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The impact of soil biological degradation: A review

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Abstract

Soil microorganisms are essential for nutrient cycling, organic matter decomposition, and pollutant detoxification, playing a vital role in ecosystem stability and soil health. This review examines the impact of agricultural practices and environmental factors on soil microbial communities and the consequent biological degradation. Intensive agriculture, characterized by monocropping and heavy use of chemical fertilizers, leads to reduced microbial diversity and activity, disrupting nutrient cycles and impairing soil fertility. Studies indicate that sustainable practices such as organic farming, conservation agriculture, and crop rotations enhance microbial activity, improve soil structure, and boost crop yields. Agroforestry systems and the use of organic amendments have shown higher microbial and enzymatic activities compared to traditional monocropping. Additionally, environmental disturbances like deforestation, mining, and fires significantly reduce microbial biomass and alter community structures, further degrading soil health. Mitigation strategies, including zero tillage and residue retention, are effective in combating biological degradation and improving soil health. This review underscores the need for sustainable land management practices to preserve soil microbial diversity, enhance soil fertility, and ensure long-term agricultural productivity. Future research should focus on promoting strategies that support microbial activity and contribute to the sustainability of agroecosystems.

Keywords: Biological degradation, soil biological indices, soil enzymes, soil microorganisms, MBN, MBC

Introduction

Soil degradation

It is defined as the rate of adverse change in soil qualities resulting in decline in productive capacity of land due to human intervention. Thus, it implies a decline in soil's productivity, deterioration in vegetative cover, qualitative and quantitative decline of water resources, degradation of soils and pollution of air. (Aulakh and Sidhu, 2004) ^[4]. Soil degradation in India is estimated to be occurring on 147 million hectares (M ha) of land including 94 M ha from water erosion, 16 M ha from acidification, 14 M ha from flooding, 9 M ha from wind erosion, 6 M ha from salinity and 7 M ha from a combination of factors. This is extremely serious because India supports 18% of the world's human population and 15% of the world's livestock population, but has only 2.4% of the world's land area (Bhattacharyya *et al.*, 2015) ^[7]. About 5000 tonnes of top soil is being eroded every year and nearly 30% (1600) tonnes is lost to sea in the form of river flow (Rakesh *et al.*, 2021) ^[53]. There are 4 types of soil degradation they are: 1. Physical degradation, 2. Chemical degradation, 3. Ecological degradation, 4. Biological degradation.

Soil biological degradation

Biological degradation of soil as described herein refers to the elimination of one or more "significant" populations of microorganisms in soil, often with a resulting change in biogeochemical processing within the associated ecosystem. This degradation adversely affects the diverse community of microorganisms which are essential for nutrient cycling, soil structure maintenance, and overall ecosystem stability. Can happen naturally or due to human activity. It includes: Loss of biodiversity, loss of soil enzymes, loss of vegetative cover, decline in soil organic matter content, loss of carbon sink capacity, loss of disease resistance, Increased soil borne pathogens.

Factors affecting soil biological degradation

1. Toxic Substances: Toxic metals like lead and cadmium can persist in soils and disrupt microbial communities (He *et al.*, 2005) [21]. Oil contamination negatively affects soil microbes and their activities (Mitchell *et al.*, 2012) [26]. Pesticides influence microbial processes such as nitrogen fixation and ammonification (Smith *et al.*, 2007) [67]. Acid mine drainage affects water with low pH from mining activities harms soil microbes (Krynock *et al.*, 2004) [29]. Coal mine drainage influence high concentrations of metals from coal mine drainage can be toxic to soil microbes. (Sanchez *et al.*, 2003) [58].

2. Land Management: Forest burning increases soil pH and alters soil structure, leading to nutrient loss and erosion. (Jones *et al.*, (2010) [27]. Deforestation reduces organic inputs, increases soil temperature and pH. (Lee *et al.*, (2011) [32]. Soil erosion removes organic matter and clay, impacting soil fertility (Sharma *et al.*, (2009) [59].

3. Soil Health Maintenance and Organic Agriculture: Organic matter supports a diverse range of soil organisms. Soil conditions improving aeration, temperature, and moisture benefits soil biodiversity (Madsen, 2008) [40]. Organic agriculture enhances soil biological activity and increases microbial mass (Tisdall and Oades, 1982) [77].

Soil microbial communities in response to global change factors:

Global change factors, such as elevated CO₂, warming, nitrogen (N) deposition, and alterations in precipitation patterns, have significant and complex impacts on soil microbial communities. Elevated CO₂ and warming are expected to enhance plant productivity, leading to increased organic matter input. This boost in substrate availability stimulates microbial activity, promoting soil organic carbon (SOC) decomposition and nutrient metabolism, although it may also alter microbial community composition (Rustad *et al.*, 2012) [55]. Increased N deposition, projected to rise significantly in the coming century, can cause soil acidification and change nutrient availability, often resulting in decreased microbial diversity and biomass due to shifts in pH and nutrient imbalances (Houlton *et al.*, 2008) [23]. Changes in precipitation, including variations in wetting-drying cycles and drought conditions, impose stress on microbial communities. However, soil microorganisms exhibit considerable physiological tolerance to these changes, maintaining microbial diversity under extreme conditions. Key microbial processes affected include the priming effect, where fresh organic inputs enhance decomposition of existing soil organic matter, and the entombing effect, where microbes immobilize organic matter (Sowerby *et al.*, 2008) [70]. Enhanced microbial activity also leads to increased soil respiration (Fierer and Jackson, 2006) [13]. Understanding these interactions is crucial for predicting future soil health and ecosystem functions.

Soil Biological Indices:

Soil biological degradation is a complex phenomenon influenced by a variety of microbial and environmental factors. Recent research has underscored the significance of several indicators in assessing soil health and degradation. Perovic *et al.* (2020) [51] highlighted that the abundance and diversity of microorganisms, including bacteria, fungi, actinomycetes, algae, and protozoa, serve as crucial indicators of soil biological degradation. Their study in the Sumadija and Western Serbia region illustrated how these microorganisms can reflect varying degrees of soil health,

with bacteria being the most prevalent. Complementing this, Milicic *et al.* (2006) [25] emphasized the importance of monitoring total microbial counts, specific groups such as ammonifiers and Azotobacter, and enzyme activities, particularly dehydrogenase, to predict soil degradation. These indicators provide insight into the microbial activity and health of the soil, which are critical for understanding degradation processes. Further exploration by Tian *et al.* (2021) [76] revealed the role of soil organic matter (SOM) composition in microbial abundance, highlighting that variations in SOM can significantly impact microbial communities. Xu *et al.* (2016) [87] expanded on this by examining how soil pH and nutrient availability affect microbial communities, adding another layer to our understanding of soil degradation. Enzyme activities, as a degradation indicator, were the focus of Zhang *et al.* (2018) [89], who demonstrated their utility in assessing soil health. Li *et al.* (2019) [35] examined how changes in land use impact microbial diversity, indicating that anthropogenic activities can influence microbial communities and soil health. The impact of climate change on soil microbial dynamics and health was explored by Wang *et al.* (2021) [82] and Zhao *et al.* (2020) [90], revealing that shifting climate conditions can alter microbial processes and contribute to soil degradation. This aligns with findings by Smith *et al.* (2017) [68] and Johnson *et al.* (2015) [26], who investigated how agricultural practices and microbial community roles affect soil degradation.

Causes of soil biological degradation

1. By change in agricultural practices

Intensive agricultural practices have been identified as a major factor contributing to soil biological degradation. Research indicates that practices such as frequent tillage, excessive use of synthetic fertilizers, and monoculture cropping significantly impact soil health. Wolinska *et al.* (2014) [86] conducted a comprehensive study to examine these effects, revealing that soils under intensive agricultural management exhibited lower microbial biomass (MB) and respiratory activity (RA) compared to non-cultivated control soils. Their findings highlight that intensive farming depletes organic matter, adversely affecting soil microbial communities. Other studies support these results, showing that reduced tillage and minimal disturbance can preserve soil structure and organic content, thus supporting healthier microbial ecosystems (Six *et al.*, 2004) [65]. Similarly, the application of high levels of chemical fertilizers and pesticides has been linked to decreased microbial diversity and activity, as these substances disrupt the natural balance of soil microorganisms (Giller *et al.*, 2009) [18]. Additionally, monoculture cropping systems, where a single crop species is grown repeatedly, contribute to nutrient imbalances and reduced microbial diversity, further exacerbating soil degradation (Liebman & Schipanski, 2003) [38]. The evidence underscores the need for a shift towards more sustainable agricultural practices, such as crop rotation, organic amendments, and reduced chemical inputs, to mitigate soil biological degradation and promote long-term soil health.

2. Fire

Fires significantly impact soil biological degradation by altering microbial biomass, community structure, and carbon dynamics. Studies reveal that fires lead to a notable decrease in microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN), though the ratio of microbial carbon to nitrogen (MCN: MBN) remains unchanged (Anderson and Domsch, 2010) [2]. This decline is due to the conversion of labile organic carbon into

more recalcitrant pyrogenic organic carbon, which is less accessible to microbes (Li *et al.*, 2021) ^[36]. As a result, microbial energy expenditure increases, particularly affecting soil fungi, whose biomass decreases by 27.17%, while bacterial biomass remains relatively stable due to bacteria's ability to form heat-resistant spores (Zhou *et al.*, 2017b) ^[92]. This shift reduces the fungal-to-bacterial biomass ratio by 29.01% and leads to a higher specific carbon dioxide emission rate (qCO_2), reflecting decreased microbial carbon utilization efficiency. In forest ecosystems, fires increase qCO_2 more significantly due to higher biomass and longer combustion times, with a greater impact observed in areas with a higher fungal-to-bacterial ratio (Zhou *et al.*, 2017) ^[91]. The response of soil organic carbon (SOC) to fires is negatively correlated with qCO_2 , indicating that reduced plant biomass and lower microbial carbon efficiency after fires contribute to SOC loss (Wardle and Ghani, 2018) ^[83].

3. Land cover

Land cover and land use changes significantly influence soil biological degradation by affecting soil microbial biomass and organic matter content across different soil depths. Tiwari *et al.* (2019) ^[78] observed that soil microbial biomass carbon (SMB-C), nitrogen (SMB-N), and phosphorus (SMB-P) were highest in the topsoil layer (0-10 cm) of natural forest soils and decreased in mixed forest, savanna, and agricultural lands, with the lowest values found in the 20-30 cm depth of agricultural soils. This decline is attributed to reduced plant residue inputs and soil organic matter content with increasing depth, a trend supported by Maharjan *et al.* (2017) ^[41], who linked decreased SMB with lower carbon inputs in sub-tropical soils. Leeuwen *et al.* (2017) ^[33] further demonstrated that land use changes significantly impact SMB, especially in the upper soil horizons, due to variations in substrate quality and quantity. The superior SMB-C, SMB-N, and SMB-P in natural and mixed forests compared to savanna and agricultural lands reflect the influence of dominant vegetation cover on soil organic matter and nutrient levels. Agricultural practices, including frequent tillage and crop cultivation, contribute to reduced SMB levels, as noted by Peixoto *et al.* (2006), who highlighted soil disturbances as key factors affecting microbial communities. Zhang *et al.* (2016) ^[88] observed higher microbial biomass in afforested and reforested sites compared to cropland, emphasizing the benefits of forested environments for microbial health. Singh and Ghoshal (2014) ^[62-63] pointed out that converting dense forests to farmland disrupts canopy cover and physical soil conditions, leading to increased soil erosion and reduced microbial biomass. Additionally, intense cattle grazing and conventional farming practices further diminish organic matter and destabilize microbial communities in savanna and agricultural lands. These findings collectively underscore the profound effects of land cover on soil biological degradation, highlighting the importance of land management practices in maintaining soil health.

4. Land use systems

Land use systems profoundly affect soil biological degradation by influencing microbial biomass, enzyme activities, and carbon cycling. Sharma *et al.* (2022) ^[60] demonstrated that microbial biomass carbon (MBC) and basal soil respiration (BSR) were significantly higher under agroforestry systems compared to mono-cropping systems, with agroforestry exhibiting 83% higher MBC and a 32% higher BSR. The respiratory quotient (qCO_2) was 2.3 times higher in mono-cropping soils, indicating faster decomposition and lower BSR compared to agroforestry. This difference is partly due to intensified tillage in mono- and

double-cropping systems, which disrupts soil aggregates and exposes soil organic matter (SOM) to microbial oxidation (Six *et al.*, 2002) ^[66]. The higher enzymatic activities, such as alkaline phosphatase (Alk-P), dehydrogenase (DHA), and fluorescein diacetate (FDA) in agroforestry soils, reflect enhanced microbial function and SOM stabilization. Conversely, mono- and double-cropping systems showed higher acid phosphatase (acid-P) activity, likely due to lower available phosphorus in these systems compared to agroforestry (Sharma *et al.*, 2022) ^[60]. This aligns with findings by Lehmann *et al.* (2015) ^[34], who emphasized that plant-mediated carbon inputs and microbial by-products are crucial in SOM stabilization. Benbi *et al.* (2012) ^[5] and Singh *et al.* (2018) ^[61, 64] highlighted that microbial residues contribute significantly to the stable mineral-associated carbon pool. Sokol *et al.* (2019) ^[69] noted that higher MBC in agroforestry promotes the assimilation and transformation of root carbon inputs into microbial necromass. Additionally, Saikia *et al.* (2019) ^[56] found that increased plant productivity in agroforestry enhances root exudates and biomass, positively influencing soil enzymatic activity. Collectively, these studies illustrate that land use systems, especially those incorporating agroforestry, tend to support higher microbial biomass and enzyme activities, thereby mitigating biological degradation and enhancing soil health compared to more intensive mono-cropping and double-cropping systems.

4.1 Impacts of land use system

Different land use systems significantly impact the distribution and stabilization of soil organic carbon (SOC) fractions and microbial biomass carbon (MBC). Sharma *et al.* (2022) ^[60] reported that total organic carbon (TOC) was significantly lower in mono-cropping systems compared to agroforestry systems, attributing higher TOC in agroforestry to greater carbon inputs and rhizodeposition. Agroforestry systems contribute 2.9-3.3 Mg ha⁻¹ year⁻¹ of leaf litter, enhancing soil organic matter (Chauhan *et al.*, 2011) ^[11]. Increased root biomass in agroforestry further releases more carbon as rhizodeposition, enhancing carbon storage (Pausch *et al.*, 2018) ^[50]. In contrast, intensive tillage in mono- and double-cropping systems reduces TOC due to disruption of soil aggregates and exposure of organic matter to oxidation. The TOC fractions, classified by oxidizability, showed that very labile carbon (Fract. 1) was the smallest fraction, while recalcitrant carbon (Fract. 4) was the largest across all land-use systems. The abundance order of these fractions was Fract. 1 < Fract. 2 < Fract. 3 < Fract. 4, indicating a greater stability of carbon in agroforestry soils due to a higher proportion of recalcitrant fractions. Labile carbon pools, with faster turnover rates, are crucial indicators of short- and medium-term changes in soil organic matter (Singh *et al.*, 2018) ^[61, 64]. The recalcitrant carbon pool, although less reactive, decomposes more readily upon interaction with decomposer microorganisms (Lehmann *et al.*, 2015) ^[34]. The active carbon pool (Fract. 1 and Fract. 2) was significantly higher in mono-cropping soils by 24%, whereas the stable carbon pool (Fract. 3 and Fract. 4) was 76% higher in agroforestry soils, underscoring the latter's role in carbon sequestration due to slower microbial alteration of these fractions (Islam *et al.*, 2003) ^[25]. The microbial biomass carbon showed the lowest percentage change in double-cropping soils (38.1%) and the highest in agroforestry soils (82.7%), highlighting the enhanced microbial activity and carbon stabilization in agroforestry systems. Land use changes significantly affect SOC and MBC dynamics, with agroforestry systems demonstrating superior carbon storage and stability due to enhanced organic matter inputs and reduced disturbance. This

effect is further supported by numerous studies: Six *et al.* (2002) ^[66] highlighted that tillage disrupts soil aggregates, exposing SOM to oxidation, while Benbi *et al.* (2012) ^[5] and Singh *et al.* (2018) ^[61, 64] emphasized the role of microbial residues in forming stable carbon pools. Sokol *et al.* (2019) ^[69] noted that increased MBC in agroforestry systems enhances carbon transformation into microbial necromass, contributing to long-term carbon sequestration. Saikia *et al.* (2019) ^[56] and Chauhan *et al.* (2011) ^[11] found that higher plant productivity and root biomass in agroforestry systems lead to increased carbon inputs and microbial activity, further stabilizing soil carbon. Pausch *et al.* (2018) ^[50] demonstrated that rhizodeposition significantly boosts carbon storage in agroforestry soils. Lehmann *et al.* (2015) ^[34] and Islam *et al.* (2003) ^[25] provided insights into the decomposition dynamics of labile and recalcitrant carbon pools, highlighting their roles in soil carbon stability.

5. Tillage

Different tillage practices significantly impact various soil properties, including microbial community composition, enzyme activity, and soil organic carbon (SOC) content. Wang *et al.* (2020) ^[81] found that long-term no-tillage with straw mulching significantly altered bacterial and fungal abundances, especially with more frequent mulching. Mangalassery *et al.* (2015) ^[42] observed that zero-tilled soils had 9% more soil carbon and 30% higher microbial biomass carbon than tilled soils due to crop residue retention and lower decomposition rates (Sainju *et al.*, 2005; Lal *et al.*, 2004) ^[57, 31]. Higher microbial enzyme activities, such as dehydrogenase, cellulase, and β -glucosidase, were reported in zero-tilled soils, indicating improved microbial functioning and carbon sequestration (Roldan *et al.*, 2005) ^[54]. Maharjan *et al.* (2017) ^[41] found increased enzyme activities in non-disturbed pasture soils due to active microbial biomass and stable extracellular enzymes, while Melero *et al.* (2009) ^[43] noted that zero-tillage creates a less oxidizing environment, enhancing enzyme stability. Katsalirou *et al.* (2010) ^[28] observed that enzymes involved in carbon metabolism were positively correlated with carbon content, supporting carbon sequestration in zero-tilled soils. Bhattacharyya *et al.* (2018) ^[6] concluded that combining residue retention with zero tillage (ZT) and permanent bed planting (PBB) significantly increased dehydrogenase (DHA) and fluorescein diacetate (FDA) activities in the topsoil. This increase in enzyme activity is linked to organic matter accumulation from crop residues, boosting microbial activity (Włodarczyk *et al.*, 2002; Green *et al.*, 2006) ^[85, 20]. Madejon *et al.* (2007) ^[39] confirmed that crop residues and zero tillage favor overall soil biological activity compared to conventional tillage (CT), highlighting the benefits of reduced soil disturbance and residue retention for maintaining soil health.

6. Imbalanced fertilization

Long-term manuring and balanced chemical fertilization have profound effects on soil biological properties, significantly enhancing microbial communities. Continuous application of farmyard manure (FYM) combined with balanced chemical fertilization (100% NPK + FYM) results in the highest populations of bacteria, fungi, and actinomycetes, outperforming other treatments (Tejada *et al.*, 2008) ^[75, 1]. This combination provides a substantial amount of readily available carbon, crucial for chemoheterotrophic microorganisms that rely on organic substances for cellular synthesis and energy. Incorporation of organic matter through FYM improves soil physical conditions, creating a conducive environment for

microbial proliferation (Suresh *et al.*, 1999) ^[73]. Conversely, imbalanced fertilization, such as 100% N or NP, significantly reduces microbial populations by depleting native nutrient reservoirs and reducing plant root biomass, which are essential carbon substrates for soil microbes (Mangalassery *et al.*, 2015) ^[42]. Lime application with optimal NPK negatively impacts fungal communities due to increased soil pH, whereas treatments like 100% NPK + HW show better microbial counts, though still lower than FYM-enhanced plots (Bhattacharyya *et al.*, 2018) ^[6]. Nitrogen-cycling bacteria, particularly ammonia-oxidizing bacteria (AOB), flourish significantly more under 100% NPK + FYM treatment, indicating the inadequacy of N or NP alone in maintaining high AOB populations (Singh *et al.*, 2014) ^[62-63]. Continuous FYM application also promotes enzyme activities, with higher dehydrogenase and fluorescein diacetate activities observed, enhancing soil biological activity (Roldan *et al.*, 2005) ^[54]. The combined approach of manuring and balanced fertilization thus optimizes soil health by sustaining robust microbial communities and enzymatic functions (Lal *et al.*, 2004) ^[31]. These practices ensure a stable and healthy soil ecosystem, contributing to sustainable agricultural productivity and mitigating climate change impacts by enhancing soil carbon sequestration (Melero *et al.*, 2009; Katsalirou *et al.*, 2010) ^[43, 28].

7. Residue management practices

Residue management practices play a crucial role in influencing soil microbial populations and enzyme activities. Kumar *et al.* (2019) ^[30] observed a significant reduction in bacterial, fungal, and actinomycete populations following residue burning, attributed to the high temperatures generated. Biederbeck *et al.* (1980) ^[8] and Mickovski (1967) ^[44] also noted a substantial decline in heterotrophic microorganisms post-burning due to soil surface heating up to 50-70°C. Conversely, Govaerts *et al.* (2008) ^[19] reported that retaining crop residues promoted higher populations of bacteria, fluorescent *Pseudomonas*, and actinomycetes under zero and conventional tillage. Soil enzyme activities, indicators of microbial growth and activity, were also affected by residue management. Kumar *et al.* (2019) ^[30] found that cellulase, β -glucosidase, xylanase, and dehydrogenase activities were significantly reduced after burning, with the highest activities observed in residue-retained plots. Nannipieri (1994) ^[48] identified dehydrogenase as a sensitive indicator of soil quality and microbial activity. Madejon *et al.* (2007) ^[39] and Tao *et al.* (2009) ^[74] also reported higher dehydrogenase activity in conservation agriculture with legume crops. Stott *et al.* (2010) ^[71] highlighted β -glucosidase as crucial for degrading crop residues and providing simple sugars for microbial populations, with its activity significantly lower after burning. Garcia-Ruiz *et al.* (2008) ^[16] demonstrated that organic amendments enhance β -glucosidase activity. Carboxymethyl cellulase (CMCase) and xylanase, enzymes acting on cellulose and hemicellulose, showed highest activities in residue-retained plots due to greater availability of substrates and increased populations of cellulose degraders. This aligns with findings from Kumar *et al.* (2019) ^[30], indicating that organic matter incorporation boosts these enzyme activities, supporting enhanced microbial function and soil quality. The Impact of Straw Mulching on Soil Microbial Networks explained wang *et al.* (2020) ^[81] conducted a detailed network analysis to investigate the effects of straw mulching on soil microbial communities. Their study revealed that straw mulching led to less complex bacterial and fungal networks compared to no mulching treatments. This was indicated by lower average connectivity and fewer links and nodes in the mulching treatments. However, mulching increased the ratio of

positive to negative links in microbial networks, suggesting enhanced cooperative interactions. Specifically, bacterial networks showed increased average path length and modularity, with decreased clustering coefficients, while fungal networks exhibited the opposite trend, indicating more cohesive fungal interactions under mulching. Supporting evidence aligns with these findings. Wang *et al.* (2020)^[81] found that organic fertilization, including straw mulching, decreased microbial network complexity. Noted that such changes could be due to reduced competition and inhibition among microbes in enriched conditions. This highlights the role of straw mulching in altering microbial interactions, which can impact soil health, nutrient cycling, and overall fertility.

8. Conservation agriculture (CA) practices

Conservation agriculture (CA) practices, including no-tillage (NT) and residue management, significantly influence soil biological properties, as highlighted by various studies. Found that NT compared to conventional tillage (CT) reduces soil physical disturbance and enriches organic matter at the soil surface, enhancing conditions for microbial life. NT showed higher total phospholipid fatty acids (PLFAs), indicating increased microbial biomass, particularly for fungi and bacteria (Helgason *et al.*, 2009). However, NT also led to lower MUFA/STFA ratios, suggesting more anaerobic conditions (Bossio *et al.*, 1998). Residue returning further boosted microbial activity, increasing total PLFAs, bacterial and fungal biomass, and reducing microbial stress by 45.9% (Zhu *et al.*, 2014). Residue incorporation improved aeration and C/N ratios, promoting higher fungal to bacterial ratios. Wang *et al.* (2020)^[81] observed the highest microbial populations in residue retention treatments under zero tillage (ZT), indicating that residue enhances microbial food sources (Govaerts *et al.*, 2008)^[19]. This is supported by findings that residue retention increases microbial counts and diversity compared to residue removal (Govaerts *et al.*, 2008; Nicolardot *et al.*, 2007)^[19, 49]. Significant interactions between tillage and residue management affect microbial dynamics, with conservation practices fostering higher fungal and bacterial populations (Ghimire *et al.*, 2014)^[17]. Additionally, the MBC/MBN ratio, which reflects microbial community structure, was lowest under ZT with residue, indicating a balanced microbial biomass (Moore *et al.*, 2000)^[47]. Overall, conservation agriculture practices, particularly residue management and no-tillage, enhance soil biological properties by improving microbial biomass, activity, and diversity, which are crucial for soil health and sustainability.

9. Biofertilizers

Biofertilizers, particularly those containing beneficial microbes, have shown significant impacts on soil biological properties, enhancing microbial abundance and activity. Demonstrated that biofertilizer (BF) treatments led to the highest levels of soil microbial populations, including bacteria, fungi, actinomycetes, and *Trichoderma*, compared to control treatments. Specifically, the total bacterial populations in BF treatments increased to Log8.6, whereas control treatments yielded lower counts (Log7.9). The actinomycete populations initially decreased but later increased in all treatments, while *Trichoderma* showed variable responses, peaking in BF and residue treatments. These findings are consistent with previous studies highlighting the benefits of biofertilizers. For instance, Adesemoye *et al.* (2009)^[1] and Huang *et al.* (2011)^[24] reported that *Trichoderma harzianum*, a common biofertilizer, enhances root growth through the secretion of auxin-like phytohormones. This

improved root growth leads to increased exudation of sugars and organic acids, which in turn stimulates soil microbial activity and nutrient availability. The enhanced microbial populations promote better soil health and nutrient cycling, benefiting plant growth. Incorporating biofertilizers can thus significantly boost soil microbial diversity and function. Their use supports sustainable agricultural practices by improving soil fertility and health, thereby enhancing overall crop productivity.

10. Co-occurrence network analysis

The co-occurrence network analysis offers valuable insights into the interactions between different fungal guilds in agricultural soils, particularly in relation to soil organic carbon (SOC) content. By categorizing soil samples into high SOC (> 20 g kg⁻¹) and low SOC (< 20 g kg⁻¹) groups, notable differences in the fungal community structure were observed. In the high SOC group, the proportion of phytopathogens was 1.40 times greater than in the low SOC group. Additionally, the proportion of positive correlated edges increased by 4.78%, and interactions within phytopathogens rose by 9.68%, although the average degree of interactions decreased by 1.98. This suggests that higher SOC content not only supports a greater prevalence of phytopathogens but also fosters more cooperative interactions among fungal taxa. Microbial interactions, often illustrated by positive (cooperation) and negative (competition) connections between nodes in a co-occurrence network, play a crucial role in regulating soil fungal phytopathogens (Faust *et al.*, 2012)^[12]. The higher proportion of positive connections in the high SOC group implies increased cooperation between fungal taxa, which may create conditions favorable for pathogens. This is consistent with the notion that a microbial community with more cooperation can produce more public goods, benefiting pathogens (Li *et al.*, 2019)^[37]. Similar findings have been reported, where microbial communities in diseased soils exhibited more positive connections compared to healthy soils (Wei *et al.*, 2019)^[84]. Furthermore, competition between pathogens and beneficial microorganisms often occurs, with the balance between these interactions influencing soil health (Raaijmakers *et al.*, 2009; Singh *et al.*, 2014)^[52, 62-63]. Thus, understanding these complex interactions in the context of SOC content is vital for managing soil health and mitigating pathogen prevalence in agricultural systems.

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