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## Valorization of kiwi waste through composting

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### ABSTRACT

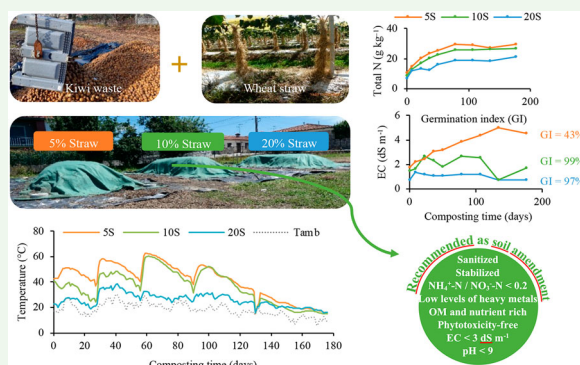
Kiwi waste from the calibration process is a major environmental problem of kiwi production due to landfill deposition. This work aims to contribute to the agronomic use of recycled kiwi waste through composting. With this objective, a composting experiment was carried out with kiwi fruit waste mixed with 5%, 10% and 20% (fresh weight) of wheat straw from bundles used to protect kiwifruit trunks from frost, as bulking agent to increase aeration, in the piles 5S, 10S and 20S, respectively. The highest temperatures for piles 5S and 10S were above 60°C, whereas the temperature did not reach 40°C in the pile with the highest straw content (20S) because the aeration increased heat loss in addition to increased C/N ratio of this pile. Also, the amount of organic matter mineralized decreased with increasing amount of straw because of the high C/N ratio of the straw. The highest total N (29.7 g kg<sup>-1</sup>) and the lowest C/N ratio (13) of the compost with 5% of straw is important from the agricultural point of view to promote N availability. In contrast, the high electrical conductivity (4.6 dS m<sup>-1</sup>) of this compost increases the risk of salt accumulation in the soil. Our results show that the compost with 10% straw, with high degree of maturation, absence of poor hygiene indicators as coliforms and pathogens as *Salmonella* sp., high organic matter content and rich in nutrients, together with the adequate compost pH and low electrical conductivity improves compost quality.

### ARTICLE HISTORY

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### KEYWORDS

Bacteria; C/N ratio; compost; fungi; phytotoxicity



## 1. Introduction

The kiwi production in Portugal has been increasing since 1970s due to the high acceptability of the kiwifruit by consumers and its market valorization [1] reaching in 2021 the production of 55,460 t of kiwifruits [2]. This involves the generation of a significant amount of kiwi-fruit waste from the calibration process. For example, the waste of the KiwiGreensun company is estimated at 5–6% of the total production. This percentage turns into approximately 3000 t of kiwi fruit waste in Portugal

each year, and the most usual process for the disposal of fruit waste is through landfill deposition which affects soil health and increases global warming due to the substantial methane and greenhouse gases emissions [3,4].

The kiwifruit is rich in vitamins, minerals and phenolic compounds that are considered great anti-oxidant and anti-inflammatory compounds that could be used for food, pharmaceutical and cosmetic industry, but the kiwi industry has not implemented strategies to valorize

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these compounds [5]. Alternatively, composting can be a suitable way to reintroduce the kiwi waste into the soil according to the principles of circular economy. The compost application allows to reduce the use of agrochemicals and their negative effects on the environment which are not only related to accumulation in the soil and leaching, but also result from the consumption of fossil energy to produce synthetic mineral fertilizers and to transport them from the factory to the field. The continuous application of mineral fertilizers contribute to decrease soil organic matter content and consequently the soil quality for the future generations [6]. Moreover, the successive application of N fertilizers increase the amount of mineral N available on soil increasing N<sub>2</sub>O emission and nitrate contamination in groundwater [7,8]. It is important to remark that the N losses are similar with synthetic N fertilizers and liquid manures such as slurry that are rapidly mineralized [8], whereas the application of compost, which mineralizes at slower rate, improves the synchrony between N mineralization and crop N demand, decreasing the risk of N losses [9]. The increasing interest in compost is also associated with climate change mitigation through C sequestration. The effectiveness of compost amendment to store carbon in soil has been underestimated. In a 9-year field experiment synthetic fertilizer caused a slight decrease in soil C content while compost application increased 30% soil organic matter content [10].

The organic matter (OM) degradation during the composting process depends on the feedstock composition [11,12]. The structure of the piles must provide adequate porosity to allow the oxygenation and preservation of moisture content in order to support aerobic decomposition and simultaneously minimize N losses, to reduce environmental atmospheric emissions and to retain N fertilizer value [13, 14]. Therefore, the reuse of kiwi fruit waste through composting requires a bulking agent to increase aeration of the composting piles in order to achieve effective composting [15]. The wheat straw from bundles used to protect kiwifruit trunks from frost offers adequate properties to promote aeration, such as low density, high porosity and high-water absorption capacity [16]. Therefore, the characteristics of the kiwi waste along with wheat straw indicate the possibility of obtaining a good quality compost. However, the composting process of kiwi waste and the potential of these composts as crop fertilizers has not been previously described in the literature. The experiment hypothesis was to develop a reliable compost with kiwi waste using a bulking agent, wheat straw, that is included in the cultural practices of kiwi production in order to avoid landfill deposition. With this purpose, the objective of this work was to study

the physical, chemical and microbiological parameters as well as the quality of the compost of kiwi waste with wheat straw during the composting process under field conditions to promote a circular approach.

## 2. Materials and methods

### 2.1. Composting experiment

Composting was carried out at Quinta das Picas (41°31'N; 8°27'W) owned by KiwiGreensun company located in the NW of Portugal. The feedstock materials used for the composting process included: (i) kiwifruit waste obtained from the kiwi calibration process, and (ii) wheat straw from bundles used to protect kiwifruit trunks from frost (Table 1). Three composting piles were built outdoors with alternate layers of kiwi and straw to approximately 1.6 m wide, 1.3 m high and 8 m long and different ratios of fresh weight kiwi: wheat straw (w:w), 95%:5%, 90%:10% and 80%:20% for the piles 5S, 10S and 20S, respectively. These percentages of fresh weight correspond to 26%, 43% and 63% of straw dry weight and 17%, 30% and 49% of straw in volume, respectively, considering that the straw has almost a dry weight content seven times higher than that of kiwis, and a density almost four times lower. The two materials were mixed with a backhoe loader and covered with TenCate Toptex® (GEOSIN) to avoid rainfall and simultaneously allow gas exchange. The composting process was monitored over a period of 176 days. During this period the piles were turned after 28, 56, 90 e 130 days from the beginning of the composting process with the backhoe loader.

Outdoor air temperature was automatically measured with a thermistor below a reflector board at 80 cm height and compost temperatures were measured also with thermistors placed in the core of three different parts of each pile. The temperatures were recorded every hour with a Data Logger DL2 from Delta Devices.

Losses of OM were calculated according to the Equation (1) [17].

$$\text{OM loss (g kg}^{-1}\text{)} = 1000 \times 1000 [x_1(1000 \cdot - \cdot x_2)] / [x_2(1000 \cdot - \cdot x_1)] \quad [1]$$

where  $x_1$  and  $x_2$  are the initial and final ash content (g kg<sup>-1</sup>), respectively.

A first-order kinetic model (Equation (2)) was fitted to OM mineralization determined by the OM lost [17].

$$\text{OM}_m = \text{OM}_0(1 - \cdot e^{-kt}) \quad [2]$$

where  $\text{OM}_m$  is the estimated mineralized OM (g kg<sup>-1</sup> DM) at time  $t$  (days),  $\text{OM}_0$  is the amount of potentially

**Table 1.** Density ( $\rho$ ), moisture content (MC) and chemical characteristics of kiwifruit waste and wheat straw.

|             | $\rho$ | H (%) | pH  | EC (dS m <sup>-1</sup> ) | OM (g kg <sup>-1</sup> ) | C/N | N (g kg <sup>-1</sup> ) | P (g kg <sup>-1</sup> ) | K (g kg <sup>-1</sup> ) |
|-------------|--------|-------|-----|--------------------------|--------------------------|-----|-------------------------|-------------------------|-------------------------|
| Kiwi waste  | 0.61   | 87    | 3.5 | 2.07                     | 915                      | 38  | 13.4                    | 1.7                     | 32.6                    |
| SD          | 0.02   | 1     | 0.1 | 0.11                     | 11                       | 4   | 1.4                     | 0.2                     | 6.2                     |
| Wheat straw | 0.16   | 12    | 5.6 | 0.39                     | 978                      | 203 | 2.7                     | 0.1                     | 7.8                     |
| SD          | 0.02   | 3     | 0.3 | 0.32                     | 2                        | 7   | 0.1                     | 0.1                     | 2.5                     |

Nutrient contents are expressed on a dry matter basis. SD = Standard deviation.

mineralizable OM, and  $k$  is the mineralization constant rate (day<sup>-1</sup>).

## 2.2 Germination test

The phytotoxicity potential was assessed by the determination of the germination index. The germination test was performed with five repetitions according to a modified procedure based on [18]. The compost extract (1:10, w/v) with 50 seeds of cress (*Lepidium sativum* L.) per treatment was incubated at 25°C for 96 h. The germination index was calculated by multiplying relative seed germination (%) and relative root growth (%).

## 2.3 Analytical methods

Dry matter content (DM), pH, electrical conductivity (EC) and OM content were determined by standard procedures [19]. The total N was determined using the modified Kjeldahl method based on a sulphuric acid digestion, using a Kjeldahl digestion unit (DK 20, VELP Scientifica) and a compact distillation unit (UDK 139, VELP Scientifica). The total organic C content in compost was estimated by dividing the OM by a factor of 1.8 [8]. Mineral N was extracted from 100 g of fresh compost with 1 M KCl 1:5 solution and determined by molecular absorption spectrophotometry using a continuous flow auto-analyser (SanPlus System, Skalar, Breda, the Netherlands). The total P was measured by UV visible spectrophotometry (UV visible espectro, Thermo Scientific) after digestion with sulphuric acid and the total K was determined by atomic spectrophotometry (Analyst 200, Perkin Elmer) after nitro-perchloric acid digestion.

## 2.4 Microbiological analysis

### 2.4.1 Collection of the microbial flora associated with wheat straw

Three samples of 10 g of wheat straw were soaked in 90 ml of 0.1% (w/v) Buffered Peptone Water (Merck, Darmstadt, Germany) containing 0.01% (w/v) Tween 80 (PanReac Applichem, Barcelona, Spain) for 30 min at room temperature. The samples were placed in an

orbital shaker (Certomat B. Braun, Melsungen, Germany) and agitated at 150 rpm. Subsequently, they were subjected to 30 s of ultrasounds at 40 kHz in an ultrasound bath (Soltec EP, Sonica, Milan, Italy). Afterwards, the suspension was centrifuged at 10,000 rpm for 10 min at 4°C, and the resulting pellet was kept at -80°C until used for DNA extraction.

### 2.4.2 Collection of the microbial flora associated with kiwi fruit

Three 10 g samples of kiwi fruit were placed in sterile stomacher bags with filters and diluted with 90 ml of 0.1% (w/v) Buffered Peptone Water containing 0.01% (w/v) Tween 80. The samples were homogenized using a stomacher (Laboratory Blender Stomacher 400; Seward, London, UK) for 1 min. After homogenization, the 10 ml of the suspension was centrifuged at 10,000 rpm for 10 min at 4°C. The resulting pellet obtained from the centrifugation step was kept at -80°C until used for DNA extraction.

### 2.4.3 Sampling and preparation of compost samples for DNA extraction

Compost from the three piles was sampled using a sterile 50 ml polypropylene tube. For maximal representativity, each sample was composed of ten sub-samples taken from different positions of each pile. The refrigerated compost samples were weighted (200 mg) and divided into three DNase-free 2.0 ml polypropylene tubes that were kept at -80°C until used for DNA extraction.

### 2.4.4 Extraction, purification and sequencing of microbial DNA

Total DNA was extracted from each sample, with three replicate tubes for each sample, using the DNeasy® PowerSoil® Pro-Kit (QIAGEN, Hilden, Germany). The extraction process followed the instructions provided by the manufacturer and involved using a bead beater (Benchmark Scientific, Sayreville, USA) at 4000 rpm for two cycles of 30 s, with at least 30 s of ice-cooling between the cycles. Subsequently, the purified DNA from the three independent sample extractions was combined and stored at -80°C for future downstream

procedures. The pooled DNA from each extraction was quantified using a UV spectroscopie (Nanodrop, manufactured by Thermo Fisher Scientific, located in Massachusetts, USA) and by fluorimetry (QUBIT 3.0, manufactured by Thermo Fisher Scientific, located in Massachusetts, USA). The 260/280 ratio was determined to assess the quality of the DNA samples, and gel electrophoresis was performed to evaluate any degradation present in the DNA. PCR amplification with the DNA from each sample was performed using the primers, 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGAC-TACNNGGTATCTAAT-3'), targeting from V3 to V4 hypervariable regions of the 16S rRNA gene for bacterial identification and the primers ITS5 (5'-GGAAG-TAAAAGTCGTAACAAGG-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC -3') for fungal DNA amplification [20]. The produced amplicons were then normalized, pooled, end-repaired, A-tailed, and ligated with Illumina adapters. The resulting libraries were sequenced on a paired-end Illumina platform at Novogene Company Limited (located in Cambridge, United Kingdom) to generate paired-end reads.

#### 2.4.5 Bioinformatics and sequencing results analysis

The paired-end reads were merged using FLASH (V1.2.7). The raw tags underwent quality filtering using the Qiime [21] and were compared with the reference SILVA138 Database [22] for rRNA sequence data, and Unite\_INSDC [23] for ITS sequence data, using the UCHIME algorithm [24] to identify and eliminate chimera sequences, and taxonomically annotated against the databases using Qiime and the Mothur method at each taxonomic rank. For sequence analysis, the UPARSE [25] software (Uparse v7.0.1090) was used, considering all the effective tags. Sequences with a similarity of  $\geq 97\%$  were assigned to the same Operational Taxonomic Units (OTUs). The abundance information of the OTUs was normalized based on the sequence number corresponding to the sample with the lowest number of sequences. Alpha diversity metrics for abundance and evenness, including Shannon index [26], observed features and Simpson diversity [27], were calculated using the normalized data.

#### 2.4.6 Nucleotide sequences accession number

Raw reads were deposited in the SRA database under BioProject PRJNA995337.

### 2.5 Statistical analyses

The OM mineralization data was fitted to the kinetic function by the Marquardt-Levenberg algorithm to

minimize the sum of the squared differences between the observed and predicted values of the dependent variable. Analysis of variance (ANOVA) was performed by the SPSS general linear model procedure. Statistical significance was indicated at a probability level of  $P = 0.05$  to compare means between treatments. All statistical calculations were performed using IBM SPSS Statistics v. 27.0 for windows (SPSS Inc.).

## 3. Results and discussion

### 3.1 Temperature

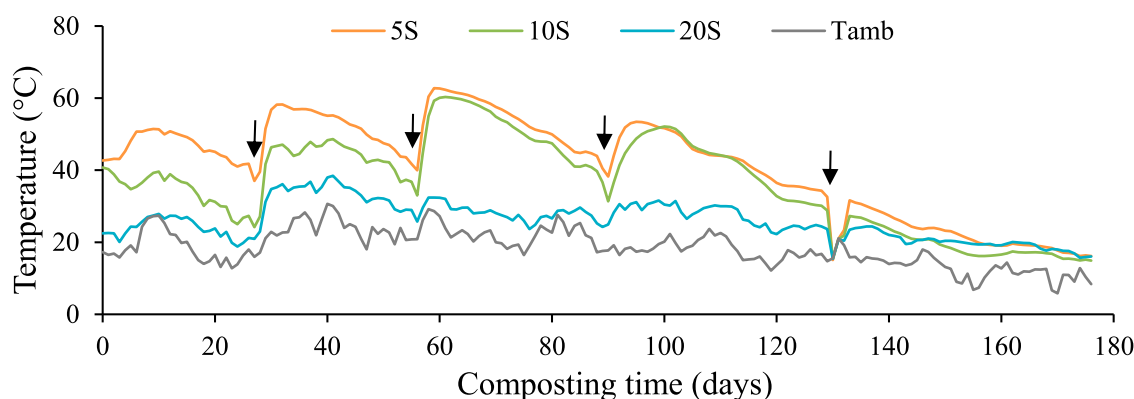
The active decomposition of organic compounds is an exothermic process that results in heat generation. The highest temperatures were registered 61 and 59 days after the start of composting for piles 5S (69.4°C) and 10S (67.5°C), respectively (Figure 1). In these piles, the temperature was above 55°C for a period of 28 days in pile 5S and for a period of 12 days in pile 10S. In contrast, the maximum temperature found for pile 20S, with a higher proportion of straw, was only 38.4°C. The lower temperature in the pile 20S was probably due to increased C/N ratio, but also to the increase in porosity that promoted heat dissipation. These results are similar to those reported by [28] who observed lower temperatures in the composting piles consisting of dairy cow manure and straw compared to dairy cow manure with sawdust, because the higher free air space in the compost with straw allowed greater heat loss. After the thermophilic phase, the temperature approached to ambient air temperature about 140 days after the commencement of the composting process.

An important characteristic for the use of compost in agriculture is its sanitary safety. According to the Portuguese mandatory guidelines [29], the sanitation requirements of temperature above 55°C at least 28 days, with a moisture content over 40% and piles turned 3 times during this period was only fulfilled for 5S. However, compliance with these requirements is more important in the presence of animal waste than for plant waste.

### 3.2 Moisture content

The appropriate moisture content for OM degradation depends on the structure of the pile, and a compromise must be made between the moisture content suitable for the microorganisms and the aeration required to allow aerobic decomposition. Here, the moisture content ranged between 69.6 and 82.5% (Table 2), which is above the maximum values between 60% and 70% suggested by [30] for maximum microbial activity.





**Figure 1.** Temperature during composting of kiwi waste with wheat straw mixed in the following proportions (w:w): pile 5S (95%:5%), pile 10S (90%:10%) and pile 20S (80%:20%). Tamb is the ambient air temperature. Arrows indicate pile turning events.

However, the amount of wheat straw added to the pile 5S (5% of the total fresh weight of the pile, corresponding to 17% of the volume and 26% of the dry weight) was enough to obtain a good structure with adequate porosity to support aerobic decomposition. Likewise, during the composting process with grape stalks and winery sludge, the amount of grape stalk in the proportion of sludge:stalk (1:1) was enough to enable oxygen diffusion to support microbial activity and temperature did not decline with the moisture content between 72% and 79% [31].

### 3.3 Ph and electrical conductivity

The pH value increased during the early days of composting (Table 2) due to the degradation of organic acids and  $\text{NH}_3\text{-N}$  production [32]. After 36 days of composting the pH value was alkaline and in the range 7.9–9.4. These conditions enhance the risk of  $\text{NH}_3$  volatilization during the thermophilic phase of composting because the high temperature and alkaline pH values

favour nitrogen volatilization, decreasing the fertilizer N value of the compost [33]. At the end of the composting process, the pH values in the piles 10S and 20S (8.8 and 7.8, respectively) were in the range acceptable for compost utilization as a soil organic amendment (5.5–9.0) [29], unlike the pH value in pile 5S (9.4).

High salt concentrations may cause phytotoxicity problems in seed germination and root growth. Therefore, the EC value is important to assess the agronomic value of the composts. As expected, the EC value decreased with the addition of straw (Table 2) because the greater amount of kiwi waste contributes to increase OM decomposition (Figure 2) and consequently release mineral salts such as phosphate and ammonium ions. The values of EC found in this experiment for the piles with higher proportion of straw 10S ( $1.7 \text{ dS m}^{-1}$ ) and 20S ( $0.7 \text{ dS m}^{-1}$ ) were below the maximum recommended value of  $3 \text{ dS m}^{-1}$  for compost application to soil [34]. In contrast, the EC value for the pile with the lowest proportion of straw 5S ( $4.6 \text{ dS m}^{-1}$ ) was over that limit. The application of the compost 5S ( $4.6$

**Table 2.** Moisture content, pH and electrical conductivity (EC) during composting of kiwi waste with wheat straw mixed in the following proportions (w:w): pile 5S (95%:5%), pile 10S (90%:10%) and pile 20S (80%:20%).

| Characteristic            | Pile | Sample day |      |      |      |      |      |      |      |      |
|---------------------------|------|------------|------|------|------|------|------|------|------|------|
|                           |      | 0          | 8    | 22   | 36   | 50   | 78   | 106  | 134  | 176  |
| MC (%)                    | 5S   | 82.2       | 82.5 | 82.0 | 80.2 | 79.4 | 76.6 | 72.1 | 70.3 | 72.1 |
|                           | 10S  | 80.7       | 80.9 | 78.4 | 81.5 | 81.1 | 78.0 | 78.5 | 82.7 | 82.7 |
|                           | 20S  | 79.1       | 77.0 | 69.6 | 74.6 | 77.7 | 80.3 | 76.1 | 82.7 | 84.6 |
|                           | LSD  | 2.3        | 4.2  | 6.0  | 4.5  | 3.0  | 3.4  | 9.0  | 3.5  | 1.0  |
| pH                        | 5S   | 3.7        | 4.3  | 4.1  | 7.9  | 8.1  | 8.7  | 8.9  | 8.9  | 9.4  |
|                           | 10S  | 3.8        | 4.6  | 6.4  | 7.9  | 8.5  | 8.7  | 8.6  | 7.6  | 8.8  |
|                           | 20S  | 3.6        | 4.4  | 7.5  | 7.9  | 8.4  | 8.0  | 7.6  | 7.6  | 7.8  |
|                           | LSD  | 0.2        | 0.2  | 1.8  | 0.5  | 0.7  | 0.2  | 0.4  | 0.2  | 0.3  |
| EC ( $\text{dS m}^{-1}$ ) | 5S   | 1.7        | 2.2  | 2.4  | 3.0  | 3.2  | 3.9  | 4.3  | 5.0  | 4.6  |
|                           | 10S  | 1.5        | 1.6  | 2.7  | 2.3  | 1.8  | 2.6  | 2.5  | 0.7  | 1.7  |
|                           | 20S  | 0.7        | 1.3  | 1.2  | 1.1  | 1.0  | 1.2  | 1.2  | 0.7  | 0.7  |
|                           | LSD  | 0.3        | 0.3  | 0.6  | 0.4  | 0.4  | 0.3  | 0.4  | 0.5  | 0.3  |

LSD = least significant difference ( $P < 0.05$ ) between mean values within columns for each characteristic.

dS m<sup>-1</sup>) with high EC promotes salt accumulation in soil that may affect the growth of plants in the future.

### 3.4 Organic matter degradation

The OM biodegradation was described by a first-order kinetic model (Figure 2). The easily biodegradable organic compounds were rapidly mineralized as the available C and N sources were used by the microorganisms during the thermophilic stage, followed by a slow decomposition of the recalcitrant fraction of OM. Here, the dry matter of kiwifruit consists mainly of compounds easily assimilated by microbes (80% carbohydrates and 5.7% protein) [35], whereas wheat straw is made up of substances resistant to microbial biodegradation (14–17% lignin, 37–38% cellulose and 28–36% hemicellulose) [36]. Thus, the largest amount of potentially mineralizable OM was found for 5S (816 g kg<sup>-1</sup>), and it decreased for 10S (720 g kg<sup>-1</sup>) and 20S (687 g kg<sup>-1</sup>) because the C/N ratio of wheat straw (203) is much higher compared to kiwi waste (38). However, the mineralization rate (day<sup>-1</sup>) increased for 10S ( $k=0.037$ ) and 20S ( $k=0.036$ ) with a higher proportion of straw compared to 5S ( $k=0.022$ ) because the straw improved the aeration conditions. These results are in agreement with those reported by [11], who showed that during the composting of cattle slurry solid fraction the OM mineralization rate increased for piles with straw or gorse, whereas the maximum amount of mineralizable OM decreased with the addition of these bulking agents, indicating that these materials had larger recalcitrant OM fractions compared to the cattle slurry solid fraction.

### 3.5 Total N content and C/N ratio

The total N content increased from initial values of 6.7–10.6 g kg<sup>-1</sup> to values between 21.2 and 29.7 g kg<sup>-1</sup>,

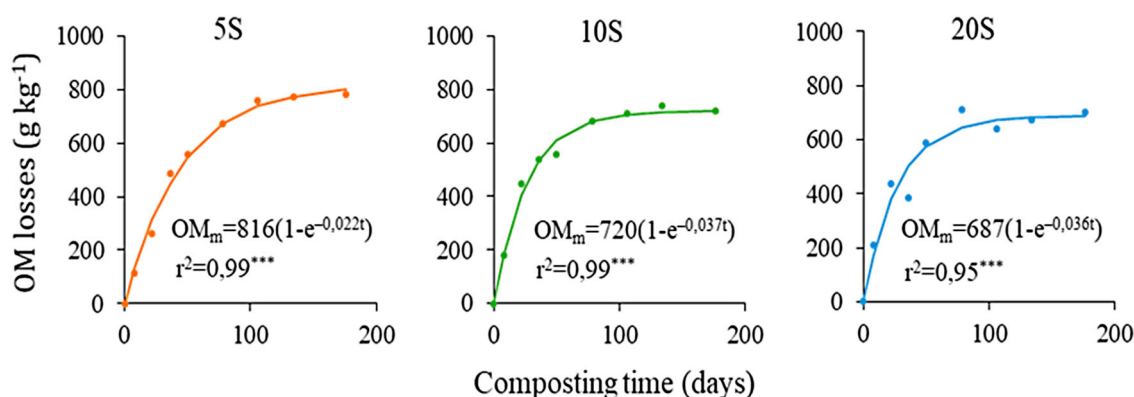
which is important from an agricultural perspective in terms of the N fertilizer value of compost, with the highest content being that of the compost 5S. The C/N ratio declined from values in the range of 49–80 at the start of composting to 13–22 in the final composts (Figure 3), and the ratio (final C/N) / (initial C/N) in the three composts (0.3) was smaller than the value suggested by [37] for matured composts (0.6). This is due to the fact that N losses by volatilization were relatively smaller compared to C losses mainly as carbon dioxide. The high C/N ratio at the beginning of the composting process promoted the retention of NH<sub>4</sub><sup>+</sup> released during the OM degradation and, therefore, contributed to decrease NH<sub>3</sub> volatilization [33]. The compost 5S with high N content (29.7 g kg<sup>-1</sup>) and low C/N ratio (13.5 g kg<sup>-1</sup>) < 15 probably promotes N availability and is a good fertilizer (Chadwick, 2000). In contrast, the compost 20S with C/N ratio > 15 generally provides less nutrients but contributes to the improvement of soil quality.

### 3.6 Microbiological composition

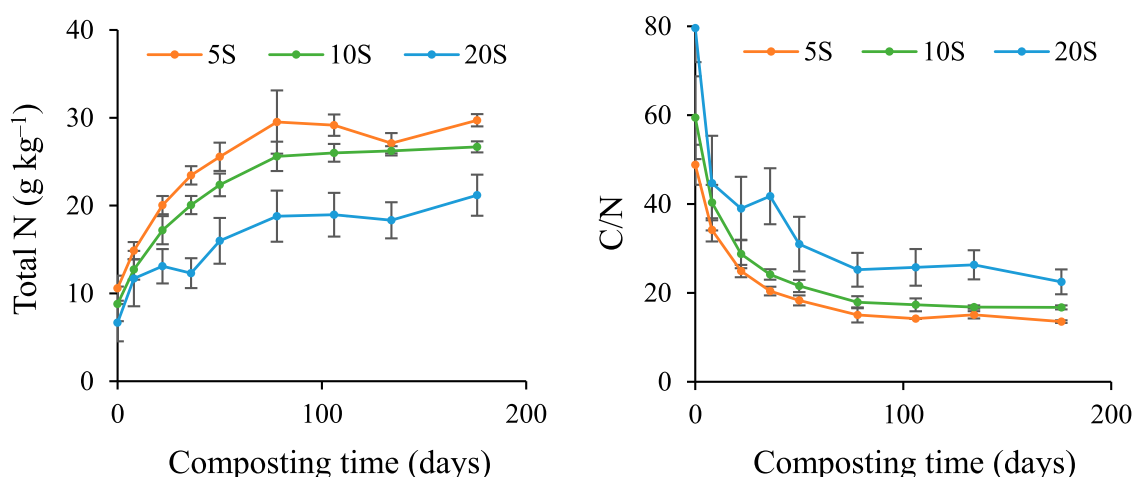
Microorganisms play a vital role in the composting process, facilitating the efficient breakdown of organic materials and the production of compost, being the presence of a robust and diverse community of native microorganisms crucial for the development of a mature, stable and desirable compost. The bacterial and fungal composition of the composting piles was analysed at two different time points: during the thermophilic phase (day 75) and during maturation (day 176).

#### 3.6.1 Bacterial and fungal community structure

The results clearly demonstrate a distinct difference in the structure of the bacterial and fungal microbiomes.



**Figure 2.** Organic matter (OM) losses (g kg<sup>-1</sup>) during composting of kiwi waste with wheat straw in the following proportions (w:w): pile 5S (95%:5%), pile 10S (90%:10%) and pile 20S (80%:20%). \*\*\* $P < 0.001$ .



**Figure 3.** Total N and C/N ratio during composting of kiwi waste and wheat straw mixed in the following proportions (w:w): pile 5S (95%:5%), pile 10S (90%:10%) and pile 20S (80%:20%).

**Table 3.** Summary of  $\alpha$ -diversity – based on taxonomic composition obtained by sequence analysis of the V3–V4 regions of the 16S rRNA gene.

| Sample  | Obs. species | Shannon | Simpson | Chao1    | ACE      | goods_coverage | PD_whole_tree |
|---------|--------------|---------|---------|----------|----------|----------------|---------------|
| Straw   | 1194         | 4.950   | 0.830   | 1375.674 | 1435.210 | 0.997          | 112.868       |
| Kiwi    | 3452         | 9.026   | 0.992   | 3645.457 | 3666.354 | 0.996          | 262.337       |
| 20S/75  | 3565         | 9.451   | 0.995   | 3768.689 | 3793.153 | 0.996          | 272.434       |
| 10S/75  | 2981         | 8.246   | 0.986   | 3225.751 | 3241.206 | 0.996          | 231.068       |
| 5S/75   | 2832         | 8.524   | 0.991   | 3057.317 | 3076.363 | 0.996          | 221.094       |
| 20S/176 | 3354         | 9.358   | 0.995   | 3585.901 | 3593.357 | 0.996          | 264.715       |
| 10S/176 | 3183         | 8.772   | 0.991   | 3446.896 | 3449.391 | 0.995          | 249.938       |
| 5S/176  | 3111         | 9.176   | 0.994   | 3305.503 | 3315.412 | 0.996          | 248.532       |

The  $\alpha$ -diversity indices (Tables 3 and 4) indicate a more complex and diverse ecosystem structure for the bacterial community compared to the fungal community.

The bacterial community exhibits a greater number of species and a more balanced distribution of abundances, suggesting a wide range of interactions and functional roles among different bacterial species, whereas the fungal community comprises fewer taxa, with two or three dominant taxa representing a significant portion (43.0% to 84.9%) of the total reads. This pattern is reflected in the ACE (Abundance-based Coverage Estimator) index, which provides an estimate of the total number of different species in a sample, as well as

the Shannon and Simpson indices, which assess the evenness of species abundance within a sample.

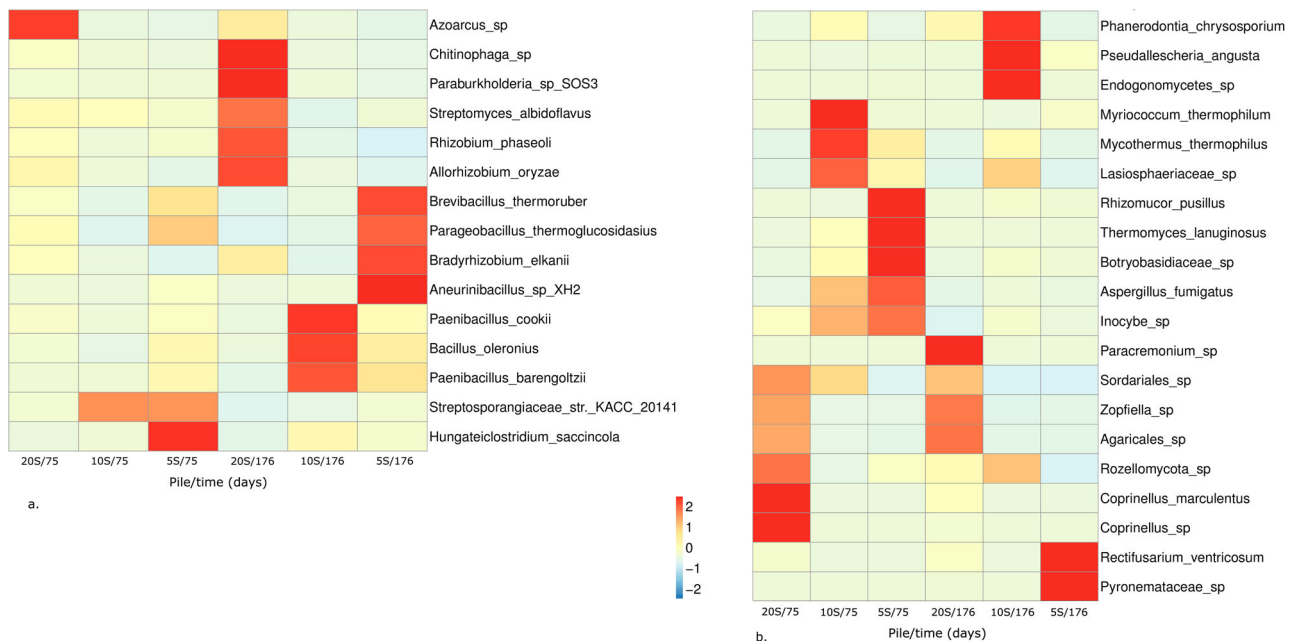
### 3.6.2 Changes in bacterial abundance during composting

Concerning the bacterial microbiome (heat-map from Figure 4(a)) observed in the three compost piles, as mentioned earlier, no specific dominant taxa were found. However, after 176 days of composting, certain bacterial species emerged as the most abundant in their respective piles. *Brevibacillus thermoruber* was the most prevalent species in pile 5S, representing 1.9% of the total relative abundance. In pile 10S, *Bacillus oleronius*

**Table 4.** Summary of  $\alpha$ -diversity – based on taxonomic composition obtained by sequence analysis of the Internal Transcribed Spacer region of the rRNA gene.

| Sample  | Obs. species | Shannon | Simpson | Chao1   | ACE      | goods_coverage | PD_whole_tree |
|---------|--------------|---------|---------|---------|----------|----------------|---------------|
| Straw   | 702          | 4.287   | 0.885   | 975.026 | 1000.737 | 0.996          | 247.267       |
| Kiwi    | 681          | 4.734   | 0.901   | 745.694 | 781.616  | 0.998          | 225.032       |
| 20S/75  | 819          | 5.219   | 0.919   | 934.532 | 959.858  | 0.997          | 291.152       |
| 10S/75  | 625          | 3.478   | 0.774   | 866.875 | 869.652  | 0.997          | 217.443       |
| 5S/75   | 741          | 5.032   | 0.918   | 920.279 | 927.620  | 0.997          | 269.240       |
| 20S/176 | 868          | 5.015   | 0.851   | 934.043 | 950.617  | 0.998          | 325.707       |
| 10S/176 | 627          | 3.751   | 0.843   | 852.653 | 886.181  | 0.997          | 231.831       |
| 5S/176  | 442          | 2.326   | 0.637   | 512.439 | 544.062  | 0.998          | 162.791       |





**Figure 4.** Taxonomic abundance cluster heatmap based on dominant sequences that were identified in samples based on partial sequence analysis of the analysis of the V3–V4 regions of the 16S rRNA gene (a) and Internal Transcribed Spacer region of the rRNA gene (b).

accounted for 1.8% of the abundance, while *Chitinophaga* sp. comprised 0.9% of the total relative abundance in pile 20S. Table 5 provides a concise characterization of the top ten bacterial taxa found in the sampled piles.

### 3.6.3 Changes in fungal abundance during composting

The heat-maps shown in Figure 4(b) depict the most prevalent taxa found in the composting piles at 75 and 176 days.

As expected, during the thermophilic phase, thermophilic fungi, such as *Mycothermus thermophilus* and *Lasio-sphaeriaceae* sp., dominate the fungal population in pile 10S, accounting for 65.4% of the total reads. In pile 5S, *Thermomyces lanuginosus*, *Mycothermus thermophilus*, and *Aspergillus fumigatus*, which are also capable of growing at high temperatures [38] were the dominant fungi. In line with the temperature readings recorded in pile 20S during composting, the dominant taxa consisted of mesophilic microorganisms, namely *Zopfiella* sp. and *Rozellomycota* sp., which constituted 51.0% of the total reads.

After 176 days, a significant divergence was observed in the fungal community of piles 10S and 5S in terms of the most abundant microorganisms. *Pseudallescheria angusta*, a ligninolytic fungus [39] emerged as the most prevalent species in pile 10S, comprising 31% of the total reads. The degradation of lignin produces

polyphenols that are precursors of humic substances, which are commonly considered to promote soil health. On the other hand, *Rectifusarium ventricosum* accounted for 44.5% of the total reads obtained in pile 5S after 176 days.

### 3.6.4 Impact of the initial pile composition on microbial development and the potential implications of specific microorganisms

The composition of the initial raw materials has a significant impact on the microbial community and the overall process of composting [40]. Although initially present in low numbers, most of the bacteria listed in Table 3 originated from kiwi fruit. In fact, 69% of the ten most abundant bacteria found in the compost piles were not detected in the straw. With the exception of *Streptomyces albidoflavus*, *Bradyrhizobium elkanii*, and *Bacillus gibsonii*, the remaining species DNA was present in very minimal amounts (Figure 5).

Actinomycetes are essential contributors to the composting process [41] as they efficiently break down complex and resilient organic compounds such as cellulose, lignin, and chitin. These microorganisms, known for their ability to produce bioactive molecules [42], form extensive, filamentous structures that permeate the compost, aiding in the decomposition of organic matter. The kiwi used in the composting piles demonstrated to be a significant source of these bacteria, initially

**Table 5.** Most prevalent taxa (based on sequence of V3-V4 regions of 16S rRNA gene) present in each pile after 75 and 176 days.

| Pile/days                                  | Taxa                                            | Gram | Aerobic/Facultative anaerobic | Anaerobic | Growth Temperature |
|--------------------------------------------|-------------------------------------------------|------|-------------------------------|-----------|--------------------|
| 5S/176                                     | <i>Aeribacillus pallidus</i>                    | +    | X                             |           | Thermophilic       |
| 20S/176                                    | <i>Allorhizobium oryzae</i>                     | -    | X                             |           | Mesophilic         |
| 5S/176                                     | <i>Aneurinibacillus</i> sp. XH2                 | +    | X                             |           | Mesophilic         |
| 20S/75 20S/176                             | <i>Azoarcus</i> sp.                             | -    | X                             |           | Mesophilic         |
| 10S/176 5S/176                             | <i>Bacillus clausii</i>                         | +    | X                             |           | Mesophilic*        |
| 20S/176                                    | <i>Bacillus gibsonii</i>                        | +    | X                             |           | Mesophilic         |
| 20S/75 10S/75 5S/75 20S/176 10S/176 5S/176 | <i>Bacillus oleronius</i>                       | -    | X                             |           | Mesophilic         |
| 20S/75 10S/75 5S/75 20S/176 10S/176 5S/176 | <i>Bradyrhizobium elkanii</i>                   | -    | X                             |           | Mesophilic         |
| 20S/75 5S/75 5S/176                        | <i>Brevibacillus thermoruber</i>                | +    | X                             |           | Thermophilic       |
| 20S/176                                    | <i>Chitinophaga</i> sp.                         | -    | X                             |           | Mesophilic         |
| 10S/176                                    | <i>Clostridium</i> sp. 6-31                     | +    |                               | x         | Mesophilic         |
| 10S/75                                     | <i>Clostridium</i> sp. TG60-81                  | +    |                               | x         | Mesophilic         |
| 5S/75 10S/176                              | <i>Hungateiclostridium saccincola</i>           | +    |                               | x         | Mesophilic         |
| 20S/75                                     | <i>Luteimonas marina</i>                        | -    | X                             |           | Mesophilic         |
| 5S/75                                      | <i>Moorella glycerini</i>                       | +    | Anerobic                      | x         | Thermophilic       |
| 10S/75 5S/75 10S/176 5S/176                | <i>Paenibacillus barengoltzii</i>               | +    | X                             |           | Mesophilic         |
| 10S/176                                    | <i>Paenibacillus cookii</i>                     | +    | X                             |           | Mesophilic         |
| 20S/176                                    | <i>Paraburkholderia</i> sp. SOS3                | -    | X                             |           | Mesophilic         |
| 5S/75 5S/176                               | <i>Parageobacillus thermoglucosidasius</i>      | +    | X                             |           | Thermophilic       |
| 10S/176                                    | <i>Porphyrionadaceae bacterium</i> NLAE-zl-C104 | -    |                               | x         | Mesophilic         |
| 20S/75                                     | <i>Proteiniphilum saccharofermentans</i>        | -    | X                             |           | Mesophilic         |
| 10S/75 20S/176                             | <i>Rhizobium phaseoli</i>                       | -    | X                             |           | Mesophilic         |
| 20S/75 20S/176                             | <i>Shinella zoogloeoides</i>                    | -    | X                             |           | Mesophilic         |
| 20S/75                                     | <i>Sporomusa sphaeroides</i>                    | -    |                               | x         | Mesophilic         |
| 20S/75 10S/75 5S/75 20S/176 10S/176 5S/176 | <i>Streptomyces albidoflavus</i>                | +    | X                             |           | Mesophilic         |
| 20S/75 10S/75 5S/75 10S/176 5S/176         | <i>Streptosporangiaceae</i> str. KACC 20141     | +    | X                             |           | Mesophilic         |
| 10S/75                                     | <i>Symbiobacterium terraclitae</i>              | -    | X                             |           | Mesophilic         |
| 10S/75 5S/75                               | <i>Thermomonospora curvata</i>                  | +    | X                             |           | Thermophilic       |
| 10S/75                                     | <i>Thermostaphylospora chromogena</i>           | +    |                               | x         | Thermophilic       |

Note: \* ability to grow at 50°C has been reported [37].

comprising approximately 20.6% of *Actinobacteria* in relation to the total bacterial population (Figure 6).

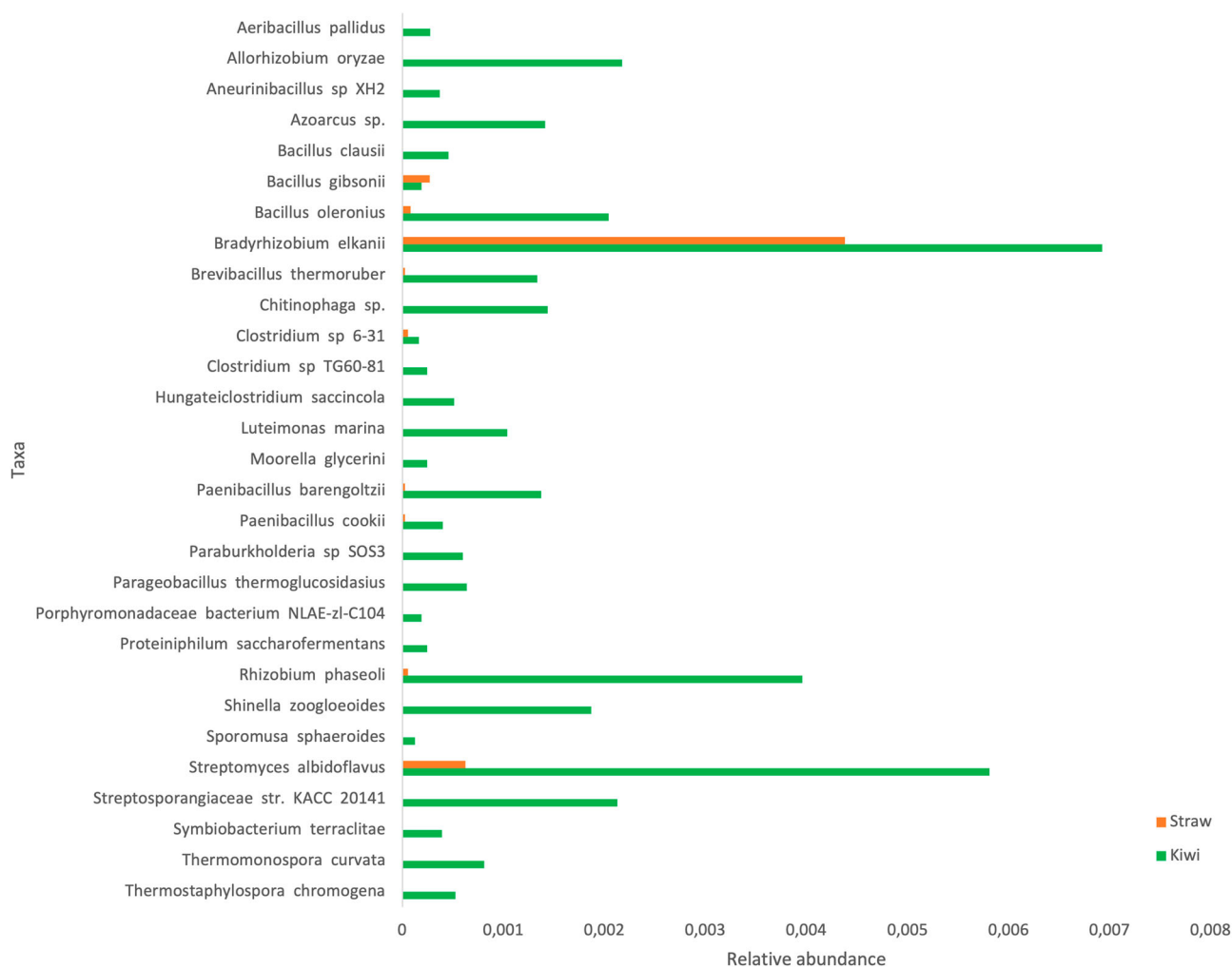
When it comes to the fungal population, the influence of straw on the prevalence of fungal species at the end of composting seems to be more pronounced. This is particularly observed in pile 20S, which had a higher proportion of straw (Figure 7).

Among the top ten most abundant taxa, pile 20S exhibited a notable prevalence of Gram-negative bacteria when compared to the other piles, accounting for 80% after 75 days and 70% after 176 days of Gram (-) bacteria. Additionally, most of the thermophilic bacteria were observed in the samples collected at 75 days, specifically in pile 5S, where four out of the ten species identified were thermophilic. After 176 days, both piles 5S and 20S displayed a bacterial community completely dominated by aerobic species. This finding suggests that even with the lowest amount of straw used, the system maintained sufficient aeration.

Each pile functions as a complex ecosystem, where the interaction between microorganisms plays a crucial role in the overall success of the composting process [43]. The presence of specific microorganisms capable of facilitating organic matter degradation and nutrient cycling is crucial. Moreover, compost may also contain

beneficial microorganisms that can suppress phytopathogens and promote plant growth [44].

The dominant bacterial taxa observed in the compost piles during the maturation phase (176 days) have the potential to play significant roles in promoting plant health, nutrient cycling, and defense against plant pathogens. *Rhizobium* and *Allorhizobium* species establish symbiotic relationships with plants, contributing to nitrogen fixation [45]. *Azoarcus* sp., a diazotrophic bacterium, fixes atmospheric nitrogen and enhances nitrogen availability in the soil, promoting plant growth [46]. *Bacillus* species contribute to nutrient cycling, organic matter decomposition, and disease suppression in the soil, mainly through the production of antibiotics, hydrolases, and siderophores [47,48]. *Bradyrhizobium elkanii* forms symbiotic relationships with plants and provides nitrogen fixation [49]. Certain *Brevibacillus* species have shown biocontrol activity against plant pathogens and soil bioremediation capabilities [50]. *Paenibacillus* species have the ability to enhance crop growth through various mechanisms, including nitrogen fixation, phosphate solubilization, production of the plant growth hormone indole-3-acetic acid (IAA), and siderophore release for iron acquisition. They also offer protection against insect herbivores and a



**Figure 5.** Relative abundance of the most prevalent bacteria based on partial sequence analysis of V3-V4 regions of 16S rRNA gene, present in the raw materials, straw and kiwi of the piles 5S, 10S and 20S, before composting.

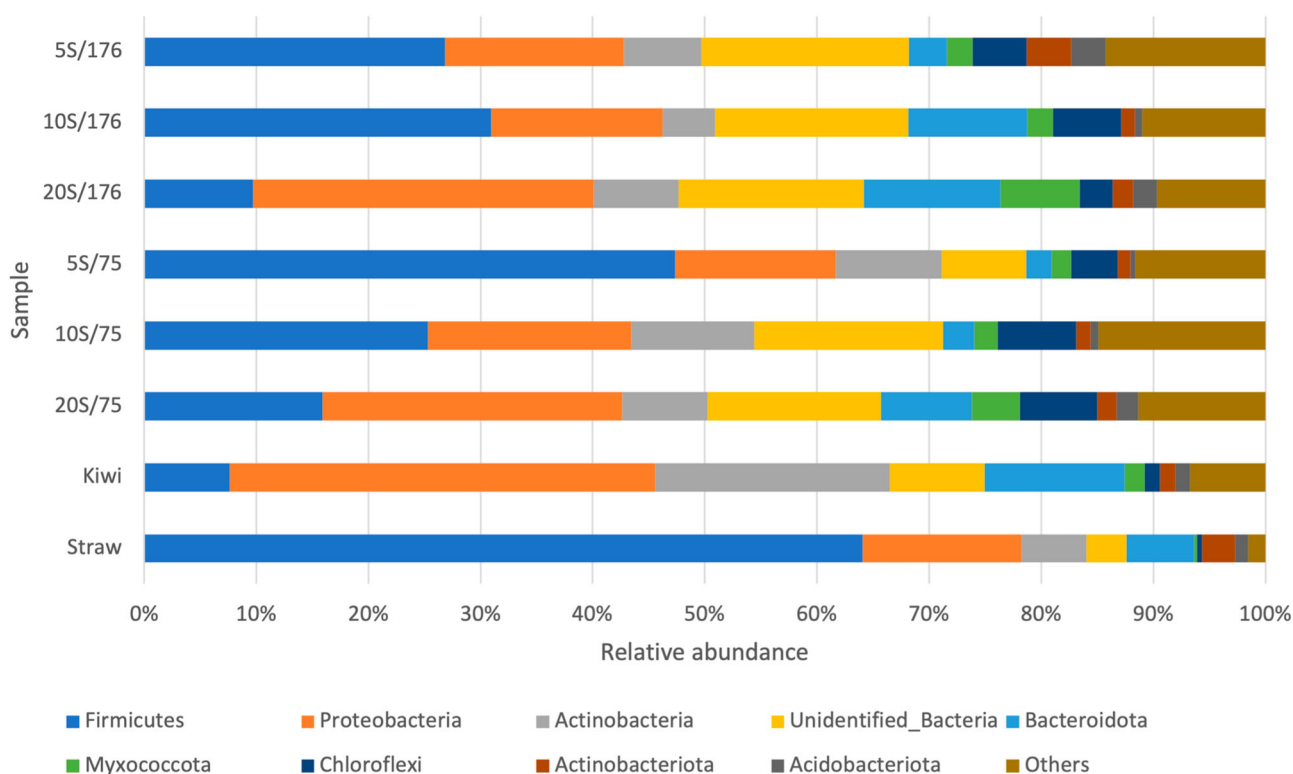
range of phytopathogens, including bacteria, fungi, nematodes, and viruses [51]. Certain *Paraburkholderia* species appear to have a role in soil priming [52]. *Streptosporangiaceae*, including *Streptomyces* species, produce antibiotics and secondary metabolites with antimicrobial properties, aiding in the suppression of plant pathogens [53]. However, it is important to confirm the potential benefits of these species through suppression and productivity tests, as specific strains may vary in their characteristics.

### 3.7. Compost quality

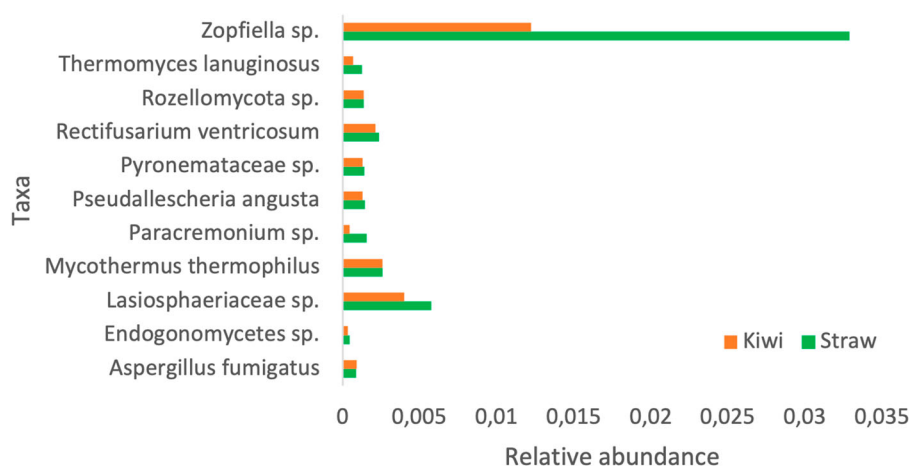
The effect of compost on soil physical, chemical and biological properties as well as the capacity of the compost to fertilize the crops defines its quality. Thus, the quality of the compost may be established in terms of stability, absence of phytotoxicity, chemical characteristics such as pH, EC, OM and nutrient contents, and, low levels of heavy metals. Compost stability refers to the degree of

OM decomposition. An unstable compost with labile C and high microbial activity can affect soil environment and may have a negative impact on plant growth [54], for example, by microbial N immobilization. In this study, the compost showed a high level of stability 176 days after the commencement of the composting process as the temperatures decreased to stable temperatures near the ambient levels. Compost stability can also be defined in terms of nitrification because after the thermophilic phase there is a progressive increase in the  $\text{NO}_3^-$ -N content [55]. Here, the ratio  $\text{NH}_4^+$ -N /  $\text{NO}_3^-$ -N (between 0.1 and 0.2) was below the upper limit of 0.5 recommended by [56] for matured composts (Table 6).

The assessment of phytotoxicity usually involves germination tests to quantify seed germination and root growth (Table 7). The germination index in the composts 10S (99%) and 20S (97%) above 80% indicated the absence of phytotoxicity. On the contrary, the germination index of the final compost 5S (42%) indicated that



**Figure 6.** Relative abundance of taxa assigned to phylum level in samples (raw and piles) that were identified in samples based on partial sequence analysis of V3-V4 regions of 16S rRNA gene.



**Figure 7.** Relative abundance of the most prevalent fungi taxa, present in the raw materials, straw and kiwi, of the piles 5S, 10S and 20S before composting, based on partial sequence analysis of the Internal Transcribed Spacer region of the rRNA gene.

**Table 6.** Chemical characteristics of final composts (mean and standard deviation).

|     | OM<br>(g kg <sup>-1</sup> ) | N<br>(g kg <sup>-1</sup> ) | C/N      | N-NH <sub>4</sub> <sup>+</sup><br>(mg kg <sup>-1</sup> ) | N-NO <sub>3</sub> <sup>-</sup><br>(mg kg <sup>-1</sup> ) | P<br>(g kg <sup>-1</sup> ) | K<br>(g kg <sup>-1</sup> ) |
|-----|-----------------------------|----------------------------|----------|----------------------------------------------------------|----------------------------------------------------------|----------------------------|----------------------------|
| 5S  | 724 ± 20                    | 29.7 ± 0.7                 | 13 ± 0.1 | 1 ± 0.1                                                  | 50 ± 1                                                   | 5.7 ± 0.8                  | 26.4 ± 6.4                 |
| 10S | 804 ± 2                     | 26.7 ± 0.6                 | 17 ± 0.1 | 46 ± 0.1                                                 | 240 ± 8                                                  | 4.8 ± 0.3                  | 27.0 ± 4.5                 |
| 20S | 858 ± 14                    | 21.2 ± 2.3                 | 22 ± 3   | 84 ± 5                                                   | 401 ± 12                                                 | 2.4 ± 0.5                  | 25.1 ± 1.8                 |

Nutrient contents are expressed on a dry matter basis.

**Table 7.** Relative seed germination (RSG; %), relative root growth (RRG; %) and germination index (GI; %) of final compost.

|     | RSG (%) | RRG (%) | IG (%) |
|-----|---------|---------|--------|
| 5S  | 112 a   | 38 b    | 42 b   |
| 10S | 127 a   | 78 a    | 99 a   |
| 20S | 115 a   | 84 a    | 97 a   |

Mean values followed by different letters within the same treatment are significantly different.

GI (%) = RSG (%) × RRG (%).

this compost may be phytotoxic. Probably, the increase in phytotoxicity for 5S was associated with high EC (4.6 dS m<sup>-1</sup>), and pH (9.4) values [57].

Nutrient-rich composts were obtained in the three piles (Table 3). The OM content increased in pile 20S (858 g kg<sup>-1</sup>) in comparison to the other piles (724–804 g kg<sup>-1</sup>) due to the incorporation of increased amount of straw more recalcitrant than the kiwifruits. In contrast, N and P contents decreased with increased amount of straw. A high level of potassium (25.1–27.0 g kg<sup>-1</sup>) was present in all three composts and phosphorus and potassium contents were within the typical ranges reported for composts produced with different feedstocks such as cattle slurry, cattle manure and winery wastes [58–60]. This, in addition to low heavy metals (Cu, Zn, Pb, Cd, Cr, Ni and Hg) contents (Table 8), below the maximum values established by the Portuguese mandatory guidelines for compost use as soil organic amendments [26], are indicative that these composts have a good quality for use as organic soil fertilizers.

According to the above-mentioned guidelines and considering the hygiene quality of the compost, the microbiological criteria for *Salmonella* spp. and *E. coli*, are very strict. However, no coliforms or *Salmonella* spp. were found associated with any of the mature composts, despite *E. coli* presence in the raw materials, although in small amounts. Regarding the presence of potential phytopathogenic fungi in mature composts, pile 20S showed substantial levels of *Fusarium* spp., accounting for approximately 1.5% of the total reads, while pile 5S had a lower presence at about 0.3%. In contrast, the mature compost of pile 10S did not contain any of the most relevant and common phytopathogenic fungi. Only trace amounts of DNA from *Alternaria*, *Fusarium*, and *Macrophomina* were found, and always in quantities of no more than 0.08% of the total reads.

**Table 8.** Heavy metal content of final composts.

|      | Cu<br>(mg kg <sup>-1</sup> ) | Zn<br>(mg kg <sup>-1</sup> ) | Pb<br>(mg kg <sup>-1</sup> ) | Cr<br>(mg kg <sup>-1</sup> ) | Ni<br>(mg kg <sup>-1</sup> ) | Cd<br>(mg kg <sup>-1</sup> ) | Hg<br>(µg kg <sup>-1</sup> ) |
|------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| 5S   | 70 ± 5                       | 94 ± 4                       | 2.3 ± 0.2                    | 7.5 ± 1.4                    | 2.9 ± 0.3                    | < 0.1                        | 1.0 ± 0.4                    |
| 10S  | 63 ± 5                       | 95 ± 11                      | 2.3 ± 0.6                    | 6.2 ± 0.9                    | 2.5 ± 0.3                    | < 0.1                        | 1.3 ± 0.5                    |
| 20S  | 60 ± 5                       | 93 ± 5                       | 1.8 ± 0.3                    | 5.2 ± 1.2                    | 2.7 ± 0.4                    | < 0.1                        | 1.0 ± 0.4                    |
| *LVL | 100                          | 200                          | 100                          | 100                          | 50                           | 0.7                          | 700                          |

\*LVL: Limit values allowed by Portuguese guidelines (Portaria n°185-2022).

No commonly known phytopathogenic bacteria were found in any of the mature composts.

## 4. Conclusions

Our data support the hypothesis that the addition of wheat straw as a bulking agent is an efficient method to recycle kiwifruit waste through composting. The high total N content (29.7 g kg<sup>-1</sup>) and low C/N ratio (13) of the compost with 5% (fresh weight) of straw promotes the availability of N to the crops, which is important from the agricultural point of view. However, the high values of pH (9.4) and EC (4.6 dS m<sup>-1</sup>) associated with a low germination index (43%) suggested that this compost may be phytotoxic. Thus, the compost with 10% straw with good hygiene quality, high level of stability, absence of phytotoxicity as well as high OM content (>800 g kg<sup>-1</sup>), pH < 9, EC < 3 dS m<sup>-1</sup> and rich in potentially beneficial microorganisms that may promote plant growth and help suppress some phytopathogenics is recommended as organic amendment to improve the sustainability of the soil fertility. This finding is particularly important for kiwi producers to reduce the negative effects of landfill deposition on the environment and demonstrates the suitability of simple techniques to compost and retain the N fertilizer value with agronomic benefits. Long-term studies are needed to make solid conclusions on the effect of compost according to the principles of circular economy.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## Data availability statement

Data presented in this study are available upon request to the corresponding author.

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