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Structural and Functional Shift in Soil Bacterial Community in Response to Long-Term Compost Amendment in Paddy Field

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Abstract: Microbial community composition and diversity of agricultural soils primarily depend on management practices. The application of compost on agricultural fields is known to increase soil fertility, which can also help to enhance agricultural productivity. The effects of long-term application of compost along with nitrogen (N), phosphorus (P), and potassium (K) (+Compost) on soil bacterial diversity and community profiles were assessed by amplicon sequencing targeting the 16S rRNA gene of bacteria and compared with those on soils that received only NPK but not compost (−Compost). Ordination plot showed treatments to cluster differently, implying changes in community composition, which were validated with taxonomical data showing Firmicutes, Actinobacteria, and their related classes to be significantly higher in +Compost than in −Compost soils. The predicted abundance of functional genes related to plant growth promotion, development, and decomposition was significantly higher in compost-amended soil than in soils without compost. The results are of particular importance as they provide insights into designing management practices to promote agricultural sustainability.

Keywords: long-term fertilization; next-generation sequencing; bacterial diversity; plant growth

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1. Introduction

The diverse and abundant population of soil bacteria plays a major role in the functioning of the ecosystem; however, different agricultural management strategies primarily drive the bacterial community composition and functioning [1,2]. Nutrient amendments in agricultural soils are usually used to improve plant productivity, but overuse of fertilizers, like inorganic N, can affect the soil quality by deteriorating soil fertility [3], decreasing crop yield [4], and affecting bacterial diversity [5,6].

With the increasing importance of soil microbes, which include bacteria, in maintaining soil quality [7], understanding soil microbial processes under different management schemes is recognized to be important for the sustainability of agricultural ecosystems. Soil fertility management based on the use of organic fertilizers can promote microbial processes and increase crop yield [8]. Increment in microbial processes can help biogeochemical cycles and nutrient cycling [9], which can assist in the enhancement of crop productivity [10]. Extensive research has indicated the beneficial effect of organic matter application in enhancing bacterial diversity and positive interaction with plants [11,12].

Livestock manure and crop residue are two major types of organic matter that have the potential to mitigate soil degradation caused by long-term chemical fertilization. Several studies have addressed the impacts of livestock manure and plant residues on soil-dwelling bacterial communities [13,14]. Crop residues are the most abundant, cheapest, and most readily available organic waste to be biologically transformed, with rice straw being one of them, in many rice-growing countries. They have been used as a common organic material as they contain numerous mineral nutrients, such as nitrogen, phosphorus, potassium, and silicate [15]. The application of straw in soil has been used to improve the activity of soil bacterial community and promote soil nitrogen and carbon sequestration potential [16]. Additionally, livestock manure has shown similar characteristics as crop residues [17]. However, there have been limited studies that concentrate on the effect of compounded compost made of rice straw and livestock manure on soil bacterial diversity and community profiles.

Thus, this study was conducted to compare and characterize the bacterial diversity and community composition of paddy soils treated with NPK combined with rice straw and livestock manure (+Compost) and that with only conventional fertilizers without compost (−Compost). We hypothesized that the additional compost amendment will result in changes in the structural and functional composition of the bacterial community.

2. Materials and Methods

The experimental field (1230 m^2) was located at the Paddy Crop Research Division, Department of Southern Area Crop Science, National Institute of Crop Science, Republic of Korea. The plots within the experiment were managed and fertilized as mentioned previously by Samaddar et al. [18]. Briefly, the fertilization trial was initiated in 1967, and rice (*Oryza sativa* cv. Hwayeong) was cultivated as a single crop. Out of several fertilization treatments, two different treatments with three replicate plots (10 m × 10 m each) for each treatment arranged in a completely randomized manner were used in this study. The treatments were (1) inorganic NPK fertilized soil sample (−Compost) and (2) NPK amended with compost (10 tons·ha^{-1}·$year^{-1}$) fertilized soil sample (+Compost). NPK fertilizers were applied as urea, fused superphosphate, and potassium chloride at the rate of 120, 80, and 80 kg·ha^{-1} from 1967 to 1972. Since 1973, N, P, and K have been applied at a higher rate of 150, 100, and 100 kg·ha^{-1}, respectively. Compost was prepared by decomposing rice straw and cattle manure for a period of 6 months, and it contained 431, 19.8, 5.2, and 29.1 g·kg^{-1} of total C, N, P, and K, respectively. A total of five individual subsamples (~20 cm depth) were collected from each of the three replicates of individual treatments in April 2016 and were pooled to make a composite sample (~100 g) for each replicate. Thus, six individual samples were collected. Soil samples were sieved (<2 mm) and stored in sterile bags and transported to the laboratory on ice. Soil DNA was extracted using 0.5 g of fresh soil sample by using a PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA), and DNA was stored at −20 °C for all downstream analysis. The 16S rRNA copy number was estimated using quantitative PCR (qPCR) with the primers 517F/1028R [19,20] following conditions described in detail by Samaddar et al. [18]. Briefly, qPCR amplification targeting the 16S rRNA gene of bacteria was performed using the primers 517F (5′-GCCAGCAGCCGCGGTAA-3′) and 1028R (5′-CGACARCCATGCASCACCT-3′) with a Rotor-Gene® Q (Qiagen, Foster City, CA, USA) in 10 µL of reaction mixture containing 5 µL of Maxima SYBR Green Master Mix (Thermo Fisher Scientific Inc., Waltham, MA, USA), 0.4 µM of each of forward and reverse primers, 1 µL of template DNA, and 3.6 µL of sterile distilled water. The reaction conditions for the qPCR were as follows: 1 cycle at 95 °C for 10 min, 45 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 40 s, and extension at 72 °C for 45 s. All sample and standard curve measurements were performed in triplicate, and negative controls were included in every qPCR run. For standard curve preparation, the 16S rRNA fragment from *Pseudomonas mendocina* PC1 was amplified with the same primer pair under the same thermal conditions. Rotor-Gene Series Software v. 2.0.2 (Qiagen, Foster City, CA, USA) and LinRegPCR

program v. 2017.0 (Academic Medical Centre, Amsterdam, Netherlands) [21] were used to analyze the obtained data. The qPCR standard curve efficiency was 1.866 ± 0.031 (n = 12). Additionally, the amplification efficiency of each sample was considered for calculating the 16S rRNA gene copy numbers. The isolated DNA was subjected to high-throughput Illumina MiSeq sequencing at ChunLab, South Korea. The V3–V4 regions of 16S rRNA genes were targeted and amplified using the primers 341F (5′-TCGTCGGCAGCGTCAG ATGT GTATAAGAGACAGCCTACGGGNGGCWGCAG-3′; underlined sequences indicating the target) and 805R (5′-GTCTCGTGGGCTCGGAGATGGTATAAGAGACAG GACTACHVGGTATCTAATCC-3′). The raw data obtained were analyzed using a Mothur pipeline v. 1.39.5 [22] similarly as performed in our previous studies [18,23]. The raw sequences were deposited in a Sequence Read Archive (SRA) dataset at NCBI (National Center for Biotechnology information) under accession numbers SRP127951 (−Compost) and SRP298800 (+Compost). Analyzed data were normalized to a minimum number of reads prior to calculation of Shannon and Chao indices in Mothur. Principal coordinate analysis (PCoA) was performed in Mothur. Tukey's test was used wherever necessary to calculate the differences between the means, which were considered significant at $p < 0.05$ using SAS version 9.4 [24]. Bacterial community was characterized using the linear discriminant analysis (LDA) effect size (LEfSe) tool for biomarker discovery (http://huttenhower.sph.harvard.edu/lefse/, accessed on 25 February 2021) [25]. The PICRUSt tool was used to predict functional profiles of bacterial communities from the bacterial 16S rRNA abundance data [26].

3. Results

The bacterial 16S rRNA abundance, as determined by qPCR, increased significantly by nearly threefold in +Compost soil compared with −Compost soil (Figure 1a). Analysis of sequence data yielded on average 782 operational taxonomic units (OTUs) for −Compost and 750 OTUs for +Compost, which were not significantly different. Additionally, no significant differences for diversity and richness estimates were observed between the treatments (Table 1). A 98% coverage from Good's coverage estimator and rarefaction curve (Figure S1) showed that sampling was sufficient to estimate those indices. Principal coordinate analysis (PCoA), performed to estimate the effects of studied treatments on bacterial community composition, showed differences in ordination patterns of the treatments with PC1 explaining 28% of variance and PC2 explaining 22% of variance (Figure 1b).

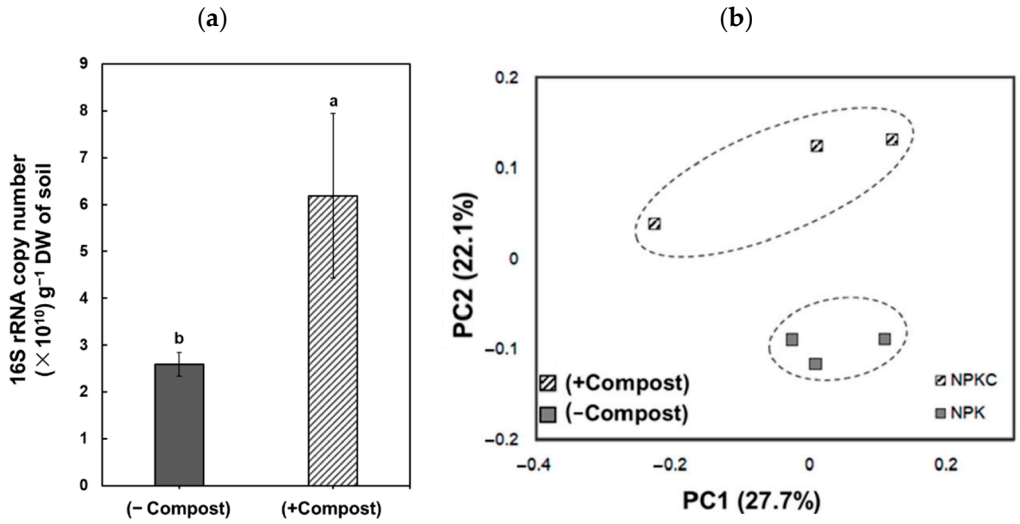


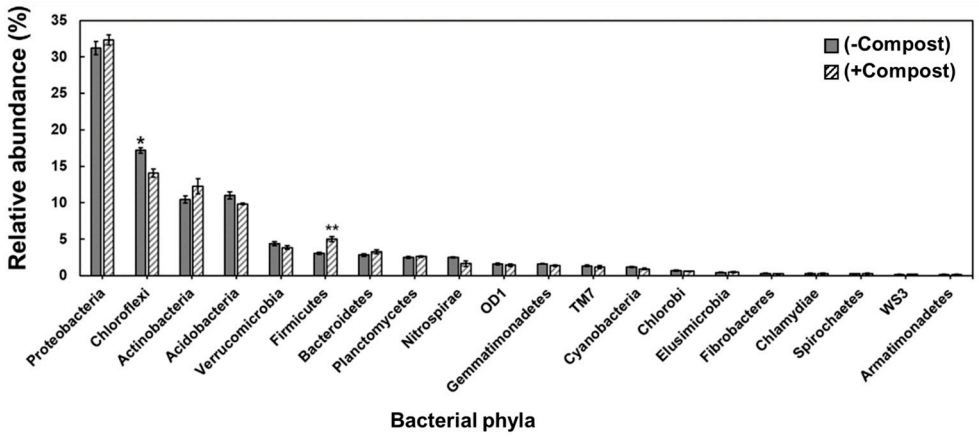
Figure 1. (**a**) The 16S rRNA copy numbers of soil bacterial communities estimated by qPCR. (**b**) The principal coordinate analysis (PCoA) showing the clustering of bacterial communities between soil samples. Values for 16S rRNA data are plotted with mean; error bars indicate standard errors; different letters on plot mean significant difference ($p < 0.05$) between treatments according to Tukey's test.

Table 1. Summary data for 16S rRNA sequencing results and α-diversity of the soil samples.

Treatment	No. of Sequences	Goods Coverage (%)	No. of OTUs	Chao	Shannon's Index
−Compost	6567	98.6	782 ± 6.71 [a]	816 ± 8.2 [a]	6.02 ± 0.02 [a]
+Compost	6567	98.8	750 ± 13.5 [a]	775 ± 15 [a]	5.98 ± 0.01 [a]

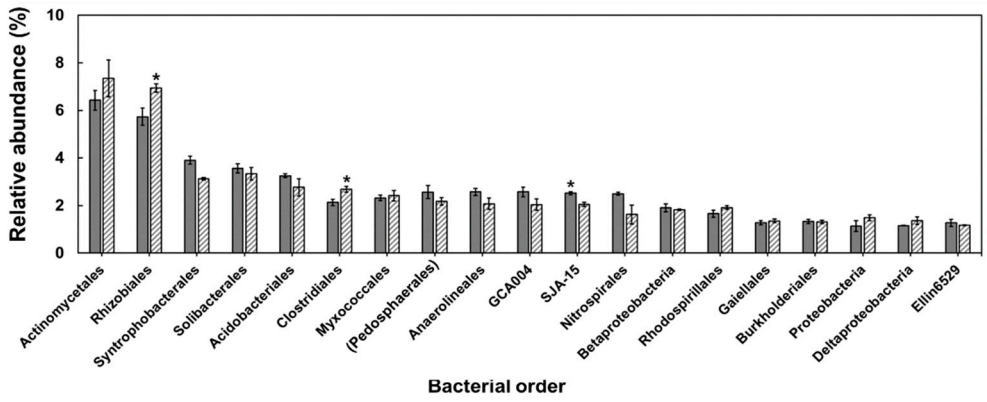
[a] Values are not significant at $p < 0.05$ among the treatments according to Tukey's test.

At taxonomic levels, the sequences from the soils were annotated to 56 different phyla, of which the 20 most abundant phyla are represented in Figure 2a. Proteobacteria were the most abundant bacterial phylum in both the studied soil groups, followed by Chloroflexi, Actinobacteria, Verrucomicrobia, Firmicutes, Bacteroidetes, Nitrospirae, uncultured-OD1, Gemmatimonadetes, uncultured-TM7, Cyanobacteria, Chlorobi, Elusimicrobia, Fibrobacteres, Chlamydiae, Spirochaetes, uncultured-WS3, and Armatimonadetes. Of all phyla, the abundance of Chloroflexi and Firmicutes varied significantly with Chloroflexi significantly abundant in −Compost soils while Firmicutes significantly dominating +Compost soils. At the bacterial order level (Figure 2b), Actinomycetales were the most abundant, followed by other groups. However, Rhizobiales and Clostridiales were significantly abundant in +Compost, whereas the abundance of uncultured-SJA-15 increased significantly in −Compost soils. LEfSe analysis (Figure 3) demonstrated that −Compost soils had significantly higher abundance of the phylum Chloroflexi and their related class Anaerolineae and the phylum Acidobacteria and their related order Acidobacteriales compared with −Compost soils. On the other hand, +Compost soils had higher abundance of Actinobacteria along with the family Micrococcaceae and genus *Arthrobacter*, and Firmicutes along with the class Bacilli and order Bacillales. Additionally, abundance of the class Alphaproteobacteria, their related order Rhizobiales, family Hyphomicrobiaceae, and genus *Rhodoplanes* increased significantly in +Compost soils compared with −Compost soils. The predicted abundance of genes encoding enzymes related to plant growth promotion, development, and fatty acid biosynthesis was significantly increased in +Compost soils when compared with −Compost soils (Figure 4).



(**a**)

Figure 2. *Cont.*



(**b**)

Figure 2. The relative abundances of the 20 most abundant bacterial (**a**) phyla and (**b**) orders in the studied soil samples. The phylum/order significantly different according to ANOVA is marked by asterisk (*: $p < 0.05$; **: $p < 0.01$).

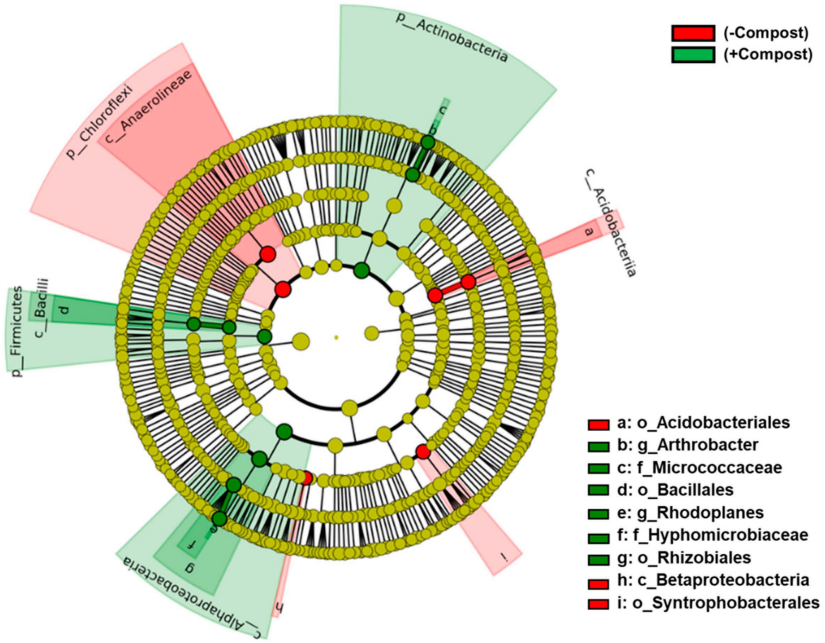


Figure 3. The significantly different taxa of bacteria between two soil samples depicted by linear discriminant analysis effect size (LEfSe) method. The significantly different abundant taxa among treatments are denoted by colored dots, and from the center to the outer circle, they represent the kingdom, phylum, class, order, family, and genus. The trends of the significantly different taxa are represented by colored dots.

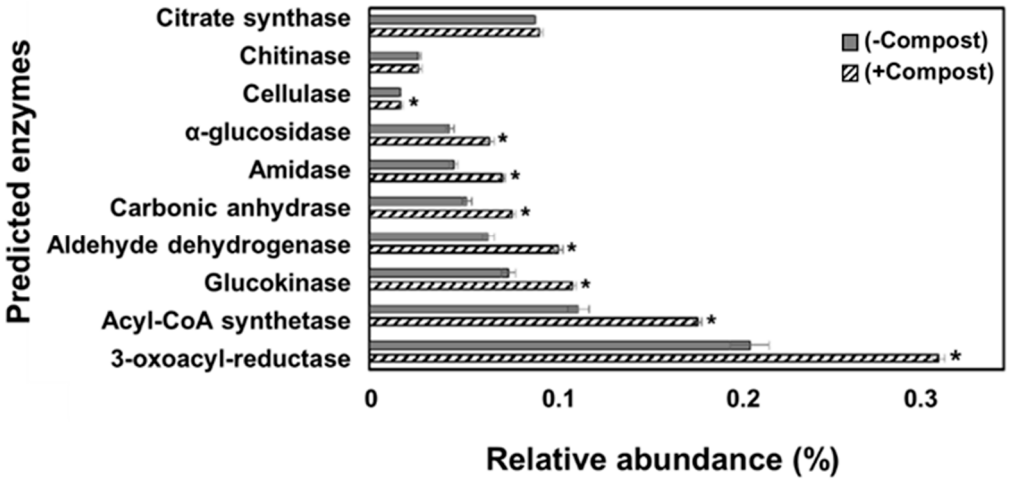


Figure 4. The abundance of genes encoding enzymes in the two soil samples related to plant growth promotion, development, and fatty acid biosynthesis predicted by the PICRUSt. Error bars indicate standard errors; the relative abundance of enzyme-encoding genes with significantly differed between treatments are marked by asterisk (*: $p < 0.05$).

4. Discussion

Investigation of soil microbial communities, considered as indicators of soil quality, [7] showed altered compositional and functional profiles of bacterial community in +Compost and −Compost soils.

The bacterial 16S RNA abundance increased in +Compost soil compared with −Compost soil, which is in agreement with the observations of Li et al. [27], where the 16S rRNA gene abundance increased in soils amended with manure and conventional fertilizer compared with conventional fertilizer alone. Besides, compost amendment did not alter the microbial diversity or richness, but differences in community composition were observed from taxonomical data as also observed in other studies [28,29]. Diversity or richness might not always alter with changes in community composition, as differences in abundance of some taxonomic groups may be compensated by differences in abundance of other taxonomic groups [30]. Additionally, it is true that these estimates are powerful tools and provide us an ecological trend, but it also important to keep in mind that univariate analyses, like alpha diversity estimates, are just a step in the line of scientific query and do not provide a definite answer to community outcomes [31] or composition, which was observed to be altered in the present study.

Proteobacteria were the most abundant phylum belonging to both the studied soil groups [32,33]. However, the abundance of Chloroflexi was observed to be significantly higher in −Compost soils along with their order, the uncultured bacterium SJA-15. Zhalnina et al. [34] recently reported that a decrease in pH and an increase in total nitrogen can contribute to a decrease in Chloroflexi population, as they might be following an oligotrophic lifestyle. Additionally, the amendment of straw and manure results in a decrease in soil pH and an increase in total nitrogen content in soil [35], which might have resulted in a decrease in Chloroflexi population in +Compost soils. The relative abundance of Firmicutes and their order Clostridiales was observed to be higher in +Compost soils, which is important as Firmicutes are regarded as the main phylum that consists of decomposers and are important for the conversion of organic matters [36]. These observations also get support from Sharmin et al. [37], where the abundance of Firmicutes increased in a sugarcane processing plant, which encompasses a large amount of plant organic matters. Actinobacteria and their related class and genus were also significantly abundant in +Compost soils, which draws support from studies where Actinobacteria were sensitive to management strategies [18,38]. A significant increase in the abundance of Rhizobiales in +Compost soil is interesting as they are one of the most important bacterial orders responsible for nitrogen fixation and the enhancement of the total nitrogen

content of the soil [39]. Changes in bacterial community composition might also have contributed to changes in the functional profiles of the bacterial community as observed from the predicted abundance of genes encoding enzymes, which were significantly higher in +Compost soil when compared with −Compost soil. One of the limitations of amplicon gene sequencing is that it does not provide information on what the microbes are doing, so the use of the PICRUSt tool [26], which uses an ancestral-state reconstruction algorithm to predict the presence of gene families and then combines them to estimate composite metagenome, is expected to provide an idea about the functional composition of microbes, which is believed to be informative. This tool has been proved to be highly accurate in predicting a community's functional capabilities from 16S abundance profiles [26,40] and has been popular recently [41–44]. The abundance of genes encoding enzymes, which were relatively higher in +Compost compared with −Compost, was mostly related to decomposition and plant growth promotion and is well known to be synthesized by Proteobacteria and Firmicutes [45]. Amidases, which increased significantly in +Compost soil, are characteristic of Actinobacteria [18] and are important for the synthesis of indole-3-acetic acid, an important plant-growth-promoting hormone [46]. Likewise, bacterial citrate synthases, which were relatively higher in abundance in +Compost soil, have shown enhanced plant growth under nutrient-limited soils [47,48]. On the other hand, cellulases, which also increased in +Compost soil, are important for breaking down cellulose into monosaccharides or shorter polysaccharides [49], which improves soil fertility and plant growth through accelerated straw decomposition [50]. The requirement of higher amount of ATP in the decomposition process might have resulted in an increase in the abundance of glucokinase in +Compost soils [51]. On the other hand, compost addition also improved aldehyde dehydrogenase activity, which is known to provide stress tolerance to plants [52]. Furthermore, enzymes known to act on plant development, like acyl-CoA synthetase, which is essential for cuticle development [53], microsporogenesis [54], and pollen development [55], also increased in +Compost soils. Lastly, the increase in the predicted abundance of the fatty acid biosynthesis-specific enzyme 3-oxoacyl reductase, which is known to improve seed yield [56], was also higher in compost-amended soils.

5. Conclusions

Long-term amendment of compost altered the bacterial community composition both structurally and functionally. The relative abundances of a few groups of bacteria, like Firmicutes, Actinobacteria, and Proteobacteria, were significantly higher in compost-amended soil, of which several are reported to be beneficial. Moreover, the predicted abundance of genes coding enzymes related to decomposition, plant growth promotion, and development increased in +Compost soils compared with −Compost soils alone. Thus, a combined application of compost and inorganic fertilizers might be a good way to keep up with the agricultural productivity while keeping the environmental balance.

Supplementary Materials: The following are available online at https://www.mdpi.com/2076-3417/11/5/2183/s1, Figure S1: Rarefaction curves of bacterial 16S rRNA sequences obtained from the studied soil samples.

Author Contributions: S.K. and T.S.: conceptualization of the study and funding acquisition; S.K. and S.S.: design of experiments and performance of experiments; S.K. and S.S.: analysis of sequencing data; P.C. and J.C. (Jongseo Choi): assistance in soil sampling and preliminary experiments; S.K., S.S., and P.C.: writing of the manuscript; A.R.C. and J.C. (Jeongyun Choi): assistance in experiments and writing of the manuscript; T.S.: critical review and editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data and analyses from the current study are available from the corresponding authors upon reasonable request. The raw reads were deposited in SRA archives and can be accessed by accession nos. SRP127951 (−Compost) and SRP298800 (+Compost).

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

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