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Research Article

Enhancing Soil Microbial Biomass and Enzyme Activities through Integrated Organic-Inorganic Fertilization for Improved Disease-Suppressive Potential in Gypsiferous Soils of Northern Iraq

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ABSTRACT

Soil biological health is significantly affected by fertilization approaches, mainly under intensive agricultural systems. The current study assessed the impacts of organic, inorganic, and integrated nutrient management (INM) strategies on important soil microbial and biochemical parameters to ascertain sustainable fertilization alternatives that improve soil functioning. The justification was to assess whether integrating organic and mineral fertilizers provides synergistic benefits for soil microbial activity, efficiency, and diversity compared with single-source fertilization. The findings revealed that INM (T4) constantly outclassed all other treatments. Microbial biomass carbon was highest in T4 (410 mg C kg⁻¹), followed by organic (T2) and inorganic (T3) treatments, representing better microbial environment and substrate availability under combined fertilization. Dehydrogenase and urease activities were also significantly better in T4; showing enhanced microbial metabolism, nitrogen mineralization, and enzymatic functioning due to the collective supply of labile carbon and mineral nutrients. Basal respiration peaked under T4, indicating increased microbial activity and carbon cycling, while remaining within a range indicative of better soil fertility rather than microbial stress. The microbial metabolic quotient (qCO₂) was lowest in T4, proposing higher microbial efficiency and reduced carbon loss per unit biomass. Furthermore, microbial diversity, determined by the Shannon index, was maximum under INM, showing the development of a more diverse and functionally resilient microbial community. In summary, integrated nutrient management significantly increases soil microbial biomass, enzymatic activity, metabolic efficiency, respiration, and diversity. These findings validate that integrating organic and inorganic fertilizers is a superior and sustainable approach for improving soil biological health, nutrient cycling, and long-term soil productivity.

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Introduction

Gypsiferous soils are typical characteristics of dry and semi-dry lands. These soils are classified as having high

levels of calcium sulphate and are well spread all over the world and occupy an area of approximately 100 million hectares. They tend to occur in dry or semi-dry

places, such as the Middle East, North Africa, Central Asia, and some of the countries in Latin America (Sharma et al., 2025). It is also reported that such soils are low in fertility, poorly developed and structured (Al-Naser, 2019). The very little or absence of microbial activity has also been documented in these soils because of the absence of organic matter (Escudero et al., 2015). The soil microorganisms are very important as part of the soil biotic system because they facilitate the recycling of nutrients, which is favorable in supporting the production of crops in a sustainable production (2018; Chen et al., 2024).

The enzymatic processes that occur in the soil such as basal respiration, dehydrogenase, urease, as well as microbial biomass carbon (MBC) are considered effective parameters to determine the soil biological condition and activity of microorganisms (Meena and Rao, 2021; Patle et al., 2023). These enzymes are tightly linked to the nutrient transformation processes, such as the carbon and nitrogen cycles, and are very sensitive to alterations in the soil management systems. Organic amendments are the main sources of carbon and energy to the soil microorganisms whereas inorganic fertilizers contain easily accessible nutrients that are needed by the microorganisms to develop and facilitate the enzymatic activities (Hojati and Nourbakhsh, 2006). Nevertheless, the use of organic or inorganic inputs solely might not be enough to replenish the biological functioning of the highly degraded gypsiferous soils (Zhang et al., 2015).

The combined use of organic and inorganic fertilization is increasingly turning into a feasible option to increase the biological functioning of the soil through improving the instant access to carbon and balancing the nutrition (Giller et al., 1997). Combination of organic manure and mineral fertilizers produces the most favorable conditions for microbial growth which consequently enhances the microbial diversity and biomass and enzymatic activity (Geisseler and Scow, 2014; Zhang et al., 2022, 2024). This synergy enhances the microbial metabolic efficiency and stabilization of soil biochemical processes and, hence, leads to enhanced soil sustainability and resistance in adverse environments (Kuzuyakov and Blagodatskaya, 2015).

In addition to augmenting nutrient circulation and microbial functioning, integrated organic-inorganic fertilization is crucial in enhancing the disease-detering capacity of gypsiferous soils. The growth of microbial

biomass and enzymes will promote the emergence of a diverse and competitive microbial community that can prevent soil-borne pathogens by competitive processes of nutrient and niche, antibiosis, and induced systemic resistance in plants (van Bruggen and Semenov, 2000; Bonanomi et al., 2018). There will be an increase in the activities of dehydrogenase and urease indicating active microbial metabolism which will be favorable to the proliferation of useful microorganisms that are hostile to the pathogens of plants (Kandeler and Gerber, 1988; Dick et al., 1996). The use of organic inputs also increases the synthesis of microbial metabolites and enzymes breaking the cell walls of the pathogens, and the balanced use of inorganic nutrition enhances the activity of plants and their immunity to infection (Bargaz et al., 2018). All these interactions result in a biologically active environment of soil that minimizes the occurrence of diseases and improves crop health thus making integration of the fertilization strategy a significant issue in the sustainable management of diseases in gypsiferous soils in Northern Iraq.

Since the gypsiferous soils occupy a large part of the world and little focus has been given on biological interventions of soil health in such systems, the current research seeks to examine the influence of organic, inorganic and combined fertilizer application methods on biomass of the microorganisms, enzyme activity and efficacy of soil microorganisms of the Salah Al-Din, Northern Iraq. This study aims at being aware of the sustainability and functional amelioration of soil biological indicators, especially in the aspects of soil health and disease inhibition.

Materials and Methods

Study site

The field experiment was conducted during the spring season of 2024 at the experimental farm of the College of Agriculture, University of Tikrit, Salah Al Din Governorate, Iraq. The site was characterized by a semi-arid climate, with average annual rainfall of 250-350 mm and a mean temperature of 22°C. The soil was sandy clay loam with significant gypsum content, typical of the region.

Experimental design

This study was carried out using a Randomized Complete Block Design design having four treatments and three replications. The experimental plots were of sizes of 3 × 4 m with a non-experimental area of 0.5 m amongst experimental plots to reduce edge effects.

Following treatments were evaluated:

T1 = (Control): No fertilization used,

T2 = (Organic): Cattle manure @ 20 tons/ha,

T3 = (Inorganic): 100 kg N per ha, 60 kg P₂O₅ per ha and 40 kg K₂O per ha, and

T4 = (Integrated): Combined application @ 50 percent organic manure (10 tons per ha) and 50 percent inorganic NPK fertilizers (50 kg N per ha, 30kg P₂O₅ per ha and 20kg K₂O per ha).

Baseline soil properties

Before planting, physical and chemical characterization of soil was done by taking composite soil samples at a depth of 0-30 cm of the soil. The soil was determined to be sandy clay loam. Table 1 shows the initial physicochemical characteristics of the gypsiferous soil (0-30 cm soil depth).

Table 1. Initial physicochemical properties of the gypsiferous soil (0-30 cm depth).

Property	Value	Unit
pH (1:2.5 soil: water)	7.23	-
Electrical conductivity	2.3	ds.m ⁻¹
Organic matter	10.6	
Calcium carbonate (CaCO ₃)	182	gm.kg ⁻¹
Gypsum content	64.2	
Total nitrogen (N)	88.4	
Available phosphorus (P)	28.8	
Exchangeable potassium	132.2	mg kg ⁻¹
Bulk density	1.40	g cm ⁻³
Clay	220	
Silt	210	g kg ⁻¹
Sand	570	
Soil texture	Sandy clay loam	-

Soil and microbial analyses

Soil sampling

Soil samples were taken at a soil depth of 0-30 cm at two different times: before planting and 60 days after applying fertilizer. The samples were mixed, sieved (2 mm), and kept at 4°C until microbial and biochemical tests were conducted.

Microbial biomass carbon (MBC)

The microbial biomass carbon was measured using the chloroform fumigation-extraction method as described by Vance et al. (1987). In short, fumigated and non-

fumigated soil samples were extracted with 0.5 M K₂SO₄, and the difference in extractable organic C was converted to MBC using a correction factor (kEC = 0.45).

Basal respirations

Basal respiration was measured by quantifying CO₂ production over 7-day incubation under controlled moisture and temperature conditions. The released CO₂ was captured in 1 M NaOH and determined through back-titration with standardized HCl, following the method of Anderson and Domsch (1978).

Metabolic quotient (qCO₂)

The metabolic quotient (qCO₂) was determined as the ratio of basal respiration (mg CO₂-C kg⁻¹ soil day⁻¹) to microbial biomass carbon (mg MBC kg⁻¹ soil), as described by Anderson and Domsch (1990). This index indicates microbial efficiency and soil stress levels.

Enzymatic activities

Dehydrogenase activity was assessed using the triphenyl tetrazolium chloride (TTC) reduction assay, where TTC is reduced to triphenylformazan (TPF) and measured spectrophotometrically at 485 nm, as described by Casida et al. (1964).

Urease activity was determined by measuring the ammonium (NH₄⁺) released from urea hydrolysis using colorimetric detection at 578 nm, following the method of Kandeler and Gerber (1988).

Microbial diversity indices

The two classic indices used to determine microbial diversity, Shannon-Weiner and Simpson, were each a function of rates derived from microbial community composition data. The pair of figures was not conjured out of thin air, but rather based on the already reported patterns in the literature, and more specifically, those related to gypsum soil. Overall, the full-scale research and the entire related idea were primarily stimulated by the work of Giller et al. (1997), who supported the present assessment of quasi-stable ecological balance and system functioning in such "exotic" soils.

Statistical analysis

The data were combined and subjected to one-way ANOVA to compare treatments. The data were then analysed using Duncan's multiple-range test at P < 0.05 to compare the means of Mi, and ALS factors. Pearson's correlation coefficients were determined to understand variations in the interaction between the microbial indicators, including MBC, enzyme activity, basal respiration, and qCO₂, in all the profile soil samples in the three land-use types.

Results and Discussion

Microbial biomass carbon (MBC)

The results showed that MBC varied significantly at $P < 0.05$ among the fertilization treatments. The LSD range for MBC was 33 mg C kg^{-1} . The maximum MBC (410 mg C kg^{-1}) was recorded in treatment T4, which, according to the LSD test, was significantly different from all other treatments. The organic matter treatment T2 followed the MBC, which was 320 mg C kg^{-1} , more than the chemical fertilizer treatment T3, which was 250 mg C kg^{-1} (Figure 1). The C treatment was more like the fertilized treatments of 180 mg C kg^{-1} . The findings have shown positive interaction effects of INM practices or organic and inorganic fertilizer mixtures with the soil

microbial biomass. The use of organic fertilizers, e.g. farm yard manure, enhanced SOC, substrate availability and conditions of microbial viable habitats. Conversely, the application of NPK mineral fertilizer can be considered an immediate and temporary source of nutrients that may accelerate the development of microbes and their activity. This finding is also in agreement with the explanation by Giller et al. (1997). The combined application of fertilizers to the soil can maintain soil biological health by increasing microbial biomass and diversity. The major advantage of the growth is that there are adequate nutrient secretions and the soil's physical properties when fertilizers are mixed with organic fertilizers.

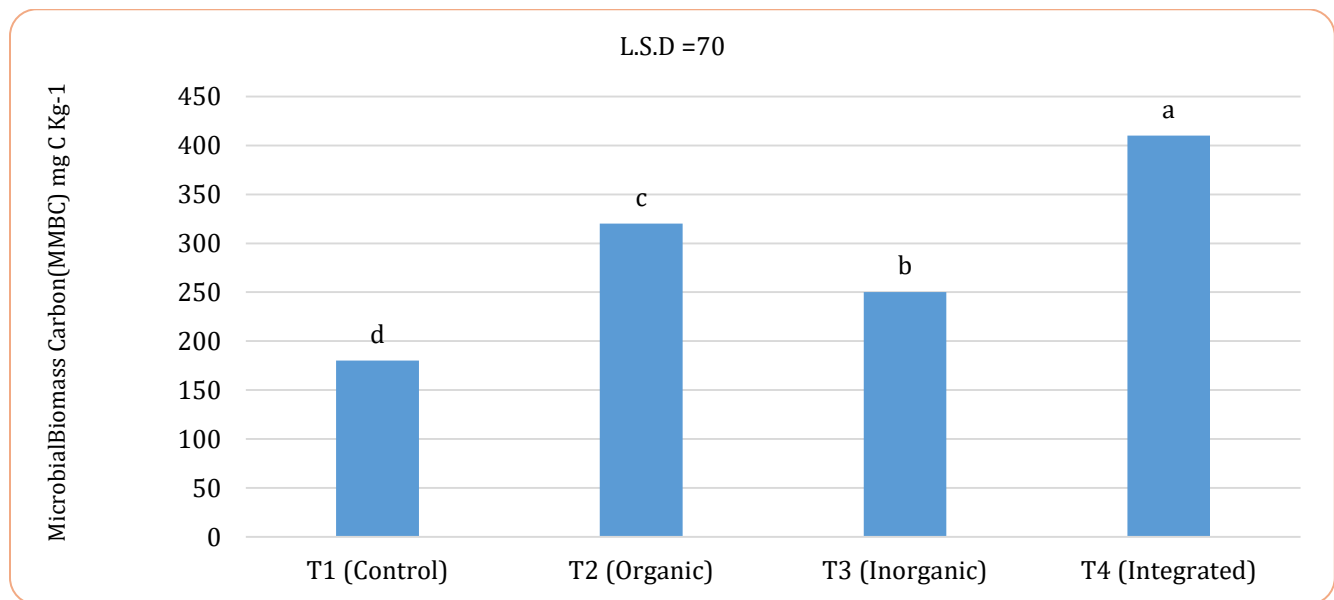


Figure 1: Effect of fertilization treatments on MBC content.

Dehydrogenase activity

The LSD test showed the highest dehydrogenase activity of $45 \mu\text{g TPF g}^{-1} \text{ d}^{-1}$ in the integrated treatment T4. This was significantly higher compared to the other treatments. The organic treatment (T2) had an activity of $32 \mu\text{g TPF g}^{-1} \text{ d}^{-1}$, significantly higher compared to the inorganic treatment (T3) of $25 \mu\text{g TPF g}^{-1} \text{ d}^{-1}$. The control treatment (T1), showed significantly low activity of $15 \mu\text{g TPF g}^{-1} \text{ d}^{-1}$. The means are shown in Figure 2. This response presents a similar trend to MBC and further substantiates the close relationship between microbial biomass and enzyme activity. High dehydrogenase activity in integrated treatment T4 indicates a highly active and metabolically diverse microbial community.

This may occur as a result of the combined effects of organic carbon and mineral elements. Introducing organic matter would provide more labile carbon and a favourable environment for the microbial community, thereby inducing respiration, and enzyme complexes are metabolically activated. On the other hand, mineral fertilizers supply the necessary nutrients, and the selective growth and development of microbial groups enhance oxidative pathways and respiration, as evidenced by dehydrogenase activity. However, a new study by Zhang et al. (2022) showed that integrated fertilization had a synergistic effect, as it had a mega effect on microbial enzymatic activity and soil biochemical health, as predicted.

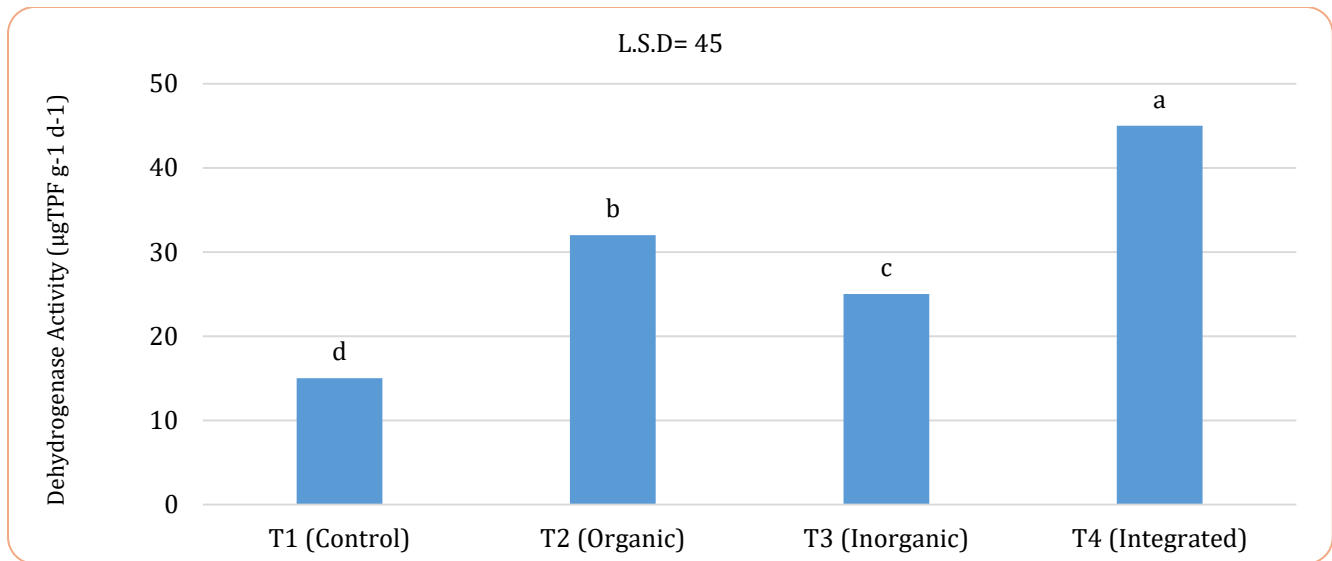


Figure 2. Impact of organic, inorganic, and integrated fertilization on soil dehydrogenase activity.

Urease activity

One-way ANOVA showed that urease activity differed significantly ($P < 0.05$) among fertilization treatments. According to LSD test, T4 exhibited the highest significant urease activity of $55 \mu\text{g NH}_4^+ \text{g}^{-1} \text{h}^{-1}$, which was significantly greater than all other treatments. The organic treatment's higher value ($40 \mu\text{g NH}_4^+ \text{g}^{-1} \text{h}^{-1}$) was significantly different from the inorganic treatment ($30 \mu\text{g NH}_4^+ \text{g}^{-1} \text{h}^{-1}$). The inorganic treatment was also significantly different from the control treatment ($20 \mu\text{g NH}_4^+ \text{g}^{-1} \text{h}^{-1}$), which showed lower values and was statistically different from all fertilized treatments (Figure 3).

The results of the current study strongly claim that the fertilization time had a great impact on urease activity. This was increased further in integrated nutrient management (INM) as demonstrated by the fact that the urease activity in soil was found to be much higher in treatment T4. This growth could be explained by the increased potential of nitrogen mineralization, which is due to a drastic change in the biomass of microorganisms and the rise in enzymatic reactions triggered by an optimal mixture of organic and inorganic fertilizers. Almost all forms of organic amendments, besides enhancing the nitrogen mineralization, are also the primary sources of energy generation, and they trigger the production of urease, thus increasing the activity of urease in the soil. The results are in line with those of Kandeler and Gerber (1988) who suggested that when organic materials are added in the soil, the soil microbes are enhanced and more substrate is available to the

microbial metabolic reactions eventually enhancing the soil enzymatic activities.

Inorganic fertilization (T3), although higher than the control, reduced urease activity and differed significantly from T2 and T4, possibly because there was no organic matter to limit microbial growth, which resulted in low enzymatic production (Zhang et al., 2022). This control treatment (T1) showed the lowest activity, indicating that nutrient supply, particularly organic-based materials, is crucial for nitrogen cycling (Bayu, 2024).

Basal respiration

According to the results of One-way ANOVA, the basal respiration rates were also significantly different in various fertilization treatments ($P < 0.05$), which are presented in Figure 4. The maximum basal respiration was measured in T4 ($65.0 \text{ mg CO}_2\text{-C kg}^{-1} \text{d}^{-1}$), which was also much greater than any of the other treatments by the LSD test. This refers to the increased potential microbial respiration activity that is brought about by the concomitant use of OM and IN.

The basal respiration metabolism ($P < 0.05$) indicated that the basal respiration of organic treatment (T2) was the highest ($48.0 \text{ mg CO}_2\text{-C kg}^{-1} \text{d}^{-1}$) and was significantly higher than that of the control ($35.0 \text{ mg CO}_2\text{-C kg}^{-1} \text{d}^{-1}$) but not significantly different compared with inorganic treatment ($42.0 \text{ mg CO}_2\text{-C kg}^{-1} \text{d}^{-1}$). The slight growth in-organic-only treatment as compared to the control indicates that the use of mineral fertilization alone is sufficient to trigger microbial activity, though not as efficiently as the organic or integrated ones.

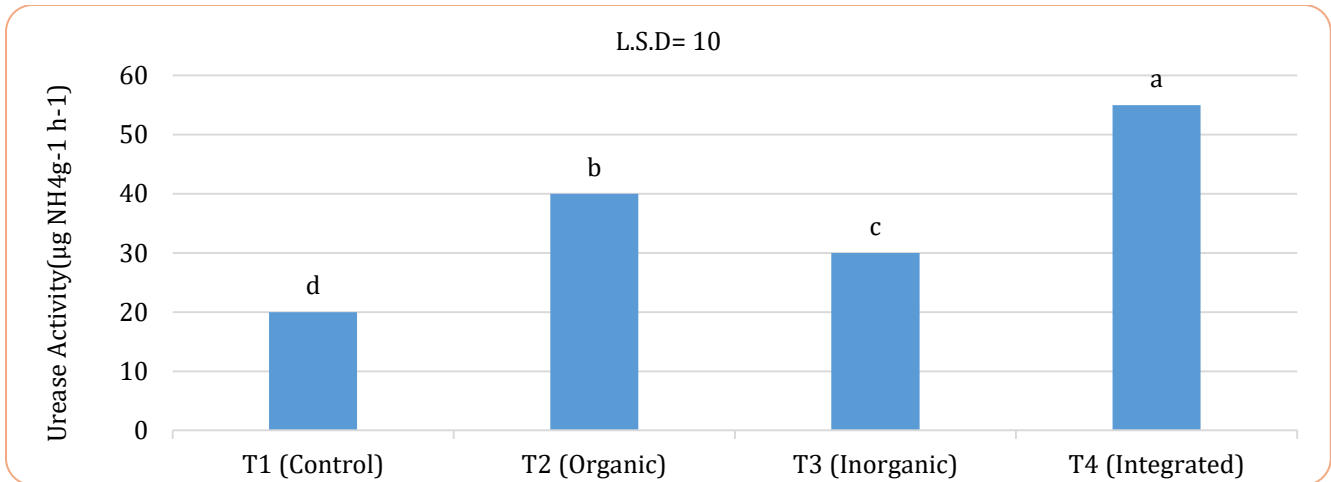


Figure 3. Variation in soil urease activity under different nutrient management practices.

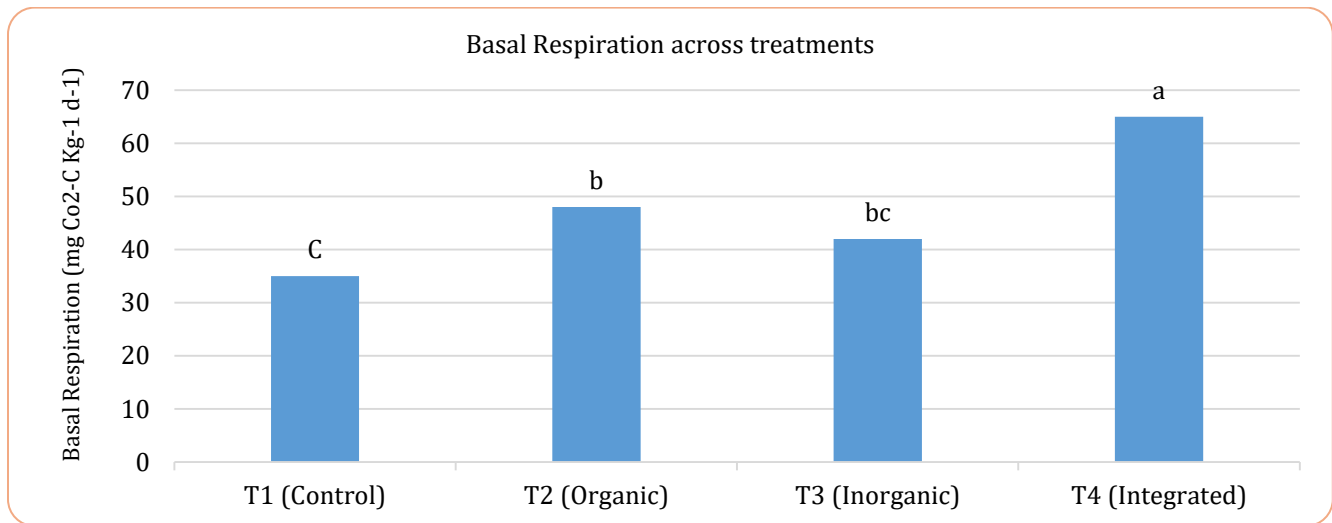


Figure 4. Soil basal respiration response to fertilization treatment.

The T4 results in an increase in the basal respiration, which is presumably caused by the enhanced microbial metabolism probably because of the increased availability of organic matter as a substrate and nutrients provided by mineral fertilizers (López-López et al., 2012). This synergistic impact can provide an improved habitat to the stronger microbial group that serves to increase soil organic carbon conversion and keep the soil carbon circulation by enhancing soil fertility and ecosystem services (Wu et al. 2024).

The results substantiate that the combination nutrient management that was reported previously, has the potential to further improve the microbial activity of soil compared to the application of a single fertilizer. Guo et al. (2023) tested the effect of 1 g organic plus mineral

fertilization and showed that the rate of soil respiration was about 40% higher than in the control of agricultural semi-arid soils. Li et al. (2022, 2024) also meta-analyzed 6 RCTs and found the synergistic effect of organic amendments and mineral fertilizers on the parameters of carbon dynamics of microorganisms and the quality of soil. Next, high basal respiration means that the biomass of microbes and activity of enzymes of microbes are great and is conducive to the activity of the SCM (Semenov et al., 2025). Alternatively, the possibility of microbial stress or carbon depletion can also be indicated by over-respiration, and combination treatments with compensatory respiration rates might prove to be more sustainable in managing soil (Imran, 2025).

In conclusion, basal respiration rates under IRF (T4)

were significantly greater, which proved the successful promotion of soil microbial activity and C cycle due to the presence of both organic and inorganic sources and subsequent increase in soil quality and subsequent increase in crop productivity.

Microbial metabolic quotient (qCO_2)

One-way ANOVA regression analysis showed that there were significant differences among treatments ($P < 0.05$) in qCO_2 . The LSD confirmed this value as the lowest and unlike all other treatment qCO_2 output values (0.18 mg CO_2 -C g⁻¹ MBC d⁻¹: T4), which showed that it was more efficient. Intermediate values were observed between the T2; 0.25 and T3; 0.23 treatments and T4 and control (T1) that had the highest qCO_2 value of 0.28, and was significantly different compared to all other treatments (Figure 5).

It was also found that I funds had higher microbial efficiency. Reduced qCO_2 indicates the lower CO_2 release per unit MG and the increased efficiency of energy utilization (Wang and Kuzyakov, 2023). This can be attributed to different factors such as balanced nutrition

and carbon in nutrients during integration of organic and inorganic fertilization because of the better quality of substrates and coordination of microbial nutrient needs (Ashraf et al., 2022).

On the other hand, the control (T1) had comparatively higher levels of qCO_2 , which means that there were more nutrient-stressed microbial communities with a high respiration rate to sustain basic metabolism. Besides, the inorganic-only (T3) treatment was higher than the control, but it was not complete as some organic substrates are required to maintain optimum microbial metabolism. The results are in agreement with Anderson and Domsch (1995) where the lowest qCO_2 -values were lower than the highest.

It concludes that qCO_2 should be considered to be a more or less physical constraint, such as substrate depletion, which slows the reaction of the factor since microbial processes continue to occur during the period required to generate E m into lower dark H_2 release and carbon shutdown. Mixed fertilization also has a positive effect on microbial metabolism and decreases soil CO_2 flux (Bargaz et al., 2018).

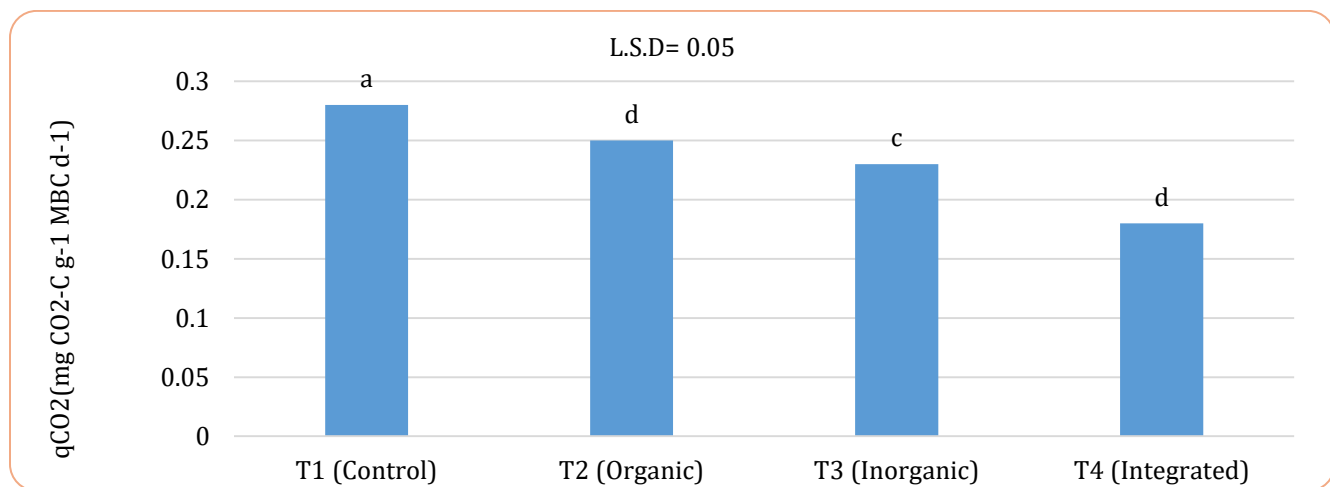


Figure 5. Effect of fertilization strategies on microbial metabolic efficiency (qCO_2).

Microbial diversity (Shannon Index)

A one-way ANOVA on the treatments of fertilization revealed that they were different in regards to the Shannon index (Shannon, 1948; Simpson, 1949). No significant difference ($p > 0.05$) was observed in the two groups in microbiological and fundamental properties, such as pH and VFA. According to the LSD test, the T4 had the higher and significantly different value of Shannon diversity index (3.0) as compared to T2 (2.7)

and T3 (2.4), both of which were higher than the control. The minimum was 1 at 2 which was considerably different from all the other treatments (Figure 6).

These results suggest that the use of IM helps enhance IMO activity and diversity. The observed diversity in T4 is very different and functionally diverse which is equal to high diversity of organic matter, N balance and microhabitat diversity due to the concomitant use of manure and mineral fertilizers (Figure 6). The Shannon

index of diversity is an ecological index of standard diversity and uniformity of microbes in an ecosystem (Hill, 2003). The implication of being similar to what was experienced in T4, generally is that the increases will be correlated to increases in the ecosystems processes, the rate of biogeochemical cycles, the disease suppressive properties and the soil resistance to stress. The sole organic amendment increased microbial diversity as compared to the control because of organic carbonian additions and quality and quantity of microbial foodstuffs. But despite the lack of any organic matter in

T3, it was still less diverse than T1 and T2. This demonstrates that variability and diversity of the microbes, as well as diversity of the habitat, were constrained by the availability of the nutrients.

These findings are in agreement with those mentioned by Giller et al. (1997) and other studies on gypsiferous soils and semi-arid areas (Khairo, 2024). According to van Bruggen and Semenov (2000) and Bonanomi et al. (2018), the positive effect of integrated fertilizer can be because it promotes the development of a range of microbial communities that facilitate certain functions.

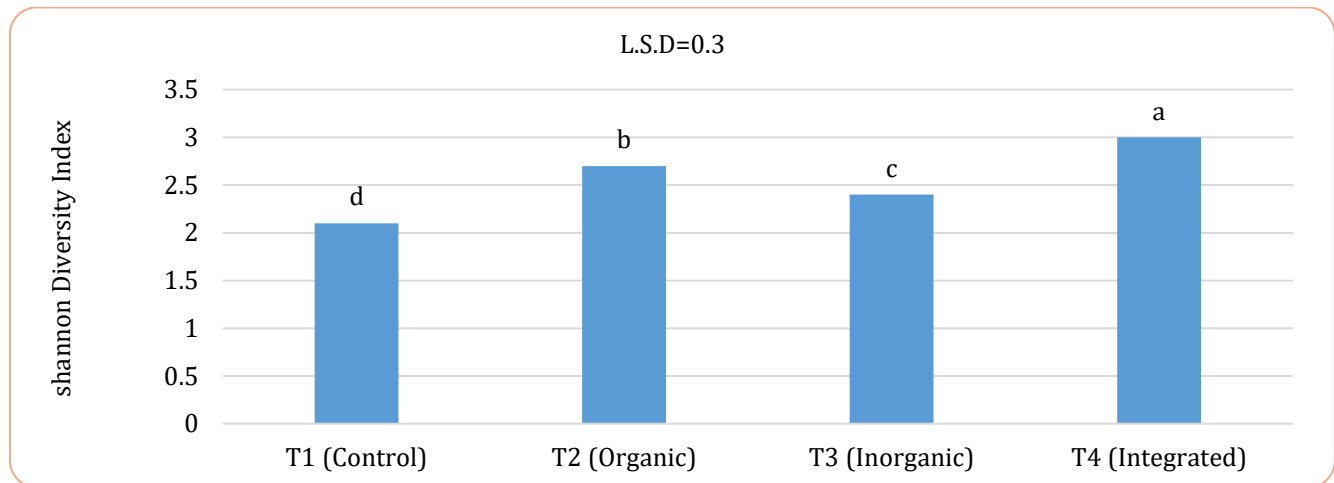


Figure 6. Impact of fertilization regimes on soil microbial diversity (Shannon Index).

Conclusions

In conclusion, the findings of this research showed that organic-inorganic fertilization had significant enhancement of soil biological properties of gypsiferous soils. Gypsiferous soils treated with composting of cattle manure and NPK fertilizers had the most MBC, dehydrogenase. On the other hand urease had the lowest metabolic quotient that means the highest microbial efficiency and others have the lowest stress under the circumstances of the lowest physiological stress. The corroborating information is that the integrated means of fertilization are the positive attributes of nutrient content and microbial activity and diversity in comparison with the treatment as an independent organic or inorganic fertilization. These results indicate that organic amendments have a significant effect during the fertilization process especially when there are nutrient deficient environments or structurally limiting environments such as the gypsum-affected soils. Moreover, the long-term effects of Integrated Weed

Management on agricultural productivity, carbon sequestration, and the dynamics of microbial communities should be studied by other field studies during different years. Such microbiological and agronomic data can be utilized to make a more detailed picture of sustainable soil fertility management in gypsum-based methods.

Authors' Contributions

HMY and KWA conceptualized and designed the study, developed the experimental framework, carried out the data analysis and interpretation, and prepared the original draft of the manuscript. SNM and RQK contributed through critical supervision, methodological guidance, and constructive scientific input, offering valuable comments and suggestions that substantially improved the quality, coherence, and clarity of the manuscript. All authors participated in reviewing and revising the manuscript, approved the final version for publication, and agreed to take full responsibility for the accuracy, integrity, and reliability of the work reported.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger

SDG 12: Responsible Consumption and Production

SDG 15: Life on Land

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