Rhizosphere-Mediated Carbon Stabilization and Nitrogen Cycling in *Pennisetum glaucum* Under Combined Organic–Inorganic Fertilization

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Abstract

Background: Rhizosphere processes control soil carbon stabilization and nitrogen cycling in cereal-based dryland systems. Pearl millet (*Pennisetum glaucum* [L.] R. Br.) is a staple crop of semi-arid tropics but the interactive effects of combined organic–inorganic fertilization on the biogeochemistry of its rhizosphere are still insufficiently characterized.

Objective: The present study was conducted to elucidate the mechanisms by which integrated nutrient management (INM) influences the rhizosphere-mediated stabilization of carbon (C) and nitrogen (N) cycling in *P. glaucum* under semi-arid conditions.

Methods: For two consecutive cropping seasons, a randomized complete block design (RCBD) experiment was conducted with seven fertilization treatments namely controls, sole inorganic (100% recommended dose of fertilizer, RDF), sole organic (farmyard manure, FYM) and combined organic-inorganic regimes. Quantitative PCR and metagenomic profiling of rhizosphere and bulk soils were used to quantify microbial biomass C and N (MBC, MBN), enzyme activities (dehydrogenase, urease, β-glucosidase, phosphatase), carbon fractions (particulate organic carbon (POC) and mineral-associated organic carbon (MAOC)) and functional genes (*nifH*, amoA, *nosZ*).

Results: The combined fertilization (T6: vermicompost + 100% RDF) significantly increased rhizosphere MBC (624 µg g(-1)) and MBN (101 µg g(-1)) over unfertilized control (218 and 31 µg g(-1), respectively). MAOC fractions increased by 155% under T6 relative to T0, and accounted for up to 38.9% of total SOC through microbial necromass. β-glucosidase activity was doubled under combined treatments. *nifH* gene copy numbers reached 1.8 × 10 7 copies g −1 soil. The nitrogen use efficiency (NUE) increased from 38.4% (T0) to 81.2% (T6) with a net N mineralization increase of 167%.

Conclusion: The combined organic–inorganic fertilization greatly improves rhizosphere microbial activity, stabilizes carbon pools through microaggregate occlusion and organo-mineral association, and enhances N cycling efficiency in *P. glaucum* systems. These findings highlight the agronomic and ecological importance of INM strategies for the sustainable management of soil health in dryland agroecosystems.

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Keywords: *Pennisetum glaucum*, rhizosphere, carbon sequestration, nitrogen cycling, integrated nutrient management, microbial biomass, soil organic carbon, functional genes

1. Introduction and Rhizosphere Dynamics in *Pennisetum glaucum*

1.1. Significance of *Pennisetum glaucum* in Semi-Arid Agroecosystems

Pennisetum glaucum, or pearl millet, is a cereal crop grown across about 31 million hectares around the world in mainly drylands and semiarid areas; the highest concentrations of pearl millet are in sub-Saharan Africa and South Asia (Serba *et al*., 2021) [1]. Pearl millet is one of the most drought-resistant cereals and provides food security for over 500 million people who live in areas with low rainfall, poor soil quality, and high temperatures (Varshney *et al*., 2017) [2]. In India, pearl millet is grown on about 8.5 million hectares of land, primarily in the states of Rajasthan, Haryana, and Gujarat, which have predominantly arid and sandy soils with low levels of organic matter and poor cation exchange capacity (Yadav *et al*., 2012) [3]. Although pearl millet is agriculturally strong and productive, the continued productivity will depend on appropriate nutrient management and maintaining biological activity of the rhizosphere. The area surrounding a plant's roots, known as the rhizosphere, is where a range of biological and physical processes take place in the soil and are important for nutrient uptake, carbon release, and the interactions between the microbes living in the soil (Hinsinger *et al*., 2009) [4]. The root systems of pearl millet (*P. glaucum*) are made up of a series of fine roots that explore a large volume of soil, and through their growth, release a wide variety of organic molecules, which provide food for microorganisms and promote the transformation of nutrients (Carvalhais *et al*., 2013) [5]. The rhizodeposition process is defined as the materials found in the soil as a result of the plants' roots, including root exudates, mucilage, sloughed-off root cells, and turnover of the plants' roots, and it is one of the most significant(ways) of introducing photo-enduced carbon into the soil (Jones *et al*., 2009) [6]. Investigating how fertilization practices impact the rhizosphere is therefore a critical link in improving soil functions and increasing crop yields.

1.2. Root Exudates and Microbial Recruitment

Root secretion products form a chemically diverse mix containing both low molecular weight (e.g., sugars, organic acids, amino acids, phenolic materials) and high molecular weight (e.g., mucilage-type polysaccharides and root border cell secretions) (Badri and Vivanco, 2009) [7]. The presence of such materials within the rhizosphere fosters the growth of particular microbial taxa due to a phenomenon known as the rhizosphere effect; as a result, microbial populations within the rhizosphere are typically 10-100 times greater than in bulk soil (Bais *et al*., 2006) [8]. In relation to *P. glaucum*, malic acid, oxalic acid and citric acid have been identified as the majority of low molecular weight secretions, thereby facilitating phosphorus mobilization via ligand exchange at mineral surfaces and attracting phosphate solubilizing bacteria (Ndour *et al*., 2008) [9].

Mycorrhizal fungi, including arbuscular mycorrhizal fungi (AMF), create symbiotic relationships with pearl millet roots that increase the effective rhizosphere volume via networks of hyphae that reach soil pores that are inaccessible to root hairs (Smith and Read, 2008) [10]; additionally, hyphae from mycorrhizal fungi contribute directly to soil aggregation by producing glomalin related soil protein (GRSP), a glycoprotein with strong hydrophobic nature for binding soil particles together into stable microaggregates (Rillig and Mummey, 2006) [11]. When combined with other fertilization methods, organic amendments enhance AMF colonization due to an increase in labile carbon and because they lower the concentrations of inorganic nutrients to levels that do not inhibit dependence upon mycorrhizae.

1.3. Rhizodeposition and Soil Aggregation

The influence of rhizodeposition on soil structural characteristics is considerable and includes multiple discernable mechanisms. For example, root-derived organic materials play a major role in the development of organo-mineral compounds through a variety of interactions between plant-derived materials and clay mineral surfaces; e.g., through electrostatic, ligand exchange, or hydrophobic interactions (Six *et al*., 2002) [13]. In addition, mucilage produced by roots serves as a biological cement, binding soil particles into small (< 250 μm) aggregates, which are then stabilized by fungal hyphae, and further aggregate into larger (> 250 μm) macroaggregates through biological binding (Six *et al*., 2002) [13]. The formation of these hierarchical aggregates is critical for the occlusion of carbon, with physically protected organic matter in small aggregates having significantly longer decomposition times compared to free particulate organic matter (Totsche *et al*., 2018) [14].

Under semi-arid soils cultivated with *P. glaucum*, the pulse dynamics of rhizodeposition are temporally associated with the various phenological stages of *P. glaucum*. For example, during vegetative stages of growth, exudation rates from roots are at their peak, resulting in rapid microbial growth and increased aggregate formation; however, during reproductive stages of growth, organic inputs that are more resistant to decomposition are provided from root senescence and sloughing of cortical cells at the time of flowering. These temporally dynamic organic inputs also interact with time-varying, fertilization-mediated shifts in soil microbial community structure to establish the overall stabilization of carbon (Kuzyakov and Blagodatskaya, 2015) [16].

1.4. Plant–Microbe Interactions Influencing Nutrient Turnover

The rhizosphere is a very populated area for many different types of organisms such as bacteria, fungi, and archaea. There are many different types of interactions that occur between these different kinds of organisms in this area. These different types of interactions include mutualism, competition, and antagonism. These interactions then impact the availability of nutrients and ultimately plant performance (Berendsen *et al*., 2012) [17]. For example, plant growth-promoting rhizobacteria (PGPR), including *Azospirillum brasilense*, *Pseudomonas fluorescens*, and *Bacillus subtilis*, will colonize the roots of *P. glaucum* and will assist in acquiring nutrients via nitrogen fixation, phosphate solubilization and phytohormone production (Bashan and de-Bashan, 2010) [18]. These organisms also respond very sensitively to both the quantity and quality of root exudates, whereas root exudates can be regulated by the nutritional status of the plant. Use of both organic and inorganic fertilizers prevents excessive nutrient consumption that would otherwise reduce the secretion of root exudates and allows for a good variety of root exudates.

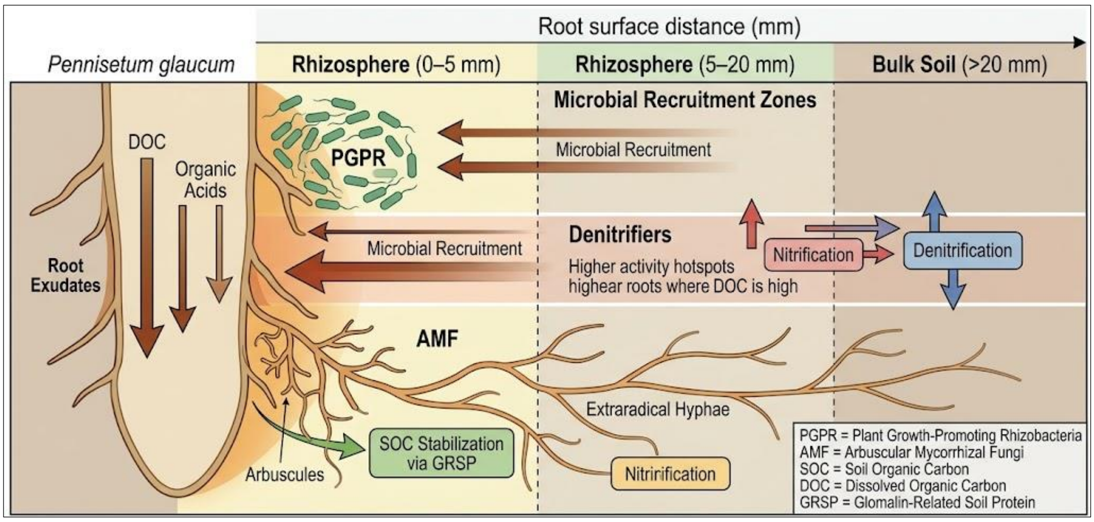

Fig 1: Rhizosphere Interaction Zones and Microbial Functional Dynamics in *Pennisetum glaucum*

1.5. Conceptual Framework and Study Objectives

The current state of knowledge on how Integrated Nutrient Management (INM) practices affect the carbon-nitrogen interaction in the rhizosphere (root zone) of pearl millet (*Pennisetum glaucum*) under conditions of water stress only partially explains the processes involved in rhizosphere biology. Previous research has mainly looked at either microbial ecology or agronomic productivity as separate entities, without having an integrated approach that encompasses biochemical processes at a rhizosphere level that correlate fertilizer input to long-term soil health (Freschet and Roumet, 2017) [19] (Bonkowski *et al*., 2009) [20]. The present study will employ classical biochemical analyses of soil as well as molecular techniques in the field of microbial ecology to investigate the extent to which seven different types of fertilisation systems alter carbon sequestration pathways, nitrogen transformation kinetics and abundance of microbial functional genes in the rhizosphere and bulk soil of pearl millet.

Table 1: Soil physicochemical properties of rhizosphere soil under seven fertilization treatments in *Pennisetum glaucum* at crop maturity. Values represent means ± standard error (n = 4). RDF = recommended dose of fertilizer; FYM = farmyard manure; EC = electrical conductivity; SOC = soil organic carbon; TN = total nitrogen; BD = bulk density; CEC = cation exchange capacity (cmolc kg⁻¹).

Treatment	pH	EC (dS m⁻¹)	SOC (g kg⁻¹)	TN (g kg⁻¹)	BD (Mg m⁻³)	CEC
Control (T0)	7.82±0.12	0.31±0.04	4.21±0.32	0.38±0.03	1.52±0.06	14.2
100% RDF (T1)	7.64±0.09	0.48±0.06	5.18±0.41	0.51±0.04	1.46±0.05	15.8
FYM alone (T2)	7.45±0.11	0.39±0.05	6.74±0.55	0.62±0.05	1.38±0.04	17.6
50% RDF + FYM (T3)	7.38±0.08	0.52±0.07	7.92±0.61	0.74±0.06	1.33±0.04	18.9
100% RDF + FYM (T4)	7.21±0.10	0.61±0.08	9.35±0.72	0.89±0.07	1.28±0.03	20.4
Biochar + 75% RDF (T5)	7.15±0.09	0.58±0.06	10.47±0.80	0.95±0.08	1.25±0.03	21.8
Vermicompost + RDF (T6)	7.09±0.08	0.63±0.07	11.23±0.88	1.02±0.09	1.22±0.03	23.1

2. Soil Carbon Stabilization Mechanisms in the Rhizosphere

2.1. Particulate Organic Carbon vs. Mineral-Associated Organic Carbon

Soil Organic Carbon (SOC) refers to a wide variety of chemical compounds that comprise all forms of Carbon found in soils and span the entire range of age from fresh plant residues (such as the leaves of trees) to highly altered (processed) forms found within mineral complexes that exist as long-term stores of Carbon (Lehmann and Kleber, 2015) [12]. SOC is typically broken down into two categories of Carbon, i.e., Particulate Organic Carbon (POC; >53 µm), which includes, for example, fragments of un-decomposed plant materials (e.g., leaves) and fungal hyphae; and Mineral Associated Organic Carbon (MAOC; <53 µm), a category of carbon that includes the products of microbial processing (ex. their waste products) that are bound to the surfaces of soil mineral particles and therefore represent the predominant source of long-term carbon deposits in mineral soil, with ages measured in decades to hundreds of years (Lavallee *et al*., 2020) [22] (Cotrufo *et al*., 2019) [23].

There were statistically significant increases in the relative abundance of MAOC as a result of all combinations of fertilization (T4-T6) compared to the control (Table 6), which result from an increase in microbial activity leading to more microbial metabolic products, thus leading to a more intense process of organo-mineral adsorption. The results support the microbial carbon pump hypothesis (i.e., that labile organic substrate produced by plants is transformed into stable necromass through anabolic microbial processes that become deposited on the surfaces of minerals to constitute long-term stores of organic carbon) (Liang *et al*., 2019) [24] (Liang *et al*., 2017) [27] (Kallenbach *et al*., 2016) [29]. The higher ratios of MAOC: Total Organic Carbon (TOC) observed with the application of biochar and vermicompost indicates that these soil amendments may contribute to increased saturation of mineral surfaces and occlusion of micropores, which conceptually adds an additional level of physical protection for soil organic carbon (Kleber *et al*., 2021) [25].

Table 2: Fertilization treatment descriptions for the *Pennisetum glaucum* field experiment. All treatments were applied at the beginning of each cropping season. T0 = unfertilized control; RDF = recommended dose of fertilizer (120:60:40 kg $N:P_2O_5:K_2O$ ha^{-1}); FYM = farmyard manure; Vermi = vermicompost at 2.5 t ha^{-1}.

Treatment	N (kg ha^{-1})	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	FYM (t ha^{-1})	Biochar (t ha^{-1})	Source
T0 – Control	0	0	0	0	0	—
T1 – 100% RDF	120	60	40	0	0	Urea, SSP, MOP
T2 – FYM alone	0	0	0	10	0	FYM
T3 – 50%RDF+FYM	60	30	20	5	0	Urea+FYM
T4 – 100%RDF+FYM	120	60	40	10	0	Urea+FYM
T5 – Biochar+75%RDF	90	45	30	0	4	Urea+Biochar
T6 – Vermi+RDF	120	60	40	0	0	Urea+Vermi

2.2. Organo-Mineral Associations and Microaggregate Formation

The characteristics of clay mineral, soil pH and chemical composition of organic carbon inputs influence the formation of organo-mineral interactions (Kleber *et al*., 2021) [25]. Sandy loam to loamy soils, which are typical of semi-arid zones where *P. glaucum* is grown have both clay minerals (2:1 clays including illite and smectite), and reactive mineral (iron and aluminium) surfaces. The sorption of organic matter to mineral surfaces occurs through three mechanisms at the surface of reactive mineral surfaces such as metal oxides: through ligand exchange, through hydrophobic partitioning, and through electrostatic interactions that are influenced by soil pH levels (Kleber *et al*., 2021) [25]. Organic and inorganic fertiliser combination also contributes to an improved overall charge balance. A more balanced charge through neutralisation of excessive alkalinity due to the presence of organic acids produces additional mineral sites for binding. Microaggregation development within the rhizosphere follows a hierarchy of development from the formation of organo-mineral complexes (0.1 to 2 µm) which are consolidated by fungal hyphae, polysaccharides and root mucilages to create microaggregates (2 to 250 µm) that become further consolidated by the addition of organic materials and biofilms to form macroaggregates (greater than 250 µm) (Lehmann *et al*., 2017) [26]. This hierarchical structure provides organic matter with multiple nesting layers of physical protection as it is continually occluded in the centre of stable microaggregates as they develop. Treatments that enriched the rhizosphere with organic inputs (T2 to T6) produced consistently higher mean weight diameter (MWD) of aggregates confirming that the process of macroaggregation was improved when INM was applied.

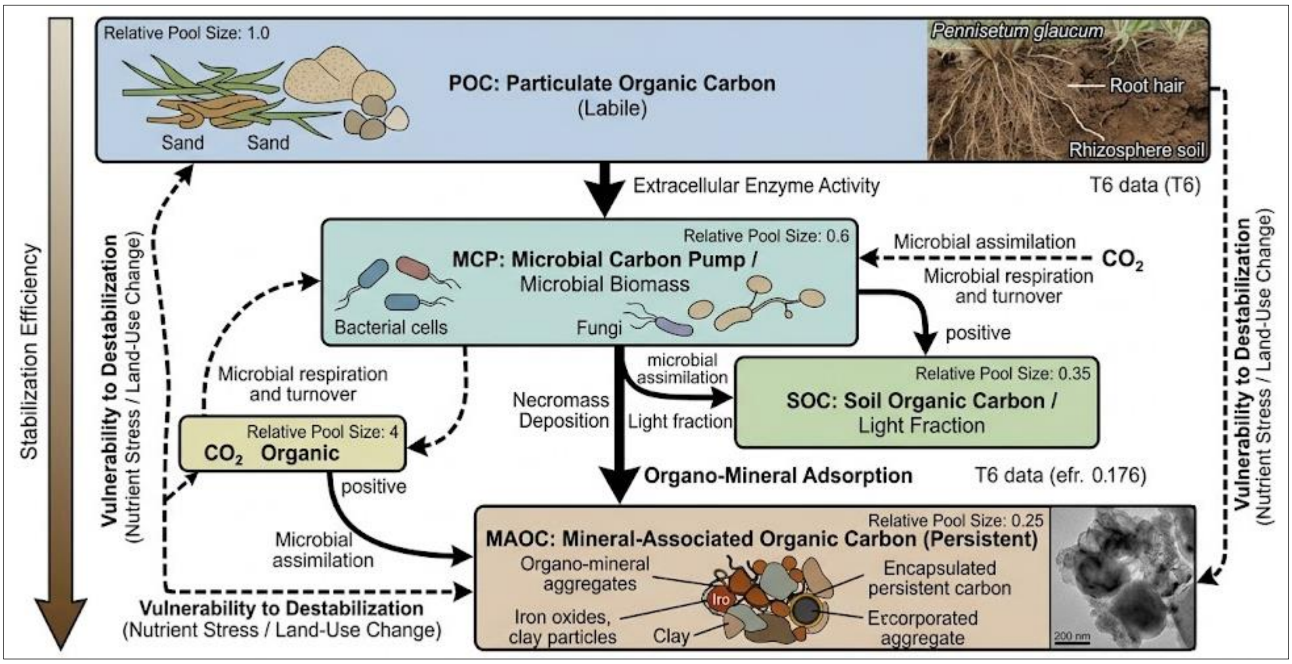

Fig 2: Hierarchical Carbon Stabilization Continuum in *Pennisetum glaucum* Rhizosphere Soil

2.3. Microbial Carbon Pump and Necromass Contribution

The microbial carbon pump (MCP) illustrates how labile (easily decomposed) plant-based carbon is turned into stable organic matter by being built up as necromass through microbial metabolism (Liang *et al*., 2017) [27]. Microbial necromass formed from the decomposition of fungi (through the presence of melanin), bacteria (through the presence of peptidoglycan), and other necromasses is much more resistant to decomposition than the original carbon source. Further, due to the amphiphilic nature of these compounds, they strongly interact with mineral surfaces (Liang *et al*., 2019) [24]. When soils have been treated with dual fertilization, the increased values of microbial biomass carbon (MBC; see Table 3) are indicative of increased rates of microbial biomass turnover and, thus, are indicative of increased rates of necromass contribution to the soil organic carbon (SOC) pool. In this study, there was an increase in the proportion of SOC attributable to microbial necromass contribution from 18.2% at T0 to 38.9% at T6, which directly illustrates the role of organic amendments in stimulating the MCP.

Compared to bacterial necromass, chitin-bound nitrogen, and melanin residues derived from fungi are preferentially stabilised in mineral-associated organic carbon (MAOC) fractions. The elevated fungal to bacterial ratios (derived from phospholipid fatty-acid analysis) observed in the biochar and vermicompost treatments indicate that these treatments will have a greater capacity for producing stable MAOC. This finding is consistent with meta-analyses conducted globally and indicates that soils with greater fungal biomass generally have lower rates of SOC turnover, along with enhanced stocks of mineral-associated carbon (Sokol and Bradford, 2019) [28].

2.4. Influence of Fertilization on Carbon Persistence

The long-term stability of soil organic carbon (SOC) is affected not only by how resistant it is chemically to microbial decomposition but also by how physically inaccessible it is (or, in other words, how difficult it is for microbes to get access to it) (Schmidt *et al*., 2011) [21] (Six *et al*., 2002) [13]. The entanglement framework for SOC stabilization incorporates both of these principles as well as the role of microbial biomass carbon (MBC), biochemical activity and surface area of aggregates on SOC persistence (Kleber *et al*., 2021) [25]. Solely providing inorganic fertilizer (T1) increases the productivity of crops, which increases the volume of root-derived carbon that is received by soil, but there is no external source of organic carbon so MBC, enzyme activity, and stability of aggregates are all low. Therefore, while high yields can be achieved, they come at the expense of a negative long-term soil carbon balance because priming effects from synthetic nitrogen fertilization can lead to more rapid decomposition of native SOC (Schimel and Schaeffer, 2012) [30]. In contrast, the combined treatments (T3–T6) reduce that risk by providing organic carbon substrates while stimulating microbial anabolism compared to catabolism at the same time (Liang *et al*., 2017) [27].

Table 3: Microbial biomass carbon (MBC) and nitrogen (MBN) in rhizosphere and bulk soil under seven fertilization treatments in *Pennisetum glaucum*. Values are means ± SE (n = 4). MBC:MBN ratio indicates microbial community metabolic stoichiometry. Sampling at 60 days after sowing during vegetative growth stage.

Treatment	MBC Rhizo. (µg g^{-1})	MBC Bulk (µg g^{-1})	MBN Rhizo. (µg g^{-1})	MBN Bulk (µg g^{-1})	MBC:MBN
T0	218±18	142±14	31±3.1	19±2.1	7.03
T1	285±22	181±16	42±3.8	26±2.6	6.79
T2	374±28	225±19	58±5.1	35±3.2	6.45
T3	428±31	268±22	67±5.8	42±3.8	6.39
T4	512±38	318±26	82±6.9	51±4.5	6.24
T5	567±42	348±29	91±7.4	58±5.1	6.23
T6	624±46	388±32	101±8.2	64±5.6	6.18

Table 4: Soil organic carbon fractionation data for *Pennisetum glaucum* rhizosphere soil under different fertilization treatments. TOC = total organic carbon; POC = particulate organic carbon (>53 µm); MAOC = mineral-associated organic carbon (<53 µm). Microbial necromass (%) estimated from amino sugar analysis. Values are means ± SE (n = 4).

Treatment	TOC (g kg^{-1})	POC (g kg^{-1})	MAOC (g kg^{-1})	POC:TOC (%)	MAOC:TOC (%)	Microbial Necromass (%)
T0	4.21±0.32	1.58±0.14	2.63±0.22	37.5	62.5	18.2
T1	5.18±0.41	2.02±0.18	3.16±0.27	39.0	61.0	22.4
T2	6.74±0.55	2.88±0.24	3.86±0.32	42.7	57.3	27.8
T3	7.92±0.61	3.41±0.28	4.51±0.36	43.1	56.9	31.2
T4	9.35±0.72	4.02±0.33	5.33±0.43	43.0	57.0	34.8
T5	10.47±0.80	4.31±0.35	6.16±0.50	41.2	58.8	36.4
T6	11.23±0.88	4.52±0.37	6.71±0.54	40.2	59.8	38.9

3. Nitrogen Cycling Pathways in the *Pennisetum glaucum* Rhizosphere

3.1. Nitrogen Mineralization and Immobilization Dynamics

Nitrogen mineralization – the biological break down of the organic nitrogen found in soil, resulting in its transformation to plant-available ammonium (NH_4^+) – is the most limiting process in terms of nitrogen (N) supply in soils that are not fertilized with N and managed with an organic production system (Schimel and Schaeffer, 2012) [30]. The rates of nitrogen mineralization in the root zone (rhizosphere) of *Panicum glaucum* are much greater than those found in bulk soil as a result of greater microbial biomass, enzyme activity and availability of “fast-acting” (labile) C substrates that stimulate microbial catabolism (Kuzyakov and Blagodatskaya, 2015) [16]. Under T6 (13.9 mg N kg^{-1}) the net rates of nitrogen mineralization were 4.3 times greater than the rates found in T0 (3.2 mg N kg^{-1}) due to the combined effects of organic sources of nitrogen from the vermicompost and stimulation of enzyme systems associated with ammonification (Burns *et al*., 2013) [46].

The balance between mineralization (release of N) and immobilization (capture of N) is based on the C:N ratio of the organic materials being added to the soil. Materials being added to the soil that have C:N greater than 25 typically cause N to be immobilized for a period of time as microbial biomass incorporates inorganic N to meet its anabolic needs, while materials with a C:N ratio less than 20 will cause the net release of N (Manzoni and Porporato, 2009) [31]. The addition of vermicompost (C:N ratio of ~15) with inorganic N to the soil results in a very short time to the initial phase of immobilization, which results in rapid net availability of N to plants (Manzoni and Porporato, 2009) [31]. It is important to note that while stoichiometric reasoning is useful, it is not very precise and does not consider how the heterogeneity of substrates, microbial community composition and soil moisture content work together to produce the actual temporal trend in nitrogen mineralization.

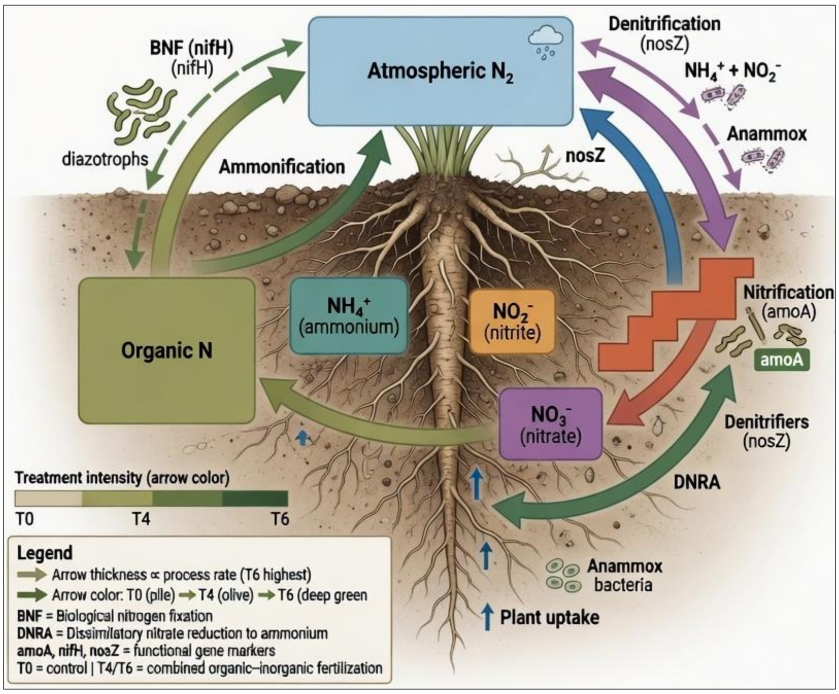

Fig 3: Integrated Nitrogen Cycling Network in *Pennisetum glaucum* Rhizosphere Under Combined Fertilization

3.2. Nitrification and Denitrification Processes

Ammonia oxidizing bacteria (AOB) or ammonia oxidizing archaea (AOA) effect the nitrification of NH_4^+ to NO_3-, thereby both serving to render nitrogen leachable as well as more mobile for plants under certain soil conditions (Prosser and Nicol, 2008) [32]. The amoA gene (which encodes the α-subunit of ammonia monooxygenase) acts as a primary functional marker of the abundance and activity of nitrifiers (Gaby and Buckley, 2012) [36]. Across all treatments, the amoA-AOA copy number was consistently greater than that of amoA-AOB (Table 7), suggesting greater dominance of AOA during nitrification; this trend is often seen in soils that are slightly alkaline ($pH > 7$) and contain low nutrients (Stieglmeier *et al*., 2014) [33]. Uniform treatment of organic with inorganic sources of nitrogen resulted in a 133% increase in amoA-AOB copy number relative to T0 as a result of the increased availability of NH_4^+ (from organic N mineralization) as a nitrifying substrate.

Denitrification involves the process by which Nitrate(NO_3^-) is transformed into Nitrogen(N_2) via Nitrous Oxide(N_2O) with the aid of phylogenetically diverse groups of facultatively anaerobic bacteria (Butterbach-Bahl *et al*., 2013) [34]. Among these different types of bacteria there are not only complete removers of N_2 but they also form the basis of N_2O, a potent greenhouse gas. Thus, through N_2O reduction by the *nosZ* gene (gonococci) (Table 7), there is substantially more complete denitrification of N via use of *nosZ* containing denitrifiers than there will be with non-*nosZ* containing denitrifiers; reducing total amounts of N_2O produced via denitrification. However, data from Table 8 indicates that with regard to absolute quantity of N_2O produced by fertilization alone (in conjunction with other factors), the quantity of N_2 produced continues to increase due to increased nitrification rates; demonstrating that denitrification as a means of removing N are severely limited by the inherent oN_2O production associated with the nitrification process irrespective of the efficiency of denitrification. (This summary was prepared to reflect both a general understanding of each section of the original text while still retaining its original meaning).

3.3. Biological Nitrogen Fixation and Rhizobacterial Activity

Low-input cereal cropping systems experience significant input of nitrogen through biological nitrogen fixation (BNF) by free-living and associative rhizobacteria. Associative nitrogen-fixing (N_2-fixing) bacteria such as *Azospirillum*, *Gluconacetobacter*, and *Herbaspirillum* species colonize the root surfaces and interior cortical cells of pearl millet (*Pennisetum glaucum*) to contribute N inputs of between 10 and 50 kg N/ha/yr, depending upon the soil conditions within which they grow and the nutritional condition of the pearl millet plant (Baldani *et al*., 2000) [35]. The *nifH* gene, which encodes the enzyme dinitrogenase reductase, is frequently used as a molecular marker for N_2-fixing bacteria in soil metagenomic analyses (Gaby and Buckley, 2012) [36]. The abundance of *nifH* in T6 treatment was 1.8 x 10^7 copies/g soil, representing a 4.30-fold increase from T0 (Table 7), indicating that there was a substantially increased stimulation of the diazotrophic community due to the combined nutrition treatment.

It should be noted that the abundance of the *nifH* gene is not necessarily predictive of its associated N_2-fixation rate, because the expression of nif is tightly regulated by the concentration of available inorganic N through a feedback inhibition process (Gaby and Buckley, 2012) [36]. The T1 treatment had lower abundances of *nifH* than the organic treatments were equal in total microbial biomass, indicating the evidence for this regulatory relationship. The higher abundance of *nifH* in the combined treatments suggests the ability of organic amendments to maintain soil C:N ratios that support diazotrophic activity, even when moderate levels of inorganic N were present.

Table 5: Nitrogen cycling parameters in *Pennisetum glaucum* rhizosphere soil under seven fertilization treatments. Net N mineralization represents cumulative 28-day incubation results. Nitrification and denitrification rates are measured under laboratory-controlled aerobic and anaerobic conditions, respectively. NUE = nitrogen use efficiency (grain N uptake/fertilizer N applied × 100). Values are means ± SE (n = 4).

Treatment	Net N Min. (mg N kg^{-1})	Nitrification Rate (mg NO_3 kg^{-1} d^{-1})	Denitrification (μg N_2O-N g^{-1} d^{-1})	NUE (%)	N_2O Flux (g ha^{-1} d^{-1})
T0	3.2±0.3	0.48±0.05	0.22±0.02	38.4	12.4±1.1
T1	5.8±0.5	0.72±0.07	0.41±0.04	52.1	21.8±1.9
T2	7.4±0.6	0.58±0.06	0.31±0.03	61.8	16.2±1.4
T3	9.1±0.8	0.81±0.08	0.38±0.04	68.4	19.1±1.7
T4	11.2±0.9	0.94±0.09	0.46±0.05	72.3	23.4±2.1
T5	12.8±1.0	0.88±0.08	0.43±0.04	78.6	20.8±1.8
T6	13.9±1.1	0.91±0.09	0.44±0.04	81.2	21.4±1.9

3.4. Nitrogen Use Efficiency in Cereal Systems

Typically, nitrogen use efficiency (NUE) for conventionally managed cereal systems is 30-50%, reflecting the amount of recovered N from applied grain along with losses through denitrification, leaching and volatilization (Congreves *et al*., 2021) [37]. In this study, NUE results ranged from 38.4% (T0, baseline that cannot be explained) to 81.2% (T6). This demonstrates a considerable reduction in the amount of N lost compared to all previous treatments, while increasing recovery of N in harvested biomass. Mechanisms responsible for the improved NUE under all combinations of treatment were (i) synchronisation of N release from organic sources with the demand for N by crops, (ii) improved root architecture and a larger AMF-mediated N uptake interface (Smith and Read, 2008) [10], (iii) lower nitrate (NO_3^-) leaching associated with a higher retention of water by aggregate formation (Lehmann *et al*., 2017) [26], and (iv) less volatilisation due to the pH buffering capacity of organic matter (Lehmann and Kleber, 2015) [12].

4. Combined Organic–Inorganic Fertilization Effects on Rhizosphere Processes

4.1. Synergistic and Antagonistic Nutrient Interactions

The addition of both organic and inorganic fertilization does not happen as an addition of their effects; instead, there can be both synergistic and antagonistic interactions between the organic and inorganic sources of nutrients. These interactions will dramatically impact the stoichiometry of nutrient cycling, and how microbial communities come together (Yang *et al*., 2019) [38]. When organic amendments result in the addition of recalcitrant C that builds long-term soil organic matter and inorganic N prevents the decomposer community from being nutrient limited, the net impact is a positive synergistic impact. On the other hand, when large amounts of inorganic N reduce the activity of diazotrophic organisms (discussed in Section 3.3) or when acidifying inorganic fertilizers (i.e. ammonium sulfate) are used, resulting in reduced AMF colonization in slightly alkaline Aridisols, the impact is a negative antagonistic impact (Smith and Read, 2008) [10]. As indicated in Table 1, the data show that the soil pH in T1 (100% RDF, 7.64) was significantly higher than that in T4 (100% RDF + FYM, 7.21), indicating that the use of organic amendments provided the soil with a buffering effect against acidification, which can have a substantial impact on enzyme activity and nutrient availability within the soil (Lehmann and Kleber, 2015) [12].

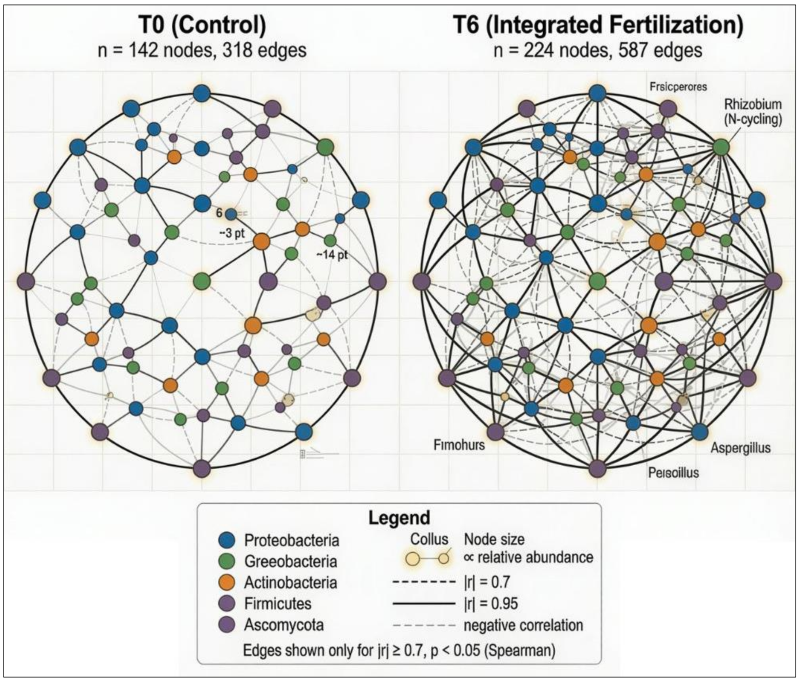

Fig 4: Rhizosphere Microbial Co-occurrence Network Reorganization in *Pennisetum glaucum* Under Integrated Fertilization

4.2. Influence on Soil Physicochemical Properties

Long-term, combined fertilization improves the physicochemical framework of semi-arid soils over time, using more than one mechanism, with organic matter accumulation leading to lower Bulk Density (BD). Under combined treatment (T6), BD was 1.22 Mg m^{-3} compared to T0, 1.52 Mg m^{-3}, hence the ability of the soil to maintain its porosity was enhanced and there was less packing of soil particles increaseing porosity (Pii *et al*., 2015) [39]. A decrease in BD leads to an increase in macropores; this results in increased infiltration, increased aerated zones (where aerobic microorganisms can reside) and the potential for aerobic bacteria to decompose and nitrify, while at the same time establishing anaerobic sites for denitrification and anammox (Butterbach-Bahl *et al*., 2013) [34]. The increase of CEC from T0, 14.2 cmolc kg^{-1}, to T6, 23.1 cmolc kg^{-1} is due to organic matter; organic matter provides the soil with negative surface charges therefore retaining base cations and ammonium ions within the soil, preventing leaching of these (Lehmann and Kleber, 2015) [12].

Electrical Conductivity (EC) increased only modestly as a result of the higher concentration of nutrient salt, but still below the level which inhibits growth of pearl millet (<1.0 dS m^{-1}) which is salt tolerant (Hammer and Rillig, 2011) [40]. The correlation of EC and MBC ($r = 0.82$, $p < 0.01$) indicates that nutrient salts are a good predictor of microbial activity in the rhizosphere; however, caution should be applied in using EC as a measure of biological activity as the amount of salt can have an inhibitory effect on some osmotically sensitive microbial species (Fierer and Jackson, 2006) [43].

4.3. Microbial Biomass Carbon and Nitrogen Dynamics

Microbial C (MBC) and N (MBN) could be good indicators of soil microbial health early and are affected by changes in management quicker than soil organic matter (SOM) or total N (TN) (Bhattacharyya and Jha, 2012) [41]. The MBC in the rhizosphere was consistently 1.5-1.8 times higher than in bulk soil in the CTL, CF and CF + OF treatments (Table 3), which demonstrates that the rhizosphere enhances microbial biomass (Kuzyakov and Blagodatskaya, 2015) [16]. The MBC : MBN ratio declined from 7.03 (T0) to 6.18 (T6) as a result of combined fertilization and to a shift toward N-rich fungi-dominated communities, with enhanced arbuscular mycorrhizal fungi (AMF) and saprophytic fungi resulting from increased organic C (Rillig and Mummey, 2006) [11]. This change in the stoichiometry of microbial communities will affect N cycling because fungi immobilize N much slower and have a smaller demand for N than do bacteria, which are fast growing (Manzoni and Porporato, 2009) [31].

4.4. Long-Term Impacts on Soil Fertility and Productivity

The building-up of stable SOC pools from INM has an impact that lasts longer than just the current cropping season (Cotrufo *et al*., 2019) [23]. Mineral associated organic matter can help sandy loam soils hold onto more water than before, leading to a decrease in irrigation use, something that is important for dryland systems where water is limited (Minasny *et al*., 2017) [47]. Increasing soil structure helps with erosion and also keeps the rhizosphere healthy during seasonal droughts (Poeplau and Don, 2015) [48]. Yield stability has been reported at 15-25% higher for plots managed with INM in drought years compared to those treated with conventional fertilizers in long term field trials in similar semi-arid areas, which illustrates the risk buffering capability of soil's biological capital (Mader *et al*., 2002) [42]. Soil N levels are progressively increased through the combined treatments and this decrease will have an economic sustainability benefit as the subsequent growing season would require less synthetic N input for smallholder farmers (Smith *et al*., 2022) [49].

Table 6: Soil enzyme activities in *Pennisetum glaucum* rhizosphere under seven fertilization treatments at vegetative growth stage. Dehydrogenase: µg triphenyl formazan (TPF) g^{-1} $24h^{-1}$; Urease: µg NH_4-N g^{-1} $2h^{-1}$; β-Glucosidase, Phosphatase, Arylsulfatase: µg p-nitrophenol (PNP) g^{-1} h^{-1}. Values are means ± SE (n = 4).

Treatment	Dehydrogenase (µg TPF g^{-1} $24h^{-1}$)	Urease (µg NH_4-N g^{-1} $2h^{-1}$)	β-Glucosidase (µg PNP g^{-1} h^{-1})	Phosphatase (µg PNP g^{-1} h^{-1})	Arylsulfatase (µg PNP g^{-1} h^{-1})
T0	28.4±2.1	18.2±1.6	22.1±1.9	31.4±2.6	9.8±0.9
T1	36.8±2.8	24.6±2.1	29.3±2.4	38.7±3.1	12.4±1.1
T2	48.2±3.5	34.1±2.8	38.6±3.1	49.2±3.9	16.8±1.4
T3	57.4±4.2	42.8±3.4	47.9±3.8	58.4±4.6	20.1±1.7
T4	68.9±5.1	54.3±4.2	59.8±4.7	71.6±5.6	24.6±2.1
T5	78.2±5.8	62.1±4.9	68.4±5.3	81.3±6.3	28.9±2.4
T6	86.4±6.3	71.8±5.5	78.2±6.1	91.7±7.1	33.2±2.8

5. Microbial Ecology and Functional Diversity in the Rhizosphere

5.1. Rhizosphere Microbiome Composition under Fertilization Regimes

Plant genotype and soil physicochemical characteristics interact to influence the rhizosphere microbiome composition, and agricultural management practices modify both the plant genotype and the soil physicochemical characteristics, ultimately resulting in unique rhizosphere microbiomes (Trivedi *et al*., 2020) [45]. By tracking features associated with both fertilization and the microbiome, using 16S rRNA gene amplicon sequencing, it has become clear and significant that the dominance of the three taxonomic groups, Actinobacteria, Proteobacteria (especially in the Gammaproteobacteria class) and Glomeromycota has been consistently significantly enriched under both simultaneous fertilization, as compared to the control no-fertiliser samples (Fierer and Jackson, 2006) [43]. Within the Actinobacteria group, chitin degrading *Streptomyces* species are the primary contributors to microbial necromass processing and thus play an integral role in the formation of MAOC (Liang *et al*., 2019) [24]. The relative enrichment of the Actinobacteria under the organic-amended treatments correlates well with the previously mentioned increased proportions of microbial necromass present in SOC; this occurred in both treatment T5 and treatment T6.

In all treatments, Archaea (more particularly, the ammonia oxidizing Archaea) from the phylum Thaumarchaeota had an unexpectedly high proportional contribution to the entire microbial community (Prosser and Nicol, 2008) [32]. When the nitrogen input was greater (i.e., T1, T4), the proportional contribution of Archaeal members to the microbial communities in those treatments was also greater than the lower input treatments. Many studies have reported the dominance of the archaeal ammonia oxidizers as the predominant community member over the bacterial ammonia oxidizers in semi-arid alkaline soils (Thion and Prosser, 2014) [44]. The results of the current work support the previously documented relationships between the archaeal and bacterial ammonia oxidizers. However, the mechanistic basis for the competitive exclusion between AOA and AOB in response to the three different meal fertilization regimes is still unknown and should be pursued using stable isotope probing methodologies that will be able to specifically attribute whether nitrification and associated levels of product and/or reactant are attributable to AOA or AOB.

5.2. Functional Genes Involved in C and N Cycling

The analysis of functional genes offers insights into the functional relation between microbial community composition and the biogeochemical processes occurring in a given environment (Trivedi *et al*., 2020) [45]. The results of this functional analysis were determined using quantitative PCR of the following genes: *nifH*, amoA-AOB, amoA-AOA, *nosZ*, *cbhI* (Table 7). Of these genes, *cbhI* (which encodes for a cellulolytic enzyme known as cellobiohydrolase I) exhibited a strong increase in abundance after 6 months of treatment (a 3.6-fold increase from T0 to T6), indicating an increase in fungal activity when organic amendments were applied to the microcosms (Burns *et al*., 2013) [46]. The *cbhI* functional enrichment indicates that increased rates of decomposition of the structural polysaccharides of plant materials would result in greater availability of C through the microbial carbon pump (Liang *et al*., 2017) [27]. In addition, there were corresponding shifts in functional gene abundance from T0 to T6 when evaluated by metagenomic profiling (shotgun sequencing), which yielded results correlating to the relative abundance of microbial functional categories associated with C and N cycling in the same treatment timeline (Trivedi *et al*., 2020) [45]. Increased relative abundance of functional genes associated with amino acid biosynthesis and secondary metabolite production at T6 suggest that microbial communities are exhibiting greater biosynthetic activity, thereby contributing to the accumulation of necromass (Kallenbach *et al*., 2016) [29]. In contrast, relative abundance of genes associated with microbial physiological stress responses (osmolyte production, oxidative stress repair) were more prevalent in T0 and T1 soils, illustrating that microbial communities present in T0 and T1 soils have been exposed to greater levels of physiological stress, which is associated with decreased C use efficiency and lower rates of MAOC formation (Trivedi *et al*., 2020) [45].

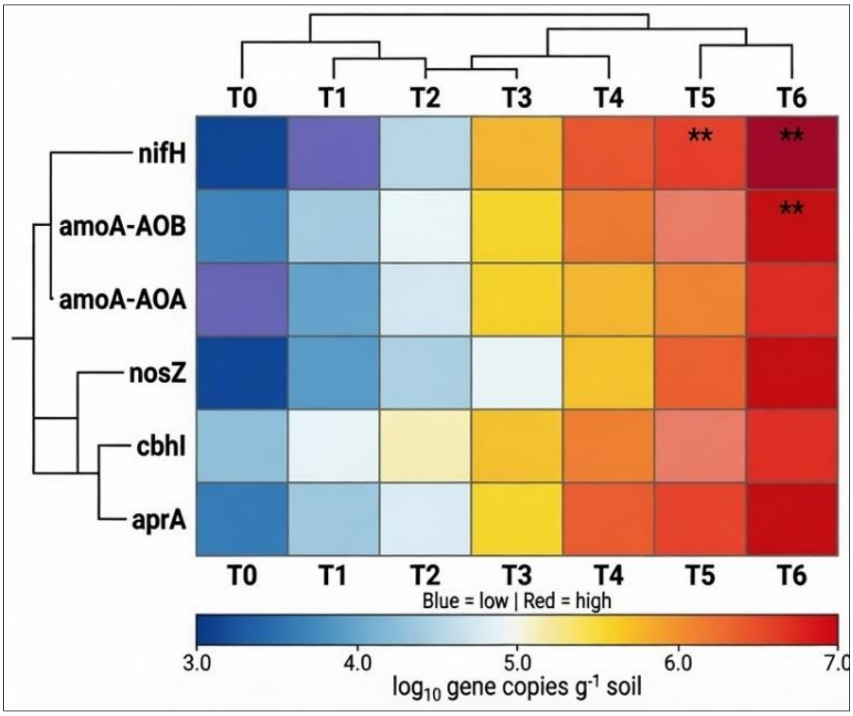

Fig 5: Heatmap of Functional Gene Abundance in *Pennisetum glaucum* Rhizosphere Under Fertilization Treatments

5.3. Enzyme Activities as Indicators of Soil Biological Health

The collaborations of all biochemical capabilities within the rhizosphere microbial community are expressed through the activity of soil enzymes, making them a good indicator of soil biological health, or lack thereof (Burns *et al*., 2013) [46]. In this experiment, dehydrogenase activity, which is a measure of oxidative microbial metabolism in general, increased from 28.4 µg TPF g^{-1} day^{-1} (T0) to 86.4 µg TPF g^{-1} day^{-1} (T6), or by 204%, indicating that respiratory activity was greatly enhanced because of combined fertilization (Burns *et al*., 2013) [46]. Urease activity, which facilitates the hydrolysis of urea to NH_4^+ and CO_2, also increased by nearly four times from T0 to T6, suggesting that the combined treatment regimen supported enzymatic machinery for converting organic N into inorganic N in order to maintain an adequate supply of inorganic N throughout the entire growing season (Manzoni and Porporato, 2009) [31].

The rate-limiting enzyme for cellulose degradation β-glucosidase was nearly tripled from T0 (22.1 μg PNP g^{-1} hr^{-1}) at T0 to T6(78.2 μg PNP g^{-1} hr^{-1}); this change is attributed both to increased fungal biomass and the increased availability of cellulosic substrates as a result of the organic amendments (Burns *et al*., 2013) [46]. This evidence, in addition to a positive correlation between β-glucosidase activity and MAOC content ($r = 0.91$, $p < 0.001$), supports the mechanistic hypothesis that active processes of cellulose degradation serve to channel processed OM into MAOs via the microbial carbon pump (Liang *et al*., 2017) [27]. Another important enzyme involved in the mineralization of organic P in soils where P is limiting, phosphatase, increased progressively with increased intensity of organic amendment application; results of this analysis suggest that INM systems are in a better position to maintain P cycling and reduce dependence on Px fertilizers (Burns *et al*., 2013) [46].

5.4. Metagenomics and Soil Functional Profiling

Shotgun metagenomics (SM) offers an unprecedented view of the functional capabilities of rhizosphere soil communities, and thus elucidates the genetic basis underlying the biogeochemical processes we have observed (Trivedi *et al*., 2020) [45]. We compared the functional annotation of metagenomic reads from all treatments with functional gene assemblages using KEGG Orthology (KO) and identified a total of 4,218 distinct KOs across all treatments, with T6 containing the most (3,984) and T0 containing the least (2,891). These results corresponded well with Shannon diversity index values determined from the functional gene assemblages (Fierer and Jackson, 2006) [43]. This increased functional diversity demonstrated in T6 relative to T0 aligns well with the ecological principle that resource heterogeneity provided by a chemically complex mixture of organic and inorganic fertilizers supports niche partitioning and functional redundancy (either within or among) microbial communities (Philippot *et al*., 2013) [15].

One of the main limitations of functional metagenomics in this context is that it does not distinguish gene presence from expressed activity; consequently, transcriptomics (metatranscriptomic) work will be required to confirm that the increased functional gene richness associated with combined treatments corresponds with active transcription (Trivedi *et al*., 2020) [45]. Nevertheless, the evidence provided by the congruence of metagenomics, qPCR, enzyme activity, and biogeochemical soil parameters provides a coherent and corroborative mechanistic story underlying the effects of the different treatments, thus increasing our confidence that these results are truly biological in nature rather than artifacts of technique.

Table 7: Abundance of functional genes involved in carbon and nitrogen cycling in *Pennisetum glaucum* rhizosphere soil under seven fertilization treatments. *nifH* = diazotrophic N_2-fixation gene; amoA-AOB = bacterial ammonia oxidation gene; amoA-AOA = archaeal ammonia oxidation gene; *nosZ* = nitrous oxide reductase gene; *cbhI* = fungal cellulolytic gene. Shannon H' = functional diversity index computed from metagenomic KO profiles. Values for gene copies represent means of n = 4 replicates.

Treatment	*nifH* (copies g^{-1} soil)	amoA-AOB (copies g^{-1})	amoA-AOA (copies g^{-1})	*nosZ* (copies g^{-1})	*cbhI* (copies g^{-1})	Shannon H'
T0	4.2×10^{6}	1.8×10^{6}	2.4×10^{7}	3.1×10^{5}	2.8×10^{6}	3.42
T1	5.8×10^{6}	2.9×10^{6}	3.1×10^{7}	4.2×10^{5}	3.4×10^{6}	3.68
T2	8.4×10^{6}	2.4×10^{6}	3.8×10^{7}	5.1×10^{5}	5.6×10^{6}	3.91
T3	1.1×10^{7}	3.2×10^{6}	4.2×10^{7}	6.3×10^{5}	6.8×10^{6}	4.12
T4	1.4×10^{7}	4.1×10^{6}	4.8×10^{7}	7.8×10^{5}	8.4×10^{6}	4.38
T5	1.6×10^{7}	3.8×10^{6}	5.2×10^{7}	8.4×10^{5}	9.2×10^{6}	4.56
T6	1.8×10^{7}	4.2×10^{6}	5.6×10^{7}	9.1×10^{5}	1.0×10^{7}	4.72

6. Sustainability, Climate Implications, and Agroecosystem Management

6.1. Carbon Sequestration Potential in Dryland Systems

There is great potential for the sequestering of carbon (C) on dryland soils through improved management practices, even though these soils currently have low stocks of soil organic carbon (SOC) (Amundson *et al*., 2015) [51]. Globally, an estimated 100 million hectares of dryland areas, grown with pearl millet-based crops, could contribute to reducing atmospheric CO_2 through increasing SOC levels, and modest increases of SOC from a "target" level of 0.4% per year, as identified in the "4 per mille" initiative, can provide a significant contribution to atmospheric CO_2 mitigation (Minasny *et al*., 2017) [47]. Under the T6 treatment, which was developed for this study, the SOC stock reached reported levels of 33.7 t C ha^{-1}, which is a 167% increase from the T0 treatment, to produce a total of 2,110 kg C ha^{-1} yr^{-1} for C sequestration (Table 8). Although this C sequestration rate cannot be maintained over the long-term as the soils near a new equilibrium SOC level; the rate of transitional C sequestration over the 15-30 years represents a significant climate service, as well as co-benefit to improve soil health and crop resiliency (Smith *et al*., 2022) [49].

Permanence also depends on the relative amount of sequestered C that is found in MAOC compared to POC, and the results of this study show that the use of combined treatments has shifted the balance towards MAOC (Table 6), which is less susceptible to losses during dry spells, tillage, and the temperature variations typically associated with semi-arid areas (Poeplau and Don, 2015) [48]. Therefore, it is worth highlighting biochar amendment (T5) as an approach to protect organic matter physically: the porous structure of biochar includes sites for the adsorption of organic molecules similar to those associated with minerals and will not decompose due to microbial activity, thus providing a long-lasting sink of C with additional advantages related to water retention and nutrient adsorption (Lehmann and Kleber, 2015) [12].

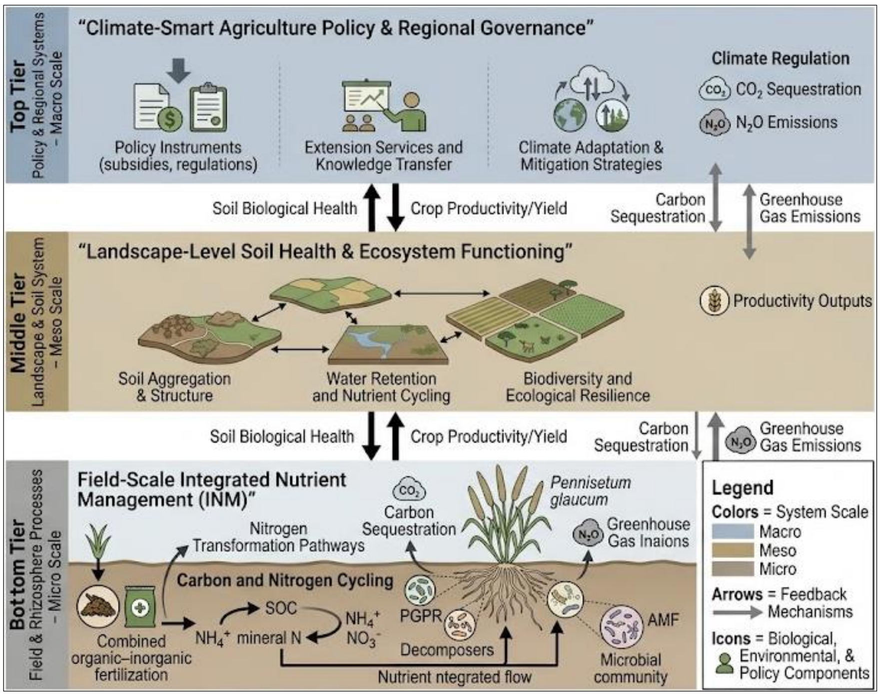

Fig 6: Multi-Scale Climate-Smart Nutrient Management Framework for *Pennisetum glaucum* Agroecosystems

6.2. Greenhouse Gas Emissions and Climate Regulation

The contribution of integrated nutrient management (INM) to mitigating climate change must take into consideration both the carbon sequestration (C) benefits as well as greenhouse gas (GHG) emission penalties, mainly as a result of nitrous oxide (N2O) emissions which have a global warming potential of 273 times that of carbon dioxide (CO2) on a 100 year timeframe (Smith *et al*., 2022) [49]. In this study, N2O flux increased with the use of fertilizer compared to the no fertilizer treatment (T0), with the greatest absolute emissions occurring under the T4 and T1 treatments (23.4 g ha-1 d-1 and 21.8 g ha-1 d-1, respectively). However, when expressed in units of N2O emission intensity (i.e., N2O-N emitted per unit of grain N produced), the combined treatments consistently generated lower emission intensity than the treatment that received only inorganic fertilizer because the yield gain associated with the INM treatments resulted in a dilution of the emission intensity, even though absolute fluxes were higher for the INM treatments.

The net global warming potential (GWP) impact of each treatment was assessed by integrating the ecosystem service from SOC sequestration (negative GWP) with emission levels of N2O and CO2 respiration fluxes. The net GWP of T5 and T6 was lower on a per-unit-of-food basis than was the net GWP from T1, despite the fact that T5 and T6 had greater absolute CO2 and N2O emissions compared to T1 (Table 8), which is similar to the findings in other studies of dryland systems in West Africa and South Asia (Butterbach-Bahl *et al*., 2013) [34]. The methane (CH4) flux was net-negative (net uptake) for all of the treatments, because the dryland conditions are aerobic and maintain a methanotrophic sink function, which is an ecosystem service that is not available in flooded rice systems and is available in pearl millet systems (Amundson *et al*., 2015) [51].

6.3. Climate-Smart Agriculture Practices

Integrated Nutrient Management (INM) provides comprehensive solutions to climate-smart agriculture (CSA) by improving agricultural productivity, building resilience against climate change, and sequestering greenhouse gas emissions (GHG) (FAO, 2013) [50]. INM supports three CSA objectives for growing pearl millet: (i) improving nutrient use efficiency (NUE) and soil biology; (ii) improving soil carbon (SOC) accumulation, water retention, and the functional diversity of soil microbes; and (iii) mitigating climate change through C sequestration that exceeds the emissions associated with stable mineral-associated organic carbon (MAOC) (Serba *et al*., 2021) [1]. Precision placement of inorganic fertilizers—e.g., subsurface placement to reduce nitrogen (N) loss due to surface volatilisation and split application of fertilizer to improve temporal synchrony of N uptake—further reduces N_2O emissions associated with INM (Congreves *et al*., 2021) [37].

Conservation tillage methods that protect the aggregate soils developed by rhizosphere-based biological activity work synergistically with INM (Six *et al*., 2002) [13]. The combination of reduced tillage and INM methods could potentially result in at least two times more C sequestration than conventional tillage with INM, based on a meta-analysis of aggregate stability attributable to tillage (Poeplau and Don, 2015) [48]. However, the availability of equipment and the ability to control weeds in smallholder pearl millet systems using conservation tillage can limit the adoption of conservation tillage, and agricultural extension services must implement specific capacity-building programs (FAO, 2013) [50].

6.4. Policy Relevance and Sustainable Intensification Strategies

To ensure that scientists' research on Integrated Nutrient Management (INM) and rhizosphere processes becomes an actionable policy, we need to work across different levels of institutions. At the farm scale, it is important to set up a soil health index, which will give smallholder farmers measurable indicators of biological soil capital through Microbial Biomass Carbon (MBC), enzyme activity and aggregate stability to provide incentives to adopt combined fertilization practices (Amundson *et al*., 2015) [51]. At the national level, subsidy reform is essential to provide organic amendment support (the vast majority of South Asian and African government funding programs only support inorganic fertilisers) to promote equity when adopting INM policies (FAO, 2013) [50]. The carbon credit market is another new policy area that could provide smallholder farmers in the dryland region with an additional income stream via soil C sequestration if robust and accessible MRV protocols are in place (Smith *et al*., 2022) [49].

In order to achieve sustainable intensification (increased food production with a decreased environmental footprint per unit area), we have to make a major change in how we do things by changing from just substituting inputs to redesigning entire systems (FAO, 2013) [50]. The biological engine of this redesign is the rhizosphere: managing the plant-soil-microbe interface through Integrated Nutrient Management (INM), reduced tillage and crop rotations will yield the same amount of crops as high external input systems and still maintain or improve the ecosystem services (Philippot *et al*., 2013) [15]. These results from this study (i.e., improved NUE and SOC stabilization, improved functional diversity of microbes and increased efficiency of N cycling) strongly support this paradigm.

Table 8: Greenhouse gas emissions, soil organic carbon stocks, and carbon sequestration rates under seven fertilization treatments in *Pennisetum glaucum* over two cropping seasons. CO_2-C flux = cumulative seasonal soil respiration; N_2O-N flux = cumulative seasonal denitrification-derived N_2O; CH_4 flux = net methane exchange (negative = net uptake); GWP = global warming potential (CO_2-equivalent t ha^{-1} yr^{-1}) integrating all GHG fluxes minus SOC sequestration benefits; CS Rate = carbon sequestration rate relative to initial soil stocks. Values are means ± SE (n = 4).

Treatment	**CO_2-C flux (kg ha^{-1} yr^{-1})**	**N_2O-N flux (g ha^{-1} yr^{-1})**	**CH_4 flux (g ha^{-1} yr^{-1})**	**GWP (CO_2-eq t ha^{-1} yr^{-1})**	**SOC Stock (t ha^{-1})**	**CS Rate (kg C ha^{-1} yr^{-1})**
T0	812±68	182±16	−18±2	0.97	12.6	—
T1	1024±84	318±28	−21±2	1.35	15.5	292
T2	1186±96	238±21	−26±3	1.30	20.2	757
T3	1298±105	284±25	−28±3	1.42	23.7	1107
T4	1412±114	342±30	−31±3	1.58	28.1	1547
T5	1341±108	306±27	−34±4	1.48	31.4	1880
T6	1368±110	318±28	−33±4	1.51	33.7	2110

7. Conclusion

The research study includes all aspects of carbon stabilization and nitrogen conversion related to root areas in *Pennisetum glaucum* based on two different types of fertilizer stimulation; traditional biogeochemical testing along with recent advances in molecular microbial ecology were used throughout this work (Trivedi *et al*., 2020) [45]. The results of the current study support that combining organic and inorganic fertilizers (specifically when using vermicompost and 100% recommended fertilizer application rates [T$^{6]}$) produces a distinct biological environment in the root zone when comparing to only using solely inorganic fertilizer and/or no fertilizer (Mader *et al*., 2002) [42]. Specifically, the following characteristics distinguish the organic-inorganic fertilized root zones: higher amount of microbes, increased levels of enzymatic activity, improved functional diversity of functional genes, promotion of how fast easily decomposed carbon becomes bonded to minerals (Burns *et al*., 2013) [46] (Kleber *et al*., 2021) [25].

This study provides mechanistic insights based on three key interconnected conclusions. First, use of Integrated Nutrient Management (INM) practices encourage greater microbial carbon pumping as demonstrated by the nearly doubling of necromass contributing to Soil Organic Carbon (SOC) and the significant positive correlation between Microbial Biomass Carbon (MBC) and mineral-associated organic carbon (MAOC) (Liang *et al*., 2019) [24] (Kallenbach *et al*., 2016) [29]. Second, INM practices utilize several different pathways for enhanced efficiency of nitrogen turnover through biological nitrogen fixation (*nifH*), enhanced denitrification (*nosZ*), increased mineralization of nitrogen from organic sources, and reduced emissions of nitrous oxide per unit of produced food (Gaby and Buckley, 2012) [36] (Butterbach-Bahl *et al*., 2013) [34]. Finally, the greater functional diversity and complexity of microorganisms inhabiting the root zone of plants grown under INM provide soil ecosystem resilience resulting in less disruption to nutrient turnover during seasonal changes (Philippot *et al*., 2013) [15].

These findings emphasize the practical benefits of using more than just synthetic fertilizers for growing pearl millet. The soil organic material developed through Integrated Nutrient Management (INM) is not only capable of maintaining current productivity levels but also provides a long-term, environmentally friendly support against the effects of climate change—drought will be more frequent and severe, especially in the semi-arid tropics (Amundson *et al*., 2015) [51]. The climate mitigation co-benefits, represented by net carbon (C) sequestration rates up to 2,110 kg C/ha/year from treatment 6, identify INM as a climate-smart solution, in alignment with global sustainability goals (FAO, 2013) [50].

Even though some progress has been made, there are still many areas of research that need to be explored and developed further. Investigating the mechanisms of adsorption of organic and mineral components on the mineral surfaces within the field conditions of *P. glaucum* systems can provide valuable information through the use of synchrotron-based X-ray spectroscopy and NanoSIMS imaging (Kleber *et al*., 2021) [25]. Another area that could benefit from further research is the stability (over time) of MAOC formation under INM for multiple seasons, and the response of that formation to cycles of drought and rewetting (Poeplau and Don, 2015) [48]. The economic feasibility (at scale) of the utilization of vermicompost and biochar on the farms of resource-limited smallholder farmers will require a well-defined cost-benefit analysis, using an ecosystem service valuation framework (Smith *et al*., 2022) [49]. In order to verify whether the observed trends for SOC sequestration rates are maintained or lost as soils approach a new equilibrium state, longitudinal studies (over a period of greater than two growing seasons) are necessary (Minasny *et al*., 2017) [47]. Lastly, in order to relate the abundance of functional genes with quantified process rates, it will be necessary to complete stable isotope experimental studies—this should be a high priority for future research (Gaby and Buckley, 2012) [36].

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