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ARTICLE

Vermicompost and Cow Dung Admixture Increases Rhizosphere Bacterial Population and Promotes Rapid Physiological Maturity in Maize (*Zea mays* L.)

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ABSTRACT

Incessant application of chemical fertilizers to the agricultural fields may alter the composition and activities of soil microbiota. Thus, the shift of cultivation practices from chemical to organic is considered to be the need of the hour in order to maintain soil health. A study was conducted in the agricultural fields of the University of Burdwan, India to observe the impact of organic manure on the rhizosphere bacterial community. The experiments were conducted on maize plants, supplemented with the recommended dose of chemical fertilizer and organic manure (vermicompost and cow dung mixture). Corresponding changes in the plant phenological events and soil health in terms of soil physico-chemical factors and rhizosphere bacterial groups up to the level of CFU g-1× 105 dry soil was noted. The results showed a significant increase in population of phosphate solubilizing bacteria during 30DAS. However, at 90 DAS, significant increase in the population of phosphate solubilizing bacteria, nitrifying bacteria, asymbiotic nitrogen-fixing bacteria and protein hydrolyzing bacteria was observed in the organically treated plots. The growth of rhizosphere bacteria was attributed to the type of organic manure supplied to the agricultural fields. In addition, a strong correlation was observed between Zn and protein hydrolyzing bacteria. The soil organic carbon and available nitrogen were strongly correlated with nitrifying, fat solubilizing and phosphate solubilizing groups of bacteria.

1. Introduction

The soil health is governed by the physico-chemical factors and the microorganisms present in the soil. The important soil processes such as organic matter decomposition, nutrient cycling and soil degradation are maintained by soil microorganisms [1,2]. The change in the soil microbial community is the most reliable indicator of the soil health of any agroecosystem [3]. For the last two decades, there has been a gradual shift towards the use of organic manures in the agricultural fields, as the manures improve the nutrient availability through soil microbial

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processes and biological interactions [4].

Moreover, the organic manures are rich in organic matter and maintain the soil moisture, thereby improving the soil microbial structure, function and shapes the crop production [5,6]. Wang et al. [7] showed that the addition of organic manures to the soil increases the levels of organic matter, soil porosity, moisture, structural stability and biological activity. The parameters of soil biological processes like the rate of decomposition, nitrification, nitrogen fixation and denitrification are dependent upon the structural stability of the soil microbial community [8,9]. Thus, a better understanding of the effect of organic manures on the soil microbial community could help to reveal the key players of soil biological interactions responsible for crop growth [10].

Here, the study was carried out on maize (*Zea mays* L.) plant. The plants were treated with chemical fertilizer and recommended dose of organic manure. Sangoi [11] studied the effect of plant density on maize yield. The study emphasized on the management practices like water availability, planting date, row spacing and soil fertility, to be maintained in the field for maximum maize production. Subedi and Ma [12] pointed out that the weed infestation is the primary limiting factor of maize yield followed by nutrient (nitrogen) application and plant population density. Milander [13] propounded that the solar irradiation, water availability and temperature are the physical factors that influence the grain yield in maize.

Vermicomposting is a process by which organic materials are converted into vermicompost that is a peat-like material and exhibit high porosity, aeration, drainage, water holding capacity and rich in microbial activity. It is a non-thermophilic and biological oxidation process in association with earthworm and soil microbes [14]. Zhong et al. [15] studied the effect of chemical fertilizer and organic manure on the phospholipid fatty acid profiles and the microbial functional diversity. The work showed that the combination of balanced dose of fertilizer (N, P, K) and organic manure promotes soil microbial biomass and activity. Li et al. [16] showed the application of green manure (*Brassica* sp.) on the soil properties that influence the microbial growth. In Asia, research efforts were made by several authors about application of organic manure and crop growth [17, 18, 19, 20, 21]. Earlier research works were focused on crop performance and productivity. Nevertheless, the underlying role of specific organic manure in application is yet to be understood.

The objective of the present research was to study the impact of chemical fertilizer and organic manure (vermicompost and cow dung mixture) on the rhizosphere bacterial community and the maize phenology.

2. Materials and Methods

2.1 Study Site

Field locations were designed at Crop Research and Seed Multiplication Farm (CRSMF), University of Burdwan, West Bengal, India (latitude, 23°14’58.04”N and 23°15’19.44”N, longitude: 87°50’34.29”E and 87°50’43.95”E). The experiments were done using one variety of *Zea mays* L. (var. *RE-55, Royal England*) during May-August of 2016 and 2017. The seeds were obtained from CRSMF, Burdwan.

2.2 Plot Design and Ttreatment

The field plots of 4×3 m^2 were divided under three treatments such as control, chemical and organic. For each treatment, three replications were arranged in a Randomized Block Design (RBD) [22]. The seeds were soaked in distilled water for 24 hours and sown in the control, chemical and organic plots on 26-05-2016 and 30-05-2017. One seed was sown per hill at a depth of 0.03 - 0.04m with row spacing of 0.5m and plant-to-plant spacing of 0.5m. The spacing was done to provide an adequate gap for horizontal expansion of maize roots. Irrigation channels of 0.45m wide were made in between the replications in order to ease the flow of irrigation for each plot. The crops were harvested after the attainment of physiological maturity (R6) stage in the organic plots (05-08-16, 08-08-17), chemical plots (25-08-16, 23-08-17) and control plots (31-08-2016, 03-09-17).

For the hybrid varieties of maize crop, the chemical plots were treated with the recommended dose of *N* in the form of urea at the rate 120 kg·ha^{-1}, phosphorus (*P*) in the form of single super phosphate at the rate of 60 kg·ha^{-1} and potassium (*K*) in the form of muriate of potash at the rate of 40 kg·ha^{-1} [23]. At the time of sowing, the fertilizers were applied in such a way that it was not in touch with the seed.

The vermicompost derived from cow dung and processed cow dung were added in the ratio of 1:1 and applied to the organic plots at the rate of 600 g m^{-2} [23]. The combination ratio was accepted and practiced by local farmers, as this combination produced better yields in other crops. No fertilizer or manure was added to the control plots. The maize cultivation was performed for the two consecutive years. The field plots were hand weeded twice, at 20 DAS (days after sowing) and 50 DAS, as the infestation of weeds might cause hindrance in maize growth and procurement of nutrients from the soil. As per the agronomical procedure of maize cultivation, the other fertilizer or manure application was done before the

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attainment of tasseling (VT) stage. The first irrigation was made after the seeds were sown. Later, the frequency of irrigation was maintained as per the requirement by the crops. No crop protection was adopted as the crops did not exhibit any sign of insect/pest attack or disease incidence.

2.3 Soil Sample Collection

Five random sites were selected from a plot for sampling, the soil was sampled through soil sample borer from the rhizosphere zone of maize plants (0.08-0.15m), mixed thoroughly and kept in sterilized polythene bags. The soil samples were collected at V6 stage, VT stage and R6 stage (physiological maturity). The microbial diversity was determined from the soil samples in the Parasitology and Microbiology Research Laboratory, The University of Burdwan.

2.4 Physico-chemical Factors

Physico-chemical variables of soil such as root temperature, T (^oC); available phosphorus, P (kg ha^{-1}); available sulphur, S (mg kg^{-1}); available boron, B (mg kg^{-1}); available zinc, Zn (mg kg^{-1}); available potassium, K (kg ha^{-1}); available nitrogen, N (kg ha^{-1}); organic carbon, OrgC (%); electrical conductivity, EC (µS/cm); soil pH (pH); and soil moisture, M (%) were measured. The T, and other variables like pH, EC and M were determined using temperature meter (LUTRON, PTM-816) and (EUTECH, multiparameter, PM-700) respectively. The data were collected at different vegetative and reproductive stages of the maize plant. Soil available N was measured following the alkaline permanganate method. The available P was estimated following Olsen et al. [24]. The available K was measured by flame photometer after calibration. The water-soluble B was estimated using an azomethine H method [25]. The available S in soil was extracted using monocalcium phosphate and the method described by Bardsley and Lancaster [26]. The available Zn was estimated following Lindsay and Norvell [27]. The OrgC was measured following modified Walkley-Black method [28].

2.5 Rhizosphere Bacteria Analysis

The non-symbiotic bacteria were analysed from the soil suspensions. These include eight groups such as asymbiotic nitrogen-fixing bacteria (ANFix), fat-hydrolysing bacteria (FHydro), heterotrophic bacteria (Het), nitrifying bacteria (Nit), nitrate-reducing bacteria (NRed), phosphate-solubilizing bacteria (PSol), protein hydrolysing bacteria (PHydro) and starch-hydrolysing bacteria (SHydro). The soil samples were kept at 4^oC, where the microbiological analysis was not performed immediately. For the determination of Het group, the soil samples were diluted up to 10^{-3} and a 20 µl soil suspension (10^{-3}) was mixed with 25 ml nutrient agar (peptone 5 g l^{-1}, beef extract 3 g l^{-1}, agar 2 g l^{-1}, pH 7) and incubated at 30 ± 1 ^oC in the BOD incubator. The soil samples were incubated on starch agar media for 24 h for the determination of SHydro group; those bacterial colonies were counted that produced halo zone after flooding with Gram's iodine. After 5-30 days (5 day intervals) from the date of incubation, the bacterial population of Nit group were enumerated on Winogradsky's medium containing $(NH_4)_2SO_4$ (1.0 g l^{-1}) and the colonies were identified (pink colour) by flooding the plates with sulphanilic acid reagent. During the determination of the PSol group, a halo zone formation was observed around the colonies on the insoluble phosphate $[Ca_3(PO_4)_2]$ containing medium. The nitrogen-free medium was used to determine the ANFix group of bacteria. The nitrate agar medium and spirit blue agar medium with TWEEN 20 were used to determine NRed and FHydro groups respectively. These groups of bacterial populations were determined after 1-3 days of incubation [29, 30, 31, 32]. PHydro group of bacteria was determined by the presence of a halo zone around the colonies by flooding with $HgCl_2$ on nutrient agar medium with 2% gelatine.

2.6 Maize Growth Stages

The phenological events of maize plant was divided into vegetative phases; VE: Emergence, V3: Third leaf collar, V6: Sixth leaf collar, V10: Tenth leaf collar, Vn: nth leaf collar visible, VT or Tasseling: last branch of tassel is completely visible; and reproductive phases; R1: Silking, silk visible outside the husk, R2: Blister, kernels are white and resemble as blister in shape, R3: Kernels are yellow with white milky fluid, R4: Dough, milky inner fluid thickens into pasty consistency, R5: Dent, all kernels have dent, R6: Physiological maturity, black abscission layer has formed [33]. These plant phenological events were measured directly from the field. Five replicates were used at each stage, and the average was considered in the present study.

2.7 Statistical Analyses

To understand the association between the bacterial population groups in the organic and chemically treated plots and the physico-chemical factors of soil, principal component analysis (PCA) was done using SPSSv.16 software. To compare the means among the control, organic and chemically treated plots at V6 and R6 stages, single-way ANOVA followed by LSD was performed using SPSS v. 16 software.

3. Results

3.1 Rhizosphere Bacterial Community Exhibit Significant Change at R6 Stage of Maize

The improvement in soil health of the maize fields was clearly indicated by the significant rise in population of some of the bacterial groups under study. The ANOVA at R6 stage of maize showed that the Nit, ANFix, PSol and PHydro groups were significantly higher in number at organic plots in comparison to the corresponding chemical and control plots (Figure 1a and 1b), p< 0.05. The LSD result confirmed that no such significant increase in other bacterial groups was observed during the period of study. The Figure 1a and 1b shows the bacterial distribution among control, organic and chemical plots at 30 DAS and 90 DAS respectively.

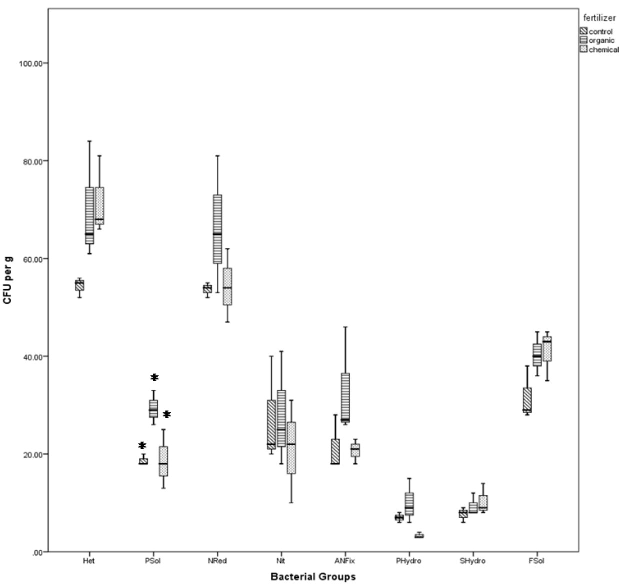

Figure 1a. Box plot showing bacterial population (CFU $g^{-1}\times 10^{5}$ dry soil) in the control, organic and chemical field plots during 30 Days. Only the phosphate solubilizing bacteria (PSol) showed the significant difference (p <0.05). The (*) over the error bars of box plot represent the significant difference among control, organic and chemical field plots.

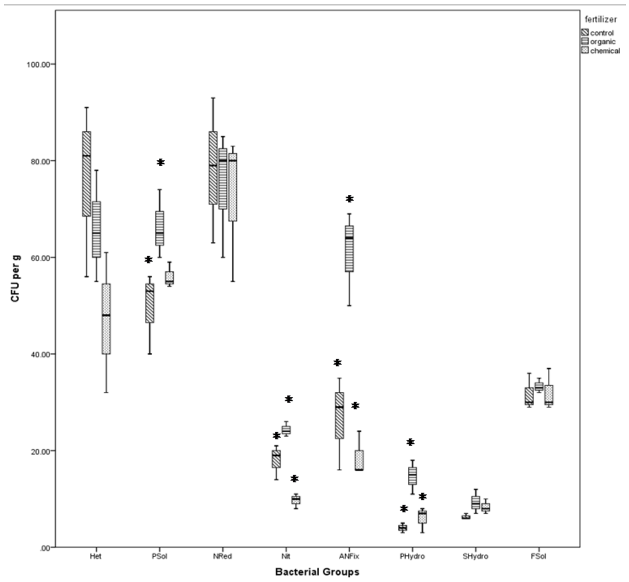

Figure 1b. Box plot showing bacterial population (CFU $g^{-1}\times 10^{5}$ dry soil) in the control, organic and chemical field plots during 90 DAS. Significant difference ($p < 0.05$) in bacterial population (phosphate solubilizing bacteria (PSol), asymbiotic nitrogen fixing bacteria (ANFix), nitrifying bacteria (Nit), and protein hydrolysing bacteria (PHydro) was observed between organic and chemical field plots. The (*) over the error bars of box plot represent the significant difference among control, organic and chemical field plots.

3.2 Changes in Physico-chemical Factors of Soil

The physico-chemical factors of the soil showed variation among control, chemical and organic field plots. The ANOVA result showed significant difference among the treatment plots. Post-hoc test through LSD showed all the physico-chemical variables except soil pH and available B had significant difference between organically treated plots and chemical plots with respect to control plots (p <0.001). The details of the distribution of physico-chemical factors are shown in Table 1.

Table 1. Physico-chemical factors of soil under organic and chemical treatments, value ± S.E.

Treatments	pH	EC(µS/cm)	T(oC)	M(%)	OrgC(%)	N(Kg/ha)	P(Kg/ha)	K(Kg/ha)	Zn(mg kg-1)	B(mg kg-1)	S(mg kg-1)
Control	5.87 ±0.07	0.078 ±0.005	29.8± 1.49	10-20	0.86 ±0.02	155.4 ±0.68	257.4 ±0.68	609.6 ±0.51	2.40 ±0.01	0.574 ±0.02	18.01± 0.03
Organic	7.12 ±0.03	0.34 ±0.01	28.6± 1.82	35-40	0.96 ±0.01	164 ±0.55	227.8 ±0.66	470 ±0.71	2.54 ±0.01	0.63 ±0.01	16.41 ±0.01
Chemical	5.80 ±0.01	0.22 ±0.01	31.7± 0.85	15-30	0.81 ±0.02	171.2 ±0.37	237.4 ±0.68	551.2 ±0.37	3.058 ±0.03	0.634± 0.05	19.68 ±0.02

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The organic manure used in the organic field plots showed individual variation in NPK and C:N ratio (Table 2). The C:N ratio was higher in cow dung (50.24) than the vermicompost. The higher values of NPK were noted in vermicompost.

Table 2. The NPK (average) of vermicompost and cow dung manures applied to maize fields

Manure	Nitrogen (%)	Phosphorus (%)	Potassium (%)	C:N ratio
Vermicompost	1.92	2.22	1.08	14.09
Cow dung	1.04	0.78	0.15	50.24

3.3 Attainment of Physiological Maturity of Maize Plants

The results of the phenological events of maize plants in the control, chemical and organic treated plots were shown in the Figure 2. Despite the similar environmental conditions, the maize plants in the organic, chemical and control field plots attained the physiological maturity or R6 stage in 86 ± 4.3 days, 95 ± 2.85 days and 107 ± 7.49 days respectively. The average maximum height of maize plants (at tasseling or VT stage) in the organic, chemical and control field plots were found to be 197.3 ± 29.59 cm, 196.38 ± 29.4 cm and 188.98 ±22.6 cm respectively. In control and chemically treated field plots, it was observed that there was a delay in the progression from one vegetative or reproductive stage to the next succeeding stage.

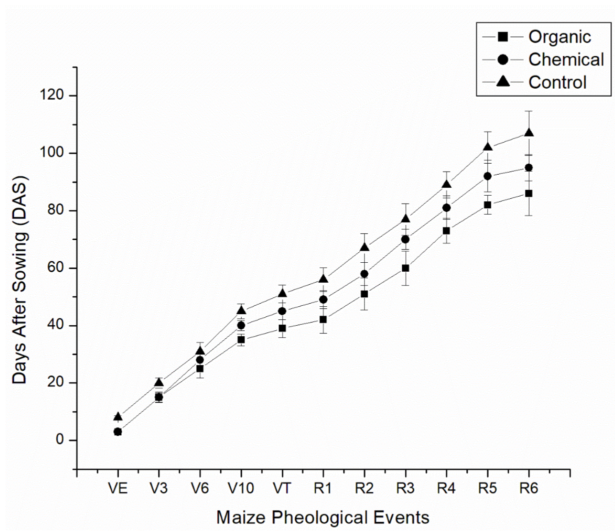

Figure 2. The appearance of phenological events of maize plant (VE: Emergence, V3: Third leaf collar, V6: Sixth leaf collar, V10: Tenth leaf collar, VT or Tasseling: last branch of tassel is completely visible; and reproductive phases; R1: Silking, R2: Blister, R3: Kernels are yellow with white milky fluid, R4: Dough, R5: Dent, and R6: Physiological maturity) in the organic, chemical and control field plots. The bars on the line graph represent the standard deviation from the mean.

3.4 Association of Rhizosphere Bacteria and Physico-chemical Factors

The PCA results of chemical and organically treated plots are shown in Figure 3 and Figure 4 respectively. In the chemical plots, factor 1 accounts for 50.13% and factor 2 accounts for 30.67 % of the variables (Figure 3). The physico-chemical factors like P, Zn, B, S, OrgC formed a close association with PSol and ANFix. On the other side, EC formed a cluster with SHydro, PHydro and FSol. A close association between soil pH and K was observed when factor 2 was considered.

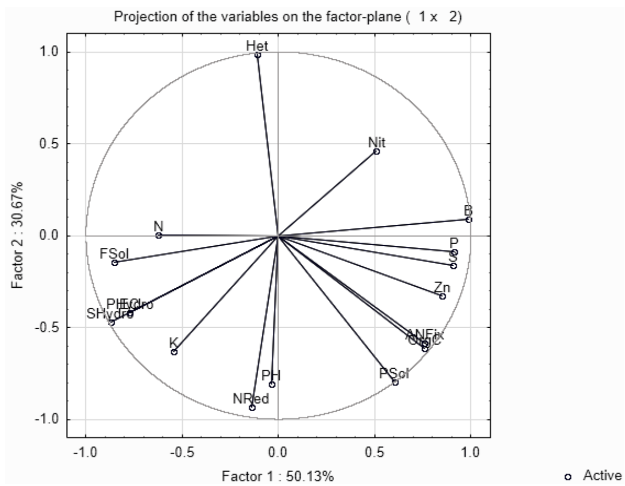

Figure 3. The PCA biplot of the chemical field plots showing the association of rhizosphere bacterial groups and physico-chemical factors.

In the organic field plots, factor 1 and factor 2 explained 46.32% and 28.39% of the variables respectively (Figure 4). A strong correlation was observed among PHydro, SHydro and Zn. Another cluster was formed by soil pH, P, K and B that appeared opposite to the above bacterial groups. The soil properties like OrgC and N were strongly correlated with Nit, FSol and PSol.

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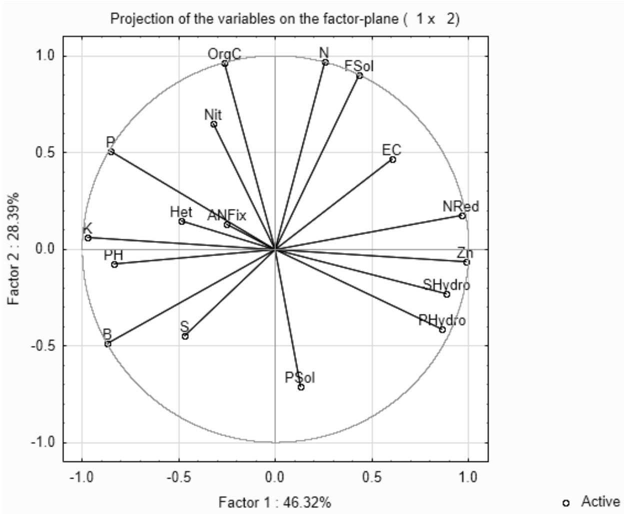

Figure 4. The PCA biplot of the organic field plots showing the association of rhizosphere bacterial groups and physico-chemical factors

4. Discussion

The roots are the indicator of soil health [34]. The maize plant treated with chemical fertilizer (NPK) and organic manure (vermicompost and cow dung) showed a remarkable difference in the growth events as well as in the soil bacterial composition. The significant increase in the Nit, ANFix, PSol and PHydro group of bacteria in the organic field plots at R6 stage were attributed to the application of recommended dose of vermicompost and cow dung composition. Alidadi et al. [35] experimented with the application of vermicompost and cow dung on tomato plants and concluded that the vermicompost enriches the soil with essential nutrients. Similar results were found by Karmakar et al. [36], while experimenting on rice plant. According to Fulhage [37], organic manure consists of the three major plant nutrients, nitrogen, phosphorus and potassium (NPK), and the essential nutrients such as Ca, Mg, S, Zn, B, Cu, Mn *etc.* for maize growth.

Adegunloye et al. [38] showed that the C:N ratio in cow dung manure is the indicator of protein that serves as an important protein source for the microbes which involved in the decomposition of organic matter. The organic manure raises the pH level of soil and accelerates the decomposition of organic matter and termite activity. The present study indicated that the population of the protein hydrolyzing bacterial group (PHydro) was significantly higher in the organically treated plots at R6 stage. Ram [39] studied the bacterial strains in cow dung responsible for plant growth-promoting traits. The study showed that the cow dung necessarily contains the bacterial strains that are responsible for Indole Acetic acid production (IAA) and phosphate solubilisation.

Atiyeh et al. [14] showed that the nitrification process in soil, resulting in the rapid conversion of ammonium-nitrogen to nitrate nitrogen are favoured by vermicompost along with cow dung manure and this nitrate nitrogen is crucial for maize or other plant growth. Besides, they also observed the increase of nitrate-nitrogen to 28 folds after 17 weeks of DAS, while in conventional compost, this increase was only 3 folds. The increase in the ash content showed rapid mineralization or organic matter. The ash hinders the H_2S formation and improves the availability of O_2, thus help the nutrient uptake by maize plants. The high phosphorus (%) was due to mineralization and mobilization of phosphorus resulting from the enhanced phosphatase activity by the microbes present in the gut epithelium of earthworm [40, 41]. The present study corroborates the above findings.

Plant growth promoting (PGP) microorganisms including PGP-Rhizobacteria (PGPR), phosphate solubilizing microorganisms (PSMs) and other symbiotic microorganisms like arbuscular mycorrhizas (AM) fungi, may play a major role in developing a sustainable use of P resources and making them solubilise in the soil [42]. The potential role of phosphate solubilizing bacteria (PSol) and P availability of vermicompost have been studied by several authors [43, 44, 45]. Their results have showed the increase P availability to plants through phosphate solubilization by PSol. Bhatt and Maheshwari [46] advocated that the cow dung consists of phosphate solubilizing bacteria that help in the early growth of plants. Moreover, the study highlighted the bacteria groups present in the cow dung promote reproductive growth in plants. The gradual increase in the PSol at 30 DAS through 90DAS in the organic plots and subsequent maize growth was attributed to the manure mixture supplied to the plants.

The vermicompost and cow dung composition not only enhances the population of free-living nitrogen fixers, but also promotes the plant growth through nitrification, phosphate solubilisation, and plant disease suppression [47,48]. Sinha et al. [49] added that this process is possible due to complex interactions between earthworm and plant growth-promoting microbes. The earthworms ingest plant growth-promoting rhizobacteria like Pseudomonas, Bacillus, Azosprillium, Azotobacter etc. along with rhizosphere soil, and they might get activated or increased due to the ideal micro-environment of the gut. When released into the environment, these microbes serve as a plant growth promoter and help the maize plant in rapid

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growth attainment. In the present study, the increased population of the ANFix group and Nit group of bacteria, observed in the organic plots were probably due to the above reasons.

The previous studies on the effect of vermicompost on soil pH, EC and C:N ratio showed that the earthworm activity reduces pH and C:N ratio in manure [14] This slightly decreased value of pH is due to mineralization of N and P, microbial decomposition of organic material into the intermediate organic substances like humic and fulvic acids [10] and simultaneous evolution of CO_2 [40]. However, the values of the soil pH of the organic field plots were a bit higher than the chemical counterparts. Soil pH is considered to be a significant factor contributing to the structure of soil microbial community [50]. Wu et al. [51] showed that the increase in pH of soil caused shifts in the microbial community. Li et al. [52] propounded that the addition of organic manure increases the soil pH, which in turn increases the soil organic matter, promotes soil maturation, and enhances the soil base saturation percentage. The present study showed that the average soil pH values were higher in organic plots in comparison to chemical plots.

The strong correlation between the Zn and PHydro group of bacteria in the soil of organic plots might be the reason for the accelerated growth of maize plants. Protein hydrolysis through PHydro releases a mixture of peptides and amino acids that are adsorbed by the roots and then translocated to the plant [53]. Ertani et al. [54] reported that the protein hydrolysates have a positive effect on plant root and shoot growth in maize plants. The PHydro present in the organic manure consists of biostimulants like IAA that promotes plant growth [55]. Zn has an essential role in the synthesis of tryptophan, which is considered to be the precursor of IAA [56]. The present study showed a strong correlation between Zn and PHydro. Besides, protein hydrolysates promote the nitrogen assimilation in plants through co-ordinated regulation between C and N metabolism [55]. Schiavon et al. [57] showed that the protein hydrolysates enhanced shoot biomass production in maize plants. They found that the biostimulants increase the activities of the nitrate reductase enzyme. In the present study, the PCA results of the organic plots showed that the PHydro and NRed groups of bacteria were under the same cluster. In the organic plots, the addition of vermicompost and cow dung mixture increased the organic carbon content, which in turn increased the moisture content and aeration. As a result, the nitrification process was promoted in the soil. Nie et al. [58] studied on the N_2O emission from the soil about soil carbon and nitrogen content and found similar results.

The chemical plots indicate strong correlation between available P and PSol group of bacteria. Studies on phosphate solubilization in soil showed that the bioavailability of P is regulated by the activity of PSol. The PSol group of bacteria converts nonlabile P through either inorganic P-solubilizing or organic P mineralization processes [59]. The study also revealed that the ANFix group of bacteria was closely associated with OrgC. Roper and Gupta [60] inferred that the non-symbiotic nitrogen fixing bacteria are largely dependent upon the carbon stock of the soil, as the organic carbon resources are essential for their enzyme activity. The present findings were in agreement with the above statement.

5. Conclusions

The present study revealed some interesting findings. The application of vermicompost and cow dung mixture promotes maize growth with significant increase in the four groups of rhizosphere bacteria like PSol, Nit, ANFix and PHydro. This clearly indicates that the process of phosphate solubilization, nitrification, nitrogenase activity and protein hydrolysis are enhanced by the application of organic manure used in the experiment. The protein hydrolysates released by the action of PHydro in the organic manure help in the release of biostimulants like IAA, which in turn increases the maize plant growth. Organic carbon associated with the organic manure is considered as the essential sites for microbial activity. The application of the recommended combination (1:1) of vermicompost and cow dung could be suggested to the farmers to raise maize production in the tropics. Thus, it can be said that the present combination of organic manure not only maintains soil health but also promotes sustainable agriculture.

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The authors have no conflict of interest to declare.

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