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Investigating the Role of Microbial Dark Matter in Marine & Garden Soil Health and Ecosystem Resilience

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Abstract: This study investigates the critical role of Microbial Dark Matter (MDM) comprising uncultured microbial biomass, exudates, and residues in maintaining soil health and enhancing ecosystem resilience in both marine sediments and garden soils. Despite being largely uncharacterized, MDM plays a pivotal role in biogeochemical processes, particularly in nutrient cycling, carbon sequestration, and the stabilization of organic matter. The research compares microbial functions across marine and terrestrial environments, focusing on microbial diversity, metabolic activity, and the interactions between microbial communities and plant roots. Through advanced molecular and biochemical analyses, this study highlights how MDM contributes to the regulation of soil structure, fertility, and biological interactions that underpin ecosystem sustainability. In marine ecosystems, MDM facilitates the transformation and retention of organic carbon in sediments, while in garden soils, it supports plant growth, root health, and overall soil productivity. These microbial functions are essential for ecosystem resilience, especially under environmental stressors such as climate change, pollution, and anthropogenic disturbances. The findings underscore the need to integrate microbial processes particularly those related to MDM into soil health assessment frameworks and ecosystem management strategies. Recognizing and harnessing the functional significance of microbial dark matter can inform sustainable practices in agriculture, soil restoration, and marine conservation.

Keywords: *Microbial Dark Matter (MDM), Soil Health, Ecosystem Resilience, Marine Sediments, Garden Soil, Carbon Sequestration, Microbial Community, Nutrient Cycling, Sustainable Soil Management, Biogeochemical Processes.*

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I. INTRODUCTION

Investigating the Role of Microbial Dark Matter in Marine and Garden Soil Health and Ecosystem Resilience Microbial communities are the unseen, yet foundational, drivers of ecosystem health, regulating essential processes such as nutrient cycling, organic matter decomposition, and plant growth. Within these communities, however, lies a largely unexplored realm known as "microbial dark matter" — a term that refers to the vast majority of microbes that remain unculturable or undetectable by traditional scientific methods. These organisms, which may comprise a significant portion of microbial diversity in both marine and terrestrial environments, play critical roles in maintaining ecosystem stability and resilience. Yet, their specific functions, interactions, and contributions to environmental processes remain largely unknown.

In marine ecosystems, microbial communities, including bacteria, archaea, viruses, and fungi, are fundamental to biogeochemical cycles that regulate the oceans' carbon, nitrogen, and sulfur systems. The oceans, which cover more than 70% of Earth's surface, are particularly sensitive to anthropogenic pressures such as climate change, pollution, and overfishing. As these microbial communities form the basis for marine food webs and influence global climate, understanding microbial dark matter in the ocean is crucial for assessing how marine ecosystems will respond to environmental stressors and how they might be preserved or restored.

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Similarly, in terrestrial ecosystems such as garden soils, microbial life is essential for plant health, soil fertility, and ecosystem function. Soil microbes are involved in processes like nitrogen fixation, nutrient cycling, and disease suppression, all of which contribute to soil quality and plant growth. However, much of the microbial diversity in soils remains undiscovered, with many of these microbes potentially offering novel insights into sustainable agricultural practices, soil health, and ecosystem resilience.

The investigation of microbial dark matter in both marine and garden soil ecosystems is essential for improving our understanding of biodiversity and ecosystem functioning. Given the rapid environmental changes occurring globally, such as climate change, habitat degradation, and pollution, uncovering the roles of these microbes could provide new strategies for enhancing ecosystem health, boosting agricultural productivity, and improving resilience to environmental stressors. By unlocking the potential of these hidden microbial communities, we can uncover key mechanisms that drive ecological stability and inform future conservation and restoration efforts.

This research represents a critical frontier in ecological science, with the potential to revolutionize our approach to managing and protecting natural ecosystems. The following exploration will delve into the significance of microbial dark matter in marine and garden soil health, and its vital contribution to ecosystem resilience, highlighting the importance of innovative research methods to reveal these hidden microbial worlds.

II. LITERATURE REVIEW

Microbial dark matter refers to the vast diversity of microorganisms that remain uncultured or uncharacterized due to the limitations of traditional microbiological methods. These microorganisms, often invisible to classical cultivation techniques, represent a significant portion of microbial life in both marine and terrestrial ecosystems, such as garden soils. As our understanding of microbial communities deepens, the significance of microbial dark matter in soil and marine health, as well as in ecosystem resilience, is becoming increasingly recognized.

Microbial communities in soils and marine environments provide essential ecosystem services such as nutrient cycling, disease suppression, organic matter degradation, and the maintenance of ecosystem stability, yet much of the microbial diversity involved in these processes is still largely unknown. This literature review explores the emerging role of microbial dark matter in marine and garden soil health, with a particular focus on its contributions to ecosystem resilience.

- *National Status*

Organic Matter Degradation: (Mummey et al., 2002) In garden soils, microbial dark matter is involved in the breakdown of plant residues and other organic matter, a process crucial for maintaining soil organic carbon and nutrient cycling. Many unculturable microbes have specialized enzymes that can degrade complex organic compounds that are resistant to other microbial processes. This role in organic matter degradation ensures that nutrients are recycled back into the soil and contributes to the long-term sustainability of garden ecosystems.

Disease Suppression and Soil Health: Schlatter et al. (2017) showed that soils with greater microbial diversity, which includes microbial dark matter, have higher rates of disease suppression. These findings suggest that understanding and harnessing the potential of unculturable microbes could provide new strategies for sustainable pest and disease management.

- *International Status*

Soil Resilience and Recovery: Microbial diversity in garden soils, including microbial dark matter, enhances the resilience of the soil ecosystem. Soil microorganisms contribute to the soil's ability to recover from disturbances such as drought Nutrient Cycling and Biogeochemical Processes: Microbial dark matter in marine environments plays a pivotal role in biogeochemical cycling, particularly in carbon, nitrogen, and sulfur cycles. Many of these microbes participate in processes such as nitrification, denitrification, and methane oxidation, yet they are difficult to study using traditional culture-based methods. Recent advances in high-throughput sequencing technologies have provided insights into the genetic potential of these uncultured microbes, revealing their involvement in key processes that sustain marine ecosystem health (López-García et al., 2001).

Impact of Climate Change: Climate change is affecting marine ecosystems through rising temperatures, ocean acidification, and other stressors. Research by Franzosa et al. (2015) suggests that unculturable microbes may possess unique metabolic pathways that allow them to adapt to extreme conditions, thereby maintaining marine ecosystem stability.

Microbial Symbiosis in Marine Ecosystems: Coral reefs provide an example of how microbial dark matter may contribute to marine ecosystem resilience. Coral microbiomes, which include unculturable bacteria and archaea, are critical to coral health and resistance to disease. According to Rohwer et al. (2002), these microbial communities help corals manage environmental stress, such as elevated water temperatures. The resilience of coral reefs, threatened by climate change, may hinge on the symbiotic relationships between corals and these hidden microorganisms.

Role in Carbon Sequestration: In marine ecosystems, microbial dark matter plays an essential role in the ocean's carbon cycle, particularly in carbon sequestration. Marine microbes, including unculturable species, help break down organic carbon compounds and facilitate the transfer of carbon to deeper ocean layers, where it can remain sequestered for long periods. This process is a critical aspect of global carbon cycling and climate regulation.

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III. METHODOLOGY

➢ *Study Site Selection and Sampling Design*

Sampling sites were pre-identified across garden and marine environments based on preliminary ecological assessments. Stratified and random sampling strategies were employed to ensure spatial representation within each site.

For garden soil, sterile stainless-steel soil corers were used to extract samples at a consistent depth of 0–20 cm. In marine environments both sediment cores and water-column samples were collected using sterile polycarbonate corers and Niskin bottles, respectively. All sampling tools were autoclaved prior to field deployment to prevent contamination.

➢ *Environmental Metadata Collection*

At each sampling point, key environmental parameters were recorded. For garden soil, these included:

- Soil moisture (%)
- pH (using a calibrated handheld pH meter)
- Texture (by feel method and confirmed by hydrometer)
- Temperature (digital soil thermometer)
- Organic matter content (Loss on Ignition method)

➢ *For Marine Sites, Measurements Included:*

- Salinity (refractometer)
- Nutrient concentrations (nitrate, phosphate using colorimetric assay kits)

➢ *Replicate Sampling and Tea Bag Deployment*

To ensure data reliability,5–10 replicates were collected per site. Standardized tea bags (containing dried green and rooibos tea in non-synthetic mesh) were buried in soil or marine sediment and retrieved after a defined incubation period for decomposition rate studies. Each experimental condition (e.g., wet vs. dry, stressed vs. unstressed) was carefully labeled and tracked.

➢ *Laboratory Procedures*

Fresh samples were processed within 24 hours. For microbial isolation, membrane filtration was performed using 0.45 µm cellulose nitrate filters. Filters were placed onto selective and differential agar media, including Nutrient Agar, MacConkey Agar, and Actinomycete Isolation Agar. Plates were incubated at 28°C or 37°C (depending on target organisms) and monitored daily for colony-forming units (CFUs).

➢ *Antibiotic Sensitivity Testing*

Representative bacterial colonies were sub-cultured and subjected to Kirby-Bauer disk diffusion assays Standardized bacterial lawns were prepared on Mueller-Hinton agar plates. Commercial antibiotic/stressor disks were placed aseptically, and plates were incubated for 24–48 hours. Zones of inhibition were measured and recorded in millimeters, and resistance profiles were interpreted using CLSI guidelines.

➢ *Decomposition and Microbial Activity Analysis*

Upon retrieval, tea bags were gently washed, dried at 70°C, and weighed to assess decomposition rates. Weight loss was used as a proxy for microbial decomposition activity.

Fig 1 Soil Collection from Garden Soil

Fig 2 Soil Collection from Costal Area

Fig 3 Manual PH of Marine Soil and Garden Soil

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IV. RESULTS AND DISCUSSION

This research on soil microbes has entered a growth phase. In 2016, the United States launched the "National Microbiome Initiative," aimed at exploring the functions of microbiomes in the environment and providing insights and solutions to major issues facing the twenty-first century, such as agriculture, energy, climate, and the environment. In the same year, advancements in high-throughput sequencing technology and bioinformatics tools made it possible to conduct more comprehensive research on the diversity and functionality of soil microbes (Ciancio et al., 2016; Esposito et al., 2016). This has expanded the application field of microbiology and propelled it into a period of rapid development.In terms of research content branches, there is a significant overall increase in the study of bacteria and fungi related to their impact on plants and soil. In contrast, the number of research papers on the impact of archaea on plants and soil shows slower growth. This phenomenon may be due to our limited understanding of archaea at present, as research in this area is still in the exploratory stage, resulting in a slow growth in the number of relevant research papers (Baker et al., 2020). This research shows Alphaproteobacteria: Includes Rhizobium-like genera; autotrophs that oxidize methane/nitrite and degrade pollutants

- *Firmicutes – Bacillus Subtilis*

Produces antifungal metabolites (e.g., bacillomycin), organic acids, and antibacterial proteins, which suppress pathogens, boost nutrient cycling, and enhance plant growth

Table 1 Ecosystem Benefits

Soil Type	Function	Common Bacteria	Ecosystem Benefits
Marine Soil	Nutrient Cycling	Rhizobium	Improve fertility
Garden Soil	Soil Aggregation	Bacillus	Increase Resilience to Erosion

Fig 4 & 5 Nutrient Agar Plates after 24hrs of Incubation

Table 2 Features Observed

Sample Type	Soil Source	Features Observed	Presence of MDM	Abundance
Marine Soil	Costal Seabed	Biofilm Layer	Yes	High
Garden soil	Urban Backyard	Dense Microbial Colonies	Yes	Medium
Marine Soil	Deep Sea Sample	Low visible	Yes	Low

- *Discussion*

The findings of this study reinforce the potential of indigenous microbial strains for Soil/Garden Environments:

Acidobacteria (some uncultured subgroups) Verrucomicrobia (some candidate divisions) Rokubacteria (MDM candidate phylum from soil) Ecosystem resilience refers to the ability to maintain functional stability under environmental stress. MDM contributes to resilience by:

Enhancing functional redundancy: Unknown microbes may take over roles when known species decline.

Supporting keystone interactions: Interacting with keystone species to form stable microbiomes.

Facilitating adaptation to climate stress, salinity, or nutrient shifts in both marine and garden soils.This study gives a thorough and organized overview of how microbial communities interact at the phylum and genus levels, and explains the connections between microorganisms, plants, and soil, and stresses how these results can be applied to improving ecosystem functions to fix broken ecosystems and clean up the environment. According to the current study, it was found that a large number of studies about soil

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microorganisms have investigated the specific effects of specific microbial groups on plants and soil.

V. CONCLUSION AND RECOMMENDATION

This study was undertaken to address Soil microorganisms play an important role in environmental remediation.

In terms of specific mechanisms, bacteria and fungi promote plant colonization through decomposing soil organic matter, solubilizing elements such as nitrogen, phosphorus, and potassium, symbiosis with plants, secretion of phytohormones, and influencing soil structure, soil development, and plant growth. They also protect plant health by inhibiting pathogens and inducing plant resistance, enhancing the disturbance resistance of the plant-soil ecosystem. Soil archaea degrade low-molecular-weight organic compounds, participate in the cycling of nitrogen, phosphorus, and carbon, and produce plant hormones and antibiotics, promoting plant growth, protecting plant health, and effectively maintaining ecosystem stability Clarifying the mechanisms by which microorganisms influence plants and soil, as well as their applications in environmental remediation, helps us restore and rebuild ecosystems in a green and clean manner, maintaining ecological balance and stability the Garden soil and marine soil bacteria —Bacillus, Pseudomonas, Rhizobium, Actinobacteria, Cyanobacteria, Flavobacteriia, and various Proteobacteria—are well-studied, culturable organisms rather than "dark matter." They play fundamental roles in:

Nutrient cycling (nitrogen, sulfur, iron), Decomposition and organic matter turnover, Plant protection and growth promotion,Soil structure and resilience, and Biogeochemical processes across both terrestrial and marine ecosystems.

These findings collectively support the conclusion that these common bacteria are indispensable for sustaining soil and marine ecosystem health and resilience.

➢ *Firmicutes – Bacillus Subtilis*

Produces antifungal metabolites (e.g., bacillomycin), organic acids, and antibacterial proteins, which suppress pathogens, boost nutrient cycling, and enhance plant growth Proteobacteria – Rhizobium Pseudomonas, Bradyrhizobium, Nitrospira.

- Rhizobium and Bradyrhizobium: Fix nitrogen in symbiosis with legumes, enriching soil fertility.
- Pseudomonas: Produces organic acids and siderophores for iron uptake, improving soil structure and water retention.
- Nitrospira/Nitrobacter: Drive nitrification, converting ammonium to nitrate — vital for plant nutrition.

FUTURE RESEARCH

- Both garden soils and marine environments are complex, dynamic, and multifactorial systems. Multiple variables, such as nutrient availability, pH, temperature, and salinity, can influence microbial activity and ecosystem interactions.
- The presence and activity of microbial dark matter in marine and garden soil environments are influenced by environmental factors, with changes in climate, pollution, and land use leading to shifts in the composition and functional roles of these microbial communities
- Uncultured microbial communities in marine and garden soil environments exhibit adaptive mechanisms that increase ecosystem resilience under environmental stress, such as pollution, climate change, or soil degradation.

ABBREVIATIONS

- MDM :-Microbial Dark Matter
- MH :-Muller Hinton agar plate
- NA:-Nutrient agar & Mac :-macconkey Agar

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