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Organic Amendments and Sampling Date Influences on Soil Bacterial Community Composition and Their Predictive Functional Profiles in an Olive Grove Ecosystem

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Abstract: A collapse of soil microbial diversity, mainly due to chemical inputs, has been reported to lead to the degradation of conventional agroecosystems. The use of compost from urban and agricultural waste management, in order to achieve a net gain in the storage of C, is an adequate management of agricultural soils, especially in rainfed conditions. However, the great variability of composts of different maturity and origins and of the soils to which they are added limits the ability to predict the impact of these amendments on the dynamics of soil microbial communities. This study was designed to gain insights on the effect of exogenous organic matter management on the soil bacterial community and its contribution to key functions relevant to agricultural soils. To achieve this, two different types of compost (alperujo or biosolids composts) at two doses were used as soil amendments twice for 3 years in a rainfed olive grove ecosystem. A metagenomic analysis was carried out to assess the abundance and composition of the soil bacterial communities and predicted functions. We only detected a minor and transitory effect on the bacterial abundance of the soil, the structure of the community and the potential functions, less related to the dose or the type of compost than to seasonal variations. Although the result suggests that the soil bacteria were highly resilient, promoting community stability and functional resilience after the addition of the two composts, more efforts are necessary to assess not only the resulting soil microbial community after organic fertilization but the intrinsic microbial community within the organic amendment that acts as an inoculum, and to what extent the changes in its dose could lead to the functionality of the soil.

Keywords: bacterial diversity; bacterial functionality; metagenomics; olive agroecosystem; organic amendments; soil management

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1. Introduction

Soils produce a range of services that are essential to human health and welfare [1]. There is a large amount of literature indicating microbial communities as those mainly responsible for providing key ecosystem services such as soil fertility, resilience and resistance to stresses [2]. Microbes impact all living organisms and play a central role in many biogeochemical cycles on earth, driving global carbon and nutrient cycling with direct feedback effects on ecosystem functioning and productivity [3]. However, soil ecosystem services are under increasing pressure because of human activities. The impacts of agricultural management on soil- and plant-associated microbiomes have been widely evidenced [4]. Land-use practices impact soil microbial functionality and biodiversity, with

reports suggesting that anthropogenic activities potentially result in reduced microbial functions and biodiversity loss. These findings provide insights into the role of farming practices in shaping soil bacterial communities and their functions in agroecosystems [5]. In general, most studies provide experimental evidence that soil microbial diversity is important for the maintenance of plant productivity [6]. However, some of them suggest that functional redundancy in soil microbial communities may be overestimated, especially in agroecological systems [7]. In this context, the study of the whole genome of soil microbiota by metagenomic approaches has an extraordinary potential to shed light on ecosystem functions of microbial communities [8,9]. However, a lack of knowledge on the basic principles of how soils function from a metagenomic perspective has been evidenced [10].

At the land-use policy level, the European Community sets a baseline for agri-environment measures to promote good farming practices that ensure basic ecosystem services to maintain land in good agricultural and environmental conditions through the common agricultural policy (CAP) [11]. In this scenario, soil management seems to be critical to sustaining fragile ecosystems such as rainfed agriculture, characterized by being located in water- and organic-matter-scarce environments. The addition of organic matter as compost to soil is a common soil-conservation management practice in olive rainfed agriculture to increase soil fertility and permeability, water retention, nutrient availability and enhance carbon sequestration [12,13]. Related to this, a positive effect of compost on microbial diversity has been often associated with an increase in soil quality and functionality [14–16]. However, the effect of organic amendments on microbial communities in degraded soils has given contrasting results so far. Regarding olive agroecosystems, it has been shown that organic amendments increase bacterial richness without affecting fungal richness [17]. Conversely, the use of fresh organic amendments such as oil mills wastewater, combined with other agricultural methods such as tillage, shows an increase in bacterial biomass but a reduction in bacterial diversity in the semiarid soils of olive groves [18] Additionally, shifts in soil microbial community composition have been associated with seasonality and sampling date in Mediterranean ecosystems, often linked to changes in soil management [19,20]. Understanding the role of both is critical to elucidate their effect on microbial biodiversity.

This work aims to elucidate to what extent sampling time and the addition of exogenous organic matter contribute to the abundance and composition of soil bacterial communities and their predictive functional profiles in an olive agroecosystem. To achieve this, two different composts at two doses were used as soil amendments twice for 3 years, and a metagenomic analysis focused on soil bacteria was then carried out.

2. Materials and Methods

2.1. Experimental Design and Edaphoclimatic Conditions

In January 2018, about 1.2 ha of the rainfed olive grove in the experimental farm "La Hampa" of the "Institute of Natural Resources and Agrobiology of Sevilla (IRNAS-CSIC)" (37°17′01.8″ N 6°03′57.4″ W) was divided into 20 plots following a completely randomized block design. Each plot included a 3 × 3 frame of olive trees ("Manzanilla de Sevilla" variety) of over 25 years old (Figure 1). The experiment lasted until October 2020. A detailed description of the experimental design and climatic condition over the study can be found in de Sosa et al. [21].

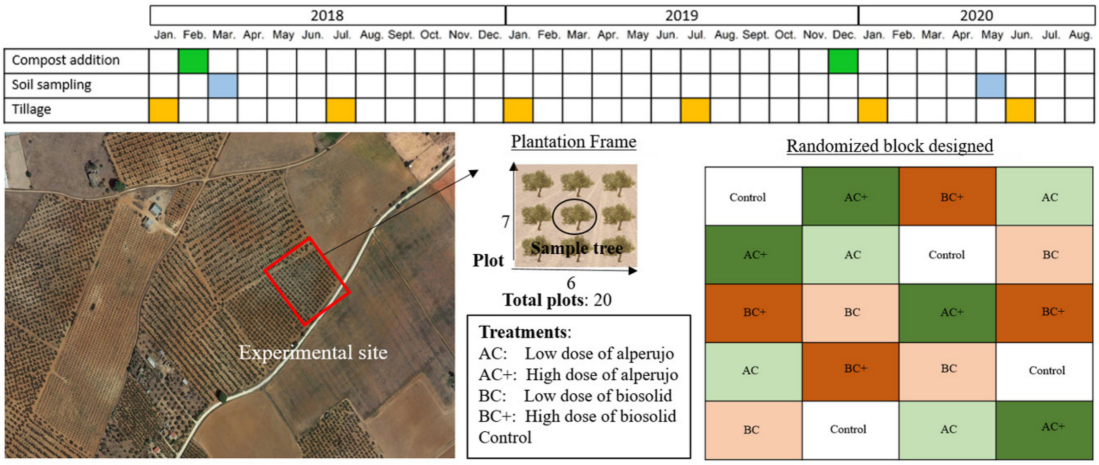

Figure 1. Description of the experimental design of the olive trees orchard and treatments applied.

Two different composts were applied twice during a three-year field study to determine their effect on bacterial community structure and functionality. The first compost (alperujo, AC hereafter) is a solid byproduct from the extraction of olive oil composted and provided by Fertiormont Company located in Málaga, Spain. The second one (biosolid compost, BC hereafter) is obtained by composting a mixture of sewage sludge and gardening prune from municipal parks, produced and provided by EMASESA, the municipal waste management entity in Seville, Spain.

Two application doses per olive tree were established: the low dose was set to 17.8 kg for AC and BC treatments, whereas the high dose was 26.7 kg for AC+ and BC+ treatments. The main characteristics of the two composts can be found in [21]

Each treatment received two compost applications, in February 2018 and in December 2019 (Figure 1). Composts were applied in circles starting at a 1 m distance from each tree trunk, then incorporated into the soil by hand tilling.

The control plots did not receive organic fertilization, only the mineral fertilization applied in previous years: a basal dressing with NPK 15-15-15 (100 kg ha^{-1}). A foliar application of KNO_3 was applied to all trees every other year and B before fruit set. Copper fungicide was applied once a year, and dimethoate insecticide was applied twice. Herbicide application was stopped at the beginning of the present experiment.

To incorporate aboveground biomass fallen from trees and from spontaneous weeds, in January and July of each year, two tillage operations involving a disc harrowing (at 0–10 cm depth) were performed.

The soil of the farm is a calcic Cambisol (pH: 7.5) characterized by a sandy clay loam texture, low organic matter content (TOC: 8 g kg^{-1}) and low fertility (N: 0.8 g kg^{-1}; Olsen P: 10 mg kg^{-1}; available K: 200 mg kg^{-1})

The farm presents a typically Mediterranean climate, with very hot and dry summers, followed by mild and rainy winters. The climatic conditions are typically Mediterranean, with mild rainy winters (504 mm mean annual rainfall) and very hot and dry summers. The mean annual daily temperature at the experimental site is around 17 °C, with maximum and minimum mean monthly temperatures of 28.9 °C and 6.3 °C registered in July and December, respectively.

2.2. Soil Sampling

Four soil samples per treatment (each composed of 3 mixed subsamples within the compost application area of the central tree of each plot) were collected from the 0–10 cm layer in March 2018 and May 2020, one month after the first and five months after the second compost application, respectively. Samples were gently sieved to pass through a 2 mm mesh sieve, kept at 4 °C during the sampling campaign and then stored at −80 °C until DNA extractions.

2.3. Molecular Analyses of Soil Bacteria

Eight 1 g aliquots from each soil sample were DNA extracted using the bead-beating method with the aid of a PowerSoil® DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA), following the manufacturer's instructions. Extractions were pooled into two groups of four subsamples and concentrated at 35 °C to a final volume of 20 µL using a Savant Speedvac® concentrator.

Real-time PCR was performed to quantify the bacterial community relative abundance in soil DNA extracts with the universal primers for the V3 hypervariable region of 16S rRNA eubacteria P1 and P2 [22], as described in [23]. All reactions were carried out in triplicate, with four replications per qPCR. The potential presence of qPCR inhibitors was tested by mixing 1 µL (4–8 ng) of soil DNA extracts with a known amount of recombinant plasmid DNA (pCR®2.1, Invitrogen, Carlsbad, CA, USA) with the appropriate primers. Controls, where DNA templates were replaced by filter-sterilized Milli-Q water, were carried out simultaneously. Ct values were not significantly different between the DNA extracts and the controls.

The V3–V4 hypervariable regions of the 16S rRNA gene were targeted by bacterial PCR primers 5′ CCTACGGGNBGCASCAG 3′ and 5′ GACTACNVGGGTATCTAATCC 3′ [24,25] to characterize the bacterial communities of two replicates per sample using the Illumina MiSeq (Illumina Inc., San Diego, CA, USA) (2 × 250 nucleotide paired-end protocol) platform at the López-Neyra Institute of Parasitology and Biomedicine's (IPBLN-CSIC) genomic facilities. The SEED2 pipeline v2.1.05 [26] was used to process raw sequences, as described in [9], and then clustered using the UPARSE method [27]. Taxonomic assignment of OTUs was achieved using the classify.seqs algorithm in mothur software v1.46.1 against the SILVA v132 database [28,29] (Table S1). An abundance OTU matrix × sample was generated using the Marker Data Profiling module in the MicrobiomeAnalyst web-based platform (https://www.microbiomeanalyst.ca/faces/home.xhtml) [30,31]. The most abundant sequence per OTU was selected as representative. All samples reached a plateau according to rarefaction curves generated by the MicrobiomeAnalyst tool.

Raw Illumina sequence data were deposited in the Sequence Read Archive (SRA) service of the European Bioinformatics Institute (EBI) database (https://www.ebi.ac.uk/) (BioProject ID: PRJNA772542).

2.4. Predictive Metagenomics Profiling

Tax4fun v0.3.1 [32], implemented in the Shotgun Data Profiling (SDP) module of MicrobiomeAnalyst, was used to predict, from the 16S datasets obtained from the SILVAngs web server, the functional pathways of soil bacterial communities based on Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations [33,34]. These were based on (1) modules, i.e., functional units of gene sets in the KEGG metabolic pathways database that can be linked to specific metabolic capacities and other phenotypic features, and (2) KEGG Orthologous (KO) corresponding to a group of orthologous genes identified by a K number that have identical functions [30,35].

2.5. Statistical and Diversity Analysis

One-way analysis of variance (ANOVA) of repeated measures, followed by Dunn's post hoc Bonferroni-corrected *p*-values, was performed to assess the treatment effect and sampling date on 16S rRNA gene abundance, bacterial classes relative abundance and diversity estimate values. Data normality and homogeneity of variance were assessed with the Shapiro–Wilk test and Levene statistics, respectively. Statistical analysis was performed using Paleontological Statistics (PAST) software program v3.14 [36]. OTU abundance information was normalized to the abundance value of the sample with the fewest sequences. Bacterial richness and diversity in soil samples were compared using alpha diversity indices generated by SEED2. Beta diversity analysis and linear discriminant analysis (LDA) effect size (LEfSe) were performed using the phyloseq package [37] to test species complexity and functional orthologs (KEGG KO) differences between groups, respectively,

using the MicrobiomeAnalyst web-based platform. For beta diversity, Bray–Curtis distance and Permutational ANOVA (PERMANOVA) were used to evaluate the distance between samples and the statistical significance of the clustering pattern, respectively. For LEfSe, KOs with an LDA score >4 were considered important biomarkers of each treatment, and a value of $p < 0.05$ indicates significant differences between them. Data were analyzed using R version 3.6.3 [38] and R Studio version 1.1.456 [39].

3. Results

3.1. Bacterial Community Abundance

At lower doses of compost and after the first addition, the number of 16S rRNA gene copies increased by almost two times in the AC treatment with respect to the control, whereas it showed a decrease of 30% in the BC treatment compared to the control (Figure 2). No changes were evidenced at high compost doses with respect to nonamended soils. At the end of the experiment, there were no main significant differences between treatments, but a decrease over time in each of them was detected, including the control.

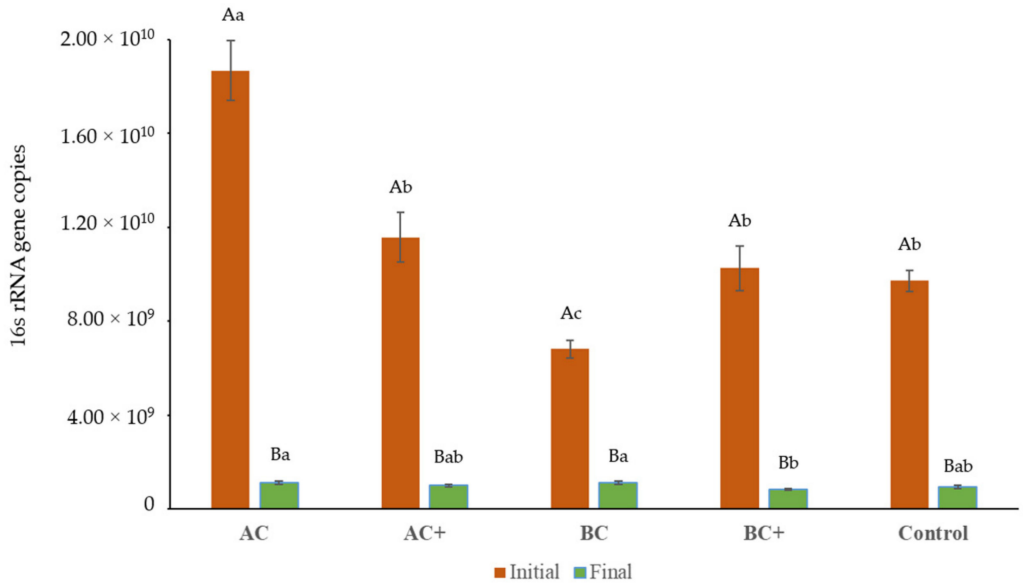

Figure 2. 16s rRNA gene copies in control soil and amended soils at low (AC and BC soils) and high doses (AC+ and BC+ soils) at two different samplings. Different lowercase letters indicate a significant difference among different treatments, and different uppercase letters indicate a significant difference between different times ($p < 0.05$, ANOVA, Dunn's post hoc Bonferroni-corrected p-values).

3.2. Bacterial Communities

Ten dominant phyla (>1% abundance) accounted for more than 99% of all bacterial sequences at the class level (Figure 3).

The total bacterial community was dominated by classes *Acidobacteria*, *Actinobacteria* and *Alphaproteobacteria*, accounting for almost 70% of the entire bacterial population in all treatments. The composition of the total bacterial community did not differ significantly between treatments. However, a common pattern in the relative abundance within bacterial classes could be observed between initial and final bacterial composition in all treatments; the relative abundance of *Actinobacteria* increased, and *Alphaproteobacteria* and *Acidobacteria* decreased over time ($p < 0.05$), the last only when compost was applied at higher doses (AC+ and BC+). Alpha diversity measures showed a comparative trend in terms of predicting the number of OTUs in all soil samples (Table 1), and no clear differences were detected among treatments at any sampling time.

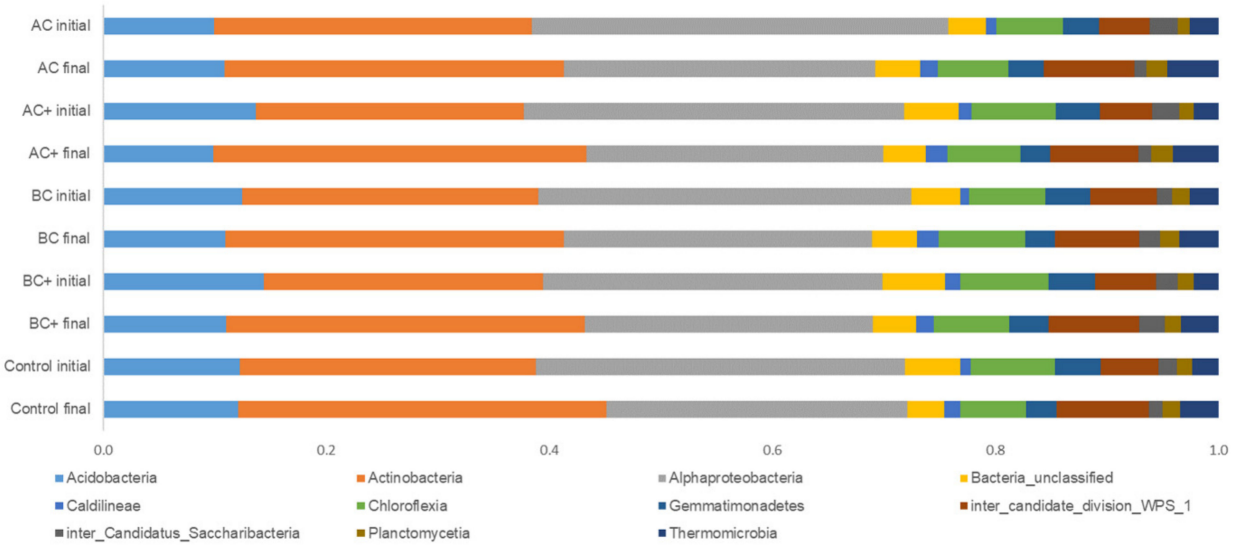

Figure 3. Relative abundance of top 10 soil bacterial phyla in control soil and amended soils at low (AC and BC soils) and high doses (AC+ and BC+ soils) at two different samplings.

Table 1. Richness estimates and diversity indices (means ± SE) for 16S rRNA libraries of soils. For each parameter, different letters indicate a significant difference among initial and final sampling ($p < 0.05$, ANOVA, Dunn's post hoc Bonferroni-corrected p-values) when they exist.

	Shannon–Wiener Diversity Index		Species Richness (S)		Simpson Diversity Index		Evenness	
	INITIAL	FINAL	INITIAL	FINAL	INITIAL	FINAL	INITIAL	FINAL
AC	5.84 ± 0.13	6.09 ± 0.03	2854 ± 246	2323 ± 34	0.013 ± 0.000a	0.008 ± 0.000b	0.735 ± 0.008b	0.786 ± 0.003a
AC+	6.05 ± 0.01	6.01 ± 0.02	3327 ± 30a	2470 ± 82b	0.013 ± 0.000	0.010 ± 0.001	0.747 ± 0.000	0.769 ± 0.004
BC	6.04 ± 0.07	6.19 ± 0.04	3435 ± 262	2637 ± 36	0.012 ± 0.002a	0.008 ± 0.001b	0.743 ± 0.002b	0.786 ± 0.003a
BC+	6.19 ± 0.03	6.11 ± 0.05	3625 ± 153	2645 ± 120	0.010 ± 0.000	0.009 ± 0.001	0.756 ± 0.000	0.776 ± 0.006
Control	6.14 ± 0.07	5.92 ± 0.13	3497 ± 146a	2318 ± 126b	0.012 ± 0.001	0.011 ± 0.002	0.753 ± 0.012	0.765 ± 0.013

Beta diversity patterns, that is, the relative differences in the composition of species between samples, were examined using various qualitative and quantitative similarity indices at the putative OTU level, pointing to a high degree of dissimilarity of the bacterial population structures between time (Figure 4b; F-value: 9.8886, R-squared: 0.26099; $p < 0.001$) but not between treatments (Figure 4a; F-value: 1.7973, R-squared: 0.44714; $p < 0.016$).

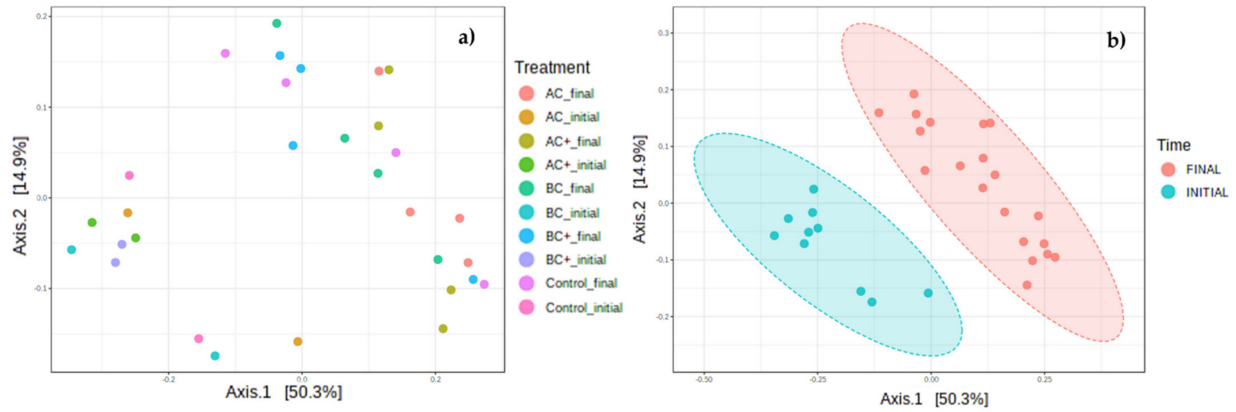

Figure 4. Effect of treatment (**a**) and time (**b**) on bacterial beta diversity in control soil and amended soils at low (AC and BC soils) and high doses (AC+ and BC+ soils). Ordination method PCoA; distance method: Bray–Curtis index; statistical method: PERMANOVA. Ellipses were drawn only when 95% of the data fell inside the ellipse.

3.3. Bacterial Community Functional Analysis

Metagenomic analysis allows predicting the functional potential of the bacterial community and exploring associated metabolic pathway networks based on Kyoto Encyclopedia of Gene and Genome (KEGG) clusters. At the functional units of gene sets level (KEGG

modules), all treatments shared all the 186 predicted functions related to soil bacteria (Table S2). On closer analysis, we detected some differences at the KO level, that is, molecular functions represented in terms of functional orthologs, and the linear discriminant analysis (LDA) showed 10 features to be mainly responsible for the differences between treatments and between doses in each treatment (Figure 5). We detected a common pattern of increase over time in the abundance of ABC.PE.S, ABC.PE.P and ABC.PE.P1 (peptide/nickel transport system substrate-binding and permease proteins; K02035, K02033 and K02034), fdoG, fdhF, fdwA (formate dehydrogenase major subunit [EC:1.17.1.9]; K00123) and coxL, cut (aerobic carbon monoxide dehydrogenase large subunit [EC:1.2.5.3]; K03520) proteins, while bglX (beta-glucosidase [EC:3.2.1.21]; K05349), GDH2 (glutamate dehydrogenase [EC:1.4.1.2]; K15371), prnA, rebH, ktzQ (tryptophan 7-halogenase [EC:1.14.19.9]; K14266), ACAT, atoB (acetyl-CoA C-acetyltransferase [EC:2.3.1.9]; K00626) proteins showed the opposite.

Finally, and similar to that observed for beta diversity, the effect of the treatment on the relative differences in the abundance of bacterial predicted functions between samples (Figure 6a) was less evident than those of the sampling time (Figure 6b).

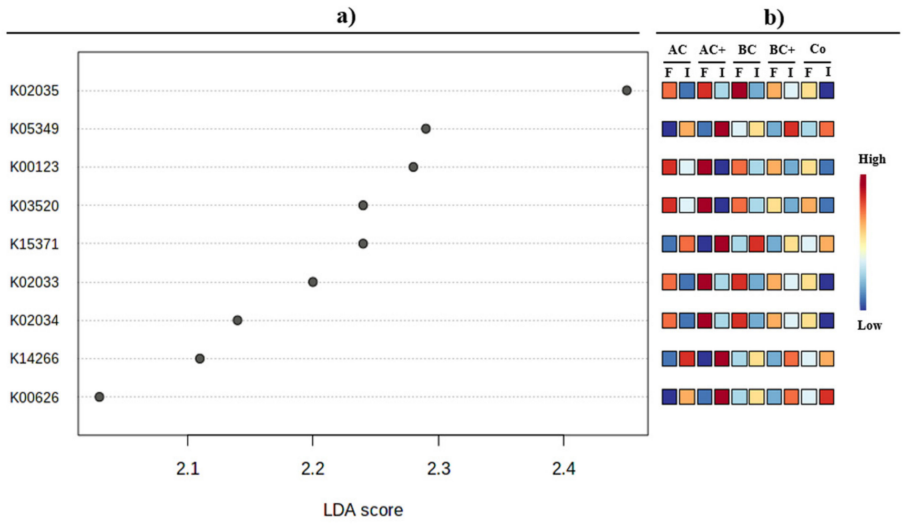

Figure 5. (**a**) Linear discriminant analysis (LDA) scores and, (**b**) heatmap from blue (low) via white to red (high) of KO relative abundances in control soil (Co) and amended soils at low (AC and BC soils) and high doses (AC+ and BC+ soils) at ini-tial (I) and final (F) sampling.

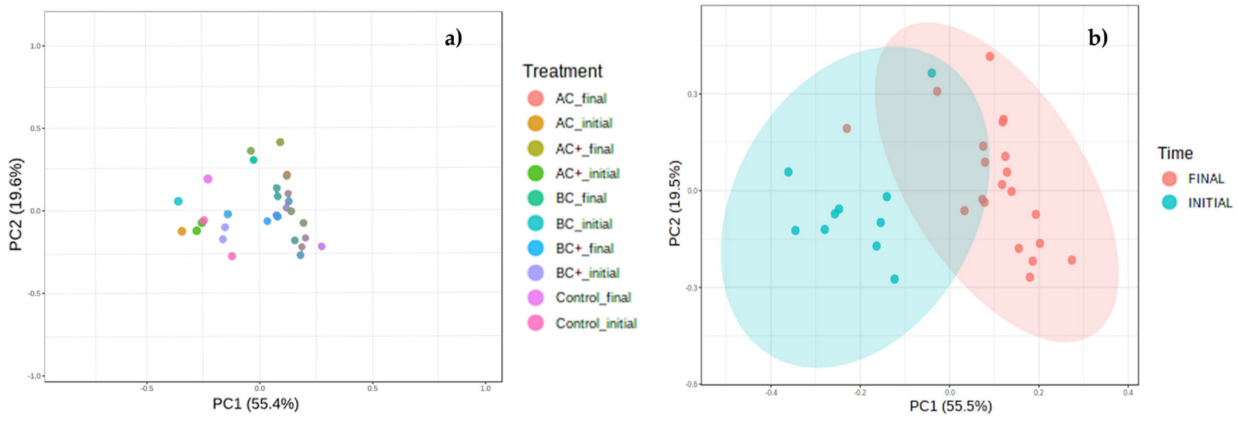

Figure 6. Principal component analysis of effect of treatment (**a**) and time (**b**) on molecular functions represented in terms of functional orthologs (KEGG KO) in control soil and amended soils at low (AC and BC soils) and high doses (AC+ and BC+ soils). Ellipses were drawn only when 95% of the data fell inside the ellipse.

4. Discussion

4.1. Bacterial Communities Composition and Abundance

In this work, we use a metagenomic approach to elucidate the effect of the addition of exogenous organic matter and sampling time influences on bacteria-driven soil-predicted functions.

The structure of the bacterial community was not particularly affected by the first input of organic matter, either alperujo or biosolid compost, although previous studies have shown the opposite. Parham and colleagues demonstrated the positive effect of cattle manure on the richness and evenness of the soil bacterial community, correlating this with soil productivity [40]. Other studies show how the incorporation of biochar or other organic fertilizers affects the abundance and the composition of the microbial community, as well as the crop yield [41], even in the short term [42]. However, a stronger effect of organic amendments not completely stabilized on microbial growth and community composition has also been widely evidenced and has been related to the greater contribution of easily available organic compounds from the former [43]. For our experiment, the use of two humified mature composts could have provided limited inputs of available C pools, therefore reducing the effect on soil microbial communities [21]. Another plausible explanation to the subtle compost effect could fall on the similarity of the microbial composition of the bacterial population introduced to the soil with the application of the composts and the soil-borne bacterial population [44]. However, these hypotheses do not fully explain the contrasting effect of the two composts on bacterial abundance. AC treatment increased significantly, and BC treatment significantly decreased bacterial abundances, while the application of a higher dose of both composts did not modify the abundance of bacteria compared to the control in the first sampling date. Furthermore, for the second sampling point, the abundance of bacteria was higher in the AC and BC treatments compared to the BC+ treatment, although no differences with the control could be detected for every treatment; therefore, the time between the compost application and sampling should be considered. It became apparent that the two different exogenous C sources induced different patterns of short-term soil disturbance, and only a better understanding of the chemistry of soil organic matter could shed light on the above trend. In fact, in-depth studies of organic C at the molecular composition level have been shown to be essential to assess soil quality and the effectiveness of soil restoration practices, particularly in degraded soils [45].

When observing each treatment separately, some changes in the relative abundance of certain phyla over time were evidenced. The phylum *Acidobacteria* is considered oligotrophs exhibiting slow growth rates in soils where the quality and/or quantity of organic C is high [46], and this may explain why the relative abundance of these groups decreased over time when compost was applied at higher doses (AC+ and BC+). This fact could suggest that both composts were rich in *Acidobacteria*. On the contrary, a greater availability of C substrates at the beginning could explain the decrease over time of *Alphaproteobacteria*, described as fast-growing copiotrophs. Additionally, *Actinobacteria* had generally been considered to be copiotrophic, and a relative increase in *Actinobacteria* over time can be related to greater decomposition of the recalcitrant carbon substrate, rather than a labile one [47].

The first addition of compost did not substantially modify the diversity indices either. However, some changes were detected at the end of the experiment both in the Simpson diversity index (D) and evenness at the OTU level in the AC and BC treatments. Given that the higher the D value, the lower the diversity, a loss of bacterial diversity was evidenced over time in these treatments. Evenness points to the similarity of OTU frequencies in bacterial populations, and the addition of compost at low doses caused a significant increase. This is consistent with a more even distribution of soil microbial communities in the context of high availability of C inputs, i.e., after compost addition, that decrease over time following the pattern of degradation of the easily available C sources, followed by microbial competition for the remaining hardly available ones.

Even though the evenness and richness of species are complementary, no differences were observed in the second; the number of OTUs was similar in the initial and final samplings (Table 1). However, the number of species per sample decreased in the non-amended control soils, as well as in the AC+ treatment, which indicates richness did not follow a behavior shape related to the addition of stabilized exogenous organic matter. Alfa diversity Chao1, the relative differences in the composition of species within a single treatment, and beta diversity patterns, the relative differences in the composition of species between samples, corroborated the lack of a clear effect of the addition of compost on soil bacteria community structure. Since the application of compost did not significantly affect the structure and diversity of the bacterial community, we hypothesized that the maturity and stability of the compost used caused in a certain way an effect similar to that evidenced for conservation or even zero tillage [48,49], in which the new C inputs are added to the soil in the form of vegetal litter in a context of low soil disturbance. Still, however, the effect of compost application on microbial resilience is unclear. It has been evidenced that most of the microorganisms transmitted by compost are outcompeted by the soil microorganisms once the compost is applied to the soil and then, the changes after compost amendment are mainly due to the input of organic matter and only marginally to the input of compost-borne microorganisms [50,51]. However, since species richness itself is not correlated with functional redundancy or microbial resistance [52], more empirical work focusing on functional groups is needed to clarify this point.

4.2. Bacterial Community Functional Potential

Metagenomic analysis was used to predict the functional potential of the bacterial community and the associated metabolic pathway network. Regarding the N cycle, although there was no difference between treatments or sampling times in urease-encoding genes (K01428_K01429_K01430_K14048), the potential abundance of denitrifying catabolic genes, norZ (K00376) and norB (K04561), increased significantly ($F = 8.442$, $p < 0.01$; $F = 3.363$, $p < 0.05$, respectively) in treatments that included alperujo compost as an organic amendment.

We also detected differences among treatments and a general increase over time on proteins related to quorum sensing pathways (K02035, K02033 and K02034) and carbon metabolism pathways (carbon monoxide dehydrogenase K00123 and carbon monoxide/quinone oxidoreductase K03520). These last two enzymes are related to the humification of soil organic matter, where it is known that catechol and o-quinones derived from biotic activity play a fundamental role in the synthesis of humic substances and/or the degradation of biopolymers such as lignin [53]. At the same time, the enzymes related to organic carbon mineralization, i.e., K05349 (beta-glucosidase [EC: 3.2.1.21]) and K15371 (glutamate dehydrogenase (GDH2) [EC: 1.4.1.2]), decreased. These results showed that the soil management under rainfed conditions proposed in this study (i.e., compost amendment and incorporation of weed biomass in an olive grove) affected some pathways of soil metabolism and environmental information processing in a manner comparable to that evidenced by other authors and for conservation management [54,55], promoting the humification of both exogenous and natural organic carbon [56]. Finally, we did not find significant differences in the global metabolic pathways related to the P cycle (K01113_K01077_alkaline phosphatase).

Despite some differences in certain biochemical pathways, all treatments shared all predicted functions related to soil bacteria (Table S2). The evidence that multiple bacterial species can fulfill a similar function (functional redundancy) must then be assumed, which would mean a high degree of stability of the resident soil bacterial community [57].

5. Conclusions

Achieving a net gain in C storage, especially under rainfed conditions, through management practices is becoming a fundamental tool to guarantee the continuity of agricultural soils. In this sense, strategies that include circular economy and zero waste

are being increasingly incentivized, urging greater utilization of organic amendments. The use of compost from urban and agricultural wastes is supposed to offer an important recycling tool to integrate these strategies. However, the vast variability of composts of different maturity and origins and of the soils to which they are added constrains the ability to predict a priori the impact of these amendments. Taken together, our results show how changes in soil bacterial community structure and potential functions were more related to sampling time than to adding compost at any dose. Overall, we only detect a minor and transient effect regardless of the dose for compost on soil microbial bacterial structure and behavior. This makes it impossible to reach firm conclusions of whether (i) the new community members originating from mature and stable composts did not strongly differ from the native soil bacteria, and therefore radical shifts cannot be detected, or (ii) the relatively conservative increase in organic matter introduced by way of the compost amendment (ca. 30%) was not enough to overcome or stimulate the native population and cause drastic shifts. Although our results support that agro-urban composts could represent a suitable alternative to inorganic amendments and that soil-borne bacteria were highly resilient, promoting community stability and functional resilience after the addition of alperujo or biosolids composts, more effort should be directed to assessing not only the soil-borne microbial community resulting after adding the fertilization but the intrinsic microbial community within the organic amendment that acts as an inoculum, and to what extent changes in its dose could drift soil functionality.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11111178/s1, Table S1: Tab-delimited taxonomy tables. Table S2: Abundance of predicted functions (KEGG modules) related to soil bacteria.

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