Anthropogenic fertilization influences a shift in barley rhizosphere microbial communities

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Abstract

Background:

Anthropogenic mediations contribute a significant role in stimulating the positive reactions of soil–plant interactions, however, methodical reports on how anthropogenic activities impact soil microorganism-induced properties and soil health are still inadequate. Here, we evaluated the influence of anthropogenic fertilization of farmland soil on barley rhizosphere microbial community structure and diversity, and the significant impacts on agro-ecosystem productivity. This will help validate the premise that soil amendment with prolonged synthetic fertilizers can lead to a significant reduction in bacterial abundance and diversity, while soils amended with organic fertilizers elicit succession of the native soil microbial community and favor the growth of copiotrophic bacteria.

Methods: The total metagenomic DNA was mined from soils obtained from barley rhizosphere under chemical fertilization (CB), organic fertilization (OB), and bulk soil (NB). These were then sequenced using an amplicon-based sequencing approach, and the raw sequence dataset was examined using a metagenomic rast server (MG-RAST).

Results: Our findings showed that all environments (CB, OB, and NB) shared numerous soil bacterial phyla but with different compositions. However, Bacteroidetes, Proteobacteria, and Actinobacteria predominated barley rhizosphere under chemical fertilization, organic fertilization, and bulk soils, respectively. Alpha and beta diversity analysis showed that the diversity of bacteria under organic barley rhizosphere ~~were~~ was significantly more and evenly distributed than when related to bacteria under chemical fertilization and bulk soil.

Conclusion: The knowledge of the influence of conventional and organic fertilizers on the structure, composition, and diversity of the rhizosphere microbiome will assist in engineering soil to increase microbial diversity in the agroecosystem.

Keywords: amplicon sequencing, crop production, metagenomics, root exudate, synthetic fertilizers

Introduction

Introduction

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Globally, crop production is presently increasing because of the high demand for animal feed, biological fuels and food. Even the increase in oil prices made bioenergy more economical and cost-effective when related to fossil fuels. At present, 47.9 million km^2 of land are dedicated to agriculture, this will certainly increase with the high population rate. The ever-increasing human population and the need to boost the production of cash and food crops ~~has~~ have ensued the anthropogenic amendment of soil with chemical fertilizers (Amoo et al. 2021; Hemathilake & Gunathilake 2022). Chemical fertilizers encompass a high dose of major nutrients like phosphorous, potassium, and nitrogen, such as inorganic salts, and microelements like sulfur, magnesium, and calcium. The nutrient value in chemical fertilizers is specified as the N:P:K rate, signifying the proportions of nitrogen, total phosphorus, and potassium. These synthetic fertilizer inputs could intensely impact farmland ecology and have additional influences on microbial variability (Enebe & Babalola 2020). Yang et al. (2021) reviewed that long-term N fertilization greatly reduced microbial biomass, which can further influence the function and structure of the soil microbial community. It was also reported by Wakelin et al. (2012) that phosphorus application reliably shifted the structure and functionality of phosphorus~~-cycling -~~-cycling-associated soil microorganisms, as the insufficiency of ~~soil~~ soil-accessible phosphorus elicits the bacterial community to develop increased phosphorus solubilization ability. It has been reviewed that too much and perpetual application of chemical fertilizers vastly impacts soil nutrients (Pahalvi et al. 2021), quickens soil acidification (Hao et al. 2020), intensifies the decline of soil fertility (Bhatt et al. 2019), influences soil microbial communities (Luan et al. 2020) and led to environmental deterioration (Kumar et al. 2019).

Owing to the decline in microbial population and environmental problems (such as greenhouse gas emissions, pollution of water, and soil ecosystems) linked with chemical fertilizer as well as the quest to realize ~~an~~ optimum farming without compromising future generations, agronomists are assessing the need ~~of~~ for using organic fertilizers to boost crop production (Trujillo-Tapia & Ramírez-Fuentes 2016). Organic fertilizers are derivatives from animal or plant-based resources or other organic elements that are the product of naturally occurring processes that contain both major and minor nutrients for plant growth (Tan et al. 2023). They can also be an active source of soil microbes, while also ~~improve~~ improving soil structure (Sharma et al. 2023). Lin et al. (2019) reported that the application of organic fertilizer can shape the microbial composition and recruit useful bacteria into the tea rhizosphere. This became evident from their results which showed a significant increase in the relative abundance of *Gemmatimonadales*, *Acidobacteriales*, *Solibacterales*, *Streptomycetales*, *Burkholderiales*, *Nitrospirales*, *Ktedonobacterales* and *Myxococcales*. Underwood et al. (2011) investigation revealed that the variability of fungi, archaea, viruses and bacteria are increased in soil due to the rich nutrients in organic manure added to soil. This claim became clear from rhizosphere microbiome research conducted by Qiao et al. (2019) where they established that organic fertilization promotes bacterial diversity when compared with chemical fertilization.

The rhizosphere of plants has evolved into diverse and complex microbial groups with different information processing systems involved in plant enlargement and growth, and plant defense response (Babalola et al. 2020). Plants induce substantial selection pressure on the development of some bacteria like Rhizobium. This they achieved through the emission of roots' exudates. The secreted root exudates contain compounds of different kinds that attract the development of specific plant microbiota. The attracted organisms utilize these exudates as sources of energy and multiply in the vicinity (Bukhat et al. 2020; Pantigoso et al. 2022).

Globally, barley is cultivated on about forty-eight million acres of land and is the fourth most grown grain. Furthermore, it is a good investigational model to study plant-microbe communications in the light of domestication and crop selection. (Escudero-Martinez et al. 2022; Giraldo et al. 2019). Barley plays a significant role in the selection, fortification, and nourishment of rhizosphere microbial composition and structure (Verstegen et al. 2014). Barley rhizosphere microbiome plays a significant function in improving plant fitness and plays a key role in suppressing disease as well as the biogeochemical cycling in the soil (Berendsen et al. 2012; Lu et al. 2018). It is also a primary caveat (pointer) of soil quality due to their quick reaction to ecological alteration (Zheng et al. 2020).

Bacterial composition, structure, and diversity are posited to underprop ecosystem functioning and a loss of it can affect the functioning of soil and food security (Bano et al. 2021). To comprehend the adverse, neutral, or positive effects of fertilization schemes on soil microbiome, we use the amplicon sequencing approach to profile the bacterial structure, richness, and variability of barley rhizosphere. The method has been used to provide complete insights into the species diversity of microbial communities in soil systems (Amoo & Babalola 2019). This study hypothesis assumes that soil amendments with chemical fertilizers are often lethal to non-target soil microorganisms and organic fertilizers elicit succession of the native soil microbial community. We also posited that organic fertilization will favor the growth of copiotrophic bacteria, while long-term chemical fertilization ~~caused~~ causes a significant reduction in bacterial abundance and biodiversity.

Materials & Methods

Study area and sample collection

Eight weeks after the germination of the barley seed, we collected twenty-four soil samples of 20 g each from barley rhizosphere under chemical fertilization (8 soil samples) **(**25°39’’32.2 “S 27°39’49.8 "E**)**, barley rhizosphere under organic fertilization (8 soil samples) (25°39'04.9"S, 27°40'46.6"E) and bulk soils (8 soil samples) at a depth of 0 –15 cm. For the inorganic field, 150 kg ha^{-1} N, 75 kg ha^{-1} P_2O_5, and 75 kg ha^{-1} K_2O were the quantities of inorganic fertilizer ~~which~~ that had been in use for more than a decade. Urea, potassium ~~sulphate~~sulfate, and calcium superphosphate were the sources of N fertilizer, K fertilizer, and P fertilizer, respectively. The organic fertilizer field has been undergoing the use of 10,625 kg ha^{-1} amount of cow dung for more than a decade. The fertilization regime employed in this study ~~were~~ was in line with the United States Department of Agriculture (USDA, 2014). The measurement (10 × 4 m) was used for the respective planting field for this research, and the cultivar planted on both fields is barley

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seed (Pume). Each of the research fields was 40 m away. Sterile plastic bags were used to temporarily store the soil samples and then moved to cooler boxes filled with ice. On getting to the laboratory, the soil samples (after debris removal) were stored in the refrigerator (–20 °C) for further assessment. The history of the soil's physical and chemical properties (Table S1) before fertilization and planting was done following the standard analytical method earlier reported by Enagbonma et al. (2021).

Molecular and downstream analysis

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We used the Nucleospin Soil kit (Macherey-Negel, Germany) to extract the DNA of the entire barley rhizosphere microbiome by using the kit’s manual as a guide. The mined DNA was later sent to the Molecular Research Laboratory, Clovis ~~road~~Road, Texas, United States. Library preparation of the16S rRNA gene was done by employing the 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') universal primers with standard Illumina barcodes and adapters. The Ampure XP beads were used to further purify the amplicons. The validation and quantification of the barcoded libraries were done by using Agilent DNA 1000 Bioanalyser and Qubit DNA BR reagent assay. Thereafter, the MiSeq was used to sequence the quantified libraries. The preprocessing and analysis of the raw sequences (FASTQ files) from Illumina Miseq were done via a public pipeline (MG-RAST server) seen at http://metagenomics.anl.gov/ (Meyer et al. 2008; Wakung’oli et al. 2020). Generated reads after quality preprocessing and deduplication (via eliminating sequences that are: (a) greater than 5 vaguely base pairs with 15 phred score limit, (b) artificial sequences made by sequencing artifacts (c) have a length of greater than 2 standard deviations from the average value) were annotated by using alignment device called BLAT against a databank that offers a nonredundant integration of numerous databases called M5NR database. Taxonomic analysis was done using the *Ribosomal*

Database Project (RDP) under the following conditions: an e-value of 1e^{-5}, an optimal alignment length of 15 bp., and a lowest identity of 60%. The metagenomic rast server technology presented an approximation of microbial abundances existing in the barley rhizosphere under chemical fertilization (CB), barley rhizosphere under organic fertilization (OB) and bulk soil (NB). After the MG-RAST analysis on the 24 individual sequences, we computed the comparative abundances of the taxa via percentages. Thereafter, the mean number of relative abundance of the 8 replicates for each site (CB, OB, and NB) was used for statistics. From this statistical analysis, an assessment was performed to evaluate the composition, structure, and diversity of the microbiome among the samples. The normalization tool in metagenomic rast server technology ~~were~~ was switched on to standardize the dataset. The rarefaction curve analysis was also prepared via the rarefaction tool in MG-RAST. PAST version 3.20 was used to evaluate alpha diversity (via Pielou Evenness, Simpson, Chao-1, and Shannon) for individual samples. The Kruskal–Wallis test was used to compare these indices among sites (Hammer et al. 2001). The principal coordinate analysis (PCoA) was used to depict the diversity between species (beta diversity) based on a Euclidean distance matrix, and the differences in community composition among the groups of samples were tested by using the one-way analysis of similarity through 999 permutations (Clarke and Green 1988). The principal component analysis (PCA) demonstrated how the bacteria at the phylum level were distributed among sites. The PCoA and PCA were designed by CANOCO 5 (http://www.canoco5.com/). The Shinyheatmap was used to plot the heatmap with a z-score converted to the relative abundance of bacterial classification (Khomtchouk et al. 2017).

Results

Sequencing information and bacterial distribution across the barley rhizosphere under different fertilization schemes and bulk soils

The sequence information of barley rhizosphere under chemical fertilization (CB), organic fertilization (OB), and Bulk soil (NB) are summarized in Table S2.

Analysis of the amplicon sequence data using the RDP database showed that 22 phyla were present in barley rhizosphere obtained from CB and OB as well as the bulk soil (NB) and the others were grouped as unclassified bacteria (Fig. 1). PCA was plotted to demonstrate how these bacteria at phylum level were distributed among the barley rhizosphere (CB and OB) and the bulk soil with PCA axes 1 and 2 elucidated 95.50% and 4.50% variance correspondingly (Fig. 3). The arm length of the PCA revealed that Bacteroidetes, Verrucomicrobia, Nitrospirae, Planctomycetes, Spirochaetes, Aquificae, Dictyoglomi, Fibrobacteres, and Synergistetes predominated barley rhizosphere under chemical fertilization (CB) while Proteobacteria, Firmicutes, Cyanobacteria, Fusobacteria, Tenericutes, Deinococcus-Thermus and Chlorobi predominated barley rhizosphere under organic fertilization (OB). The bulk soil was predominated with Actinobacteria, Acidobacteria, Chloroflexi, Chlamydiae, Thermotogae, and Thermodesulfobacteria. The relative abundance (phylum level) of *Proteobacteria* (18.95%) in barley rhizosphere under organic fertilization (OB) was significantly higher than the *Proteobacteria* (18.58%) of barley rhizosphere under chemical fertilization (CB) and the bulk soil (NB) (17.52%). The relative abundance of Actinobacteria where significantly higher in NB (22.42%) than the relative abundances of Actinobacteria in CB (19.14%) and OB (18.41%) while the relative abundance of Bacteroidetes was significantly higher in CB (4.17%) than the relative abundances of OB (3.87%) and NB (2.71%) (Fig. 1). At the species level, 2320 species were recorded in the barley rhizosphere under chemical fertilization (CB), while 2393 species were recorded in the barley rhizosphere under organic fertilization (OB) and 2197 species were observed in bulk soil (NB). *Rubrobacter radiotolerans* dominated in CB while *Bacillus megaterium* dominated in OB and *Rubrobacter xylanophilus* dominated the bulk soil NB (Fig. 2).

Assessment of bacterial diversity from the barley rhizosphere and the bulk soil

The alpha diversity for the barley rhizosphere under fertilization and bulk soil was calculated (Chao-1) to be 2320 species in CB, 2393 species in OB, and 2197 species in NB. The Simpson, Shannon index and Evenness values were significantly higher in OB followed by CB and NB (Table 1). The rarefaction curve (Fig. S1) shows that the bacterial richness was higher in OB when compared with CB and NB. Contrasting among any duo of bacterial societies (beta diversity) using Principal Coordinate Analysis (Fig. 4) revealed no clustering in the compared environments. The analysis of similarity calculated the ~~p~~ p-value to be 0.02 and the ~~R~~ R-value to be 0.67, suggesting that the separation of sites is strong. For example, sample OB is separate and far away from sample NB, meaning that its bacterial society and structure are distinctive from those of the bulk soils. Sample OB was away from CB, signifying that its bacterial society and structure between the two samples differ (Fig. 4).

Discussion

This study used the amplicon sequencing technique to profile the bacterial composition, abundance, and diversity of barley rhizosphere under different fertilization regimes (CB and OB) and to see if there was a marked shift from comparable bulk soils. Soil fertilization is an old farming scheme targeted at promoting the fertility of soils for high growth and yield of crops (Bitire et al. 2022; Seenivasagan & Babalola 2021). Lately, ecologists have concentrated on revealing fertilisation regimes' influence on the soil's microbial societies (Ajilogba et al. 2022a; Masowa et

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Also, addressing the mechanisms involved in changing the diversity of the microbial community under different inoculations requires more details.

al. 2021). This investigation was able to establish that some substantial differences occur in bacteria from barley rhizosphere under chemical fertilization, organic fertilization, and bulk soil. PCA (Fig. 3) supported our assumption that each site (CB, OB, and NB) has a predominant bacteria phyla. This bacterial variation could be linked to the fertilization scheme which adds nutrients to the soil but alters the soil pH (Ajilogba et al. 2022b; Zhang et al. 2017). This variation was also seen in bacterial composition with the relative abundance of Bacteroidetes, Proteobacteria and Actinobacteria predominated CB, OB and NB respectively (Fig. 1). The alterations in bacterial dominance among the sites may perhaps impact the ecosystem functions contributed by these bacteria (Enagbonma et al. 2019; Enebe & Babalola 2022). It also indicated a clear shift in the bacterial community structure in the rhizosphere under different fertilization practices, however, it is quite undecided if this temporal selection is triggered by the barley plants' selective pressure or the bacteria. Rhizosphere soil under organic fertilization showed the highest alpha diversity (evenness and richness) of bacteria (Table 1, Fig. S1). This supported a previous study that reported that organic fertilization commonly increases bacterial abundance in soils with manure (Cheng et al. 2020; Uzoh et al. 2021). This was also reflected in the total number of species recorded in each site, with 2393 species found in OB, 2320 species found in CB, and 2197 species in bulk soil (Fig. 2). This pattern was also observed by Enebe & Babalola (2020) when they used shotgun metagenomics to profile the bacterial richness and structure in maize rhizosphere under different fertilization schemes. These bacterial variations among CB, OB, and NB were further supported by the beta diversity analysis via PCoA (Fig. 4), suggesting that the separation of sites is significantly strong ($p < 0.05$). For example, sample OB were separate and far away from sample NB, meaning that its bacterial community and structure are unique from those of the bulk soils. Sample OB was away from CB, signifying that its bacterial society and structure between the two samples differs (Fig. 4). Our rarefaction curve (Fig. S1) shows that the metagenome of the organic fertilizer-treated rhizosphere soil and the control samples were alike, this suggests that organic fertilizer showed the most stable community than the chemical fertilizer. Although, we are aware of the barley plant selection effect, which could be credited to chemical signaling compounds secreted by the plants' roots through rhizodeposition (Bouhaouel et al. 2019; Liljeroth et al. 1990). The correlation between the microbial community enrichment effects of organic manure and barley plants are both capable of promoting the soil organic carbon needed by bacteria. We expected the bacterial diversity in CB to be lower than the NB, but this was not the case, as revealed by the alpha diversity analysis (Table 1). So, care must be taken in relating inorganic versus organic amended soils. In all, this study revealed that effects on the abundance, structure, and diversity of the rhizosphere microbiomes are governed by fertilization. Fertilization supervision is recommended to manipulate rhizosphere bacterial communities to farmers' advantage.

Conclusions

Taken together, this research unveiled and supported the various studies that stated that soil fertilization brings about a microbial shift. These bacterial differences were also supported by the alpha and beta diversity analysis, which showed that bacteria under organic fertilization were more diverse and evenly distributed when related to the bacteria under chemical fertilization and bulk soils. Moreover, this study revealed the dominance of Bacteroidetes, Proteobacteria, and Actinobacteria in barley rhizosphere under chemical fertilization, organic fertilization, and bulk soils respectively suggesting the shift in their ecological function played by these bacteria. Results from this study and with the quest to feed the human population that is ever increasing, there is a need to sustainably employ integrated fertilizer tactics to achieve optimal outputs in maintaining the microbial communities and promoting plant health and yield.

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