Rhizosphere Microbial Regulation of Carbon Mineralization and Nitrogen Transformation in Zea mays Systems Under Integrated Nutrient Management

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Abstract

Introduction: The rhizosphere is the soil region affected by plant roots, where microorganisms play a role in regulating C and N cycling. Integrated Nutrient Management (INM) with chemical fertilizers and organic sources in maize (Zea mays L.) system improves soil fertility, nutrient-use efficiency and crop productivity.

Methodology: The present study evaluated INM effects on maize rhizosphere processes through metagenomics, enzyme analysis and functional gene studies and microbial and biochemical approaches.

Results and Discussion: INM resulted in significantly higher microbial biomass, soil enzyme activities and nutrient availability than sole chemical fertilization. The enhanced nitrogen transformation and reduced N_2O emissions were observed for functional genes such as nifH, amoA, nirK, nirS and nosZ. Long-term INM also increased soil organic C, nutrient use efficiency and maize yield.

Conclusion: Integrated nutrient management enhances rhizosphere microbial activity and carbon and nitrogen cycling in maize systems. It improves soil health, crop productivity and environmental sustainability and therefore an effective measure for sustainable maize production.

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1. Rhizosphere Ecology and Functional Characteristics in Maize Systems

1.1. Morphological and Physiological Characteristics of Zea mays

Zea mays L. (maize or corn), a member of the Poaceae family, is a C4 annual grass that has high photosynthetic efficiency, rapid biomass production, and a fibrous, extensive root system (Flint-Garcia *et al*., 2005) [1]. Maize's architecture enables it to meet the water, nutrient, and habitat needs of its roots and soil microsystems through rhizosphere processes (Lynch, 2007) [2]. Maize grows nodal roots that include; Seminal primary roots, crown, brace, and lateral roots; all types of roots help maize establish access to water and nutrients at varying depths in the soil (Lynch, 2007) [2]. Maize's C4 photosynthesis is responsible for allocating approximately 20-40% of the carbon fixed by photosynthesis below ground, creating large energy subsidies to rhizosphere-associated microbial communities through root exudation and cellular turnover (Philippot *et al*., 2013) [3].

Maize has a relatively high volume of transpiration that generates strong gradients of nutrient concentration in the rhizosphere, especially with respect to nitrate and potassium, which places considerable selective pressure on microbial communities that are adapted to use root-derived substrates as food resources. The sensitivity of maize to nitrogen supply is reflected by the fact that high yield production systems need between 150-250 kg N ha-1, making it a critical component of productivity to improve nitrogen cycling efficiency (Bashan and de-Bashan, 2010) [4]. In addition, maize produces a variety of secondary metabolites, including benzoxazinoids, which inhibit nitrification (biological nitrification inhibition, BNI) and influence the nitrifier community present in the rhizosphere, with this trait varying greatly between genotypes (Subbarao *et al*., 2015) [5].

1.2. Root Architecture and Rhizosphere Characteristics

The rhizosphere of maize can extend approximately 0–2 mm into the bulk soil from root surfaces, but the functional effects of root exudates may extend further through diffusion. The structure of maize's root system, which has many fine, highly surficial lateral roots, creates a rhizosphere volume that is much greater relative to root biomass than that of many other crops (Lynch, 2007) [2]. For example, root length densities of greater than 5 cm cm^{-3} in topsoils produce an extensive interface for root-microbe exchanges. Rhizosphere soils have a lower bulk density and higher aggregate stability than their bulk soil counterparts, and they typically have pH values 0.5–2 units lower than those of bulk soils due to the release of protons from roots and CO_2 from the soil; they also have higher concentrations of dissolved organic C and exhibit altered moisture regime (Philippot *et al*., 2013) [3].

The rhizosphere effect is expressed as the relative microbial density of microorganisms in the rhizosphere versus the bulk soil (the ratio of rhizosphere to bulk (R:S)) — values for bacteria usually range from 5–50 compared to bulk, and those for fungi are usually 2–10 compared to bulk. Individual R:S ratios vary with crop management and growth stage and soil type (Hinsinger *et al*., 2009) [6]. This preferential enrichment of microorganisms within the rhizosphere reflects the very high energy and substrate (organic C) levels available in rhizospheres from exuded root material, thereby providing an area of concentrated biogeochemical activity. Mycorrhizae will increase the functional size of the rhizosphere through hyphal networks (mycorrhizoshpere), thereby enhancing the mobilization of phosphorus and improving physical soil aggregation (Smith and Read, 2008) [7].

1.3. Root Exudation Patterns and Rhizodeposition Processes

Root exudates are a large and varied collection of chemicals – low-molecular-weight compounds such as organic acids (e.g., citric, malic, and oxalic acids) and sugars (e.g., glucose, fructose, and sucrose), amino acids, phenolics, and phytohormones, as well as high-molecular-weight mucilage, exoenzymes, and border cells that collectively make up what is referred to as rhizodeposits (Philippot *et al*., 2013) [3]. Maize (Zea mays L.) directs a substantial portion of net photosynthetically-fixed carbon (10 to 40%) to rhizodeposition; the greatest rate of exudation occurs at the maximum vegetative growth stage (V6 to V12) and during the grain-filling phase of the plant (Carvalhais *et al*., 2011) [8]. The composition of root exudates is highly variable, changing rapidly based on factors such as nutrient status, moisture regime in the soil, ambient temperature, biological interactions affecting the morphology or physiology of the maize plant, and herbivory.

When nitrogen is deficient, maize increases the exudation of organic acids, especially citric acid, to enhance the solubilization of phosphorous and iron from their mineral forms. In response to phosphorous deficiency, maize improves the solubility of phosphate by increasing the amount of protons (H+) that are exuded and/or carboxylates (negatively charged organic molecules) that are released, both of which decrease the acidity (pH) of the rhizosphere and improve the solubility of phosphorous (Richardson *et al*., 2009) [9]. The changes in chemistry that result from the increased exudation of root exudates produce a shift in the density of microbes belonging to different functional guilds, indicative of the capacity of the plant to exert 'microbiome engineering' in an effort to maximize its nutritional environment. Rhizodeposits also include sloughed cells from the border layer of the root and mucilage from the root tip. These materials constitute a major source of carbon that represent diverse microbial assemblages that colonize these materials and differ from any that colonize the epidermal layer of the root (Hinsinger *et al*., 2009) [6].

1.4. Interactions Between Roots and Microbial Communities

The relationship between root and microbes is varied for maize, ranging from mutualism to pathogenism, as determined by plant genetics and the edaphic properties of the site; e.g., the "rhizobiome" of the maize reflects both the plant's genetics and the edaphic properties of the site (Bulgarelli *et al*., 2013) [10]. The most common types of mutualistic interactions are with arbuscular mycorrhizal fungi (AMF) of Glomus spp. and Rhizophagus spp., which colonize maize roots under a wide range of soil conditions and improve the uptakes of immobile phosphorus from soil in exchange for photosynthate. Mutualistic bacteria known as plant growth-promoting rhizobacteria (PGPR) including Azospirillum brasilense, Pseudomonas fluorescens, and Bacillus subtilis produce IAA (indole-3-acetic acid), gibberellins and solubilize phosphates, thus enhancing root architecture and nutrient uptake by Maize (Bashan and de-Bashan, 2010) [4]

Niche-based processes, such as the root exudates, are also involved in the assembly of microbial communities within roots, through deterministic filtering and stochastic effects. Studies utilizing 16S rRNA amplicon and ITS metabarcoding studies of the maize microbiome show that the maize microbiome has been identified as having consistent microbial signatures (core rhizobiome), so it has been determined that maize is actively selecting for specific taxa from the surrounding bulk soil (Bulgarelli *et al*., 2013) [10]. Of the dominant bacterial phyla in the maize rhizosphere, Proteobacteria, especially the Gammaproteobacteria and Bacteroidetes, and Actinobacteria, have been consistently enriched when compared to bulk soil, while Acidobacteria, which are the dominant bacterial phylum in bulk soil, have been less abundant compared to the bulk soil (Hinsinger *et al*., 2009) [6].

1.5. Temporal Changes in Rhizosphere Activity Across Crop Developmental Stages

The rhizosphere in maize has changing microbial populations across time and associated with phenological growth stages of maize throughout its life cycle (e.g., germination & seedlings (V1–V6), vegetative (V7–VT), anthesis/pollination (R1–R2), and grain filling/maturity (R3–R6)). During the initial phase when seedlings are developing, microbial communities are created from the microbial content of seeds and germination exudates. After seeds germinate and seedlings are growing, root exudation increases rapidly and therefore the accumulation of microbial biomass and metabolic diversity within the rhizosphere is occurring more rapidly during vegetative growth.

The peak of the microbial biomass in the rhizosphere usually occurs at the same time as the peak of root length density (V10–VT), when maximal rates of root exudation are occurring, and at that time, the total surface area that root is in contact with soil is also maximized. After anthesis, subsequent vegetative growth shifts toward the grain filling

stage causing a reduction in the amount of carbon (C) allocated below ground and a decrease in root exudate flow to the microbes in the rhizosphere. Through the late growing season, microbial biomass and microbial activity decline as plant root growth declines; however, senescing roots and mucilage contribute to microbial activity. The temporal dynamics of this microbial activity directly affect the timing of nitrogen (N) in relation to the time at which the crop will require N, and represents one of the greatest obstacles to optimizing integrated nutrient management (INM).

1.6. Role of Rhizosphere Processes in Regulating Nutrient Availability

Various mechanisms alter macronutrient availability from the rhizosphere including acidification (which increases the solubility of iron and phosphorus), alteration in the redox state of the soil (which will affect the cycling of the soil nutrients such as iron, manganese and sulphur), the production of various enzymes (such as phosphatases, proteases and ureases) and the ability of microorganisms to transmute nitrogen from their natural state (Smith and Read, 2008) [7]. As maize roots release organic acids and protons they create a region of differing pH surrounding them (2–5 mm from the root surface). This will have a large impact on the availability of phosphate-nutrients, immobilization of the toxic aluminum compound and cation exchange capacity. The number of available nitrogen within the rhizosphere is dependent on the balance between the immobilization of nitrogen by microorganism (through uptake from soil mineral cohorts) and the mineralisation net of nitrogen (through decomposition of organic matter), where the factors in both are based on the C:N ratio of root exudates and residues (Schimel and Bennett, 2004) [11].

Maize dependant systems are especially reliant on the rhizosphere "priming effect", where fresh labile carbon inputs from roots cause a stimulation of the decomposition of the native SOM (Subbarao *et al*., 2015; Kuzyakov, 2010) [5, 16]. With large amounts of below-ground C inputs to the system, there is the potential for "accelerating" the release of plant available N from within stabilized SOM pools within the soil and therefore creating an improved synchrony of nutrient release with that of crop demand. Conversely, high C:N ratio exudates may create temporary N immobilization, creating "short-term" competition for available N between microorganisms and plants, ultimately decreasing NUE (Subbarao *et al*., 2015) [5]. The science that underpins and explains how to manipulate these processes in the rhizosphere provides the basis for optimally developing and implementing INM strategies for maize production.

2. Carbon Mineralization Mechanisms in Agricultural Soils

2.1. Definition and Significance of Carbon Mineralization

Carbon Mineralization is the process of microbial respiration with O2 (oxygen) to produce CO2 (carbon dioxide) and H2O (water); this will provide energy for the growth of microbes and return carbon to the atmosphere (Paul, 2015) [12]. Agricultural soils are the principal means by which organic materials such as plant residues, manure, and root-derived organic matter are recycled and their nutrients (N, P, and S) are released through carbon mineralization. The rate of C (Carbon) mineralization determines the amount of time organic C remains in soil, the amount of nutrients bound in organic matter that can be used by plants, and the difference between documenting net C gain versus net C loss from soils. From an agronomic perspective, the importance of C mineralization to agriculture includes the release of N, P and S from their organic form into a form that plants can utilize, and to help support soil biological activity and soil physical structure (Fauci and Dick, 1994) [13]. However, when organic carbon is subjected to excessive amounts of C mineralization, soil organic carbon (SOC) stock levels can be diminished, resulting in degradation of soils, decreasing their ability to hold water, and contributing to the increase in CO2 concentration in the atmosphere. Balancing C inputs and C outputs from soil carbon mineralization is critical to the concept of sustainable soil C management and climate-smart agriculture.

2.2. Biochemical Pathways of Organic Matter Decomposition

The process of decomposing organic materials works up through a series of biochemical transformations, which start with the extracellular hydrolysis of the polymeric substrates into their monomeric units (cellulose, hemicellulose, starch, proteins, and lipids) through the functions of a variety of soil enzymes (cellulase, hemicellulase, amylase, proteases, and lipase) (Paul, 2015) [12]. After the transformation, the monomeric units are absorbed by microorganisms and metabolised via general metabolic routes. When there is molecular oxygen present, microbial oxidation of glucose and similar monosaccharides proceeds aerobically through glycolysis, the tricarboxylic acid cycle (TCA cycle), and oxidative phosphorylation, resulting in both the production of carbon dioxide (CO_2) and ATP, being made efficiently.

Microbial fermentation and anaerobic respiration dominate in anaerobic environments (such as saturated microsites and soil aggregates), producing intermediate compounds like volatile fatty acids, alcohols, and methane, among others (Strickland and Rousk, 2010) [14]. The aerobic/anaerobic ratio of C mineralization in soils is influenced by the total amount of pore space, aggregate structure, and moisture content, which are strongly affected by organic amendments in INM systems. The widely used measure of the potential for labile C mineralization within agricultural soils is beta-glucosidase (the enzyme responsible for the final step in cellulose hydrolysis (cellobiose → glucose) (Fauci and Dick, 1994) [13].

2.3. Microbial Mediation of Soil Organic Matter Turnover

Soil microbes constitute the primary agents for SOM turnover; they account for about 90% of all soil respiration for the majority of terrestrial ecosystems (Sinsabaugh *et al*., 2013) [15]. Bacteria versus fungi decompose materials using fundamentally different strategies: Bacteria usually dominate the decomposition of labile and highly degradable substrate (sugar; simple amino acids) because of their rapid growth rates and high levels of enzyme production; fungi, with their hyphal networks and enzymes involved in degrading lignin (laccase, peroxidase, manganese peroxidase), primarily decompose the recalcitrant lignocellulosic substrates (Paul, 2015) [12].

The composition of predominate decomposer communities will have a profound effect on biochemistry and stoichiometric balance of SOM transformation processes. Therefore, the decomposition of a high-quality substrate (low C:N ratio, easily mineralizable) will predominantly result in bacterial decomposition with a high CUE value; thus, these

substrates will support substantially higher microbial biomass accumulation and N mineralization. However, the decomposition of low-quality and high-C:N substrates will mainly result in fungal decomposition with diminished CUE values, thus promoting N immobilization and substantially lower decomposition rates than the former (Strickland and Rousk, 2010) [14]. Under INM, the application of organic amendments together with the application of inorganic N fertilizers will decrease the C:N ratios of the substrates and, thus, stimulate bacterial activity and increase net N mineralization — this phenomenon is thought to be a key mechanism to explain the synergistic yield benefits of organic/inorganic management systems.

2.4. Labile and Recalcitrant Carbon Pools

The categories of soil organic carbon contain different pools much like the two different C pools are referred to as: the labile (active C pool), and the recalcitrant (passive C pool). These two pools have very different characteristics; for example, the labile C pool consists of; microbial biomass C, particle organic matter (POM), dissolved organic C (DOC), and readily available mineralizable C from freshly added plant material. The recalcitrant C consists of chemically stabilized humus, char (pyrogenic C) and mineral associated organic matter (MAOM) that has been physically protected from microbial access by means of either adsorption to clay minerals or physical entrapment within soil aggregates.

After six years - INM has enabled the addition of organic matter to be used repetitively, and in the short-term, organic additions will primarily increase the labile C pools (microbial biomass C; particulate organic matter (POM); etc.). Humification processes will gradually transfer a portion of the labile organic C pools to stable (mineral associated) pools (Fauci and Dick, 1994) [13]. The period of time that organic C remains in stable organic C pools will depend on the quality of the added organic C, the texture of the soil being amended, and the environmental conditions present at the time of the application. Typically, the humification coefficient of beef animal manures ranges from 10 – 30% and the humification coefficient of corn or soybean residue is between 5 - 15%. Therefore, for achieving both increased productivity as well as enhancement of sustainability, a successful INM strategy must strive to maximize both the labile and stable C pools (labile carbon supports microbial activity/nutrient cycling, and stable C improves long-term fertility/climate change mitigation).

2.5. Rhizosphere Priming Effects on Carbon Transformation

The RPE, or rhizosphere priming effect, is when the addition of organic matter from newly established root growth during crop cultivation increases or decreases SOM decomposing. This is one area of soil biogeochemistry that has a lot of importance, but there are several uncertainties about how it works (Kuzyakov, 2010) [16]. Positive RPE occurs most often: Labile C-rich root exudates stimulate higher-rate microbial activity and extracellular enzyme production, creating an increase in the decomposition of old (root-exuded) SOM (a.k.a., SOM that has already been mineralized by previous crops), compared to what the decomposition rate would have equaled if there were no roots present.

Negative RPE occurs infrequently under a very specific set of conditions, when microbial communities take up more of the organic-rich root exudates than native SOM, temporarily reducing the rate of SOM mineralization.

In maize, the amount of RPE can be large: ^{13}C-depleted and ^{13}C-enriched maize isotope-tracer studies show that RPE in maize systems can be as high as +20% to +300% of the baseline SOM mineralization rate (depending on root exudate type and the quality of the preexisting SOM) (Kuzyakov, 2010) [16]. Three different mechanisms drive the RPE; they are: co-metabolic stimulation, microbial nitrogen (N) mining of SOM, and enzyme upregulation of copiotrophic rhizosphere-associated bacteria. In systems utilizing INM, the magnitude and direction of RPE are influenced by the quantity and type of organic materials being added, which, in turn will change microbial community composition and availability of substrates for the microorganisms. The massive increase in the magnitude and direction of RPE will have very significant impacts on SOC pool sizes and synchrony between nutrient release from SOM and macro- and micro- nutrient plant uptake.

2.6. Short-Term Versus Long-Term Carbon Mineralization Processes

The short-term dynamics of carbon mineralization (days to weeks) associated with organic matter additions, predominately driven by the decomposition of labile fractions (simple sugars, amino acids, dissolved organic carbon (DOC)), are highly influenced by soil temperature, moisture, and disturbance. The long-term dynamics of carbon mineralization (months to years) associated with the progressive decomposition of more stable fractions (cellulose, hemicellulose, lignin) are regulated by both the physical protection of soil (i.e., aggregate encapsulation and mineral association) (Sinsabaugh *et al*., 2013) [15]. However, the relationship between short-term dynamics and long-term dynamics has implications for management practices regarding fertilizer application. Rapid N mineralization in the short-term from the application of fresh manure may not coincide with maize N demand curves throughout the growing season and may result in excess N supply in the early-season and a deficit in the late-season.

Long-term integrated nutrient management (INM) studies consistently demonstrate that the combination of organic fertilizers and inorganic fertilizers generates superior C mineralization dynamics compared to either method applied individually, resulting in higher levels of microbial biomass and enzyme activity during the growing season (Fauci and Dick, 1994) [13]. Additionally, the introduction of biofertilizers in combination with organic and/or inorganic inputs modifies C turnover rates due to the introduction of specific microbial catalyzers (cellulolytic bacteria and mycorrhizal fungi) that enhance the rate of organic matter decomposition and nutrient release. Finally, these synergistic interactions occur within rhizosphere microbial networks, and facilitate improved agronomic efficiency through the use of INM.

3. Nitrogen Transformation Processes and Microbial Regulation

3.1. Nitrogen Cycle Dynamics in Agricultural Soils

Agricultural soil nitrogen cycles consist of numerous transformations through a variety of microbes at different times and places (Robertson and Groffman, 2015) [17]. Nitrogen is typically the limiting nutrient in crop production globally, and therefore, proper nitrogen use in maize production systems is critical from both an economic and

environmental standpoint. The ways in which nitrogen enters maize growing soils will be primarily inorganic (urea, ammonium, or nitrate) through chemical fertilizer applications, but nitrogen also enters soils through organic sources while implementing INM. To become available for plant uptake, nitrogen must undergo a series of biological transformations following the breakdown of organic material.

Agricultural soil nitrogen loss through leaching of nitrate, ammonia volatilization, and nitrous oxide and nitrogen gas emissions through denitrification present economic and environmental challenges (Treseder, 2008) [18]. Crops contribute to approximately 60 percent of total N2O emissions on a global scale, and N2O is a potent greenhouse gas with a global warming potential that is 298 times that of CO2 over a 100-year time period. Composition of microbial communities and abundance of functional genes have been analyzed as mechanistic predictors of greenhouse gas N2O emissions, providing options for the development of INM-based GHG mitigation strategies.

3.2. Biological Nitrogen Fixation

The nitrogenase enzyme encoded by the nifH gene cluster catalyzes biological nitrogen fixation (BNF), which converts atmospheric nitrogen gas (N 2) into ammonium ions (NH 4 +). The process of BNF requires 16 ATP and 8 electrons for every N 2 molecule that is converted to NH 4 + (Zehr *et al*., 2003) [19]. In maize production systems, BNF is conducted mainly by free-living diazotrophs (e.g., Azospirillum, Gluconacetobacter, Burkholderia) and by associative nitrogen-fixers that are present in or on the maize plant's roots (rhizosphere and rhizoplane). Unlike legumes, maize does not form a root nodule, but N source (N vs. fixed N) through associative BNF can contribute 10-50 kg N/ha/yr, primarily in low-input tropical systems (Bashan and de-Bashan, 2010) [4].

The nifH gene is the most widely used molecular indicator of diazotrophic communities in soils. Studies evaluating integrated nutrient management (INM) support higher numbers of nifH gene copies and greater phylogenetic diversity of nitrogen-fixing communities with combined organic and inorganic management systems than with chemical fertilization alone (Zehr *et al*., 2003) [19]. This increase in N-fixing potential is likely a result of the utilization of additional carbon substrates from organic amendments to support the energetically demanding BNF process. Biofertilizers containing Azospirillum brasilense and Gluconacetobacter diazotrophicus have shown to enhance yield in smallholder maize systems (15-25%) through the combined effects of BNF, phytohormone production, and nutrient solubilization (Bashan and de-Bashan, 2010) [4].

3.3. Ammonification and Nitrification

By way of archaea, heterotrophic microbes break down the organic nitrogen (N) into ammonium (NH_4+) through a process called ammonification (also referred to as organic N mineralisation). Deaminases, peptidases, ureases and proteases are produced by these microbes in an extracellular form (Robertson and Groffman, 2015) [17]. The process is based on the substrates being used; the mineralisation of N is dictated by temperature, moisture content in the soil, and the C:N ratio of inputs. Generally, if the substrate has a C:N ratio of <20-25:1 then the minerals will be released to the environment (ammonification > microbial immobilisation) while if the substrate has a C:N ratio of >30:1 then the minerals will be bound to the biomass (microbial immobilisation > ammonification). The optimal growth environment for N-mineralisation throughout the cropping period is achieved by mixing high C:N crop residues with low C:N organic manures and inorganic (N) in INM, therefore optimising the substrate stoichiometry of N and C (Schimel and Bennett, 2004) [11].

Nitrification is the process of converting NH_4^+ (ammonium) into NO_2^- (nitrite) and finally into NO_3^- (nitrate), making this an important process in agriculture; it converts the poorly mobile form (NH_4^+) to a highly mobile form (NO_3^-) that is readily available for plant uptake, but loses through leaching and denitrification (Prosser and Nicol, 2008) [20]. The key enzyme responsible for the first reaction in the nitrification pathway (the conversion of NH_4^+ to NO_2^-) is ammonia monooxygenase (the amoA gene). The use of quantitative PCR to analyse the amoA genes of archaeal and bacterial nitrifiers has demonstrated that AOA generally outnumber AOB in bulk soil samples; however, AOB can dominate in the rhizosphere of maize fields treated with INM when NH_4^+ concentrations are high and favour fast-growing, substrate saturated kinetics (Prosser and Nicol, 2008) [20]. The activity of nitrifiers is influenced by pH, temperature and the presence of inhibitory compounds in organic amendments.

3.4. Denitrification and Gaseous Nitrogen Losses

Microbial respiration called "denitrification" takes place when microbes use NO3- (nitrate) as their electron source for anaerobic respiration by successively converting NO3- into N2 to produce nitrogen gas in four steps via four different types of reductases each represented by a different type of gene: narG catalyzes nitrate reductase, nirK/nirS makes nitrite reductase, norB makes nitric oxide reductase, and nosZ produces nitrous oxide reductase. The primary mechanism by which agricultural soils lose fixed nitrogen is through denitrification, which has both to do with nitrogen being inefficiently used by crops and with the production of N2O (greenhouse gas). The amount of N2O produced relative to the amount of N2 produced depends on how complete the denitrification reaction has been (i.e., essentially, does the reaction stop before reaching the end product of N2) and this is dependent on availability of carbon (C) and oxygen (O) for supporting microbial growth, resulting in either insufficient substrate for complete denitrification (i.e., producing N2O) or sufficient substrate for complete denitrification (i.e., producing N2) (Wallenstein *et al*., 2006) [21].

Using Integrated Nutrient Management (INM) practices, adding labile carbon through organic products and moderate amounts of nitrogen fertilizer can lead to increases in Nitrous Oxide (N_2O) as well as decrease and suppress the amount of N_2O emitted, depending upon how the crop is managed. Research indicates that organic amendments lead to an increase in the nosZ:nirS gene ratio indicating a shift from partial denitrification to complete denitrification with lower ratios of N_2O:N_2, which suggests that more N than is needed is being converted into N_2O (Wallenstein *et al*., 2006) [21]. Integrated Nutrient Management has shown to reduce the cumulative amount of N_2O emitted from the soil by 15–30% over time when compared to strictly chemical fertilization in maize systems, resulting in substantial co-benefits to the climate (Wallenstein *et al*., 2006) [21]. To achieve these results through Integrated Nutrient Management, it is necessary to

maintain sufficient soil aeration and avoid over-application of quickly mineralizable organic inputs during periods of anaerobic conditions.

3.5. Nitrogen Immobilization and the Microbial N Pool

Temporarily removing nitrogen from a plant-available pool through the process of nitrogen immobilization (by the uptake and holding of inorganic nitrogen by microbes) can be accomplished with nitrogen immobilization occurred with microbes (as biological organisms, all biological organisms are considered as by “a temporary removal;” not having the issue of the nitrogen, as an example of N, nitrogen would be in a form of ammonium and/or nitrate since the presence of the microorganisms in the soil). By the process of nitrogen immobilization, it occurs through the incorporation of the nitrogen into the biomass of the microbes. Under normal circumstances, the nitrogen that is present in the biomass of the microbes is referred to as microbial biomass nitrogen or MBN. When soils are properly managed, the range of MBN is typically from 20 µg N g −1 soil (20 µg N g soil, which is twenty micrograms of nitrogen with respect to a gram of soil) to 200 µg N g −1 soil (200 µg N g soil, which is two hundred micrograms of nitrogen with respect to a gram of soil), and when properly managed, MBN is a very dynamic reserve of N, as the MBN can be made available as a nitrogen source through the process of remineralization when microbes die and turn over (the turn over of the microbes are on the order of weeks to months). Thus, the incorporation of MBN into the soil, through the process of remineralization, allows for the MBN to serve as a continuously available N source for plants' uptake (through active mineralization) that can serve as a source of N for plants during active mineralization, since MBN is a slow released form of nitrogen (Schimel and Bennett, 2004) [11].

3.6. Environmental Controls on Nitrogen Transformations

There are several environmental factors that will influence rates of N transformation in maize rhizospheres. These factors include soil temperature (for most nitrifiers ranges from 25 - 35 ° C; above 15 ° C, denitrification is increased exponentially), soil moisture (nitrification is maximized when the soil is between 50% and 70% WFPS; when WFPS is above 80%, denitrification is accelerated), soil pH (nitrification is inhibited at pH levels less than 5.5; nitrification is maximized at a pH of 6.5–8.0), and carbon availability (Butterbach-Bahl *et al*., 2013) [22]. These environmental factors do not interact on a linear fashion and all display variability according to the spatial scale from individual soil aggregates to the field landscape. This variability leads to great heterogeneity in rates of N transformation.

Climate change will alter these environmental controls in regions producing maize through increased temperature, altered precipitation patterns, and increasing CO2; these changes will present predominantly negative impacts upon N Use Efficiency and may increase N2O emissions (Butterbach-Bahl *et al*., 2013) [22]. Practices of INM designed to maintain high soil organic matter; increase the retention of soil moisture; and develop diverse, functionally redundant microbial communities may provide more resilience to climate-driven shifts in N cycling. The interaction of climate-driven forces and INM-related changes in microbial communities present an area that is critical to future research involving sustainable maize production.

4. Integrated Nutrient Management and Soil Health

4.1. Principles and Objectives of Integrated Nutrient Management

The concept of Integrated Nutrient Management (INM) is defined as maintaining fertility of soils and supply of nutrients to crops to an optimal level so as to maximise crop production using "judicious" or "conjunctive" use of organic, inorganic, and biological sources of nutrients (Manna *et al*., 2005) [23]. The principles of INM are at odds with the paradigm established by the 'green revolution', where there was a total reliance on synthetic fertiliser; in contrast, INM believes that the long-term maintenance of soil fertility includes maintaining the biological activity, the organic matter, and the physical structure of soil which synthetic fertiliser will not accomplish by itself. INM reflects principles of circular biobased economy therefore it promotes recycling of all organic waste materials produced by crops, animals and municipalities while also addressing many of the Sustainable Development Goals set out in the Agenda 2030 (specifically, SDG 2: No Hunger; SDG 15: Life on land; SDG 13: Climate action).

Specific objectives for INM within maize production systems include; maximising Nitrogen Use Efficiency (NUE) by ensuring there is synchronous supply of nutrients and demand curves throughout the growing season; building and maintaining sufficient SOC stocks above the minimum critical threshold (i.e. greater than 1.5% in the tropics) necessary to support biological activity; reduce the dependence and therefore the costs associated with synthetic fertilisers; mitigate the adverse environmental consequences (e.g. N_2O emissions, NO_3^- leaching, eutrophication of P) of using synthetic fertiliser; and; increase biodiversity (e.g. micro-organism) within soils, thereby enabling production systems to be sustainable (Nardi *et al*., 2007) [24]. All of these objectives can only be achieved at the systems level through the use of a systematic approach and adaptive management, based upon the monitoring of soil health, including all associated indicators.

4.2. Components of INM and Their Soil Effects

Table 2 shows that the main ingredients of INM all have an influence on soil characteristics and microbial populations. Chemical fertilizers (e.g., urea, DAP, NPK) supply soluble (immediately available to plants) nutrients, but do not help build soil structure or soil organic carbon (SOC). Continued exclusive use of chemical fertilizers over a period of time has been shown to lessen soil acidity and contribute to a decrease in microbial biomass and diversity in both temperate and tropical soils (Doran and Zeiss, 2000) [25]. Organic manures (e.g., animal farmyard manure, poultry manure) supply macro and micronutrients and labile/recalcitrant carbon (C) substrates, which provide inputs to support many types of microorganisms (heterotrophs). Composts (products/outputs of thermophilic decomposition of various organic substrate wastes), which contain more stabilized C than fresh manures with a lower C:N ratio than either, significantly contribute to building of SOC long term (Bernal *et al*., 2009) [26].

Microbial inoculants—biological conditioners that include diazotrophic (nitrogen-fixing) bacteria (Rhizobium, Azospirillum), phosphate-solubilising (PSB) and plant growth-promoting (PGPR) bacteria—are intended to supplement specific microbial groups that play a critical role

in soil nitrogen or phosphorus cycling. The effectiveness of biofertilizers depends largely upon how well inoculated bacteria can establish themselves and maintain competitiveness with indigenous soil microbiota, which is affected by levels of soil organic matter and how the integrated nutrient management (INM) is carried out (Bashan and de-Bashan, 2010) [4]. The management of crop residues (i.e., whether it is removed from the field, tilled into soil, used for mulching or composting) is typically the largest addition of carbon to the soil within agricultural production systems and can have a major impact on the short-term immobilisation of nitrogen, as well as on long-term soil organic carbon (SOC) dynamics.

4.3. Effects of Long-Term INM on Soil Fertility and Carbon

Long-term integrated nutrient management (INM) studies (>10 years) consistently exhibit better soil health outcomes than either exclusively chemical or exclusively organic management. Meta-analyses of global INM studies show that compared with chemical fertilizer-only control farms, INM-produced organic-inorganic combinations of management exhibit increases in soil organic carbon (SOC) by 15–35%, C from microbial biomass (mic) by 20-50%, and maize yields by 20-40% (Mäder *et al*., 2002) [27]. These improvements are derived from accumulated (cumulative) organic carbon (building labile and stable carbon pools), improved microbial populations (better nutrient cycling), improved physical characteristics (increased aggregate stability, improved water retention, decreased bulk density).

The long-term use of INM also improves cation exchange capacity (CEC) which allows for increased SOC, which also promotes clay-humus complex formation, to improve the soil's ability to hold and buffer nutrient cations (Nardi *et al*., 2007) [24]. The ability to manage soil pH is one benefit that is often overlooked regarding organic components found in INM. Cationic alkalinity from the use of manures and composts are effective in neutralizing the acidity created by nitrification and the application of synthetic fertilizers, and these cations buffer soil pH to maintain a range of 6.0 to 7.0, which is ideal for the availability of nutrients and activity of microbes.

Furthermore, the buffering ability of the SOC aids in decreasing the sensitivity of the soil microbial community to environmental perturbations, including drought, flood, and temperature changes.

4.4. Synergistic Effects Between Organic and Inorganic Inputs

INM treatments have been shown to produce greater agronomic and biological results than the individual organic and inorganic components added together-the result of complementary mechanisms. The addition of inorganic nitrogen decreases the carbon:nitrogen ratio in organic materials in the soil, and thus accelerates the microbial decomposition of that organic material and the net mineralization of nitrogen from it. At the same time, adding organic matter to the soil supplies labile C energy sources that fuel the microbial fixation of N and nitrifying and denitrifying pathways important to the inorganic N cycle. In addition, the mycorrhizal fungi stimulated by a decrease in the amount of phosphate supplied through chemical fertilizers, as required by INM protocols, increase the surface area of the plant that can be actively used to search for phosphorus when the available phosphorus is less (i.e., through chemical fertilization) (Smith and Read, 2008) [7].

Stable isotope studies (labelled with ^{15}N isotopes) have demonstrated that the recovery of N from combined organic/inorganic INM is significantly greater than that from chemical fertilizers alone, particularly during the growing season following the organic input. The slow-release mechanism associated with microbial activity helps to guarantee that N is available for peak crop growth demand over time. This illustrates the importance of determining the INM management system as an integrated biological entity as opposed to a simple blend of organic and inorganic ingredients (rather than as just organic or inorganic).

4.5. Microbial Biomass as an Indicator of Soil Health Under INM

Microbial biomass nitrogen (MBC) and microbial biomass carbon (MBN) have been identified as sensitive and early indicators of soil health under different nutrient management practices, generally reflecting management changes within one to three years prior to the detection of any changes in total SOC (Manna *et al*., 2005) [23]. For instance, MBC in INM-treated maize soils generally ranges from approximately one-third of a microgram of biomass carbon (C) to four-fifths of a microgram of biomass Ca per gram of soil (the mean range of total MBC versus average C in sole chemical fertilizer vs average MBC versus average C in all INM [C & B]) – consistent with the previous data. On average, total MBC in maize agroecosystems treated with INM utilizes more of its resources than comes in as inputs; this is evidenced by a consistently lower ratio for the metabolic (qCO_2=basal respiration/MBC) in systems that utilized INM versus all others, indicating an overall greater efficiency in the utilization of biomass C and nutrients by microorganisms in soil treated using INM versus soil that has only been treated with chemical fertilizers alone.

In addition to MBC and MBN, dehydrogenase, urease, phosphatase, and beta-glucosidase enzyme activities can all be used as a means to measure microbial activity in soil, and can all respond to INM treatments very quickly, often incorporating the cumulative effects of soil management on biological health. Table 4 provides comparative data from two replicated field experiments showing that INM-treated soils consistently demonstrated improved microbial activity across an increasing intensity of INM use. The relationship between the overall dosing of organic inputs (amounts and quality), and the biological vitality of the soil system indicates clear dose-dependent relationships between the microbial environment and nutrient management use in maize agroecosystems as indicated by the microbial biomass use data presented in tables 5 through 9 of this publication.

Table 2: Components of integrated nutrient management (INM) and their effects on soil properties, microbial responses, and maize crop performance.

INM Component	Examples	Effects on Soil Properties	Microbial Response	Crop Benefit
Chemical fertilizers	Urea, DAP, NPK, KCl	Short-term nutrient surge; may acidify soil; reduce SOC if used alone	Transient increases in fast-growing copiotrophs; suppressed oligotrophs	Rapid yield response; risk of leaching losses
Organic manure	Farmyard manure, poultry manure, slurry	Improve CEC, WHC, soil structure; raise SOC; reduce bulk density	Stimulate broad microbial diversity; increase microbial biomass C and N	Slow-release nutrients; improved root environment
Compost	Vermicompost, green compost, biosolid compost	Long-term SOC stabilization; buffering of pH; improved aggregate stability	Enrich fungal communities; enhance enzymatic activity (urease, dehydrogenase)	Sustained nutrient supply; suppress soilborne pathogens
Biofertilizers	Rhizobium, Azospirillum, PSB, Trichoderma	No direct chemical change; indirect improvement via microbial activity	Augment targeted microbial guilds (fixers, solubilizers, PGPR)	Reduce chemical fertilizer need; enhance NUE and PUE
Crop residues	Maize stover, wheat straw, legume residues	Add labile + recalcitrant C; mulching reduces evaporation; may temporarily immobilize N	Priming effect on SOM; boost decomposer communities; shift fungal:bacterial ratio	Long-term fertility; erosion control; GHG mitigation potential
Green manures	Sesbania, Crotolaria, Tithonia	Rapid N addition; improve soil physical structure	Stimulate N-cycling microbes; increase enzyme activity	Pre-crop N supply; weed suppression; C input
Combined INM (organic + inorganic)	50% RDF + FYM + biofertilizer	Maximum SOC, CEC, WHC, and aggregate stability improvements	Highest microbial biomass, diversity, and functional redundancy	Optimum yield, NUE, and long-term soil health

FYM = farmyard manure; RDF = recommended dose of fertilizer; CEC = cation exchange capacity; WHC = water-holding capacity; SOC = soil organic carbon; NUE = nitrogen-use efficiency; PUE = phosphorus-use efficiency; PGPR = plant growth-promoting rhizobacteria.

5. Rhizosphere Microbial Community Dynamics Under Integrated Nutrient Management

5.1. Effects of INM on Bacterial and Fungal Diversity

The nutrient management system greatly alters how diverse and structured the microbial communities are in the rhizosphere. Multiple studies of rhizosphere microbial composition utilizing high-throughput Ilam-based 16S rRNA gene sequencing consistently show that INM over several years has a higher total measure of alpha diversity (Shannon H', Observed OTUs, Faith's PD) than chemical fertilizer alone; additionally, the combination of these two types of fertilization maximizes diversity (Hartmann *et al*., 2015) [32]. At the phylum level, different INM treatments will increase the number of Actinobacteria, Chloroflexi, and Verrucomicrobia present while decreasing the number of Proteobacteria that grow as a result of the use of chemicals that contain soluble nutrients.

The response of fungi communities to INM is mediated through multiple routes. Adding organic matter results in the growth of saprophytic fungi (such as Trichoderma, Penicillium, Aspergillus) that decompose lignocellulose; on the other hand, the decreased addition of chemical P fertilizers under INM leads to increased colonization of AM Fungi. Estimates of the fungal:bacterial biomass ratio are often made based on ergosterol:PLFA data, and will be higher under organic and integrated management systems because of the larger proportion of fungal decomposition pathways in the total ecosystem that includes the decomposition of large quantities of carbon (C) (Strickland and Rousk, 2010) [14]. These changes to the biochemical environment of the soil will therefore affect how much SOM accumulates since more stable, mineral-associated organic matter is produced from the necromass (dead biomass) of fungi than from the necromass of bacteria.

5.2. Community Assembly Mechanisms in Rhizosphere Environments

Factors affecting microbial community assembly within the rhizosphere may include both deterministic processes, e.g., filtering effects due to the chemical composition of root exudates; pH; nutrient limitations, as well as stochastic processes. Both deterministic factors and stochastic factors will alter the community assembly mechanism over gradations of the rhizosphere, with deterministic factors dominating within the rhizosphere immediately adjacent (0 – 0.5 mm) to the root where there are higher concentrations of root exudate and stochastically influencing beyond the immediate rhizosphere; into the outer rhizosphere; then, into the transition; and ultimately, into the bulk soil (Bulgarelli *et al*., 2013) [10]. The greater chemical heterogeneity created by integrated nutrient management (i.e., multiple organic substrate types, spatially random nutrient "hot spots") allows for the potential for greater numbers of ecological niches, hence leading to greater partitioning and resource use among many diverse groups of microbes (the "diversity-niche hypothesis"). Supporting the "diversity-niche hypothesis" is the positive correlation between microbial diversity indices and the SOC index, which is improved by the use of integrated nutrient management (Fierer and Jackson, 2006) [30].

5.3. Functional Diversity and Ecological Interactions

Functional diversity is the full range of metabolic capabilities shown by a microbial community within an ecosystem has a strong influence on process rates compared to solely using taxonomic diversity (Bell *et al*., 2005) [29]. The INM approach enhances functional diversity because there is an inclusion of multiple guilds of microorganisms that are able to perform complementary functions (i.e. carbon decomposers, nitrogen

fixers, nitrifiers, denitrifiers, phosphate solubilizers, and mycorrhizal symbionts) within one community of microorganisms; these different guilds create a network of redundant functions that help buffer against changes in the environment. The use of substrate-induced respiration (SIR) where multiple carbon substrates are used to stimulate respiration (also know as community-level physiological profiling (CLPP)), has shown that microbial communities found in pasture soils being managed under the INM technique have broader metabolic profiles compared to chemically managed soils (Doran and Zeiss, 2000) [25].

Ecological interactions, such as mutualism, competition, and cross-feeding, that occur naturally between different types of microorganisms who reside in close proximity to other microorganisms within the plant rhizosphere create a complex network of inter-dependencies that stabilize nutrient cycling processes. One type of network that occurs between fermentative microorganisms and their terminal electron acceptor microorganisms that reside within soil microaggregates, as well as between primary degradative microorganisms and the secondary consumers of the metabolic by-products produced during primary digestion, creates emergent properties that cannot be predicted solely from studying the physical characteristics of the individual microorganisms making up the population (Bell *et al*., 2005) [29]. Therefore, understanding these complex ecological interactions will be essential to developing microbial inoculation strategies appropriate to the INM framework.

5.4. Microbial Network Structures and Ecological Stability

Microbial co-occurrence networks created from 16S rRNA amplicon sequencing data of INM treatments demonstrate a greater level of complexity and connectedness when compared to chemical-only applied fertilization (Deng *et al*., 2012) [31]. Network complexity indices (i.e., node degree, clustering coefficient, modularity) are significantly correlated to soil microbial diversity and SOC levels, while they are negatively correlated to the level of environmental disturbance. To be ecologically stable to disturbances, networks need high connectivity, with most of the connections being positive (mutualism and cross-feeding) and modular (functional guilds with limited connections to other functional guilds).

Important taxa that connect hubs between INM rhizosphere co-occurrence networks include Streptomyces (Actinobacteria), Pseudomonas and Rhizobium (Proteobacteria), and Trichoderma (Fungi). If hub taxa are removed from co-occurrence networks they significantly disrupt overall connectivity of those networks, indicating that they are important for stability of the community (Deng *et al*., 2012) [31]. Therefore, INM practices that maintain habitat and substrate diversity allowing for hub taxa success will enhance ecological resilience of soil microbial communities facing climate variability.

5.5. Shifts in Microbial Populations Under Long-Term Nutrient Management

Numerous studies show long-term divergence in microbial community composition, particularly when compared across different nutrient management systems. For example, INM and chemical fertilization in maize and other cereal systems for periods of 10 to 25 years demonstrate distinct structures of microbial communities, often showing unique taxonomic signatures for each treatment (Hartmann *et al*., 2015) [32]. The impacts of these treatment regimes manifest as legacy effects related to accumulating changes in chemical, physical, and biological properties of the soil (e.g., soil organic carbon, pH, cation exchange capacity, aggregate stability, and pore connectivity) resulting from the ongoing selective pressure exerted on soil microbial communities due to the repeated addition of organic substrates.

Importantly, shifts in microbial community composition from long-term use of INM represent more than an additive response to each management element, but also represent an authentic ecological succession as the microbial community themselves engineer their habitats. For instance, soils subject to long-term INM undergo a progressive buffering of soil pH, aggregation of soil particles, and humus formation that provides increasing favorability for the establishment of slow-growing, oligotrophic taxa associated with high amounts of soil organic carbon, thereby positively reinforcing the relationship between microbial community quality and soil habitat quality and creating a ‘biological route to soil health’ that is initiated and maintained by INM (Hartmann *et al*., 2015) [32].

5.6. Relationship Between Microbial Diversity and Ecosystem Functioning

Many debates within the scientific community have revolved around the effects of microbial diversity on rates of ecosystem processes in soils. There is, however, an abundance of empirical evidence that supports a positive, saturating relation between microbial diversity and most of the carbon (C) and nitrogen (N) cycling processes (Bell *et al*., 2005; Fierer and Jackson, 2006) [29, 30]. Soils with a high level of microbial diversity may have a greater functional redundancy (the ability for multiple taxa to perform the same function) which helps to maintain the rate of soil processes in the event that one of the taxa is lost from the community. The insurance effect of having a high level of functional redundancy in soils is especially important for soils that are disturbed frequently by agricultural activities, such as tillage, pesticide application and drought conditions, as it plays an important role in the resilience of nutrient cycling processes.

Research has shown, through meta-analysis of experiments manipulating the diversity of soil microbial communities, that communities with between 5 and 10 functional groups (which have a range of taxonomic diversity within each functional group) consistently outperformed low-diversity communities when looking at metrics such as C mineralization, N cycling and biomass production, particularly under variable environmental conditions (Bell *et al*., 2005) [29]. By supporting the development of diverse, multifunctional soil microbial communities, INM (Integrated Nutrient Management) is not just an approach to manage nutrient supply to plants, but is a form of biological engineering that uses the diversity-function relationship to maximize the benefits provided to crops through the services of the rhizosphere microbiome.

Table 1: Characteristics and functional roles of major rhizosphere microorganisms involved in carbon and nitrogen cycling in Zea mays systems.

Microbial Group	Representative Taxa	Function	Key Genes/Enzymes	Ecological Role in C/N Cycling
Nitrogen-fixing bacteria	Azospirillum, Rhizobium, Bradyrhizobium, Frankia	Biological N fixation	nifH, nifD, nifK; nitrogenase complex	Convert atmospheric N2 to NH4+; reduce dependence on synthetic N fertilizers
Ammonia-oxidizing bacteria (AOB)	Nitrosomonas, Nitrosospira	Nitrification (step 1)	amoA, amoB, amoC; ammonia monooxygenase	Oxidize NH4+ to NO2-; first step in nitrification; key in N availability
Ammonia-oxidizing archaea (AOA)	Nitrososphaera, Nitrosotalea	Nitrification under low pH	amoA (archaeal); Thaumarchaeota-type	Dominant nitrifiers in acidic and low-ammonia soils; often exceed AOB in rhizospheres
Nitrite-oxidizing bacteria (NOB)	Nitrobacter, Nitrospira	Nitrification (step 2)	nxrA, nxrB; nitrite oxidoreductase	Oxidize NO2- to NO3-; complete nitrification pathway with AOB/AOA
Denitrifying bacteria	Pseudomonas, Paracoccus, Thiobacillus	Denitrification	narG, nirK, nirS, nosZ; reductase enzymes	Reduce NO3- → NO2- → NO → N2O → N2; N loss from soil system
Ammonifying bacteria	Bacillus, Clostridium, Proteus	Ammonification	Urease, protease, deaminase	Decompose organic N compounds to NH4+; link organic N to plant-available N pools
Cellulose-decomposing bacteria	Cytophaga, Cellulomonas, Trichoderma (fungi)	C mineralization	Cellulases, hemicellulases, beta-glucosidase	Decompose plant residues; release CO2 and labile C; drive soil C turnover
Mycorrhizal fungi	Glomus, Rhizophagus, Funneliformis	P solubilization, C sequestration	Phosphatases, glomalin	Extend root surface area; stabilize soil aggregates; facilitate C transfer
Phosphate-solubilizing bacteria	Pseudomonas, Bacillus, Penicillium	P mobilization	Gluconic acid, citric acid, phosphatases	Release bound P; coupled to C exudate mineralization; improve P-use efficiency

AOB = ammonia-oxidizing bacteria; AOA = ammonia-oxidizing archaea; NOB = nitrite-oxidizing bacteria; PSB = phosphate-solubilizing bacteria; AMF = arbuscular mycorrhizal fungi; PGPR = plant growth-promoting rhizobacteria. Enzyme names indicate key functional markers for each group.

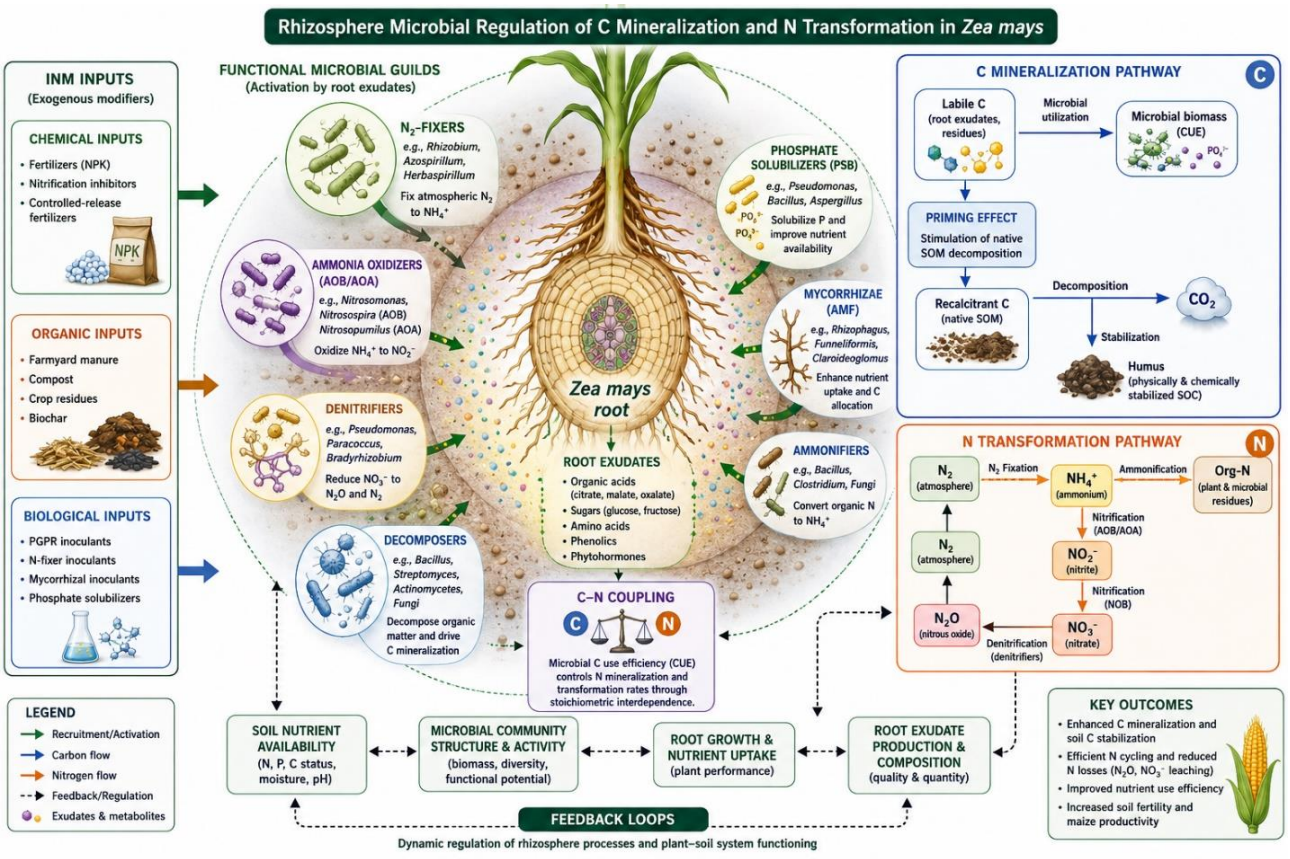

Fig 1: Rhizosphere Microbial Regulation of Carbon Mineralization and Nitrogen Transformation in *Zea mays* under Integrated Nutrient Management (INM)

6. Interactions Between Carbon Mineralization and Nitrogen Transformation

6.1. Coupling Between Carbon and Nitrogen Cycling

Microbial metabolism links the carbon (C) and nitrogen (N) cycles in soil through a tight coupling with the stoichiometric (elemental) requirements of microbial biomass. Microbial biomass has an average carbon to nitrogen (C:N) ratio of 6:1 to 12:1 depending on the composition of the community. This coupling means that the rate of nitrogen transformation is limited by the availability of cellulose as an energy source for the heterotrophic microbial processes such as ammonification and denitrification. Conversely, the availability of N controls enzyme synthesis and limits microbial biomass growth. Thus, at the microbial biomass pool, the intersection of both of these nutrient cycles makes it dynamic and managing one cycle will also have an effect on the other cycle (Sinsabaugh *et al*., 2013; Manzoni and Porporato, 2009) [15, 34].

With respect to integrated nutrient management (INM) in maize\rhizospheres, the addition of both organic C sources (such as manures and residues) and inorganic N sources (such as fertilizers) at the time of planting provides the ability to create a precisely controlled substrate stoichiometry for optimal conditions of net N mineralization and minimum loss of gaseous emissions of N such as N_2O (Manzoni and Porporato, 2009) [34]. When the C:N ratio of organic inputs is 20-25, the demand for N by microorganisms is approximately equal to the supply of inorganic N, thus minimizing both immobilization of N (eliminating temporary shortages in nitrogen) and N_2O emissions (thereby limiting the N substrate for denitrification). This concept of stoichiometric optimization is the mechanistic basis to the rate/timing recommendations developed by INM practitioners based on empirical yield data.

6.2. Carbon Availability and Its Effects on Nitrogen Transformations

The main driver of N transformation in soils is C supply through biological mechanisms such as denitrification. Denitrification — the main vector for the loss of gaseous N — will be strongly limited by the availability of C in well-aerated agricultural soils, and the application of labile C substrates will usually stimulate N_2O and N_2 production from denitrification (Wallenstein *et al*., 2006) [21]. The quality of the C substrate is also critical; for example, simple and readily decomposable substrates such as glucose or acetate lead to immediate high levels of denitrification upon application, whereas more complex substrates such as cellulose (and lignocellulose) can sustain a reduced level of denitrification over a longer time frame as a result of providing a longer term but lower rate of C supply for the complete denitrification of N_2O to N_2 via nosZ.

The availability of C will also stimulate the fixation of N through biological N fixation (BNF). BNF requires approximately 6 to 7 mol of C to be supplied through rhizosphere exudates per mol of N_2 that is fixed. This creates an energetic link between the supply of C in the rhizosphere and N inputs via biological means (Zehr *et al*., 2003) [19]. Higher amounts of C in the rhizosphere, as a consequence of either high-C exuding maize genotypes or organic amendments via INM, have been shown to correlate with higher levels of nifH gene transcription and diazotrophic diversity, suggesting that the ability of plants to transfer C to their rhizosphere could be a promising approach to enhancing BNF without the need for the addition of inoculants. Plant-driven microbiome management represents a new frontier in plant breeding and INM integration (Subbarao *et al*., 2015) [5].

6.3. Nitrogen Regulation of Microbial Carbon Utilization

Nitrogen availability provides feedback regulation for microbial C utilization via microbial growth efficiency (CUE) and enzyme production of C decomposition. Less nitrogen means limited microbial biomass synthesis, making microbial organisms respire a larger portion of assimilated C. Adequate nitrogen enables efficient C incorporation in biomass and SO organic carbon accumulation. This N limitation on CUE is important in soil microsites with the application of high C:N organic inputs without supplemental nitrogen, leading to crop residue-only management. Under INM, the dual application of inorganic N together with high-C organic inputs alleviates microbial N limitation, which increases CUE and thus promotes greater microbial biomass accumulation per unit C mineralized. This has a dual advantage; more C remains in the soil system (i.e. less CO_2 emission per unit C input) and more N is immobilized in microbial biomass, a fraction of which is protected from leaching and made available gradually during the subsequent mineralization stage. This is a combination of reasons explaining why INM is more efficient than the either single form of management in building soil C and improving NUE (Bhattacharyya *et al*., 2008) [28].

6.4. Rhizosphere Feedback Mechanisms Affecting Nutrient Cycling

There are both positive and negative feedback loops present in maize rhizospheres that regulate nutrient cycling processes. Examples of positive feedback loops are: (1) exudates released from maize roots enhance the activity of microbes → increased rates of nutrient mineralization → enhanced nutrient availability to maize → enhanced root growth and exudation of N-fixing bacteria; (2) Mycorrhizal fungi colonising the maize roots provide enhanced phosphorus (P) availability → increased rates of photosynthesis → increased amounts of C moved below ground → increased growth of mycorrhizal fungi and their ability to mobilise P (Smith and Read, 2008) [7].

There are also examples of negative feedback that regulate these interactions: (1) high concentrations of nitrogen (N) in the soil reduce the competitive ability of N-fixing bacteria, thus suppressing biological nitrogen fixation (BNF); (2) high rates of nitrification reduce the concentrations of ammonium (NH_4^+), and subsequently generate a plant-mediated BNI response that will suppress activity of organisms that nitrify (Subbarao *et al*., 2015) [5].

The complex interactions between these feedback mechanisms produce emergent regulatory dynamics within rhizospheres, which can be manipulated through Integrated Nutrient Management (INM) to achieve desired outcomes. For example, maintaining moderate to low rates of inorganic N application while providing high rates of organic C and N avoids suppression of N-fixing bacteria and also provides adequate energy for BNF. Similarly, combining maize genotypes with strong BNI capacity and developing INM systems that maintain high levels of nitrifier diversity will optimise N retention relative to nitrification (Subbarao *et al*., 2015) [5].

6.5. Implications for Nutrient-Use Efficiency

Microbial interactions between C and N cycles in maize cropping systems under integrated N management (INM), determine the final amount of N (nitrogen) uptake (nitrogen use efficiency (NUE)) based on the composition of rhizosphere microorganisms and activity levels. In meta-analyses, NUE values were found to be 60-80% under INM when compared to 30-50% under sole chemical fertilizers. The difference in NUE is primarily attributed to microbial processes. For example, under INM, there is less nitrate (NO3-) leaching, due to being immobilized by the microbes; reduced nitrous oxide (N2O) emissions as a result of having more complete denitrification; and more synchronization between the release of N and crop demand because of sustained mineralization from organic materials or pools (Mäder *et al*., 2002) [27].

The connection between microbial diversity in INM systems and NUE is facilitated through functional complementarity, wherein diverse microbial communities with multiple N-cycling guilds functioning on a variety of substrates and in multiple environments, provide more reliable temporally buffered (i.e., steady) N release compared to low-diversity microbial communities characterized primarily by fast-growing copiotrophic microorganisms that have transient (i.e., burst and crash) dynamics in response to nutrient pulse input (Fierer and Jackson, 2006) [30]. This supports the need for managing the quantity, diversity and functional structure of the rhizosphere microbial communities as an explicit goal in INM for maize farming.

6.6. Modeling C-N Interactions in Maize Rhizospheres

The area of research on mathematical modeling for combined dynamics of carbon & nitrogen (C/N) in maize rhizospheres using Integrated Nutrient Management (INM) is new and seeks to integrate the biogeochemical theories with the ecology of microorganisms (Manzoni and Porporato, 2009) [34]. Examples of models currently in use vary from simple, two-pool systems of C and N (active & passive organic matter) to multi-pool models such as CENTURY and RothC that will denote the differences in the classes of organic matter and their rates of mineralization. In addition, C/N models look at the role of microbial communities as 'black boxes', devoid of representation of microbial diversity and/or community composition thus limiting their predictive abilities across a range of INM practices.

The use of emerging trait- and individual-based models to represent functional metrics of microbial populations such as carbon use efficiency (CUE) enzymatic activity and substrate affinity, dynamics of microbial population growth, death and predation, as well as trait-environment coupling will present one pathway for mechanistic, accurate predictions regarding C mineralization and the transformation of N, that will improve predictive capabilities for fermentation under varying INM practices (Manzoni and Porporato, 2009) [34]. The combination of this modeling work with multi-omics datasets (i.e., gene abundance & process rates combined using structural equation modeling) represents a computational frontier for developing quantitative, real-time measures of how maize production impacts soil quality; thus when using INM practices we will ultimately produce more sustainable systems.

Table 3: Major microbial genes associated with nitrogen transformation pathways in agricultural soils, their host organisms, enzymatic functions, and relevance to INM research.

Gene	Full Name	Microbial Hosts	Function in N Cycle	Significance in INM Studies
nifH	Nitrogenase reductase	Rhizobium, Azospirillum, Frankia, Cyanobacteria	Encodes Fe protein of nitrogenase; N2 fixation; reduces N2 to NH4+	Universal marker for diazotrophic communities; increases under organic INM
amoA (bacterial)	Ammonia monooxygenase subunit A	Nitrosomonas, Nitrosospira (AOB)	First step in nitrification; oxidizes NH4+ to NH2OH then NO2-	Indicator of nitrification potential; suppressed by high C:N organic inputs
amoA (archaeal)	Archaeal ammonia monooxygenase subunit A	Nitrososphaera, Nitrosotalea (AOA)	Performs ammonia oxidation in acidic/low-N soils; distinct from bacterial amoA	Dominant in unfertilized soils; ratio to bacterial amoA shifts with INM
nirK	Copper-containing nitrite reductase	Rhizobium, Paracoccus, Bradyrhizobium	Reduces NO2- to NO; first step of gaseous N production in denitrification	Indicates N loss risk; differentiates denitrifier communities in soil
nirS	Cytochrome cd1 nitrite reductase	Pseudomonas, Thiobacillus, Sulfurimonas	Alternative NO2- → NO reductase; functionally equivalent to nirK but phylogenetically distinct	Co-exists with nirK in soils; relative abundance affected by C availability
nosZ	Nitrous oxide reductase	Paracoccus denitrificans, Dechloromonas, Anammox bacteria	Reduces N2O to N2; final step in complete denitrification; reduces GHG emissions	Higher nosZ:nirS ratio indicates complete denitrification; promoted by organic C additions
narG	Nitrate reductase subunit alpha	Pseudomonas, Paracoccus, Thiobacillus	Reduces NO3- to NO2-; initiates dissimilatory nitrate reduction (DNRA and denitrification)	Marker of anaerobic N processing; increases under waterlogged or high C conditions
hzo	Hydrazine dehydrogenase	Candidatus Kuenenia, anammox bacteria	Key enzyme in anaerobic ammonium oxidation (anammox); converts N2H4 to N2	Relevant in flooded or waterlogged maize soils; largely underexplored in INM contexts

AOB = ammonia-oxidizing bacteria; AOA = ammonia-oxidizing archaea; BNF = biological nitrogen fixation; DNRA = dissimilatory nitrate reduction to ammonium; GHG = greenhouse gas; INM = integrated nutrient management.

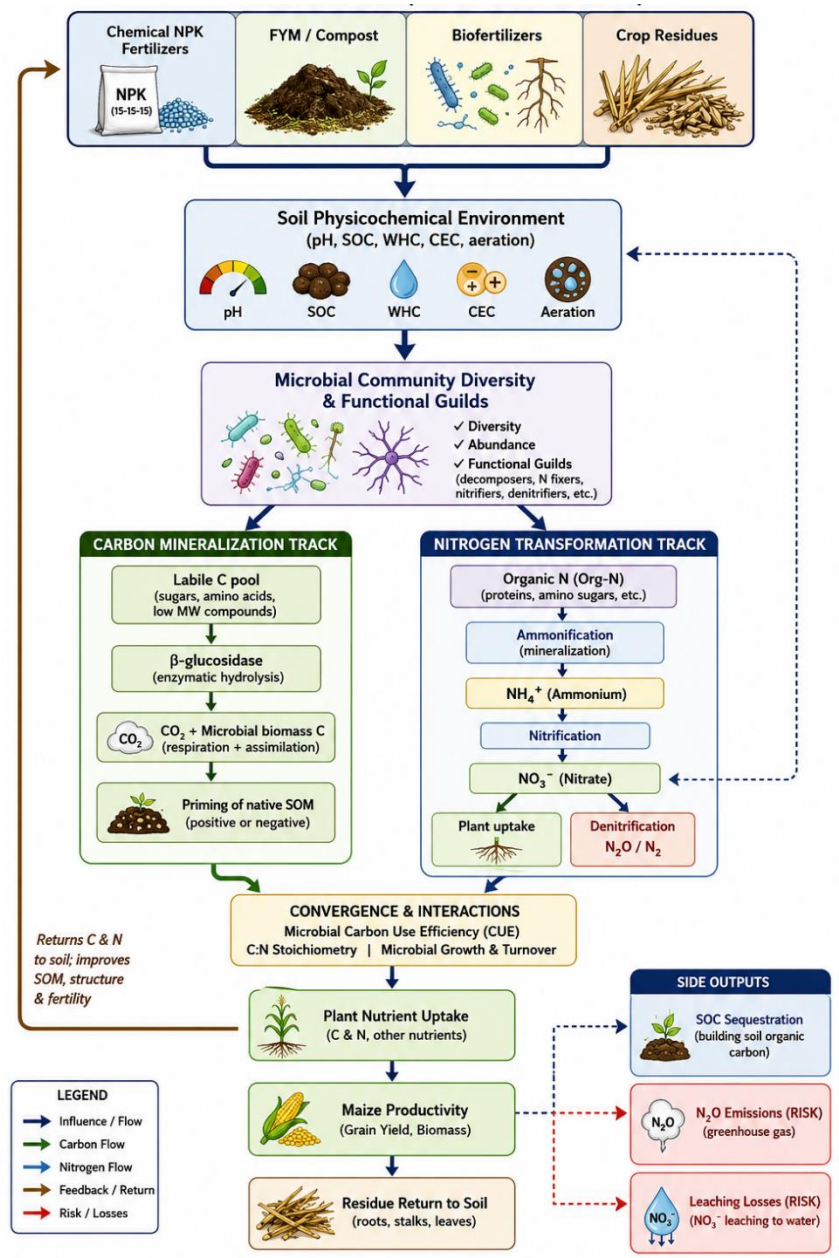

Fig 2: Integrated Nutrient Management–Driven Regulation of Rhizosphere Microbial Communities and Coupled Carbon–Nitrogen Cycling in *Zea mays*

7. Soil Physicochemical Responses and Crop Performance Under INM

7.1. Effects of INM on Soil Physical Properties

Soil physical properties that support both microbial habitat quality and crop root growth are greatly impacted by integrated nutrient management (INM), especially through the regular addition of organic materials (manure/compost/crop residues) (Manna *et al*., 2005; Doran and Zeiss, 2000) [23, 25]. Repeated additions of organic materials via INM ultimately reduce bulk densities of tropical soils from approximately 1.45-1.55 g/cm3 under chemical-only management to roughly 1.25 to 1.35 g/cm3 under long-term INM (Manna *et al*., 2005; Bhattacharyya *et al*., 2008) [23, 28]. The reduction in bulk density improves the aeration of soils, depth of root penetration, and level of soil microbial activity, thus providing for a positive feedback loop between organic matter management and biological function.

INM significantly enhances soil aggregate stability (a primary indicator of soil structural health) (Bhattacharyya *et al*., 2008; Manna *et al*., 2005) [28, 23]. This is accomplished through four mechanisms: aggregate binding by fungal hyphae; AMF glomalin-related soil protein (GRSP) production; soil microbial biomass and associated microbial activity increasing the cohesion among soil particles; chemical bridging between organic materials and mineral surfaces in soil. Additionally, aggregate stability minimizes the likelihood of erosion; maintains soil aeration within the aggregates; and protects soils from the rapid mineralization of stored soil organic matter (SOM) via physical encapsulation, thus contributing to the long-term sequestration of carbon (C) (Lal, 2004) [33].

Moreover, water-holding capacity (which is essential for producing maize in water-limited environments) is significantly enhanced by INM as a result of both the increase of soil organic carbon (SOC) improving soil water retention and enhanced aggregation of soils (Bhattacharyya *et al*., 2008; Lal, 2004) [28, 33].

7.2. Effects on Soil Chemical Properties

Soil chemical properties may be moderated by Integrated Nutrient Management (INM), thereby enhancing nutrient availability and providing a conducive environment for microorganisms. Soil pH, which tends to decrease as a result of high inputs of ammonium nitrogen through nitrification when chemical fertilizers are used exclusively as the only source of nutrients, has been found to be stabilized or increased slightly where INM management is implemented because of the alkaline nature of the products formed by the decomposition of organic matter and the ability of high SOC soils to act as a pH buffer (Nardi *et al*., 2007; Doran and Zeiss, 2000) [24, 25]. Managing pH levels to range from 6.0 to 7.0 is important for multiple nutrient availability (N, P, K, S, Mg, Ca, Mo, B) and optimal rates of nitrification.

Cation Exchange Capacity (CEC), or the soil's ability to retain positively charged nutrient cations from leaching, has been shown to increase progressively under INM management as SOC accumulates in high humic and fulvic acid fractions with a high negative surface charge (Bernal *et al*., 2009; Nardi *et al*., 2007) [26, 24]. The evidence from 10 years of INM experimentation in various tropical soils suggests CEC increases between 2 and 5 cmol(+) kg^{-1} CEC for improving nutrient retention efficiency significantly (Bhattacharyya *et al*., 2008) [28]. Phosphorus and potassium availability have also been enhanced through the use of organic fertilization and the microbial activity responsible for mineral nutrient solubilization (Richardson *et al*., 2009) [9].

7.3. Nutrient Availability and Microbial Habitat

The use of INM (integrated nutrient management) allows for increased nutrient cycling and improved growth of various types of bacteria (Doran and Zeiss, 2000; Manna *et al*., 2005) [25, 23]. By providing a high level of SOC (soil organic carbon), there is a source of energy and carbon for bacteria that is available from the decomposition of organic materials. Since there is moderate levels of inorganic nitrogen available, bacteria have enough nitrogen to grow without causing excessive amounts of nitrification or denitrification.

Additionally, the presence of easily decomposable carbon sources in the soil of an integrated nutrient management (INM) system results in a greater dehydrogenase activity and microbial respiration quotient (Table 4; ref. 23) than what would occur in soils that only had mineral fertiliser applied (Manna *et al*., 2005) [23].

The spatially heterogeneous nature of nutrient concentrations found in an INM system results from both an unequal decomposition rate of organic amendments used to improve the soil (manures versus composts versus residues) as well as the difference in where bacteria are growing and metabolizing. The areas adjacent to roots (rhizosphere) are where most of the bacterial activity occurs. Therefore, in an INM system, there are numerous places for the bacteria to grow and have their own distinct niche. The habitat heterogeneity that occurs within an INM system must be the main reason for the greater diversity of microbial species than would typically be found if only mineral fertiliser was applied (Bell *et al*., 2005; Fierer and Jackson, 2006) [29, 30].

7.4. Effects on Maize Growth and Yield Parameters

The cumulative impacts on soil health resulting from INM (integrated nutrient management) lead to increased maize yields and improved maize growth (Bhattacharyya *et al*., 2008; Manna *et al*., 2005) [28, 23]. Maize grown in INM (integrated nutrient) soils has greater root biomass accumulation than maize produced in soils treated by conventional agricultural practices. The increase in root biomass from INM leads to a more substantial rhizosphere volume and surface area for microbial interactions, which generates a positive feedback that increases the benefits of microbially mediated nutrient cycling. Shoot biomass and leaf area index showed similar benefits from INM practices, indicating improved nutrient uptake and nutrition of plants from the combination of chemical and organic activities associated with INM management.

The expected long-term maize grain yields resulting from field-based INM are between 8.5–9.5 t ha^{-1} and are higher than yields from only chemical (6.5–7.5 t ha^{-1}) or only organic (5.0–6.0 t ha^{-1}) management systems, as well as yields from untreated controls (3.5–4.5 t ha^{-1}) according to Table 4 (Manna *et al*., 2005; Bhattacharyya *et al*., 2008; Mäder *et al*., 2002) [23, 28, 27]. The expected increase in grain yield from INM versus conventional agricultural practices results from improved synchrony of N (nitrogen) supply and demand (reducing early-season N deficits and late-season N surpluses), improved water retention capacity of soils (reducing the potential for droughts) and the utilization of PGPR (plant growth promoting rhizobacteria) for improving root exploration of soil (improving the potential for late-season nutrient foraging) (Bashan and de-Bashan, 2010; Bhattacharyya *et al*., 2008) [4, 28]. Under INM management, FNUE (fertilizer nitrogen uptake efficiencies) are 30% to 40% higher than those from only chemical management, which reflects improved spatial and temporal synchrony of actual N supply and demand created through microbially mediated slow-release mechanisms (Bhattacharyya *et al*., 2008; Manna *et al*., 2005) [28, 23].

7.5. Long-Term Sustainability and Soil Degradation Prevention

The sustainability of maize production with Integrated Nutrient Management is characterized by maintaining or increasing yields over time. Over periods of 10 to 25 years, yields at experimental sites continuously increase under Integrated Nutrient Management, while sole chemical fertiliser systems typically exhibit yield stagnation or decline after 10 years (Hartmann *et al*., 2015; Manna *et al*., 2005) [32, 23]. The indicators of soil degradation caused by sole chemical fertilisation, such as compaction, acidification, decreasing soil organic carbon, and decreasing microbial diversity, are often eliminated or reversed through Integrated Nutrient Management (Hartmann *et al*., 2015; Doran and Zeiss, 2000) [32, 25]. In addition, trajectory analyses of soil health indicators have shown that there have been further improvements over the first 15 to 20 years of implementing Integrated Nutrient Management practices (Hartmann *et al*., 2015) [32].

The economic analysis of long-term Integrated Nutrient Management and sole chemical fertiliser systems should take into account the compounding benefits of the improvement of soil capital. These benefits include, but are not limited to, a reduction in the amount of fertilizer needed in the future (as biological nitrogen cycling becomes more effective), a reduction in the amount of irrigation needed in the future (as water retention improves), a reduction in the amount of pest and disease pressure (as the development of soil suppressive microbial communities occurs), and a greater ability of the soil to withstand extreme weather events (as soil structure and increased water retention buffers agricultural production).

Life cycle assessment studies of Integrated Nutrient Management systems consistently demonstrate that they have better environmental performance metrics (reduced global warming potential, reduced eutrophication potential, and decreased energy consumption per tonne of grain produced) than high-input chemical fertiliser systems (Lal, 2004) [33].

Table 4: Comparative impacts of different nutrient management treatments on soil physicochemical properties, microbial indicators, and maize productivity parameters. Values represent means±standard error from replicated field experiments (n = 4).

Parameter	Control (No Input)	Chemical Only (RDF)	Organic Only (FYM)	INM (50% RDF + FYM)	INM + Biofertilizer	Statistical Significance
Soil organic C (g kg^{-1})	7.2±0.3	7.5±0.4	9.8±0.6	11.3±0.5	12.1±0.7	$p < 0.001$; INM > organic > chemical = control
Microbial biomass C (µg g^{-1})	185±12	210±18	310±22	395±28	430±31	$p < 0.001$; progressive increase with organic inputs
Microbial biomass N (µg g^{-1})	24±2	28±3	40±4	52±5	58±4	$p < 0.001$; highest under integrated management
Soil basal respiration (µg CO_2-C g^{-1} d^{-1})	1.8±0.2	2.1±0.2	3.2±0.3	4.1±0.4	4.5±0.5	$p < 0.001$; reflects microbial metabolic activity
Dehydrogenase activity (µg TPF g^{-1} h^{-1})	38±4	45±5	72±6	98±8	110±9	$p < 0.001$; best indicator of overall microbial activity
Urease activity (µg NH_4^+-N g^{-1} h^{-1})	12.3±1.2	14.5±1.4	22.8±2.1	29.4±2.5	33.1±2.8	$p < 0.001$; linked to N mineralization capacity
Bacterial diversity (Shannon H')	3.12±0.08	3.25±0.09	3.68±0.11	4.02±0.12	4.18±0.13	$p < 0.001$; INM treatments highest
Maize grain yield (t ha^{-1})	4.1±0.3	7.2±0.4	5.8±0.4	8.6±0.5	9.2±0.6	$p < 0.001$; synergistic effect in INM treatments
N-use efficiency (%)	—	42±3	51±4	68±5	74±6	$p < 0.001$; dramatic improvement under integrated management
Soil pH	6.8±0.1	6.3±0.1	6.9±0.1	6.8±0.1	6.9±0.1	$p < 0.05$; chemical fertilizer causes acidification

RDF = recommended dose of fertilizer; FYM = farmyard manure; MBC = microbial biomass carbon; MBN = microbial biomass nitrogen; TPF = triphenyl formazan; WFPS = water-filled pore space; NUE = nitrogen-use efficiency. Different letters within rows indicate statistically significant differences at $p < 0.05$ (Tukey's HSD). Data compiled from representative long-term field experiments in tropical and subtropical maize production systems.

8. Emerging Analytical Approaches and Future Perspectives

8.1. Molecular and Sequencing Approaches

Utilization of high-throughput sequencing technologies for rhizosphere microbial ecology has transformed previously culture-dependent techniques into answers regarding the diversity and the character of microbial communities without the need to rely on cultures. Currently, the primary means of examining the bacterium community composition is to use 16S rRNA gene amplicon sequencing, which analyzes a subset of the 16S gene and can provide both taxonomic identification down to the genus or at a species level (with either V3-V4 or V4-V5 hypervariable regions) and cover thousands of individual samples, enabling regional INM comparisons across various soil types, climatic conditions, and management practices (Jansson and Hofmockel, 2020) [35]. The limitations of amplicon sequencing consist of primer bias during amplification, the potential for chimeric sequences to be formed during PCR reactions, and that they do not allow for the direct linking of known taxonomy to functional characteristics.

Shotgun metagenomics addresses some of the limitations associated with amplicon sequencing, as it obtains functional gene inventories from the total DNA in any given soil sample (including genes related to N cycling like nifH, amoA, nirK, nirS, and nosZ) as well as taxonomic information, with the advantage of not being impacted by PCR amplification bias (Jansson and Hofmockel, 2020) [35]. Metatranscriptomics, which involves sequencing of all of mRNA in a soil sample, goes even farther than shotgun metagenomics by quantifying both the presence and expression (i.e., actual activity) of genes in situ, which allows for direct linking of the composition of the microbial community to the rate of metabolic activity of that microbial community. For example, linked to how N-cycling genes are expressed within rhizospheres of INM field trials have been shown to change significantly in terms of expression (not just concentration) due to different management practices and can result in incorrect process rate predictions (Jansson and Hofmockel, 2020) [35].

8.2. Stable Isotope Probing

Stable Isotope Probing (SIP) provides an effective way to link the identity of nonphotosynthetic microorganisms with the functional outputs of those organisms using heavy isotopes and density gradient methods to separate labeled nucleic acids and/or lipids from soil communities. By utilizing DNA-SIP with ^{13}C labeled root exudates, such as glucose, citrate and glycine, researchers have identified bacteria that metabolically utilize root exudates and those specific taxa comprise a "core" community of rhiizosphere organisms in contrast to the larger community of rhizosphere organisms measured by 16S abundance (Kuzyakov, 2010) [16].

SIP has been used to evaluate how different practices affect microbial metabolism based on three different ways in which it lets you link the identity with function of microorganisms under intensive nutrient management (INM): Under INM there is a smaller number of taxa that are metabolically active in metabolizing labile carbon, but with a larger diversity of taxa than in soils only fertilized with chemicals; N-cycling functional groups (nitrifiers and denitrifiers) are more metabolically active, even with similar levels of gene abundances as compared to soils treated with fertilizer; the factors behind the "priming effect" found in maize rhizospheres are driven by a focused group of copiotrophic microorganisms that respond intimately to organic inputs in INM. The findings produced from these SIP studies support targeting specific functional groups of microorganisms in

developing biofertilizer based on the practices of INM (Kuzyakov, 2010) [16].

8.3. Multi-Omics Integration

Combining all four layers of omics data with soil-based biogeochemical data will offer novel insights into how and why multiple layers of omics data integrate to create a functioning rhizosphere ecosystem (Jansson and Hofmockel, 2020) [35]. The integration of multiple omics data types allows us to build multi-level models that explain how ecosystem services occur; linking taxonomic composition (genomics) through the transcription of genes (transcriptomics), production of enzymes (proteomics), metabolism of substrates (metabolomics), and rate of biogeochemical cycling of nutrients through the ecosystem as a function of time (e.g., CO_2 flux, N_2O emission, and nutrient cycling rates) has not been accomplished yet.

A multi-omics approach can answer fundamental questions about how organic amendments to the soil enhance the efficiency of nitrogen cycling; how changes induced by INM in microbial population characteristics alter the production of N_2O; and, what novel taxa and functional genes can be developed into biofertilizers. All of these advances rely on multi-omics integration. However, the limitations of multi-omics integration include throughput constraints, the sheer volume of data generated, and the need for expanded computational power to process the data, as well as determining how molecular data relates to biogeochemical fluxes over various spatial and temporal scales (Jansson and Hofmockel, 2020) [35].

8.4. Statistical and Ecological Modeling Techniques

Research has demonstrated that statistical methods for analyzing rhizosphere microbial ecology data have greatly improved over the last few decades, evolving from simple diversity indices and pairwise comparisons to advanced methods such as multivariate ordination (e.g., Principal Component Analysis (PCA), Principal Coordinate Analysis (PCoA), Non-Metric Multidimensional Scaling (NMDS), Redundant Analysis (RDA)), network analysis and structural equation modeling (SEM) (Deng *et al*., 2012) [31]. RDA and constrained ordination methods appropriately account for the variance associated with the environmental measurements (e.g., the soil organic carbon (SOC), soil pH, soil texture, and management practices) and the unexplained variance associated with microbial community composition. This creates a useful framework for estimating the relative contributions of various INM components to the ecological dynamics of microbial communities.

SEM also provides an excellent tool to analyze both the intricate and bidirectional causal relations among INM systems, soil properties, microbial communities, and crop productivity by evaluating theoretical causal models to empirical data and identifying indirect pathways of influence. Empirical results from using SEM on INM datasets have revealed that the majority of organic amendments' effects on maize yield occur indirectly due to the contribution of soil microbial biomass and enzyme activity, rather than due to direct contributions from the nutrient supply provided by the amendment. This finding has significant implications regarding the interpretation of INM mechanisms and future experimental design. Machine learning approaches such as random forests and neural networks are frequently employed to develop predictive models of the composition of microbial communities or the rates of ecosystem processes (e.g., decomposition, primary production) from the physical and chemical characteristics of soil, providing empirically-based predictive capability when mechanistic understanding is less than complete (Deng *et al*., 2012; Jansson and Hofmockel, 2020) [31, 35].

8.5. Challenges and Limitations in Rhizosphere Research

Despite remarkable recent progress in understanding how rhizosphere microbes regulate carbon (C) and nitrogen (N) cycling in the context of integrated nutrient management (INM), many fundamental limitations continue to impede the mechanistic understanding of these processes. In particular, the scale problem – reconciling microbial processes occurring at micrometer-scale (individual cells and soil aggregates) with observed field-scale nutrient cycling and crop performance – has not been solved (Jansson and Hofmockel, 2020) [35]. Additionally, most of the sequencing and molecular biology methodologies used to study microbial communities require bulk soil or rhizosphere soil sample collection, which removes the enormous spatial heterogeneity that exists in soils; therefore, they may not accurately represent the critical nature of microbial hotspots (i.e. root tips, rhizosphere-bulk interface, aggregate surfaces) for driving ecosystem-level process rates.

The distinction between causal and correlational studies in observational research on INM continues to be a challenge: there are many documented instances of correlations between INM treatments, changes in microbial community structure, and enhanced N cycling; however, demonstrating that specific changes in microbial community structure are responsible for improvements in N cycling (as opposed to simply being correlated with one another) requires experimental manipulation (e.g. inoculation, selective suppression) that is often challenging to accomplish in the highly heterogeneous and dynamic soils of commercial agricultural fields (Bell *et al*., 2005) [29]. In addition, the temporal resolution of almost all studies using INM focuses on annual sampling of soils, which does not allow for capture of the higher-frequency dynamics of rhizosphere activity that underlie nutrient cycling throughout the growing season for crop plants. Future investigations will require increased temporal resolution (e.g. weekly to monthly sampling) and spatial resolution (e.g. micro-scale imaging, microfluidic platforms) in order to fully document the processes occurring within the rhizosphere.

8.6. Future Research Priorities and Technological Innovations

The following are the high priority areas for future research on rhizosphere microbial ecology in maize systems under INM. These areas include area: (1) Investigation into the mechanisms of carbon:nitrogen (C:N) coupling at the microbial community level using Stable Isotope Probing (SIP) and multi-omics; (2) Development of predictive models for N2O emissions from INM systems incorporating microbial community composition data; (3) Characterization of the function of archaeal communities (AOA, methanogens, Thaumarchaeota) in INM rhizospheres, which have not previously been adequately studied; (4) Identification of hub taxa within INM rhizosphere networks and their roles, with a goal of promoting the conservation or augmentation of these taxa through management; and (5) Assessment of the impact of INM on the virome of the rhizosphere (phage

communities) and their effect on regulating the growth of microbial communities (Jansson and Hofmockel, 2020; Deng *et al*., 2012) [35, 31].

Recent advancements in technology that have enabled these objectives are; NanoSIMS for single-cell isotope tracking in soil matrix; microfluidic root-on-chip systems designed to carry out controlled rhizosphere research; CRISPR-based functional screening of soil metagenomes; and real-time environmental sensors allowing the in situ monitoring of CO_2, N_2O and NH_3 fluxes at very short time intervals. By using these new technologies in conjunction with precision agriculture data systems (e.g. remote sensing, soil sensors, and yield monitoring) we can begin to envision digitally-enabled, microbially-informed precision nutrition management of maize that could lead to dramatic improvements in NUE while also reducing the environmental impact of maize production on a large scale (Jansson and Hofmockel, 2020) [35].

Table 5: Research gaps, methodological limitations, and future directions in the study of rhizosphere microbial regulation of carbon and nitrogen cycling under integrated nutrient management in maize systems.

Research Domain	Current Limitation	Methodological Constraint	Recommended Future Direction
Rhizosphere C priming	Mechanisms of negative vs. positive priming poorly resolved in maize systems	Isotope dilution methods cannot distinguish primed vs. mineralized native SOM	Combine 13C-SIP with metatranscriptomics to resolve active decomposers in priming events
N2O emission pathways	Relative contributions of nitrifier denitrification vs. heterotrophic denitrification under INM unclear	Gene-based approaches conflate potential with actual activity; transcript levels needed	Metatranscriptomic analysis of nosZ and nirS/nirK across growing season under INM treatments
Mycobiome-INM interactions	Fungal communities underrepresented relative to bacteria in INM studies	ITS sequencing less standardized; reference databases incomplete for soil fungi	Targeted ITS2 amplicon sequencing with ectomycorrhizal and saprotrophic guild analysis
Plant-microbe metabolic signaling	Root exudate composition under INM barely characterized in tropical maize varieties	Exudate collection methods distort rhizosphere chemistry; GC-MS/LC-MS not standardized	Apply HPLC-MS exudate profiling combined with rhizosphere microbiome sequencing at multiple crop stages
C-N coupling mechanisms	Microbial carbon use efficiency (CUE) rarely measured in field INM experiments	CUE estimates from batch incubations poorly predict field conditions	Develop in situ CUE measurement using 18O-water and 13C-glucose dual-labeling under varied INM
Long-term INM effects	Most INM studies <5 years; decadal effects on microbial community assembly unknown	Replication and site comparability issues in long-term experiments; confounding effects	Establish multi-site, replicated decadal INM experiments with archived soil banks for retrospective omics
Modeling and prediction	Current biogeochemical models (CENTURY, RothC) poorly integrate microbial diversity data	Trait-based models require parameterization data that are rarely available for diverse microbial guilds	Develop trait-based microbial explicit models integrating metagenomics output for C and N cycle prediction

SIP = stable isotope probing; SEM = structural equation modeling; SOM = soil organic matter; INM = integrated nutrient management; CUE = carbon use efficiency; ITS = internal transcribed spacer; HPLC-MS = high-performance liquid chromatography–mass spectrometry; AOA = ammonia-oxidizing archaea; omics = genomics, transcriptomics, proteomics, metabolomics.

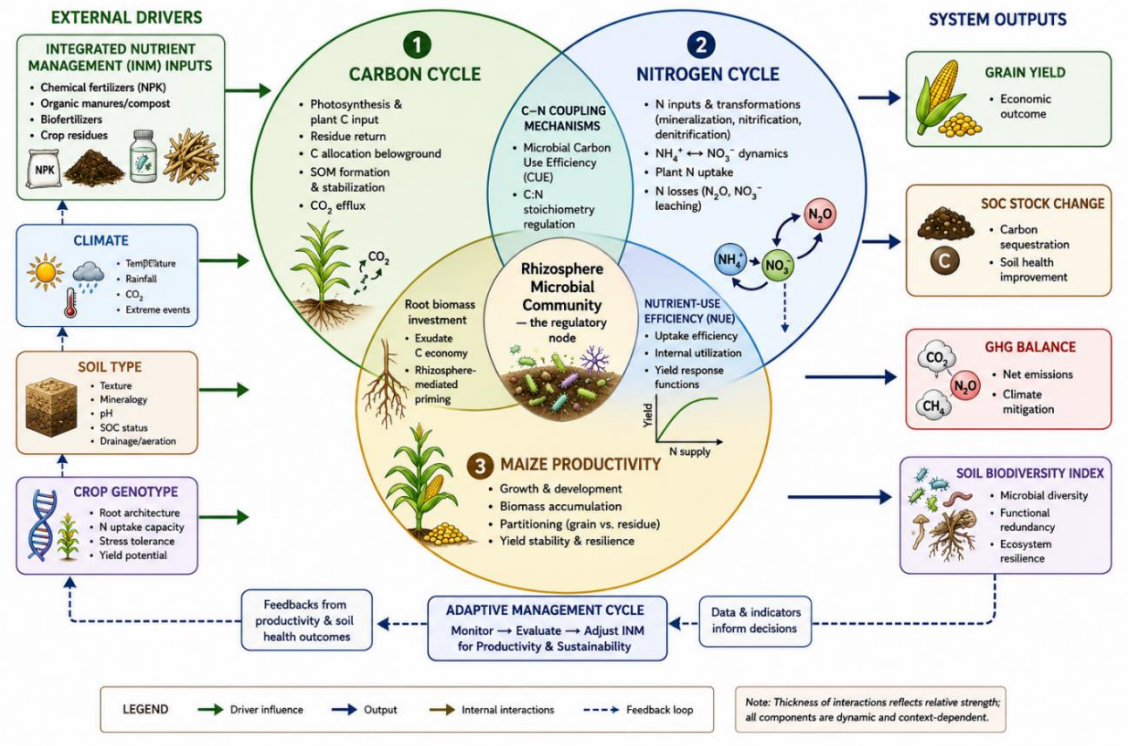

Fig 3: Integrated Framework of Carbon–Nitrogen Coupling, Rhizosphere Microbial Feedbacks, and Maize Productivity under Integrated Nutrient Management

9. Conclusion

This entire analysis shows that rhizosphere microbial communities are actively involved in influencing how soils change with respect to nutrient availability; they serve as biological agents regulating the processes of carbon mineralization and nitrogen transformations occurring within Zea mays (corn) production systems. The findings presented suggest, without question, that employing integrated nutrient management strategies to supply organic carbon substrates; inorganic mineral nutrients; and specific microbial inoculants (e.g., through inoculation of soil) provides for an enhanced rhizosphere environment where diverse, functionally redundant populations of microorganisms will carry out all the essential functions associated with the cycling of both carbon and nitrogen; and do so at a level of efficiency and environmental sustainability that either organic or conventional agricultural production methods are unable to achieve independently.

The summary of findings from this comprehensive review are as follows. First, maize rhizospheres display increased microbial activity due to exudation of labile carbon compounds; the timing of these increases correlates well with developmental stages of maize plants, which are affected largely by inputs from INM. Second, processes responsible for mineralization of carbon in the maize rhizospheres include both labile and recalcitrant substrates as sources; additionally, priming effects associated with the rhizosphere are quantitatively significant but sensitive to management strategies - the three examples above link root activity with the decomposition of native soil organic matter. Third, different guilds and functional genes exist for mediating nitrogen transformation processes (fixation, ammonification, nitrification/denitrification, immobilization) — as such they represent unique microbial communities; furthermore, their abundance and activity will change greatly according to type and intensity of INM application. Fourth, the cycling of C and N are tightly linked through microbial carbon use efficiencies and stoichiometric control via microbial biomass, thereby establishing a coherent mechanistic context in which to understand how organic matter management influences both C and N cycling simultaneously. Long-term Integrated Nutrient Management (INM) significantly improves soil physicochemical properties (e.g., soil organic carbon (SOC), cation exchange capacity (CEC), and bulk density), as well as microbial indicators (e.g., microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and enzyme activities) and agronomic performance metrics (e.g., root biomass, grain yield, and nitrogen use efficiency (NUE)). Long-term INM treatments that combine organic and inorganic sources of nutrients and biofertilizers provide the **most** consistent improvements in these measures.

These findings have important implications for sustainable nutrient management. In practical terms, transitioning from purely chemical sources of nutrients to a diversified INM approach that combines organic amendments (from local sources) at a 25-50% reduction in overall nutrient inputs with biofertilizers targeting key functional groups/ guilds (e.g., diazotrophs (nitrogen-fixing bacteria), PSB (phosphate-solubilizing bacteria), and arbuscular mycorrhizal fungi), can result in comparable or higher yields relative to full mineral nutrient inputs while reducing fertilizer costs, providing long-term benefits to soil health, and achieving substantial co-benefits for mitigating nitrous oxide (N_2O) emissions and sequestering soil organic carbon (SOC) consistent with climate-smart agricultural objectives.

The research indicates that MBC, MBN, dehydrogenase activity, and microbial diversity metrics collectively serve to determine the degree and rate with which management-induced effects alter soil health; therefore making them effective early indicators of soil health change for inclusion into routine soil health monitoring programs and may be useful in making decisions regarding adaptive INM management. The concept of a 'microbial health threshold,' where soil biological activity is insufficient to maintain sustainable crop production without additional input, warrants further quantitative development and field validation across many production environments of maize.

Research priorities for the next years will include: (1) multi-omics integration to better determine the molecular mechanisms for how INM affects C-N coupling; (2) development of trait-based, microbially explicit biogeochemical models to predict INM effectiveness across soil types and climates; (3) longitudinal studies of 15 to 25 years that will be able to document the full trajectory of microbial community succession with INM; (4) farmer-participatory action research to convert findings from microbial ecology to a practical decision-making tool for crop production by smallholder producers of maize in tropical climates; and (5) interdisciplinary collaborations between soil microbiologists, agronomists, plant breeders, and climate scientists to develop INM systems that optimize production, soil health, and climate benefit. The rhizosphere microbiome has long been neglected as an “invisible” driver of soil fertility but is now recognized increasingly as a target and tool for sustainable maize production as our world changes.

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