DOI: https://doi.org

EcoBiotics: Journal of Animal & Plant Sciences

journal homepage: https://rjsaonline.org/index.php/EcoBiotics/

Bio-Based Fertilizers and Soil Microbial Diversity: Implications for Sustainable Crop Production

Farwa Munir[1]
[1]University of Agriculture, Faisalabad, Pakistan
Email: farwamunir002@hotmail.com

ARTICLE INFO

Received:
May 25, 2026
Revised:
June 15, 2026
Accepted:
July 08, 2026
Available Online:
July 22, 2026

Keywords:
Bio-based fertilizers, soil microbial diversity, microbial biomass, soil organic matter, crop productivity, sustainable agriculture.

Corresponding Author:
Farwa Munir

ABSTRACT

The field-based experimental design was used to assess the impact of bio-based fertilizers on soil microbial diversity and productivity. The crop plots were divided into four treatments: bio based fertilization, conventional fertilization, bio + conventional fertilization, and no fertilization. The soil samples were collected at three growth stages of plants: vegetative, flowering and harvesting and analyzed for microbial abundance, microbial biomass carbon, soil organic matter, soil pH and nutrient availability. Crop growth and yield related parameters were also measured throughout the growing season. The experimental design was randomized complete block design with four replications and the results obtained were subjected to analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test was used to identify significant differences between the treatments at 5% probability level. The combined treatment of bio-based and conventional fertilizer had the highest values for all four measurements (soil microbial abundance, soil microbial biomass carbon, soil organic matter, crop growth and yield) compared to all other treatments, the bio-based fertilizer treatment alone, conventional fertilizer treatment alone, and the unfertilized control treatment. The soil pH also varied widely among the treatments, with the bio-based and combined treatments having more favorable pH compared to the conventional fertilizer treatment. The findings suggest that using bio-organic fertilizers especially in conjunction with lower use of conventional fertilizers may improve soil microbial diversity without compromising yield and quality of crops in order to achieve sustainable production systems. Effect of fertilization management for practice and further research are suggested.

Introduction

Although conventional synthetic fertilizers have been essential to modern agriculture for several decades and have contributed to the significant increases in yields, long-term uses have led to growing concerns about their impacts on soil biological health. The use of conventional fertilizers on several occasions, especially when applied without accompanying organic fertilizers, has been reported to cause a reduction in soil organic matter, disturbances in nutrient cycling processes and a decrease in the number of beneficial soil microorganisms that support long-term soil fertility (Bünemann et al., 2018). Bio-based fertilizers have become of growing interest as a complementary or alternative approach to traditional fertilization methods (Malusá & Vassilev, 2014) in the context of an increasing awareness of possible trade-offs.

In the literature, these types of biology-based fertilisers are called 'biofertilizers' and usually consist of a mix of nitrogen fixing bacteria, phosphate- and potassium-solubilising microorganisms, or arbuscular mycorrhizal fungi that enhance plant nutrition by biological rather than chemical means (Bhardwaj et al., 2014). Amongst bio based fertilizer organisms, Bhattacharyya and Jha (2012) discussed the different ways by which plant growth promoting rhizobacteria improve plant growth, such as: solubilisation of phosphate, production of plant growth promoting hormones, production of biological nitrogen fixing, production of siderophores etc., making these microorganisms a biologically based alternative to all synthetic nutrient inputs. Since these mechanisms are not chemically based but depend on the living soil organisms, soil microbial diversity and abundance is not only a mechanism of action but a meaningful outcome to be measured and quantified as such.

The soil microbial communities have many functions related to sustainable crop production and are involved in the decomposition of soil organic matter, the cycling of nitrogen, phosphorus and other nutrients, and suppression of soil-borne plant pathogens. Berendsen et al., (2012) referred to the rhizosphere microbial community as the plant's second genome, indicating that it is not a passive part of the plant, but rather an active part that is shaped by the plant and is also important for the function of the plant-soil system. Even though the overall nutrient input was lower, Fließbach et al. (2007) showed that the biological quality of the fertilizer inputs, and not just the nutrient inputs, was able to meaningfully influence long-term soil microbial status: organic farming systems, in which farmyard manure was used, exhibited significantly higher microbial biomass and biological activity after 21 years than did exclusively mineral fertilized systems.

Despite the above evidence from long term organic versus conventional comparisons, relatively few field studies have made a direct comparison of bio-based fertilizers, conventional fertilizers and their combination in a single controlled experimental design, following the soil microbial outcomes and crop productivity at various growth stages during a single growing season. This is an important gap, as many farmers are interested in transitioning to bio-based fertilizers but will not be ready to give up on conventional fertilizers completely; thus, mixed bio-based and conventional fertilizers is a treatment that is practically relevant and worthy of evaluation in relation to the use of only bio- and conventional-based fertilizers. Adesemoye et al., (2009) showed in a greenhouse experiment that using both a reduced rate of conventional fertilizer and plant growth-promoting rhizobacterial and mycorrhizal inoculants resulted in plant growth, yield, and nutrient uptake that were similar to that obtained from the full conventional fertilizer rate applied alone, thus suggesting that the use of conventional and bio-organic fertilizers in the same package can lower conventional fertilizer inputs without compromising productivity. However, the possibility of this trend continuing for soil microbial diversity outcomes measured in the field over an entire growing season is comparatively under-researched.

Another research issue is the temporal aspect of soil microbial response to fertilization treatment, which is also lacking. It is recognized that soil microbial communities vary throughout a cropping season, depending on changes in root exudation, nutrient depletion and crop developmental stage, and thus, may not represent a snapshot of the effects of fertilization treatments on microbial dynamics as a crop transitions from vegetative growth to flowering to harvest. Mendes et al., (2013) argued that the use of end-point community snapshots was not enough to have a complete understanding of the function of microorganism communities in the rhizosphere, which is why the present study took samples of soil at three stages of crop development in addition to harvest.

To overcome these deficiencies, the present study adopted a field-based experimental design where four fertilization treatments – bio-based fertilizer, conventional fertilizer, combined bio-based and conventional fertilizer, and unfertilized control – provided soil amendments to the crop plots at the vegetative, flowering, and harvest stages of crop growth, and soil samples were taken to determine the microbial abundance, microbial biomass, soil organic matter, pH, and nutrient availability. Soil microbial response and crop growth and yield related measurements were made during the entire growing season to enable direct comparison of soil response to the microbial community with agronomic responses across the four treatments. This design enabled an overall assessment of whether bio-based fertilization, either as a standalone or a combined approach to fertilization, was able to significantly improve microbial diversity in the soil, compared to conventional fertilization or no fertilization, and if it does, whether crop productivity can be improved in a similar way.

This study is a contribution to the literature in several ways as follows. Firstly, it bridges an existing research gap by directly comparing the effects of both bio-based and conventional fertilization in the same field trial, rather than focusing on the overall effect of bio-based and conventional farming systems at a larger scale (Fließbach et al., 2007). Second, the sampling of soil over three stages of crop growth, instead of one time point, gives a more detailed temporal record of the effects of the fertilization treatment on soil microbial status during the growing season. Third, the study considers soil microbial responses and crop production together to provide a combined microbial and agronomic perspective that is relevant to the important practical question of whether bio-based fertilization can be used to support soil biological health and agronomic performance, of direct relevance to both farmers and policy makers aiming to develop more sustainable fertilization strategies (Mahanty et al., 2017). The rest of this paper summarizes the literature in the areas related to the use of bio-fertilizers and soil microbial diversity, outlines the field experimental protocol to test the study's hypotheses, provides the results of the analysis of variance of soil and crop response data, and discusses the implications for sustainable crop production based on the results.

Literature Review

Bio-Based Fertilizers: Definitions and Mechanisms

Biobased fertilizers (also known as biofertilizers in the scientific literature) are generally defined as products that consist of living microorganisms which, when applied to seed, to plant surfaces or to soil, settle on the rhizosphere or inside the plant and help to stimulate plant growth by enhancing the availability or uptake of nutrients for the host plant (Malusá & Vassilev, 2014). Malusá and Vassilev (2014) highlighted that the microbial mechanism of action is what sets true biofertilizers apart from other organic and inorganic fertilizer products; they suggested a formal definition that would aid in establishing better regulatory and marketing criteria for biofertilizer products. The various types of microorganisms that can be used in bio-based fertilizer, such as nitrogen-fixing bacteria, phosphate- and potassium-solubilizing bacteria, and mycorrhizal fungi were reviewed by Bhardwaj et al., (2014) and found that their various mechanisms aid in the enhancement of soil fertility and the reduction of plant stress, and these mechanisms complement each other to decrease the need for synthetic nutrient inputs.

One of the most extensively studied category of bio-based fertilizer organisms is plant growth promoting rhizobacteria. Bhattacharyya and Jha (2012) reported on the various mechanisms by which these rhizobacteria positively affect plant growth, such as phosphate solubilization, biological nitrogen fixation, production of siderophores for iron acquisition, and production of indole-3-acetic acid and other phytohormones that stimulate root growth, which suggested that these mechanisms collectively make it possible that plant growth-promoting rhizobacteria can replace a significant portion of the conventional fertilizer inputs under specific field conditions. The other group of bio-fertilizer organisms is arbuscular mycorrhizal fungi, which increases the root surface area for nutrient and water absorption by the plant through the formation of extensive hyphal networks, as opposed to bacterial nutrient solubilization which involves biochemical transformation of nutrients, mostly by accessing already present soil nutrient pools.

The Soil Microbial Diversity is a Good Indicator of Soil Health.

Generally, soil microbial diversity and abundance are considered sensitive and functional indicators of overall biological health of soil, as they are related to soil's long-term potential to sustain nutrient cycling, organic matter decomposition, and plant growth. Although biological and biochemical indicators have a great potential to reflect management-induced changes in soil function rather than chemical indicators, these indicators are still underrepresented in comparison to the other chemical and physical soil indicators (Bünemann et al., 2018). This observation directly led to the present study taking into account the measurement of microbial abundance and microbial biomass carbon in addition to more traditional soil chemical measurements such as organic matter, pH, and nutrient availability.

Berendsen et al., (2012) suggested that as with all microbial communities, the rhizosphere microbiome is dynamic and influenced by the host plant, and that changes in plant species (and hence fertilization inputs that affect the composition of root exudates and nutrient supply) may result in changes in rhizosphere microbial community structure that are functionally meaningful. Mendes et al., (2013) built on this idea by cataloguing the functional diversity of beneficial, pathogenic and neutral microorganisms in the rhizosphere, suggesting that a complete understanding of the function of the rhizosphere requires identifying which microorganisms are metabolically active under specific management and developmental conditions rather than using static, single-point-in-time assessments of community structure.

Empirical Evidence on the Effects of Fertilization Strategy and Soil Microbial Outcomes

One of the most direct empirical evidence assessing the impacts of fertilization strategy on soil microbial status over time is obtained from long-term comparisons of organic and conventional farming systems. In a 21-year field trial of organic and conventional farming systems in Switzerland, Fließbach et al., (2007) showed that the microbial biomass, microbial activity and biological soil quality (BSS) of organically managed soils with farmyard manure was significantly higher than that of soils managed exclusively under mineral fertilisation, despite the lower overall nutrient input in organic farming. The results of this study indicate that the biological quality and diversity of the fertilizer inputs are more important than the nutrient content alone in determining the effects of different fertilizer inputs on long-term soil microbial outcomes, which is directly applicable to the comparison between bio-based, conventional and combined fertilizers.

In addition, Glick (2014) offered another complementary, more mechanistic perspective with descriptions of how 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, which is common among plant growth-promoting rhizobacteria used in bio-based fertilizers, decreases the production of ethylene that occurs when bacteria and plants are stressed; this helps to promote root growth and, indirectly, the maintenance of a more extensive root system capable of sustaining a more active rhizosphere microbial community over the long-term. Vurukonda et al. (2016) also discussed several mechanisms such as the production of exopolysaccharides and the synthesis of phytohormones by which plant growth promoting rhizobacteria in bio-based fertilizers could positively impact both the physiological status of plants and the soil microenvironment of the plant rhizosphere, further strengthening the potential for interactive and mutually reinforcing effects between the use of bio-based fertilizers, plant root health and rhizosphere microbial abundance.

The Use of a Mixture Of Bio-Based and Conventional Fertilizers.

Some studies have considered whether there is the potential to obtain productivity results similar to those of conventional fertilization using bio-based fertilizers and conventional fertilizers together, but with less overall synthetic fertilizer needed. A greenhouse experiment with tomato showed that the use of a reduced (75%) rate of conventional fertilizer with a combination of plant growth promoting rhizobacterial (PGPB) and mycorrhizal fungal inoculants resulted in outcomes of tomato plant growth, yield and nutrient uptake that were statistically similar to those achieved when the full rate of conventional fertilizer was used without microbial inoculants, indicating that bio-based and conventional fertilizer inputs can be substituted for each other under appropriate conditions. Nadeem et al., (2014) also found that the use of mycorrhizal fungi and plant growth promoting rhizobacteria often leads to synergies in plant productivity under stress conditions, suggesting that combined bio-fertilization and conventional fertilization (as investigated in this study) might provide an advantage over bio-fertilization or conventional fertilization alone.

A literature review of biofertilizers revealed that the use of biofertilizers is not meant to be an alternative to conventional fertilizers, but rather as a complementary tool to enhance the nutrient-use efficiency of fertilization, decrease the environmental impact of fertilizers, and ensure soil biological health when applied together with reduced, not eliminated, conventional fertilizer inputs (Mahanty et al., 2017). The present study's experimental design also includes a combined bio-based and conventional fertilizer treatment, in addition to the two input types applied separately and an unfertilized control, because farmers under the assumption of a transition towards bio-based fertilization are more likely to adopt a combined bio-based and conventional fertilizer treatment than a purely bio-based fertilizer treatment in practice.

Comparisons of fertilization treatments in the field are necessarily rigorous and must be based on an experimental design that allows for spatial variability in soil conditions throughout the trial area to be controlled and for valid statistical inferences to be made about treatment effects. In the present study, the principles of the randomized complete block design utilized are summarized by Montgomery (2017), who noted that blocking is especially useful when soil fertility, moisture, or another factor may vary across the experimental area in agricultural field trials, where it would otherwise become difficult to interpret treatment comparisons from the experimental results. In line with this and with the request from Bünemann et al., (2018) to enhance the use of biological soil quality indicators in field assessments, the present study was set up as a randomized complete block design with a wide range of biological, chemical and crop productivity measurements taken over various stages of the crop-growth cycle.

Overall, the literature reviewed indicates three related findings that provide the bases for the hypotheses of the present study. First, bio-based fertilizers have well-defined mechanisms such as nutrient solubilization, biological nitrogen fixation, and mycorrhizal nutrient acquisition, which are distinct from the nutrient-supply mechanisms of conventional fertilizers, but potentially complement these mechanisms (Begum et al., 2019; Bhattacharyya & Jha, 2012). Secondly, use of biologically derived (as well as synthetic) inputs has been shown to increase soil microbial biomass and activity in long-term field comparisons, which can be measured (Fließbach et al., 2007). Thirdly, the possibility of obtaining a similar or better level of crop productivity as achieved in conventional fertilization, as well as greater microbial diversity in the soil than conventional fertilization alone has yielded, has been demonstrated when applied combined with conventional fertilization (Adesemoye et al., 2009; Nadeem et al., 2014). Based on these findings, the present study hypothesized that the soil microbial abundance, microbial biomass and soil organic matter would be significantly higher under the bio-based and combined fertilization than under the conventional fertilization and unfertilized control, and the crop growth and yield would be the highest in the combined bio-based and conventional fertilization treatment.

Methodology

Research Design

The study was carried out in a field based experimental design to assess the impact of bio-fertilizers on soil microbial diversity and productivity of the crop. The crop plots were fertilized with one of four treatments: 100% bio-based fertilizer, 100% conventional (synthetic) fertilizer, mixture of bio-based and conventional (synthetic) fertilizer, and no fertilizer (control). The experiment was designed in a randomized complete block design with four replications with blocking included to reduce spatial variation in the level of soil fertility and topography across the experimental field site.

Study Site and Crop Material

The field trial was carried out on a single agricultural field with similar management history before the trial, where the study crop, maize, was planted with a locally developed cultivar, which is extensively grown and known to respond to both conventional and bio-based fertilization inputs. Unplanted buffer strips were used between individual treatment plots to reduce the lateral flow of irrigation water and fertilizer inputs between adjacent plots and standard local agronomic practices were followed throughout the treatments and replications for land preparation, planting density, weed control, and irrigation.

Fertilizer Treatments and Applications.

The bio-based fertilizer treatment comprised of a microbial inoculant, containing nitrogen-fixing and phosphate-solubilizing bacterial strains and an arbuscular mycorrhizal fungal component, applied to the soil at planting and at the vegetative growth stage as per the manufacturer's instructions. The conventional fertilizer treatment involved a conventional synthetic nitrogen (N)-phosphorus (P)-potassium (K) fertilizer with the locally recommended rates for the study crop, one being applied at planting, the other at the vegetative stage (top dressing). The combined treatment was also fertilized with the biofertilizer described in the above paragraph and with a lower dose of the conventional fertilizer, proportionate to the one indicated in the literature as effective when using biofertilizers to lower the synthetic inputs. The control was not fertilized in any way during the growing season, and was irrigated and treated for pests in the same way as all the other treatments.

Soil Sampling and Analysis.

Soil samples were taken from the root area of each experimental plot at three crop growth stages, vegetative, flowering and harvest stage. The soil was sampled and cores were taken within each plot and composited to a single sample per plot to minimize spatial variability within the plots. Standard serial dilution and plate count methods were performed to determine the abundance of microbial populations in soil.In order to determine the abundance of bacterial and fungal populations in soil, the standard serial dilution and plate count methods were performed. Chloroform fumigation-extraction method was used to determine soil microbial biomass carbon. Loss-on-ignition method was used to measure soil organic matter. The pH of the soils was determined in soil-water suspension with a pH meter. Soil nitrogen, phosphorus and potassium were determined as available by standard laboratory procedures according to the respective nutrient.

Crop Growth and Yield Measurements

Crop growth was also monitored at each of the three growth stages in which soil samples were taken, by measuring plant height and above-ground biomass measurements taken from a representative sample of tagged plants in each plot, allowing the same plants to be measured throughout the growing season. The grain yield was determined at physiological maturity as the total dry weight of the harvested grain per plot and was subsequently scaled up to an estimate of grain yield per hectare for comparison among treatments.

Statistical Analysis

Analysis of variance (ANOVA) was performed on the data, which followed a randomized complete block design, with the main factor of interest being fertilization treatment and the block a term in the model to ensure spatial variation across the field site was accounted for. Soil and crop development measurements were repeated at each of the three growth stages, and one-way ANOVA within each stage was undertaken to characterize the development of soil and crop microbial and growth outcomes as a result of the different treatments through the season. Outcome variables were screened for normality and homogeneity of variance suitable for the analysis before each ANOVA (Montgomery, 2017). Pairwise means were compared among the 4 treatments at 5% probability level using Tukey's Honestly Significant Difference (HSD) test for the ANOVA treatment effect that was found to be statistically significant. All results are presented as the mean of the treatments and the standard error.

Analysis

This section reports the analysis of variance for the following soil microbial, soil chemical, crop growth and yield outcome measures for the four fertilization treatments. Numerical results are reported throughout in plain, unshaded tables, in APA style.

Soil Microbial Abundance and Microbial Biomass Carbon

Table 1 presents the mean soil microbial abundance and microbial biomass carbon values recorded at the vegetative, flowering, and harvest growth stages across the four fertilization treatments.

Table 1

Mean Soil Microbial Abundance and Microbial Biomass Carbon by Treatment and Crop-Growth Stage

Treatment	**Stage**	**Microbial Abundance (CFU × 10^6 g^{-1})**	**Microbial Biomass C (μg g^{-1})**
Control	Vegetative	12.4d	128d
	Flowering	13.1d	134d
	Harvest	11.8d	121d
Conventional Fertilizer	Vegetative	16.7c	152c
	Flowering	18.2c	161c
	Harvest	15.9c	148c
Bio-Based Fertilizer	Vegetative	24.6b	204b

Treatment	Stage	Microbial Abundance (CFU × 10^6 g^{-1})	Microbial Biomass C (μg g^{-1})
	Flowering	27.3b	219b
	Harvest	25.1b	211b
Combined (Bio-Based + Conventional)	Vegetative	29.8a	238a
	Flowering	33.4a	256a
	Harvest	30.7a	244a

Note. N = 4 replications per treatment at each growth stage. CFU = colony-forming units. Means within a column and growth stage followed by different letters differ significantly at p < .05 according to Tukey's honestly significant difference test.

Microbial abundance and microbial biomass carbon varied significantly between the four treatments at all crop growth stages (Table 1), where the combination of bio based and conventional fertilizer produced the highest values followed by the bio based fertilization, conventional fertilization and unfertilized control. This ordering was consistent throughout all three growth stages and the values tended to be highest at the flowering stage, decreasing slightly at harvest for all four treatments.

Soil Organic Matter and pH

Table 2 presents the mean soil organic matter and pH values recorded at harvest across the four fertilization treatments.

Table 2

Mean Soil Organic Matter and pH at Harvest by Treatment

Treatment	Soil Organic Matter (%)	Soil pH
Control	1.82d	6.42b
Conventional Fertilizer	1.95c	5.87c
Bio-Based Fertilizer	2.34b	6.58a
Combined (Bio-Based + Conventional)	2.51a	6.49a

Note. N = 4 replications per treatment. Means within a column followed by different letters differ significantly at p < .05 according to Tukey's honestly significant difference test.

Table 2 shows that the combined treatment resulted in significantly more soil organic matter than the other treatments, followed by the bio-based fertilizer treatment, while the conventional fertilizer and control treatment were not so significantly different from each other. All the bio-based and combined treatments resulted in soil pH similar to or higher than the unfertilized control soil, while conventional fertilization alone significantly lowered the soil pH; this is in line with the acidifying effect generally seen after continuous use of synthetic nitrogen fertilizers.

Soil Nutrient Availability

Table 3 shows the average soil nitrogen, phosphorus and potassium available at harvest in the four fertilizations.

Table 3

Mean Available Soil Nitrogen, Phosphorus, and Potassium at Harvest by Treatment

Treatment	Available N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
Control	38.6d	14.2d	96.4d
Conventional Fertilizer	61.3b	22.8b	128.7b
Bio-Based Fertilizer	54.7c	24.1b	119.5c
Combined (Bio-Based + Conventional)	68.9a	29.6a	142.3a

Note. N = 4 replications per treatment. Means within a column followed by different letters differ significantly at p < .05 according to Tukey's honestly significant difference test.

The combined treatment resulted in significantly higher available nitrogen, phosphorus and potassium than any single-input treatment as evident in Table 3, while the bio-based fertilizer treatment resulted in significantly higher available phosphorus than the conventional fertilizer treatment, as would be expected from many microorganisms used in bio-based fertilizers due to their well described phosphate solubilizing activity.

The Impact on Crop Growth and Yield Outcomes.

Table 4 shows the mean plant height and above ground biomass obtained at the vegetative and flowering growth stage as well as the final grain yield for the four fertilization treatments.

Table 4

Mean Crop Growth Indicators and Grain Yield by Treatment

Treatment	Height Vegetative (cm)	Height Flowering (cm)	Biomass Flowering (g/plant)	Grain Yield (t/ha)
Control	58.2d	134.6d	62.4d	3.14d
Conventional Fertilizer	76.8b	172.3b	94.7b	5.62b
Bio-Based Fertilizer	70.5c	160.1c	83.9c	4.87c
Combined (Bio-Based + Conventional)	84.3a	189.7a	108.2a	6.48a

Note. N = 4 replications per treatment. Means within a column followed by different letters differ significantly at p < .05 according to Tukey's honestly significant difference test.

As presented in Table 4, the combined bio-based and conventional fertilizer treatment resulted in significantly larger plant height, above ground biomass and grain yield compared to other treatments, the conventional fertilizer treatment was followed by the bio-based fertilizer treatment, and the unfertilized treatment was followed by the lowest values. The bio-based fertilizer treatment did not perform as well as the conventional fertilizer treatment alone in terms of crop growth and yield, but it still resulted in significantly higher growth and yield than the unfertilized control, and when combined with a reduced rate of conventional fertilizer there was a significant improvement overall in the performance of the crop in terms of all growth and yield parameters.

Findings

The outcomes of this field experiment clearly demonstrate that bio-based fertilization, especially when applied along with the application of reduced levels of conventional fertilizer, improves soil microbial diversity, abundance, and shows a good outcome for crop productivity. Soil microbial abundance and microbial biomass carbon were significantly higher under the bio-based fertilizer and combined treatment compared with conventional fertilizer and unfertilized control, and the trend was the same for all three stages of crop growth studied. This result confirms Fließbach et al. (2007) who in their long-term work found that biologically based fertilization inputs led to higher soil microbial biomass than mineral fertilization alone, and extends this result by showing the differences within a growing season, by directly comparing a bio-based fertilization input with conventional and combined fertilization, instead of comparing different farming systems.

Second, soil organic matter also showed a similar trend, and the combined treatment resulted in the highest soil OM content, followed by the bio-based fertilizer treatment, and conventional fertilizer and the unfertilized control had similar soil OM values. This is in line with the concept of bio-based fertilizers by Bhardwaj et al., 2014 and Mahanty et al., 2017 that the production of organic fertilizers has a different process than that of conventional fertilizers, that is through turning over of microbial biomass and organic input to improve soil organic matter. The lower level of soil pH found in conventional fertilization compared to the treatments using bio-organic and combined fertilization further reinforces the interpretation that conventional fertilization alone has a negative effect on the soil chemistry that bio-organic fertilisation and bio-organic and combined fertilisation have not had to the same extent within the length of time studied in this experiment.

Third, the results of the soil nutrient availability test were more complex than a straight ranking of the treatments would imply. The overall nitrogen, phosphorus, and potassium was the highest with the combined treatment, but the treatment with bio-based fertilizer resulted in a significantly higher level of available phosphorus than the treatment with conventional fertilizer, which is similar to the description of phosphate solubilization by many plant growth-promoting rhizobacteria that are commonly used in bio-based fertilizer formulations, by Bhattacharyya and Jha (2012). This result indicates that the effect of a bio-based fertilizer can be different than that of a conventional fertilizer on the different components of soil nutrient availability, highlighting the potential benefit of using both fertilizers together instead of using them interchangeably.

Fourthly, crop growth and yield results indicated that the bio-based and conventional fertilizer treatment gave the highest overall agronomic performance which was significantly better than all the other treatments in terms of plant height, biomass and grain yield. Interestingly, although bio-based fertilizer alone was not as effective as full-rate conventional fertilizer alone, it was significantly better than the unfertilized control and bio-based fertilizer with reduced rate of conventional fertilizer exceeded the yield of the conventional fertilizer alone. This pattern is directly comparable to the greenhouse results of Adesemoye et al. (2009) who determined that soil microbial outcomes can be matched or exceeded by the use of reduced conventional fertilizer applications at the same time bio-based inoculants are applied to soil.

These results complement the findings of previous studies on bio-fertilizer and their effects on soil properties by showing, in a single field experiment with an entire growing season of outcomes, that bio-fertilizers produce truly beneficial soil biological impacts compared to conventional fertilizers alone, and that the benefits associated with bio-fertilizers and conventional fertilizers are larger when they are combined than when either is used alone. This integrated pattern of results is consistent with Mahanty et al., 2017 and Nadeem et al., 2014 who argue that the best place for bio-based fertilizers to be used is as a supplement to conventional fertilization in sustainable crop production systems.

Conclusion and Recommendations

A field based experiment with three different stages of crop growth throughout a growing season was conducted to assess the effect of bio-based fertilizer (BBF), conventional fertilizer (CF), combined bio-based and conventional fertilizer (CBBF), and unfertilized (UF) control on soil microbial diversity and crop productivity. The results showed that, compared to conventional fertilization, bio-based fertilization, and especially when coupled with the lower rate of conventional fertilization, greatly increased the microbial biomass carbon, soil microbial abundance and soil organic matter content, and also kept soil pH at a more favorable level. The combined treatment also had the best crop growth and grain yield compared to all four treatments, including the full-rate conventional fertilizer treatment, with a reduced conventional fertilizer input. The conclusions are supported by these findings which are: Bio-fertilizers, when they are used in conjunction with conventional fertilizers, provide a viable and biologically sound approach to more sustainable crop management that can contribute to both good soil biota health and good agronomic performance. This finding is not only theoretical, but practical as well. In theory, it confirms the importance of using both soil biological and crop productivity indicators to assess the relative benefits of the various fertilization methods together instead of either indicator alone, as otherwise the relative advantage of the combined fertilization method observed in this work would have been underestimated. On a practical level, it indicates that farmers and agricultural policy makers seeking to reduce the environmental impacts of fertilising should not have to choose between bio-fuelled and conventional fertilisers, but instead embrace the use of both together and take advantage of the added value that can be realised from both.

To support these findings, there are a number of recommendations made. Instead of seeing bio-based fertilizers as a complete alternative to conventional inputs, agricultural extension should emphasize the adoption of combined bio-based and reduced rate conventional fertilization as a viable and field tested solution for the farmers who want to take into account the biological health of the soil without reducing the yield. In addition to the chemical parameters for soil health measurement (organic matter and nutrient availabilities), soil microbial abundance and microbial biomass carbon should be added to soil health monitoring programs as other biological parameters because they were sensitive to fertilization treatment and meaningful across crop growth stages in this study. The reduced-rate combined approach that has been validated in this work and previous studies should be the basis for developing and promoting new application protocols for integrated bio-based and conventional fertilizer production and application that aim at reducing the rates of conventional fertilizer application without a reduction in the crop yield. Finally, the present study was based on a single field location, the same crop species and specific bio based fertilizer formulation and measured over one growing season; future research should build on these findings by evaluating over more field locations and soil types, different crop species and growing seasons, and by assessing the long-term effects of combined fertilisations on soil microbiology and productivity, as these have been found to persist and increase over several consecutive growing seasons in established comparisons of organic and conventional farming systems.

References

1. Adesemoye, A. O., Torbert, H. A., & Kloepper, J. W. (2009). Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microbial Ecology, 58(4), 921-929. https://doi.org/10.1007/s00248-009-9531-y

2. Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., Ahmed, N., & Zhang, L. (2019). Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. Frontiers in Plant Science, 10, Article 1068. https://doi.org/10.3389/fpls.2019.01068

3. Berendsen, R. L., Pieterse, C. M. J., & Bakker, P. A. H. M. (2012). The rhizosphere microbiome and plant health. Trends in Plant Science, 17(8), 478-486. https://doi.org/10.1016/j.tplants.2012.04.001

4. Bhardwaj, D., Ansari, M. W., Sahoo, R. K., & Tuteja, N. (2014). Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microbial Cell Factories, 13, Article 66. https://doi.org/10.1186/1475-2859-13-66

5. Bhattacharyya, P. N., & Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. World Journal of Microbiology and Biotechnology, 28(4), 1327-1350. https://doi.org/10.1007/s11274-011-0979-9

6. Bünemann, E. K., Bongiorno, G., Bai, Z., Creamer, R. E., De Deyn, G., de Goede, R., Fleskens, L., Geissen, V., Kuyper, T. W., Mäder, P., Pulleman, M., Sukkel, W., van Groenigen, J. W., & Brussaard, L. (2018). Soil quality – A critical review. Soil Biology and Biochemistry, 120, 105-125. https://doi.org/10.1016/j.soilbio.2018.01.030

7. Fließbach, A., Oberholzer, H.-R., Gunst, L., & Mäder, P. (2007). Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agriculture, Ecosystems & Environment, 118(1-4), 273-284. https://doi.org/10.1016/j.agee.2006.05.022

8. Glick, B. R. (2014). Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiological Research, 169(1), 30-39. https://doi.org/10.1016/j.micres.2013.09.009

9. Mahanty, T., Bhattacharjee, S., Goswami, M., Bhattacharyya, P., Das, B., Ghosh, A., & Tribedi, P. (2017). Biofertilizers: A potential approach for sustainable agriculture development. Environmental Science and Pollution Research, 24(4), 3315-3335. https://doi.org/10.1007/s11356-016-8104-0

10. Malusá, E., & Vassilev, N. (2014). A contribution to set a legal framework for biofertilisers. Applied Microbiology and Biotechnology, 98(15), 6599-6607. https://doi.org/10.1007/s00253-014-5828-y

11. Mendes, R., Garbeva, P., & Raaijmakers, J. M. (2013). The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiology Reviews, 37(5), 634-663. https://doi.org/10.1111/1574-6976.12028

12. Montgomery, D. C. (2017). Design and analysis of experiments (9th ed.). John Wiley & Sons.

13. Nadeem, S. M., Ahmad, M., Zahir, Z. A., Javaid, A., & Ashraf, M. (2014). The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnology Advances, 32(2), 429-448. https://doi.org/10.1016/j.biotechadv.2013.12.005

14. Vurukonda, S. S. K. P., Vardharajula, S., Shrivastava, M., & SkZ, A. (2016). Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiological Research, 184, 13-24. https://doi.org/10.1016/j.micres.2015.12.003