Bacterial and fungal community dynamics during different stages of agro-industrial waste composting and its relationship with compost suppressiveness

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Abstract

Composting is an advantageous and efficient process for recycling organic waste and producing organic fertilizers, and many kinds of microorganisms are involved in obtaining quality compost with suppressive activity against soil-borne pathogens. The aim of this work was to evaluate the main differences in the effects of three composting piles on the whole bacterial and fungal communities of baby-leaf lettuce crops and to determine the specific communities by high-throughput sequencing related to suppressiveness against the soil-borne plant pathogen *Pythium irregulare*- (*P.*

irregulare). Compost pile A was composed of 47% vineyard pruning waste, 34% tomato waste and 19% leek waste; pile B was composed of 54% vineyard pruning waste and 46% tomato waste; and pile C was composed of 42% vineyard pruning waste, 25% tomato waste and 33% olive mill cake. The temperature and the chemical properties of the piles were monitored throughout the composting process. In addition, the potential suppressive capacity of the three composts (C_A, C_B and C_C) against *P. irregulare* in baby-leaf lettuce was assessed. We found that the bacterial community changed according to the composting phases and composting pile and was sensitive to chemical changes throughout the composting process. The fungal community, on the other hand, did not change between the composting piles and proved to be less influenced by chemical properties, but it did change, principally, according to the composting phases. All composts obtained were considered stable and mature, while compost C_C showed higher maturity than composts C_A and C_B. During composting, the three piles contained a greater relative abundance of Bacterioidetes, Proteobacterias and Actinobacterias related to the suppression of soil-borne pathogens such as *Pythium irregulare.* Composts C_A and C_B, however, showed higher suppressiveness against *P. irregulare* than compost C_C. Deeper study showed that this observed suppressiveness was favored by a higher abundance of genera that have been described as potential suppressive against *P. irregulare*, such as *Aspergillus, Penicillium, Truepera* and *Luteimonas.*

Keywords: composting process, microbial community, agro-industrial wastes, chemical factors, *Pythium irregulare* suppressiveness

1. Introduction

48 The bioconversion of agro-industry wastes through the composting process
49 results in a stabilized end-product known as compost (Meng et al., 2019). This is an
50 efficient and environmentally friendly method for managing and recycling agro-industry
51 waste and turning it into something that can be used as a soil amendment or as a
52 component of growing media (Blaya et al., 2015; Zhang et al., 2018b; Ding et al.,
53 2020). Such composts are used due to their high level of organic matter and nutrients,
54 lack of pathogens and low heavy metal content (Meng et al., 2019). Furthermore, they
55 have demonstrated a potentially suppressive effect against soil-borne pathogens
56 (Morales et al., 2016).

57 Microorganisms play a key role during the whole composting process and
58 compost maturation. The presence of certain microorganisms with different enzymatic
59 capabilities reflects the evolution of the composting process and results in added-value
60 quality compost (Meng et al., 2019; Zhao et al., 2019; Zhong et al., 2020). Bacteria and
61 fungi are both functional communities in this process. Bacteria are characterized as
62 having high metabolic versatility (Ma et al., 2020), while fungi have the ability to use
63 many different carbon substrates as food sources and survive under different conditions,
64 including dry and acidic conditions or low nitrogen conditions (Meng et al., 2019).

65 Microbial abundance and diversity during composting depends on (among other
66 factors) the initial feedstock materials, the temperature, the oxygen concentration, the
67 moisture content and the pH and carbon/nitrogen ratio (Zhang et al., 2018a). The initial
68 feedstock carries the nutrients necessary for feeding the microorganisms and providing
69 a suitable environment for them. These microorganisms are responsible for the increase
70 in temperature during the composting process that reduces plant and animal pathogens
71 and weeds. The initial feedstock materials used are also responsible for the degree of
72 pile aeration (Meng et al., 2019). Temperature also controls the composting process due

to its effect on the microbial metabolic rate and population structure, which defines the different composting phases, such as the mesophilic, thermophilic and maturation phases (Jiang et al., 2017). Many difficulties in the composting process can be traced to insufficient oxygen levels for supporting the decomposition process, a lack of optimal moisture levels and insufficient turning frequency; all of these issues are influenced by the type of initial feedstock (Tiquia, 2005; Bernal et al., 2009).

Quality compost must be mature—a quality linked to plant-growth potential and phytotoxicity—and stable—a quality linked to the compost microbial activity (Azim et al., 2018). Moreover, apart from these basic characteristics, composts can show added-value properties, such as suppressive activity against phytopathogens and a biostimulant and/or biofertilizing effect (Morales et al., 2016; De Corato, 2020). Composts made from specific agro-industrial wastes and by-products have been proven to suppress a wide variety of soil-borne plant pathogens (Bonanomi et al., 2007). Particularly, composts made from tomato or leek waste from agri-food production processes, olive mill cakes from olive oil industries and vineyard pruning residues from the wine industry have been used to suppress *Pythium irregulare* (*P. irregulare*) in baby-leaf lettuce (Hernández-Lara et al., 2021). This is one of the crops that is most affected by this oomycete (Giménez et al., 2019), which mainly causes damping off and significant reductions in plant growth (Van Beneden et al., 2009; Hernández-Lara et al., 2021).

The microbiota in composts have been found to be the main factor responsible for suppressiveness. According to De Corato et al., (2018) composts from green wastes are colonized by different fungi and bacteria belonging to highly diversified taxonomic groups, such as *Bacillus*, *Trichoderma*, *Fusarium*, and other Eukarya belonging to Ascomycota and Basidiomycota—all are potentially effective in controlling *P. irregulare* and other soil-borne pathogens. Monitoring microbial succession is therefore

important in effectively managing the composting process (Zhang et al., 2018a). Currently, the 16sRNA/ITS high-throughput sequencing technology is widely used for exploring microbial diversity. It has been used to analyze the microbial communities in samples from several different environments, including soils (Sun et al., 2015), wastewater treatment plants (Prevost et al., 2015), water systems (Tiwari et al., 2021), and composts (Holman et al., 2016).

The aim of this study was to determine the main differences between the microbial communities in mixtures of different feedstocks (vineyard pruning waste, tomato waste, leek waste and olive mill cake) during the different phases of composting that could influence the suppressive activity of three specific composts against *P. irregulare* in a baby-leaf lettuce crop. In this study, we considered the following hypotheses: 1. Mixtures from different organic wastes and proportions can produce composts with different microbiota and chemical characteristics, and consequently, show different suppressive activity against *P. irregulare;* 2. Differences in the microbial community between the different phases can be higher in the initial and maturation phases; and 3. The stability and maturity of composts can influence suppressive activity against *P. irregulare*.

2. Materials and methods

2.1 Composting procedure and sampling

Three open-air composting piles (10 m^3) using the turning composting system were established at Miguel Hernández University (Orihuela, Alicante, Spain). The piles were composed of different proportions of agro-industrial wastes (dry weight basis), as follows: Pile A: 47% vineyard pruning waste, 34% tomato waste and 19% leek waste;

Pile B: 54% vineyard pruning waste and 46% tomato waste; and Pile C: 42% vineyard pruning waste, 25% tomato waste and 33% olive mill cake. The characteristics of the initial wastes are shown in Table 1. Mechanical turnings were carried out weekly until the end of the bio-oxidative phase, and irrigation was conducted periodically to keep the moisture of each composting pile at around 65%. The temperature was automatically measured every six hours with a temperature data logger (HOBO-Data Logger U12-006) at 30 cm from the surface. The composting process was monitored for 226 days in the three piles. The samples were collected from three different sites throughout the length of the composting pile, and for each of the three samples, seven different sub-samples from the whole profile (from the top to the bottom of the pile) were taken and mixed. The compost sampling was determined by the temperature conditions of the pile: a) at the beginning of the composting process (initial); b) during the thermophilic phase (thermophilic); c) when piles had cooled to ambient temperature and the temperature did not increase (end of the bio-oxidative phase); and d) after the maturation phase (maturation). The samplings correspond, respectively, to days 1, 70, 157 and 226 for pile A; 1, 73, 157 and 226 for pile B; and 1, 60, 166 and 216 for pile C.

Each sample was sub-divided into two parts: one was stored at -80º C for DNA extraction and the other was stored at 4º C for the later determination of physicochemical and chemical properties.

2.2 Chemical properties

The pH and electrical conductivity (EC) of the composts were measured in a 1:10 (w/v) water-soluble extract, after shaking the fresh samples at 120 r/min for 60 min, using a pH meter and conductivity meter (Crison), respectively. Total nitrogen (TN) and total organic carbon (TOC) were determined by dry combustion at 950 ºC

147 using an Elemental Analyzer (C/N Flash EA 112 Series-Leco Truspec). The principle of
148 this method is that a microportion of the sample is injected into a heated reaction
149 chamber packed with an oxidative catalyst, where the water is vaporized and the
150 nitrogen and organic carbon is oxidized and transported via carrier gas streams to be
151 measured by the analyzer.
152
153 **2.3 DNA extraction, PCR amplification and sequencing**

154 Soil DNA was extracted from 0.5 g of compost using the DNeasy PowerSoil kit
155 (Qiagen, Germany) and purified with a QIAquick Gel extraction kit (Qiagen) following
156 the manufacturer's instructions. DNA extraction was performed in triplicate. The
157 quality of the DNA was examined by electrophoresis in 1.5% agarose gel. In addition, a
158 Qubit 3.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, USA) and NanoDrop
159 2000 fluorospectrometer (Thermo Fisher Scientific, Waltham, MA, USA) were used,
160 respectively, to quantify the DNA samples, prior to sequencing on an Illumina MiSeq
161 platform at the Institute of Microbiology of the Czech Academy of Sciences (Prague,
162 Czech Republic). The V4 region of bacterial 16S rRNA was PCR-amplified using the
163 barcoded primers 515F and 806R (Argonne National Laboratory) (Caporaso et al.,
164 2012), and the fungal ITS2 region was amplified using the barcoded primers gITS7 and
165 ITS4 (Ihrmark et al., 2012). Three PCRs per sample were used for sequencing purposes.
166 The PCR mix and thermocycling conditions are shown in Table S1. The PCR products
167 were purified using a MinElute PCR purification kit (QIAgen) according to the
168 manufacturer's instructions. A TruSeq PCR-Free kit (Illumina) was used for library
169 preparation. The sequencing of fungal and bacterial amplicons was performed in an
170 Illumina MiSeq C4SYS facility at the Institute of Microbiology of the Czech Academy
171 of Sciences (Prague, Czech Republic).

2.4 Bioinformatics analysis

Raw sequence data generated from Illumina Miseq were processed in QIIME2 v 2020.2.0 (Caporaso et al., 2010) through Oracle Virtualbox v 6.1. Fastq files were imported into QIIME2 with the '*single end method*' and the sequences were then denoised and filtered with the *dada2* pipeline to remove noisy and chimeric sequences (Callahan et al., 2016). Operational Taxonomic Units (OTUs) were generated by the VSEARCH plugin at 99% identity (Rognes et al., 2016). Taxonomic assignation of OTUs was performed using "*feature-classifier*" against the Silva 132 database for bacteria and the Unite database for fungal sequences. Mitochondria and Chloroplast sequences were removed from the bacteria database, as were low confident OTUs. The data were rarefied to the depth of sample with the least reads, 2,000 sequences per sample.

The sequences have been deposited in the ENA database with the accession code PRJEB44354 and the project (study) name Microorganisms in agro-industrial waste compost.

2.5 *In vivo* assessment of compost as a growing medium against *Pythium irregulare*

A pot experiment was performed to evaluate compost suppressiveness against *P. irregulare*. The red baby-leaf lettuce (*Lactuca sativa L.*) cultivar 'Antoria' (Rijk Zwaan, De Lier, The Netherlands) was used as a host plant. The composts were mixed with either commercial peat 315 [Blond/black 60/40 (Turbas y Coco Mar Menor S.L.)] at a ratio of 3:1 (w:w) or peat alone (control treatment). The main physico-chemical and chemical characteristics of the peat were as follows: a pH of 5.6; an EC of 1.0 dS m^{-1}; 466.8 g kg^{-1} total C; 9.4 g kg^{-1} total N; 0.3 g kg^{-1} total P; and 0.9 g kg^{-1} total K.

A total of 54 washed lettuce seeds were planted in 26-cm^3 pots. Nine replicates per compost were inoculated with 1.5 mL of *P. irregulare* (8.23 log copies ITS g^{-1}) and another nine were not inoculated. *P. irregulare* was isolated from lettuce plants with damping-off symptoms and quantified by qPCR according to Giménez et al., (2019). The inoculum of *P. irregulare* was obtained by blending 50 mL of sterile water on a 4-day growth of *P. irregulare* in PDA. The pots were placed in a germination chamber at 18±1 ºC in the dark with a relative humidity of 80% for 48 hours. After this, the pots were randomly distributed in a growth chamber at 24/18 ºC day/night temperatures with a relative humidity range of 60-70%. The lettuce plants were collected 25 days after planting, and their aerial parts were weighed to obtain the fresh biomass. For the plants infected with *P. irregulare*, the suppressiveness index (%) was also calculated using the following formula:

(%) suppressiveness = (FBL inoculated/ non-FBL inoculated) * 100

FBL inoculated: the fresh biomass of the lettuce grown on *P. irregulare*-infected growing media; non-FBL inoculated: the fresh biomass of lettuce grown on growing media not infected with *P. irregulare.*

2.6 Statistical analysis

The statistical package R (RStudio Team, 2021) was used to perform the different statistical analyses. The normality and homogeneity of variance assumptions were assayed by Shapiro-Wilk and Levene's test in the car package (Fox et al., 2007). The mean comparison was performed using one-way analysis of variance (ANOVA) followed by post-hoc tests, Tukey's honestly significant difference (HSD). When homoscedasticity was not met a Kruskal-Wallis test was performed followed by Dunn test with a 'Benjamine-Hochberch' p-value adjust. Bacterial and fungal alpha diversity

indices (Chao1 and Shannon) were estimated using the vegan package (Oksanen et al., 2019). The effect of the different compost piles on alpha diversity was evaluated using one-way ANOVA or Kruskal-Wallis, and the differences were tested by Tukey's HSD or Dunn's post-hoc test, respectively. To visualize the indices, boxplot was performed in R using the ggplot2 package v 1.4-5 (Wickham, 2009) .

Principal coordinates analysis (PCoA) was performed to visualize the variation in community composition based on the Bray-Curtis distance. To evaluate differences between piles, a Permutation Multivariate Analysis of Variance (PERMANOVA) was conducted using the 'betadisper' and 'adonis' functions with 999 permutations from the vegan package assuming the homogeneity of the variance. In the cases in which homoscedasticity was not fulfilled, an Analysis of Similarities (ANOSIM) was carried out instead. Relationships between the bacterial community and the rest of the parameters were determined using the 'bioenv' function from the vegan package to find the best subset of parameters (using Euclidean distance) that had a maximum correlation with the community dissimilarity matrix (Clarke and Ainsworth, 1993).

Redundancy analysis (RDA) was performed to visualize the correlation between OTUs and chemical parameters (Vegan package). The OTU abundance was Hellinger transformed prior to analysis with the retained variables from the bioenv procedure (Legendre and Gallagher, 2001), which was performed via the 'bioenv' function based on Spearman's rank correlation coefficient.

3. Results

3.1 Temperature during composting

The temperature profiles of the three piles are shown in Fig. 1. The temperature in the piles increased gradually for 80 days, reaching > 65º C (thermophilic phase); peak

thermophilic temperatures of between 70ºC-75ºC were attained. Once the thermophilic phase was finished, the temperature progressively decreased (bio-oxidative phase) and eventually reached constant values close to the external temperature (maturation phase). Pile C showed the highest temperature values during the process, followed by pile B and pile A (Fig. 1).

3.2 Microbial diversity during the composting stages

A total of 5,677,000 high-quality bacterial reads and 1,566,000 high-quality fungal reads were obtained after quality filtering. In total, 1,215 bacterial OTUs and 40 fungal OTUs were identified, with 97% similarity.

Both bacterial and fungal diversity indices (Chao and Shannon) were calculated based on the observed OTUs (Fig. 2, Table S2), respectively. In general, both bacterial diversity indices showed an increase during the three composting processes, with significant differences between piles during the first three stages (initial, thermophilic and bio-oxidative). In the maturation phase, the piles showed similar values. On the other hand, the fungal diversity indices of the three piles decreased from the initial to the thermophilic phase and gradually increased in the two subsequent phases (Fig. 2, Table S2).

Principal coordinate analysis (PCoA) using Bray-Curtis dissimilarity revealed differences in the bacterial communities between the three piles that were independent of the composting phase (Fig. 3a-d). PCoA of the fungal communities revealed different community groups in the three piles in the initial phase (Fig. 3e-h), while in the other phases, pile A and pile B were grouped together, completely separate from pile C (Fig. 3e-h).

3.3 Community composition of different composting stages

At the phylum level, 11 bacterial phyla were observed in the different composting phases for the three composting processes (Fig. 4a, Table S3). In the three piles, at the initial phase, the dominant phylum was Firmicutes (53.78%-70.59%), followed by Proteobacteria and Actinobacteria. Bacteroidete was only observed in piles A and B. In the thermophilic phase, the dominant phylum was Proteobacteria (41.60%-57.07%), followed by Firmicutes, Actinobacteria, Chloroflexi, Bacteroidete and Gemmatimonadetes. After the bio-oxidative and maturation phases, the most abundant phyla were Proteobacteria (19.08%-30.59%), Bacteroidetes (22.22%-30.37%) and Actinobacteria (17.11%-22.35%), followed by Chloroflexi, Firmicutes, Gemmatimonadetes and Acidobacteria. Planctomycetes and Thaumarchaeota were only observed in the maturation phase (Fig. 4a, Table S3). The phylum Firmicutes significantly decreased through the composting phases, while Bacteroidetes and Chloroflexi increased significantly (Fig. 4a, Table S3).

At the genus level, 55 classified bacterial genera were observed (Fig. 4b, Table S4). In the initial phase, different genera like *Lactobacillus* (27.22%- 62.95%), *Leuconostoc* (2.34%-41.42%), *Acetobacter* (12.02%-15.48%) and *Pseudomonas* (3.08%-4.26%) were observed in all piles, and the first three disappeared after this phase. Genera such as *Brevibacillus*, *Chryseobacterium*, *Paenibacillus*, *Sphingobacterium* and *Stenotrophomonas* were observed in pile A and pile B only, and, like the other genera mentioned above, they also disappeared after the initial phase. In the thermophilic phase, genera such as *Acinetobacter* (24.57%-68.10%), *Bacillus* (3.36%-5.09%) and *Pseudomonas* (3.52%-16.06%) were observed in the three piles (Table S4), while genera such as *Pseudoxanthomonas*, *Solibacillus*, *Chryseolinea*, *Planifilum*, *Thermomonospora* and *Thermopolyspora* were only observed in piles B and

pile C. After the bio-oxidative phase, the following three genera were found in the three piles: *Chyseolinea* (21.59%-64.71%), *Bacillus* (13.64%-16.75%) and *Truepera* (3.35%-11.85%) (Table S4). After maturation, the following four genera were common to the three piles: *Pseudofulvimonas* (3.73%-12.51%), *Planktosalinus* (4.04%-8.47%), *Chryseolinea* (9.96%-44.67%) and *Cellvibrio* (6.11%-21.57%).

The fungal community dynamics are shown in Fig. 4c-d, Table S5-S6. At the phylum level (>1%), four fungal phyla were observed in the different composting phases (Fig. 4c). The phylum Ascomycota was dominant in all phases and in the three piles (58.34%-99.89%), except in pile B in the initial phase: in this case, Mucoromycota was the most abundant phylum (96.13%) (Fig. 4c, Table S5), although it practically disappeared after this phase. Basidiomycota was the second most abundant phylum and was present in practically all phases and piles. Mortierellomycota was only observed in all piles in the maturation phase.

At the genus level, 23 different fungal genera were observed (Fig. 4d, Table S6). In the initial phase, different genera were observed in the three piles *Alternaria* (0.15%-4.36%), *Candida* (0.74%-62.12%), *Issatcheukia* (0.22%-10.39%), *Kazachstania* (0.0%-4.64%), *Kluveromyces* (0.34%-2.26%), *Vishniacozyma* (0.57%-5.18%), and *Mucor* were observed in pile B only (70.77%); *Stemphylium* (1.93%), *Clostridium* (4.30%), *Corynebacterium* (8.17%) and *Enterococcus* (6.87%) were observed in pile A only; and *Clonostachys* (0.49%) was observed in pile C only. All of these genera disappeared after the initial phase. *Aspergillus* (0.14%-7.75%), *Penicillium* (0.63%-3.06%) and *Cladosporium* (6.0%-13.58%) were also observed in the three piles. In the thermophilic and bio-oxidative phases, six fungal genera were dominant: in all piles, *Thermomyces* showed the highest values (68.75%-89.27%), followed by *Mycothermus*, *Myceliophthora*, *Coprinus*, *Aspergillus* and *Coprinopsis*. In the maturation phase,

316 *Thermomyces* still remained in high percentage in all piles (56.92%-65.99%), followed
317 by *Mycothermus*, *Aspergillus*, *Myceliophthora*, *Copripnopsis* and *Coprinus* in piles B
318 and C. Some genera, including *Scedosporium* (0.43%-2.17%) and *Coprinellus* (26.5%-
319 2.88%), appeared in the maturation phase in the three piles, while other genera appeared
320 only in certain piles, like *Morterierella*, which appeared in piles B and C (2.33% and
321 0.82%, respectively) (Table S6).

322 **3.4 Chemical changes during the composting process**

323 The main physico-chemical and chemical characteristics of the different raw
324 materials used to produce the three composts are shown in Table 1. The pH and EC
325 increased throughout the process in the three piles: the pH values increased from 5.51-
326 6.69 to 8.94-8.41, and the EC values increased from 3.31-4.07 to 3.65-5.98 dS m^{-1}. Pile
327 A showed the highest pH and EC values during the whole composting process (Table
328 2).

329 The total organic carbon decreased in the three piles throughout the composting
330 process (Table 2). Out of the three piles, pile C showed the highest total organic C
331 during the four composting phases (Table 2). The total N content, on the other hand,
332 increased during composting, and pile A showed significantly higher nitrogen levels in
333 the initial phase (Table 2). In the three piles, the C/N ratio decreased gradually during
334 composting, while pile C showed the highest C/N ratio throughout the composting
335 process (Table 2).

336 In terms of microorganisms considered to be human pathogens, the composts in
337 our study showed levels below the maximum limit allowed in composts used as
338 fertilizers according to Spanish legislation (Real Decreto 506/2013; US EPA
339 regulations) (Hernandez-Lara et al., 2021). They can therefore be used as fertilizers

according to Spanish legislation (BOE 2013) and considered Class A Biosolid composts (EPA 2003; EC, 2011)

3.5 Correlation between microbial communities and chemical parameters

Redundancy analysis (RDA) was performed to further analyze the relationship between the chemical properties of the composts and the bacterial and fungal communities during the different composting phases (Fig. 5). The bacterial community was clearly separated in each phase according to the different piles (Fig. 5a, b, c and d). The first and second axis (RDA1 and RDA2) explained 62.85%, 55.22%, 45.44% and 32.32% of the variation in the bacterial community composition in the initial, thermophilic, bio-oxidative and maturation phases, respectively (Fig. 5a, b, c and d). The length of the arrow in the RDA plot indicates the degree of correlation between the environmental factor and sample distribution. The analysis indicated that the most significant correlations with the bacterial community changed according to the different phases. In the initial and thermophilic phases, pH, EC, TN, TC and C/N were parameters that correlated the most with the bacterial community (Fig. 5a, b). In the bio-oxidative phase, however, temperature was also incorporated (Fig. 5c). In the maturation phase, only EC, pH and temperature remained significant (Fig. 5d).

On the contrary, the fungal community was not clearly separated in each phase according to the different piles (Fig. 5e, f, g, h). The first and second axis explained 40.65%, 38.87%, 27.97% and 27.70% of the variation in the fungal community in the initial, thermophilic, bio-oxidative and maturation phases, respectively (Fig. 5e, f, g, h). In the initial phase, the parameters that correlated the most with the fungal community were EC, temperature and TOC, and in the thermophilic phase, NT was also incorporated (Fig. 5e, f). In the bio-oxidative stage, the correlated parameters were

364 temperature and C/N, while in the maturation phase, the correlated parameters were pH,
365 temperature and TOC (Fig. 5g, h).

366 **3.6 Compost suppressiveness against Pythium *irregulare***

367 Once the composting process was finished, the three composts obtained were
368 tested to evaluate their suppressiveness, and they showed a higher suppressiveness
369 index against *P. irregulare* than peat for baby-leaf lettuce crops (Fig. 6). Out of the
370 three composts, composts A (C_A) and B (C_B) showed higher suppressiveness
371 indexes than compost C (C_C).

372

373 **4. Discussion**

374 **4.1 Assessment of the composting process**

375 The selection of the materials and effective management of the composting
376 process are key in obtaining an added-value compost, i.e., stable organic matter with a
377 suppressive capacity and without phytotoxic compounds or plant or animal pathogens
378 (Morales et al., 2016). The temperature profiles of the different piles are indicators of
379 the microbial activity involved during the composting process, considering that
380 microorganisms play key roles in the transformation of the raw materials into compost
381 and in their suppressive activity (Hadar and Papadopoulou, 2012; Morales et al., 2016).
382 Pile C showed higher temperatures than the other piles during the process and a longer
383 bio-oxidative phase due to the high percentage of olive mill cake, a recalcitrant material
384 that slows down the composting process and may hinder the ability of microorganisms
385 and their enzymes to degrade the cake (Alburquerque et al., 2004).

386 The bacterial diversity increased during the composting process, probably due to
387 a higher species richness or equitability according to the availability of easily available

388 organic substances (Wang et al., 2018; Meng et al., 2019). Moreover, the piles showed
389 different bacterial community structures during composting, probably due to the nature
390 of the different mixes of starting feedstock and their transformation throughout the
391 different composting stages (Estrella-González et al., 2019; Jurado et al., 2020). On the
392 other hand, fungal diversity indices have been found to decrease in the thermophilic
393 phase and increase in the bio-oxidative and maturation phases, according to Galitskaya
394 et al., (2017). This is likely due to the fact that some fungal species are less specialized
395 than bacteria and are not thermo-tolerant so cannot survive at high temperatures (>65
396 ºC). This suggests a self-purification process (Zhong et al., 2020). Fungal species are
397 often recovered in the maturation phase, where temperatures are moderate (<45ºC),
398 explaining in part the highly similar fungal communities shared between piles (Meng et
399 al., 2019).

400 During the composting process, Proteobacteria, Bacteroidetes, Firmicutes,
401 Chloroflexi and Actinobacteria were the most abundant bacteria, and Ascomycota and
402 Basidiomycota were the most abundant fungi, indicating that these were the major
403 players in the composting processes studied (Wang et al., 2018; Meng et al., 2019:
404 Zhong et la., 2020).

405 In the initial phase, in accordance with Tian et al. (2013), Firmicutes was the
406 most abundant phyla, followed by Proteobacteria. The low pH in the initial phase,
407 principally in pile C (pH = 5.4), could be due to the high amount of lactic acid bacteria
408 such as *Lactobacillus* species. This species forms lactic acid and other organic acids and
409 also possesses the ability to produce antibiotic compounds (Partanen et al., 2010;
410 Nakasaki et al., 2019). The high level of lactic acid would explain the presence of
411 *Acetobacter*, which uses lactic acid as a main substrate (Partanen et al., 2010). The

presence of *Pseudomonas* can also indicate an ability to inhibit soil-borne pathogens (Haas and Défago, 2005).

Ascomycota and Mucoromycota were the dominant microbial communities in the initial phase, which is similar to results obtained by Liu et al. (2021). Ascomycota dominate all the phases because they can secrete a variety of cellulose- and hemicellulose-degrading enzymes and efficiently utilize nutrients in compost (Meng et al., 2019). The highest abundance of Mucoromycota found in pile B could be due to a high abundance of *Mucor*, a filamentous fungi normally dominant in fresh organic wastes (Mehta and Satyanarayana, 2013; Wang et al., 2018). The presence of certain yeasts able to metabolize cellulose and lignin—such as *Candida, Issatchekia or Kluveromyces*, found in all piles, and *Vishniacozyma or Kazachstania*, found in piles A and B—indicates that yeasts were followed by the growth of thermophilic bacteria (Choi and Park, 1998; Partanen et al., 2010). In addition, some plant and human pathogens, such as *Stemphylum, Clostridium, Corynebacterium* and *Enterococcus*, and soil-borne pathogens, such as *Alternaria*, disappeared with the high temperatures reached in the thermophilic phase (Neher et al., 2013; Zhang et al., 2017)).

In the thermophilic phase, the genera belonging to the phyla Proteobacteria, Firmicutes and Actinobacteria were the bacteria groups involved in the turnover of organic matter, including the extensive degradation of cellulose and lignocellulose residues (Steger et al., 2007) . Moreover, these bacteria are characterized by a high tolerance to unfavourable conditions or an ability to live under environmental stress, mainly through endospore formation (Li et al., 2019; Meng et al., 2019). *Pseudomonas* were also observed that can play important roles in the denitrification and degradation of pollutants (Lalucat et al., 2006), and it is known that many strains could also promote plant growth and suppress plant disease (Haas and Défago, 2005). Other genera,

437 including *Pseudoxanthomonas*, *Solibacillus*, *Chryseolinea*, *Planifilum*,
438 *Thermomonospora* and *Thermopolyspora*, were only observed in piles B and C, which
439 showed the highest temperature values during the composting process. Some of these
440 genera, e.g. *Solibacillus* and *Thermomonospora*, have been observed by other authors in
441 this phase (Antunes et al., 2016).

442 There was a high diversity of thermophilic and thermotolerant fungi
443 [*Thermomyces*, *Myceliophtora* and *Mycothermus* (*Mycothermus thermophiles)*] in all
444 piles. These fungi are capable of withstanding temperatures above 45 ºC for a long time
445 (Zhang et al., 2015; 2018b). Other thermophilic fungal genera such as *Aspergillus*, have
446 been observed in accordance with (Sebők et al., 2016), this genus has been found to be
447 a common saprophytic fungi on food wastes in the initial phase (Neher et al., 2013).
448 During the bio-oxidative phase and maturation phase, Firmicutes decreased
449 significantly in accordance with other studies (Ren et al., 2016; Meng et al., 2019), and
450 Proteobacteria, Bacteriodetes, Chloroflexi and Actinobacteria became the four major
451 phyla. The genus *Truepera*, able to live under thermophilic temperatures (Krishnan et
452 al., 2017), joined *Pseudomonas*, *Bacillus* and thermotolerant fungi such as
453 *Thermomyces*, *Mycothermus* and *Aspergillus* that were still maintained during the bio-
454 oxidative phase.

455 RDA analysis showed that the bacterial community was clearly more affected by
456 chemical parameters than the fungal community during the first three stages of
457 composting, indicating that the former community was more sensitive to environmental
458 fluctuations than the fungal community (Jiang et al., 2017; Meng et al., 2019).
459 Therefore, composts are a potentially profitable source of microbiota to be used as a
460 suppressive soil amendment or growing media.

In addition, the rates of nutrient transformation and compost maturation are processes mainly sponsored by bacteria and fungi (Meng et al., 2019). The biodegradation of the organic matter increases electrical conductivity and pH during composting due to the degradation of acid compounds and the liberation of ammonia. (Alburquerque et al., 2006; Wang et al., 2019). Pile C showed the highest levels of TOC throughout the composting process due to its high percentage of olive mill cake as starting feedstock, which extended the bio-oxidative phase in this pile (Alburquerque et al., 2004). These processes also involved a decrease in the C/N ratio, which has been widely mentioned as an index of compost maturity, with values below 15–20 being indicative of mature compost (Morales et al., 2016).

4.2 Determination of the suppressive properties of the composts against *Pythium irregulare*

The composting process produces an ideal environment for the growth of microbes involved in controlling different plant diseases (Mehta et al., 2014) and that are the key in understanding the suppressive process (Blaya et al., 2016; De Corato et al., 2018; Scotti et al., 2020). This process could occur through competition among microbial populations, antibiosis, hiperparasitism and/or systemic acquired resistance and induced systemic resistance (De Corato, 2020). The degree of decomposition of the organic matter and the nature of the substrates critically affects the composition of bacterial taxa, as well as the populations and activities of the biocontrol agents contained therein, which are considered key factors in disease suppression (Ros et al., 2005). During composting, the three piles in the current study contained a greater relative abundance of Bacterioidetes, Proteobacterias and Actinobacterias, all of which have been well documented to be correlated with the suppression of soil-borne

pathogens that cause plant diseases such as *Pythium irregulare* (De Corato et al., 2018; Scotti et al., 2020). *Bacillus or Pseudomonas*, for instance, have been found to effectively control pathogens.

In the maturation phase, composts C_A and C_B showed greater suppressive activity against *P. irregulare* than compost C_C. It has been shown that the genera *Aspergillus* and *Penicillium*, which were present in the highest abundance in both piles A and B, promote suppression against soil-borne pathogens like *Pythium spp*. (De Corato et al., 2018). In addition, the *Trupera* and *Luteimonas* genera were relatively highly represented in all three piles. The higher abundance of these genera in piles A and B than in pile C, could potentially be linked to the higher suppressiveness of these composts, even though they do not have a well described suppressive role in literature (Scotti et al., 2020). On the other hand, it has been shown that extremely stable and mature compost shows lower suppressiveness (Van Elsas and Postma, 2007), as is the case in compost C.

In addition, it has been described that plant-based composts can show suppressive activity due to the presence of aromatic compounds or soluble organic molecules, such as potentially bioactive soluble components released by the dissolved humic substances (Pascual et al., 2000). This suppressiveness depends on the compost composition (Morales et al., 2016), as we observed in our study in which the compost with the highest proportions of tomato waste (34%-46%)—mainly tomato pulp waste and peels—and with antimicrobial compounds, such as phenolic and carotenoid compounds (e.g. lycopene, β-carotene and lutein), showed the highest suppressiveness.

Conclusions

509 This study showed that the selection of raw materials and effective management
510 of the composting process are important factors in obtaining mature compost that has
511 the added-value of being suppressive against plant pathogens such as *P. irregulare*. The
512 specific raw material feedstock selected produced different levels of bacterial and
513 fungal diversity and abundance throughout the composting phases, and significant
514 differences were observed among the composting piles. Feedstock materials combining
515 vineyard pruning waste, tomato waste and leek waste favored the presence of some
516 suppressive microorganisms in the final product, including *Aspergillus* and *Penicillium*,
517 and other microorganisms like *Trupera* and *Luteimonas* that could be responsible for
518 the observed Suppressiveness. Incorporating olive mill cake into the pile resulted in a
519 more stable but less suppressive compost.

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