Soil Physicochemical Properties and Bacterial Community Composition in *Cicer arietinum* Systems Following Organic Amendment: A Long-Term Perspective

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Abstract

Long-term organic amendments represent a cornerstone strategy in sustainable agroecosystem management, yet the cumulative effects on soil physicochemical properties and bacterial community dynamics in chickpea (*Cicer arietinum* L.) cropping systems remain incompletely characterized. This comprehensive review synthesizes current scientific evidence pertaining to the multifaceted interactions between organic amendment application, soil physical and chemical attributes, and the composition, diversity, and functional capacity of soil bacterial communities in chickpea-based agricultural systems across extended time horizons. Soil physicochemical parameters, including pH, electrical conductivity, bulk density, water-holding capacity, cation exchange capacity (CEC), and soil organic carbon (SOC), are demonstrably modified by prolonged organic amendment inputs, with vermicompost and compost exhibiting the most pronounced improvements in soil structural and nutritional quality. Bacterial community composition, characterized through advanced molecular methodologies including 16S rRNA gene sequencing, metagenomics, and metatranscriptomics, undergoes substantial restructuring under long-term amendment regimes, evidenced by increased alpha-diversity indices, enhanced microbial biomass, and shifts in the relative abundance of dominant phyla including Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes, and Bacteroidetes. In the chickpea rhizosphere specifically, root exudation patterns and legume-specific rhizodeposition processes create unique selective pressures that modulate bacterial community assembly and augment the functional guilds responsible for biological nitrogen fixation, phosphorus solubilization, and organic matter decomposition. The mechanistic linkages between soil physicochemical modifications and microbial community responses are mediated through pH-driven niche differentiation, organic carbon substrate availability, and shifts in nutrient stoichiometry. Critically, contradictory findings exist in the literature regarding the magnitude and directionality of amendment-induced community shifts across soil types, amendment rates, and temporal scales, underscoring substantial methodological and ecological knowledge gaps. This review identifies priority research frontiers, including the need for coordinated multi-decadal field experiments, integrated multi-omics approaches, and climate-responsive amendment strategy modeling, with the overarching objective of harnessing soil microbiome engineering for enhanced chickpea productivity and agroecosystem sustainability.

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1. Introduction

1.1. Global Significance of Soil Health in Agricultural Systems

The health of soil is the basis for the sustainable production of food, maintaining ecosystems, and achieving global security of food supply (FAO and ITPS, 2015) [1]. Soil health, which is characterised as the ability of a soil to act like an ecological system capable of supporting the production of both plants and animals while maintaining or improving the quality of water and air and supporting the health of plants and animals, goes beyond the conventional framework of soil fertility (Nannipieri *et al*., 2003)

[18]. During the twentieth century, agricultural intensification resulted in the significant increase in food crop production but at the same time caused considerable degradation of soils through losses of organic matter, structural integrity, loss of biodiversity, and disruption of biogeochemical cycles (Lehmann and Kleber, 2015) [3]. Estimates indicate that 33% of the Earth's land surface has been degraded through erosion, salinization, compaction, and chemical contamination, thus creating an unprecedented challenge to the continued viability of food production systems (FAO and ITPS, 2015) [1].

As a result of this realization, it has been widely accepted that soils are home to the highest number and most diverse array of microorganism types (bacteria, fungi, protozoa, etc.) on Earth, with one gram of productive agricultural soil containing 10^8 to 10^9 bacterial cells representing thousands of different taxa (Delgado-Baquerizo *et al.*, 2018) [4]. These microorganisms do not merely passively inhabit the soil but rather actively contribute to soil fertility through their roles in nutrient cycling, organic matter decomposition, stabilization of aggregates, and promotion of plant growth. Therefore, any soil management practices that disturb the physical and chemical properties of soil will result in changes to the composition and abundance of soil's resident microorganisms and, as a result, will have extreme consequences on ecosystem services provided by soils and the ability of soils to grow food.

1.2. Chickpea as a Model Legume in Sustainable Agriculture

Chickpea or *Cicer arietinum* L. has a very important role in agriculture and nutrition as the 3rd most cultivated food legume in the world with yearly totals exceeding 14 million metric tons and occurring in over 50 countries (Jukanti *et al.*, 2012) [5] out of which over 60% is found in countries such as India, Australia, and Turkey (Philippot *et al.*, 2013) [6]. As a legume, chickpeas are capable of biological nitrogen fixation (BNF) via symbiotic relationships with the bacteria Mesorhizobium ciceri and Mesorhizobium mediterraneum and can produce an amount of N equal to 40 to 140 kg N ha^{-1}/season on average during optimal growing conditions (Jukanti *et al.*, 2012) [5] while decreasing reliance on chemical nitrogen fertilizers and therefore reducing greenhouse gas emissions associated with chemical fertilizer application. In addition to its inherent nutritional value as a source of both protein and micronutrients, the chickpea is also a valuable component to sustainable crop rotations, increasing soil N availability for the next cereal crop grown, contributing to soil organic matter increase from root biomass and rhizodeposition.

Chickpea rhizospheres (the zone of soil surrounding plant roots that are directly impacted by the activities of those roots) are prominent sources of bacterial activity and abundance, as well as diverse (by means of their species) microbial communities that can arise in a hotspot due to the continual production by roots of organic compounds (i.e., organic acids; amino acids; sugars; secondary metabolites) that stimulate microbial growth in this part of the soil (Philippot *et al.*, 2013) [6]. The excess growth in these microbial communities as a result of organic compounds produced by plant roots serves to improve the availability of nutrients, reduce pathogenic pressure upon chickpeas (by acting as a barrier to the infection of chickpeas by microorganisms), and promote plant growth. Microbial communities within the chickpea rhizosphere are influenced by several factors, including the genetic makeup of the chickpea plant, the environmental conditions surrounding the rhizosphere, and the history of land management practices or inputs (e.g., fertilizer type, cover crops) that have impacted the soil within the rhizosphere. Thus, understanding how long-term applications of organic amendments affect the physicochemical properties of chickpea rhizospheres and subsequently influence the structure and function of the bacterial communities associated with the chickpea rhizosphere is essential for developing evidence-based agricultural management practices that are ecologically sustainable.

1.3. Organic Amendments and Soil Microbiome Interactions

Composting, composted animal waste or animal excrement, vermicomposted animal excrement (vermicomposting), green manures, residue from crops, and biochar-based materials are some examples that fall under the umbrella of organic amendment materials. Organic amendments are put into soil to increase its fertility, structure and biological activity (Bernal *et al.*, 2009) [7]. Many studies have documented the short-term effects of specific organic amendment applications on soil properties and microbial communities; however, research on the effects of long-term and cumulative use of organic amendments on soil physicochemical dynamics and the assembly of bacterial communities is ongoing and highly contested. As the response of soil microbial communities to organic amendments occurs on a range of time scales, the timing aspect of organic amendment research is especially important. Time scales for response to organic amendments can occur from days to weeks (rapid r-strategist responses) for the first influx of labile carbon to months to years (gradually changing community composition) and, finally, for decades (before major changes to community structure and network topology occur) (Lehmann and Kleber, 2015) [3].

1.4. Objectives and Scope of This Review

Objectives of this review are to: (i) review past and present understanding of physicochemical characteristics and ecology of agricultural soils and their relationship to chickpea production systems; (ii) review ecology of the rhizosphere of *Cicer arietinum* with emphasis on interactions between roots and the microbiome; (iii) evaluate organic amendments used over long periods of time for type, composition and effects on soils; (iv) evaluate composition, diversity and functional roles of soil-bacterial communities in amended soils; (v) evaluate the long-term effects of organic amendments on the structure and function of soil-bacterial communities; (vi) provide mechanistic insights of how soil characteristics, microbial communities, and plant performance are linked in chickpea production; (vii) explain how advanced molecular and analytical techniques are enhancing the study of the soil microbiome; and (viii) recognize critical knowledge gaps and future research directions. By synthesizing data from the disciplines of soil physics, soil chemistry, microbial ecology, plant physiology, and agronomy, this review will develop a complete and critically analyzed conceptual schematic of long-term effects of organic amendment on restored agroecosystems in chickpea.

2. Soil Physicochemical Characteristics and Agricultural Relevance

2.1. Fundamental Physicochemical Properties of Agricultural Soils

Agricultural soils are complicated and ever-changing systems whose characteristics, including their physical, chemical, and biological properties, are interconnected to one another in such a way that they affect the soil's ability to produce plants and to sustain the ecosystem. There is a long list of different parameters that can be used to assess soil quality, but the following properties—pH, electrical conductivity (EC), bulk density, texture, porosity, water-holding capacity, and cation exchange capacity (CEC)—are among the most common physicochemical property measurements used in agricultural or ecological assessments, as they have measurable and direct impacts upon the soil's availability for nutrients to plants; on the microbial activity in soil; on the development of roots of plants in soil; and on the movement of water through soil (FAO and ITPS, 2015) [1].

The summary of the physicochemical properties of agricultural soil, including the range values typically found in agricultural soils, the optimal ecological value of these properties for soils, and the functional implications of these properties for soils, is presented in Table 1.

Table 1: Major physicochemical properties of agricultural soils and their ecological significance in agroecosystem functioning.

Property	Typical Range	Optimal Range	Ecological Significance	Impact on Crop Productivity
Soil pH	4.0–9.0	6.0–7.5	Controls nutrient availability and microbial activity	Determines N, P, K availability; affects enzyme activity
Electrical Conductivity (EC)	0.1–4.0 dS/m	< 2.0 dS/m	Reflects salt concentration and ionic strength	High EC causes osmotic stress, limits root uptake
Bulk Density	1.0–1.8 g/cm³	1.1–1.4 g/cm³	Indicates soil compaction and pore structure	Affects root penetration, aeration, and water movement
Soil Texture	Sand/Silt/Clay	Loam to clay-loam	Determines water retention and nutrient-holding capacity	Influences moisture availability and workability
Porosity	30–60%	45–55%	Governs gas exchange and water infiltration	Critical for root respiration and microbial aerobiosis
Water-Holding Capacity (WHC)	20–60%	35–55%	Reflects soil capacity to retain plant-available water	Directly influences drought tolerance and yield stability
Cation Exchange Capacity (CEC)	5–50 cmolc/kg	> 20 cmolc/kg	Measures soil's ability to retain cations	Reduces nutrient leaching; supports sustained nutrient supply
Soil Organic Carbon (SOC)	0.5–3.0%	> 2.0%	Central to nutrient cycling and soil structure	Enhances microbial biomass, aggregation, and fertility

There are multiple and complex relationships amongst multiple physical and chemical parameters. Water pH not only affects plant nutrient availability due to the pH control of mineral solubility, but will also modify EC as a result of the effect of water pH on the reaction rates of minerals breaking down into the soil and ion exchange reactions and at the same time will impose different selective pressures on taxonomic and functional groups of microorganisms within the soil environment (Fierer and Jackson, 2006) [2]. All of these interplay to create a combination of influences which will combine to provide an assessment of soil health and therefore, need to be assessed together instead of focusing on single attributes alone.

2.2. Soil pH: Master Variable of Soil Chemistry and Biology

Numerous studies over the years, including comprehensive reviews of these studies, have concluded that soil pH is by far the most important chemical property of soils that affects the amount of nutrients available for plants and microorganisms (Fierer and Jackson, 2006) [2]. In soils with a pH less than 5.5 (strongly acidic soils), the solubility of aluminum (Al^{3+}) and manganese (Mn^{2+}) is increased and can reach levels that are phytotoxic to plants while reducing phosphorus (P), calcium (Ca) and magnesium (Mg) availability. In contrast, in soils with a pH greater than 7.5 (alkaline soils), there is a higher occurrence of micronutrient deficiencies (iron (Fe), zinc (Zn), copper (Cu), and boron (B)) due to precipitation of these elements in the form of hydroxides and/or carbonates. In the case of chickpeas, the optimal soil pH for chickpeas is from 6.0 to 7.5, which corresponds to the optimal pH of the symbiotic Mesorhizobium for plant nutrition and nitrogen-fixation (Jukanti *et al*., 2012) [5].

Bacterial communities' structure is greatly affected by soil pH and is a reliable indicator of the diversification of soil bacterial communities on a global scale. Soil pH is the leading predictor of soil bacterial community beta-diversity and accounts for more variation (on average) in soil bacterial communities than other features of soils (Fierer and Jackson, 2006) [2]. Generally, Acidobacteria will comprise the largest proportion of bacterial taxa in acidic soils, while with the increased pH, Acidobacteria will decrease and be replaced by Actinobacteria and Bacteroidetes; the relative abundance of these two groups will increase as pH increases. The pH response of Proteobacteria will vary between different classes of Proteobacteria; however, during the overall response to pH changes by many bacteria, the response is attributed to differences in response to pH among taxonomic groups, differences in substrate availability due to pH, and indirect effects of community composition and root exudate chemistry associated with shifts in plant communities.

2.3. Soil Organic Carbon Dynamics and Nutrient Retention

Soil organic carbon (SOC) is vital to fertility and biota activity. It is a food source to heterotrophic microbes; forms part of soil aggregate structure; is the source of naturally bound nutrients; and it is the major soil property of CEC and soil moisture retention (Lehmann and Kleber, 2015) [3].

SOC is the range of organic substances in continuous fashion: starting with labile (years) to extremely recalcitrant (centuries) organic compounds. The organic substances vary from fresh dead plant materials or live microbial products (days to weeks) as the most labile organic compounds, to partially decayed organic material (years to decades), and finally, very old (>1000 yr/) accumulations of organic material that are still active in the soil environment.

Inputs from organic amendments enhance soil carbon stocks through augmentation, but the rate and duration of SOC enhancement are highly dependent on the biochemical resistance of the organic amendment(s), the chemical composition and physical characteristics of the receiving soil, and the composition and activity levels of the decomposer community (Diacono and Montemurro, 2010) [8]. Vermicompost, with its high level of humic acids and microbially-processed organic matter, decomposes at a slower rate and has greater persistence within the soil than either raw manure or newly harvested crop residues. Biochar, on the other hand, is an extreme example of an organic carbon that has resisted decomposition for thousands of years in the soil and, at the same time, enhances stabilisation of easily-decomposable organic matter via physical protective and chemical adsorptive processes (Lehmann *et al*., 2011) [12].

2.4. Physical Soil Properties: Structure, Porosity, and Water Dynamics

The physical characteristics of soil, such as texture, structure, bulk density, porosity, and water-holding ability, shape the way that both water and gases move through and are held in a soil profile. These physical characteristics play a significant role in root growth, nutrient fluxing, and microbial activity, and combine to control the movement, retention, and allocation of water and gases in a given soil profile (FAO and ITPS, 2015) [1]. More specifically, soil texture, which will always be defined as the ratio of sand, silt, and clay, has a larger role than most other aspects of physical characteristics in limiting how soils are likely to respond to management changes. Generally, soils with higher clays will hold water much better than sand, will have proportionately higher CECs, and will likely have smaller pore sizes. Therefore, clay-rich soils will usually have lower aeration compared to sandy soils. In comparison to clay-rich soils, sandy soils will drain quickly, have lower nutrient retention potential, have larger pore sizes, and provide greater aeration for aerobic microbes; however, sandy soils will likely dry out rapidly.

Bulk density (g/cm^3) and porosity of soils are considered dynamic properties that respond to organic amendments on time scales of years to decades. Bulk density of soils that receive long-term organic amendments is normally reduced between 0.05 and 0.20 g/cm^3 compared to soils that did not receive such amendments, representing the addition of lower-density organic matter to the total soil volume and, more fundamentally, enhancing soil aggregate structure and macroporosity through aggregates being cemented together by microbial processes and organic materials and the soil being burrowed into by organisms (Bhogal *et al*., 2009) [9].

The increase of porosity resulting from organic amendments improves root penetration in soils; improves gas exchange in soils; increases the rate of infiltration of water into soils; and enhances the heterogeneity of microhabitats (aerobic and microanaerobic) to support a diverse community of microorganisms.

2.5. Cation Exchange Capacity and Nutrient Retention Mechanisms

Cation exchange capacity (CEC) reflects the ability of a soil 7to absorb and exchange cations on its negatively charged surface, making it a vital buffering system against nutrient leaching and a supply of plant-available nutrients. CEC is provided by both the mineral component (mainly clay minerals and iron and aluminum oxides) of the soil and its organic components. Humus has a CEC of 200-400 cmolc kg-1 and much higher than the majority of clay minerals. As a result, when organic amendments increase soil organic carbon (SOC), the resulting increase in CEC relates directly to the increase in nutrient retention capacity, especially for calcium, magnesium, potassium, and ammonium ions.

Multiple mechanisms govern the relationship between CEC and microbial community composition in soils. Soils with high CEC have a higher capacity for buffering pH fluctuations than those with low CEC. This helps keep the chemical environment constant despite the lowering of pH by nitrification and root-exuded organic acids and supports communities of diverse bacteria. As well, soils with high CEC retain nutrients better than soils with low CEC; thus, nutrients are less susceptible to being limited, which would favour oligotrophic (adapted to nutrient-scarce) microbial communities. As a result, many copiotrophic (adapted to nutrient-rich) microbial communities will dominate the soils and will have the highest metabolic activity and growth rates. (Delgado-Baquerizo *et al*., 2018) [4]

2.6. Long-Term Impacts of Soil Quality Changes on Agroecosystem Functioning

The approach to improving soil quality through continuous use of organic amendments represents a form of biological capital accumulation. The benefits accumulate and complement each other and have cumulative effects upon agroecosystem function. The results of studies that have used organic amendments for a period of 5 to 20 years demonstrate both quantitative increases in individual soil characteristics, as well as qualitative changes in soil ecosystem function, including increased nutrient-use efficiency, increased drought tolerance, increased carbon sequestration, and increased capacity for disease suppression. All of these functional benefits result from the complex interactions between improved physical soil structure, increased chemical fertility, and enrichment of beneficial soil microbe communities and represent a positive feedback system where improved soil conditions allow for increased microbial activity, which then allows for further improvement in soil condition.

3. Rhizosphere Ecology of Chickpea (*Cicer arietinum*)

3.1. Morphological and Physiological Characteristics of *Cicer arietinum*

The chickpea (*Cicer arietinum* L.) is an annual cool-season food legume that belongs to the Fabaceae family and the Papilionoideae subfamily. There are two primary types of cultivated chickpeas: desi and kabuli. Desi chickpeas have small, angular, diced seeds and have rough seed coats. Kabuli chickpeas have large, round, cream-coloured seeds and have smooth seed coats (Jukanti *et al*., 2012) [5]. Chickpeas are a diploid species ($2n = 2x = 16$) with genome sizes of approximately 738 Megabase pairs and have a root system that is composed of a primary taproot that can reach depths > 100 cm under favourable soil conditions and a lateral root system that is concentrated primarily in the upper 20-40 cm of the soil profile.

The following physiological characteristics of chickpea have a direct effect on the ecology of its rhizosphere: the ability to fix nitrogen biologically in symbiosis with Mesorhizobium species; the ability of chickpea to tolerate moderate soil water deficit, which is reflected in root architecture and exudate chemistry; and the ability of the chickpea to produce a variety of secondary metabolites (malic acid, citric acid, oxalic acid, and isoflavonoids) that act as both nutrient-mobilizing compounds and as signalling molecules for interactions with microorganisms in the rhizosphere (Jukanti *et al.*, 2012) [5]. Chickpea roots also contain large amounts of proteins, polysaccharides, and border cell populations, which contribute to the high levels of organic substrate available in the rhizosphere of legumes.

3.2. Root Architecture and Rhizosphere Characteristics

Soil physicochemical properties have a major influence on chickpea root architecture, which has cascading effects on the distribution of rhizosphere-associated microorganisms and the three-dimensional structure of the rhizosphere. Chickpea roots in high-bulk-density soils exhibit reduced branching of roots, shallower rooting depth, and changes in the lateral distribution of roots such that chickpea root activity and associated rhizosphere impacts are concentrated in the surface soil layer (Jukanti *et al.*, 2012) [5]. Organic amendments will reduce soil bulk density and increase soil porosity, which will indirectly affect the structure of the chickpea rhizosphere by facilitating deeper, more extensively branched root systems that penetrate and exploit deeper soil layers and create larger volumes of total rhizosphere.

The chickpea rhizosphere differs dramatically from the surrounding bulk soil in terms of pH, oxygen concentration, organic substrate availability, and ionic strength, resulting in very steep gradients for these parameters (Philippot *et al.*, 2013) [6]. The production of acidic conditions in the rhizosphere by the root system (e.g., through the release of protons during uptake of cations) can result in a rhizosphere pH 0.5–2.0 lower than the bulk soil pH and can enhance the solubility of micronutrients as well as inorganic phosphorus. The high respiration rate of roots creates localized depletion of oxygen surrounding actively respiring root surfaces and creates microanaerobic habitats that promote nitrogen-fixing bacteria and other anaerobic or microaerophilic groups, thereby contributing to the functional diversity of microbial communities in the rhizosphere.

3.3. Root Exudation Patterns and Rhizodeposition Processes

Root exudates include both passive and active processes for the release of low-molecular-weight organic compounds from the living cells of root tissue into the surrounding soil. These exudates constitute a large portion of how chickpea plants allocate their photosynthetically fixed carbon for rhizosphere priming effects and recruitment of microorganisms (Philippot *et al.*, 2013) [6]. Estimates suggest that between 5 and 21% of net fixed carbon from legumes is allocated to root exudation by legumes, with the amount and composition of root exudates differing considerably as a function of plant developmental stage, nutritional status, pathogen challenge and physicochemical properties of the soil.

Chickpeas, specifically, predominantly release four different types of organic acids (malic, citric, oxalic and succinic acids), three types of amino acids (glutamine, aspartate and glycine), three types of sugars (glucose, fructose and sucrose) and various flavonoid molecules that provide critical signalling functions during the legume-rhizobium recognition process (Jukanti *et al.*, 2012) [5]. Isoflavonoids and flavones released by chickpea roots induce the expression of nodulation (Nod) genes in compatible strains of Mesorhizobium, providing a molecular cue to initiate nodule organogenesis and the establishment of nitrogen-fixing symbiosis. In addition to their role in the establishment of symbionts, these signalling compounds also influence the overall composition of the rhizosphere bacterial community by preferentially enriching bacterial taxa capable of metabolising flavonoids as carbon sources while simultaneously inhibiting the growth of pathogens via antimicrobial properties.

Rhizodeposition, not just defined solely by root exudates (also defined by root border cell sloughing, mucilage production, turnover of root hairs, and turnover of fine roots), represents an additional contribution of greater amounts of organic carbon to the rhizosphere than solely from liquid exudates (position (Philippot *et al.*, 2013) [6]). Root border cells are active cells that were purposely separated from their root cap meristem and can actively contribute to the rhizosphere after being released as living cells that are capable of further polysaccharide, protein, and secondary metabolite secretion. In chickpea, root border cell populations express a unique gene expression pattern compared to root cap cells indicating a unique secretory function for modifying the rhizosphere and interacting with microorganisms.

3.4. Legume-Rhizosphere Interactions and Nitrogen Fixation

Chickpeas exhibit the most well-documented and intense level of study in their rhizosphere ecology, as well as being one of the main ways that they add nitrogen to soil. When the shoot provides photosystems to provide energy to the batiroids within the peribacteroid membranes of infected root hair cells, they develop into differentiated root nodules.

In the active nodule, the ATP energy required for each M. cicero to fix one nitrogen molecule is 16 within the nitrogenase enzyme complex, as N_2 is reduced to NH4.

Nodulation and the effectiveness of nitrogen fixation in chickpea are influenced by soil conditions, including the availability of soil nitrate (which reduces the ability to nodulate at high levels), soil pH (which affects competition for host plants), soil water potential, and phosphorus (which is very necessary for nodule function) levels. The application of long-term organic amendments (which improve soil conditions for both Mesorhizobium and nodule function through pH buffering, improved moisture- and phosphorus-retaining ability, and better phosphorus availability through organic matter decomposition) is a prominent means by which organic amendments assist nitrogen fixation by chickpeas.

3.5. Root-Associated Microbial Recruitment Mechanisms

Plant hosts play an interactive role in influencing rhizosphere bacterial communities by preferentially selecting for certain microorganisms based on various criteria, including exudate-derived substrate preferences, the production of antimicrobial metabolites, and immune-related changes (Philippot *et al.*, 2013) [6]. The resulting stimulus from the plant after the microbiota of the soil colonizes the rhizosphere can be referred to as the rhizosphere recruitment effect, which is why rhizosphere bacteria are usually more abundant and have distinct taxonomic identities than bacteria from non-rhizosphere soil. Certain nitrogen-cycling bacteria, such as Mesorhizobium, Bradyrhizobium, Azospirillum, etc., are commonly found to be enriched by 10 to 1000 times in comparison to non-rhizosphere bacterial communities in chickpea.

3.6. Influence of Chickpea Growth Stages on Microbial Community Assembly

The composition and quantity of chickpea root exudates can differ greatly between stages of development from the point of germination through the vegetative stage to flowering, pod filling and senescence. This variation has been found to be reflected in the composition of the associated, dynamic rhizosphere microbial communities. High amounts of sugars and amino acids are produced by the roots at the germination and early seedling establishment period, therefore allowing for rapid growth of copiotroph-dominated microbial communities. At flowering and early Pod Fill, when nitrogen demand is greatest, there is a peak of isoflavonoids being exuded by the roots and maximum activity of Mesorhizobium of the nodules. Isoflavonoid exudation is also coincident with the development of nitrogen-cycling functional guilds in the rhizosphere. Decay of the root biomass of chickpea plants during senescence releases a pulse of highly diverse organic substrates, supporting the establishment of taxonomically distinct decomposer communities enriched in Firmicutes and Actinobacteria.

4. Organic Amendments and Their Effects on Soil Properties

4.1. Types and Characterization of Organic Amendments

Chickpea crop production has numerous uses for organic amendments, which consist of a wide range of organic matter, each having unique chemical/nutritional composition, rate of decomposition and modifying effects on soil properties. Compost is created through aerobic thermophilic/composting of a combination of organic feedstocks (manures, food waste, plant residues). Therefore, compost is a permanent/humus-like soil amendment with an average C:N ratio of 15 to 25:1, and with a large population of thermophilic actinomycetes and other beneficial microorganisms. Farmyard manure (FYM) also has an organic material mixture of livestock excreta, bedding materials, and feed residues but generally has much higher amounts of nitrogen than compost and less biological stabilization than compost; therefore, it decomposes more quickly and releases nutrients faster. Table 2 illustrates the comparison of the chemical/biological characteristics of the major organic amendments used in chickpea production systems.

Table 2: Characteristics and nutrient composition of major organic amendments used in *Cicer arietinum* production systems.

Amendment Type	C:N Ratio	N (%)	P (%)	Microbial Activity	Persistence in Soil
Compost	15–25:1	1.5–3.5	0.5–1.5	High – diverse microbial consortium	Moderate (6–24 months)
Farmyard Manure (FYM)	20–30:1	0.5–2.5	0.2–1.0	Moderate – plant-available nutrients	Low–Moderate (3–12 months)
Vermicompost	10–15:1	2.0–4.5	1.0–2.5	Very high – rich in humic acids	Moderate–High (12–36 months)
Crop Residue Incorporation	40–80:1	0.3–1.5	0.1–0.5	Low initially; increases with decomposition	Variable (3–18 months)
Biochar-Enriched Amendment	50–400:1	0.1–1.0	0.1–0.8	Moderate – improves habitat for microbiota	Very high (100–1000 years)

Vermicomposting is the process that produces vermicompost as eathworms and microbes work together via the consumption of organic materials and create an organic substance that can be used on plants. Therefore, vermicompost is characteristic of having a unique place as one of the organic amendments due to the number of plant available nutrients, highly available humic acids, and substances that promote growth such as auxins and cytokinins along with the many beneficial microbial communities are associated with vermicompost. In addition to the numerous components found in vermicompost, the way that organic substrates are ingested, digested, and passed out through the castings of earthworms serves to intimately mix and interact the microbial communities and organic substrates, create oxidative chemical reactions, and produce castings that have a tremendous amount of surface area for the retention of moisture and hold water; all in all it would be difficult to determine any other organic amendment with more biological richness than vermicompost (Lazcano *et al*., 2008) [10].

Through the pyrolysis of biomass, a process that occurs under low oxygen conditions at temperatures ranging between 300–700 °C, biochar is produced. Biochar is completely different from other organic amendments because of its chemical inertness; its porous nature; its large surface area; and its alkaline pH when predominant (approximately 25% carbon content) (Lehmann *et al*., 2011) [12].

When used as the sole source of soil amendment, biochar primarily affects the soil pH and neutralizes acidic soils; creates cation exchange capacity over time via the formation of oxygen-containing functional groups on the biochar surface; contributes to soil moisture retention; and provides habitats for soil microorganisms in biochar micropores. When combined with other organic amendments such as compost or FYM, the physicochemical properties and microbial communities of the combined organic amendments are improved above and beyond either individual organic or compost/FYM amendment's contribution.

4.2. Long-Term Effects of Organic Amendments on Soil Fertility

Long-term field studies (5 to 30 years) have demonstrated considerable improvement in SOC, nutrient availability, and soil biological activity due to the accumulated effect of repeated use of organic amendments on soil fertility. The effects of the major amendment types on key soil physicochemical properties have been summarized in Table 3 using a combination of meta-analysis studies and long-term field experiments (Diacono and Montemurro, 2010; Bhogal *et al*., 2009) [8, 9].

Table 3: Long-term effects of organic amendments on soil physicochemical properties based on multi-year field trials and meta-analytical studies.

Amendment	pH Change	EC Change	Bulk Density	SOC Increase (%)	Key Reference
Compost (5–10 yr)	+0.3 to +0.7	+0.2 to +0.8 dS/m	−0.05 to −0.15 g/cm³	+25–45%	Diacono & Montemurro [8]
Farmyard Manure (5–10 yr)	+0.2 to +0.5	+0.1 to +0.5 dS/m	−0.05 to −0.12 g/cm³	+15–35%	Bhogal *et al.* [9]
Vermicompost (3–7 yr)	+0.1 to +0.4	+0.1 to +0.3 dS/m	−0.08 to −0.18 g/cm³	+30–55%	Lazcano *et al.* [10]
Crop Residue (5–10 yr)	−0.1 to +0.2	Minimal change	−0.03 to −0.10 g/cm³	+10–20%	Powlson *et al.* [11]
Biochar Amendment (3–5 yr)	+0.5 to +1.5	Minimal change	−0.10 to −0.20 g/cm³	+40–80%	Lehmann *et al.* [12]

When compost is applied at a rate of 10 to 20 Mg ha^{-1} yr^{-1} for 5 to 10 years, the soil organic carbon (SOC) content typically increases by 25 to 45% compared to control soils without compost, and soil aggregate stability, water holding capacity, and cation exchange capacity (CEC) all improve accordingly (Diacono and Montemurro, 2010) [8]. Improvements in the physical structure of soils facilitate positive feedback loops that result in decreased soil erosion, increased infiltration of water into soils and greater root penetration, which together help maintain yield stability during periods of variable climatic conditions. The pH-buffering properties of compost prevent two major forms of soil pH change (acidification due to nitrification of ammonium and alkalinization due to carbonate additions), thus maintaining pH levels that are close to optimal for the availability of nutrients to plants and for the activity of soil microorganisms.

4.3. Soil Aggregate Stability and Physical Structure

The stability of soil aggregates, which is defined as the resistance of soil aggregates to destruction through mechanical and hydrological forces, is an important characteristic of soil health, and long-term organic amendments greatly increase their stability. The processes by which organic amendments increase aggregate stability take place at a variety of scales: Microaggregate (< 250 μm) stability is increased by fungal hyphae and bacterial exopolysaccharides, which bind primary particles together; Macroaggregate (250 μm -2 mm) stability is enhanced by plant root networks, hyphae of fungi, and earthworm activities that develop large structural units that create communities of pores (i.e., macropores) for front-loading nutrients, and HO-SUBSTANCES derived from organic matter stabilize through ligand exchange with clay mineral surfaces and bridging divalent cations, the largest structural scale of stability. This study consistently estimates mean weight-diameter (MWD) of aggregates in long-term organic amended soils to be 15-35% higher than their corresponding unamended control after 5-10 years of organic amendment, and of all organic amendments, vermicompost provided the most significant improvements to aggregate stability, illustrated by the comparatively higher levels of acid soluble exopolysaccharides and humic substances derived from earthworm castings found in vermicompost. Agreate stability provides several ecosystem services including: decreases in the potential for soil erosion; increases in the rate at which water infiltrates soils due to retention of connectivity between macropores in soils; reduces an area's overall amount of runoff and loss of associated nutrients; creates heterogeneous microhabitats for the establishment of diverse plant and animal species; and provides a long-term storage system for carbon.

4.4. Organic Matter Decomposition and Carbon Stabilization Pathways

The conversion of organic matter that has been added to soils into persistent soil organic matter is a complex operation that consists of a series of biological, chemical, and physical events at separate times that together dictate the long-term stability and ecological functioning of organic carbon in soils. Organic amendments (organic material that is added to soil) are exposed to colonization by a variety of different types of soil decomposer microorganisms that eventually utilize the labile carbon sources, such as soluble sugars, amino acids, and short-chain organic acids, within days or weeks and give off carbon dioxide as they recycle the associated nutrients to inorganic forms. The decomposer microorganisms use different specialized enzymes to convert resisting structural materials (i.e., cellulose, hemicellulose, and lignin) to degraded materials over a longer time period of several months or years.

When microorganisms decay their biomass as they decompose organic matter, they create a lot of stable soil organic matter by depositing microbial necromass. This is now being viewed as more important (intended to be larger) than the conversion of recalcitrant plant derived compounds, via chemical means, into stable humus. Amino sugars such as muramic acid when found in association with humus are thought of as indicators of the role that microorganisms play in stabilizing soil organic carbon (SOC) through the deposition of their dead cells (necromass). Long term organic amendments to soil that result in enhanced production of microbial biomass will indirectly promote SOC stabilization via increased deposits of microbial necromass.

4.5. Amendment-Induced Changes in Soil Nutrient Stoichiometry

Soil composition is influenced by organic amendment characteristics, elemental composition, and other factors. Each of these factors affects carbon, nitrogen, phosphorus, and sulfur availability in relation to these substrates (organic amendments) and has implications for microbial community structure and activity (Bernal *et al.*, 2009) [7]. When high C:N ratio amendments (crop residues, biochar) are used, the initial net immobilization of nitrogen will cause a temporary decrease in nitrogen availability to plants due to the initial increase in microbial biomass associated with expanding access to labile C. However, when low C:N ratio amendments (vermicompost, slurry manures) are used, ammonium and nitrate will be rapidly mineralized and available for plant uptake. This could also create a temporary surplus of nitrogen that favors selection of copiotrophic bacteria. As microbial communities become acclimated over longer periods of time to the stoichiometric characteristics of amendments, copiotrophic communities develop with C:N ratios compatible with the subtended stoichiometry of available substrates; this is in accordance with ecological stoichiometry theory.

4.6. Water Retention, Salinity, and EC Dynamics

Organic amendments improve the WHC of soil, increasing it by 15–40% from the application of sustained organic amendments, which is particularly beneficial in semi-arid chickpea growing areas, since soil moisture deficits are the principal limiting factor to yield during dry periods in these areas. Organic amendments (particularly in conjunction with the use of some types of manure or biosolid) do not limit the viability or competitiveness of beneficial rhizophere microorganisms, but can raise the EC of the soil, and thus cause osmotic stress for chickpeas, when sufficient quantities of NC and/or salinity-affected amendments are applied (saline materials such as municipal biosolids or poultry manure). The EC of soils receiving conventional rates of manures increased by 0.2–0.8 dS m^{-1} over periods of 5–10 years of application and represent a manageable increase relative to the range of EC values typically encountered with most soil types for chickpea production.

5. Bacterial Community Composition and Functional Diversity

5.1. Soil Bacterial Diversity in Agricultural Ecosystems

Soil bacteria communities are extraordinarily taxonomically diverse in nature, having estimates of about 10^3 to 10^4 different kinds of species per gram of soil found in productive agricultural soils; the vast evolutionary time scheme, billions of years of evolution, has taken place within one of Earth's most aged and highly complex environments that include many types of microbially derived habitats (Delgado-Baquerizo *et al*., 2018) [4]. Therefore, the diversity present in soil-based bacterial communities can include representatives from every type known to the bacterial domain, that is, from each of the dozens of individual bacterial phyla, with each class exhibiting broad variations in their metabolic capabilities, ecological strategies, and ranges of environmental tolerances. While these bacteria contain an overwhelming amount of cumulative diversity, however, the majority of all bacteria (by number of cells) within most agricultural soils belong to a few dominant bacterial phyla, whose relative abundance will change systematic environmental conditions in productivity; for example: soil pH, soil organic carbon content, nutrient content in the soil, and soil management history (Fierer and Jackson, 2006) [2]. Agricultural management practices can greatly alter soil bacterial community composition; the transformation of natural ecosystems into agriculture has typically resulted in decreased bacterial diversity and changes in bacterial community composition to an assemblage of bacteria that is dominated by copiotrophic bacteria, which are well adapted to accepting pulsed nutrient inputs found in cultivated ecosystems (Delgado-Baquerizo *et al*., 2018) [4]. Long-term organic amendments are a means of counteracting the loss of biodiversity experienced in agricultural systems due to the pressure created by systems implementing intensive forms of agriculture; through the continued addition of statistically diverse types of organic substrates, it has helped enable the coexistence of both taxonomically and functionally diverse groups of bacteria that would typically be out-competed within low-nutrient, simplified agricultural systems.

5.2. Dominant Bacterial Phyla and Their Ecological Functions

A typical combination of phyla dominates the agricultural soil bacterial community, as evidenced by the relative abundance and functional contribution of each phylum listed in Table 4. Of the major bacterial phyla present in agricultural soils, Proteobacteria are consistently ranked first in both phylogenetic and metabolic diversity and represent some of the most abundant groups in agricultural soils, including aerobic heterotrophs, nitrogen fixers, sulfur oxidizers, iron reducers, and plant growth-promoting rhizobacteria (Delgado-Baquerizo *et al*., 2018) [4]. Within the Proteobacteria phylum, the Alphaproteobacteria class is also of greatest importance in the chickpea rhizosphere as it includes both the Mesorhizobium and Bradyrhizobium genera, which contain nitrogen-fixing species.

Table 4: Major bacterial phyla and their ecological functions in agricultural soils, with reference to their abundance and responses to organic amendments.

Bacterial Phylum	Relative Abundance (%)	Key Functional Roles	Response to Amendment	Representative Genera
Proteobacteria	15–40%	N-fixation, P-solubilization, C-cycling, plant growth promotion	Increase with labile C inputs	Rhizobium, Bradyrhizobium, Pseudomonas, Burkholderia
Actinobacteria	10–35%	Organic matter decomposition, antibiotic production, chitinase activity	Stable; enriched by compost	Streptomyces, Arthrobacter, Frankia, Micromonospora
Acidobacteria	10–30%	Cellulose degradation, biogeochemical cycling in acidic soils	Decrease with pH increase	Candidatus Koribacter, Acidobacterium
Firmicutes	2–15%	Spore formation, fermentation, stress tolerance	Enrich in manure-amended soils	Bacillus, Clostridium, Paenibacillus
Bacteroidetes	2–20%	Polymer degradation, complex carbohydrate breakdown	Increase with FYM and compost	Flavobacterium, Sphingobacterium, Prevotella
Planctomycetes	1–5%	Anammox N-cycling, particulate organic matter decomposition	Moderate response to amendments	Planctomyces, Gemmata

Actinobacteria are a functionally important part of agricultural soil communities and are particularly important for the cycling of organic matter and the production of biologically active secondary metabolites (Delgado-Baquerizo *et al*., 2018) [4]. The filamentous growth habit of many taxa of actinobacteria facilitates their penetration into soil aggregates that are not accessible to unicellular bacteria. This allows actinobacteria to utilize organic matter that was physically protected. The Streptomyces genus provides about 70% of all known antibiotics derived from soil suggesting that actinobacteria play an important role in the disease-suppressive properties of soils with an abundance of organic matter. Long-term applications of organic amendments (in particular, compost) continue to enrich actinobacterial communities in agricultural soils and have been shown to cause suppression of disease in composted soils.

Acidobacteria are one of the most abundant yet least physiologically characterized phyla of bacteria present in soil, ranging from 15-30% of the total bacterial 16S rRNA gene sequences in neutral to moderately acidic cropland soils and dropping significantly with an increase in pH (Fierer and Jackson, 2006) [2]. Most acidobacteria are oligotrophic and slow-growing organisms that have a physiological connection to limited nutrient sources and are capable of processing recalcitrant organic materials and influence biogeochemical cycling within stable soil systems. Acidobacteria have a notable negative reaction to the addition of organic amendments, thereby shifting the community to more copiotrophic taxa, making it a reliable indicator of the amount of organic carbon and the amount of metabolic activity after the addition of organic amendments to soil.

5.3. Functional Roles in Nitrogen Cycling

Bacteria are responsible for carrying out several stages of the process known as "nitrogen cycling" or "agricultural nitrogen cycling". The processes performed by bacteria during agricultural nitrogen cycling include the transformation of nitrogen into biologically usable forms through processes such as "biological nitrogen fixation", "nitrification", "denitrification", "reduction of nitrate to ammonium (DNRA)" and "ammonification" (Jukanti *et al*., 2012) [5]. In an agricultural sample of chickpea plants, the major source of biologically fixed nitrogen comes from the bacteria Mesorhizobium ciceri that fix nitrogen in plant roots that form nodules. Additionally, other groups of bacteria that live independently from plant roots and fix nitrogen include Azospirillum, Azotobacter, Cyanobacteria, and also include some nitrogen-fixing members of the Proteobacteria that may fix nitrogen in the soil surrounding the roots of chickpea. The functional marker gene "nifH" is expressed in symbiotic and non-symbiotic nitrogen-fixing bacterial communities and is involved with the production of a nitrogen-fixing enzyme called "nitrogenase"; the gene has been shown to have a 2-5 fold increase in copy number in soils with long-term organic amendment versus soils that have not been amended with organic material.

Nitrification represents an essential part of the nitrogen cycle as it is the two-step process where ammonium is oxidized to nitrate through a nitrite intermediate by both ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). This step determines how nitrogen will be partitioned into either leachable or plant-available forms as nitrate or ammonium respectively. Organic amendments regulate how fast nitrification occurs by affecting the availability of ammonium as a substrate for nitrification, soil pH, and the composition of the bacterial community involved in nitrification (Bernal *et al*., 2009) [7]. Using compost as an amendment results in lower rates of nitrification than using mineral fertilizers due to the competition for ammonium between the heterotrophic bacteria that are present in compost and the nitrifying bacteria; because the pH in compost is buffered such that it maintains conditions that are less than optimal for the AOB; and because there are potentially inhibitory compounds present in compost, including some allelochemicals from plant residues, which inhibit nitrifying bacteria from growing.

5.4. Phosphorus Solubilization and Carbon Cycling Functions

The primary constraint on chickpea yield in weathered tropical and subtropical soils is often how much available phosphorus there is in the soil. This is likely because there is little available phosphorus due to most of the phosphorous that exists within the soil being in a mineral form and bound within aggregates of soil, which are not available for plant roots to access (Jukanti *et al*., 2012) [5]. Phosphate Solubilizing Bacteria (PSB) help relieve this limitation by releasing organic acids that dissolve calcium based phosphorous minerals and also secreting phosphatases which help to hydrolyze organic phosphate esters so that the liberated orthophosphates can be utilized by plants. For example, genera such as Pseudomonas, Bacillus, Rhizobium, Burkholderia and Enterobacter have shown to have good capacity to solubilize phosphorus from amended soils, with the addition of vermicompost providing a consistent increase in PSB numbers because of the high concentration of humic acids present which encourages the growth of microbial communities producing organic acids.

The fundamental ecological role of carbon-cycling microbiota is the decomposition of organic material. They decompose complex organic compounds into carbon dioxide and water, thus producing a secondary set of nutrition elements that will also become available to all other biota in an ecosystem. The effectiveness of the overall process of decomposition depends on the functional diversity among various communities of carbon-cycling physicological processes; this functional diversity helps determine the overall level of success and efficiency of organic matter decomposing or becoming stabilized as long term, or non-decomposed, soil organic matter. The addition of organic amendments over time has been demonstrated to enhance both the functional diversity and level of activities of carbono-cycling communities as a result of providing a continuous supply of organic complexes from which all communities can derive energy; thus allowing those communities to co-exist and make up any entire community of carbon-cycling organisms from sugar-forms of organic carbon to very hard-to-decompose carbon complexes such as lignins.

5.5. Rhizosphere Versus Bulk Soil Bacterial Communities

One of the most consistent findings in soil microbiology is the contrast between the bacterial communities in the rhizosphere and bulk soils; thus demonstrating that root activity has a large effect on local bacterial ecology (Philippot *et al*., 2013) [6]. The R:S ratio (ratio of microbial population density in rhizosphere soil compared with that for bulk soil) quantifies the rhizosphere effect and typically is between 5 and 50 for total bacterial numbers, but can be >100 for particular functional groups (e.g., Mesorhizobium in chickpea rhizospheres). In general, the rhizosphere bacterial community has lower alpha-diversity but is more functionally active than the bulk soil communities; because high-quality root exudates selectively enrich copiotrophic, root-associated functional specialists.

5.6. Functional Redundancy and Ecological Resilience

The concept of functional redundancy, i.e., that multiple taxonomically different species of bacteria can do the same ecological function (in other words, if one of those species is lost or suppressed, it doesn't necessarily mean that there will be less overall functional output) is critical to understanding the ways that organic amendments can affect microbial ecology. Having high functional redundancy will give soil microbial communities the resilience necessary to perform their important functions (e.g., nitrogen cycling, organic matter decomposition, phosphorus solubilization), even when environmental changes such as drought, pollution, or changes in management practices occur. Long-term addition of organic amendments is thought to have increased the overall diversity of the microbial community, which could potentially lead to an increase in functional redundancy and higher levels of ecological resilience for the soils treated with organic amendments vs. soils that have not received organic amendments. However, the empirical evidence required to support this hypothesis has been limited under field conditions.

6. Long-Term Effects of Organic Amendment on Bacterial Communities

6.1. Shifts in Bacterial Diversity and Abundance Over Time

Temporal dynamics of bacterial communities in response to organic amendment demonstrate distinct phases of community composition change related to the short-term (labile carbon addition) and long-term (pH and substrate structure) substrate changes [4]. In the short term (0 to 3 months), there is a rapid increase in abundance of bacteria due to the addition of labile carbon (i.e., copiotrophic Proteobacteria and Firmicutes dominate the early arrangement); in the long term (3 to 12 months), more recalcitrant substrate utilization generates increased community structural complexity (as well as increased niche space), and hence greater representation of Actinobacteria and Bacteroidetes will occur. Table 5 summarizes bacterial community variables for the various organic amendment treatments as described in the published literature.

Table 5: Comparative bacterial community responses under different organic amendment treatments in agricultural soils, based on 16S rRNA gene-based diversity analyses.

Parameter	Control	Compost	FYM	Vermicompost	Biochar Amendment
Shannon Diversity (H')	3.2–3.8	4.0–4.6	3.8–4.4	4.2–4.9	3.9–4.5
Species Richness (OTUs)	800–1200	1100–1600	1000–1500	1200–1800	1050–1550
Microbial Biomass C (mg/kg)	120–180	250–380	200–320	280–420	220–360
Proteobacteria (%)	18–25%	28–35%	25–33%	30–38%	26–34%
Actinobacteria (%)	20–28%	25–34%	22–30%	26–35%	23–31%
Acidobacteria (%)	20–30%	14–22%	16–24%	12–20%	15–23%
N-cycling Gene Abundance	Low	High	Moderate–High	High	Moderate
P-solubilizing Bacteria	Low	Moderate–High	Moderate	High	Moderate

For years or decades, the composition of bacterial communities becomes stable over time, with specific groups of bacteria becoming abundant in soils treated with different amendments. Bacterial community composition on different sized scales (from Phylum down to Genus) diverges also from that found in unamended control soils, evidenced by lower Alpha Diversity Index values. The difference in Shannon Diversity Index value between long-term amended vs. unamended control soils is consistent: they are generally 0.4 to 1.2 units greater than those found in the control soil (1.5 to 3.3-fold increase in effective number of species). The increase in bacterial diversity within the amendment-treated communities is driven by several simultaneous mechanisms: pH buffering has increased the abundance of acid-sensitive bacteria; increased variety of organic carbon substrates in the form of amendment materials has allowed for the development of more specialized decomposers, and improved soil physical characteristics such as greater physical stability have created greater spatial heterogeneity in the soil.

6.2. Alterations in Microbial Biomass and Community Structure

Carbon from microbial biomass (MBC – the total amount of carbon in microbial biomass) has been estimated using chloroform fumigation-extraction or phospholipid fatty acid (PLFA) analysis. Under long-term application of organic amendments, MBC increased generally by a factor of 1.5–3.5 on the amended soils after 5–10 years compared to unamended controls (Bhogal *et al*., 2009) [9]. The increase in MBC is attributed to the fact that organic amendments have provided more substrates for microbial growth and improved the physical structure of the soil to reduce the amount of desiccation stress to the soil, thereby allowing microbes to remain active and to have a higher moisture content, which favors microbial activity. The stoichiometry of microbial biomass, especially the C:N ratio of microbial biomass, changes with the use of organic amendments, typically becoming lower when using organic amendments because the substrates contained in organic amendments have a higher nutritional quality than the organic matter contained in the native soil.

PLFA (Phospholipid Fatty Acid) analysis, which offers a group level of resolution of microbial community structure without the need for DNA extraction, has shown that there are consistent changes in the relative abundance of bacterial and fungal biomass across different types of amendments (Lazcano *et al*., 2008) [10]. For example, gram-positive bacteria typically decline in relative abundance under organic amendments; gram-negative bacteria typically increase in abundance. These gram-positive organisms contain iso- and anteiso-branched fatty acids, whereas gram-negative organisms contain monounsaturated and cyclopropyl fatty acids. In addition, the studies have shown that fungal communities, measured by ergosterol or 18:2ω6,9 PLFA, have generally increased higher in relative amount than bacterial communities when compost and vermicompost were added to the system. This indicates that fungi have a competitive advantage over bacteria when it comes to breaking down structural plant polymers.

6.3. Effects on Beneficial Microbial Populations

The long-term application of organic amendments to the soil has a variety of positive impacts on the populations of beneficial microorganisms that are associated with improving the growth of plants, including plant growth-promoting rhizobacteria (PGPRs), phosphate-solubilizing bacteria, biological control agents, and nitrogen-fixing symbiotic and free-living diazotrophs (Bernal *et al*., 2009) [7]. The genera of PGPRs (e.g., Pseudomonas, Bacillus, Azospirillum, and Burkholderia) in organically amended soils tend to have consistently higher frequency of occurrence than either those amended with mineral fertility or not amended soils. PGPRs exhibit beneficial functional traits, such as indole-3-acetic acid (IAA) production, siderophore production, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, and phosphate solubilization, with all showing enhanced frequency of occurrence in association with organic amendments.

When soils are amended with organic fertilizers over a long-term basis, there will be an increase in the populations of beneficial microorganisms that are associated with controlling plant diseases (i.e., biocontrol agents), which is an important variable in the suppression of soil-borne diseases caused by soil-borne pathogens because soils containing higher amounts of organic matter with diverse microbial populations will have lower levels of disease incidence than soils containing lower amounts of organic matter with less diverse microbial populations (Diacono and Montemurro, 2010) [8]. Actinobacteria (particularly Streptomyces) and certain members of the Proteobacteria (e.g., Pseudomonas fluorescens sensu lato) that produce fluorescent siderophores play disproportionately large roles in suppressing the occurrence of plant diseases by means of their ability to produce antibiotics, compete with plant pathogens for iron and carbon substrates, and induce systemic resistance in plants. Long-term application of compost to soils has resulted in the development of biologically suppressive soils for several important soil-borne pathogens that affect chickpeas, including Fusarium oxysporum f. sp. ciceris and Phytophthora medicaginis.

6.4. Microbial Adaptation Under Prolonged Amendment Practices

Long-term application of organic amendments generates selective pressures that lead to adaptive changes both within bacterial communities (i.e., changes in both relative abundance of taxa) and also through functional trait evolution, physiological acclimation, and/or changes in community interaction networks (Lehmann and Kleber, 2015) [3]. For example, compared to their counterparts occurring in unamended soils but with otherwise similar characteristics, bacteria from long-term amended soils have substantially increased expression levels of genes related to enzymes involved with the biological degradation of organic matter (including ligninases, cellulases, hemicellulases, and xylanases). This indicates a functional adaptation at the level of transcription. Furthermore, the profile of the extracellular enzyme activity within the communities of bacteria in amended soils is systematically different from those found in control (unamended) soil and typically shows a high activity of oxidative enzymes (e.g., phenol oxidase and peroxidase) in the compost-amended soils. These results reflect the functional adaptation of bacteria to process the aromatic (e.g., lignin-derived) substrates found in greater concentrations in compost [41] than in non-compost, due to their respective stages of degradation.

6.5. Community Succession and Ecological Stability

There is much evidence supporting the intermediate disturbance hypothesis, which suggests that nature has developed different types of ecosystems through a series of disturbances over time, including disturbances created by people, and the restoration of those ecosystems following these disturbances. The theory of intermediate disturbance states that periodic intrusions of humans to perturb ecosystems will prevent competitive exclusion and produce greater diversity in the communities that develop after these disturbances. After applying amendments to the soil for a minimum of three years, the community composition becomes relatively stable and will only change slightly as amendments continue to be added; therefore, each year’s addition represents a small perturbation. The data suggest that communities of copiotrophic (high-nutrient) and oligotrophic (low-nutrient) organisms will continue to be established as long as the rate of disturbance remains consistent with previously disturbed ecosystems. Data collected over long time periods indicates that the composition stability of the community will be correlated to the diversity and structural complexity of the substrate used as an amendment (Bhogal *et al*., 2009) [9]. A low amount of diversity (such as single crops) will create less stable communities than will the addition of organic amendments that contain many different substrata at different levels of degradation. Thus, the creation of a stable and heterogeneous environment will increase the potential for diverse types of organisms to be present in a community.

6.6. Soil Microbial Network Interactions

Soil bacterial co-occurrence patterns have been studied for decades. They show that long-term application of organic amendments has fundamental effects on the structure of microbial ecological networks; increasing the overall connectivity, modulating and complexity of positive co-occurrence relationships among microbes. In unamended soils, microbial networks typically have few "hubs" (highly interconnected nodes), many weak pairwise associations and low density (i.e., few connections per node) overall. A greater diversity of substrate resources and physical microhabitats support the formation of additional mutualistic and commensal relationships amongst bacterial taxa, resulting in more complex (ecologically redundant) and robust network structures. More connected networks are expected to be less susceptible to external disturbances and contribute to the increased ecological resilience of amended soils.

7. Soil–Microbe–Plant Interactions and Nutrient Cycling

7.1. Mechanisms of Nutrient Transformation in Chickpea Systems

Chickpea croplands' nutrient cycles are intricate entanglements of biological changes which rely on differing vertebrate species/strains. Altogether they will control nutrient usage efficiency and soil fertility sustainability (see [5]). The biggest macronutrients that affect agriculture are nitrogen (N), phosphorus (P), and potassium (K) where each type of nutrient undergoes separate transformations. In each case different soil physical/chemical conditions affect nutrient type transformation. There are also many different strains/species of microbes that can transform these three nutrients depending on where the nutrients are in the soil profile, what type of nutrient they are transforming, and the microbial community structure associated with the nutrient type.

In chickpeas specifically, by combining biological nitrogen (N), microbial phosphorus (P), and potassium solubilizing bacteria into an integrated nutrient acquisition system, through the use of properly managed organic amendments, significant external nutrient input can be eliminated without compromising, or improving, chickpea production.

7.2. Biological Nitrogen Fixation and Nutrient Acquisition

Clinical evidence suggests that biotic nitrogen fixation through both symbiotic Mesorhizobium and free-living diazotrophic bacteria found in rhizosphere and bulk soil environments can contribute to chickpea (Cicer ae-tabulinum) nutrition, primarily in the form of N, via symbiotic and free-living heterotrophic bacteria associated with chickpea root systems (Jukanti *et al*., 2012) [5]. When chickpeas are well-inoculated, symbiotic N2 fixation can contribute between 40–140 kg N/hectare/cropping season under optimal soil conditions, while the use of organic soil amendments has been shown to consistently provide a positive effect on the number of nodules, the weight of nodules, and the rate of N2 fixation [acetylene reduction assay values] per unit of nodule weight. The favorable effects of organic soil amendments on symbiotic N fixation are attributed to improved soil conditions that enable the survival and competitive capacity of Mesorhizobium and availability of phosphorus, which is beneficial to maintaining high levels of ATP during N fixation, and to maintaining optimal soil moisture conditions during the period that nodulation occurs. Free-living N fixation, as mediated by Azospirillum, Azotobacter, Cyanobacteria, Herbaspirillum, and other free-living diazotrophic organisms, can also provide additional amounts of N (5–30 kg N/hectare/year) under favourable soil conditions, while higher levels of contribution occur when soil amendments are applied owing to the fact that carbon substrates and energy necessary for N fixation are available for these free-living diazotrophic organisms to utilize (Jukanti *et al*., 2012) [5]. Under long-term organic soil amendment conditions, nifH gene abundance in the rhizosphere of chickpeas is generally present at levels 2 to 5 times greater than in non-amended soils, which reflects the relatively larger diversity of diazotrophic organisms present in the rhizosphere of chickpeas under organic amendment conditions compared to non-amended soils and supports the concept that greater population densities of diazotrophic organisms exist in soils enriched with organic matter when examined at a comparable depth in relation to the rhizosphere of chickpeas.

7.3. Microbial Mediation of Nutrient Availability

In addition to their function(s) related to the nitrogen cycle in soils, the presence of rhizosphere bacteria in soil ecosystems also plays a role in increasing phosphorus (P), potassium (K), sulfur (S), iron (Fe), and zinc (Zn) availability to plants through enzymatic mineralization (the breakdown of organic material), acidolysis (the hydrolysis of minerals and organic matter), and chelation (the formation of stable complexes with metal ions), mechanisms that are greatly increased when organic amendment (i.e. compost or animal waste) is added to the soil [7]. Phosphate-solubilizing bacteria (PSB) produce/secreted low molecular weight organic acids such as gluconic acid (from bacteria), citric acid (from fungi), and oxalic acid (from Rhizobium) which dissolve calcium and iron phosphate minerals to release orthophosphate into the soil solution. In chickpea rhizospheres, PSB population density can be > 10^7 CFU/g of soil when grown under long-term compost amendments compared to 10^5 - 10^6 CFU/g of soil in soils that are not amended, indicating that PSB are 10-100 more times effective at solubilizing phosphorus than bacteria that survive in unamended soils.

7.4. Interactions Among Soil Properties, Bacteria, and Crop Productivity

There is a complex interplay of relationships between soil physicochemical properties, the composition of the bacterial community, and productivity of chickpea crops that impact, influence and impact each other within their respective networks simultaneously [5, 7]. Organic amendments can improve soil pH, the quantity of organic carbon, and soil structure and all three support increased bacterial diversity and biomass. In turn, these improved bacterial communities promote faster nutrient cycling, create better soil structure through exo-polysaccharide generation, and promote direct plant growth through phytohormone generation, which contributes positively to chickpea roots' ability to develop, uptake necessary nutrients and produce higher grain yields than those achieved in the absence of organic amendments. Multiple research publications cite an increase in chickpea yield from 15-40% greater than un-amended controls over an extended timeframe due to organic amendments. The increase in yield corresponding with an increase in qualitative improvement of the soil physicochemical quality and the soil microbial community functional capacity.

7.5. Feedback Mechanisms Influencing Soil Ecosystem Functioning

The long-term amendment of soil with organic materials results in the generation of positive feedback loops in the soil ecosystem that progressively amplify the improvements in the quality of the soil over time [3] as they all develop in a progressive manner over time. Increased organic carbon content leads to an increase and diversification of microbial biomass which in turn helps to accelerate the decomposition of organic materials and the cycling of nutrients which, in turn, allows for increased production of biomass by plants which produces greater amounts of organic amending materials through the turnover of their roots and the return of crop residues. In addition, as the aggregation of soil improves, it helps to reduce losses of surface soil organic matter due to erosion, while the increased retention of water reduces the frequency and intensity of events that dry out the soil; both of these things promote a higher level of microbial activity. These positive feedback mechanisms result in an ongoing trend of increasing soil improvement resulting from continuous organic management practices that can last for decades (thus, resulting in a higher level of long-term benefits from a continuous supply of organic materials than from their initial application).

7.6. Impacts on Sustainable Agricultural Productivity

The use of long-term organic amendments in the production of chickpeas provides a means for improving fertility in the long term, with a focus on sustainability of agriculture, while also addressing the problems of soil degradation, inefficient use of nutrient resources, problems related to climate change and increase biodiversity [1, 11]. Organic amendments typically have less reliance on synthetic fertilizers and pesticides (energy-intense) providing equal or greater yields than synthetic-based systems, and contribute to lower greenhouse gas emissions per unit of food produced, reduce agricultural pollution, as well as have greater economic benefits for the smallholder farmer, who typically has to face the greatest cost when using inputs as a primary means of production.

8. Advanced Molecular and Analytical Approaches

8.1. 16S rRNA Gene Sequencing for Bacterial Community Profiling

Carl Woese's pioneering work established the foundational use of the 16S ribosomal RNA gene as a universal phylogenetic marker for identifying bacteria and profiling communities. The development of high-throughput next-generation sequencing (NGS) platforms has transformed the use of 16S rRNA genes in soil microbial ecology in modern times (Handelsman, 2004) [13]. The typical workflow for sequencing 16S rRNA amplicons includes PCR amplification of hypervariable regions (usually the V3–V4 or V4 region alone) of the 16S rRNA gene with universal primers, followed by paired-end sequencing with Illumina MiSeq or NovaSeq or similar platforms to generate millions of sequence reads for each sample to provide sufficient depth to observe rare taxa (<0.1% of community members). Bioinformatics pipelines are then used to process sequence data to assign operational taxonomic units (OTUs) or amplicon sequence variants (ASVs) using either clustering algorithms or exact sequence matching to reference databases (e.g., SILVA, Greengenes, RDP) to assign taxonomic identities.

The use of 16S rRNA sequencing has revealed highly consistent, ecologically important differences in bacterial communities that are not detectable through traditional culture-dependent or low-resolution molecular methods; essentially allowing comparisons between two different treatment types where prior approaches were unable to demonstrate this ability. While 16S rRNA amplicon sequencing does provide only taxonomic data on bacterial communities and cannot give detailed functional information, the data from these sequencing methods must be interpreted in conjunction with functional data or there is a presumption that the different phylogenetic groups of bacteria have similar functions. This additional knowledge can be obtained using a combination of PICRUSt2 or Tax4Fun (Handelsman, 2004) [13]. The most significant challenge associated with this work is the use of PCR amplification bias which occurs through differences in primer binding affinities among different bacterial phyla when their 16S rRNA gene sequences have significant divergence, thus resulting in potentially large discrepancies in the estimated composition of bacterial communities.

8.2. Metagenomics and Functional Gene Analysis

Through the use of shotgun metagenomics, it is possible to directly sequence the total extracted DNA from soil samples without having to perform amplification of specific target genes and thus eliminate the amplification bias associated with 16S rRNA gene sequencing. This approach provides simultaneous information about both the taxonomic composition of the microbial community and the functional gene repertoire present in that community (Handelsman, 2004) [13]. In the case of soil metagenomics datasets generated from organically amended soils, the datasets consistently demonstrate enrichments in functional genes associated with nitrogen cycling (e.g., nifH, amoA, nirK, nirS, and nosZ), phosphorus mobilization (e.g., phoD, phoC, and gcd), carbon degradation (e.g., cbhI, abfA, and ligB), and plant growth promotion (e.g., ipdC – producing IAA, acdS – producing ACC deaminase) that are associated with soil amendment. Patterns of the functional gene abundance in the amended soils provide a mechanistic understanding of the biochemical capacity of amended soil communities that cannot be determined from only taxonomic-based analyses.

Metatranscriptomics uses extracted RNA (particularly messenger RNA) sequenced from soil to identify the actively expressed genes of a community of microbes. This information about the active genes complements the compositional information from metagenomics by adding an important time dimension to the gene expression activity. Because only a small fraction of the genes that are present in a community are actively expressed at any one time, metatranscriptomics identifies the subset of actively functioning genes and is able to differentiate between genes that are always expressed (housekeeping genes) from those that are expressed only in response to a change in the environment.

When it comes to researching organic amendments, metatranscriptomics allows for the identification of which decomposition pathways are being activated by different organic amendment substrates, as well as how gene expression changes over time through the decomposition process from labile to recalcitrant forms of carbon.

8.3. Statistical Frameworks for Microbial Diversity Analysis

Analyses of the statistical data regarding soil bacterial communities will require specialized methods that are suited to the unique characteristics of microbiome data: i.e., the inability to observe absolute abundance and measure only relative proportions; the high dimensionality of species-rich community datasets; and also the complex nature of the multivariate relationship between the environmental variables and the community composition. α-Diversity metrics including species richness (i.e., numbers of observed OTUs/ASVs), Shannon entropy (H'), Simpson's index (D), and Faith's phylogenetic diversity (PD) quantify the α-Diversity of the observed unique samples; comparison of α-Diversity across amendment treatments are typically performed with ANOVA or non-parametric equivalents. β-Diversity, which is a measure of the compositional dissimilarity of the observed unique samples, is typically measured using either Bray-Curtis dissimilarity or UniFrac distances (which incorporate phylogenetic information) and visualized using ordination methods such as principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS).

There are two constrained ordination techniques for use with multivariate data: RDA (redundancy analysis) for linear response models, and CCA (canonical correspondence analysis) for unimodal response models. Both types of analysis can identify systematic relationships between bacterial populations and physicochemical characteristics of soil (Bates *et al*., 2011) [15]. In addition to measuring species abundance (population structure), both analyses can be utilized to explain and partition the total community variance (containing an "explained component" due to physicochemical variables and an "unexplained component" due to the residuals of an explanation) enabling researchers to determine which physicochemical variables are most responsible for creating the structure of the community. In long-term amendments analyses, RDA analysis has shown that pH, SOC, and C:N are the three major predictors of bacterial community structure and together account for between 30 and 60 percent of total community variance.

8.4. Microbial Network Analysis and Ecological Inference

The use of co-occurrence network analysis has become a major tool for studying bacterial taxa relationships and community organization patterns that would not be apparent by conventional diversity analysis methods. Co-occurrence networks, where each node represents a taxon and each edge represents a statistically significant correlation (positive or negative) between taxa across samples, are becoming popular methods of evaluating ecological relationships via the use of statistical methods. As the co-occurrence correlation characteristics of microbial communities can be further understood using network metrics such as degree distribution, clustering coefficient, modularity, and centrality, these attributes may also identify differences in community architecture between treatment groups based on amendment-induced changes to these networks. In particular, hub taxa normally have disproportionately large numbers of co-occurring relationships, thus making them very important as potential keystone or organizing species in a community capable of causing changes to overall community function upon response to amendments.

Caution must be exercised when interpreting microbial co-occurrence patterns as ecological interactions based solely on the presence of statistical correlation because statistical correlations in abundance data do not provide sufficient information to separate similarities of environmental preference between organisms from direct mutualistic or antagonistic relationships or indirect interactions that occur through common resources or cross-feeding and therefore, it is important to validate any relationships established through the use of co-occurrence network analysis using other techniques. There is potential to use synthetic community studies, stable isotope probing and in situ fluorescence in situ hybridization (FISH) spatial proximity measurements to validate the ecological significance of the relationships determined by utilizing the co-occurrence network analysis approach.

8.5. Multi-Omics Integration and Emerging Technologies

The ability to connect the characteristics of a soil microbial community with the functions performed by that community and with soil processing using an integrated multi-omics approach to evaluate different levels or omics of living organisms is an exciting opportunity. For example, the integration of genomics, transcriptomics, proteomics, and metabolomics data collected on microorganisms present in a soil environment creates an opportunity to better understand how metabolic pathways and enzymes are linked to the production, transport, and impact of metabolically active microorganisms living in the soil. Ultimately, this multi-omics approach can help researchers to define the mechanisms that underlie the production of organic amendments and the factors affecting the pathways, efficiencies, and activities by which an organic amendment enhances crop production in a long-term magnitude.

The advent of novel sequencing technologies, including next-generation long-read (such as Pacific Biosciences' SMRT and Oxford Nanopore Technologies) sequencing technologies are better suited for the characterization of soil microbiomes when compared to conventional short-read sequencing methodologies (such as 454, Illumina). The new long-read technologies provide: better performance in the formation of chimeric sequences when using 16S amplicons (PCR products of the variable regions between the 16S rRNA gene's conserved regions), better read length for assembling metagenome-assembled genomes (MAGs), and the ability to sequence entire (full-length) 16S rRNA genes that yield improved phylogenetic and taxonomic resolution at both the genus and species level. (Handelsman, 2004) [13]. The recent trend of decreasing costs and improving accuracy associated with long-read sequencing strongly suggests that long-read technologies will continue to replace short read methodologies for soil metagenomic research over the next decade.

8.6. Bioinformatics and Data Integration Challenges

Analyzing data from soil metagenomics presents considerable computational challenges; however, each sequencing run produces an incredible amount of raw sequence data (on the order of 50-500 GB), necessitating highly advanced bioinformatic analysis if all the steps (quality control, assembly, gene prediction, functional annotation, and taxonomic classification) are to be performed efficiently (Handelsman, 2004) [13]. A major issue is the lack of standardized bioinformatic analysis protocols between different research groups, which hampers the ability to make cross-sectional comparisons. These differences result from the various options for assemblers, gene callers, functional databases, and taxonomic classifiers within each bioinformatic pipeline, leading to significantly different estimates of community composition and functional gene abundance based upon identical input data. The development and use of standardized community bioinformatic platforms: QIIME2, Mothur, and MGnify (offered by the European Bioinformatics Institute) represent critical steps toward improving cross-sectional comparability within studies of soil microbiomes.

9. Knowledge Gaps and Future Research Directions

9.1. Contradictory Findings in Long-Term Amendment Studies

This review shows the general findings from studies of organic amendments and their effects on soil physical, chemical, and biological properties to be consistent with each other. However, there is a large number of studies within the scientific literature that present contradictory findings. These contradictions are due to real and complex ecological factors (e.g., soil type, pre-existing conditions of the site, rates and frequency of amendments, chemical composition of the amendments) in addition to methodological errors [7, 11].

There is wide variation in the effects of organic amendments on specific groups of bacteria among studies, with some studies reporting a significant increase in the abundance of Proteobacteria in the treatment with compost while others show no significant changes at the phylum level or improvement in α-diversity indices.

In Table 6, the key gaps in knowledge, research limitations and future directions for research resulting from this review of the long-term organic amendment literature regarding chickpea and related legumes are summarized.

Table 6: Knowledge gaps, methodological limitations, and recommended future research directions in long-term organic amendment microbiome research.

Knowledge Gap / Limitation	Affected Research Domain	Current Challenge	Recommended Future Direction
Causality vs. correlation in amendment-microbiome links	Microbial ecology	Observational studies dominate; mechanistic pathways unclear	Controlled microcosm experiments with isotope tracing (13C, 15N)
Long-term (>10 yr) field trial scarcity	Agronomy, soil science	Most studies < 5 years; short-term dynamics extrapolated	Establish coordinated international long-term field networks
Amendment rate and timing optimization	Agronomic management	Varied dosing regimes limit cross-study comparisons	Factorial design experiments with standardized protocols
Soil type-specific microbial responses	Pedology, microbiology	Most research on agricultural soils of temperate zones	Include tropical, arid, and semi-arid soil ecosystems
Functional redundancy versus gene expression	Metagenomics	Taxonomic composition ≠ functional activity	Integrate metatranscriptomics and proteomics
Plant–microbiome feedback dynamics	Rhizosphere biology	Plant-host specificity poorly characterized at long time scales	Multi-year rhizosphere metatranscriptomic profiling
Climate change interaction effects	Climate–soil–microbiome nexus	Amendment effects under future climate scenarios poorly understood	Integrate climate projections into long-term amendment trials

9.2. Methodological Limitations in Bacterial Community Analysis

Recent molecular approaches used in the study of soil bacterial communities are much more powerful than culture-based methods but also have limitations and biases that affect how reliable the ecological interpretations are of the derived data (Handelsman, 2004) [13]. These limitations are primarily due to differences in the extraction efficiencies of DNA from different bacterial taxa, since certain taxa, such as gram-positive or spore-formers, generally require more adverse conditions for extraction than gram-negative bacteria, thereby leading to an underestimation of the contribution of these bacterial taxa to the overall community composition. In addition, PCR amplification with universal 16S rRNA primers introduces taxonomic bias through the differences in primer binding efficiency on different bacteria. For example, Acidobacteria and Verrucomicrobia may potentially be underrepresented because of the presence of primer mismatches.

The difficulties experienced in translating sequence-based data into functional interpretations create another set of methodological challenges. Metabolic pathway predictions based on phylogenetic marker genes using tools such as PICRUSt2, rely upon the assumption that functional conservation occurs within all the phylogenetic lineages, and this assumption will probably be violated by both horizontal gene transfer and convergent functional evolution of soil bacteria (Bates *et al*., 2011) [15]. Additionally, since the endemicity and genomic novelty of soil bacterial populations is very high and many of the identified taxa are new lineages that are not closely related to cultivated relatives. Therefore, the use of phylogeny as a predictor of functional potential is not a reliable approach. Consequently, there is a need for a functional approach independent of culture, such as metagenomic or metatranscriptomic.

9.3. Challenges in Establishing Causative Relationships

Long-term amendment research is faced with a basic epistemological dilemma: it can be challenging to establish a causal link between changes in physicochemical properties of soils (e.g., pH, soil organic carbon [SOC], cation exchange capacity [CEC], and microbial biomass) due to the observational nature of long-term studies. Researchers commonly observe the changes in their field experiments; however, there are multiple variables involved that can create confounding correlations (i.e., how these variables interact). The use of multivariate statistical models to identify the independent effects of any one of the physicochemical parameters on the composition of the microbial communities is hampered by high colinearity between the physicochemical parameters (all improvements in the physicochemical parameters of soil are made synchronously and interdependently) in amended soils. Therefore, complementary experimental methods are needed to examine how to decompose the complex and interdependent relationships observed in field studies (i.e., create mechanistic cause-and-effect relationships): through controlled pH and organic carbon manipulation experiments using replicated standardized soils; through the transplantation of soil microbial communities (from amended to unamended and vice versa), and through synthetic communities of defined composition.

9.4. Need for Long-Duration Field Experiments

The scientific community is highly limited in its understanding of how organic amendments affect soil bacterial communities over the long-term because there are very few long term (greater than 10 - 15 years) experiment conducted in the field [9]. The vast majority of the long-term amendment studies have been published as short-term (3 -8 years), and may lead to an inability to detect significant delayed responses across mature oligotrophic bacterial communities, establish and stabilize microbial network architecture, develop soil disease suppressive capacity, and provide climate change mitigation services through the stabilization of deep SOC. The establishment of a coordinated network of international long-term research on soil biology similar to the Long-Term Ecological Research (LTER) model would result in the data required to examine bacterial community dynamics over the entire time-line from the initial application of the amendment through long-term equilibration.

9.5. Integration of Microbial Ecology with Agronomic Management

One of the major challenges of the translated organic amendment research is the development of evidence-based guidelines for management of amendments that are based on an understanding of the way the community of microorganisms in the soil responds to the use of the amendment as opposed to the empirical yield response alone [7, 11]. The recommendations made currently regarding the type, rate, timing, and method of incorporating the amendment are derived primarily from agronomic response trials that measure crop yield and soil physicochemical parameters without including microbial data. Developing an approach for amendment management using knowledge of the microbiome, where there is targeted enhancement of the functional guilds of microorganisms by careful selection and application of amendments, represents an emerging area of promise to maximize the biological efficiency of organic amendment use in chickpea production systems.

9.6. Recommendations for Future Research

The present synthesis of all current available literature provides an overall data base from which the following research priorities were identified. Coordinated field experiments that examine multi-decadal and multi-site amendments and their impact on soil bacterial communities will use standardized molecular and agricultural methods to enable true comparative studies on diverse soil types, climates, and management systems. A second priority will be to use multi-omics approaches (metagenomics, metatranscriptomics, metaproteomics, and metabolomics) to examine the functional consequences of community shifts due to the use of organic amendments, and thus create mechanistic links between community composition and soil process rates. A third priority will involve using isotopic tracer techniques (^{13}C, ^{15}N, ^{33}P), in combination with molecular analysis of microbial communities, to measure the contribution of microbial taxa and functional guilds in the nutrient cycling of organically amended soils. The fourth priority will be to assess the interaction effects between climate variables (increased CO_2, temperature, and precipitation variability) and organic amendments on both soil bacterial community composition and function. Finally, the fifth research priority will be the development of predictive models that utilize soil physicochemical, microbial community composition, and agronomically pertinent data to predict the long-term effects of organic amendment application on soil health and chickpea yield under current and future predicted climate scenarios.

Figures

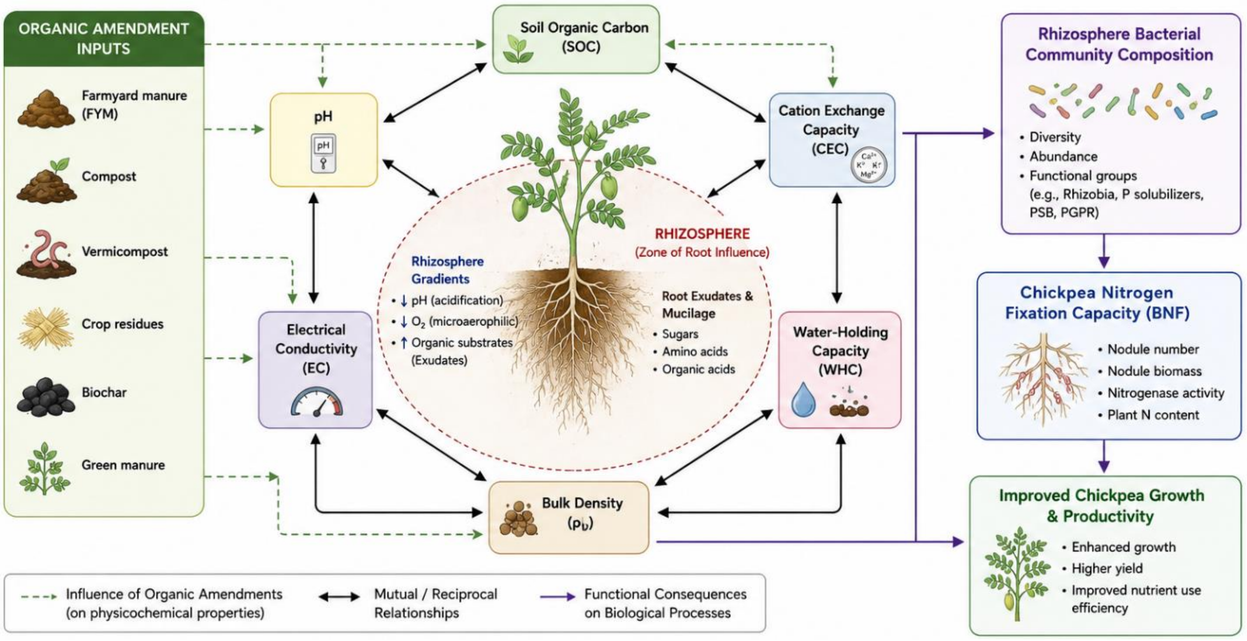

Fig 1: Conceptual Diagram – Soil Physicochemical Interactions in Chickpea Rhizosphere

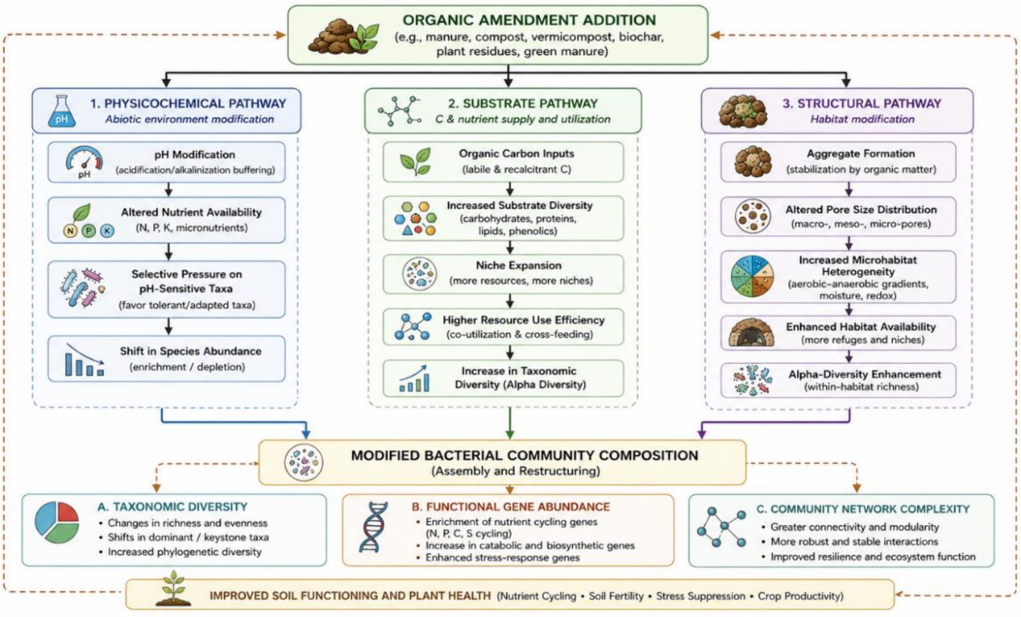

Fig 2: Flowchart – Effects of Organic Amendments on Bacterial Community Assembly

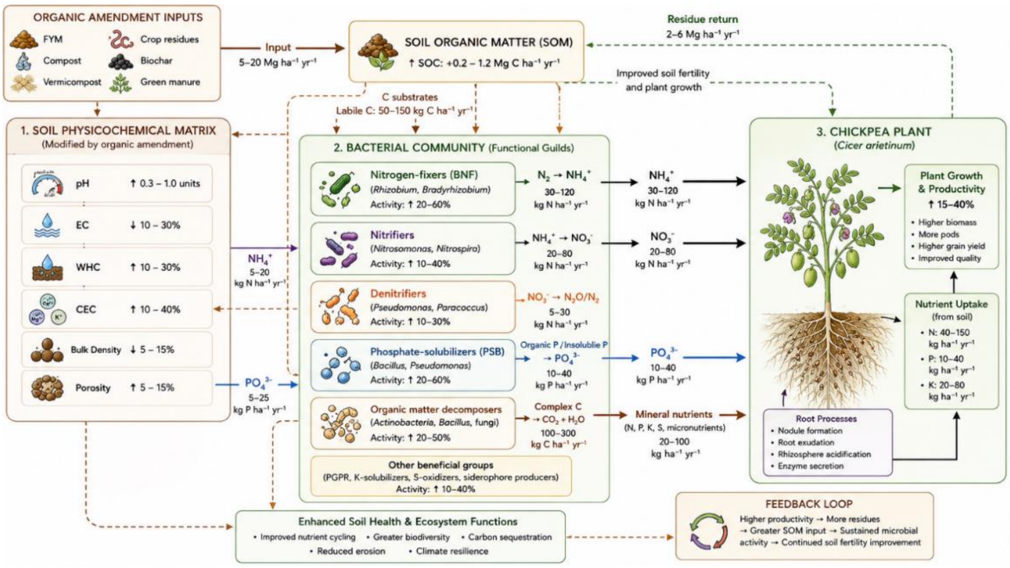

Fig 3: Mechanistic Model – Soil–Microbe–Plant Interactions and Nutrient Cycling

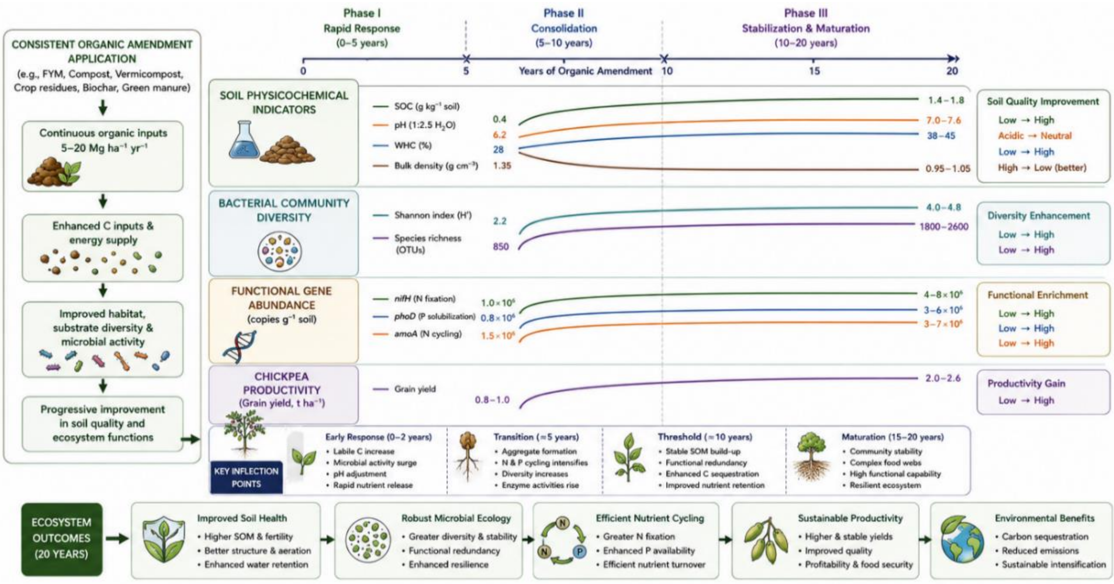

Fig 4: Integrated Framework – Long-Term Impacts on Soil Quality and Bacterial Ecology

10. Conclusion

A thorough review of the long-term impacts of organic amendments on the properties of soils (physically and chemically) and bacterial community structure/functional groups in chickpea growing systems has produced a consistent body of evidence that there is ongoing improvement in soil quality and ongoing population growth in bacterial communities as a result of organic agricultural technology. These improvements make organic agriculture possible and provide both agronomic and environmental sustainability benefits. Long-term input of organic amendments (vermicomposts and composts at agronomically appropriate rates) significantly increases soil pH capacity to resist change, reduces bulk density, increases aggregate stability, increases ability to retain water and have cation exchange capacity, and maintains soil organic carbon levels both through direct transfer of organic matter and through increased accumulation of organic matter in microbial biomass. Improvements to physical and chemical properties of soils collectively contribute to a more favorable growing environment for chickpea roots, nitrogen fixation as facilitated by Mesorhizobium, and the recruitment and persistence of diverse groups of rhizosphere bacteria.

Evidence at the microbe level extensively shows that amendments made to soil over longer periods can produce increases of alpha-diversity of bacteria, microbial biomass carbon, and the abundance of beneficial functional groups of bacteria (e.g., phosphate-solubilizing bacteria, plant growth-promoting rhizobacteria, actinomycetes that are acting as biocontrol agents, and free-living diazotrophic nitrogen-fixing bacteria). These changes in microbial communities are connected mechanistically to improvements seen at the physicochemical level with soils because of: expansion of niches for bacteria due to pH differences; diversity of organic substrates influencing the make-up of different types of communities; and improvements made at the soil structure level, which create a range of different micro-environments that are needed to sustain diversity - both taxonomic and functional. The changes on the phylum level in the bacterial communities associated with long-term organic amendments exhibit trends that can be predicted, in that the number of Proteobacteria & Actinobacteria typically increase with long-term organic amendment, while the number of Acidobacteria decreases. This suggests that the long-term organic amendments shift the bacterial communities from primarily oligotrophic environments (those with low nutrients) to more copiotrophic (higher nutrient) communities.

These findings have significant implications for continuing development of sustainable agricultural systems. Use of organic amendments on long-term basis (in terms of years) in chickpea production systems provides a means to simultaneously improve food production; improve the health (and thus productivity) of soils; create stocks of carbon in the soil, which has the potential to mitigate climate change; limit the use of synthetic (e.g., chemical) inputs; and increase the ecological resilience of agricultural soils to disturbances from environmental changes.

The positive feedback dynamics (e.g., between the application of the amendments to improve the conditions of the soil, and, therefore, create enriched microbial communities) are the basis for a truly sustainable intensification of chickpea production via managing the soil to improve the natural capital inherent within soils, rather than depleting and degrading it. However, the full potential from microbial contributions to chickpea production will be realized through the development of specific management strategies that target the enrichment of specific function-based (bacterial) guilds; optimize the physicochemical conditions that support specific guild activity; and ensure that there are (long-term) sustainable populations (and interactions) of those guilds over multi-year to multi-decade timeframes.

The gaps in knowledge still basically remain in terms of how to describe the mechanisms of contradictory outward appearances from the use of soil amendments to build soil types; understanding the long-term (> 10 years) dynamics associated with the progression and stabilization of the respective bacterial communities; understanding the functional implications and outcomes related to amendment-induced changes in bacterial community composition within the associated soil environments or functions/possibilities; as well as understanding how the interactive nature of organic management(s) and climate change variables affect one another. To address these knowledge deficiencies will necessitate development of long-term field studies with standardized methods etc.; developing predictive models that can relate soil physiochemical and microbiological data to actionable management recommendations; coordinating the implementation of multi-omic approaches and ultimately allowing for the ability to correlate community composition with functional activity. The convergence of soil sciences; microbial ecology; plant physiological responses; and systems biological response on how to develop and improve inorganic (expected outcome will be; regardless of the methodology used) will provide the basis for developing good evidence, science readable and applicable organic amendments that are needed to successfully meet the global sustainable development goals regarding food security; climate change mitigation/impacts/impacts; and protection of biodiversity.

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