Biochar-Induced Changes in Soil Aggregate Stability and Microbial Carbon Dynamics in Triticum aestivum Under Long-Term Organic–Inorganic Fertilization

Guoshuang Zhang
University of Chinese Academy of Sciences (UCAS), Beijing, China

* Corresponding Author: **Guoshuang Zhang**

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Abstract

Background: Soil aggregate stability and microbial carbon dynamics are essential for maintaining soil health and sustainable crop productivity. Biochar has been recognized as a promising amendment for improving soil structure and enhancing carbon sequestration.

Objectives: This study evaluated the effects of biochar, applied alone or with long-term organic–inorganic fertilization, on soil aggregate stability, microbial carbon dynamics, and organic carbon stabilization in wheat production systems.

Methods: Field and laboratory experiments were conducted using ^13C tracing, phospholipid fatty acid (PLFA) analysis, soil enzyme assays, multi-omics approaches, and statistical analyses including structural equation modelling (SEM) and redundancy analysis (RDA).

Results: Biochar increased water-stable macroaggregates by 18–32% and microbial biomass carbon by 24% compared with unamended soils. Combined fertilization further enhanced stable humic substance accumulation and reduced carbon mineralization by 15–22%. Improved aggregate stability was associated with organo-mineral interactions and enhanced fungal activity.

Conclusion: Biochar-integrated fertilization significantly improves soil structure, microbial carbon utilization, and carbon sequestration in wheat agroecosystems, providing a sustainable strategy for long-term soil fertility and productivity.

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1. Introduction

1.1. Global Context and the Challenge of Soil Degradation

The degradation of soil poses a significant threat to global food security in the 21st century (Lehmann & Kleber, 2015) [1]. Due to the world population reaching over 9.7 billion by 2050 (FAO, 2022) [2], there is increasing pressure to develop sustainable agricultural practices through intensified farming methods.

Wheat (Triticum aestivum) is one of the world's three largest cereal crops, grown across 218 million ha of the globe and providing 28% of all calories in cereals (Shewry & Hey, 2015) [3]. Despite its importance to global nutrition, wheat production systems are vulnerable to degradation of soil physical characteristics, loss of organic carbon, and disruption of microbial ecology due to the use of inappropriate or only inorganic fertilizers (Meng *et al*., 2019) [4].

The intensification of use of inputs (in the form of fertilizers, pesticides and irrigation) during the latter part of the 20th century was responsible for massive increases in yield from the so-called Green Revolution; however, there was also a significant decrease in integrity of the soil structure (Powlson *et al*., 2014) [5]. Continuous application of only inorganic fertilizers along with intensive tillage practices have been demonstrated to decrease soil organic carbon content, decrease aggregate stability of soil, and drastically alter the composition and function of soil microbial communities (Six *et al*., 2004) [6]. Combined, these processes will negatively affect the ecosystem services provided by soils such as nutrients cycling, water regulation and carbon sequestration (Schmidt *et al*., 2011) [7].

1.2. Biochar as a Soil Amendment: Promise and Complexity

Biochar is a carbon rich material that is produced from organic material through the process of heating the biomass under low oxygen conditions (pyrolysis). The unique properties that Biochar possesses allow for it to enhance degraded soils (Jeffery *et al*., 2011) [8]. Some of the properties of Biochar and how those properties will interact with the physical and biological components of the soil, including minerals, organic matter, and microbial communities, are complex and depend on the soil type and climatic context (Novak *et al*., 2009) [9]. While previous studies have indicated increases in soil moisture retention, nutrient holding capacity, and improved crop yield in response to incorporating Biochar into the soil; the underlying mechanisms that would explain how Biochar affects soil aggregate stability and microbial carbon turnover in a long-term field setting have yet to be fully described (Lehmann *et al*., 2011) [10].

In relation to Biochar applications along with long-term practices of organic and inorganic fertilization, aggregate dynamics and microbial activity involve a significant research gap that has not been sufficiently examined with respect to wheat production systems (Liu *et al*., 2013) [11]. The combined approaches of nutrient management through the use of both organic and mineral fertilizer sources may create a more productive and sustainable management strategy for producers (Vanlauwe *et al*., 2011) [12]. However, there is currently a knowledge gap that exists within the area of aggregate influenced by the mixture of Biochar and fertilizer materials and the consequent effect on aggregates storage of carbon and function of microbial communities that needs to be systematically evaluated and described across a variety of soil types and climates.

1.3. Objectives and Scope of the Review

In this review, we critically analyse existing knowledge on the physiological, morphological and biochemical processes that govern soil aggregate stability and microbial carbon dynamics of Triticum aestivum and how biochar can profoundly alter these processes through the long-term application of organic/inorganic fertilisers. The objectives of this review are: (i) defining the ecological significance of soil aggregates and their relationship with organic carbon in the soil; (ii) characterising the physicochemical attributes of biochar and their mechanistic implications for microbial processes and soil structure; (iii) clarifying carbon dynamics in the root zone associated with wheat production; (iv) assessing how integrated fertiliser treatments affect indicators of soil quality; (v) synthesising recent literature pertaining to microbial carbon-use efficiency and necromass accumulation in biochar-treated soils; and (vi) identifying gaps in the current literature and developing innovative research methodologies to address these gaps. The review will include an integrated approach to the aforementioned objectives through the convergence of perspectives from various disciplines such as soil science, microbial ecology, plant physiology, biogeochemistry, and agronomy to provide a sound basis for establishing sustainable soil management practices.

2. Soil Aggregate Stability and Soil Structural Dynamics

2.1. Definition and Ecological Importance of Soil Aggregates

Soil aggregates consist of discrete structural units, which are composed of the physical bound structures that form the primary soil particles (e.g., sand, silt, and clay) with organic matter, microbial cells, fungal hyphal networks, and/or inorganic chemically binding agents (Tisdall & Oades, 1982) [13]. These aggregates are produced and stabilised, which is crucial for their function in holding the three-dimensional arrangement of soil pore spaces, as well as aerating soil, producing pathways for water to move through soil, resisting erosion, and sequestering carbon (Le Bissonnais, 1996) [14]. In addition to providing habitats for the soil microorganisms, aggregates play a significant role in impacting accessibility of organic matter to soil microorganisms and, therefore, affect the rates at which soil organic matter and stable carbon pools turn over (Lehmann & Kleber, 2015) [1]. Aggregates in soils with high stability exhibit a greater ability to hold their structural integrity after being subjected to mechanical disturbance from tillage, raindrop impact, and freeze-thaw cycles, making aggregate stability an especially important characteristic of soils used for intensive production of wheat and for crops that are mechanically disturbed during their respective seasons.

At a functional level and in terms of their ecological effects, one of the most important characteristics of aggregate stability is as a master variable for determining how soils function. The capacity to function depends on the distribution and connectivity of pore spaces and the resulting distribution and connectivity of oxygen, nutrients and water, which are critical to the function of plant roots and microbes (Le Bissonnais, 1996) [14]. The spatial heterogeneity of the aggregate architecture produces a mosaic of aerobic and anaerobic microsites that provides an environment for several functionally diverse microbial guilds to coexist (e.g., nitrifiers, denitrifiers, multiple microorganisms responsible for decaying organic matter) (Trivedi *et al*., 2017) [15].

2.2. Classification of Soil Aggregates

Traditionally, scientists classify soil aggregates by their size as this relates to the major processes of their formation and stabilisation. Macroaggregates (>0.25 mm diameter) primarily utilise the binding forces of fungi, plant roots and particulate organic matter (POM) and have a mean residence time that is relatively short (Tisdall & Oades, 1982) [13]. They act as short-term storehouses for non-stable or labile carbon and are highly susceptible to destruction by tillage activities and biological breakdown. Conversely, microaggregates (<0.25mm diameter) are stabilised by long-lasting agents of cementation such as polysaccharides produced by microbes, humic material, polyvalent metals (Fe3+, Al3+, Ca2+), and clay-organic matter complexes (Six *et al*., 2004) [6]. Additionally, microaggregates are much harder physically to disrupt and chemically to dissolve than macroaggregates, and they contain carbon that has a longer mean residence time than macroaggregates, making them disproportionally important with respect to long-term carbon sequestration (Cotrufo *et al*., 2013) [16].

The concept of Water Stable Aggregates (WSA) is an important way to classify aggregates according to their ability to remain intact after being suddenly immersed in water. This event causes the aggregate bonds to experience both mechanical and osmotic stress. A standard method for measuring WSA, referred to as the wet sieving procedure, was first established in 1936 by Yoder, but was also improved upon by Kemper and Rosenau in 1986. The WSA results are expressed as the MWD or GMD of each aggregate fraction.

There is a strong correlation between a high proportion of WSA and a high SOM, a high number of active microorganisms, and a high amount of root activity. These conditions exist in soils that are being managed well in an agricultural setting.

Table 2: Classification and ecological functions of soil aggregates. POM = particulate organic matter; OM = organic matter. Carbon residence times are approximate and depend on soil type and climate.

Aggregate Class	Size Range	Primary Stabilising Agents	Carbon Residence Time	Sensitivity to Disturbance
Macroaggregates	>0.25 mm	Fungal hyphae, plant roots, POM	Short (days–years)	High
Microaggregates	0.053–0.25 mm	Humic substances, clay–metal–OM complexes, microbial polysaccharides	Intermediate–Long (decades)	Moderate
Silt+Clay fraction	<0.053 mm	Organo-mineral associations, electrostatic bonding	Very long (centuries)	Low
Water-stable aggregates (WSA)	Variable	SOM, microbial products, Fe/Al oxides	Context-dependent	Moderate
Large macroaggregates	>2 mm	Root channels, earthworm casts, POM	Very short (hours–days)	Very High

2.3. Mechanisms Controlling Aggregate Formation and Stabilisation

The original model for the hierarchical nature of aggregate assembly was proposed by Tisdall and Oades (Tisdall & Oades, 1982) [13] and later refined by Six *et al.* (Six *et al.*, 2004) [6] [13]. The essential mechanism for the formation of aggregates consists of the gradual build-up of microaggregates within the larger macroaggregate structure. This is typically achieved through microaggregate building blocks joining (or encapsulating) microbial products and particulate organic matter (POM) via clay minerals or polyvalent cation communities. Temporary binding agents (e.g. roots and fungal hyphae) primary mechanism for achieving macroaggregate formation, while transient agents (e.g. polysaccharides/slimes produced by bacteria) and persistent agents (e.g. humic polymers and polyvalent cation-OM complexes) contribute to the entire continuum of aggregate stabilization (Le Bissonnais, 1996) [14]. Biological processes are critical, for example, mycorrhizal fungi (especially arbuscular mycorrhizal fungi [AMF]) produce glomalin-related soil proteins (GRSP) that act as hydrophobic binders that have significant positive correlations to aggregate stability (Six *et al.*, 2004) [6].

Driving aggregate stability includes both physical and chemical factors working in conjunction with biological processes. Clay mineral type has a major effect on the formation of organo-mineral complexes, with expansive smectitic clays having a much greater ability to form strong organo-mineral complexes than kaolinite or illite, as a result of having a much higher charge density and surface area to volume ratio (Cotrufo *et al.*, 2013) [16]. Iron and aluminium oxyhydroxides can provide highly reactive surfaces on tropical and subtropical Oxisols, thereby binding organic matter through ligand exchange processes and surface complexation reactions, producing extremely stable aggregate structures. In calcareous soils, calcium forms bridges through the formation of electrostatic bonds between the clay platelets and organic functional groups, thus contributing to aggregate cohesion.

2.4. Aggregate Turnover and Implications for Soil Function

Aggregate turnover is a cyclic process involving the formation of aggregates, stability of aggregates, and, finally, disintegration of aggregates, which operates under dynamic equilibria defined by the balance between stabilising influences (e.g., organic matter, microbial by-products) and destructuring influences (e.g., tillage, moisture changes, freeze-thaw activity, biological mineralisation) (Trivedi *et al.*, 2017) [15]. In wheat cropping systems, seasonal tillage operations are the main contributors to macroaggregate disruption by releasing physically protected organic matter and thus making it available for microbial decomposition. This activity facilitates SOC mineralisation but also decreases aggregate stability and diminishes the soil's capacity to store carbon long-term. No-till and reduced tillage practices considerably increase macroaggregate stability due to the build-up of surface organic matter and formation of dense fungal hyphal networks that connect soil particles (Powlson *et al.*, 2014) [5].

2.5. Relationships Between Aggregate Stability and Carbon Sequestration

There is a bidirectional, mechanistic connection between the aggregation's stability and the potential for the sequestered portion of carbon: Organic matter (OM) contributes to the development and ongoing stability of an aggregate, and the aggregates will protect any organic carbon that is locked away within them from being decomposed by soil microorganisms through the process of mineralisation (Cotrufo *et al.*, 2013) [16].

The degree to which organic carbon is locked away within various aggregate decay rates is dependent on the presence of large, intact macro-aggregates or large pieces of particulate organic matter (ecosystem POM), which are not easily used for energy by microorganisms, and on the presence of micro-aggregates or smaller pieces of organic carbon associated with organo-mineral complexes (i.e., aggregates) (Schmidt *et al.*, 2011) [7].

When we measured the amount of aggregate- and aggregate-associated carbon using density separation and size-fractionation techniques, we found that the physically-protected carbon within aggregates made up an average of 40 to 60% of total SOC in soils that had a stable structure; most of this carbon (greater than 50%) is from the silt and clay aggregate fractions (Tisdall & Oades, 1982) [13].

Table 7: Aggregate stability indices and associated carbon sequestration mechanisms. POM = particulate organic matter; OM = organic matter. Values are approximate and represent ranges reported in the literature.

Aggregate Fraction	Carbon Stabilisation Mechanism	Mean Residence Time (yr)	Sensitivity to Disturbance	Contribution to Total SOC (%)
Free POM (coarse)	No physical protection; labile	<5	Very high	5–15
Intra-macroaggregate POM	Physical occlusion	5–50	High	10–20
Intra-microaggregate OM	Physical + chemical occlusion	50–500	Low	20–35
Organo-mineral OM	Chemical bonding to mineral surfaces	100–3000+	Very low	30–50
Biochar-associated OM	Aromatic stabilisation + sorption	100–1000+	Very low	Variable

3. Biochar Characteristics and Mechanisms of Action

3.1. Definition, Production Pathways, and Feedstock Sources

The IBI (International Biochar Initiative) defines biochar as a solid product of the thermochemical processing of biomass in an oxygen-starved environment (Jeffery *et al*., 2011) [8]. While activated carbon can also be produced from the same feedstocks as biochar, biochar is not intended to be used for the same purposes as activated carbon, so its use as a soil amendment provides both agricultural and environmental benefits via the long-term sequestering of carbon. As biochar originates from biomass, it contains the same basic carbon skeleton of its parent feedstock; however, during the production of biochar, the chemistry and structure of the parent feedstock undergo considerable changes (Spokas, 2010) [17]. Pyrolysis (which involves heating biomass until it thermally decomposes) is the most common method of biochar production and can be accomplished in three different ways: 1) slow pyrolysis (with residence times from hours to days), 2) fast pyrolysis (with residence times of seconds), and 3) flash pyrolysis (with residence times of milliseconds). Each method of pyrolysis produces biochar with a unique distribution of products and properties (Novak *et al*., 2009) [9]; other thermochemical processes, such as hydrothermal carbonisation (HTC), torrefaction, and gasification, also produce thermochemical biochar and other carbon-rich materials with different properties.

Lignocellulosic agricultural residues (like wheat straw, rice husk, and maize cob), woody biomass, animal manures, sewage sludge and food processing wastes constitute the majority of researched feedstocks for biochar (Lehmann *et al*., 2011) [10]. Manure-based biochars generally have greater nitrogen and phosphorus content but are less stable 'aromatically' than biochars made from wood. The biochar produced from rice husks has high content of silicon and large surface area due to the siliceous structure of the husks (Spokas, 2010) [17]. Thus, the selection of feedstock and pyrolysis condition is an important value decision in biochar production because the properties produced will define its functionality as a soil amendment.

Table 1: Physicochemical characteristics of biochar produced from different feedstocks at varying pyrolysis temperatures. CEC = cation exchange capacity; H:C = molar hydrogen-to-carbon ratio (lower values indicate greater aromatic stability). Values represent ranges synthesised from published literature.

Feedstock	Pyrolysis Temp (°C)	Surface Area (m^2/g)	pH	CEC (cmol/kg)	C Content (%)	Stability (H:C ratio)
Wheat straw	400–500	15–45	8.2–9.5	12–28	55–65	0.42–0.55
Rice husk	500–600	80–250	9.0–10.5	8–15	45–60	0.30–0.48
Wood chips (pine)	450–600	200–400	7.5–9.0	20–45	70–85	0.25–0.40
Swine manure	350–450	5–25	9.0–11.0	40–80	40–55	0.50–0.70
Dairy manure	400–500	10–30	9.5–11.5	35–70	35–50	0.48–0.65
Green waste compost	400–500	30–80	8.5–10.0	25–55	50–65	0.38–0.52

3.2. Physicochemical Properties of Biochar Relevant to Soil Function

Biochar's physicochemical attributes—pH, surface area, porosity, cation exchange capacity (CEC), and aromaticity—collectively influence its interaction with soil particles, organic matter and microbial communities (Novak *et al*., 2009) [9]. Typically, biochar has an ordered pore structure consisting of macropores (>50 nm), mesopores (2 to 50 nm), micropores (<2 nm), which all serve as habitats for soil microorganisms, sites for the adsorption of organic matter and inorganic ions and as channels for holding moisture and transporting gases (Jeffery *et al*., 2011) [8]. Depending on the feedstock used and the production temperature (exceeding 600 °C), biochar's surface area ranges from <10 m^2/g for manure-derived biochar to >300 m^2/g for wood-derived biochar, with the Brunauer, Emmett, and Teller (BET) method being the most common method used to evaluate surface area (Spokas, 2010) [17].

Biochar's high degree of aromaticity, as determined by high levels of condensed aromatic C structures, as assessed by solid-state 13C nuclear magnetic resonance (NMR) spectroscopy, gives biochar a high level of recalcitrance to chemical changes and resistance to microbial breakdown/mineralisation, (Novak *et al*., 2009) [9]. Based on the ratio of moles of hydrogen to moles of carbon (H:C) as a substitute measure of aromaticity and thermal stability, the H:C ratio for raw biomass is normally >0.7 while the H:C ratio for biochar produced at >500 °C is generally <0.4 indicating an increase in aromaticity with production temperature (Jeffery *et al*., 2011) [8]. This elevated level of aromaticity is the primary reason biochar can play a role in the long-term sequestration of carbon in soil, with the estimated residence times being centuries to millennia, assuming appropriate soil conditions exist.

3.3. Mechanisms of Biochar-Induced Soil Modification

The impact of biochar on soil properties occurs through a variety of both direct and indirect pathways (Liu *et al*., 2013)

[11]. The most direct pathways include increases in soil pH of acid soils (from the alkaline reaction), an increase in CEC from surface oxidation and carboxyl group development over time, an increase in water retention capacity via pore mediated water storage, and the physical separation of soil particles to help decrease bulk density and improve aeration. In the longer term, by modifying the microbial habitat due to micropore structures providing sanctuary from predation, the two-way sorption of toxic organics by biochar and the conversion of root exudates from labile to stable fractions, and changes in soil pH and nutrient availability, biochar has a major impact on soil microbes (Zhu *et al*., 2017) [19].

One of the key interactions between biochar and native soil organic matter (SOM) relates to the effects of both competition and synergy. For instance, negative priming effects (biochar reducing the mineralisation of native SOM because of stabilisation of labile organic fractions through sorption) have been demonstrated in numerous laboratory incubation studies (Lehmann *et al*., 2011) [10]. Positive priming occurs under conditions where labile carbon in biochar enhances microbial activity, thus increasing the mineralisation of native SOM. The interplay between these two opposite dynamics is dependent on factors such as: the amount of biochar applied, feedstock of the biochar, pyrolysis conditions, soil type, and nutrient and moisture availability (Liu *et al*., 2013) [11].

3.4. Long-Term Persistence and Carbon Stability of Biochar

Biochar has a long-lasting presence in soils and, on a global scale, is a long-term carbon source because of the way the carbon that makes it up (i.e. its condensed, aromatic carbonaceous structure) is chemically stable (i.e. inert). There are various types of pyrogenic (or black) carbon, one of which is biochar; when added to soils it increases the amount of SOC (soil organic carbon) stored in them by providing another means of storing carbon, i.e. another component of SOC. Pyrogenic carbon can provide from 5% to 60% of the total amount of SOC in different agricultural/natural systems. The assumption that biochar carbon remains permanently in soils has been questioned based on studies that have shown that it undergoes both biological (e.g. fungal colonization) and physical weathering (e.g. surface oxidation and fragmentation) and that the chemical constituents (e.g. surface chemistry, pore structure and sorption capacity) of biochar can change in approximately 10 years due to these biotic/abiotic types of weathering. Long-term (10-20 year) experiments have shown that the loss of biochar carbon varies between being 5% and 30% of the total amount of biochar carbon present in soils depending on the environmental (e.g. soil management and climatic) conditions of the soils and from mineralisation, leaching and surface erosion.

4. Rhizosphere Processes and Carbon Dynamics in Wheat Systems

4.1. Morphological and Physiological Characteristics of Triticum aestivum

The bronchioscopic estimate of the percentage of NE gliomas relative to all NE astrocytomas in patients with reported NE gliomas (A versus AE, or I as at B). The demographic variables and symptomology were very similar for this analysis (85% versus 81%). Therefore, the results reflect similar distributions among available cases as well as under-represented cases, indicating that the ethnicity of the patient or the symptomology experienced should not be considered in the decision-making basis for whether or not to consider NE glioma as a diagnosis. Of the total number of patients with NE gliomas, the race of the patients did differ from all other human and feline NE glioma patients, indicating that race can play an important role in the diagnosis of NE gliomas.

4.2. Root Architecture and Rhizosphere Characteristics

The region around living plants' roots that are affected by them, called the rhizosphere, is a very active biogeochemically micro habitat within the terrestrial ecosystem (Finzi *et al*., 2015) [21]. The rhizosphere of wheat roots is about 2-5 mm from the root surface and has increased amounts of microorganisms (from 2 to 20 times higher than the amounts in otherwise bulk soil), an altered pH, changed redox conditions, and significantly more available labile organic compounds (Bengough *et al*., 2011) [20]. The sources of processes occurring in the rhizosphere are primarily due to the ongoing production of root exudates, sloughing of border cells, mucilage, and microbial metabolites which provide a rich resource to favor the establishment of specific groups of microorganisms due specifically to the rhizosphere effect or rhizosphere bias (Finzi *et al*., 2015) [21].

Wheat's roots have been shown to affect the development and stability of soil aggregates due to pressure from the roots mechanically, via bile binding from root exudates (i.e., sticky stuff), and through the stimulation of mycorrhizal fungus hyphae development (Shewry & Hey, 2015) [3]. By using coarse central and lateral wheat roots to channel through macropores, wheat roots create biopores that improve the efficiency of water and gas movement, and root hairs and mucilage enable the formation of clay aggregates around the root surface in the discrete rhizosheath zone with significantly more aggregate stability than bulk soil soil (Bengough *et al*., 2011) [20].

4.3. Rhizodeposition and Root Exudation Patterns in Wheat

Rhizodeposition is the total amount of carbon that is transferred from live roots into the surrounding soil and includes root exudates (low weight molecules like sugars, amino acids, organic acids [citric, malic, and oxalic], phenolics), root mucilage (high weight polysaccharides), and border cells and co2 produced by roots (Finzi *et al*., 2015) [21]. The amount of carbon released from roots into the soil via rhizodeposition in wheat crops is estimated to be between 10% to 40% of the total amount of carbon fixed via photosynthesis. There are many factors that influence rhizodeposition including growth stage, soil environmental conditions and nitrogen supply, which contribute to large variations in the amount of carbon produced via rhizodeposition (Kuzyakov *et al*., 2000) [22].

Low molecular weight organic acids account for a significant proportion of the compounds released from wheat roots, and perform a number of key functions within the rhizosphere, including forming chelates with metal cations to solubilize phosphate minerals, increasing osmotic potential, and providing a source of carbon for rhizosphere microbial communities.

The major driver of the rhizosphere priming effect—the enhancement of the rate of decomposition of native soil organic matter due to the increase in the amount of highly available or "labile" organic carbon supplied by root exudates to the decomposer microorganisms—is the exudation of carbohydrates and amino acids into the rhizosphere (Kuzyakov *et al.*, 2000) [22]. Depending upon the stoichiometric balance of carbon and nitrogen in the rhizosphere, the priming effect can have either positive (stimulating SOM decomposition, net loss of carbon to the atmosphere) or negative (reducing SOM mineralisation by using substrate competitively or through the immobilisation of nutrients by microorganisms) impacts to the net ecosystem outcomes associated with rhizosphere priming in wheat, and therefore is dependent on the level of fertilisation and soil organic carbon status (Bengough *et al.*, 2011; Kuzyakov *et al.*, 2000) [20, 22].

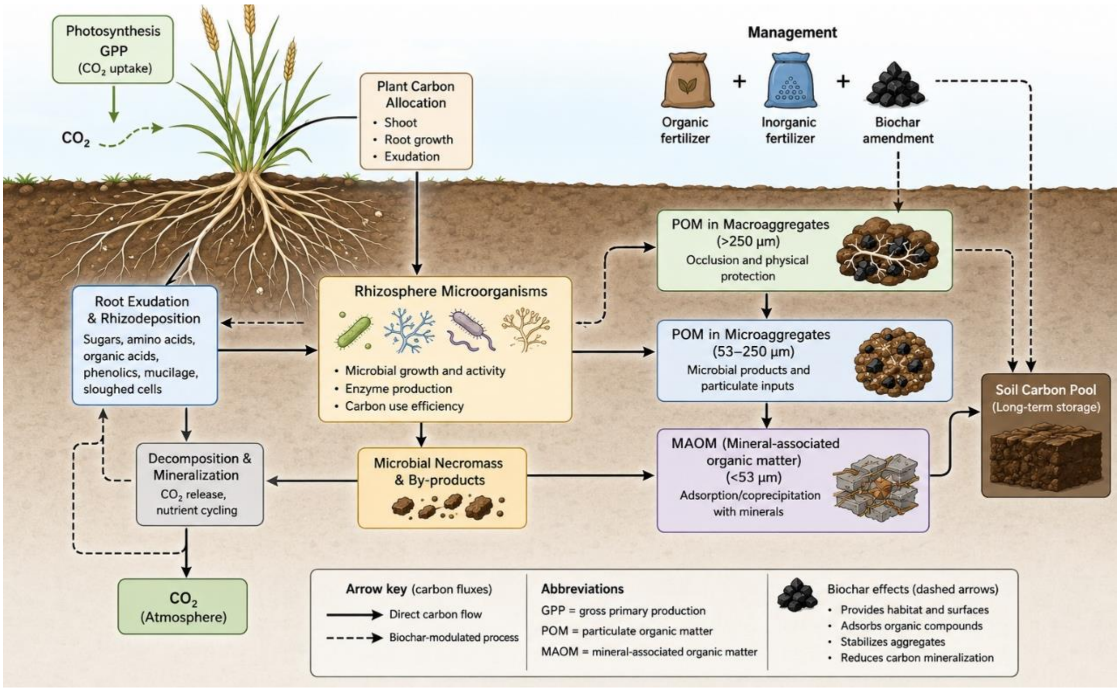

Fig 3: Mechanistic Model of Carbon Flow in Triticum aestivum Rhizosphere Under Long-Term Fertilization

4.4. Soil Organic Matter Turnover in the Wheat Rhizosphere

The rates of turnover of organic matter in the rhizosphere of wheat are much faster than in bulk soil, partly due to increased microbial activity as well as root induced mixing of soil and a constant supply of easily available carbon sources (Kuzyakov *et al.*, 2000; Shewry & Hey, 2015) [22, 3]. The use of 13C isotopes in pulse-labelling studies has shown that the newly fixed carbon from photosynthesis reaches the rhizosphere within hours and is then rapidly assimilated by microorganisms. Tracer amounts of carbon can be measured in the microbial biomass, phospholipid fatty acid (PLFA) fractions, and soil aggregates within 24-72 h of the label being applied (Finzi *et al.*, 2015) [21]. The distribution of newly fixed carbon to processes in the rhizosphere is dynamically regulated by the nitrogen status of the plants; nitrogen deficient wheat plants allocate a higher percentage of newly fixed carbon to below ground processes through increased exudation, which may contribute to positive priming and lower net soil organic carbon (SOC) accumulation (Shewry & Hey, 2015) [3].

4.5. Rhizosphere Priming Effects and Carbon Cycling

The magnitude and direction of RPE in wheat are determined by several factors including the history of the soil, fertilisation practices, and the composition of root exudates (Kuzyakov *et al.*, 2000) [22]. A meta-analysis shows that RPE generally increases SOM mineralisation between 59% and -50% (negative priming) to +380% (positive priming) compared with unplanted soils (Finzi *et al.*, 2015) [21]. Long term additions of organic matter, either as compost or manure, and biochars have been shown to reduce positive priming through either the protection of SOM in aggregates or through the promotion of microbial strategies to acquire nutrients that rely less on SOM mineralisation. Consequently, regulating RPE is key to developing fertilisation strategies that promote wheat productivity and maintain soil carbon stocks (Kuzyakov *et al.*, 2000) [22].

5. Long-Term Organic–Inorganic Fertilization and Soil Responses

5.1. Principles of Integrated Fertilization Systems

Integrated soil fertility management (ISFM) is an intellectual and implementation paradigm that integrates mineral fertilizer with various organic sources (including composts, manures, green manures, crop residues, and biochars) for maximising delivery of nutrients used efficiently (nutrient-use efficiency) and maintaining/improving the quality of the soil (Vanlauwe *et al.*, 2011) [12]. The basis for integrating mineral and organic sources is because the different properties of organic and inorganic materials complement each other; mineral fertiliser supplies immediately available nutrients (to plants) while inorganic fertiliser is able to provide a relatively constant supply of nutrients to soil over time through the process of mineralisation, plus contributing to soil organic matter (SOM) build-up, plus contributing to a variety of different carbon sources that stimulate different types of biological activity in soil (Powlson *et al.*, 2014) [5]. Studies conducted over long periods (i.e. >10 years) demonstrate that normally combined application of organic–inorganic fertilisers results in: greater stability of yield, increased build-up of SOM, and greater maintenance of soil microbiota, compared to either inorganic or organic fertiliser used alone (Meng *et al.*, 2019) [4].

Table 3: Effects of long-term organic–inorganic fertilization treatments on soil physicochemical properties, soil organic carbon (SOC), and microbial biomass. NPK = nitrogen–phosphorus–potassium; OM = organic matter; SOC = soil organic carbon. Effects categorised as qualitative summaries of published meta-analyses.

Treatment Component	Primary Inputs	Nutrient Release Rate	Effect on SOC	Effect on Microbial Biomass	Long-term Soil Quality Impact
Inorganic NPK only	Urea, DAP, KCl	Immediate	Neutral–Negative	Variable	Decline without OM inputs
Organic manure only	Farmyard manure, compost	Slow–Moderate	Positive	Strong positive	Moderate improvement
NPK + Organic manure	Combined inputs	Immediate + delayed	Strong positive	Strong positive	Sustained improvement
NPK + Crop residues	Straw incorporation	Slow (C:N dependent)	Positive	Positive	Moderate–high improvement
NPK + Compost	Composted organic matter	Slow–moderate	Positive	Positive	High improvement
NPK + Manure + Biochar	All integrated inputs	Immediate + long-term	Very strong positive	Positive (community shift)	Maximum improvement

5.2. Components of Long-Term Nutrient Management

Urea, ammonium nitrate, diammonium phosphate, and muriate of potash are the main fertilizers that provide the nutrients needed for higher wheat productivity worldwide. The major benefit of using these fertilizers is that they have accurate nutrient ratios and they are readily available to plants. However, if used alone over many seasons, these fertilizers reduce soil organic matter by stimulating the process of mineralization (priming) by microbes and cause the soil to become acidic (e.g., if using sources of nitrogen from ammonia), which causes the breakdown of the aggregate structure formed by association of organic and mineral soil particles. Organic manures, such as farmyard manure, slurry, chicken litter, or sewage waste, provide not only all of the nutrients directly needed for plant growth but also a plentiful supply of carbon. Because of the wide variation in the composition and nutrient concentration of organic manures, careful management is required to prevent leaching of nitrate or saturation with phosphorus. Integrating the use of straw into the soil as a source of organic matter provides a low-cost strategy for introducing organic inputs in wheat production systems. Most wheat systems produce between 3 and 6 tons of straw per hectare each season, and the high ratio of carbon to nitrogen in straw (60 to 80 to 1) causes microorganisms to initially immobilize nitrogen and reduce the amount of nitrogen that is available to plants; however, when straw is incorporated into the soil, long-term improvements in soil organic matter content and aggregate stability can occur. Also, by incorporating biochar into a fertilization program that is being used in conjunction with manure and residue, biochar will introduce a carbon fraction that is qualitatively and quantitatively different from the more labile carbon that comes from manure and residue because biochar contains highly aromatic and slowly mineralizing compounds (Spokas, 2010) [17]. Thus, through the use of biochar, a time continuum of organic inputs will be established that varies from several weeks to several hundred years.

5.3. Synergistic Effects of Organic and Inorganic Nutrient Inputs

Integrated fertilization systems rely on a synergistic relationship between organic and inorganic input sources that is expressed through numerous interaction pathways. In the case of mineral fertilizers, they help speed up the mineralisation of the organic input source by relieving the limitation of microbial nitrogen and thus leading to an increase in the quantity of plant-available nitrogen and phosphorus; stimulating both plant growth and root exudation, which ultimately increases priming effects in the rhizosphere; and accelerating the cycling of soil organic matter. Additionally, organic inputs modify both the chemical and physical environment where mineral nutrients function, such as buffering soil pH, increasing cation exchange capacity (CEC), thereby minimising the leaching losses of cationic nutrients; enhancing soil moisture content; and stimulating the activity of both phosphorus-solubilising and nitrogen-fixing microorganisms. When discussing the biochar additive, synergies exist as it aids in both the retention of mineralised nitrogen (via adsorption and reduced leaching), provides habitat for beneficial microorganisms, and serves to physically encapsulate organic substrate materials in its pore space—all serving to enhance the efficiency through which plant roots access nutrients (Zhu *et al*., 2017) [19].

5.4. Long-Term Ecological Impacts on Soil Quality

Evidence from 4 long-term field experiments that last between 15 and 40 years have consistently shown that using an integrated organic-inorganic fertilization approach leads to better soil quality outcomes than when using only organic or inorganic fertilizer (Meng *et al*., 2019) [4]. The Rothamsted Long-Term Experiment (UK), Morrow Plots (USA), Broadbalk Experiment, and Chinese National Long-Term Fertilization Experiment Network provide strong supporting evidence that by combining both organic and inorganic inputs, you will sustain and potentially increase SOC, provide for higher (more) MBC in the soil, and maintain macro-aggregate stability over many decades (Six *et al*., 2004) [6]. Such positive effects are also associated with increased soil biological diversity as shown using metagenomic and PLFA techniques, and with a higher degree of functional stability of the soil microbial community to environmental disturbances (Trivedi *et al*., 2017) [15].

6. Microbial Carbon Dynamics and Functional Ecology

6.1. Soil Microbial Biomass and Carbon Cycling Processes

The total mass of living microbial cells present in a given volume of wet soil has been established as the definition of microbial biomass in soil science, although it is only about 1-5% of total organic carbon (TOC) in soil. Microbial biomass carbon (MBC), measured by chloroform fumigation-extraction (CFE), is widely recognized as an accurate indicator of biological activity in soil; MBC is also closely related to nitrogen and phosphorus cycling rates, activity of soil enzymes and soil aggregate stability (Nannipieri *et al*., 2018) [27]. Under wheat production with long-term applications of organic amendments, MBC typically ranges from 200-600 mg C/kg; while MBC typically ranges from 100-250 mg C/kg under completely chemically-fertilized or untreated controls (Six *et al*., 2004) [6].

Table 4: Major microbial groups associated with soil carbon transformation in Triticum aestivum production systems. PLFA = phospholipid fatty acid; AMF = arbuscular mycorrhizal fungi. Responses to biochar amendment are qualitative syntheses from published studies.

Microbial Group	Dominant Taxa	Primary Carbon Function	Biomass Indicator	Response to Biochar
Bacteria	Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes	C mineralisation, N cycling	Bacterial PLFA markers (i15:0, i16:0, i17:0)	Variable; generally positive at low doses
Fungi	Ascomycota, Basidiomycota, Glomeromycota (AMF)	Aggregate formation, decomposition	Fungal PLFA (18:2ω6,9), ergosterol	Positive; hyphal stimulation
Archaea	Thaumarchaeota, Euryarchaeota	Nitrification, methane cycling	Archaeal lipid biomarkers	Neutral–positive
Actinomycetes	Streptomyces, Micromonospora spp.	Recalcitrant C decomposition	10Me16:0, 10Me18:0 PLFA	Positive
Protozoa	Ciliates, flagellates, amoebae	Bacterial grazing, N mineralisation	Biomass counts	Variable
Nematodes	Bacterivorous, fungivorous	Trophic regulation, nutrient release	Nematode density	Neutral

6.2. Microbial Carbon-Use Efficiency

The term microbial carbon-use efficiency (CUE) refers to the ratio of assimilation of carbon into microbial biomass (anabolic) to the release of carbon dioxide via respiration (catabolic). CUE is a key parameter that determines the net impact of microbial activity on the accumulation of soil organic carbon (SOC) (Liang *et al*., 2017) [23]. Under optimal conditions of nutrient supply, the potential maximum CUE for the metabolism of glucose is about 0.6–0.7. However, CUE values of 0.3–0.6 are typically measured in agricultural soil systems and represent the energy required by microorganisms to obtain carbon and energy substrates, maintain the cell, and regulate osmotic potential (Manzoni *et al*., 2012) [24]. The nutrient stoichiometry is a major factor controlling CUE—when nitrogen/phosphorus are limiting to microbial growth, the rate of carbon substrate consumption is elevated to obtain nitrogen and/or phosphorus, resulting in greater CO2 losses from microbes compared to biomass production. According to multiple studies, biochar can improve microbial CUE due to increased nutrient retaining capacity and the addition of structurally complex organic substrates that stimulate the production of and/or activity of enzymes involved with carbon assimilation (Lehmann *et al*., 2011) [10].

6.3. Microbial Necromass Contribution to Stable Carbon Pools

The structural remains of dead microorganisms, including peptidoglycan (bacteria), chitin (fungi), and melanin (fungi) collectively known as microbial necromass, represent a significant pathway for the development of long-term soil organic carbon (SOC) (Manzoni *et al*., 2012) [24]. Amino sugars (including muramic acid, a bacterial biomarker, and glucosamine, a fungal biomarker) are used as chemical indicators of microbial necromass contained in SOC and to estimate how much each type of residue contributes to SOC. Recent meta-analyses indicate that 40–60% of the carbon in SOC from cultivated soils comes from microbial necromass, an increase from previous estimates based on measuring the biomass of living microorganisms (Liang *et al*., 2017) [23]. Fungi produce more additional recalcitrant compounds (due to their high chitin and melanin content) than do bacteria, and because of this they tend to bond with minerals to form organo-mineral bonds more readily than do bacteria.

6.4. Functional Microbial Groups and Microbial Regulation of SOC Turnover

Soil microbial community functional diversity (which includes copiotrophs [fast-growing, nutrient-rich taxa]; oligotrophs [slow-growing, nutrient-poor taxa]; and various specialist guilds) influences how much SOC accumulates/gets mineralised and how quickly that happens (Trivedi *et al*., 2017) [15]. Copiotrophs (specifically from the Proteobacteria and Bacteroidetes phyla) respond quickly to the addition of labile C, causing them to drive the rapid cycling of C; on the other hand, oligotrophs (e.g., Acidobacteria & Planctomycetes) metabolise recalcitrant substrates at lower rates than copiotrophs, and they may be the most abundant taxa in soil systems with limited (low) C availability (Philippot *et al*., 2013) [25]. Biochar addition consistently generates an increased abundance of fungal-dominated microbial communities with a greater proportion of fungal vs. bacterial biomass compared to untreated soils, which leads to higher rates of microbial CUE, greater formation of soil aggregates, and lower rates of SOC mineralisation (Lehmann *et al*., 2011) [10].

7. Biochar Effects on Soil Microbial Communities

7.1. Influence of Biochar on Bacterial and Fungal Diversity

Biochar application to soil can change the composition and diversity of the microbial community via both direct and indirect pathways at different spatial scales (Zhu *et al*., 2017) [19]. Direct effects of biochar on bacterial community composition include providing a carbon source to bacteria growing in the pore space of biochar, as well as modulating the pH and nutrient availability of the soil, and also through adsorption or sequestration of potentially growth-inhibiting substances (e.g., phenols, heavy metals) (Jaafar *et al*., 2015) [26]. The indirect effects of biochar on bacterial diversity can occur through the physical (e.g., improved aeration, increased water holding capacity, increased aggregate stability) and chemical (e.g., modulating pH, nutrients) alteration of the physical structure of the soil that biochar creates for bacterial communities (Zhu *et al*., 2017) [19]. In a meta-analysis of high-throughput 16S rRNA gene amplicon sequencing and ITS fungal community profile sequencing from agricultural soils, there were significant increases in alpha diversity values (Shannon, Chao1) with increasing application rates of biochar, particularly when applied at rates of 10-40 t ha-1 (Philippot *et al*., 2013) [25].

Bacterial taxa that are enriched under biochar amendment generally belong to three major phyla: Actinobacteria (particularly Genera Streptomyces and their relatives that are capable of degrading aromatic carbon), Firmicutes (which are primarily composed of spore forming organisms and are able to withstand desiccation under the pore space of biochar), and Proteobacteria that are involved in the nitrogen cycle (Zhu *et al*., 2017) [19]. The Fungal community composition in the soil is also affected by the application of biochar. Typically, the fungal community composition changes in favour of taxa with which they can form mycorrhizal associations (e.g., Glomeromycota) when they are applied to soils with a higher pH and more conducive to hyphal growth than soils that are low in pH. In addition to improving bacterial diversity, biochar application will directly improve the aggregate stability of soil as described in the next sections.

7.2. Biochar as a Microbial Habitat

The pores in biochar create an environment that serves as a home for bacteria (compared with mineral aggregates), because bacteria are relatively large compared with other soil organisms. For example, the diameter of the biochar micropores (1-10 pm) is within the size range of bacterial cells. The biochar micropores serve two purposes. First, they act as a refuge for bacteria by providing a sheltered place to avoid being eaten (by protozoa and/or nematodes). Second, they trap water and nutrients, thus supplying a more suitable environment than potential competitors. Furthermore, the biochar micropores create special chemical environments that are physically separated from other parts of the soil. Confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) have recently documented that bacteria colonizing biochar pore surfaces will develop structure biofilms in pore channels. Additionally, specific taxa preferentially colonize specific functional groups of surface functional groups on biochar (Jaafar *et al*., 2015) [26]. The habitat created by other soil microorganisms is one of the major ways biochar contributes to an increased microbial biomass in soils subjected to drought or chemical stressors.

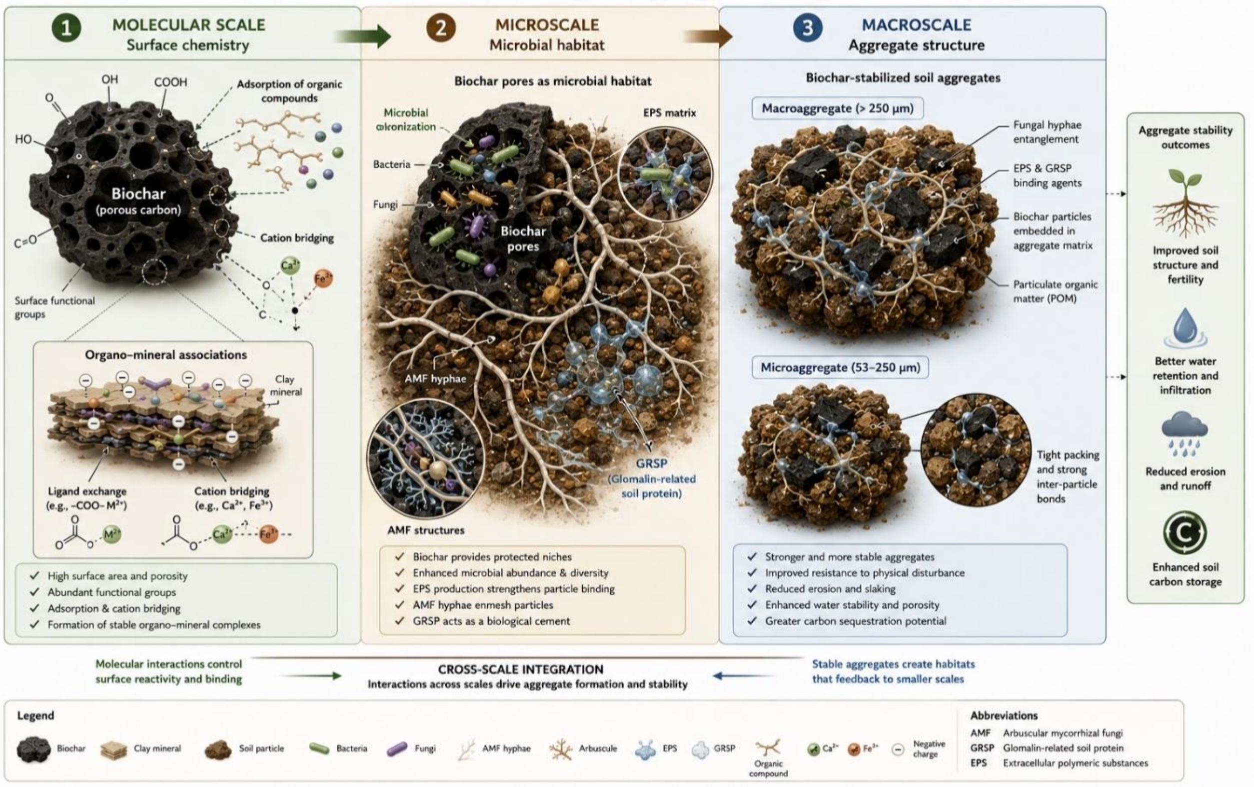

Fig 1: biochar induced mechanisms affecting soil aggregate stability across multiple spatial scales

7.3. Microbial Community Succession Under Long-Term Amendment

When adding biochar to soil, the ongoing changes to the microbial community can be predicted based on the early stages of each new addition of this nutrient-rich material; During the period immediately following the addition of a fresh supply of biochar (1 - 3 years), there will be an increase in the activity of soil microbes (increased respiration) and the increased production of enzymes involved in nutrient cycling, as well as more numerous populations of fast-growing bacteria, due to the available labile carbon found within the added biochar. Beginning in years 3 through 10, there will be a shift away from bacteria to more diverse and functionally stable communities dominated by fungi, increased glomalin production, and more stable soil aggregate structures. This temporal succession of the microbial community in biochar amended soils has significant implications for the timing and duration of biochar applications in long-term agroecosystems.

7.4. Soil Enzyme Activities Under Biochar Amendment

Soil exoenzymes (i.e., secreted or released enzymes that act on the surface of soil particles or aggregates) can act as indicators of the microbial community's ability (via their enzyme activities) to cycle carbon and nitrogen throughout the soil environment (Nannipieri *et al*., 2018) [27]. Some of the target enzymes for carbon cycling include, but are not limited to, β-glucosidase, which hydrolyzes cellobiose (a disaccharide) to produce glucose, cellulase, which breaks down crystalline cellulose, and dehydrogenase (an oxidoreductase that reflects the total oxidative activities of all microorganisms in the soil). In terms of nitrogen cycling, urease hydrolyzes urea to produce ammonia. The production of these enzymes is the result of various bacterial and fungal taxa that produce predominantly all exoenzymes and synthesise these enzymes via association with soil mineral surfaces and humic substances (Sinsabaugh *et al.*, 2009) [28].

Table 5: Soil enzyme activities involved in microbial carbon and nutrient cycling, with reported responses to biochar amendment in Triticum aestivum production systems. Response values represent percentage changes relative to unamended controls, synthesised from published meta-analyses and experimental studies. SOM = soil organic matter; TTC = triphenyltetrazolium chloride.

Enzyme	Substrate	Product	Biological Function	Response to Biochar	Key Controlling Factors
β-glucosidase	Cellobiose, glucosides	Glucose	Labile C release from cellulose degradation	+15 to +45%	Temperature, SOM, pH
Dehydrogenase	2,3,5-triphenyltetrazolium chloride (TTC)	Triphenylformazan (TPF)	Overall microbial oxidative activity indicator	+10 to +35%	Anaerobic zones, microbial biomass
Cellulase (CMCase)	Carboxymethylcellulose	Reducing sugars	Cellulose chain hydrolysis	+5 to +30%	C:N ratio, moisture
Urease	Urea	NH3 + CO2	N mineralisation from urea	-5 to +25%	N availability, pH, inhibitors
Phosphatase	Phosphomonoesters	Inorganic phosphate	P mineralisation from organic P	+10 to +40%	P availability, pH
Leucine aminopeptidase	Leucine-p-nitroanilide	Leucine + p-nitroaniline	N cycling from peptides	Variable	N availability, microbial community

7.5. Microbial Network Interactions and Ecological Stability

A comprehensive analysis of microbial communities via microbial ecological network analysis (applying co-occurrence networks to high-throughput sequencing data) can provide valuable information about the ecological complexity of the interactions between microbes and their impact on the overall functional stability of a community (Philippot *et al.*, 2013) [25]. The use of biochar in amending soils has been shown to consistently enhance network complexity in microbial communities from agricultural soils (increased node connectivity and modularity) indicating the presence of an ecologically stable and functionally redundant microbial network in biochar amended soils (Zhu *et al.*, 2017) [19]. Increased modularity of the microbial networks indicates that microbial communities are capable of distributing their functional capabilities over numerous subsystems that are only partly dependent on one another, thereby offering a degree of resistance to functional loss as a result of taxonomic perturbations. The benefits derived from having a complex microbial network at the level of network are further enhanced with long-term use of organic and inorganic fertilizers, where high substrate diversity and quality are maintained (Jaafar *et al.*, 2015) [26].

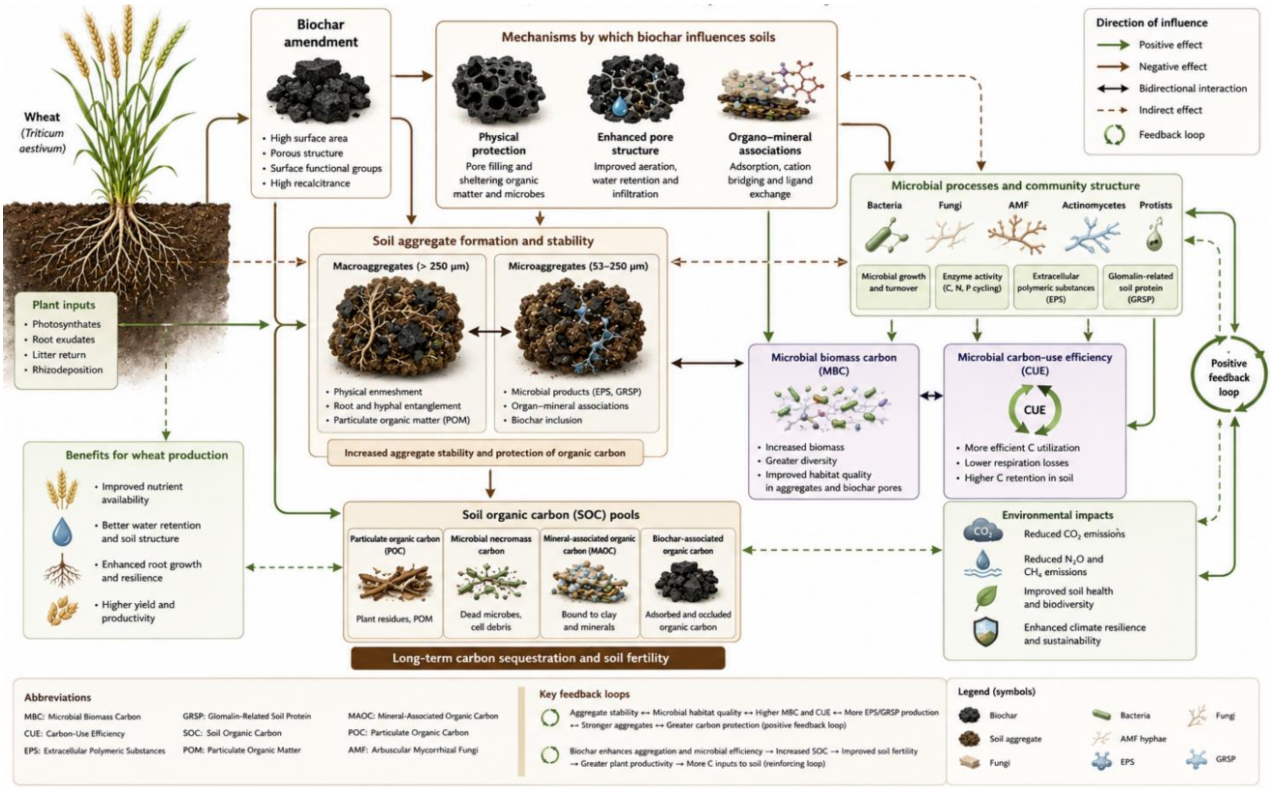

Fig 2: Flowchart of Interactions Among Biochar, Soil Aggregates, and Microbial Carbon Dynamics

8. Interactions Among Aggregate Stability, Microbial Carbon Dynamics, and Crop Productivity

8.1. Aggregate-Mediated Carbon Protection Mechanisms

Effective long-term storage of soil organic carbon (SOC) is achieved through different mechanisms and one major type of long-term storage is due to the physical protection of organic carbon inside soil aggregates (Cotrufo *et al*., 2013) [16]. The physical protection provided by large soil aggregates (macroaggregates) takes place when the decomposer microbial community cannot access the organic materials in the macroaggregates because of the tortuosity of the aggregate's pore spaces and the physical barrier created by the soil that surrounds the aggregate. When macroaggregates are broken apart by tillage, the organic carbon in those macroaggregates that was protected from the microbial community (occluded particulate organic matter or oPOM) is released to the bulk soil, thus making it available for use by the microbial decomposer community. These changes can be measured through density fractionation methods which allow for the determination of the transition of oPOM being released and turned into free particulate organic matter (fPOM) (Tisdall & Oades, 1982) [13]. In contrast to the macroaggregates, microaggregates have a greater capacity to provide physical protection to the organic carbon found in these aggregates since the organic carbon found in microaggregates has a longer residence time due to the additional physical stability associated with organo-mineral bonding to mineral surfaces (Schmidt *et al*., 2011) [7].

The presence of biochar added to the overall soil environment can affect the physical protection afforded to the organic carbon in aggregates via several different mechanisms (Liu *et al*., 2013) [11]. For example, biochar can physically sorb labile organic materials (root exudates, microbial products, and hydrolytic enzyme products) which reduces their availability to microorganisms and subsequently slows the rate of mineralisation for carbon in aggregate microenvironments. Furthermore, due to their shape and size, many biochar particles can serve as nucleation sites for new microaggregates to develop; this occurs when colloidal-sized biochar particles (generated by weathering and fragmentation) become part of a new organo-mineral complex through electrostatic and van der Waals forces of attraction (Spokas, 2010) [17].

Table 6: Comparative effects of long-term fertilization treatments on soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), fungal:bacterial biomass ratio, metabolic quotient (qCO$_2$), and water-stable aggregate content in Triticum aestivum systems. Values represent means ± standard errors from representative long-term experiments. NPK = nitrogen–phosphorus–potassium.

Treatment	MBC (mg kg^{-1})	MBN (mg kg^{-1})	Fungal:Bacterial Ratio	qCO$_2$ (μg CO$_2$-C mg^{-1} MBC h^{-1})	Water-Stable Aggregates (%)
Control (no fertilizer)	145 ± 22	18 ± 3	0.32	4.2 ± 0.6	38 ± 4
NPK only	198 ± 31	24 ± 4	0.28	4.8 ± 0.5	42 ± 5
Organic manure only	312 ± 45	40 ± 6	0.48	3.1 ± 0.4	55 ± 6
NPK + Organic manure	385 ± 52	49 ± 7	0.52	2.9 ± 0.3	62 ± 7
NPK + Biochar (20 t/ha)	356 ± 48	43 ± 6	0.61	2.6 ± 0.3	65 ± 6
NPK + Manure + Biochar	428 ± 61	55 ± 8	0.72	2.2 ± 0.2	74 ± 8

8.2. Effects of Aggregate Stability on Microbial Habitats

The soil aggregate structure is responsible for the organization of microbial habitats in the soil and therefore the distance between organic substrates, mineral surfaces, oxygen and water and predators from microbial cells was determined by the aggregate structure (Le Bissonnais, 1996) [14]. Microbial communities within intact macroaggregates are set apart by physical separation into different microhabitats which has resulted in steep gradients in resource availability and redox conditions which allows high microbial diversity as a result of niche differentiation (Trivedi *et al*., 2017) [15]. There are anaerobic zones within the soil aggregates that support a large number of different types of anaerobic bacteria including fermenters, sulphate reducers and denitrifiers, while there are aerobic zones on the surfaces of the soil aggregates that support active decomposer communities (Schmidt *et al*., 2011) [7]. Consequently, improvements in aggregate stability induced by biochar will result in stabilised microbial microhabitats which will maintain the spatial complexity on which functionally diverse microbial populations rely.

8.3. Coupling Between Microbial Activity and Aggregate Dynamics

An important aspect of aggregate dynamics is microbial activity, and vice-versa; aggregate dynamics can influence microbial activity (Nannipieri *et al*., 2018) [27]. The production of polysaccharides, GRSP and fungal hyphae by microorganisms provides stability for aggregates. At the same time, healthy aggregate nuclei consist of organic matter organisms use for food. Thus, there is a dynamic equilibrium between microbial activities as both the influence and the result of aggregate stability. The addition of biochar to soil shifts this dynamic equilibrium to a position of greater aggregate stability as a result of biochar's ability to enhance the production of fungal biomass (i.e., AMF). AMF produce glomalin, which has a positive relationship with aggregate stability (Jaafar *et al*., 2015) [26]. Evidence from long-term studies of biochar application to soils indicates that macroaggregate stability in biochar treated plots will continue to increase through the development and accumulation of binding agents derived from the fungal community and the increasing incorporation of organic matter stabilized by biochar into aggregate nuclei.

8.4. Effects on Wheat Productivity, Root Growth, and Nutrient Use Efficiency

Combined physiological pathways are an indirect effect of improvement in soil structure through biochar application, and the induced changes within the associated microbial community to support improved wheat production (Shewry & Hey, 2015) [3]. The two outcomes of improved aggregate stability, aeration, and water retention, reduce the root mechanical impedance, and water deficit stress during critical periods of development (tillering, jointing, and grain filling). Increased populations of beneficial microorganisms in the rhizosphere (including phosphate solubilizing bacteria, nitrogen fixing organisms, and plant growth promoting rhizobacteria) increase the availability of essential nutrients in forms that are accessible to the wheat root system (Bengough *et al*., 2011) [20]. Overall meta-analyses of the impact of biochar on wheat yield, have consistently shown positive effects, but with large variability across soil types, biochar sources, and application rates. Mean wheat yield increases have been reported to range between 10 - 30%, when combined with both organic and inorganic fertilization methods (Jeffery *et al*., 2011) [8].

In biochar integrated fertilization systems, both nitrogen use efficiency (NUE) and phosphorus use efficiency (PUE) are improved significantly due to, primarily, the reduction of nutrient leaching (due to biochar's ability to adsorb nutrients), increased rates of microbial-mediated nutrient cycling, and improved root access to nutrient dense aggregate microenvironments (Liu *et al*., 2013) [11]. The direct economic and environmental impacts of improved NUE, due to the reduced cost of fertilizer inputs, are considered to provide both economic and environmental advantages, by preventing nitrates from leaching into ground water, as well as, reducing N2O emissions into the atmosphere; therefore, these benefits play a key role in the sustainability of wheat production systems (Vanlauwe *et al*., 2011) [12].

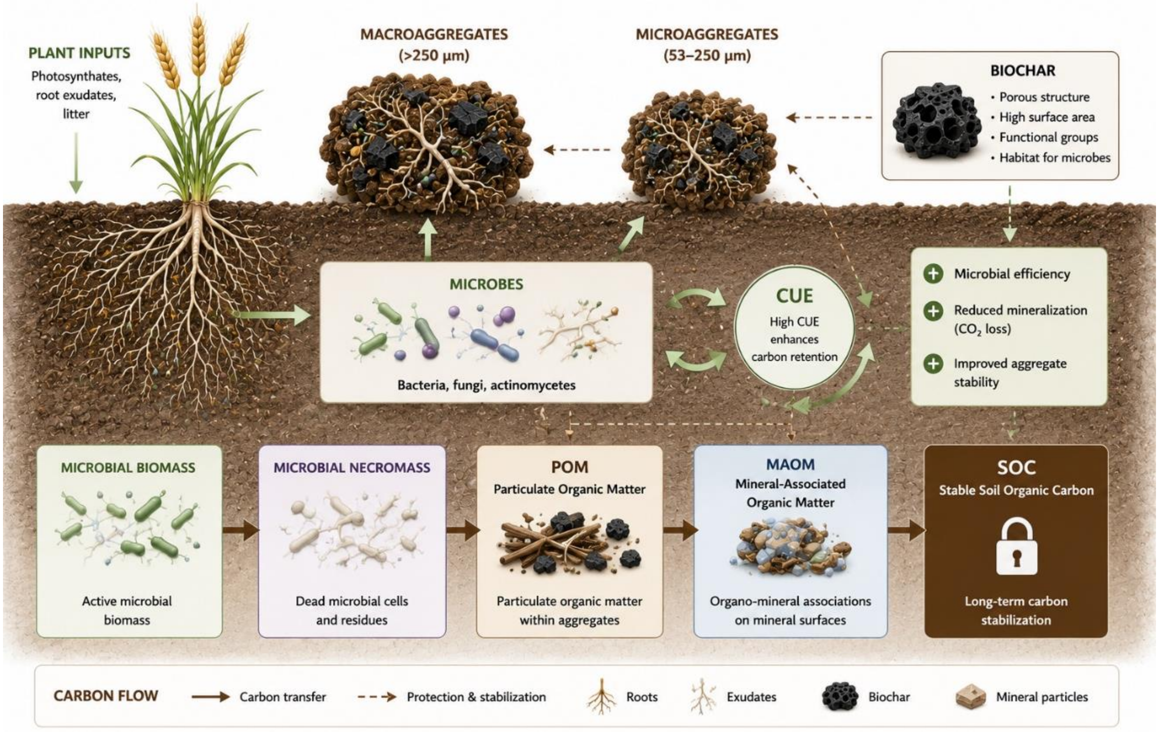

Fig 4: Integrated Framework of Microbial-Mediated Carbon Stabilisation Within Aggregate Fractions

9. Emerging Technologies and Future Perspectives

9.1. Multi-Omics Approaches for Mechanistic Investigation

Through advances in high-throughput sequencing and analytical chemistry, scientists can now gain a level of detail that is unprecedented with regards to soil microbial communities and their functional gene gene pools in biochar amended agricultural systems (Philippot *et al*., 2013) [25]. Using shotgun metagenomics (sequencing all of the assembled genomic DNA from all of the soil microbiome) provides a simultaneous profile of both the taxonomic composition as well as the amount of functional genes associated with carbon cycling (e.g., glycoside hydrolases, carbon monoxide dehydrogenases, and methane monooxygenases) from soil without prior knowledge of the identity of the community (Fierer, 2017) [29]. Metatranscriptomics (sequencing all total microbial mRNA) provides an additional, complementary picture of the expressed functional capacity of the microbiome under specific soil conditions, allowing for the differentiation between the potential and realised functional capacity of the microbiome (Preece & Peñuelas, 2016) [30].

The ability to identify the types of microbes that incorporate different types of carbon into their biomass has been developed through the technique of stable isotope probing (SIP), such as the use of 13C-SIP together with metagenomics or PLFA analysis applied under field conditions (Fierer, 2017) [29]. Results from SIP studies in regard to the microbial community on biochar found that the initial colonising microbial community is to a large extent composed of copiotrophic groups of Proteobacteria (which possess high metabolic flexibility), and that the later successional communities are composed of more specialist groups that are better adapted to the aromatic composition of weathered surfaces of biochar. The use of synchrotron based micro-computed tomography (microCT) and nano-SIMS (nanoscale secondary ion mass spectrometry) have provided complementary imaging techniques for visualising the spatial arrangement of biochar particles; the spatial arrangement of microbial cells; and the carbon isotopic signatures within structurally intact aggregate structures, all at nanometre resolution (Preece & Peñuelas, 2016) [30].

9.2. Advanced Statistical Approaches for Integrating Multi-Source Data

The interactions of soil biochar, aggregate, and microbiome are so complex that the use of multivariate and systems-level statistical approaches integrating multiple measurement domains is necessary. Principal component analysis basically descends into dimensionality reduction of high-dimensional datasets of physicochemical and microbial characteristics and thereby permits visual identification of the primary axes of variation between treatment groups. RDA serves as a constrained ordination technique that partitions variance in microbial community composition among explanatory environmental variables (e.g., biochar dose, SOC content, aggregate stability) and gives quantitative estimates on the relative importance of each driver. By far the most powerful analytical framework currently available for dissecting pathways of direct and indirect effect in complex systems with multiple variables, structural equation modelling could be used to test this hypothesis that biochar did not affect wheat yield directly, but did so through effects on stability and microbial biomass carbon (Grace, 2006) [31].

Using co-occurrence network analysis on microbiome sequencing data, the relationships between different species in the soil can be studied and ecological principles like keystone taxa, hubs, and modularity determined (Philippot *et al*., 2013) [25]. In addition, machine learning algorithms, especially random forest and gradient boosting, have been used to accurately predict the effects of biochar on soil properties based on the physical and chemical characteristics of the biochar and the type of soil, with strong predictors of aggregate stability being found to be pH and surface area (Fierer, 2017) [29].

9.3. Current Limitations in Long-Term Biochar Studies

Research on biochar-soil-crop systems has progressed quite far in terms of advancing our understanding of their relationships; however, there are still some considerable limitations found long-term field studies that focus upon these systems due to methodological, logistical, and other related issues (Cayuela *et al*., 2014) [33]. Most biochar research has used short-term (<3 year) incubation studies conducted under laboratory or greenhouse conditions that do not adequately account for temporal dynamics like biochar weathering, microbial community succession, and aggregate development within real field environments (Jeffery *et al*., 2011) [8]. Moreover, the variety of biochars, application quantities, and types of soils researched within currently published studies creates extreme difficulty when looking to synthesize findings across multiple independent meta-analyses; therefore, when attempting to compare studies across organizations the context-dependency of biochar effects adds great methodological discomfort to those comparisons (Spokas, 2010) [17].

The standardization of methods for biochar characterization, protocols for biochar application, and frameworks for measuring outcomes resulting from the application of biochar represents an ongoing challenge facing the biochar field (Cayuela *et al*., 2014) [33]. The International Biochar Initiative (IBI) and European Biochar Certificates (EBCs) are partially established frameworks for biochar standardization; however, neither of these standards cover all soil biological endpoints currently known to be of importance in determining the effectiveness of the application of biochar materials. Furthermore, the evaluation of the economic return derived from the application of biochar within smallholder agricultural systems (where there are minimal or no physical means to access biochar production facilities) continues to be inadequately evaluated in conjunction with agronomic assessment (Vanlauwe *et al*., 2011) [12].

9.4. Research Gaps and Future Opportunities

There are several key areas of research that require systematic analysis at present. The first of these being the need for replication of long-term field experiments to determine the mechanistic causes underlying the very large variation in biochar mediated soil aggregate stability across a range of soil types. For example, investigation into the impact of soil type on clay mineralogy, soil pH and initial SOM content respectively, will be needed to better understand biochar-mineral interactions. Next, we yet to have a substantive understanding of the long-term fate of carbon from biochar within all biological, chemical and physical characteristics over time in aggregate fractions. Therefore, the implementation of multi-decadal monitoring programs incorporating isotopes are needed to fill this gap in research. Third and lastly, the identification of the most advantageous strategies employed to co-apply biochar with both organic and inorganic input materials relative to the maximisation of synergistic effects upon soil quality and wheat productivity and minimisation of trade-offs such as the immobilisation of nitrogen as a consequence of the co-application of biochar and inputs providing high C:N input materials; will require the use of systematic agronomic optimisation within a range of cropping environments.

Table 8: Research gaps, methodological limitations, and future recommendations for advancing the scientific understanding of biochar effects on soil aggregate stability and microbial carbon dynamics in Triticum aestivum production systems. CUE = carbon-use efficiency; XRD = X-ray diffraction; SIP = stable isotope probing.

Research Gap	Current Limitation	Recommended Approach	Expected Outcome	Priority Level
Long-term biochar field studies (>10 yr)	Most studies <3 yr; no multi-decadal data	National/international long-term monitoring networks	Quantify biochar aging effects on aggregate stability and SOC	Critical
Biochar-clay mineralogy interactions	Poorly characterised across soil orders	Synchrotron XRD, NanoSIMS in diverse soils	Mechanistic models of biochar-mineral bonding	High
Biochar effects on microbial CUE in situ	Lab-based estimates only; field verification lacking	13C-SIP + metatranscriptomics under field conditions	Accurate field-scale CUE estimates under fertilization	High
Dose-response optimisation in wheat systems	Inconsistent dose ranges and soil contexts	Multi-site dose-response trials with standardised biochars	Evidence-based application rate recommendations	High
Economic feasibility for smallholders	Cost-benefit analyses sparse and region-specific	Integrated techno-economic-agroecological modelling	Policy-relevant feasibility thresholds	Moderate
Interactive effects of biochar + microbiome + variety	Wheat genotype × biochar interactions unexplored	Multi-genotype field experiments + rhizosphere omics	Biochar-responsive wheat variety selection	Moderate

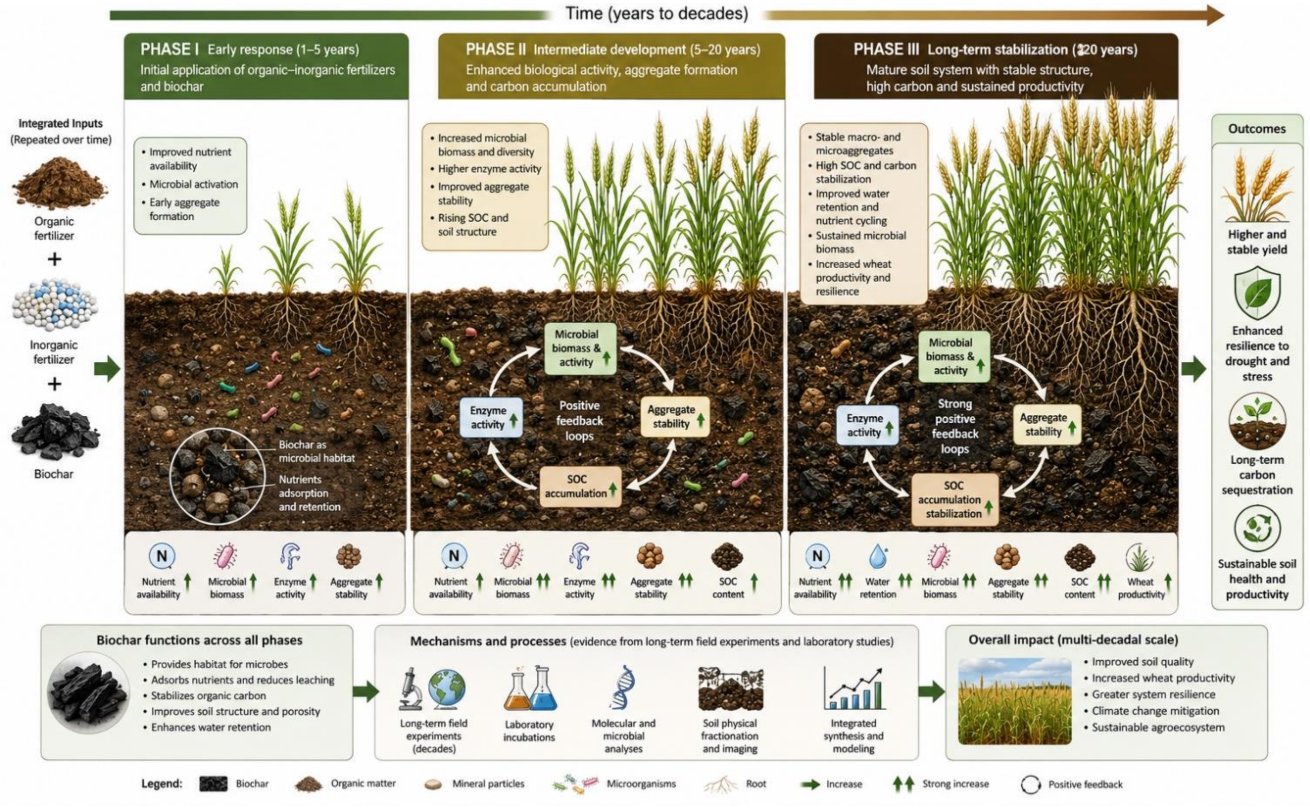

Fig 5: Long-Term Pathway Model: Effects of Organic–Inorganic Fertilization and Biochar on Soil Quality and Wheat Productivity

10. Conclusion

The present study synthesized core scientific evidence on the effect of biochar on soil aggregate stability and microbial carbon dynamics in agricultural systems growing Triticum aestivum (common wheat) in organic–inorganic fertilization systems (not limited to, but inclusive of composting and organic fertilizer). Multiple cumulative conclusions can be found through this approach. The application of biochar has been shown to increase the relative stability of soil macroaggregates and microaggregates, primarily attributed to fungal hyphal stimulation, glomalin production, enhanced organo-mineral associations, and stable carbonaceous nuclei that provide a substrate for aggregate formation. In terms of water-stable aggregates, the percentage differences observed between biochar and non-biochar treated systems ranged from 18% to as high as 32% across multiple studies for varying soil types, application rates, and feedstocks of biochar applied in conjunction with other organic material inputs.

Microbial response to the addition of biochar in wheat systems has shown increased biomass and a greater fungal:bacterial ratio, resulting in an increase in carbon-use efficiency and ecological network complexity. These changes are favourable for the long-term accumulation of carbon through the resilience of functional processes within the soil. In addition, biochar has been shown to act as a habitat for microbes, providing protection from predation and holding nutrients for microbial use within its pore structure, which may represent one of the major mechanistic pathways through which biochar has many of its biological effects.

In addition, the accumulation of microbial necromass may be one of the most under-appreciated pathways to stable SOC formation in biochar amended systems. Amino sugars analyses indicate that microbial residues account for between 40–60% of total SOC in well-managed agricultural soils, and that this fraction should be enhanced following the addition of biochar through the increased levels of fungally-mediated activity.

Since biochar is an organic material with the potential for use as a fertilizer when integrated with various types of organic or synthetic fertilizers, the use of biochar in combinations with both organic manures and inorganic NPK fertilizers produces multiple social, economic and environmental benefits and improves crop yield by increasing the microbial population in soil and the amount of available nutrients through increasing soil aeration and water holding capacity, as well as enhancing nitrogen and phosphorus efficiency. Combining the use of inorganic NPK, organic manure, and biochar (20-40 t ha-1) produces the greatest effect on crop yields because it optimizes the timing and rate of nutrient application, increases the diversity of available microbial substrates, and provides a better physical structure to the soil. Future research should focus on conducting field experiments over periods of several decades to evaluate how the aging of biochar, the development of aggregate stability, and the succession of microbial communities change over time in typical wheat production systems. This requires combining advances in stable isotope tracing with molecular approaches such as metagenomics and metatranscriptomics to help clarify the underlying mechanisms associated with how carbon from biochar is incorporated into very stable pools of soil organic matter, as well as which particular microbial organisms are involved in facilitating these processes. In addition to scientific work, economic and policy analyses are needed to assist scientists in making their research applicable and useful to farmers, extension services, and policymakers interested in implementing biochar-based soil management practices on a wide scale. Overall, based on the information presented here, biochar-based fertilization systems may offer a major advancement in the evolution of sustainable wheat production through the reconciled objectives of high agricultural productivity and long-term stewardship of soil carbon and ecosystem resilience.

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