Microbial Diversity in Soil and Sediment and the Impacts of Climate Change and Pollution.

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Abstract

Climate change and pollution are two of the biggest threats to the microbial communities that underpin soil ecosystems. Yet, these threats are rarely studied in tandem, limiting our ability to predict their future impacts on soil microbiome. Macronutrients, like carbon, nitrogen and phosphorus, that circulate through biogeochemical and hydrogical cycles, are driven by adaptable and diverse soil microbial communities. This study examines the impact of temperature change on soil and sediment microbial communities, focusing on their diversity after pollution. Prediction is that the soil and sediment pollution will lead to an increase in AMR bacteria. Also, it is predicted that with added pollution the diversity will decrease in warmer temperatures the longer time will pass. Soil samples were collected from the Roman River Valley Nature Reserve and sediment samples from Brightlingsea Colne River estuary in Essex and subjected to chemical contamination under two temperature conditions (16^{o}C and 20^{o}C). For culturing, Extended Spectrum Beta Lactamase plates were done and to study wider microbial community, 16S and 18S amplicon sequencing was conducted. The results were analysed using R studio. It was found that soil and sediment microbial community responded differently to warming and chemical stressors, with soils showing compositional stability and sediments displaying early sensitivity. Resource enrichment and warming increased microbial biomass, yet functional resilience may be compromised due to keystone taxa loss. These findings highlight the need for long-term, genomic-based studies to predict ecosystem responses to climate change and pollution.

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1. Introduction

1.1 Background

Deterring the impacts of environmental changes on freshwater systems, and delivering appropriate mitigation strategies, requires an integrative understanding of ecological and hydrological response of these changes. In 1961 it was proposed by the International Hydrological Association (IHA), that hydrology and ecology should be combined (Langbein, 1962). Ecohydrological connectivity studies how water in different forms, like runoff, groundwater, rainfall, and soil moisture, influences and is influenced by the distribution and functioning of the ecosystems. Likens and Bormann (1974) presented a framework to understand the link between aquatic and terrestrial ecosystems. The authors discuss how disruption of nutrient cycling and land-use change, breaks the connectivity between the aquatic and terrestrial ecosystems (Likens and Bormann, 1974). Hydrological pathways were determined as a key mechanism for ecohydrological connectivity. By the end of 20th century ecohydrology had a complete definition, and its importance was determined by several researchers (Wassen and Grootjans, 1996; Baird and Wilby, 1999).

A conceptual framework was developed to enhance the understanding across multiple spatial and temporal dimensions (Wang et al., 2012). The research agreed with previous work done by Likens and Bormann (1974) that water and nutrient fluxes can create a connectivity of landscapes.

However, Wang studied it deeper and included biogeochemical connectivity, introducing a framework that could be crucial for ecosystem management and conservation. The framework integrated connectivity across different landscapes at multiple scales (regional and global) and explored movement of nutrients across different landscapes using electrical analogy. In the framework Wang et al. (2012), not only included multiple scales but also highlighted landscape heterogeneity and human impacts. Their work emphasized the ability

to use the conceptual framework to analyze connectivity patterns and understand the impacts of human activity or other disturbances on the functions of ecosystems (Wang et al., 2012). The need to consider ecohydrology across crossing habitats at a different scale was later discussed by Krause et al in 2017.

Krause et al. (2017) did research on ecohydrological interfaces being the hotspots for ecosystem processes. They highlighted the significance of habitat connectivity and discussed how climate change and land use modification can influence the functions of ecohydrological interfaces. However, their work has a bias when it comes to ecohydrological interfaces especially when it comes to freshwater ecosystems. Also, it does not cover microbial changes during pollution and temperature fluctuations. Work done by Krause et al (2017) agreed with the research done by Baigun et al. (2008) that challenges in ecohydrology demand for the interdisciplinary approaches and different methodologies. Ecohydrological interface zones provide unique conditions for biodiversity and habitat connectivity by providing support and corridors for the species movement. Considering different scales is important as that contributes to the overall function of these zones. Krause et al. (2017) concluded that a deeper recognition of ecohydrological interfaces could advance understanding of conceptual frameworks of ecosystem processes in freshwater ecosystems when it comes to groundwater and surface water interactions, and benthic and pelagic interfaces. This was supported by Fang et al. (2018) who defined the connectivity as an interaction between terrestrial and marine ecosystems through physical, biological, and chemical processes including human impact. In water management, biodiversity conservation, and land use planning ecohydrological connectivity is recognizing hydrology role in shaping habitats and supporting diverse species.

Biogeochemical processes involve the cycling and transformation of elements such as carbon, nitrogen and phosphorus through the ecosystems and are closely linked to hydrological processes. Water drives the transport and transformation of these elements, influencing carbon fluxes, nutrient availability, and ecosystem productivity (Lohse et al., 2009). Clark et al (2022) highlighted the importance of hydrological processes in shaping microbial communities that participate in methane and nitrogen cycles regulation in rivers.

The variability in hydrology can affect both methane consuming and denitrifying bacterial communities, which leaves a lasting impact for greenhouse gasses and nutrient removal (Clark et al., 2022). Rain is responsible for nutrient runoff from agricultural fields, which causes nitrogen and phosphorus act like a fertilizer in water bodies like lakes and rivers, causing excessive growth of algae. Even though recent studies have highlighted the importance of understanding ecohydrological and biogeochemical processes, there is still limited understanding in how changes in different environmental conditions affect biogeochemical cycles and therefore microbial taxa.

Microbes have crucial role in carbon, nitrogen and phosphorus cycling (Graham et al., 2016). They not only influence nutrient uptake but also soil structure and therefore overall plant health. Microbe functional diversity within microbial communities in the presence of chemicals and environmental changes is not well understood. Creating a study combining chemical and ecohydrological data including terrestrial and marine ecosystems are required. Also, this kind of study could help predicting in how changes in one ecosystem could affect the other.

1.2. Factors influencing microbial diversity

1.2.1 Pollution

In 2024 48 million km^2 classified as agricultural land, which is 44% of the world's habitable land (https://ourworldindata.org/global-land-for-agriculture). The intensive anthropogenic land use increases the abundance of microbial genes involved in carbon degradation, potentially leading to higher CO_2 emissions from soil (Malik et al., 2018). It is done by changing soil structure, nutrient availability and microbial community composition. These conditions favour fast growing taxa that specialises in organic matter decomposition. Their increased activity accelerates carbon mineralization, resulting in higher CO_2 emissions (Li et al., 2024; Yadav et al., 2022). Microorganisms play a central role in soil greenhouse gas dynamics through processes like respiration, methanogenesis, and denitrification. These

microbial-driven pathways significantly influence carbon, nitrogen, and sulphur cycling, thereby shaping global climate feedback. Due to excessive fertilizer and pesticide use, agricultural pollution has profound impacts on soil ecosystems (Kalia, A. and Gosal, S.K., 2011). These changes affect not only microbial communities but also the functional integrity and resilience of ecosystems (Yadav et al., 2022). Most agricultural chemicals are nitrogen-based, which lower soil pH levels through nitrification, leading to soil acidification and release of greenhouse gasses into the atmosphere. Excess fertilizers and pesticides modify microbial community structure and function, accelerating carbon and nutrient cycling, creating soil acidification, and ultimately enhancing greenhouse gas emissions (Li et al., 2024; Lo, 2010; Yadav et al., 2022).

Acidification alters bacterial community assembly processes, thereby reducing microbial diversity and affecting ecosystem functions (Li et al., 2024). This shift favours acid-tolerant taxa and leads to the loss of more sensitive microbial groups, disrupting the equilibrium necessary for balanced nutrient cycling.

The use of pesticides has a similarly complex impact. As Lo (2010) outlines, pesticide effects vary widely depending on their chemical composition and application conditions. Some compounds, like carbofuran, may stimulate certain microbial groups, while others, such as fenamiphos, suppress beneficial organisms like nitrifying bacteria. Lo (2010) emphasized that the extent of stimulation or suppression depends on factors like pesticide concentration, persistence, frequency of application and organic matter content. Over time, microbial communities may adapt to certain pesticides through the development or acquisition of degradation pathways, but functional losses can persist if toxic effects dominate (Malhotra et al., 2021). These disruptions can alter microbial-mediated nutrient transformations and community structure, with long-term consequences for soil fertility and ecosystem stability. Although some microbes capable of degrading pesticides may proliferate, this often comes at the cost of reduced overall diversity and functional balance. As tolerant or specialised taxa become dominant, sensitive microbial groups involved in key ecological processes may decline, reducing functional redundancy and weakening ecosystem resilience. Such shifts can disrupt microbial-mediated nutrient transformations, including carbon and nitrogen

cycling, ultimately affecting soil fertility, organic matter turnover, and long-term ecosystem stability.

Dutta and Dutta (2016) outlined, and Abatehn et al. (2018) summarised, that in terrestrial soils and aquatic environments, microbial communities act both as sinks and sources of these gases. Photosynthetic microbes sequester carbon, while heterotrophs decompose organic matter and release GHGs, directly influencing atmospheric concentrations. These impacts extend beyond microorganisms. The increased use of agricultural chemicals negatively affects larger soil organisms, such as earthworms, reducing functional diversity and altering nutrient cycling. The decline in soil biota further weakens ecosystem services, such as aeration and organic matter breakdown (Pelosi et al., 2014).

Yadav et al. (2022) emphasize the critical role of beneficial microbes—particularly those in rhizospheric and endophytic niches—in supporting sustainable agriculture and environmental health. These microbes enhance nutrient availability, suppress pathogens, and improve plant resilience to stress. The authors advocate for microbial-based technologies like biofertilizers and biocontrol agents as eco-friendly alternatives to synthetic chemicals, potentially reversing some of the harmful trends caused by conventional agriculture. Additionally, extremophilic microbes from diverse environments hold promise for remediation efforts in polluted or degraded soils. This is particularly important, because as Li et al. (2024) showed, microbial diversity loss directly constrains soil's ability to mitigate climate change by reducing carbon sequestration and mitigating greenhouse gasses.

Sediments also play an essential role in nutrient cycling, organic matter decomposition, and pollutant degradation. However, anthropogenic pollution, including agricultural runoff, profoundly affects sediment microbial diversity, altering community structure, function, and resilience (Zoppini et al., 2020). Organic pollutants, such as pesticides and pharmaceuticals, enter sediments via runoff and wastewater, favouring the growth of specialized degraders, therefore displacing more sensitive taxa, leading to reduced microbial diversity and altered community assembly (Zhou et al., 2020). While these microbes help break down pollutants, they often do so at the expense of broader microbial diversity, disrupting microbial community balance and ecosystem stability. This imbalance can impair critical

biogeochemical processes, particularly those involved in the nitrogen, carbon, and sulphur cycles. These findings also highlight the gap in understanding the cumulative effects of multiple stressors acting simultaneously on microbial structure and function.

Furthermore, pollution-driven shifts in microbial communities often result in the increased abundance of pathogenic and opportunistic microorganisms, which pose risks to both aquatic organisms and human health.

Phosphorus, a key nutrient limiting primary productivity in both terrestrial and aquatic systems, is typically immobile in soils. However, heavy rainfall and runoff can mobilize phosphorus, introducing it into aquatic environments alongside nitrogen. In 2020, Kebedew and colleagues studied phosphorus distribution in Lake Tana—the largest freshwater lake in Ethiopia. They found that phosphorus concentrations have risen over the last five decades, supporting the proliferation of invasive water hyacinths in shallow, phosphorus-rich zones. A bathymetric survey revealed that lake morphometry played a significant role in nutrient accumulation. Hydropower plants were identified as major phosphorus sources, with prevailing winds and currents transporting phosphorus to the northern part of the lake and into the Blue Nile. Kebedew concluded that reducing inputs from the power plants and strengthening river connectivity could mitigate phosphorus buildup and curb water hyacinth spread (Kebedew et al., 2020).

Research done in 2016, focused on a pesticide spill in the river Kennet, suing a multilevel bioassessment approach to link gene level microbial changes to ecosystem impacts on nutrient cycling and productivity (Thompson et al., 2016). It was demonstrated that contamination effects can trigger immediate and cascading effects across biological hierarchies, underscoring the need for both broad scale synthesis and fine scale assessments in freshwater ecosystem management. As a contrast another research

provided a broader review of how multiple chemical stressors, like pesticides and pharmaceuticals, affect freshwater microbiomes (Bani et al., 2022). Bani et al. (2022) emphasised that addressing the cumulative and interactive effects of chemical stressors is critical for predicting microbial community responses and for guiding freshwater ecosystem management and conservation. The complexity of synergistic and antagonistic interactions was highlighted together with a knowledge gap on understanding combined effects and long-term ecosystem consequences.

1.2.2 Climate change

Climate change through warming, precipitation shifts, ocean acidification, and deoxygenation is reshaping microbial diversity and function across ecosystems. In terrestrial ecosystems, especially in cold and temperate biomes, soil microbial diversity decreases due to the loss of cold-adapted taxa and accelerated organic matter turnover, which is driven by rising temperatures (Dutta and Dutta, 2016). Microorganisms play a pivotal yet often underestimated role in the global climate system. Importantly, Abatehn et al. (2018) emphasized that microbial communities can adapt to changing environmental conditions, exhibiting functional resilience that modulates ecosystem responses and feedback loops under climate change. Such microbial plasticity allows certain taxa to maintain critical biogeochemical functions even under stress, although shifts in community composition can amplify greenhouse gas emissions if keystone taxa are lost. Therefore, climate change alters microbial community structure and function, potentially creating feedback loops that can intensify warming. However, microbial processes remain underrepresented in climate models, highlighting a critical research gap.

Recent findings by Li et al. (2024) further reinforce this perspective. Their research demonstrates that losses in soil microbial diversity constrain the soil’s ability to buffer against climate change. Specifically, reduced microbial diversity diminishes the thermal adaptation of

soil respiration, leading to increased CO_2 emissions under warming conditions. The loss of keystone microbial taxa—those critical for regulating respiration sensitivity—undermines the resilience of microbial communities and their role in long-term carbon stabilization. This emphasizes the ecological importance of conserving microbial diversity for climate regulation. The research done by Li et al. (2024), supports the findings of Pold and DeAngelis (2013) who demonstrated that warming-driven changes in microbial community composition—especially the loss of functionally significant microbial taxa—can lead to altered decomposition rates and carbon cycling dynamics. They highlighted that microbial responses to warming are often ecosystem-specific and can evolve over time, sometimes mitigating or amplifying carbon release depending on microbial adaptation. They also pointed that keystone microbes, though often low in abundance, can have disproportionate impacts on carbon feedback, stressing the need for their consideration in climate projections. The authors advocate integrating microbial traits and diversity metrics into Earth system models to improve the accuracy of carbon cycle feedback predictions. As climate change significantly influences the hydrological cycle, it is often examined together with ecohydrological connectivity. The interactive effects of multiple stressors on microbial communities have been further elucidated in experimental and observational studies. Sayer et al. (2021) showed that chronic drought induces microbial legacy effects that modify soil microbial responses to plant phytohormones, illustrating how historical climate stress reshapes nutrient cycling and microbe-plant interactions. In a broader multi-stressor context, Yang et al. (2022) demonstrated that the combined effects of climate change, pollution, and land-use modification can eliminate the positive effects of soil microbial diversity on ecosystem multifunctionality.

Altered river flows and groundwater recharge can trigger cascading effects across ecosystems. The interplay between ecological and hydrological processes—such as soil moisture regulation, nutrient cycling, and vegetation dynamics—is essential for sustaining ecosystem function and resilience. Ecohydrological connectivity has thus become a central

focus in conservation, particularly in response to increased terrestrial pollutants like herbicides and antibiotics found in freshwater systems globally (Urbaniak, 2021). Ecological connectivity involves both the physical and functional linkages among ecosystems. Key drivers include precipitation patterns, infiltration rates, surface runoff, and extreme weather frequency—all of which are being reshaped by climate change. These changes directly influence hydrological functions like river discharge and groundwater replenishment. Research indicates that altered precipitation regimes affect soil moisture dynamics and, consequently, ecosystem productivity. Coelho et al. (2013) emphasized that in marine sediments, climate stressors interact with pollutants to reduce microbial diversity and resilience, often selecting for tolerant or opportunistic taxa and reducing functional redundancy. Complementing this, research a couple of years later showed that organic pollutants under future climate changes, restructure sediment bacterial communities, producing non-additive effects on diversity and biogeochemical processing (Rodriguez et al., 2018).

Rising temperatures further reduce water availability and increase drought frequency, intensifying stress on both plant and microbial communities. In 2009, Hodgson and colleagues advocated for a “back to basics” conservation strategy centered on landscape connectivity. They emphasized that facilitating species movement across landscapes is crucial for adapting to changing climate conditions. Though primarily focused on terrestrial ecosystems, their work aligns with broader conservation goals that account for dynamic land use and climate-driven pressures. This approach supports earlier studies identifying anthropogenic impact as a major threat to biodiversity (Baigun et al., 2008; Hannah, 2011; LaPoint et al., 2015). Microbial taxa play vital roles in biogeochemical processes including decomposition, nitrogen fixation, and nutrient mineralization. Climate-induced shifts in temperature, moisture, and substrate availability alter microbial community composition and activity, thereby affecting the pathways and efficiency of nutrient cycling (Zalewski, 2015; Zhu et al., 2020). When combined with the insights of Dutta & Dutta (2016) and Li et al.

(2024), as well as the review of Abatehn et al. (2018)**,** it becomes evident that microbial processes are not only affected by climate change but are also central to understanding and mitigating its impacts. Integrating microbial dynamics into climate science and conservation planning is essential for effective ecosystem management in a warming world. Evidence from research papers suggest that climate change and pollution together interact to reshape microbial diversity and undermine ecosystem function in both terrestrial and marine sediments (Coelho et al., 2013; Rodriguez et al., 2018; Sayer et al., 2021; Yang et al., 2022). Across systems, stressors act non-additively and legacy from past stress alter present responses – together constraining the buffering capacity typically attributed to high microbial diversity.

1.3 Differences in microbial diversity

1.3.1 Sediment background

Even though coastal zones are challenging habitat due to tidal fluctuations, anthropogenic activity and seasonal changes, they are one of the most productive marine environments (Underwood et al., 2022). The microorganisms inhabiting these environments are important in primary production, nitrogen and carbon cycling. However, the sediment is vulnerable to different pollutants, including heavy metals, excess nutrients and hydrocarbons. The advancement in sequencing technologies and bioinformatics have enhanced the understanding of these microorganisms.

Krause et al. (2017) did research on ecohydrological interfaces being the hotspots for ecosystem processes. They highlighted the significance of habitat connectivity and discussed how climate change and land use modification can influence the functions of ecohydrological interfaces. Improved study of freshwater ecohydrological interfaces could provide a more integrated understanding of ecosystem connectivity, biodiversity, and microbial ecosystem functioning under environmental stress. In 2017 Griffin and colleagues characterized microbial diversity and dispersal connectivity in heavily managed soil, sediment, and water communities. The results showed high dispersal between environments and influence of factors like flooding, drainage and stream mixing in lotic environments. Griffin et al., (2017) suggested future work in quantifying connectivity during flooding events,

dry conditions and different types of hydrological networks as that could help in understanding whether the results are influenced by connectivity or habitat selection. They also observed similarities in soil and sediment communities, however, there was difference in richness and Shannon index. Significant proof of dispersal between soil and sediment and the aquatic environments was not found, therefore more research on the role of dispersal and connectivity in shaping communities during spring and early summer was suggested. Building on this microbial perspective, Chen et al. (2019) examined the effects of a pollution gradient in marine sediments and demonstrated that chemical stressors significantly reshape microbial community composition and function. Polluted sites showed elevated levels of sulphate-reducing bacteria and an expansion of the resistome, including genes linked to antibiotic resistance. Likewise, Xu et al. (2025) found that chemical pollution in marine sediments led to taxonomic and functional shifts**,** including the enrichment of potentially pathogenic genera, and increased resistance genes and altered nutrient cycling. These microbial changes were also shown to influence benthic metazoans, reinforcing that pollution-induced microbial shifts have ecosystem-wide implications. Zhang et al. (2021) reported similar patterns in riverine systems subjected to long-term agricultural contamination. They observed reduced microbial richness and evenness in areas with high nutrient loads from animal waste, along with the emergence of functionally specialized bacteria involved in phosphorus cycling. Network analyses in their study revealed distinct microbial modules corresponding to different pollution types,

These studies share several key insights with each other and with the work of Griffin et al. (2017). First, all highlight how pollution acts as a strong environmental filter, selecting for microbial taxa with specialized survival functions such as nutrient metabolism and antibiotic resistance. Second, they collectively emphasize that microbial community assembly is shaped by both connectivity and environmental selection, depending on local hydrology and pollution levels. Third, both the microbiome studies and Krause et al. (2017) ecohydrological framework underscore the critical role of interface zones — whether terrestrial-aquatic or sediment-water — as dynamic regions where biological, chemical, and physical processes converge.

Crucially, earlier in the decade, research showed that climate change stressors (warming, acidification, hypoxia) and pollutants, like nutrients and hydrocarbons, interact to erode microbial diversity and resilience in marine sediments, reducing functional redundancy and threatening ecosystem stability (Coelho et al., 2013). This conceptual framework was later supported experimentally, by Rodriguez et al. (2018), who showed that organic pollutants and future climate scenarios (warming, increased organic output) interact in non-additive ways to restructure bacterial communities in Baltic Sea sediments. Their results revealed reduction in diversity, selection for pollutant tolerant taxa, and altered biogeochemical processing.

Together, these studies converge on several themes. First, pollution acts as a strong environmental filter, selecting for taxa with specialized survival functions such as nutrient metabolism, sulphate reduction, or resistance to chemical stress. Microbial community assembly in sediments is shaped by both hydrological connectivity and environmental filtering, though their relative influence is context- and scale-dependent (Griffin et al., 2017; Chen et al., 2019; Rodriguez et al., 2018). Ecohydrological frameworks recognize sediment–water interfaces as key zones of biogeochemical exchange, but they often emphasize physical connectivity while giving limited consideration to microbial functional dynamics (Krause et al., 2017). In contrast, microbiome studies show that these interfaces are highly heterogeneous and sensitive to pollution and climatic stressors, which can rapidly restructure community composition and function (Coelho et al., 2013; Xu et al., 2025). Although anthropogenic stressors consistently drive microbial shifts, the predictability of resulting functional changes remains uncertain across spatial and temporal scales (Griffin et al., 2017; Rodriguez et al., 2018; Chen et al., 2019; Zhang et al., 2021). This reveals a gap between process-based ecohydrological models and empirical evidence of microbial sensitivity and non-linear responses. Integrating microbial functional ecology into ecohydrological frameworks is therefore essential for improving predictions of sediment ecosystem responses under increasing anthropogenic pressure.

1.3.2 Soil background

Climate change is expected to significantly affect soils and ecosystems through rising temperatures and altered precipitation patterns, including changes in rainfall amount and frequency. These shifts will, in turn, disrupt hydrological and biogeochemical cycles. Key macronutrients such as carbon, nitrogen, and phosphorus circulate through the biosphere via biogeochemical cycles sustained by diverse and functionally adaptable soil microbial communities (Crowther et al., 2016). However, the combined pressures of climate change, biological invasions, and other anthropogenic disturbances can disrupt soil environments and the microbial processes that regulate nutrient availability, with consequences for ecosystem functioning. Human activity, both directly and indirectly, contributes to climate change, thereby altering the composition of the global atmosphere. Global average temperatures have risen by 1.1°C since the preindustrial era and could potentially increase by up to 4°C by the end of the 21st century due to the escalating concentration of greenhouse gases. Since soils are interconnected with the climate system through nutrient and hydrological cycles, global climate change is anticipated to affect soil fertility through its physical, chemical, and biological properties.

Weather modifications, including the introduction of greenhouse gases into the atmosphere, heavily depend on the compositional structure and functional changes in the soil microbiome. Understanding how microorganisms regulate the flux of greenhouse gases is crucial for improving climate models, which requires comprehending the complex interactions between microorganisms and other biotic and abiotic factors. Recent research by Zhou et al. (2020)**,** through a global meta-analysis of over 1,200 observations, reveals that the effects of global change factors—such as warming, altered precipitation, nitrogen deposition, and land-use change—on soil microbial diversity and function are complex and context-dependent. While microbial alpha diversity does not always decrease under global change pressures, shifts in community structure and biomass are more consistent drivers of changes in soil functionality.

Additionally, rare microbial taxa tend to be more sensitive to environmental changes, and soil pH was identified as a key environmental variable mediating these effects. These findings emphasize the importance of looking beyond simple diversity metrics to assess microbial contributions to soil processes and climate feedback.

Agricultural ecosystems are under increasing pressure from human activities and environmental disruptions like climate fluctuations, plant invasion, and the buildup of pollutants, pesticides, and antibiotics. Maintaining soil health is vital for agricultural sustainability and reflects the productivity of agro-ecosystems. Soil resources are under severe threat from human activities and climate change. Connecting the distribution of microbial diversity with ecosystem functioning is essential for understanding ecosystem responses to environmental changes. Soil microbial communities are crucial in the context of global climate change as they play significant roles in plant growth and carbon sequestration. Research by Jiao et al. (2019) demonstrated that soil microbiomes exhibit strong resilience to chemical contamination, with taxonomic profiles responding more sensitively than functional profiles to selection processes induced by different legume plants. However, they indicated that the recovery of soil microbial alpha diversity to a stable state is greatly influenced by pollutants. Their 16S rRNA data showed richer microbial diversity in the presence of plants after 90 days. Nonetheless, their study did not account for climate change. Without considering climate change, alpha diversity showed recovery patterns by day 30. Future research will include a 15-day incubation period in a water bath with a 4°C temperature increase, potentially slowing recovery and reducing microbial richness. Building on these findings, Sayer et al. (2021) showed that chronic drought adaptation modifies soil microbial community responses to plant phytohormones, indicating that climate-driven stressors alter microbe-plant interactions and could reshape nutrient cycling feedback in terrestrial ecosystems. In broader experimental context, Yang et al. (2022) demonstrated that the combined effects of multiple anthropogenic pressures – including climate change and pollution - can eliminate the positive effects of microbial diversity on ecosystem functioning, underscoring that diversity alone does not guarantee resilience under compound global change drivers.

Microbes exhibit high growth rates and can rapidly adapt through horizontal gene transfer, allowing their functions to shift even if their composition does not change under environmental disturbances. Soil microbiomes regulate the biogeochemical cycling of macronutrients, micronutrients, and other elements essential for the growth of plant and animal life. The work of Zhou et al. (2020), together with insights from Sayer et al. (2021) and Yang et al. (2022), reinforces the importance of integrating both microbial structure and functional potential into climate resilience strategies and soil management practices

1.4 Aims

Understanding and predicting the impact of climate change on soil microbiomes and the ecosystem services they provide both a significant challenge and a major opportunity. Given the importance of the soil and sediment microbiome, it is vital to determine its functions in response to heavy contamination in the context of climate change. Quite a few previously mentioned scientific studies highlighted the knowledge gap in understanding microbiome response in ecosystem level during both climate change and pollution. This scientific study will fill some of the gap and could therefore offer insights into restoring polluted ecosystems and returning healthy soil to benefit plant growth.

There was a hypothesis put forward that soil pollution, particularly from chemical contaminants such as antibiotics and agricultural runoff, will promote the proliferation of antimicrobial-resistant (AMR) bacterial. This is supported by studies showing that chemical stressors act as strong environmental filters, selecting for microbial taxa capable of surviving in polluted conditions and often enriching the soil resistance (Chen et al., 2019); Xu et al., 2025). Furthermore, it is predicted that the combined effects of pollution and elevated temperatures will reduce overall microbial diversity over time. Rising temperatures accelerate organic matter turnover and stress microbial communities, while persistent chemical inputs impose additional selective pressures, favoring tolerant or opportunistic taxa at the expensive of sensitive ones (Abatehn et al., 2018; Li et al., 2024; Yang et al., 2022).

Over extended periods, this interactions between warming and pollution are expected to diminish microbial richness and evenness, potentially disrupting key ecosystem functions such as nutrient cycling and carbon stabilization. This research will investigate whether temperature difference and pollution act to restructure soil and sediment microbial communities. These interactions are expected to enhance the prevalence of AMR while reducing microbial diversity and ecosystem resilience.

2. Methods

Metabarcoding and eDNA extraction is a common method used for assessing microbial diversity. Even though papers do not always mention the specific kits, the most common one is Qiagen DNeasy Powersoil Kit or similar. It is known for its reliability and efficiency in extracting high-quality DNA suitable for downstream applications like PCR amplification and sequencing. However, it also noted that protocols that come with these kits often require optimization or adjustments, for example additional purification to remove inhibitors (Pawloski et al., 2022). Pawloski et al (2022) reviewed sediment sampling and DNA extraction methods for benthic monitoring. The authors noted that delaying processing samples can lead to DNA degradation, but if the immediate processing is not possible then freezing samples or adding chemical preservatives, like ethanol, can stabilize the eDNA. Effective removal of inhibitors, that can be PCR amplification, is mentioned as one of the challenges for successful DNA analysis. It is also mentioned that distinguishing between living and dead organisms, as well as between species residing in situ and those that have passively settled from the water column, presents a significant challenge. As a solution using RNA has been proposed, however eDNA provides better accuracy and it is simpler. Using RNA, means stricter storage/preservation techniques like deep freezing and it is also more time-consuming and expensive compared to eDNA (Wood et al., 2020; Pawlowski, et al., 2022).

Metabarcoding is a widely used approach. It non-invasive and broadly applicable, highly sensitive, detects multiple taxa and is useful for poorly characterized environments. Emami-Khoyi et al. (2025) used it for eDNA assessment of eukaryotic biodiversity in supratidal microbialite pools. They noted its usefulness in uncovering taxa in habitats like microbialites that are often under-surveyed. However, the limitations of metabarcoding were also noted, as its database had an underrepresentation of many known macroinvertebrates, therefore many reads could not be assigned beyond phylum or class level. Also, there is a possibility of high number of false positives, as DNA in water can persist beyond the presence of organisms, like legacy DNA. It was noted that to improve future ecological assessments, there is a need for more comprehensive barcoding of local taxa for macroinvertebrates (Emami-Khoyi et al., 2025).

Metabarcoding employs next generation sequencing technologies to sequence a multiplexed library pool. In this approach, specific genetic markers in the sample are amplified with target specific primers that bind to conserved region of the genome shared across the taxa. While these regions are conserved, they also contain species specific variation, enabling taxonomic resolution. By sequencing these amplified regions it's possible to identify organisms in a community, typically at the genus or species level.

Quite a few papers mention methodological inconsistency, highlighting the need for standardized protocol and therefor broader application of eDNA methods (Pawlowski, et al., 2022; Banerjee et al., 2021). Due to differences in characteristics of environment (pH, temperature, oxygen level and others) the abundance of DNA in ecosystems varies. Now, there is a risk of generating a false biodiversity information, for example a false detection of a species may change their conservation status. The standardization of protocols not only need to include small scale but also need to be feasible on a large scale, particularly when considering thresholds. There is also a need for further expansion of reference databases, so that species can be classified to the smallest levels of taxa (Emami-Khoyi et al., 2025).

2.1 Field sampling and preparation for lab analysis

Terrestrial soil was collected from Roman River Valley nature reserve and marine sediment from River Colne estuary, from the intertidal at Brightlingsea, during May / June 2024 during low tide. Both times only the upper 5-10cm were collected from the field in the bucket and then taken to the lab. In the lab soil and sediment were homogenized by putting them through the sieve and small leaves, branches and stones were taken out. Two water baths were prepared one at 16^{o}C (ambient) and the other at 20^{o}C (warm). 4^{o}C degree difference was chosen to replicate possible temperature increase in the UK in late spring/early summertime. Temperature condition was chosen as it is one of the strongest environmental drivers for ecological and microbial processes. 40 250ml cylinders were filled halfway with soil and placed in each water bath. When 10-day soil microcosm experiments were done, the cylinders were rinsed with water and then sediment experiment was done. Soil and sediment each had four different treatments – control, chemicals, organic fertilisers, and chemicals and organic fertilizer.

For sediment the chemicals that were used were herbicide (diflufenican), fungicide (chlorothalonil and tebuconazole), and antibiotic (oxytetracycline). For soil the chemicals were herbicide (glyphosate), fungicide (tebuconazole), antibiotic (amoxicillin), and insecticide (imidacloprid). Tebuconazole and chlorothalonil were found in the Fungus Fighter Plus, glyphosate and diflufenican in Roundup. Oxytetracycline, amoxicillin and imidacloprid were found as powders in the laboratory. Chemicals were chosen based on most abundant ones in UK soil and sediment which are also used in agronomy. To replicate the organics, fertilizer mix 1x Hoagland's solution was used. It was made sure that each chemical was used in concentration 0.01 g/l and then mixed together before use. Each treatment had 10 replicates in both water baths. Sampling was done after 0h, 24h, 48h, 96h and 168h. Both soil and sediment were collected in 2ml Eppendorf tubes. Samples were stored in the

freezer for further molecular analysis.

2.2 Lab work

2.2.1 Quantification of AMR bacteria

The following experiment was designed to test for the hypothesis that chemical contaminants promote the proliferation of AMR bacteria. To measure the abundance of AMR bacteria, samples used were 168h after the treatment. Five replicates from each treatment were taken for Extended Spectrum Beta Lactase (ESBL) plates. To prepare the plates 0.5g of soil or sediment was diluted with 5ml of water in the laminar flow cabinet. The mixture was vortexed for 20sec and then 100μl was sampled and spread on the Agar plate. The plates were incubated at 36^{o}C for 48h after which bacterial colonies were counted.

2.2.2 Molecular methods

The following experiment was designed to test for the hypothesis that combined effects of pollution and elevated temperatures will reduce microbial diversity over time. To quantify the diversity and composition of the microbial communities, four replicates from each timepoint were randomly selected. DNA was extracted following the protocol of QIAGEN Powersoil DNeasy Pro kit. Then PCR 96 well plates were prepared. For the PCR, Golay4 341F and 805R primers with Illumina attachment and DNA concentration 1:10 was used to target 16S and 18S DNA (Klindworth et al., 2013). Targeting 16S was chosen to analyse bacterial abundance. 18S was chosen to understand invertebrate abundance. First the mastermix was made using 1250μl of Appleton wood AppTaq Red mix (2x), 800μl of H_2O, 100μl of reverse and 100μl of forward primer. The primers concentration was 1 in 10. 22,5μl of mastermix was pipetted into each well and then 2,5μl of DNA was added. The plate was sealed, centrifuged at 500rpm for 30-40sec. The thermocycling conditions were different for both 16S and 18S. For 16S denaturing stage was 3min at 95^{o}C. The stage 2 was 30 cycles long. It started with 30sec of 95^{o}C, the annealing stage of 30sec at 55^{o}C and

finished with 30sec at 72ºC. It finished with stage 3 with extending for 7min at 72ºC and was held at 20ºC until next step could be done. The samples meant for 18S analysis were first held for 3min at 95ºC. The 2nd stage was 25 cycles long and started with 15sec at 95ºC, which was followed by anealling for 30sec at 58ºC. That was finished by 30sec at 72ºC. The last, extension step was 7min at 72ºC. At the end of thermocycling, the PCR plate was kept at 20ºC till the next step could be done. To check for successful and specific amplification, immediately after thermocycling PCR product was visualized on the gell, which was made using 1% TAE buffer and 1μl of SYBR Safe DNA Gel Stain. Randomly choosing 5μl of DNA from the PCR plate were put in the wells and run at 70V for 30min. Then purification was done using Agencourt AMPure XP beads and following the protocol of their use. However, the protocol was slightly adjusted. 8μl of beads was used per 10μl of PCR product, instead of protocol suggested 18μl of beads. As the elution buffer, 26.25μl of the EB buffer 6 from Qiagen Powersoil DNeasy Pro kit was used, instead of 40μl. After the purification, e-gel was done using 15μl of TE Buffer and 5μl of DNA from the PCR plate. For the wells that had no DNA, just 20μl of TE Buffer was used. The ladder was used for the first and last well of each row, but it was diluted first using 50μl of ladder and 50μl of TE Buffer. After e-gel, indexing was done. Before use, indexes were spun for 10sec. Indexing was done under the floom hood and tips were changed after index was added to the row or column. Indexing was done using Nextera XTsample prep kit. For 16S indexes B, C and D were used. For 18S, indexes A, C and D were used. After the indexing, normalization was done using Pico Green dsDNA kit and the samples were pooled in equimolar concentration. The PCR, purification, indexing and normalization was done for 16S and 18S DNA.

2.2.3 Bioinformatics analysis and sequencing library preparation.

After normalization PCR was done. First, the standard curve for 16S and 18S was created. Soil and sediment samples for qPCR were chosen randomly based on 4 treatments and 3 time points (24h, 48h and 168h) from 2 temperature conditions. Mastermix was made using autoclaved water, forward and reverse primers with no Illumina, and taq from SensiFAST SYBR No-ROX kit by Meridian Bioscience. 10 μl was chosen as the final volume, therefore 9μl of mastermix and 1μl of DNA sample before the extractions were pippeted in the PCR plate. The plate was then put in the centrifuge to spin for 30sec at 1000 x g. The plate was put in the PCR machine and then sent for sequencing. Sequence data were first demultiplexed, and primer sequences were removed using cutadapt v4.4 (Martin, M., 2011), allowing no mismatches in the primer region and removing poly-G tails. Sequences were then quality controlled using fastp (Chen, S., 2023). Low quality sequences in which >20% of bases are lower than q20 and sequences shorter than 200nt (bacterial 16S) or 100nt (eukaryal 18S) in length after trimming were removed. Forward and reverse sequences were pair-end aligned with a minimum overlap of 15nt also using fastp. Sequences were then clustered into ASVs using VSEARCH v 2.30 (Rognes et al., 2016). Briefly, sequences were first dereplicated and sequences with fewer than 8 occurrences across the dataset were discarded, before being denoised with the UNOISE3 algorithm (Edgar, R. C., 2016) implemented in VSEARCH. Chimeric sequences were discarded using the UCHIME3 algorithm (Edgar, R. C., 2016), and sequences were mapped to ASVs at 97% identity. Taxonomy was assigned to ASVs using the RDP classifier 2.14 using training set 19 for bacterial taxonomy (Wang, Q., and Cole J. R., 2024) and the PR2 database for eukaryal taxonomy (Guillou et al., 2012). Data analysis was done in the R studio using ggplot (Wickham, H., 2011), dplyr, vegan and tidyr packages (Wickham et al., 2023; Wickham et al., 2024;

Oksanen et al., 2025).

3. Results

3.1 Culturing

Bacterial growth, measured as colony-forming units (CFU), in soil and sediment samples showed distinct visual patterns across both temperature treatments and through all four chemical treatments (Fig 3.1.1). In soil, CFU values indicated higher abundance of antibiotic-resistant bacteria when fertilizer (org, org w) was added compared to chemical-only (ch, ch w) treatments. This pattern is evident during 20°C temperature treatments, when added chemicals and fertilizer (ch + org) are combined and showed highest bacterial colony counts. Similarly, sediment samples show elevated colony counts when fertilizer was added, with the samples that were kept in 20°C temperatures, having high bacterial growth compared to control (h2o) or chemical only samples. However, despite visually apparent differences in CFU values, Kruskal-Wallis test did not reveal any significant effects of combined treatments. When done on soil samples to evaluate joint temperature and chemical treatments, statistical test indicated no significant variation in bacterial abundance amongst the treatment groups ($x^2 = 7.0$, df = 7, p = 0.429). An equivalent analysis for sediment samples produced the same outcome ($x^2 = 7.0$, df = 7, p = 0.429). These results point high within-treatment variability that mask any consistent treatment effects. Bacterial growth, measured as colony-forming units (CFU), in soil and sediment samples showed distinct visual patterns across both temperature treatments and through all four chemical treatments (Fig 3.1.1). In soil, CFU values indicated higher abundance of antibiotic-resistant bacteria when fertilizer (org, org w) was added compared to chemical-only (ch, ch w) treatments. This pattern is evident during 20°C temperature treatments, when added chemicals and fertilizer (ch + org) are combined and showed highest bacterial colony counts. Similarly, sediment samples show elevated colony counts when fertilizer was added, with the samples

that were kept in 20°C temperatures, having high bacterial growth compared to control (h2o) or chemical only samples. However, despite visually apparent differences in CFU values, Kruskal-Wallis test did not reveal any significant effects of combined treatments. When done on soil samples to evaluate joint temperature and chemical treatments, statistical test indicated no significant variation in bacterial abundance amongst the treatment groups (x^2 = 7.0, df = 7, p = 0.429). An equivalent analysis for sediment samples produced the same outcome (x^2 = 7.0, df = 7, p = 0.429). These results point high within-treatment variability that mask any consistent treatment effects.

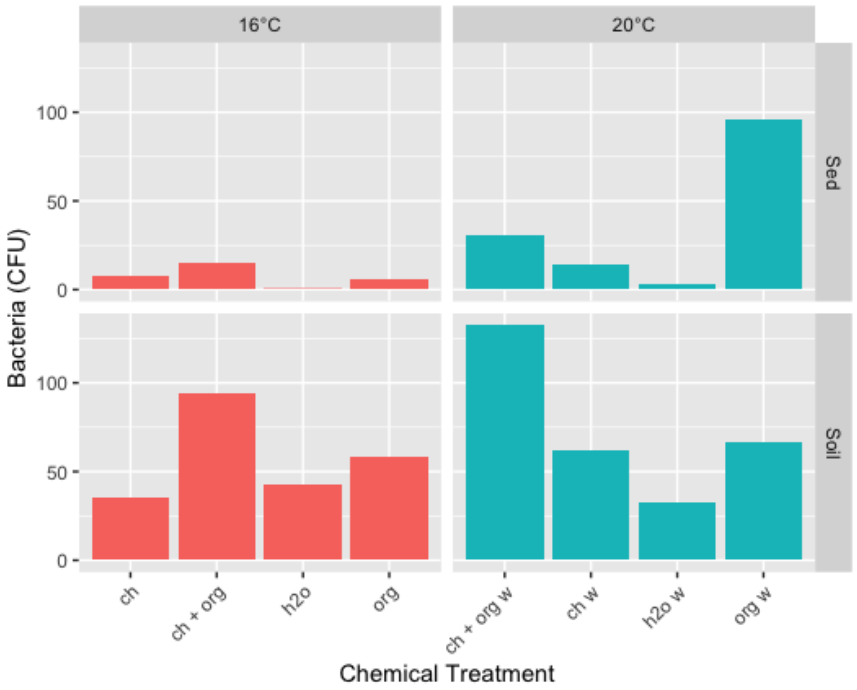

Fig 3.1.1 Shows bacterial colony abundance during two temperature treatments and four chemical treatments – fertilizer (org), chemicals (ch), chemicals and fertilizer (ch + org) and control (h2o) – using ESBL plates.

3.2 16S Sequencing

16S gene sequencing revealed diverse bacterial communities across all soil and sediment samples. Diversity patters as measured by richness, Shannon diversity, and evenness indices, varied strongly between the two habitats and were further shaped by added chemicals or fertilizer mix and temperature treatments. While both habitats contained a broad range of microbial taxa, soils and sediments responded differently to experimental conditions. Soils were generally more stable, with relatively consistent alpha diversity across treatments, whereas sediments exhibited stronger responses, including significant reductions in richness under specific conditions of temperature and added chemicals.

3.2.1 Soil microbiome

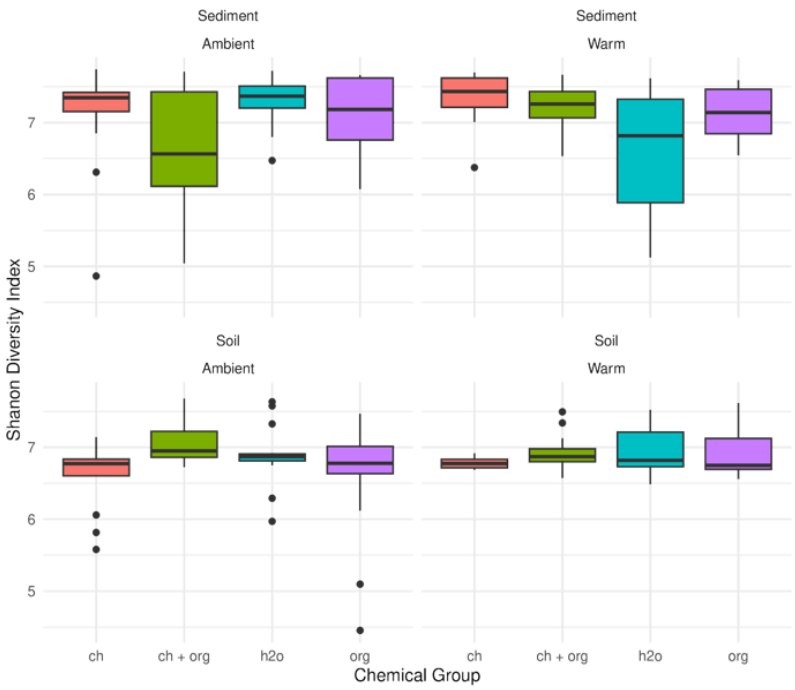

Fig 3.2.1 Microbial diversity in soil and sediment during two temperature conditions (ambient and warm) and four chemical treatments: chemicals (ch), chemicals and fertilizer (ch + org), control (h2o) and fertilizer (org).

Shannon diversity in soil shows overall stability across all treatments (Fig. 3.2.1). Diversity values remained consistent between ambient and warmed conditions, regardless of added chemical type. When only chemicals were added, soil exhibited slightly lower Shannnon diversity under warming, but this effect was not statistically significant. The general lack of variation in Shannon diversity suggests that soil communities maintained both taxonomic richness and evenness of species abundance across treatments, buffering against the effects of environmental stressors. However, performing Wilcoxon test, soil shows lower diversity than sediment ($W = 11.258$, $p < 0.001$; Fig 3.2.1).

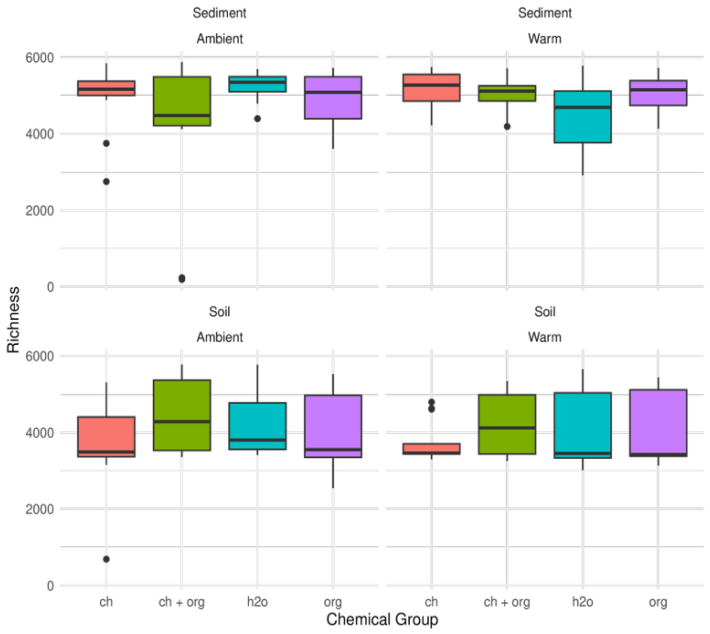

Fig 3.2.2. Richness in soil and sediment during two temperature conditions (ambient and warm) and four chemical treatments: chemicals (ch), chemicals and fertilizer (ch + org), control (h2o) and fertilizer (org).

Microbial richness in soils remained relatively stable across all tested treatments and temperatures (Fig. 3.2.2). A Kruskall-Wallis test revealed no significant differences in richness between added chemicals-temperature combinations ($x^2 = 12.97$, $df = 8$, $p = 0.113$). This result indicates that despite the addition of different chemicals and the influence of warming, soil microbial communities retained comparable levels of richness. Boxplots show overlapping distributions across treatments with no strong directional shifts. Although, no

statistically significant, some minor patterns were apparent. When only chemicals (ch) were added, under warming conditions soil microbiome showed a slight reduction in richness compared to ambient conditions. In contrast, when fertilizer mix (org, ch + org) was added in ambient temperature treatment, microbiome tended to maintain richness values that were slightly higher than in samples were only chemicals (ch) added or control (h2o). However, these differences were small and did not reach statistical significance, reinforcing the resilience of soil microbial communities.

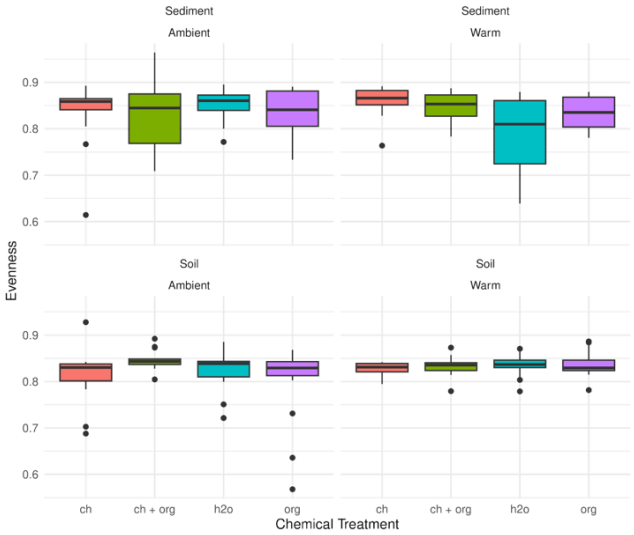

Fig 3.2.3. Evenness in soil and sediment during two temperature conditions (ambient and warm) and four chemical treatments: chemicals (ch), chemicals and fertlizer (ch + org), control (h2o) and ferilizer (org).

Soil microbial evenness (Fig. 3.2.3) was consistently higher across treatments and did not vary significantly with temperature or added chemical type (Kruskall-Wallis, $x^2 = 11.56$, df = 7, p= 0.116 when all samples were considered). When analysed separately, soil evenness showed no evidence of treatment effects, same as richness and Shannon diversity results.

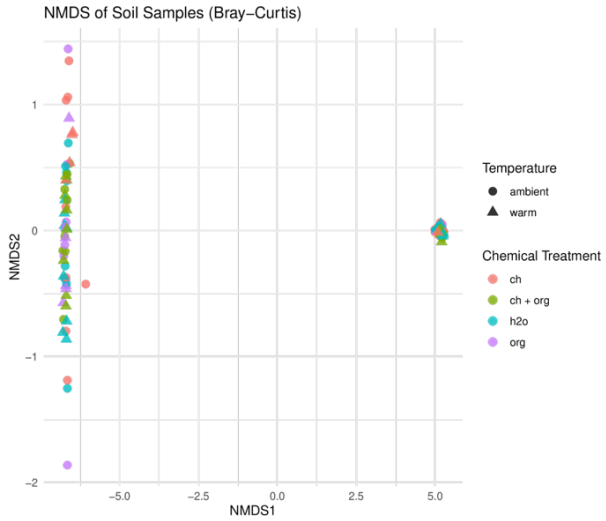

Fig 3.2.4 Soil sample NMDS graph doing Bray-Curtis analysis

NMDS analysis indicated that soil microbial communities were stable across treatments. Permanova revealed no significant effects of temperature (R^2 = 0.021, p = 0.214), added chemicals (R^2 = 0.022, p = 0.427) or their interactions (R^2 = 0.014, p = 0.740) on soil microbiome. Residual variation accounted for 93% of the total variance. These findings confirm that soil microbiome is robust to warming and chemical treatments, consistent with the stability observed in richness, evenness and diversity graphs and statistics.

3.2.2. Sediment microbiome

Shannon diversity in sediments showed reduction in warming treatment and water only added, compared to ambient (16^{o}C) conditions (Fig 3.2.1). In both cases when fertilizer mix (org, ch + org) was added, sediment samples showed higher Shannon diversity in both temperature conditions, indicating that these amendments buffered against diversity loss.

In contrast to the stability observed in soils, sediment microbial richness was strongly influenced by the temperature and added chemicals or fertilizer (Fig 3.2.2). A Kruskal-Wallis test detected significant differences in richness among treatment combinations (x^2 = 18.96,

df = 8, p = 0.015). Graph shows that sediments subjected to warming conditions with only water (h2o) added exhibited the lowest richness values, while when fertilizer mix (org, ch + org) was added exhibited higher richness.

Evenness in sediments was generally lower than in soils and showed greater variability across treatments (Fig 3.2.3). A Kruskal-Wallis test was performed, and it indicated no significant treatment effect (x^2 = 11.89, df = 7, p = 0.104). Control samples in warming conditions showed reduction in evenness, with communities unevenly dominated by fewer taxa. By contrast, when fertilizer was added, sediments maintained higher and more stable evenness under both ambient and warming temperature treatments. The Wilcoxon test performed between soils and sediments confirmed that sediments exhibited significantly lower evenness than soils overall (W = 10.305, p = 0.0009), highlighting fundamental structural differences.

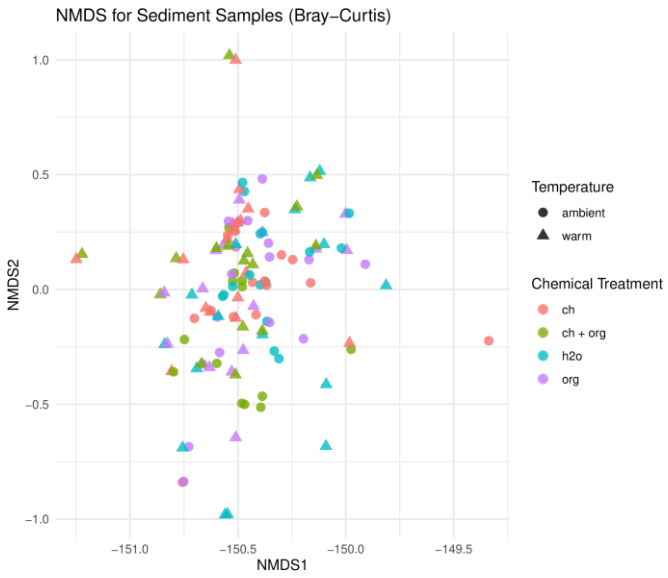

Fig 3.2.5 Sediment sample NMDS graph doing Bray-Curtis analysis

In NMDS analysis, sediment communities show significant shift in response to temperature treatments (Fig 3.2.5) with Permanova result R^2 = 0.043, p = 0.001. Added chemicals showed a smaller variation (R^2 = 0.029, p = 0.055). The interaction between

temperature and chemicals showed higher significance (R^2 = 0.037, p = 0.003), indicating that chemicals act different across different temperatures. Collectively, these factors accounted for 11% of variance, while 89% are explained by different factors. Warmer treatments, particularly when only water was added tend to cluster along the x axis, while samples that had fertilizer mix added to them (org, ch + org) seem to cluster more around ambient temperature samples. These results indicate that sediments are more compositionally responsive to environmental change than soils, with warming and added chemicals driving shifts in community structure.

3.3 18S sequencing

3.3.1. Soil microbiome

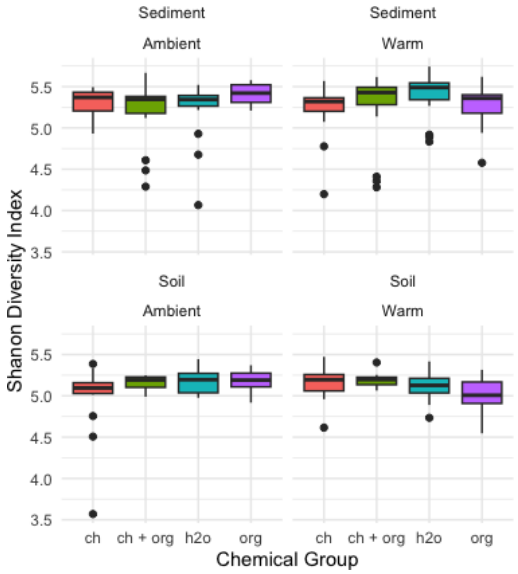

Fig. 3.3.1. Microbial diversity in soil and sediment during two temperature conditions (ambient and warm) and four chemical treatments: chemicals (ch), chemicals and fertlizer (ch + org), control (h2o) and fertilizer mix (org).

Kruskal–Wallis results showed no significant differences across treatments or temperature regimes (χ^2 = 7.0, df = 7 p > 0.05). Pairwise Wilcoxon tests further supported this lack of treatment-driven variation (p > 0.1).

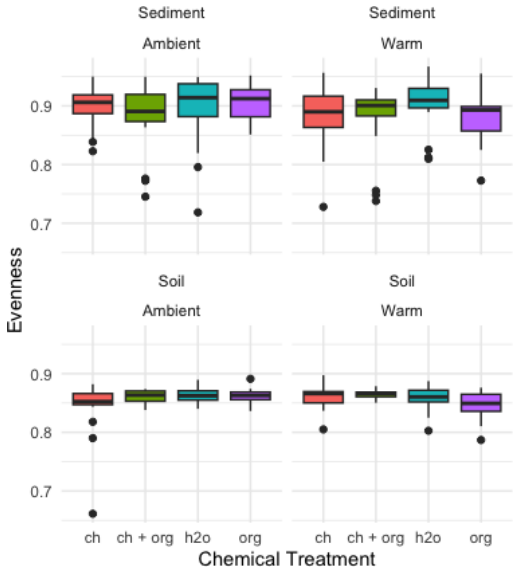

Fig 3.3.3. Evenness in soil and sediment during two temperature conditions (ambient and warm) and four chemical treatments: chemicals (ch), chemicals and fertlizer (ch + org), control (h2o) and fertilizer mix (org).

Soil microbial evenness was generally lower than in sediments, with medians clustered between 0.80 and 0.85 (Fig 3.3.3). Within soils, Wilcoxon test showed that temperature and chemical treatment significantly influenced evenness ($W = 13{,}216$, $p = 3.11 \times 10^{-16}$).

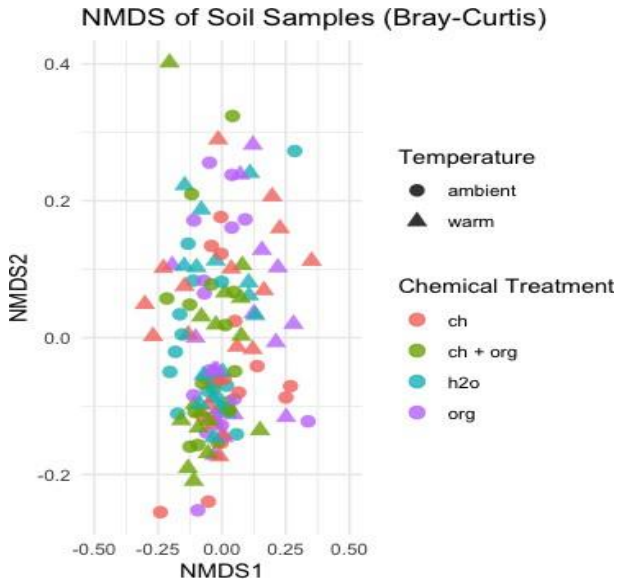

Fig 3.3.4 Soil sample NMDS graph doing Bray-Curtis analysis

Soil NMDS graph revealed strong treatment-related clustering (Fig 3.3.4). Although some overlap persisted, samples separated more distinctly by added chemical groups, particularly under warming conditions. For example, samples, where chemicals and fertilizer mix (ch + org) were added, clustered apart from control samples (h2o), suggesting that added pollutants exerted selective pressure on microbial community composition. These visual differences were supported by Permanova test. Both temperature (R^2 = 0.024, F = 1.59, p = 0.008) and chemical treatment (R^2 = 0.032, F = 1.44, p = 0.001) significantly explained community variance, with pollutants exerting a stronger influence than warming. No interaction was observed between the two factors (R^2 = 0.022, F = 1.00, p = 0.454) suggesting additive but independent effects.

3.3.2. Sediment microbiome

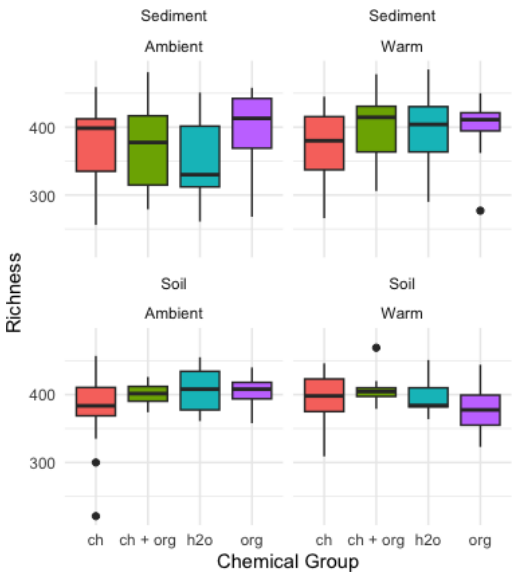

Fig 3.3.2 Richness in soil and sediment during two temperature conditions (ambient and warm) and four chemical treatments: chemicals (ch), chemicals and fertilizer (ch + org), control (h2o) and fertilizer mix (org).

Richness in sediments ranged from ~ 300 to ~500 observed taxa (Fig 3.3.2). A Kruskal–Wallis test suggested marginal treatment effects when both temperature and chemical groups were considered together (χ^2 = 13.656, df = 8, p = 0.0912). Although not statistically

significant, fertilizer-only treatments showed a tendency to support higher richness compared to chemical-only and water-only treatments, particularly under warming.

Sediment microbial communities exhibited consistently high evenness across chemical treatments and temperature regimes, with median values generally above 0.85 (Fig 3.3.3). Organic-only treatments tended to reduce evenness slightly, especially under warming, though Kruskal–Wallis tests did not detect significant treatment effects.

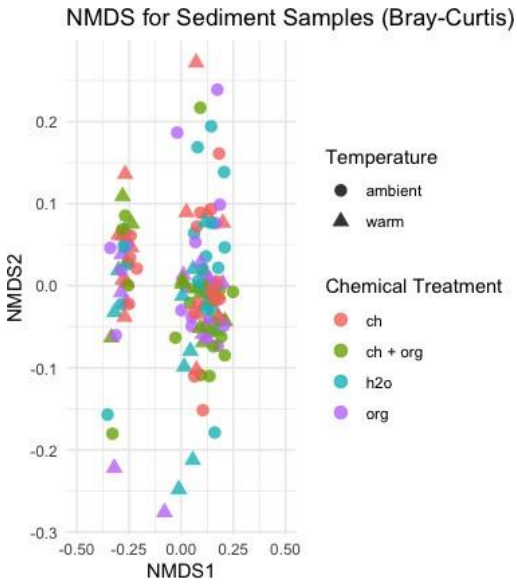

Fig 3.3.5 Sediment sample NMDS graph doing Bray-Curtis analysis.

The NMDS graph for sediment samples (Fig 3.3.5) showed moderate overlap across chemical treatments, with only weak clustering by either pollutant exposure or temperature. In both temperatures (ambient and warm) samples were distributed along similar gradients, suggesting broad community stability. While certain warm-treated samples (particularly ch + org) displayed subtle displacement ordination space, there was no strong segregation. This pattern is also seen in the Permanova results. Temperature explained small but significant proportion of variance (R^2 = 0.026, F = 1.71, p = 0.012) whereas chemical treatments had no delectable effect (R^2 = 0.021, F = 0.92, p = 0.581). No significant interaction between warming and chemical stress was detected (R^2 = 0.024, F = 1.03, p = 0.343). Thus,

sediment microbial communities are primary structured by temperature rather than pollutants, though the effect size remains small (~ 2.6% of total variance explained).

4. Discussion

Understanding and predicting the impacts of climate change on soil and sediment microbiomes represents both a pressing challenge and a unique opportunity. This study set out to test the hypothesis that combined warming and chemical contamination would restructure microbial communities, favour pollution-tolerant and antimicrobial-resistant (AMR) taxa while reducing overall diversity, richness, and evenness. If these shifts were analysed deeper, they could be expected to compromise key ecosystem functions, including nutrient cycling and carbon stabilization, with sediments predicted to be particularly vulnerable due to higher hydrological connectivity and pollutant exposure (Chen et al., 2019; Xu et al., 2025; Abatehn et al., 2018; Li et al., 2024; Yang et al., 2022).

The results partly support these hypotheses but also reveal important nuances. AMR bacterial abundance, measured as colony-forming units (cfu), responded strongly to warming and organic inputs, especially when combined, with both soils and sediments showing elevated counts under these conditions. This aligns with earlier studies showing that warming accelerates microbial metabolic rates and organic carbon turnover, stimulating microbial proliferation (Pold & DeAngelis, 2013; Li et al., 2024). However, the lack of statistically significant treatment effects suggests high within-treatment variability, potentially masking consistent abundance responses. Similarly, the bacterial culturing on ESBL plates showed increased abundance, particularly in samples kept in warm temperature conditions, therefore supporting previously documented responses of enhanced abundance of microbial communities in high nutrient availability in agricultural soils (Yadav et al., 2022; https://ourworldindata.org/global-land-for-agriculture). Nutrient-rich inputs stimulate bacterial proliferation, a mechanism central to carbon cycling and often associated with accelerated

CO_2 release in intensively managed soils. It could be similar to stimulation of spring bacterial diversity. Bacterial proliferation is driven by the alleviation of nutrient limitation, which provides microbes with readily available resources for growth and metabolism. Increased substrate availability allows populations to expand and enhances metabolic processes such as decomposition and respiration (Zhou et al., 2020). Despite this rise in abundance, community richness, evenness, and diversity remained largely stable, indicating that higher resource levels did not trigger strong competitive exclusion. This stability likely reflects functional redundancy, where multiple taxa exploit the same resources without displacing one another (Jiao et al., 2019). Additionally, soil micro-niches and spatial heterogeneity support co-existence, allowing substrate diversity, metabolic versatility, and resource partitioning to promote biomass growth without diminishing overall diversity. The sediment response to chemical and temperature treatment is consistent with the results in aquatic ecosystems, where organic enrichment from agricultural runoff enhances microbial growth, but can reduce functional diversity and ecosystem stability. However, despite the apparent trends in results, the lack of statistically significant treatment effects suggests considerable within treatment variability, which may have masked consistent abundance responses across treatments.

In contrast, microbial richness, evenness, and Shannon diversity remained remarkably consistent across treatments in soils, whereas sediments exhibited greater sensitivity. This stability in soils likely reflects functional redundancy—the presence of multiple taxa performing similar ecological roles—which buffers diversity against environmental stressors (Jiao et al., 2019; Zhou et al., 2020). Yet, as emphasized by Li et al. (2024), stable diversity metrics may conceal the loss of keystone taxa with disproportionate roles in regulating greenhouse gas fluxes and nutrient cycling. The NMDS results support these findings, as the ordination reveal subtle compositional shifts, indicating that certain microbial species are replacing others in functional roles within community. Their removal can weaken long-term ecosystem resilience, even when overall richness appears unaffected. However, sediment microbiomes exhibited stronger compositional sensitivity in NMDS analyses, echoing

findings from Coelho et al. (2013) and Rodriguez et al. (2018). Both researchers suggested that aquatic sediments are particularly vulnerable to multi-stressor interactions, often restructuring toward opportunistic or stress-tolerant taxa.

The lack of significant diversity differences across treatments also underscores that persistence of richness does not guarantee functional stability (Li et al., 2024). Keystone taxa, though often rare and not captured by broad diversity metrics, can disproportionally regulate processes like respiration and nutrient cycling.

Environmental change did not cause changes in microbial diversity (Fig 3.2.1-3.2.3), the bacterial abundance data during the research revealed that functional responses to resource inputs can be highly dynamic. When organics and chemicals were added in warm temperature conditions (ch + org w), they showed the highest colony abundance, exceeding when only water (h2o), chemicals (ch) or fertilizers (org) where added. These results suggests that it is the resource availability that shifts microbial biomass, rather than restructuring of communities, which may have consequences in nutrient cycles during climate change. The results may suggest that compositional changes reflect differences in substrate utilization, causing species turnover instead of competitive exclusion. The observed patterns of biomass and function being more sensitive to environmental change align with findings of Zhou et al., 2020. For example, when only chemicals (ch) were added the bacterial abundance was low, however during warmer temperatures, the abundance doubled, highlighting the importance of temperature in regulating microbial activity. Similarly, when only fertilizers (org) were added in warmer temperature, the bacterial abundance was higher than during ambient temperature. These results emphasize that soil / water balance and organic substrate act synergistically to shape microbial responses, making microbes particularly sensitive during altered rainfalls and warmer temperatures.

The control samples (h2o and h2o w) showed low bacterial abundance during both temperature treatments, resulting in nutrient limitation constrains microbial proliferation, even

though diversity remains stable. That supported the statement that structural resilience does not guarantee functional stability (Yang et al., 2022). Soil microbiomes may retain evenness and richness under stress, but it is likely that their contributions to carbon or nutrient cycling can shift as dramatically as fluctuation of biomass levels. These findings support the idea that climate change impact on soils will show not only through compositional changes but also through altered microbial activity and biomass production. By showing that bacterial abundance is tightly linked to interaction of moisture and organic inputs, the results highlight the vulnerability of microbial driven nutrient cycles to climate induced shifts in precipitation regimes and anthropogenic inputs. This is consistent with evidence from previous years that drought adaptation alters microbial interactions with plants and that combined stressors can undermine ecosystem resilience (Sayer et al., 2021; Yang et al., 2022). Therefore, while microbial communities may appear stable, their functional responses in abundance and ability to cycle nutrients must be considered when predicting soil resilience during climate change.

The bacterial abundance patterns observed in sediment samples support the broader understanding of ecohydrological interfaces as dynamic hotspots of microbial activity (Griffin et al., 2017; Krause et al., 2017). The highest abundance was shown during warm temperature treatment when both organics and chemicals (ch + org w) were added. These results show that resource enrichment in sediment can stimulate microbial proliferation, potentially intensifying biogeochemical cycling during nutrient and temperature change. Beyond soil, abundance dynamics link to freshwater and sedimentary ecosystems. Agricultural runoff carrying excess pesticides and pharmaceuticals, introduces selective pressures that reorganize sediment microbiomes (Coelho et al., 2013; Rodriguez et al., 2018). The increased microbial growth observed in enriched treatments may therefore serve as a microcosm of nutrient driven microbial expansion in aquatic systems.

While increased bacterial abundance in organic fertilizer – water treatments suggest higher microbial activity, high biomass alone does not guarantee functional resilience. Reduced diversity under chemical pressure and the loss of sensitive taxa can compromise long-term

soil health, even when microbial abundance appears high. As emphasized by Yadav et al. (2022), sustainable practices leveraging beneficial rhizospheric and endophytic microbes – rather than relying solely on chemical inputs – may offer pathway to reconcile microbial productivity with ecosystem resilience.

The NMDS ordination revealed contrasting community-level response between soil and sediment environments. Soil microbial communities appeared relatively compositionally stable across treatments, despite the observed fluctuations in bacterial abundance. This suggests that soils may harbor greater functional redundancy, where shifts in abundance of individual taxa do not necessarily translate into broader community restructuring. These findings are consistent with research that demonstrated that soil microbiomes can recover taxonomic stability even under chemical perturbation, with functionally potential often maintained despite community turnover. In contrast sediment microbial communities displayed more pronounced compositional shifts, indicating higher sensitivity to both temperature and chemical / organic treatments. That aligns with studies done by Coelho et al. (2013) and Rodriguez et al. (2018).

The NMDS analyses further highlight these ecosystem-specific differences in microbial community responses. Soil communities clustered closely regardless of treatment, suggesting compositional resistance to both warming and chemical inputs. By contrast, sediments exhibited clear treatment-driven shifts, particularly under warming combined with pollutant inputs. These shifts suggest that aquatic and sedimentary systems are more vulnerable to multi-stressor interactions, which is consistent with previous observations where chemicals and thermal stressors acted synergistically to reshape microbial composition and function (Coelho et al., 2013; Rodriguez et al., 2018). Hydrological connectivity likely amplifies these effects by facilitating pollutant and nutrient transport across the landscape, reducing the buffering capacity typical of terrestrial soils. Enhanced connectivity can accelerate contaminant dispersal, nutrient enrichment, and organic matter

inputs, thereby influencing microbial activity and the overall resilience of the ecosystem (Griffin et al., 2017; Krause et al., 2017). These patterns underscore the importance of considering ecosystem connectivity and multi-stressor dynamics when assessing microbial response to climate change and anthropogenic pressures, as the same treatment produce different outcomes, depending on whether the microbial community resides in soil, sediment or water.

Importantly, the expected synergistic interactions between warming and contamination—predicted to erode microbial diversity and enrich AMR taxa—were not strongly supported in the short-term diversity metrics but remain ecologically plausible over longer timescales. Previous studies demonstrate that chronic exposure rather than acute treatments often drives selection for pollutant-tolerant or AMR-associated taxa, leading to functional simplification of microbial communities (Chen et al., 2019; Xu et al., 2025). Our results, therefore, align with growing evidence that early community responses manifest first as shifts in abundance and composition (e.g., NMDS patterns in sediments), while losses in richness, evenness, and functional diversity may emerge only under prolonged or repeated stress (Yang et al., 2022; Li et al., 2024).

Taken together, these findings highlight contrasting responses across ecosystems: soils exhibit strong taxonomic buffering despite fluctuations in abundance, whereas sediments show early compositional sensitivity to combined climate and pollution stressors. This suggests that sediments may serve as sentinel systems for detecting early microbial responses to global change, with potential implications for nutrient cycling, carbon stabilization, and ecosystem resilience under future climate scenarios.

Despite providing valuable insights into how pollution and warming shape soil and sediment microbial communities, the research had several limitations that constrained the interpretation of its findings. First, high within-treatment variability likely reduced statistical power, masking treatment effects on bacterial abundance, a challenge noted in other microbial ecology studies where subtle environmental gradients require greater replication to detect consistent patterns (Griffin et al., 2017). Even with high replication which was present

in the research, this variability highlights the heterogeneity and context dependence of microbial responses to environmental stressors, providing valuable insight into the resilience, functional redundancy, and adoptive capacity of bacterial communities under warming and pollutant exposure. Second, the short experimental duration may have overlooked legacy and delayed responses to stressors, as Sayer et al. (2021) demonstrated that historical drought stress can produce long-term shifts in microbial functioning. However, Aslam et al. (2016) showed that microbial responses to environmental perturbations, like rainfall, are strongly dependent on soil compartment, with surface community responding rapidly and subsurface community exhibiting greater resilience. Third, while NMDS and PERMANOVA revealed compositional changes, the exclusive reliance on diversity indices and colony-forming units (CFU) limited functional interpretation. Studies by Chen et al. (2019) and Xu et al. (2025) highlight the importance of linking taxonomic shifts to functional genes, especially antimicrobial resistance and nutrient cycling pathways. Furthermore, the lack of observed interactive effects between warming and pollutants contrasts with findings by Rodriguez et al. (2018) and Yang et al. (2022), who reported strong non-additive impacts of multiple stressors on microbial diversity and ecosystem function, suggesting that experimental design or treatment resolution may have constrained detection here. With more time for further investigation Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PiCRUST2) analysis could be used. Finally, culture-based approaches likely underestimated microbial diversity, excluding unculturable but functionally significant taxa central to biogeochemical processes and climate feedback (Abatehn et al., 2018; Li et al., 2024). Together, these limitations emphasize the need for longer-term, multi-site studies integrating molecular tools and ecosystem process measurements to fully capture how climate change and pollution jointly reshape microbial communities and the services they provide.

4.1 Future Work

Future research could benefit from a more genomic approach and should prioritize longer-term experiments to capture legacy effects and delayed microbial responses to warming and pollution, as Sayer et al. (2021) highlighted that past climate stress can produce persistent alterations in microbial function. Integrating high-throughput sequencing and metagenomic approaches, as recommended by Chen et al. (2019) and Xu et al. (2025), would enable the identification of functional genes linked to antimicrobial resistance, nutrient cycling, and stress adaptation, providing deeper insights into ecosystem consequences beyond taxonomic diversity. Multi-factorial designs incorporating realistic climate scenarios—such as fluctuating temperatures, altered precipitation, and pollutant pulses—could help reveal the non-additive effects observed by Rodriguez et al. (2018) and Yang et al. (2022), which were not captured in this study. Additionally, extending experiments across spatial scales and ecohydrological gradients, as suggested by Griffin et al. (2017) and Krause et al. (2017), would clarify how hydrological connectivity mediates microbial responses to stressors in soil–sediment interfaces. Finally, coupling microbial community data with ecosystem process measurements, such as greenhouse gas fluxes and nutrient turnover rates (Abatehn et al., 2018; Li et al., 2024), would improve predictions of how climate change and anthropogenic pollution jointly affect ecosystem resilience and climate feedback. Supporting this, Li et al. (2024), supported by the findings of Pold and DeAngelis (2013) advocated that integrating microbial traits and diversity metrics into Earth system models to improve the accuracy of carbon cycle feedback predictions. As climate change significantly influences the hydrological cycle, it is often examined together with ecohydrological connectivity. The interactive effects of multiple stressors on microbial communities have been further elucidated in experimental and observational studies, underscoring the need for integrative, multiscale approaches in climate and ecosystem research.

4.2 Conclusion

Overall, this study highlights contrasting microbial responses across ecosystems, with soils exhibiting strong taxonomic buffering despite fluctuations in abundance, while sediments showed early compositional sensitivity to combined warming and pollution stressors. These patterns suggest that sediments may function as sentinel systems for detecting early microbial responses to global change, carrying significant implications for nutrient cycling, carbon stabilization, and long-term ecosystem resilience.

Microbial abundance increased sharply under combined organic and chemical enrichment, particularly in sediments, reflecting the strong influence of resource availability and temperature on microbial activity. However, greater biomass did not necessarily equate to functional resilience, as losses of keystone taxa and longer-term diversity declines can undermine ecosystem stability even when overall richness appears unchanged. This aligns with evidence that functional redundancy in soils can initially buffer diversity metrics, yet rare taxa often play disproportionate roles in regulating greenhouse gas fluxes and nutrient cycling.

The results further demonstrate that short-term stressor interactions primarily manifest as shifts in microbial abundance and composition rather than outright diversity loss. Yet, chronic or repeated exposures documented in previous studies tend to drive synergistic effects, enriching pollutant-tolerant and antimicrobial-resistant taxa while simplifying microbial functional networks. Hydrological connectivity amplifies these risks in aquatic and sedimentary systems by accelerating nutrient and contaminant transport, thereby weakening the natural buffering capacity observed in terrestrial soils.

Finally, the study's limitations—including high biological variability, short experimental duration, and reliance on culture-based methods—underscore the need for long-term, multi-site research integrating high-throughput genomic tools, functional gene analyses, and

ecosystem process measurements. Such approaches are essential to capture delayed microbial responses, disentangle synergistic stressor effects, and improve predictions of how climate change and anthropogenic pollution jointly shape microbial communities and the ecosystem services they provide.

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