Article

Short-Term Dynamics of Bacterial Community Structure in Restored Abandoned Agricultural Soils under Semi-Arid Conditions

Natalia Rodríguez-Berbel [ID], Rocío Soria [ID], Ana B. Villafuerte, Raúl Ortega *[ID] and Isabel Miralles *

Department of Agronomy & Center for Intensive Mediterranean Agrosystems and Agri-Food Biotechnology (CIAIMBITAL), University of Almeria, E-04120 Almería, Spain
* Correspondence: rortega@ual.es (R.O.); imiralles@ual.es (I.M.)

Abstract: The restoration of unproductive abandoned agricultural soils under a semi-arid climate in southeastern Spain was evaluated. Four organic amendments from different composted wastes (greenhouse crop residues; worm compost from sheep–cow manure and plant remains; chicken manure; and sheep–chicken manure and plant remain) were applied, untreated control plots were installed, and natural unexploited soils were selected as a reference ecosystem. Through three sampling campaigns (initial, 3 months, and 12 months), significant changes were observed in soil physico-chemical properties, nutrient content (carbon -C-, nitrogen -N-, and phosphorus -P-), and bacterial composition of the restored soils with respect to control and natural soil, especially at 3 months. The increase of labile nutrients caused the proliferation of copiotrophic phyla at 3 months, which, after their consumption, were replaced by oligotrophic phyla at 12 months. Specific taxa involved in C, N, and P cycles were identified for each soil. For the soil bacterial composition of vermicompost, only chicken and sheep–chicken manures were more dissimilar to control and natural soils after 12 months, while greenhouse crop compost showed an intermediate position between them. Results indicated that composted greenhouse crop remains could be an optimal treatment for the short-term recovery of physico-chemical properties, nutrient content, and bacterial composition of agriculture-degraded soils in semi-arid areas.

Keywords: ecological restoration; carbon; nitrogen; phosphorus; metagenomic analysis; bacterial community composition

1. Introduction

The exploitation of fertile soils for agricultural activities generates degraded soils susceptible to abandonment due to lack of productivity. These soils tend to lose their capacity to regulate the ecosystem, which, together with functional deterioration, leads to serious biodiversity declines [1]. In arid and semi-arid areas, the particular climatic factors, such as low rainfall and weather irregularity [2] as well as the low organic matter content of soils [3,4], aggravate this problem, making natural restoration processes more difficult [5]. In agroecosystems, a crucial determinant of soil quality is the organic matter content, given that it is intimately linked to biological activity, nutrient cycling, and crop productivity [6]. For these reasons, to successfully recover these soils, restoration techniques must be employed that are oriented not only to repairing the physical and chemical soil properties, but also to re-establishing the biological communities [2], as there is increasing evidence of the fundamental role played by the microbial composition in soil fertility [7]. Therefore, the application of composted organic wastes could be a good strategy to avoid the abandonment of non-productive land under a semi-arid climate, because their high organic matter content [8] favors soil fertility [9] and could accelerate the recovery of these degraded soils, in addition to contributing to the European Circular Economy Strategy through the reuse of organic waste produced by local industrial activities in the restoration

of semi-arid degraded soils. The addition of organic amendments benefits the physical, chemical, and biological soil properties [10–13], favoring the supply of nutrients and improving soil functionality [11,12,14,15]. The organic compounds that make up these composted residues depend on their organic provenance and are directly related to their nutrient content (C, N, and P) [16], causing stoichiometric variations in the C:N:P ratio [17] of the amended soils.

Previous studies have suggested significant differences in the structure of soil bacterial communities after soil restoration, which together with soil characteristics determine the bacterial structure of the soil [10,13,18]. Thus, soil bacterial composition is explained not only by edaphic variables [11,19] but also by the nutrients provided from the different origins of the organic amendments used [20,21]. As a consequence, the behavior of the soil microbiome responds to the availability of nutrients [20], and these changes will alter the C, N, and P cycles affecting the biological and physico-chemical reactions carried out by soil micro-organisms [22]. Consequently, the type of organic matter [21] together with the capacity and efficiency of the micro-organisms in the decomposition of the organic matter provided [16] could have a differential effect on the structure of the soil bacterial community, influencing the proliferation of some taxa versus others better adapted to the applied amendment [18]. Therefore, bacterial succession induced by substrate availability could have possible beneficial effects on the functionality and stability of the agroecosystem [23]. However, there are few studies that support short-term monitoring of restorations with the organic amendment of semi-arid abandoned agricultural soils. Likewise, the differential effect of the type of organic amendment applied on the physico-chemical properties and nutrient content of the soil as well as their impact on soil bacterial communities at the genus level have not yet been explored.

Therefore, three sampling campaigns were carried out at the beginning, 3 and 12 months of the restoration of an abandoned agricultural soil using different organic amendments with the main objective of studying the changes in soil physico-chemical properties and nutrient content, and their impact on soil bacterial composition. The working hypothesis was that the application of organic composted residues will improve soil quality and fertility by enhancing the physico-chemical properties and nutrient content of the soil, and consequently, altering the structure of the soil bacterial community involved in C, N, and P cycling. This research should result in a better understanding of the changes produced after the application of different organic amendments, facilitating their appropriate selection for the recovery of the bacterial community in abandoned agricultural soils under a semi-arid climate.

2. Materials and Methods

2.1. Site Details and Field Experimental Design

An old dry farming area located in the Sierra de Gádor range (Almería, SE Spain) was selected to conduct the restoration task. The experimental site was located in abandoned cultivation terraces without slope, with soils developed on limestones, dolomite, and phyllites rocks classified as Leptosols [24]. Grapes, almonds, and cereals were grown on these soils, being abandoned due to lack of productivity more than 50 years ago. It is located at an altitude of 977 m. a. s. l. (36°53′20″ N, 02°36′14″ W) and at approximately 10 linear km from the sea. The climate of the area corresponds to the semi-arid Mediterranean climate (with rainfall of 398.50 mm yr^{-1} over the last 24 years [25]; temperatures of 17 °C yr^{-1} with long dry periods and high solar radiation). Through randomly distributed linear transects, a vegetation inventory was carried out in the natural areas that have never undergone agricultural exploitation. The vegetation was mainly composed of *Retama sphaerocarpa* L. Boiss., *Anthyllis cytisoides* L., *Stipa tenacissima* L., *Thymus zygis* L., *Cistus albidus* L., and some spare specimens of *Pinus halepensis* Mill., among others.

The restoration work was carried out in the first week of October 2020. Initially, soil decompaction was performed, and subsequently, 15 experimental plots of 35 m^2 (7 m × 5 m) were designed, 3 replicates per treatment, and 3 untreated control plots (CON). A previous

selection of amendments from different types of organic wastes was carried out in order to determine if the composition of nutrients derived from the source of origin affect in a dissimilar way the recovery of semi-arid restored soils. A compost of different plant remains was used as resilient organic matter [11,12]. Animal manure rich in labile organic matter, such as chicken manure, was used to provide organic matter rich in N and P [26]. A vermicompost from the bio-oxidative digestion of some species of earthworms and their digestive microbiota was chosen because of its benefits in promoting plant growth and against pathogens [27] as well as for the contribution of diverse organic matter rich in N, P, and micronutrients [28]. Lastly, an amendment consisting of a mixture of animal manure and plant debris was chosen in order to obtain a balanced composition between labile and resilient organic matter. Finally, four types of composted organic amendments were added, mixed, and homogenized to a depth of 20 cm using a rotary tiller. The composted amendments consisted of both plant and animal wastes: (i) compost from fruit and vegetable greenhouse crop residues (GC); (ii) organic compost derived from floor-raised chicken manure on ecological farms (CM); (iii) vermicompost from sheep and cow manures with vegetable remains (VC); and (iv) certified organic compost derived from sheep manure, chicken manure, and vegetable waste from grass and straw (SC). A single dose was used to increase the initial organic matter content by 1.5% above the degraded soil without amendment. To contribute to soil restoration, four species of native aromatic plants, such as *Rosmarinus officinalis* L. Schleid., *Lavandula latifolia* Medik., *Thymus zygis* L., and *Anthyllis cytisoides* L., were selected for their ability to adapt to climatic conditions and for their biotechnological potential in the pharmaceutical and cosmetic industry [29]. In addition, the suitability of the introduced plants was consulted with the flora technicians of the region and contrasted with the Forest Restoration Models of the Andalusian Government [30]. Subsequently, 15 plants of each aromatic species were planted in each experimental plot (60 plants per plot) using a planting frame of 60 cm × 60 cm. In addition, they were planted adjacent to natural vegetation that has never been submitted to agricultural activities, and preserved natural vegetation soils were selected as reference ecosystem (NAT) [31]. One week after completion of the restoration (19 October 2020; t1), three months (29 January 2021; t3), and one year later (22 October 2021; t12), soil samples composed of 10 random subsamples were collected from each experimental plot at a depth of 0–10 cm. The samples were transported to the laboratory cold, and DNA extraction analyses were immediately performed. Then, soil samples were air-dried, sieved (Ø 2 mm) and stored at 4 °C for physico-chemical property and nutrient content (C, N, and P) analyses.

2.2. Soil Physico-Chemical Properties Measurements

The pH and electrical conductivity (EC) were determined in a distilled water solution (1:2.5 and 1:5 w/v, respectively) measured with a pH meter (LAQUA PH1100, Horiba, Tokyo, Japan) and conductivity meter (LAQUA EC1100, Horiba, Tokyo, Japan). Total organic carbon (TOC) was determined by colorimetric method described by Mingorance et al. [32] and measured with a spectrophotometer, Spectronic Helios Gamma UV-Vis (Thermo Fisher Scientific, Waltham, MA, USA), and total nitrogen (TN) with an elemental analyser TCD detector (ELEMENTAR Rapid N; Elemental Analysen systems GmbH, Hanau, Germany). Available phosphorus (AP) was determined by Olsen and Watanabe (1957) method [33]. The results of TOC, TN, and AP contents were used to calculate the carbon–nitrogen (C:N), carbon–phosphorus (C:P), and N:P ratios [34]. Finally, available water (AW) was calculated from the pF values at −33 and −1500 KPa using the Richards membrane method [35].

2.3. Bacterial Community Composition and Sequencing Data Processing

Total soil DNA was extracted from each sample (n = 18) using the commercial DNA Power Soil kit (QIAGEN, Hilden, Germany) and quantified by ND-2000 Nanodrop spectrophotometer (Thermo Scientific, Waltham, MA, USA), owned by the "Nucleic Acid Analysis Service" located at the "Central Research Services" of the University of Almería.

The V4–V5 regions of the bacterial 16S rRNA were amplified by polymerase chain reaction (PCR) and sequenced by Illumina MiSeq platform [36]. Then, QIIME2 software (version 2019.7) was used for sequence processing following the protocol established by Comeau et al. [37] to identify the soil bacterial community at phylum level and genus level or the next upper taxonomic level identified in the different soils analyzed at each sampling time (t1, t3, and t12). Soil bacterial taxa with a relative abundance $\geq 0.1\%$ in all samples were selected.

2.4. Statistical Analysis

Measurements of selected physico-chemical and bacterial soil properties analyzed were performed in triplicate for each treatment. A permuted multivariate and univariate analysis of variance (PERMANOVA) was used to determine the differences as a function of treatment and elapsed time for chemical properties and nutrient stoichiometry (Euclidean distance) as well as for bacterial community structure (Bray–Curtis) in restored, natural, and control soils [38], which included two factors: soil treatment and time of field sampling. In cases where PERMANOVA detected a significant effect ($p < 0.05$), the origin of differences was assessed by comparing treatment pairs with post PERMANOVA (Monte Carlo) test pairs, and results with $p < 0.05$ were reported as significant. Differences in physico-chemical soil properties and stoichiometric relationships between treatments were evaluated by principal component analysis (PCA). A previous distance-based linear modelling analysis (DistLM) was performed on genus-level relative abundance data of bacterial taxa and abiotic variables (physico-chemical properties and nutrient stoichiometry) of the soil to determine the relative importance in the interrelationships between soil bacterial composition and edaphic conditions in the different sampling campaigns (t1, t3, and t12). Then, a distance-based redundancy analysis (dbRDA) was employed on bacterial taxa data to build a regression model against the abiotic variables. The Akaike Information Criterion (AICc) was employed for selecting the best model, and the stepwise approach for building the model was followed [39]. PERMANOVA, PCA, DistLM, and dbRDA statistical analysis were performed with PRIMER-E + PERMANOVA software (PRIMER-E Ltd., Plymouth Marine Laboratory, Plymouth, UK). To identify those bacterial taxa at the genus or the next upper taxonomic level identified that were specifically associated with each treatment (restored, control, and natural reference soil), an indicator species analysis (ISA) was conducted using the IndicSpecies R package, using the *multipatt* function (9999 perm) [40]. Pearson correlations ($p < 0.05$) were calculated using *corrplot* and *RColorBrewer* R packages in order to evaluate the interrelationships between the bacterial taxa identified by species-index analysis and soil physico-chemical properties and stoichiometric components.

3. Results

3.1. Changes in Soil Physico-Chemical Properties and Stoichiometry Nutrients over Time

The application of organic amendments progressively changed the physico-chemical soil properties (EC, pH, and AW), increased nutrient content (TOC, TN, and AP), and modified the stoichiometry of nutrients (C:N, C:P, and N:P). The multivariate PERMANOVA analysis applied to soil properties and nutrients stoichiometry showed that the treatments were significantly different ($p < 0.05$) for both time and treatment factors, but not in the interaction between them (Table S1a). All treated soils showed a significantly higher ($p < 0.05$) EC than CON and NAT in all soil sampling campaigns, being significantly higher at t1 just after amendment application (Table 1). The treatments significantly decreased the pH with respect to CON soils, with SC and CM having significantly lower values ($p < 0.05$) at t1, while the pH of GC and VC was similar to CON and NAT (Table 1). t3 and t12 had similar trends, except for CM showing the lowest significant pH values at t12. Restored and control (CON) soils showed significantly lower water availability (AW) than in the reference soils (NAT) just after the start of the experiment (t1); however, this became similar over time (Table 1). All restored soils had significantly higher TOC, TN, and AP content

than CON, and were similar to NAT in all sampling campaigns, except for AP, which also showed significantly higher values ($p < 0.05$) than NAT during all sampling campaigns (Table 1). The TOC values were maintained in VC and GC soils in all sampling times, while there was a significant decrease in CM over time and a slight increase in SC at t12 (Table 1). VC and CM presented significantly higher TN content, followed by SC, and then, lastly, GC. This trend was maintained in the restored soil during all sampling campaigns. On the other hand, VC, SC, and CM had the significantly highest AP values and GC the smallest in all periods of study, but significantlu lower values were presented at t1, while its content increased at t3 and t12 (Table 1). Regarding the stoichiometric nutrient ratios, all restored soils showed a C:N ratio significantly superior to CON and similar to NAT, and so, the results at t1, t3, and t12 experienced a comparative decline over time (Table 1). NAT and CON presented high C:P values together with GC, being significantly lower in VC, SC, and CM, which decreased further through time. This trend was clearest at t1, but GC had C:P values similar to CON and below NAT at t3 and t12 (Table 1).

Table 1. Soil physico-chemical properties and main nutrient ratios of newly reclaimed abandoned agricultural soils with incorporation of different organic amendments.

		GC	VC	SC	CM	CON	NAT
t1	EC (mS cm^{-1})	0.98 ± 0.01 aA	0.63 ± 0.8 bA	0.93 ± 0.09 abA	1.02 ± 0.11 aA	0.10 ± 0.00 cA	0.09 ± 0.00 cA
	pH	8.13 ± 0.04 abA	8.25 ± 0.01 acA	8.07 ± 0.02 bA	8.01 ± 0.01 bA	8.44 ± 0.08 cAB	8.31 ± 0.07 acA
	TOC (%)	2.55 ± 0.24 aA	4.01 ± 0.55 aA	2.81 ± 0.30 aAB	2.80 ± 0.13 aA	1.78 ± 0.12 bA	4.25 ± 0.59 aA
	TN (%)	0.29 ± 0.02 aA	0.39 ± 0.02 bA	0.367 ± 0.04 abA	0.370 ± 0.01 bA	0.210 ± 0.01 cA	0.393 ± 0.04 abA
	AP (%)	0.004 ± 0.00 aA	0.028 ± 0.0 b0A	0.019 ± 0.00 bA	0.023 ± 0.00 bA	0.002 ± 0.00 aA	0.004 ± 0.00 aA
	C:N	8.63 ± 0.45 aA	10.25 ± 0.86 bA	7.67 ± 0.25 abA	7.56 ± 0.19 bA	8.52 ± 0.20 cA	10.78 ± 0.48 abA
	C:P	581.75 ± 148.35 aA	141.75 ± 5.77 bA	141.84 ± 10.34 bA	120.35 ± 8.87 bA	730.80 ± 132.85 aA	1022.07 ± 100.00 aA
	N:P	66.15 ± 13.31 aA	13.95 ± 0.82 bA	18.54 ± 1.65 bA	15.87 ± 0.79 bA	86.57 ± 17.95 aA	95.69 ± 12.89 aA
	AW (%)	9.66 ± 0.26 aA	9.19 ± 0.39 aA	9.52 ± 0.24 aA	9.47 ± 0.30 aA	9.12 ± 0.37 aA	13.02 ± 1.01 bA
t3	EC (mS cm^{-1})	0.20 ± 0.01 aAB	0.24± 0.01 abB	0.43 ± 0.06 bB	0.72 ± 0.16 bA	0.09 ± 0.01 cA	0.07 ± 0.01 cA
	pH	8.38 ± 0.14 abcA	8.35 ± 0.05 aA	8.04 ± 0.04 bA	8.05 ± 0.08 bcB	8.34 ± 0.07 acB	8.03 ± 0.05 bB
	TOC (%)	2.17 ± 0.23 abA	3.20 ± 0.13 cA	2.48 ± 0.04 aA	2.74 ± 0.27 acAB	1.64 ± 0.18 bAB	3.44 ± 0.14 cA
	TN (%)	0.27 ± 0.02 aA	0.35 ± 0.00 bA	0.34 ± 0.01 bA	0.32 ± 0.04 abA	0.14 ± 0.01 cA	0.33 ± 0.01 abA
	AP (%)	0.015 ± 0.00 aB	0.12 ± 0.00 bB	0.05 ± 0.00 cB	0.07 ± 0.02 abcB	0.007 ± 0.00 dA	0.004 ± 0.00 dA
	C:N	8.12 ± 0.43 aA	9.15 ± 0.23 bA	7.29 ± 0.38 bA	8.68 ± 0.54 abA	8.84 ± 0.43 cA	10.36 ± 0.03 abA
	C:P	139.91 ± 3.24 aB	26.58 ± 0.96 bB	54.05 ± 7.14 cB	42.63 ± 9.13 bc B	246.07 ± 40.96 aB	840.35 ± 184.24 dA
	N:P	17.3 ± 1.04 aB	2.90 ± 0.16 bB	7.35 ± 0.58 cB	4.90 ± 1.03 bcB	28.43 ± 6.31 aB	81.18 ± 18.03 dA
	AW (%)	10.70 ± 0.36 aA	8.42 ± 2.13 abA	9.65 ± 0.41 aA	10.08 ± 0.27 aA	10.43 ± 1.68 abA	13.12 ± 0.24 bA
t12	EC (mS cm^{-1})	0.32 ± 0.06 aB	0.45 ± 0.08 abA	0.47 ± 0.04 aB	1.30 ± 0.30 bA	0.10 ± 0.01 cA	0.08 ± 0.00 cA
	pH	8.76 ± 0.16 aB	8.55 ± 0.14 abB	8.11 ± 0.00 cA	7.67 ± 0.11 dC	8.54 ± 0.01 aA	8.19 ± 0.02 bAB
	TOC (%)	2.61 ± 0.22 abA	3.47 ± 0.20 cA	2.86 ± 0.05 aB	2.23 ± 0.12 bB	1.31 ± 0.05 cB	3.03 ± 0.03 aA
	TN (%)	0.27 ± 0.01 aA	0.39 ± 0.01 bA	0.34 ± 0.01 bA	0.33 ± 0.04 abA	0.16 ± 0.00 cA	0.28 ± 0.00 aA
	AP (%)	0.01 ± 0.00 aB	0.158 ± 0.02 bB	0.055 ± 0.00 cB	0.141 ± 0.02 bB	0.003 ± 0.00 dA	0.004 ± 0.00 dA
	C:N	9.51 ± 0.29 aA	8.70 ± 0.36 bA	8.42 ± 0.27 bA	6.86 ± 0.66 abA	8.14 ± 0.26 cA	10.7 ± 0.37 aA
	C:P	214.96 ± 41.2 aAB	23.22 ± 4.40 bB	51.78 ± 4.08 cB	16.42 ± 2.43 bC	457.89 ± 141.61 adAB	728.85 ± 133.25 dA
	N:P	22.36 ± 3.75 aB	2.63 ± 0.42 bB	6.13 ± 0.29 cB	2.48 ± 0.52 bB	55.19 ± 15.86 adAB	67.34 ± 11.16 dA
	AW (%)	10.63 ± 1.59 abA	8.91 ± 1.33 abA	8.42 ± 1.23 abAB	7.37 ± 0.67 aB	8.49 ± 1.17 abA	11.02 ± 0.07 bA

EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; AP: assimilable phosphorus; C:N: carbon-to-nitrogen ratio; C:P: carbon-to-phosphorus ratio; N:P: nitrogen-to-phosphorus ratio; AW: available plant water. GC: greenhouse crop residues compost; VC: vermicompost; SC: organic compost derived from the mixture of chicken and sheep manure; CM: manure from chickens raised on the soil of organic farms; CON: unamended control soils; NAT: natural reference soil. t1: initial soil sampling; t3: soil sampling at 3 months; t12: soil sampling at 12 months. The lowercase letter indicates significant differences ($p < 0.05$) between treatments at the same sampling time. The uppercase letter indicates significant differences ($p < 0.05$) of the same treatment between different sampling times (t1, t3, and t12).

These results were confirmed by the PCA performed shown in Figure 1. The first two component axes accounted for 40.7% and 25.7% of the total variance of the soil properties and stoichiometry of nutrients, explaining a total of 66.4% (Figure 1). The samples for soil physico-chemical properties and nutrients stoichiometry were approximately grouped, where the distance between samples represented the extent of the differences. PC1 clearly differentiated soils treated with compost derived from animal waste (CM, SC) and vermicompost (VC). On the opposite side (negative PC1 values), NAT and CON soils were clearly located, while vegetable composts (GC) were in an intermediate position between the two previous clusters (Figure 1a). On the other hand, PC2 separated the control soils in its positive values, and practically all the samples of the GC treatment were separated from

the rest of the treatments and NAT soils, which were mainly located in the negative values of PC2 (Figure 1a). Therefore, the groups emphasized that, in the short term (Figure 1b), an improvement of soil quality is related to the treatment, as they were positioned closer to NAT soils. In addition, the arrow lines of AP, TN, TOC, and EC influenced mainly SC, CM, and VC, while the AW and stoichiometric ratios (C:N, C;P, and N:P) were declined for NAT, and the pH for CON soils (Figure 1; Table S2).
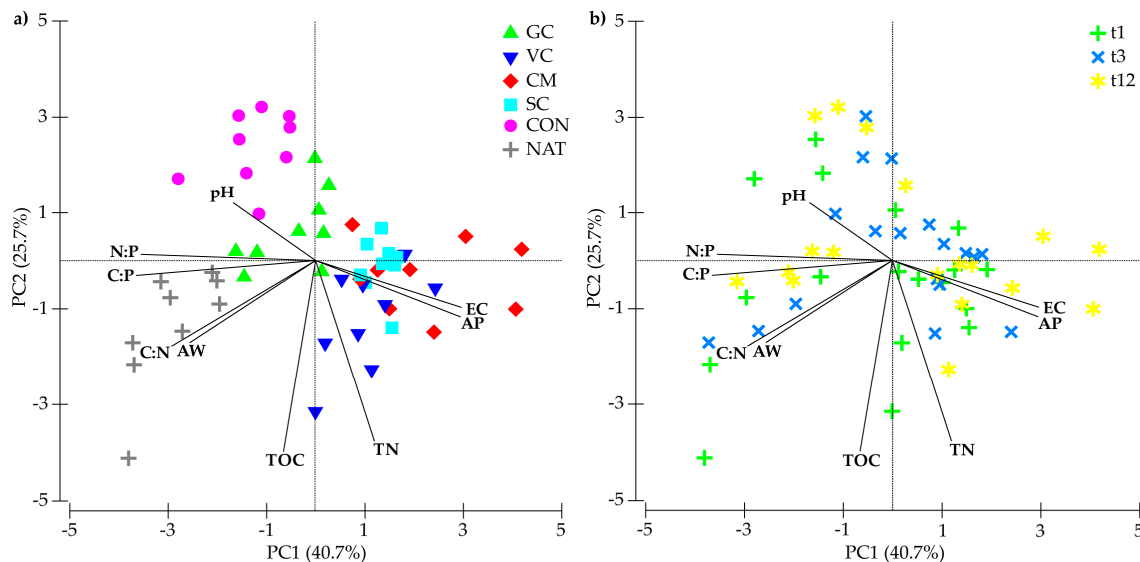

Figure 1. Principal component analysis (PCA) of soil physico-chemical properties and stoichiometry of nutrients (C, N, and P) between treatments (**a**) and between soil sampling campaign (**b**) EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; AP: assimilable phosphorus; C:N: carbon-to-nitrogen ratio; C:P: carbon-to-phosphorus ratio; N:P: nitrogen-to-phosphorus ratio; AW: available plant water. GC: greenhouse crop residues compost; VC: vermicompost; SC: organic compost derived from the mixture of chicken and sheep manure; CM: manure from chickens raised on the soil of organic farms; CON: unamended control soils; NAT: natural reference soil. t1: initial soil sampling; t3: soil sampling at 3 months; t12: soil sampling at 12 months.

3.2. Bacterial Community Composition

The composition of the bacterial soil community at the phylum level varied significantly ($p < 0.05$) between the different treatments (GC, VC, SC, CM, CON, and NAT) and campaigns (t1, t3. and t12; Table S1b). Proteobacteria (31.9%), Bacteroidetes (21.8%), Planctomycetes (13%), Actinobacteria (10.6%), Acidobacteria (10.1%) and Firmicutes (4.1%) were the most abundant bacterial phyla in the soil samples (Figure S1). The phylum Proteobacteria was the most abundant in all treatments, being the most abundant at 3 months of the restoration (t3) in vegetable compost soils (GC; 37.9%), and t3 and t12 in SC soils (35.4% and 38.6%, respectively). Bacteroidetes phylum showed the highest abundance, mainly at t3 in soils treated with manures (SC = 47.5% and CM = 48.9%). GC and vermicompost (VC) treatments favored the presence of Planctomycetes and Acidobacteria (Figure S1). The abundance of Planctomycetes in GC and VC soils increased over time (18.1% and 16.2%, respectively), but without reaching the values recorded in CON (19.8%) and NAT soils (15%). The VC treatment mainly favored the phylum Chloroflexi (t1 = 5.4%; t3 = 2.9%; t12 = 6.1%) with respect to the rest of restored, CON (t12 = 3.7%), and NAT (3.5%) soils, while Actinobacteria decreased at t3 (6.4%) and increased at t12 (18.2%) in VC soils, approaching the values of the reference soils (NAT; 18.5%). The application of GC (23.9%), SC (12.4%), and CM (11.9%) promoted the presence of Firmicutes at t1 with respect to CON (2.2%) and NAT soils (1.3%; Figure S1). Finally, CON and NAT soils showed scarce changes in the relative abundance of over time.

At the genus level or the next upper taxonomic level identified, a total of 181 bacterial taxa were found. The bacterial soil membership at the genus level was significantly different ($p < 0.05$). both among the different treatments and among three monitoring campaigns performed (Table S1c).

3.3. Relationships among Soil Chemical Properties, Nutrient Stoichiometry, and Bacterial Communities

The DistLM analysis in combination with the best linear distance model ($R^2 = 0.37$; AICc = 345.14) explained that all abiotic soil variables studied (physico-chemical properties and nutrient stoichiometry) were key in the development of the soil bacterial community (Table S3). Moreover, dbRDA results revealed a distinct clustering of soil bacterial communities for soils restored with organic amendment, control, and natural reference soils (Figure 2 and Table S3). The soil bacterial taxa of the VC, CM, and SC treatments were separated from NAT and CON soils (positive and negative dbRDA1 values, respectively; Figure 2a), while GC-treated soils were placed in an intermediate position between the two previous groups. Furthermore, the results of dbRDA1 in this study revealed that EC and PA exerted a significant influence on the bacterial composition of soils treated with animal-derived composts (VC, CM, and SC; positive dbRDA1 values). In contrast, for negative dbRDA1 values, samples collected at t1 and t3 from exclusively plant-derived compost (GC) and CON and NAT soils were influenced by pH, TN, TOC, and nutrient stoichiometry (C:N, C:P, and N:P; Figure 2b). Regarding the dbRDA2 axis, control and VC-treated soils had positive values influenced by AP, C:N, pH, and TN, while N:P, TOC, and AW were negatively influencing GC-treated soils, and SC and CM in campaign t1 (Figure 2a). Regarding the timing of the different campaigns, the soil bacterial community of the treatments at t1 showed a more similar behavior, clustering around the origin of the co-ordinates (Figure 2b). However, at t3 and t12, the bacterial composition was better differentiated among treatments, especially in soils treated with VC, CM, and SC (Figure 2b).

3.4. Indicator Bacterial Communities in Different Restored, Control, and Reference Soils

Soil bacterial taxa sensitive to restoration strategies using organic amendments were identified through the analysis of indicator species for the total number of sampling campaigns over time (Figure 3). The results showed that, among the bacterial taxa identified, 53 were potential indicator species for soils treated with organic amendments employed together with CON and NAT soils (Table S4). Specifically, five indicator bacterial taxa belonged to CG treatment, seven to VC, 10 to CM treatment, and three to SC treatment. Also, five bacterial taxa were obtained as indicator species from the control soils (CON), while the reference soils (NAT) showed 23 different taxa (Figure 3 and Table S4).

3.5. Correlation Analysis between Bacterial Taxa from Indicspecies Analysis and Soil Parameters

Different correlation patterns were found among the 53 selected as treatment indicator physico-chemical properties (EC, pH, TOC, TN, AP, AW) and stoichiometric nutrient ratios (C:N, C:P, N:P) in the different sampling campaigns (t1, t3, and t12) (Figure 4). Overall, the highest number of significant correlations ($p < 0.05$) was found at time t3, followed by t12, while it was lower at t1. The bacterial taxa of the amended soils clearly differed from those belonging to the natural reference soils (NAT) in terms of their relationship with the soil properties studied (Figure 4). Specifically, in CM-treated soils, the bacterial groups identified with the highest significant positive correlations were related to EC for sampling times t3 and t12 (Figure 4). *Alcaligenes, Atopostipes,* and *Sphingobacterium* showed high significant positive correlations with EC. Other bacteria indicative of CM treatment in addition to EC also showed correlations with AP, especially at t12, such as *Arthrobacter, Nitrosospira, Pontibacter, Leifsonia,* and *Myroides,* the last two being also correlated with TOC at t1 (Figure 4). Similarly, in VC-treated soils, bacteria such as *Truepera, Alistipes, o_SBR1031_g_anaerobic bacterium MO-CFX2, o_Ardenticatenales_bacterium YC-LK-LKJ3,* and genera belonging to the Xanthomonadaceae and Flavobacteriaceae families had an intense

positive significant correlation with AP during the whole study period. In addition, the last four also correlated with TOC at t1. Regarding CG and SC treatments, the trends were clear regarding the high number of negative correlations with stoichiometric ratios, coinciding in this aspect as VC and CM; however, GC showed that some bacterial taxa such as *Staphylococcus* had significant positive correlations with pH in t3. Other taxa, such as *UTCFX1* and c_Subgroup 6_o_unknown_f_unknown, followed the same trend for pH in CON soils (Figure 4). On the other hand, the bacterial taxa belonging to the NAT soils had an opposite affinity with the soil properties. The NAT soil indicator bacteria showing the highest significant correlations with the stoichiometric ratios C: N, C:P, and N:P (Figure 4), and which were also negatively correlated with EC and AP, included the genera *Blastococcus*, *RB41*, *Segetibacter*, *Tychonema CCAP 1459-11B*, *Psychroglaciecola*, *PMMR1*, *Reyranella*, *Rhodoplanes*, *Zavarzinella*, and *Ellin6067* (Figure 4).
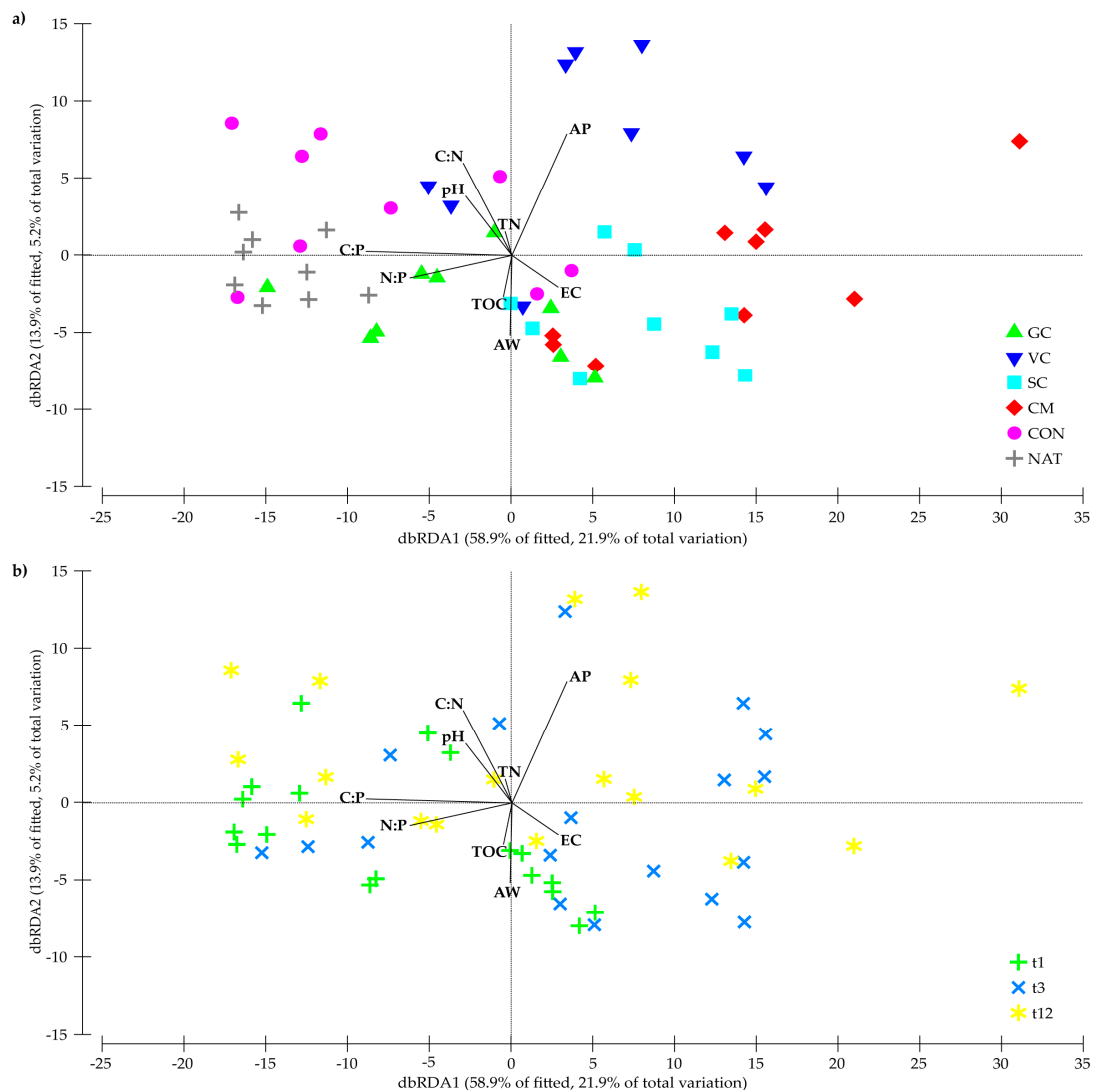
Figure 2. Distance-based redundancy analysis (dbRDA) of soil bacterial community and physico-chemical properties for restored, control, and natural reference soils (**a**), and for soil sampling campaign (**b**). EC: electrical conductivity; AW: available plant water; TOC: total organic carbon; TN: total nitrogen; AP: assimilable phosphorus; C:N: carbon-to-nitrogen ratio; C:P: carbon-to-phosphorus ratio; N:P: nitrogen-to-phosphorus ratio. GC: greenhouse crop residues compost; VC: vermicompost; SC: organic compost derived from the mixture of chicken and sheep manure; CM: manure from chickens raised on the soil of organic farms; CON: unamended control soils; NAT: natural reference soil. t1: initial soil sampling; t3: soil sampling at 3 months; t12: soil sampling at 12 months.
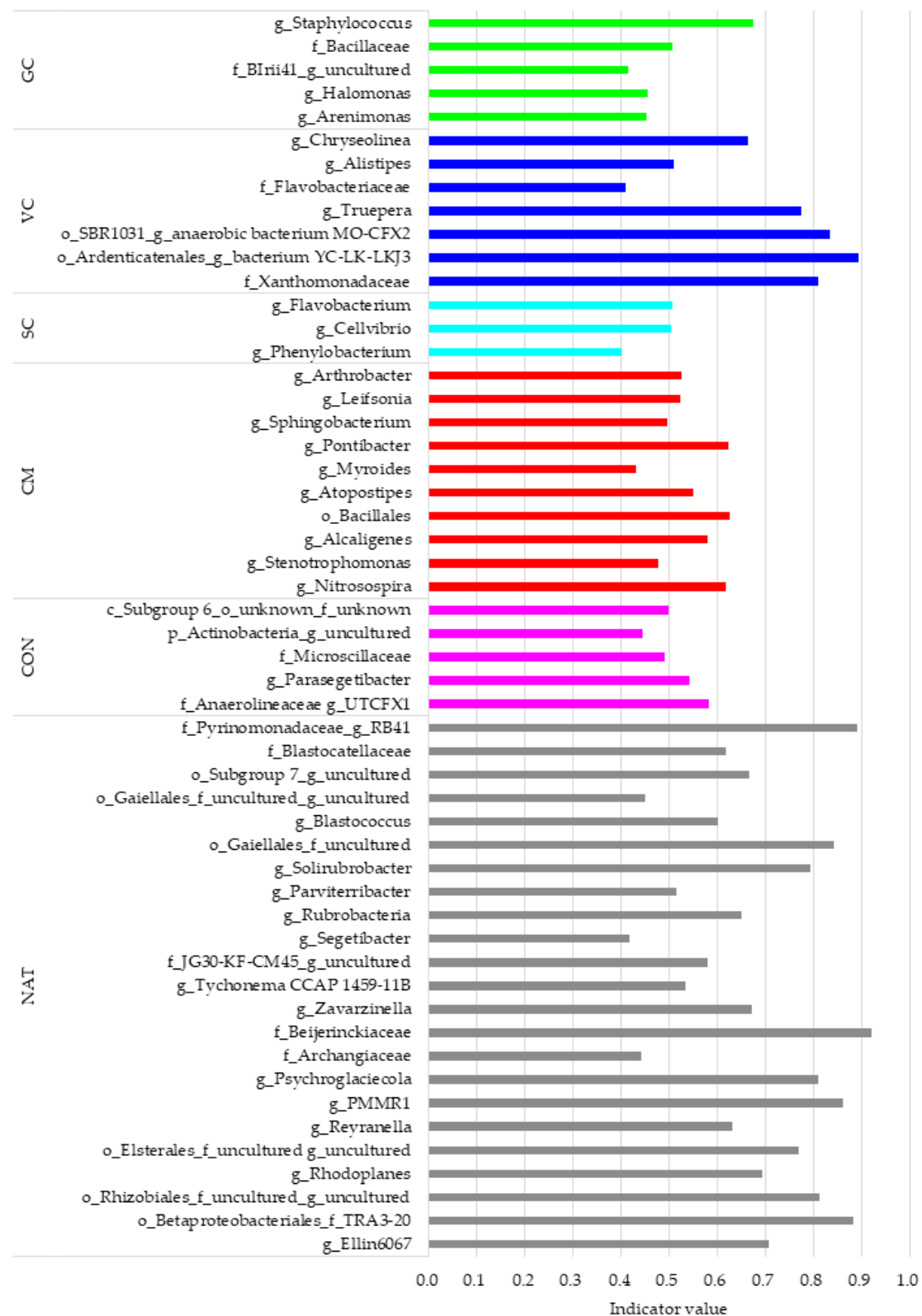
Figure 3. Soil bacterial taxa selected by indicator species analysis for different treatments (restored, control, and natural reference soils). GC: greenhouse crop residues compost; VC: vermicompost; SC: organic compost derived from the mixture of chicken and sheep manure; CM: manure from chickens raised on the soil of organic farms; CON: unamended control soils; NAT: natural reference soil.
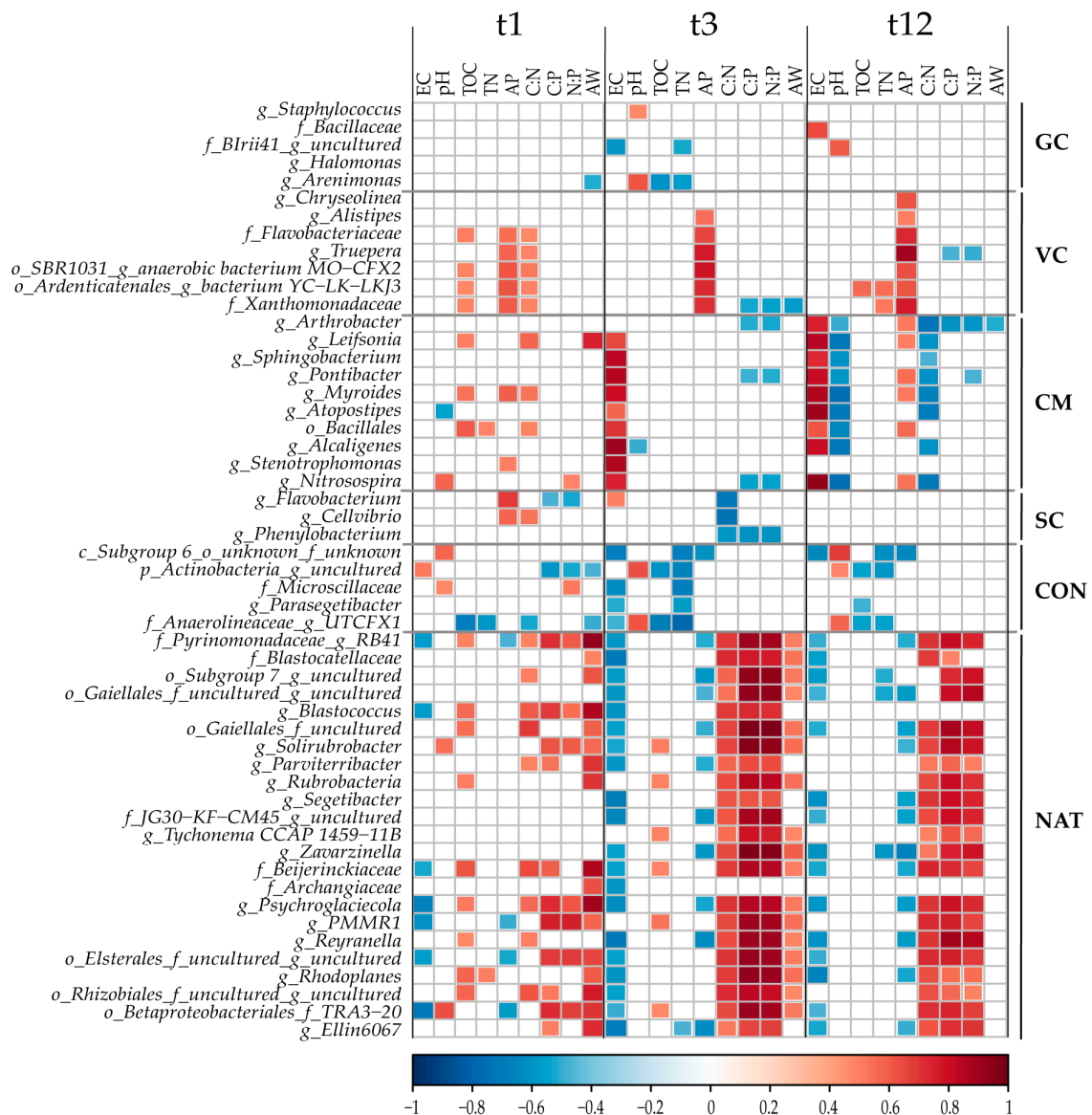
Figure 4. Significant Pearson correlation ($p < 0.05$) between soil physico-chemical properties and the groups of bacterial taxa selected as indicators of each restored, control, and natural reference soils, for each sampling campaign (t1, t3, and t12). EC: electrical conductivity; AW: available plant water; TOC: total organic carbon; TN: total nitrogen; AP: assimilable phosphorus; C:N: carbon-to-nitrogen ratio; C:P: carbon-to-phosphorus ratio; N:P: nitrogen-to-phosphorus ratio. GC: greenhouse crop residues compost; VC: vermicompost; SC: organic compost derived from the mixture of chicken and sheep manure; CM: manure from chickens raised on the soil of organic farms; CON: unamended control soils; NAT: natural reference soil.

4. Discussion

Soil physico-chemical properties and bacterial metagenomic analysis at the phylum and genus level provided evidence on the impact of the addition of organic amendments from different origins on soil bacterial communities. Our results provide information to facilitate the selection of organic amendments which could favor the recovery of abandoned agricultural soils in a semi-arid climate by studying the short-term response of edaphic bacterial populations.

The organic amendment application modified the physico-chemical properties of the restored soils, differentiating them from control soils and natural soils over time (Table S1a). The most marked changes were observed between the beginning and 3 months after

restoration (t1 and t3), with the greatest alterations in soil bacterial communities (Figure 2b). Calcareous soils in semi-arid regions, such as the study area, are characterized by a low nutrient content [3,4]. Thus, the composted waste used produced a significant increase in TOC, TN, and AP for all treatments, causing a nutrient stoichiometric imbalance (C:N, C:P, and N:P; Table 1). These changes were more notable in soils amended with animal compost (VC, SC, and MC), which also suffered an increase in salinity compared to untreated (CON) and reference soils (NAT; Table 1). Decomposition of added organic matter could lead to immobilization of available nutrients, causing competition for mineral N and available P between soil organisms, and leading to modifications in the bacterial community [41]. Previous studies have shown that compost from plant waste has a higher organic matter resilience than compost from animal waste [12,42]. This could explain the milder changes in nutrient content in the very short term (t1 and t3) after plant compost (GC) application, while after 12 months of restoration, the values of the stoichiometric nutrient ratios were in an intermediate position between the rest of the restored soils and the NAT soils (Table 1 and Figure 1).

Total and available nutrient contents could have contributed to the change in the bacterial community composition of the restored soils, mainly between t1 and t3 (Table S1b,c). Changes in the bacterial community structure favor the decomposition of organic amendments [16,43], consequently favoring the relationships in mineral nutrients [23]. Therefore, the bacterial communities of the soils together with the new characteristics of the restored soils could have generated notable impacts over time in the soils of our study. These were more evident in the soils amended with compost from manure (VC, CM, and SC) after 12 months of restoration (Figure 2a,b). The influence exerted by the stoichiometry of soil nutrient content may have produced changes in the composition and functionality of the soil decomposer bacterial community [16], which could reflect differences in nutrient demand and nutrient availability [44]. The use of composted waste to reclaim degraded soils could not only favor a more efficient soil decomposer community, but could also benefit the presence of genera categorized as plant-growth-promoting rhizobacteria (PGPR) [45]. In this study, some of the taxa identified as indicator species for restored soils, such as *Alcaligenes*, *Arthrobacter*, *Staphylococcus*, or *Leifsonia*, are considered PGPR [28,46–48].

The increase of the copiotrophic phyla Proteobacteria and Bacteroidetes [49] three months after the application of organic amendments, as well as the high abundance of Firmicutes at the beginning of the restoration, suggests high rates of mineralization of nutrients easily assimilated by the bacterial community [50], in agreement with other authors who have previously observed similar behaviors after the addition of organic wastes [8,21]. The application of GC favored the presence of indicator taxa involved in the nitrogen cycle such as the Proteobacteria *Halomonas* and the Firmicutes Bacillaceae [51,52], as well as the Proteobacteria *Arenimonas* that present different catalytic activities capable of metabolizing a large number of substrates [21]. The presence of these bacteria could have favored the improvement in stoichiometric ratios, bringing these soils closer to the values of natural soils. The increase in TN and AP after the application of CM and SC composts could have benefited the presence of bacterial taxa with copiotrophic lifestyles, such as the genera *Sphingobacterium* and *Flavobacterium* belonging to the phylum Bacteroidetes [53], capable of degrading organic compounds [54]. Likewise, the positive correlations with nutrient content (TOC, TN, and AP) presented by Proteobacteria *Cellvibrio* (SC indicator species; Figure 4 and Table S4) could indicate that the SC treatment favored the proliferation of micro-organisms with a heterotrophic metabolism due to their involvement in the degradation of organic compounds [55] such as polysaccharides [26] or cellulose [56], suggesting a high degradation of the organic matter contributed 3 months after restoration (Figure 4). The CM treatment may have led to a selection of bacterial taxa adapted to increased salinity and decreased pH (Figure 4), as EC and pH together with nutrient content are the most influential chemical properties on the soil bacterial composition [10,44,50,57], suggesting that soil characteristics were the main driving forces behind edaphic biodiversity [58]. Moreover, positive correlations with TN and AP in CM soils of the Proteobacteria *Alcaligenes*

(order Burkholderiales) able to degrade both labile and recalcitrant C [50] and fix N [59]; as well as of the order Bacillales (Firmicutes) able to secrete organic compound degrading enzymes [60] and involved in C and P cycling [61] could favor biogeochemical cycles and consequently improve plant yield by repairing soil fertility. Similarly, the indicator bacterial taxa for the VC treatment showed significantly positive correlations with AP, as well as positive correlations with TOC and TN (Figure 4). Among the VC indicator taxa, the Flavobacteriaceae (Bacteroidetes) and Xanthomonadaceae (Proteobacteria) families are distinguished for their involvement in the digestion of organic macromolecules and lignin, respectively [50,62]. Li et al. (2017) [21] found that unclassified members of the family Xanthomonadaceae increased with amendments of manure and straw. In addition, the significantly positive correlations between VC indicator taxa with PA, together with their high content, could suggest that the vermicompost presented a high stage of maturation, since mature composts represent an important source of available P [63]. Regarding the copiotrophic phyla detected in untreated soils and natural soils (CON and NAT, respectively), the indicator bacterial taxa belonging to the phylum Bacteroidetes as the family Microscillaceae in CON soils, and in NAT soils, the genus *Segetibacter* or the Proteobacteria *Psychroglaciecola* have been previously observed in Mediterranean soils near the study area [10,57].

Despite the fact that most of the indicator bacterial taxa of the study soils belonging to oligotrophic phyla were identified in the untreated and natural reference soils (Table S4), the restoration also produced changes in oligotrophic phyla such as Actinobacteria and Planctomycetes, which suffered a decrease at t3 and proliferated at t12 in the restored soils (Figure S1). These changes in relative abundance between copio- and oligotrophic phyla could indicate a succession of soil bacterial communities towards more oligotrophic metabolisms [13,21,64] due to short-term consumption of easily assimilable nutrients, mainly in soils treated with animal-derived compost (VC, SC, and CM), given that in these same soils, the phylum Acidobacteria decreased in abundance over time, increasing only in GC soils (Figure S1), suggesting a decrease in the mineralization of C [50,64]. Soil enrichment with poultry manure compost (CM soils) favored the presence of PGPR genera (phylum Actinobacteria) such as *Arthrobacter* and *Leifsonia* involved in N fixation and P cycling, respectively [47,59]. The presence of these indicator bacteria together with those previously described in these soils could suggest that metabolisms involved in N and P cycling are taking place in CM soils that could be reflected in the slight loss of N and increase of assimilable P over time (Table 1). As for the indicator taxa of the VC treatment, bacteria belonging to the oligotrophic phyla Deinococcus-Thermus and Chloroflexi were found, which could be involved in the decomposition of recalcitrant organic matter [50]. In the control soils, all those bacteria that were not able to be identified at lower levels taxonomically were encompassed in the slow-growing oligotrophic phylum Actinobacteria [3,65]. This indicator taxon presented significantly negative correlations with TOC and TN, and positive correlations with pH, suggesting the adaptation of the bacterial community to the higher pH and low nutrient conditions of untreated soils (Table 1). As for the indicator taxa from natural soils, most of them presented significantly positive correlations with stoichiometric nutrient ratios (C:N, C:P, and N:P) and useful water, suggesting greater adaptation to the conditions of the reference soils. Some of these bacterial taxa, such as *Blastococcus,* were also frequently found in other semi-arid soils characterized by low organic matter content and adaptations to extreme conditions [11,57].

The results of this experimental case for the reclamation of abandoned agricultural soils have shown that the bacterial community structure of the soils was influenced by the type of organic amendment applied. The most drastic changes occurred between the beginning and 3 months after restoration, as a consequence of the alterations in the abiotic properties generated (nutrient content and stoichiometric ratios). It was also observed that the organic origin of the composted residues used generated differences in the bacterial composition of the restored soils, control soils, and natural reference soils.

5. Conclusions

Studies of the evolution of abandoned soil restorations through the application of organic amendments are important to reveal soil bacterial patterns and to identify edaphic factors and nutrient availability that could influence soil biodiversity. The results of this research revealed that the addition of composted residues modified the physico-chemical properties of the soils by increasing the nutrient content and varying their stoichiometric ratios with respect to untreated soils and natural reference soils, especially during the 3 months of the restoration. These alterations could have interfered with the development of the bacterial community in the different soils studied. This could be explained by the fact that the bacterial composition of each treatment was significantly different from the rest. Additionally, the results revealed that throughout the study period the soils restored with plant compost (GC) presented an intermediate behavior between the reference soils (untreated and natural soils) and the soils treated with vermicompost and animal compost (SC and CM), the latter behaving differently from the former mainly at 12 months of restoration. At the phylum level, the results suggested the existence of a short-term bacterial succession between the increase after 3 months in the relative abundance of faster-growing phyla (Proteobacteria, Bacteroidetes, and Firmicutes), which, after 12 months of restoration, were replaced by slower-growing phyla (Actinobacteria, Planctomycetes, and Acidobacteria). Likewise, the organic matter provided by each amendment could have favored the presence of specific bacterial taxa for each of the study soils with important implications in the C, N, and P cycles, as well as the establishment of bacterial genera considered PGPR such as *Alcaligenes*, *Arthrobacter*, *Staphylococcus*, or *Leifsonia*. In conclusion, the results indicated that composted greenhouse crop residues favored a soil bacterial community and nutrient content closer to reference soils than other organic amendments applied. Therefore, it could be an optimal treatment for the short-term recovery of agriculturally degraded soils in semi-arid areas.

References

1. Stanturf, J.A. Landscape Degradation and Restoration. In *Soils and Landscape Restoration*; Elsevier: Amsterdam, The Netherlands, 2021; pp. 125–159.
2. Navarro, J.A.C.; Estellés, M.G.; González, G.G.; Sánchez, V.M.C.; del Campo, M.V. *Restauración Ecológica En Ambientes Semiáridos. Recuperar Las Interacciónes Biológicas y Las Funciones Ecosistémicas*; CSIC: Madrid, Spain, 2017.
3. Bastida, F.; Torres, I.F.; Hernández, T.; Bombach, P.; Richnow, H.H.; García, C. Can the Labile Carbon Contribute to Carbon Immobilization in Semiarid Soils? Priming Effects and Microbial Community Dynamics. *Soil Biol. Biochem.* **2013**, *57*, 892–902. [CrossRef]
4. García, C.; Hernández, T.; Costa, F. Microbial Activity in Soils under Mediterranean Environmental Conditions. *Soil Biol. Biochem.* **1994**, *26*, 1185–1191. [CrossRef]
5. Lasanta, T.; Arnáez, J.; Nadal-Romero, E. *Soil Degradation, Restoration and Management in Abandoned and Afforested Lands*; Elsevier: Amsterdam, The Netherlands, 2019; Volume 4, pp. 71–117.
6. Li, Y.; Gong, J.; Liu, J.; Hou, W.; Moroenyane, I.; Liu, Y.; Jin, J.; Liu, J.; Xiong, H.; Cheng, C.; et al. Effects of Different Land Use Types and Soil Depth on Soil Nutrients and Soil Bacterial Communities in a Karst Area, Southwest China. *Soil Syst.* **2022**, *6*, 20. [CrossRef]
7. Gao, W.; Xu, J.; Zhao, J.; Zhang, H.; Ni, Y.; Zhao, B.; Tebbe, C.C.; Zhang, J.; Jia, Z. Prokaryotic Community Assembly after 40 Years of Soda Solonetz Restoration by Natural Grassland and Reclaimed Farmland. *Eur. J. Soil Biol.* **2020**, *100*, 103213. [CrossRef]
8. Calleja-Cervantes, M.E.; Menéndez, S.; Fernández-González, A.J.; Irigoyen, I.; Cibriáin-Sabalza, J.F.; Toro, N.; Aparicio-Tejo, P.M.; Fernández-López, M. Changes in Soil Nutrient Content and Bacterial Community after 12 Years of Organic Amendment Application to a Vineyard. *Eur. J. Soil Sci.* **2015**, *66*, 802–812. [CrossRef]
9. Wijesekara, H.; Bolan, N.S.; Vithanage, M.; Xu, Y.; Mandal, S.; Brown, S.L.; Hettiarachchi, G.M.; Pierzynski, G.M.; Huang, L.; Ok, Y.S.; et al. Utilization of Biowaste for Mine Spoil Rehabilitation. *Adv. Agron.* **2016**, *138*, 97–173. [CrossRef]
10. Rodríguez-Berbel, N.; Ortega, R.; Lucas-Borja, M.E.; Solé-Benet, A.; Miralles, I. Long-Term Effects of Two Organic Amendments on Bacterial Communities of Calcareous Mediterranean Soils Degraded by Mining. *J. Environ. Manag.* **2020**, *271*, 110920. [CrossRef]
11. Rodríguez-Berbel, N.; Soria, R.; Ortega, R.; Bastida, F.; Miralles, I. Quarry Restoration Treatments from Recycled Waste Modify the Physicochemical Soil Properties, Composition and Activity of Bacterial Communities and Priming Effect in Semi-Arid Areas. *Sci. Total Environ.* **2021**, *774*, 145693. [CrossRef]
12. Soria, R.; González-Pérez, J.A.; de la Rosa, J.M.; San Emeterio, L.M.; Domene, M.A.; Ortega, R.; Miralles, I. Effects of Technosols Based on Organic Amendments Addition for the Recovery of the Functionality of Degraded Quarry Soils under Semiarid Mediterranean Climate: A Field Study. *Sci. Total Environ.* **2022**, *816*, 151572. [CrossRef]
13. Bastida, F.; Torres, I.F.; Hernández, T.; García, C. The Impacts of Organic Amendments: Do They Confer Stability against Drought on the Soil Microbial Community? *Soil Biol. Biochem.* **2017**, *113*, 173–183. [CrossRef]
14. Tejada, M.; Hernandez, M.T.; Garcia, C. Application of Two Organic Amendments on Soil Restoration: Effects on the Soil Biological Properties. *J. Environ. Qual.* **2006**, *35*, 1010–1017. [CrossRef] [PubMed]
15. Li, X.; Zhu, W.; Xu, F.; Du, J.; Tian, X.; Shi, J.; Wei, G. Organic Amendments Affect Soil Organic Carbon Sequestration and Fractions in Fields with Long-Term Contrasting Nitrogen Applications. *Agric. Ecosyst. Environ.* **2021**, *322*, 107643. [CrossRef]
16. Wang, Q.; Gao, W.; Bol, R.; Xiao, Q.; Wu, L.; Zhang, W. Microbial Regulation of Net N Mineralisation Is Driven by C, N, P Content and Stoichiometry. *Eur. J. Soil Sci.* **2022**, *73*, e13257. [CrossRef]
17. Xiao, C.; Janssens, I.A.; Zhou, Y.; Su, J.; Liang, Y.; Guenet, B. Strong Stoichiometric Resilience after Litter Manipulation Experiments; A Case Study in a Chinese Grassland. *Biogeosciences* **2015**, *12*, 757–767. [CrossRef]

18. Bastida, F.; Kandeler, E.; Moreno, J.L.; Ros, M.; García, C.; Hernández, T. Application of Fresh and Composted Organic Wastes Modifies Structure, Size and Activity of Soil Microbial Community under Semiarid Climate. *Appl. Soil Ecol.* **2008**, *40*, 318–329. [CrossRef]

19. Xue, L.; Ren, H.; Brodribb, T.J.; Wang, J.; Yao, X.; Li, S. Long Term Effects of Management Practice Intensification on Soil Microbial Community Structure and Co-Occurrence Network in a Non-Timber Plantation. *For. Ecol. Manag.* **2020**, *459*, 117805. [CrossRef]

20. Singh, D.P.; Prabha, R. *Microbial Interventions in Agriculture and Environment*; Springer: Singapore, 2019; Volume 3, ISBN 9789813290846.

21. Li, F.; Chen, L.; Zhang, J.; Yin, J.; Huang, S. Bacterial Community Structure after Long-Term Organic and Inorganic Fertilization Reveals Important Associations between Soil Nutrients and Specific Taxa Involved in Nutrient Transformations. *Front. Microbiol.* **2017**, *8*, 187. [CrossRef]

22. Neemisha; Sharma, S. Soil Enzymes and Their Role in Nutrient Cycling. *Struct. Funct. Pedosph.* **2022**, 173–188. [CrossRef]

23. Francioli, D.; Schulz, E.; Lentendu, G.; Wubet, T.; Buscot, F.; Reitz, T. Mineral vs. Organic Amendments: Microbial Community Structure, Activity and Abundance of Agriculturally Relevant Microbes Are Driven by Long-Term Fertilization Strategies. *Front. Microbiol.* **2016**, *7*, 1446. [CrossRef]

24. Peinado, F.M.; Sierra, A.M. Proyecto LUCDEME. Mapa de Suelos a Escala 1:100.000 Provincia de Almería 2004. Available online: https://www.miteco.gob.es/es/biodiversidad/temas/desertificacion-restauracion/memoriamapasueloslucdemealmeria_tcm30-512287.pdf (accessed on 3 November 2022).

25. SAIH Hidrosur Demarcación Hidrográfica de Las Cuencas Mediterráneas Andaluzas. *Informe Hidrológico 2020–2021. Consejería de Agricultura, Ganadería, Pesca y Desarrollo Sostenible*; Dirección General de Infraestructuras Del Agua: Sevilla, Spain, 2021.

26. Wongkiew, S.; Koottatep, T.; Polprasert, C.; Prombutara, P.; Jinsart, W.; Khanal, S.K. Bioponic System for Nitrogen and Phosphorus Recovery from Chicken Manure: Evaluation of Manure Loading and Microbial Communities. *Waste Manag.* **2021**, *125*, 67–76. [CrossRef]

27. Munoz-Ucros, J.; Panke-Buisse, K.; Robe, J. Bacterial Community Composition of Vermicompost-Treated Tomato Rhizospheres. *PLoS ONE* **2020**, *15*, e0230577. [CrossRef] [PubMed]

28. Naseer, I.; Ahmad, M.; Nadeem, S.M.; Ahmad, I.; Najm-ul-Seher; Zahir, Z.A. *Biofertilizers for Sustainable Agriculture and Environment*; Springer: Cham, Switzerland, 2019; Volume 55, ISBN 978-3-030-18932-7.

29. Gupta, A.K.; Tomar, J.M.S.; Kaushal, R.; Kadam, D.M.; Rathore, A.C.; Mehta, H.; Ojasvi, P.R. Aromatic Plants Based Environmental Sustainability with Special Reference to Degraded Land Management. *J. Appl. Res. Med. Aromat. Plants* **2021**, *22*, 100298. [CrossRef]

30. Costa Pérez, J.C.; Valle Tendero, F. *Modelos de Restauración Forestal: Datos Botánicos Aplicados a La Gestión Del Medio Ambiente Andaluz*; Consejería de Medio Ambiente; Junta de Andalucía, Consejería de Medio Ambiente: Sevilla, Spain, 2004; ISBN 84-95785-97-8.

31. Miralles, I.; Ortega, R.; Almendros, G.; Sánchez-Marañón, M.; Soriano, M. Soil Quality and Organic Carbon Ratios in Mountain Agroecosystems of South-East Spain. *Geoderma* **2009**, *150*, 120–128. [CrossRef]

32. Mingorance, M.D.; Barahona, E.; Fernández-Gálvez, J. Guidelines for Improving Organic Carbon Recovery by the Wet Oxidation Method. *Chemosphere* **2007**, *68*, 409–413. [CrossRef]

33. Olsen, S.R.; Watanabe, F.S. A Method to Determine a Phosphorus Adsorption Maximum of Soils as Measured by the Langmuir Isotherm. *Soil Sci. Soc. Am. J.* **1957**, *21*, 144–149. [CrossRef]

34. Boudjabi, S.; Chenchouni, H. Soil Fertility Indicators and Soil Stoichiometry in Semi-Arid Steppe Rangelands. *CATENA* **2022**, *210*, 105910. [CrossRef]

35. Richards, A. Pressure-Membrane Extraction Apparatus for Soil Solution. *Soil Sci.* **1941**, *51*, 377–386. [CrossRef]

36. Olsen, S.R. *Estimation of Available Phosphorus in Soils by Extraction with Sodium*; US Department of Agriculture: Washington, DC, USA, 1954.

37. Comeau, A.M.; Douglas, G.M.; Langille, M.G.I. Microbiome Helper: A Custom and Streamlined Workflow for Microbiome Research. *mSystems* **2017**, *2*, e00127-16. [CrossRef]

38. Anderson, M.J. Permutational Multivariate Analysis of Variance (PERMANOVA). In *Wiley StatsRef Statistics Reference Online*; Wiley: New York, NY, USA, 2017; pp. 1–15. [CrossRef]

39. Akaike, H. A New Look at the Statistical Model Identification. *IEEE Trans. Automat. Contr.* **1974**, *19*, 716–723. [CrossRef]

40. De Caceres, M.; Jansen, F. Package "indicspecies" Type Package Title Relationship Between Species and Groups of Sites. Version 1.7.6. Available online: https://cran.r-project.org/web/packages/indicspecies/index.html (accessed on 26 September 2022).

41. Lv, X.-M.; Shao, M.-F.; Li, C.-L.; Li, J.; Gao, X.; Sun, F.-Y. A Comparative Study of the Bacterial Community in Denitrifying and Traditional Enhanced Biological Phosphorus Removal Processes. *Microbes Environ.* **2014**, *29*, 261–268. [CrossRef]

42. González-Ubierna, S.; Jorge-Mardomingo, I.; Carrero-González, B.; de la Cruz, M.T.; Casermeiro, M.Á. Soil Organic Matter Evolution after the Application of High Doses of Organic Amendments in a Mediterranean Calcareous Soil. *J. Soils Sediments* **2012**, *12*, 1257–1268. [CrossRef]

43. Li, J.; Cooper, J.M.; Lin, Z.; Li, Y.; Yang, X.; Zhao, B. Soil Microbial Community Structure and Function Are Significantly Affected by Long-Term Organic and Mineral Fertilization Regimes in the North China Plain. *Appl. Soil Ecol.* **2015**, *96*, 75–87. [CrossRef]

44. Khan, K.S.; Mack, R.; Castillo, X.; Kaiser, M.; Joergensen, R.G. Microbial Biomass, Fungal and Bacterial Residues, and Their Relationships to the Soil Organic Matter C/N/P/S Ratios. *Geoderma* **2016**, *271*, 115–123. [CrossRef]

45. Kadoglidou, K.; Chalkos, D.; Karamanoli, K.; Eleftherohorinos, I.G.; Constantinidou, H.I.A.; Vokou, D. Aromatic Plants as Soil Amendments: Effects of Spearmint and Sage on Soil Properties, Growth and Physiology of Tomato Seedlings. *Sci. Hortic.* **2014**, *179*, 25–35. [CrossRef]

46. Zheng, X.; Aborisde, M.A.; Wang, H.; He, P.; Lu, S.; Cui, N.; Wang, S.; Zhang, H.; Ding, H.; Liu, K. Effect of Lignin and Plant Growth-Promoting Bacteria (Staphylococcus Pasteuri) on Microbe-Plant Co-Remediation: A PAHs-DDTs Co-Contaminated Agricultural Greenhouse Study. *Chemosphere* **2020**, *256*, 127079. [CrossRef]

47. Pratap, D.; Harikesh, S.; Singh, B.; Prabha, R. *Microbial Inoculants in Sustainable Agricultural Productivity*; Springer: Berlin/Heidelberg, Germany, 2016; Volume 1, ISBN 9788132226451.

48. Ortega, R.; Miralles, I.; Domene, M.A.; Soriano, M. Functions and Applications of Plant Growth Promoting Bacteria (PGPR) in Highly Technified Crops. *Agric. Res. Technol.* **2017**, *12*, 10–13.

49. Fierer, N. Embracing the Unknown: Disentangling the Complexities of the Soil Microbiome. *Nat. Rev. Microbiol.* **2017**, *15*, 579–590. [CrossRef]

50. Goldfarb, K.C.; Karaoz, U.; Hanson, C.A.; Santee, C.A.; Bradford, M.A.; Treseder, K.K.; Wallenstein, M.D.; Brodie, E.L. Differential Growth Responses of Soil Bacterial Taxa to Carbon Substrates of Varying Chemical Recalcitrance. *Front. Microbiol.* **2011**, *2*, 94. [CrossRef]

51. Guo, Y.; Zhou, X.; Li, Y.; Li, K.; Wang, C.; Liu, J.; Yan, D.; Liu, Y.; Yang, D.; Xing, J. Heterotrophic Nitrification and Aerobic Denitrification by a Novel Halomonas Campisalis. *Biotechnol. Lett.* **2013**, *35*, 2045–2049. [CrossRef]

52. Xu, J.; Xu, X.; Han, Y.; Sheng, S.; Meng, Q.; Wang, Z.; Liu, W.; Di, L. Evaluation of a Novel Thermophilic Nitrifying Bacillaceae Species Aliibacillus Thermotolerans BM62T Promoting Nitrogen Retention in Livestock Manure Compost. *Environ. Prog. Sustain. Energy* **2019**, *39*, e13357. [CrossRef]

53. Neher, D.A.; Limoges, M.A.; Weicht, T.R.; Sharma, M.; Millner, P.D.; Donnelly, C. Bacterial Community Dynamics Distinguish Poultry Compost from Dairy Compost and Non-Amended Soils Planted with Spinach. *Microorganism* **2020**, *8*, 1601. [CrossRef] [PubMed]

54. Zhang, W.; Yu, C.; Wang, X.; Hai, L.; Hu, J. Increased Abundance of Nitrogen Fixing Bacteria by Higher C/N Ratio Reduces the Total Losses of N and C in Cattle Manure and Corn Stover Mix Composting. *Waste Manag.* **2020**, *103*, 416–425. [CrossRef] [PubMed]

55. Akyol, Ç.; Ince, O.; Ince, B. Crop-Based Composting of Lignocellulosic Digestates: Focus on Bacterial and Fungal Diversity. *Bioresour. Technol.* **2019**, *288*, 121549. [CrossRef] [PubMed]

56. Miranda-Carrazco, A.; Chávez-López, C.; Ramírez-Villanueva, D.A.; Dendooven, L. Bacteria in (Vermi)Composted Organic Wastes Mostly Survive When Applied to an Arable Soil Cultivated with Wheat (*Triticum* Sp. L.). *Environ. Monit. Assess.* **2022**, *194*, 363. [CrossRef] [PubMed]

57. Miralles, I.; Soria, R.; Lucas-Borja, M.E.; Soriano, M.; Ortega, R. Effect of Biocrusts on Bacterial Community Composition at Different Soil Depths in Mediterranean Semi-Arid Ecosystems. *Sci. Total Environ.* **2020**, *733*, 138613. [CrossRef] [PubMed]

58. Liang, X.; Wang, X.; Zhang, N.; Li, B. Biogeographical Patterns and Assembly of Bacterial Communities in Saline Soils of Northeast China. *Microorganisms* **2022**, *10*, 1787. [CrossRef]

59. Miralles, I.; Ortega, R.; Montero-Calasanz, M.C. Studying the Microbiome of Cyanobacterial Biocrusts from Drylands and Its Functional Inuence on Biogeochemical Cycles. 2021. Available online: https://www.researchgate.net/publication/349600353_Studying_the_Microbiome_of_Cyanobacterial_Biocrusts_From_Drylands_and_Its_Functional_Influence_on_Biogeochemical_Cycles (accessed on 3 November 2022).

60. Ye, G.; Lin, Y.; Liu, D.; Chen, Z.; Luo, J.; Bolan, N.; Fan, J.; Ding, W. Long-Term Application of Manure over Plant Residues Mitigates Acidification, Builds Soil Organic Carbon and Shifts Prokaryotic Diversity in Acidic Ultisols. *Appl. Soil Ecol.* **2019**, *133*, 24–33. [CrossRef]

61. Lin, Y.; Ye, G.; Kuzyakov, Y.; Liu, D.; Fan, J.; Ding, W. Long-Term Manure Application Increases Soil Organic Matter and Aggregation, and Alters Microbial Community Structure and Keystone Taxa. *Soil Biol. Biochem.* **2019**, *134*, 187–196. [CrossRef]

62. McBride, M.J. The Family Flavobacteriaceae. In *The Prokaryotes: Other Major Lineages of Bacteria and The Archaea*; Springer: Berlin/Heidelberg, Germany, 2014; Volume 9783642389, pp. 643–676, ISBN 9783642301230.

63. Elbl, J.; Maková, J.; Javoreková, S.; Medo, J.; Kintl, A.; Lošák, T.; Lukas, V. Response of Microbial Activities in Soil to Various Organic and Mineral Amendments as an Indicator of Soil Quality. *Agronomy* **2019**, *9*, 485. [CrossRef]

64. Fierer, N.; Bradford, M.A.; Jackson, R.B. Toward an Ecological Classification of Soil Bacteria. *Ecology* **2007**, *88*, 1354–1364. [CrossRef]

65. Zhang, C.; Li, J.; Wang, J.; Liu, G.; Wang, G.; Guo, L.; Peng, S. Decreased Temporary Turnover of Bacterial Communities along Soil Depth Gradient during a 35-Year Grazing Exclusion Period in a Semiarid Grassland. *Geoderma* **2019**, *351*, 49–58. [CrossRef]