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Changes in the Bacterial Community Composition of Cultivated Soil after Digging up Operations for Laying a Pipeline

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Abstract: Our study aimed to evaluate the impact of the pipeline installation on the bacterial composition in cultivated soil by metagenomic analyses performed before the excavation and in the following three years. Differential abundance analysis was obtained using DESeq2 from the GAIA pipeline to verify the bacteriological diversity in soils collected after the reference year (2013). Soil samples presented a different distribution of taxa, especially in 2014, in which a further allocation at the phylum and family levels was observed compared to the previous year (2013). The phyla Bacteroidetes and Firmicutes increased significantly, while the phylum Actinobacteria, most abundant in 2013, showed reduced abundance; moreover, Chloroflexi and Planctomycetes decreased considerably, and Verrucomicrobia was absent. The significant differences in the taxonomic composition and structure of the soil microbial community were due to critical stress conditions following the soil excavations. The bacterial communities were capable of profound physiological and genetic changes, implementing different mechanisms for survival and adaptation to an environment with changed conditions. The implication of changes in microbial diversity before and after the mechanical insult of soil has been determined.

Keywords: soil; bacterial composition changes; pipeline installation; metagenomic approach

Citation: Bonomo, M.G.; Scrano, L.; Mang, S.M.; Scalese, B.E.; Bufo, S.A.; Modley, L-A.; Buongarzone, E.; Salzano, G. Changes in the Bacterial Community Composition of Cultivated Soil after Digging up Operations for Laying a Pipeline. *Agriculture* **2023**, *13*, 1189. https://doi.org/10.3390/agriculture13061189

Academic Editors: Cristina Abbate and Miguel Soriano

Received: 26 March 2023
Revised: 18 May 2023
Accepted: 31 May 2023
Published: 2 June 2023

1. Introduction

The soil microbial community is essential in maintaining ecosystem services; it promotes plant growth and nutrition and consolidates the transfer of matter and energy in the various terrestrial environments [1,2].

In recent years, the soil microbial community has received more attention as it can influence soil's heterogeneous ecosystem and dynamics and interact with soil properties and processes [3–6]. The soil biota interacts with soil minerals and organic particles leading to the development and stabilization of macro-aggregates, clay-organic matter complexes, and micropores, which characterize the structure of the soil matrix and influence the microbial community [1]. The microbial communities are randomly spread out, following nutrient gradients, moisture content, etc., determining the so-called "hot-spot" distribution and acquiring complex functions and diversity [1].

Most soil microorganisms are still unidentified and represent a vast unexplored pool of metabolic and genomic diversity. Therefore, characterizing and conserving soil microorganisms' diversity is critical, as they contain an extensive reservoir of unknown genes encoding new proteins and enzymes [1].

Because of the importance of the soil microbial community to ecosystem services, understanding how different disturbance practices (such as contamination and pollution) affect the soil microbial community is essential. As already demonstrated, variations in the microbial community structure or physiological status and its functions inhibition can change the soil ecosystem [3,6,7].

Salt and water stresses have been proven to significantly influence the soil microbiota composition with increased bacterial communities that can survive in arid or salty environments [2,5]. Wildfires also select for heat-resistant microbial populations in forest soils [6]. Nevertheless, metal contaminants have been indicated as responsible for the most critical modifications in soil microbiota, especially in the presence of toxic elements or radionuclides [3,4,7].

Over the years, researchers have driven the development of culture-independent methods [8–13], including the metagenomics approach. The resulting discovery of new functional molecules and the consequent better understanding of soil complexity and heterogeneous microbial spatial distribution have permitted an accurate link of microbial diversity with soil functions [14–19]. Therefore, it is essential to investigate the genetic structure and function of the microorganisms at every monitored site to understand the possibility of bioremediation processes and management of such areas [20,21]. Furthermore, restoring soil microbial communities is crucial as they are responsible for physiological and metabolic functions of great importance for soil quality [22–24].

The Soil Biodiversity platform has invited researchers, politicians, decision-makers, industries, and states to seriously consider soil biodiversity conservation, including all kind of microorganisms: 'The maintenance of soil biodiversity is essential to both the environment and agricultural industries' [25]. Moreover, since 2015, the 'Cross-Sector Biodiversity Initiative (CSBI)' has presented a platform for the development and sharing of 'good practices' to be implemented concerning the protection and restoration of 'biodiversity' in extractive activities. This platform is a collaborative tool to access the knowledge and collective experience of experts in the sector. It provides practical guidance, innovative approaches, and examples to support mitigation operationalizing. In addition, CSBI has developed some guides to limit, as far as possible, the negative impacts on the biodiversity of development projects and activate the processes of mitigating the damage and recovery of biodiversity [26].

Our study aimed to assess the severity of soil disturbance due to the digging-up operations for laying a pipeline through the changes in the bacterial community composition determined before the excavation and in the following three years. The analysis of bacterial diversity and the identification of predominant taxa have proven to be helpful tools for obtaining indicators of soil stress. To our knowledge, this is the first attempt to assess the extent of soil disturbance by comparing microbial diversity and identifying predominant taxa before and after such a deep mechanical soil insult.

2. Materials and Methods

2.1. Site Description and Sampling

A farmhouse located in Southern Italy was the sampling site of this study. Two agricultural sectors (cultivated areas) characterize the farmland (i) arable area and (ii) wood area. The pipeline was installed into an excavation 1500 m long, 4 m wide, and 4 m deep (Figure S1). Soil samples were taken in June and July of 2013 (reference year, before the excavation) and in the same months in the three years (2014–2016) following the pipeline installation (post-installation monitoring). Eight sampling sites were identified along the length of the excavated site. Soil samples were collected from 0 to 20 cm (surficial) and 20

to 40 cm (deep) layers at all sampling sites. From each sampling site and layer, 2 kg of soil was taken per year.

The specification drawn up by CSBI recommends respecting the stratigraphy of the excavation by placing the soil in the order in which the layers were removed. Given the vastness of the work, it is possible that this recommendation was not followed to the letter and that some mixing of the soil layers occurred in parts of the excavation.

2.2. The 16S rDNA Amplicon Sequencing and Sequence Processing

The DNA was isolated from each sample of 250 mg soil by using ZR Soil Microbe DNA MicroPrep™ Kit (Zymo Research, Italy), according to the manufacturer's instructions, and it was visualized by electrophoresis on agarose gel (1.0%, *w*/*v*) and quantified by Nanodrop Spectrophotometer ND-1000 (Thermo Fisher Scientific, Rodano-MI, Italy). The extraction of microbial genomic DNA was performed in triplicate.

The 16S gene amplicon libraries at IGA technology (Udine, Italy) were prepared using the isolated DNA, based on the Illumina protocol ©16S Metagenomic Sequencing Library Preparation protocol to sequence the V3 and V4 variable regions using the primers 16S-341F 5′-CCTACGGGNGGCWGCAG-3′ and 16S-805R 5′-GACTACHVGGGTATCTAATCC-3′.

Subsequently, sequences were indexed by an amplification performed on the sequencing cell (NexteraXT Index Kit, FC-131-1001/FC-131-1002). Then, the MiSeq Illumina platform was used to sequence the libraries and to obtain 300 bp paired reads.

The read quality was evaluated using FASTQC (Version 0.11.1), and the process was necessary to remove the sequencing primers and the poor-quality reads using the Trimmomatic program. Further quality control was carried out with FASTQC (0.11.1).

2.3. Bioinformatics Analysis

A GAIA automation workflow (pipeline) was acquired from Sequentia Biotech SL (Barcelona, Spain) for the metagenomic and differential analysis of microorganisms. This pipeline uses superior mapping features by comparing the Burrows–Wheeler Alignment (BWA) tool vs. the National Center for Biotechnology Information (NCBI) database to find their complete taxonomic lineage and implement the Low Common Ancestor (LCA) algorithm for resolving the complex data structure. Individual entries are employed to arrange readings into Operational Taxonomic Units (OTUs) at species, genus, family, phylum, and domain levels.

The LCA process permitted the characterization at the resulting ratios: identities ranging from 0% to 70%: reads allocated at the domain level; identities ranging from 71% to 73%: assigned readings at the phylum level; identities from 74% to 85%: assigned readings at the household level; equivalence ranging 86–93% of published readings at the genre level; identity rating 94–97%: readings attributed to species level; reads recorded to a single species have been categorized into the strain of referring species they are included; however, reads that were not mapped were reported as '*unknown*' [20,21].

The total richness (described as the total reads/pair amounts) and comparative abundance (stated as a ratio) were estimated for each found taxa. Data were reported in a bar plot that allows the detection of taxa at each collecting site and their relative amount. Taxa with less than 0.1% presence in all samples have been aggregated as '*others*' for convenience. The experiments are ordered by sampling site (DEFGH), by year (pursuing the chronological order of soil sampling), and by depth, indicating for each set the sampling dept 0–20 cm (1) or 20–40 cm (2).

Alpha and beta diversities were calculated using Phyloseq to indicate the abundance level (taxa number) and evenness level (taxa relative abundance) in the separate soil samples and to calculate the distance between the pairs of samples as a matrix of Bray–Curtis dissimilarities.

Moreover, a disparity richness analysis using DESeq2 was also achieved by GAIA to recognize taxa differentially copious in soils grouped by site and sampling year and

assessed in comparison: cultivated areas 2014 vs. cultivated areas 2013; cultivated areas 2015 vs. cultivated areas 2013; cultivated areas 2016 vs. cultivated areas 2013.

For each comparison, the counts obtained from the recording of reads against the reference database have been standardized to allow comparison between diverse samples; a Principal Component Analysis (PCA) was conducted to study the variation existing within the compared sample sets and to observe how they were distributed according to their distance. A Volcano and a MA plot were built to denote the assessed degree of difference in the richness levels.

Lastly, the taxa for each taxonomic level were estimated, and their abundances were matched to the reference year (2013). The taxa abundances are reported as the decimal logarithm of the fold-change (log FC), i.e., the number of times the loads are significantly different compared to the reference).

3. Results

A total of 56 taxa were identified in the sole arable area at the phylum class, 485 at the family similarity, 1190 at the genus type, and 23,232 at the species status. Soils presented different taxa distributions, specifically in their groups, in 2014. Considering overall the variations in the taxonomic rank of phylum, Figure 1 shows that in the samples from 2014: - The phyla Bacteroidetes and Firmicutes increased significantly and represented the most abundant taxa and the reasonably constant phylum Proteobacteria. - The most abundant phylum in 2013 (Actinobacteria) showed reduced abundance in 2014. - *Chloroflexi* and Planctomycetes decreased considerably. - Verrucomicrobia was (apparently) absent.

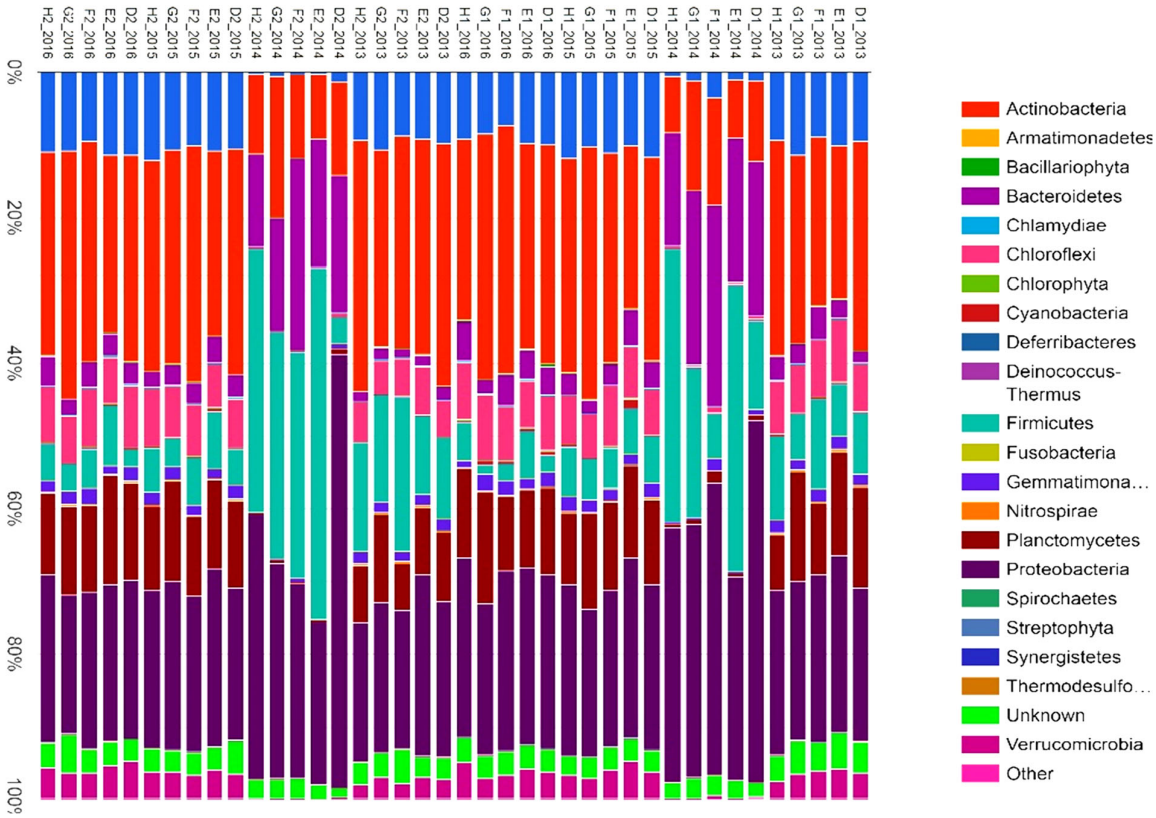

Figure 1. Distribution of taxa in soil samples (arable area) at the taxonomic ranking of the phylum. The samples are described in order of sampling sites (DEFGH), by year (following the chronological order of sampling), and by depth, remarking for each group the sampled depth 0–20 cm (D1-H1) and subsequently at 20–40 cm (D2-H2).

Furthermore, at the taxonomical family ranking, *Acidobacteriaceae*, *Pseudomonadaceae*, *Bacillaceae*, *Hymenobacteriaceae*, *Planctomycetaceae*, *Micrococcaceae*, *Propionibacteriaceae*, *Oxalobacteriaceae*, *Paenibacillaceae*, and *Rubrobacteriaceae* were the variable and abundant taxa (Figure S2).

The heatmaps were constructed for easy and instant data visualization and showed the most outstanding results at the taxonomic levels of phylum, family, and genus; Figures S3–S5 show blocks of different taxa abundance in all samples (wood and arable areas) in the three years succeeding the pipeline installation, suggesting changes in the biodiversity over time, especially for the taxa distinguished at the phylum and family groups.

A change in the abundance of taxa at the phylum level is evident, especially for Proteobacteria, Planctomycetes, Firmicutes, Actinobacteria, and Bacteroidetes (Figure S3), and also a change in the abundance of taxa at the family level (Figure S4), while a difference in the abundance of taxa at the genus level, especially for *Bacillus* is observed; noted as the proportion of unknown increased (Figure S5).

Since changes in microbiological diversity were detected in the samples after 2013, the identification of taxonomies, with significantly changed abundance over time, was conducted by comparative analysis. Therefore, GAIA accomplished a differential copiousness analysis using DESeq2 to detect differentially characterized taxa.

The microbial composition was evaluated by comparing the year 2014 vs. 2013. At the systematic phylum recognition, PCA presented considerable changeability amongst samples from 2014, particularly alongside the principal component 1, which accounts for more than 87% of the irregularity; however, the samples from 2013 grouped themselves, showing a minimum change (Figure 2a). In total, 56 phyla were identified in these samples, and 33 had differential abundance in 2014; 14 were more copious, and 19 were less abundant in 2014 (Table S1). At the taxonomical family ranking, the PCA presented more variability among the soils collected in 2014 (Figure 2b).

A total of 484 families were identified; 262 had differential abundance in 2014, 123 were present in larger quantities, and 137 were present in reduced amounts (Table S1). The Volcano and MA plots show the taxa differentially present in these samples with an overall differential abundance (Figure 3).

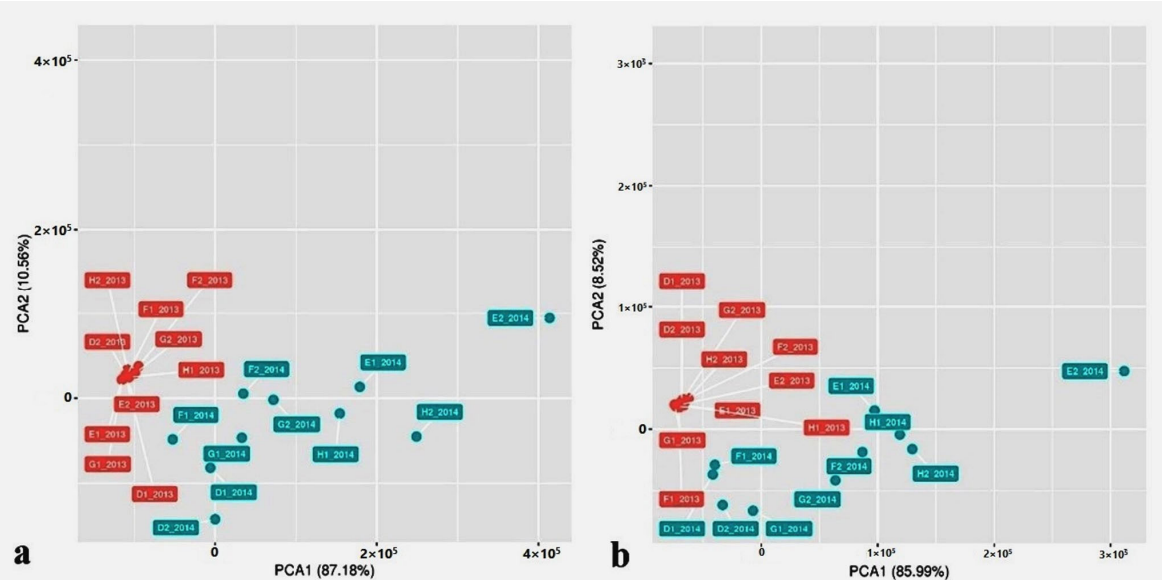

Figure 2. PCA distribution according to their distance of the comparison 2014 vs. 2013 (arable area) at the phylum (**a**) and family (**b**) taxonomic level.

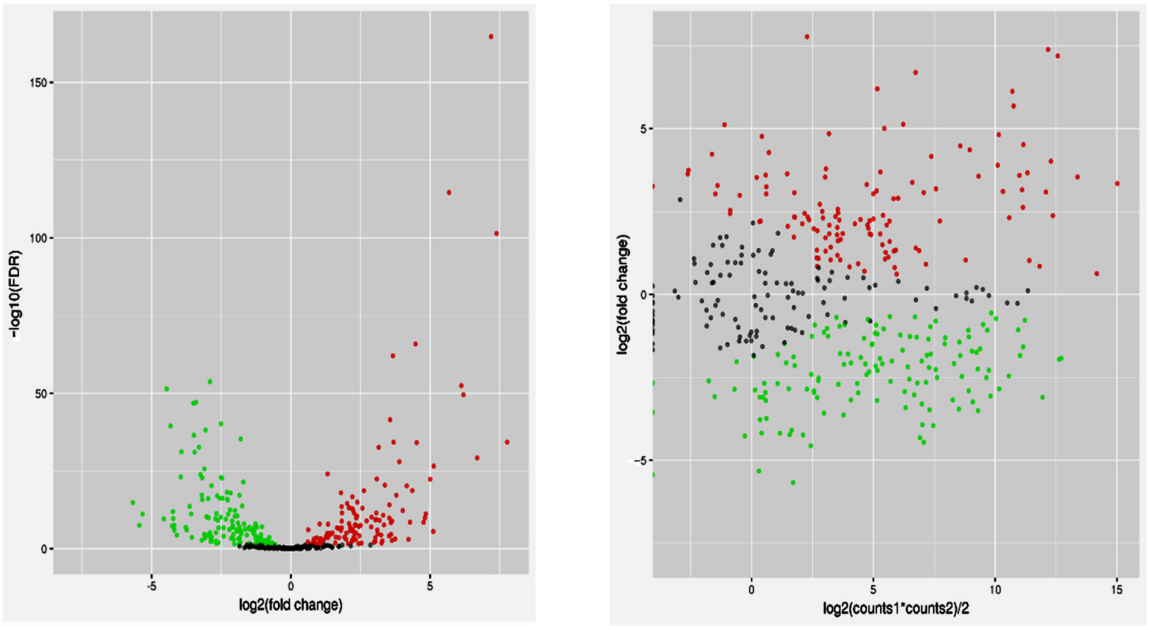

Figure 3. Volcano plot (**left**) e MA plot (**right**) of the comparison 2014 vs. the refer ence year 2013 (arable area) with differential abundance at the family taxonomic level: under-represented taxa (green points), over-represented taxa (red points), not statistically significant (blackpoints).

Table S2 shows the taxa identification and their relative abundance. *Ktedonobacteraceae, Methanosarcinaceae, Nitrososphaeraceae, Flatidae, Hymenobacteraceae*, and *Oxalobacteraceae* are the families that showed the most differential relative abundance.

It was also possible to identify among the differential taxa families typically correlated with the microbiological activity in the soil and to obtain a good resolution at the genus taxonomic level, in which 316 taxa were over-represented and 426 under-represented with variable fold-change. At the taxonomic level of genus, PCA presented good changeability among the soils collected in 2014, principally alongside the principal component 1, which describes more than 74% of changeability; while the 2013 samples were grouped and showed the lowest variability (Figure 4).

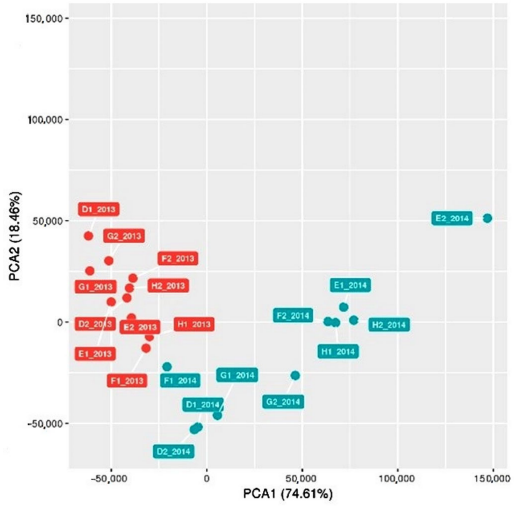

Figure 4. PCA distribution according to their distance of the comparison 2014 vs. 2013 (arable area) at the genus taxonomic level.

The Volcano and the MA plots show the taxa with a vast differential abundance at the genus level (Figure 5).

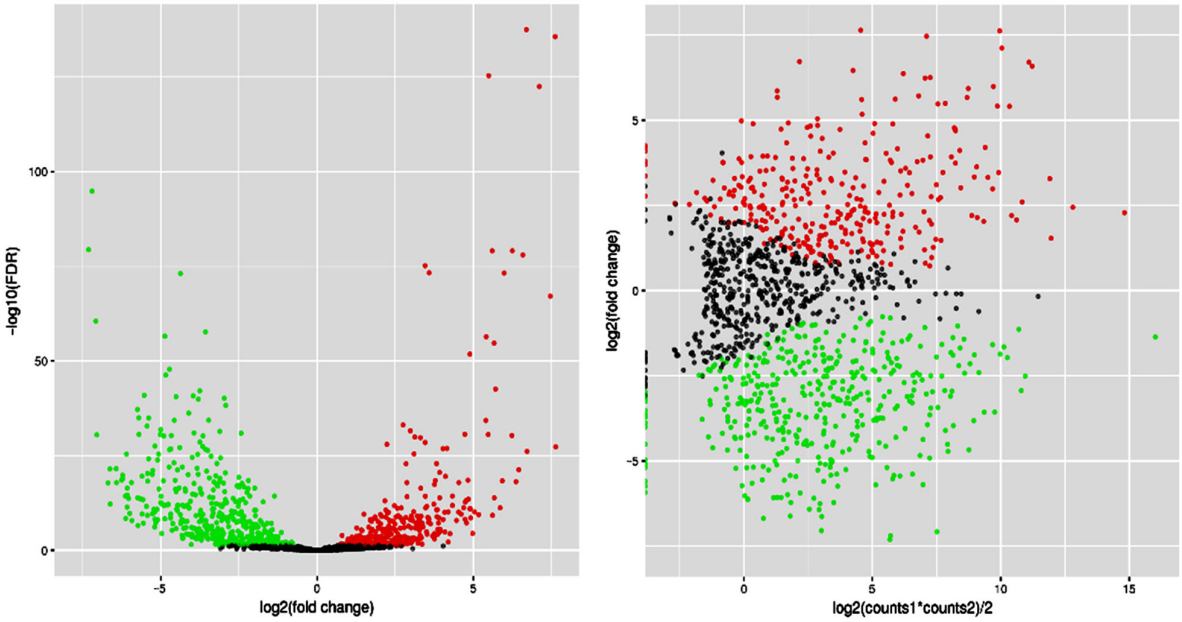

Figure 5. Volcano plot (**left**) e MA plot (**right**) of the comparison 2014 vs. the refer ence year 2013 (arable area) with differential abundance at the genus taxonomic level: under-represented taxa (green points), over-represented taxa (red points), not statistically significant (blackpoints).

When comparing 2015 vs. 2013, the abundance of identified taxa was assessed, and at the taxonomical phylum status, PCA presented a lower changeability in soils collected in 2015 compared to the reference year; this is shown in the graph in which the X-axis corresponds to the main component 1 that supports 64% of the variability (Figure 6a). Amongst the 56 phyla recognized, 14 seem to be drastically more abundant in 2015 and 19 less abundant. At the taxonomical family status, the PCA presented an unrelated irregularity in soils sampled in 2015 (Figure 6b).

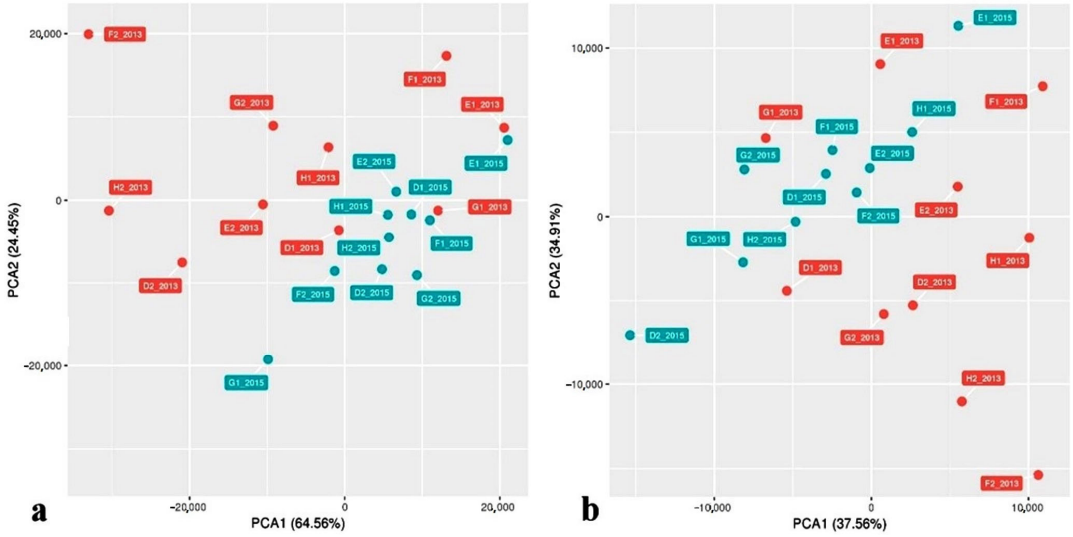

Figure 6. PCA distribution according to their distance of the comparison 2015 vs. 2013 (arable area) at the phylum (**a**) and family (**b**) taxonomic level.

The system identified 485 families; 29 were more abundant, and 139 were less abundant in 2015, with fold-changes ranging between −5.67 and 4.01 (Figure 7).

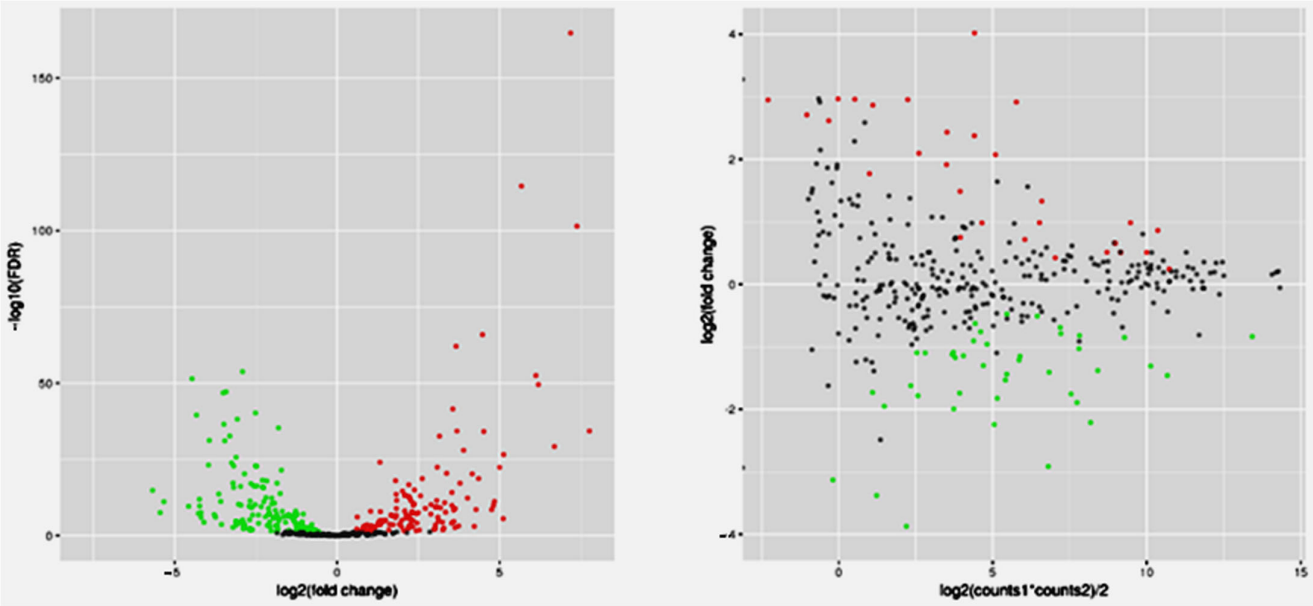

Figure 7. Volcano plot (**left**) e MA plot (**right**) of the comparison 2015 vs. the refer ence year 2013 (arable area) with differential abundance at the family taxonomic level: under-represented taxa (green points), over-represented taxa (red points), not statistically significant (blackpoints).

Ktedonobacteraceae, Methanosarcinaceae, Nitrososphaeraceae, and *Williamsiaceae* show the most differential relative abundance (Table S2) comparing biodiversity between 2016 and 2013 at the taxonomical phylum level. The PCA revealed a reasonable variability degree both in the samples from 2016 (it accounts for 32% of the total changeability along the principal component 2; not shown) and in those from 2013 (summing up to 52% of the variability along the principal component 1; Figure 8a). The comparative analysis indicated that 33 of the 56 detected phyla revealed a significantly different abundance, as 14 were more abundant and 19 were less abundant in 2016. At the taxonomic family level, the PCA presented a high diversity degree for samples taken in 2013 and 2016 (Figure 8b).

Of 485 families identified, 81 showed a meaningfully augmented richness (especially *Coccomyxaceae, Fragilariaceae, Williamsiaceae, Stephanopyxidaceae*, and *Chattonellaceae*) and 63 a reduced abundance (particularly, *Methanosarcinaceae, Defluviitaleaceae, Erysipelotrichaceae*, and *Prolixibacteraceae*) in 2016 compared to 2013, with fold changes varying in logarithmic scale, varying between −5.31 and 5.38 (Table S2).

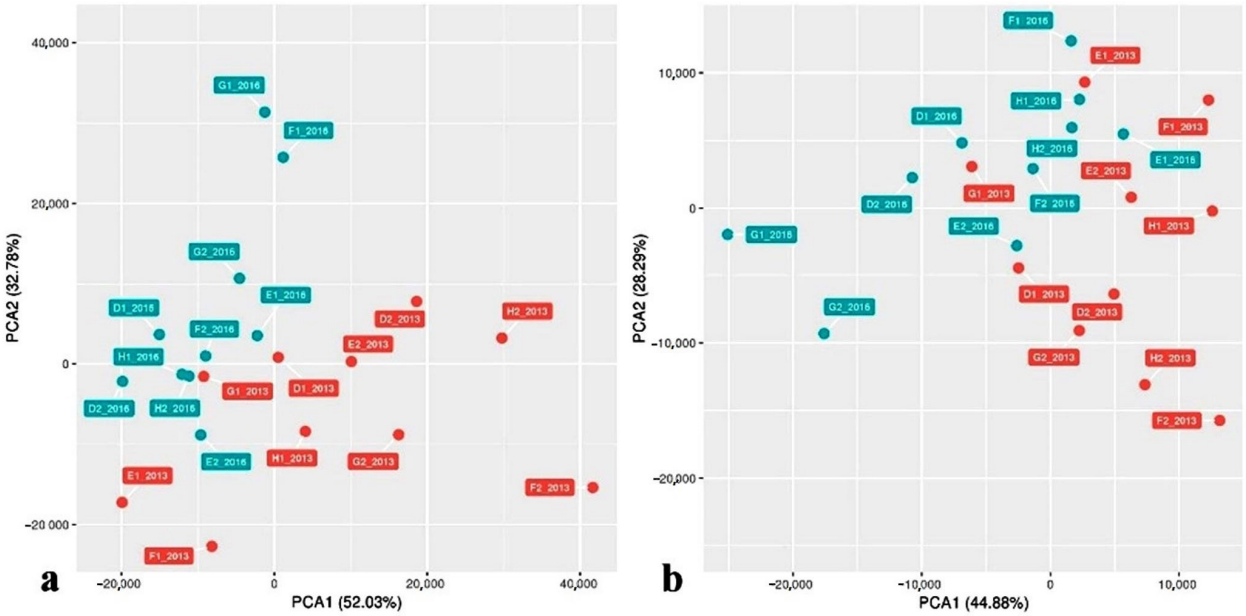

Figure 8. PCA distribution according to their distance of the comparison 2016 vs. 2013 (arable area) at the phylum (**a**) and family (**b**) taxonomic level.

4. Discussion

The soil microorganisms and their biodiversity are crucial in sustaining efficient ecosystem functionality, as there are multiple chemical-metabolic processes. A balance is established in the soil's microbial ecosystem due to stabilizing the functional interrelationships between the various microorganisms. This stability positively affects the plants and the overlying animal community.

One of the prescriptions reported in the guide produced by CSBI for implementing the mitigation hierarchy suggests storing the different layers removed during the excavation separately and closing the excavation after laying the pipelines, arranging the layers in the reverse order in which they were taken. Ultimately, the guide prescribes being careful not to mix up the layers of soil removed. This procedure should minimize the impact on the living beings that populate different soil layers. Therefore, alterations in the bacterial population reported in the results of this investigation may be due to an imperfect observance of the prescription mentioned earlier. This problem is also highlighted by the great irregularity of the chemical–physical characteristics of the soil samples taken in the years of observation (Table S3), which did not allow for any significant elaboration of correlations with the metagenomic data.

Today, metagenomic sequencing provides access to soil microbial diversity, enabling the complexity of the soil microbiome to be resolved and providing necessary data to understand soil microbial diversity and its functions. The molecular approach includes information on agricultural issues, such as soil fertility and sustainability, plant health and biotechnological processes, and interesting biomolecules or microbial strains. It can also assess soil microbiome composition and physiological and genetic mechanisms indispensable for the efficacious microbes' adaptation in specific environments, such as contaminated and disturbed soils. Under stressful conditions caused by adverse anthropic activities, soil microorganisms' development, and biochemical behaviors undergo various alterations [1,2].

In this study, we obtained results in agreement with previous studies, proving differences in the taxonomic composition and structure of the soil microbial community due to several stressing conditions [2,24,27–31]. Significant alterations of the microbiological dynamics were observed in the years following the pipeline implantation compared to the

reference year 2013. The environmental restoration processes induced microcosm evolution. Variations detected over the years highlight a precise adaptation to the soil-disturbed conditions by selected bacterial groups. Post-installation analyses presented significant variations in bacterial communities, demonstrating changes in bacterial physiological and genetic response to cope with soil stresses and implement different mechanisms for survival and adaptation to an environment with changed conditions.

The taxa connected to the biological diversity of the sampled soils were identified through differential analysis. This assessment highlighted substantial differences over time, particularly in the first year of post-installation monitoring (2014), representing the period of most considerable perturbation of biodiversity. Significantly differentiated taxa, especially for the phylum and family taxonomic levels, were identified in the sampling areas by a comparative analysis, highlighting the most influenced taxonomies and potentially involved in the soil remediation process. In particular, the taxa with more significant changes were identified. In addition, the analysis highlighted the level of taxa abundance correlated with soil microbial activity, including bioremediation, demonstrating how their presence has changed over time.

Many of the changes that occurred in the composition of the bacterial populations may be due to the partial mixing of the soil layers removed during the excavation. The heterogeneity of these modifications found at the various sampling sites indicates how different the conditions created are for each site upon repositioning the soil. More excellent aeration of the soil or, on the contrary, greater compactness, perhaps also due to the passage of excavation machines, has created various micro-environments in which different bacterial species have developed.

The PCA already showed a smaller biological gap between the samples collected in 2015 and 2016 years in comparison to 2013 (reference year), indicating a specific grade of soil recovery following the general alteration detected in the soil taken in 2014, the first year of monitoring subsequent the pipeline installation in which an intense alteration of the ecosystem balance is evident. The pipeline installation modified the bacterial communities' composition and the soil metabolic activities due to the appearance of diverse bacterial families. Bacteria commonly present in the soil were identified; due to their diversification, they can facilitate new specific biochemical processes and play a central role in the metabolic functioning of soil-living organisms and their stability. However, the critical outcome of this analysis was the identification of bacteria belonging to the natural population of microorganisms that are present in soils with particular conditions, such as soils contaminated with arsenic or polluted with creosote [20,23] or soils subjected to extreme salt and drying conditions, or rivers containing chemicals, such as calcium and magnesium (for example bacterial groups belonging to *Micrococcaceae, Xanthomonadaceae, Sporolactobacillaceae*, and *Flavobacteriaceae* family). Our personal opinion is that the development of these kinds of bacterial populations is due to the transit and stationing of the excavation vehicles, with the probable losses of lubricating liquids or fuel, rather than to the mixing of deep soil layers with the more superficial ones as in the case of soil erosion and downstream accumulation [24].

Many reports have examined the effects of different contaminants, such as heavy metals and organic substances. Their immediate impact on soil is a decrease in microbial biomass and the loss of biochemical activities essential for soil function [32,33]. However, over a more extended period, there can be a gradual change in the microbial composition in which natural selection, gene exchange, and immigration can contribute to a microbial community adapted to the actual conditions [34,35]. Therefore, a deeper knowledge of these communities should be obtained for future assessment of bioremediation potential at disturbed sites [36–39].

In the case of exogenous substance fallout, the differences in soil source and properties have caused the occurrence of different bacterial isolates, such as *Acinetobacter*, *Aeromonas*, *Aureobacterium*, *Bacillus*, *Escherichia*, *Klebsiella*, *Micrococcus*, *Pseudomonas*, *Rhodococcus* and *Stenotrophomonas* [40,41]. Mathè et al. [42] revealed how exogenous substances

influence the activity and variety of endogenic microorganisms. They tested particular isolates able to destroy the structure of several aliphatic and aromatic hydrocarbons, resist toxic metals, or develop despite the presence of antibiotics. Researchers revealed that increased activity of hydrocarbonoclastic bacteria in contaminated sites occurs, indicated by large CO_2 production. Furthermore, Alisi et al. [43] and Sprocati et al. [44] demonstrated the remediation possibility of soil polluted with toxic metals and diesel fuel, employing a bioaugmentation plan centered on the development of a microbial mix designed with selected natives, which can efficiently enable and accelerate the bioremediation of environments co-polluted by toxic metals and hydrocarbons.

In our study, through the 2014 analysis, bacterial groups were observed, and differential abundance levels of specific families were estimated.

Rhodobacteraceae, Alphaproteobacteria, Acetobacteraceae, Sphingomonadaceae, and *Rhizobiaceae* families were detected in the samples. In addition, bacteria belonging to *Sphingomonadaceae, Vibrionaceae, Mycobacteriaceae, Moraxellaceae, Nocardiaceae, Flavobacteriaceae*, and *Bacillaceae* families were observed to use hydrocarbons as the only carbon source [45]. Furthermore, families of *Actinomycetaceae, Rhizobiaceae, Streptomycetaceae, Nocardiaceae*, and *Bacillaceae* have also been related to petroleum and hydrocarbon degradation [45–48].

A differential abundance was also observed for *Methylobacteriaceae,* which can utilize methanol and several other substances containing a single carbon atom as energy sources, and *Geobacteraceae,* involving numerous strains that oxidize monoaromatic hydrocarbons (benzene and methylbenzene). The family *Methanosarcinaceae*, the most versatile of methanogenic Archaea, was also observed concerning the variety of materials used to obtain energy. These are located in many anaerobic environments, producing methane (thermal environments, freshwater, wetlands, marine environments, hypersaline sediments, petroleum wells, anaerobic composting of waste, and gastric and intestinal animal systems) [49–51]. These results allow hypothesizing that the analyzed field is a modified soil biosystem in which the reduction or growth of specific bacterial communities attended to the natural biodegradation/bioremediation process to reestablish optimal conditions.

5. Conclusions

In conclusion, the significant differences in taxonomic composition and structure of the soil microbial community due to critical stress conditions demonstrate the fundamental role of microorganisms in the soil and how bacterial communities are capable of profound physiological and genetic changes to cope with soil stresses and implement different mechanisms for survival and adaptation to an environment with changed conditions.

It is not easy to combine the heterogeneity of the results obtained and the complexity of the microbial populations, as found with the analysis of the bacterial pool extracted from the sixteen soil samples taken annually for four consecutive years.

We can summarize the causes of differentiating bacterial populations into mechanical and chemical events.

Among the mechanics, we indicate the possible (i) mixing of soil layers positioned differently from the actual depth; (ii) surface compaction of the soil due to the repeated passage of excavation machines. Among the chemical events, we can include (i) the loss of lubricating oils or fuels from the vehicles used during the works; (ii) the carryover of salts and metals from deep layers to the surface; (iii) the diffusion in the deeper layers of organic material from the soil surface.

In light of previous considerations, we can assert that further research needs to be conducted to understand and study the activities and dynamics of soil bacterial populations and the associations between microbial diversity and bacterial functionality that trigger substantial revival and renovation of bio-processes.

Even if a large number of species is required to preserve stable processes in everchanging ecosystems, a small number of species is essential for the functioning of an ecosystem in balance conditions. Therefore, taxonomic diversity has the ecological role of ensuring that some species can perform specific functions in the presence of perturbations.

The greater the grade of biodiversity of an ecosystem, the more extended its resilience to stresses, showing more chances due to new genotypes or species that can perform the functions of disappeared organisms.

Knowledge of bacterial community structure and its influence on ecosystem processes must be improved. This lack of knowledge is partly attributable to belowground ecosystems' high species richness and complexity. Future research can reveal hidden mechanisms in more complex ecosystems by identifying reduced-complexity ecosystems. Results can also be used to predict the response of ecosystems to increasing environmental change and disturbance.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13061189/s1, **Figure S1.** Google earth picture of the sampling site. **Figure S2**. Distribution of taxa in soil samples (arable area) at the taxonomic level of the family. The samples are reported in order by sampling sites (DEFGH), by year (following the chronological order of sampling), and by depth, remarking for each group the sampled sites at 0-20 cm (1) and subsequently those at 20- 40 cm (2). **Figure S3.** Heatmap for data visualization at the taxonomic level of phylum; blocks of different taxa abundance in all samples (wood and arable areas) in the years before (2013) and following (2014-2016) the pipeline installation. **Figure S4.** Heatmap for data visualization at the taxonomic level of the family; blocks of different taxa abundance in all samples (wood and arable areas) in the years before (2013) and following (2014-2016) the pipeline installation. **Figure S5**. Heatmap for data visualization at the genus taxonomic level; blocks of different taxa abundance in all samples (wood and arable areas) in the years before (2013) and following (2014-2016) the pipeline installation. **Table S1.** Taxa were identified at the phylum taxonomic level in all cultivated soil area samples collected during the three years following the pipeline installation (2014-2016). Taxa with more abundance are reported as over-represented, and those with less abundance are reported as under-represented in comparison to the 2013 (reference year); the abundance was defined as the log FC, the decimal logarithm of the fold-change that represents the times' number that the abundance is significantly modified compared to the reference. **Table S2.** Taxa were identified at the family taxonomic level in all cultivated soil area samples collected in the three years following the pipeline installation (2014, 2015, 2016). Taxa with more abundance are reported as over-represented, and those with less abundance are reported as under-represented in comparison to the 2013 (reference year); the abundance was defined as the log FC, the decimal logarithm of the fold-change that represents the times' number that the abundance is significantly modified compared to the reference. **Table S3.** Ranges of chemical, physical-chemical and biological properties of collected samples.

Author Contributions: Conceptualization, G.S. and S.A.B.; methodology, M.G.B., L.S., and L.-A.M.; validation, M.G.B., G.S., and S.A.B.; formal analysis, M.G.B., S.M.M., and B.E.S.; writing—original draft preparation, M.G.B.; writing—review and editing, L.S., G.S. and S.A.B.; supervision, G.S.; funding acquisition, E.B. All authors have read and agreed to the published version of the manuscript.

Funding: SAIPEM—S.p.a.: a private company supported the monitoring activity (Grant No. 5000026003).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We are grateful to Mr. Lorenzo Montinaro who collected the soil samples and performed the chemical analyses.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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