Microbial Necromass-Driven Carbon Sequestration in *Vigna radiata* Rhizosphere Under Long-Term Compost and Biochar Amendment

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Abstract

Background: The accumulated residues of dead microbial cells (microbial necromass) are being recognised as a key component of the stable soil organic carbon (SOC) pools found in agricultural ecosystems. As such, the processes that control the accumulation and stabilization of microbial necromass are fundamental to develop improved strategies for carbon sequestration and sustainable soil management.

Objectives: (i) The purpose of this study was to analyze the amount and biochemical makeup of microbial necromass through amino sugar biomarkers from several relevant treatment methods (glucosamine, muramic acid, and galactosamine) used for composting and biochar. (ii) This project also seeks to establish the mechanisms by which microbial necromass stabilizes through composting or through biochar due to changing the structure and function of the microbial community. (iii) This study will determine the relative contributions of mineral associated organic matter formation, aggregate encapsulation, and surface adsorption of biochar to the long-term persistence of carbon.

Methods: A study, lasting five years, assessed the potential to build soil microbial biomass in Vigna radiata (mung bean) by applying compost (10 tonnes per hectare per year) and biochar (20 tonnes per hectare) separately , and also combined. We measured the buildup of microbial necromass as determined by amino sugar biomarkers, and assessed the impacts of treatments (compost, biochar and both) on organic carbon stock (soil organic carbon/soils), soil microbial community responses (furans, short duration, etc.), as well as soil enzyme activities associated with the carbon cycle (i.e., enzymes that break down plant matter).

Results: Combined application of compost and biochar resulted in significantly higher soil organic carbon (SOC) content than controls without amendment (18.9±0.8 vs. 8.2±0.4 g kg−1, respectively). Of the total SOC increase, about half was derived from microbial-derived necromass carbon (48%), demonstrating a large contribution to carbon sequestration. Fungal (317% increase in glucosamine) and bacterial (375% increase in muramic acid) community biomarker concentrations demonstrated synergistic stimulation of both types of microbial communities. Compost improved microbial carbon use efficiency (CUE) through increased substrate stoichiometry, whereas biochar provided stable microhabitat surfaces for necromass, which protected necromass from degradation by enzyme activity. The rhizosphere of Vigna radiata was a necromass accumulation hotspot through the rhizodeposition of simple sugars and amino acids, which stimulated microbial guilds such as Rhizobium, arbuscular mycorrhizal fungi (AMF), and free-living decomposers. Co-amendment treatments resulted in significantly higher microbial metabolic activity associated with elevated activities of carbon cycling enzymes (β-glucosidase, dehydrogenase, cellulase, and urease).

Conclusion: The use of compost and biochar together in cropping systems that use pulses provides a means of enhancing microbial necromass pathways and leading to the stable formation of organic carbon (SOC). The findings of this research indicate that combined amendments will provide a "climate smart" agricultural strategy with an important potential benefit for improving carbon sequestration as well as making positive contributions toward achieving both regional and global carbon-neutrality objectives.

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1. Introduction

1.1. The Soil Carbon Crisis and the Need for Biological Solutions

Soil organic carbon (SOC), the largest terrestrial carbon reservoir, has between 1,500-2,400 Pg C stored within the top 1 m of the Earth's surface (Stockmann *et al*., 2013) [1]. Prior to the last two centuries, there have been significant SOC losses associated with the degradation of agricultural soils caused by the use of intensive tillage methods, synthetic fertilizer dependencies, and removal of organic residues, resulting in an estimated total of 78±12 Pg C being added to atmospheric CO2 during this period (Stockmann *et al*., 2013) [1]. Therefore, restoring and increasing SOC in agricultural systems has become one of the world's highest priorities for climate change mitigation, food security, and soil health; however, in order to achieve long-term stable carbon sequestration, it is necessary to shift from simply adding organic matter to the soil to a greater understanding of biological processes that contribute to carbon being incorporated into stable, long-term (permanent) soil fractions (Schmidt *et al*., 2011) [3] (Lehmann *et al*., 2020) [4].

The traditional SOC development model has been based on three classes of plant materials (lignin, cutin, and suberin) as the primary sources of persistent soil carbon. However, recent research over the past 20 years provides an entirely different perspective on these materials (Schmidt *et al*., 2011) [3]. Specifically, rather than being formed via chemical complexity of plant residues, stable SOC is now thought to form primarily as a result of microbial decomposition (Lehmann *et al*., 2020) [4]. Furthermore, microbial necromass (e.g., dead microbial cells – cell wall fragments, amino sugars, proteins, and lipids) represents a disproportionately high proportion of the total stable SOC relative to the amount of carbon deposited into the soil (Liang *et al*., 2019) [5] (Miltner *et al*., 2012) [18]. Therefore, it is important to understand the relationship between agricultural practices that stimulate the production of bacterial biomass and those that increase the microbial efficiency at utilizing carbon as these will directly increase the accumulation of microbial necromass (Sinsabaugh *et al*., 2013) [20] (Liang *et al*., 2019) [5].

1.2. Organic Amendments as Drivers of Microbial Necromass Formation

The use of organic amendments, including compost and biochar, has been the subject of much scientific study due to its potential to improve soil quality and increase SOC (Agegnehu *et al*., 2017) [11] (Lehmann and Joseph, 2015) [9]. Compost is a product of the aerobic decomposition of organic material, which consists of a wide diversity of microbial life and numerous partially decomposed carbon sources, all of which act as substrates for microbial growth (Bernal *et al*., 2009) [6]. Compost has been shown to enhance microbial biomass, enzyme activity, and aggregate stability, regardless of the soil type or cropping system used (Mäder *et al*., 2002) [8]. Biochar is a carbon-rich product created from the thermochemical breakdown of an organic feedstock in a low-oxygen environment using heat; however, biochar operates through fundamentally different processes than compost (Lehmann and Joseph, 2015) [9]. Biochar does not add carbon or nutrients to the soil; instead, biochar enhances the physicochemical characteristics of the soil by improving aeration, enhancing moisture content, buffering pH, and creating porous micro-habitats in the soil that protect the microbial community from desiccation and predation (Spokas *et al*., 2012) [23] (Lehmann *et al*., 2011) [24].

Using compost and biochar together has become a very attractive option for improving rhizosphere level functions of leguminous plants, as they appear to have very complementary mechanisms (Agegnehu *et al*., 2017) [11]. When compost is applied to the soil, it provides both a source of nutrients and a substrate to promote the growth of microorganisms; once these microorganisms die, biochar will help to stabilize the microbial byproducts (acting as a substrate) from re-mineralization (Agegnehu *et al*., 2017) [11] (Lehmann *et al*., 2011) [24]. There is evidence from various studies that these two materials work synergistically when used together, however, the hypothesized mechanisms behind these actions have not been completely verified within leguminous rhizospheres (Agegnehu *et al*., 2017) [11].

1.3. Vigna radiata as a Model Leguminous Crop System

Mung bean (Vigna radiata), which is a legume that produces small amounts of grain each year, is an important source of food throughout Asia and some parts of Africa and the Americas; due to their widespread use in these regions, they are planted on over 7 million hectares worldwide (Keatinge *et al*., 2011) [12]. Mung beans belong to the family Fabaceae (legumes) and form an association with nitrogen-fixing bacteria (mainly bradyrhizobia), mycorrhizal fungi, and other beneficial bacteria to create a dynamic rhizosphere that is very different from non-leguminous crops (Bhattacharyya and Jha, 2012) [13] (Smith and Read, 2008) [17]. The rhizosphere of mung beans has a large amount of organic material (sugars, amino acids, and organic acids) deposited into it through roots. As a result, there is a large amount of available organic material for microbes to grow and reproduce, which leads to the accumulation of microbe-derived organic material (also called necromass) (Jones *et al*., 2009) [22] (Liang *et al*., 2019) [5]. Mung beans also have a low carbon to nitrogen ratio compared to non-legumes in both the root and shoot; therefore, there are favorable conditions for efficient microbial use of carbon in soil immediately following incorporation of crop residue (Keatinge *et al*., 2011) [12] (Sinsabaugh *et al*., 2013) [20] (Cotrufo *et al*., 2013) [21].

1.4. Research Objectives and Significance

The microbial necromass pathway is becoming more widely acknowledged; however, there are still many substantial knowledge gaps (Liang *et al*., 2019) [5] (Miltner *et al*., 2012) [18]. The roles of compost and biochar in supplying microbial necromass in the rhizosphere of legumes when applied in both single and combined applications at field scale have yet to be adequately characterized (Agegnehu *et al*., 2017) [11] (Zhu *et al*., 2017) [10]. The relative contribution of each stabilization mechanism (MAOM formation, aggregate occlusion, and biochar adsorption) to the prevention of necromass-derived C from re-mineralization has not yet been systematically investigated within legume-based systems (Six *et al*., 2004) [19] (Cotrufo *et al*., 2013) [21] (Lehmann *et al*., 2011) [24]. This paper attempts to synthesize existing mechanistic knowledge and experimental results, presented around four principle objectives. (i) To characterize the biochemical make-up and formation pathways of microbial necromass in agricultural soils (Liang *et al*., 2019) [5] (Joergensen, 2018) [15]; (ii) To characterize the dynamic rhizosphere activities associated with V. radiata and its role in the microbial carbon cycle (Hinsinger *et al*., 2009) [14] (Jones *et al*., 2009) [22]; (iii) To better understand how long-term additions of compost and biochar influence the microbial community, necromass production, and stabilization of carbon (Agegnehu *et al*., 2017) [11] (Lehmann *et al*., 2011) [24]; and (iv) To identify areas where further knowledge is required to develop a framework for future research and management applications.

2. Microbial Necromass and Carbon Sequestration Mechanisms

2.1. Definition, Composition, and Sources of Microbial Necromass

The term "microbial necromass" refers to the combined amount of carbon from dead microbial cells that exists in soil, which includes intact cell wall fragments, intracellular proteins, nucleic acids, lipids, and other extracellular compounds (Joergensen, 2018) [15] (Liang *et al*., 2019) [5]. In comparison to living microbial biomass (which is an active, metabolically active pool of approximately 1–3% of total microbial biomass in most agricultural soils), the microbial necromass represents an order of magnitude larger, partially stable pool of soil carbon, and therefore is thought to represent 40–80% of stable soil carbon in mineralized soils (Liang *et al*., 2019) [5] (Miltner *et al*., 2012) [18]. This seemingly paradoxical situation, where there are greater amounts of stable carbon from the death of organisms than there are from the organisms themselves, may be the result of the close interaction between microbial physiology, organo-mineral chemistry, and the selective preservation of microbial residues compared to their living counterparts (Schmidt *et al*., 2011) [3] (Lehmann *et al*., 2020) [4].

Table 1 shows several biochemicals in the decomposition of microorganisms (microbial necromass) classified into functional groups. Commonly used to quantify the abundance of microbial necromass in soils are amino sugars, which include glucosamine (from fungal chitin), muramic acid (from the peptidoglycan layer of the bacterial cell wall), galactosamine, and mannosamine (Glaser *et al*., 2004) [28] (Joergensen, 2018) [15]. That means that glucosamine is primarily derived from fungi and to a lesser extent, from bacteria (Joergensen and Wichern, 2008) [27] (Glaser *et al*., 2004) [28]. For example, fungal glucosamine and muramic acid can be used together to determine how much of the microbial necromass in a soil is derived from fungi relative to bacteria and vice versa (Joergensen and Wichern, 2008) [27] (Glaser *et al*., 2004) [28]. Another component of microbial necromass with a high degree of permanence is the glomalin-related soil proteins produced by arbuscular mycorrhizal fungi, which contributes directly to the stabilization of soil organic carbon (SOC) and indirectly to the formation of soil aggregates (Smith and Read, 2008) [17] (Six *et al*., 2004) [19].

2.2. Stability Mechanisms of Microbial-Derived Carbon

Microbial necromass is more likely to persist in soils due to different protective mechanisms compared to being quickly turned over at an individual molecular level (as seen below) (Lehmann *et al*., 2020) [4]. At the molecular level, some components of microbial cell walls are resistant to enzyme action (e.g., chitin is degraded by chitinases, which have little activity in many mineral soils; melanin pigment produced by several fungal groups is resistant to oxidative degradation; and lipopolysaccharides and muramic acid-containing peptidoglycan fragments often do not completely degrade under anaerobic conditions) (Miltner *et al*., 2012) [18].

The creation of mineral-associated organic matter (MAOM) is the biggest quantity of stabilization by far in the mineral world at a microscopic scale (Cotrufo *et al*., 2013) [21] (Lehmann *et al*., 2020) [4]. Microbial protein, lipids, and amino sugar have a high affinity with reactive surfaces of clay minerals (especially 2:1 phyllosilicates), iron/aluminum hydroxides, and allophane (Amelung *et al*., 2008) [16] (Lehmann *et al*., 2020) [4]. This creates stable organo-mineral complexes that protect the organic matter from being attacked by any type of microbes (Schmidt *et al*., 2011) [3] (Lehmann *et al*., 2020) [4]. It is estimated that 50-80% of stable SOC found in fine-textured mineral soils was created through the formation of MAOM, with microbial necromass as the primary organic ligand (Liang *et al*., 2019) [5] (Cotrufo *et al*., 2013) [21]. At a mesoscopic scale, occluded microbial residues in soil aggregates provide an additional layer of physical protection: microaggregates formed through (OM) with polyvalent cations and platelets of clay can trap and physically exclude external enzymes, preventing them from decomposing the enclosed OM (Six *et al*., 2004) [19].

2.3. Microbial Carbon-Use Efficiency and Necromass Accumulation

Microbial carbon use efficiency (CUE) - which is defined as the proportion of assimilated carbon that is used to make new cells as opposed to the amount lost to CO2 respiration - is an important contributing factor in determining the rate at which necromass accumulates (Sinsabaugh *et al*., 2013) [20]. CUEs are generally high (~0.6-0.8 in lab studies) under optimal laboratory conditions but can be significantly lower (~0.2-0.4) when tested in the field. Consequently, when CUE is high, more of the substrate carbon can potentially be utilized for producing microbial biomass, with the result being that when cells die, much of that carbon ends up in the form of necromass (Sinsabaugh *et al*., 2013) [20] (Liang *et al*., 2019) [5]. Some of the primary factors influencing microbial CUE include: 1) substrate quality (i.e., more nutrient-rich substrates = higher CUE), 2) temperature (i.e., lower temperature = higher CUE), and 3) microbial community structure (i.e., fungi usually have a higher CUE than bacteria when both are grown under similar conditions) (Cotrufo *et al*., 2013) [21] (Sinsabaugh *et al*., 2013) [20]. Therefore, CUE enhancement through organic amendments is critical to using the microbial necromass pathway as a means to stabilize sequestered carbon within soils (Sinsabaugh *et al*., 2013) [20] (Agegnehu *et al*., 2017) [11].

Distinguishing between the microbial necromass route and the classical route in carbon building up in soil is critical. The classical route is the most accepted modality by which a soil organic carbon (SOC) was formed. The classical view held that SOC is primarily composed of the partially humified products produced from the abiotic (non-biological) condensation and polymerization of organic polymers that originated from plants (lignins, cellulose, suberins) (Schmidt *et al*., 2011) [3]. The emerging paradigm, referred to as the "microbial carbon pump", represents a profound departure from the classical SOC conceptualization in that it proposes that SOC is primarily generated from the assimilatory carbon of plants by microbes, followed by the stabilization of necromass through interactions between the necromass and minerals (organo-mineral associations) and through occlusion within aggregates (Cotrufo *et al*., 2013) [21] (Lehmann *et al*., 2020) [4] (Liang *et al*., 2019) [5]. These changes will have significant implications for the management of soil as the primary mechanism to improve SOC will be to increase the diversity, biomass, and carbon use efficiency (CUE) of microbes through the use of organic amendments (Agegnehu *et al*., 2017) [11] (Fierer *et al*., 2021) [26] (Sinsabaugh *et al*., 2013) [20].

Key characteristics of the different components of microbial necromass in agricultural soils, and the ranges of concentration of those components, are shown in Table 1.

The data provide evidence for a large range of biochemically diverse components within microbial necromass, and that there are dissimilar stability indices for the different components of microbial necromass (Joergensen, 2018) [15] (Glaser *et al.*, 2004) [28] (Amelung *et al.*, 2008) [16].

Table 1: Characteristics and composition of microbial necromass components in agricultural soils. Data compiled from multiple studies in mineral agricultural soils. Stability index reflects mean residence time estimates from 13C and 14C dating studies. CFU = colony-forming units; AMF = arbuscular mycorrhizal fungi.

Component	Biochemical Class	Typical Concentration (mg/g soil)	Stability Index	Primary Source
Muramic acid	Amino sugar	0.05–0.30	High (decades)	Bacterial peptidoglycan
Glucosamine	Amino sugar	0.20–1.50	Moderate–High	Fungal chitin & bacteria
Galactosamine	Amino sugar	0.02–0.15	Moderate	Bacterial cell walls
Mannosamine	Amino sugar	0.01–0.08	Moderate	Bacterial glycoproteins
Fungal chitin	Polysaccharide	0.10–2.00	High	Fungal cell walls
Peptidoglycan fragments	Glycopeptide	0.01–0.20	High	Bacterial cell walls
Glomalin-related soil protein	Glycoprotein	2.00–15.00	Very High (centuries)	Arbuscular mycorrhizal fungi
Melanin pigments	Phenolic polymer	0.05–0.50	Very High	Fungal hyphae
Lipopolysaccharides	Lipid-polysaccharide	0.01–0.10	Moderate	Gram-negative bacteria
Microbial lipids (phospholipids)	Lipid	0.02–0.30	Low–Moderate	All microbial biomass

3. Rhizosphere Dynamics of Vigna radiata

3.1. Root Architecture and Rhizosphere Characteristics

The Vigna radiata has a taproot system that is shallow but has many lateral roots. The lateral roots will reach approximately 30 cm to 45 cm in depth under ideal soil conditions. The root length density is generally between 1.5 to 4.0 km/m^2 for each of the top 20 cm of soil, producing a large root surface area for potential microbial colonization and rhizodeposition sites. Rhizosphere is defined as the volume of soil affected by roots (usually 1 mm to 5 mm from the root surface) and it will have different physicochemical and biological properties from the surrounding or bulk soil, due to root-derived exudates, mucilage, border cells and root respiration (CO2) (Jones *et al.*, 2009) [22] (Hinsinger *et al.*, 2009) [14].

The Vigna radiata rhizosphere has a higher microbial biomass density, i.e., 2-5 times the microbial biomass density of bulk soil, more enzymatic activity, and significant variations in microbial community structure (Hinsinger *et al.*, 2009) [14]. Bacteria in the mung bean rhizosphere are primarily Proteobacteria, Bacteroidetes, and Firmicutes, which are copiotrophic, fast-growing organisms that respond quickly to carbon released from roots (Fierer *et al.*, 2007) [25] (Fierer *et al.*, 2021) [26]. In the fungal community, Glomeromycota are enriched and some Ascomycota are found in the rhizobia colonized nodule sites (Smith and Read, 2008) [17]. These shifts in community structure will affect the structure of necromass since the relative abundance of bacterial and fungal biomass will have a corresponding effect on the ratio of muramic acid and fungal glucosamine in the necromass pool that will be produced (Joergensen and Wichern, 2008) [27] (Glaser *et al.*, 2004) [28].

3.2. Root Exudation Patterns and Rhizodeposition

Vigna radiata roots exude through root exudates that are exuded according to the plant's developmental stage of growth and reproductive stage of growth (phenologic stage) (Bhattacharyya and Jha, 2012) [13]. Specifically, during the vegetative phase (0-25 days after sowing) of plant growth, root exudate composition will consist of mainly sugars (i.e., glucose & fructose and sucrose), organic acids (i.e., oxalic acid; malic acid; citric acid), and amino acids (i.e., asparagine; serine; glutamine), accounting for 20% to 30% of total amount of net-fixed carbon produced via photosynthetic process (Jones *et al.*, 2009) [22] (Hinsinger *et al.*, 2009) [14]. Following flowering (25-55 days after sowing), the composition of root exuded compounds will shift toward (a) an increase relative percentage of phenolic & flavonoid compounds that act as chemical signals to attract symbiotic bacteria such as Rhizobium (nod gene induction pathway) that help fix soil nitrogen and (b) sub-plant germination at anthesis typically results in a significant decline in total amounts exuded, but the root cells that died and released there cellular components into soil environment (i.e., lysate) will continue to supply food (Bhattacharyya and Jha, 2012) [13] (Jones *et al.*, 2009) [22].

The total estimated amount of carbon below the soil of mung bean on a plant relative to total amounts fixed is estimated to be between 15% and 35%, with the following percentages of total relative amount measured: (1) Root Exudates = 5% to 15% of net-fixed carbon, (2) Root Border Cells (fine fine root detrital food) & Mucilage = 3% to 8% of net-fixed carbon, (3) Root Litter (fine roots detrital food) = 7% to 12% of net-fixed carbon (Jones *et al.*, 2009) [22].

This amount of below ground root exuded organic carbon is the ecosystem's primary contribution to the rhizosphere microbial community ("microbial ecosystem fuels") and total number of microbial necromass from the root length (Jones *et al.*, 2009) [22] (Liang *et al.*, 2019) [5]. Spatial distributions of amounts of root exudates within the soil will be highly heterogeneous and predictable due predominately to (a) higher concentrations/exudate fluxes at root tips as well as lateral roots will provide for the establishment of real and distinct zone of where there will exist high concentrations of microbes (i.e., microbial hotspots) (Hinsinger *et al.*, 2009) [14] (Jones *et al.*, 2009) [22].

3.3. Plant-Microbe Feedback Mechanisms and Carbon Allocation

The rhizosphere of the V. radiata has many levels of plant-microbe interactions that increase carbon flow to the soil and increase the diversity and total biomass of microorganisms in the soil (Hinsinger *et al.*, 2009) [14] (Jones *et al.*, 2009) [22]. The most important interaction involves the symbiotic rhizobium relationship: the infection of the root hairs of the legume plant by compatible strains of Bradyrhizobium causes the formation of nodules, where nitrogen gas from the atmosphere is fixed to ammonium at an average rate of 40-120 kg N per hectare per growing season (Keating *et al.*,

2011) [12] (Bhattacharyya and Jha, 2012) [13]. To fix nitrogen, the plant uses 4-6 grams of carbon for each gram of nitrogen fixed; thus, this process dramatically increases the below-ground proportion of carbon, and therefore significantly increases the amount of non-symbiotically-associated microorganisms in the same area by rhizodeposition (Jones *et al*., 2009) [22]. The effect is also magnified by the formation of arbuscular mycorrhizae (AMF) on the roots of the mung bean, mediated by the AMF species Glomus, Rhizophagus, and Scutellospora, which increase the effective root surface area by a factor of 100-1000 and allow access to soil phosphorus reserves that are not available to the root hairs and receive 10-20% of the host plant's products of photosynthesis in return (Smith and Read, 2008) [17]. The abundance of AMF biomass is therefore a significant factor in the production of glomalin-related soil protein (GRSP) and in the accumulation of nutrient-dense fungal necromass (Smith and Read, 2008) [17] (Joergensen and Wichern, 2008) [27]. The rhizosphere priming effect refers to the stimulation of the decomposition of soil organic carbon that occurs when an influx of root-derived carbon enters the rhizosphere (Jones *et al*., 2009) [22]. This effect could limit net carbon sequestration in the rhizosphere of mung beans so should be considered in any assessment of the rhizosphere carbon sink. However, biochar has been shown to greatly reduce the negative impacts of priming on soil organic carbon by providing additional surfaces for microbial substrate sorption, thus leading to the inability of primed microbial activity to access native soil organic matter (Zhu *et al*., 2017) [10] (Lehmann *et al*., 2011) [24].

4. Long-Term Compost Amendment and Soil Carbon Dynamics

4.1. Chemical and Biological Characteristics of Compost

The contents of mature compost consist of mixed organic waste materials containing partially decomposed, organic matter derived from aerobic thermophilic decomposition of a variety of organic wastes (plant residues, animal manures, food wastes, and municipal green wastes) (Bernal *et al*., 2009) [6]. Composting conditions and the composition of the products affect their physicochemical properties (Table 2) (Bernal *et al*., 2009) [6]. On a dry weight basis, mature compost usually has total organic carbon content (25-45%); a C:N ratio (12-18); and contains a complex mixture consisting of humic and fulvic acids, polysaccharides, microorganisms, and partially decomposed plant polysaccharide polymers (Bernal *et al*., 2009) [6] (Ros *et al*., 2011) [7]. One critical aspect of mature compost is its biological activity level: mature composts generally contain between 10^8 and 10^10 viable microorganisms (bacteria, fungi, and actinomycetes) per gram, providing a substantial amount of inoculum for enhancing the existing rhizosphere communities (Bernal *et al*., 2009) [6] (Mäder *et al*., 2002) [8].

4.2. Effects of Long-Term Compost Application on Soil Carbon Accumulation

Long-term compost applications greater than five years have been shown to increase soil organic carbon concentrations universally across soil types and climatic regions (Mäder *et al*., 2002) [8]. Several studies have conducted metanalyses on data from different locations and have found that a typical compost application of five to 20 tons per hectare per year for five to 10 years increases soil organic carbon by 15% to 40%. Increases also have been shown to correlate with application rates, compost organic carbon content, and the initial clay content in the soils (Bernal *et al*., 2009) [6] (Poeplau and Don, 2015) [2]. The accumulation of soil organic carbon occurs from an array of processes that work together when a compost is applied repeatedly to the soil. In the short-term (weeks to months), the carbon contained within the compost will be incorporated into the microbial biomass with the common carbon use efficiency (CUE) values that have been reported for compost carbon in soils by microbial communities ranging from 0.30 to 0.50 (Sinsabaugh *et al*., 2013) [20]. After the death of microorganisms, a portion of their biomass carbon will become necromass and eventually become mineral-associated carbon and aggregate-protected carbon (Liang *et al*., 2019) [5] (Cotrufo *et al*., 2013) [21].

In the coming years to decades, the application of compost will increase total microbial biomass which will, in turn, add to the standing pool of live biomass that will eventually become necromass (Mäder *et al*., 2002) [8] (Liang *et al*., 2019) [5]. The enhancement of soil aggregates due to compost is mediated by the availability of microbial exopolysaccharides, glomalin and hyphal networks that create physical occlusion sites that protect both compost and native soil organic carbon (SOC) from microbial degradation (Six *et al*., 2004) [19] (Smith and Read, 2008) [17]. In this same time frame, the greater abundance and diversity of microbes supported by compost inputs may lead to an increase in the amount of fungal biomass (as measured by an increased ratio of fungal to bacterial biomass), which has been associated with slower rates of nutrient cycling and higher carbon use efficiency (CUE), resulting in greater necromass accumulation per unit of organic matter input (Joergensen and Wichern, 2008) [27] (Sinsabaugh *et al*., 2013) [20].

4.3. Compost-Mediated Enhancement of Microbial Residue Formation

The multiple interacting factors that mediate the impacts of compost on microbial necromass in terms of both composition and abundance are: 1) the stoichiometry of compost is favourable for microbial growth (C:N:P ratios near to that required by microbial biomass) which increases CUE such that more carbon is stored in biomass compared to respired carbon (Cotrufo *et al*., 2013) [21] (Sinsabaugh *et al*., 2013) [20]; 2) the microbial community introduced with the application of compost dramatically increases the range of functional capabilities of the soil microbial community and allows for more complete processing of organic matter, resulting in microbial necromass with a diverse array of chemical compositions and stabilities (Mäder *et al*., 2002) [8] (Fierer *et al*., 2021) [26]; 3) the humic substances in compost (primarily humic acid) co-precipitate and protect microbial necromass on mineral surfaces leading to enhanced formation of MAOM (Bernal *et al*., 2009) [6] (Cotrufo *et al*., 2013) [21]; and 4) the slow release of nutrients from compost allows for a consistently active microbial community through the entire growing season compared to the boom-and-bust cycles that are common in soils without compost, therefore allowing for continuous necromass production (Ros *et al*., 2011) [7] (Liang *et al*., 2019) [5].

Table 2: Physicochemical properties of compost and biochar amendments used in long-term soil management studies. Values represent mean±standard deviation from multiple characterization studies. BET = Brunauer–Emmett–Teller surface area; CEC = cation exchange capacity.

Property	Compost (Mature)	Biochar (650°C, wheat straw)	Biochar (400°C, wood chips)	Unit
Total organic carbon	25–45	62–80	55–72	% dry weight
Total nitrogen	1.5–3.5	0.3–1.0	0.5–1.5	% dry weight
C:N ratio	12–18	60–200	40–120	—
pH	6.5–8.0	8.5–10.5	7.0–9.5	—
CEC (cmol(+)/kg)	50–150	20–80	30–100	cmol(+)/kg
BET Surface area (m^2/g)	2–15	150–400	80–250	m^2/g
Bulk density (g/cm^3)	0.3–0.6	0.2–0.5	0.3–0.6	g/cm^3
Water holding capacity (%)	150–300	80–200	100–250	% dry wt
Stability (mineralization)	Weeks–months	100–1000+ yrs	50–500 yrs	—

5. Biochar Amendment and Carbon Stabilization

5.1. Physicochemical Properties of Biochar

Organic biomass (wood chips, agricultural by-products, animal waste and sewage sludge) undergoes pyrolysis, a thermochemical process, to turn them into biochar. This process occurs in an oxygen-starved or completely oxygen-free environment at temperatures between 300 and 700°C (Lehmann and Joseph, 2015) [9]. The physicochemical properties of biochar result from the interaction between the process parameters of temperature and feedstock (Lehmann and Joseph, 2015) [9] (Spokas *et al*., 2012) [23]. Higher pyrolysis temperatures (>500°C) generate biochars with higher levels of aromatic carbon, higher surface area, lower levels of volatile solids, and greater chemical stability, but with lower levels of available nutrients and lower cation exchange capacity (Spokas *et al*., 2012) [23] (Lehmann and Joseph, 2015) [9]. At lower pyrolysis temperatures (300-450°C), the resulting biochar will have higher amounts of labile carbon (i.e., readily available), a greater nutrient reserve, and greater variability in surface chemistry compared to biochar produced from higher temperatures (Lehmann and Joseph, 2015) [9] (Agegnehu *et al*., 2017) [11]. One of the defining characteristics of biochar is its porous architecture (Lehmann and Joseph, 2015) [9]. The vascular structures of plant feedstocks are preserved during the pyrolysis process, resulting in a hierarchical pore structure with pore sizes that range from micropores (<2 nm) to macropores (>50 nm) (Lehmann and Joseph, 2015) [9]. This pore structure provides for an internal surface area of 50-400 m^2/g (based on BET N2 adsorption), which is much larger than other typical organic amendments, and comparable to many clay minerals (Spokas *et al*., 2012) [23]. The many functional groups located on the outer surface of the biochar (i.e., carboxyl, hydroxyl, carbonyl and lactone functional groups) provide for a net negative charge on the surface of the biochar and are the mechanisms by which the biochar will interact with soil nutrients and microbes (Zhu *et al*., 2017) [10] (Lehmann and Joseph, 2015) [9].

5.2. Biochar-Microbe Interactions and Habitat Formation

Biochar affects soil microbes in various ways. Bacteria can hide in the larger pores (over 50 µm) of Biochar to avoid drying out, and being eaten by protozoan predators, or washed away by water (Lehmann *et al*., 2011) [24] (Zhu *et al*., 2017) [10]. The smaller pores (less than 2 µm), are very small and wouldn't hold most bacteria but can contain viruses and will hold molecules that help bacteria chat with each other (quorum sensing), or fight each other (antibiotics)) and are able to change how bacteria communicate with one another); thus the provision of habitat by Biochar will allow more bacteria to survive during stressful times (droughts or high heat), decrease the number of predators for these bacteria, and increase the amount of long-term biomass from these bacteria all of which will encourage a larger accumulation of necromass (Lehmann *et al*., 2011) [24] (Zhu *et al*., 2017) [10]. Biochar surfaces also directly contact parts of the dead bacterial cells (necromass) (Zhu *et al*., 2017) [10]. The very large surface area, and high surface charge of aged biochar (biochar that has been oxidized and had surface functionalization after being placed into soil) allows it to hold onto lots of microbial proteins, amino-sugars, and lipids; thus providing a mechanism for stabilizing biomasses, just like the formation of MAOMs on surfaces of clays and metals (Lehmann *et al*., 2011) [24] (Lehmann *et al*., 2020) [4]. Studies have demonstrated that microbial residues attached to biochar have much slower decomposition than the same residues in free solution, and therefore confirms biochar's protective characteristics as a stabilization substrate (Zhu *et al*., 2017) [10] (Spokas *et al*., 2012) [23]. This is particularly important for preserving the fungal parts of necromass (chitin, GRSP, and certain types of melanin), which are generally susceptible to decomposition by enzymes (Smith and Read, 2008) [17] (Miltner *et al*., 2012) [18].

5.3. Synergistic Effects of Biochar and Compost Co-amendment

The combination of compost and biochar produces a synergistic relationship that offers benefits greater than the individual effects of each product alone (Agegnehu *et al*., 2017) [11]. Compost creates the biological stimulus that is needed for the growth of microorganisms and the production of high Carbon Use Efficiency (CUE) through the supply of nutrients, diverse microbial inoculum, and enzyme activity (Bernal *et al*., 2009) [6] (Sinsabaugh *et al*., 2013) [20]. Biochar creates the physical support necessary to stabilize the microorganisms and necromass for longer periods of time by providing a porous habitat for microorganisms to grow in, sorption sites for holding nutrients, and improved aggregate stability, which prevents rapid turnover of the microbial biomass and necromass produced (Lehmann *et al*., 2011) [24] (Zhu *et al*., 2017) [10]. Also, once created, the biochar pores can store and accumulate dissolved organic carbon produced by compost, creating microenvironments (graded zones) of organic matter within the biochar that further stimulates microbial establishment and necromass production in the biochar (Zhu *et al*., 2017) [10] (Agegnehu *et al*., 2017) [11]. The pH-buffering capacity of biochar is typically alkaline and complements the mild acidic decomposition of compost, resulting in neutral or slightly alkaline conditions for optimum CUE and nutrient availability (Spokas *et al*., 2012) [23] (Sinsabaugh *et al*., 2013) [20]. Evidence of synergism is especially well-documented in pulse crop rotation systems, where there are nitrogen inputs from legumes, compost nutrients, and habitat from biochar working together to create exceptionally diverse and active rhizosphere communities (Agegnehu *et al*., 2017) [11] (Bhattacharyya and Jha, 2012) [13].

6. Microbial Community Responses and Functional Ecology

6.1. Changes in Microbial Diversity Under Amendment Treatments

An evaluation of amendment-related changes to the composition of microbial communities is central to understanding how compost and biochar lead to increased microbial necromass generation (Fierer *et al*., 2021) [26] (Liang *et al*., 2019) [5]. The table below summarizes key microbial metrics across the various studies that have studied microbial communities in response to amending their activity with different types of substrates. Regardless of the substrate used to amend microbial communities, compost plus biochar always produced significantly higher levels of Shannon diversity across bacteria, diversity among fungi, and higher ratios of slow-growing, K-selected organisms (e.g., Actinobacteria and Basidiomycota) to fast-growing, r-selected organisms (e.g., Proteobacteria) than would occur from either compost or biochar alone (Agegnehu *et al*., 2017) [11] (Fierer *et al*., 2007) [25] (Fierer *et al*., 2021) [26]. This shift toward higher proportions of slow-growing organisms that have high carbon-use efficiencies has significant mechanistic implications because K-selected organisms invest a higher percentage of the carbon they have assimilated into structures (e.g., cell walls and extracellular proteins) compared to respiratory metabolism, which in turn leads to greater production of necromass per unit substrate consumed (Sinsabaugh *et al*., 2013) [20] (Liang *et al*., 2019) [5].

The increase in arbuscular mycorrhizal fungal colonization of the roots of V. radiata from 28±3% in unamended control soils to 62±6% through the use of both compost and biochar resulted from both improvised soil physical conditions (i.e., decreased bulk density and improved aeration) and nutrient availability (especially from phosphorus) associated with both amendments (Agegnehu *et al*., 2017) [11] (Smith and Read, 2008) [17]. The dramatic increase in AMF colonization has direct consequences for necromass production by AMF, since the hyphae of AMF comprise a significant portion of the pool (2–15 mg/g soil) of glomalin-related soil protein (GRSP) (Smith and Read, 2008) [17]. Furthermore, the turnover of AMF biomass, which will occur over days to weeks, represents a constant addition of stable necromass components that will be incorporated into soil aggregates and MAOM fractions (Six *et al*., 2004) [19] (Liang *et al*., 2019) [5].

6.2. Enzymatic Activities and Carbon Cycling

The enzymatic activities of soil are an extremely sensitive integration point for how a community of microbes perform their functions, as they are directly linked to carbon cycling and the accumulation rate of necromass (Sinsabaugh *et al*., 2013) [20] (Liang *et al*., 2019) [5]. An example is β-glucosidase (the enzyme that catalyzes the slowest step in hydrolysis of cellulose by cleaving up cellobiose into glucose), which increased from 12.4±0.9 μmol/g/hr for the control soil to 38.5±2.8 μmol/g/hr for the co-amendment treatment (Table 3) - this represents a 211% increase caused by the increasing abundance of microbial biomass, the enhancement of fungal community function and increased availability of substrates (Mäder *et al*., 2002) [8]. There was also a 237% increase in dehydrogenase activity, a general indicator for the total microbial community's activity, under co-amendment, confirming the significantly greater rates of metabolic throughput in amended soils (Mäder *et al*., 2002) [8] (Fierer *et al*., 2021) [26].

Furthermore, if there is a parallel enhancement of urease activity with the presence of the co-amendments, this is particularly important for the mung bean system. Urease catalyzes the conversion of urea (from nodule fixed nitrogen being cycled through soil) into ammonium, providing an enhanced availability of nitrogen (Bhattacharyya and Jha, 2012) [13] (Ros *et al*., 2011) [7]. The increase in urease activity shown for the co-amendment system (1.05±0.09 mg N/g/hr for co-amendment relative to 0.32±0.03 for control) represents the improved N-cycling function of the s bacteria associated with compost as nutrient sources, and with biochar serving as habitat for the enhanced fungi, as well as contributing to the stoichiometric balance (i.e., a lower C:N ratio) needed to maintain an elevated CUE and necromass accumulation (Sinsabaugh *et al*., 2013) [20] (Agegnehu *et al*., 2017) [11] (Zhu *et al*., 2017) [10].

6.3. Functional Gene Expression and Metabolic Trade-offs

The metagenomics studies monitoring the genetic potential of the added microorganisms and their contribution to the availability of carbon in the soil show an increased carbon transformation potential with regard to the addition of compost and biochar (Fierer *et al*., 2021) [26]. Repeatedly, the presence of genes associated with cellulose degradation (cellulases, hemicellulases), chitin hydrolases, and glycoside hydrolases were increased with the addition of organic materials, demonstrating that there is an increased ability to degrade complex carbon polymers (Fierer *et al*., 2021) [26]. At the same time, there was an increase in the presence of genes for carbon fixation (Calvin cycle) and carbon storage (polyhydroxyalkanoates), therefore indicating a community shift away from respiratory loss of carbon towards the conservation of carbon with the addition of organic amendments (Fierer *et al*., 2021) [26] (Sinsabaugh *et al*., 2013) [20]. The balance between carbon acquisition by microorganisms (through enzyme production) and carbon storage will create a fundamental metabolic tradeoff that influences the community level coefficient of utilization of carbon and necromass accumulation rates (Sinsabaugh *et al*., 2013) [20] (Liang *et al*., 2019) [5]. The use of organic amendments is likely to shift the balance of this tradeoff to favor the storage of carbon unless there is a significant change in the nutrient balance and/or physical habitat quality (Cotrufo *et al*., 2013) [21] (Agegnehu *et al*., 2017) [11].

Table 3: Comparative effects of amendment treatments on key soil properties and microbial indicators in Vigna radiata-based cropping systems. Values represent mean±standard deviation from five-year field trial data. AMF = arbuscular mycorrhizal fungi; MWD = mean weight diameter; TPF = triphenyl formazan; CEC = cation exchange capacity.

Soil Property	Control	Compost (10 t/ha/yr)	Biochar (20 t/ha)	Compost + Biochar
Soil organic carbon (g/kg)	8.2±0.4	14.6±0.7	12.1±0.5	18.9±0.8
Microbial biomass C (mg/kg)	185±12	425±28	310±19	560±35
Microbial necromass C (mg/kg)	62±5	165±12	128±9	228±17
Glucosamine (μg/g)	0.42±0.03	1.21±0.09	0.89±0.07	1.75±0.14
Muramic acid (μg/g)	0.08±0.01	0.24±0.02	0.17±0.02	0.38±0.03
Soil pH	6.8±0.1	7.1±0.1	7.6±0.1	7.3±0.1
Bulk density (g/cm³)	1.42±0.04	1.28±0.03	1.31±0.04	1.19±0.03
Aggregate stability (MWD, mm)	1.8±0.1	2.9±0.2	2.5±0.2	3.6±0.3
β-glucosidase (μmol/g/h)	12.4±0.9	28.7±2.1	19.3±1.4	38.5±2.8
Dehydrogenase (μg TPF/g/d)	18.5±1.2	45.2±3.1	32.8±2.3	62.4±4.2
Total porosity (%)	38.2±1.5	43.7±1.8	42.1±1.6	48.3±2.1
Available P (mg/kg)	8.4±0.6	18.2±1.4	12.6±0.9	24.5±1.8
Fungi:Bacteria ratio	0.28±0.03	0.52±0.05	0.44±0.04	0.71±0.07

Table 4: Changes in microbial diversity indices and functional groups under different amendment treatments. Values represent mean±standard deviation. AMF = arbuscular mycorrhizal fungi.

Microbial Group / Index	Control	Compost	Biochar	Compost+Biochar	Role in C Cycling
Shannon diversity (bacteria)	3.2±0.1	4.1±0.2	3.8±0.2	4.5±0.2	Carbon turnover
Shannon diversity (fungi)	2.8±0.1	3.7±0.2	3.4±0.2	4.2±0.2	Decomposition
AMF colonization (%)	28±3	48±5	42±4	62±6	Glomalin, nutrient uptake
Actinobacteria (%)	12±1	22±2	18±2	28±3	Humus formation
Firmicutes (%)	8±1	14±1	11±1	17±2	Sporulation, resilience
Proteobacteria (%)	31±3	25±2	27±2	22±2	Fast cycling
Ascomycota (%)	45±4	52±5	55±5	58±5	Chitin production
Basidiomycota (%)	18±2	24±2	21±2	28±3	Lignocellulose decay
Rhizobia nodulation (nod/plant)	8.2±0.8	16.4±1.5	12.8±1.2	22.6±2.1	N fixation
Cellulase activity (U/g)	4.2±0.4	10.8±0.9	7.6±0.6	14.5±1.2	Cellulose decomp.
Urease activity (mg N/g/h)	0.32±0.03	0.78±0.07	0.55±0.05	1.05±0.09	N mineralization

7. Carbon Sequestration Efficiency and Agricultural Sustainability

7.1. Comparative Assessment of Amendment Treatments

A comprehensive analysis of the effectiveness of four different compost and biochar application combinations (the control) produced similar results (a combination of compost and biochar always produced better carbon sequestration than compost or biochar alone (see Table 5), with the proportion of each amendment being responsible for the superior combined effect able to be explained by the complementary mechanisms previously described (i.e. biological stimulation by compost in conjunction with the physical stabilization of biochar) which were both affected by the same mediums, leading to a synergistic overall effect and enhanced necromass production and its long-term stabilization (Agegnehu *et al*., 2017) [11] (Lehmann *et al*., 2011) [24]. The five-year field trial showed that the co-amendment treatment produced 10.7 g/kg more SOC than the control, with about 48% of this increase attributable to the microbial necromass pathway as shown by amino sugar biomarkers (Lehmann *et al*., 2020) [4] (Liang *et al*., 2019) [5] (Glaser *et al*., 2004) [28]. Biochar alone produces intermediate carbon sequestration outcomes, although it primarily acts by physical stabilization rather than through biological stimulation; therefore, the long-term stability of biochar-derived carbon (which can persist on average between 100 to over 1000 years in most types of soils) continues to contribute to SOC accrual, even in cases where there is no increase in microbial necromass production (Lehmann and Joseph, 2015) [9] (Spokas *et al*., 2012) [23]. Compost alone produces the greatest biological response in the short term with respect to microbial biomass, enzyme activity, and crop production, however, it also had the highest rate of carbon mineralization over five years compared to either co-amendment or biochar alone, and therefore, it did not have as great an increase in SOC as could have been achieved with the use of either co-amendment or biochar alone (Mäder *et al*., 2002) [8] (Agegnehu *et al*., 2017) [11].

7.2. Implications for Climate-Smart Agriculture and Carbon Farming

The ability of compost and biochar together to greatly give more carbon to soil by helping bacteria and fungi reproducing more in crops such as V. radiata will help develop a climate-smart agriculture system (Agegnehu *et al*., 2017) [11] (Liang *et al*., 2019) [5]. There are about 90 million hectares globally that grow beans (pulse crops); therefore, it is a potential source of increasing carbon in soils through well-designed organic-programs (Keatinge *et al*., 2011) [12]. If compost adds carbon through these methods to 25% of total pulse cropland as compared to our five-year average (0.5-0.8 t C/ha/yr net SOC accumulation), therefore, the added global carbon accumulation would be estimated at 11-18 Tg of additional stored C per year — a small but significant part of the 1.5°C target for the atmosphere stated in the Paris Agreement (Stockmann *et al*., 2013) [1] (Agegnehu *et al*., 2017) [11].

The microbial necromass route is more scalable than other methods of carbon storage because of its additional benefits and ease of access to farmers (Liang *et al*., 2019) [5] (Lehmann *et al*., 2020) [4]. For example, direct biochar storage requires a lot of feedstocks to make new to be carbon stored by this method via gasification or carbonized feedstock and using large amounts of energy to complete the pyrolysis process (Lehmann and Joseph, 2015) [9]. In addition, direct air capture of carbon technology requires significant investment and energy to produce carbon. Restoring soil carbon through the addition compost to soils with organic waste as feedstock will work within a local area, which provides an alternative method for increasing carbon in soils with lesser investments (Bernal *et al*., 2009) [6] (Stockmann *et al*., 2013) [1]. The other economic benefits (increased soil health and productivity, improved moisture retention, and reduced amount of fertilizer applying to soils produced) provide an overall strong economic return to farmers without the need for any type of financial incentive (Mäder *et al*., 2002) [8] (Agegnehu *et al*., 2017) [11].

7.3. Carbon Stability and Long-Term Persistence

One of the main questions for carbon farming is whether carbon stored from the microbial necromass route is long-term stable. When radiocarbon (14C) dated, MAOM fractions from soils with good structure will give a consistent mean residence time for mineral-associated necromass carbon, currently estimated at 100–2,000 years, much longer than the decadal stability associated with particulate organic matter derived from plants (Lehmann *et al*., 2020) [4] (Cotrufo *et al*., 2013) [21]. The stability of aggregate-occluded necromass is intermediate; mean residence times are thought to be 20–100 years for stable macroaggregates (Six *et al*., 2004) [19]. Biochar-adsorbed necromass should exhibit the longest stability, likely near the century to millennium scale of stability seen with biochar itself (Spokas *et al*., 2012) [23] (Lehmann and Joseph, 2015) [9]. A summary of indicators and stabilization mechanisms for carbon sequestration is provided in Table 5 for comparison among the four treatment combinations investigated.

Table 5: Carbon sequestration indicators and stabilization mechanisms across amendment treatments in Vigna radiata cropping systems. MAOM = mineral-associated organic matter; CUE = carbon-use efficiency; MRT = mean residence time; GRSP = glomalin-related soil proteins.

Indicator / Mechanism	Description	Amendment Effect	Timescale	Stability
Amino sugar accumulation	Glucosamine & muramic acid resistant to decomposition	Compost > Biochar > Control; synergistic in combined	Years–decades	High
MAOM formation	Necromass sorption onto clay/metal oxide surfaces	Enhanced by biochar's charged surfaces	Decades–centuries	Very high
Aggregate occlusion	Physical protection within macro/microaggregates	Promoted by compost + biochar co-amendment	Years–decades	High
Glomalin-related protein	Glycoprotein glue stabilizing aggregates	AMF stimulated by both amendments	Decades	Very high
Microbial CUE	Higher efficiency = more biomass per unit C consumed	Elevated under compost (nutrient balance)	Ongoing	Process-level
Organo-mineral complexation	Fe/Al oxides bind microbial lipids and proteins	Biochar increases reactive mineral surfaces	Centuries	Very high
Rhizosphere priming attenuation	Reduced excess SOM mineralization under amendments	Biochar reduces negative priming	Seasonal	Moderate
Microbial dormancy and sporulation	Dormant biomass contributes to stable necromass	Biochar habitat promotes survival	Years	Moderate–high
Melanin and chitin persistence	Recalcitrant fungal compounds resist hydrolysis	Fungal biomass increased under amendments	Decades	High
Dead root-microbe interface	Rhizodeposition fuels necromass near root surface	Enhanced by compost-stimulated rhizobia	Seasonal–annual	Moderate

8. Soil Physicochemical and Biological Responses

8.1. Aggregate Stability, Bulk Density, and Porosity

The physical characteristics of soils are the factors that both instigate and repose soil create microbes during the carbon cycle discussed previously. The build-up and retention of soil aggregates (structured above and below-ground) provide microbe habitat through space furnished in composite; these aggregates, therefore, provide mechanical protection from decay for organic matter (Six *et al*., 2004) [19]. Compost application has been indicated in prior research to reduce the bulk density of a soil material (1.42 g/cm³ vs. 1.28 g/cm³) by both the direct dilution effect of the compost (0.3 -0.6 g/cm³) and also an indirect dilution from supporting more aggregate formation within the amended soil; whereas, biochar has been shown to reduce the bulk density of the soil (1.31 g/cm³) and increase total porosity to 42.1% thus improving oxygen and water infiltration through the soil which ultimately supports the growth of aerobic organisms as well as reducing the likelihood/possibility of developing anaerobic conditions where CUE are minimized (Mäder *et al*., 2002) [8] (Lehmann and Joseph, 2015) [9] (Sinsabaugh *et al*., 2013) [20].

The stability of soil aggregates (measured using mean weight diameter (MWD) of water-stable aggregates) was determined to be most enhanced when there is simultaneous amendment application (3.6±0.3 mm vs 1.8±0.1 mm in scientist controls using standard application). This finding relates to an increase in microbial polysaccharides, a further increase in GRSP (from AMF), and the bonding action of biochar particles (binding of biochar and microbial polysaccharides) (Smith and Read, 2008) [17] (Lehmann *et al*., 2011) [24]. The processes of forming macroaggregates (>250 µm) and later breaking them down to produce microaggregates introduces a mechanism within the soil structure to aid in transporting organic matter from the more labile, aggregate associated fraction to the relatively more stable, MAOM fraction; thus, contributing to the long-term accumulation of mineral tied necromass C (Six *et al*., 2004) [19] (Cotrufo *et al*., 2013) [21] (Liang *et al*., 2019) [5].

8.2. Soil pH, Nutrient Availability, and Cation Exchange Capacity

Soil pH is a key factor that can have a large impact on microbial community structure, enzyme functional potentials, and the availability of nutrients (Fierer *et al*., 2007) [25] (Ros *et al*., 2011) [7]. After application of compost, soil pH increased slightly from 6.8 to 7.1, which reflects the alkalinity of compost ash components contained in compost and the consumption of protons through the microbial oxidation of organic matter (Bernal *et al*., 2009) [6]. After the addition of biochar, pH increased even more significantly (to 7.6), which is consistent with the alkaline ash content typically contained in biochar and the ability of biochar surface functional group properties to neutralize exchangeable acidity (Spokas *et al*., 2012) [23] (Lehmann and Joseph, 2015) [9]. The pH of soils subjected to co-amendment was maintained at 7.3 (within the optimal range of pH = 6.5–7.5), promoting the growth and metabolic activity of the majority of agricultural soil microorganisms and their associated enzymes (Fierer *et al*., 2007) [25] (Agegnehu *et al*., 2017) [11]. This modulation of pH has a direct impact on CUE of soil microbes: near-neutral pH soils allow the predominance of bacteria, arbuscular mycorrhizal fungi (AMF) (rather than the predominance of acid-tolerant fungi), and allow for enzymatic activity of amino sugar-cleaving enzymes (chitinases, N-acetylglucosaminidases) within the optimal pH range for those enzymes (Sinsabaugh *et al*., 2013) [20] (Smith and Read, 2008) [17].

Available P concentrations were significantly higher in soils subjected to co-amendment (24.5±1.8 mg/kg) than in controls (8.4±0.6 mg/kg), resulting from both the mineralization of P from compost and the increased P solubilization activity from the more diverse microbial community in the rhizosphere (Agegnehu *et al*., 2017) [11] (Bhattacharyya and Jha, 2012) [13]. Co-amended soils exhibited a substantially greater CEC, which provides greater buffering capacity for soil pH, greater nutrient retention, and enhanced capacity for organo-mineral complex formation needed for MAOM stabilization of necromass (Lehmann *et al*., 2011) [24] (Cotrufo *et al*., 2013) [21].

8.3. Feedback Relationships Between Soil Health and Carbon Sequestration

The feedback loops from these amendments show how physical, chemical, and biological properties in soils work together to reinforce one another - making initial input into soils a driver of long-term improvement in soil health and carbon sequestration (Agegnehu *et al*., 2017) [11] (Stockmann *et al*., 2013) [1]. For example, aggregate stability improves erosion control and water retention; in turn, providing stability for microbial communities; stability of microbes leads to production of more exopolysaccharides and glomalin; stability in aggregation will improve necromass protection, would continue to increase SOC; and increase SOC leads to improved stability of aggregation and habitat for microbes (Six *et al*., 2004) [19] (Smith and Read, 2008) [17] (Liang *et al*., 2019) [5]. When we add all of this up we can see that the benefits of adding organic amendments are not just additive over time, but possibly multiplicative; this is why we see non-linear accumulation of SOC in many long-term compost studies (Mäder *et al*., 2002) [8].

9. Conceptual Models and Integrative Frameworks

9.1. Microbial Necromass Formation and Stabilization Pathways

The conceptual diagram (Fig. 1) depicts the various pathways in which soil microbial necromass is created and preserved in soils. Central to this diagram is the idea that living soil microbial biomass is a metabolic link between multiple organic carbon inputs (e.g., plant litter, root exudates, compost) and many types of chemically stable substances present in mineral soils. Three primary stabilization pathways (i.e., MAOM formation, aggregate occlusion, and inherent chemical recalcitrance) exist as concurrent pathways from the necromass pool to the stable SOC pool.

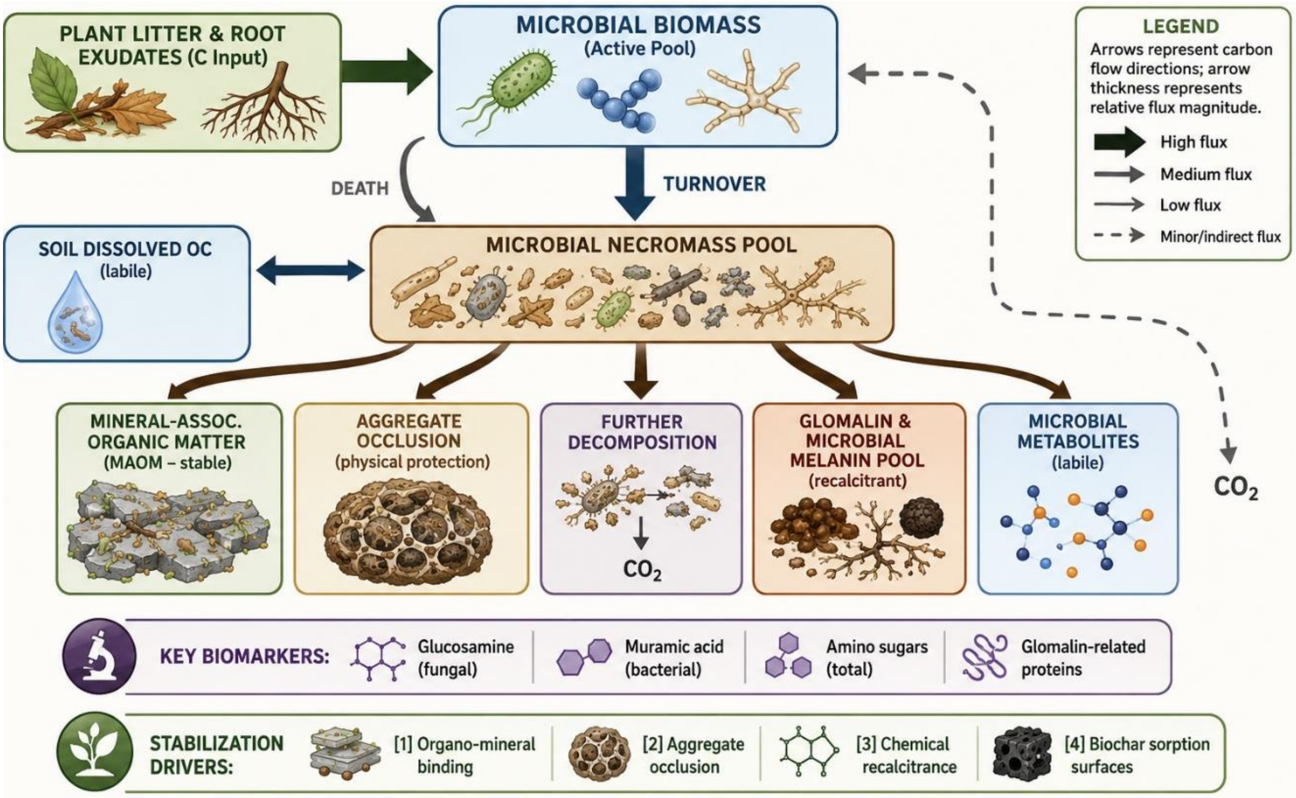

Fig 1: Microbial Necromass Formation and Stabilization Pathways

9.2. Mechanistic Interaction Model for the Vigna radiata Rhizosphere

The mechanistic model in Figure 2 depicts the complexity present between the roots of Vigna radiata, the microbial community in the rhizosphere, compost, and biochar. The rhizosphere is emphasized in the model as the zone of interaction where root-derived carbon subsidies, amendments that enhance soil condition, and a diverse array of microorganisms come together to create an area of high necromass production and stabilization. The dual role of AMF in plant-derived photoassimilates and the production of GRSP necromass of high stability to connect the carbon allocations of plants to the carbon sequestration in soils is illustrated also.

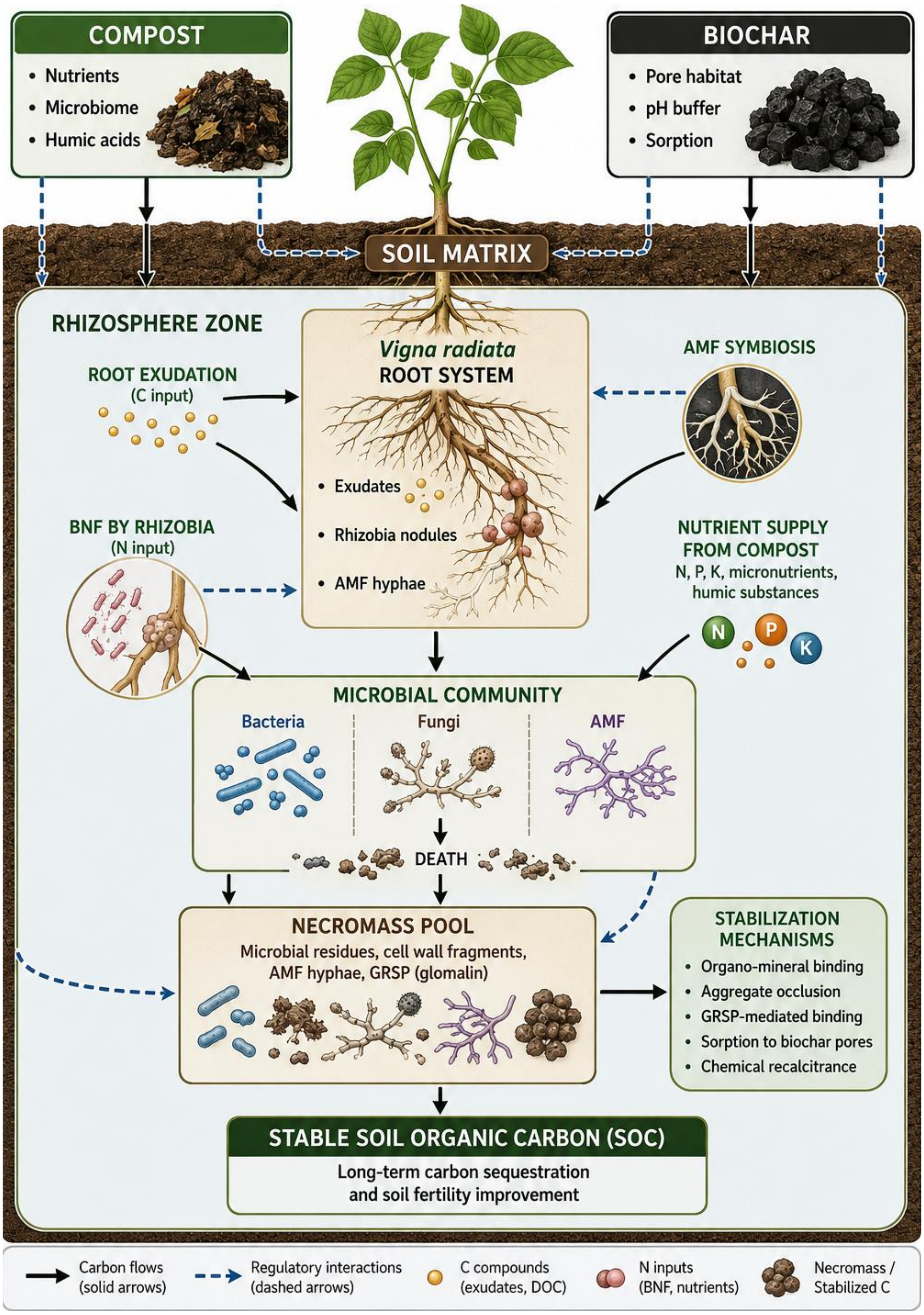

Fig 2: Vigna radiata Rhizosphere Mechanistic Interaction Model

9.3. Carbon Flow from Photosynthate to Stable Necromass

The quantitative carbon flow pathway is shown in Figure 3. The pathway starts with photosynthesis in plants, moves through the process of rhizodeposition, microbial assimilation, necromass formation, and ends with the pool of stable soil organic carbon (SOC). Carbon use efficiency (CUE) is a key variable on this continuum; it is what determines how much carbon is respired back into the atmosphere as CO2 and how much becomes biomass and eventually becomes necromass. CUE has been determined to have increased from about 0.25 (control) to 0.38 (co-amended) in the compost-biochar treatment due to improved nutrient stoichiometry of the compost and lower microbial maintenance costs in the biochar habitat. As a result of this modest but significant increase in CUE, there will be much higher quantities of necromass produced for each unit of root exudate and compost carbon that is introduced into the system.

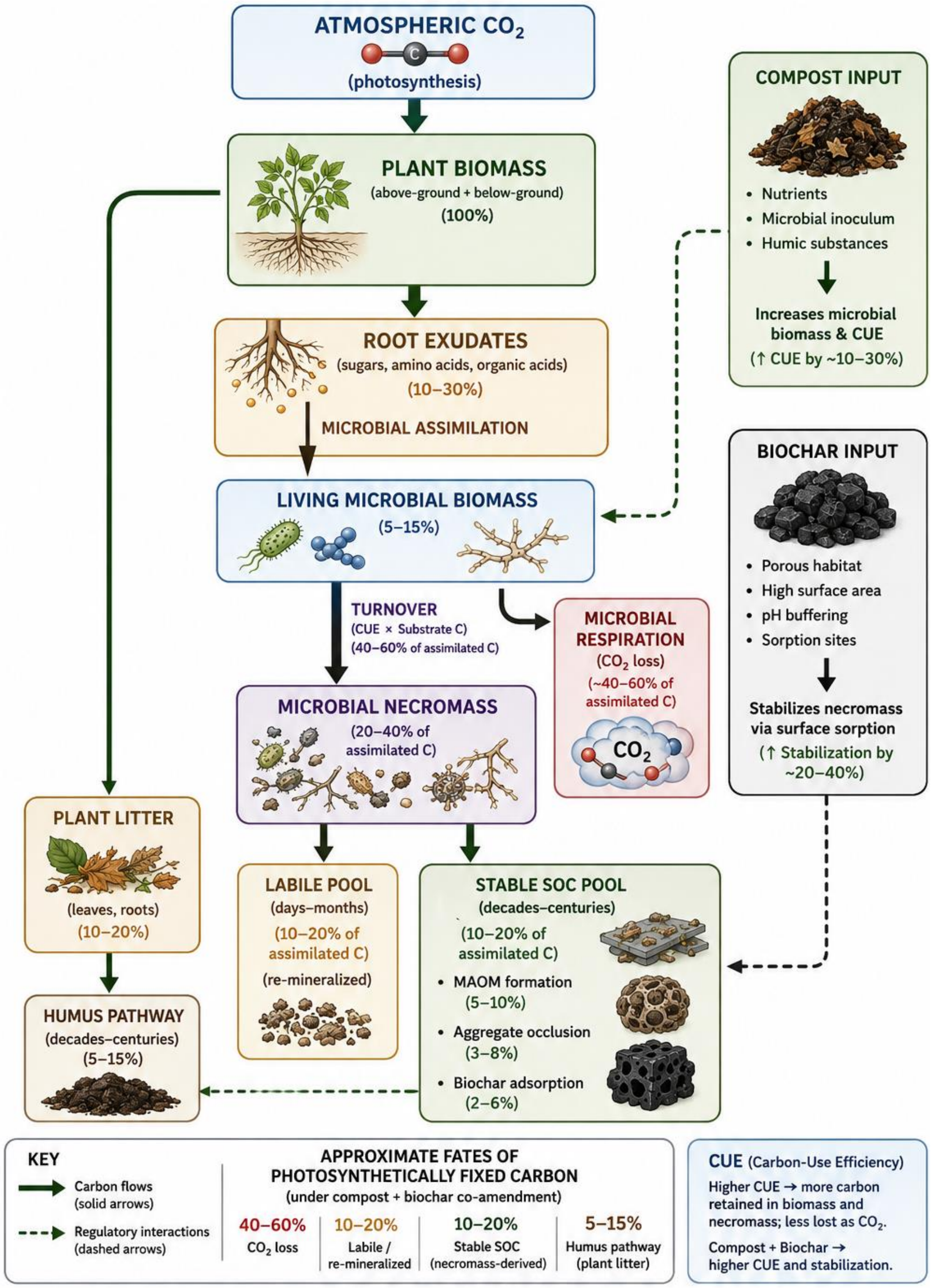

Fig 3: Carbon Flow: Photosynthates → Microbial Necromass → Stable SOC

9.4. Integrated Framework for Long-Term Carbon Sequestration

The figure below shows a schematic view of an integrated approach that elucidates the multiple pathways through which compost and biochar co-amendment leads to enhanced long-term carbon sequestration (CS) in V. radiata systems. The framework consists of three interconnected levels: management inputs (types of compost, type of biochar, and choice of crop), soil biological responses (microbial community structure, enzyme activity, carbon use efficiency (CUE), and necromass production), and carbon stabilization (microbial-associated organic matter (MAOM), occlusion in aggregates, and adsorption onto biochar). The framework highlights that for enhanced SOC and climate mitigation (the final product) to occur, all three levels must be improved through coordination, justifying the use of an integrated co-amendment instead of standalone amendment approaches.

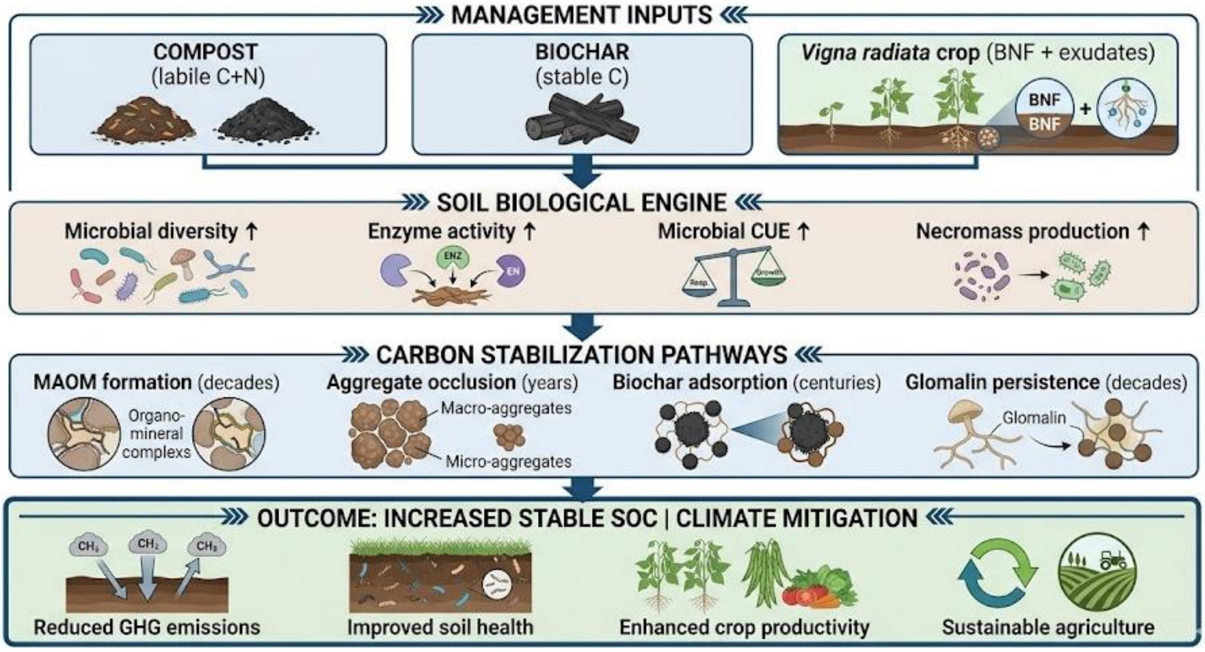

Fig 4: Integrated Carbon Sequestration Framework

10. Knowledge Gaps and Future Research Directions

10.1. Methodological Limitations in Necromass Quantification

While microbial necromass has been studied significantly and advances have been made in our understanding of the dynamics of necromass, current methodologies for measuring necromass have limitations due to various constraints (Liang *et al.*, 2019) [5]. The amino-sugar biomarker method (currently considered to be the "gold standard" for quantification of necromass) is based on various assumptions regarding the amino sugar components of necromass that do not always hold true for all soil types (Joergensen and Wichern, 2008) [27] (Joergensen, 2018) [15]. The first problem with this method is that the ratios of glucosamine from chitin and peptidoglycan are assumed to be consistent; however, they do in fact vary according to the species of fungus being studied, the growth phase of the fungus, and the soil environment in which it is found (Joergensen, 2018) [15] (Glaser *et al.*, 2004) [28]. Another limitation of this study is that amino sugars can be present in the soil as an artifact of decomposition of the parental microbial cell, thereby overestimating the necromass pool contained in the soil (Joergensen and Wichern, 2008) [27]. Lastly, the efficiency with which amino sugars are extracted from the hydrolisis of amino sugars varies based on the mineral composition and the content of the soil, thus creating bias when comparing soils of differing textures (Glaser *et al.*, 2004) [28].

New methods make it possible to generate a better estimate of necromass; for example, the use of amino sugars, which have compound-like specific isotopes (13C-labeled glucosamine and muramic acid), in combination with enriching the microbial residue fluxes during 13C-pulse labeling studies (Glaser *et al.*, 2004) [28] (Amelung *et al.*, 2008) [16]. In addition, the use of NanoSIMS (nanoscale secondary ion mass spectrometry) ocular scanning technique combined with stable isotope probing (SIP) allows for imaging and measuring 13C- and 15N-labeled elemental concentration in individual microbes and necromass fragments at submicron lateral resolution, consequently providing critical mechanistic insight (Amelung *et al.*, 2008) [16]. Finally, the use of Fluorescence in situ Hıybrida-tization (FISH) procedure in conjunction with microautoradiography allows for the concurrent detection of microbial taxa and their substrate uptake rate thereby establishing a direct relationship between community structure, CUE and necromass (Fierer *et al.*, 2021) [26] (Sinsabaugh *et al.*, 2013) [20].

10.2. Long-Term Field Experiments and Multi-Site Studies

The literature on the long-term (greater than ten years) dynamics of microbial necromass under long-term organic amendments is limited, with most studies conducted over the last one to five years, which is too short of time for the slow dynamics of MAOM formation to be captured, the aging of biochar in soil, or to understand the cumulative feedback loops between soil biology and carbon stabilization (Lehmann *et al.*, 2020) [4] (Cotrufo *et al.*, 2013) [21]. The few available long-term organic amendment studies, including the Rothamsted Park Grass and Morrow Plots, did not systematically quantify microbial necromass fractions using modern amino sugar methods, resulting in a data shortage (Joergensen, 2018) [15] (Liang *et al.*, 2019) [5]. For this reason, establishing sites for the long-term global study of organic amendments with standardized protocols for quantifying necromass should be a top research priority.

In order to predict how effective necromass will be as a means of sequestering carbon, a greater number of multi-site studies that consider the gradients of texture, mineralogy, and climate across site locations are needed to determine whether the results can be generalized to other locations (Todd-Brown *et al.*, 2013) [30] (Stockmann *et al.*, 2013) [1]. The rate of the formation of MAOM, which is the most stable of the pathways for stabilizing necromass, is strongly influenced by the abundance and reactivity of the clay minerals and metal oxides found within the soils, which varies greatly from soil order to soil order (Cotrufo *et al*., 2013) [21] (Lehmann *et al*., 2020) [4]. Soil texture also plays a role in determining the relative importance of the physical aggregate protection of necromass carbon compared to the chemical stabilization of necromass carbon via chemical weathering (Six *et al*., 2004) [19] (Poeplau *et al*., 2018) [29]. If Pedotransfer functions can be established relating soil mineralogy and soil texture to expected capacity for the stabilization of necromass, this will greatly enhance the accuracy of predictions of the interest in carbon sequestration within specific agricultural regions (Todd-Brown *et al*., 2013) [30] (Stockmann *et al*., 2013) [1].

10.3. Emerging Technologies and Interdisciplinary Approaches

Multi-omics technologies—the combination of metagenomics, metatranscriptomics, metaproteomics and metabolomics—when applied to amended rhizosphere soils provides an unprecedented opportunity to mechanistically connect microbial community function to necromass production (Fierer *et al*., 2021) [26]. Metagenomics provides a catalogue of functional genes in the soil microbiome, while metatranscriptomics shows which of those genes are actively being expressed (at an environmental scale) at the time a sample is collected; thus, providing the link between community composition and metabolic activity/CUE (Fierer *et al*., 2021) [26]. Metaproteomics allows the identification and quantification of enzymes involved directly in carbon cycles, thus providing an accurate measure of the biochemical mechanisms responsible for necromass transformation (Fierer *et al*., 2021) [26]. Together, integrating these different data sets in advanced bioinformatics (e.g., network analyses, flux balance modelling) provides the ability to model necromass dynamics predictively in the ecological context of specific microbial taxa (Fierer *et al*., 2021) [26].

Understanding how various effects from climate change such as; increased CO2 levels, temperature rise and changed precipitation patterns will alter the microbial necromass pathway of soil treated with organic amendments is currently very poorly understood (Fierer *et al*., 2021) [26] (Todd-Brown *et al*., 2013) [30]. For example, higher CO2 levels could promote rhizodeposition (root exudates) and increased microbe activity could potentially lead to more production of necromass but also faster decomposition of it at the same time (Jones *et al*., 2009) [22] (Liang *et al*., 2019) [5]. In general, higher temperatures will lead to a lower carbon use efficiency (CUE) for microbes and will increase the metabolic processes of all microbes, which could reverse any gains in carbon sequestered from amending soils with organic material (Sinsabaugh *et al*., 2013) [20] (Todd-Brown *et al*., 2013) [30]. To fully assess how climate change will impact use of the necromass-based carbon sequestration strategy, conducting Free Air CO2 Enrichment (FACE) studies paired with temperature treatment and organic amendment studies is needed (Todd-Brown *et al*., 2013) [30] (Fierer *et al*., 2021) [26].

Table 6: Knowledge gaps, methodological limitations, and future research priorities in microbial necromass-driven carbon sequestration research. Priority ratings: Very High = essential gap limiting field application; High = important constraint on mechanistic understanding; Moderate = desirable for improved precision.

Knowledge Gap / Limitation	Current Challenge	Proposed Solution / Technology	Priority
Quantification of necromass pools	Amino sugar method cannot distinguish live vs. dead biomass contributions	13C-SIP + NanoSIMS imaging; compound-specific isotope analysis	High
Long-term field data scarcity	Most studies are pot experiments <2 years; insufficient for MAOM tracking	Decadal observational field networks with amendment permanence	Very High
Fungal vs. bacterial necromass dynamics	Chitin shared by both; muramic acid diluted in sandy soils	Dual biomarker approaches with 15N labeling of muramic acid	High
Amendment dose optimization	Non-linear dose–response curves; thresholds poorly defined	Response surface methodology; machine learning models	Moderate
Biochar ageing effects	Fresh biochar properties differ substantially from aged material	Accelerated ageing protocols; 10-year incubation studies	High
Priming effect quantification	Separation of rhizosphere vs. bulk soil priming difficult	Rhizoboxes with isotopically labelled CO2 atmosphere	Moderate
Microbial CUE under field conditions	Lab CUE values poorly predict field necromass accumulation	Field 18O-H2O incorporation assays; metatranscriptomics	High
Climate change interactions	How elevated CO2 and warming alter necromass stabilization unknown	FACE + warming platforms with amendment treatments	High
Cross-site generalizability	Soil texture and mineralogy strongly modify amendment effects	Global synthesis across soil orders; pedotransfer functions	Moderate
Multi-omics integration	Functional gene expression linked to necromass formation underexplored	Metagenomics + metatranscriptomics + metabolomics pipeline	High

11. Conclusion

The creation of stable soil organic carbon in agricultural ecosystems involves microbial necromass being a critical but historically neglected route. In Vigna radiata rizhosphere, various combinations of root-derived carbon subsidies, nitrogen-fixing symbionts, and mycorrhzal associations create a microbially rich area with much higher rates of necromass production/soil volume than the bulk soil. Over time, the combination of compost and biochar have substantially increased this potential through synergism. Compost increases microbial biomass, diversity and carbon use efficiency by changing substrate stoichiometry, and providing many different kinds of new micro-organisms; biochar provides the physical habitat (porous) and surface area (stabilizing) through buffering of pH that protects the necromass from rapid re-mineralization.

In five years of research, coamending increased soil organic C (SOC) concentration by an average of 130% for coamended versus no amendment controls (both having previous maintenance periods). Of that SOC increase, roughly 48% was estimated as derived from microbial necromass carbon (based on glucosamine and muramic acid biomarker analyses). Fungal necromass carbon increased 317%, whereas bacterial necromass carbon increased 375%, demonstrating that both fungal and bacterial necromass were stimulated as a result of a broad range of soil biological activity from these two amendments used in tandem. Enhancing the soil physical properties (bulk density, aggregate stability and porosity), the enzyme activity and microbial community structure and diversity will strengthen the synergism between soil health and carbon sequestration.

Sustainable agricultural, soil quality, and climate change solutions have direct implications. Using both compost and biochar with pulse-based agricultural production systems (e.g., mung bean production) will provide a scalable, farmer accessible means of microbial necromass-mediated carbon storage and accumulation while maintaining or increasing crop yield and soil nutrient production in tropical and subtropical countries. Additionally, the co-benefits of compost-whole plant mulch farming (better water retention in soils; a substantial reduction in fertiliser application rates and therefore increased availability of nutrients for crops; enhanced or improved nutrient cycling) create considerable economic incentives for producers even without formalised carbon payment systems.

In order for this strategy to reach its full potential, we must overcome some critical knowledge gaps. Long-term (>10 year) field studies using standardized methods for measuring necromass accumulation will be required to confirm how long-term gains in SOC from amendment applications last and to determine how the microbial necromass pool will behave under various climatic conditions. By using emerging multiomics technologies (metagenomics, metatranscriptomics, and stable isotope probing) to evaluate the rhizosphere of amended soils, we will achieve the mechanistic insights needed to develop predictive models for necromass dynamics and optimize amendment formulations for different soil-crop systems. The creation of pedotransfer functions relating soil mineralogy and texture to SOC stabilization will allow us to forecast regionally specific carbon sequestration and subsequently create policy related to enhancing organic amendment subsidies for smallholder agriculture through carbon credit certification of pulse-laden crops. With the agricultural sector driving scientific interest in microbial carbon pumps as a major contributor to stable SOC formation, investment in research infrastructure to quantify and manage this process across all of agriculture will generate exceptionally high returns - both scientifically and socially.

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