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Soil microbiome responses to climate change: Implications for regenerative agriculture and environmental sustainability

Bochu Meghana[1*], Bommakanti Shivani[2], Dr. Raju Chavan[3]
[1] Department of Microbiology, College of Agriculture, UAS Dharwad, Karnataka, India
[2] Department of Human Development and Family Studies, College of Community Science, UAS Dharwad, Karnataka, India
[3] Professor, Department pf Environmental Science, College of Agriculture, UAS Dharwad, Karnataka, India
Corresponding Author: Bochu Meghana
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Abstract
Climate change significantly affects soil microbial diversity, abundance, activity, and community structure through rising temperatures, drought, flooding, salinity, elevated atmospheric carbon dioxide, and extreme weather events. Soil microbiomes, comprising bacteria, fungi, archaea, and actinomycetes, play essential roles in nutrient cycling, nitrogen fixation, phosphorus solubilization, organic matter decomposition, carbon sequestration, and plant–microbe interactions. Climate-induced disturbances can alter these functions, influencing soil fertility, agricultural productivity, and ecosystem resilience. This review examines soil microbiome responses to climate change and explores regenerative agriculture as a strategy for improving soil health and environmental sustainability.
The review highlights microbial adaptation mechanisms and the contributions of regenerative practices, including cover cropping, reduced tillage, crop diversification, organic amendments, biochar, and microbial bio-inputs, in enhancing microbial diversity, nutrient availability, and soil organic carbon. Microbial indicators and emerging technologies such as metagenomics, metabolomics, artificial intelligence, and microbiome engineering are discussed for evaluating soil regeneration and developing sustainable soil management strategies. However, challenges include limited long-term field studies, inconsistent microbial indicators, complex microbiome data interpretation, and biosafety concerns. Integrating soil microbiome research with context-specific regenerative practices and long-term field validation can strengthen climate-resilient agriculture, improve soil health, and support environmental sustainability under changing climatic conditions.

Keywords: Soil microbiome, climate change, regenerative agriculture, soil health, microbial diversity, environmental sustainability

Introduction
A mechanistic understanding requires distinguishing microbial community composition from realized ecosystem function. Taxonomic shifts do not necessarily imply proportional changes in nutrient transformation because organisms differ in a physiological state, substrate access, and functional redundancy. Equally, an increase in microbial biomass is not automatically evidence of net carbon sequestration: microbial growth, respiration, residue production, and stabilization must be considered together. This review therefore adopts a process-based framework linking climate exposure, microbial traits, plant–soil feedbacks, and measurable soil outcomes.
Climate change has emerged as one of the most significant global challenges affecting agricultural productivity, ecosystem stability, and environmental sustainability (IPCC, 2023). Increasing temperatures, irregular precipitation patterns, drought, flooding, salinity, and extreme climatic events are profoundly influencing soil ecosystems and their biological functionality. Soil serves as a dynamic living system that supports nutrient cycling, carbon sequestration, plant productivity, and ecosystem resilience, with soil microorganisms playing central roles in maintaining these ecological processes (van der Heijden *et al*. 2008) [20].
The soil microbiome, comprising bacteria, fungi, archaea, actinomycetes, and other microbial communities, represents one of the most diverse and functionally important components of terrestrial ecosystems (Fierer, 2017) [8]. These microorganisms regulate critical biogeochemical processes including nitrogen fixation, phosphorus solubilization, organic matter decomposition, greenhouse gas regulation, and rhizosphere interactions (Nannipieri *et al*. 2020) [13]. However, climate-induced environmental stress can significantly alter microbial diversity, abundance, metabolic activity, and community structure, thereby disrupting soil ecosystem stability and agricultural sustainability (Jansson and Hofmockel, 2020) [10].
Recent studies indicate that climate change-driven disturbances may reduce beneficial microbial populations, impair nutrient transformation processes, destabilize soil carbon dynamics, and compromise plant–microbe interactions (Bardgett and Caruso, 2020) [2]. Simultaneously, certain stress-adapted microbial groups may exhibit resilience mechanisms that enable survival under adverse environmental conditions. Understanding these microbial responses is therefore essential for developing sustainable and climate-resilient agricultural systems.
Regenerative agriculture has gained increasing attention as a sustainable management approach capable of restoring soil biodiversity, enhancing microbial functionality, improving soil organic carbon, and strengthening ecosystem resilience (Lal, 2020) [11]. Practices such as cover cropping, reduced tillage, organic amendments, crop diversification, and microbial bioinputs may positively influence soil microbiome composition and functionality under climate stress conditions (Toju *et al*. 2018) [18].
This review critically examines the effects of climate change on soil microbiome dynamics and explores the role

of regenerative agriculture in restoring microbial ecosystem functionality. Particular emphasis is placed on microbial indicators of soil regeneration, microbiome-mediated resilience mechanisms, emerging omics technologies, and future microbiome engineering strategies for sustainable agricultural and environmental management.

Soil Microbiome and Ecosystem Functionality

Microbial carbon-use efficiency (CUE), the fraction of assimilated carbon allocated to growth rather than respiration, provides a useful conceptual bridge between decomposition and soil carbon persistence. Microbial residues, including cell-wall compounds and other necromass constituents, can become protected through association with reactive minerals or occlusion within aggregates. The resulting mineral-associated organic matter (MAOM) differs from particulate organic matter (POM) in formation pathways and vulnerability to disturbance. Neither a high fungal-to-bacterial ratio nor a high diversity index alone establishes superior carbon storage; mineralogy, moisture, substrate chemistry, and microbial turnover all matter. In nitrogen cycling, the balance among mineralization, immobilization, nitrification, denitrification, and dissimilatory nitrate reduction to ammonium determines nutrient availability and gaseous losses.

The soil microbiome represents a highly complex and dynamic biological system composed of bacteria, fungi, archaea, actinomycetes, protozoa, and other microscopic organisms that collectively regulate essential ecosystem processes (Fierer, 2017) [8]. Soil microorganisms are fundamental drivers of terrestrial ecosystem functionality and play critical roles in maintaining soil fertility, plant productivity, nutrient cycling, and environmental sustainability (van der Heijden *et al*. 2008) [20]. The rhizosphere serves as a hotspot for microbial interactions and biochemical activities that strongly influence ecosystem resilience and agricultural performance (Philippot *et al*. 2013) [14].

One of the primary ecological functions of soil microorganisms involves nutrient cycling and biogeochemical transformations. Microbial communities' mediate nitrogen fixation, nitrification, denitrification, phosphorus solubilization, sulfur transformation, and organic matter decomposition, thereby controlling nutrient availability within agricultural and natural ecosystems (Nannipieri *et al*. 2020) [13]. Beneficial microorganisms such as plant growth-promoting rhizobacteria (PGPR), mycorrhizal fungi, and phosphate-solubilizing bacteria contribute significantly to plant nutrition and stress tolerance through multiple biochemical mechanisms (Compant *et al*. 2019) [4].

Soil microbiomes also play an important role in carbon sequestration and climate regulation. Microbial decomposition of plant residues influences soil organic carbon dynamics, greenhouse gas emissions, and long-term carbon stabilization (Six *et al*. 2006) [17]. Certain microbial communities contribute to soil aggregation and structural stability through production of extracellular polymeric substances and biofilm formation, which improve soil water retention, aeration, and resistance to erosion.

In addition to nutrient regulation, soil microorganisms contribute to suppression of soil-borne pathogens and maintenance of ecological balance within the rhizosphere. Competitive microbial interactions, antimicrobial metabolite production, and induced systemic resistance mechanisms help protect plants against pathogenic invasion and environmental stress (Philippot *et al*. 2013) [14].

Microbial diversity is widely recognized as an important indicator of soil health and ecosystem multifunctionality. Greater microbial diversity generally enhances ecological resilience, functional redundancy, and adaptive capacity under environmental disturbances (Bastida *et al*. 2021) [3]. Consequently, disturbances affecting microbial community composition may significantly influence ecosystem stability, agricultural sustainability, and regenerative soil functionality.

Advances in molecular biology and omics technologies have greatly improved understanding of soil microbiome complexity and ecological interactions. Metagenomics, metatranscriptomics, metabolomics, and high-throughput sequencing approaches now enable detailed characterization of microbial diversity, functional genes, and ecosystem processes associated with soil health and climate resilience.

Climate Change Impacts on Soil Microbiomes

Climate drivers rarely act independently. Warming can intensify water deficits, whereas heavy rainfall can generate transient anaerobic microsites even in nominally well-drained soils. Consequently, factorial studies and repeated measurements are preferable to single-factor, single-date comparisons. Response magnitude also depends on baseline soil pH, texture, organic matter, crop identity, and management history.

Climate change is significantly altering soil ecosystems through increasing temperatures, changing precipitation patterns, elevated atmospheric carbon dioxide concentrations, salinity intrusion, flooding, and frequent extreme weather events. These environmental disturbances strongly influence soil microbial diversity, metabolic activity, community structure, and ecosystem functionality (Jansson and Hofmockel, 2020) [10]. Since soil microorganisms are highly sensitive to environmental fluctuations, climate-induced stress can disrupt critical biological processes associated with nutrient cycling, carbon sequestration, soil fertility, and plant–microbe interactions.

1. Elevated Temperature

Rising global temperatures directly affect microbial metabolism, enzymatic activity, and soil respiration rates. Increased temperature may accelerate organic matter decomposition and microbial carbon turnover, leading to enhanced carbon dioxide release from soils (Bardgett and Caruso, 2020) [2]. Long-term warming can alter microbial community composition by favoring thermotolerant microbial populations while reducing temperature-sensitive taxa.

2. Drought Stress

Drought is considered one of the most significant climate-related factors affecting soil microbial ecosystems (Schimel, 2018) [15]. Water limitation reduces microbial biomass, enzyme activity, nutrient diffusion, and microbial metabolic processes. Drought conditions often decrease bacterial diversity while selecting for stress-tolerant microorganisms capable of surviving desiccation stress through dormancy,

osmolyte production, and biofilm formation (de Vries *et al*. 2018) [6].

3. Flooding and Waterlogging

Flooding alters soil oxygen availability and creates anaerobic conditions that significantly influence microbial community dynamics. Waterlogged soils promote growth of anaerobic microorganisms including methanogens and denitrifying bacteria while suppressing aerobic microbial populations. These changes may increase greenhouse gas emissions such as methane and nitrous oxide (Bardgett and Caruso, 2020) [2].

4. Salinity Stress

Climate change-associated salinity intrusion can adversely affect soil microbial ecosystems. High salinity levels reduce microbial diversity, alter enzyme activities, and suppress beneficial rhizosphere microorganisms. Salinity stress can impair nutrient uptake, microbial respiration, and organic matter decomposition, ultimately reducing soil productivity and biological functionality.

5. Elevated Atmospheric CO_2

Increased atmospheric carbon dioxide concentrations influence plant physiology, root exudation patterns, and rhizosphere microbial interactions. Elevated CO_2 may stimulate microbial activity by increasing carbon inputs into the rhizosphere through enhanced root biomass and exudates (Jansson and Hofmockel, 2020) [10].

6. Extreme Weather Events

Frequent heatwaves, storms, wildfires, and irregular climatic fluctuations can rapidly disturb soil microbial communities and ecosystem processes. Extreme events often reduce microbial diversity, destabilize soil structure, and impair nutrient transformation pathways.

Table 1: Climate stressors and ecosystem consequences

Climate Stressor	Effects on Soil Microbiome	Effects on Ecosystem Processes	Major Consequences
Elevated temperature	Alters microbial community structure, reduces temperature-sensitive taxa, increases thermotolerant microbes	Accelerates organic matter decomposition and soil respiration	Increased carbon loss and reduced soil stability
Drought stress	Reduces microbial biomass and diversity, suppresses enzyme activities	Impairs nutrient cycling and rhizosphere interactions	Reduced soil fertility and lower crop productivity
Flooding/Waterlogging	Promotes anaerobic microorganisms and suppresses aerobic microbes	Alters nitrogen cycling and increases methane production	Greenhouse gas emissions and soil degradation
Salinity stress	Decreases microbial diversity and metabolic activity	Disrupts nutrient transformation and microbial respiration	Reduced ecosystem multifunctionality
Elevated CO_2	Alters rhizosphere microbial interactions and carbon allocation	Influences carbon cycling and microbial metabolism	Changes in soil organic carbon dynamics
Extreme weather events	Disturbs microbial ecological balance and ecosystem stability	Reduces resilience and ecosystem recovery capacity	Ecosystem instability and productivity decline

Microbial Adaptation Mechanisms under Climate Stress

At the community scale, resilience may arise through physiological acclimation within taxa, selection among taxa, dispersal from refugia, and changes in trophic interactions. Resistance denotes the magnitude of change during disturbance; resilience denotes the trajectory and extent of recovery. These should not be used interchangeably. Trait-based approaches, including growth rate, osmotic tolerance, extracellular enzyme investment, and substrate-use breadth, can explain responses more directly than broad taxonomic labels.

Soil microorganisms possess diverse physiological, metabolic, and ecological adaptation mechanisms that enable survival under climate-induced environmental stress conditions. Despite significant disturbances caused by elevated temperature, drought, flooding, salinity, and nutrient fluctuations, many microbial communities exhibit resilience through functional plasticity, stress-responsive metabolism, and cooperative ecological interactions.

1. Stress Tolerance Mechanisms

Microorganisms respond to climate stress through activation of stress tolerance pathways that protect cellular integrity and metabolic functionality. Under drought and salinity conditions, many bacteria and fungi accumulate compatible solutes such as proline, trehalose, glycine betaine, and exopolysaccharides that help maintain osmotic balance and cellular hydration.

2. Biofilm Formation

Biofilm formation is an important microbial survival strategy under environmental stress. Soil microorganisms produce extracellular polymeric substances (EPS) that facilitate microbial aggregation and surface attachment, creating protective microenvironments around cells.

3. Dormancy and Sporulation

Many soil microorganisms survive unfavorable environmental conditions through dormancy and sporulation. Spore-forming bacteria such as Bacillus species possess highly resistant structures capable of surviving extreme temperature, dehydration, and nutrient limitation.

4. Functional Redundancy

Functional redundancy refers to the presence of multiple microbial taxa capable of performing similar ecological functions within soil ecosystems. Even when certain microbial groups decline under climate stress, other functionally similar microorganisms may compensate and maintain nutrient cycling and ecosystem functionality.

5. Metabolic Plasticity and Genetic Adaptation

Microbial communities exhibit remarkable metabolic flexibility that enables utilization of diverse carbon and nutrient sources under fluctuating environmental conditions. Horizontal gene transfer, mutation, and rapid evolutionary adaptation may further enhance microbial survival under long-term climate stress.

Regenerative Agriculture and Soil Microbiome Restoration

Regenerative agriculture is best evaluated as a set of testable practices rather than a uniform treatment category. Management outcomes depend on climate, soil type, crop sequence, nutrient balance, and time since adoption. Assessments should jointly consider yield stability, soil carbon stocks, nutrient-use efficiency, biodiversity, and greenhouse-gas trade-offs rather than relying on a single microbial endpoint.

Regenerative agriculture has emerged as a sustainable land management approach aimed at restoring soil health, improving ecosystem functionality, enhancing biodiversity, and increasing agricultural resilience under climate change conditions (Lal, 2020) [11]. Soil microbiomes play a central role in these regenerative systems by supporting nutrient cycling, carbon sequestration, soil aggregation, and plant health.

1. Cover Cropping and Soil Microbiome Restoration

Cover cropping is widely recognized as an effective regenerative agricultural practice capable of improving soil biological activity, nutrient cycling, and ecosystem resilience under climate stress conditions (Lal, 2020) [11]. Cover crops enhance soil organic carbon inputs through root exudates and plant residues, thereby stimulating microbial growth and increasing rhizosphere microbial diversity (Toju *et al*. 2018) [18]. Several studies have demonstrated that diversified cover crop systems significantly improve microbial biomass, enzyme activities, and abundance of beneficial microorganisms involved in nitrogen fixation and phosphorus solubilization (Bastida *et al.* 2021) [3].

The rhizosphere associated with cover crops serves as a biologically active niche that supports complex microbial interactions and ecological stability (Philippot *et al.* 2013) [14]. Increased microbial diversity under cover cropping systems may enhance nutrient transformation efficiency, suppress soil-borne pathogens, and improve soil aggregation through microbial extracellular polymeric substances production (Nannipieri *et al.*, 2020) [13].

2. Reduced Tillage and Microbial Stability

Conventional intensive tillage practices can severely disrupt soil microbial habitats, reduce fungal biomass, accelerate organic matter decomposition, and destabilize soil structure (Six *et al*. 2006) [17]. In contrast, reduced tillage and conservation agriculture practices help preserve soil microbial communities and maintain ecosystem functionality under climate stress conditions.

Reduced tillage systems generally support greater microbial biomass, fungal abundance, and soil aggregation compared to conventionally tilled soils (Bastida *et al*. 2021) [3]. Preservation of fungal hyphal networks and microbial microhabitats contributes to improved nutrient cycling and soil structural integrity (Nannipieri *et al*., 2020) [13].

3. Organic Amendments and Compost Application

Application of organic amendments such as compost, farmyard manure, vermicompost, crop residues, and biochar has been widely recognized as an effective strategy for improving soil biological activity and restoring microbial ecosystem functionality under climate stress conditions (Lehmann *et al.* 2020) [12]. Organic inputs serve as major carbon and nutrient sources for soil microorganisms, thereby stimulating microbial growth, enzymatic activity, and nutrient cycling processes (Nannipieri *et al*., 2020) [13]. Increased availability of organic substrates enhances microbial biomass and promotes establishment of beneficial microbial communities involved in decomposition, mineralization, and soil aggregation.

Compost application significantly improves rhizosphere microbial diversity by enriching populations of beneficial bacteria, fungi, actinomycetes, and plant growth-promoting microorganisms (Bastida *et al*. 2021) [3]. Organic amendments may also suppress soil-borne pathogens through microbial competition, antibiotic production, and enhanced antagonistic microbial interactions (Compant *et al*. 2019) [4]. Furthermore, increased microbial metabolic activity following compost addition contributes to improved nitrogen cycling, phosphorus solubilization, and organic matter stabilization within soil ecosystems.

Biochar amendments have gained considerable attention due to their ability to improve soil physicochemical properties and microbial habitat stability under climate stress conditions (Lehmann *et al*. 2020) [12]. The porous structure of biochar provides protective microhabitats for microorganisms while enhancing soil aeration, moisture retention, and nutrient availability. Biochar-associated microbial communities may therefore contribute to improved soil resilience against drought and temperature fluctuations.

Several studies have reported that combined application of compost and biochar positively influences microbial enzyme activities, carbon sequestration, and ecosystem multifunctionality (Bardgett & Caruso, 2020) [2]. Organic amendment-mediated restoration of microbial diversity and soil functionality is therefore considered an important component of regenerative agriculture and climate-smart soil management.

4. Crop Diversification and Rotation

Crop diversification and rotational farming systems are important regenerative agricultural strategies that positively influence soil microbial diversity, ecosystem stability, and long-term agricultural sustainability (Lal, 2020) [11]. Continuous monocropping often leads to depletion of soil nutrients, accumulation of pathogens, reduction of microbial diversity, and destabilization of rhizosphere ecological interactions. In contrast, diversified cropping systems support a broader range of microbial communities through variation in root exudates, organic substrates, and nutrient availability (Philippot *et al*. 2013) [14].

Different plant species release distinct root exudates that selectively influence rhizosphere microbial composition and functionality. Crop rotation therefore promotes microbial heterogeneity and functional diversity by supporting multiple ecological niches within soil ecosystems (Toju *et al*. 2018) [18]. Diverse microbial communities may improve nutrient transformation, suppress pathogenic microorganisms, and enhance soil structural stability through microbial-mediated aggregation processes.

Rotational systems involving legumes can significantly enrich nitrogen-fixing microbial populations and improve nitrogen availability within agricultural soils (Compant *et al*. 2019) [4]. Similarly, diversified cropping practices may stimulate beneficial fungal populations including arbuscular mycorrhizal fungi that contribute to phosphorus acquisition and plant stress tolerance. Enhanced microbial interactions

within diversified systems may therefore improve ecosystem resilience against climate-induced disturbances such as drought, salinity, and heat stress.

Crop diversification has also been associated with improved carbon sequestration, reduced greenhouse gas emissions, and greater ecosystem multifunctionality (Bastida *et al*. 2021) [3]. By improving microbial diversity and ecological resilience, crop rotation and diversified farming systems contribute significantly to sustainable regenerative agriculture and long-term environmental sustainability.

5. Biofertilizers and Plant Growth-Promoting Rhizobacteria (PGPR)

Biofertilizers and plant growth-promoting rhizobacteria (PGPR) have emerged as important microbiome-based tools for improving soil fertility, plant productivity, and climate resilience within regenerative agricultural systems (Singh *et al*. 2020) [11]. PGPR include beneficial bacterial genera such as Bacillus, Pseudomonas, Azospirillum, and Rhizobium, which enhance plant growth through multiple direct and indirect mechanisms including nutrient mobilization, phytohormone production, stress tolerance induction, and pathogen suppression (Compant *et al*. 2019) [4].

Many PGPR contribute to biological nitrogen fixation, phosphorus solubilization, potassium mobilization, and siderophore production, thereby improving nutrient availability under nutrient-deficient or climate-stressed soil conditions (Philippot *et al*. 2013) [14]. Certain microbial inoculants also stimulate root growth and rhizosphere development through production of phytohormones such as indole-3-acetic acid, gibberellins, and cytokinins. These microbial interactions enhance plant nutrient uptake efficiency and overall agricultural productivity.

Under drought and salinity stress conditions, PGPR may improve plant resilience through osmolyte accumulation, antioxidant enzyme induction, and modulation of stress-responsive signaling pathways (Schimel, 2018) [15]. Beneficial microorganisms can additionally improve soil aggregation and moisture retention through biofilm formation and extracellular polymeric substance production, thereby contributing to rhizosphere stability under adverse environmental conditions.

Recent advances in microbiome engineering and synthetic microbial consortia development have further expanded the potential applications of microbial bioinputs in climate-smart agriculture (Jansson and Hofmockel, 2020) [10]. Multifunctional microbial consortia capable of combining nutrient mobilization, stress tolerance, pathogen suppression, and carbon stabilization are increasingly being explored as sustainable alternatives to excessive chemical fertilizer use.

Overall, biofertilizers and PGPR-based interventions represent promising strategies for restoring soil biological functionality and supporting resilient regenerative agricultural systems under future climate change scenarios.

Microbial Indicators of Soil Regeneration

Indicator selection should follow an explicit conceptual model. Biomass, enzyme potential, gene abundance, and realized process rates answer different questions. Robust assessment combines biological indicators with soil pH, electrical conductivity, texture, moisture, organic carbon stocks, nutrient availability, and crop outcomes. A multivariate index requires calibration against independent functional endpoints and validation across seasons and sites.

Microbial indicators are increasingly recognized as sensitive and reliable tools for evaluating soil health, ecosystem recovery, and regenerative agricultural sustainability (Doran and Zeiss, 2000) [7]. Unlike conventional physical and chemical soil parameters that often provide static information, microbial indicators reflect dynamic biological processes occurring within soil ecosystems. Since soil microorganisms respond rapidly to environmental changes, agricultural management practices, and ecological disturbances, microbial parameters can serve as early predictors of soil degradation, regeneration, and ecosystem resilience (Lehmann *et al*. 2020) [12].

The concept of microbial indicators is based on the premise that regenerative soils exhibit distinct microbial signatures characterized by greater microbial diversity, enhanced metabolic activity, improved nutrient cycling, and increased ecological stability. Changes in microbial biomass, enzyme activities, microbial community composition, and functional gene abundance may therefore provide valuable insights into soil restoration processes and long-term ecosystem sustainability.

1. Microbial Biomass Carbon and Soil Respiration

Microbial biomass carbon (MBC) is widely used as an important indicator of soil biological activity and microbial abundance (Nannipieri *et al*. 2020) [13]. Increased microbial biomass generally reflects greater organic matter availability, nutrient cycling potential, and ecosystem functionality. Regenerative agricultural practices such as cover cropping, reduced tillage, and organic amendment application often increase microbial biomass and improve soil biological stability (Bastida *et al*. 2021) [3].

Soil respiration is another important biological indicator associated with microbial metabolic activity and carbon cycling. Enhanced respiration rates may indicate active decomposition and nutrient transformation processes, whereas reduced respiration may reflect microbial stress or ecosystem degradation (Schimel, 2018) [15]. However, interpretation of soil respiration must consider environmental variables such as moisture, temperature, and substrate availability.

Table 2: Regenerative practices and microbial responses

Regenerative Practice	Effects on Soil Microbiome	Ecological Benefits
Cover cropping	Enhances microbial diversity and rhizosphere activity	Improved nutrient cycling and soil structure
Reduced tillage	Preserves microbial habitats and fungal networks	Improved carbon sequestration and soil stability
Organic amendments	Stimulates beneficial microorganisms and enzyme activities	Enhanced soil fertility and water retention
Crop diversification	Promotes functional microbial diversity	Increased ecosystem resilience
Biofertilizers and PGPR	Improves nutrient mobilization and stress tolerance	Sustainable crop productivity and soil regeneration
Biochar application	Provides microbial habitat and stabilizes soil carbon	Improved moisture retention and climate resilience

2. Soil Enzyme Activities

Soil enzymes produced by microorganisms are directly involved in nutrient transformation and organic matter decomposition. Enzyme activities are therefore considered highly sensitive indicators of soil functionality and regenerative capacity (Nannipieri *et al.* 2020) [13]. Key enzymes commonly used as microbial indicators include:
Dehydrogenase — indicator of overall microbial oxidative activity

1. Urease — associated with nitrogen cycling
2. Phosphatase — involved in phosphorus mineralization
3. β-glucosidase — linked to carbon cycling
4. Arylsulfatase — involved in sulfur transformation

Enhanced enzyme activities generally indicate improved microbial metabolism and soil ecosystem recovery under regenerative agricultural systems.

Table 3: Microbial indicators

Microbial Indicator	Biological Significance	What It Indicates
Microbial biomass carbon	Overall microbial abundance and activity	Soil biological health
Soil respiration	Microbial metabolic activity	Carbon cycling efficiency
Dehydrogenase activity	Oxidative microbial metabolism	Total microbial activity
Urease activity	Nitrogen transformation	Soil fertility status
Phosphatase activity	Phosphorus mineralization	Nutrient availability
β-glucosidase activity	Carbon decomposition	Organic matter turnover
Microbial diversity	Ecological stability and resilience	Ecosystem multifunctionality
Functional genes	Specific microbial metabolic pathways	Soil regenerative capacity

3. Microbial Diversity and Community Structure

Microbial diversity is strongly associated with ecosystem multifunctionality, ecological resilience, and stability under environmental stress conditions (Bastida *et al.* 2021) [3]. High microbial diversity improves functional redundancy, nutrient cycling efficiency, and resistance to ecosystem disturbances. Climate stress and intensive agricultural practices may reduce microbial diversity and destabilize ecological interactions within the rhizosphere.
Advances in metagenomics and next-generation sequencing technologies now enable detailed characterization of microbial community structure and diversity. Analysis of bacterial, fungal, and archaeal populations may therefore provide important information regarding soil regeneration status and ecosystem functionality (Fierer, 2017) [8].

4. Functional Microbial Groups as Indicators

Specific microbial functional groups are increasingly being utilized as indicators of regenerative soil health and ecosystem sustainability. These include:

1. Nitrogen-fixing bacteria
2. Phosphate-solubilizing microorganisms
3. Plant growth-promoting rhizobacteria (PGPR)
4. Mycorrhizal fungi
5. Cellulose-degrading microorganisms
6. Stress-tolerant microbial taxa

The abundance and activity of these functional groups may reflect nutrient transformation efficiency, soil fertility, stress resilience, and rhizosphere ecological stability.

5. Molecular and Omics-Based Indicators

Emerging molecular approaches including metagenomics, metatranscriptomics, metabolomics, and proteomics have significantly improved the ability to identify functional microbial biomarkers associated with soil regeneration and ecosystem resilience (Jansson and Hofmockel, 2020) [10]. Functional genes associated with carbon cycling, nitrogen metabolism, stress tolerance, and microbial communication may serve as predictive indicators of regenerative soil functionality.

Machine learning and AI-assisted analysis of microbiome datasets are also being explored for development of predictive soil health models and climate-smart agricultural monitoring systems. Integration of omics technologies with microbial indicator frameworks may therefore provide powerful tools for future regenerative agriculture and environmental sustainability assessment.
Collectively, microbial indicators provide biologically responsive and ecologically meaningful approaches for monitoring soil regeneration and climate-resilient agricultural sustainability. Their integration into regenerative agricultural systems may support development of precision soil management strategies aimed at improving long-term ecosystem functionality and environmental resilience.
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Machine learning and AI-assisted analysis of microbiome datasets are also being explored for development of predictive soil health models and climate-smart agricultural monitoring systems. Integration of omics technologies with microbial indicator frameworks may therefore provide powerful tools for future regenerative agriculture and environmental sustainability assessment.

Collectively, microbial indicators provide biologically responsive and ecologically meaningful approaches for monitoring soil regeneration and climate-resilient agricultural sustainability. Their integration into regenerative agricultural systems may support development of precision soil management strategies aimed at improving long-term ecosystem functionality and environmental resilience.

Emerging Technologies and Future Microbiome Engineering Approaches

A tiered workflow can improve cost-effectiveness: routine physicochemical and enzyme measurements for all plots, targeted qPCR and amplicon sequencing for selected contrasts, and shotgun or other multi-omics approaches for mechanistically informative samples. Sampling design, biological replication, negative controls, extraction blanks, and batch randomization are as important as sequencing depth.

Recent advances in molecular biology, bioinformatics, artificial intelligence, and microbiome engineering have transformed understanding of soil microbial ecosystems and their role in sustainable agriculture under climate change conditions. Emerging technologies are enabling detailed characterization of microbial diversity, functional interactions, metabolic pathways, and ecological responses within complex soil environments. These innovations are increasingly supporting development of climate-smart regenerative agricultural systems and precision soil management strategies.

1. Metagenomics and High-Throughput Sequencing

Metagenomics has revolutionized soil microbiome research by enabling direct analysis of microbial genetic material from environmental samples without the need for culture-dependent techniques (Fierer, 2017) [8]. High-throughput sequencing technologies allow comprehensive characterization of bacterial, fungal, archaeal, and viral communities within soil ecosystems. These approaches provide detailed information regarding microbial diversity, functional genes, stress adaptation pathways, and ecosystem-level interactions.

Metagenomic studies have revealed that climate stress conditions can significantly alter microbial functional potential associated with nutrient cycling, carbon metabolism, and stress tolerance (Jansson and Hofmockel, 2020) [10]. Functional gene profiling may therefore serve as a

powerful tool for identifying microbial biomarkers of soil regeneration and climate resilience.

2. Metatranscriptomics, Metabolomics, and Proteomics

While metagenomics provides information regarding microbial genetic potential, metatranscriptomics and metabolomics help identify active microbial functions and metabolic responses under changing environmental conditions. Metatranscriptomic approaches analyze gene expression patterns within microbial communities, thereby revealing functional microbial responses to drought, temperature stress, salinity, and agricultural management practices (Bardgett and Caruso, 2020) [2].

Metabolomics enables characterization of microbial metabolites involved in nutrient cycling, signaling, stress adaptation, and rhizosphere interactions. Similarly, proteomics helps identify functional proteins associated with microbial metabolism and ecological adaptation. Integration of multi-omics approaches therefore provides a comprehensive understanding of soil microbiome functionality and ecosystem resilience.

3. Artificial Intelligence and Predictive Soil Monitoring

Artificial intelligence (AI), machine learning, and big-data analytics are increasingly being utilized for analysis of complex microbiome datasets and development of predictive soil health models. AI-assisted systems can identify microbial patterns associated with soil degradation, climate stress, nutrient imbalance, and regenerative agricultural performance.

Predictive microbiome models may support precision agriculture by enabling real-time monitoring of soil biological status and ecosystem functionality. Integration of microbial indicators with remote sensing technologies, biosensors, and digital agriculture platforms may further improve climate-smart soil management and regenerative farming decision-making.

4. Microbiome Engineering and Synthetic Microbial Consortia

Microbiome engineering involves deliberate manipulation of microbial communities to improve soil health, plant productivity, and environmental resilience (Toju *et al*. 2018) [18]. Advances in microbial ecology and systems biology have facilitated development of synthetic microbial consortia capable of performing multifunctional ecological roles including nutrient mobilization, pathogen suppression, stress tolerance induction, and carbon stabilization.

Engineered microbial communities may enhance agricultural sustainability by restoring beneficial rhizosphere interactions and improving soil ecosystem stability under climate stress conditions. Future microbiome engineering approaches may also involve targeted microbial inoculation, rhizosphere modulation, and ecological niche management.

5. CRISPR and Genetic Engineering Approaches

Genome editing technologies such as CRISPR-Cas systems are emerging as potential tools for studying and modifying microbial functional traits associated with climate resilience and nutrient cycling. Genetic engineering approaches may help develop microorganisms with enhanced stress tolerance, biodegradation capability, and agricultural functionality.

However, application of genetically engineered microorganisms within agricultural ecosystems requires careful biosafety evaluation, ecological risk assessment, and regulatory consideration to prevent unintended environmental consequences.

6. Biosensors and Smart Soil Monitoring Systems

Biosensor technologies are increasingly being explored for rapid detection of soil biological activity, microbial metabolites, nutrient status, and environmental stress indicators. Integration of biosensors with AI-based analytics and wireless monitoring systems may support development of real-time soil health assessment platforms for precision regenerative agriculture.

Smart monitoring systems capable of integrating microbial, chemical, and environmental data may therefore improve ecosystem management and climate adaptation strategies in future agricultural systems.

Collectively, emerging technologies and microbiome engineering approaches are transforming soil microbiology research and regenerative agricultural management. Integration of omics technologies, artificial intelligence, microbial indicators, and synthetic biology may provide powerful solutions for developing climate-resilient agricultural ecosystems and sustainable environmental management strategies under future climate change scenarios.

Table 4: Emerging technologies

Technology	Application	Potential Benefits
Metagenomics	Microbial community analysis	Identification of functional microbial groups
Metatranscriptomics	Analysis of active microbial genes	Understanding stress-response mechanisms
Metabolomics	Detection of microbial metabolites	Evaluation of ecosystem functionality
Artificial intelligence	Predictive soil health modeling	Precision regenerative agriculture
Biosensors	Real-time soil monitoring	Early detection of soil degradation
Synthetic microbial consortia	Microbiome engineering	Enhanced stress tolerance and nutrient cycling
CRISPR technologies	Functional microbial modification	Development of climate-resilient microbial systems

Challenges and Research Gaps

Priority gaps include disentangling climate from management effects, linking molecular potential to measured process rates, quantifying carbon persistence rather than short-term respiration, and testing whether microbial signatures predict outcomes beyond a single site. Future studies should predefine primary outcomes, incorporate temporal replication, and report uncertainty and effect sizes.

Despite significant advances in soil microbiome research and regenerative agricultural practices, several scientific, technological, and practical challenges remain in

understanding and managing soil microbial ecosystems under climate change conditions. The complexity of soil microbial communities, environmental variability, and limitations in methodological standardization continue to restrict the large-scale application of microbiome-based regenerative agriculture and soil health monitoring systems.

1. Complexity of Soil Microbial Ecosystems

Soil microbiomes represent highly diverse and dynamic ecological systems influenced by multiple interacting factors including soil type, climate, vegetation, agricultural management, moisture availability, and nutrient status (Fierer, 2017) [8]. The enormous diversity and functional redundancy within microbial communities make it difficult to identify universal microbial indicators applicable across different ecosystems and geographical regions.

Microbial responses to climate stress are often ecosystem-specific, resulting in inconsistent findings among studies. Consequently, understanding the precise relationships between microbial diversity, ecosystem functionality, and climate resilience remains a major scientific challenge.

2. Lack of Standardized Microbial Indicators

Although microbial biomass, enzyme activities, and microbial diversity are widely used as indicators of soil health, there is currently no universally accepted framework for microbial indicator-based soil regeneration assessment (Doran and Zeiss, 2000) [7]. Different studies utilize varying methodologies, sampling strategies, analytical techniques, and interpretation criteria, thereby limiting comparability among datasets.

Standardization of microbial indicator selection, molecular methodologies, and ecological interpretation is therefore necessary for development of reliable regenerative soil monitoring systems.

3. Limited Long-Term Field Studies

Most current studies evaluating climate change impacts on soil microbiomes are based on short-term laboratory experiments or controlled greenhouse conditions (Bardgett and Caruso, 2020) [2]. Long-term field-scale investigations examining microbial adaptation, ecosystem resilience, and regenerative agricultural performance under real environmental conditions remain limited.

Longitudinal studies are particularly important for understanding temporal shifts in microbial communities and long-term effects of regenerative agricultural practices on ecosystem sustainability.

4. Challenges in Omics Data Interpretation

Advanced omics technologies such as metagenomics, metatranscriptomics, and metabolomics generate extremely large and complex datasets that require sophisticated bioinformatics tools and computational analysis (Jansson & Hofmockel, 2020) [10]. Interpretation of microbial functional data and ecological interactions remains challenging due to incomplete microbial databases and limited understanding of microbial gene functionality.

Additionally, the presence of unculturable and poorly characterized microorganisms further complicates accurate ecological interpretation.

5. Translation from Laboratory to Field Application

Many microbiome-based technologies and microbial inoculant studies show promising results under controlled experimental conditions but exhibit inconsistent performance under field environments. Variability in soil physicochemical properties, climatic conditions, and native microbial communities may affect survival and functionality of introduced microorganisms.

Scaling microbiome engineering approaches from laboratory research to practical agricultural application therefore remains a major challenge for sustainable regenerative agriculture.

6. Biosafety and Ecological Concerns

Emerging microbiome engineering and genetic modification approaches raise important biosafety and ecological concerns regarding unintended environmental impacts, microbial imbalance, horizontal gene transfer, and ecological disruption. Introduction of engineered microbial communities into agricultural ecosystems may influence native microbial diversity and long-term ecosystem stability. Comprehensive ecological risk assessment and biosafety evaluation are therefore essential before large-scale implementation of synthetic microbial systems and genetically engineered microorganisms.

7. Climate Uncertainty and Ecosystem Variability

Future climate scenarios involve complex and unpredictable environmental interactions including fluctuating temperature, drought frequency, flooding intensity, and extreme weather events. These uncertainties make it difficult to predict long-term microbial responses and ecosystem adaptation patterns under changing environmental conditions.

Development of predictive models integrating climate data, microbial indicators, and ecosystem functionality remains an important research priority for future climate-smart agriculture.

Overall, addressing these scientific and technological gaps will be essential for advancing soil microbiome research and developing reliable regenerative agricultural systems capable of supporting environmental sustainability and climate resilience. Future interdisciplinary research integrating microbiology, ecology, biotechnology, artificial intelligence, and environmental science may significantly improve understanding and practical application of microbiome-based regenerative agriculture.

Future Perspectives

Future work should integrate replicated field experiments with process-based models and multi-omics measurements to identify management-responsive, climate-robust indicators. Particularly valuable are paired measurements of microbial necromass, POM and MAOM fractions, soil carbon stocks at equivalent soil mass, nitrogen transformation rates, and greenhouse-gas fluxes. The strongest claims will arise from convergent evidence across molecular, biochemical, and agronomic measurements.

Future agricultural sustainability will increasingly depend on the ability to develop climate-resilient soil management systems capable of maintaining productivity, ecosystem functionality, and environmental stability under changing climatic conditions. Soil microbiomes are expected to play central roles in future regenerative agriculture due to their involvement in nutrient cycling, carbon sequestration, stress adaptation, and ecosystem resilience. Advances in microbiome research, biotechnology, artificial intelligence,

and ecological engineering are therefore likely to transform future approaches toward sustainable soil and agricultural management.

One of the most promising future directions involves development of climate-smart microbiome engineering strategies designed to enhance beneficial microbial functions within agricultural soils. Targeted manipulation of rhizosphere microbial communities may improve nutrient acquisition, drought tolerance, salinity resistance, pathogen suppression, and carbon stabilization under climate stress conditions (Jansson and Hofmockel, 2020) [10]. Synthetic microbial consortia capable of performing multiple ecological functions may become important components of future sustainable farming systems.

Integration of omics technologies with artificial intelligence and predictive analytics is also expected to revolutionize soil health assessment and regenerative agriculture monitoring. Machine learning-based microbial models may enable early detection of soil degradation, prediction of ecosystem stress responses, and development of precision regenerative management strategies. Real-time monitoring systems integrating microbial indicators, biosensors, and digital agriculture technologies may further improve decision-making and resource management within climate-smart agricultural systems.

Future research is also likely to focus on identification of universal microbial biomarkers associated with soil regeneration and ecosystem multifunctionality. Such indicators could support development of standardized soil health indexing systems for evaluating sustainability and regenerative agricultural performance across different ecosystems and climatic regions.

In addition, increasing interest in carbon farming and nature-based climate solutions may further emphasize the importance of soil microbiomes in long-term carbon sequestration and greenhouse gas regulation. Microbial-mediated stabilization of soil organic carbon may therefore become a critical component of global climate mitigation strategies.

Interdisciplinary collaboration among microbiologists, ecologists, agronomists, environmental scientists, data scientists, and policymakers will be essential for advancing microbiome-centered regenerative agriculture. Future policy frameworks may increasingly incorporate biological soil health indicators and microbiome-based sustainability assessment into agricultural management and climate adaptation programs.

Overall, the integration of microbial ecology, regenerative agriculture, biotechnology, and climate science offers significant potential for developing resilient agricultural ecosystems capable of supporting future food security and environmental sustainability under global climate change scenarios.

Conclusion

The central challenge is to move from describing who is present in soil toward determining which microorganisms are active, what processes they perform, and whether those processes produce durable agronomic and environmental benefits. Regenerative practices have substantial potential, but their effectiveness must be evaluated with context-sensitive, long-term evidence and explicit attention to trade-offs.

Climate change represents a major global threat to soil ecosystem stability, agricultural productivity, and environmental sustainability. Increasing temperatures, drought, salinity, flooding, and extreme climatic disturbances significantly influence soil microbial diversity, metabolic functionality, nutrient cycling, and rhizosphere interactions. Since soil microorganisms regulate essential ecological processes including carbon sequestration, nutrient transformation, plant growth promotion, and ecosystem resilience, climate-induced disruption of soil microbiomes may have profound consequences for long-term agricultural sustainability and global food security.

Emerging evidence demonstrates that regenerative agricultural practices such as cover cropping, reduced tillage, crop diversification, organic amendment application, and microbiome-based bioinputs can significantly improve soil microbial diversity and restore ecosystem functionality under climate stress conditions. These regenerative strategies contribute to enhanced soil resilience, improved nutrient cycling, stabilization of soil organic carbon, and restoration of beneficial plant–microbe interactions.

Microbial indicators including microbial biomass, enzyme activities, soil respiration, microbial diversity, and functional microbial groups have gained increasing importance as biologically responsive tools for assessing soil regeneration and ecosystem recovery. Integration of molecular approaches, omics technologies, artificial intelligence, and predictive microbiome modeling is further transforming soil health monitoring and climate-smart agricultural management.

Despite considerable advances, important challenges remain regarding standardization of microbial indicators, interpretation of complex microbiome datasets, long-term field validation, and translation of microbiome engineering approaches into practical agricultural systems. Addressing these challenges will require interdisciplinary research integrating microbiology, ecology, biotechnology, environmental science, and data analytics.

Overall, soil microbiomes represent a critical component of future climate-resilient regenerative agriculture and sustainable ecosystem management. Improved understanding of microbial responses to climate change and strategic integration of microbiome-based technologies may provide innovative solutions for restoring soil functionality, improving agricultural sustainability, and supporting environmental resilience under future global climate scenarios.

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