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Composts Obtained by Mixing Hop Leaves with Wheat Straw or Farmyard Manure Improved Soil Properties and Increased Microbial Communities

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Abstract: Hop (*Humulus lupulus* L.) leaves are rich in nutrients, particularly nitrogen (N). After harvest, they can be recycled through composting for use as a soil amendment. In this study, we report the effect of composts obtained from mixtures of hop leaves with other organic materials (wheat straw, farmyard manure, and ash from hop stems) at different ratios on soil properties and microbial diversity. Data on total N, total organic carbon (TOC), microbial N (Mic-N), microbial C (Mic-C), soil basal respiration (SBR), metabolic quotient (qCO_2), Mic-C/TOC ratio, acid phosphatase activity (APA), microbial density, and species identification were assessed after each one of the two growing seasons of potted lettuce (*Lactuca sativa* L.). The diversity of microbial species was evaluated using Simpson and Shannon diversity indexes, and the interactions between soil properties and the microbial community were explored. Higher microbial activity was found among the soils amended with leaves plus straw (HS), which exhibited higher levels of TOC, APA, Mic-N, and total N in the first growing cycle and higher levels of Mic-C, Mic-C/TOC, SBR, TOC, and Mic-N in the second growing cycle. Fungi identified belong to the Ascomycota and Zygomycota phyla, while bacteria belong to the Actinobacteria, Bacillota, Bacteroidetes, Firmicutes, and Proteobacteria phyla. Differences in the prevalent microbial genera were observed between compost treatments and growing cycles. Correlation analysis revealed significant relationship between soil bacteria and fungi abundance and higher levels of N and C in the soils, indicating the relevance of specific microbial genera, such *Acrostalagmus*, *Doratomyces*, *Talaromyces*, and *Aspergillus* fungi, as well as *Gordonia* and *Bacillus* bacteria. Overall, the results indicate that hop leaves-based compost, particularly with a higher proportion of leaves and straw, influenced the composition of the soil microbial community, ultimately enhancing soil N availability for plant development.

Keywords: *Humulus lupulus*; waste reconversion; soil quality; microbial profile; nutrient cycling

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1. Introduction

The reconversion and valorisation of agricultural waste present significant challenges at the present time. In the case of hops, a crop that is destined mostly for the brewing industry [1,2], a considerable quantity of organic residues is produced every year all over the world. The main world producers are Germany and the United States, though commercial production is spread over many countries [3,4]. Recently, the craft brewing movement expanded the interest in hop production to new emerging areas of production such as Italy and Brazil [5–7]. The increasing interest in hop production also increased

the interest in valuing the waste resulting from hop harvest [8,9] and from the brewing industry [10,11].

Composting is a process that allows the management of agricultural waste in a sustainable way, decreasing the negative environmental impact. The organic waste is transformed into composts that can be used as soil amendments [12,13]. The benefits of the application of these organic materials on soil quality result from the increase in soil available nutrients, soil organic matter, and beneficial microorganisms [14,15].

Soil biochemical and biological parameters such as microbial biomass C and N, soil respiration, enzyme activity, and mineralizable N are widely used to assess the effects of composts on soil properties. These parameters have the sensitivity to reflect slight modifications that soil can undergo after the application of an organic substrate [16–18]. Organic amendments are usually rich in organic C and N, which promote microbial activity and growth [19–21]. Microbial biomass represents the living component of soil organic matter, while microbial respiration reflects the basic turnover rates, and both respond rapidly to changes in soil management [16,21]. The decomposition of complex organic compounds through microorganisms requires the activity of enzymes, which will be reflected in the mineralization rates [18,22]. Enzyme phosphatases, for instance, are involved in the process of mineralizing of phosphate, increasing the availability of phosphorus (P), an important nutrient for soil microbes' growth, which consequently influences C decomposition and cycling [23,24]. The mineralization of N is also carried out through microorganisms, and the rate at which this occurs depends on the content of C and N in organic residues [24,25].

The application of organic substrates to the soil not only influences microbial activity and growth but can also lead to changes in microbial diversity and in the dominance of functional groups. Soil properties such as nutrient content, pH, total N, organic C, and C/N ratio are major factors affecting soil microbial community composition [26–30]. These changes will in turn be reflected in the quality of the soil [31–33]. Pospíšilová et al. [34] showed that the application of organic amendments resulted in chemical and structural changes in the humic substances of soil organic matter.

Hop waste resulting from hop harvest is mostly leaves and stems since the flowers (cones) are the most valuable commercial part of the plant. The hop plants are able to grow nearly 7 m high, producing a large quantity of biomass. In the hop fields of the north-eastern of Portugal, Afonso et al. [35] measured an average dry matter yield of 14.4 t ha^{-1}. In addition, this considerable amount of biomass is very rich in nutrients, particularly N [35]. Therefore, the composts obtained from hop leaves may have adequate levels of nutrients and organic C to improve soil quality. For the present study, we hypothesised that hop leaves-based composts may improve soil properties and enhance soil microbial biomass and its diversity. Thus, the main purpose of the study was to evaluate the effect of composts obtained through suitable mixtures of hop leaves with other local organic resources (wheat straw, farmyard manure, and ash from hop stems) on soil properties and microbial composition from a pot experiment with lettuce carried out during two growing seasons. The relationships between soil properties and the microbial community among the different compost treatments were also explored. The results of the agronomic behaviour of lettuce were already reported in a previous paper [8].

2. Materials and Methods

2.1. Experimental Trials

The composts were obtained after 9 months of composting of hop leaves mixed with other raw materials (wheat straw, farmyard manure, and ash from hop stems) at different ratios (Table 1). The composting process included seven domestic composters that were prepared with mixtures of hop leaves and cow manure and hop leaves and wheat straw at different ratios. Additionally, one of the compost mixtures included hop stem ash. The hop leaves used as raw materials originated from the hop harvesting process and resulted from the separation of cones. The ash also originated from the hop harvest, resulting from the burning of hop stems, a common practice among hop farmers. The cow manure and wheat

straw were obtained from local farmers and employed without further processing. The raw materials were introduced into the composters in intercalated layers at predetermined rates to achieve different C/N ratios (Table 1). The process was regularly monitored, including the physical turning of piles, temperature recording, and watering when necessary to ensure adequate moisture. The final composts obtained were used in a pot experiment with lettuce, cv. Wonder of Summer, carried out outdoors under a tile roof during two growing cycles (June–August 2018; April–July 2019). The experiment included nine fertilised treatments, an untreated control (C), and four replicates per treatment. Each pot (~3 L) was filled with 3 kg of dry and sieved soil (2 mm), sampled from the 0–0.20 m layer of a fallow plot. The initial soil had a sandy clay loam texture (soil separates, 23.9% clay, 21.8% silt, and 54.3% sand), a pH (H_2O) of 6.51, organic C (Walkley–Black method) of 5.2 g kg^{-1}, total N (Kjeldahl) of 1.28 g kg^{-1} and extractable P and K (Egner–Riehm method) of 47.8 mg P_2O_5 kg^{-1} and 53.3 mg K_2O kg^{-1}. The composts were previously mixed with the soil at rates of 20 t ha^{-1} (dry weight basis) (D1) and 40 t ha^{-1} (D2), considering that the dry mass of the <2 mm soil fraction of the arable layer (0.2 m) is 2240 t ha^{-1}. The final compost samples were collected in triplicate and subjected to analysis to determine total organic C (Walkley–Black method) and N (Kjeldahl method) for the calculation of the C/N ratio. All the composts were applied at the single (D1) rate, and the composts with the highest and lowest C/N ratios were also applied at the double rate (D2) (Table 1). The pots were watered regularly to maintain optimal moisture levels for plant growth. The amount of water applied varied over time and between pots, since evapotranspiration and the need for watering depend on the development stage of the plants. In the second cycle, the soils were maintained within each pot and were the same soils used in the first cycle, with no additional compost added.

Table 1. Compost mixtures, respective mixture ratios, carbon/nitrogen (C/N) ratio, soil application rate, and treatment abbreviation.

Compost Mixtures	Ratio	C/N	Soil Application Rate	Abbreviation
Hop leaves + cow manure (HM)	1:5	11.58	20 t ha^{-1} dry weight (D1)	HM1:5D1
Hop leaves + cow manure (HM)	1:3	12.03	20 t ha^{-1} dry weight (D1)	HM1:3D1
Hop leaves + cow manure (HM)	1:1	10.53	20 t ha^{-1} dry weight (D1)	HM1:1D1
Hop leaves + wheat straw (HS)	1:2	27.75	20 t ha^{-1} dry weight (D1)	HS1:2D1
Hop leaves + wheat straw (HS)	1:1	21.95	20 t ha^{-1} dry weight (D1)	HS1:1D1
Hop leaves + wheat straw + hop stems ash (HSA)	1:1:0.04	25.29	20 t ha^{-1} dry weight (D1)	HSA1:1:0.04D1
Hop leaves + wheat straw (HS)	1:0.5	15.79	20 t ha^{-1} dry weight (D1)	HS1:0.5D1
Hop leaves + wheat straw (HS)	1:2	27.75	40 t ha^{-1} dry weight (D2)	HS1:2D2
Hop leaves + wheat straw (HS)	1:0.5	15.79	40 t ha^{-1} dry weight (D2)	HS1:0.5D2

2.2. Soil Analysis

Soil samples were collected in each pot after the lettuce harvest in the two growing seasons and were split into two subsamples. One subsample was frozen in fresh and used for microbial analysis. The other subsample was oven-dried at 40 °C (Memmert UFE 800-Germany) and used for the determination of total organic C (TOC), total N, and acid phosphatase activity (APA). TOC was determined by dry combustion (Thermolyne 6000 furnace- Germany) and total N by the Kjeldahl method (FOSS Kjeltec^{TM} 8400). APA was determined by measuring p-nitrophenol released from 1 g of soil after incubation with 0.2 mL toluene, 4 mL MVB (Modified Universal Buffer) adjusted to pH 6.5, and 1-mL *p*-nitrophenylphosphate, at 37 °C for 1 h [36] (T80 + UV/VIS spectrometer, PG Instruments-Leicestershire, UK).

Microbial analyses were performed in three replicates for each treatment. Soil microbial biomass C (Mic-C) and N (Mic-N) were determined using the chloroform fumigation–extraction method [37]. The soil samples, adjusted to 60% water holding capacity (WHC), were weighed (20 g) in bottles, fumigated directly with 1 mL of ethanol-free chloroform, and incubated in the dark for 24 h at 25 °C. The extraction from fumigate and non-fumigated

samples was performed with 0.5 M K_2SO_4 (1:4, soil/solution). The extracts were frozen until further analysis. The Mic-C and Mic-N were calculated using a KEC (non-extractable biomass N fraction) factor of 0.33 [38] and a KEN (extractable biomass N fraction) factor of 0.54 [39], respectively. All results are expressed on a dry weight (105 °C) basis. Soil basal respiration (SBR) was measured, according to Pereira et al. [40], in a 7-day laboratory incubation. Briefly, a moist sample (25 g) adjusted to 60% WHC was placed in a 1.5 L glass jar with a plastic vial containing 20 mL of NaOH (1M) solution. After incubation at 25 °C for 1, 3, and 7 days, $BaCl_2$ (1.5 M) solution was added to a plastic vial and then titrated with 2M HCl until the colour changed from pinkie to transparent. Four jars without soil served as controls. SBR was calculated as the accumulative CO_2-C released from the soil during 7 days' laboratory incubation. The metabolic quotient (qCO_2) was calculated as the ratio of respiration (mg C-CO_2 mg^{-1} d^{-1}) to Mic-C. The reagents currently in use in the above-mentioned analyses are PanReac p.a.

The densities of cultivable bacteria and fungi were estimated in soil samples by using a standard dilution-plating procedure on agar media. The bacteria were quantified on plate count agar (PCA, Liofilchem, Roseto degli Abruzzi Italy) supplemented with cycloheximide (100 mg L^{-1}; Sigma-Aldrich, Milan, Italy) to suppress fungal growth and incubated at 30 °C for 3 days. Fungi were quantified using rose bengal agar medium (RBA, Liofilchem, Italy), and the plates were incubated at 25 °C for 5 days. Actinomycetes were quantified using Difco™ Actinomycete isolation agar medium, supplemented with cycloheximide (100 µg mL^{-1}) and nalidixic acid (10 µg mL^{-1}), and incubated at 30 °C for 4 to 10 days. After incubation, the colonies were counted as colony-forming units (cfu). Results were expressed as log cfu g^{-1} of dry soil. Selected colonies were then inoculated onto yeast malt extract agar (MEA, Liofilchem, Italy) in the case of fungi and plate count agar (PCA, Liofilchem, Italy) in the case of bacteria, for purification, and stored in 30% glycerol at −18 °C.

2.3. DNA Extraction, PCR Amplification, and Sequences Analysis

Total genomic DNA from bacteria was extracted using the REDExtract-N-Amp™ Blood PCR Kit (Sigma-Aldrich), following the manufacturer's instructions. Total genomic DNA from fungi was extracted according to Martins et al. [41]. The extractions were performed on the soil samples collected after the harvest of lettuce from the first and second growing cycles.

PCR amplification of the 16S rDNA gene was performed using the primer pairs 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 518R (5′-ATTACCGCGGCTGCTGG-3′) for the V3 region [42], and the primer pairs 338F and 797R (5′-GCGTGGACTACCAGGGTATCTAATCC-3′) for the V3-V4 regions of the gene [43]. PCR amplification of the 18S rDNA for the eukaryota ITS1 region was performed using the primer pairs ITS5F (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS1R (5′-GCTGCGTTCTTCATCGATGC-3′) [26]. The PCR mixture was prepared in a 50 µL volume containing 100 ng of DNA extract, 0.4 µM of each primer, 0.2 mM of each dNTP, 2 U of Taq polymerase, 5X PCR buffer (Promega), 3 mM of $MgCl_2$, 400 µg of bovine serum albumin, and adjusted to the final volume with sterile deionized water. The PCR amplification of 16S rDNA was performed as follows: initial denaturation step of 3 min at 98 °C, followed by 30 cycles (95 °C for 30 s, 57 °C for 45 s, and 72 °C for 50 s), with a final elongation step of 8 min at 72 °C, for primers 338F-518R; initial denaturation step of 2 min at 95 °C, followed by 30 cycles (95 °C for 30 s, 60 °C for 30 s, and 72 °C for 50 s), with a final elongation step of 5 min at 72 °C, for primers 338F-797R. The 18S rDNA PCR programme was carried out with an initial denaturation step at 98 °C for 2 min, followed by 30 cycles (98 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s), and a final elongation step at 72 °C for 5 min. Amplified PCR products were checked by gel electrophoresis on a 1% agarose gel. PCR amplifications were first extracted from a 0.8% *w*/*v* agarose gel and then purified with the Geneclean II Kit (MP Biomedicals). The DNA was quantified by a nano-spectrophotometer (mySPEC Twin 732-2535, VWR, Darmstadt, Germany). The DNA was sequenced with the Sanger method using an automatic sequencer, ABI Prism 377™ (Salamanca University, Salamanca, Spain), that performed the electrophoretic separation and detection of the

fluorescence-labelled DNA fragments. Four different fluorescence colours identify the four didexoxynucleotides incorporated in the extension reaction (ATCG).

2.4. Data Analysis

Data were subject to analysis of variance to identify significant differences between treatments, according to the experimental design, with a one-way ANOVA using the SPSS v. 25.0 program. When significant differences were found among experimental treatments, the means were separated by the Tukey–Kramer HSD test ($\alpha = 0.05$). The analysis of DNA sequences was carried out through the BioEdit programme, and the sequence identity was determined by a BLAST and FASTA nucleotide search from the NCBI and EMBL databases, respectively. It was chosen the closest match sequence, considering the identification percent, score bits, and expected value. The diversity indexes, Simpson and Shannon, were determined using PAST (paleontological statistics software package) version 2 [44], and the data were subjected to analysis of variance according to the procedure described above.

3. Results

3.1. Soil Chemical and Microbiological Evaluation

After the first lettuce cycle, it was found that the composts prepared with a higher proportion of leaves increased the total N and TOC in the soil, particularly the HS2:1D2 treatment, which corresponds to a double rate of leaves (Table 2). The mixture of leaves plus straw tended to increase Mic-N compared to the mixture of leaves plus manure, particularly when a high proportion of leaves (HS2:1D1) was used, which was also reflected in the treatment applied at a double rate (HS2:1D2). Mic-C was particularly high in the treatment in which an equivalent amount of leaves and straw (HS1:1D1) was used and was much lower in all the other treatments. SBR did not vary significantly between treatments. The metabolic coefficient showed very high values in the control treatment and very low values in the HS1:1D1 treatment. The opposite was observed with the Mic-C/TOC ratio. The highest average value of APA was observed in the HS2:1D2 treatment, which was significantly higher than those of the other treatments, except for that of the HS1:2D2 treatment. After the second plant cycle, all values related to the microbial activity of the soil maintained, in some way, the trends between treatments observed in the previous cycle, but the differences faded in a general way (Table 2). SBR was the variable that appeared out of the standard. In the first cycle, there were no significant differences between treatments, while in the second cycle, treatments with a double rate of compost (HS1:2D2 and HS2:1D2) showed significantly higher values than the other treatments.

Table 2. Total nitrogen (N), total organic carbon (TOC), microbial nitrogen (Mic-N), microbial carbon (Mic-C), soil basal respiration (SBR; cumulative respiration during a 7-day incubation), metabolic quotient (qCO^2), Mic-C/TOC ratio and acid phosphatase activity (APA) from soil samples collected in August 2018 after the harvest of lettuce of the first and second growing cycle (HM, hop leaves+cow manure at the ratios 1:5, 1:3, and 1:1; HS, hop leaves + wheat straw at the ratios 1:2, 1:1, and 2:1; and HSA, HS + ash from hop stems at the ratios 1:1:0.04; D1 and D2, 20 and 40 kg dw ha^{-1}).

Treatment	Total N	TOC	Mic-N	Mic-C	SBR	qCO_2	Mic-C/TOC	APA
	-------- g kg^{-1} -------		---------- mg kg^{-1} ----------			mg CO_2-C g^{-1}	mg g^{-1}	µg PNP g^{-1}
2018								
Control	0.94 c	22.0 e	15.5 c	55.1 b	84.8 a	92.3 a	2.5 b	195.6 bc
HM1:5D1	1.50 b	21.9 e	32.6 bc	257.8 b	127.6 a	23.1 ab	11.9 b	193.6 bc
HM1:3D1	1.32 bc	23.8 de	36.7 abc	98.6 b	86.8 a	39.5 ab	4.1 b	193.2 bc
HM1:1D1	1.77 b	21.0 e	27.4 bc	262.5 b	91.2 a	14.5 b	12.9 ab	199.5 bc
HS1:2D1	1.30 bc	26.6 cd	43.9 abc	321.7 b	95.1 a	22.2 ab	12.1 ab	188.4 c
HS1:1D1	1.47 b	30.4 b	40.1 abc	776.2 a	92.7 a	5.1 b	26.5 a	198.6 bc
HS2:1D1	1.68 b	30.7 b	67.3 ab	425.6 ab	93.6 a	12.7 b	13.8 ab	207.6 bc
HSA1:1:0.04D1	1.60 b	29.7 bc	51.8 abc	210.5 b	97.4 a	21.0 ab	7.2 b	217.7 bc
HS1:2D2	1.56 b	32.3 ab	49.0 abc	280.6 b	107.5 a	16.1 b	8.7 b	243.6 ab

Table 2. *Cont.*

Treatment	Total N	TOC	Mic-N	Mic-C	SBR	qCO_2	Mic-C/TOC	APA
	-------- g kg^{-1} -------		---------- mg kg^{-1} ----------			mg CO_2-C g^{-1}	mg g^{-1}	µg PNP g^{-1}
HS2:1D2	2.34 a	35.4 a	74.8 a	178.8 b	108.0 a	29.4 ab	5.0 b	276.1 a
Prob. > F	<0.0001	<0.0001	0.0017	0.0004	0.2713	0.0309	0.0007	0.0125
Standard error	0.06	0.92	3.80	41.59	3.65	5.85	1.43	6.35
2019								
Control	1.31 f	23.0 d	24.4 d	78.3 c	56.8 b	117.1 a	3.4 bc	173.8 b
HM1:5D1	1.52 de	27.6 bc	49.4 bcd	113.4 c	55.7 b	72.3 a	4.1 bc	189.9 b
HM1:3D1	1.59 cde	26.9 c	27.3 cde	72.4 c	50.3 b	114.0 a	2.7 c	183.1 b
HM1:1D1	1.55 e	27.7 bc	31.4 cde	123.9 c	54.3 b	65.7 a	4.5 bd	195.6 ab
HS1:2D1	1.52 e	28.2 bc	45.4 bcde	127.1 c	59.9 b	68.6 a	4.5 bc	199.6 ab
HS1:1D1	1.64 bcd	28.3 bc	37.8 bcde	135.3 c	51.2 b	55.7 a	4.8 bc	192.4 b
HS2:1D1	1.69 bc	28. bc	54.3 abc	120.3 c	56.0 b	67.6 a	4.3 bc	186.8 b
HSA1:1:0.04D1	1.48 e	27.3 c	44.4 bcde	137.1 bc	56.0 b	66.4 a	5.0 bc	189.0 b
HS1:2D2	1.73 b	30.1 ab	60.0 ab	262.0 a	121.1 a	67.3 a	8.7 a	244.8 a
HS2:1D2	2.08 a	31.9 a	73.3 a	204.6 ab	150.1 a	106.6 a	6.4 ab	186.9 b
Prob. > F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0534	<0.0001	0.0084
Standard error	0.03	0.38	2.69	9.48	19.76	5.54	0.290	4.16

Means followed by the same letter are not statistically different by Tukey HSD test ($\alpha = 0.05$).

3.2. Microbial Community Composition and Species Identification

3.2.1. Effect of Compost Treatments on Soil Microbial Composition

The numbers of culturable heterotrophic bacteria differed significantly between compost treatments in the soils collected after the first growing cycle, while significant differences were not found for the culturable actinomycetes population and fungi. The average soil fungi population was higher in the treatments with leaves plus straw applied at the double rate (HS1:2D2 and HS2:1D2) and in the treatment with the same proportion of leaves plus straw (HS1:1D1). The lowest average value was found in the control treatment. The average soil bacteria population was higher in the treatment with a higher leaves proportion plus straw and a single rate of application (HS2:1D1). The average soil actinomycetes population did not show significant differences between treatments. The average population of fungi and bacteria in the soils collected after the second growing cycle was higher in the treatment with a double rate of leaves and a double rate of application (HS2:1D2). The average soil actinomycetes population was higher in the treatment with leaves plus manure with a higher proportion of manure (HM1:5D1). The lower average levels for the fungi population were found in the treatments with leaves plus manure and the control treatment. The lower average levels of bacteria soil population were registered in the control and HM treatments, which were also significantly lower than the HS2:1D2 treatment. The differences between treatments were not significant for the actinomycetes soil population. The proportion of actinomycetes in relation to total bacteria was the lowest in the treatments with leaves plus straw applied at the double rate of application (HS1:2D2 and HS2:1D2) in the soils collected after the first cycle. In the soils collected after the second cycle, the proportion of actinomycetes in relation to total bacteria was significantly lower in the HS2:1D2 treatment in comparison with the HM1:1D1 and control treatments (Table 3).

Table 3. Effect of different compost treatments on microbial populations of the soils samples collected in August 2018 and July 2019 after the harvest of lettuce of the first and second growing cycle, respectively (HM, hop leaves + cow manure at the ratios 1:5, 1:3, and 1:1; HS, hop leaves + wheat straw at the ratios 1:2, 1:1, and 2:1; and HSA, HS+ash from hop stems at the ratios 1:1:0.04; D1 and D2, 20 and 40 kg dw ha^{-1}).

	Treatment	Fungi	Bacteria	Actinomycetes	Act./Bacteria (%)
		---------- [log (cfu g^{-1})] ----------			
2018	Control	4.52 a	7.04 ab	6.78 a	56.06 a
	HM1:5D1	5.01 a	7.39 ab	7.13 a	56.20 a
	HM1:3D1	5.08 a	7.00 b	6.61 a	43.08 a
	HM1:1D1	5.00 a	7.23 ab	6.92 a	52.78 a
	HS1:2D1	4.93 a	7.14 ab	6.74 a	45.80 a
	HS1:1D1	5.11 a	7.12 ab	6.85 a	54.95 a
	HS2:1D1	5.03 a	7.44 a	7.11 a	46.85 a
	HSA1:1:0.04D1	4.90 a	7.25 ab	7.02 a	61.60 a
	HS1:2D2	5.11 a	7.21 ab	6.69 a	30.45 a
	HS2:1D2	5.11 a	7.23 ab	6.72 a	30.89 a
	Prob. > F	0.0733	0.0353	0.0701	0.3741
	Standard error	0.25	0.18	0.24	17.57
2019	Control	4.44 bcde	6.63 c	6.31 a	48.89 a
	HM1:5D1	4.24 de	6.88 bc	6.49 a	42.39 ab
	HM1:3D1	4.05 e	6.77 bc	6.29 a	33.77 ab
	HM1:1D1	4.32 cde	6.86 bc	6.47 a	50.45 a
	HS1:2D1	4.52 abcde	6.99 abc	5.97 a	13.86 ab
	HS1:1D1	4.83 abc	7.06 abc	6.39 a	22.96 ab
	HS2:1D1	4.88 ab	7.15 ab	6.26 a	14.48 ab
	HSA1:1:0.04D1	4.62 abcd	7.05 abc	6.18 a	13.65 ab
	HS1:2D2	4.75 abcd	7.14 ab	6.24 a	13.01 ab
	HS2:1D2	5.00 a	7.32 a	6.25 a	8.96 b
	Prob. > F	>0.0001	0.0010	0.6545	0.0029
	Standard error	0.33	0.23	0.29	19.15

Means followed by the same letter are not statistically different by Tukey HSD test ($\alpha = 0.05$).

3.2.2. Correlation Analysis between Microbial Population and Soil Properties

The relation between microbial population and soil properties was analysed through Spearman's correlation coefficient. Positive and significant correlations were identified for the bacteria and actinomycetes populations in the soils collected after the first growing cycle (2018). The abundance of bacteria and actinomycetes was positive and significantly correlated with the levels of total N (Table 4). Correlation results were more expressive in relation to the soils collected after the second growing cycle (2019). The abundance of fungi and bacteria was positively and significantly correlated with total N, TOC, Mic-N, and Mic-C. Significant correlations were not found for the actinomycetes population.

Table 4. Spearman's correlation coefficients between microbial communities and soil properties— total nitrogen (N), total organic carbon (TOC), microbial nitrogen (Mic-N), microbial carbon (Mic-C), soil basal respiration (SBR; average daily respiration), metabolic quotient (qCO_2), and acid phosphatase activity (APA)—of the soil samples collected in August 2018 and July 2019, after the harvest of lettuce of the first and second growing cycle, respectively.

	Total N	**TOC**	**Mic-N**	**Mic-C**	**SBR**	**qCO_2**	**APA**
	---------- g kg^{-1} --------		**---------- mg kg^{-1} ----------**			**mg CO_2-C g^{-1}**	**µg PNP g^{-1}**
2018							
Fungi	0.344	0.264	0.118	0.346	0.058	−0.345	0.174
Bacteria	0.524 **	0.161	0.317	0.317	0.261	−0.237	0.246
Actinomycetes	0.394 *	−0.090	0.225	0.303	0.183	−0.207	0.106
2019							
Fungi	0.494 **	0.509 **	0.463 *	0.555 **	0.161	−0.202	0.105
Bacteria	0.534 **	0.486 **	0.588 **	0.574 **	0.154	−0.314	0.084
Actinomycetes	0.159	−0.064	−0.158	−0.153	−0.056	0.086	−0.113

Correlation are significant at the <0.05 level * and 0.01 level **.

3.2.3. Microbial Species Identification

A total of 10 fungi colonies and 23 bacteria colonies were identified from the isolates obtained from the soil samples collected after the harvest of lettuce in the first and second growing cycles. Between the fungi colonies isolated, the species identified were found to belong to two phyla, namely Ascomycota and Zygomycota. Between the bacteria colonies isolated, the species identified were found to belong to five different phyla, namely Actinobacteria, Bacillota, Bacteroidetes, Firmicutes, and Proteobacteria (Figure 1).

In the soil samples collected after the first growing cycle, among the fungi communities, Ascomycota was the predominant phyla in all treatments. The genus *Aspergillus* and *Mortierella*, respectively, from the Ascomycota and Zygomycota phylum's, were the most abundant in the control and HM treatments. The dominance among genera varied depending on the treatment. In this regard, *Aspergillus* was prevalent in the control treatment, exhibiting the highest average values among treatments (38.46%), while *Mortierella* had the highest rate of abundance in the HM1:3D1 treatment (26.03%). The predominant genera in the treatments with leaves plus straw (HS) were *Acrostalagmus*, *Aspergillus*, *Doratomyces*, and *Mortierella*. Among these groups, the higher average values were associated with the treatments applied at a double rate, particularly for *Doratomyces* (45.62%) and *Acrostalagmus* (31.86%), respectively, in the treatments with a higher proportion of straw (HS1:2D2) and a higher proportion of leaves (HS2:1D2). Among bacterial communities found in the soil samples collected after the first growing cycle, Actinobacteria and Firmicutes were the predominant phyla in all treatments (Figure 1). For most treatments, both phyla shared the dominance with variations, though Firmicutes clearly dominated in HS treatments applied at a double rate. Between these phyla, the main genera were *Arthrobacter* and *Bacillus*, respectively, from Actinobacteria and Firmicutes. The higher average values found for *Arthrobacter* were recorded in the control treatment (58.87%) and the lower in the treatment with a higher leaves proportion plus straw at a double rate of application (HS2:1D2; 25.32%). Conversely, the higher average value found for *Bacillus* was recorded in the HS2:1D2 treatment (62.25%) and the lower in the control treatment (33.59%).

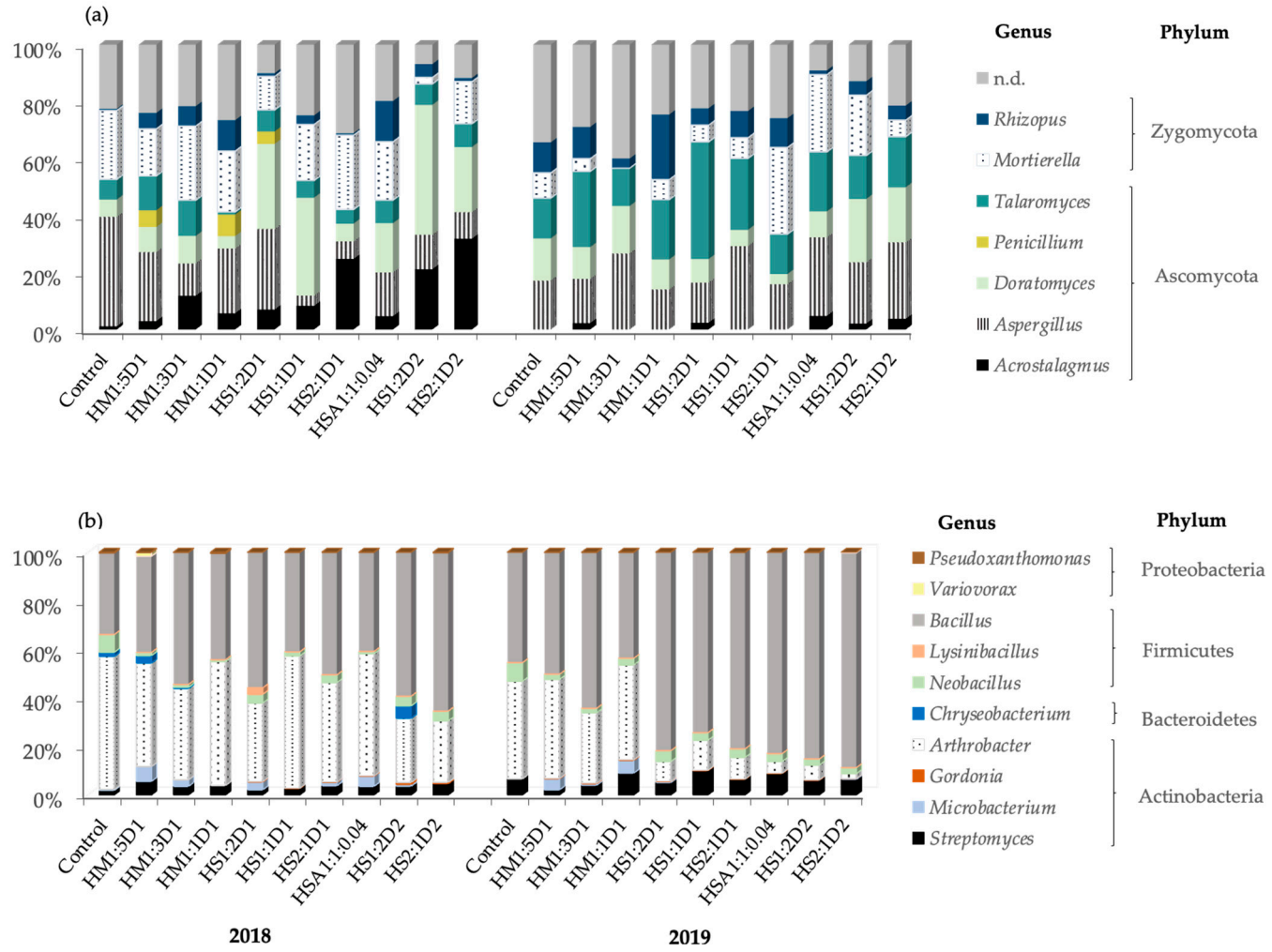

Figure 1. Fungi (**a**) and bacteria (**b**) community composition at the genus and phylum level in the soils samples collected in August 2018 and July 2019 after the harvest of lettuce of the first and second growing cycle, respectively (HM, hop leaves + cow manure at the ratios 1:5, 1:3, and 1:1; HS, hop leaves + wheat straw at the ratios 1:2, 1:1, and 2:1; and HSA, HS+ash from hop stems at the ratios 1:1:0.04; D1 and D2, 20 and 40 kg dw ha^{-1}).

In soil samples collected after the second growing cycle, among the fungi communities, Ascomycota continued to be the predominant phylum in all treatments, but no clear dominance was found between the different genera of this phylum. The higher average values were found for the genus *Talaromyces* (41.18%) and *Mortierella* (30.41%), respectively, in the treatment with a higher proportion of straw (HS1:2D1) and in the treatment with a higher proportion of leaves (HS2:1D1), both at a single rate of application. Between bacteria communities, Firmicutes continued to be the dominant phyla on the soil samples collected after the second growing cycle in all treatments. The genus *Bacillus* belonging to the Firmicutes phylum was the dominant genus in all treatments, and markedly in the treatments with leaves plus straw (HS). The higher average value for *Bacillus* (88.56%) was recorded in the treatment with a higher proportion of leaves plus straw at a double rate of application (HS2:1D2), and the lower value was recorded in the control treatment (45.53%), highlighting the trend found in the first growing cycle.

Between the fungi species identified, six species belong to the Ascomycota phylum (*Acrostalagmus luteoalbus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Cephalotrichum asperulum*, *Penicillium restrictum*, and *Talaromyces pinophilus*), and two species belong to the Zygomycota phylum (*Mortierella alpina* and *Rhizopus arrhizus)*. The majority of the bacterial species identified belong to the Actinobacteria phylum (six species) and Firmicutes phylum

(nine species). *Streptomyces* and *Bacillus* were the predominant species identified, respectively, in the Actinobacteria and Firmicutes phylums. The other species identified were *Neobacillus endophyticus* (Bacillota phylum), *Chryseobacterium* sp. (Bacteroidetes phylum), *Pseudoxanthomonas* sp., and *Variovorax guangxiensis* (Proteobacteria phylum).

3.2.4. Correlation Analysis between Microbial Taxonomic Distribution and Soil Nitrogen and Organic Carbon Content

The abundance of fungi and bacteria genus, soil total N, and TOC were analysed by Spearmen's correlation coefficients to determine their relationships (Table 5). Positive and significant correlations were identified for some fungi and bacteria taxa in the soils collected after the first growing cycle (2018). The abundance of *Acrostalagmus* and *Doratomyce*s Ascomycota fungi was significantly and positively correlated with soil TOC (r = 0.544 and 0.547, respectively; $p < 0.01$). Significant and positive correlations were also found between the abundance of *Acrostalagmus* and total N (r = 0.390; $p < 0.05$). Between bacteria communities, the abundance of *Gordonia* Actinobacteria was also significantly and positively correlated with soil TOC (r = 0.627; $p < 0.01$). Positive and significant correlations were also found for different fungi and bacteria taxa in relation to the soils collected after the second growing cycle (2019). The abundance of *Aspergillus* and *Talaromyces* fungi and of *Streptomyces* and *Bacillus* bacteria was shown to be positively and significantly correlated with both soil indicators, total N and TOC, at $p < 0.05$ and $p < 0.01$.

Table 5. Spearman's correlation coefficients between fungi and bacteria Genus taxa level and soil total nitrogen (N) and total organic carbon (TOC) of the soil samples collected in August 2018 and July 2019, after the harvest of lettuce of the first and second growing cycle, respectively.

Phylum	Genus	2018		2019	
		Total N	TOC	Total N	TOC
		---------- g kg^{-1} --------		---------- g kg^{-1} --------	
Fungi					
Ascomycota	*Acrostalagmus*	0.390 *	0.544 **	0.091	0.263
Ascomycota	*Aspergillus*	−0.146	−0.361	0.420 *	0.460 *
Ascomycota	*Doratomyces*	0.235	0.547 **	0.226	0.197
Ascomycota	*Penicillium*	−0.118	−0.323	n.d.	n.d.
Ascomycota	*Talaromyces*	0.110	0.206	0.413 *	0.432 *
Zygomycota	*Mortierella*	0.167	−0.067	0.195	0.222
Zygomycota	*Rhizopus*	0.126	−0.175	0.249	0.184
	n.d.	0.222	−0.155	0.318	0.266
Bacteria					
Actinobacteria	*Arthrobacter*	0.332	−0.150	−0.091	−0.308
Actinobacteria	*Gordonia*	0.319	0.627 **	0.034	0.117
Actinobacteria	*Microbacterium*	−0.147	−0.085	0.093	0.185
Actinobacteria	*Streptomyces*	0.304	0.243	0.460 *	0.478 **
Bacteroidetes	*Chryseobacterium*	−0.138	−0.224	n.d.	n.d.
Firmicutes	*Bacillus*	0.389 *	0.308	0.541 **	0.524 **
Firmicutes	*Lysinibacillus*	−0.236	−0.032	n.d.	n.d.
Firmicutes	*Neobacillus*	0.136	0.339	−0.071	0.017
Proteobacteria	*Pseudoxanthomonas*	0.112	−0.262	0.082	0.064
Proteobacteria	*Variovorax*	0,118	−0.182	0.291	0.290

Correlation are significant at the <0.05 level * and 0.01 level **: n.d. (fungi/bacteria not present in soil samples collected in 2019).

3.2.5. Diversity Indices of Soil Fungi and Bacteria Communities

The diversity of fungi and bacteria among the compost treatments was estimated by Simpson and Shannon indexes, which indicated, respectively, the evenness and richness of the communities. As shown in Figure 2, significant differences were not found among fungi and bacterial communities in the soils collected after the first growing cycle and after the second growing cycle. However, a more consistent trend was found in the treatments

with leaves plus straw at the double rate of application (HS1:2D2 and HS2:1D2). Overall, these treatments presented the highest and lowest average index values and in the opposite direction when comparing fungi and bacteria results. For instance, in the soils collected after the first growing cycle, the higher Simpson's index average registered between bacteria communities (0.783), indicating lower species evenness, was found in the treatment with the higher proportion of straw and at the double rate of application (HS1:2D2). Conversely, the lower Simpson's index average registered between fungi communities (0.697), indicating higher species evenness, was found in the same treatment. Similarly, in the soils collected after the second growing cycle, the higher and lower average values of both indexes were found in the treatment with a higher proportion of leaves plus straw and at the double rate of application (HS2:1D2). The average values recorded in that treatment indicated lower evenness (Simpson index, 0.814) and higher richness (Shannon index, 1.82) of species between the fungal communities, whereas higher evenness and lower species richness were observed among the bacterial communities (Simpson index, 0.473; Shannon index, 1.0.6).

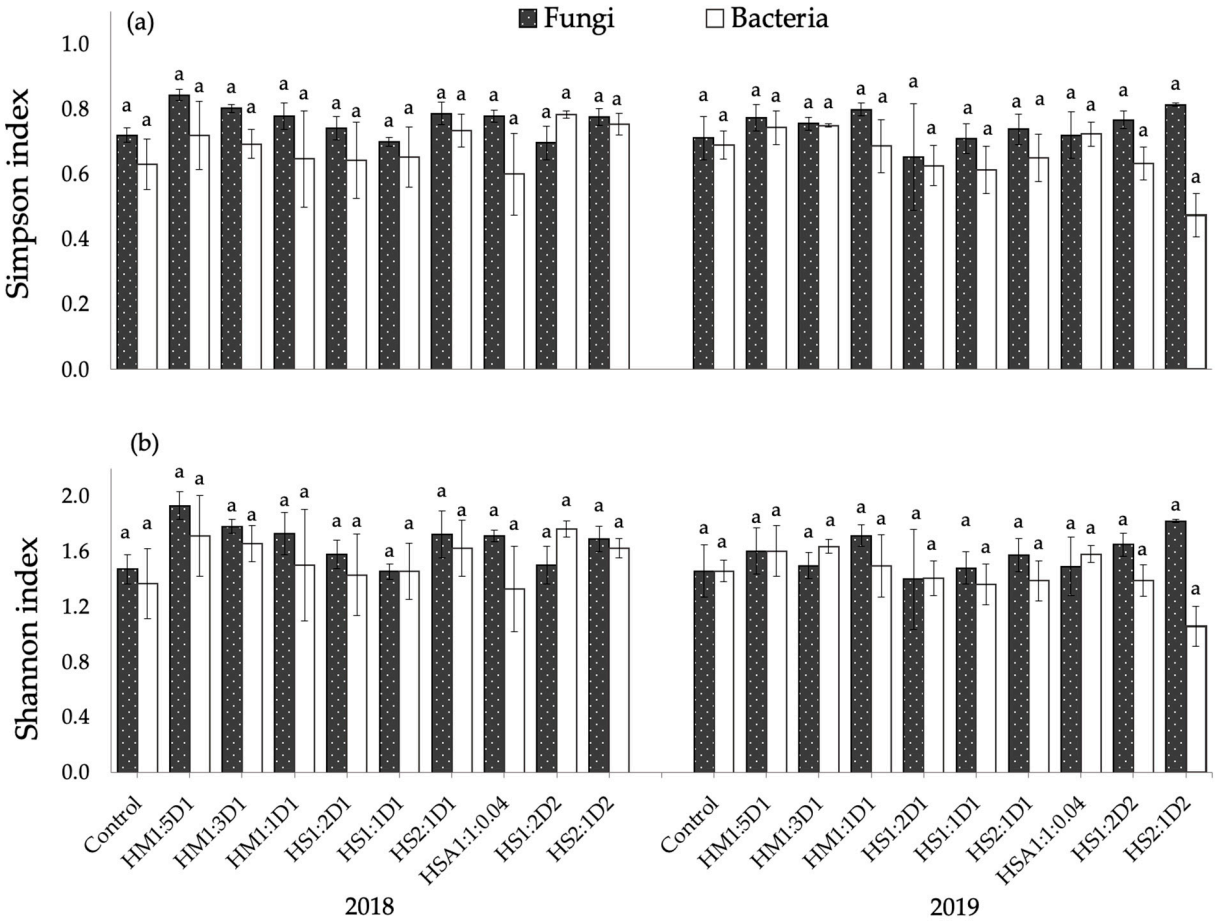

Figure 2. Simpson (**a**) and Shannon (**b**) diversity indexes of fungi and bacteria species identified in the soils samples collected in August 2018 and July 2019 after the harvest of lettuce of the first and second growing cycle, respectively (HM, hop leaves + cow manure at the ratios 1:5, 1:3, and 1:1; HS, hop leaves + wheat straw at the ratios 1:2, 1:1, and 2:1; and HSA, HS + ash from hop stems at the ratios 1:1:0.04; D1 and D2, 20 and 40 kg dw ha^{-1}). Means followed by the same letter are not statistically different by Tukey HSD test ($\alpha = 0.05$).

4. Discussion

The effect of composts on soil properties differed between treatments and more clearly between growing cycles. In the soils collected after the first growing cycle, the lower levels of TOC and Mic-N in the treatments with leaves plus manure (HM) indicate a higher

stabilisation of OM due to the high maturity of HM compost. On the contrary, some of the treatments with leaves plus straw (HS), in particular with high straw proportions, presented high levels of Mic-C and Mic-C/TOC, which suggests a high availability of C for microbial biomass production [45]. The microbial biomass C is a soil quality indicator, and the increase in soils due to organic manure application has been reported in other studies [46–48]. The treatment leaves plus straw with a higher leaves proportion at the double rate of application (HS2:1D2) differentiated from all due to the high levels of TOC, APA, Mic-N, and total N, suggesting an intense activity of microorganisms in the degradation of OM. Thus, the higher availability of organic C likely stimulates phosphatase enzyme activity (APA) due to P demand by microorganisms [18]. At the same time, the degradation of OM by microorganisms resulted in high mineralization and immobilisation of N by microbe biomass, increasing N availability (total N and Mic-N) for plant uptake.

In the soils collected after the second growing cycle, the treatments with the lower and higher straw proportions at the double rate of application, respectively, HS2:1D2 and HS1:2D2, diverge from all due to the high levels of microbiologic activity (Mic-C, Mic-C/TOC, SBR, TOC, Mic-N) and higher availability of total N. As previously discussed, the higher levels of OM, particularly easily degradable OM (since in the second year the composts in soil were more degraded), increased microorganisms' activity, as reported in other studies [46,49]. On the opposite, the high levels of qCO_2 in the control treatment in both growing cycles, along with the lower levels of Mic-C/TOC, indicate a high maintenance energy requirement and a low efficiency in the use of energy for microbial growth [45]. Probably this was due to the lower levels of OM and thus lower organic C availability, resulting in less microbial N and C biomass. In the comparison between the treatments with ash (HSA1:1:0.04D1) and without (HS1:1D1), the addition of ash resulted in lower C availability for microbial growth (lower levels of Mic-C and Mic-C/TOC) and higher availability of N (higher levels of Mic-N and total N), suggesting an enhanced effect on the mineralization rate. It is known that under laboratory conditions, the growth of ammonia-oxidising microorganisms is inhibited at pH values less than 6.5 [50]. The buffering effect of ash may have increased nitrifying microorganisms' growth, thus increasing the nitrification rate.

Concerning the effect of composts on soil microbial composition, in the soils collected after the first growing cycle, no significant differences between treatments were observed for soil fungi and actinomycetes. However, for soil bacteria, the treatments with leaves plus straw (HS2:1D1) exhibited the highest average (7.44 log cfu g^{-1}), while the treatments with leaves plus manure (HM1:3D1) showed the lowest (7.00 log cfu g^{-1}), and the differences were statistically significant. When straw-based composts were incorporated into the soil, they were less mature than manure-based composts, thereby promoting the continuation of the decomposition process [8]. The high prevalence of bacteria in the soil may be associated with the decomposition of leaves and straw from immature composts, as bacteria tend to dominate when large amounts of organic carbohydrates are available [51].

The effect of composts on soil microbial composition was more pronounced in the second growing cycle for bacteria and fungi, revealing significant differences between treatments. However, no significant differences were observed for soil actinomycetes. The HS treatments exhibited a higher abundance of soil fungi and bacteria compared to the HM and control treatments, with the highest average values recorded in the HS2:1D2 treatments, respectively, of 5.00 log cfu g^{-1} and 7.32 log cfu g^{-1}. Overall, the results indicate that HS treatments, especially those with higher proportions of leaves, fostered a greater abundance of fungi and bacteria. This outcome could be attributed to the higher concentration of nutrients, particularly N in leaves, along with a higher concentration of C in straw, which likely promoted the growth of bacteria and fungi [52,53]. The results of the correlation analysis between microbial populations and soil properties support this hypothesis, as they revealed a significant relationship between the abundance of soil bacteria and soil fungi with higher levels of N and C in the soils. This result aligns with the findings of Afonso et al. [8], suggesting a period of N immobilization in the first growing cycle followed by a

period of net mineralization in the second growing cycle. The activity of bacteria and fungi is closely associated with the rates of soil N transformation [53]. The abundance of C and N in the soil strongly influences the growth of bacteria and fungi, driving the response of heterotrophic nitrification [52,53].

The fungi colonies isolated from the soil samples collected after the harvest of lettuce from the first and second growing cycles were identified as belonging to the Ascomycota and Zygomycota phyla. The bacteria colonies included Actinobacteria, Bacillota, Bacteroidetes, Firmicutes, and Proteobacteria phyla. In the soil samples collected after the first growing cycle, the dominant fungus phylum was Ascomycota. Ascomycota are prominent decomposers among fungi and have been identified as major phyla in soils treated with sewage sludge compost [54]. Ma et al. [55] also recognised Ascomycota members as dominant during various stages of fungal succession while decomposing straw residues in arable soil. Voříšková and Baldrian [56] studied fungal communities during litter decomposition and found Ascomycota to be the most abundant during the early stages of this process. These findings align with our results. The genera *Acrostalagmus, Aspergillus*, and *Doratomyces* from the Ascomycota phylum, along with *Mortierella* from the Zygomycota phylum, were the most prevalent in the HS treatments. Within this group, *Doratomyces*, followed by *Acrostalagmus*, showed higher abundance in the treatments with leaves plus straw applied at a double rate (HS1:2D2; HS2:1D2). On the other hand, the genus *Aspergillus* (Ascomycota) was most abundant in the control treatment, while *Mortierella* (Zygomycota) was most abundant in the HM treatments. *Acrostalagmus*, *Aspergillus*, and *Mortierella* are commonly found in soils [24]. In accordance with our findings, *Acrostalagmus* and *Doratomyces* genera were identified during the composting of organic material [57]. *Aspergillus* is known for its role in phosphate and potassium solubilization in the soil [24,58]. Some *Aspergillus* species, such as *Aspergillus niger*, are commonly used in bioleaching for heavy metal removal [59], and can enhance the mineralization of lignocellulose [60,61]. *Mortierella*, on the other hand, was found to be associated with systems exclusively receiving organic fertilisers such as farmyard manure and slurry [28]. This genus is known to promote plant growth and exhibit high cellulose and hemicellulose degradation activity [62]. Hence, it aligns with the observation that these genera were abundant in the soil samples, particularly in the treatments of leaves plus straw, with *Mortierella* also being the most abundant in the treatments of leaves plus manure. The correlation analysis revealed that the abundance of *Acrostalagmus* and *Doratomyces* fungi correlated with high levels of organic C, and for *Acrostalagmus*, it was also related to high levels of N, likely indicating their role in straw degradation and N cycling. However, further information is needed to fully understand the specific roles of these genera in the decomposition process.

Regarding bacteria, in the soil samples collected after the first growing cycle, Actinobacteria and Firmicutes were the most abundant phyla across all the treatments. This is consistent with other studies that reported these phyla as abundant in various types of organic waste compost [54,63,64]. Among the different genera, *Arthrobacter* (Actinobacteria) and *Bacillus* (Firmicutes) were the most prevalent in all the treatments. These genera are commonly found in soil and are known to colonise the rhizoplane, promoting plant growth [24]. The highest rate of *Arthrobacter* was found in the control treatment (58.87%), and the lowest was found in the treatment of leaves plus straw, with a higher proportion of leaves at a double rate (HS2:1D2: 25.32%). Conversely, *Bacillus* abundance was highest in the treatments of leaves plus straw applied at a double rate (HS2:1D2: 62.5% > HS1:2D2: 59.3%) and lowest in the control treatment (33.59%). *Arthrobacter* bacteria are typically abundant in mature compost [57], which may explain their highest presence in the control treatment, where OM was more stabilized. Regarding *Bacillus* species, they possess the ability for N fixation [65], P and K solubilisation [58], and the capability to degrade cellulose and starch [66]. Hence, it is reasonable that they were found at higher rates in the treatments of leaves plus straw at a double rate. The correlation analysis indicated that the abundance of the genus *Gordonia*, which belongs to the phylum Actinobacteria, was related to high levels of organic C. Although this genus had low rates in all treatments, the highest levels were

found in the treatments of leaves plus straw at a double rate, particularly with higher levels of straw (HS1:2D2: 0.76%; HS2:1D2: 0.54%). *Gordonia* species are known to contribute to soil quality improvement and plant growth promotion. They produce several enzymes and are capable of degrading lignin [67], which may explain their higher presence in treatments with higher proportions of straw and leaves.

In the soil samples collected after the second growing cycle, Ascomycota remained the predominant fungus phylum across all treatments. Similarly, Tan et al. [61] found Ascomycota to be the dominant phylum throughout all stages of wheat straw composting. Although there was no clear dominance between fungi genera, higher rates were found for the *Talaromyces* (41.18%) and *Mortierella* (30.41%) genera in the treatments of leaves plus straw, with higher proportions of straw (HS1:2D1) and leaves (HS2:D1), respectively. Our findings align with those of Xu et al. [68], who demonstrated that *Talaromyces* is a crucial fungus in promoting the degradation of lignin and humification during lignocellulosic waste composting. *Mortierella*, along with *Talaromyces*, likely played a relevant role in the decomposition of these subtracts, maybe related to the degradation of cellulose or hemicellulose [62]. The correlation analysis revealed a connection between the abundance of *Talaromyces* and *Aspergillus* with high levels of organic C and N in the soil. Talaromyces species, including *T. pinophilus* identified in this study, are frequently employed in phytoremediation and can enhance nutrient availability in soil with organic amendments [69]. Microorganisms can utilise lignin as a source of C, contributing to an increase in organic C availability in the soil [70]. This process seems to be influenced by interactions between lignin decomposition products (microbial biomass and necromass) and soil minerals [71]. As previously stated, *Aspergillus* can mineralize metals and lignocellulose [59,61]. Therefore, both genera most likely played a role in lignin breakdown and contributed to the increase in soil organic C and N.

Regarding bacteria, in the soil samples collected after the second growing cycle, Firmicutes emerged as the most abundant phylum across all treatments, with *Bacillus* being the dominant genus, particularly in the treatments with leaves plus straw. These findings may be related to the role of Firmicutes, especially *Bacillus*, in the degradation of cellulose rich materials such as straw [66,72] as discussed earlier. Moreover, Firmicutes tend to dominate during the late stage of cellulose degradation, operating at high ammonia levels [72]. This observation can explain the positive correlation found between *Bacillus*, the dominant genus within Firmicutes, and the concentration of organic C and N in the soil. Positive relationships between soil C and N were also observed for *Streptomyces*, a genus belonging to the Actinobacteria phylum. Actinobacteria, in general, and particularly *Streptomyces*, are known for their ability to decompose plant litter and more recalcitrant compounds like lignin. They are typically present during the final stages of decomposition [24,66].

The diversity indexes, Simpson and Shannon, which, respectively, estimated the evenness and richness of the fungi and bacteria communities, did not exhibit significant differences for soil microbe communities in either the first or second growing cycle. Conversely, other studies have reported changes in soil microbial communities after compost amendments, leading to improved microbial diversity [32,54]. However, a consistent trend was observed in the treatments of leaves plus straw, at the double rate of application. In the first growing cycle, the treatment with a higher proportion of straw exhibited high fungi species evenness and low bacterial species evenness. The distribution of fungi was less equitable, with a clear dominance of *Doratomyces* fungi, likely related to straw decomposition at early stages. Among bacteria, dominance was shared between *Arthrobacter* (Actinobacteria) and *Bacillus* (Firmicutes). In the second growing cycle, the treatments with a higher proportion of leaves plus straw showed lower evenness and higher richness of fungi, indicating a more balanced distribution among the taxa present. For bacteria, higher evenness and lower richness were observed, likely due to the clear dominance of *Bacillus* species and their importance in the decomposition of cellulose straw at late stages [66,72]. Furthermore, previous research with lettuce pots and field experiments in soil fertilised with different types of organic compost demonstrates that composts can

improve soil chemical, physical, and microbiological properties, ultimately promoting plant growth [73–75]. Likewise, the results of our short-term pot experiment also revealed that hop leaves-based composts have the potential to be used in crop fields to enhance soil fertility, soil microbiology, and plant growth while contributing to improved circular management of crop residue. However, further research with long-term field experiments is required to substantiate the preliminary findings of the current study.

5. Conclusions

The present study explored the effect of hop leaves-based compost on soil properties and microbial communities during two growing cycles of lettuce. Compost with leaves plus straw increased microbial abundance compared to leaves plus manure composts and control treatments. This was likely due to the increased N availability from leaves, C from straw, and the presence of degradable OM from immature compost. The treatment with leaves plus straw, especially during the first growing cycle, exhibited intense microbial activity and stimulated phosphatase enzyme activity, leading to substantial mineralisation and immobilisation of N by microbial biomass, ultimately enhancing N availability for plant uptake. The findings indicate that the higher availability of C and N in the soil played a crucial role in driving the growth and activity of bacteria and fungi, as well as influencing the dominance between microbial genera.

The changes in fungi and bacteria genera in response to compost treatments were more pronounced in the treatments with leaves plus straw, particularly during the first growing cycle when the decomposition process was more intense. The relationship found between soil properties and microbial communities revealed that the activity of bacteria and fungi was closely associated with the abundance of N and C in the soil, likely related to straw decomposition and N cycling at different stages of the growing cycles. The microbial genera found in these associations included *Acrostalagmus*, *Doratomyces*, *Talaromyces*, and *Aspergillus* fungi, as well as *Gordonia*, *Bacillus*, and *Streptomyces* bacteria. While significant effects on microbial diversity due to compost treatments were not observed, the dominance of certain genera, such as *Doratomyces* fungi in the first growing cycle and *Bacillus* bacteria in the second growing cycle, suggested their relevance in early-stage straw decomposition and decomposition of cellulose straw at later stages, respectively. However, further research is needed to uncover the specific functions and interactions of these microorganisms in the context of OM transformation.

Overall, our findings contribute to the understanding of compost-induced changes in soil microbial communities and underscore the importance of organic compost to promote soil health and crop productivity, suggesting the potential applicability of hop-leaves based compost in agricultural fields.

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