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Effect of organic amendments obtained from different pretreatment technologies on soil microbial community

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ABSTRACT

The application of organic amendments (OAs) obtained from biological treatment technologies is a common agricultural practice to increase soil functionality and fertility. OAs and their respective pretreatment processes have been extensively studied. However, comparing the properties of OAs obtained from different pretreatment processes remains challenging. In most cases, the organic residues used to produce OAs exhibit intrinsic variability and differ in origin and composition. In addition, few studies have focused on comparing OAs from different pretreatment processes in the soil microbiome, and the extent to which OAs affect the soil microbial community remains unclear. This limits the design and implementation of effective pretreatments aimed at reusing organic residues and facilitating sustainable agricultural practices. In this study, we used the same model residues to produce OAs to enable meaningful comparisons among compost, digestate, and ferment. These three OAs contained different microbial communities. Compost had higher bacterial but lower fungal alpha diversity than ferment and digestate. Compost-associated microbes were more prevalent in the soil than ferment- and digestate-associated microbes. More than 80% of the bacterial ASVs and fungal OTUs from the compost were detected 3 months after incorporation into the soil. However, the addition of compost had less influence on the resulting soil microbial biomass and community composition than the addition of ferment or digestate. Specific native soil microbes, members from Chloroflexi, Acidobacteria, and Mortierellomycota, were absent after ferment and digestate application. The addition of OAs increased the soil pH, particularly in the compost-amended soil, whereas the addition of digestate enhanced the concentrations of dissolved organic carbon (DOC) and available nutrients (such as ammonium and potassium). These physicochemical variables were key factors that influenced soil microbial communities. This study furthers our understanding of the effective recycling of organic resources for the development of sustainable soils.

1. Introduction

Biological pretreatment technologies convert organic residues into recovered resources. Composting, anaerobic digestion (AD), and lactic acid fermentation are widely used pretreatment technologies. Composting is an aerobic process that involves groups of decomposers in different phases (Insam and De Bertoldi, 2007). Compost-associated microbes are often found in topsoil (Mehta et al., 2014), and we recently reported that the majority of compost bacteria and fungi are detected after incorporation into the soil (Luo et al., 2022). The

composting process removes labile organic fractions and concentrates more recalcitrant fractions, thereby stabilizing organic matter (OM) with low nutrient content (Tambone et al., 2007). In contrast to composting, AD is an anaerobic process that produces CH₄ and less decomposable OM (digestate) with a higher proportion of mineral N and P than compost (Tambone et al., 2009; Li et al., 2023). AD leads to a diverse group of saccharolytic, proteolytic, and lipolytic bacteria and fungi that are responsible for degrading refractory organics. These microbes can secrete extracellular enzymes to enhance the hydrolysis of bacteria and methanogenic archaea (Amani et al., 2010; Chen et al.,

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Table 1

Physicochemical properties and alpha diversity of organic amendments (OAs) obtained from composting, lactic acid fermentation, and anaerobic digestion (AD). EC: electrical conductivity; WC: water content; OM: organic matter content; DOC: dissolved organic carbon; TC: total carbon; TN: total nitrogen; TP: total phosphorus; TK: total potassium. NA: below the detection limit.

Parameters	Compost	Ferment	Digestate
Physicochemical properties			
pH	9.0 ± 0.0	3.9 ± 0.0	8.2 ± 0.0
EC	1.7 ± 0.0	2.3 ± 0.1	4.5 ± 0.0
WC	29.6% ±	66.1% ±	78.7% ±
OM	0.8% ±	0.1% ±	1.2% ±
	27.0% ±	94.4% ±	78.7% ±
	0.3% ±	1.0% ±	0.2% ±
DOC g/g	6.2 ± 0.1	82.2 ± 1.8	85.5 ± 1.6
TC g/kg	145.1 ± 20.1	507.9 ± 16.6	484.7 ± 6.8
TN g/kg	16.5 ± 1.8	36.0 ± 1.4	49.9 ± 2.6
Nitrite g/kg	NA	NA	NA
Nitrate g/kg	0.03 ± 0.00	0.03 ± 0.00	0.09 ± 0.03
Ammonium g/kg	1.3 ± 0.0	1.1 ± 0	29.3 ± 1.3
Phosphate g/kg	0.6 ± 0.0	11.9 ± 0.1	1.6 ± 0.2
Potassium g/kg	4.0 ± 0.1	7.8 ± 0.1	17.4 ± 0.8
Alpha diversity			
Bacterial Shannon index	5.5 ± 0.0	1.7 ± 0.1	3.0 ± 0.0
Observed bacterial ASV richness	635 ± 36	247 ± 18	375 ± 21
Fungal Shannon index	0.6 ± 0.1	0.2 ± 0.0	2.7 ± 0.4
Observed Fungal OTU richness	43 ± 4	70 ± 3	61 ± 9

2016; Fan et al., 2022; Zhuang et al., 2021). Fermentation is also an anaerobic process, characterized by the accumulation of fatty acids (e.g., lactic, acetic, and succinic acids) by specific acid fermenters such as *Lactobacillus* rather than CH₄ as the end product of metabolic activity (Madigan et al., 2019; Gao et al., 2023).

The products obtained from these processes are commonly applied to agricultural soils as organic amendments (OAs) to sustain soil fertility and quality (Ansari et al., 2023; Lohri et al., 2017; Valentinuzzi et al., 2020). The provision of OM, nutrients, and specialized microorganisms has the potential to modify soil physicochemical properties and microbial communities, thereby regulating soil function. However, it remains unclear to what extent microbes associated with particular OAs (hereafter referred to as OA microbes) can survive and affect soil microbial communities, and how this corresponds to different pretreatment technologies. To the best of our knowledge, there is a lack of studies using standardized organic feed materials to connect the effects of pretreatment technology to soil quality enhancement (Chavez-Rico et al., 2023). This knowledge is essential for developing effective waste recycling processes and sustainable soil management strategies.

Compost, digestate, and lactic acid ferment can affect soil physicochemical and biological properties in different ways, as they differ in the stability of OM, nutrient status, and microbial compositions. First, the nutrient level in compost may be insufficient to meet the requirements of agricultural soil (especially during the growing season); however, compost has a greater potential to increase soil organic matter (SOM) and benefit the soil in the long term than digestate and ferment (Odlare et al., 2011). Secondly, the C content in the digestate can be more readily metabolized than that in the compost because of less efficient mineralization during AD than during composting. Therefore, it is more likely that digestate promotes soil microbial biomass in the short term than does compost (Gómez-Brandón et al., 2016; Odlare et al., 2011). Lastly, the effects of the addition of ferment to soil reported in the scientific literature are mainly derived from studies that use bokashi, a fermented product originating from animal manure, vegetables, or grass, with the addition of a commercial microbial inoculum, which is dominated by lactic acid bacteria (LAB) (Andreev et al., 2018; Shin et al., 2017). LAB can increase the availability of P by solubilizing P in the soil (Hug et al., 2013). LAB metabolites (carbohydrates, amino acids, and organic acids) also benefit soils by stimulating beneficial soil microorganisms and

suppressing soil pathogens (Zlotnikov et al., 2013). Ferments typically have notably lower pH than compost and digestate, which may lead to stagnation or even a decrease in soil microbial activity or biomass. In contrast, the growth of acid-tolerant bacteria in the soil may be stimulated by the addition of ferment (Andreev et al., 2018).

The response of soil microbes to OAs and the survival of OA-associated microorganisms in the soil greatly depend on the soil type and quality (Shin et al., 2017). Soil microbes co-evolve and can be highly organized and biologically accommodated (Connell, 1978). This leads to a strong resilience (i.e., the capacity to return to its original state) of native soil microbes, especially in clayey soils and soils with high microbial diversity and OM content (Mayer et al., 2010; Messiha et al., 2007; van Vliet et al., 2006). This study investigated the presence of OA microbes in the soil and their effects on soil abiotic and biotic properties. The OAs used in this study were compost, digestate, and ferment. These OAs were produced from a model organic residue, which enabled us to study and compare the effects of different biological pretreatment technologies on the characteristics of the OAs. This study focused on microbial aspects and aimed to uncover the prevalence of OA-associated microbes in the soil, and how these microbes interact with the native soil microbial community. We expected that the OAs resulting from different biological pretreatment technologies would have distinct physicochemical properties and microbial communities, reflecting the aerobic and anaerobic conditions of the technologies. We hypothesized that different OAs with different levels of nutrients, OM stability, specialized microorganisms, and pH would influence soil microbial community differently. We also expected that compost-associated microbes would be more prevalent in the resulting soil community than ferment- and digestate-associated microbes due to the fact that compost has similar microbial composition as soil.

2. Material and methods

2.1. Experimental set-up

Compost, digestate, and ferment were produced from a model organic residue containing 6.9% meadow hay (Pet's Own Choice, NL), 27.2% dog food (Hill's Science Diet, USA), and 65.9% water. The use of this type of model organic residue was inspired by Fernandez-Bayo et al. (2018), who used (the same brand of) dog food as a model organic residue with a long-term representative and stable composition for different biological treatment processes. Model organic residues covered a wide range of compounds, ranging from more recalcitrant OM (lignin and hemicellulose in meadow hay) to more easily degradable OM (carbohydrates, proteins, and lipids in dog food). The model organic residue had a homogeneous composition, ideal for unbiased comparisons among the different pretreatments. The model organic residue has pH = 6.1, electrical conductivity (EC) = 2.2 mS/cm, total C = 443.5 g/kg dry, total N = 34.3 g/kg dry, total P = 4.6 g/kg dry, NO₂⁻ = 0.01 g/kg dry, NO₃⁻ = 0.05 g/kg dry, NH₄⁺ = 0.73 g/kg dry, PO₄³⁻ = 4.21 g/kg dry, K⁺ = 4.72 g/kg dry, and C:N ratio = 12.9. The detailed characteristics of the model organic residues can be found in Chavez-Rico et al. (2022).

The OAs were produced in three identical glass-jacketed reactors (12 L working volume) at controlled temperatures. The compost was produced in a packed-bed reactor after 60 d in a two-stage process. The first stage lasted for 30 d with a gradual increase in temperature, as in the standard composting process. The following curing/maturation stage lasted for 30 d at 30 °C. A 1.7 kg commercial mature compost (Attero, The Netherlands) was used as the inoculum and mixed with 3.3 kg of the model organic residue. AD was carried out under mesophilic conditions (30 °C) in a fed-batch bioreactor for 165 d. The digestate from a reactor fed with sugar beet residue was used as the inoculum and added three times to the AD reactor. In total, 3 kg of the inoculum was added to 10 kg of the model organic residue. Fermentation was performed under anoxic conditions at 25 °C for 60 d 10 kg of model organic residue was

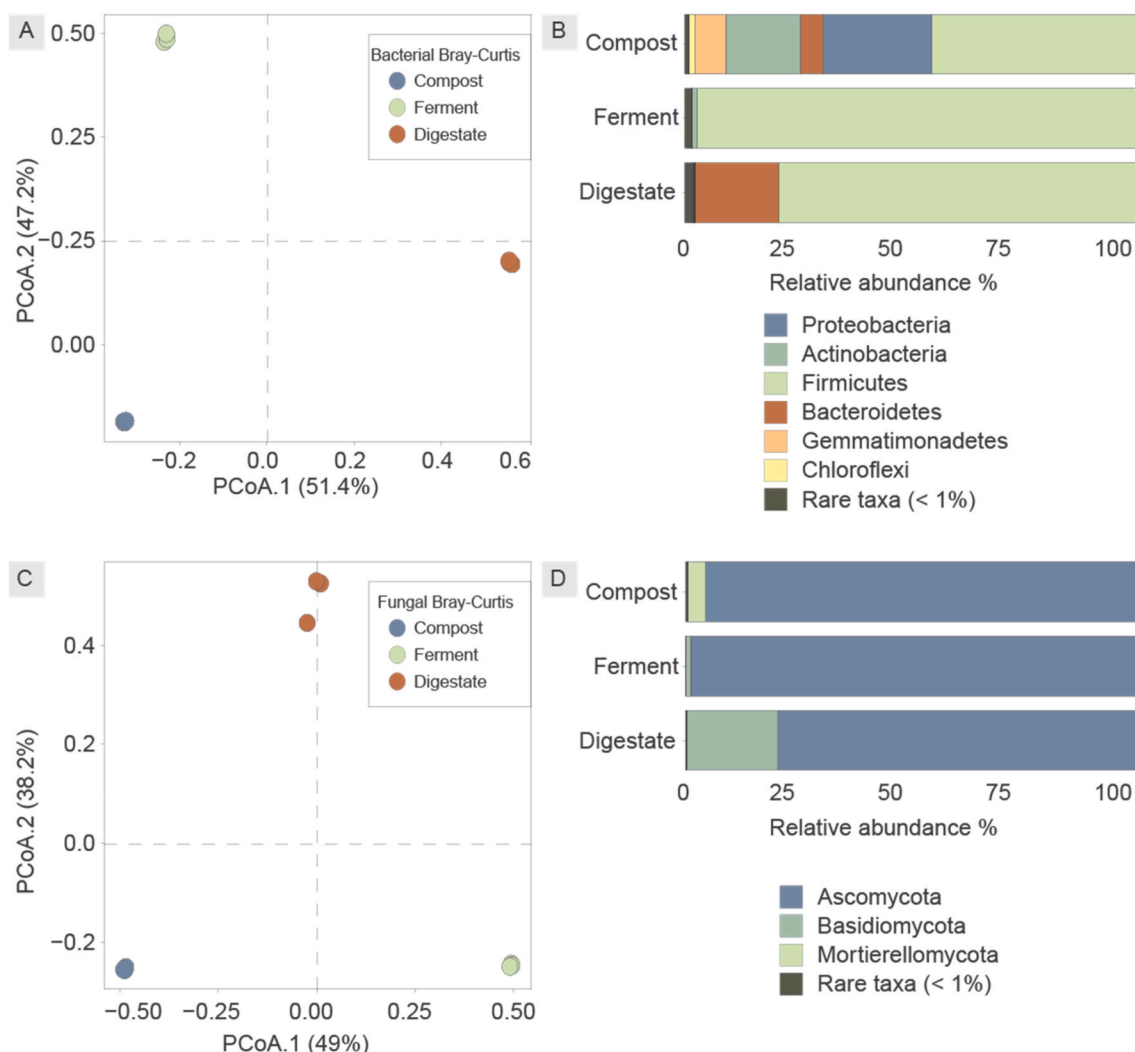


Fig. 1. Beta-diversity of bacteria (A) and fungi (C) and their relative abundance (B and D, respectively) in compost, ferment, and digestate. Beta diversity is expressed as Bray-Curtis dissimilarities based on PCoA ordination. Relative abundance of bacteria and fungi is shown at the phylum level, and phyla with <1% relative abundance are grouped into “Rare taxa”. Experimental replication = 3.

inoculated with 29 g of commercial microbial inoculum (EM-1®, Agriton, The Netherlands). Detailed biowaste conversion technologies for obtaining OAs can be found in [Chavez-Rico et al. \(2023\)](#). Soil was collected from an agricultural field in Kollumerzwaag (Friesland, The Netherlands) and had a Loamy sand texture (78% sand, 14% silt, and 4% clay). Of the collected field soil, 90% was heated in a furnace at 550 °C for 4 h and then mixed with the remaining original field soil. Heat treatment was performed to achieve a low OM content as well as to destroy the soil structure and aggregates because this experiment was part of a larger study on soil aggregation. Heat treatment also destroyed the native soil microbial community; therefore, we mixed heat-treated soil with live soil from the same source. This resulted in soil with a community that resembled an agricultural soil microbial community, whereas the OM content was low. Water was added to reach 60% of field capacity. The C:N ratio of the soil was then set to 17.7 by adding ammonium nitrate so that the soil resembled the C:N ratio of the original field soil. Before OA incorporation, the soil was pre-incubated for 14 d to stabilize the microbial community.

We added 20.5 g (dry weight equivalence) of the OAs to 1.5 kg of modified soil, and then the soil was homogeneously mixed and placed into PVC cylinders with a diameter of 10 cm and a height of 30 cm (hereafter referred to as the soil columns). There were four treatments: control without the addition of OAs, compost-amended soil, digestate-

amended soil, or ferment-amended soil. Each treatment was replicated thrice. The soil columns were incubated in a dark climate room at 22 °C (16 h) and 14 °C (8 h), with 60% humidity. The water content (WC) of the soil columns was maintained at 60% of the field capacity, and water loss was compensated weekly by measuring the weight of the soil columns. The incubation lasted for 3 months. Approximately 50 g of soil was collected from each column at the beginning (day 0) and end (day 90) of the experiment. The collected soil samples were divided into three sub-samples. The subsamples were stored at 4 °C, -20 °C, or dried at 65 °C until a constant weight was reached.

2.2. Physicochemical characterization of soil and OAs

Soil samples stored at 4 °C were used to measure WC and OM content. Dried soil samples (at 65 °C) were first ground and sieved (<2 mm) and then used to measure pH, EC, water available nutrients, total nutrients, and dissolved organic carbon (DOC) as described by [Luo et al. \(2022\)](#). The effects of treatments on soil physicochemical properties were tested using one-way ANOVA ($\alpha = 0.05$), followed by pairwise comparison (Tukey HSD, family wise error rate 5%).

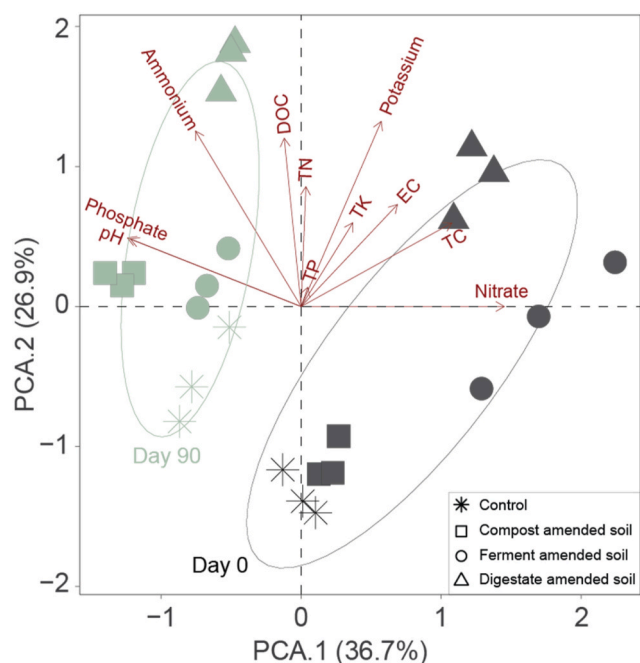


Fig. 2. PCA of the physicochemical properties of the unamended (control) and OA-amended soils at the beginning (day 0) and end of the experiment (day 90). EC: electrical conductivity; DOC: dissolved organic carbon; TC: total carbon; TN: total nitrogen; TP: total phosphorus; TK: total potassium.

2.3. Characterization of bacteria and fungi

DNA was extracted from 0.25 g of soil using the DNeasy Power Soil Kit (Qiagen) following the manufacturer's protocol. The qPCR assays were performed in triplicates with a total volume of 20 μ L reactions using iQ SYBR Green master mix (Bio-rad) on a CFX96 Real-Time System (C1000 Touch, Bio-rad). The bacterial qPCR efficiency was 93.8% with R^2 value of 0.999. The fungal qPCR efficiency was 94.6% with R^2 value of 0.996. The qPCR primers and amplification protocols are listed in Table S1. The effects of treatments on microbial gene abundance were tested using one-way ANOVA ($\alpha = 0.05$), followed by pairwise comparison (Tukey HSD, family wise error rate 5%).

The DNA samples were sequenced (MrDNA, TX, USA) using a MiSeq (Illumina). Raw sequence data were processed as described by Luo et al. (2022). The alpha diversity of bacteria and fungi was assessed on rarefied datasets by calculating the Shannon index and observed bacterial ASV/fungal OTU richness. The difference in alpha diversity between treatments was tested using the nonparametric Wilcoxon test and the Kruskal-Wallis test, with pairwise adjusted (Holm) P -values. Bray-Curtis dissimilarities of bacterial and fungal communities were visualized using principal coordinate analysis (PCoA). Redundancy analysis (RDA) was used to investigate the relationships between soil microbial communities at the phylum level (>1% relative abundance) and soil physicochemical properties across the treatments on day 90. Raw sequence data can be accessed from the European Nucleotide Archive (ENA) (<https://www.ebi.ac.uk/ena/browser/view/PRJEB61524>).

3. Results

3.1. Physicochemical and microbial properties of OAs

The properties of the compost, digestate, and ferment produced from the model organic residue were analyzed before incorporation into the soil. The compost and digestate had an alkaline pH (up to 9), whereas the ferment had an acidic pH (3.9). The ferment and digestate contained

higher amounts of OM, DOC, and nutrients than the compost (Table 1).

The compost, ferment, and digestate differed distinctly in bacterial and fungal composition (Fig. 1A and C). Moreover, compost had higher bacterial alpha diversity than the other OAs, whereas ferment and digestate had higher fungal diversity than compost (Table 1). The Shannon index showed that the ferment-associated fungi were highly diverse, but with an unevenly distributed abundance.

Firmicutes was the dominant bacterial phylum in all the three OAs (Fig. 1B). However, the orders belonging to Firmicutes differed between OAs (Fig. S1). Bacillales (50% relative abundance) were dominant in the compost, ferment mainly contained Lactobacillales (90% relative abundance), and digestate mainly contained Lactobacillales (50% relative abundance) and Clostridiales (30% relative abundance). Proteobacteria, Bacteroidetes, Actinobacteria, Chloroflexi, and Gemmatimonadetes were more abundant (>1% relative abundance) in the compost than in the ferment and digestate. Among the fungi, Ascomycota was the dominant phylum in all the three OAs (Fig. 1D). The compost mainly contained Microascales (80% relative abundance) from Ascomycota, whereas the ferment contained Eurotiales (90% relative abundance) from Ascomycota. The digestate contained more diverse orders from Ascomycota, among which Hypocreales (30% relative abundance) were dominant (Fig. S1). Mortierellomycota were only detected in the compost with >1% relative abundance, whereas Basidiomycota were only detected in the ferment and digestate with >1% relative abundance.

3.2. Soil characterization after OA incorporation

3.2.1. Physicochemical properties of soil after OAs incorporation

The physicochemical properties of the soil on days 0 and 90 after OA incorporation were analyzed using principal component analysis (PCA) (Fig. 2). The first two axes of the PCA model explained 63.6% of the data variability. The unamended control and compost-amended soils shared similar initial physicochemical properties on day 0 (immediately after OA application), whereas the ferment- and digestate-amended soils had higher EC, DOC, and nutrients (i.e., nitrate, ammonium, and potassium) than the control and compost-amended soils. After an incubation period of 90 d, the physicochemical properties of the soil changed regardless of the treatment. The pH and the concentrations of phosphate and ammonium were higher on day 90 than on day 0 for all treatments. Moreover, the physicochemical properties of digestate-amended soil differed the most on day 90 compared to the other treatments, with higher DOC, ammonium, and potassium concentrations. The physicochemical data of the soil samples are listed in Table S2.

3.2.2. Prevalence of OA microbes in the soil after incorporation

Compost-associated microbes were more prevalent in the soil than ferment- and digestate-associated microbes after incorporation into the soil. Most of the microbes initially present in the compost, particularly 88% of bacterial ASVs (Fig. 3A) and 83% of fungal OTUs (Fig. 3B), were detected in the soil 90 d after incorporation. Some bacteria (28% of ASVs) and fungi (81% of OTUs) were present in the soil prior to compost incorporation. The prevalence of digestate- and ferment-associated microbes in the soil was similar. Approximately 50% of bacterial ASVs and 70% of fungal OTUs associated with digestate and ferment were found in the soil after incorporation. Some OA microbes, especially those from digestate and ferment, were no longer detected after their incorporation into the soil. The relative abundances of these microbes in the original OAs were also low (Fig. S2). In addition, more bacterial ASVs (990 ASVs) and fungal OTUs (91 OTUs) associated with soil were detected 90 d after the addition of compost than after the addition of digestate and ferment, suggesting that compost increases the chance of survival of native soil microbes in compost-amended soil.

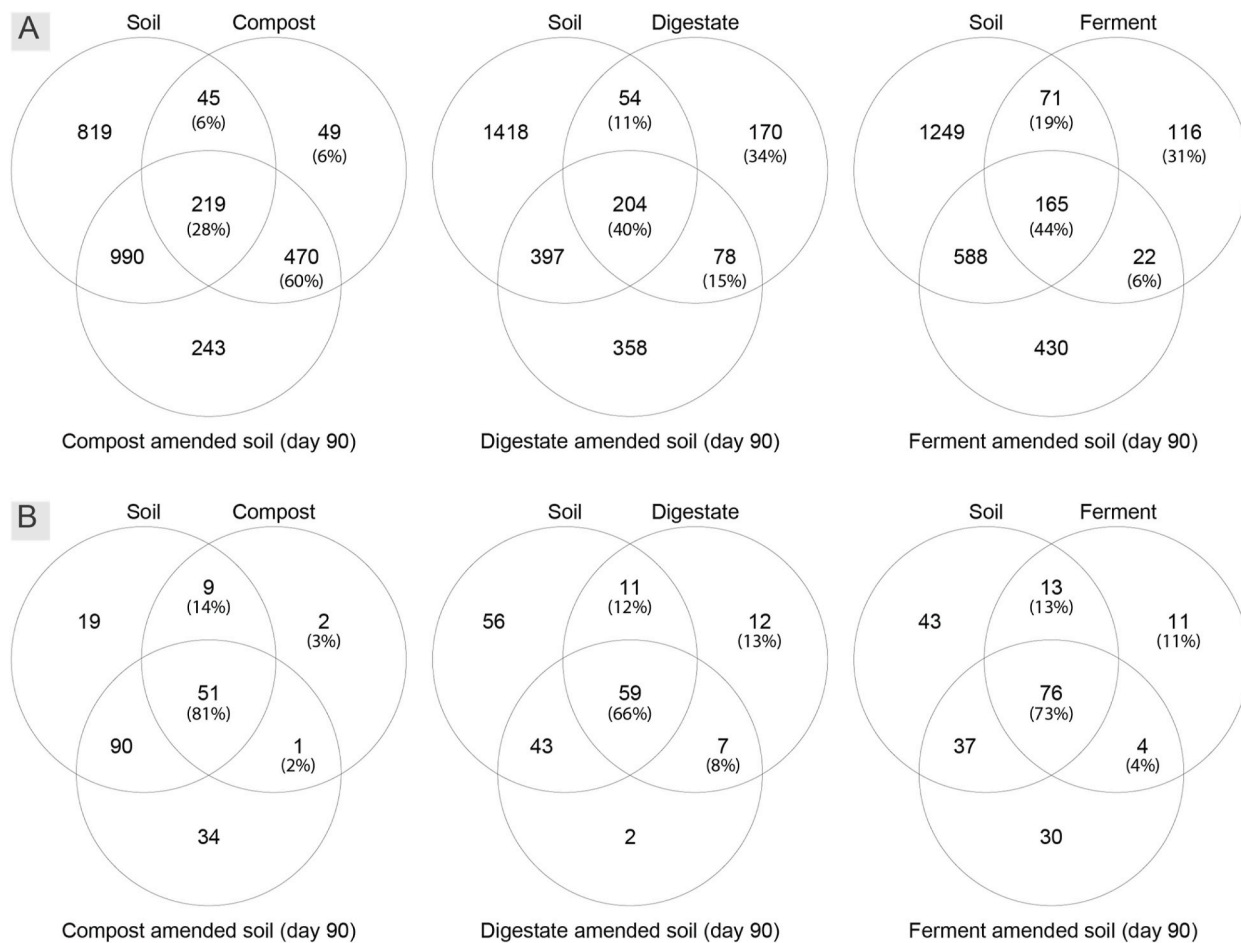


Fig. 3. Venn diagrams illustrating the number of the unique and shared bacterial ASVs (A) and fungal OTUs (B) in the soil, OAs, and OA-amended soils after 90 d of incorporation. The numbers in parentheses are the percentages of the unique and shared ASVs or OTUs in OAs.

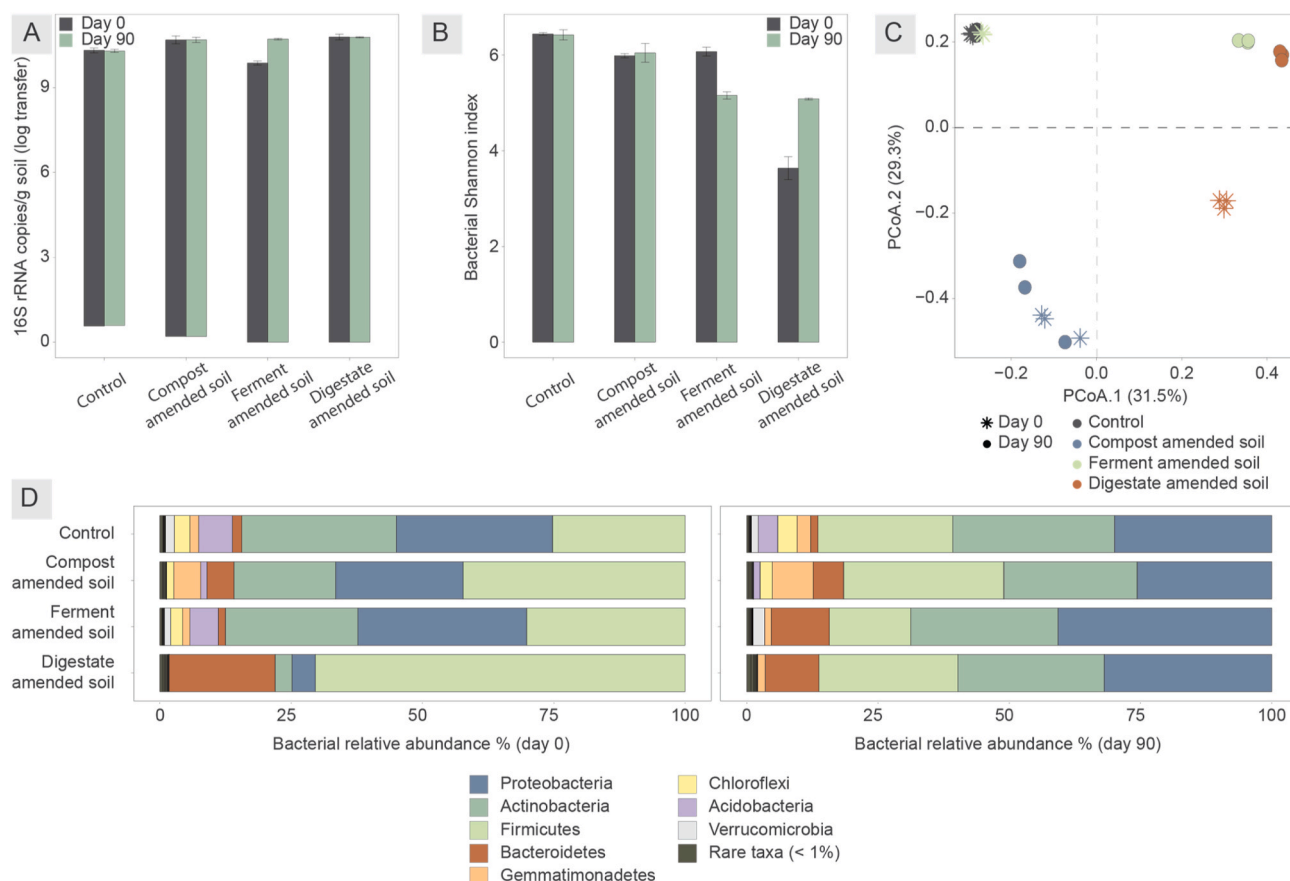


Fig. 4. Effects of OAs on soil bacteria after 90 d of incubation. A) Density of 16S rRNA gene copies in log transfer. B) Bacterial alpha diversity expressed as the Shannon index. C) Beta diversity is indicated by Bray-Curtis dissimilarity based on a PCoA ordination. D) Relative abundance of bacteria at the phylum level on day 0 (left) and day 90 (right). The error bars in the bar plot represent the standard deviation of three replicates.

3.2.3. Microbial dynamics of soil after OAs incorporation

In general, adding OAs increased bacterial and fungal biomass compared with the control soil but decreased Shannon diversity (Fig. 4A and B, and 5A and B). After 90 d of incubation, the bacterial and fungal biomasses were higher in the ferment-amended soil than what was recorded in the initial biomass on day 0 (Figs. 4A and 5A), with decreased bacterial Shannon diversity and increased fungal Shannon diversity (Figs. 4B and 5B). The digestate-amended soil had no increase in bacterial biomass but had a higher bacterial Shannon diversity index on day 90 compared with day 0, and fungal biomass increased with decreased Shannon diversity index. No significant changes in microbial biomass or Shannon diversity index were observed in the compost-amended soil on day 90 compared with day 0.

In contrast to the compost-amended soil, after 90 d, the bacterial and fungal composition in the ferment- and digestate-amended soils differed from the initial microbial composition in the soil on day 0 (Figs. 4C and 5C). The addition of ferment and digestate changed the bacterial and fungal composition compared to the control soil, whereas compost only affected the bacterial communities. Comparing the relative bacterial abundance on days 90 and 0 (Fig. 4D), the relative abundance of Firmicutes decreased, whereas the relative abundances of Proteobacteria and Actinobacteria increased in the ferment- and digestate-amended soils after 90 d. Moreover, Acidobacteria were abundant in the soil before OA addition, but were no longer abundant (<1% relative

abundance) 90 d after ferment application; instead, Bacteroidetes thrived. Regarding fungal composition, the relative abundance of Ascomycota increased and that of Basidiomycota decreased in the ferment-amended soil and vice versa for the digestate-amended soil (Fig. 5D).

Compared to the control soil, on day 90, Bacteria Chloroflexi and Acidobacteria, and fungi Mortierellomycota were not detected as major phyla (>1% relative abundance) in the ferment- and digestate-amended soils. Gemmatimonadetes were more abundant in the compost-amended soil than in the control soil. Chloroflexi, Gemmatimonadetes, and Mortierellomycota were only detected as major phyla (>1% relative abundance) in the unamended soil and compost (before incorporation), whereas Acidobacteria were only detected in the unamended soil (Figs. 1, 4D and 5D).

Soil microbial communities were correlated with specific soil physicochemical properties based on multivariate redundancy analysis (RDA; Fig. 6). Overall, 86.2% (adjusted = 74.6%) of the microbial community variation was explained by soil physicochemical variables, and the first two RDA axes explained 67.9% and 17.7% of this variation, respectively. In particular, soil pH, DOC, and available nutrients (nitrate, ammonium, and potassium) were significantly correlated with the soil microbial community composition.

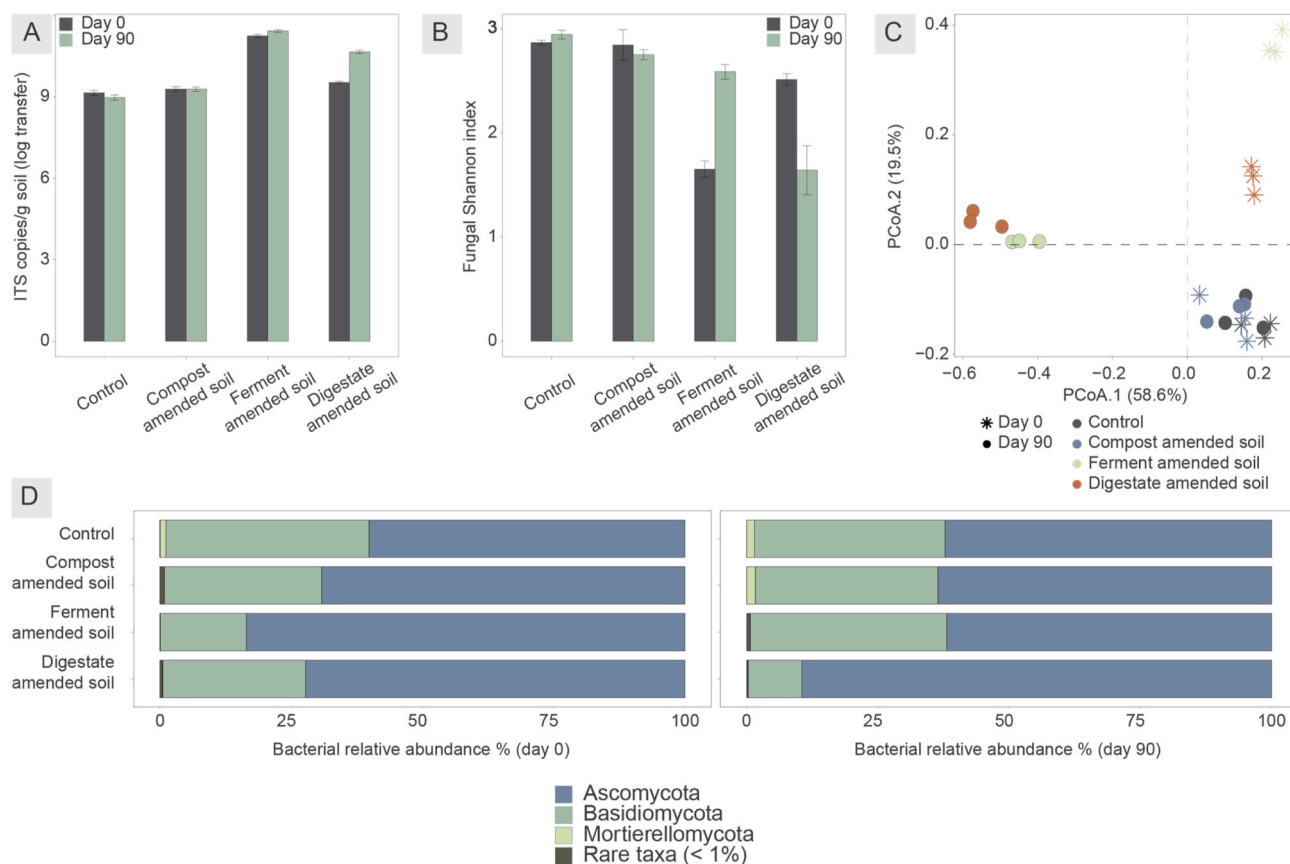


Fig. 5. Effects of OAs on soil fungi after 90 d of incubation. A) Density of ITS gene copies in log transfer. B) Fungal alpha diversity expressed as the Shannon index. C) Beta diversity is indicated by Bray-Curtis dissimilarity based on a PCoA ordination. D) Relative abundance of fungi at the phylum level on day 0 (left) and day 90 (right). The error bars in the bar plot represent the standard deviation of three replicates.

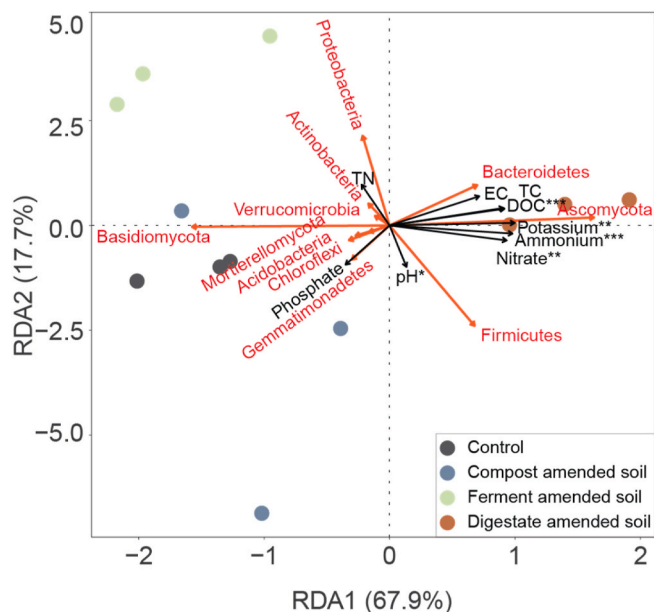


Fig. 6. Constrained ordination based on redundancy analysis (RDA) visualizing the relationships between soil physicochemical properties and soil microbial communities on day 90. EC: electrical conductivity; DOC: dissolved organic carbon; TC: total carbon; TN: total nitrogen. Asterisks *, **, and *** indicate significant correlations at $P < 0.05$, 0.01 , and 0.001 , respectively.

4. Discussion

In line with our hypothesis, the OAs differed in their physicochemical properties and microbial community compositions. The compost showed significantly lower OM and DOC than the ferment and digestate, which is in line with the results of other studies (Fernandez-Bayo et al., 2018; Gómez-Brandón et al., 2016; Odlare et al., 2011). We found that the compost contained groups of decomposers that metabolized and mineralized complex and recalcitrant OM. For instance, Chloroflexi and Gemmatimonadetes were abundant in the compost but not in the ferment and digestate. Chloroflexi and Gemmatimonadetes play essential roles in organic carbon turnover from complex sources with high levels of recalcitrance (Barton et al., 2014; Hug et al., 2013; Liu et al., 2020; Nielsen et al., 2009). Proteobacteria and Actinobacteria were also found in the compost, with a high relative abundance. These bacteria are commonly present in the composting and vermicomposting processes (Ashraf et al., 2007) and are the most common phyla detected in soil (Wolińska, 2018). Proteobacteria are critical drivers of S, N, and C cycles (Wei et al., 2018; Zhong et al., 2018). Actinobacteria are often aerobic bacteria involved in nutrient cycling (such as nitrogen fixation and phosphate solubilization) (Hamdali et al., 2008; Jog et al., 2014; Sellstedt and Richau, 2013). These bacteria consumed carbon and nutrients during the composting process, which could explain the lower concentrations of carbon and nutrients found in the compost than in the ferment and digestate.

Mortierellomycota fungi were abundant in the compost but not in the ferment and digestate, whereas Basidiomycota occurred vice versa. However, the cause of this pattern remains unclear. Interestingly, several fungi belonging to the phylum Mortierellomycota are “sugar fungi” that metabolize labile compounds (Clocchiatti et al., 2020).

Members of the phylum Basidiomycota are major degraders of lignin during rumen fermentation (Xing et al., 2020), indicating that Basidiomycota may be responsible for lignocellulose breakdown during lactic acid fermentation and biowaste digestion.

Firmicutes was the major bacterial phylum in all OAs. However, members of the phylum Firmicute differed among OAs. Bacillales were the main Firmicutes in compost and were also commonly found in soil (de Andrade Cavalcante et al., 2019; Earl et al., 2008; Nakasaki et al., 2019). They can survive at high temperatures owing to their endospore-forming capacity (Secaira-Morocho et al., 2020). They can also break down complex polymers that can be used as carbon sources and electron donors (Arkatkar et al., 2010; Marvasi et al., 2010). Lactobacillales were the dominant Firmicutes in the ferment and digestate. They are fermentative organisms that produce lactic acid as the major end-product of metabolism (Andreev et al., 2018; Shin et al., 2017). This explains the low pH observed in the ferment. Lactobacillales and Clostridiales were also dominant Firmicutes in digestate. These are extremely acid-tolerant and can produce volatile fatty acids that ensure subsequent processes in AD (Wainaina et al., 2019).

The compost had the least effects on soil microbial biomass and composition compared to the ferment and digestate. It is not surprising that the majority of the compost microbes were detected 3 months after incorporation into the soil since compost and soil share similar aerobic conditions as well as the majority of the microbes (Mehta et al., 2014). However, this contradicts other research, which found that compost had more extensive effects on soil microbial structure than digestate, and that digestate mainly affected the abiotic properties of soil (Akari and Uchida, 2021). Ferment and digestate are notably different from soil in terms of their physicochemical properties, leading to fewer ferment and digestate microbes being detected in the soil compared with compost and the selection of native soil microbes after incorporation (Andreev et al., 2018). This may explain the reduction in alpha diversity compared to the compost-amended and the control soils on day 90.

Some native soil bacteria, members of the phyla Chloroflexi, Acidobacteria, and Mortierellomycota, were not detected as major taxa (>1% relative abundance) 3 months after ferment and digestate application. As discussed above, Chloroflexi are heterotrophic oligotrophs found in the soil. They usually contain numerous exoenzymes including chitinases, glucuronidase, galactosidases, and proteases (Kragelund et al., 2007; Xia et al., 2007), suggesting that Chloroflexi grow primarily on complex polysaccharides and proteins (Barton et al., 2014; Hug et al., 2013; Nielsen et al., 2009). We observed that Chloroflexi were negatively correlated with TC, DOC, and available nutrients, including nitrate, ammonium, and potassium. Acidobacteria are primarily aerobic bacteria (Madigan et al., 2019), which could explain why they were not abundant in the soil after ferment and digestate application. The provision of more available carbon and nutrients by ferment and digestate may favor "sugar microbes" and other microbes that fit well into the ferment- and digestate-like habitat, and these may have out-competed Chloroflexi and Acidobacteria. It is not clear why Mortierellomycota were not abundant after ferment and digestate application, although they were abundant in the original soil. They were negatively correlated with DOC, TC, and available nutrients (except for phosphate). This contradicts other studies that have reported that Mortierellomycota are positively correlated with nutrients (Clocchiatti et al., 2020; Zhang et al., 2020).

In contrast to Chloroflexi, Acidobacteria, and Mortierellomycota, Proteobacteria thrived in all treatments, although they were not detected as major phyla in the ferment and digestate before incorporation. Proteobacteria is the largest and most metabolically diverse phylum of bacteria (Madigan et al., 2019). Their metabolic characteristics range from strictly aerobic to facultatively aerobic and obligate anaerobic, making them capable of inhabiting different environments. In addition, they are the key drivers of nutrient cycling (Wei et al., 2018; Zhong et al., 2018). The addition of ferment and digestate provided more labile nutrients to the soil and, therefore, may have led to a higher abundance

of Proteobacteria than in the compost-amended and control soils. The Ascomycota and Basidiomycota fungi were dominant in all treatments. Ascomycota are the main decomposers of plant residues in the early successional stages of several soil ecosystems (Klaubauf et al., 2010), and members of Ascomycota are involved in numerous soil functions. They may form symbiotic relationships with mycorrhizae or endophytes that promote plant growth (Fröhlich-Nowoisky et al., 2015). Basidiomycota are important lignin degraders under anaerobic conditions, and their negative correlations with DOC and available nutrients indicate that they can be enriched by the provision of more stable organic compounds.

This study focused on the changes in soil microbes after 3 months of OA application. The long-term effects of OAs on soil microbes merit further investigation, because the soil microbial community is usually viewed as being strongly resilient. In addition, this study uncovered correlations between the biotic and abiotic characteristics of the soil, and how this influences plant growth after OA application requires further investigation.

5. Conclusion

In this study, we investigated the effects of compost, ferment, and digestate originating from a model organic residue and obtained from controlled biological pretreatment technologies. As expected, the compost contained less carbon and nutrients than the ferment and digestate. The addition of ferment and digestate provided more labile nutrients to the soil than compost, increasing soil microbial biomass. Owing to their distinct nature (such as pH and microbial composition), the addition of ferment and digestate had relatively large effects on soil microbes and reduced the soil alpha diversity. Some native soil microbes, including Chloroflexi, Acidobacteria, and Mortierellomycota, were not detected after the addition of the ferment and digestate. This study is the first step in designing and implementing sustainable agricultural management using OAs by understanding the effects of pretreatment technologies on the properties of OAs and their subsequent roles in modifying soil microbial communities. These results will be valuable for designing efficient waste recycling processes and implementing sustainable soil management strategies.

Credit author statement

Yujia Luo: Data collection, formal analysis, methodology, visualization, writing original draft, writing revision. Vania Scarlet Chavez-Rico: Conceptualization, methodology, data collection, and formal analysis. Valentina Sechi: Conceptualization, supervision of data interpretation, writing, and revision. T. Martijn Bezemer: supervision of data interpretation, writing, and revision. Cees J.N. Buisman: funding, supervision of data interpretation, writing, and revision. Annemiek ter Heijne: supervision of data interpretation, writing, and revision.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2023.116346>.

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