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Effects of sterilization and maturity of compost on soil bacterial and fungal communities and wheat growth

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ABSTRACT

Composts are commonly used as soil amendments to sustain and improve the functionality of agricultural soil. Compost has abiotic (organic matter [OM], nutrients) and biotic characteristics (microorganisms) and both can influence the soil microbiome. The abiotic and biotic characteristics of compost, in turn, depend on properties of the compost such as maturity. Few studies have investigated the relative effects of abiotic and biotic components of compost on the soil microbial community and crop growth. To bridge this gap, we used a full-factorial design with sterile and live composts that differed in maturity (fresh, intermediate, mature) that were added to sterile and live soil to investigate the separate role of abiotic and biotic characteristics of composts on the resulting soil microbial community and on wheat growth. We found that the changes in the soil microbial community were mainly due to the input of compost with the presence of microorganisms rather than due to the abiotic properties of compost. The majority of the compost-associated microorganisms (more than 70% for bacteria and 90% for fungi) were detected in the soil in the presence of native soil microorganisms. Elimination of native soil microorganisms by sterilization enhanced the prevalence and abundance of compost-associated microorganisms. Adding fresh compost increased wheat biomass production, but the positive effects of compost on plant growth were strongest when sterile composts were used. Hence, our study reports that compost-associated microorganisms are essential to modify soil microbial community but may not benefit crop growth. This highlights the importance of understanding the role of abiotic and biotic properties of composts as common soil amendments on improving the functioning of agricultural soil.

1. Introduction

Due to intensive agricultural practices worldwide, soils suffer from erosion, nutrient depletion, declining biodiversity, and consequently from declining agricultural productivity (Agegnehu et al., 2017; Kirschenmann, 2010). Soil microbial community composition and function are sensitive to changes in the soil microenvironment (Liu et al., 2021), and applying soil amendments, such as composts, has long been used to sustain and improve the functionality of agricultural soils (Garcia and García, 2015; Rivero et al., 2004; Xu et al., 2020). However, the relative effects of abiotic (organic matter [OM] and nutrients) and biotic (microbiota) properties of composts, on the soil microbial community and on crop growth remain poorly understood.

As it is rich in OM and nutrients, addition of compost can increase the growth of soil microorganisms and plants via provision of nutrients. The concentration of labile and humic OM compounds in compost depends on the maturity of the compost (Aguiar et al., 2013; Cozzolino et al., 2016), and an important question is how maturity of the compost influences the composition of the microbial community that is present in the soil. Fresh compost contains relatively high concentrations of readily available nutrients and higher amounts of biolabile OM, which can be easily accessed by microorganisms and plants (Cozzolino et al., 2016; Spaccini and Piccolo, 2009). However, amending soil with fresh compost can also reduce the availability of nutrients to plants as it can promote immobilization of nutrients in rapidly growing soil microbial populations (Cozzolino et al., 2016).

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Mature compost has concentrated hydrophobic alkyl and aromatic compounds, and this reduces the accessibility of biolabile hydrophilic carbon, and thus limits the nutrient immobilization for soil microbial communities (Spaccini and Piccolo, 2007, 2009). Compost maturity can be defined based on physical, chemical, or biological characteristics of compost (Bernal et al., 2009). In this paper, we classify the maturity of compost based on the duration of an aerobic process that the compost was exposed to air (0, 20, 60 days in this study).

Apart from being a carrier of organic matter, compost contains a wealth of microbes and can act as a microbial inoculum. A number of studies show that compost-associated microorganisms can change the composition or activity of soil microorganisms and improve soil health. These effects disappear after sterilization or pasteurization of the compost (Antoniou et al., 2017; Bonanomi et al., 2010; Mendes et al., 2011; Papasotiriou et al., 2013). However, the role of compost-associated microorganisms in modifying soil microbial communities is debated. Compost-associated microorganisms that are incorporated in the soil may be outcompeted by native soil microorganisms, and changes in the characteristics of the soil microbial community may be mainly due to the input of OM rather than due to the microbial characteristics of compost (Saison et al., 2006). Eliminating the active native soil microbial community by sterilization is therefore expected to benefit the establishment of compost-associated microorganisms in the soil (Saison et al., 2006).

The majority of studies examining the influence of compost as soil amendment on the soil microbial community focus either on the OM and nutrients present in the compost, or on the composition of compost-associated microorganisms (Aguiar et al., 2013; Antoniou et al., 2017; Bernal et al., 2009; Bonanomi et al., 2010; Cozzolino et al., 2016; Mendes et al., 2011; Papasotiriou et al., 2013). However, the OM/nutrients and the microbiota of compost determine its characteristics. The aim of the current study was to disentangle the effects of abiotic and biotic properties of composts on the soil microbial community. We used three different compost maturities to vary the quality and microbial composition of the compost. We hypothesized that: 1) compost addition would change soil microbial community composition, and that addition of live composts would affect soil microbial composition more than addition of sterile composts which only provide OM and nutrients; 2) eliminating the native soil microbial community would enhance the prevalence of compost-associated microorganisms in the soil; 3) fresh compost would influence soil microbial community composition to a greater extent than more mature compost, since fresh compost provides microorganisms with more easily available nutrients and OM. Furthermore, we tested how these compost-induced changes in the soil influenced wheat growth. To test our hypotheses, we used a full-factorial design with live and sterile composts that differed in maturity and added them to live and sterile soil. Using live and sterile composts, we can examine the impact of compost-associated microorganisms and abiotic properties of composts on the soil and on wheat growth. Elimination of the native soil microorganisms by sterilization enables us to examine the role of the soil microbiome in these interactions. In this study, compost-associated microorganisms refer to the microorganisms detected in compost prior to incorporation into the soil.

2. Materials and methods

2.1. Soil and compost collection

A microcosm experiment was performed with sandy soil comprising a mixture of agricultural soil (10%, w/w) and sterile background soil (90%, w/w). The agricultural soil (~5% OM content) was collected from the surface layer (0–10 cm) of an organically managed agricultural field in Wageningen, in The Netherlands. The sterile background soil (~2% OM content) was used to reduce the organic matter content of the agricultural soil and amplify the effects of amended exogenous OM (i.e., compost). The background soil was sterilized by Gamma irradiation (STERIS, Ede, The Netherlands) with a dose of > 25 kGy.

The compost was provided by Attero (Apeldoorn, The Netherlands), and consisted of 25% organic household waste and 75% of yard waste. The compost went through standard composting processes before arriving in the laboratory. In the laboratory the compost went through an additional aerobic maturation process of 0, 20 and 60 days to obtain three different compost maturities indicated as C0, C20 and C60. This aerobic maturation process was conducted in four open containers (as four replicates) with around eight kg of compost in each container. The compost was manually turned over once per day (except weekends) to ensure sufficient airflow. We collected compost from the four containers at different days as mentioned above and then stored at 4 °C until use in the experiment. Half of the amount of each compost, and half of the amount of agricultural soil were sterilized by Gamma irradiation (> 25 kGy). Agricultural soil was then mixed with sterilized background soil and the mixture was pre-incubated for two weeks prior to starting the microcosm experiment. The physical and chemical characteristics of soil and composts are shown in Table S1.

2.2. Microcosm experiment

A microcosm experiment with wheat (*Triticum aestivum*) was conducted in a growth chamber at Unifarm, Wageningen University, The Netherlands. Microcosms for growing wheat were made from grey PVC cylinders, with a diameter of 12.5 cm and a height of 25 cm (hereafter referred to as pots). The experiment consisted of three factors: soil sterilization (live or sterile soil, indicated as soil.L or soil.S), compost sterilization (live compost or sterile compost, indicated as compost.L or compost.S), and compost maturity (fresh compost C0, intermediate mature compost C20, and mature compost C60). A fully cross-design yielded twelve treatments (soil.L + C0.L, soil.L + C0.S, soil.S + C0.L, soil.S + C0.S, soil.L + C20.L, soil.L + C20.S, soil.S + C20.L, soil.S + C20.S, soil.L + C60.L, soil.L + C60.S, soil.S + C60.L, and soil.S + C60.S) and two additional unamended soils as controls (soil.L and soil.S). Treatments and controls were replicated four times, resulting in a total of 56 pots (Fig. S1). Each pot was filled with three kg of soil or soil-compost mix. The compost fraction was 5% (w/w) for C0, C20 and C60. The ambient temperature in the growth chamber was 22 °C during the day (16 h) and 14 °C during the night (8 h), with a daytime light intensity of 200 μmol/m^2/s artificial sunlight, and an air humidity of 70%.

To grow wheat in the pots, wheat seeds were first soaked in 5% sodium hypochlorite for 5 min, and then rinsed with distilled water six times. The washed seeds were placed in petri dishes with filter paper moistened with distilled water. The seeds were germinated in the dark at 25 °C. After germination (four days), each pot was planted with three healthy (> 4 mm) wheat seedlings of uniform size. The seedlings were thinned to one per pot after four days. During plant growth, soil water content was maintained at 60% of field capacity by periodically (three times per week) adding water to compensate evapotranspiration loss.

Soil and wheat samples were collected 90 days after sowing. Wheat shoots and roots were separated, and roots were collected after gently shaking off the soil and careful washing. Subsequently, soil was collected and sieved using a 2 mm sieve. Each soil sample was divided into two subsamples: one subsample was stored at −20 °C for molecular microbial analysis, and the other subsample was forced-air dried at 40 °C for one week and stored at room temperature for physico-chemical analysis.

2.3. Soil characterization and wheat dry biomass

Soil pH and electrical conductivity (EC) were measured using a Mettler Toledo SevenExcellence^{TM} in a 1:5 soil/water suspension (w/v) after 1 h shaking at 25 °C. The water content (WC) of soil was measured after drying in a forced-air oven at 105 °C for 8 h, and samples were burned subsequently at 550 °C for 2 h to quantify OM content (OM). Total carbon (TC) and total nitrogen (TN) were determined with an elemental analyzer (Interscience FlashSmart CHNSO). Total phosphorus (TP) and total potassium (TK) were analyzed by inductive coupled plasma optical emission spectrometry (Perkin Elmer Optima 5300 DV) after microwave acid digestion (Milestone Ethos Easy SK-15). NO_3^-, NO_2^-, NH_4^+, and K^+ were measured in a 1:10 soil/0.01 M $CaCl_2$ suspension (w/v). The suspension was centrifuged at 3750 *g* after 2 h

shaking at 25 °C. The supernatant was filtered through a 0.45 μm membrane filter and then analysed using ion chromatography (Metrohm Compact IC 761). PO_4^{3-} was analysed in the same way but was suspended in a 1:10 soil/water suspension (w/v). Dissolved organic carbon (DOC) was extracted and prepared in the same way with PO_4^{3-}, and analyzed by a TOC analyzer (Shimadzu TOC-L). Humification index (HI) of composts was analysed according to Zbytniewski and Buszewski (Zbytniewski and Buszewski, 2005). Briefly, soil was suspended in a 1:50 soil/0.5 M NaOH suspension. The suspension was shaken for 2 h and left overnight. The next day the suspension was centrifuged at 3750 *g* for 25 min, and HI was determined as the ratio between the absorbance of the supernatant at $\lambda = 472$ nm and 664 nm, which were measured by a UV/Vis spectrophotometer (Shimadzu UV-1800). Shoot and root dry weight were quantified after drying in a forced-air oven at 70 °C for one week.

2.4. Microbial community analysis of soil and compost

Soil was sampled after 90 days and analysed for bacterial and fungal community structure. DNA was extracted from 0.5 g of soil or 0.25 g of compost using the DNeasy Power Soil Kit (Qiagen) following the manufacturer's protocol. DNA concentration and purity were quantified by Quantus (Promega) and a Nanodrop spectrophotometer (Thermo Scientific NanoDrop 1000 spectrophotometer) with OD_{260} and OD_{280}, respectively. DNA samples were stored −20 °C before downstream analysis. DNA samples were normalized to 20 ng/μl for library preparation and sequencing (MrDNA, TX, USA) on a MiSeq (Illumina). Libraries for bacteria were constructed using primers 338F (ACTCCTACGGGAGGCAGCAG) (Fierer et al., 2005) and 806R (GGACTACHVGGGTWTCTAAT) (Caporaso et al., 2011), and for fungi using primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2R (GCTG CGTTCTTCATCGATGC) (Adams et al., 2013). The raw sequence data can be accessed at the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB43411 (https://www.ebi.ac.uk/ena/browser/view/PRJEB43411).

2.5. Bioinformatic analysis and statistics

For bacteria, each of the two 16S rRNA gene library pools were separately imported, demultiplexed, and processed with QIIME2 (version 2019.10). Primer sequences were trimmed from the reads using *q2-cutadapt* (Martin, 2011). Then, quality filtering of paired-end reads (i.e., denoising, error-correction, and chimera removal) was performed, and feature tables and representative sequences for unique Amplicon Sequence Variants (ASVs) were inferred for each data set using the *DADA2* q2-plugin (Callahan et al., 2016). Parameters for quality filtering were set as follows: forward and reverse reads were trimmed at position 225 and 225 (median Phred ≥ 30) and the first five bases were trimmed from all reads. The two feature tables and representative sequence sets were then merged, respectively. ASVs with a total frequency lower than four (< 0.01% of the mean frequency per sample) that were observed in less than five samples were removed from the merged feature table. All representative sequences for ASVs were *de novo* aligned using *MAFFT* (Katoh and Standley, 2013), filtered for gaps, and used to construct a phylogenetic tree with *Fasttree2* (Price et al., 2010). Taxonomy assignment was performed on representative sequences using the scikit-learn naive Bayesian classifier (Pedregosa et al., 2011) trained on primer set-specific 16S rRNA sequences from the SILVA database (version 132) (Quast et al., 2013).

For fungi, internal transcribed spacer-1 (ITS1) regions were subtracted from demultiplexed fastq files using ITSxpress (Rivers et al., 2018) for each of the two library pools separately, and then imported into QIIME2. The forward reads were processed using the *DADA2* q2-plugin without truncation of the reads due to the variable length of ITS fragments and median quality scores consistently higher than Phred = 30 along the reads. The two feature tables and two representative sequence sets were then merged, respectively. Rare features were removed following criteria used for bacteria. All representative sequences for each fungal ASV were clustered (closed-reference clustering) into OTUs (Operational Taxonomic Units) based on a 97% similarity threshold using *VSEARCH* (Rognes et al., 2016) based on the UNITE database v8.0 (UNITE Community, 2019). Taxonomy assignment was performed on representative sequences using a scikit-learn naive Bayesian classifier trained on the UNITE database (version 8.0) (UNITE Community, 2019). A phylogenetic tree was constructed using a pre-built ghost tree based on the same database used for closed-reference clustering (Fouquier et al., 2016).

Mitochondria, chloroplast, and archaea were removed in QIIME2 before the downstream analyses of bacterial and fungal communities in RStudio 4.0.2 using *phyloseq* package (McMurdie and Holmes, 2013) and *vegan* package (Oksanen et al., 2019). Alpha diversity of bacteria and fungi was assessed on rarefied datasets (at depth of 8655 and 11,581 reads per sample respectively, in Fig. S2) by calculating the Shannon diversity and observed richness. The effects of experimental factors (compost maturity, compost sterilization and soil sterilization) on alpha diversity were tested using a nonparametric Wilcoxon test and a Kruskal-Wallis test, with pairwise adjusted (Holm) p-values. Bray-Curtis dissimilarities among bacterial and among fungal communities were visualized using principal coordinates analysis (PCoA). Permutational multivariate analysis of variance (PERMANOVA, number of permutations = 999) was used to evaluate the effects of compost maturity, compost sterilization and soil sterilization on Bray-Curtis dissimilarities of bacterial and fungal communities. Differences in relative abundance (> 1%) across treatments at phylum, class, and order levels were analysed using the *Songbird* q2-plugin (Morton et al., 2019). Fitted multinomial models with experimental parameters were compared against null models (intercept only) to explore associations between experimental factors and microbial taxon abundances (Fig. S3).

The effects of the experimental factors on wheat growth and soil physico-chemical properties were tested with the three-way ANOVA (alpha = 0.05), followed by pair-wise comparison (TukeyHSD, family-wise error rate 5%). The effects of physico-chemical properties of soil (including pH, EC, DOC, TC, TN, TP, TK, AN, PO_4^{3-} and K^+) on wheat biomass were analysed based on forward stepwise selection and then tested with the ANOVA model (alpha = 0.05). The parameters which had high variance inflation factors (> 10) were removed before testing with the ANOVA model. The assumptions of normality and equality of variances were checked for each ANOVA model. To assess potential associations between the biomass of wheat and soil microorganisms (at order level, > 1% relative abundance), Spearman correlation coefficients were used. Variance partitioning was performed to test the experimental factors (compost maturity, compost sterilization and soil sterilization) and physico-chemical properties of soil on soil microbial community composition using the *vegan* package in R (Oksanen et al., 2019). Prior to variance partitioning analysis, forward stepwise selection was used to filter out the irrelevant parameters of the experimental factors and physico-chemical properties, and parameters with high variance inflation factors (> 10) were also removed.

3. Results

3.1. Effects of composts on soil physico-chemical properties

Compost maturity, compost sterilization and soil sterilization significantly affected specific physico-chemical properties (mainly nutrients) of soil 90 days after compost application (Table 1; detailed statistical results are shown in Table S2 and Table S3). Compost maturity affected total nitrogen (TN, $P < 0.001$), PO_4^{3-} ($P < 0.0001$) and K^+ ($P < 0.0001$). However, the effect of compost maturity on TN depended on whether the soil was sterilized (interaction $P < 0.05$), and the effect of compost maturity on K^+ depended on whether compost was sterilized (interaction $P < 0.05$). TN was significantly lower in the sterile soil amended with C0 than in the live soil amended with C0 ($P < 0.01$). K^+ was significantly lower in the soil amended with sterile C60 than in the soil amended with live C60 ($P < 0.0001$). PO_4^{3-} was significantly lower in the C0 amended soil compared to the C20 amended soil ($P < 0.0001$) and the C60 amended soil ($P < 0.01$), and sterilization of soil decreased PO_4^{3-} ($P < 0.0001$). Soil sterilization affected available nitrogen (AN) across all treatments, with lower AN in soils that were sterilized than in soils that were

not sterilized ($P < 0.0001$). Compared to the control soils, the compost amended soils, regardless of compost maturity, had higher DOC ($P < 0.0001$), TK ($P < 0.05$), PO_4^{3-} ($P < 0.0001$) and K^+ ($P < 0.0001$), and more mature compost amended soils had lower TN ($P < 0.001$). pH was slightly but significantly higher in the C60 amended soils than in the control soils ($P < 0.05$).

3.2. Effects of composts on soil microorganisms

Based on variance partitioning, we found that variation of the soil microbial community could be largely explained by experimental factors instead of physico-chemical soil properties (Table S4).

3.3. Effects of composts on soil bacterial communities

We observed 3855 bacteria ASVs in soil samples after quality filtering and removal of rare reads. Addition of composts with different maturities to soil did not influence Shannon diversity or observed ASV richness compared to the control soils (Fig. 1A,B; detailed statistical results are shown in Table S5). As expected soil sterilization and compost sterilization decreased alpha diversity. Based on the PCoA (Fig. 1C; statistical results are shown in Table S6), live composts, regardless of compost maturity and soil sterilization, had stronger effects on soil bacterial community composition than sterile composts. This highlights the importance of compost-associated bacteria in modifying soil bacterial community composition. In addition, the more mature live composts (C20 and C60) had stronger effects on soil bacterial community composition than the fresh live compost (C0).

According to the UPGMA-based dendrogram (Fig. 1D), soil bacterial communities across all treatments were first clustered into groups belonging to the sterile and the live soil. Soil sterilization affected the relative abundances of five bacterial orders of three phyla (multinomial regression: Pseudo $Q^2 = 0.4$, Fig. S3 and Table S7): Nitrospirales (Nitrospirae), Nostocales (Cyanobacteria), Oxyphotobacteria (Cyanobacteria), Tepidisphaerales (Planctomycetes), and Isosphaerales (Planctomycetes). Phyla Nitrospirae and Cyanobacteria were more abundant in the live soil, and Planctomycetes were more abundant in sterile soil. Within each of the two main dendrogram clades, soil bacterial communities were clustered into subclades based on compost sterilization. Compost sterilization affected the relative abundances of four bacterial orders of four phyla: Bacteroidales (Bacteroidetes), Cellvibrionales (Proteobacteria), Actinomarinales (Actinobacteria), and Chloroflexales (Chloroflexi). The soils amended with live composts had higher Bacteroidales, Cellvibrionales, and Actinomarinales, whereas Chloroflexales were more abundant in the sterile compost amended soils, especially in the soils amended with sterile C20 and C60.

Pairwise comparison of taxon abundances among the C20 and the C60 amended soils revealed no differential abundance at the bacterial order level, but the C0 amended soil had proportionally fewer Chloroflexales (Chloroflexi) than the C20 and C60 amended soils (Table S7). No orders differed between the C0 amended soil and the control soils. However, Actinomarinales (Actinobacteria) were more abundant in the C20 and the C60 amended soils than in the control soils when C20 and C60 were not sterilized.

3.4. Effects of composts on soil fungal communities

We detected 2072 fungal ASVs after quality filtering and removal of rare reads, and they were clustered into 219 OTUs. The Shannon index was lower in the C0 amended soil than in the other treatments, and the observed OTU richness was higher in the C0 amended soil than in the C60 amended soil (Fig. 2A,B; statistical results are shown in Table S5). Similar to the bacterial PCoA (Fig. 2C; statistical results are shown in Table S6), adding live composts affected soil fungal community composition more than adding sterile composts, with sterile C0 as an exception.

Soil fungal communities across treatments were clustered into four groups according to the UPGMA tree (Fig. 2D): soil amended with sterile C0, soil amended with live C0, live soil amended with C20 and C60, and sterile soil amended with C20 and C60. Soil sterilization affected five fungal orders

Table 1
Physico-chemical properties of the control soils and the compost amended soils 90 days after application (mean ± sd). Significant effects of soil sterilization, compost maturity and compost sterilization are indicated by lowercase letters, capital letters and asterisks respectively (Three-way ANOVA: $P < 0.05$). Significant difference between the control soils and the compost amended soils are indicated by √(ANOVA: $P < 0.05$). Lowercase letters indicate significant difference between sterile soil and live soil. Capital letters indicate significant difference between the soil amended with fresh, median mature, and mature composts. Asterisks * and ** indicate significant difference between the soil amended with sterile composts and the soil amended with live composts. Experimental replication n = 4. EC: electrical conductivity; DOC: dissolved organic matter; TC: total carbon; TN: total nitrogen; TP: total phosphorus; TK: total potassium; AN: available nitrogen, sum of NO_3^-, NO_2^-, and NH_4^+.

Treatment	Compost maturity	Compost sterilization	Soil sterilization	pH	EC	DOC	TC	TN	TP	TK	AN	PO_4^{3-}	K^+
					μs/cm	mg/kg	g/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Control	/	/	Live	7.6 ± 0.0	119 ± 23	127 ± 12	11.3 ± 2.5	665 ± 180	305 ± 21	818 ± 193	26 ± 41	11 ± 1	9 ± 7
			Sterile	7.7 ± 0.0	97 ± 4	134 ± 9	7.7 ± 1.8	453 ± 117	266 ± 36	664 ± 127	3 ± 2	9 ± 1	4 ± 2
C0 amended soil	C0	Live	Live	7.8 ± 0.1	117 ± 7	185 ± 24√	9.3 ± 0.2	503 ± 17^a	270 ± 27	908 ± 107√	7 ± 3^a	17 ± 1^{Aa}√	87 ± 14^a√
		Sterile	Live	7.8 ± 0.0	111 ± 8	197 ± 27√	9.0 ± 1.1	533 ± 76^a	339 ± 56	1044 ± 201√	4 ± 1^a	21 ± 2^{Aa}√	72 ± 10^a√
			Sterile	7.7 ± 0.1	107 ± 9	178 ± 33√	8.8 ± 0.8	374 ± 182^b	339 ± 56	988 ± 189√	3 ± 1^b	16 ± 1^{Ab}√	70 ± 13^b√
		Sterile	Sterile	7.7 ± 0.1	104 ± 10	194 ± 13√	11.5 ± 2.2	110 ± 112^b	1217 ± 1243	1198 ± 683√	5 ± 2^b	15 ± 1^{Ab}√	68 ± 2^b√
C20 amended soil	C20	Live	Live	7.6 ± 0.1	133 ± 11	205 ± 33√	9.7 ± 3.1	41 ± 80√	342 ± 80	1204 ± 143√	12 ± 7^a	23 ± 1^{Ba}√	143 ± 19^a√
		Sterile	Live	7.7 ± 0.1	116 ± 170	203 ± 22√	11.8 ± 3.4	101 ± 118√	329 ± 17	1108 ± 128√	10 ± 1^a	23 ± 3^{Ba}√	136 ± 28^a√
		Live	Sterile	7.7 ± 0.1	104 ± 5	181 ± 12√	13.7 ± 8.0	230 ± 381√	289 ± 46	993 ± 149√	5 ± 1^b	19 ± 1^{Bb}√	118 ± 15^b√
		Sterile	Sterile	7.7 ± 0.2	114 ± 23	195 ± 18√	9.0 ± 1.8	4 ± 9√	301 ± 86	1217 ± 201√	4 ± 1^b	18 ± 2^{Bb}√	112 ± 21^b√
C60 amended soil	C60	Live	Live	7.7 ± 0.1√	116 ± 18	196 ± 8√	10.7 ± 2.4	163 ± 90√	358 ± 63	1279 ± 159√	6 ± 3^a	21 ± 2^{Ba}√	145 ± 16^{a*}√
		Sterile	Live	7.8 ± 0.1√	102 ± 15	191 ± 41√	11.1 ± 4.3	217 ± 169√	329 ± 76	908 ± 75√	7 ± 1^a	21 ± 4^{Ba}√	115 ± 14^{a*}√
		Live	Sterile	7.8 ± 0.0√	118 ± 13	217 ± 12√	12.7 ± 6.7	292 ± 423√	305 ± 27	1005 ± 86√	8 ± 5^b	19 ± 1^{Bb}√	140 ± 16^{b*}√
		Sterile	Sterile	7.8 ± 0.1√	110 ± 8	204 ± 13√	11.7 ± 1.9	227 ± 132√	295 ± 42	1074 ± 105√	3 ± 1^b	17 ± 3^{Bb}√	88 ± 18^{b*}√

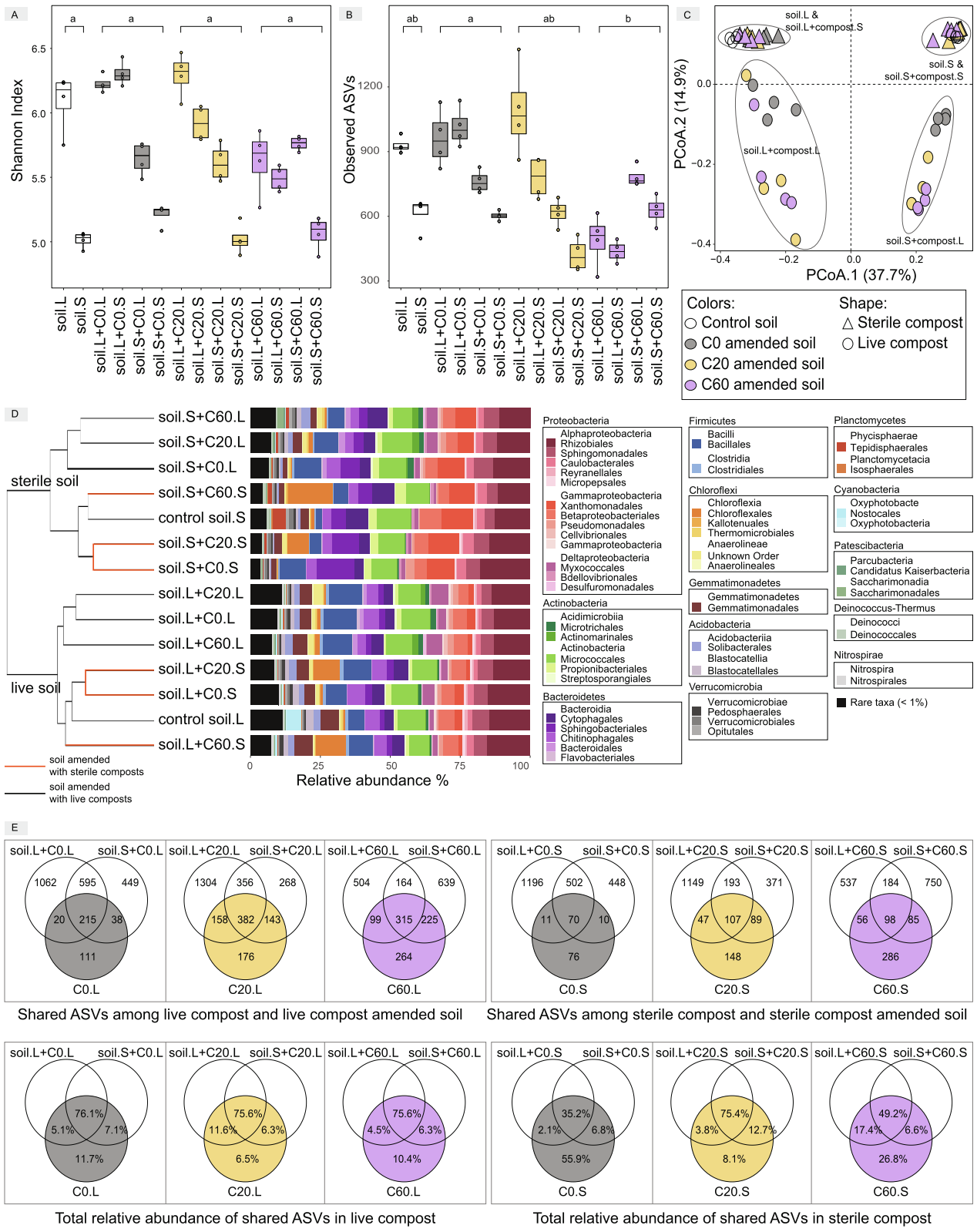

Fig. 1. Effects of composts on soil bacteria 90 days after compost application. Alpha diversity is indicated by A) Shannon diversity and B) Observed ASVs richness. The box in the boxplot represents the first and the third quartile, and the horizontal line in the boxplots represents the median of the four replicates that are shown as dots. Identical lowercase letters indicate no significant difference in the alpha diversity based on a Kruskal-Wallis test. C) Beta diversity in the control soils and the compost amended soils. A PCoA ordination is presented based on a Bray-Curtis dissimilarity matrix. D) Relative abundance of soil bacteria at order level in the control soils and the compost amended soils. Orders with < 1% relative abundance are grouped into "Rare taxa". Orders belonging to the same phylum are grouped within the boxes. Treatments in the y-axis are clustered based on the Bray-Curtis matrices using the unweighted pair group method with arithmetic mean (UPGMA). Experimental replication n = 4. E) Venn diagrams illustrating the number of unique and shared bacterial ASVs in composts and the compost amended soils, and the total relative abundance of these shared bacterial ASVs in composts.

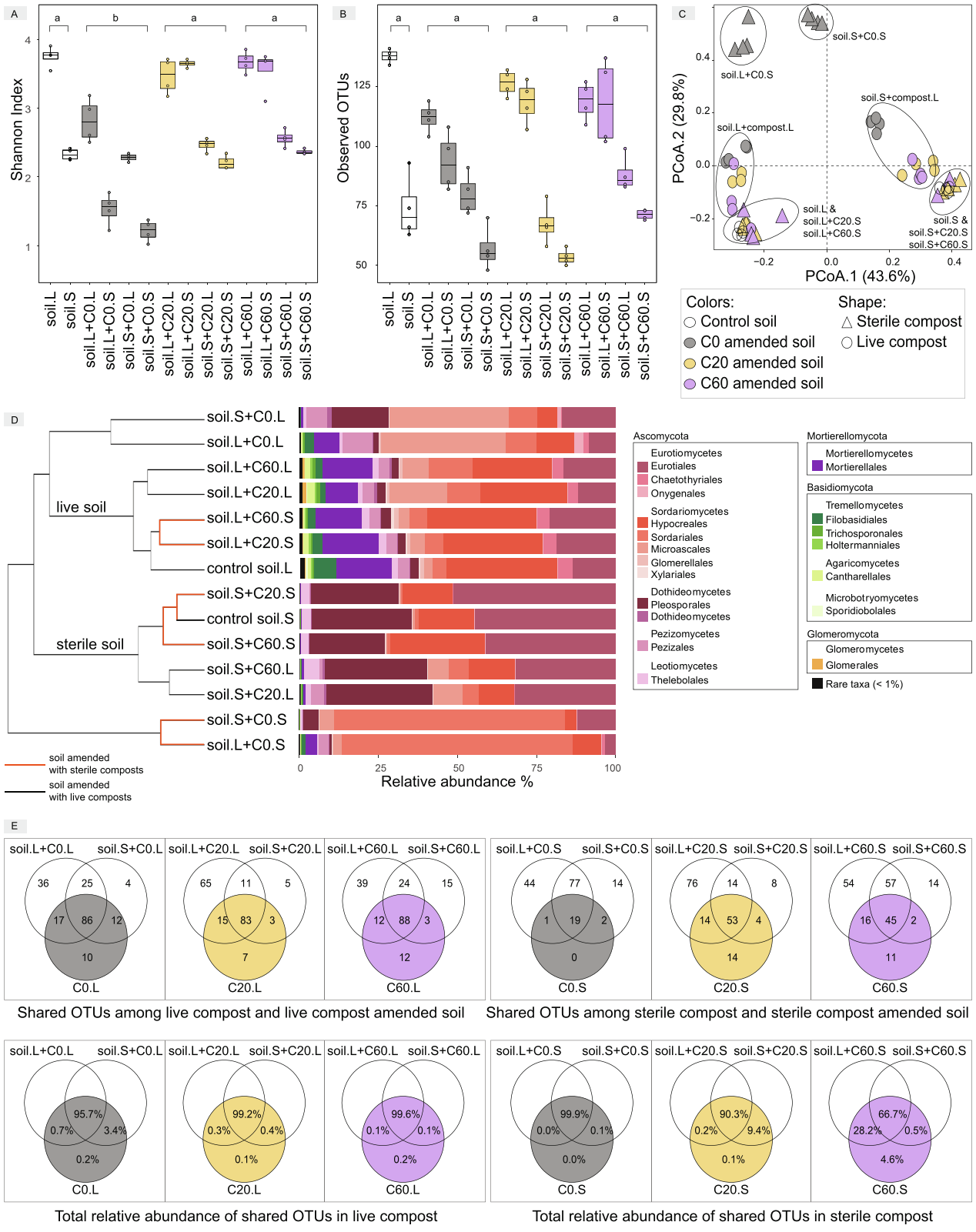

Fig. 2. Effects of composts on soil fungi 90 days after compost application. Alpha diversity is indicated by A) Shannon diversity and B) Observed OTUs richness. The box in the boxplot represents the first and the third quartile, and the horizontal line in the boxplots represents the median of the four replicates that are shown as dots. Identical lowercase letters indicate no significant difference in the alpha diversity based on a Kruskal-Wallis test. C) Beta diversity in the control soils and the compost amended soils. A PCoA ordination is presented based on a Bray-Curtis dissimilarity matrix. D) Relative abundance of soil fungi at order level in the control soils and the compost amended soils. Orders with < 1% relative abundance are grouped into "Rare taxa". Orders belonging to the same phylum are grouped within the boxes. Treatments in the y-axis are clustered based on the Bray-Curtis matrices using the unweighted pair group method with arithmetic mean (UPGMA). Experimental replication n = 4. E) Venn diagrams illustrating the number of unique and shared fungal OTUs in composts and the compost amended soils, and the total relative abundance of these shared fungal OTUs in composts.

belonging to two phyla (multinomial regression: Pseudo $Q^2 = 0.5$. Fig. S3 and Table S8): Eurotiales (Ascomycota), Chaetothyriales (Ascomycota), Pleosporales (Ascomycota), Thelebolales (Ascomycota) and Cantharellales (Basidiomycota). In general, sterilization of soil resulted in a higher relative abundance of Eurotiales, Pleosporales, and Thelebolales; while Chaetothyriales and Cantharellales were more abundant in live soil. Onygenales (Ascomycota) were more abundant in the C0 amended soil than other treatments, and adding sterile composts increased its relative abundance in the soil than adding live composts.

3.5. Shared microbiome between composts and compost-amended soils

A large number of bacterial ASVs were found both in live composts and in the live compost amended soils (Fig. 1E, left panel). These shared ASVs accounted for more than 70% of the relative abundance of bacteria in the live composts. This highlights that the majority of the compost-associated microorganisms were able to be prevalent in soil in the presence of the native soil microorganisms. They belonged mainly to the phyla Proteobacteria, Actinobacteria, and Bacteroidetes. Fewer bacterial ASVs were detected in sterile composts than in live composts, with fewer shared bacterial ASVs in sterile composts and in the sterile compost amended soils (Fig. 1E, right panel). It is important to note that the majority of bacterial ASVs detected in live and sterile composts were also detected in the control soils (accounted for ~20% of the relative abundance of soil bacteria). The highest total relative abundance of compost-associated bacterial ASVs in soil was found when composts were added to the sterile soils (Fig. S4). This indicates that sterilization of soil enhanced the prevalence and relative abundance of compost-associated bacteria in soil. This trend was also found for fungi (Fig. 2E; Fig. S5). The only difference with bacteria was that fungal OTUs found in composts were also detected in the control soils with high relative abundance.

3.6. Effects of composts on wheat dry biomass

Only fresh compost increased wheat dry biomass (Fig. 3; statistical results are shown in Table S9 and Table S10). Sterilization of soil did not affect wheat biomass ($P > 0.05$), while sterilization of compost, and especially so for the most mature compost C60, significantly promoted wheat biomass production ($P < 0.001$). Wheat dry weight was significantly correlated with TN ($P < 0.001$), PO_4^{3-} ($P < 0.001$), and K^+ ($P < 0.05$) in a forward stepwise selection model (Table S9). Positive and negative correlations were also found between wheat dry weight and specific soil microorganisms at order level (>1% relative abundance, Table S11).

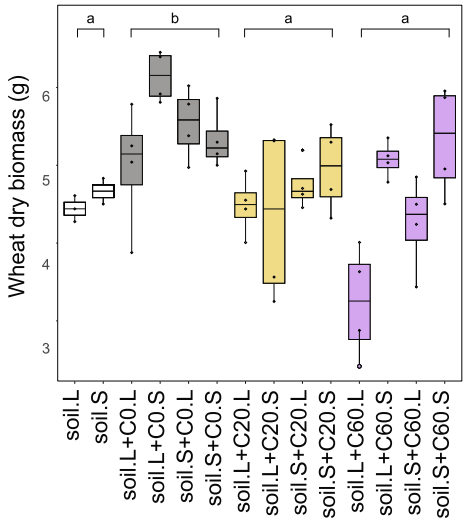

Fig. 3. Wheat dry biomass in the control soils and the compost amended soils 90 days after application. The box in the boxplot represents the first and the third quartile, and the horizontal line in the boxplots represents the median of the four replicates that are shown as dots. Boxes with identical lowercase letters are not significantly different based on a Tukey HSD test. Experimental replication n = 4.

4. Discussion

There is no consensus in the literature to what extent compost-associated microbes change the composition of the microbial community in the soil and whether this promotes plant growth. We hypothesized that compost addition would change the soil microbial community composition considerably, and that live composts would have stronger effects on the soil microbial community than sterile composts. Our microcosm experiment shows indeed that 90 days after addition of live compost the bacterial and fungal communities in the soil differed considerably from those in the control soil. Overall, soils amended with sterile composts had similar bacterial and fungal communities as the control soils, and this suggests an important role for compost-associated microorganisms and only a marginal role for the abiotic properties of compost. This contrasts previous studies, which suggested that there is strong resilience of soil microbial communities to invasions, and reported that compost-associated microorganisms are outcompeted by native soil microorganisms after application (Saison et al., 2006). Remarkably, in our study, more than70% and 90% relative abundance of compost-associated bacteria and fungi, respectively, were detected in the soil 90 days after application, even when a microbial community was already present in the soil. This suggests that a large part of the compost-associated microbes were not out-competed by resident soil microorganisms, though the resident soil microorganisms are usually viewed to be resistant and tend to exclude exotic populations (Levine and D'Antonio, 1999). Eliminating the activity of native soil microorganisms by sterilization enhanced the prevalence and relative abundance of these compost-associated bacteria and fungi in the soil even more and this is in agreement with our hypothesis.

The compost-associated bacterial ASVs that were detected in the soils after incorporation belonged mainly to the phyla Proteobacteria, Actinobacteria, and Bacteroidetes, all of which are key drivers in nutrient-cycling (Diánez et al., 2005; He et al., 2019; Kuypers et al., 2018; Maia and Moura, 2014; Wang et al., 2014; Wei et al., 2018; Zhong et al., 2018). These compost-associated bacterial ASVs were also present in the unamended soils, but only accounted for around 20% of the relative abundance of the bacterial community. Some compost-associated bacterial ASVs were not detected in the soil after incorporation. This could be because they are weak competitors or because the soil environment we used may not constitute a suitable habitat for these microorganisms, even in the absence of native soil microorganisms.

In our study, changes in soil bacterial and fungal communities depended on the maturity of the compost. Readily available C and nutrients in fresh compost are supposed to benefit bacterial populations, while C and nutrients constrained in stable compounds of mature compost may favour the growth of slow-growing fungi (Börjesson et al., 2012; Koranda et al., 2014). Therefore, we expected that fresh compost would benefit bacterial communities, and mature compost would benefit fungal communities. However, in our study, addition of more mature compost had stronger effects on soil bacteria than addition of fresh compost, and the effects on soil fungi were opposite. A potential reason for this unexpected result is that since we measured the bacterial and fungal composition 90 days after compost incorporation into the soils, the initial nutrient flush that we expected to occur particularly in fresh compost had already disappeared by then. It is known from other work that the effects of soil disturbances on microorganisms depend on the duration of the experiment (Bender et al., 1984; Shade et al., 2012).

Compost addition also affected wheat biomass but only addition of fresh compost increased wheat dry biomass. We hypothesized that compost addition would increase wheat biomass, and that addition of fresh compost would result in more wheat biomass than addition of

more mature composts, so our hypothesis was only confirmed partly. Interestingly, fresh compost did not contain more available nutrients than more mature composts in our study. This indicates that lower wheat growth in the mature compost amended soils may not be due to lower availability of nutrients compared to the fresh compost amended soils. Cozzolino and co-authors (2016) reported that a specific molecular composition of the OM in compost may be detrimental to plant growth and that this can counter balance the positive effects caused by provisioning of C and nutrients.

Compared with the control soils, the more mature compost amended soils contained a higher relative abundance of bacterial Actinomarinales, and the fresh compost amended soil had a higher relative abundance of fungi Sordariales. Actinomarinales belong to phylum Actinobacteria, which is widely reported as a plant growth-promoting bacterial taxon. They can benefit plant growth via multiple mechanisms, such as nitrogen fixation (Sellstedt and Richau, 2013), phosphate solubilisation (Hamdali et al., 2008; Jog et al., 2014), iron acquisition (Wang et al., 2014), and production of phytohormones (Vijayabharathi et al., 2016). Actinobacteria can minimize the deleterious effects of external stresses via competing for nutrients with e.g. pathogens (Sathya et al., 2017). Sordariales are Saprotrophic fungi that usually grow on dung or decaying plant biomass (Huhndorf et al., 2004). Their presence has been reported in agricultural soils (Klaubauf et al., 2010). Furthermore, Sordariales are dominant in the early successional stages of residue decomposition (Ma et al., 2013). Sordariales have been found to have antifungal activity and can control soil diseases (Rajini et al., 2020; Tang et al., 2020), and a positive correlation between wheat growth and Sordariales has been reported earlier (Wang et al., 2017). In our study, there was a negative correlation between Actinomarinales and wheat growth and a positive correlation between Sordariales and wheat growth. It should be noted that soils generally consist of diverse bacterial and fungal subpopulations that co-exist, co-evolve, and synergistically provide ecosystem functions (Bezemer et al., 2010; Fuhrman, 2009; Haq et al., 2014). Further studies should aim to examine the complex intra- and inter-kingdom interactions among soil microbes and their effects on plant growth.

Addition of sterile composts enhanced wheat dry biomass more than addition of live composts, and this was particularly true for the most mature compost. Multiple mechanisms could be involved and we cannot draw conclusions about this from our study. However, we observed that the soils amended with live composts had a higher relative abundance of bacteria involved in nutrient recycling (i.e., denitrification) than the soils amended with sterile composts. This indicates that nutrient availability may play a role, but further studies are needed to examine these processes. Bacteroidales and Cellvibrionales were more abundant in the soils amended with live composts than the soils amended with sterile composts, and they were negatively correlated with wheat growth. Bacteroidales (phylum Bacteroidetes) are denitrifying bacteria and have been found in composts and soil (He et al., 2019; Kuypers et al., 2018; Maia and Moura, 2014). Cellvibrionales (phylum Proteobacteria) are also commonly found in compost (Ma et al., 2018), and play an important role in S, N and C recycling (Wei et al., 2018; Zhong et al., 2018).

It should be noticed that Gamma sterilization is commonly used to eliminate the activity of soil microorganisms, but it will not eliminate all of their DNA. Hence it is possible that relic DNA remains present in the sterile soil or compost. This relic DNA could then later be detected and analyzed as live DNA and create noise or false results. We measured DNA concentrations in the live and sterile samples. Although Gamma sterilization did not completely degrade DNA, it was roughly two third lower in soil and compost after sterilization (Table S12). Further we also sequenced the sterile soil and compost and compared the ASVs/OTUs with those that we detected at the end of the experiment after 90 days. Sterile soil and compost had a lower number of ASVs/OTUs than live soil and compost samples (Table S12). A large part of the bacterial ASVs found in the experimental soils after 90 days, were not found in the original sterile soil or compost samples (Fig. S6), and the microorganisms in these experimental soils were also very different from those at day 0 (Figure S7). This suggests that the patterns we observed were not due to relic DNA. However, it is important to note that extracellular DNA from dead microorganisms can persist and can still yield products from PCR amplification and that this can obscure the treatment effects (Carini et al., 2016). Therefore, we recommend that future studies with sterile soil or compost, should focus on the active microbial community, e.g. via RNA extraction or should remove relic DNA before DNA extraction, e.g. via applying propidium monoazide (Carini et al., 2016; Nocker et al., 2007).

5. Conclusion

In our study the effects of composts on the soil microbial community were mainly related to the input of compost-associated microorganisms rather than the input of organic matter and nutrients. The majority of the compost-associated bacteria and fungi were detected in the soil, and sterilization of the native soil microorganisms enhanced the prevalence and the total relative abundance of compost-associated microorganisms in the soil. Addition of fresh compost increased wheat biomass relative to biomass in the control soil, but adding sterile compost, especially mature compost, promoted wheat biomass more than adding live compost. Hence, our study highlights the importance of compost-associated microorganisms for modifying soil microbial communities, but also that eliminating the activity of compost-associated microorganisms by sterilization contributes to wheat growth. This advances our understanding of the effects of abiotic and biotic properties of composts on soil microbial communities and wheat growth with implications for soil management. We conclude that compost addition can influence the biotic and abiotic properties of soil in multifaceted ways. This is important when generalizing how compost affects soil functioning and plant growth. More insights into the role of fungi and bacteria, and the interactions (i.e., nutrient competition) between native soil microbes and introduced microbes in structuring soil communities can help to further unravel the mechanisms influencing soil quality and plant growth.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2021.115598.

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