Microbiota Characterization of Agricultural Green Waste-Based Suppressive Composts Using Omics and Classic Approaches

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Abstract: While the control of soil-borne phytopathogenic fungi becomes increasingly difficult without using chemicals, concern over the intensive use of pesticides in agriculture is driving more environmentally sound crop protection managements. Among these approaches, the use of compost to suppress fungal diseases could have great potential. In this study, a multidisciplinary approach has been applied to characterize microbiota composition of two on-farm composts and assess their suppress and biostimulant activities. The on-farm composting system used in this study was able to produce two composts characterized by an antagonistic microbiota community able to suppress plant pathogens and biostimulate plant growth. Our results suggest a potential role for Nocardiopsis and Pseudomonas genera in suppression, while Flavobacterium and Streptomyces genera seem to be potentially involved in plant biostimulation. In conclusion, this study combines different techniques to characterize composts, giving a unique overview on the microbial communities and their role in suppressiveness, helping to unravel their complexity.

Keywords: Metagenomics; 16S rDNA; 18S rDNA; Whole genome shotgun sequencing; microbiome; sustainable agriculture

1. Introduction

Soil-borne phytopathogenic fungi are some of the more destructive plant pathogens, affecting different plant portions of a wide hosts range and they can be difficult to control without use of chemicals. However, concerns over the intensive use of pesticides in agriculture are driving increasing interest in more environmentally sound crop protection methods, such as biological control practices. Among these, compost could have a great potential in suppress fungal diseases [1].

Compost is a mature and stable organic matter derived by the bio-oxidation of several feedstocks, including agricultural green wastes. The composting process is normally performed in industrial plants or, alternatively, in farm based composting plants, using simple agricultural tools already present in the farm [2]. In agricultural management where green waste for composting is recommended, such as organic farming, compost with suppress and biostimulant activities would be helpful to substitute the not eco-friendly chemical managements.

Although its effectiveness in disease control can be variable [3], compost has been considered a suitable and sustainable method to manage several plant pathogens, including Pythium spp., Thielaviopsis sp. Rhizoctonia solani, Phytophthora spp., Fusarium spp., and Sclerotinia spp., in many horticultural cropping systems [4,5]. The biotic component, represented by microbes involved in organic matter decomposition, plays a main role in compost suppressiveness against plant pathogens.
The suppressive microbiota may have an antagonistic interactions with detrimental microbes and/or induce the systemic resistance in plants [6]. Moreover, microbiota decomposition of complex feedstocks during compost maturation may, indirectly, produce natural biostimulants, as humic substances, which may have a suppressive activity against some pathogens [7]. Compost microbiome structure is closely related to the organic matter composition and, therefore, to the input materials used for the composting process. Shifts in the feedstocks affect microbial community and, as consequence, its suppression efficacy [8].

Relatively few studies describe both bacterial and fungal compost communities, even though both groups are important in plant pathogen suppression activity. Most of the studies have been conducted using culture-based methods [9–11], capturing only a small portion of the microbial diversity. Next generation sequencing (NGS) techniques can be a powerful tool to resolve microbial community composition and diversity at greater depth.

This study represents a multidisciplinary approach using chemical, biochemical, biological and NGS techniques, to characterize two composts produced on-farm from different agricultural wastes and to evaluate their biostimulant and suppressive activities against two soil-borne pathogens, Sclerotinia minor Jagger and Rhizoctonia solani Kühn. Both pathogens were chosen because of their wide host range and worldwide distribution, representing a reliable damping-off disease model [1,5]. Our hypothesis was demonstrated that on-farm composting is a useful agricultural practices to produce compost with effective biostimulant/suppressive properties. In detail, our investigation aimed to characterize (i) microbiota composition associated with suppressiveness and biostimulation and (ii) give new insights on mechanisms governing these abilities in compost.

2. Materials and Methods

2.1. Composting Process and Sampling

In this study, two composts, named L5/6A and L2A, produced in an on-farm composting plant located in Southern Italy (40°34’36.538” N, 15°1’30.932” E), were used. Both composts were obtained through composting of raw organic materials (see Table 1 for details), previously chipped, on static piles (1.5m × 30m) aerated by mechanical turning and by basal forced ventilation along 45-day active phase, followed by a two months-curing period. Pile wetting was through an irrigation system, manually activated when gravimetrically determined relative humidity was <50%. Composting temperatures were measured by PT100 thermo-sensors placed in the core of the pile. During the thermophilic phase, pile heating exceeded 55 °C for at least 5 days, to achieve biomasses sanitation.

For all chemical and biological characterizations and for the DNA extraction, compost samples of approximately 1 kg were collected by pooling and mixing 10 subsamples taken from 10 different points of each compost pile. For chemical and biological analyses, samples were stored at 4 °C, in cold room while, for metagenomic study, a representative aliquot was stored at ~80 °C until DNA extraction.
Table 1. Compost raw organic material content. For composts L5/6A and L2A, raw material compositions (percentage of fresh weight), in terms of species, is reported.

| Raw materials       | Species               | L5/6A (%) | L2A (%) |
|---------------------|-----------------------|-----------|---------|
| Rocket salad        | Eruca sativa          | 58        | 8       |
| Endive              | Cichorium endivia     | 9         | 48      |
| Lettuce             | Lactuca sativa        | 2         | 25      |
| Fennel              | Foeniculum vulgare    | 14        | -       |
| Mandarin orange     | Citrus reticulata     | 6         | -       |
| Broccoli            | Brassica oleracea     | 2         | -       |
| Pumpkin             | Cucurbita pepo        | -         | 1       |
| Basil               | Ocimum basilicum      | -         | 11      |
| Wood scraps         |                       | 9         | 7       |
| Total               |                       | 100       | 100     |

2.2. Chemical and Biological Compost Characterization

Total N was determined according to the Kjeldahl method. Electrical Conductivity (EC) and pH were determined according to the standard official methods [12]. Suppressive compost assays were performed using two fungal plant pathogens: Rhizoctonia solani and Sclerotinia minor. Fungi were maintained on potato dextrose agar (PDA, Oxoid) and each isolate was preliminarily tested for pathogenicity. Both pathogens were artificially inoculated onto cress plants (Lepidium sativum L.), which is recognized as a sensitive and reliable plant test [13,14]. Fungal inoculums were prepared, according to a previous report [15], as follows: 100 g common millet seeds were placed in 1 L capacity flasks and saturated with a potato dextrose broth (PDB) solution (1/10 w/w) and, after that, autoclaved twice. Flasks were inoculated with fungi previously cultured on PDA for 15 days and then were incubated for 21 days at 20 °C. The resulting millet colonized by fungal mycelia was air-dried for 3 days, powdered in a mortar and mixed at a concentration of 0.5% (w/w, dry weight), into a potting substrate of sterilized peat.

Pots (7 cm diameter and 100 mL volume capacity) were filled with 80% inoculated peat and 20% compost (v/v) and, after, sown with 20 L. sativum seeds cv. Comune (Blumen). Pots were moistened to field capacity and arranged in greenhouse (25 °C) following a complete randomized design; pot distribution was rearranged randomly every 2 days to avoid the effects of environmental heterogeneity into greenhouse. After 15 days, disease incidence was recorded as percentage of diseased plants. Damping-off percentage was calculated as described by Veeken et al. [16] % DO = (HPo-HPi)/HPo × 100 (%), where HPo is the number of healthy plants in the non-inoculated control mixture and HPi is the number of healthy plants in the inoculated potting mixes. Overall, the experimental design included two composts, with two different fungi inoculum and ten replications. The experiment has been repeated twice. Not amended control plots inoculated with both fungi were included.

Compost biostimulation/phytotoxicity activity was assessed by measuring germination and root elongation of cress plants (Lepidium sativum L.). Composts water extracts (CWEs) were prepared by vigorously shaking of compost/water mixture (50 : 50 v/v) and poured on a petri dish containing 30 cress seeds incubated on sterilized glass fiber filter paper moistened. CWEs, diluted in three concentrations (50, 16.6, and 5 g l\(^{-1}\)), and only water, as control, were used. For each CWE concentration, 10 petri dishes were replicated. The number of seeds germinated and root length were recorded after 36 h following germination. GI% that was directly affected by phytotoxicity, was then obtained by multiplying the number of germinated seeds by the relative mean root length, expressed as percentage of control, accordingly to the following formula [17] % GI = ((N\(^0\) seeds germinated on CWEs)/(N\(^0\) seeds germinated on water)) × ((Mean root length on CWEs )/(Mean root length on water)) × 100.

Diversity of microbial metabolism were evaluated by BIOLOG EcoPlates™ method based on carbon substrate utilization. BIOLOG EcoPlates™ consist of 96 wells containing 31 carbon sources and one blank, in triplicate. As the carbon source is utilized, the tetrazolium violet dye is reduced,
developing a purple color. The plates were incubated at 25 °C for 4 days and color development in each well was recorded, 96 h post inoculum, at optical density 590 nm, using the Bio-Rad Microplate Reader 550 (Biorad, USA). The assay was conducted as previously described by Bartelt-Ryser et al. [18]. Average well color development (AWCD) was calculated as the sum of activities measured in all wells of each plate, divided by the 31 carbon sources. The substrates were subdivided into six categories: amines and amides, aminoacids, carboxylic acids, carbohydrates, phenolic compounds and polymers. Shannon’s index was calculated as H’ = Σpi ln pi, where pi is the ratio of the activity on a particular substrate and Σpi is the sum of activities on all substrates [19].

2.3. Microbial DNA Isolation, Amplification and Sequencing

For metagenomic analysis, for both composts four sequencing replicates from four different DNA extractions were performed. For each extraction, DNA was extracted from approximately 1.5 g of compost using the DNeasy PowerMax Soil Kit (MOBIO Laboratories Inc., Carlsbad, CA, USA) and stored at −20 °C until required. Final yield and quality of extracted DNA was determined by using NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA) and Qubit Fluorometer 1.0 (Invitrogen Co., Carlsbad, CA). PCR amplification was performed with the following primers: i) Forward: 5’-CCTACGGGNGGCWGCAG-3’ and Reverse: 5’-GACTACVGGGTATCTAATCC-3’ [20], which target the hypervariable V3-V4 regions of the 16S rRNA gene; and ii) Forward: 5’-GTAGTCATATGCTTGTCTC-3’ and Reverse: 5’-GGCTGCTGGCACCAGACTTGC-3’ [21] which target the NS1 and NS2 region of the 18S rRNA gene. Each PCR reaction was assembled according to Metagenomic Sequencing Library Preparation (Illumina, San Diego, CA, USA). Libraries were quantified by Qubit fluorometer (Invitrogen Co., Carlsbad, CA, USA) and pooled to an equimolar amount of each index-tagged sample to a final concentration of 2nM, including the Phix Control Library (Illumina; expected 25%). Pooled samples were subject to cluster generation and sequenced on MiSeq platform (Illumina, San Diego, CA, USA) in a 2 × 300 paired-end, 1.5 M reads, format at a final concentration of 18 pmol.

For the whole genome shotgun (WGS) sequencing, indexed libraries were prepared from 1 ng/μl DNA with Nextera XT DNA Prep Kit (Illumina), according to the manufacturer’s instructions. Libraries were quantified using Qubit fluorometer (Invitrogen Co., Carlsbad, CA, USA) and pooled to an equimolar amount of each index-tagged sample to a final concentration of 4 nM. Pooled samples were subject to cluster generation and sequenced on the MiSeq platform (Illumina, San Diego, CA, USA) in a 2 × 300 paired-end format, 10 M reads.

The DNA libraries were produced and sequenced by Genomix4Life S.r.l (Salerno, Italy, http://www.genomix4life.com) for the 16S amplicon and WGS sequencing, and by Microgem s.r.l (Naples, Italy, http://www.microgem.it) for the 18S amplicon sequencing. The raw sequence files generated (fastq files) underwent quality control analysis with FastQC.

All sequences have been deposited at European Nucleotide Archive (ENA, http://www.ebi.ac.uk/ena) under project number PRJEB14766.

2.4. Computational Study

The analyses of the NGS datasets were performed through the EBI Metagenomics service pipeline [22], that includes quality control and taxonomic analysis based on SSU rDNA sequences and assembling.

2.5. Statistical Analysis

Statistical analysis of compost biological data was carried out using JMP 8 Software (JMP®, Version 8, 1989). To assess the normality of distributions and variance homogeneity between groups, variables were firstly checked with Kolmogorov–Smirnov and Levene’s tests, respectively. When necessary, to satisfy the assumptions of normality, a logarithmic transformation was applied to the variables.
Once the assumption of sphericity was verified, one-way analysis of variance (ANOVA) was performed over all biological variables (damping-off, germination index and BIOLOG EcoPlates™ substrates), to assess effects arising from the different types of compost. For damping-off incidence and germination index, differences were tested using a one-way ANOVA followed by a Tukey’s HSD post-hoc test. Significance was evaluated in all cases at $p < 0.01$.

All statistical analyses for metagenomic datasets were executed using custom code in R statistical software (version 3.5.2, packages “phyloseq”, “vegan”, “DESeq2” and “ggplot2” [23–26]). For downstream analyses, OTUs represented by <10 reads and/or making up <2% of the total number of OTUs identified in a given sample, were removed prior to statistical analyses. For microbial diversity indices calculations, sequence data were rarefied to the highest sequencing depth at which all study samples were retained. Then, differences in bacterial alpha diversity (Chao1, Shannon, and Simpson indices) between the microbiota sequence data generated from L5/6A and L2A compost samples, were calculated.

The average relative abundance of bacterial phyla and genera was calculated as mean across all replicates belonging to the same compost. In comparative analyses between both comports, only the top ten (in the case of 16S rRNA gene analysis) and 5 (for 18S rRNA gene analysis) most abundant phyla and 20 most abundant genera were considered. Significant differences ($p < 0.05$) between the two comports were calculated using the non-parametric Wilcoxon rank-sum test. Further exploratory analysis was performed using the negative binomial distribution method in DESeq2 to identify OTUs that made up a significantly different proportion of the microbiota in one comparison compost versus another.

### 3. Results

#### 3.1. Compost Chemical and Biological Properties

Rocket (*Eruca sativa*) and endive (*Cichorium endivia*) plant residues were used as the main feedstocks for compost L5/6A and L2A, respectively. Lettuce (*Lactuca sativa*), fennel (*Foeniculum vulgare*), basil (*Ocimum basilicum*) residues and wood chips were matrices also added in smaller amounts. The full compositions of the two composting piles, are listed in Table 1.

The chemical features of the comports are reported in Table 2. L5/6A compost samples exhibited a neutral pH value (6.95), while L2A compost was characterized by an alkaline pH (9.02). Both comports showed relative high level in terms of electrical conductivity (1950 $\mu$S cm$^{-1}$ and 1514 $\mu$S cm$^{-1}$ in L5/6A and L2A, respectively) and a good nitrogen content (1.32% and 1.68% in L5/6A and L2A, respectively).

| Compost Chemical Properties. For comports L5/6A and L2A, pH, electrical conductivity (EC, $\mu$S/cm) and total nitrogen content (%) are reported. |
|-----------------|-----------------|-----------------|
| **Compost**     | **L5/6A**       | **L2A**         |
| pH              | 6.95 ± 0.07     | 9.02 ± 0.02     |
| EC              | 1950 ± 25       | 1514 ± 31       |
| Total nitrogen  | 1.32 ± 0.07     | 1.68 ± 0.22     |

In this study, the bioassay indicated that damping-off caused by two pathogens (*Rhizoctonia solani* and *Sclerotinia minor*) was significantly affected by compost type (ANOVA, $p < 0.01$).

In control pots, seedling mortality was reported after 15 days post inoculum, at about 91% and 54%, due to *R. solani* and *S. minor*, respectively (Figure 1A). Compared to non-amended control pots, L5/6A compost reduced disease caused by both fungal plant pathogens, while L2A showed no significant differences (Figure 1A).

Water extracts from the two comports showed different effects on cress germination index percentage (GI%) (Figure 1B). While L5/6A did not show any biostimulation activity (100% GI), L2A increased the cress germination index up to 150%.
The use of different carbon sources by microbial communities, as determined by BIOLOG Ecoplates™, was significantly different between the two composts for 16 out of 31 analysed substrates: β-Methyl-D Glucoside, L-Arginine, pyruvic acid methyl ester, D-galacturonic acid, tween 40, i-erythritol, L-phenylalanine, tween 80, D-mannitol, 4-hydroxy benzoic acid, L-serine, itaconic acid, glycil-L-glutamic acid, D-cellobiose, glucose-1-phosphate, D,L-α-glycerol phosphate (Table 3, Figure 2). In general, the L5/6A microbial community was able to metabolize easily degradable substrates, as well as complex matrices, more quickly than L2A. Based on the BIOLOG Ecoplates™, L5/6A compost reported higher AWCD and Shannon’s diversity index (H’) values compared to L2A (Figure 2).

Figure 1. (A) Lepidium sativum damping-off incidence at 15 days post inoculum with Rizoctonia solani and Sclerotinia minor. Bars within inoculum with the same letters are not significantly different according to the Tukey’s HSD post-hoc test (P < 0.01). (B) Lepidium sativum germination index on water extracts of composts (50, 16.6 and 5 g L⁻¹). Bars within compost with the same letters are not significantly different according to the Tukey’s HSD post-hoc test (P < 0.01). Values are mean ± standard deviation (n = 10).
Table 3. Carbon substrates utilized by microorganisms in BIOLOG EcoPlate™ plates. Significant effects of type of compost, according to one-way ANOVA (1 d.f.) $P<0.01$, are reported in bold.

| Type                | Substrates            | Number |
|---------------------|-----------------------|--------|
| Amines/amides       | Phenylethyl-amine     | G4     |
| Amines/amides       | Putrescine            | H4     |
| Amino acids         | L-Arginine            | A4     |
| Amino acids         | L-Asparagine          | B4     |
| Amino acids         | L-Phenylalanine       | C4     |
| Amino acids         | L-Serine              | D4     |
| Amino acids         | L-Threonine           | E4     |
| Amino acids         | Glycil-L-Glutamic Acid| F4     |
| Caboxylic acid      | Pyruvic Acid Methyl Ester| B1 |
| Caboxylic acid      | D-Galacturonic Acid   | B3     |
| Caboxylic acid      | γ-Hydroxybutyric Acid | E3     |
| Caboxylic acid      | D-Glucosaminic Acid   | F2     |
| Caboxylic acid      | Itaconic Acid         | F3     |
| Caboxylic acid      | -Ketobutyric Acid     | G3     |
| Caboxylic acid      | D-Malic Acid          | H3     |
| Carbohydrates       | -Methyl-D Glucoside   | A2     |
| Carbohydrates       | D-Galactonic Acid -Lactone| A3 |
| Carbohydrates       | D-Xylose              | B2     |
| Carbohydrates       | i-Erythritol          | C2     |
| Carbohydrates       | D-Mannitol            | D2     |
| Carbohydrates       | N-Acetil-D-glucosamine| E2     |
| Carbohydrates       | D-Cellobiose          | G1     |
| Carbohydrates       | Glucose-1-Phosphate   | G2     |
| Carbohydrates       | -D-Lactose            | H1     |
| Carbohydrates       | D,L - -Glycerol Phosphate| H2 |
| Phenolic compounds  | 2-Hidroxy Benzoic Acid| C3     |
| Phenolic compounds  | 4-Hydroxy Benzoic Acid| D3     |
| Polymers            | Tween 40              | C1     |
| Polymers            | Tween 80              | D1     |
| Polymers            | -Cyclodextrine        | E1     |
| Polymers            | Glycogen              | F1     |
3.2. Compost Microbial Composition

We performed an NGS-based metagenomic analysis of the two composts to characterize bacterial and eukaryotic microbial communities using amplicon and WGS approaches. Compost samples were collected from both compost piles at the end of composting time. DNA was extracted from 8 samples (2 composts × 4 biological replicates) and subjected to paired-end Illumina sequencing, as described in the material and methods section. After filtering out low-quality reads, an average of 1,938,089 and 1,400,736 of high quality reads spanning the V3-V4 region of the bacterial 16S rRNA gene, were recovered for the L5/6A and L2A samples, respectively (Table 4). Meanwhile, for the NS1-NS2 region of the 18S rRNA gene, an average of 1,891,223 and 1,866,587 of high quality reads for L5/6A and L2A samples, respectively, were obtained.
Table 4. Overall count of 16S and 18S amplicon sequencings. Data are mean of four replicates for each compost. It is reported the number of raw reads from Illumina paired-end sequencing, the number of high quality reads resulting from the pre-processing step and the number of reads successfully assigned to the reference database (SILVA SSU/LSU version 128). In brackets are reported the percentage with respect to high quality reads.

| Sample | Amplicon | Raw reads | High quality reads | Total number of reads assigned to the reference database | Assigned OTUs |
|--------|----------|-----------|--------------------|----------------------------------------------------------|--------------|
| L5/6A  | 16S      | 2,578,978 | 1,938,089          | 1,934,201                                                 | 2884         |
| L2A    | 16S      | 1,868,462 | 1,400,736          | 1,397,531                                                 | 2880         |
| L5/6A  | 18S      | 1,891,223 | 1,757,231          | 1,748,980                                                 | 1375         |
| L2A    | 18S      | 1,866,587 | 1,692,091          | 1,677,857                                                 | 1696         |

Table 4 also shows the percentage of high-quality reads assigned to the reference database (SILVA SSU/LSU version 128): it is higher than 99% for all samples. These reads were assigned to ~2,800 different OTUs, for bacterial 16S rRNA gene in both compost samples, and to 1,375 and 1,696 OTUs for the 18S rRNA gene for L5/6A and L2A, respectively (Table 4). Both bacterial and eukaryotic alpha diversities were lower in L5/6A than in L2A samples (Figure 3).

![Figure 3](image_url)  
**Figure 3.** Box plots showing Chao1, Shannon and Simpson diversity indices based on bacterial (above) and eukaryotic (below) communities in the compost samples analysed in this study.

16S rRNA gene amplicon sequences for the 10 most abundant phyla, covering 95% of taxonomic annotations, outlined high abundances of Proteobacteria, Bacteroidetes, Actinobacteria, and Deinococcus-Thermus, all of them more abundant in the L5/6A compost. On the other hand, Verrumicrobia, Gemmatimonadetes, Acidobacteria, and Planctomycetes phyla was more abundant in the L2A compost (Figure 4).
Figure 4. Mean relative abundance (bacterial community) for the ten most abundant phyla observed in both compost samples analysed in this study. Asterisks indicate significant differences ($p < 0.05$) between the two composts calculated with the non-parametric Wilcoxon rank-sum test.

Further, 18S rRNA gene amplicon analyses showed lower diversity compared to the 16S results. Less than 60% of sequences were classified at the phylum level. Two main phyla were identified, Basidiomycota (more abundant in the L5/6A compost) and Ascomycota (more abundant in the L2A compost) (Figure 5).

Figure 5. Mean relative abundance (eukaryotic community) for the five most abundant phyla observed in both compost samples analysed in this study. Asterisks indicate significant differences ($p < 0.05$) between the two composts calculated with the non-parametric Wilcoxon rank-sum test.

Figure 6 showed differences between the two composts for the twenty most abundant bacterial genera. The differences are plotted as Log2 Fold change of the relative abundance, using the DeSeq2 R package. Three genera, belonging to the Actinobacteria phylum, were reported in both composts: Nocardiopsis (1.17% in L5/6A compost and 3.8 Log2FC), Glycomyces and Streptomyces (2.56% in L2A compost and 2.3 Log2FC). Bacteroidetes phylum showed the genera with highest relative abundance, as Gallibacter (4.7% in L5/6A compost and 4.9 Log2FC), Parapedobacter and Flavobacterium (2.2% and 3.15%, in L6/5A and L2A compost, respectively). Trupera was the only genus reported for the Deinococcus-Thermus phylum, showing 3.42% and 1.35% of relative abundance in compost L5/6A and L2A, respectively. Proteobacteria phylum showed generally genera with a relative abundance
lower than 1%, except for Luteimonas and Pseudomonas, 2.4% and 1%, more abundant in L5/6A compost.

**Figure 6.** Heatmap of the relative abundance (% of bacterial community) for the twenty most abundant genera observed in both compost samples analysed in this study. For each genus, it is reported the difference abundance estimate as Log2 Fold-change, calculated by DESeq2. Asterisks indicate statistical significance values between the two composts that meet threshold criteria (padj < 0.05). α = Actinobacteria; β = Bacteroidetes; δ = Deinococcus-Thermus; κ = Planctomycetes; π = Proteobacteria.

WGS reads were assembled using metaSPAdes [27] and metagenome assemblies producing contigs were binned by MetaBAT [28], generating a total of 19 and 20 bins, for L5/6A and L2A respectively. Quality metrics were using CheckM [29] to estimate the level of genome completeness and contamination. Based on these metrics, two high quality metagenome-assembled genomes (MAGs) with more than 90% completeness and less than 5% contamination, were obtained for each compost (Table 5).

We also generated 10 and 5 medium-quality MAGs (at least 50% completeness and less than 10% contamination [30] for L5/6A and L2A, respectively). In general, assemblies confirmed the 16S results. In both composts, two MAGs were assigned to the same genera, Sphingobacterium and Luteimonas.
Table 5. Assembling binning in metagenome-assembled genome (MAG) and relative taxonomic assignment. Only MAGs defined as high (>90% completeness and <5% contamination) and medium (≥50% completeness and <10% contamination) by minimum information about a metagenome-assembled genome (MIMAG) standards are showed.

| L5/6A  | MAG Id | CMPL  | CNTM  | #UM | Taxonomy                                                                 |
|--------|--------|-------|-------|-----|--------------------------------------------------------------------------|
| MAG.1  | 74.45  | 2.2   | 29    |     | k__Bacteria                                                              |
| MAG.10 | 62.28  | 1.3   | 33    |     | k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae |
| MAG.11 | 98.62  | 1.13  | 43    |     | k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae |
| MAG.13 | 93.23  | 7.54  | 43    |     | k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadales;g__Luteimonas |
| MAG.14 | 83.26  | 5.79  | 42    |     | k__Bacteria;p__Deinococcus-Thermus;c__Deinococcic;o__Deinococciales;f__Deinococcaceae |
| MAG.15 | 60.55  | 9.12  | 11    |     | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Paracoccus |
| MAG.17 | 85.16  | 7.57  | 41    |     | k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__Sphingobacterium |
| MAG.18 | 99.14  | 1.19  | 43    |     | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales |
| MAG.2  | 57.23  | 5.17  | 35    |     | k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cyclobacteriaceae |
| MAG.4  | 66.22  | 6.43  | 17    |     | k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__Sphingobacterium |
| MAG.6  | 53.38  | 4.7   | 24    |     | k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudomonosporaceae |
| MAG.8  | 89.47  | 6.36  | 42    |     | k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae |

| L2A    | MAG Id | CMPL  | CNTM  | #UM | Taxonomy                                                                 |
|--------|--------|-------|-------|-----|--------------------------------------------------------------------------|
| MAG.10 | 74.89  | 2.62  | 35    |     | k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae |
| MAG.11 | 97.88  | 1.19  | 43    |     | k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales |
| MAG.12 | 98.81  | 4.88  | 40    |     | k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__Sphingobacterium |
| MAG.13 | 85.16  | 3.58  | 39    |     | k__Bacteria;p__Deinococcus-Thermus;c__Deinococcic;o__Deinococciales;f__Deinococcaceae |
| MAG.14 | 86.51  | 2.28  | 36    |     | k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae |
| MAG.15 | 85.2   | 4.45  | 39    |     | k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadales;g__Luteimonas |

CMPL: completeness; CNTM: contamination; #UM: # unique markers.
The EBI analysis pipeline offers functional analysis for predicted protein coding sequences in metagenomic data sets using InterProScan [31] and Gene Ontology [32] terms. In our study, functional annotation has been performed using the WGS raw reads, because the low number of reads sequenced did not allow for comprehensive functional annotation using MAGs; all the results were reported as relative abundance percentage (Figure 7). Both composts showed almost the same level of annotation for all the Gene Ontology categories. In particular, the metabolic process, biosynthetic process, nitrogen compound metabolic process, and transport were the most abundant categories for biological process category. Meanwhile, catalytic activity, oxidoreductase activity, ion binding, and nucleic binding were the most abundant for molecular function category.
Figure 7. Summary of Gene Ontology (GO) terms derived from InterPro matches for both composts. The heights of the bars represent the annotated reads (relative abundance, %) found for each functional category.
4. Discussion

Green waste composting is a sustainable practice to transform a by-product into a useful product. In our study, the on-farm composting produced two composts with appreciable content of nitrogen.

Suppressive activity against soil-borne plant diseases is considered an added value for composts and organic amendments [4]. In the present study, suppressive tests showed that only one of the two on-farm composts, rocket and fennel-derived L5/6A, was highly suppressive and able to reduce significantly the incidence of cress damping-off caused by *R. solani*, as compared to the control plots. The same compost also showed the same suppressive behaviour against Sclerotinia disease (Figure 1A).

The reason why L5/6A was able to suppress both diseases may be due to the different organisms that it contains, which may produce different mechanisms of suppressivity [33]. In this regard, microbiota composition has been well described to be one the main factor in compost for control of plant diseases [34]. L5/6A contained greater relative abundance of Bacteriodetes, Proteobacteria and Actinobacteria, all of them have been well documented to be correlated with plant disease suppression [33,35].

We found large numbers of Ascomycota and Basidiomycota sequences in both composts. In previous studies, fungal populations have been reported as the main contributors to the biological suppressiveness of compost [36]. During the composting process, usually bacterial populations decrease, due to the reduction of substrate quality, while the fungal community increases [37]. Moreover, the incorporation of wood scrap wastes may have led to the development of fungi associated with hardwood compost, as was previously reported by Neher et al. [8]. Unfortunately, the lower variability of the 18S rRNA gene, did not provide detailed resolution for the fungal community. Further investigation using an alternative approach targeting the internally transcribed spacer (ITS) regions, may provide finer-grained analysis; however, this was outside of the scope of this study.

Looking at bacterial genera, previous studies have reported that *Sphingobacterium, Parapedobacter, Nocardiopsis, Flavobacterium,* and *Truepera,* play a role in the decomposition of complex organic matter, such as starch and cellulose [38–41]. These were some of the most abundant genera identified in both composts, supporting the idea of their involvement in the composting processes and in breaking down complex organic compounds.

Actinobacteria are widely studied for their applicability in biocontrol [42,43]. In L5/6A compost *Nocardiopsis,* a genus belonging to the Actinobacteria phylum, was significantly more abundant than in L2A (1.17% in L5/6A compost and 3.8 Log2FC). This genus has been reported to produce several secondary metabolites, such as antimicrobial and antifungal compounds [44]. Several species belonging to the *Pseudomonas* genus (1% in L5/6A compost and 0.95 Log2FC) also possess plant fungi antagonistic activities [45,46]. In L5/6A compost, both genera were present and were more abundant than in L2A compost: therefore, taking into account their well described role in plant disease control, they could be involved in the higher suppression activity of this compost against *Rizoctonia solani* and *Sclerotinia minor* found in this study. In L5/6A compost, *Galbibacter, Truper,* and *Luteimonas* genera were relative quite represented and more abundant than in L2A compost too. Potentially, they could be involved in the higher suppression activity of this compost, even though they do not have a well described suppressive role in literature, as occur for the two previous mentioned genera.

*Streptomyces* spp. are well known to influence soil fertility through the involvement of many components. Some streptomycetes are reported to show a plant growth-promoting activity in their host plants [47]. Dochhil et al. [48] described two *Streptomyces* spp. strains, showing plant growth-promoting activity and a higher percentage of seed germination due to the synthesis of indole acetic acid. On the other hand, species belonging to the *Flavobacterium* genus have been positively correlated with increased plant biomass and stimulation [49,50]. Moreover, some members of the *Flavobacterium* genus can synthesize plant-growth hormones [51]. *Flavobacterium* is the most abundant genus reported in compost L2A (3.15% and 0.5 Log2FC) and, together with *Streptomyces* genus (2.56% and
2.3 Log2FC), is relatively more abundant than in L5/6A compost. Therefore, these genera could be potentially involved in the higher germination activity reported for the L2A compost on cress seed germination.

Finally, the combined use of NGS technologies and biochemical methods for characterizing microbial actives, gave us an unparalleled opportunity to study compost microbiota composition. The functional comparison using two different approaches, one biochemical using BIOLOG Ecoplates™ and the other based on NGS sequencing, showed different results. The higher metabolic activity showed by the BIOLOG Ecoplates™ in L5/6A, where microbial communities were able to break down easily degradable substrates, as well as complex matrices, quicker than L2A, is partially confirmed by the functional analysis for Gene Ontology categories. Therefore, although the two compost microbiomes were characterized by the same functional annotations, only the L5/6 compost showed a higher metabolic activity, probably due to different environmental conditions (pH, EC, or nutrient contents) present in both composts.

5. Conclusions

This work shows that an on-farm composting system is able to produce green waste composts characterized by an antagonistic microbiota community able to suppress plant pathogens and biostimulate plant growth.

Although composts are characterized by abundant microbial communities, not all of them are able to control plant disease or to biostimulate in the same way. These differences are correlated to both the chemical and microbiota composition. In this paper, we investigated the microbial composition of two on-farm composts and correlated their microbiota with suppressive activity. Our results suggest a role for *Nocardiopsis* and *Pseudomonas* genera in suppression, while *Flavobacterium* and *Streptomyces* genera are potentially involved in biostimulation.

Finally, combining different techniques, including omics approaches (NGS sequencing), to characterize composts, gave an overview of the complexity of compost microbial communities and their role in suppressiveness. Nevertheless, further study will be necessary to clarify the role of each microorganism and of microbial networking in the compost activities.

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