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Effects of Green Manure Combined with Fertilizer on Microbial Communities in Poor Red Soil: A Case Study of Milk Vetch and Ryegrass

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Microbial Biomass,
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Immature Red Soil.

Even though green manuring is regarded as one of the promising tactics in the sustainable agricultural system, there is scanty investigate on effects integrating green manure with fertilizers, particularly in the red, unfertile soils. In study, influence of two green manure form of plants (Milk vetch besides Ryegrass) along with applied fertilizers to the total microbial populations, the microbial activity, the microbial biomass and the potential predicted function in the growth, and the incorporation time periods were evaluated. The fertilizer management strategies that were taken into the consideration in this study include the organic manure, humic acid, and mineral fertilizers. The results indicated that integrating green manure with organic fertilizers enhanced the bacterial community's α-diversity while the fungal community diversity was suppressed. Observations were made showing that green manure affected the growth and incorporation periods of the varying microbial populations that were found in the soil whereby taxa such as sulfur, nitrogen and carbon group were affected. Thus, this study shows the utilization of varieties of green manure plants integrated with fertilizers on the boost of infertile red soil's microorganisms, and the fertilization process.

1. Introduction

The global population and urbanization rates continue to increase at a fast pace, putting a lot of pressure on limited resources such as fertile land hence the justification for sustainable and efficient ways of increasing crop yields and soil fertility. Despite the farmers' desire towards improved crop production as a way of catering for the growing food demand, their efforts are derailed by the severs biological inactivity, poor nutrients and organic matters, particularly the immature red soil reclaimed (Bao et al., 2019). This puts an emphasis on the use of fertilizers and green manure in the contemporary farming as it boosts crop yield and charges the fertility of the soils (Ren et al., 2021). Higher rank green manure is a more environmentally friendly system of convenient agriculture where plantations are purposefully grown and utilized for improving and supporting soil properties (Bao et al., 2019).

Green manure has been identified by research to improve the chemistry and physics of soil through the formation as well as installation of the soil macro aggregates, and improving nutrient and also the organic matter status (Adetunji et al., 2020; Ren et al., 2021). The Green manure which is also known to improve the microbial populations, until density and function within the agricultural soils; thus, making it an organic input in that of the sustainable form of agricultural systems (Das, Liptzin, & Maharjan, 2023; He et al., 2020). The Green manure brings in beneficial microorganisms such as that of the agroecosystem that increases the rate of activities such as that of decomposition of various form of organic matter, among others, thus, benefiting the soil and plants. Green manure helps in enriching microbial population and microbial enzyme in the soils hence, increasing the cycling rates of nitrogen and carbon within soils (Brtnicky et al., 2021; Kolesnikov, Kazeev, & Akimenko, 2019).

1.1 Problem Statement

Literature review reveals that the effect of green manure upon the enzyme activities varies with the type of that of green manure used despite that green manure is usually linked with improvement of soil fertility and crop yield (Ye et al., 2014; Zhang et al., 2020). Nevertheless, research on influence of various form of green manure and also its method of incorporation on the biological potential for the created new arable land is rather limited. For this reason, farmers are compelled to apply fertilizers, namely, humic compounds and mineral fertilizers apart from planting green manure to enhance the quality of these soils in newly cultivated lands (Han, Dong, & Zhang, 2021; Kätterer et al., 2019). It also has been reported that the use of fertilizers with green manure changes the microorganism's population, intensity, and mass (Lori et al., 2017). In the recent past, Kamran et al. (2021) acknowledge that grain yield stability and carbon increases are higher when the mineral fertilizer is applied together with green manure than when the two practices are used independently. Similarly, Si et al. (2023) affirmed that regardless of the fertilizer type used, an application of a mixture of the three applied sources, particularly mineral fertilizers, organic manure, and humic acid, enhanced the soil fertility and led to a huge rise in the rice grain production and hence raised the profit by 17 – 33 %. Although effective in promoting improvement in the quality of the soil and biomass characteristics in combination with green manure fertilizer varieties, studies assessing the relationship between microbial populations' functionality and characteristics inoculated in fresh and with low fertility features have been relatively fewer.

Some of the green manure species popularly used in winter-based green manure–rice rotation systems are Milk vetch (scientifically known as *Astragalus sonics*) and Ryegrass (whose scientific name is *Lolium perennial*) (He et al., 2020; Lei, Wang, & Yao, 2022). Ryegrass is particularly described by deep root structures and good competencies in poor quality soil that enhances the biomass yields in such soils (He et al., 2020). While with milk vetch, it possesses a sound nitrogen fixation potential. This means that, the carbon to nitrogen ratio is higher in residues from the milk vetch. Because of the different functionalities along with the variations in the content of the ECMs, the actual effects of the two green manure form of composition as well as property of the soil might also differ in the processes of incorporation and plant growth. In this respect, this particular study aims to evaluate the main form of effects of these two kinds of that of the green manures incorporated with mineral fertilizers on some physical and chemical properties of microbial community of newly cultivated red soils. The assessment encompasses the function, the population, the level of activity and the biogeographical development of microorganisms in infertile, young reclaimed soil.

1.2 Objective of the Study

In order to assess the influence of vatiosu form of green manure and also the fertilizer mixtures on soil microbial biomass form of activity, communities and also the functional predictions, as well as the correlations with soil properties at the time of growth and also the incorporation periods.

1.3 Hypotheses

In line with the above objective, this study hypothesized the following:

1. Growing and incorporating green manure can stimulate soil microbial community functional groups associated with nutrient cycling.
2. Combining mineral fertilizer with that of green manure has the ability to enhance the biological and physiochemical properties of new red soils by modifying the microbial form of community.

3. The influence of that of green manure on that of microbial community of the soil depend on the plant species used.

2. Literature Review

Scant literature researches on practices in the sustainable agriculture, which combines the use of mineral fertilizers and green manure or organic manure. Ren et al. (2021) studied some of short-term physicochemical and enzyme activity changes of the maize rhizosphere soil after little amount of substitution of chemical fertilizers with that of the organic manure. The impact of organic manure treatment also recorded an initial rise in the strength of soils' Alkaline phosphatase, invertase, and urease in addition to the nutrient levels when contrasted with a fall in the electrical conductivity, pH, and soil's bulking factor. Organic substitution also enhanced the count of bacterial as compared to fungal from the original count. In the same way, Iqbal et al. (2021) revealed the actual application of that of the inorganic fertilizer in the presence of manure enhances the functionality of the soil, yield of rice, and the foliar physiological characteristics of rice plants in a paddy field. Similar findings were reported in cross sectional studies done by Wu et al. (2024) and Chang et al. (2014).

Some other researches have also evaluated the effects of incorporating green manure or using it with mineral fertilizers on the soil characteristics and other factors. For example, He et al. (2020) used Italian ryegrass, which is a green manure plant to analyze the on impact on soil characteristics and bacteria in the Italian ryegrass – rice rotation. Here, six levels if Italian ryegrass were incorporated into red soil for rice cultivation and allowed for 40 days of decay; soil properties, nutrients release and bacterial population were quantified. In examining the outcomes, the investigations proved that improved nutrient levels and richness of microbes that are involved in the nutrient cycling can be attributed to green manure; Also, improved bacterial diversities and nutrient cycling were observed in the bacterial communities. Another study by Sharma et al. (2017) has also revealed that through the uptake of green manure, the concentrations of various form of organic carbon within soils are very much boosted. Fernandez (2015), investigated on how the cover crop – organic fertilizer combinations change the physicochemical properties, nutrient cycling functions, bacterial communities. Thus, Fernandez's study showed that the cover-crop-organic fertilizer combinations enhanced enzyme activities and respiration. However, the study found out that such effects as these depended with the places, which means that the kind of soil influenced these effects.

Islam et al. (2019) conducted research on the effect of the actual green manure-nitrogen form of fertilizer mixtures within soil characteristic, rice yield, and physiological and morphological properties of the rice crop. The study proved that this combination increased the nutrient values of the soil after harvest of rice, hence showing the essence of green manure, especially in solving weaknesses associated with using nitrogen fertilizers only. Naz et al. (2023) also made similar observations, where they revealed that a mixture of the green manure as well as NPK fertilizers enhanced the production of the rice-beseem (Trifolium alexandrine) system along with an increase in soil porosity and the N, P, K contents.

Kamran et al. (2021) also discussed the effect on that of organic carbon within soil as well as the stability of the different fractions in Southern China's fluvio-aquic paddy soil regarding the NPK fertilizer- green manure (Chinese milk vetch) application and concluded that the integration of fertilizers along with green manure helped in enhancing the macroaggression and the stability aspects in relation to soil. Alam et al. (2016) told that the inclusion of green manure and organic amendments enhanced the content of soil mineral N in a potato yield in a potato field. de Jesus Souza et al. (2019) likewise provided useful information to the body of knowledge on sustainable agriculture on their works about the effects of fertilization and jack bean (Canavalia ensiform is) on soil fertility and humic fractions. De Jesus I Souza and colleagues had shown that the combination of fertilizers with that of green manure often raises the the actual fertility of soils as well as humic fractions; the scholars observed a significant increase of Nitrogen and Carbon stock of humic fractions and soils.

In the above-reviewed studies, it is evident that the actual using of the green manure in conjunction with various types of fertilizer augments the quality and fertility of soil, leading to improved crop production. Among these studies, none of them have evaluated the green manure fertilizer combinations for newly cultivated immature red soil, where nutrient deficiency is comparatively high. The closest study to the current research was carried out by He et al. (2020) but discuss Italian ryegrass-fertilizer combinations in red soil within a rice filed. Lastly, the red soil that was used in this study was a mature one; therefore, the conclusions that have been arrived at in this study cannot be used to recommend a given combination of these parameters on immature red soils. Moreover, none of the above-mentioned research works evaluated the implications of two forms of green manure with fertilizer on immature red soils. Regarding this, this research aims at filling this research gap by evaluating the impacts of two species of the green manure namely milk vetch and ryegrass of the features of soil form of microbial community.

3. Methodology

This study was conducted in a greenhouse using soils using immature red soils randomly collected from hilly parts of India. The chemical and also the physical characteristics of that of the soil are actually very much mainly summarized in Table 1 below.

Table 1: Physical as well as Chemical features of the Soil Used in the Experiment.

Property	Value/Features
% soil	Comprised of Red glutamate, sand shale, and red sandstone
Level of pH (water: soil, 5:1)	5.31
Organic matter: Soil	2.52g/kg
n	0.29g/kg
Phosphorus level	0.69g/kg
K Level	54mg/kg
Hydrolysable plus nitrogen alkali	21.3g/kg

3.1 Sampling and Experimentation

In addition to the green manure, like milk vetch or ryegrass, the control treatment was a fallow, and plant response contrasts with an organically-manured fallow, or a fallow treated with "humic acid" form of fertilizer, or the mineral fertilizer. A control treatment was also incorporated into the study; it did not have fertilizer or green manure species. This led to 10 treatments, namely (1) Milk vetch accompanies with the organic manure (MO), (2) milk vetch with that of humic acid fertilizer (MH), (3) a combination of milk vetch and mineral fertilizer (MG), (4) Ryegrass plus organic manure (RO), (5) Ryegrass with various form of humic acid fertilizer (RH), (6) Ryegrass plus mineral fertilizer (Treatments A, B, C were repeated three times each, as illustrated in the following figure 1.

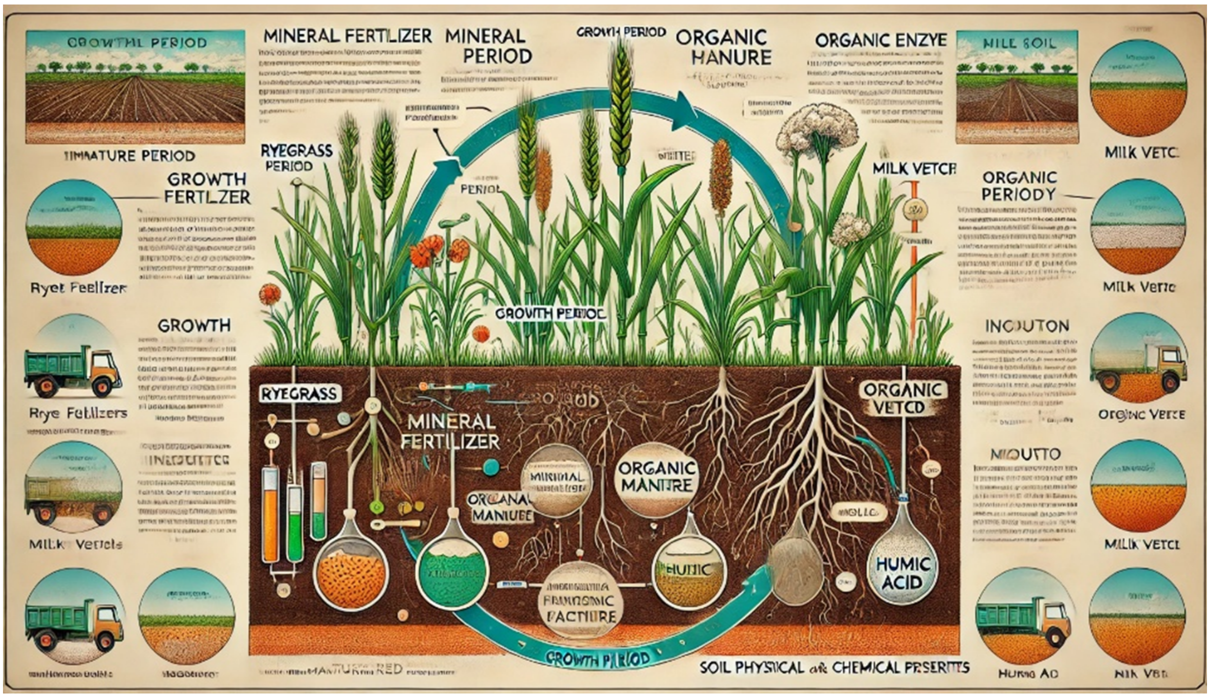

Figure 1: Experimental Diagram.

Note. The fallow form of treatment lacked green manure, unlike the milk vetch and ryegrass treatments. Nonetheless, all three treatments included the three fertilizers (humic acid, organic, and mineral fertilizers). The control treatment without fertilizer and manure was also included.

The percentage of organic manure was 29.2%, and humic acid fertilizer was 75.3%, whose nutritional content is summarized in Table 2 below.

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Table 2: Nutritional Content of the Two Fertilizers Used.

Soil Properties	Humic Acid Fertilizer	Commercial Organic Fertilizer
pH	6.16	7.42
Organic matter (g kg^{-1})	753.94	291.98
Total N (g kg^{-1})	9.031	14.37
Phosphorus levels (g kg^{-1})	7.871	17.91
Potassium Levels (g kg^{-1})	1.31	33.1

The red soil was put in pots of 10kg each. Before the seeding of the green manure, all the treatments, included mineral fertilizers, except the control treatment (CK). The organic manure and humic acid were incorporated in AFH, RH, as well as the MH or the OMF, RO, and also the MO to obtain a level of 20g/kg. Before the time of sowing, the seeds were very properly disinfected, and also was properly washed with that of the sodium hypo-chlorite. In post germination, the seedlings were then properly thinned to about fifteen for every pot. The growth phase took place at 7.2 to 15°C, while the incorporation phase occurred at 16-25°C. Concerning the green manure, it was cut and inserted within the soil after about five months of continuing the process of growth. Throughout the harvest, the actual form of green manure plant shoots (used in six treatments with milk vetch and ryegrass) were severed, and their actual roots were very much meticulously removed. The soil surrounding the actual roots within various crops' rhizosphere was gathered by gently shaking the soil off the roots situated within the actual upper layer of that of the soil (approximately 2-9cm).

The soil which was collected was homogeneous due to the use of a soil corer; the sterile tubes were also used to take the samples to the laboratory in liquid nitrogen. It has to be pointed out that they were subjected to temperatures below 80oC prior to and during analysis. The green manures' fresh shoots were cut into approximately pieces of 3 cm and evenly incorporated within each of the three replicates' mixture. In the case of the experiment, about 200g of fresh residues from each of the available treatment were measured and was inserted into the pots for each of the soil; the ad that the soil was preserved in a proper flooded throughout this period, which lasted for about 8 weeks. After that, about 0.5kg of that of the soil examples which were properly occupied after the about 10 obtained form of samples using the soil corer at 10cm from the surface of the pot and then sub-sampled to about three parts. The initial sample which were collected within the 50ml sterile tubes and then was stored at about 80°C was actually and properly used to get DNA; the second subsample which was sieved using a sieve of 4mm and was stored at 4°C was the properly which were cast-off to properly evaluate the actual enzyme movement and microbial biomass within 7 days, while the third subsample collected in the sterile tubes was air dried then-ground into fine particles for the chemical and physical analysis.

3.2 Statistical Analyses and Measurements

Various methods, techniques, and tools were used to analyze the collected data. Table 3 below describes the various analyses conducted in this study.

Table 3: Statistical Analysis Methods and Techniques Employed in the Research.

Analysis Method, Tool, or Technique	Purpose or property analyzed
SPSS Software (version 21.0)	Examination goes the properties of the green manure as well as the fertilizer mixtures on enzyme activities, microbial form of biomass carbon, as well as the soil properties. The actual least significant difference (LSD) method was then properly used to mainly compare the data at a 5% of that of the confidence level, while the actual variance homogeneity was established using Levene's test.
Chalo Index	"Microbial communal richness"
Shannon index	"Microbial communal diversity"
Principal coordination analysis (Coa)	To determine the level of difference among fungal as well as the bacterial communities
Redundancy analysis (RDA)	To inspect relationship among soil fungal besides bacterial societies
Distance-based RDA (db.-RDA)	Achieved within the fungal as well as the bacterial genus level to understand the association between soil examples derived from respectively action besides the soil's chemical and physical belongings.
Analysis of similarities (ANOSIM)	Hand-me-down to properly evaluate the actual statistical import among behaviors.
Welch's T-test	Utlized to analyze and compare fungal or bacterial taxa assemblies
linear discriminant analysis (LDA) effect size (Levees)	This remained properly castoff to create the actual noteworthy alterations in fungal or bacterial taxonomic group within the "green manure conducts" as well as the fallow treatments, regardless of fertilizer combination.
Functional annotation of prokaryotic taxa (FAPROTAX) database	This was utilized to properly forecast the actual bacterial community's practical clusters.

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4. Results

4.1 Effects of Combining Green Manure with Fertilizers on the Soil Infective Community on the Rhizosphere

This study aimed to evaluate the impacts of that of the intermingling green manure besides also the fertilizers scheduled that of the phylogenetic and functional attributes, alpha diversity, and soil microbial assemblages. About 1.4 million operative bacterial and also the 1.8 fungal ITS sequences were derived from the samples. The net bacterial phyla which were recognized were about 38 and proteobacteria dominated the all treatments comprising 30-49%, with the exception of CK treatment (Figure 2). Both milk vetch and ryegrass registered a huge relative form of richness of actinobacteria. The richness of the firmicutes was 3 folds more in the ryegrass rhizosphere than milk vetch form of rhizosphere as well as the fallow soil (as depicted in Figure 3).

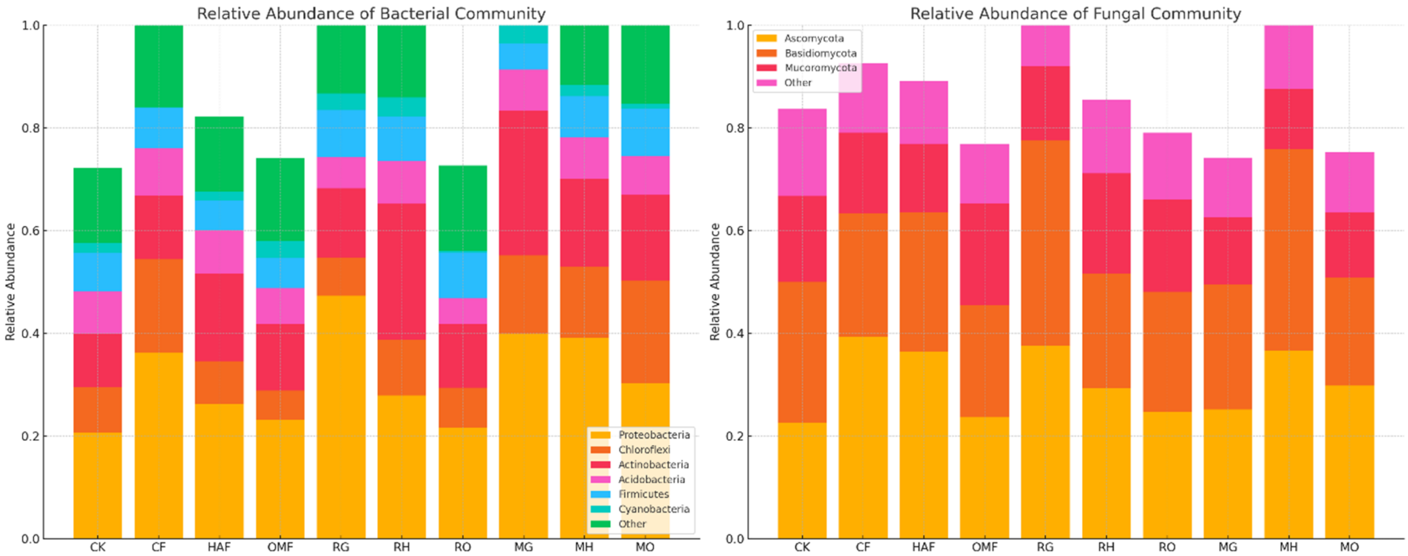

Figure 2: Relative Richness of (a) Bacterial and (b) Fungal Community.

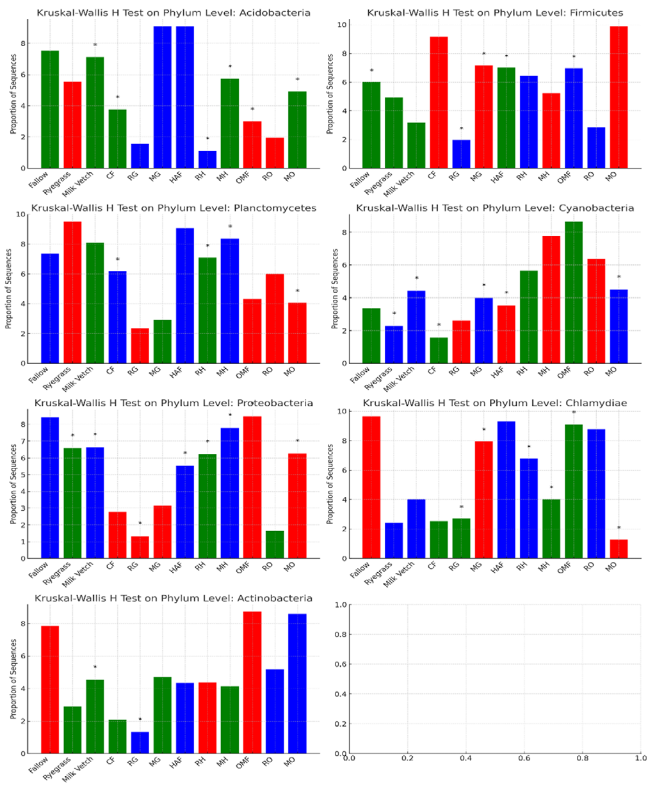

Figure 3: A Comparative Analysis of the Relative Abundance of Bacterial Taxa in Green Manure Crops vs Fallow Treatments During the Growing Period.

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On soil microbial alpha diversity, it was initiate that a mixture of green manure in combination with mineral fertilizer enhances bacterial variety and wealth of the rhizosphere soil, as was properly demonstrated by the Shannon and Chao1 indices (see Table 4). The RG and MG treatments witnessed a 39.7% and 60% surge, correspondingly, in the Chao1 index, while the Shannon index indicated a 21.5% surge in RG and about 28.1% increase in the MG treatment. However, the fungal community was quite lower in RG than in MG treatment.

Table 4: Treatment Combinations Across Growth and Incorporation Periods.

Treatment Combination	Growth Period Bacterial Community	Fungal Community	Incorporation Period Bacterial Community	Fungal Community
	Chao1	Shannon	Chao1	Shannon
CK	115,455.61 ± 14.20a	4.63 ± 0.54a	578.34 ± 44.78abc	3.16 ± 0.45ab
CF	1,101.29 ± 72.99a	5.45 ± 0.04a	386.43 ± 41.96abc	3.42 ± 0.534abc
HAF	1,734.05 ± 138.59bc	7.19 ± 0.45b	688.70 ± 83.34cd	3.94 ± 0.341c
OMF	2,017.00 ± 90.93de	9.59 ± 0.23cd	234.41 ± 54.29d	4.00 ± 0.256c
RG	1,464.64 ± 73.17b	5.57 ± 0.44bc	455.10 ± 49.90a	2.89 ± 0.445a
RH	1,446.11 ± 42.756cd	5.78 ± 0.cd	4,565.73 ± 78.98ab	3.69 ± 0.547bc
RO	2,015.50 ± 129.75cde	5.60 ± 0.18cd	530.23 ± 104.03abc	3.75 ± 0.09bc
MG	1,760.00 ± 98.62cd	5.19 ± 0.10cd	552.89 ± 96.16bc	3.49 ± 0.11bc
MH	1,978.34 ± 36.05cde	5.75 ± 0.09cd	499.93 ± 26.90abc	3.48 ± 0.12abc
MO	2,235.82 ± 100.31e	5.66 ± 0.06d	517.30 ± 53.54abc	2.67 ± 0.25a

Note. Chao1 data depicts soil microbial community richness while Shannon shows diversity.

The soil portion of the bacteriological communal structure, which remained properly analyzed by process of using Coa, was found to be gathered conferring the green manure plants (ANOSIM: R=0.71, p. 0.001) higher than collective form of fertilizer forms (see Figure 4).

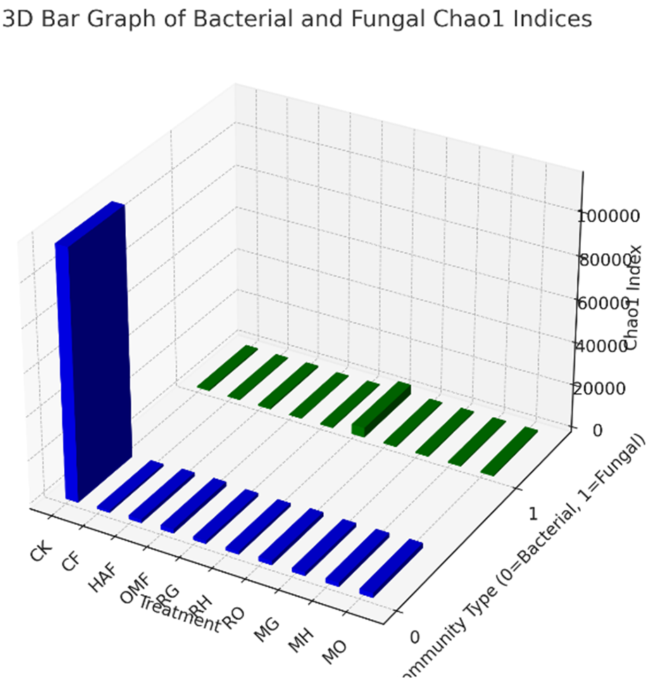

Figure 4: Coa Results for Bacterial and Fungal Community.

Based on the FAPROTAX, 66 functions of bacterial communities were annotated and the results indicated that those functions were affected by the green manure species. Majority of the functions were pertaining to sulfur, nitrogen, and carbon cycling (Figure 5). Resources utilized through aerobic chemoheterotrophic such as milk vetch had a relative abundance difference with ryegrass in the functional groups related to carbon. The same was noted for the vast reproduction of the bacterial groups associated with N cycling process to include urea lysis, nitrogen and nitrate respiration, nitrate reduction, aerobic ammonia oxidation, nitrogen fixation and nitrification. However, when it comes to the S cycling-related functional groups, their abundance was higher during growth of the ryegrass than when the milk vetch was dominant.

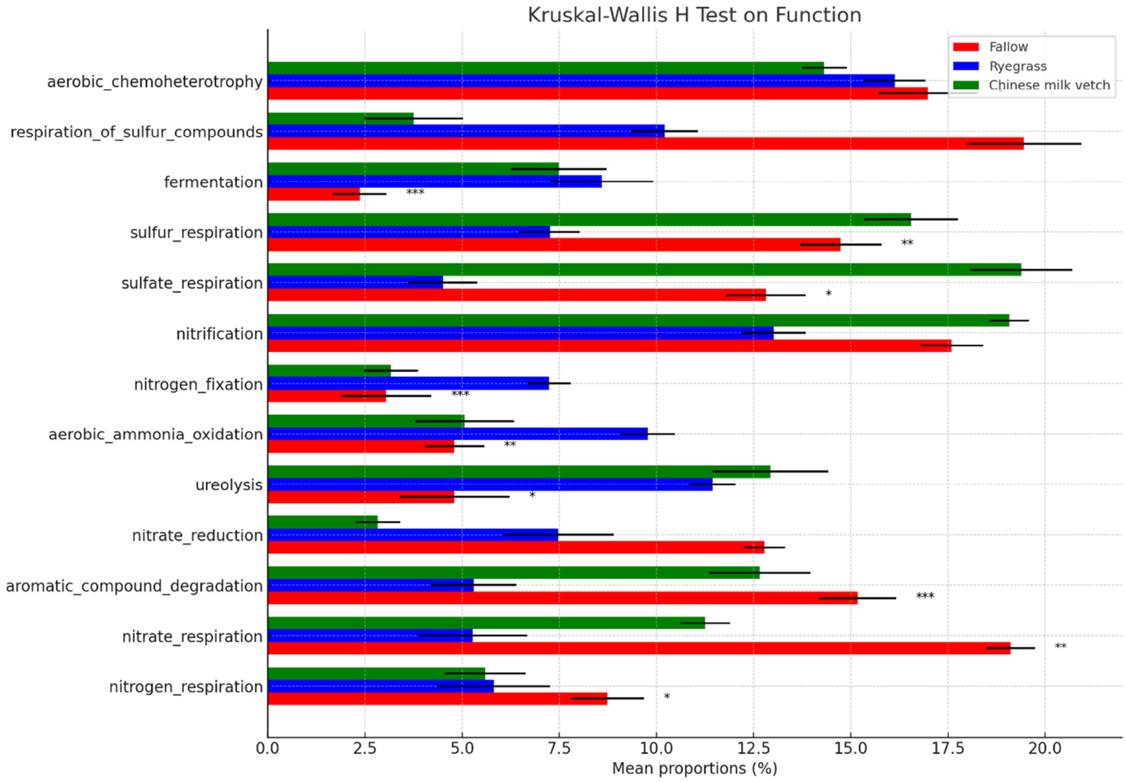

Figure 5: Bacteria Community Functional Prediction.

4.2 Green Manure form of Incorporation as Well as the Fertilizer Mixtures Effects on Soil Properties

Results of this study include investigations on how the green manure-fertilizer combination influences the properties like physicochemical, biomass C, and enzyme activity in the soil. Concerning the soil physicochemical characteristics, this study revealed that a combination of green manure and fertilizer had a substantial impact on soil AN, TN, SOM, and pH as presented in Table 5.

Application of ryegrass raised pH from 9% to 12% in comparison to the fallow treatments while milk vetch raised the soil pH by 5% in MH and a 4% in MG but not in MO. The contents of TN in MG and RG were 14% higher than those in CF treatments Signals 7 and 8. SOM had a stronger content increment when treated with the humic acid fertilizer than in the action of organic manure both under the effects of the fallowing practice and of the various form of green manure form of treatments.

Table 5: Influence of Green Manure-fertilizer Mixtures on Tat of the Soil Properties.

Treatment	pH	SOM (g/kg)	TN (g/kg)	TP (g/kg)	AN (mg/kg)	OP (mg/kg)
CK	5.01 ± 0.04b	3.53 ± 0.44a	0.56 ± 0.44a	1.03 ± 0.44a	52.56 ± 3.76a	4.29 ± 0.04a
CF	4.84 ± 0.43a	2.62 ± 0.22a	0.51 ± 0.22a	1.19 ± 0.74ab	55.50 ± 1.22ab	4.43 ± 0.21ab
HAF	3.17 ± 0.22b	18.86 ± 1.02c	2.64 ± 0.02c	6.14 ± 0.07abc	32.91 ± 1.83abc	7.36 ± 0.21b
OMF	4.15 ± 0.11b	10.44 ± 0.73b	1.71 ± 0.53de	1.18 ± 0.63abc	18.34 ± 2.63c	2.49 ± 0.17ab
RG	5.44 ± 0.22c	8.23 ± 0.62a	6.47 ± 0.76b	2.12 ± 0.73a	87.33 ± 3.73d	6.46 ± 0.25ab
RH	7.23 ± 0.22d	20.14 ± 0.73c	5.66 ± 0.64cd	4.22 ± 0.73abc	30.63 ± 6.73abc	6.92 ± 0.63b
RO	2.75 ± 0.44d	10.85 ± 0.73b	3.71 ± 0.53e	2.23 ± 0.72abc	82.73 ± 7.83d	2.42 ± 0.73ab
MG	2.02 ± 0.25b	71.81 ± 0.73a	1.47 ± 0.84b	6.12 ± 0.76ab	25.36 ± 2.73bc	1.82 ± 0.63b
MH	3.22 ± 0.82c	38.66 ± 0.83c	0.68 ± 0.63cde	4.28 ± 0.83c	14.70 ± 5.83abc	2.81 ± 0.63ab
MO	5.27 ± 0.89b	20.77 ± 0.63b	1.74 ± 0.73cd	2.23 ± 0.83bc	25.47 ± 3.52bc	2.31 ± 0.63ab

The effect revealed for SMBC suggested that this parameter significantly increased for both combined fertilizer application and green manure incorporations as well as their interactions. Marked by an enhancement of 40, the SMBC rose. The percentage of males was also significantly higher in RG compared to CF constituting 6% only in RG. In MH and RH, the SMBC was 73% and 44. At the same time, they are 70% higher, respectively, than the AHF treatments. The experiment findings indicated that actual green manure which is enhanced the actual β-glucosidase as well as the urease in the soil though the fallow treatments; nevertheless, it is reduced the catalase activity in the combined manure and mineral fertilizer. Nonetheless, the three enzymes' activity had no variation concerning green manure application and the treatments involving fallowing that with the actual humic acid form of fertilizer.

4.3 Soil Microbial Community Done the Application of Green Manure

Approximately 2,160 fungal OTUs (12 phyla) and 4,360 bacterial OTUs (43 bacterial phyla) were identified after the incorporation of green manure. \Dominant form of the bacterial phyla was Chlorolipid (11-44%), Proteobacteria (11-22%), and Actinobacteria (8-28%), among others (Figure6). Likened to the various form of fallow remedies, the incorporation of ryegrass remarkably increased Firmicutes' relative abundance (see Figure 7 below).

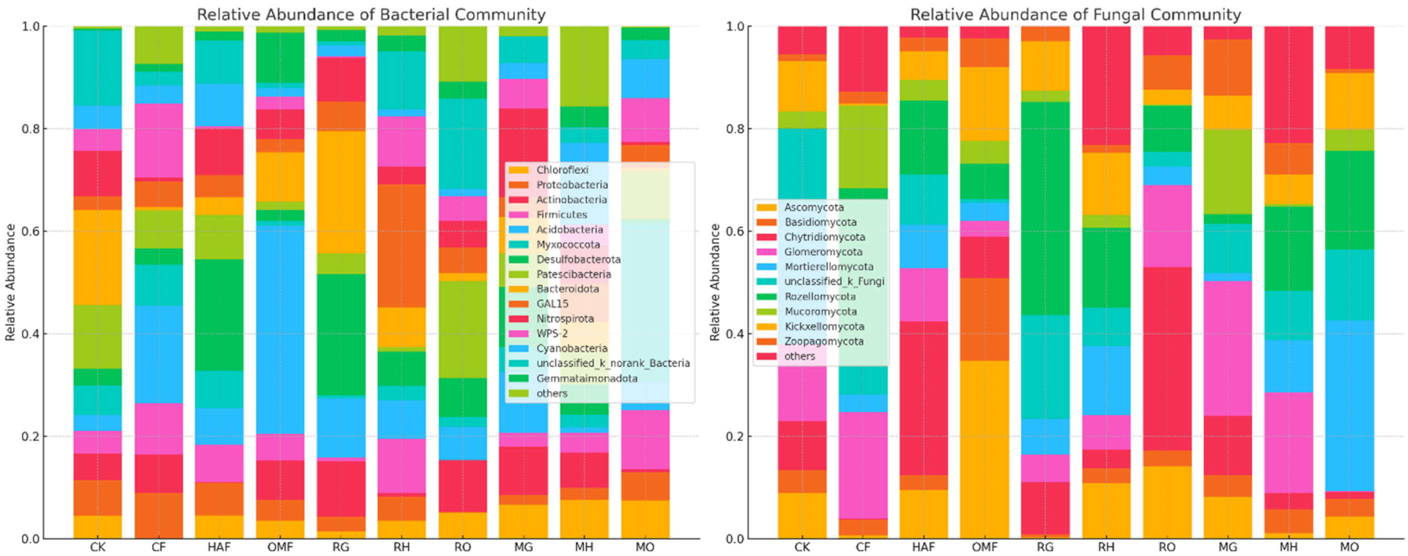

Figure 6: Relative Abundance of Infectious and Mycological Communal.

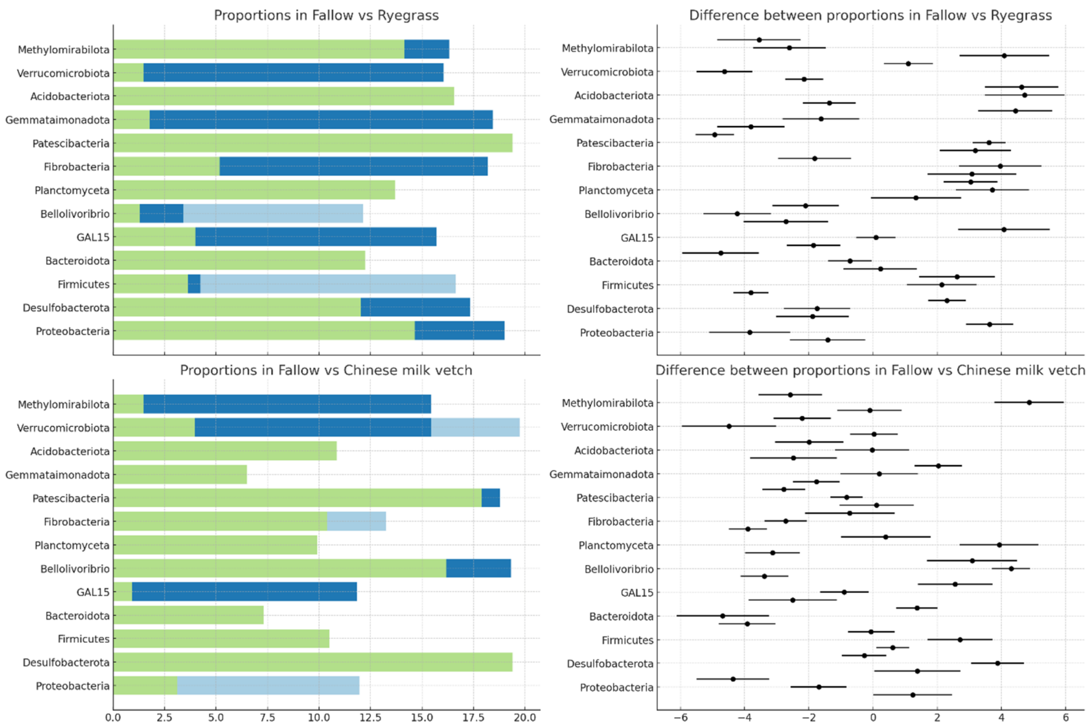

Figure 7: Bacterial Taxa Relative Abundance.

With regard to the soil microbial form of alpha-diversity, within this specific as well as the particular study, the actual form of results presented that the various form of soil microorganisms community alpha-diversity remained very much significantly much high in that of the manure incorporation treatments than that of fallow treatments for all the parameters except Chao1 of MO and RH. They estimated the chao1 index went up by 23. 6 – 38. 8% relative to the use of green manure, and Shannon type of diversity index increased by 7% compared with the fallow treatments. An interaction with organic manure or humic acid fertilizer was not possible to yield any difference compared to the fallow conducts (as noted on Table 4 above). Within this context of the microbial community structure and function prediction, the results highlighted the samples' significant clustering referring to the green manure treatment. The FAPROTAX functional form of prediction point out that the functions expressed, namely, fermentation, nitrogen fixation as well as the chemoheterotrophic were very much strikingly boosted by the application of green manure in regard to N and C cycling (Figure 8).

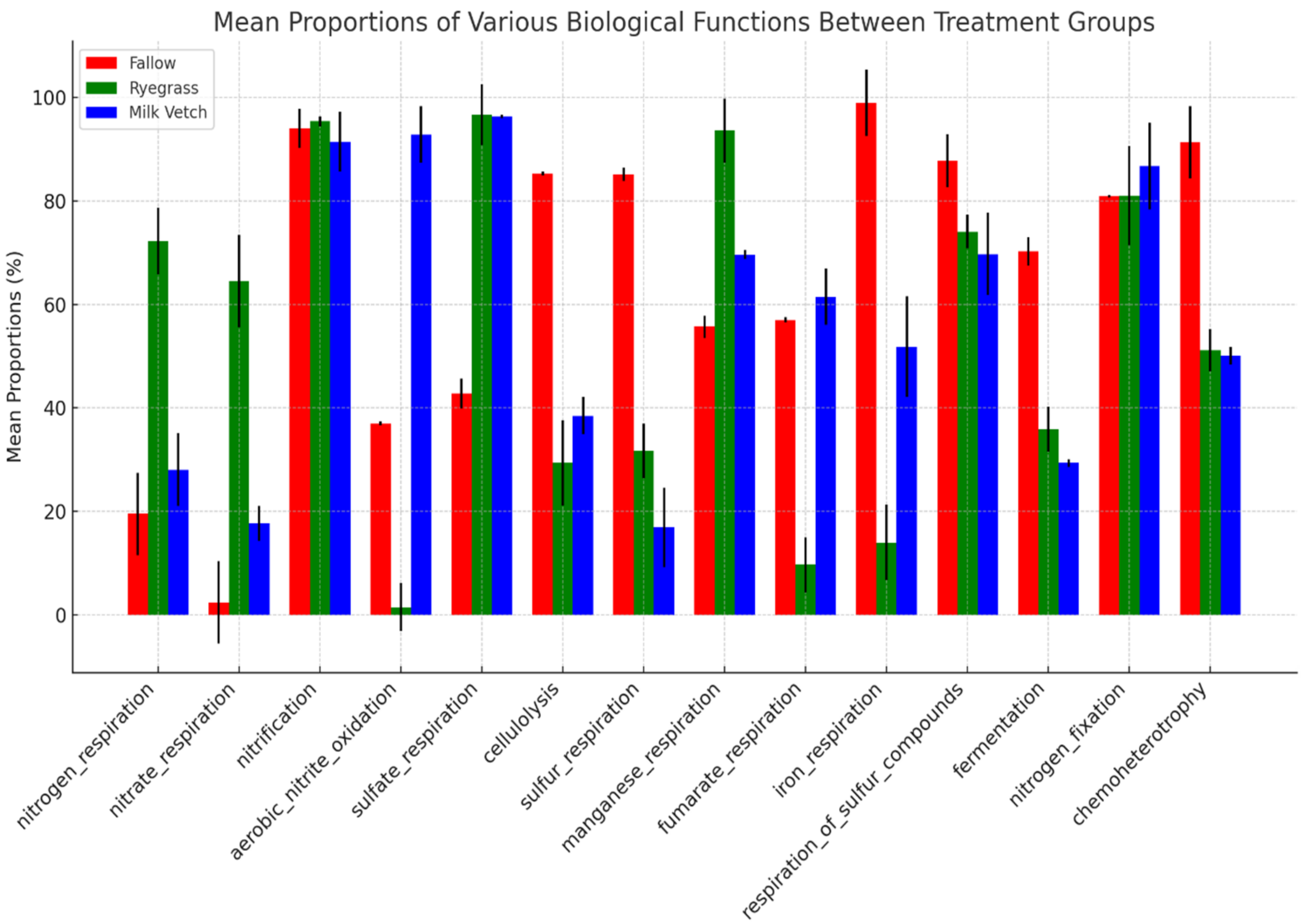

Figure 8: Kruskal H-test Function.

Lastly, this study assessed the actual form of relationships among that of the microbial community enzyme activities and soil properties. Based on the Db-RDA results, this study established a significant correlation between bacterial communities in green manure applications and TN and SOM (in organic manure-humic acid combinations). TP strongly and positively correlated with RG, MH, and RH bacterial communities, while AN and pH strongly correlated with MO and RO (see Figure 9).

Besides, as demonstrated in Figure 10 above, nearby was a noteworthy connection among soil fungal communities and OP, TN, AN, SOM, and ph. Furthermore, this study also established a strong and distinct relationship between soil enzyme activities and fungal and bacterial communities, depending on the various green manure treatments (Figure 10 (C) (D)).

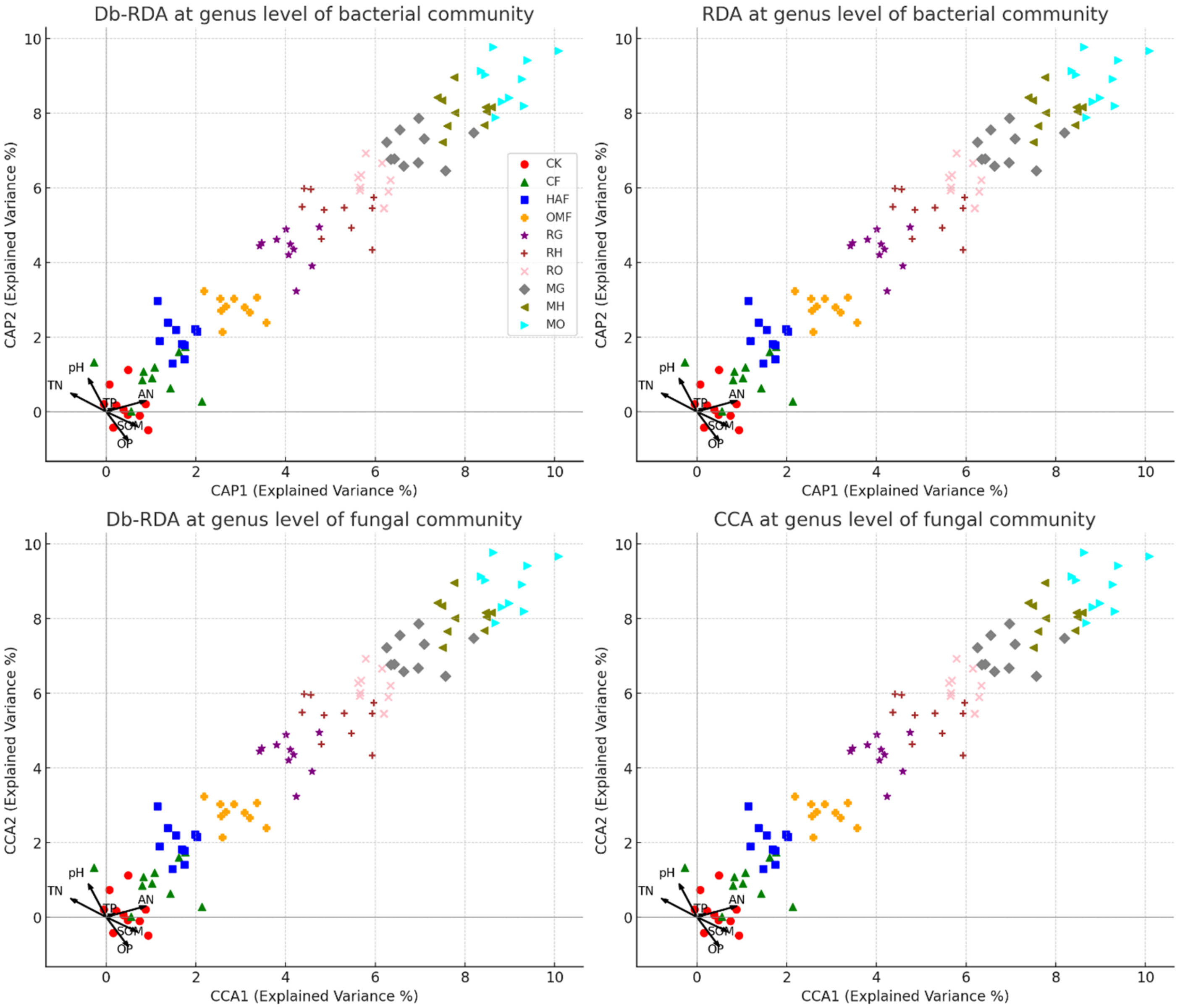

Figure 9: Db-RDA at the Bacterial and Fungal Community Genus Level.

5. Discussion

The various results indicated that the actual treatment of green manure help to properly improved the SM on both growth and incorporation phases. The amplification of bacterial richness within present study after the use of green manure also corresponds to earlier studies carried out by Songjuan, Weidong, & Guopeng (2021) and He et al. (2020)". The rhizosphere is to a great extent responsible for improving nutrient traffic between the soil and plants during green manure plant growth; therefore, it influences the microbial variety and other characteristics of the microbial community in the region. It is noteworthy that swings in diversity of fungi as we;; as bacteria shown this study can be attributed to the competition and mutualistic relationships between microorganisms and green manure species (Liu et al., 2019). Findings within this research have shown that the actual form of characteristics of the chemical reactions composed with the various form of organic materials that are applied significantly influence the growth period of green manure and the resulting microorganisms (Liu et al., 2020). Accordingly, microbial taxa which are associated with processing of the organic matter can also stand to be supported by the green manure residues. Fungal societies are also recognized as validating the biodegradation of lignin and cellulose containing organic matters (Liu et al., 2020). This explains why the actual negative impacts of that of the green manure on the fungal richness lesser during the incorporation of green manure in this study. This study also made findings that there exists a more enhanced, positive relationship between green manure plants and bacteria; as compared to the

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use of fertilizers particularly, due to the root system of ryegrass. These are as follow: In a study conducted by Lovell et al. (2001), similar conclusions were arrived at.

Concerning the response of that of actual level of the green manure on composition of that of the soil microbial diversities; distinctively, milk vetch and ryegrass amended actinobacteria content increased relative to the fallow treatment. It helps in increasing the rates of nitrogen as well as the carbon cycling and metabolism in the rhizosphere (Fierer, 2017). Ryegrass was also identified to increase the relative frequency of phylum Firmicutes because of ability known to increase rate of turnover of carbon by degradation of plant polysaccharides. Ryegrass is also connected with increasing the fungal Dothideomycetes and Sordariomycetes commonly metabolizing various lignocellulosic, hemicelluloses, and cellulases (Fierer, 2017; Wang et al., 2021). Hence, the findings are supplementIng other surveys that reveal that the act of growing and incorporating green manure plants including the ryegrass increases the functionality of the fungal and bacterial groups, which is most favorable when integrated to for improving the carbon metabolisms of the immature red soil. However, milk vetch increases the relative incidence of bacterial phyla that cycles nitrogen in the soil. In support of these findings, Khan et al. (2020) found that the incorporation of legumes (barley) enhanced the activity of the enzyme. Błońska & Lasota (2017) also observed that incorporating ryegrass, which has a high ratio of carbon to nitrogen as well as cellulose matter, substantially enhances the fungal and bacterial communities correlating with the activity of β-glucosidase, which is essential for the cycling of organic matter, particularly carbon degradation. Incorporation of ryegrass was found to promote nitrogen-cycling-related functions such as nitrification, nitrite oxidation, and nitrogen fixation, a finding that is supported by He et al. (2020), who found a significant correlation between Italian ryegrass incorporation and the growth of N cycle-related microbes.

It was also realized from the findings that the combination of that of the green manure with fertilizers affected the physical characteristics as well as microbial activities of the soil. These combinations affected the presence of the compounds in the soils, therefore enhancing the availability of nutrients for microbial growth. These results are quite similar with the study of Yang et al., (2018) where various form of green manure improved the pH and nitrogen regardless of the immaturity of the red soil like those included in this research. Regarding the factors associated with soil pH, it should be noted that green manure has alkaline nature, which helps to neutralize protons in the acidic rhizosphere (Rukshana et al., 2014). As in this study, utilization of that of the green manure together with the actual form of organic fertilizer elevated TN, AN, and pH; which are important for the fungus and bacteria. These findings are in the actual form of agreement with a proper as well as a particular study which is actually conducted by Yu et al. (2019) that identified that matter org, nutrients, and pH are among the soil parameters that are controlled by green manure and/or organic fertilizer. It makes substantiated sense that SOM, AN, TN, and pH augment with carbon and nitrogen cycling- related bacteria; Zheng et al. (2018) are of the same opinion as they 2018 have identified that bacteria taxa, which is initiated by green manure applications, is accountable for decomposition processes in the soil. Furthermore, Songjuan et al. (2021) also noted that green manuring enhanced the pH values; therefore, assisted in preventing the immature red soil acidification.

6. Conclusion, Recommendation, Limitations, and Future Research

This research evaluated the influence of that of the actual application of green manure as well as fertilizer treatment for the characteristics of new red soil microbial properties. The outcomes of this specific study showed that there is an increase in ASM, bacterial populations, nitrogen status, and pH when green manure is used. The results of the study established that various aspects of the soil are closely linked with microbial community as well as thus supported the main function of green manure in altering the microbial community and consequently improving on the functionality of the ecosystem and soil fertility. Therefore, according to the observations made in this specific research, the using of the green manure in conjunction with fertilizers should be promoted as an agricultural practice that enhances the sustainability of the actions that alter biological characteristics and fertility of immature red soils, all of which would help increase crop yield. Taken together, this research shows the significance of looking at the place of the soil microbes in modulating the functionality of ecosystems, as well as in coming up with practice agriculture techniques.

Nevertheless, despite the above contribution, this study was limited in that it did not observe the effects of the green manuring practices on the growth of the crops and productivity levels. It was also specific to the red soils and only investigated two forms of green manures. According to these limitations, further research can be conducted to evaluate other kinds of soils or other kinds of green manure. However, more studies are required to establish the extension of the above impacts of green manure-fertilizer combinations on the subsequent series of food producing plants.

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