1894	495	612	1603	4908	1352	880	1291	2132	5256
	Longimicrobiales		0	82	38	0	0	27	37	179	182	25	240	43	132	3	10	98	87	45	123	126
	M55-D21		374	220	4	576	0	0	4	49	32	532	189	96	53	84	46	26	10	27	21	66
	MBA03		332	219	6	1202	0	0	9	126	116	924	46	0	16	34	132	109	70	16	29	45
	Methanobacteriales		0	0	0	0	0	0	0	0	0	0	0	12	0	0	21	0	20	0	0	0
	Methanosarcinales		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0
	Methylococcales		430	2244	1081	163	229	1433	571	2827	1842	695	1013	435	446	559	252	420	386	143	294	462
	Micavibrionales		0	0	0	0	0	0	0	54	0	51	0	0	0	0	0	0	0	12	0	0
	Micrococcales		1709	624	1170	1083	310	1961	7903	6433	14361	2440	9870	20275	4700	10457	9068	2591	4007	3451	1391	2729
	Micromonosporales		223	1517	337	117	273	467	138	508	849	771	794	93	300	139	360	151	110	350	284	1056
	Microtrichales		112	2203	941	80	65	1351	2011	1599	3902	929	5442	1055	2324	590	1458	3488	2731	1726	2879	4561
	Mollicutes_RF39		0	7	0	9	0	7	6	35	16	0	9	12	0	0	7	5	0	0	0	0
	Myxococcales		821	6728	3557	524	998	3157	1748	3087	5751	1782	7418	1051	3403	855	1332	3125	4639	1869	5630	3930
	NB1-j		0	30	10	0	0	0	0	0	196	0	480	11	234	21	0	10	47	354	1841	2040
	Nitrococcales		0	8	3	0	0	76	10	0	0	0	0	0	0	0	0	0	14	0	0	0
	Nitrosococcales		0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	2	0
	NRB23		32	12	0	10	0	0	0	0	31	36	0	0	0	0	25	6	10	0	0	0
	Obscuribacterales		49	23	0	6	0	0	138	178	449	42	63	0	11	39	22	9	27	0	0	23
	Oceanospirillales		37	0	0	0	0	0	0	475	169	22	155	107	117	0	12	11	75	186	3	0
	Oligoflexales		6	29	8	0	0	0	18	108	55	21	12	15	30	3	11	27	38	27	9	134
	OPB56		13	117	0	9	0	0	0	25	10	12	0	0	3	9	26	30	19	0	0	0
	Opitutales		0	0	0	0	0	0	16	37	16	15	3	28	0	0	0	3	0	41	0	2
	Paracaedibacterales		0	0	0	0	0	0	0	25	21	0	37	2	0	0	5	0	0	0	0	5
	Parvibaculales		13	20	9	0	0	0	48	133	21	19	57	97	27	12	13	19	21	69	5	32
	PB19		0	34	61	10	0	30	53	185	209	83	83	168	41	15	46	143	123	65	30	93
	Pedosphaerales		0	0	2	0	0	0	5	9	42	0	537	0	394	5	0	24	52	67	413	153
	Petrotogales		0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0
	Phycisphaerales		0	129	111	3	0	15	209	34	269	8	822	9	320	0	4	211	232	47	311	190
	Pirellulales		4	96	39	4	0	0	43	144	152	46	166	30	87	10	25	56	79	73	81	184
	Pla1_lineage		0	0	0	0	0	0	0	0	0	0	22	0	4	0	4	4	0	4	27	23
	Planctomycetales		0	7	7	6	0	0	21	36	65	17	48	56	54	9	10	45	44	25	9	29
	PLTA13		0	0	0	0	0	0	0	0	0	0	12	0	17	0	0	3	11	0	11	16
	Propionibacteriales		41	0	4	0	0	0	80	113	81	22	31	552	24	134	0	23	0	25	0	0
	Pseudomonadales		63	15	14	284	0	0	63	2101	510	2571	216	3059	82	7448	3159	39	72	1515	122	194
	Pseudonocardiales		197	123	32	123	0	13	96	19	25	46	336	33	151	39	0	0	0	107	173	301
	Puniceispirillales		0	0	8	0	0	0	4	25	57	5	90	0	40	0	0	0	0	7	45	26
	R7C24		42	257	291	13	0	390	287	546	1275	122	1033	128	537	39	177	723	1859	522	447	438

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
	Rhizobiales		2984	7704	6552	1274	3823	31399	13319	10483	16234	5169	11448	11125	3895	7236	2698	4092	3592	5151	3071	6446
	Rhodobacterales		45	24	7	16	0	0	0	495	29	67	24	626	8	1012	68	0	18	262	0	15
	Rhodospirillales		0	91	97	0	0	0	96	85	211	27	176	10	78	11	0	20	35	27	81	29
	Rhodothermales		1766	777	663	131	0	528	428	491	1132	780	3662	236	1479	831	334	466	443	476	1320	1817
	Rhodovibrionales		56	162	242	23	0	0	233	550	552	173	1234	18	575	55	48	216	270	68	28	99
	Rickettsiales		0	0	0	0	0	0	0	26	9	0	23	4	4	0	13	0	4	10	2	5
	S085		0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0	2	0	0
	Saccharimonadales		71	441	167	23	22	28	281	1487	974	289	396	1952	199	307	117	354	368	646	121	372
	SAR202_clade		0	3	5	0	0	0	0	0	0	0	32	0	16	0	0	8	21	0	0	16
	SAR324_clade(Marine_group_B)		0	0	0	0	0	0	0	24	46	0	20	12	13	0	0	2	57	5	0	0
	SBR1031		50	1145	1412	75	0	325	984	818	4758	1407	7212	151	2540	332	861	3643	3183	1827	8028	11532
	Selenomonadales		4	0	0	24	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0
	Sneathiellales		0	0	28	0	88	0	34	0	65	6	304	9	125	7	0	15	206	73	0	0
	Solibacterales		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
	Solirubrobacterales		152	627	240	100	32	248	196	405	1454	636	662	371	317	146	230	322	313	180	308	489
	Sphingobacteriales		737	220	562	35	7063	446	10342	1980	1551	32	457	2613	497	3831	1298	341	413	715	0	0
	Sphingomonadales		84	167	88	13	201	505	219	1182	533	33	723	1416	457	248	32	16	53	403	59	25
	Spirochaetales		0	0	0	0	0	0	0	40	0	0	0	0	0	0	0	0	0	109	0	0
Order	Steroidobacterales		83	1665	1629	53	293	591	944	818	3008	591	3031	70	1580	397	298	1997	2420	676	3077	2785
	Streptomycetales		29	29	0	40	246	0	11	20	0	26	3328	49	2043	92	0	0	0	84	51	154
	Streptosporangiales		1025	5400	4272	649	1551	3199	2602	2242	7692	3565	7387	449	4788	994	692	836	1181	1917	2631	3996
	Synergistales		7	0	0	0	0	0	0	7	0	0	3	0	0	0	0	0	5	2	0	0
	Tepidisphaerales		13	84	48	2	5	2	49	35	56	23	156	39	63	10	22	37	36	0	48	45
	Thalassobaculales		3	28	93	3	39	0	63	21	61	4	110	14	55	15	0	0	0	8	5	0
	Thermales		14981	718	25	8214	0	194	25	657	142	5909	245	227	67	1305	1961	114	153	103	96	221
	Thermoanaerobacterales		958	276	0	833	0	0	21	57	44	403	33	10	0	25	52	17	11	5	4	16
	Thermodesulfobacteriales		892	81	0	1700	0	0	0	10	0	415	0	0	0	11	49	10	31	0	12	22
	Thermomicrobiales		1042	1978	642	441	0	252	528	1051	2400	2063	2524	1051	1161	1039	1266	2038	1641	1461	2341	5009
	Thermophilales		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
	Thermotogales		344	7	0	77	0	211	0	43	0	66	0	3	0	10	0	0	5	0	0	0
	Thiomicrospirales		0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
	Unknown_Order		0	0	27	0	0	0	36	51	357	0	594	68	184	13	18	198	198	44	44	54
	Vampirovibrionales		0	0	0	0	0	0	0	0	12	0	15	0	11	0	0	4	0	0	0	0
	Verrucomicrobiales		31	73	81	0	85	270	60	2412	524	233	310	458	91	89	125	187	217	318	4	177
	Vibrionales		0	0	0	0	0	0	0	10	0	0	0	0	0	20	0	0	0	0	0	0
	WN-HWB-116		10	18	27	0	0	0	14	173	24	38	17	57	8	36	14	13	12	0	0	0
	Xanthomonadales		438	7969	17909	77	15837	24611	34233	14465	20179	677	6280	13220	2669	4817	1869	11428	14139	1901	1314	2545
	0319-6G20		6	0	0	0	0	0	0	47	0	2	12	0	6	3	0	8	25	7	5	5
Family	37-13		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0
	67-14		11	255	105	14	0	242	103	75	625	200	461	154	260	64	93	140	155	138	248	349

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
Family	A4b		24	341	449	7	0	86	513	208	1797	362	2143	60	1111	126	323	1223	971	661	2428	2348
	Acetobacteraceae		10	39	45	0	0	0	39	27	13	30	181	11	40	16	14	63	83	22	48	58
	Acholeplasmataceae		0	138	12	2	0	0	76	65	63	204	26	59	25	6	8	25	13	11	0	0
	Acidiferrobacteraceae		0	3	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	5
	Actinomycetaceae		38	0	0	8	0	0	0	0	22	22	0	0	0	0	0	0	0	0	0	0
	Aerococcaceae		0	0	0	45	0	0	6	1587	0	642	0	160	0	168	52	0	0	0	0	0
	Aeromonadaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0
	AKYG1722		10	30	55	5	0	0	83	89	282	53	888	75	394	44	15	155	200	178	553	475
	Alcanivoracaceae		30	0	0	0	0	0	0	103	0	4	14	11	25	0	0	0	0	9	0	0
	Alicyclobacillaceae		17	0	0	0	0	0	0	82	0	0	26	0	0	0	0	7	0	0	0	7
	Alteromonadaceae		0	0	0	0	0	0	0	0	0	0	0	5	0	0	11	0	0	180	0	0
	Anaerolineaceae		0	30	26	0	0	0	0	0	162	27	443	9	78	22	13	86	115	101	724	496
	Aquificaceae		48	0	0	4	0	0	0	0	0	13	0	0	0	0	22	0	0	0	0	0
	Archangiaceae		4	2	0	2	61	0	9	33	18	16	90	96	0	2	0	0	29	5	8	6
	Arcobacteraceae		0	0	0	0	0	0	0	188	0	551	0	5	0	10	0	0	0	71	0	0
	Ardenticatenaceae		6	0	7	0	0	0	6	0	26	26	74	15	38	0	10	29	37	40	137	129
	Azospirillaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	24	0	0	0
	Babeliaceae		0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
	Bacillaceae		11698	4612	1495	8395	3003	3507	1949	5157	4257	21821	5378	2146	1550	2599	4377	2507	2130	1575	2380	5610
	Bacteriovoracaceae		22	21	0	0	0	0	0	477	56	93	94	353	14	29	18	46	21	174	16	54
	Bacteroidaceae		0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Balneolaceae		0	0	0	0	0	0	0	36	0	0	0	7	0	0	0	0	0	5	0	0
	Bdellovibrionaceae		65	579	596	48	0	0	777	750	1289	469	1018	112	498	85	143	1059	903	173	349	446
	Beggiatoaceae		0	11	7	2	0	0	9	4	0	96	8	0	0	0	0	0	0	0	8	4
	Beijerinckiaceae		346	808	2155	90	3302	21638	3526	1075	2633	213	2865	439	661	766	149	300	260	223	159	435
	Bifidobacteriaceae		0	0	0	4	0	0	0	7	0	17	0	8	0	11	0	0	0	0	0	0
	BIrii41		130	510	879	64	344	697	631	580	2006	163	2990	158	1755	100	288	1599	1892	991	2001	936
	Blastocatellaceae		0	0	0	0	0	0	0	0	0	0	709	0	423	34	0	0	45	464	109	93
	Blfdi19		0	0	0	0	0	0	3	92	100	0	30	51	5	8	0	32	60	7	0	0
	Bogoriellaceae		228	44	94	64	0	300	432	432	240	134	167	467	38	190	10	9	0	48	0	9
	Bradymonadaceae		0	0	0	0	0	0	0	19	52	0	0	0	0	0	0	0	0	0	0	0
	Brevibacteriaceae		108	21	0	99	0	30	0	8	0	21	6	6	0	17	24	0	0	0	0	0
	Burkholderiaceae		329	967	1348	36	1527	2033	2470	4584	2402	520	1729	2512	843	986	977	2749	2282	1183	385	887
	Caldicoprobacteraceae		7836	976	76	2963	319	384	33	346	412	1959	389	127	100	330	456	131	185	88	82	153
	Caldilineaceae		17	287	291	5	105	305	377	521	1314	172	2097	146	857	86	175	824	864	288	916	1102
	Carnobacteriaceae		206	33	8	110	0	81	0	899	302	1191	0	395	0	654	867	0	0	104	0	0
	Caulobacteraceae		42	12	0	0	0	0	200	90	183	13	132	85	15	73	5	16	114	64	0	35
	Cellulomonadaceae		255	141	860	156	238	845	7006	1214	11848	574	8665	5597	4037	1057	164	1406	1639	726	496	1084
	Cellvibrionaceae		7	32	0	11	0	0	0	7467	157	1011	66	5659	32	7132	444	9	8	7029	7	16
	Chitinibacteraceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	4

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Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
Family	Chitinophagaceae		68	340	639	12	923	626	1833	2738	2231	68	1586	1091	1393	808	529	520	966	1059	8	25
	Chloroflexaceae		34	98	1500	0	235	1089	2703	311	3357	16	21337	105	13198	292	222	1429	2245	1454	296	303
	Christensenellaceae		13	19	0	6	44	0	4	120	19	9	33	0	0	6	0	0	0	64	0	0
	Chthoniobacteraceae		0	73	0	0	0	0	15	107	14	5	37	71	0	5	36	30	29	61	9	19
	Clostridiaceae_1		377	366	55	190	1539	405	52	2505	182	1458	151	27	37	127	260	188	153	256	61	208
	Clostridiaceae_2		14	0	0	0	32	0	0	12	0	0	0	0	0	0	0	0	0	0	0	18
	Clostridiaceae_4		71	92	6	191	0	5	51	0	0	96	103	11	35	17	33	0	9	22	0	20
	Clostridiales_Incertae_Sedis		0	9	0	0	0	0	4	29	11	27	44	10	8	0	0	0	0	12	0	16
	Clostridiales_vadinBB60_group		0	139	9	3	391	209	20	4	10	12	25	0	18	8	0	0	4	0	0	8
	Coriobacteriales_Incertae_Sedis		4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Corynebacteriaceae		744	121	14	375	0	287	10	393	41	442	51	179	0	185	57	22	0	52	0	44
	Corynebacteriales_Incertae_Sedis		7	0	6	0	0	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Crocinitomicaceae		0	0	0	0	0	0	0	16	0	0	0	152	0	233	674	2	0	148	0	0
	Cryomorphaceae		0	0	0	0	0	0	0	11	0	0	0	68	0	27	0	0	0	162	0	0
	Cyclobacteriaceae		0	0	5	0	0	0	4	149	55	0	314	497	96	241	0	0	0	1380	0	0
	Cytophagaceae		12	14	5	9	0	101	30	469	234	10	159	1338	35	410	61	175	114	568	11	28
	Deferribacteraceae		0	2	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0
	Defluviitaleaceae		733	5532	25	2309	686	521	142	355	250	500	51	30	70	369	295	419	202	23	18	61
	Demequinaceae		104	76	11	57	0	260	22	2418	743	819	442	3314	453	1328	387	235	1208	1243	89	807
	Dermabacteraceae		434	109	0	307	0	23	0	94	0	128	0	37	0	62	81	0	0	24	7	0
	Desulfarculaceae		0	0	0	0	0	0	0	0	0	0	118	0	50	4	0	0	5	10	50	15
	Desulfobulbaceae		0	0	0	0	0	0	0	24	0	0	0	0	0	0	0	0	0	16	0	0
	Desulfomicrobiaceae		4	16	0	13	0	0	0	0	60	50	10	3	19	0	17	51	61	22	5	0
	Desulfovibrionaceae		0	0	0	0	0	0	0	5	0	0	5	0	0	0	0	0	0	4	0	0
	DEV007		0	0	0	0	0	0	0	555	35	53	20	105	10	11	0	5	0	93	0	121
	Devosiaceae		77	41	0	71	0	0	0	1149	184	186	51	4077	10	2073	117	17	11	1551	48	129
	Dietziaceae		104	31	8	44	0	0	0	24	12	40	0	0	0	15	22	35	0	20	0	10
	Dysgonomonadaceae		59	0	0	9	0	0	0	657	40	252	190	187	49	60	16	0	48	331	8	12
	Eggerthellaceae		0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0
	Enterobacteriaceae		5	3	0	0	0	0	0	15	328	0	0	0	0	4	0	0	0	0	0	9
	Enterococcaceae		51	6	0	53	73	0	0	2633	0	1980	0	146	0	891	109	0	0	115	0	0
	Erysipelotrichaceae		0	0	0	0	0	0	0	43	5	297	0	0	0	3	50	0	0	31	0	0
	Eubacteriaceae		87	69	6	66	0	0	0	105	90	116	40	14	30	21	14	7	0	35	30	53
	Euzebyaceae		5	71	35	0	0	353	53	27	191	41	332	21	144	37	57	329	240	88	253	361
	Family_III		584	245	0	574	0	0	21	57	37	288	33	0	0	15	24	17	11	0	4	7
	Family_X		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
	Family_XI		1636	639	2	2615	0	0	21	708	253	3209	166	104	44	151	313	98	57	97	117	296
	Family_XII		0	0	0	0	14	0	0	92	12	162	0	847	0	332	30	0	0	43	0	0
	Family_XIII		10	11	0	7	0	0	0	45	12	22	32	0	5	14	8	16	20	21	0	0
	Family_XIV		0	0	0	0	0	0	0	0	4	19	0	0	0	0	11	11	8	0	0	0

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
Family	Family_XVII		422	15	0	75	0	0	0	6	56	85	20	16	0	98	42	7	0	0	5	14
	Family_XVIII		1363	338	17	581	244	555	30	56	116	469	191	95	94	135	389	295	128	127	142	450
	Fervidobacteriaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
	Fibrobacteraceae		0	0	0	0	0	0	0	124	0	7	0	43	0	0	0	0	0	415	0	0
	Flavobacteriaceae		56	0	0	59	0	0	0	3574	51	417	70	2599	204	7414	3730	2	746	1961	456	172
	Fodinicurvataceae		46	149	160	14	0	0	186	536	317	160	103	15	69	24	48	85	74	4	16	83
	Gemmataceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	Gemmatimonadaceae		0	49	27	0	0	0	13	0	4	0	36	32	0	0	0	14	33	18	0	0
	Geobacteraceae		0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0
	Geodermatophilaceae		0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0
	Glycomycetaceae		59	25	10	13	0	0	0	2	0	9	8	0	0	0	9	0	0	2	0	6
	Gracilibacteraceae		37	3	0	85	0	0	0	7	0	262	29	0	9	5	52	21	0	9	8	5
	Halanaerobiaceae		151	355	48	2170	0	0	35	61	36	386	143	55	47	54	9	4	0	13	4	70
	Haliangiaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0
	Halieaceae		0	0	0	0	0	0	0	25	0	0	0	0	0	8	0	0	0	0	0	0
	Halomonadaceae		0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	11	0	0
	Haloplasmataceae		262	236	2	276	29	0	23	35	25	842	63	3	9	22	61	15	6	14	24	31
	Halothiobacillaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0
	Heliobacteriaceae		448	1093	32	4944	3	64	15	28	254	697	280	25	35	67	280	153	130	44	51	156
	Hydrogenedensaceae		0	0	0	0	0	0	9	0	41	0	149	0	44	5	0	14	54	9	17	18
	Hydrogenophilaceae		1881	72	6	1627	0	16	4	56	0	398	0	0	0	102	97	29	31	12	16	0
	Hymenobacteraceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
	Hyphomicrobiaceae		483	2187	1010	332	51	582	680	924	2663	1662	2326	299	925	492	923	1575	1032	826	1481	2694
	Hyphomonadaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0
	Iamiaceae		22	1215	308	28	0	674	471	273	1801	232	2675	210	1090	304	296	1549	1140	365	1278	1456
	Idiomarinaceae		0	0	0	0	0	0	0	82	0	60	0	39	0	52	13	0	0	11	0	0
	Ilumatobacteraceae		9	0	28	0	0	38	28	127	0	0	316	41	147	8	20	59	67	80	46	64
	Inquilinaceae		0	0	0	0	0	0	0	79	39	0	27	18	24	0	9	0	33	11	0	0
	Intrasporangiaceae		35	0	0	4	0	0	66	173	246	73	156	583	49	124	167	253	271	165	274	434
	JG30-KF-CM45		38	160	118	52	0	0	169	293	744	236	1046	536	437	153	200	665	570	516	1101	1523
	Jonesiaceae		0	0	0	18	0	0	0	212	12	0	0	215	0	57	0	0	0	0	0	0
	Kallotenuaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0
	Kiloniellaceae		10	13	82	9	0	0	47	14	235	13	1131	3	506	31	0	131	196	64	12	16
	Lachnospiraceae		100	675	58	62	474	222	127	272	239	183	580	120	117	168	114	112	102	139	71	154
	Lactobacillaceae		219	8	0	129	0	0	12	12	9	6	0	3	0	0	0	0	0	0	0	2
	Legionellaceae		0	0	0	0	0	0	0	0	0	0	12	43	25	5	0	0	0	7	4	0
	Lentimicrobiaceae		0	8	0	0	0	0	9	0	29	0	0	0	0	0	0	54	78	0	0	0
	Leptospiraceae		11	6	4	5	0	0	0	24	18	196	11	0	15	15	0	9	42	5	0	16
	Leuconostocaceae		110	10	0	39	0	0	0	40	0	19	0	10	0	11	0	0	0	0	0	0
	Limnochordaceae		11047	4856	1000	3064	602	1126	541	1981	4222	14509	1870	487	604	1583	4862	1349	880	1283	2128	5241

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
Family	Longimicrobiaceae		0	82	38	0	0	27	37	179	182	25	240	43	132	3	10	98	87	45	123	126
	Marinilabiliaceae		83	12	27	19	0	0	84	2451	1423	118	572	1601	995	258	30	60	955	783	4	8
	Methanosarcinaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0
	Methanothermobacteraceae		0	0	0	0	0	0	0	0	0	0	0	12	0	0	21	0	20	0	0	0
	Methylococcaceae		430	2244	1081	163	229	1433	571	2827	1842	695	1013	435	446	559	252	420	386	143	294	462
	Methylophagaceae		0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0
	Methylophilaceae		0	0	0	0	0	0	0	140	0	0	28	28	47	0	0	42	210	27	0	0
	Micavibrionaceae		0	0	0	0	0	0	0	39	0	40	0	0	0	0	0	0	0	0	0	0
	Microbacteriaceae		144	69	37	98	0	37	15	784	342	371	218	4368	43	2188	169	73	174	628	96	310
	Micrococcaceae		178	81	0	189	0	39	0	109	31	44	0	1963	0	2409	7812	20	0	421	386	22
	Micrococcales_Incertae_Sedis		0	0	0	0	0	0	0	256	0	74	0	303	0	1048	0	0	0	0	0	0
	Micromonosporaceae		223	1517	337	117	273	467	138	508	849	771	794	93	300	139	360	151	110	350	284	1056
	Microscillaceae		0	11	23	0	0	0	57	60	1136	3	2730	30	1980	70	7	507	1159	518	194	337
	Microtrichaceae		40	485	384	32	60	450	1401	881	1143	244	861	473	396	120	281	640	643	219	152	391
	Midichloriaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
	ML635J-40_aquatic_group		0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
	Moraxellaceae		7	0	14	244	0	0	0	362	338	907	52	2841	20	7070	2734	3	25	476	24	5
	MWH-CFBk5		0	0	0	0	0	0	0	715	0	13	0	282	0	26	0	0	0	105	0	0
	Mycobacteriaceae		0	13	9	0	0	0	15	31	44	9	113	18	57	21	23	55	37	0	34	46
	Nakamurellaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0
	Nannocystaceae		0	0	0	0	0	0	0	0	24	0	133	0	21	13	7	306	899	38	83	18
	Nitrosococcaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
	Nitrosomonadaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0
	Nocardiaceae		4	0	0	2	0	0	0	0	0	11	0	0	0	0	0	0	3	0	0	0
	Nocardioidaceae		41	0	4	0	0	0	80	113	81	22	31	552	24	134	0	23	0	25	0	0
	Nocardiopsaceae		449	2987	1268	279	1063	2614	877	875	1181	1328	676	177	359	646	280	171	117	195	129	481
	NS11-12_marine_group		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0
	NS9_marine_group		0	0	0	0	0	0	0	687	0	10	5	495	0	25	163	17	134	380	0	10
	Oceanibaculaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
	Oligoflexaceae		0	29	8	0	0	0	18	61	55	19	0	15	24	0	11	19	13	20	4	129
	Opitutaceae		0	0	0	0	0	0	0	6	0	0	3	0	0	0	0	0	0	31	0	0
	P3OB-42		0	0	0	0	0	0	0	0	0	0	15	0	7	0	10	36	58	0	5	10
	Paenibacillaceae		4582	4037	1515	647	1268	6288	1993	5282	4450	3178	8677	3413	1791	2190	1287	1321	1186	1014	1338	2788
	Paludibacteraceae		5	0	0	0	0	0	0	0	0	19	0	17	0	6	0	0	0	22	0	0
	Paracaedibacteraceae		0	0	0	0	0	0	0	25	21	0	37	2	0	0	5	0	0	0	0	5
	Parachlamydiaceae		0	0	0	0	0	0	0	0	0	0	3	6	0	0	0	0	0	0	0	0
	Parvibaculaceae		13	20	9	0	0	0	48	133	21	19	57	97	27	12	13	19	21	69	5	32
	Pedosphaeraceae		0	0	2	0	0	0	5	9	42	0	537	0	394	5	0	24	52	67	413	153
	Peptococcaceae		801	916	53	1649	860	598	135	316	285	744	566	116	125	284	166	134	90	88	53	134
	Peptostreptococcaceae		9	7	6	6	550	0	0	365	58	89	8	0	0	0	0	0	0	124	0	15

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Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
Family	Petrotogaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0
	Phaselicystidaceae		108	2289	591	86	79	0	234	236	439	541	532	96	302	138	368	349	339	165	411	834
	Phycisphaeraceae		0	129	111	3	0	15	209	34	269	8	822	9	320	0	4	211	232	47	311	190
	Pirellulaceae		4	96	39	4	0	0	43	144	152	46	166	30	87	10	25	56	79	73	81	184
	Planococcaceae		756	1120	85	651	879	830	3513	1118	1171	6189	2279	4858	689	5000	12858	1884	823	1024	1471	7975
	Polyangiaceae		0	5	0	0	0	0	0	0	0	0	109	0	71	0	0	28	537	152	2171	337
	Porticoccaceae		0	0	0	4	0	0	0	532	26	59	7	77	6	69	8	0	5	11	0	0
	Prolixibacteraceae		0	0	0	0	0	0	0	12	0	0	0	12	0	0	0	0	0	51	0	0
	Promicromonosporaceae		0	0	0	14	0	0	0	14	0	10	0	357	0	92	0	0	0	20	0	0
	Pseudohongiellaceae		0	0	0	0	0	0	0	226	162	7	121	90	82	0	12	0	53	161	3	0
	Pseudomonadaceae		56	15	0	40	0	0	63	1739	172	1664	164	218	62	378	425	36	47	1039	98	189
	Pseudonocardiaceae		197	123	32	123	0	13	96	19	25	46	336	33	151	39	0	0	0	107	173	301
	Puniceicoccaceae		0	0	0	0	0	0	16	13	0	15	0	25	0	0	0	3	0	10	0	2
	Puniceispirillales_Incertae_Sedis		0	0	8	0	0	0	4	25	57	5	90	0	40	0	0	0	0	7	45	26
	Rhizobiaceae		2038	4651	3357	770	470	9179	9066	7173	10583	3080	6045	6162	2132	3853	1393	2148	2233	2453	1305	3161
	Rhizobiales_Incertae_Sedis		0	0	0	0	0	0	0	12	13	8	0	82	0	12	3	0	0	23	0	0
	Rhodanobacteraceae		160	55	357	13	13	50	308	1926	3066	123	142	527	133	38	220	1099	1323	92	5	41
	Rhodobacteraceae		45	24	7	16	0	0	0	495	29	67	24	626	8	1012	68	0	18	262	0	15
	Rhodobiaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
	Rhodocyclaceae		35	30	29	20	0	0	32	290	0	11	42	23	14	21	10	4	0	114	22	34
	Rhodopirillaceae		0	91	97	0	0	0	96	44	211	27	176	0	78	6	0	20	35	27	81	29
	Rhodothermaceae		1766	777	663	131	0	528	428	491	1132	780	3662	236	1479	831	334	466	443	476	1320	1817
	Rickettsiaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
	Rikenellaceae		0	0	0	0	0	0	0	50	0	7	0	0	0	0	0	0	0	20	0	0
	Roseiflexaceae		26	69	603	0	0	292	1335	165	1015	7	4116	34	1518	59	301	3296	4501	1474	4006	2336
	Ruaniaceae		20	6	0	0	0	0	6	8	0	0	0	0	0	0	0	0	0	0	0	0
	Rubinisphaeraceae		0	7	7	6	0	0	21	36	65	17	48	54	51	9	10	45	44	25	9	23
	Rubritaleaceae		0	0	0	0	0	0	0	338	0	15	4	59	0	55	6	0	0	83	0	0
	Ruminococcaceae		16242	9645	158	8622	1860	1231	560	1778	1915	7143	1521	313	547	887	1814	2481	1837	375	188	686
	Saccharimonadaceae		27	22	0	10	0	0	86	755	145	62	29	1368	0	214	18	25	25	190	10	14
	Saccharospirillaceae		0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0
	Sandaracinaceae		89	2442	1528	23	355	1823	502	1332	2045	164	2538	531	896	369	174	502	598	199	304	598
	Sanguibacteraceae		0	0	0	0	0	0	0	94	0	30	0	145	0	120	83	0	0	0	0	0
	Saprospiraceae		0	0	0	0	0	0	0	620	40	0	4	88	4	37	0	0	0	754	0	0
	SC-I-84		0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0
	Shewanellaceae		0	0	0	0	0	0	0	0	0	5	0	0	0	0	10	0	0	5	0	0
	SM2D12		0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	4	0	0
	Sneathiellaceae		0	0	28	0	88	0	34	0	65	6	304	9	125	7	0	15	206	73	0	0
	Solibacteraceae_(Subgroup_3)		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
	Solirubrobacteraceae		141	372	135	86	32	6	93	330	829	436	201	217	57	82	137	182	158	42	60	140

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
Family	Sphingobacteriaceae		737	212	562	35	7063	446	10333	1899	1522	22	457	2613	479	3831	1268	246	270	696	0	0
	Sphingomonadaceae		84	167	88	13	201	505	219	1182	533	33	723	1416	457	248	32	16	53	403	59	25
	Spirochaetaceae		0	0	0	0	0	0	0	40	0	0	0	0	0	0	0	0	0	109	0	0
	Spirosomaceae		35	0	0	18	0	0	0	2697	44	43	124	1299	242	307	131	8	125	570	0	8
	Spongiibacteraceae		0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0
	Sporolactobacillaceae		6	0	0	0	0	0	7	0	0	5	0	0	0	0	0	0	0	0	0	0
	SRB2		11	0	0	0	0	0	0	0	7	2	0	0	0	0	0	0	0	5	0	0
	SS1-B-06-26		7	0	0	0	0	0	0	146	7	0	20	0	10	0	0	11	22	5	0	0
	Staphylococcaceae		145	22	0	145	0	0	0	21	25	23	2	245	0	69	30	0	0	3	0	0
	Stappiaceae		0	0	0	0	0	0	0	0	13	0	88	0	115	12	0	0	0	11	0	0
	Steroidobacteraceae		83	1665	1629	53	293	591	944	818	3008	591	3008	70	1566	397	298	1997	2420	676	3037	2772
	Streptococcaceae		126	21	0	135	18	120	0	151	11	0	15	30	0	50	0	0	0	0	0	0
	Streptomycetaceae		29	29	0	40	246	0	11	20	0	26	3328	49	2043	92	0	0	0	84	51	154
	Streptosporangiaceae		427	2121	2818	281	232	520	1611	1173	6289	1330	5860	192	3890	245	283	585	998	1194	2002	2409
	Synergistaceae		7	0	0	0	0	0	0	7	0	0	3	0	0	0	0	0	5	2	0	0
	Syntrophomonadaceae		130	177	3	517	0	0	0	38	35	159	47	11	6	14	7	22	48	0	0	19
	Tannerellaceae		0	0	0	0	0	0	0	8	0	14	0	0	0	0	0	0	0	5	0	0
	Terrimicrobiaceae		0	0	0	0	0	0	0	0	0	0	32	0	0	0	0	6	13	0	0	0
	Thermaceae		14981	718	25	8214	0	194	25	657	142	5909	245	227	67	1305	1961	114	153	103	96	221
	Thermoactinomycetaceae		514	1926	195	272	1681	372	740	1105	1508	967	975	196	206	275	662	752	775	261	228	682
	Thermoanaerobacteraceae		350	31	0	259	0	0	0	0	0	113	0	0	0	10	28	0	0	0	0	9
	Thermodesulfobacteriaceae		892	81	0	1700	0	0	0	10	0	415	0	0	0	11	49	10	31	0	12	22
	Thermoleophilaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
	Thermomicrobiaceae		994	1788	469	384	0	252	276	669	1374	1774	590	440	330	842	1051	1218	871	767	687	3011
	Thermomonosporaceae		149	292	186	89	256	65	114	194	222	907	851	80	539	103	129	80	66	528	500	1106
	Thermopetrobacteraceae		0	0	0	0	0	0	3	0	0	0	10	0	0	0	0	0	0	0	13	0
	Thermotogaceae		344	7	0	77	0	211	0	43	0	66	0	3	0	10	0	0	0	0	0	0
	Thioalkalispiraceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0
	Thiomicrospiraceae		0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
	TRA3-20		0	0	0	0	0	0	0	0	0	0	56	0	34	0	0	10	7	0	48	24
	Trueperaceae		167	702	799	48	0	95	773	2446	4809	1751	4804	1040	1599	308	401	1005	818	909	1951	2350
	Unknown_Family		5	22	63	0	221	99	101	64	439	0	1587	309	461	17	29	266	269	64	63	54
	Veillonellaceae		0	0	0	13	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
	Verrucomicrobiaceae		31	0	0	0	0	0	0	1408	51	118	9	280	0	15	44	0	23	104	4	26
	Vibrionaceae		0	0	0	0	0	0	0	10	0	0	0	0	0	20	0	0	0	0	0	0
	Vulgatibacteraceae		490	1480	556	349	159	637	369	803	1119	898	981	119	341	225	485	263	227	312	641	1191
	WD2101_soil_group		13	84	48	2	5	2	49	35	56	23	156	39	63	10	22	37	36	0	48	45
	Weeksellaceae		78	12	16	20	0	0	32	2029	313	163	50	716	57	224	1204	40	191	555	534	241
	Woeseiaceae		0	0	0	0	0	0	0	0	0	0	23	0	14	0	0	0	0	0	40	13
	Xanthobacteraceae		29	0	0	0	0	0	17	22	81	0	46	0	32	0	99	24	34	17	59	22

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Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
Family	Xanthomonadaceae		278	7914	17548	64	15824	24561	33925	12539	17113	554	6138	12691	2536	4779	1649	10329	12816	1766	1309	2504
	Xiphinematobacteraceae		0	11	5	0	173	29	26	15	67	0	1779	20	789	37	0	48	107	155	9	19
Genus-Species	**Acetanaerobacterium**		**10**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Acetivibrio		**20**	**479**	**65**	**10**	**816**	**429**	**176**	**0**	**381**	**0**	**139**	**0**	**74**	**0**	**92**	**245**	**186**	**45**	**29**	**83**
	Acetoanaerobium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**200**	**0**	**13**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**78**	**0**	**0**
	Acholeplasma		**0**	**138**	**12**	**2**	**0**	**0**	**76**	**65**	**63**	**204**	**26**	**59**	**25**	**6**	**8**	**25**	**13**	**11**	**0**	**0**
	laidlawii		0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0
	Acidibacter		**0**	**13**	**36**	**0**	**221**	**99**	**65**	**0**	**43**	**0**	**987**	**0**	**277**	**0**	**0**	**22**	**25**	**11**	**19**	**0**
	Acidovorax		**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**15**	**0**	**0**
	radicis		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0
	Acinetobacter		**7**	**0**	**0**	**241**	**0**	**0**	**0**	**231**	**329**	**828**	**2**	**2647**	**0**	**6205**	**105**	**0**	**13**	**156**	**24**	**0**
	indicus		0	0	0	0	0	0	0	0	0	0	0	44	0	0	0	0	0	0	0	0
	johnsonii		0	0	0	0	0	0	0	0	98	0	0	0	0	0	0	0	0	0	0	0
	lwoffii		0	0	0	0	0	0	0	113	12	402	0	434	0	1927	43	0	0	133	0	0
	towneri		0	0	0	0	0	0	0	57	0	0	0	0	0	0	0	0	0	0	0	0
	Actinocatenispora		**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Actinomadura		**17**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**16**
	Actinomyces		**13**	**0**	**0**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Actinotalea		**218**	**124**	**860**	**134**	**238**	**835**	**7006**	**1071**	**11502**	**529**	**8437**	**3432**	**3826**	**892**	**162**	**1406**	**1630**	**393**	**496**	**1084**
	Advenella		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**9**	**0**	**25**	**4**	**0**	**0**	**10**	**0**	**0**	**0**	**6**	**0**	**0**
	alkanexedens		0	0	0	0	0	0	0	9	0	25	4	0	0	10	0	0	0	6	0	0
	Aequorivita		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**30**	**0**	**0**	**0**	**0**	**0**	**0**	**9**	**0**	**0**	**2**	**0**	**0**
	Aeribacillus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**61**	**20**	**9**	**2**	**0**	**0**	**0**	**0**	**0**	**0**
	pallidus		0	0	0	0	0	0	0	0	0	0	22	0	9	0	0	0	0	0	0	0
	Aerococcus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**26**	**0**	**10**	**0**	**0**	**0**	**0**	**0**	**0**
	Aeromicrobium		**41**	**0**	**4**	**0**	**0**	**0**	**68**	**53**	**81**	**22**	**23**	**308**	**0**	**54**	**0**	**17**	**0**	**6**	**0**	**0**
	Aeromonas		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**8**	**0**	**0**
	Aerosphaera		**0**	**0**	**0**	**29**	**0**	**0**	**6**	**1525**	**0**	**642**	**0**	**134**	**0**	**158**	**0**	**0**	**0**	**0**	**0**	**0**
	taetra		0	0	0	29	0	0	0	577	0	214	0	49	0	48	0	0	0	0	0	0
	Aestuariicella		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**21**	**0**	**0**	**0**	**0**	**0**	**0**
	AKYG587		**0**	**6**	**26**	**0**	**0**	**0**	**49**	**0**	**29**	**0**	**59**	**0**	**30**	**0**	**0**	**25**	**21**	**0**	**40**	**9**
	Alcanivorax		**30**	**0**	**0**	**0**	**0**	**0**	**0**	**103**	**0**	**4**	**14**	**11**	**25**	**0**	**0**	**0**	**0**	**9**	**0**	**0**
	Algoriphagus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**5**	**0**	**0**	**0**	**98**	**0**	**0**
	Alishewanella		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**18**	**0**	**0**
	Alkalibacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**9**	**0**	**0**
	Alkalibaculum		**0**	**9**	**0**	**0**	**0**	**0**	**0**	**13**	**22**	**19**	**16**	**0**	**7**	**0**	**0**	**0**	**0**	**0**	**0**	**9**
	Alkaliphilus		**14**	**0**	**0**	**0**	**32**	**0**	**0**	**12**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**18**
	Allorhizobium-Neorhizobium-Pararhizob		**32**	**9**	**0**	**7**	**0**	**38**	**0**	**81**	**0**	**6**	**15**	**723**	**0**	**668**	**23**	**0**	**0**	**36**	**0**	**6**
	Altererythrobacter		**12**	**0**	**0**	**4**	**0**	**0**	**117**	**440**	**217**	**33**	**615**	**1123**	**423**	**121**	**16**	**0**	**9**	**278**	**54**	**9**
	Alterococcus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors	Compost yard	A							B			C				D			E		
	Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level	Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
	Amaricoccus	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Aminobacter	**90**	**59**	**0**	**38**	**0**	**0**	**0**	**224**	**132**	**87**	**121**	**469**	**37**	**290**	**51**	**24**	**18**	**259**	**17**	**73**
	Ammoniibacillus	**224**	**139**	**12**	**32**	**0**	**0**	**36**	**50**	**130**	**200**	**103**	**37**	**54**	**52**	**35**	**21**	**0**	**43**	**40**	**81**
	agariperforans	224	139	12	32	0	0	36	50	130	200	103	37	54	52	35	19	0	43	40	81
	Ammoniphilus	**231**	**310**	**47**	**57**	**36**	**26**	**97**	**392**	**248**	**305**	**431**	**100**	**130**	**133**	**94**	**112**	**134**	**80**	**111**	**272**
	oxalivorans	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Amphibacillus	**11**	**0**	**0**	**0**	**0**	**0**	**0**	**13**	**56**	**7**	**0**	**0**	**0**	**0**	**10**	**17**	**0**	**0**	**0**	**0**
	Anaerobacillus	**91**	**95**	**15**	**41**	**0**	**78**	**14**	**17**	**77**	**176**	**135**	**35**	**17**	**43**	**14**	**0**	**14**	**0**	**15**	**22**
	Anaerocella	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**17**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**12**	**0**	**0**
	Anaerococcus	**14**	**0**	**0**	**22**	**0**	**0**	**0**	**7**	**15**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Anaerofustis	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**
	Anaerolinea	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**15**	**0**	**69**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Anaeromyxobacter	**4**	**2**	**0**	**2**	**61**	**0**	**9**	**33**	**18**	**16**	**90**	**96**	**0**	**2**	**0**	**0**	**29**	**5**	**8**	**6**
	Anaerosalibacter	**0**	**7**	**0**	**6**	**0**	**0**	**0**	**28**	**0**	**22**	**0**	**0**	**0**	**0**	**3**	**10**	**0**	**9**	**21**	**44**
	bizertensis	0	7	0	6	0	0	0	28	0	22	0	0	0	0	3	10	0	9	21	44
	Anaerosporobacter	**17**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Anaerovorax	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**39**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**21**	**0**	**0**
	Aneurinibacillus	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**12**
	Anoxybacillus	**0**	**0**	**11**	**0**	**0**	**0**	**20**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**9**
Genus-	**Anseongella**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Species	**Antricoccus**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Aquamicrobium	**413**	**220**	**144**	**165**	**0**	**837**	**698**	**2061**	**1224**	**799**	**607**	**2412**	**260**	**708**	**350**	**367**	**401**	**731**	**212**	**466**
	aerolatum	0	0	0	0	0	164	0	130	0	0	0	193	0	160	0	0	0	102	0	0
	Arcobacter	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**188**	**0**	**551**	**0**	**5**	**0**	**10**	**0**	**0**	**0**	**71**	**0**	**0**
	cryaerophilus	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0
	skirrowii	0	0	0	0	0	0	0	188	0	531	0	0	0	10	0	0	0	18	0	0
	Arcticibacter	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Arenibacter	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**0**	**0**	**0**	**10**	**0**	**0**
	Arenimonas	**10**	**0**	**0**	**5**	**0**	**0**	**0**	**662**	**0**	**65**	**0**	**54**	**0**	**33**	**0**	**0**	**0**	**45**	**0**	**5**
	Arthrobacter	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**9**	**353**	**0**	**0**	**48**	**96**	**0**
	agilis	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0
	psychrolactophilus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	61	0	0	0	0	0
	Asticcacaulis	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**33**	**0**	**0**
	Atopostipes	**140**	**18**	**0**	**81**	**0**	**0**	**0**	**28**	**0**	**25**	**0**	**0**	**0**	**5**	**5**	**0**	**0**	**0**	**0**	**0**
	suicloacalis	128	18	0	81	0	0	0	28	0	25	0	0	0	5	5	0	0	0	0	0
	Azoarcus	**17**	**0**	**0**	**0**	**0**	**0**	**0**	**186**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**82**	**0**	**0**
	Bacillus	**1292**	**1208**	**300**	**928**	**1586**	**1363**	**1162**	**1061**	**801**	**3195**	**1919**	**658**	**636**	**786**	**722**	**642**	**442**	**391**	**786**	**1640**
	borbori	46	13	11	0	0	108	0	0	0	62	141	66	171	82	76	93	50	43	44	118
	coagulans	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0
	foraminis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
	fordii		0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0
	galactosidilyticus		0	0	0	0	0	54	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	gottheilii		0	0	0	0	0	0	71	0	0	0	0	0	0	0	0	0	0	0	0	0
	graminis		99	37	7	33	0	0	41	43	43	169	26	37	27	21	0	15	13	0	10	11
	infernus		0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
	maritimus		0	0	0	0	0	0	0	0	21	33	0	0	0	0	0	0	0	0	0	0
	phocaeensis		0	0	0	0	0	0	0	0	0	235	30	0	0	76	0	0	0	0	0	0
	smithii		0	0	0	0	0	0	0	0	0	0	42	0	9	16	0	0	0	0	0	0
	thermolactis		0	44	0	30	0	152	0	0	0	70	94	0	0	0	0	0	0	0	0	0
	Bacteriovorax		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**0**
	Bacteroides		**0**	**0**	**0**	**11**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	graminisolvens		0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Bauldia		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**12**	**13**	**8**	**0**	**82**	**0**	**12**	**3**	**0**	**0**	**23**	**0**	**0**
	BD1-7_clade		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**
	Bdellovibrio		**25**	**147**	**53**	**15**	**0**	**0**	**112**	**339**	**254**	**103**	**147**	**71**	**70**	**13**	**51**	**205**	**190**	**68**	**140**	**252**
	bacteriovorus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	Bergeyella		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**178**	**0**	**0**	**0**	**0**	**0**
	Bhargavaea		**0**	**12**	**0**	**0**	**0**	**0**	**0**	**0**	**22**	**39**	**0**	**0**	**0**	**15**	**11**	**0**	**5**	**0**	**0**	**0**
	Bifidobacterium		**0**	**0**	**0**	**4**	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Genus-	**BIyi10**		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**226**	**162**	**7**	**121**	**82**	**82**	**0**	**9**	**0**	**53**	**161**	**3**	**0**
Species	**Blastocatella**		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**709**	**0**	**421**	**34**	**0**	**0**	**45**	**464**	**109**	**93**
	Blautia		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Bordetella		**20**	**86**	**286**	**0**	**360**	**895**	**190**	**342**	**304**	**0**	**191**	**37**	**71**	**35**	**21**	**26**	**27**	**24**	**29**	**30**
	Bosea		**5**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**11**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Brachybacterium		**434**	**109**	**0**	**307**	**0**	**23**	**0**	**94**	**0**	**128**	**0**	**37**	**0**	**62**	**81**	**0**	**0**	**24**	**7**	**0**
	paraconglomeratum		0	0	0	0	0	0	0	0	0	0	0	0	0	0	62	0	0	0	0	0
	Bradyrhizobium		**29**	**0**	**0**	**0**	**0**	**0**	**17**	**22**	**63**	**0**	**0**	**0**	**0**	**0**	**99**	**6**	**0**	**0**	**0**	**0**
	Brevibacillus		**46**	**27**	**20**	**10**	**144**	**140**	**59**	**84**	**19**	**48**	**138**	**50**	**20**	**0**	**0**	**25**	**0**	**0**	**0**	**18**
	thermoruber		0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Brevibacterium		**108**	**21**	**0**	**99**	**0**	**30**	**0**	**8**	**0**	**21**	**6**	**6**	**0**	**17**	**24**	**0**	**0**	**0**	**0**	**0**
	avium		108	21	0	79	0	0	0	8	0	21	0	0	0	0	24	0	0	0	0	0
	Brevundimonas		**7**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**37**	**0**	**54**	**0**	**0**	**0**	**11**	**0**	**0**
	faecalis		0	0	0	0	0	0	0	0	0	0	0	10	0	20	0	0	0	0	0	0
	terrae		0	0	0	0	0	0	0	0	0	0	0	0	0	34	0	0	0	0	0	0
	BRH-c8a		**11**	**63**	**0**	**66**	**0**	**0**	**10**	**0**	**18**	**77**	**40**	**0**	**7**	**13**	**10**	**25**	**11**	**0**	**7**	**10**
	Brockia		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**13**	**0**	**0**	**0**	**0**	**0**
	Brumimicrobium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**5**	**0**	**0**	**0**	**0**	**0**
	Bryobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**
	C1-B045		**0**	**0**	**0**	**4**	**0**	**0**	**0**	**532**	**26**	**59**	**7**	**77**	**6**	**69**	**8**	**0**	**5**	**11**	**0**	**0**
	Caldalkalibacillus		**317**	**101**	**24**	**170**	**0**	**0**	**7**	**107**	**106**	**307**	**97**	**66**	**28**	**33**	**74**	**41**	**43**	**33**	**40**	**91**

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors	Compost yard	A							B			C				D			E		
	Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level	Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
Genus-Species	**Caldanaerobacter**	**223**	**10**	**0**	**50**	**0**	**0**	**0**	**0**	**0**	**12**	**0**	**0**	**0**	**5**	**5**	**0**	**0**	**0**	**0**	**0**
	subterraneus	21	0	0	7	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0
	Caldibacillus	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**27**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Caldicoprobacter	**7836**	**976**	**76**	**2963**	**319**	**384**	**33**	**346**	**412**	**1959**	**389**	**127**	**100**	**330**	**456**	**131**	**185**	**88**	**82**	**153**
	Caldinitratiruptor	**62**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**0**	**0**	**0**	**0**	**0**
	Calditerricola	**28**	**31**	**11**	**19**	**0**	**135**	**0**	**0**	**16**	**28**	**14**	**7**	**8**	**0**	**0**	**26**	**15**	**5**	**14**	**34**
	yamamurae	28	31	11	19	0	0	0	0	16	28	14	7	8	0	0	26	15	5	14	34
	Camelimonas	**65**	**18**	**0**	**22**	**0**	**0**	**9**	**31**	**0**	**0**	**0**	**36**	**0**	**11**	**15**	**9**	**0**	**0**	**0**	**0**
	Caminicella	**21**	**21**	**0**	**76**	**0**	**0**	**0**	**0**	**0**	**17**	**0**	**11**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Candidatus_Berkiella	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**241**	**0**	**0**	**0**	**0**	**0**	**9**	**0**	**0**
	Candidatus_Chloroploca	**6**	**85**	**197**	**0**	**4**	**13**	**130**	**53**	**1429**	**0**	**19084**	**89**	**12306**	**221**	**177**	**1196**	**1894**	**1320**	**296**	**303**
	Candidatus_Desulforudis	**5**	**9**	**0**	**0**	**0**	**0**	**0**	**13**	**39**	**0**	**6**	**0**	**0**	**4**	**11**	**46**	**46**	**0**	**0**	**0**
	Candidatus_Dichloromethanomonas	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**16**	**0**	**15**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Candidatus_Jidaibacter	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**0**
	Candidatus_Paracaedibacter	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**25**	**21**	**0**	**37**	**2**	**0**	**0**	**5**	**0**	**0**	**0**	**0**	**5**
	Candidatus_Xiphinematobacter	**0**	**11**	**5**	**0**	**173**	**29**	**26**	**15**	**67**	**0**	**1779**	**20**	**789**	**37**	**0**	**48**	**107**	**155**	**9**	**19**
	Caproiciproducens	**0**	**0**	**0**	**5**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**18**	**5**	**0**	**10**	**0**	**0**	**0**	**2**	**3**
	Carnobacterium	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**79**	**0**	**0**	**20**	**0**	**0**	**66**	**0**	**0**	**0**	**0**	**0**
	Caryophanon	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**19**	**0**	**41**	**0**	**0**	**0**	**78**	**0**	**0**	**0**	**0**	**4**	**0**
	Castellaniella	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**35**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	ginsengisoli	0	0	0	0	0	0	0	29	0	0	0	0	0	0	0	0	0	0	0	0
	Catabacter	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Caulobacter	**27**	**6**	**0**	**0**	**0**	**0**	**29**	**27**	**63**	**5**	**14**	**0**	**0**	**9**	**0**	**11**	**102**	**0**	**0**	**9**
	Cellulomonas	**37**	**17**	**0**	**22**	**0**	**10**	**0**	**141**	**293**	**45**	**204**	**1638**	**204**	**46**	**0**	**0**	**9**	**40**	**0**	**0**
	Cellulosibacter	**0**	**24**	**2**	**3**	**0**	**0**	**0**	**0**	**0**	**2**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Cellulosimicrobium	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**14**	**0**	**10**	**0**	**357**	**0**	**92**	**0**	**0**	**0**	**20**	**0**	**0**
	cellulans	0	0	0	0	0	0	0	14	0	10	0	357	0	92	0	0	0	20	0	0
	Cellvibrio	**0**	**0**	**0**	**7**	**0**	**0**	**0**	**2606**	**63**	**107**	**21**	**4130**	**27**	**6993**	**396**	**0**	**8**	**6033**	**7**	**6**
	Cerasibacillus	**34**	**20**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	quisquiliarum	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0
	Cerasicoccus	**0**	**0**	**0**	**0**	**0**	**0**	**16**	**7**	**0**	**15**	**0**	**10**	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**
	Cereibacter	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**107**	**0**	**0**
	Chelativorans	**1249**	**4137**	**2972**	**492**	**316**	**7130**	**6477**	**2829**	**8062**	**1604**	**3624**	**682**	**1266**	**1705**	**805**	**1404**	**1102**	**467**	**946**	**1962**
	composti	1195	3336	2261	487	103	3287	4786	2603	5870	1515	2442	671	940	1659	768	1219	857	412	463	1437
	Chelatococcus	**276**	**777**	**2136**	**68**	**3302**	**21638**	**3501**	**999**	**2572**	**207**	**2849**	**296**	**645**	**733**	**134**	**287**	**260**	**203**	**159**	**421**
	daeguensis	264	777	2132	68	3302	21171	3501	999	2572	207	2849	296	645	733	134	287	260	203	159	421
	Chitiniphilus	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**14**	**0**	**4**
	Chloronema	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**
	Christensenellaceae_R-7_group	**13**	**0**	**0**	**6**	**0**	**0**	**0**	**82**	**0**	**0**	**17**	**0**	**0**	**6**	**0**	**0**	**0**	**64**	**0**	**0**
	Chryseobacterium	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**20**	**909**	**0**	**0**	**413**	**534**	**212**

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors	Compost yard	A							B			C				D			E		
	Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level	Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
antarcticum		0	0	0	0	0	0	0	0	0	0	0	0	0	0	86	0	0	244	0	0
haifense		0	0	0	0	0	0	0	0	0	0	0	0	0	20	561	0	0	23	523	212
Chryseolinea		**0**	**11**	**23**	**0**	**0**	**0**	**57**	**0**	**1136**	**3**	**2581**	**27**	**1854**	**63**	**7**	**507**	**1151**	**486**	**194**	**337**
Chryseomicrobium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**117**	**0**	**394**	**2325**	**0**	**0**	**0**	**0**	**0**
Citrobacter		**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
CK06-06-Mud-MAS4B-21		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**0**
Clostridium_sensu_stricto_1		**95**	**98**	**10**	**61**	**1431**	**298**	**19**	**35**	**108**	**63**	**91**	**0**	**26**	**56**	**71**	**38**	**40**	**27**	**33**	**126**
butyricum		0	0	0	0	0	0	0	0	30	0	10	0	0	7	0	0	0	0	0	0
isatidis		64	86	10	53	61	22	19	0	78	31	51	0	26	49	71	38	40	27	33	111
Clostridium_sensu_stricto_13		**9**	**0**	**0**	**0**	**102**	**0**	**0**	**0**	**28**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Clostridium_sensu_stricto_15		**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**
cochlearium		0	6	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	5
Clostridium_sensu_stricto_18		**13**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**21**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Clostridium_sensu_stricto_3		**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
intestinale		0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clostridium_sensu_stricto_6		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**
Clostridium_sensu_stricto_7		**203**	**247**	**39**	**76**	**0**	**0**	**33**	**62**	**16**	**48**	**28**	**11**	**11**	**9**	**96**	**132**	**70**	**18**	**26**	**47**
thermobutyricum		0	55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clostridium_sensu_stricto_8		**29**	**15**	**6**	**12**	**0**	**107**	**0**	**0**	**9**	**0**	**32**	**0**	**0**	**0**	**55**	**18**	**43**	**11**	**2**	**18**
Genus- putrefaciens		0	0	6	0	0	0	0	0	0	0	32	0	0	0	22	18	25	11	0	18
Species **Cohnella**		**87**	**29**	**17**	**0**	**126**	**53**	**17**	**90**	**75**	**12**	**85**	**23**	**17**	**0**	**0**	**0**	**14**	**24**	**44**	**12**
Comamonas		**13**	**0**	**0**	**0**	**0**	**0**	**0**	**89**	**0**	**234**	**0**	**43**	**0**	**191**	**38**	**0**	**0**	**40**	**0**	**0**
aquatica		0	0	0	0	0	0	0	0	0	126	0	15	0	97	38	0	0	26	0	0
denitrificans		0	0	0	0	0	0	0	0	0	42	0	0	0	16	0	0	0	14	0	0
Conexibacter		**137**	**372**	**133**	**83**	**32**	**6**	**76**	**330**	**794**	**436**	**170**	**200**	**57**	**82**	**137**	**182**	**158**	**42**	**29**	**137**
Confluentibacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**13**	**0**	**0**	**0**	**138**	**0**	**68**	**6**	**0**	**0**	**412**	**0**	**0**
Constrictibacter		**0**	**0**	**8**	**0**	**0**	**0**	**4**	**25**	**57**	**5**	**90**	**0**	**40**	**0**	**0**	**0**	**0**	**7**	**45**	**26**
Corticibacterium		**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**7**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**
Corynebacterium		**177**	**0**	**0**	**93**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
frankenforstense		19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vitaeruminis		36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corynebacterium_1		**567**	**121**	**14**	**282**	**0**	**287**	**10**	**393**	**41**	**442**	**51**	**179**	**0**	**185**	**57**	**22**	**0**	**52**	**0**	**44**
glutamicum		14	0	0	22	0	0	0	0	0	23	31	106	0	60	0	0	0	0	0	0
marinum		0	0	0	0	0	0	0	0	0	0	20	55	0	92	0	0	0	13	0	0
maris		0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
nuruki		84	0	14	0	0	0	0	148	41	230	0	0	0	0	33	0	0	0	0	0
stationis		375	71	0	196	0	245	0	83	0	97	0	0	0	0	24	0	0	0	0	0
Craurococcus		**10**	**39**	**45**	**0**	**0**	**0**	**39**	**27**	**13**	**30**	**176**	**0**	**40**	**14**	**14**	**63**	**83**	**22**	**48**	**58**
Crocinitomix		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**26**	**0**	**0**
Cryomorpha		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**44**	**0**	**15**	**0**	**0**	**0**	**22**	**0**	**0**

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
	Cytophaga		**12**	**14**	**0**	**9**	**0**	**101**	**15**	**444**	**115**	**10**	**5**	**1330**	**0**	**410**	**61**	**63**	**46**	**568**	**0**	**14**
	Defluviicoccus		**0**	**91**	**97**	**0**	**0**	**0**	**96**	**44**	**211**	**27**	**176**	**0**	**78**	**6**	**0**	**20**	**35**	**27**	**81**	**29**
	Defluviitalea		**733**	**5532**	**25**	**2309**	**686**	**521**	**142**	**355**	**250**	**500**	**51**	**30**	**70**	**369**	**295**	**419**	**202**	**23**	**18**	**61**
	raffinosedens		0	0	0	0	268	114	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Defluviitoga		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**
	Demequina		**104**	**76**	**11**	**57**	**0**	**260**	**22**	**2398**	**743**	**819**	**442**	**3141**	**453**	**615**	**372**	**235**	**1208**	**1022**	**89**	**807**
	Desemzia		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**172**	**0**	**124**	**0**	**121**	**0**	**130**	**0**	**0**	**0**	**0**	**0**	**0**
	incerta		0	0	0	0	0	0	0	172	0	124	0	121	0	130	0	0	0	0	0	0
	Desulfitibacter		**547**	**254**	**0**	**1181**	**0**	**114**	**38**	**99**	**53**	**405**	**46**	**22**	**8**	**31**	**64**	**29**	**28**	**3**	**0**	**0**
	Desulfitobacterium		**10**	**32**	**6**	**0**	**0**	**0**	**17**	**52**	**9**	**17**	**22**	**6**	**8**	**2**	**0**	**0**	**0**	**0**	**0**	**2**
	Desulfobulbus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**24**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**14**	**0**	**0**
	Desulfomicrobium		**4**	**16**	**0**	**13**	**0**	**0**	**0**	**0**	**60**	**50**	**10**	**3**	**19**	**0**	**17**	**51**	**61**	**22**	**5**	**0**
	aestuarii		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0
	Desulfonispora		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**13**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Desulfotomaculum		**180**	**372**	**47**	**317**	**770**	**51**	**62**	**72**	**93**	**182**	**290**	**67**	**86**	**183**	**81**	**69**	**34**	**39**	**38**	**72**
	ferrireducens		40	193	16	18	259	51	45	0	0	17	141	0	51	135	41	25	19	0	13	0
	peckii		133	132	31	299	0	0	17	72	53	165	102	67	35	48	40	44	15	39	25	72
	Desulfovibrio		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**5**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**0**
	alaskensis		0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0
Genus-	**Desulfuribacillus**		**3**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Species	**Desulfurispora**		**0**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Dethiobacter		**92**	**116**	**3**	**411**	**0**	**0**	**0**	**0**	**12**	**137**	**38**	**6**	**0**	**0**	**7**	**22**	**36**	**0**	**0**	**14**
	Devosia		**77**	**41**	**0**	**61**	**0**	**0**	**0**	**941**	**142**	**110**	**51**	**3874**	**10**	**1999**	**87**	**6**	**0**	**1456**	**48**	**124**
	albogilva		0	0	0	0	0	0	0	8	28	0	12	206	0	62	0	0	0	40	0	0
	insulae		0	8	0	7	0	0	0	0	0	0	18	73	10	36	13	0	0	107	24	39
	psychrophila		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	42	0	0
	riboflavina		0	0	0	0	0	0	0	0	0	0	0	143	0	50	0	0	0	0	0	0
	Dialister		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Dietzia		**104**	**31**	**8**	**44**	**0**	**0**	**0**	**24**	**12**	**40**	**0**	**0**	**0**	**15**	**22**	**35**	**0**	**20**	**0**	**10**
	Diplosphaera		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**17**	**0**	**0**
	Domibacillus		**96**	**105**	**0**	**47**	**0**	**118**	**99**	**53**	**102**	**480**	**218**	**23**	**72**	**56**	**77**	**64**	**68**	**33**	**56**	**116**
	robiginosus		0	0	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0
	DSSF69		**54**	**162**	**68**	**9**	**201**	**505**	**15**	**584**	**50**	**0**	**0**	**46**	**8**	**25**	**9**	**0**	**17**	**0**	**0**	**3**
	Dysgonomonas		**13**	**0**	**0**	**0**	**0**	**0**	**0**	**9**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Echinicola		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**13**	**0**	**0**
	Ellin6055		**0**	**0**	**20**	**0**	**0**	**0**	**69**	**86**	**266**	**0**	**91**	**15**	**24**	**0**	**7**	**16**	**27**	**10**	**0**	**0**
	Empedobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Enteractinococcus		**0**	**0**	**0**	**48**	**0**	**0**	**0**	**35**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**20**	**0**	**7**	**0**	**0**
	Enterobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**44**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Enterococcus		**45**	**6**	**0**	**53**	**73**	**0**	**0**	**2633**	**0**	**1980**	**0**	**146**	**0**	**891**	**109**	**0**	**0**	**115**	**0**	**0**

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
	aquimarinus		0	0	0	0	0	0	0	149	0	116	0	0	0	71	0	0	0	0	0	0
	asini		14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	cecorum		31	6	0	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	eurekensis		0	0	0	0	0	0	0	522	0	421	0	63	0	142	0	0	0	0	0	0
	saccharolyticus		0	0	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Epulopiscium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**15**	**0**	**0**	**0**	**0**	**4**	**0**	**0**	**0**	**0**
	Ercella		**41**	**0**	**0**	**22**	**0**	**0**	**0**	**290**	**0**	**77**	**0**	**30**	**0**	**3**	**0**	**0**	**0**	**216**	**0**	**0**
	Erysipelothrix		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**36**	**0**	**264**	**0**	**0**	**0**	**3**	**50**	**0**	**0**	**31**	**0**	**0**
	Escherichia/Shigella		**5**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**0**	**0**	**0**	**0**	**0**
	Eubacterium		**17**	**8**	**4**	**7**	**0**	**0**	**0**	**47**	**12**	**29**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**
	aggregans		0	8	0	0	0	0	0	27	0	0	0	0	0	0	0	0	0	0	0	0
	Euzebya		**0**	**0**	**0**	**0**	**0**	**0**	**9**	**8**	**7**	**0**	**42**	**0**	**12**	**0**	**0**	**0**	**11**	**0**	**11**	**6**
	Exiguobacterium		**0**	**0**	**0**	**0**	**14**	**0**	**0**	**92**	**12**	**162**	**0**	**847**	**0**	**332**	**30**	**0**	**0**	**22**	**0**	**0**
	Facklamia		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**52**	**0**	**0**	**0**	**0**	**0**
	Falsochrobactrum		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**14**	**31**	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	ovis		0	0	0	0	0	0	0	14	31	10	0	0	0	0	0	0	0	0	0	0
	Family_XIII_UCG-001		**4**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Fastidiosipila		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**91**	**0**	**33**	**0**	**8**	**0**	**0**	**0**	**0**	**0**	**24**	**0**	**0**
	Fermentimonas		**24**	**0**	**0**	**0**	**0**	**0**	**0**	**259**	**29**	**67**	**144**	**77**	**18**	**29**	**0**	**0**	**0**	**68**	**8**	**12**
Genus-	caenicola		14	0	0	0	0	0	0	235	19	67	144	61	18	29	0	0	0	68	8	12
Species	**Ferrovibrio**		**0**	**0**	**16**	**0**	**88**	**0**	**26**	**0**	**65**	**3**	**291**	**9**	**100**	**7**	**0**	**7**	**189**	**70**	**0**	**0**
	Ferruginibacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**26**	**0**	**0**	**0**	**0**	**0**	**79**	**0**	**0**
	Fervidobacterium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**0**
	FFCH5858		**0**	**0**	**19**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Fibrobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**104**	**0**	**4**	**0**	**5**	**0**	**0**	**0**	**0**	**0**	**350**	**0**	**0**
	Fictibacillus		**0**	**0**	**0**	**0**	**0**	**0**	**37**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Filomicrobium		**426**	**1815**	**820**	**286**	**9**	**552**	**499**	**669**	**1963**	**1417**	**1994**	**291**	**770**	**427**	**822**	**1374**	**862**	**753**	**1272**	**2321**
	Finegoldia		**0**	**0**	**0**	**4**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	magna		0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Flaviflexus		**14**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Flavitalea		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**9**	**0**	**0**	**0**
	Flavobacterium		**56**	**0**	**0**	**59**	**0**	**0**	**0**	**2080**	**37**	**415**	**70**	**2282**	**204**	**7202**	**3712**	**2**	**746**	**1161**	**456**	**172**
	luticocti		0	0	0	0	0	0	0	0	0	0	0	46	0	133	509	0	0	410	0	0
	psychrolimnae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	0	0
	Fluviicola		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**16**	**0**	**0**	**0**	**152**	**0**	**230**	**669**	**2**	**0**	**122**	**0**	**0**
	Fonticella		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**9**
	Fusibacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**21**	**0**	**0**
	Galbitalea		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**25**	**0**	**35**	**0**	**0**	**0**	**0**	**0**	**0**
	Gallicola		**275**	**51**	**0**	**93**	**0**	**0**	**0**	**38**	**0**	**25**	**8**	**50**	**0**	**91**	**0**	**0**	**0**	**0**	**0**	**0**
	Garciella		**70**	**52**	**2**	**59**	**0**	**0**	**0**	**45**	**56**	**68**	**24**	**14**	**23**	**19**	**14**	**4**	**0**	**26**	**30**	**44**

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors	Compost yard	A							B			C				D			E		
	Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level	Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
Genus-Species	**Gelria**	**115**	**19**	**0**	**200**	**0**	**0**	**0**	**0**	**0**	**95**	**0**	**0**	**0**	**5**	**10**	**0**	**0**	**0**	**0**	**0**
	Gemmatirosa	**0**	**49**	**27**	**0**	**0**	**0**	**13**	**0**	**4**	**0**	**36**	**32**	**0**	**0**	**0**	**14**	**33**	**0**	**0**	**0**
	Gemmobacter	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**127**	**11**	**0**	**0**	**183**	**0**	**710**	**33**	**0**	**10**	**69**	**0**	**0**
	intermedius	0	0	0	0	0	0	0	57	0	0	0	34	0	232	33	0	10	0	0	0
	nectariphilus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0
	Geoalkalibacter	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Geobacillus	**135**	**99**	**0**	**43**	**0**	**120**	**23**	**143**	**206**	**274**	**740**	**295**	**285**	**371**	**244**	**164**	**103**	**137**	**115**	**269**
	stearothermophilus	0	36	0	0	0	0	0	74	45	74	125	0	44	40	45	56	34	22	31	48
	thermodenitrificans	0	0	0	0	0	0	0	0	0	0	73	0	0	43	0	0	0	0	0	0
	Georgenia	**228**	**44**	**94**	**64**	**0**	**300**	**432**	**432**	**240**	**134**	**167**	**467**	**38**	**190**	**10**	**9**	**0**	**48**	**0**	**9**
	GKS98_freshwater_group	**0**	**0**	**0**	**0**	**0**	**0**	**9**	**59**	**0**	**0**	**0**	**12**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Globicatella	**0**	**0**	**0**	**16**	**0**	**0**	**0**	**62**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Glutamicibacter	**0**	**0**	**0**	**0**	**0**	**39**	**0**	**0**	**8**	**6**	**0**	**1818**	**0**	**2350**	**7401**	**0**	**0**	**207**	**133**	**0**
	Glycomyces	**59**	**25**	**10**	**13**	**0**	**0**	**0**	**2**	**0**	**9**	**8**	**0**	**0**	**0**	**9**	**0**	**0**	**2**	**0**	**6**
	Gordonia	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**0**
	paraffinivorans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
	Gottschalkia	**12**	**0**	**0**	**0**	**0**	**0**	**0**	**74**	**0**	**34**	**13**	**0**	**0**	**7**	**2**	**0**	**3**	**0**	**0**	**0**
	Gracilibacillus	**9**	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	halotolerans	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Gracilibacter	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**11**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Gulosibacter	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Haliangium	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**
	Haliea	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**25**	**0**	**0**	**0**	**0**	**0**	**8**	**0**	**0**	**0**	**0**	**0**	**0**
	Haloactinobacterium	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Halobacteriovorax	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**7**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**8**	**0**	**0**
	Halocella	**151**	**355**	**48**	**2170**	**0**	**0**	**35**	**61**	**36**	**386**	**143**	**55**	**47**	**54**	**9**	**4**	**0**	**13**	**4**	**70**
	Halomonas	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Haloplasma	**262**	**236**	**2**	**276**	**29**	**0**	**23**	**35**	**25**	**842**	**63**	**3**	**9**	**22**	**61**	**15**	**6**	**14**	**24**	**31**
	Helcococcus	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**
	Herbinix	**71**	**675**	**58**	**58**	**474**	**222**	**127**	**70**	**200**	**167**	**546**	**114**	**117**	**168**	**114**	**108**	**102**	**89**	**67**	**152**
	luporum	56	276	25	23	103	84	32	48	113	62	285	65	46	40	29	0	44	0	0	0
	Herminiimonas	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**11**	**0**	**0**
	Homoserinibacter	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**
	Huakuichenia	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**47**	**0**	**0**	**0**	**60**	**0**	**50**	**0**	**0**	**0**	**0**	**0**	**0**
	soli	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0
	Hydrogenispora	**448**	**1093**	**32**	**4944**	**3**	**64**	**15**	**28**	**254**	**697**	**280**	**25**	**35**	**67**	**280**	**153**	**117**	**44**	**51**	**156**
	Hydrogenobacter	**48**	**0**	**0**	**4**	**0**	**0**	**0**	**0**	**0**	**13**	**0**	**0**	**0**	**0**	**22**	**0**	**0**	**0**	**0**	**0**
	subterraneus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	0	0	0	0	0
	Hydrogenophaga	**13**	**0**	**8**	**0**	**0**	**0**	**0**	**77**	**7**	**0**	**15**	**27**	**9**	**0**	**25**	**7**	**13**	**286**	**14**	**22**
	temperata	0	0	0	0	0	0	0	50	0	0	0	0	0	0	25	0	0	144	0	0

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
	Hydrogenophilus		**1875**	**72**	**6**	**1627**	**0**	**16**	**0**	**56**	**0**	**398**	**0**	**0**	**0**	**102**	**97**	**29**	**13**	**12**	**16**	**0**
	Hymenobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**
	Hyphomicrobium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**57**	**0**	**14**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Hyphomonas		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**11**	**0**	**0**
	Iamia		**22**	**1215**	**308**	**28**	**0**	**674**	**471**	**273**	**1801**	**232**	**2675**	**210**	**1090**	**304**	**296**	**1549**	**1140**	**365**	**1278**	**1456**
	Idiomarina		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**82**	**0**	**60**	**0**	**39**	**0**	**52**	**13**	**0**	**0**	**11**	**0**	**0**
	Ilumatobacter		**9**	**0**	**0**	**0**	**0**	**0**	**10**	**78**	**0**	**0**	**33**	**41**	**32**	**8**	**0**	**0**	**14**	**45**	**0**	**0**
	IMCC26207		**23**	**450**	**235**	**17**	**0**	**148**	**467**	**336**	**453**	**91**	**362**	**125**	**185**	**70**	**199**	**451**	**443**	**106**	**104**	**340**
	Imperialibacter		**0**	**0**	**5**	**0**	**0**	**0**	**4**	**0**	**55**	**0**	**314**	**0**	**96**	**9**	**0**	**0**	**0**	**12**	**0**	**0**
	Inquilinus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**79**	**39**	**0**	**27**	**18**	**24**	**0**	**9**	**0**	**33**	**11**	**0**	**0**
	Isobaculum		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**
	Isoptericola		**0**	**0**	**0**	**14**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Jahnella		**0**	**5**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Janibacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**27**	**0**	**0**	**0**	**0**	**0**
	JCM_18997		**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Jeotgalibaca		**22**	**0**	**0**	**14**	**0**	**0**	**0**	**679**	**0**	**997**	**0**	**173**	**0**	**362**	**796**	**0**	**0**	**0**	**0**	**0**
	arthritidis		0	0	0	0	0	0	0	101	0	21	0	0	0	38	0	0	0	0	0	0
	dankookensis		0	0	0	0	0	0	0	99	0	497	0	33	0	112	721	0	0	0	0	0
	Jeotgalicoccus		**40**	**0**	**0**	**16**	**0**	**0**	**0**	**8**	**0**	**3**	**2**	**0**	**0**	**7**	**0**	**0**	**0**	**0**	**0**	**0**
Genus-	**Jonesia**		**0**	**0**	**0**	**18**	**0**	**0**	**0**	**212**	**12**	**0**	**0**	**215**	**0**	**57**	**0**	**0**	**0**	**0**	**0**	**0**
Species	denitrificans		0	0	0	18	0	0	0	212	12	0	0	215	0	57	0	0	0	0	0	0
	Kallotenue		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**
	Ketogulonicigenium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**28**	**0**	**0**	**0**	**0**	**0**	**0**
	Klebsiella		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**15**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Kocuria		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**103**	**0**	**14**	**18**	**0**	**0**	**10**	**0**	**0**
	Kurthia		**15**	**0**	**0**	**0**	**0**	**0**	**0**	**15**	**0**	**2**	**0**	**307**	**0**	**91**	**0**	**0**	**0**	**0**	**0**	**0**
	gibsonii		0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0
	Lachnoclostridium_5		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**11**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Lachnospiraceae_UCG-010		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Lachnotalea		**5**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**23**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Lactobacillus		**219**	**8**	**0**	**129**	**0**	**0**	**12**	**12**	**9**	**6**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**
	agilis		33	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	coryniformis		0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
	delbrueckii		0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	fermentum		0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	pontis		0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0
	vaccinostercus		0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Lactococcus		**35**	**0**	**0**	**9**	**0**	**0**	**0**	**119**	**0**	**0**	**0**	**12**	**0**	**13**	**0**	**0**	**0**	**0**	**0**	**0**
	lactis		16	0	0	0	0	0	0	0	0	0	0	12	0	13	0	0	0	0	0	0
	Lautropia		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**65**	**0**	**21**	**0**	**0**	**23**	**26**	**27**	**21**	**17**

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
	LD29		**0**	**73**	**0**	**0**	**0**	**0**	**15**	**65**	**14**	**5**	**37**	**43**	**0**	**5**	**36**	**30**	**29**	**43**	**9**	**13**
	Leadbetterella		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**25**	**0**	**0**	**0**	**39**	**0**	**23**	**0**	**0**	**0**	**65**	**0**	**0**
	Legionella		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**12**	**43**	**25**	**5**	**0**	**0**	**0**	**7**	**4**	**0**
	londiniensis		0	0	0	0	0	0	0	0	0	0	12	0	25	5	0	0	0	0	4	0
	Leifsonia		**0**	**0**	**35**	**25**	**0**	**37**	**0**	**214**	**0**	**20**	**37**	**696**	**0**	**812**	**40**	**0**	**0**	**134**	**0**	**57**
	Lelliottia		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**136**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Lentibacillus		**0**	**4**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Leptonema		**11**	**6**	**4**	**5**	**0**	**0**	**0**	**24**	**18**	**0**	**11**	**0**	**15**	**4**	**0**	**7**	**42**	**5**	**0**	**0**
	Leucobacter		**80**	**42**	**0**	**58**	**0**	**0**	**0**	**178**	**204**	**190**	**71**	**1620**	**25**	**831**	**84**	**0**	**48**	**169**	**16**	**142**
	aridicollis		0	0	0	0	0	0	0	0	0	0	0	66	0	123	48	0	0	0	0	44
	salsicius		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	128	0	48
	Limisphaera		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Limnobacter		**0**	**115**	**90**	**9**	**177**	**74**	**244**	**379**	**296**	**17**	**586**	**169**	**302**	**67**	**47**	**156**	**183**	**110**	**209**	**439**
	Limnochorda		**52**	**95**	**25**	**188**	**0**	**0**	**0**	**0**	**103**	**201**	**13**	**10**	**0**	**16**	**98**	**0**	**0**	**16**	**16**	**24**
	Litorilinea		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**158**	**24**	**47**	**6**	**35**	**94**	**174**	**75**	**6**	**30**
	Longispora		**13**	**80**	**6**	**14**	**0**	**0**	**0**	**0**	**31**	**0**	**14**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**18**
	Luedemannella		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**264**	**0**	**90**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Luteimonas		**157**	**287**	**593**	**35**	**930**	**560**	**1379**	**4730**	**4375**	**102**	**608**	**1221**	**343**	**392**	**437**	**2864**	**2386**	**524**	**171**	**311**
	Luteitalea		**0**	**0**	**27**	**0**	**0**	**0**	**36**	**51**	**357**	**0**	**594**	**68**	**184**	**13**	**18**	**198**	**198**	**44**	**44**	**54**
Genus-	**Luteolibacter**		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**338**	**0**	**15**	**4**	**59**	**0**	**55**	**6**	**0**	**0**	**83**	**0**	**0**
Species	**Lutispora**		**37**	**3**	**0**	**85**	**0**	**0**	**0**	**7**	**0**	**262**	**18**	**0**	**9**	**5**	**52**	**21**	**0**	**9**	**8**	**5**
	Lysinibacillus		**544**	**950**	**76**	**478**	**830**	**712**	**981**	**591**	**813**	**5142**	**1802**	**430**	**481**	**486**	**593**	**1679**	**600**	**757**	**885**	**5179**
	halotolerans		117	132	33	42	296	0	359	135	335	390	339	187	209	158	155	346	195	175	203	746
	manganicus		55	114	0	76	0	97	76	75	76	588	88	46	84	75	48	67	0	46	60	252
	massiliensis		0	0	0	0	0	0	0	0	0	66	94	40	0	0	136	468	152	134	110	405
	Lysinimicrobium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**85**	**0**	**33**	**0**	**0**	**0**	**184**	**0**	**0**
	Macellibacteroides		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**14**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Macrococcus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**210**	**0**	**62**	**14**	**0**	**0**	**0**	**0**	**0**
	Marinagarivorans		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**14**	**0**	**0**
	Marinibaculum		**0**	**0**	**12**	**0**	**0**	**0**	**8**	**0**	**0**	**0**	**13**	**0**	**15**	**0**	**0**	**5**	**11**	**3**	**0**	**0**
	Marinospirillum		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**11**	**0**	**0**
	Marmoricola		**0**	**0**	**0**	**0**	**0**	**0**	**12**	**0**	**0**	**0**	**0**	**37**	**11**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Melghirimyces		**11**	**12**	**0**	**0**	**0**	**18**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**6**	**0**	**0**	**0**	**11**
	Membranicola		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**462**	**40**	**0**	**4**	**88**	**4**	**24**	**0**	**0**	**0**	**332**	**0**	**0**
	Mesorhizobium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**14**	**0**	**37**	**13**	**58**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Methanosarcina		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**8**	**0**	**0**	**0**
	Methanothermobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**12**	**0**	**0**	**21**	**0**	**20**	**0**	**0**	**0**
	Methylobacterium		**0**	**0**	**0**	**0**	**0**	**0**	**16**	**14**	**43**	**6**	**5**	**0**	**0**	**11**	**0**	**0**	**0**	**0**	**0**	**0**
	radiotolerans		0	0	0	0	0	0	0	0	43	0	0	0	0	0	0	0	0	0	0	0
	Methylophaga		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
	Methyloterrigena		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**0**	**0**	**0**	**0**
	Micavibrio		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**32**	**0**	**40**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Microbispora		**22**	**43**	**55**	**0**	**33**	**148**	**51**	**68**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**25**	**0**	**28**
	Micrococcus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**31**	**0**	**0**	**32**	**0**	**0**	**0**	**0**	**0**
	Microvirga		**0**	**13**	**0**	**0**	**0**	**0**	**0**	**20**	**18**	**0**	**0**	**52**	**7**	**11**	**0**	**4**	**0**	**20**	**0**	**3**
	Millisia		**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Moheibacter		**78**	**12**	**16**	**20**	**0**	**0**	**32**	**2029**	**308**	**163**	**50**	**716**	**57**	**204**	**117**	**40**	**191**	**142**	**0**	**29**
	Mycobacterium		**0**	**13**	**9**	**0**	**0**	**0**	**15**	**31**	**44**	**9**	**113**	**18**	**57**	**21**	**23**	**55**	**37**	**0**	**34**	**46**
	arupense		0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0
	parafortuitum		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
	phlei		0	0	0	0	0	0	0	0	0	0	21	0	0	0	0	0	0	0	0	11
	thermoresistibile		0	0	0	0	0	0	0	31	15	0	0	0	0	9	0	11	0	0	0	0
	Mycoplana		**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Nannocystis		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**24**	**0**	**133**	**0**	**21**	**13**	**7**	**306**	**899**	**26**	**83**	**18**
	Natranaerovirga		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Neochlamydia		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Neorhizobium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Neoscardovia		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**7**	**0**	**13**	**0**	**8**	**0**	**11**	**0**	**0**	**0**	**0**	**0**	**0**
	arbecensis		0	0	0	0	0	0	0	0	0	0	0	8	0	11	0	0	0	0	0	0
Genus-	**Niabella**		**10**	**47**	**43**	**0**	**170**	**395**	**596**	**71**	**982**	**9**	**649**	**0**	**68**	**10**	**24**	**49**	**80**	**9**	**0**	**0**
Species	**Nitrosomonas**		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**15**	**0**	**0**
	europaea		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0
	Nocardioides		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**60**	**0**	**0**	**0**	**202**	**8**	**66**	**0**	**6**	**0**	**19**	**0**	**0**
	solisilvae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	0	0
	Nocardiopsis		**138**	**96**	**28**	**93**	**0**	**30**	**21**	**129**	**151**	**110**	**73**	**13**	**29**	**19**	**43**	**39**	**38**	**6**	**0**	**9**
	listeri		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
	Nonomuraea		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**18**
	Nosocomiicoccus		**0**	**0**	**0**	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Novibacillus		**10**	**22**	**0**	**0**	**0**	**0**	**0**	**0**	**10**	**6**	**6**	**0**	**0**	**0**	**0**	**4**	**0**	**0**	**0**	**8**
	thermophilus		0	7	0	0	0	0	0	0	0	6	6	0	0	0	0	0	0	0	0	8
	Noviherbaspirillum		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**17**	**12**	**3**	**0**	**7**	**12**	**0**	**0**	**0**	**0**	**4**	**0**	**0**
	Novosphingobium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**96**	**0**	**45**	**0**	**0**	**0**	**56**	**0**	**0**
	Nubsella		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**
	Oceanibaculum		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**0**
	Oceanobacillus		**0**	**27**	**8**	**9**	**0**	**0**	**0**	**73**	**70**	**146**	**17**	**14**	**0**	**0**	**45**	**32**	**32**	**11**	**6**	**41**
	caeni		0	0	0	0	0	0	0	16	24	52	0	0	0	0	19	21	15	0	6	0
	chironomi		0	0	8	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	indicireducens		0	15	0	0	0	0	0	23	34	16	17	0	0	0	26	11	17	11	0	23
	Oceanobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**11**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Ochrobactrum		**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**13**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
	Ohtaekwangia		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**
	OM27_clade		**40**	**432**	**543**	**33**	**0**	**0**	**665**	**411**	**1035**	**366**	**871**	**41**	**428**	**72**	**92**	**854**	**713**	**105**	**209**	**194**
	Opitutus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**0**
	Ornithinibacillus		**78**	**39**	**27**	**7**	**29**	**0**	**0**	**43**	**66**	**146**	**63**	**38**	**9**	**24**	**13**	**12**	**18**	**0**	**8**	**0**
	Ornithinicoccus		**18**	**0**	**0**	**0**	**0**	**0**	**66**	**144**	**246**	**73**	**156**	**117**	**49**	**56**	**140**	**253**	**271**	**111**	**274**	**434**
	Ornithinimicrobium		**0**	**0**	**0**	**4**	**0**	**0**	**0**	**29**	**0**	**0**	**0**	**435**	**0**	**57**	**0**	**0**	**0**	**23**	**0**	**0**
	Orrella		**0**	**3**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**2**	**0**	**0**	**0**	**0**	**0**
	Oxobacter		**4**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**10**	**0**	**0**	**0**	**0**	**0**
	Paenibacillus		**312**	**138**	**40**	**24**	**230**	**266**	**386**	**874**	**456**	**359**	**738**	**287**	**211**	**347**	**125**	**168**	**102**	**184**	**312**	**1014**
	barengoltzii		0	0	0	0	0	106	0	0	0	16	71	41	0	0	0	0	0	0	12	0
	camelliae		0	14	0	0	0	59	70	0	17	0	43	35	15	76	0	12	14	11	30	28
	woosongensis		0	0	0	0	0	0	0	0	0	12	0	0	0	10	0	0	0	0	10	0
	Paeniglutamicibacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**147**	**157**	**22**
	antarcticus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0
	Paenisporosarcina		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**98**	**0**	**0**	**0**	**0**	**10**
	quisquiliarum		0	0	0	0	0	0	0	0	0	0	0	0	0	0	98	0	0	0	0	0
	Paenochrobactrum		**18**	**0**	**0**	**0**	**0**	**0**	**0**	**19**	**0**	**0**	**0**	**0**	**0**	**86**	**0**	**0**	**0**	**0**	**0**	**0**
	Pajaroellobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**83**	**0**	**46**	**0**	**0**	**8**	**19**	**23**	**50**	**7**
	Paludibacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**19**	**0**	**17**	**0**	**6**	**0**	**0**	**0**	**22**	**0**	**0**
Genus-	**Pantoea**		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**9**
Species	agglomerans		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
	Parablastomonas		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**
	Paracoccus		**8**	**0**	**0**	**0**	**0**	**0**	**0**	**23**	**0**	**0**	**0**	**133**	**0**	**94**	**17**	**0**	**0**	**15**	**0**	**0**
	koreensis		0	0	0	0	0	0	0	23	0	0	0	65	0	0	0	0	0	15	0	0
	thiocyanatus		0	0	0	0	0	0	0	0	0	0	0	49	0	41	0	0	0	0	0	0
	Parafrigoribacterium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**25**	**0**	**0**	**0**	**66**	**0**	**0**	**0**	**0**	**0**	**31**	**0**	**0**
	Paraherbaspirillum		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**0**	**0**	**0**	**0**	**5**	**0**
	Parapedobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**44**	**18**	**17**	**0**	**0**	**3**	**39**	**0**	**0**
	Paraperlucidibaca		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**
	Parapusillimonas		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**9**	**0**	**0**	**0**	**0**	**0**	**41**	**0**	**0**	**0**	**48**	**0**	**0**
	Parvibaculum		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**21**	**0**	**3**	**0**	**13**	**0**	**0**	**0**	**0**	**0**	**44**	**0**	**5**
	Parviterribacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**25**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**14**	**0**
	Patulibacter		**4**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**12**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Paucisalibacillus		**0**	**0**	**0**	**0**	**0**	**0**	**15**	**0**	**0**	**0**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**24**
	globulus		0	0	0	0	0	0	15	0	0	0	8	0	0	0	0	0	0	0	0	24
	Pedobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**186**	**544**	**0**	**0**	**438**	**0**	**0**
	bauzanensis		0	0	0	0	0	0	0	6	0	0	0	0	0	0	251	0	0	41	0	0
	Pedomicrobium		**57**	**372**	**190**	**46**	**42**	**30**	**181**	**255**	**643**	**245**	**296**	**8**	**155**	**65**	**101**	**201**	**170**	**73**	**209**	**373**
	Pelagibacterium		**0**	**0**	**0**	**8**	**0**	**0**	**0**	**200**	**42**	**76**	**0**	**203**	**0**	**69**	**30**	**11**	**11**	**85**	**0**	**5**
	Pelagibius		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**7**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
	Pelobium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**
	Pelomonas		**8**	**0**	**0**	**0**	**0**	**0**	**7**	**13**	**8**	**0**	**7**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**9**
	Peredibacter		**22**	**21**	**0**	**0**	**0**	**0**	**0**	**470**	**56**	**93**	**94**	**353**	**14**	**29**	**18**	**46**	**21**	**161**	**16**	**54**
	Persicitalea		**35**	**0**	**0**	**18**	**0**	**0**	**0**	**2672**	**44**	**43**	**124**	**1254**	**242**	**250**	**131**	**8**	**125**	**505**	**0**	**8**
	Petrimonas		**22**	**0**	**0**	**9**	**0**	**0**	**0**	**90**	**11**	**13**	**46**	**30**	**31**	**0**	**16**	**0**	**33**	**50**	**0**	**0**
	mucosa		22	0	0	9	0	0	0	90	11	13	46	30	31	0	16	0	33	50	0	0
	Phaselicystis		**108**	**2289**	**591**	**86**	**79**	**0**	**234**	**236**	**439**	**541**	**532**	**96**	**302**	**138**	**368**	**349**	**339**	**165**	**411**	**834**
	Phenylobacterium		**0**	**0**	**0**	**0**	**0**	**0**	**11**	**10**	**0**	**0**	**32**	**27**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**16**
	Pigmentiphaga		**0**	**16**	**51**	**0**	**93**	**0**	**103**	**26**	**57**	**0**	**36**	**0**	**9**	**3**	**0**	**18**	**8**	**0**	**0**	**0**
	daeguensis		0	16	51	0	93	0	103	26	57	0	36	0	9	3	0	18	8	0	0	0
	Pirellula		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**22**	**0**	**0**	**10**	**0**	**5**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Planctomicrobium		**0**	**7**	**7**	**6**	**0**	**0**	**21**	**36**	**65**	**17**	**48**	**54**	**51**	**9**	**10**	**45**	**44**	**25**	**9**	**23**
	Planifilum		**89**	**63**	**0**	**48**	**0**	**0**	**13**	**4**	**48**	**60**	**149**	**42**	**38**	**36**	**11**	**0**	**7**	**13**	**0**	**88**
	composti		0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30
	Planktosalinus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1426**	**14**	**0**	**0**	**143**	**0**	**100**	**0**	**0**	**0**	**292**	**0**	**0**
	Planococcus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**40**	**0**	**0**	**0**	**1053**	**0**	**233**	**553**	**0**	**0**	**0**	**0**	**0**
	psychrotoleratus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0
	Planomicrobium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**85**	**0**	**0**	**17**	**2719**	**0**	**951**	**8456**	**0**	**0**	**106**	**0**	**28**
	chinense		0	0	0	0	0	0	0	0	0	0	0	147	0	0	101	0	0	0	0	0
Genus-	glaciei		0	0	0	0	0	0	0	13	0	0	0	537	0	211	109	0	0	21	0	0
Species	koreense		0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0
	okeanokoites		0	0	0	0	0	0	0	0	0	0	0	173	0	89	1540	0	0	0	0	28
	stackebrandtii		0	0	0	0	0	0	0	0	0	0	0	105	0	0	711	0	0	0	0	0
	Povalibacter		**0**	**0**	**4**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**2**	**0**	**4**	**0**	**0**	**0**	**2**	**0**	**0**	**9**
	Prosthecobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**13**	**0**	**0**
	Proteiniborus		**0**	**9**	**0**	**0**	**0**	**0**	**4**	**29**	**11**	**27**	**44**	**10**	**8**	**0**	**0**	**0**	**0**	**12**	**0**	**16**
	Proteiniclasticum		**24**	**0**	**0**	**41**	**0**	**0**	**0**	**2408**	**0**	**1344**	**0**	**16**	**0**	**62**	**28**	**0**	**0**	**200**	**0**	**0**
	ruminis		0	0	0	0	0	0	0	121	0	133	0	0	0	0	0	0	0	65	0	0
	Proteiniphilum		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**277**	**0**	**119**	**0**	**80**	**0**	**31**	**0**	**0**	**15**	**203**	**0**	**0**
	Proteocatella		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**165**	**0**	**76**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**33**	**0**	**0**
	Pseudactinotalea		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**46**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Pseudaminobacter		**34**	**25**	**0**	**0**	**0**	**464**	**207**	**453**	**194**	**83**	**122**	**440**	**69**	**42**	**32**	**40**	**127**	**169**	**33**	**135**
	Pseudarthrobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**42**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Pseudobacteriovorax		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**
	Pseudobacteroides		**0**	**0**	**0**	**3**	**0**	**0**	**0**	**12**	**57**	**0**	**34**	**0**	**0**	**4**	**0**	**10**	**172**	**0**	**0**	**11**
	Pseudochelatococcus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**11**	**0**	**0**	**0**	**32**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Pseudochrobactrum		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**39**	**0**	**0**
	Pseudoclavibacter		**0**	**27**	**0**	**0**	**0**	**0**	**0**	**28**	**56**	**96**	**26**	**106**	**0**	**0**	**0**	**17**	**0**	**0**	**0**	**16**
	Pseudofulvimonas		**160**	**55**	**357**	**13**	**13**	**50**	**308**	**1926**	**3066**	**123**	**142**	**527**	**133**	**38**	**220**	**1099**	**1323**	**92**	**5**	**41**
	Pseudogracilibacillus		**156**	**87**	**10**	**52**	**0**	**87**	**10**	**92**	**8**	**36**	**0**	**0**	**8**	**13**	**18**	**29**	**17**	**3**	**0**	**28**

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors	Compost yard	A							B			C				D			E		
	Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level	Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
Pseudohoeflea		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**7**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Pseudohongiella		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**8**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**0**
Pseudomonas		**56**	**15**	**0**	**40**	**0**	**0**	**63**	**1694**	**172**	**1565**	**164**	**218**	**62**	**378**	**425**	**36**	**47**	**1037**	**98**	**189**
caeni		0	0	0	0	0	0	0	33	0	72	0	0	0	20	43	0	0	0	0	0
formosensis		26	0	0	32	0	0	0	565	0	383	0	102	4	238	47	0	0	46	0	0
pseudoalcaligenes		0	0	0	0	0	0	0	0	0	0	0	41	0	34	0	0	0	0	0	0
saudiphocaensis		0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0
taetrolens		0	0	0	0	0	0	0	0	0	0	0	0	0	0	146	0	0	0	0	0
thermotolerans		0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0
Pseudonocardia		**0**	**0**	**0**	**0**	**0**	**0**	**52**	**0**	**0**	**0**	**28**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
thermophila		0	0	0	0	0	0	52	0	0	0	28	0	0	0	0	0	0	0	0	0
Pseudopedobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**12**	**0**	**0**	**0**	**0**	**0**	**0**
Pseudoxanthomonas		**95**	**7614**	**16896**	**24**	**14894**	**24001**	**32385**	**6905**	**12634**	**369**	**5436**	**11367**	**2130**	**4274**	**1200**	**7376**	**9968**	**1124**	**1121**	**2179**
suwonensis		0	0	0	0	0	0	0	99	0	0	123	800	0	189	0	0	0	319	0	51
taiwanensis		95	7472	15746	24	13971	22017	11236	4684	8414	286	3950	3554	1280	3892	752	2981	3143	397	1121	2058
Psychrobacillus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**74**	**102**	**873**
psychrodurans		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	74	0	353
Psychrobacter		**0**	**0**	**0**	**3**	**0**	**0**	**0**	**69**	**9**	**79**	**0**	**176**	**0**	**856**	**2626**	**0**	**0**	**300**	**0**	**5**
luti		0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0
Genus- maritimus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	259	0	0	49	0	0
Species nivimaris		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	61	0	0
phenylpyruvicus		0	0	0	3	0	0	0	0	0	33	0	0	0	0	0	0	0	0	0	0
Pusillimonas		**162**	**16**	**61**	**0**	**611**	**54**	**390**	**3025**	**238**	**55**	**400**	**1190**	**165**	**542**	**351**	**111**	**119**	**250**	**0**	**0**
noertemannii		0	0	0	0	0	0	0	0	0	0	0	103	0	57	0	0	0	105	0	0
Qingshengfania		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**13**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Ralstonia		**0**	**0**	**0**	**0**	**0**	**481**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Ramlibacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**18**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**12**	**7**	**0**	**0**
Renibacterium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**
Rhabdobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**10**	**0**	**0**	**0**	**0**	**0**	**0**
Rheinheimera		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**11**	**0**	**0**	**162**	**0**	**0**
aquimaris		0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0
Rhodobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**29**	**0**	**0**	**0**	**0**	**0**	**34**	**0**	**0**
gluconicum		0	0	0	0	0	0	0	0	0	0	0	29	0	0	0	0	0	34	0	0
Rhodococcus		**4**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**11**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Rhodoferax		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**35**	**0**	**0**
ferrireducens		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	0	0
Rhodoligotrophos		**13**	**20**	**9**	**0**	**0**	**0**	**48**	**112**	**21**	**16**	**57**	**84**	**27**	**12**	**13**	**19**	**21**	**25**	**5**	**27**
Rhodopirellula		**4**	**72**	**28**	**0**	**0**	**0**	**19**	**30**	**56**	**38**	**44**	**0**	**32**	**7**	**18**	**30**	**36**	**29**	**37**	**105**
Rhodoplanes		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**46**	**0**	**32**	**0**	**0**	**12**	**16**	**17**	**59**	**22**
Rhodothermus		**1731**	**185**	**10**	**106**	**0**	**94**	**0**	**44**	**232**	**709**	**1594**	**148**	**523**	**705**	**289**	**184**	**78**	**369**	**780**	**1314**

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors	Compost yard	A							B			C				D			E		
	Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level	Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
	Romboutsia	**9**	**7**	**6**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**13**	**0**	**15**
	Roseimarinus	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**12**	**0**	**0**	**0**	**12**	**0**	**0**	**0**	**0**	**0**	**49**	**0**	**0**
	Roseimaritima	**0**	**0**	**0**	**4**	**0**	**0**	**0**	**46**	**35**	**0**	**0**	**24**	**0**	**0**	**0**	**0**	**0**	**33**	**0**	**0**
	Roseomonas	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**11**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**
	Ruania	**20**	**6**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Rubrivirga	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**8**	**0**	**25**	**0**	**25**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Ruminiclostridium	**11608**	**4039**	**29**	**4945**	**670**	**281**	**172**	**455**	**518**	**5080**	**617**	**95**	**196**	**460**	**920**	**1110**	**807**	**62**	**70**	**261**
	Ruminiclostridium_1	**496**	**2647**	**12**	**1038**	**0**	**215**	**65**	**413**	**385**	**757**	**167**	**0**	**76**	**166**	**337**	**613**	**164**	**13**	**41**	**174**
	Ruminococcaceae_UCG-007	**0**	**0**	**0**	**0**	**0**	**0**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**
	Ruminococcaceae_UCG-010	**13**	**54**	**0**	**27**	**23**	**0**	**0**	**0**	**35**	**28**	**11**	**0**	**6**	**0**	**9**	**22**	**3**	**2**	**3**	**13**
	Ruminococcaceae_UCG-012	**179**	**2005**	**42**	**604**	**52**	**0**	**67**	**64**	**162**	**137**	**78**	**23**	**16**	**52**	**262**	**245**	**299**	**0**	**23**	**61**
	Ruminococcaceae_UCG-013	**76**	**70**	**6**	**53**	**24**	**0**	**13**	**0**	**12**	**34**	**28**	**7**	**5**	**13**	**0**	**5**	**0**	**4**	**9**	**18**
	Ruminococcaceae_UCG-014	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**
	Ruminococcus_1	**13**	**35**	**0**	**27**	**104**	**0**	**11**	**57**	**26**	**33**	**38**	**12**	**12**	**4**	**10**	**11**	**39**	**2**	**6**	**6**
	Ruminofilibacter	**76**	**0**	**3**	**15**	**0**	**0**	**0**	**2045**	**278**	**36**	**312**	**1414**	**693**	**190**	**30**	**6**	**916**	**551**	**4**	**8**
	xylanolyticum	23	0	0	15	0	0	0	187	74	0	33	173	102	26	30	0	214	127	0	0
	Rummeliibacillus	**0**	**11**	**9**	**11**	**0**	**0**	**0**	**0**	**0**	**0**	**37**	**24**	**29**	**0**	**36**	**44**	**48**	**0**	**35**	**41**
	pycnus	0	11	9	11	0	0	0	0	0	0	37	24	29	0	36	44	48	0	35	41
	Saccharomonospora	**116**	**74**	**14**	**65**	**0**	**0**	**31**	**0**	**0**	**18**	**51**	**17**	**22**	**26**	**0**	**0**	**0**	**20**	**18**	**16**
Genus-	glauca	0	0	0	0	0	0	0	0	0	0	51	17	22	26	0	0	0	0	0	0
Species	viridis	116	74	14	65	0	0	31	0	0	18	0	0	0	0	0	0	0	20	18	16
	Saccharopolyspora	**62**	**34**	**16**	**41**	**0**	**13**	**13**	**7**	**0**	**23**	**13**	**16**	**0**	**13**	**0**	**0**	**0**	**71**	**106**	**225**
	flava	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	16	16
	rectivirgula	62	34	16	41	0	0	0	7	0	23	0	16	0	0	0	0	0	56	68	143
	Salinibacterium	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**20**	**0**	**0**
	Salinicoccus	**13**	**0**	**0**	**31**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Salinispira	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**13**	**0**	**0**
	Salinispora	**154**	**1406**	**172**	**81**	**233**	**467**	**53**	**460**	**553**	**748**	**231**	**93**	**123**	**98**	**351**	**118**	**82**	**338**	**228**	**971**
	Sandaracinus	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**8**	**44**	**21**	**273**	**0**	**178**	**0**	**0**	**0**	**0**	**31**	**66**	**143**
	Sanguibacter	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**94**	**0**	**30**	**0**	**145**	**0**	**120**	**83**	**0**	**0**	**0**	**0**	**0**
	marinus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	83	0	0	0	0	0
	Savagea	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Schlegelella	**55**	**633**	**782**	**24**	**257**	**508**	**1496**	**336**	**1361**	**150**	**351**	**985**	**227**	**60**	**466**	**2371**	**1876**	**267**	**69**	**187**
	SD04E11	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Sedimentibacter	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**49**	**16**	**16**	**42**	**7**	**4**	**0**	**7**	**0**	**6**	**17**	**0**	**9**
	Sediminibacterium	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**7**	**0**	**0**	**0**
	salmoneum	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
	Sediminihabitans	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Shewanella	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**0**	**0**	**10**	**0**	**0**	**5**	**0**	**0**
	morhuae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	5	0	0

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Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
	Shinella		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**10**	**0**	**0**
	Simplicispira		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**34**	**0**	**0**
	piscis		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34	0	0
	Sinibacillus		**417**	**134**	**36**	**190**	**247**	**85**	**21**	**315**	**137**	**186**	**75**	**30**	**11**	**73**	**25**	**8**	**26**	**11**	**21**	**27**
	soli		34	10	9	0	236	0	0	15	0	56	29	0	0	0	5	0	0	11	16	21
	SM1A02		**0**	**104**	**60**	**0**	**0**	**15**	**126**	**0**	**123**	**4**	**653**	**9**	**238**	**0**	**4**	**143**	**183**	**35**	**242**	**141**
	SN8		**0**	**13**	**47**	**0**	**0**	**0**	**0**	**8**	**0**	**2**	**0**	**5**	**18**	**2**	**0**	**2**	**35**	**0**	**17**	**0**
	Sneathiella		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**10**	**0**	**0**	**3**	**6**	**0**	**0**	**0**
	Soehngenia		**14**	**0**	**0**	**0**	**0**	**0**	**0**	**52**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**27**	**0**	**0**
	Solibacillus		**74**	**42**	**0**	**107**	**0**	**0**	**2433**	**305**	**234**	**485**	**194**	**185**	**107**	**2696**	**703**	**97**	**92**	**52**	**386**	**1653**
	Solirubrobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**12**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Sorangium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**26**	**0**	**25**	**0**	**0**	**20**	**518**	**129**	**2115**	**328**
	Sphaerimonospora		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Sphaerisporangium		**0**	**0**	**27**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**16**	**12**	**16**	**0**
	Sphaerobacter		**247**	**1632**	**452**	**208**	**0**	**252**	**257**	**635**	**1256**	**1479**	**447**	**185**	**284**	**232**	**686**	**991**	**743**	**596**	**569**	**2506**
	thermophilus		247	1614	441	206	0	210	247	581	1235	1435	417	185	284	232	686	991	741	596	569	2262
	Sphingoaurantiacus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Sphingobacterium		**737**	**212**	**562**	**35**	**7063**	**446**	**10330**	**1842**	**1522**	**19**	**457**	**2437**	**461**	**3227**	**704**	**246**	**267**	**217**	**0**	**0**
	alimentarium		0	0	0	0	0	0	0	18	0	0	0	858	0	2272	292	0	0	42	0	0
Genus-	hotanense		0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0
Species	mizutaii		0	0	0	0	0	0	0	0	0	0	0	0	0	21	0	0	0	0	0	0
	thermophilum		0	0	0	0	0	0	128	0	0	0	0	0	0	0	0	34	29	0	0	0
	wenxiniae		0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0
	Sphingobium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**16**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**
	Sphingomonas		**14**	**5**	**0**	**0**	**0**	**0**	**18**	**0**	**0**	**0**	**12**	**0**	**0**	**11**	**0**	**0**	**0**	**0**	**0**	**7**
	Sphingopyxis		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**30**	**0**	**0**	**0**	**84**	**0**	**26**	**0**	**0**	**0**	**24**	**0**	**0**
	Sphingorhabdus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**9**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Sporacetigenium		**0**	**0**	**0**	**0**	**40**	**0**	**0**	**0**	**36**	**0**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	mesophilum		0	0	0	0	40	0	0	0	36	0	0	0	0	0	0	0	0	0	0	0
	Sporanaerobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Sporobacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**11**	**0**	**10**	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Sporocytophaga		**0**	**0**	**5**	**0**	**0**	**0**	**15**	**25**	**119**	**0**	**154**	**8**	**35**	**0**	**0**	**112**	**68**	**0**	**11**	**14**
	Sporosarcina		**27**	**0**	**0**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**71**
	globispora		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	57
	pasteurii		6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	14
	Sporotomaculum		**8**	**39**	**0**	**0**	**0**	**0**	**8**	**0**	**5**	**0**	**83**	**8**	**0**	**28**	**0**	**0**	**0**	**0**	**0**	**0**
	Stackebrandtia		**56**	**7**	**0**	**22**	**0**	**0**	**0**	**24**	**0**	**10**	**0**	**0**	**0**	**0**	**9**	**9**	**0**	**12**	**7**	**20**
	Staphylococcus		**92**	**22**	**0**	**88**	**0**	**0**	**0**	**13**	**25**	**20**	**0**	**35**	**0**	**0**	**16**	**0**	**0**	**3**	**0**	**0**
	fleurettii		0	0	0	0	0	0	0	0	20	0	0	0	0	0	16	0	0	0	0	0
	lentus		39	0	0	37	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
	Stenotrophomonas		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**14**	**0**	**17**	**0**	**67**	**0**	**0**	**0**	**0**	**0**	**0**
	koreensis		0	0	0	0	0	0	0	0	0	14	0	0	0	44	0	0	0	0	0	0
	maltophilia		0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
	Steroidobacter		**0**	**83**	**179**	**0**	**35**	**0**	**114**	**118**	**688**	**0**	**2158**	**33**	**1096**	**33**	**61**	**930**	**1353**	**140**	**487**	**386**
	Streptococcus		**91**	**21**	**0**	**126**	**18**	**120**	**0**	**32**	**11**	**0**	**15**	**18**	**0**	**37**	**0**	**0**	**0**	**0**	**0**	**0**
	henryi		14	0	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	parauberis		0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0
	Streptomyces		**29**	**29**	**0**	**40**	**246**	**0**	**11**	**20**	**0**	**26**	**3328**	**49**	**2043**	**92**	**0**	**0**	**0**	**84**	**51**	**154**
	thermocoprophilus		0	0	0	0	0	0	0	0	0	0	3274	49	2043	92	0	0	0	56	0	10
	Subsaxibacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**53**	**0**	**0**
	Sulfurifustis		**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**
	Sva0996_marine_group		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**44**	**0**	**0**	**0**	**0**	**17**	**17**	**0**	**30**	**18**
	Symbiobacterium		**1292**	**314**	**10**	**581**	**80**	**555**	**12**	**46**	**110**	**469**	**179**	**81**	**78**	**117**	**336**	**247**	**98**	**112**	**116**	**416**
	ostreiconchae		86	76	0	167	0	0	0	0	68	107	0	0	0	0	54	0	0	0	0	157
	terraclitae		0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	thermophilum		177	89	0	134	0	0	12	33	0	110	128	81	78	97	135	169	84	96	116	188
	Syntrophaceticus		**12**	**2**	**0**	**9**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Syntrophococcus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**7**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Syntrophomonas		**22**	**61**	**0**	**97**	**0**	**0**	**0**	**38**	**0**	**22**	**9**	**5**	**6**	**14**	**0**	**0**	**10**	**0**	**0**	**5**
Genus-	**Tagaea**		**10**	**13**	**82**	**9**	**0**	**0**	**47**	**14**	**235**	**13**	**1124**	**3**	**506**	**31**	**0**	**131**	**196**	**64**	**12**	**16**
Species	**Taibaiella**		**17**	**0**	**0**	**7**	**620**	**0**	**0**	**1091**	**30**	**16**	**75**	**423**	**490**	**423**	**286**	**0**	**92**	**169**	**4**	**11**
	Tepidanaerobacter		**179**	**234**	**0**	**481**	**0**	**0**	**21**	**26**	**34**	**240**	**27**	**0**	**0**	**15**	**22**	**17**	**11**	**0**	**4**	**7**
	Tepidimicrobium		**1233**	**520**	**0**	**2404**	**0**	**0**	**10**	**227**	**156**	**2997**	**84**	**47**	**40**	**51**	**301**	**83**	**48**	**40**	**90**	**229**
	xylanilyticum		0	0	0	0	0	0	0	0	0	182	0	0	0	0	0	0	0	0	0	0
	Tepidiphilus		**6**	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**18**	**0**	**0**	**0**
	Terrimicrobium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**32**	**0**	**0**	**0**	**0**	**6**	**13**	**0**	**0**	**0**
	Terrisporobacter		**0**	**0**	**0**	**0**	**510**	**0**	**0**	**0**	**22**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Tetrasphaera		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**27**	**0**	**11**	**0**	**0**	**0**	**31**	**0**	**0**
	Thermaerobacter		**422**	**15**	**0**	**75**	**0**	**0**	**0**	**6**	**40**	**85**	**12**	**16**	**0**	**98**	**42**	**7**	**0**	**0**	**5**	**14**
	Thermasporomyces		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**8**	**5**	**5**	**14**	**0**	**0**	**0**	**0**	**0**	**0**
	composti		0	0	0	0	0	0	0	0	0	0	8	5	0	14	0	0	0	0	0	0
	Thermicanus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**7**
	Thermincola		**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**6**	**0**	**0**	**8**
	Thermoactinomyces		**42**	**30**	**12**	**30**	**0**	**0**	**14**	**27**	**0**	**9**	**27**	**19**	**0**	**0**	**6**	**7**	**0**	**43**	**56**	**159**
	daqus		0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0
	vulgaris		37	22	12	30	0	0	0	0	0	0	27	19	0	0	0	0	0	26	32	86
	Thermobacillus		**2660**	**3022**	**1302**	**470**	**607**	**4217**	**956**	**2908**	**3313**	**1731**	**5704**	**1144**	**1216**	**1518**	**936**	**846**	**792**	**585**	**722**	**1159**
	composti		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0
	Thermobifida		**311**	**2891**	**1240**	**186**	**1063**	**2584**	**856**	**746**	**1030**	**1218**	**603**	**164**	**330**	**627**	**237**	**132**	**79**	**189**	**129**	**470**
	alba		0	0	0	0	0	135	0	286	90	0	0	0	0	0	0	0	0	0	0	0

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
	cellulosilytica		78	448	555	52	577	2048	608	33	570	36	201	9	82	13	18	34	24	18	12	42
	fusca		233	2440	683	134	486	401	248	427	370	1182	402	155	248	614	219	98	55	171	117	426
	Thermobispora		**73**	**78**	**25**	**54**	**0**	**65**	**0**	**15**	**21**	**775**	**173**	**46**	**99**	**63**	**120**	**68**	**50**	**168**	**126**	**520**
	bispora		55	46	9	25	0	65	0	0	21	441	80	24	51	34	56	50	21	89	73	336
	Thermocrispum		**0**	**4**	**0**	**0**	**0**	**0**	**0**	**0**	**25**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	agreste		0	4	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0	0	0	0
	Thermodesulfobacterium		**892**	**81**	**0**	**1700**	**0**	**0**	**0**	**10**	**0**	**415**	**0**	**0**	**0**	**11**	**49**	**10**	**31**	**0**	**12**	**22**
	commune		883	81	0	1698	0	0	0	10	0	415	0	0	0	11	49	10	31	0	12	22
	Thermoflavimicrobium		**304**	**1577**	**87**	**164**	**1662**	**210**	**662**	**1033**	**1404**	**818**	**736**	**135**	**151**	**183**	**631**	**724**	**768**	**205**	**146**	**347**
	Thermogutta		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**28**
	Thermoleophilum		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**12**
	Thermomicrobium		**747**	**156**	**17**	**176**	**0**	**0**	**19**	**34**	**118**	**295**	**143**	**255**	**44**	**610**	**365**	**227**	**128**	**171**	**118**	**505**
	Thermomonospora		**59**	**214**	**161**	**35**	**256**	**0**	**114**	**179**	**201**	**132**	**678**	**34**	**440**	**40**	**9**	**12**	**16**	**360**	**374**	**570**
	chromogena		26	23	62	14	256	0	44	0	0	0	358	13	250	8	0	0	0	188	143	267
	curvata		0	59	0	0	0	0	19	45	56	50	91	0	43	20	0	0	0	0	37	57
	Thermopetrobacter		**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**13**	**0**
	Thermophagus		**0**	**12**	**24**	**0**	**0**	**0**	**84**	**18**	**1145**	**4**	**260**	**0**	**302**	**16**	**0**	**7**	**0**	**0**	**0**	**0**
	Thermopolyspora		**405**	**2032**	**2650**	**269**	**133**	**372**	**1497**	**1078**	**6162**	**1330**	**4391**	**168**	**3334**	**234**	**283**	**585**	**982**	**1155**	**1965**	**2363**
	flexuosa		366	1885	2619	269	0	372	1497	940	5956	1166	2827	107	2269	165	281	585	982	886	1629	1914
Genus-	**Thermosediminibacter**		**233**	**11**	**0**	**72**	**0**	**0**	**0**	**21**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Species	**Thermosyntropha**		**16**	**0**	**0**	**9**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**
	Thermotoga		**344**	**7**	**0**	**77**	**0**	**211**	**0**	**43**	**0**	**66**	**0**	**3**	**0**	**10**	**0**	**0**	**0**	**0**	**0**	**0**
	petrophila		344	7	0	77	0	107	0	43	0	66	0	0	0	10	0	0	0	0	0	0
	Thermovorax		**69**	**0**	**0**	**0**	**0**	**0**	**0**	**10**	**0**	**48**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**
	Thermus		**14981**	**718**	**25**	**8212**	**0**	**194**	**25**	**657**	**142**	**5906**	**245**	**227**	**67**	**1305**	**1961**	**114**	**150**	**103**	**96**	**221**
	Thioalkalimicrobium		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Thioalkalispira		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**
	Thiofaba		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**
	Thiopseudomonas		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**79**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Timonella		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**256**	**0**	**74**	**0**	**303**	**0**	**1048**	**0**	**0**	**0**	**0**	**0**	**0**
	senegalensis		0	0	0	0	0	0	0	46	0	0	0	12	0	23	0	0	0	0	0	0
	Tissierella		**50**	**32**	**0**	**58**	**0**	**0**	**0**	**175**	**22**	**82**	**13**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**14**
	creatinini		0	0	0	0	0	0	0	6	0	0	7	0	0	0	0	0	0	0	6	8
	Tomitella		**7**	**0**	**6**	**0**	**0**	**29**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Treponema		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**21**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**59**	**0**	**0**
	Treponema_2		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**19**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**37**	**0**	**0**
	Trichococcus		**44**	**15**	**8**	**15**	**0**	**81**	**0**	**20**	**223**	**45**	**0**	**81**	**0**	**155**	**0**	**0**	**0**	**104**	**0**	**0**
	Tropicihabitans		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**
	Truepera		**167**	**702**	**799**	**48**	**0**	**95**	**773**	**2446**	**4809**	**1751**	**4804**	**1040**	**1599**	**308**	**401**	**1005**	**818**	**909**	**1951**	**2350**
	Tuberibacillus		**6**	**0**	**0**	**0**	**0**	**0**	**7**	**0**	**0**	**5**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Supplementary Table 1. Taxonomic summary of filtered and assigned 16S sequences of end-Phase I, mid-Phase II and end-Phase II compost samples from five compost yards in Australia. The summary is ordered by compost yard, phase, crop and taxonomic levels (Kingdom to Species). ASVs not assigned to any taxonomic rank are not shown.

Factors		Compost yard	A							B			C				D			E		
		Phase	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase II	End-Phase I	End-Phase II	End-Phase I	End-Phase I	End-Phase II	End-Phase I	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II	End-Phase I	Mid-Phase II	End-Phase II
Taxonomic level		Crop	1	1	1	2	2	2	3	1	1	2	1	1	2	2	1	1	1	1	1	1
	calidus		6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Tumebacillus		**17**	**0**	**0**	**0**	**0**	**0**	**0**	**82**	**0**	**0**	**26**	**0**	**0**	**0**	**0**	**7**	**0**	**0**	**0**	**7**
	UBA6140		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**140**	**0**	**0**	**28**	**28**	**47**	**0**	**0**	**42**	**210**	**27**	**0**	**0**
	Ureibacillus		**2757**	**1525**	**846**	**1982**	**826**	**1170**	**483**	**2047**	**1479**	**14609**	**1405**	**680**	**334**	**737**	**2736**	**1226**	**1184**	**827**	**1213**	**2993**
	defluvii		473	554	291	453	123	842	192	683	675	7990	407	105	72	171	0	0	28	135	208	544
	suwonensis		116	27	22	101	0	0	0	104	48	89	43	50	0	42	70	136	87	56	86	142
	thermophilus		363	52	0	113	0	0	0	145	0	535	51	66	59	77	144	0	71	0	64	72
	thermosphaericus		553	523	332	307	267	0	155	321	206	2212	267	181	133	161	286	138	140	204	319	748
	Veillonella		**0**	**0**	**0**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Verruc-01		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Genus-	**Verrucomicrobium**		**20**	**0**	**0**	**0**	**0**	**0**	**0**	**797**	**27**	**57**	**9**	**162**	**0**	**0**	**29**	**0**	**14**	**54**	**0**	**0**
Species	**Verticia**		**58**	**96**	**61**	**0**	**29**	**0**	**31**	**154**	**101**	**34**	**63**	**12**	**19**	**31**	**26**	**37**	**18**	**0**	**32**	**183**
	Vibrio		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**10**	**0**	**0**	**0**	**0**	**0**	**20**	**0**	**0**	**0**	**0**	**0**	**0**
	Virgibacillus		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**9**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	soli		0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0
	Vitellibacter		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**17**	**0**	**0**
	Vulgatibacter		**490**	**1480**	**556**	**349**	**159**	**637**	**369**	**803**	**1119**	**898**	**981**	**119**	**341**	**225**	**485**	**263**	**227**	**312**	**641**	**1191**
	W5053		**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Weissella		**110**	**10**	**0**	**39**	**0**	**0**	**0**	**40**	**0**	**19**	**0**	**10**	**0**	**11**	**0**	**0**	**0**	**0**	**0**	**0**
	paramesenteroides		63	0	0	23	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0
	Yaniella		**178**	**81**	**0**	**141**	**0**	**0**	**0**	**32**	**23**	**38**	**0**	**11**	**0**	**32**	**0**	**0**	**0**	**0**	**0**	**0**
	ZOR0006		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Supplementary Table 2. Strain collection of bacterial isolates from mushroom compost. Restriction fragment length polymorphism (RFLP) pattern, colony morphology, growth at 30 and 50 °C, and enzyme activities

			RFLP		Colony morphology					Growth temperature		Enzyme activity				
Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
Bordetella petrii	EP2451	end-PII	670, 320, 140	570, 440, 300, 110	white	circular	raised	entire	1	Y	Y	N	N	Y	N	ND
Pseudoxanthomonas taiwanensis	EP245211	end-PII	500, 450, 210, 180, 140	960, 370, 150, 110	creamy pale yellow to pale orange	circular	raised	entire	2	W	Y	N	N	Y	N	ND
Pseudoxanthomonas taiwanensis	EP245212	end-PII	500, 450, 210, 180, 140	940, 350, 150, 110	transparent yellow to orange	circular	raised	entire	2	N	Y	N	N	Y	Y	ND
Pseudoxanthomonas taiwanensis	EP245213	end-PII	500, 450, 210, 180, 140	960, 370, 150, 110	transparent yellow to orange	circular	raised	entire	2	N	Y	N	N	Y	Y	ND
Serratia ureilytica	EP24541	end-PII	*	*	white	circular	undulate	entire	5 to 6	Y	Y	N	N	Y	Y	ND
Pseudoxanthomonas taiwanensis	EP24542	end-PII	500, 450, 210, 180, 140	980, 370, 150, 110	creamy pale yellow to pale orange	circular	raised	entire	2	N	Y	N	N	Y	Y	ND
Staphylococcus epidermidis	EP245421	end-PII	980, 650, 340, 180	420, 350, 260, 160	pale yellow	circular	convex	entire	2	Y	Y	N	N	Y	Y	ND

Supplementary Table 2. Strain collection of bacterial isolates from mushroom compost. Restriction fragment length polymorphism (RFLP) pattern, colony morphology, growth at 30 and 50 °C, and enzyme activities

Best Hit (BLAST)	Strain Name	Phase	RFLP		Colony morphology					Growth temperature		Enzyme activity				
			HinfI	*HhaI*	Colour	Shape	Elevation	Margin	Diameter (mm)	30 °C	50 °C	Cellulase	Xylanase	Peroxidase	Laccase	Chitinase
Staphylococcus epidermidis	EP245422	end-PII	1000, 310, 170	430, 360, 260, 250, 100	white	circular	flat	entire	1 to 2	ND	ND	N	N	ND	ND	ND
Pseudoxanthomonas taiwanensis	EP24543	end-PII	500, 450, 200, 160, 130	950, 360, 150, 110	pale yellow	circular	raised	entire	1	ND	ND	N	N	Y	Y	ND
Parapusillimonas granuli	EP2455	end-PII	650, 320, 150, 70	480, 380, 270, 110	white	circular	raised	smooth	1	N	Y	N	N	N	N	ND
Pseudoxanthomonas taiwanensis	EP24561	end-PII	500, 450, 200, 160, 130	950, 350, 150, 110	pale yellow	circular	convex	entire	1	ND	ND	N	N	Y	Y	ND
Pseudoxanthomonas taiwanensis	EP24562	end-PII	500, 450, 200, 160, 130	1030, 350, 140, 90	cream/pale yellow	circular	raised	entire	1	ND	ND	N	N	ND	ND	ND
Pseudoxanthomonas taiwanensis	EP2457	end-PII	500, 450, 200, 160, 130	980, 340, 140, 90	cream/ pale yellow	circular	flat	entire	1	ND	ND	N	N	ND	ND	ND

Supplementary Table 2. Strain collection of bacterial isolates from mushroom compost. Restriction fragment length polymorphism (RFLP) pattern, colony morphology, growth at 30 and 50 °C, and enzyme activities

			RFLP		Colony morphology					Growth temperature		Enzyme activity				
Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
Pseudoxanthomonas taiwanensis	EP24571	end-PII	500, 450, 200, 160, 130	970, 340, 140, 90	transparent yellow to orange	circular	raised	entire	1	N	Y	N	N	N	Y	ND
*	EP24591	end-PII	500, 450, 200, 160, 130	980, 330, 160, 130	white	circular	flat	undulate	5 to 6	Y	Y	N	N	Y	Y	ND
Bacillus safensis	EP245182	end-PII	*	*	white to cream	circular	umbonate	undulate	5	Y	Y	N	N	Y	Y	ND
Pseudoxanthomonas suwonensis	EP2451911	end-PII	980, 260, 230, 190	710, 340, 90	pale yellow	circular	convex	entire	5	Y	Y	N	N	N	N	ND
Pseudoxanthomonas suwonensis	EP245192	end-PII	980, 260, 230, 190	700, 340, 90	pale yellow	circular	flat	entire	5 to 6	Y	Y	N	N	Y	Y	ND
*	EP24520	end-PII	980, 260, 230, 190	720, 350, 80	White	circular	convex	entire	3 to 5	Y	Y	N	N	N	N	ND
Pseudoxanthomonas suwonensis	EP24521	end-PII	980, 260, 230, 190	720, 360, 80	pale yellow	circular	flat	entire	3 to 8	W	Y	N	N	Y	Y	ND
Pseudoxanthomonas taiwanensis	EP245241	end-PII	480, 430, 200,	940, 330, 110, 70	creamy yellow	circular	convex	entire	2	W	Y	N	N	Y	Y	ND

Supplementary Table 2. Strain collection of bacterial isolates from mushroom compost. Restriction fragment length polymorphism (RFLP) pattern, colony morphology, growth at 30 and 50 °C, and enzyme activities

			RFLP		Colony morphology					Growth temperature		Enzyme activity				
Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
Enterobacter hormaechei	EP245242	end-PII	160, 130 1000, 340	980, 320, 230, 170, 60	white to cream	circular	umbonate	undulate	3 to 5	Y	W	N	N	N	Y	ND
Pseudoxanthomonas suwonensis	EP24525	end-PII	990, 340, 220, 200, 140	720, 350, 80	pale yellow	circular	convex	entire	2	Y	Y	N	N	N	Y	ND
*	EP245281	end-PII	990, 210, 190, 140	720, 360, 80	white	circular	flat	entire	4 to 5	Y	W	N	Y	ND	N	ND
Sphingobacterium thermophilum	EP24529	end-PII	840, 310, 170	960. 410, 110	white	circular	convex	entire	4	Y	N	N	N	ND	N	ND
Enterobacter hormaechei	EP245292	end-PII	480, 420, 310, 200, 180, 130	920, 330, 110, 70	white	circular	umbonate	undulate	3 to 5	Y	N	N	N	N	Y	ND
Pseudoxanthomonas taiwanensis	EP24530	end-PII	480, 430, 320, 200, 180, 130	910, 320, 110, 60	pale yellow	circular	convex	entire	1	N	Y	N	N	N	N	ND

Supplementary Table 2. Strain collection of bacterial isolates from mushroom compost. Restriction fragment length polymorphism (RFLP) pattern, colony morphology, growth at 30 and 50 °C, and enzyme activities

			RFLP		Colony morphology					Growth temperature		Enzyme activity				
Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
Sphingobacterium thermophilum	EP245321	end-PII	830, 340, 220, 190	980, 390, 120, 80	cream	circular	flat	entire	3 to 5	Y	W	N	N	Y	N	ND
*	EP24533	end-PII	960, 480, 440, 320, 200, 170, 130	1050, 920, 300, 210, 160, 70	*	*	*	*	*	Y	Y	N	N	Y	N	ND
Pseudoxanthomonas suwonensis	EP24534	end-PII	990, 210, 190, 140	690, 350, 80	pale yellow	circular	flat	entire	3 to 8	ND	ND	N	N	ND	ND	ND
Microbacterium maritypicum	EP191	end-PI	510, 460, 230, 140	390, 230, 160, 130, 90	clear yellow	circular	umbonate	entire	4 to 5	Y	N	N	N	N	N	ND
Dermacoccus nishinomiyaensis	EP193	end-PI	470, 220, 210, 100	630, 400, 240, 150, 100	pale orange	irregular	convex	lobate	1 to 3	ND	ND	N	N	ND	ND	ND
Dermacoccus nishinomiyaensis	EP194	end-PI	500, 230, 200, 100	700, 400, 230, 140, 90	orange	circular	convex	entire	1 to 5	Y	W	N	N	N	N	ND

Supplementary Table 2. Strain collection of bacterial isolates from mushroom compost. Restriction fragment length polymorphism (RFLP) pattern, colony morphology, growth at 30 and 50 °C, and enzyme activities

			RFLP		Colony morphology					Growth temperature		Enzyme activity				
Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
Microbacterium maritypicum	EP196	end-PI	490, 420, 200, 180, 140, 80	360, 210, 150, 110, 80	opaque yellow	circular	raised	entire	3	Y	W	N	N	N	N	ND
Bacillus safensis	EP1-5911	mid-PII	630, 370, 90	890, 400, 240, 80	white	circular	raised	entire	1 to 3	Y	Y	N	Y	Y	N	ND
Sphingobacterium thermophilum	EP1-592	mid-PII	820, 290, 170, 100	850, 350, 120, 100	cream	circular	convex	entire	3	W	Y	N	N	N	N	ND
*	EP1-5931	mid-PII	*	*	cream	circular	flat	entire	6	Y	Y	N	N	N	Y	ND
*	EP1-5932	mid-PII	*	*	yellow	circular	flat	entire	2 to 3	Y	N	N	N	N	Y	ND
Pseudoxanthomonas suwonensis	EP1-594	mid-PII	920, 200, 170, 130	690, 360, 100	creamy yellow	circular	convex	entire	2	W	Y	N	N	Y	N	ND
Pseudoxanthomonas suwonensis	EP1-595	mid-PII	940, 200, 180, 130, 70	720, 340, 80	pale yellow	circular	convex	entire	4	W	Y	N	N	Y	N	ND
Microbacterium maritypicum	EP1-596	mid-PII	490, 430, 200, 150, 80	350, 200, 140, 110, 80	white to yellow	circular	raised	entire	1 to 2	Y	N	N	N	N	N	ND

Supplementary Table 2. Strain collection of bacterial isolates from mushroom compost. Restriction fragment length polymorphism (RFLP) pattern, colony morphology, growth at 30 and 50 °C, and enzyme activities

			RFLP		Colony morphology					Growth temperature		Enzyme activity				
Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
Microbacterium foliorum	EP1-597	mid-PII	500, 430, 190, 50	400, 320, 150, 80	yellow to white	circular	convex	entire	1	Y	N	Y	N	N	Y	ND
Lysinibacillus acetophenoni	EP1-5981	mid-PII	890, 340, 280, 200	360, 310, 250, 200	white	circular	umbonate	undulate	2	W	N	N	N	N	N	ND
Dermacoccus nishinomiyaensis	EP1-5991	mid-PII	480, 220, 180, 70	680, 390, 220, 130, 80	Orange	circular	umbonate	undulate	1 to 3	W	N	N	N	N	Y	ND
Staphylococcus succinus	EP1-5992	mid-PII	910, 310, 170	360, 220, 120, 80	cream	circular	raised	entire	1	Y	N	N	N	Y	Y	ND
Pseudoxanthomonas suwonensis	EP1-59101	mid-PII	900, 320, 210, 180, 140	660, 330, 80	pale yellow	circular	flat	entire	5 to 6	Y	Y	N	N	N	Y	ND
Pseudoxanthomonas suwonensis	EP1-59102	mid-PII	1000, 220, 200, 160	740, 360, 110	pale yellow	circular	convex	entire	5 to 6	W	Y	N	N	N	Y	ND
*	EP1-5911	mid-PII	*	*	Creamy Yellow	circular	raised	entire	1 to 2	Y	N	N	N	N	Y	ND
Pseudoxanthomonas suwonensis	SP1242	end-PI	980, 200, 170, 140, 80	710, 320, 90	Creamy Yellow	circular	flat	entire	2 to 6	W	Y	N	N	Y	N	ND

Supplementary Table 2. Strain collection of bacterial isolates from mushroom compost. Restriction fragment length polymorphism (RFLP) pattern, colony morphology, growth at 30 and 50 °C, and enzyme activities

Best Hit (BLAST)	Strain Name	Phase	RFLP		Colony morphology					Growth temperature		Enzyme activity				
			HinfI	*HhaI*	Colour	Shape	Elevation	Margin	Diameter (mm)	30 °C	50 °C	Cellulase	Xylanase	Peroxidase	Laccase	Chitinase
*	SP12442	end-PI	1040, 210, 170, 130	▲	pale yellow	circular	convex	entire	3 to 4	W	Y	N	N	Y	N	ND
*	SP1245	end-PI	*	*	light yellow	circular	raised	entire	2 to 3	Y	W	N	N	N	Y	ND
Sphingobacterium alimentarium	SP1246	end-PI	820, 180, 120, 80	990, 390, 120, 90	orange/dark yellow	circular	convex	entire	3	Y	W	N	N	N	Y	ND
*	SP12482	end-PI	*	*	white to yellow	circular	raised	entire	1	Y	N	N	N	N	N	ND
Arthrobacter psychrochitiniphilus	SP1249	end-PI	830, 210, 180, 80	460, 390, 240, 160, 80	opaque yellow	circular	raised	entire	2 to 3	Y	N	N	N	N	N	ND
*	SP12492	end-PI	*	*	yellow/shiny when held to light	circular	flat	entire	1 to 2	Y	N	N	N	ND	ND	ND
Staphylococcus epidermidis	SP12512	end-PI	1000, 330, 170, 80	420, 350, 250, 90	white	circular	flat	entire	2	ND	ND	N	N	ND	ND	ND
Microbacterium maritypicum	SP1252	end-PI	500, 430, 190, 140, 80, 40	410, 230, 170, 140, 100	white	circular	raised	entire	1	Y	N	N	N	N	Y	ND

Supplementary Table 2. Strain collection of bacterial isolates from mushroom compost. Restriction fragment length polymorphism (RFLP) pattern, colony morphology, growth at 30 and 50 °C, and enzyme activities

			RFLP		Colony morphology					Growth temperature		Enzyme activity				
Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
Microbacterium natoriense	SP12531	end-PI	470, 410, 190, 170, 140, 80	370, 300, 150, 80	translucent yellow	circular	convex	entire	1 to 2	Y	N	N	N	N	Y	ND
*	SP1254	end-PI	490, 420, 190, 80	420, 240, 170, 90	clear yellow	circular	raised	undulate	2 to 5	Y	N	N	N	N	Y	ND
*	SP1255	end-PI	510, 440, 70	400, 320, 150, 80	translucent yellow	circular	convex	entire	1	Y	N	N	N	N	Y	ND
*	SP12551	end-PI	*	*	yellow	circular	raised	entire	2 to 3			N	N	ND	ND	ND
Pseudoxanthomonas suwonensis	SP2242	end-PII	980, 260, 230, 190	740, 380, 90	white	circular	flat	entire	3	Y	W	N	N	Y	N	ND
Cronobacter condimenti	SP224411	end-PII	660, 320, 150	540, 360, 210, 110	white	circular	raised	undulate	4	Y	W	N	N	Y	N	ND
Pseudoxanthomonas suwonensis	SP224511	end-PII	990, 220, 190, 140	700, 350, 80	pale yellow	circular	convex	entire	2	Y	Y	N	N	N	Y	ND
Acinetobacter johnsonii	SP2245121	end-PII	1000, 210, 190	620, 390, 230, 200, 70	pale yellow	circular	raised	entire	2	Y	Y	N	N	N	N	ND

Supplementary Table 2. Strain collection of bacterial isolates from mushroom compost. Restriction fragment length polymorphism (RFLP) pattern, colony morphology, growth at 30 and 50 °C, and enzyme activities

			RFLP		Colony morphology					Growth temperature		Enzyme activity				
Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
Pseudoxanthomonas suwonensis	SP2245122	end-PII	990, 210, 180, 140	740, 350, 70	yellow	circular	raised	entire	1 to 3	W	Y	N	N	Y	N	ND
*	SP22461	end-PII	*	*	white	circular	flat	entire	1	N	Y	N	Y	N	Y	ND
*	SP22462	end-PII	*	*	yellow	irregular	flat	undulate	8	Y	Y	N	N	N	Y	ND
Pseudoxanthomonas taiwanensis	SP22463	end-PII	510, 460, 210, 180, 140, 70	1160, 340, 70	white	circular	raised	entire	2 to 4	Y	N	N	N	N	N	ND
*	SP22471	end-PII	*	*	creamy yellow/pale yellow	circular	flat	entire	2	W	Y	N	Y	Y	N	ND
Pseudoxanthomonas taiwanensis	SP224711	end-PII	490, 450, 210, 180, 140	1100, 360, 90	white	circlar	convex	entire	2	Y	Y	N	Y	Y	N	ND
*	SP22472	end-PII	*	*	white	circular	flat	entire	6	Y	W	N	N	Y	N	ND
Pseudoxanthomonas suwonensis	SP2248	end-PII	1010, 210, 180, 140	770, 350, 70	pale yellow	circular	raised	entire	3	Y	Y	N	N	Y	Y	ND
*	SP22491	end-PII	*	*	white	irregular	raised	enite	4 to 5	Y	Y	N	Y	Y	N	ND

Supplementary Table 2. Strain collection of bacterial isolates from mushroom compost. Restriction fragment length polymorphism (RFLP) pattern, colony morphology, growth at 30 and 50 °C, and enzyme activities

			RFLP		Colony morphology					Growth temperature		Enzyme activity				
Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
Pseudoxanthomonas suwonensis	SP22492	end-PII	1010, 210, 180, 140	720, 340, 70	pale yellow	circular	convex	entire	5 to 6	Y	Y	N	N	N	N	ND
Pseudoxanthomonas suwonensis	SP224111	end-PII	930, 320, 200, 180, 140	720, 360, 70	white	circular	flat	entire	3 to 4	Y	W	N	N	N	Y	ND
Pseudoxanthomonas suwonensis	SP224112	end-PII	920, 310, 200, 170, 130	720, 350, 70	Creamy Yellow	circular	convex	entire	2 to 3	Y	Y	N	N	Y	N	ND
*	SP22412	end-PII	*	*	white	circular	flat	entire	1 to 3	Y	Y	N	N	Y	Y	ND
Bergeyella porcorum	CP111	end-PI	*	*	*	*	*	*	*			N	N	ND	ND	ND
Microbacterium foliorum	CP112	end-PI	490, 420, 200, 140, 80	390, 320, 170, 90, 50	yellow	circular	raised	entire	2	Y	W	N	N	N	N	ND
Pseudoxanthomonas suwonensis	CP1-512	mid-PII	1000, 220, 190, 150	690, 330, 80	light yellow	circular	raised	entire	2 to 3	Y	Y	N	N	Y	N	ND
Pseudoxanthomonas suwonensis	CP2111	end-PII	1000, 210, 190, 140	740, 350, 80	creamy/light yellow	circular	raised	entire	3 to 4	W	Y	N	N	Y	N	ND

Supplementary Table 2. Strain collection of bacterial isolates from mushroom compost. Restriction fragment length polymorphism (RFLP) pattern, colony morphology, growth at 30 and 50 °C, and enzyme activities

			RFLP		Colony morphology					Growth temperature		Enzyme activity				
Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
Microbacterium maritypicum	CP2132	end-PII	460, 410, 200, 150, 90, 50	400, 230, 170, 140, 100	yellow	circular	raised	entire	2	Y	N	N	N	N	N	ND
Acinetobacter variabilis	CP214	end-PII	*	*	white	circular to irregular	raised	undulate	2	Y	N	N	N	Y	Y	ND
Mycovorax composti	CP216	end-PII	*	*	orange	circular	convex	entire	2	W	Y	N	Y	Y	N	Y
*	CP219	end-PII	*	*	translucent, shiny cream	circular	convex	entire	1.5	ND	ND	N	N	ND	ND	ND
*	CP2191	end-PII	*	*	translucent white	circular	convex	entire	1 to 2	W	N	N	N	N	N	ND
*	CP2192	end-PII	*	*	Orange	circular	convex	entire	2	W	Y	N	N	Y	Y	ND
Caulobacter segnis	CP21111	end-PII	*	*	shiny cream	circular	convex	entire	2 to 3	Y	N	N	N	N	Y	ND
Mycovorax composti	CP21121	end-PII	*	*	dark yellow/orange	circular	convex	entire	1	W	Y	N	N	N	Y	Y
Leadbetterella byssophila	MP1281	end-PI	760, 280, 150, 130, 80	▲	dark yellow/light orange	circular	raised	entire	3 to 5	ND	ND	N	N	N	N	ND
Leadbetterella byssophila	MP1282	end-PI	720, 290, 160, 140, 80	▲	yellow	circular	raised	entire	1 to 3	Y	N	N	N	N	Y	ND

Supplementary Table 2. Strain collection of bacterial isolates from mushroom compost. Restriction fragment length polymorphism (RFLP) pattern, colony morphology, growth at 30 and 50 °C, and enzyme activities

			RFLP		Colony morphology					Growth temperature		Enzyme activity				
Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
Microbacterium maritypicum	MP1284	end-PI	480, 430, 210, 190, 150, 90	420, 240, 170, 110	pale yellow	circular	convex	entire	3 to 5	Y	W	N	N	Y	N	ND
Cellulomonas flavigena	MP1285 1	end-PI	510, 360, 320, 200, 150, 140	▲	pale yellow	irregular	raised	undulate	3 to 4	Y	N	N	N	N	Y	ND
*	MP1285 2	end-PI	1010, 330, 180, 50	340, 260, 80	white	circular	flat	entire	1	Y	N	N	N	Y	Y	ND
Nitratireductor lucknowense	MP1286 1	end-PI	1000, 190	310, 260, 170, 140, 120, 80	white	circular	raised	entire	1 to 2	N	Y	N	N	N	Y	ND
*	MP1286 2	end-PI	950, 190, 120	300, 250, 170, 140, 130, 80	white	circular	raised	entire	1 to 2	N	Y	N	N	N	Y	ND
Sphingobacterium thermophilum	MP1287	end-PI	770, 280, 170, 140, 80	900, 370, 110, 90	white	circular	raised	entire	2	N	Y	N	N	Y	Y	ND
Glumacibacter arilaitensis	MP12911	end-PI	*	*	yellow	circular	flat	entire	1	Y	N	N	N	Y	N	ND

Supplementary Table 2. Strain collection of bacterial isolates from mushroom compost. Restriction fragment length polymorphism (RFLP) pattern, colony morphology, growth at 30 and 50 °C, and enzyme activities

			RFLP		Colony morphology					Growth temperature		Enzyme activity				
Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
*	MP12912	end-PI	▲	740, 360, 110	cream	circular	flat	entire	1	W	N	N	N	N	N	ND
*	MP12931	end-PI	740, 190, 140, 120, 80	390, 350, 260, 160, 80	opaque white	circular	umbonate	entire	4 to 5	Y	N	N	N	Y	Y	ND
*	MP12952	end-PI	▲	330, 300, 260, 160, 130, 80	opaque white	circular	raised	entire	1 to 2	Y	N	N	N	N	Y	ND
Acinetobacter movanagherensis	MP12961	end-PI	1000, 220, 190, 140, 110	340, 300, 240, 220, 190	white	circular	raised	entire	1	Y	N	N	N	Y	N	ND
*	MP12971	end-PI	*	*	red	circular	convex	entire	1 to 5	Y	N	N	N	Y	N	ND
*	MP2281	end-PII	500, 430, 190, 140	390, 220, 150, 80	yellow	circular	raised	entire	2 to 4	Y	N	N	N	N	N	ND
*	MP2282	end-PII	500, 430, 190, 80	400, 330, 170, 110	yellow	circular	convex	entire	1	Y	N	N	N	N	N	ND
Staphylococcus succinus	MP2283	end-PII	1070, 330, 180, 80	390, 220,	white	circular	convex	entire	<1	Y	N	N	N	N	Y	ND

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Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
				120,80												
Pseudoxanthomonas koreensis	MP2284	end-PII	980, 180, 150, 120, 80	650, 310, 80	opaque yellow	circular	convex	entire	6 to 7	Y	Y	N	N	N	Y	ND
Staphylococcus succinus	MP22851	end-PII	1030, 340, 190, 50	380, 220, 120, 80	Yellow	circular	raised	entire	0.5 to 1	Y	N	N	N	N	Y	ND
Staphylococcus succinus	MP22852	end-PII	1000, 330, 180, 50	380, 220, 120, 80	Yellow	circular	raised	entire	1 to 5	Y	N	N	N	N	Y	ND
Microbacterium maritypicum	MP2212	end-PII	*	*	Yellow	circular	convex	entire	2	Y	N	N	Y	Y	Y	ND
Staphylococcus succinus	MP2213	end-PII	960, 330, 190, 50	400, 250, 150, 100	pale yellow	circular	convex	entire	5	Y	Y	N	N	Y	N	ND
Pseudoxanthomonas suwonensis	MP22912	end-PII	1000, 200, 170, 80	740, 350, 80	creamy yellow	circular	convex	entire	2	N	Y	N	N	Y	Y	ND
Pseudoxanthomonas suwonensis	MP22921	end-PII	1000, 220, 190, 140, 50	780, 370, 110	creamy yellow	circular	convex	entire	2 to 3	W	Y	N	N	N	N	ND
Pseudoxanthomonas koreensis	MP2294	end-PII	980, 200, 170, 130, 80	540, 360, 110	yellow to orange	circular	convex	entire	5	Y	N	N	N	Y	Y	ND

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Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
Niabella terrae	MP22951	end-PII	*	*	Orange	circular	flat	entire	1	Y	Y	N	Y	N	Y	ND
*	MP22952	end-PII	*	*	*	*	*	*	*			N	N	ND	ND	ND
Solimonas soli	MP229721	end-PII	*	*	pale orange	irregular	flat	undulate	5	N	Y	N	N	Y	N	ND
Streptomyces sudanensis	MP22992	end-PII	*	*	Pale orange with white spores	irregular	flat	undulate	3	Y	Y	N	N	N	N	ND
Caulobacter segnis	MP22993	end-PII	▲	600, 320, 140, 80	Shiny cream	circular	convex	entire	2 to 3	Y	N	N	N	Y	N	ND
Streptomyces griseorubiginosus	MP22910	end-PII	*	*	Pale orange with white spores	circular	convex	undulate	2 to 3	Y	Y	N	Y	Y	N	ND
Cellulomonas iranensis	MP22911	end-PII	*	*	shiny yellow/orange	circular	convex	entire	1 to 1.5	Y	Y	N	N	Y	Y	ND
Lysinibacillus composti	TP116	end-PI	1000, 340, 190, 50	380, 320, 220, 180, 80	translucent yellow	circular	flat	entire	1 to 2	Y	N	N	N	N	N	ND
Microbacterium maritypicum	TP117	end-PI	450, 400, 200,	350, 200, 140,	yellow	circular	raised/convex	entire	1 to 5	Y	N	N	N	N	Y	ND

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			RFLP		Colony morphology					Growth temperature		Enzyme activity				
Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
			180, 140, 90	110, 70												
Pseudoxanthomonas suwonensis	TP11812	end-PI	1000, 200, 180, 130	730, 350, 80	creamy yellow	circular	convex	entire	2 to 4	Y	Y	N	N	N	Y	ND
*	TP119	end-PI	940, 200, 180, 130, 70	1100, 180, 140, 80	dark orange	circular	convex	entire	1 to 3	N	Y	N	N	Y	Y	ND
Pseudomonas fluvialis	TP1111	end-PI	*	*	transparent white	circular	raised	entire	1	Y	N	N	N	N	Y	ND
*	TP1112	end-PI	*	*	*	*	*	*	*	Y	Y	N	N	ND	ND	ND
Pseudoxanthomonas suwonensis	TP11111	end-PI	1000, 200, 180, 130	730, 350, 80	Creamy Yellow	circular	flat	entire	5 to 6	Y	Y	N	N	N	Y	ND
Pseudomonas sihuiensis	TP11112	end-PI	*	*	translucent white	circular	convex	entire	2	Y	N	N	N	Y	Y	ND
Pseudoxanthomonas suwonensis	TP11131	end-PI	1000, 200, 180, 130	730, 350, 80	cream with beige centre	circular	convex	undulate	2	Y	Y	N	N	N	N	ND
*	TP11141	end-PI	*	*	translucent white	circular	convex	entire	2	Y	N	N	N	N	N	ND
Pseudomonas fluvialis	TP11142	end-PI	*	*	translucent white	circular	convex	entire	2	Y	N	N	N	N	N	ND

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Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
Lysinibacillus chungkukjangi	TP1-513	mid-PII	1000, 340, 70	390, 330, 230, 180, 80	white	circular	flat	undulate	3	Y	W	N	N	N	N	ND
Methylobacterium radiotolerans	TP1-5161	mid-PII	*	*	pale red to intense red	circular	convex	entire	1	W	N	N	N	N	N	ND
Pseudoxanthomonas taiwanensis	TP1-5162	mid-PII	500, 450, 200, 170, 130, 70	1210, 360, 80	opaque creamy yellow with dark centre	circular	convex	entire	3 to 4	ND	ND	N	N	ND	ND	ND
Staphylococcus epidermidis	TP1-5171	mid-PII	1010, 330, 180, 70	590, 400, 330, 230, 130, 70	translucent white	irregular	flat	undulate	1	W	N	N	N	N	N	ND
Caulobacter segnis	TP1-5172	mid-PII	*	*	translucent white	circular	umbonate	entire	1 to 3	Y	N	N	N	N	N	ND
Pseudoxanthomonas suwonensis	TP1-518	mid-PII	880, 200, 170, 130	660, 340, 80	cream with beige centre	circular	raised	undulate	4 to 5	Y	Y	N	N	Y	Y	ND
Caulobacter segnis	TP1-519	mid-PII	*	*	shiny, translucent white/cream	circular	convex	entire	1	Y	N	N	N	N	Y	ND
Novosphingobium aromaticivorans	TP1-51101	mid-PII	*	*	shiny yellow/orange	circular	convex	entire	2 to 3	W	W	N	N	Y	Y	ND

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Best Hit (BLAST)	**Strain Name**	**Phase**	***HinfI***	***HhaI***	**Colour**	**Shape**	**Elevation**	**Margin**	**Diameter (mm)**	**30 °C**	**50 °C**	**Cellulase**	**Xylanase**	**Peroxidase**	**Laccase**	**Chitinase**
*	TP213	end-PII	1000, 320, 170, 70	590, 330, 140, 80	white	circular	convex	entire	2	Y	N	N	N	N	Y	ND
*	TP21411	end-PII	*	*	pale red/salmon	circular	raised	entire	1 to 2	Y	Y	N	N	Y	Y	ND
Gordonia westfalica	TP21412	end-PII	*	*	orange/pale red/salmon	circular	convex	entire	1 to 2	Y	Y	N	N	Y	N	ND
Pseudoxanthomonas taiwanensis	TP2142	end-PII	500, 450, 200, 170, 130, 70	1210, 360, 80	translucent yellow	circular	convex	entire	1	N	Y	N	N	Y	Y	ND

* – 16S sequencing/RFLP not yet completed

▲ – Fragment lengths could not be confirmed.

Y – Yes

N – No

W – Weak

ND – Not determined