JMB
Journal of Microbiology and Biotechnology

Online ISSN : 1738-8872
Pimt ISSN : 1017-7825

JMB Papers in Press. First Published online Jan 29, 2019
DOI: 10.4014/jmb.1811.11018

Manuscript Number: JMB18-11018

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Article Type: Note

Keywords: Bacterial diversity, compost levels, long-term fertilization, paddy soils

Changes in structural and functional responses of bacterial community under different levels of long-term compost application in paddy soils

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Running title: Bacterial community at different compost levels

Abstract

Soils amended for long-term with high levels of compost demonstrated greater abundance of bacterial members of the phylum Bacteroidetes whereas a decreasing trend in the relative abundance of phylum Acidobacteria was noted with increasing levels of compost. Metabolic profiles predicted by PICRUSt demonstrated differences in functional responses of the bacterial community according to the treatments. Soils amended with lower compost levels were characterized by abundance of genes encoding enzymes contributing to membrane transport, cell growth whereas genes encoding enzymes related to protein folding, transcription were enriched in soils amended with high levels of compost. Thus the results of the current study provided an extensive evidence of the influence of different compost levels on bacterial diversity and community structure in paddy soils.

Keywords: bacterial diversity; long-term fertilization; compost levels; paddy soils

Experiments that monitor changes in microbial diversity and community structure over a span of decades are critical for predicting future soil productivity and understanding interactions in the soil environment. Soil microbial communities can be influenced by several biotic and abiotic factors [1–3] and also agricultural management practices, which include the use of organic/inorganic fertilizers and crop rotation [4]. Several reports have indicated that long-term organic farming practices have positive effects on soil microbial diversity and soil processes [5,6] , but there is not much detailed evidence of how different levels or dosage of compost impact the bacterial community. In this study, we used pyro-sequencing tags between V1-V3 regions of the 16S rRNA gene to analyse and compare the effect of long-term application of different compost levels on the composition of paddy soil bacterial communities. Soil samples were collected from Gangseo series

paddy soils at the National Institute of Agricultural Science and Technology experimental area located in Suwon city (37°16′0″ N, 127°1′0″ E), Gyeonggi Province, Republic of Korea where rice was cultivated as a single crop since 1954 [7]. Five different treatments comprising of inorganic chemical fertilizers and different levels of compost application were considered for the present study. The treatments included: (i) inorganic nitrogen (N), phosphorus (P) and potassium (K) fertilizers (NPK) (ii) NPK and organic compost (C) (NPKC750) (iii) NPKC1500 (iv) NPKC2250 (v) NPKC3000. Chemical fertilizers (NPK) were applied at different rates as N-P_2O_5-K_2O kilograms per hectare at the rate of 75-75-75 from 1954 to 1970, 100-75-75 from 1971 to 1978, 150-86-86 from 1979 to 1985 and 110-70-80 from 1986 till sampling date. Organic compost was prepared by fermenting rice straw and then added at the rate of 7.5, 15.0, 22.5, and 30.0 Mg ha^{-1} in NPKC750, NPKC1500, NPKC2250, and NPKC3000 treatments, respectively. Three subsamples were collected from individual treatments at 0 to 20 cm depth in October 2009, after the rice crop harvest. Subsamples were pooled to make a composite sample, thus a total of 5 individual samples were collected. The physicochemical properties of the soils studied were as reported earlier [8]. DNA was extracted from approximately 0.5 g of soil using UltraClean™ Soil DNA Isolation Kit (Mo Bio, Solana Beach, CA, USA), following the manufacturer's instructions. The extracted DNA was amplified using primers targeting the bacterial 16S rRNA gene (9F: 5'-*X*-ACG AGT TTG ATC MTG GCT CAG -3' and V3-541R: 5'-*X*-ACW TTA CCG CGG CTG CTG G-3', where *X* denotes an 8 nucleotide long barcode uniquely designed for each sample followed by a common linker AC) and were pyro-sequenced in a 454 GS FLX Titanium Sequencing System (Roche), according to the manufacturer's instructions at Chunlab, Seoul, South Korea. Raw data are available in Mendeley data repository: http://dx.doi.org/10.17632/pgr2hc2d9k.1. Raw sequences obtained were analyzed as described [9]. After processing the raw sequences, they were

clustered at 3% cut off level and a total of 188 OTUs were observed from all the studied samples. The rarefaction curve (Fig. S1) indicated that the sampling effort was sufficient to get a full extent of taxonomic diversity. This derives high importance as number of species found in a sample at any given phylogenetic level is strongly affected by the number of sequences analyzed [10]. After analysing the sequence data and normalizing it to minimum number of reads a total of 787 sequences were obtained (Table 1). The number of OTUs observed ranged from 160-168 per sample and Goods coverage estimator demonstrated coverage ranging from 95.7- 97.1 % which implies majority of phylotypes were considered for this study. The diversity estimators Shannon and Inverted-simpson index were relatively higher in NPKC1500 amended soils compared to other treatments and was observed to be lowest in NPKC3000 amended soils. The richness estimator showed a similar trend where NPKC3000 amended soils displayed lower species richness compared to other treatments which were nearly similar. The results are very similar with the observations by where diversity estimates from community level physiological profiles (CLPP) showed lower values in higher level of compost amended field compared to low level of compost application [7]. Changes in community composition among compost application levels were evident from ordination plot (Fig. 1A) constructed from Bray-Curtis dissimilarity matrix where higher levels of compost amended soils (NPKC2250 and NPKC3000) separated from NPK, NPKC750 and NPKC1500 along the first axis which explained 29% of the variation in community composition. The results derive support from several studies as compost application has been reported widely to impact the size and composition of soil microbial community [8,11,12]. A weakness of the study was that there were no replicates of the treatments in the field and thus no replicates were added in the analyses. On that note, the results can be regarded as tentative. Nevertheless, as these fields are part of early trials established in 1954 by Government of Korea

to evaluate the effect of long-term fertilization, these sites are worth studying. The changes in community composition were attributed to differences in abundance profiles of certain groups of bacteria. Relative abundance of bacterial community (Fig. 1B) at phylum level, showed dominant groups (> 1%) across all the soil samples were Proteobacteria, Chloroflexi, Bacteroidetes, Acidobacteria, Nitrospira, Actinobacteria, Chlorobi, Firmicutes, Planctomycetes, Cyanobacteria, Gemmatimonadetes, Armatimonadetes, Verrucomicrobia, and TM7 which falls in line with earlier studies [13,14]. The most dominant phylum observed in the current study was Proteobacteria, which agrees with the earlier reports [15]. A distinct trend was observed in the relative abundance profiles of phylum Bacteroidetes where increase in levels of compost induced the abundance of the particular phylum whereas abundance of Acidobacteria decreased with increase in compost levels. At class level (Fig. 2A), members of bacterial phylum Bacteroidetes (Saprospirae, Bacteroidia, Sphingobacteriia, Flavobacteriia, Cytophagia) and Firmicutes (Bacilli and Clostridia) were relatively abundant in NPKC2250 and NPKC3000 when compared to other treatments. On the contrary, members of phylum Actinobacteria namely Thermoleophiloa, Acidimicrobiia and Actinobacteria dominated the NPKC750 and NPKC1500 whereas their abundance decreased with increase in levels of compost. Interestingly, members of phylum Acidobacteria such as iii1-8, Acidobacteria-6, BPC102, Solibacteres and Acidobacteria were abundant in NPK amended soils and with compost application their abundance decreased considerably. Cluster analysis by ward method also validated the changes in bacterial community composition in response to levels of compost application. The observations are in accordance with the results from a long-term fertilized paddy soil in Japan where phylogenetic analysis based on DGGE bands showed abundance of Bacteroidetes phylum under different treatments which include rice straw and rice straw compost application in soil. From the same field, Acidobacterial population was seen to be

less abundant with no Acidobacterial members observed in soil applied with rice straw compost [16] which corroborates with our observations. Investigation of bacterial community in cucumber plant seed and rhizosphere in response to compost application showed dominance of Bacteroidetes population [17] which further confirms our observation. Nowadays, molecular techniques can identify microbes with key functions and this molecular information can be tuned to yield markers for evaluating soil quality [18]. Thus identifying microbial groups and their functions has become an important task microbial ecology [19]. On that note, PICRUSt is highly regarded because of its high quality functional predictions using marker genes, such as those of 16S rRNA, and the databases KEGG (Kyoto Encyclopedia of Genes and Genomes) and COG (Clusters of Orthologous Groups of proteins) [20]. Thus, the current study employed PICRUSt to predict the functional profile of bacterial communities in the studied treatments (Fig. 2B) as compost management has been widely documented to influence the soil enzymatic activity and functionality of the microbial community [7,21]. Clustering of the pathway abundance profiles showed bacterial community from soils with higher compost levels clustered separately from soils applied with lower levels of compost. Bacterial members originating from NPKC2250 and NPKC3000 were characterized by higher abundance of genes encoding enzymes related to nucleotide metabolism, translation machineries, replication and repair processes, carbohydrate metabolism, process related to protein folding and general enzymatic activities compared to soils treated with low levels of compost. These results are supported by our previous observations where common soil enzymatic activities like dehydrogenase, cellulase, acid and alkaline phosphatase were significantly higher in NPKC3000 amended plots compared to other treatments [7]. On the other hand, bacterial community inhabiting the NPK750 and NPK1500 were clustered separately and were characterized by abundance of enzymes contributing to membrane transport, amino acid

metabolism and cell growth which are in line with the observations where application of farm manure is documented to increase the quantity of soil microbes [22]. The results observed imply the differential abundances of enzymes derived from differential responses of bacterial phylotypes in response to varying levels of compost. Alterations or shifts in the abundance or composition of a particular taxon as observed can explain observable conditions as well as underlying physiological processes taking place in the soil ecosystem.

Acknowledgements

The authors would like to thank the Basic Science Research Program of the National Research Foundation (NRF) under the Ministry of Education, Science and Technology (2015R1A2A1A05001885), South Korea for providing funding support towards the completion of this study.

Conflict of Interest

The authors disclose no potential conflicts of interest associated with this manuscript.

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Main text figure legends

Fig. 1 (A) Principal coordinate analysis (PCoA) constructed from Bray-Curtis dissimilarity matrix showing the bacterial community differences among the studied treatments (B) The relative abundance of bacterial community observed at phylum level

Fig. 2 (A) Heatmap illustrating relative abundance of different bacterial classes in studied treatments with the major phyla labeled. Cluster analysis indicated shifts in bacterial community in response to different levels of compost application (B) Heatmap demonstrating clustering of studied soil samples based on relative percentage of PICRUSt derived metabolic profiles of the bacterial community

Supplementary Figure Legends

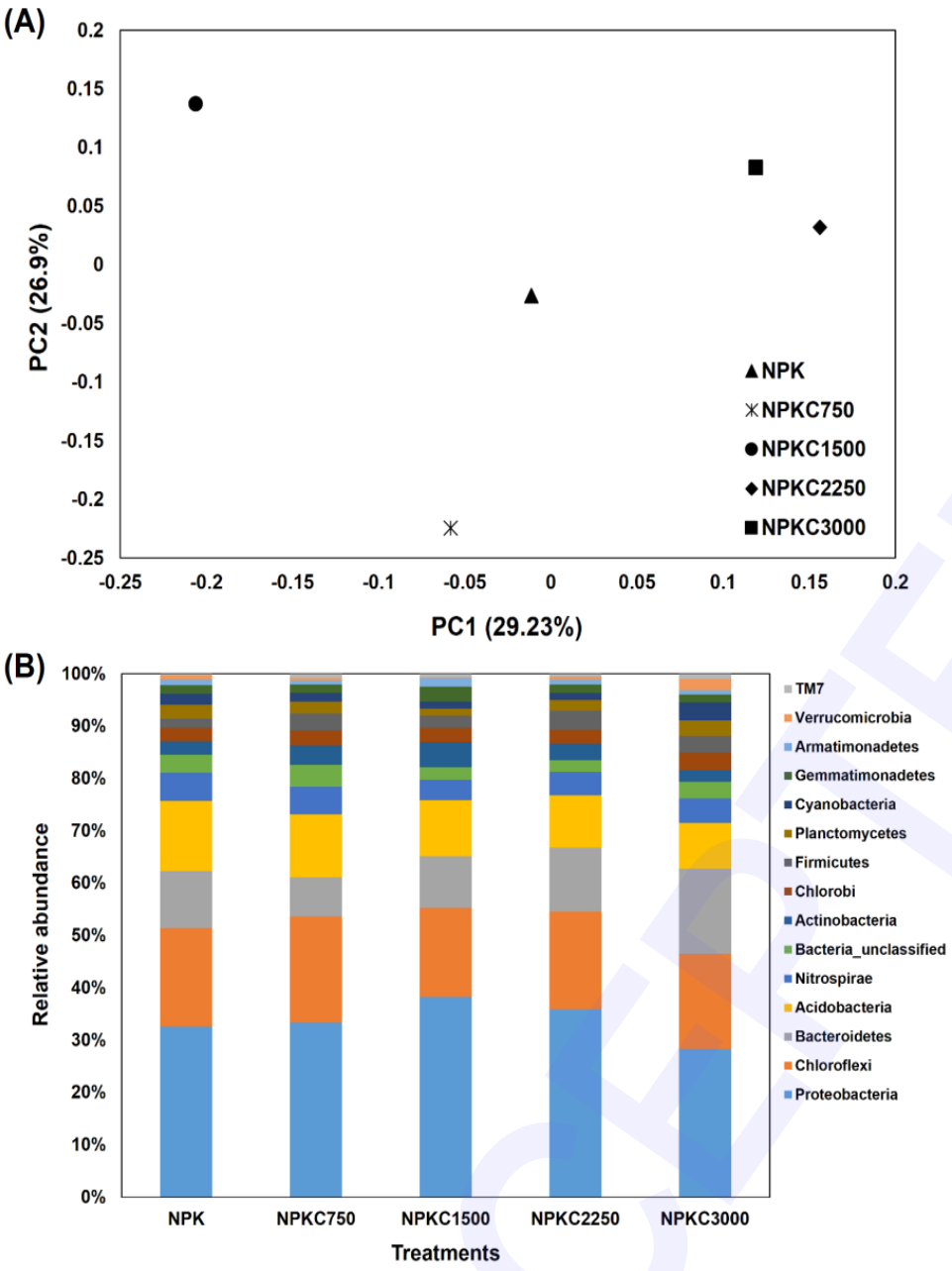
Fig. S1 Rarefaction curve based on V1-V3 16S rRNA pyro-sequencing data obtained from studied soil samples under long-term fertilizer management practices

Table

Table 1: Summary data for 16S rRNA sequencing results from the samples used in this study

Treatments	Number of sequences after normalization	sobs	Goods coverage (%)	Shannon Index	Inv-simpson Index	Chao
NPK	787	168	96.05	4.81	109.32	185.42
NPKC750	787	165	95.8	4.76	98.19	183.05
NPKC1500	787	166	96.3	4.82	110.34	182.91
NPKC2250	787	168	95.7	4.78	103.63	184.75
NPKC3000	787	160	97.1	4.75	92.8	168.45

The species observed (Sobs), coverage percentage (Good), richness estimators (Chao), and diversity indices (Shannon and inv-Simpson) were calculated using Mothur.



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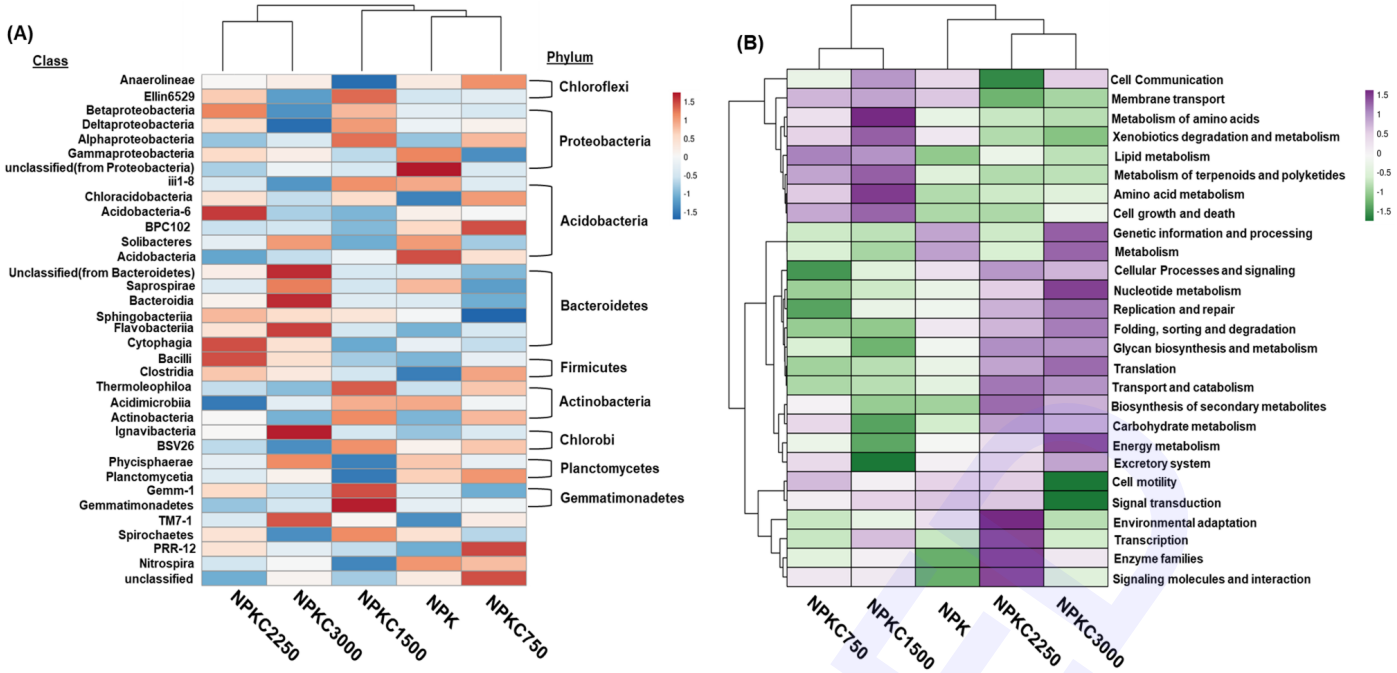

Fig. 2. (A) Heatmap illustrating relative abundance of different bacterial classes in studied treatments with the major phyla labeled. Cluster analysis indicated shifts in bacterial community in response to different levels of compost application (B) Heatmap demonstrating clustering of studied soil samples based on relative percentage of PICRUSt derived metabolic profiles of the bacterial community