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Impacts of Sugarcane Vinasses on the Structure and Composition of Bacterial Communities in Brazilian Tropical Oxisols

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Abstract

This study explored how different sugarcane vinasses influence the structure and composition of soil bacterial communities in two tropical Oxisols with contrasting textures. In a controlled microcosm experiment with sugarcane seedlings, two concentrations of three vinasse types were applied, and bacterial communities were monitored over 10, 30, and 60 days using T-RFLP and 16S rRNA gene sequencing. Across all treatments, vinasse application led to clear changes in bacterial community structure in both soils, regardless of the time point. Certain bacterial groups, such as *Sphingobacteriia*, *Alphaproteobacteria*, and *Gammaproteobacteria*, became more abundant—likely responding to increased carbon availability, higher pH, and greater soil moisture. At the same time, other groups declined, possibly due to excess nutrients like potassium and sulfur. Notably, these shifts occurred even when standard biochemical indicators suggested no major impact, highlighting the sensitivity of microbial community-level responses. These findings point to the importance of looking beyond traditional soil quality metrics when assessing the environmental effects of organic residue applications. Incorporating microbial indicators can offer a more nuanced understanding of how practices like vinasse reuse affect soil functioning in tropical agroecosystems.

Keywords: fertigation; microbial community; organic amendment; Oxisol; waste disposal

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1. Introduction

Sugarcane vinasse is a liquid waste derived from the production of ethanol. For each liter of ethanol produced, 8–15 L of vinasse is generated, whose primary destination is the

fertigation of agricultural soils [1]. In soils, vinasses can improve physical characteristics, increase mineral nutrients and organic matter (OM) [1–3], and promote increases in the growth of crops [4].

On the other hand, at high concentrations, sugarcane vinasse can cause salt saturation in the soil and leaching of cations to groundwater [5], resulting in salinization and sodification of soils, as well as increased soil instability [6]. Other negative environmental impacts of the application of vinasse on agricultural soils are also known, such as increases in the emission of greenhouse gases and toxicity to soil microorganisms, invertebrates, and plants [1,6–10]

In a previous study [11], we observed that the addition of sugarcane vinasses from different sources favored microbial growth (based on microbial biomass carbon—MBC) and activity in tropical Brazilian soils, even at concentrations higher than those recommended for application in agricultural soils in Brazil [5]. Some other authors have also reported increases in plant growth and microbial activity after applying this effluent to the soil [4,12–17]. These results suggest that the fertigation of agricultural soils with sugarcane vinasses does not adversely affect the soil microbiota and may even contribute to improving soil health [4,17]. However, there is evidence that the application of vinasse (single application, or in a mixture or with other fertilizers) in agricultural soils can significantly alter soil microbial communities [3,18–23].

Such differences in the nature of the effects on the soil microbial community are due to the assessment endpoints used to assess the impacts of this type of effluent on the soil [19]. Traditional microbial studies, based on biochemical analyses (e.g., MBC [24]), do not always allow for detecting changes in the profile of the soil microbial community since there may be compensation in the growth or activity of some groups to the detriment of others. The next-generation DNA methods allow the identification of changes in microbial communities, which can indicate the adverse effects of vinasse disposal on the terrestrial ecosystem's good functioning [20]. Among those is the reduction in bacterial taxa responsible for regulating the biogeochemical cycles of carbon, nitrogen, phosphorus and sulfur, among other ecosystem services [25]. In this sense, studies based on DNA and RNA methods have increased in recent years to help reduce uncertainties about the ecological risks of the disposal of wastes on agricultural soils [26–28].

Despite the evidence that the disposal of sugarcane vinasse on agricultural soils can change the soil bacterial communities, it is known that the impacts of this type of effluent on soil organisms depend on many factors. Here, we can mention the applied concentrations, soil type, effluent composition, exposure time, as well as the presence of other stressors, such as the use of fertilizers, pesticides, drought periods, among others [9,18–21]. We have found no studies that systematically assessed the changes in the structure and composition of soil bacterial communities to understand the isolated influences of different vinasse types, doses and exposure times in different soil types. To achieve such a set of variables, it is necessary to carry out experiments under experimental conditions that allow better control of external factors (e.g., those existing in field assays) that can influence the individual response of each study factor.

Therefore, to clearly understand the effects produced by sugarcane vinasses on soil bacterial communities, this study assessed the changes in the structure and composition of the soil bacterial communities in two Brazilian tropical soils with contrasting clay contents when exposed to two concentrations of three sugarcane vinasses (from different sources) over time (up to 60 d). Experiments were set up on a microcosm scale with sugarcane seedlings. The tested hypotheses were (i) sugarcane vinasse alters bacterial community composition and diversity in a concentration-dependent manner; (ii) these effects differ between soils of contrasting texture and source of vinasses; and (iii) the shifts in microbial

community structure disappear quickly after a short period of application to soils (up to 60 d), indicating short-term resilience. Furthermore, given the functional roles of soil microorganisms, we expected that such shifts could have implications for soil sustainability, particularly through their contributions to nutrient cycling and plant–soil interactions. Therefore, the study provides insights into how vinasse application may affect these ecological functions mediated by soil bacteria.

2. Materials and Methods

The changes promoted by the sugarcane vinasses on soil bacterial communities were assessed in a microcosm experiment under greenhouse conditions, previously described in [11]. In brief, a completely randomized factorial design (2 × 3 × 2) with three replicates was utilized, where two doses of three different sugarcane vinasses were applied to two distinct Oxisols. Experimental units were pots (4 L) containing 3.5 kg of soil (dry weight) and a sugarcane seedling (*Saccharum* sp., variety CTC-02). Soil samples were collected 10, 30 and 60 days after the beginning of the experiment to perform the DNA extraction and subsequent analyses.

2.1. Sampling and Characterization of the Soils and Vinasses

Two tropical Oxisols (Soil Taxonomy [29]), with Sandy Clay Loam (henceforth called RL) and Sandy Loam (RYL) textures, were sampled at the top layer (0–20 cm) of the soil profile in areas with sugarcane plantations in the state of São Paulo, Brazil (22°41′ S 47°38′ W and 20°58′ S 40°03′ W, respectively). The sampling sites had been free from the application of sugarcane vinasses for more than ten years.

Soils were air-dried, sieved to 2 mm, and kept at room temperature until the experiment was set up. The water holding capacity (WHC) and pH (1M KCl—1:5, *w*/*v*) were determined for both soils following Annex C of ISO 11268-2 [30]. The other chemical [31] and textural [32] properties of the soils before vinasses application (Table A1) were previously described in Alves et al. [11].

Two sugarcane vinasses were taken in different alcohol distilling plants (VA and VB), and a third vinasse was obtained from ethanol production at a laboratory scale (VC) without the additives generally used during the process [33]. Vinasses were stored in a cold chamber (4 °C), and their chemical characterization (Table 1) was performed following Kiehl [34]. The pseudo-total concentrations of potentially toxic elements (PTE) in soils (before vinasses application) and vinasses (Table A2), including As, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb and Zn, were determined following the EPA 3051A and 3015A methods [35,36], as described in [9].

Table 1. Chemical properties of the three vinasses (VA, VB and VC) used in the microcosm experiment. Adapted from [11].

Parameter	Vinasses		
	VA	VB	VC [a]
pH (1M KCl)	4.6	4.9	5.2
OM (g L^{-1})	28	17.4	13.7
TC (g L^{-1})	15.6	9.7	7.6
TN (g L^{-1})	0.6	0.7	0.3
C:N ratio	28	13	22
P (g L^{-1})	0.42	0.1	0.9
S (g L^{-1})	1.59	0.99	0.41
K (g L^{-1})	9.2	8.3	8.5
Ca (g L^{-1})	0.98	1.27	0.65

Table 1. *Cont.*

Parameter	Vinasses		
	VA	**VB**	**VC** [a]
Mg (g L^{-1})	1	0.48	0.52
Cu (mg L^{-1})	1	1	1
Fe (mg L^{-1})	8	33	28
Mn (mg L^{-1})	2	7	4
Zn (mg L^{-1})	1	1	1
EC (mS cm^{-1})	20.7	20.2	10.6
Density (g cm^{-3})	0.9	0.9	1
Ethanol (%)	0.2	0	0

[a] K_2SO_4 was added to keep K levels similar to the samples from distilleries. OM—Organic Matter; TC—Total Carbon; TN—Total Nitrogen; EC—Electric Conductivity.

2.2. Experimental Procedures

Immediately before starting the microcosm experiments, vinasses concentrations (Table 2) or control (only deionized water) treatments were applied (single application) toward the soil surface in volumes required to reach 60 or 45% of the WHC of the RL and RYL soils, respectively. The lower concentration (C1) was prepared by diluting vinasses in deionized water. The C1 was calculated based on the soil's cation exchange capacity (CEC) and K content (Table A1), alongside the K content in the vinasses (Table 1), following the Brazilian technical standard P4.231 [5]. The higher concentration (C2) represented a worst-case scenario, where pure vinasse was applied to achieve 60 or 45% of the WHC of the RL and RYL soils.

Table 2. Concentrations (C1 and C2) of the vinasses (VA, VB and VC) applied to two Oxisols (RL and RYL) in a greenhouse microcosm experiment. Values are expressed in mL of pure vinasse per kg of dry soil (mL kg^{-1}) and m^3 of pure vinasse per hectare (m^3 ha^{-1}; considering 20 cm of the soil layer).

Soil	Vinasses	Control	mL kg^{-1}		m^3 ha^{-1}	
			C1	**C2**	**C1**	**C2**
RL	VA	0	67	200	134	400
	VB	0	74	200	148	400
	VC	0	72	200	144	400
RYL	VA	0	38	120	76	240
	VB	0	42	120	84	240
	VC	0	60	120	120	240

The greenhouse conditions during the experiment were: average maximum and minimum temperatures of 30.3 ± 1.7 and 18.5 ± 1.9 °C, respectively; and the average insolation was 6.8 ± 0.5 h d^{-1}. Soil moisture was adjusted with deionized water every two days by the weight difference in the test vessels. After 10, 30 and 60 days from the beginning of the tests, soil samples (three replicates per treatment) were taken to measure soil moisture and pH, and to perform soil DNA extractions (samples were stored at −80 °C).

2.3. Assessment of the Soil Bacterial Communities

The total soil DNA was extracted from 400 mg of soil using a DNeasy PowerSoil® Kit (Qiagen, Germany). To check the integrity and to quantify the extracted DNA, an aliquot of 5 µL of the extraction product, as well as 2 µL of "Low mass DNA Ladder" (Invitrogen, Carlsbad, CA, USA), were submitted to agarose gel electrophoresis (0.8%).

Two molecular techniques were used to meet the objectives under study, and both were based on the extracted DNA. First, we used a fingerprinting technique (terminal restriction fragment length polymorphism—T-RFLP) to assess the structure of the bacterial community, using three biological replicates per treatment. Then, high-throughput sequencing was performed to identify the bacterial community's taxonomic composition. In the first step, we assessed the changes in the bacterial community structure considering the complete factorial design, i.e., vinasses (types and concentrations), soil types, and exposure times. Since we verified no difference between the three exposure times (10, 30 and 60 days) via the T-RFLP technique (see results section), samples from the different sampling times were pooled together in each treatment (composite sample) to perform the sequencing (three technical replicates per concentration).

2.3.1. Analysis of Soil Bacterial Community Structure Using Terminal Restriction Fragment Length Polymorphism (T-RFLP)

The extracted DNA was subjected to amplification of the 16S rRNA gene of bacteria, through PCR, with specific primers ("FAM-8fm-AGAGTTTGATCMTGGCTCAG" and "926r-CCGTCAATTCCTTTRAGTTT") as indicated by Schütte et al. [37]. Amplification was in a solution containing: 33.6 µL of sterile ultrapure water (Milli-Q), 5 µL of 1 × PCR buffer, 6 µL of MgCl2 (3 mM), 4 µL of dNTP (0.2 mM), 0.10 µL of each primer (0.20 pmol μL^{-1}), 0.2 µL of Taq polymerase (0.02 U μL^{-1}) and 1 µL of soil DNA sample (ca. 50 ng). Amplification conditions were: 95 °C for 4 min (min.); 30 cycles at 95 °C for 30 s (s); 57 °C for 30 s; 72 °C for 45 s; and a final extension at 72 °C for 10 min.

After amplification, the PCR products were purified with 75% (v v^{-1}) isopropanol. Then, approximately 100 ng of the products were subjected to cleavage reactions with five endonuclease HhaI (GCGˆC) units, following the manufacturer's protocol (Fermentas, São Paulo, Brazil). The final product of the amplified 16S rRNA restriction fragments was precipitated with sodium acetate (3 M) and EDTA (125 mM), resuspended in Hi-Di™ Formamide with the LIZ 600 marker (Applied Biosystems, Life Technologies, Foster City, CA, USA) and analyzed on an ABI 3500 sequencer (Applied Biosystems, Life Technologies, Foster City, CA, USA). The readings of the generated T-RFLP fingerprint profiles were transformed into peaks through the GeneMapper software version 4.1 (Applied BioSystems, Life Technologies, Foster City, CA, USA). For this, we considered the baseline of 50 fluorescence units to discriminate the "background" of the samples. In addition, T-RFLP peak heights (fluorescence units) were transformed relative to the abundance (percentage) of each terminal restriction fragment (T-RF).

2.3.2. Analysis of Soil Bacterial Community Composition Using High-Throughput Sequencing

High-throughput sequencing of the V4 hypervariable region from the 16S rRNA gene was performed using the 515F and 806R primers [38], as described in [39]. The amplification conditions were the same as described for the T-RFLP methods, and the sequencing was conducted in a MiSeq System (Illumina) with a MiSeq Reagent v2 (500 cycles) kit. This analysis was performed using a composite sample, in which all the microcosm experiments' sampling times were pooled together for each treatment.

2.4. Data Analyses

Similarity matrices, generated based on T-RF data (using the Bray–Curtis distance), were submitted to a principal coordinate analysis ordination (PCoA). This allowed us to identify the changes in the structure of soil bacterial communities (separation of T-RF clusters) promoted by vinasses concentrations along the time in each soil. The pairwise similarities ($p \leq 0.01$) between T-RF clusters observed in soils with vinasses (VA, VB, or

VC) concentrations (C1 and C2) and controls, regardless of the sampling times, were tested through similarity analysis (ANOSIM; $p \leq 0.01$). The dissimilarities between sampling times within each treatment were assessed using Bray–Curtis distances, which are suitable for non-normally distributed ecological data, and analyzed with ANOSIM ($p \leq 0.01$). The PCoA and ANOSIM analyses were based on these distance matrices and were performed using PAST 3.0 software [40].

The 16S rRNA sequencing data were analyzed using the QIIME platform, a widely used and robust platform for microbial community analysis [38], as described in [39]. The operational taxonomic units (OTUs) generated in the sequencing were gathered into 19 Classes of greater representation. The other classes with lower relative frequency (<1%) were grouped as "others". OTUs with no correspondence to the known classes were grouped as "unclassified". Percentages of changes (increase or decrease, compared to controls) in the relative frequency of each Class after exposure to vinasses were calculated for RL and RYL soils. Linear correlations between the relative frequency of each bacterial Class and soil parameters (pH, moisture, and K content) 60 days after the application of vinasses (VA, VB, and VC) were established using Pearson's correlation coefficient (r) ($p < 0.05$) through software package R (version 3.3.2).

3. Results

The soils (Table A1) and vinasses (Table 1) differed in several chemical and physical properties. Even considering the highest vinasses concentration applied on the tested soils (C2), the input of PTEs (Table A2) was lower than the prevention values for these PTEs in Brazilian soils [41]. After vinasse application, the pH and moisture of both soils increased significantly (Figure A1). Since these results were previously presented by Alves et al. [11], the current study mainly focuses on the effects of the vinasses on the structure and composition of the soil bacterial communities.

3.1. Analysis of the Structure of Soil Bacterial Communities

In general, the T-RF clusters from the control treatments showed that the structural profile of the bacterial community in the RL soil was dissimilar to that observed in the RYL (Table 3). The differences remained even after applying vinasse concentrations (C1 and C2) when considering a pool of all treatments within each soil (Figure A2).

Specifically, it was found that the intra-treatment distribution of T-RFs in RY and RYL was not influenced by the exposure time (10, 30 and 60 days) to vinasse concentrations (Table A3). On the other hand, the PCoA showed differences in the profile of the bacterial community in soils treated with C1 and C2 concentrations of the vinasses VA, VB and VC (Figure 1), being these dissimilarities confirmed for both soils by the ANOSIM test (Table 3). The percentages of explanation of the PCoA (sum of the "PCoA 1" and "PCoA 2" axes) varied from 41.7 to 48.7% in RL soil and from 50.7 to 53.1% in RYL soil (Figure 1). Clusters from VA concentrations were arranged opposite to the control treatment in the vector plane in RL soil. The concentrations of VA in RY, and VB in both soils also presented a different distribution from the control, but the T-RFs profile was more dispersed in the vector plane. There were also differences in the distribution profiles of T-RFs between the C1 and C2 concentrations of vinasse VA and VB in RY and RYL, which did not occur for vinasse VC (Table 3).

Table 3. Similarity analysis (ANOSIM) between T-RF clusters observed in RL and RYL soils after the application of the vinasses (VA, VB and VC) concentrations (C1 and C2) and controls. Comparisons were performed individually for each soil type, and the three sampling times (10, 30 and 60 days) were pooled together for each treatment. *p*-values ≤ 0.01 (in black) indicate significant dissimilarities between treatments.

Soil Type	Treatment	Control		VA-C1		VA-C2		VB-C1		VB-C2		VC-C1	
		***p*-Value**	**R**	***p*-Value**	**R**	***p*-Value**	**R**	***p*-Value**	**R**	***p*-Value**	**R**	***p*-Value**	**R**
RL	Control	0.0001 [a]	0.931 [a]										
	VA-C1	0.0001	0.889										
	VA-C2	0.0001	0.625	0.0003	0.320								
	VB-C1	0.0002	0.603	0.0002	0.402	0.0016	0.341						
	VB-C2	0.0002	0.744	0.0006	0.332	0.0302	0.184	0.0055	0.275				
	VC-C1	0.0001	0.69	0.167	0.054	0.001	0.363	0.0024	0.215	0.0005	0.407		
	VC-C2	0.0001	0.465	0.0018	0.209	0.385	0.003	0.0836	0.091	0.0581	0.112	0.0212	0.132
RYL	Control	0.0001 [a]	0.93 [a]										
	VA-C1	0.0001	0.616										
	VA-C2	0.0003	0.609	0.0026	0.270								
	VB-C1	0.0002	0.492	0.0001	0.477	0.0001	0.506						
	VB-C2	0.0001	0.777	0.001	0.465	0.0157	0.225	0.0021	0.458				
	VC-C1	0.0002	0.382	0.1688	0.061	0.0001	0.329	0.0002	0.352	0.0036	0.357		
	VC-C2	0.0001	0.396	0.0097	0.154	0.0043	0.164	0.0014	0.257	0.0103	0.241	0.0343	0.120

[a] Results from comparing T-RF clusters observed in RL and RYL control treatments. The data in grey font identify the non-significant results in the table.

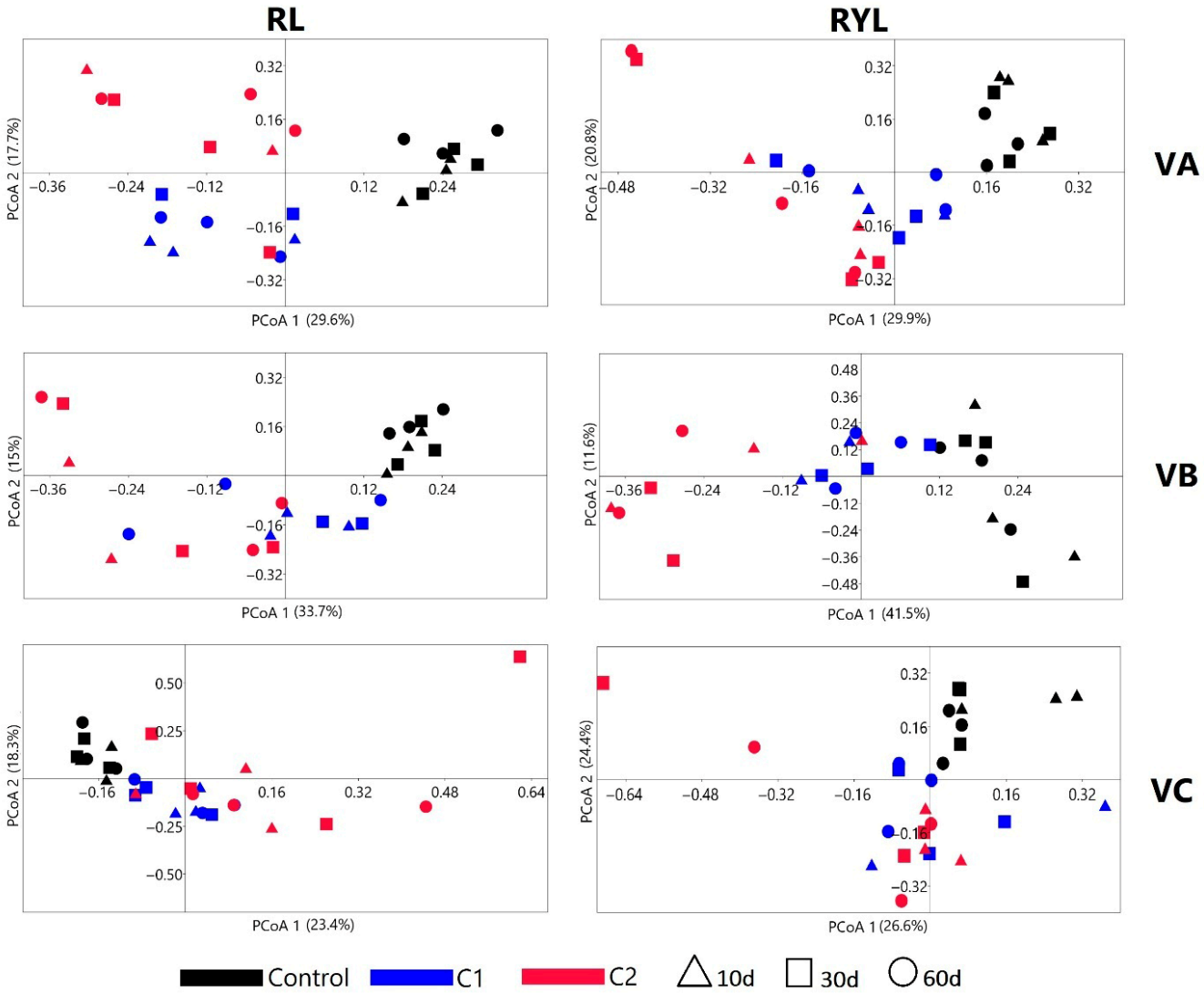

Figure 1. Principal coordinate analysis ordination (PCoA) of the bacterial community structure based on T-RFs (Bray–Curtis index) from soils RL and RYL exposed to different treatments in a microcosm experiment with sugarcane seedlings. Each panel represents one compound (VA, VB, or VC) under two soil conditions (RL and RYL). Symbols indicate sampling times (10 days = triangles, 30 days = squares, 60 days = circles), and colors represent treatments (black = control, blue = C1, red = C2). Axes show the percentage of explained variation, and clustering patterns illustrate differences in bacterial community composition among treatments and sampling times, with some soil–compound combinations showing clearer separations (e.g., RL–VA).

3.2. Analysis of Soil Bacterial Community Composition

Differences in the composition of the bacterial community between soils were found in control treatments (Figure A3). Although the highest relative abundance of OTUs in the control treatments of both soils has been assigned to those Classes with lower representation ("Others") and *Alphaproteobacteria*, which together represented about 30% of the relative abundance of OTUs in RL and RYL, the Classes *Gemmatimonadetes*, *Solibacteres*, "Unclassified" and *Acidobacteriia* had a higher contribution in RL soil bacterial composition. In contrast, the Classes *Bacilli*, *Chloracidobacteria*, *Sphingobacteriia*, and *Anaerolineae* presented higher relative abundance in RYL. The relative abundance (%) of each Class in the different treatments/soils is available in Figure A3.

Changes in bacterial community composition were found for both soils after exposure to vinasse concentrations (Figure 2). Of the 19 bacterial classes with the highest contribution

to RL soil composition, 11 were depleted whilst eight were enriched compared to the control. The Classes with lower representation or no correspondence to the known classes ("Others" and "Unclassified", respectively) were depleted after the application of the three vinasses in RL. Similar results were found in RYL soil, where the relative abundance of 10 Classes and "Others" was depleted, whilst eight Classes were enriched compared to the control. However, the "Unclassified" OTUs increased in RYL. For both soils, the concentrations of the three vinasses generally increased the relative abundance of OTUs assigned to *Betaproteobacteria*; however, it was found that the C2 of VA generated minor percentage reductions (<10%) for the Class (Supplementary Table S1).

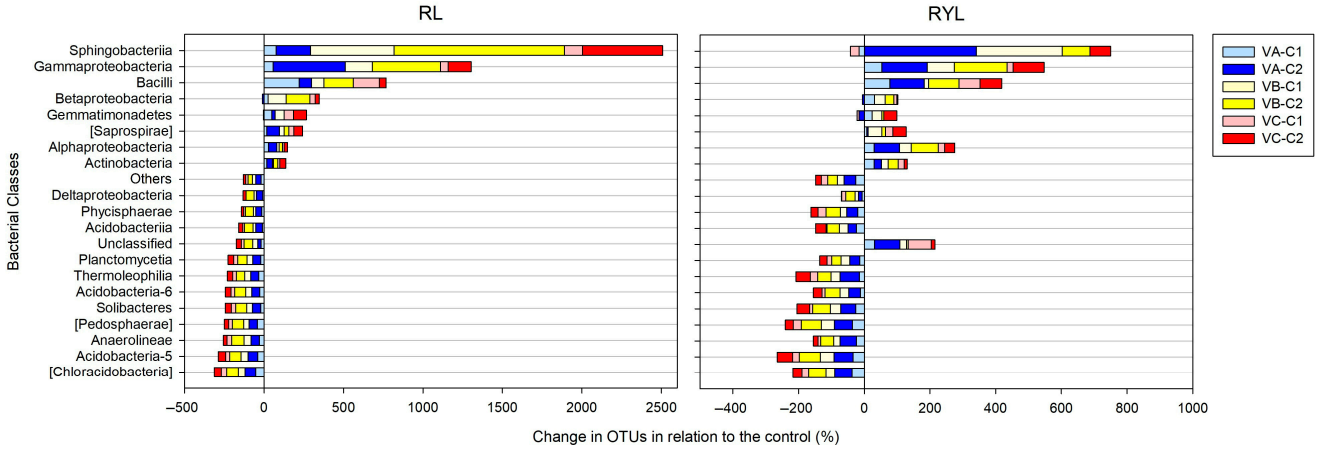

Figure 2. Percentage (%) of change (decrease or increase, compared to control) in the number of Operational Taxonomic Units (OTUs) assigned to bacterial classes found in RY and RYL soils after exposure to the concentrations (C1 and C2) of vinasses VA, VB and VC. The three sampling times (10, 30 and 60 days) were pooled together for each treatment.

The five bacterial Classes with higher reduction in the presence of vinasse concentrations in RL soil were *Chloracidobacteria*, *Acidobacteria-5*, *Solibacteres*, *Anaerolineae*, [*Pedosphaerae*]. The higher reductions caused by vinasses in RYL soil were observed for the Classes *Acidobacteria-5*, [*Pedosphaerae*], *Chloracidobacteria*, *Thermoleophilia* and *Solibacteres* (Figure 2 and Supplementary Table S1). After applying vinasse concentrations, the Classes with the higher enrichment in both soils were *Sphingobacteriia* > *Gammaproteobacteria* > *Bacilli*, although increases in the relative abundance of [*Saprospirae*], *Alphaproteobacteria*, *Actinobacteria*, *Gemmatimonadetes* and *Betaproteobacteria* have also been observed. The changes (reductions/increases) in the relative abundance of bacterial Classes promoted by the higher concentration (C2) of vinasse VA, VB and VC in RL were 2.3, 2, and 1.7 (on average, respectively) times higher than those observed in the presence of C1 (Supplementary Table S1). In RYL, the C2 concentrations of VA, VB and VC promoted changes 1.6, 1.7 and 1.4 times higher than the lowest concentrations (C1).

4. Discussion

Soil microbial communities are sensitive to changes in abiotic and biotic factors, which makes them an important indicator of soil alterations and disturbances [42]. Soil characteristics such as pH, quality, and concentration of OM, and availability of mineral nutrients (e.g., N and P) and trace metals are strictly related to the changes in soil microbial activity and composition [43,44]. Because of this, the differences found in the structure and composition of bacterial communities between the control treatments of the two soils tested in this study were expected since they were sampled in different agricultural areas, where soil properties (Table A1), climatic conditions, relief, and land-use history, among other factors, have modulated the resident microbiota [24]. Furthermore, we noticed that the dissimilarity between the bacterial structural profiles in RL and RYL was maintained

after exposure to the vinasses (Figure A2). This indicates that the invasive bacteria present in the vinasse microbiome [45] were not the main modulating factor for the changes in the structure, since they were unable to make the bacterial communities similar in the two tested soils. According to Lourenço et al. [21], the exogenous microorganisms present in sugarcane vinasses are unable to survive in soil conditions, and most of them disappear within a few days after application to soils.

According to the literature, the effects of sugarcane vinasse application on soil bacterial communities are mainly due to changes in soil pH and moisture content, the input of OM and other organic or mineral compounds, as well as the introduction of exogenous microbes [18,21,46]. Similar effects as those caused by the vinasse application on the soil bacterial communities found in the present study (Figure 1) were observed by other authors [18,20,21]. These alterations usually occur when residues deposited in the soil modify specific characteristics of the environment [47,48] and consequently, there is an increase/reduction in certain microbial taxa, which depends on the concentration and type of compounds present in the residues [49].

In this study, the changes in the structure of the resident bacterial communities in RL and RYL soils (Figure 1) are due to increases and reductions in the relative abundance of some bacterial Classes (Figure 2; Supplementary Table S1). This is likely a result of a set of environmental changes promoted by the vinasses in soils, such as increases in OM, pH, moisture content, and the concentration of mineral nutrients. For example, a strong positive correlation ($r = 0.99$; $p < 0.05$) was observed between *Bacilli*, *Gammaproteobacteria*, and *Alphaproteobacteria* and soil pH, while the latter two classes were also positively correlated with soil K levels. However, it should also be considered that approximately 50% (PCoA explanation) of the microbial community variation was explained by vinasse concentrations in the soil (Figure 1), which indicates that unmeasured biotic or abiotic factors explain about half the variation.

Regarding the variation explained by vinasse concentrations, part of the increases in some bacterial Classes was probably due to the increase in OM in the soils [50]. Vinasses contain a high content of soluble organic carbon (Table 1, and according to Pramanik and Chung [13]. This is rapidly assimilated by microorganisms and favors the predominance of those copiotrophic microorganisms (r-strategists)—where few species will show high growth rates [51,52]—resulting in competitive stress [12] and preferential selection of certain microbial groups [53]. The enrichment of *Proteobacteria* (Figure 2 and Supplementary Table S1) is consistent with their copiotrophic nature and capacity to exploit readily available resources, which may indicate stimulation of nutrient cycling.

In this study, among the classes with the higher frequency increase after adding vinasse to the soils (Figure 2), *Sphingobacteriia* and *Gammaproteobacteria* belong to phyla *Bacteroidetes* and *Proteobacteria*, respectively, which are positively correlated with C mineralization rates [23,54]. The increases in their abundance agree with the increased basal soil respiration ($C–CO_2$) after the vinasse's disposal in RL and RYL found in our previous study [11]. According to Ferguson et al. [55], these classes are common in biofilms and are capable of degrading complex biomolecules, but they are also adaptable and able to utilize various organic compounds [56]. Likewise, members of Class *Bacilli* can grow on a variety of simple compounds, inhabiting terrestrial environments where OM is found in great quantities [57] and therefore, with a quick response to the available organic C of the vinasses [21].

It is also likely that the observed increases in soil pH and moisture content (Figure A1 and [11]) have favored increases in some bacterial Classes, such as *Alphaproteobacteria* and *Gammaproteobacteria* (Figure 2), which found a suitable environment for rapid growth [11,48]. In contrast, the higher soil pH and moisture content may also have conditioned the survival of acidophilic (e.g., *Acidobacteriia*) and aerobic bacterial Classes [14,48,54]. Despite its acidic

nature (Table 1), sugarcane vinasse increases soil pH after application (Figure A1) due to the reduction in the soil cations (e.g., H^+, Al^{3+}, Fe^{3+}) by the OM functional groups (e.g., OH^-) of the effluent. According to Rousk et al. [48], the composition of the bacterial communities can be closely defined by soil pH since the changes in soil pH ranges can directly affect the growth capacity and niche specificity of some bacterial taxa because their cells have hundreds of pH-dependent enzymes [58]. The increase in soil moisture content is also directly related to microbial activity and growth. However, the saturation of the soil water pores can limit O_2 diffusion, favoring anaerobic microenvironments [59], where the presence of aerobic respiration-dependent taxa may be limited. Lourenço et al. [21] found soil moisture as the best explanatory factor for the changes in the bacterial community under a field vinasse disposal. In addition, the introduction of a few families (e.g., *Lactobacillaceae*) present in the vinasse microbiome, which can adapt to soil conditions, can also be considered to explain the increases in some bacterial Classes [21,45].

Sugarcane vinasse is also considered a potential soil pollutant due to its high concentrations of K, P, S, Fe, Mn, Zn, Cu and trace metals [1,60], as well as alcohols and recalcitrant compounds (e.g., phenols and polyphenols), among others [61]. These substances/elements can be toxic to certain soil bacterial groups [47,62]. However, the concentrations of ethanol (Table 1) and PTEs (Table A2) introduced into the soil through vinasse application were negligible in this study. Therefore, it is likely that the high concentrations of other mineral nutrients—especially K and S (Table A1, and [20])—and/or unmeasured compounds (e.g., antibiotics) [1,61] were primarily responsible for the reduction in bacterial Classes observed in RL and RYL soils. The excess of these minerals is considered a stressful factor for microorganisms [48,63–65]. These findings can be reinforced by observing that higher reductions in frequencies of Classes were found after the exposure to the doses of the vinasses VA and VB, compared to doses of VC (Supplementary Table S1), where the electrical conductivity, which represents a measure of salts concentration (salinity), was lower compared to VA and VB (Table 1). The differences in the chemical composition between vinasses are probably due to their different origins since the industrial production of ethanol (VA and VB samples) is based on methods and additives [33] distinct from those used in a laboratory (VC sample), which certainly reflects in the composition of the effluents generated. Furthermore, although the dynamics of mineral nutrients over time were not measured in this study, we recognize that such data would provide valuable insights into their effects on bacterial community structure.

All the changes in the structure of bacterial communities in both soils of this study (Figure 1) were detected shortly after the application of effluents to the soil (10 d), and the dissimilarities between vinasse treatments and controls (Table A3) remained over time (up to 60 d). These results suggest that the native soil communities were neither resistant nor resilient to the vinasses' changes, and the effluent's impact can be long-term. However, Lourenço et al. [21] demonstrated that the bacterial communities were resilient to sugarcane vinasse in an Oxisol, despite not being resistant to the application of 100 m^3 ha^{-1}. In their study, the differences between the treated samples and the control became similar after 113 days of application. These authors suggested long-term stability of the bacterial community on the time scale of 1 year when a new microbiota balance occurs. However, it is possible that the changes in microbial communities could be extended in soils where consecutive applications occur, which could not be verified in this study or by the literature data. We must consider that the impacts of vinasse on soil bacteria communities can take two to 12 months to recover and that other disturbances, such as the application of pesticides and fertilizers, periods of drought, and other agricultural management, can co-occur.

It is necessary to assess the ecosystem processes, which can be damaged during this period of vulnerability of this agroecosystem, to reduce the uncertainties about the ecological and functional impacts of the vinasse disposal on agricultural soils. For instance, the shifts observed in bacterial community composition may also have relevance for plant–microbe interactions, including those related to plant health. Certain bacterial taxa that appeared to be favored by vinasse application may be related, in other contexts, with plant growth promotion and disease suppression through mechanisms such as competition with pathogens, production of antimicrobial compounds, and induction of systemic resistance [66–68]. On the other hand, an increase in certain taxa (e.g., *Gammaproteobacteria*) may include pathogenic species [69], indicating that while some vinasse-favored taxa may benefit soil and plant health, their higher abundance could also signal a potential increase in plant pathogens. Thus, the observed shifts may reflect both beneficial contributions to soil functioning and possible risks for plant health, underscoring the need for further integrative studies.

Although our study did not directly assess plant health outcomes, these community-level changes suggest that vinasse application may influence plant-associated bacteria, highlighting the need for future research specifically designed to link community composition with functional roles, since community structure may remain stable while key functions shift [44].

Finally, we noticed that changes in the bacterial classes were found at concentrations (C1) calculated following the Brazilian technical standard P4.231 [5] and at those simulating the worst application scenario (C2). Although this study was not designed to obtain dose–response relationships, it was clear that the impacts of vinasse on bacterial communities increased as a function of the increasing doses, regardless of the tested soil type. A clear dose–response for the vinasse toxicity to soil fauna was found by Alves et al. [9], which indicated that concentrations calculated on the technical standard P4.231 did not protect two bioindicator species from the significant toxic effects of these same vinasses in tropical soils. Many literature reports attest that vinasse is beneficial for the growth and diversity of soil microbiota [4,14,16–18]. Even our previous study [11] showed that the tested vinasses favored the growth and microbial metabolism of RY and RYL soils. Nevertheless, in the same experiment, the analyses based on the 16S rRNA gene of the present study showed that this type of management could lead to an imbalance in the soil microbiota of agricultural soils because its application promotes the growth of certain bacterial classes at the expense of others. These results are alarming, especially considering that the use of sugarcane vinasses may be indiscriminate in Brazilian agricultural soils, and indicate that the management should be done with caution. Our findings also suggest that, although moderate vinasse applications may improve soil fertility, repeated or high-dose inputs could promote microbial imbalances. Therefore, sustainable reuse will require the monitoring of soil properties and microbial indicators, as well as long-term field studies to establish safe thresholds for application.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/soilsystems9030102/s1, Supplementary Table S1. Percentage (%) of change (decrease or increase, compared to control) in the number of Operational Taxonomic Units (OTUs) assigned to bacterial classes found in RY and RYL soils after exposure to the concentrations (C1 and C2) of vinasse VA, VB and VC. The three sampling times (10, 30 and 60 days) were pooled together for each treatment. The C2:C1 ratio indicates the magnitude of the change in the bacterial community considering undiluted vinasses (C2) divided by diluted vinasses (C1). Green and red arrows indicate the increase and decrease, respectively, in the number of OTUs assigned to bacterial classes; Supplementary Table S2. Raw data of the abundance of bacterial classes and orders from

16S rRNA sequencing in RY and RYL soils after exposure to vinasse treatments VA, VB, and VC at concentrations C1 and C2.

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Institutional Review Board Statement: The procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (Brazilian regulation for the scientific use of animals—Law no. 11.794).

Informed Consent Statement: Consent to participate and publication is not applicable once this study does not involve humans.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. The raw sequencing data supporting the findings of this study are available for download as a Supplementary File (Supplementary Table S2).

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Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Appendix A contains supplemental data (tables and figures). Tables are presented as "Tables A1–A3" and Figures are presented as "Figures A1–A5". Additionally, the complete dataset (including all taxonomic levels assessed) is available for download as a Supplementary File.

Table A1. Physical and chemical properties of the two natural Oxisols (RL and RYL) used in the microcosm experiment (before vinasses application). Adapted from Alves et al. [11]. OM—Organic Matter; CEC—Cation Exchange Capacity; WHC—Water Holding Capacity.

Parameter	Soil	
	RL	RYL
pH (1M KCl)	4.4 ± 0.1	4.9 ± 0.4
OM (g dm^{-3})	25	18
P (mg dm^{-3})	15	12
S (mg dm^{-3})	10	5
K (mg dm^{-3})	52	80
Ca (mg dm^{-3})	560	340
Mg (mg dm^{-3})	120	84
Cu (mg dm^{-3})	0.8	1
Fe (mg dm^{-3})	55	116
Mn (mg dm^{-3})	15.4	4.3
Zn (mg dm^{-3})	4.9	2.4
B (mg dm^{-3})	0.1	0.1
Al (mg dm^{-3})	<9	9
H + Al ($mmol_c$ dm^{-3})	25	28
CEC ($mmol_c$ dm^{-3})	63.9	54.3

Table A1. *Cont.*

Parameter	Soil	
	RL	**RYL**
Sand (g kg^{-1})	502	800
Silt (g kg^{-1})	172	24
Clay (g kg^{-1})	326	176
WHC (%)	33	20
Texture	Sandy Clay Loam	Sandy Loam

Table A2. Pseudo-total concentrations of potentially toxic elements (PTE) found in the vinasses VA, VB and VC, and RL and RYL soils (before vinasses application) used in the microcosm experiment.

PTE	Vinasses (mg L^{-1})			Soils (mg kg^{-1})	
	VA	**VB**	**VC**	**RL**	**RYL**
As	0.056	0.011	<0.01 [a]	1.3	2.15
Cd	<0.002 [a]	<0.002 [a]	<0.002 [a]	1.025	0.025
Co	0.104	0.043	0.017	8.425	1.55
Cr	0.034	0.068	0.071	51.65	18.62
Cu	0.317	0.954	0.209	75.87	13
Hg	<0.01 [a]	<0.01 [a]	<0.01 [a]	<0.01 [a]	<0.01 [a]
Mo	0.02	0.025	0.013	0.8	0.625
Ni	0.043	0.064	0.047	11.57	3.85
Pb	0.024	0.028	0.014	14.55	4.575
Zn	0.520	0.222	0.337	42.95	15.25

[a] values were lower than the limit of quantification (LQ).

Table A3. Similarity analysis (ANOSIM) between T-RF clusters observed at the three sampling times (10, 30 and 60 days) after the application of the vinasses (VA, VB and VC) concentrations (C1 and C2) on RL and RYL soils. p-values ≤ 0.01 indicate significant dissimilarity between the three sampling times for the same treatment.

Soil	Treatment	p-Value	R
RL	Control	0.84	−0.242
	VA-C1	0.91	−0.210
	VA-C2	0.82	−0.193
	VB-C1	0.95	−0.152
	VB-C2	0.89	−0.238
	VC-C1	0.95	−0.202
	VC-C2	0.97	−0.202
RYL	Control	0.56	−0.037
	VA-C1	0.98	−0.317
	VA-C2	0.88	−0.131
	VB-C1	0.94	−0.162
	VB-C2	0.66	−0.075
	VC-C1	0.40	−0.048
	VC-C2	0.93	−0.111

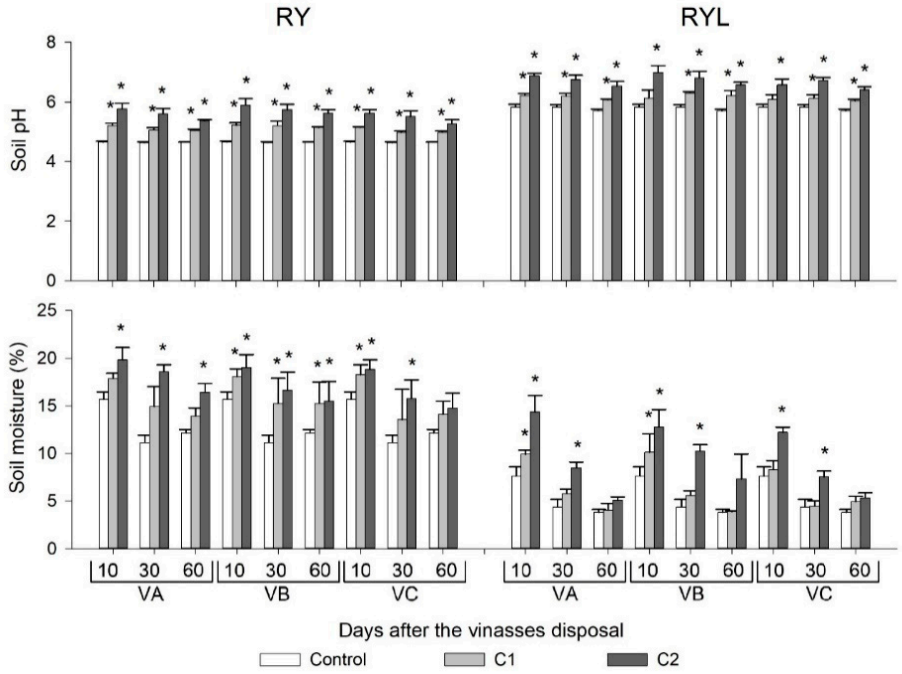

Figure A1. pH and moisture of RY and RYL Oxisols after 10, 30 and 60 days of application of the vinasses (VA, VB and VC) concentrations in the microcosm experiments (mean ± standard deviation; $n = 4$). Asterisks denote a significant difference ($p \leq 0.01$). Data adapted from Alves et al. [11].

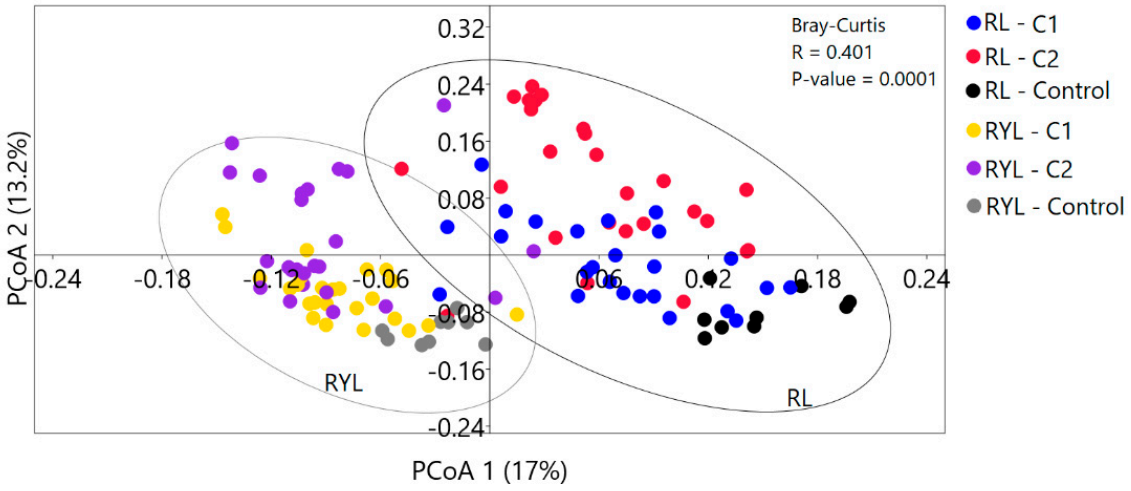

Figure A2. Principal coordinate analysis ordination (PCoA) of the T-RFs matrix (*Bray–Curtis* index) from bacterial communities. Data were obtained after applying the concentrations C1 (blue and yellow) and C2 (red and violet) of the three sugarcane vinasses (VA, VB and VC pooled together in each concentration) on RL and RYL soils, respectively, in a microcosm experiment with sugarcane seedlings. The 95% ellipses were drawn for grouping T-RFs found in each soil type. R and *p*-values were obtained through ANOSIM.

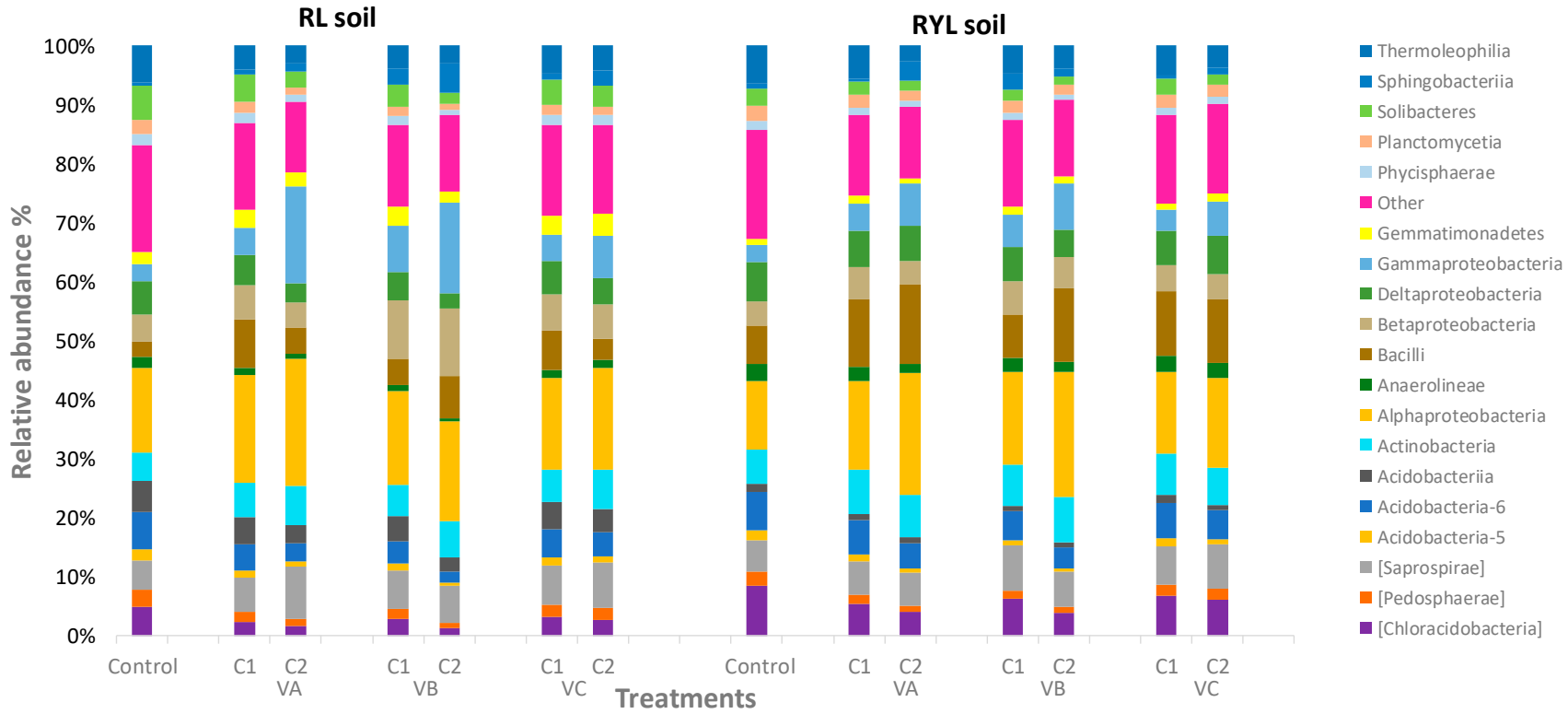

Figure A3. Relative abundance of bacterial classes from 16S rRNA sequencing data found in RY and RYL soils after exposure to the concentrations (C1 and C2) of vinasses VA, VB and VC. The three sampling times (10, 30 and 60 days) were pooled together for each treatment.

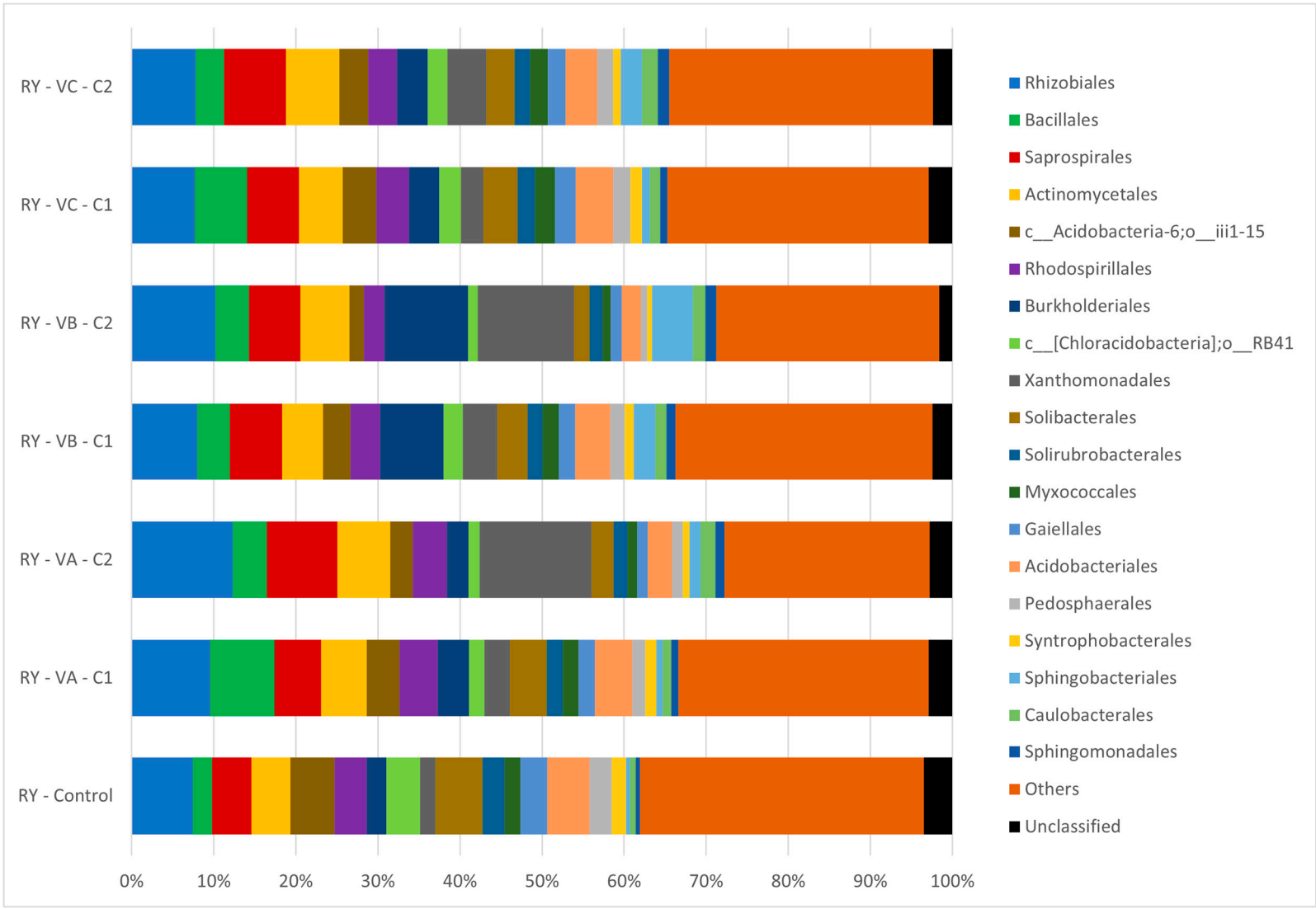

Figure A4. Relative abundance of bacterial orders from 16S rRNA sequencing data found in RY soil after exposure to the concentrations (C1 and C2) of vinasses VA, VB and VC. The three sampling times (10, 30 and 60 days) were pooled together for each treatment.

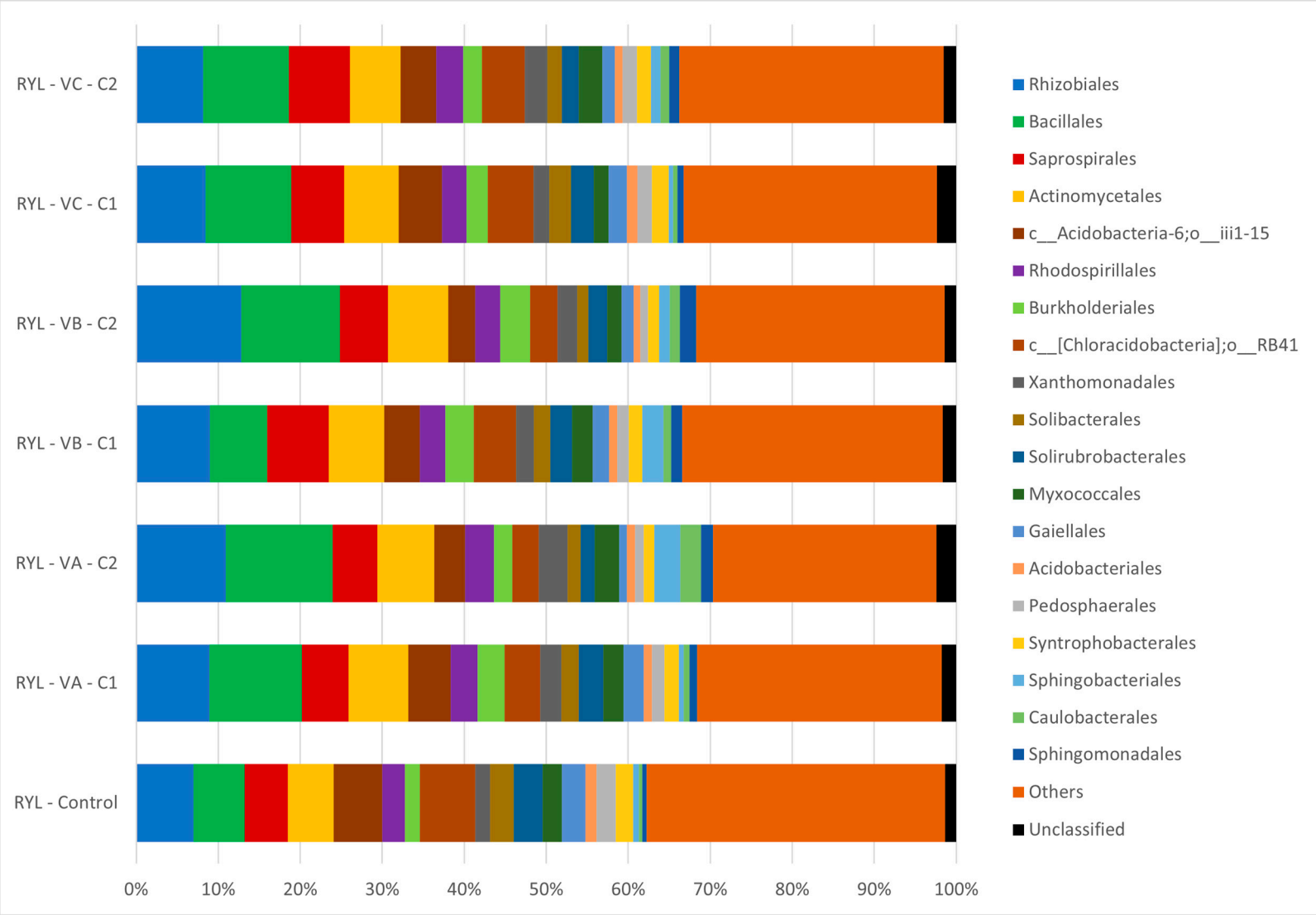

Figure A5. Relative abundance of bacterial orders from 16S rRNA sequencing data found in and RYL soil after exposure to the concentrations (C1 and C2) of vinasses VA, VB and VC. The three sampling times (10, 30 and 60 days) were pooled together for each treatment.

References

1. Christofoletti, C.A.; Escher, J.P.; Correia, J.E.; Marinho, J.F.U.; Fontanetti, C.S. Sugarcane Vinasse: Environmental Implications of Its Use. *Waste Manag.* **2013**, *33*, 2752–2761. [CrossRef]
2. Jiang, Z.-P.; Li, Y.-R.; Wei, G.-P.; Liao, Q.; Su, T.-M.; Meng, Y.-C.; Zhang, H.-Y.; Lu, C.-Y. Effect of Long-Term Vinasse Application on Physico-Chemical Properties of Sugarcane Field Soils. *Sugar Tech* **2012**, *14*, 412–417. [CrossRef]
3. Carpanez, T.G.; Moreira, V.R.; Assis, I.R.; Amaral, M.C.S. Sugarcane Vinasse as Organo-Mineral Fertilizers Feedstock: Opportunities and Environmental Risks. *Sci. Total Environ.* **2022**, *832*, 154998. [CrossRef]
4. Yang, S.D.; Liu, J.X.; Wu, J.; Tan, H.W.; Li, Y.R. Effects of Vinasse and Press Mud Application on the Biological Properties of Soils and Productivity of Sugarcane. *Sugar Tech* **2013**, *15*, 152–158. [CrossRef]
5. CETESB. *Norma Técnica P4.231: Vinhaça—Critérios e Procedimentos Para Aplicação No Solo Agrícola*; CETESB: São Paulo, Brazil, 2015.
6. Fuess, L.T.; Garcia, M.L. Implications of Stillage Land Disposal: A Critical Review on the Impacts of Fertigation. *J. Environ. Manag.* **2014**, *145*, 210–229. [CrossRef]
7. de Oliveira, B.G.; Carvalho, J.L.N.; Cerri, C.E.P.; Cerri, C.C.; Feigl, B.J. Soil Greenhouse Gas Fluxes from Vinasse Application in Brazilian Sugarcane Areas. *Geoderma* **2013**, *200–201*, 77–84. [CrossRef]
8. Christofoletti, C.A.; Pedro-Escher, J.; Fontanetti, C.S. Assessment of the Genotoxicity of Two Agricultural Residues after Processing by Diplopods Using the Allium Cepa Assay. *Water Air Soil. Pollut.* **2013**, *224*, 1523. [CrossRef]
9. Alves, P.R.L.; Natal-da-Luz, T.; Sousa, J.P.; Cardoso, E.J.B.N. Ecotoxicological Characterization of Sugarcane Vinasses When Applied to Tropical Soils. *Sci. Total Environ.* **2015**, *526*, 222–232. [CrossRef] [PubMed]
10. Moran-Salazar, R.G.; Sanchez-Lizarraga, A.L.; Rodriguez-Campos, J.; Davila-Vazquez, G.; Marino-Marmolejo, E.N.; Dendooven, L.; Contreras-Ramos, S.M. Utilization of Vinasses as Soil Amendment: Consequences and Perspectives. *SpringerPlus* **2016**, *5*, 1007. [CrossRef]
11. Alves, P.R.L.; Estrada-Bonilla, G.A.; Bini, D.; Cardoso, E.J.B.N. Changes in the Microbial Metabolism of Agricultural Tropical Soils Amended with Sugarcane Vinasses. *Sugar Tech* **2019**, *21*, 364–369. [CrossRef]
12. Santos, T.M.C.; Santos, M.A.L.; Santos, C.G.; Santos, V.R.; Pacheco, D.S. Efeito da fertirrigação com vinhaça nos microrganismos do solo. *Rev. Caatinga* **2009**, *22*, 155–160.
13. Pramanik, P.; Chung, Y.R. Changes in Fungal Population of Fly Ash and Vinasse Mixture during Vermicomposting by *Eudrilus eugeniae* and *Eisenia fetida*: Documentation of Cellulase Isozymes in Vermicompost. *Waste Manag.* **2011**, *31*, 1169–1175. [CrossRef]
14. Prado, R.D.M.; Caione, G.; Campos, C.N.S. Filter Cake and Vinasse as Fertilizers Contributing to Conservation Agriculture. *Appl. Environ. Soil. Sci.* **2013**, *2013*, 581984. [CrossRef]
15. Aleixo, A.P.; Kaschuk, G.; Alberton, O. Soil Fungal and Bacterial Biomass Determined by Epifluorescence Microscopy and Mycorrhizal Spore Density in Different Sugarcane Managements. *Ciência Rural.* **2014**, *44*, 588–594. [CrossRef]
16. Stephen, G.S.; Shitindi, M.J.; Bura, M.D.; Kahangwa, C.A.; Nassary, E.K. Harnessing the potential of sugarcane-based liquid byproducts—molasses and spentwash (vinasse) for enhanced soil health and environmental quality. A systematic review. *Front. Agron.* **2024**, *6*, 1358076. [CrossRef]
17. da Luz, F.B.; Gonzaga, L.C.; Cherubin, M.R.; Castioni, G.A.F.; Carvalho, J.L.N. Soil Health Impact of Long-term Sugarcane Vinasse Recycling. *Biofuels Bioprod. Biorefin.* **2024**, *18*, 2064–2077. [CrossRef]
18. Omori, W.P.; Camargo, A.F.D.; Goulart, K.C.S.; Lemos, E.G.D.M.; Souza, J.A.M.D. Influence of Vinasse Application in the Structure and Composition of the Bacterial Community of the Soil under Sugarcane Cultivation. *Int. J. Microbiol.* **2016**, *2016*, 2349514. [CrossRef]
19. Senatore, D.; Queirolo, A.; Monza, J.; Bajsa, N. Using sugar cane vinasse as a biofertilizer: Effects on soil microbial community due to periodical application. *Environ. Sustain.* **2023**, *6*, 173–182. [CrossRef]
20. Navarrete, A.A.; Diniz, T.R.; Braga, L.P.P.; Silva, G.G.Z.; Franchini, J.C.; Rossetto, R.; Edwards, R.A.; Tsai, S.M. Multi-Analytical Approach Reveals Potential Microbial Indicators in Soil for Sugarcane Model Systems. *PLoS ONE* **2015**, *10*, e0129765. [CrossRef]
21. Lourenço, K.S.; Suleiman, A.K.A.; Pijl, A.; van Veen, J.A.; Cantarella, H.; Kuramae, E.E. Resilience of the Resident Soil Microbiome to Organic and Inorganic Amendment Disturbances and to Temporary Bacterial Invasion. *Microbiome* **2018**, *6*, 142. [CrossRef]
22. Sanchez-Lizarraga, A.L.; Arenas-Montaño, V.; Marino-Marmolejo, E.N.; Dendooven, L.; Velazquez-Fernandez, J.B.; Davila-Vazquez, G.; Rodriguez-Campos, J.; Hernández-Cuevas, L.; Contreras-Ramos, S.M. Vinasse Irrigation: Effects on Soil Fertility and Arbuscular Mycorrhizal Fungi Population. *J. Soils Sedim.* **2018**, *18*, 3256–3270. [CrossRef]
23. de Alcântara Neto, F.; Pinheiro, D.A.; Rocha, S.M.B.; Leite, M.R.L.; Costa, R.M.; da Silva, J.M.; Ventura, S.H.; Costa, M.K.L.; Sousa, T.K.D.S.A.; Miranda, R.D.S.; et al. Bacterial Community in Sugarcane Rhizosphere Under *Bacillus subtilis* Inoculation and Straw Return. *Soil. Syst.* **2025**, *9*, 44. [CrossRef]
24. Bini, D.; Santos, C.A.D.; Bernal, L.P.T.; Andrade, G.; Nogueira, M.A. Identifying Indicators of C and N Cycling in a Clayey Ultisol under Different Tillage and Uses in Winter. *Appl. Soil. Ecol.* **2014**, *76*, 95–101. [CrossRef]

25. Basu, S.; Kumar, G.; Chhabra, S.; Prasad, R. Role of Soil Microbes in Biogeochemical Cycle for Enhancing Soil Fertility. In *New and Future Developments in Microbial Biotechnology and Bioengineering*; Elsevier: Amsterdam, The Netherlands, 2021; pp. 149–157. [CrossRef]
26. Ouyang, Y.; Reeve, J.R.; Norton, J.M. The Quality of Organic Amendments Affects Soil Microbiome and Nitrogen-Cycling Bacteria in an Organic Farming System. *Front. Soil. Sci.* **2022**, *2*, 869136. [CrossRef]
27. Meng, J.; Li, Y.; Qiu, Y.; Luo, Y.; Fang, Y.; Van Zwieten, L.; Wang, H.; Chen, H. Biochars Regulate Bacterial Community and Their Putative Functions in the Charosphere: A Mesh-Bag Field Study. *J. Soils Sediments* **2023**, *23*, 596–605. [CrossRef]
28. Yaghoubi Khanghahi, M.; Curci, M.; Cazzato, E.; Lasorella, C.; Traversa, A.; Crecchio, C.; Spagnuolo, M. Shifts in Soil Bacterial Communities under Three-Year Fertilization Management and Multiple Cropping Systems. *Soil. Syst.* **2024**, *8*, 5. [CrossRef]
29. Soil Survey Staff. *Soil Survey Staff*, 12th ed.; USDA—Natural Resources Conservation Service: Washington, DC, USA, 2014.
30. *ISO 11268-2*; Soil Quality—Effects of Pollutants on Earthworms—Part 2: Determination of Effects on Reproduction of Eisenia fetida/Eisenia andrei. International Organization for Standardization: Geneva, Switzerland, 2012.
31. Raij, B.V.; Andrade, J.C.D.; Cantarella, H.; Quaggio, J.A. *Análise Química para Avaliação da Fertilidade de Solos Tropicais*; Instituto Agronômico de Campinas (IAC): Campinas, Brazil, 2021.
32. Dane, J.H.; Topp, C.G. *Methods of Soil Analysis*; Soil Science Society of America: Madison, WI, USA, 2002. [CrossRef]
33. Compart, D.M.P.; Carlson, A.M.; Crawford, G.I.; Fink, R.C.; Diez-Gonzalez, F.; DiCostanzo, A.; Shurson, G.C. Presence and Biological Activity of Antibiotics Used in Fuel Ethanol and Corn Co-Product Production1. *J. Anim. Sci.* **2013**, *91*, 2395–2404. [CrossRef]
34. Kiehl, E.J. *Fertilizantes Organicos*; Kiehl, E.J., Ed.; Ceres: Piracicaba, Brazil, 1985.
35. United States Environmental Protection Agency. *Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils*; Method 3051A; United States Environmental Protection Agency: Washington, DC, USA, 2007.
36. United States Environmental Protection Agency. *Microwave Assisted Acid Digestion of Aqueous Samples and Extracts*; Method 3015A; United States Environmental Protection Agency: Washington, DC, USA, 2007.
37. Schütte, U.M.E.; Abdo, Z.; Bent, S.J.; Williams, C.J.; Schneider, G.M.; Solheim, B.; Forney, L.J. Bacterial Succession in a Glacier Foreland of the High Arctic. *ISME J.* **2009**, *3*, 1258–1268. [CrossRef] [PubMed]
38. Caporaso, J.G.; Lauber, C.L.; Walters, W.A.; Berg-Lyons, D.; Huntley, J.; Fierer, N.; Owens, S.M.; Betley, J.; Fraser, L.; Bauer, M.; et al. Ultra-High-Throughput Microbial Community Analysis on the Illumina HiSeq and MiSeq Platforms. *ISME J.* **2012**, *6*, 1621–1624. [CrossRef]
39. Estrada-Bonilla, G.A.; Lopes, C.M.; Durrer, A.; Alves, P.R.L.; Passaglia, N.; Cardoso, E.J.B.N. Effect of Phosphate-Solubilizing Bacteria on Phosphorus Dynamics and the Bacterial Community during Composting of Sugarcane Industry Waste. *Syst. Appl. Microbiol.* **2017**, *40*, 308–313. [CrossRef]
40. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological Statistical Software Package for Education and Data Analysis. *Palaeontol. Electron.* **2001**, *4*, 1–9.
41. CONAMA. Resolução n°420 de 28 dez 2009. *Diário Of. União* **2009**, *1*, 81–84.
42. Tajik, S.; Ayoubi, S.; Lorenz, N. Soil Microbial Communities Affected by Vegetation, Topography and Soil Properties in a Forest Ecosystem. *Appl. Soil. Ecol.* **2020**, *149*, 103514. [CrossRef]
43. Matteoli, F.P.; Silva, A.M.M.; Feiler, H.P.; de Araújo, V.L.V.P.; Cardoso, E.J.B.N. Predicting Soil Farming System and Attributes Based on Soil Bacterial Community. *Appl. Soil. Ecol.* **2022**, *171*, 104335. [CrossRef]
44. Silva, A.M.M.; Estrada-Bonilla, G.A.; Lopes, C.M.; Matteoli, F.P.; Cotta, S.R.; Feiler, H.P.; Rodrigues, Y.F.; Cardoso, E.J.B.N. Does Organomineral Fertilizer Combined with Phosphate-Solubilizing Bacteria in Sugarcane Modulate Soil Microbial Community and Functions? *Microb. Ecol.* **2022**, *84*, 539–555. [CrossRef]
45. Cassman, N.A.; Lourenço, K.S.; Do Carmo, J.B.; Cantarella, H.; Kuramae, E.E. Genome-Resolved Metagenomics of Sugarcane Vinasse Bacteria. *Biotechnol. Biofuels* **2018**, *11*, 48. [CrossRef] [PubMed]
46. Suleiman, A.K.A.; Gonzatto, R.; Aita, C.; Lupatini, M.; Jacques, R.J.S.; Kuramae, E.E.; Antoniolli, Z.I.; Roesch, L.F.W. Temporal Variability of Soil Microbial Communities after Application of Dicyandiamide-Treated Swine Slurry and Mineral Fertilizers. *Soil. Biol. Biochem.* **2016**, *97*, 71–82. [CrossRef]
47. Gomes, N.C.M.; Landi, L.; Smalla, K.; Nannipieri, P.; Brookes, P.C.; Renella, G. Effects of Cd- and Zn-Enriched Sewage Sludge on Soil Bacterial and Fungal Communities. *Ecotoxicol. Env. Saf.* **2010**, *73*, 1255–1263. [CrossRef]
48. Rousk, J.; Bååth, E.; Brookes, P.C.; Lauber, C.L.; Lozupone, C.; Caporaso, J.G.; Knight, R.; Fierer, N. Soil Bacterial and Fungal Communities across a PH Gradient in an Arable Soil. *ISME J.* **2010**, *4*, 1340–1351. [CrossRef]
49. Siles, J.A.; Rachid, C.T.C.C.; Sampedro, I.; García-Romera, I.; Tiedje, J.M. Microbial Diversity of a Mediterranean Soil and Its Changes after Biotransformed Dry Olive Residue Amendment. *PLoS ONE* **2014**, *9*, e103035. [CrossRef]
50. Zhang, X.; Li, D.; Liu, Y.; Li, J.; Hu, H. Soil Organic Matter Contents Modulate the Effects of Bacterial Diversity on the Carbon Cycling Processes. *J. Soils Sedim.* **2023**, *23*, 911–922. [CrossRef]

51. Insam, H.; Hutchinson, T.C.; Reber, H.H. Effects of Heavy Metal Stress on the Metabolic Quotient of the Soil Microflora. *Soil. Biol. Biochem.* **1996**, *28*, 691–694. [CrossRef]
52. Guo, Q.; Yin, Q.; Du, J.; Zuo, J.; Wu, G. New Insights into the r/K Selection Theory Achieved in Methanogenic Systems through Continuous-Flow and Sequencing Batch Operational Modes. *Sci. Total Environ.* **2022**, *807 Pt 1*, 150732. [CrossRef]
53. Gumiere, T.; Durrer, A.; Bohannan, B.J.M.; Andreote, F.D. Biogeographical Patterns in Fungal Communities from Soils Cultivated with Sugarcane. *J. Biogeogr.* **2016**, *43*, 2016–2026. [CrossRef]
54. Fierer, N.; Bradford, M.A.; Jackson, R.B. Toward an Ecological Classification of Soil Bacteria. *Ecology* **2007**, *88*, 1354–1364. [CrossRef] [PubMed]
55. Ferguson, R.M.W.; O'Gorman, E.J.; McElroy, D.J.; McKew, B.A.; Coleman, R.A.; Emmerson, M.C.; Dumbrell, A.J. The Ecological Impacts of Multiple Environmental Stressors on Coastal Biofilm Bacteria. *Glob. Change Biol.* **2021**, *27*, 3166–3178. [CrossRef] [PubMed]
56. Guo, T.; Zhang, Q.; Ai, C.; Liang, G.; He, P.; Lei, Q.; Zhou, W. Analysis of Microbial Utilization of Rice Straw in Paddy Soil Using a DNA-SIP Approach. *Soil. Sci. Soc. Am. J.* **2020**, *84*, 99–114. [CrossRef]
57. Mandic-Mulec, I.; Stefanic, P.; van Elsas, J.D. Ecology of Bacillaceae. *Microbiol. Spectr.* **2015**, *3*, TBS-0017-2013. [CrossRef]
58. Cardoso, E.J.B.N.; Andreote, F.D. *Microbiologia do Solo*, 2nd ed.; ESALQ: Piracicaba, Brazil, 2016.
59. Yan, N.; Marschner, P.; Cao, W.; Zuo, C.; Qin, W. Influence of Salinity and Water Content on Soil Microorganisms. *Int. Soil. Water Conserv. Res.* **2015**, *3*, 316–323. [CrossRef]
60. Kannan, A.; Upreti, R.K. Influence of Distillery Effluent on Germination and Growth of Mung Bean (*Vigna radiata*) Seeds. *J. Hazard. Mater.* **2008**, *153*, 609–615. [CrossRef]
61. España-Gamboa, E.; Mijangos-Cortes, J.; Barahona-Perez, L.; Dominguez-Maldonado, J.; Hernández-Zarate, G.; Alzate-Gaviria, L. Vinasses: Characterization and Treatments. *Waste Manag. Res. J. A Sustain. Circ. Econ.* **2011**, *29*, 1235–1250. [CrossRef] [PubMed]
62. Pan, J.; Yu, L. Effects of Cd or/and Pb on Soil Enzyme Activities and Microbial Community Structure. *Ecol. Eng.* **2011**, *37*, 1889–1894. [CrossRef]
63. Rietz, D.N.; Haynes, R.J. Effects of Irrigation-Induced Salinity and Sodicity on Soil Microbial Activity. *Soil. Biol. Biochem.* **2003**, *35*, 845–854. [CrossRef]
64. Crecchio, C.; Gelsomino, A.; Ambrosoli, R.; Minati, J.L.; Ruggiero, P. Functional and Molecular Responses of Soil Microbial Communities under Differing Soil Management Practices. *Soil. Biol. Biochem.* **2004**, *36*, 1873–1883. [CrossRef]
65. Gu, Y.; Zhang, X.; Tu, S.; Lindström, K. Soil Microbial Biomass, Crop Yields, and Bacterial Community Structure as Affected by Long-Term Fertilizer Treatments under Wheat-Rice Cropping. *Eur. J. Soil. Biol.* **2009**, *45*, 239–246. [CrossRef]
66. Mendes, R.; Garbeva, P.; Raaijmakers, J.M. The Rhizosphere Microbiome: Significance of Plant Beneficial, Plant Pathogenic, and Human Pathogenic Microorganisms. *FEMS Microbiol. Rev.* **2013**, *37*, 634–663. [CrossRef]
67. Compant, S.; Samad, A.; Faist, H.; Sessitsch, A. A Review on the Plant Microbiome: Ecology, Functions, and Emerging Trends in Microbial Application. *J. Adv. Res.* **2019**, *19*, 29–37. [CrossRef]
68. Carrión, V.J.; Perez-Jaramillo, J.; Cordovez, V.; Tracanna, V.; De Hollander, M.; Ruiz-Buck, D.; Mendes, L.W.; van Ijcken, W.F.; Gomez-Exposito, R.; Elsayed, S.S.; et al. Pathogen-Induced Activation of Disease-Suppressive Functions in the Endophytic Root Microbiome. *Science* **2019**, *366*, 606–612. [CrossRef]
69. Ntambo, M.S.; Meng, J.-Y.; Rott, P.C.; Royer, M.; Lin, L.-H.; Zhang, H.-L.; Gao, S.-J. Identification and characterization of *Xanthomonas albilineans* causing sugarcane leaf scald in China using multilocus sequence analysis. *Plant Pathol.* **2019**, *68*, 269–277. [CrossRef]