



Article Effect of Soil Aggregate Size on Vineyard Bacterial Communities under Organic and Conventional Agro-Managements

Yosef Steinberger ^{1,*}, Tirza Doniger ¹, Chen Sherman ¹, Itaii Applebaum ¹ and Gil Eshel ²

- The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 5290002, Israel
 Soil Erosian Research Station, Ministry of Agriculture and Rural Development, HaMaccohim Road
- ² Soil Erosion Research Station, Ministry of Agriculture and Rural Development, HaMaccabim Road, Rishon LeZion 5020000, Israel
- * Correspondence: yosef.steinberger@biu.ac.il; Tel.: +972-35-318-571

Abstract: Soil microorganisms are an indispensable component of natural ecosystems and play an important role in agro-management ecosystems. However, the function of soil microbial communities is still a black box. The present study aimed to investigate the effect of organic and conventional agro-management practices in a vineyard on the soil's bacterial community and its composition in three different soil aggregate sizes using functional profiles derived using 16S rDNA metagenomics analysis for elucidating the metabolic capabilities of soil microbial communities. Soil samples were compared in terms of community composition and functionality. A clear distinction was found between the two managements. The soil samples contained 12 phyla and 45 orders, where Proteobacteria was the most common phylum in all treatments. Twenty-three functional profiles were obtained for both treatments and three aggregate sizes, showing similarity in their function, suggesting that functionality is due to the community's composition and environmental conditions. The results indicate that organic farming systems have a beneficial effect on microbial diversity and encourage ecosystem multifunctionality.

Keywords: soil aggregates; soil bacteria; organic agriculture

1. Introduction

As part of the soil biota, soil microorganisms fulfill important roles (e.g., organic matter decomposition, regulation of nutrient availability, etc.), together composing an organized unit that constitutes one of the most important cooperative factors in soil formation. Together with anthropogenic and abiotic components, they are responsible for soil function over time [1]. The soil bacterial community is among the most dynamic components of soil biota and is affected by many biotic and abiotic parameters [2–4]. Due to its heterogeneous environment, the soil microbial community is strongly dependent on organic carbon, moisture, and temperature and is affected by their availability, as well as by soil physical and chemical components that control aggregate formation and stability [5–7]. Due to their involvement and activity in organic matter decomposition, microbial communities must share their space and compete for energy sources [8–10]. Nutritional resources are among the most important elements for which the microbial community competes. Bacteria can even inhabit soil aggregate pores with a diameter of less than 3 µm and form spider web networks that develop into "microbial villages", as defined by Wilpiszeski et al. [11]. Approximately 90% of soil bacteria are associated with macroaggregates, while other communities colonize the exterior parts of the aggregates [12,13].

Thanks to recent developments in molecular methods, it has also become more feasible to predict bacterial functions, in addition to bacterial community composition and diversity [14,15]. Such developments allow us to learn about the functional capabilities



Citation: Steinberger, Y.; Doniger, T.; Sherman, C.; Applebaum, I.; Eshel, G. Effect of Soil Aggregate Size on Vineyard Bacterial Communities under Organic and Conventional Agro-Managements. *Land* **2022**, *11*, 1517. https://doi.org/10.3390/ land11091517

Academic Editor: Guangju Zhao

Received: 11 August 2022 Accepted: 5 September 2022 Published: 8 September 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). of bacterial communities using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt)—a computational approach that predicts the functional composition of the metagenome. This is a key tool that enables the profiling of marker genes for studying functional capacities of the microbial community [16,17]. Relating the soil microbial community structure to its functionality is challenging and could enable great progress in structural community analysis [18,19]. The power of determining the bacterial diversity and functional capabilities in aggregate microsites that form unique niches in response to management will elucidate the specific interplay between niches and inhabitants [20,21]. In both cases, aggregates serve as a habitat, and the inhabitants of aggregates are always determined by soil food and moisture availability. According to Watt et al. [22], soil aggregate pores may retain enough moisture to allow biological activity to flourish for long periods. In many managed agroecosystems, the microbial communities in soil are structured on numerous spatial scales [23], where the aggregate size, structure, quality, origin, and amount of organic matter determine the microbial community assemblage [24].

In vineyards, as in other agroecosystems, soil microbial community diversity performs a variety of ecological services in promoting fertility, including nutrient cycling, aggregate formation, detoxification of noxious chemicals, acting as bioindicators for soil quality, and preventing soil erosion [25,26]. The bacterial and the fungal rhizosphere microbiomes associated with the grapevine are known for their roles in the carbon biogeochemical cycle and the production of secondary metabolites [27] that contribute to the above-ground biomass, yield production, and fruit qualities.

The present study examined the soil microbial community composition and structure for two vineyard agro-management practices: organic and conventional; in two sampling locations: between vineyard rows and in-rows; associated with three soil aggregate sizes: macro, meso, and micro. The main objective of the study was to understand the connection between aggregate size and bacterial community composition and function. Specifically, we attempted to answer the following questions: (1) How do agro-management practices, sampling locations, and aggregate sizes affect bacterial communities? (2) Is there a relationship between aggregate size and the diversity of the bacterial community's composition? (3) Does agro-management determine microbial functions?

Based on the above, we hypothesized that the following:

- 1. Vineyard management and aggregate size will strongly affect soil bacterial diversity, and a significant change will occur in bacterial diversity (Shannon (H') index) in soil samples near vine plants and in open spaces between vine plant rows.
- 2. Vineyard management, sampling location, and aggregate size will determine the soil bacterial functionality in soil samples near vine plants and in open spaces between vine plant rows.

Such screening information will improve our knowledge and understanding of the factors that may regulate soil microbial communities and their functions in vineyard agroecosystem management.

2. Materials and Methods

2.1. Study Site

The two sampling sites are long-term agricultural management vineyard sites, one organically managed and the other conventionally managed. Both sites are located near each other in the Binyamina agricultural region of Israel (32°32′12″ N 125 34°57′11″ E), with a mean multiannual rainfall of 585 mm between October and May and a mean annual temperature of 20.2 °C. The soil is a clay-rich (55%) (soil known as Vertisol (FAO)). Further information about the study site and different management practices can be found in our previous paper regarding the site [28].

2.2. Sampling Method

The soil samples at each site were collected randomly in the early morning from the upper 0 to 10 cm soil layer (as per [29]) from each of the long-term agricultural practices—organic management (Y) and conventional management (Ba)—between (B) the plant rows and in the vine rows (R) in the vicinity of the vine plant. Each of the three sample replicates (rep) was composed of three subsamples. At the field site, the soil samples were fractionated by progressively finer mesh for 1. macro-aggregate sizes of 9000–12,000 μ m; 2. meso-aggregates of 250–2000 μ m; and 3. micro-aggregates of <250 μ m according to [30]. They were placed in individual plastic bags (1 L volume) according to the aggregate size and sampling location, and then placed in an insulated box for transport to the laboratory.

The samples were placed in the laboratory and kept at 4 °C until biological and chemical analysis. From each sample, 2 g of soil sample was placed in an Eppendorf tube and stored at -20 °C until used for DNA extraction. Subsamples of each sample were used for the evaluation of abiotic parameters, including soil moisture (SM), organic matter (OM), pH, and electrical conductivity (EC) [31,32].

2.3. Biota Analysis

The soil microbial community was determined by DNA extraction from 0.5 g of each soil aggregate size, using an Exgene soil SV kit from GeneAll (Songpa-gu, Korea), 550 μ L SL buffer (extraction buffer), 50 μ L RH buffer, 300 μ L PD buffer, 900 μ L TB buffer (tissue binding), 500 μ L NW buffer (wash buffer N), and 50 μ L elution buffer. Centrifugation was carried out between each extraction step on a 5810 R Eppendorf centrifuge (Eppendorf, Hamburg, Germany).

Soil DNA was extracted from 0.5 g of soil using an Exgene soil DNA mini kit from GeneAll (Seoul, Korea) and stored at -20 °C until use in PCR amplification, using a SimpliAmpTM thermal cycler (Thermo Fisher Scientific, Walham, MA, USA), by mixing 12.5 µL HS Taq Mix Red (PCR Biosystems, London, UK), 9.5 µL ultrapure water, 1.0 µL extracted DNA, 1 µL CS1-515F (ACACTGACGACATGGTTCTACAGTGCCAGCMGCCGCGGT), and 1 µL CS2-806R (TACGGTAGCAGAGACTTGGTCTGGACTACHVGGGTWTCT). The thermal cycling program was set to 95 °C for 3 min, 24 cycles of 98 °C for 10 s, 55 °C for 10 s, 72 °C for 20 s, and after the cycles, 72 °C for 1 min. The amplified DNA was stored at -20 °C until sequencing.

All final PCR products were run on an agarose gel to verify amplification specificity and quality, in parallel with a negative control. The final PCR products were conducted by Hylabs Inc. using the Fluidigm Access Array primers for Illumina to generate libraries compatible for sequencing on the Miseq. Samples were measured for concentration by Qubit and size by Tapestation and then sequenced on the Illumina Miseq using a Miseq V2 sequencing kit (500 cycles) to generate 2×250 paired end reads. The data were demultiplexed using the Illumina base space cloud to generate two FASTQ files for each sample. The FASTQ files were imported into CLC-bio and analyzed as follows: Reads were trimmed for quality and adaptor sequences, merged, and then subjected to OTU picking to generate abundance tables. The database used for the OTU picking was Greengenes v13_5 at 97% sequence identity [33].

The raw sequence data were submitted to the NCBI Sequence Read Archive database with accession number: **PRJNA843903**.

PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states) functional profiling of microbial communities was predicted using the 16 S rRNA marker [15], which relies on operation taxonomic units (OTUs) for each sample.

We used the term "relative abundance" assessed as a percentage of the total abundance in order to provide a standardized comparison of the functional contribution of different features of the bacterial communities. In this sense, different proportions of functionality were established in the community, with genera's dominance classified as submediant (>10%), dominant (5–10%), and subdominant (<5%).

2.4. Statistical Analysis

All data sets underwent statistical analysis (SAS). Analysis of variance (ANOVA) was performed to test for significance between the means of sampling location and treatment. The means for the individual treatments were compared at the 5% probability level with significant differences from the Duncan test.

The soil microbial community structure was analyzed by PERMANOVA (permutational multivariate analysis of variance) to determine the effect of treatment, site, and aggregate-size fraction of each abiotic factor on the microbial community.

3. Results

The sampling site as well as the location significantly (p < 0.001) affected soil moisture (Figure 1, Table 1). Significant differences in soil moisture were observed between the three aggregate sizes. Soil organic matter differed significantly (p < 0.001) between sites, but not between the sampling locations or aggregate sizes. Soil pH ranged from 7.6 to 7.8 without significant differences between sites, aggregate sizes, and sampling locations.

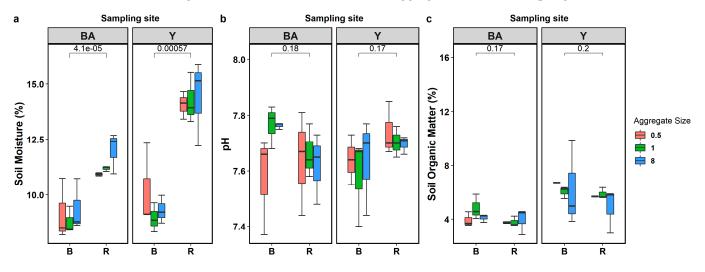


Figure 1. Changes in mean values of abiotic parameters: (**a**) soil moisture, (**b**) pH, and (**c**) soil organic matter at different agro-management: organic (Y) and conventional (Ba); at different sampling locations: between (B) the plant rows and in the vine rows (R); and with different aggregate sizes (0.5, 1, and 8 mm). The horizontal line in the box plot indicates the median, and the box indicates the upper and lower quartiles, with vertical lines representing the minimum and maximum values.

Table 1. Univariate analysis of variance (ANOVA) for soil properties and soil bacterial orders from different sampling sites, locations, aggregate size, and the interactions between them. Site indicates treatment, location indicates sampling location (between or within rows), and agg indicates aggregate size.

	Site	Loc	Agg	Site * Loc	Site * Agg	Loc * Agg	Site * Loc * Agg
	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
Soil moisture	0.0004	<0.0001	NS	0.0033	NS	NS	NS
Organic matter	0.0002	NS	NS	NS	NS	NS	NS
pH	NS	NS	NS	NS	NS	NS	NS
Shannon Index	< 0.0001	NS	0.0095	NS	0.0214	NS	NS
iii1-15	0.0011	NS	0.0004	NS	NS	NS	NS
Sva0725	NS	NS	NS	NS	NS	NS	NS
RB41	< 0.0001	0.0348	NS	NS	NS	NS	NS
Acidimicrobiales	0.0078	NS	0.0238	NS	0.0237	NS	NS
Actinomycetales	< 0.0001	NS	NS	NS	0.0129	NS	NS
Micrococcales	NS	NS	NS	NS	NS	NS	NS

Table 1. Cont.	Tab	le 1.	Cont.	
----------------	-----	-------	-------	--

	Site	Loc	Agg	Site * Loc	Site * Agg	Loc * Agg	Site * Loc * Agg
	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
0319-7L14	NS	NS	0.0002	NS	NS	NS	NS
Rubrobacterales	NS	NS	0.0026	NS	NS	NS	NS
Gaiellales	NS	NS	0.0003	NS	NS	NS	NS
Solirubrobacterales	0.0234	0.0127	0.0033	NS	0.0372	NS	NS
Cytophagales	< 0.0001	NS	NS	NS	NS	NS	NS
Flavobacteriales	NS	NS	NS	NS	NS	NS	NS
Sphingobacteriales	0.0454	NS	NS	NS	NS	NS	NS
[Saprospirales]	< 0.0001	NS	NS	NS	NS	NS	NS
H39	NS	NS	NS	NS	NS	NS	NS
SBR1031	< 0.0001	NS	NS	NS	NS	NS	NS
c:Ellin6529	< 0.0001	NS	NS	NS	NS	NS	NS
c:Gitt-GS-136	0.0001	0.0158	NS	0.0158	NS	NS	NS
AKYG885	NS	NS	NS	NS	NS	NS	NS
JG30-KF-CM45	0.0437	NS	0.01	NS	NS	NS	NS
Streptophyta	NS	NS	NS	NS	NS	NS	NS
Bacillales	NS	NS	NS	NS	NS	NS	NS
c:Gemm-1	NS	0.0393	NS	NS	NS	NS	NS
Nitrospirales	NS	NS	0.0437	NS	NS	0.0437	NS
WD2101	0.0104	NS	NS	NS	NS	NS	NS
Gemmatales	NS	NS	NS	NS	NS	NS	NS
with	0.0001	NS	NS	NS	NS	NS	NS
Planctomycetales	NS	NS	NS	NS	NS	NS	NS
Caulobacterales	NS	NS	NS	NS	NS	NS	NS
Rhizobiales	0.0043	NS	NS	NS	NS	NS	NS
Rhodobacterales	NS	NS	NS	NS	NS	NS	NS
Rhodospirillales	0.0014	NS	NS	NS	NS	NS	NS
Sphingomonadales	< 0.0001	NS	NS	NS	NS	NS	NS
c:Betaproteobacteria	NS	NS	NS	NS	NS	NS	NS
Burkholderiales	< 0.0001	NS	0.0149	NS	NS	NS	NS
Ellin6067	NS	NS	NS	NS	NS	NS	NS
MND1	0.0027	NS	0.0177	NS	NS	NS	NS
Myxococcales	< 0.0001	NS	NS	NS	0.0102	NS	NS
Enterobacteriales	NS	NS	NS	NS	NS	NS	NS
Pseudomonadales	< 0.0001	NS	0.0352	NS	0.0282	NS	NS
Xanthomonadales	< 0.0001	NS	NS	NS	0.0413	NS	NS
c:TM7-3	NS	NS	NS	NS	NS	NS	NS
Opitutales	0.0029	NS	NS	NS	NS	NS	NS
[edosphaerales]	0.0002	NS	0.0227	NS	NS	NS	NS
[Chthoniobacterales]	NS	NS	NS	NS	NS	NS	NS

Multiple factors separated by an * indicate a two- or three-way ANOVA of said factors.

Analysis of variance (ANOVA) (Table 1) yielded a significant effect (between p < 0.0004 and 0.003) for site, sampling location, and the interaction between the two. A significant difference (p < 0.0002) was found for organic matter (OM) between the sampling sites. The pH was not affected by sampling site, location, or the interplay between them.

PERMANOVA showed that the vineyard agro-management method is one of the most significant (p < 0.0001) factors affecting the bacterial community, followed by aggregate size (p < 0.01). ANOVA showed significant site (p < 0.0004), sampling location (p < 0.0001), and site and location (p < 0.0033) effects for soil moisture. The organic matter was significantly (p < 0.0002) affected (Table 2).

A total of 12 phyla, followed by 45 orders, were present in the samples. Proteobacteria was the most widespread phylum in all treatments, with conventional treatments reaching over 99% in the conventional (Ba) B 1, 0.5 mm, and (Ba) R 1 mm aggregate sizes. Actinobacteria, Chloroflexi, Bacteroidetes, and Acidobacteria phyla were present in a relatively high abundance, ranging from 17 to 30%, 6 to 15%, 4 to 9%, and 8%, respectively, in organic treatments and were significantly higher compared to the conventional treatment (Figure 2). A significant (p < 0.001) increase in the number of phyla in the 1.0 mm aggregate size was obtained in the organic management (Y) both between (B) and within rows (R) compared to conventional (B) management (Figure 2).

Table 2. Permutational multivariate analysis of variance (PERMANOVA) table showing the significant effect of vineyard agro-management and aggregate size in comparison with other abiotic factors on soil microbiota. The number of permutations = 999. Terms are added sequentially (from first to last).

	Df	Sums of Sqs	Mean Sqs	F.Model	R2	Pr (>F)	
Vineyard	1	1.2355	1.23548	24.32	0.372	0.001	**
Treatment	1	0.0977	0.09772	1.924	0.029	0.153	
Size	2	0.4258	0.21291	4.191	0.128	0.031	*
Replicate	1	0.0635	0.0635	1.25	0.019	0.265	
SM	1	0.0315	0.03151	0.62	0.01	0.471	
SOM	1	0.0387	0.03871	0.762	0.012	0.419	
pH _	1	0.0092	0.00915	0.18	0.003	0.737	
EC	1	0.0397	0.03967	0.781	0.012	0.406	
MWD	1	0.1565	0.15651	3.081	0.047	0.095	
Residuals	24	1.2193	0.0508		0.368		
Total	34	3.3173			1		
		**— <i>p</i> ≤ 0.001, *— <i>p</i>	$\leq 0.05.$				

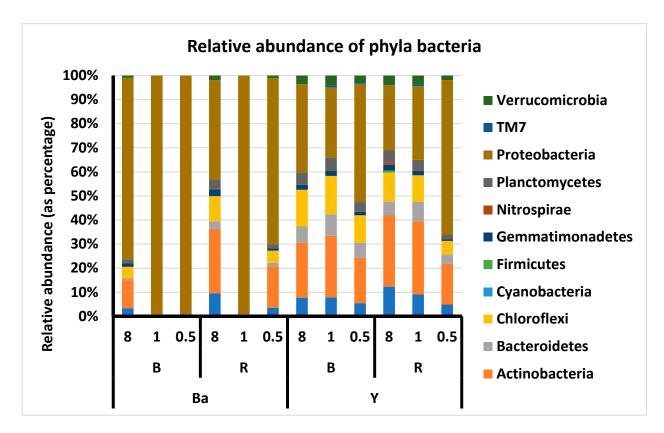
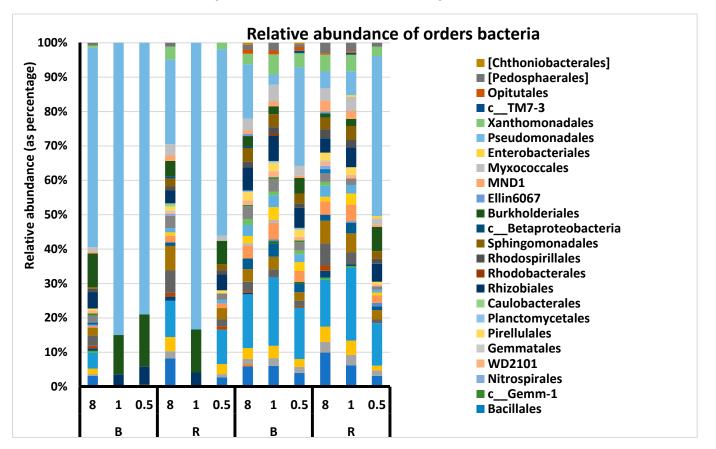


Figure 2. Relative abundance of bacterial phyla at different agro-management: organic (Y) and conventional (Ba); at different sampling locations: between (B) the plant rows and in vine rows (R) with different aggregate sizes (0.5, 1, and 8 mm).

From a total of 45 orders of bacteria, the *Pseudomonadales* order was the most abundant in both sampling sites, followed by *Rhizobiales* and *Burkholderiales*. Organic sites had significantly higher numbers of *Actinomycetales*, [*Saprospirales*], SBR1031, and c_Ellin6529 than conventional sampling sites (Figure 3). As with the Phyla, the number of orders



increased significantly in the smaller aggregate size, e.g., 1.0 and 0.5 mm, in the organic management versus the conventional management.

Figure 3. Relative abundance of bacterial orders at different agro-management: organic (Y) and conventional (Ba); at different sampling location: between (B) the plant rows and in the vine rows (R) with different aggregate size (0.5, 1, and 8 mm).

From the total of forty-five orders of bacteria, only twenty-four were significantly correlated with sampling sites, four with sampling locations, one with site and location, fourteen with aggregate size, eight orders with aggregate size and sampling sites, and one with sampling location and aggregate size. No significant differences were found between any of the orders when using a three-way ANOVA of treatment * location * aggregate size (Table 1).

The Shannon Index (order level) showed a significant difference between the treatments in site (p < 0.0001), aggregate (p < 0.01), and site*aggregate (p < 0.03), with higher values in the organic management (Table 1) and no significant difference between the sampling locations (B and R) (Figure 4).

The profiling method of bacterial communities based on gene sequences yielded a list of 24 functional groups (Figure 5) fulfilling 97% of all functions. All 23 functions were present as potential functions, with no significant differences between managements. The bacterial metabolism function included twelve functions, of which amino acid and carbohydrate metabolism fulfilled the major functions and were observed as performing ten ecologically relevant functions. These functions included various cycles, which were classified as functions related to the following: (1) cellular processes, (2) folding, (3) genetic information processing, (4) membrane processes, (5) metabolism functions, (6) functions defined as not well-characterized (unknown functions), (7) replication, (8) signal transduction, (9) translation, and (10) xenobiotic functions (Table 3). Based on their contribution, these bacterial community functions (Table 3) were divided into three groups: (a) Subdominant, (b) Dominant, and (c) Eudominant. These three functions are potentially different in their

contribution—the subdominant group is mainly associated with intercellular processes, whereas the dominant group determines cell maintenance, and the eudominant group contributes to the communication interface between the cell and the environment. Although changes in bacterial community composition were obtained due to treatment and sampling location, no significant differences in microbial functions were obtained.

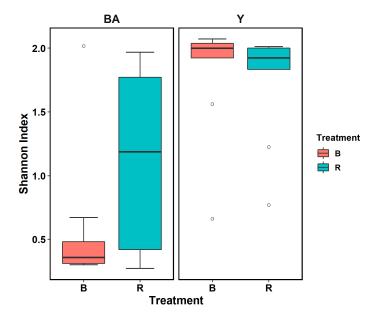


Figure 4. Box plots illustrating the alpha diversity index (Shannon diversity) in bacterial microbiomes from different agro-management: organic (Y) and conventional (Ba); and at different sampling locations: between (B) the plant rows and in the vine rows (R). The horizontal line in the box plot indicates the median, and the box indicates the upper and lower quartiles, with the vertical lines representing the minimum and maximum values.

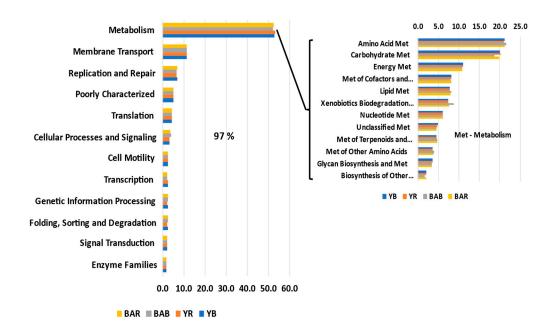


Figure 5. Functional predictions of all samples from different agro-management: organic (Y) and conventional (Ba); and at different sampling locations: between (B) the plant rows and in the vine rows (R) using PICRUSt.

Cell	Cell Motility				
Folding	Folding, Sorting and Degradation				
Genetic	Genetic Information Processing				
Metabolism	Metabolism—Enzyme Families				
Metabolism	Biosynthesis of Other Scondary Metabolites				
Metabolism	Glycan Biosynthesis and Metabolism				
Metabolism	Metabolism				
Metabolism	Metabolism of Other Amino Acids				
Metabolism	Metabolism of Terpenoids and Polyketides				
Signal	Signal Transduction				
Transcription	Transcription				
Translation	Translation				
Cell	Cellular Processes and Signalling				
Metabolism	Energy Metabolism				
Metabolism	Lipid Metabolism				
Metabolism	Metabolism of Cofactors and Vitamins				
Nucleotide	Nucleotide Metabolism				
Poorly	Poorly Characterized				
Xenobiotics	Xenobiotics Biodegradation and Metabolisn				
Replication	Replication and Repair				
Membrane	Membrane Transport				
Metabolism	Amino Acid Metabolism				
Metabolism	Carbohydrate Metabolism				
	Folding Genetic Metabolism Metabolism Metabolism Metabolism Metabolism Metabolism Signal Transcription Translation Cell Metabolism Metabolism Metabolism Nucleotide Poorly Xenobiotics Replication Membrane Metabolism				

Table 3. Table describing the bacterial community functions divided into three groups: a. Subdominant, b. Dominant, and c. Submediant.

4. Discussion

The analysis of soil variables showed a clear distinction between the two field managements. Although they were located at a homogeneous site, local climate, and hydrology, the differences in soil physical and chemical properties were found to be similar to Wei et al.'s results [34,35]. Soil moisture and organic matter increased the soil's OM content compared to the conventional fields, consistent with previous studies [36]. The pH values were similar, without any significant differences between treatments and sampling locations. These results clearly differentiate between the two sampling locations, without distinguishing between aggregate sizes.

The number and size of phyla in organic management were higher in all aggregate sizes compared to conventional management. The increase in the Verrucomicrobia's phylum abundance is related to different environmental aspects such as soil chemical factors [37,38] and impact on the nitrogen availability [39,40]. An additional phylum found to increase in abundance in the organic amendments management compared to the conventional management was the Chloroflexi phylum, which is known to be a diverse group of bacteria that, according to Speirs et al. [41], play an important role in carbohydrate degradation and soil nitrogen and phosphorous processes. Actinobacteria comprised one of the phyla that were found in all organic management samples, contrary to conventional management, which according to Bundy et al. [42] is described as "the good, the bad and the ugly", playing an important role in biochemical cycling and soil development [43]. The Proteobacteria phylum was the dominant phylum and contained five classes, and Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria were the most abundant in soil samples amongst the five classes, without any effects of treatment and sampling location, as supported by previous studies by Spain et al. [44].

Long-term land use causes minimum disturbance and tends to maximize a long-term new balance that may lead to a new soil health paradigm elucidated by the soil microbial community. As the study's goal was to determine the effects of the two managements and three aggregate sizes, the higher bacterial taxa organization level increases our hope for a better understanding and differentiation between the managements. There was a 10-fold increase in the number of orders found in the 1 and 0.5 mm size aggregates, while a 25% increase in the number of orders was observed in the 8 mm aggregate size. The increase in phylogenetic richness, diversity, and heterogeneity in the organically managed system is in a similar direction to that obtained by Lupatini et al. [45] and Lori et al. [46]. These results indicate that organic farming has a beneficial effect on microbial diversity and encourages ecosystem multifunctionality.

Microorganisms play an important role and function in terrestrial natural and manmade ecosystems. Therefore, interest in their functional profiles has been extended, similarly to aquaculture systems [47]. The uniqueness of the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) developed by Langille et al. [16] is that it allows the prediction of the functional profile based on microbial metagenomic data. Based on this new tool, our data could be used to interpret the functional profiles of the two managements. The 23 main functional profiles generated for both treatments and aggregate sizes showed similar pathways, without any significant differences triggered by management. The two primary metabolic functions were the amino acid and the carbohydrate metabolisms, followed by 10 additional metabolic functional components. Amino acid metabolism plays an important role in supporting the growth and survival of bacteria by regulating energy and protein homeostasis, while carbohydrate metabolism is known as a fundamental process in supplying continuous energy for cell function [48,49]. These two functions ensure nutrient supplies that enable the rapid adaptation of bacterial metabolic capabilities in changing habitats [50].

Based on this assumption, we can assume that management in this case will strongly affect the soil microbial community composition, density, and diversity, in which the basic functional units of the microbial population will preserve their functionality. More studies are required on this issue to provide adequate predictions regarding the functionality of microbial communities in response to agricultural activities and climate change.

5. Conclusions

Overall, conventional and organic management in vineyard agroecosystems both support a variety of ecological services. The bacterial-based ecological diversity parameters were more reliable at describing land management, albeit organically managed plots had a higher number of phyla in all aggregate sizes in comparison to conventional management. The number of phyla abundance has been found to be related to different aspects of soil abiotic components. It plays an important role in carbohydrate degradation, and soil nitrogen and phosphorus cycle. Using the new PICRUSt Phylogenetic tool for the two managements and aggregate size, we discovered that amino acids and carbohydrate metabolism are the main fundamental processes that supply continuous energy that are ultimately the criteria for a successful survival model. Thus, the best assessment of management indicates that organic farming has a beneficial effect and encourages ecosystem multifunctionality.

Author Contributions: Conceptualization, Y.S.; methodology, Y.S. and T.D.; software, T.D. and Y.S.; validation, C.S., I.A. and G.E.; formal analysis, Y.S.; investigation, C.S.; resources, G.E.; data curation, T.D.; writing—original draft preparation, Y.S.; writing—review and editing, I.A.; visualization, Y.S., T.D. and I.A.; supervision, Y.S.; project administration, C.S. and G.E.; funding acquisition, G.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The raw sequence data were submitted to the NCBI Sequence Read Archive database with accession number: **PRJNA843903**.

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

References

- 1. Paul, E.A.; Clark, D.R. Soil Microbiology and Biochemistry; Academic Press: San Diego, CA, USA, 1989.
- Barness, G.; Rodriguez Zaragoza, S.; Shmueli, I.; Steinberger, Y. Vertical Distribution of a Soil Microbial Community as Affected by Plant Ecophysiological Adaptation in a Desert System. *Microb. Ecol.* 2009, 57, 36–49. [CrossRef] [PubMed]
- Shamir, I.; Steinberger, Y. Vertical Distribution and Activity of Soil Microbial Population in a Sandy Desert Ecosystem. *Microb. Ecol.* 2007, 53, 340–347. [CrossRef] [PubMed]
- Gobbi, A.; Acedo, A.; Imam, N.; Santini, R.G.; Ortiz-Álvarez, R.; Ellegaard-Jensen, L.; Belda, I.; Hansen, L.H. A Global Microbiome Survey of Vineyard Soils Highlights the Microbial Dimension of Viticultural Terroirs. *Commun. Biol.* 2022, 5, 241. [CrossRef] [PubMed]
- Buyanovsky, G.; Dicke, M.; Berwick, P. Soil Environment and Activity of Soil Microflora in the Negev Desert. J. Arid. Environ. 1982, 5, 13–28. [CrossRef]
- Parker, L.W.; Freckman, D.W.; Steinberger, Y.; Driggers, L.; Whitford, W.G. Effects of Simulated Rainfall and Litter Quantities on Desert Soil Biota: Soil Respiration, Microflora, and Protozoa. *Pedobiologia* 1984, 27, 185–195.
- Barral, M.T.; Arias, M.; Guérif, J. Effects of Iron and Organic Matter on the Porosity and Structural Stability of Soil Aggregates. Soil. Tillage Res. 1998, 46, 261–272. [CrossRef]
- 8. Hibbing, M.E.; Fuqua, C.; Parsek, M.R.; Peterson, S.B. Bacterial Competition: Surviving and Thriving in the Microbial Jungle. *Nat. Rev. Microbiol.* **2009**, *8*, 15–25. [CrossRef] [PubMed]
- Garbeva, P.; Silby, M.W.; Raaijmakers, J.M.; Levy, S.B.; de Boer, W. Transcriptional and Antagonistic Responses of Pseudomonas Fluorescens Pf0-1 to Phylogenetically Different Bacterial Competitors. *ISME J.* 2011, *5*, 973–985. [CrossRef]
- 10. van Ooij, C. First Come, First Served? Nat. Rev. Microbiol. 2011, 9, 699. [CrossRef]
- Wilpiszeski, R.L.; Aufrecht, J.A.; Retterer, S.T.; Sullivan, M.B.; Graham, D.E.; Pierce, E.M.; Zablocki, O.D.; Palumbo, A.V.; Elias, D.A. Soil Aggregate Microbial Communities: Towards Understanding Microbiome Interactions at Biologically Relevant Scales. *Appl. Environ. Microbiol.* 2019, 85. [CrossRef]
- Ranjard, L.; Poly, F.; Combrisson, J.; Richaume, A.; Gourbière, F.; Thioulouse, J.; Nazaret, S.; Ecol, M. Heterogeneous Cell Density and Genetic Structure of Bacterial Pools Associated with Various Soil Microenvironments as Determined by Enumeration and DNA Fingerprinting Approach (RISA). *Microb. Ecol.* 2000, 39, 263–272. [CrossRef] [PubMed]
- 13. Rillig, M.C.; Muller, L.A.H.; Lehmann, A. Soil Aggregates as Massively Concurrent Evolutionary Incubators. *ISME J.* 2017, 11, 1943–1948. [CrossRef] [PubMed]
- 14. Xia, F.; Zhou, X.; Liu, Y.; Li, Y.; Bai, X.; Zhou, X. Composition and Predictive Functional Analysis of Bacterial Communities Inhabiting Chinese Cordyceps Insight into Conserved Core Microbiome. *BMC Microbiol.* **2019**, *19*, 105. [CrossRef] [PubMed]
- 15. Morgan, X.C.; Segata, N.; Huttenhower, C. Biodiversity and Functional Genomics in the Human Microbiome. *Trends Genet.* **2013**, 29, 51–58. [CrossRef] [PubMed]
- Langille, M.G.I.; Zaneveld, J.; Caporaso, J.G.; McDonald, D.; Knights, D.; Reyes, J.A.; Clemente, J.C.; Burkepile, D.E.; Vega Thurber, R.L.; Knight, R.; et al. Predictive Functional Profiling of Microbial Communities Using 16S RRNA Marker Gene Sequences. *Nat. Biotechnol.* 2013, 31, 814–821. [CrossRef]
- Sevigny, J.L.; Rothenheber, D.; Diaz, K.S.; Zhang, Y.; Agustsson, K.; Bergeron, R.D.; Thomas, W.K. Marker Genes as Predictors of Shared Genomic Functions. *BMC Genom.* 2019, 20, 1–13. [CrossRef]
- Callaham, M.A.; Stanturf, J.A. Soil Ecology and Restoration Science. In Soils and Landscape Restoration; Elsevier: Amsterdam, The Netherlands, 2021; pp. 39–62.
- 19. Bach, E.M.; Williams, R.J.; Hargreaves, S.K.; Yang, F.; Hofmockel, K.S. Greatest Soil Microbial Diversity Found in Micro-Habitats. *Soil Biol. Biochem.* **2018**, *118*, 217–226. [CrossRef]
- Nannipieri, P.; Ascher-Jenull, J.; Ceccherini, M.T.; Pietramellara, G.; Renella, G.; Schloter, M. Beyond Microbial Diversity for Predicting Soil Functions: A Mini Review. *Pedosphere* 2020, 30, 5–17. [CrossRef]
- Unc, A.; Eshel, G.; Unc, G.A.; Doniger, T.; Sherman, C.; Leikin, M.; Steinberger, Y. Vineyard Soil Microbial Community under Conventional, Sustainable and Organic Management Practices in a Mediterranean Climate. *Soil Res.* 2021, 59, 253. [CrossRef]
- Watt, M.; Silk, W.K.; Passioura, J.B. Rates of Root and Organism Growth, Soil Conditions, and Temporal and Spatial Development of the Rhizosphere. Ann. Bot. 2006, 97, 839–855. [CrossRef]
- Ritz, K.; McNicol, J.W.; Nunan, N.; Grayston, S.; Millard, P.; Atkinson, D.; Gollotte, A.; Habeshaw, D.; Boag, B.; Clegg, C.D.; et al. Spatial Structure in Soil Chemical and Microbiological Properties in an Upland Grassland. *FEMS Microbiol. Ecol.* 2004, 49, 191–205. [CrossRef] [PubMed]
- Grayston, S.J.; Campbell, C.D.; Bardgett, R.D.; Mawdsley, J.L.; Clegg, C.D.; Ritz, K.; Griffiths, B.S.; Rodwell, J.S.; Edwards, S.J.; Davies, W.J.; et al. Assessing Shifts in Microbial Community Structure across a Range of Grasslands of Differing Management Intensity Using CLPP, PLFA and Community DNA Techniques. *Appl. Soil Ecol.* 2004, 25, 63–84. [CrossRef]
- 25. Pulleman, M.; Creamer, R.; Hamer, U.; Helder, J.; Pelosi, C.; Pérès, G.; Rutgers, M. Soil Biodiversity, Biological Indicators and Soil Ecosystem Services—an Overview of European Approaches. *Curr. Opin. Environ. Sustain.* **2012**, *4*, 529–538. [CrossRef]
- 26. Wagg, C.; Bender, S.F.; Widmer, F.; van der Heijden, M.G.A. Soil Biodiversity and Soil Community Composition Determine Ecosystem Multifunctionality. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 5266–5270. [CrossRef]
- Jenkins, S.N.; Waite, I.S.; Blackburn, A.; Husband, R.; Rushton, S.P.; Manning, D.C.; O'Donnell, A.G. Actinobacterial Community Dynamics in Long Term Managed Grasslands. *Antonie Van Leeuwenhoek* 2009, 95, 319–334. [CrossRef]

- Schlüter, S.; Gil, E.; Doniger, T.; Applebaum, I.; Steinberger, Y. Abundance and Community Composition of Free-Living Nematodes as a Function of Soil Structure under Different Vineyard Managements. *Appl. Soil Ecol.* 2022, 170, 104291. [CrossRef]
- Freckman, D.W.; Whitford, W.G.; Steinberger, Y. Effect of Irrigation on Nematode Population Dynamics and Activity in Desert Soils. *Biol. Fertil. Soils* 1987, 3, 3–10. [CrossRef]
- Jiang, Y.; Qian, H.; Wang, X.; Chen, L.; Liu, M.; Li, H.; Sun, B. Nematodes and Microbial Community Affect the Sizes and Turnover Rates of Organic Carbon Pools in Soil Aggregates. *Soil Biol. Biochem.* 2018, 119, 22–31. [CrossRef]
- 31. Yu, J.; Liu, F.; Tripathi, B.M.; Steinberger, Y. Changes in the Composition of Soil Bacterial and Fungal Communities after Revegetation with Caragana Microphylla in a Desertified Semiarid Grassland. *J. Arid. Environ.* **2020**, *182*, 104262. [CrossRef]
- 32. Tripathi, B.M.; Moroenyane, I.; Sherman, C.; Lee, Y.K.; Adams, J.M.; Steinberger, Y. Trends in Taxonomic and Functional Composition of Soil Microbiome Along a Precipitation Gradient in Israel. *Microb. Ecol.* **2017**, *74*, 168–176. [CrossRef]
- McDonald, D.; Price, M.N.; Goodrich, J.; Nawrocki, E.P.; Desantis, T.Z.; Probst, A.; Andersen, G.L.; Knight, R.; Hugenholtz, P. An Improved Greengenes Taxonomy with Explicit Ranks for Ecological and Evolutionary Analyses of Bacteria and Archaea. *ISME J.* 2011, 6, 610–618. [CrossRef] [PubMed]
- Wei, X.; Shao, M.; Fu, X.; Horton, R.; Li, Y.; Zhang, X. Distribution of Soil Organic C, N and P in Three Adjacent Land Use Patterns in the Northern Loess Plateau, China. *Biogeochemistry* 2009, *96*, 149–162. [CrossRef]
- Probst, B.; Schüler, C.; Joergensen, R.G. Vineyard Soils under Organic and Conventional Management—Microbial Biomass and Activity Indices and Their Relation to Soil Chemical Properties. *Biol. Fertil. Soils* 2008, 44, 443–450. [CrossRef]
- Guo, J.; Wu, Y.; Wu, X.; Ren, Z.; Wang, G. Soil Bacterial Community Composition and Diversity Response to Land Conversion Is Depth-Dependent. *Glob. Ecol. Conserv.* 2021, 32, e01923. [CrossRef]
- Jesus, E.D.C.; Marsh, T.L.; Tiedje, J.M.; Moreira, F.M.D.S. Changes in Land Use Alter the Structure of Bacterial Communities in Western Amazon Soils. *ISME J.* 2009, *3*, 1004–1011. [CrossRef]
- Pan, Y.; Cassman, N.; de Hollander, M.; Mendes, L.W.; Korevaar, H.; Geerts, R.H.E.M.; van Veen, J.A.; Kuramae, E.E. Impact of Long-Term N, P, K, and NPK Fertilization on the Composition and Potential Functions of the Bacterial Community in Grassland Soil. FEMS Microbiol. Ecol. 2014, 90, 195–205. [CrossRef]
- Navarrete, A.A.; Soares, T.; Rossetto, R.; van Veen, J.A.; Tsai, S.M.; Kuramae, E.E. Verrucomicrobial Community Structure and Abundance as Indicators for Changes in Chemical Factors Linked to Soil Fertility. *Antonie Van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* 2015, 108, 741–752. [CrossRef]
- 40. Christou, M.; Avramides, E.J.; Jones, D.L. Dissolved Organic Nitrogen Dynamics in a Mediterranean Vineyard Soil. *Soil Biol. Biochem.* **2006**, *38*, 2265–2277. [CrossRef]
- 41. Speirs, L.B.M.; Rice, D.T.F.; Petrovski, S.; Seviour, R.J. The Phylogeny, Biodiversity, and Ecology of the Chloroflexi in Activated Sludge. *Front. Microbiol.* **2019**, *10*, 2015. [CrossRef]
- 42. Bundy, A.; Shannon, L.J.; Rochet, M.J.; Neira, S.; Shin, Y.J.; Hill, L.; Aydin, K. The Good(Ish), the Bad, and the Ugly: A Tripartite Classification of Ecosystem Trends. *ICES J. Mar. Sci.* 2010, *67*, 745–768. [CrossRef]
- Zhang, B.; Wu, X.; Tai, X.; Sun, L.; Wu, M.; Zhang, W.; Chen, X.; Zhang, G.; Chen, T.; Liu, G.; et al. Variation in Actinobacterial Community Composition and Potential Function in Different Soil Ecosystems Belonging to the Arid Heihe River Basin of Northwest China. *Front. Microbiol.* 2019, 10, 2209. [CrossRef] [PubMed]
- 44. Spain, A.M.; Krumholz, L.R.; Elshahed, M.S. Abundance, Composition, Diversity and Novelty of Soil Proteobacteria. *ISME J.* **2009**, *3*, 992–1000. [CrossRef] [PubMed]
- 45. Lupatini, M.; Korthals, G.W.; de Hollander, M.; Janssens, T.K.S.; Kuramae, E.E. Soil Microbiome Is More Heterogeneous in Organic Than in Conventional Farming System. *Front. Microbiol.* **2017**, *7*, 2064. [CrossRef]
- 46. Lori, M.; Symnaczik, S.; Mäder, P.; de Deyn, G.; Gattinger, A. Organic Farming Enhances Soil Microbial Abundance and Activity—A Meta-Analysis and Meta-Regression. *PLoS ONE* **2017**, *12*, e0180442. [CrossRef] [PubMed]
- Ortiz-Estrada, A.M.; Gollas-Galván, T.; Martínez-Córdova, L.R.; Martínez-Porchas, M. Predictive Functional Profiles Using Metagenomic 16S RRNA Data: A Novel Approach to Understanding the Microbial Ecology of Aquaculture Systems. *Rev. Aquac.* 2019, 11, 234–245. [CrossRef]
- 48. Fraenkel, D.G.; Vinopal, R.T. Carbohydrate Metabolism in Bacteria. Annu. Rev. Microbiol. 1973, 27, 69–100. [CrossRef]
- Metges, C.C. Contribution of Microbial Amino Acids to Amino Acid Homeostasis of the Host. J. Nutr. 2000, 130, 1857S–1864S. [CrossRef]
- 50. Durica-Mitic, S.; Göpel, Y.; Görke, B. Carbohydrate Utilization in Bacteria: Making the Most Out of Sugars with the Help of Small Regulatory RNAs. *Microbiol. Spectr.* **2018**, *6*. [CrossRef]