Land use and soil structure impacts on soil microbial community response to flooding

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Abstract

Climate change is leading to increased flooding coinciding with more land use conversion to agriculture production in eastern Canada. Microbial community functioning and activity depend on environmental factors that can influence their habitat in soil pore space and accessibility to resources. Interactions between land use and flooding may alter soil abiotic and microbial processes, with uncertain consequences to soil nutrient and C dynamics. Understanding how microbial communities respond to flood events across land uses will help to better explain the biotic mechanisms for soil C and nutrient shifts during flooding. This thesis examines how microbial extracellular enzyme activities (EEA) within a land use gradient vary over different spatial and temporal scales and how soil structure and land use affect soil microbial community diversity and activity within a seasonal floodplain and flooded soils. My thesis has two overall objectives within the context of flooding: 1) to determine how a land use intensity gradient influences microbial EEA, and 2) to examine how land use modifies the effects of soil structure on microbial community recovery to flooding. First, using a field-based land use gradient, I look at the relationship between soil EEA and land use across spatial and temporal scales. I collected soil samples across a land use gradient, replicated four times around Saint-Pierre Lake, Quebec, Canada. I measured soil EEA related to C, nitrogen (N) and phosphorus (P) cycling, soil nutrients and C. To capture spatial and temporal variation in flood response I sampled at three different elevations within each land use and three times over the growing season. My results demonstrated that a relationship between EEA and land use remained an important source of variability across spatial and temporal scale. Thus, in addition to abiotic site characteristics, land use intensity influences microbial-mediated C and nutrient cycling within an ecosystem that experiences seasonal flooding. For my second objective, I took a lab-based approach where I

manipulated soil structure heterogeneity, and examined how land use, soil structure and flooding impacted microbial activity and diversity. Intact soil cores from a natural grassland and agriculture site were sampled. Half the cores were sieved to modify soil structure, then a subset of cores from each structural treatment were flooded. All cores were incubated for three weeks, then dried to field-moist conditions to determine patterns in recovery. During and after the incubation, I sampled for, 16S rRNA and ITS gene sequences and PCR (bacteria and fungi), carbon dioxide fluxes, EEA, nutrients and soil C, and microbial biomass. I found that soil heterogeneity increased microbial CO_2 respiration and EEA recovery to flood in the agricultural soils. Regardless of soil structure, the grassland generally exhibited lower functional recovery compared to the agricultural soil. The observed functional recovery in the agriculture may be accounted for by the higher species richness and Shannon diversity compared to the grassland ($p<0.05$). Bacterial and fungal abundances recovered from flood in the grassland with no differences in structure, however in the agriculture, full recovery only occurred within intact soils. Soil structure influenced community composition in both land uses ($p<0.05$). In the flooded treatment grassland community composition shifted over time with recovery of composition dependent on structure whereas the agriculture communities only exhibited a difference between pre- and post-flood community compositions with no effect of structure. Thus, we found that with flooding microbial community recovery was dependent on land use and soil heterogeneity, predominately in the agriculture. In conclusion, land use influences soil microbial function and diversity within a seasonal floodplain and both land use and soil heterogeneity influence microbial response to flooding.

Résumé

Le changement climatique entraîne une augmentation des inondations qui coïncide avec une plus grande conversion de l'utilisation des terres à la production agricole dans l'est du Canada. Le fonctionnement et l'activité des communautés microbiennes dépendent de facteurs environnementaux qui peuvent influencer leur habitat dans l'espace poreux du sol et l'accessibilité aux ressources. Les interactions entre l'utilisation des terres et les inondations peuvent modifier les processus abiotiques et microbiens du sol, avec des conséquences incertaines sur la dynamique des nutriments et du carbone du sol. Comprendre comment les communautés microbiennes réagissent aux inondations en fonction de l'utilisation des sols permettra de mieux expliquer les mécanismes biotiques des changements de C et de nutriments dans le sol pendant les inondations. Cette thèse examine comment les activités enzymatiques extracellulaires microbiennes (AEE) au sein d'un gradient d'utilisation des terres varient sur différentes échelles spatiales et temporelles et comment la structure du sol et l'utilisation des terres affectent la diversité et l'activité de la communauté microbienne du sol au sein d'une plaine d'inondation saisonnière et de sols inondés. Ma thèse a deux objectifs généraux dans le contexte des inondations : 1) déterminer comment un gradient d'intensité d'utilisation des sols influence l'AEE microbienne, et 2) examiner comment l'utilisation des sols modifie les effets de la structure du sol sur le rétablissement de la communauté microbienne en cas d'inondation. Tout d'abord, en utilisant un gradient d'utilisation des sols sur le terrain, j'étudie la relation entre l'AEE du sol et l'utilisation des sols à travers des échelles spatiales et temporelles. J'ai prélevé des échantillons de sol sur un gradient d'utilisation des terres, répété quatre fois autour du lac Saint-Pierre, au Québec, au Canada. J'ai mesuré l'AEE du sol en relation avec le C, le cycle de l'azote (N) et du phosphore (P), les nutriments du sol et le C. Pour saisir les variations spatiales et

temporelles de la réponse aux inondations, j'ai prélevé des échantillons à trois altitudes différentes au sein de chaque utilisation des terres et à trois reprises au cours de la saison de croissance. Mes résultats ont démontré qu'une relation entre l'AEE et l'utilisation des terres restait une source importante de variabilité à travers l'échelle spatiale et temporelle. Ainsi, outre les caractéristiques abiotiques du site, l'intensité de l'utilisation des sols influence le cycle microbien du carbone et des nutriments dans un écosystème soumis à des inondations saisonnières. Pour mon deuxième objectif, j'ai adopté une approche en laboratoire où j'ai manipulé l'hétérogénéité de la structure du sol et examiné comment l'utilisation des terres, la structure du sol et les inondations ont eu un impact sur l'activité et la diversité microbiennes. Des carottes de sol intactes provenant d'une prairie naturelle et d'un site agricole ont été prélevées. La moitié des carottes ont été tamisées pour modifier la structure du sol, puis un sous-ensemble de carottes de chaque traitement structurel a été inondé. Toutes les carottes ont été incubées pendant trois semaines, puis séchées jusqu'à ce qu'elles soient humides pour déterminer les schémas de récupération. Pendant et après l'incubation, j'ai prélevé des échantillons de séquences de gènes ARNr 16S et ITS et de PCR (bactéries et champignons), de flux de dioxyde de carbone, d'AEE, de nutriments et de C du sol, ainsi que de biomasse microbienne. J'ai constaté que l'hétérogénéité du sol augmentait la respiration microbienne du CO_2 et la récupération de l'AEE jusqu'à l'inondation dans les sols agricoles. Indépendamment de la structure du sol, la prairie présentait généralement une récupération fonctionnelle plus faible que le sol agricole. Le rétablissement fonctionnel observé dans l'agriculture peut s'expliquer par la richesse des espèces et la diversité de Shannon plus élevées que dans la prairie ($p<0,05$). Les abondances bactériennes et fongiques se sont rétablies après l'inondation dans les prairies sans différence de structure, alors que dans l'agriculture, le rétablissement complet ne s'est produit qu'à l'intérieur de sols intacts. La structure

du sol a influencé la composition de la communauté dans les deux utilisations du sol ($p<0,05$). Dans les prairies inondées, la composition des communautés s'est modifiée au fil du temps, le rétablissement de la composition dépendant de la structure, tandis que les communautés agricoles n'ont présenté qu'une différence entre les compositions des communautés avant et après l'inondation, sans effet de la structure. Ainsi, nous avons constaté qu'en cas d'inondation, le rétablissement des communautés microbiennes dépendait de l'utilisation des terres et de l'hétérogénéité du sol, principalement dans le cas de l'agriculture. En conclusion, l'utilisation des terres influence la fonction et la diversité microbienne du sol dans une plaine d'inondation saisonnière et l'utilisation des terres et l'hétérogénéité du sol influencent la réponse microbienne aux inondations.

List of Abbreviations

Abbreviation	*Full Description*
BG	Beta-Glucosidase
C	Carbon
EEA	Extracellular Enzyme Activity
LAP	Leucine Amino Peptidase
MBC	Microbial Biomass Carbon
MBN	Microbial Biomass Nitrogen
N	Nitrogen
NAG	N-acetylglucosaminidase
NMDS	Non-Metric Dimensional Scaling
P	Phosphorous
PEP	Peptidase
PER	Peroxidase
PHE	Phenol oxidase
SMC	Soil Moisture Content
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
TAP	Tyrosine Amino Peptidase
WEOC	Water Extractable Organic Carbon
WEON	Water Extractable Organic Nitrogen

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(pairwise post-hoc test with FDR adjustments) among land uses, NS indicates no significant differences. The line within the boxplot indicates the median, the limits of the boxes indicate the 25^{th} and 75^{th} quartiles. The whiskers indicate 1.5 x the quartile range.

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significant differences occur within time (based on PERMANOVA), soil structure is denoted by circles and triangles for intact and sieved soil. Environmental parameters that significantly associated with axis 1 or 2 are shown by vectors, with the following abbreviations: WEOC = water extractable organic carbon, SMC = soil moisture content, MBC = microbial biomass carbon, MBN = microbial biomass nitrogen, TAP = tyrosine amino peptidase and NAG = N-1,4-acetylglucosaminidase.

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structure treatment (tukeyHSD), NS signifies no significant result from tukeyHSD. Intact structure is in dark green (grassland) and dark orange (agriculture) and sieved structure is in light green (grassland) and yellow (agriculture). The line within the boxplot indicates the median, the limits of the boxes indicate the 25th and 75th quartiles. The whiskers indicate 1.5 times the quartile range.

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signifies no significant result from tukeyHSD. Intact structure is in dark green (grassland) and dark orange (agriculture) and sieved structure is in light green (grassland) and yellow (agriculture). The line within the boxplot indicates the median, the limits of the boxes indicate the 25^{th} and 75^{th} quartiles. The whiskers indicate 1.5 times the quartile range.

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Contribution of Authors

This thesis contains an abstract, general introduction, literature review, two original research chapters written in manuscript format, a general discussion and a conclusion all written with the guidelines of the McGill Graduate and Postdoctoral Studies Office and the Natural Resources department. The abstract provides a broad summary of the research presented in this thesis, and the general introduction includes a brief background and justification of the research objectives. The literature review provides a more thorough background on microbial community functions in soils, community response to disturbances, flooding, land use, and scale. This literature review provides context to the justification and objectives of my research.

Chapter 2 presents the results from an ecosystem scale study and addresses the first two objectives and Chapter 3 presents the results from an incubation experiment. Chapter 2 is followed by a transition paragraph that explains the connection between Chapters 2 and 3.

The literature review was written by the candidate and minimally edited by her two supervisors Dr. Cynthia Kallenbach and Dr. Mary-Cathrine Leewis. Chapter 2 was written by the candidate and edited by her two supervisors Dr. Kallenbach and Dr. Leewis and Ms. Hannah Lieberman who is a co-author on this paper. The research objectives and hypotheses were planned by the candidate with guidance from her supervisors, however the experimental design for Chapter 2 was already in place, planned by Dr. Kallenbach as it was part of a bigger study conducted in 2021. Grace McDougall-Vick, while conducting a USRA, contributed to the enzyme activity assays and foundational interpretation of enzyme activities across the land use gradient and is also a co-author on this paper. Chapter 3 was written by the candidate and edited by her two supervisors Dr. Kallenbach and Dr. Leewis. The candidate planned the experiment objectives,

hypotheses and experimental design with guidance from her supervisors. The candidate conducted the lab work for both chapters with occasional technical assistance. Undergraduate students working in the lab assisted with soil processing and extractions for Chapter 3. In Chapter 3 the soil core imaging was conducted by Dr. Pierre Dutilleul and Mr. Li Wen Han. Dr. Pierre Dutilleul further conducted statistical analyses and provided support on data interpretation. At the Agriculture and Agri-Food Canada Research and Development laboratory in Québec City, Ms. Josée Michaud provided guidance for qPCR lab work and data analysis, and Mr. David Gagne and Mr. Mario Laterriere provided assistance with bioinformatics processing and trouble-shooting. Findings were interpreted by the candidate with guidance from Dr. Kallenbach and Dr. Leewis.

General Introduction

In eastern Canada we see both climate change and human development altering *in-situ* conditions that shape soil nutrient and carbon (C) processes resulting in unknown consequences for ecosystem productivity. Flooding is predicted to increase in eastern North America (Jeong *et al.*, 2014) as climate change alters precipitation patterns. Soil microbial communities drive soil nutrient and C cycles, however, the effects of flood on soil microbial community activity and composition remain largely unknown in mineral soils. Microbial communities are linked to soil nutrient and C cycles by producing extracellular enzymes that catalyze the depolymerization of organic matter into bioavailable forms (Sinsabaugh and Shah, 2012). Flooding alters nutrient, C, and oxygen availability within the soil matrix, thus impacting soil microbial metabolic rates and access to substrates (Peralta *et al.*, 2014, Keiluweit *et al.*, 2017). It is expected that microbial communities will respond to short term flood events, however the degree of microbial resiliency and recovery to flood may be regulated by characteristics of their environment such as land use and soil structure heterogeneity. Using an ecosystem scale study, I determined how extracellular enzyme activity (EEA) varies across a land use intensity gradient within a seasonal floodplain. In addition, through a laboratory incubation, I examined how soil microbial community activity and diversity respond to experimental flooding and how this response is mediated by soil structure and land use.

Soil microorganisms produce EEAs that catalyze the depolymerization of organic matter. Products from different enzyme reactions contribute to various C, nitrogen (N), and phosphorous (P) cycles which are important for soil health and plant productivity (Sinsabaugh and Shah, 2012). Understanding constraints on EEAs can give insight into nutrient and C cycling processes

across different land uses, and whether the impacts of seasonal flooding moderate the characteristics of land use that regulate soil microbial communities. For instance, we expect higher EEA in natural ecosystems which are associated with higher resource availability and less soil disruption from cultivation (Wallenius *et al.*, 2011, Burns *et al.*, 2013, Weintraub *et al.*, 2013, Mace *et al.*, 2016). Further, EEA is influenced by abiotic soil factors such as soil moisture and texture, which vary from micrometer to regional scales. Soil moisture mobilizes substrates, thus increasing interaction between organic matter and EEA (Bailey *et al.*, 2017). Finer soil textures increase organic matter, moisture, and enzyme retention, therefore increasing enzymatic potential under optimal conditions (Nannipieri *et al.*, 2018, Lehmann *et al.*, 2020). Thus, determining the main drivers of EEA variability will assist with optimal land management within seasonal floodplains.

The ability of soil microbial communities to resist or recover from disturbances has been linked to community richness and the taxonomic connectivity of the community (de Vries *et al.*, 2018, Philippot *et al.*, 2021). One way higher microbial taxonomic diversity would buffer against disturbances is through the 'insurance hypothesis' (Griffiths and Philippot, 2013), such that in systems with high species diversity, some species will be more resilient, replacing those that are more sensitive to the disturbance (Bargett and Caruso, 2020). In addition, higher microbial taxonomic diversity has been correlated with ecosystem functional potential, supporting ecosystem multifunctionality (Wagg *et al.*, 2019, Philippot *et al.*, 2023). Microbial community networks have been used to compare community stability, where lower connections and higher modularity in networks is associated with greater stability (de Vries *et al.*, 2018, Philippot *et al.*, 2021). Shifts in taxonomic diversity or community network parameters can give insight into the microbial community stability in response to disturbances. For example, in response to drought

and tillage disturbances, fungal networks exhibited stronger stability to drought than bacteria (de Vries *et al.*, 2018), but fungal community abundance was less stable over time in response to tillage compared to bacteria (Wagg *et al.*, 2018). Thus, the microbial response to flood disturbance may vary between fungal and bacterial communities and may depend on both community composition and connectivity.

Soil structure is important for developing niche space for soil microorganisms (Erktan *et al.*, 2020). Pore space, which varies in size and connectivity, is defined by the arrangement of macro- and micro-aggregates. Microorganisms inhabit pore spaces and their speciation and activity are influenced by the arrangement and isolation of pore space which affect microbial access to resources and biotic interactions (Bailey *et al.*, 2012, Keiluweit *et al.*, 2017, Erktan *et al.*, 2020). Soil practices such as tillage which mechanically disturb macroaggregates, homogenize the soil to become microaggregate dominated (Six *et al.*, 2000). I expect that this homogenizing effect will negatively impact microbial community resiliency to flood disturbance because of likely reductions in niche space (e.g., anaerobic and aerobic microsites), decreasing community diversity, and changes to resource availability.

Soil microbial community activity and diversity response to flood is likely influenced by starting community and resource availability. Land use can influence microbial starting community by dictating plant assemblages and soil management practices that impact the quality and quantity of microbial resources (Furhmann, 2021). For example, chemical variations in plant inputs will require specific enzymes to be produced, thus selecting for organisms that are better suited to depolymerize those substrates (Fanin and Betrand, 2017). Regardless of chemistry, the concentration of soil organic matter (SOM) is positively correlated with extracellular enzyme activity, respiration and microbial biomass (Qin *et al.*, 2010, Kallenbach *et al.*, 2015). Natural,

uncultivated, ecosystems demonstrate higher microbial activity and biomass due to more perennial and diverse plant assemblages as well as greater concentrations of SOM (Crews and Rumsey, 2017). Further, natural ecosystems likely support more heterogenous soil structure thus increasing niche space and microbial diversity. Agricultural practices that include tillage homogenize the soil which can decrease the diversity of microbial habitats (Six *et al.*, 2000). Thus, we would expect higher enzyme activity and microbial community diversity with decreasing land use intensity due to decreased physical disturbance, larger microbial population size, and greater substrate availability and diversity fueling enzymatic production and respiration (Wallenius *et al.*, 2011, Burns *et al.*, 2013, Weintraub *et al.*, 2013, Mace *et al.*, 2016).

Although land use creates variability in soil enzyme dynamics, it is not clear whether the importance of land use is robust across both time and spatial scales. For example, plant development stage varies throughout the growing season, changing foliage inputs and root exudates. As root exudates can influence microbial community activity and diversity in the rhizosphere (Huang *et al.*, 2014, de Vries and Wallenstein, 2017), resiliency to flood may be mediated through the higher resource supply and functional redundancy related to higher diversity (Philippot *et al.*, 2021, Cui *et al.*, 2019, Francioli *et al.*, 2021). Further, soil texture which varies across regional scales can impact retention of substrates and enzymes. For example, soil textures with high clay content are associated with more structured soils, decreasing heterogeneity (Nunan *et al.*, 2020), higher moisture and greater concentrations of organic matter (Lehmann *et al.*, 2020, Finley *et al.*, 2021), and thus likely have greater enzyme activities. Therefore, whether soil characteristics determined by land use are associated with enzyme activities across spatial variability associated with time and space is not clear.

Microbial activity and diversity are intimately linked with soil properties ranging from micrometer to regional scales. Thus, determining how ecosystem levels controls such as land use intensity and soil heterogeneity impact microbial level mechanisms determining resiliency and recovery to flood events is important for maintaining ecosystem services. The objectives of my second chapter are; 1) to determine which soil characteristics are associated with microbial enzyme activity across a land use intensity gradient within a floodplain, and 2) to determine whether relationships between land use characteristics and enzyme activity are consistent across time and spatial scales. I expect that moisture will be a dominant control on enzyme activity and that enzyme activities will decline with increasing land use intensity regardless of time and spatial scales. The objectives of my third chapter are; 1) to determine if soil heterogeneity supports greater microbial functional or compositional resiliency or recovery to experimental flooding, and 2) to determine if microbial responses to flooding differ between two land uses. I expect that more heterogeneous soils will be associated with higher microbial community diversity and thus greater resiliency and recovery to flooding. Further, I expect that the microbial community under a natural grassland land use will have greater recovery to flood compared to an agriculture land use.

Literature Review

1.1 *Microbial communities within the soil environment*

The soil environment is a habitat for a diverse assemblage of organisms that perform essential ecosystem functions (FAO *et al.*, 2020, Sokol *et al.*, 2022). Soil microbial communities are ubiquitous within the soil with up to one billion bacterial cells and 200 metres of fungal hyphae within one gram of soil (FAO *et al.*, 2020). Microbial communities are sensitive to their surrounding habitat, leading to unique community structures and adaptations based on small- and large-scale variations within their environment (Sokol *et al.*, 2022). How these often extensively diverse soil microbial communities respond to environmental changes is highly variable and can be difficult to assess given the interactive effects that occur within the soil matrix (Griffiths and Philippot, 2013, Philippot *et al.*, 2023).

Bacteria and fungi are, by biomass, the most dominant soil microorganisms (Six *et al.*, 2006, Fierer, 2017). These microorganisms have high diversity with a large range in life strategies, allowing for complex and extensive roles within soil nutrient and C cycles. Bacteria, due to their plasticity and diversity, perform a variety of metabolic processes under various conditions in the soil environment (Fierer *et al.*, 2007). Bacteria have been found in extreme environments demonstrating the breadth of their metabolic capabilities (Shu and Huang, 2022). Fungi take many forms, from single-celled yeasts to filamentous networks, and largely exist as saprotrophs, feeding off dead organic matter, and as plant symbionts such as mycorrhizal fungi (Taylor *et al.*, 2014, Taylor and Sinsabaugh, 2015). Due to the high diversity of soil microorganisms, the connection between community structure and assemblage to community function can be complex and context dependent (Fierer *et al.*, 2007, Fierer 2017).

Bacteria and fungi are both associated with primary decomposition (Fuhrmann, 2021); however, the two groups differ in environmental specialization. Bacteria and fungi are both capable of heterotrophic metabolism, which derives C and other nutrients such as nitrogen (N) and phosphorous (P) from soil organic matter (SOM), temporarily immobilizing molecules into their biomass (microbial biomass, MB) (Fuhrmann, 2021). Bacteria and fungi produce extracellular enzymes which catalyze the transformation of organic polymers to inorganic monomers. This is a crucial step in the decomposition of organic matter, allowing for nutrients to become more bioavailable for both soil organisms and plants (Schimel and Bennett, 2004, Burns *et al.*, 2013).

Differences in environmental specialization results in bacterial and fungal dominance in certain systems. Studies have attempted to attribute environmental variables to explain patterns in bacterial and fungal prevalence, finding pH, nutrient and C content and soil moisture to be important factors (Fierer, 2017, Philippot *et al.*, 2023). For example, Rousk *et al.* (2010) found a positive correlation between pH, ranging from 4 – 8, and bacterial gene copies. Not only was the bacteria community orders of magnitude larger than fungi, but the fungal community demonstrated no relationship to pH within that range (Rousk *et al.*, 2010). Fungi dominate in acidic soils with litter that contains relatively high C:N compounds (Taylor and Sinsabaugh, 2015). As pH can influence community composition this has implications for C and nutrient cycling. Soil microbial communities both influence and are influenced by their soil environment, thus soil nutrient and C status can also affect microbial community structure.

Bacteria and fungi activity and community composition respond differently to soil C, N and P content. Fungi that form a symbiotic association with plants are allocated photosynthesis-derived C and therefore often target N- and P-rich compounds (Taylor and Sinsabaugh, 2015, Frey, 2019). Fungi have been associated with higher C use efficiency (Six *et al.*, 2006) and ecosystems

whose nutrient cycles are dominated by fungal processes are generally considered slower cycling (Bargett and Caruso, 2020). For example, fungi are the main producers of oxidative enzymes that degrade plant lignin, although the degree of effectiveness varies widely by different taxa. This results in ecosystems that have high proportions of lignin, such as forests, to have nutrient cycles driven by fungal communities (Sinsabaugh *et al.*, 2008, Frey, 2019). Further, fungal mycelium provides a unique ability to translocate nutrients allowing for increased fitness in low nutrient environments (Ritz and Young, 2004). Related to fungal biomass stoichiometry (C:N:P) and their oxidative enzyme production, fungi typically dominate under nutrient limitations relative to bacteria. Bacteria, on the other hand, are often associated with faster nutrient cycling and higher levels of soil inorganic nutrients, or in highly disrupted soils, associated with agricultural fertilization and management (Young and Ritz, 2000, Zhang *et al.*, 2016, Bargett and Caruso, 2020). Thus, soil nutrient conditions can influence the prevalence of either bacteria or fungi, potentially changing the rates of nutrient cycling.

Soil moisture conditions also affect the composition of bacteria and fungi within the soil. Moisture is a common source of stress for microorganism functioning (Schimel *et al.*, 2007, Schimel, 2018). The heterogeneous nature of soils results in uneven moisture distribution, thus both extremes of too high or low moisture exist, which can cause physiological and metabolic stress. Bacterial and fungal communities have varying tolerance for moisture stress (Schimel *et al*., 2007, de Vries *et al.*, 2018, Philippot *et al.*, 2023). Bacteria are limited in their mobility, relying on soil moisture to connect them to resources and other organisms (Schimel, 2018). This is less the case for fungi, whose mycelium allows them to bridge larger pore spaces making them dominant in low moisture conditions (Drenovsky *et al.*, 2004, Pajor *et al.*, 2010, Witzgall *et al.*, 2021). Witzgall *et al.* (2021) found that between coarse- and fine-textured soils, fungi dominated

decomposition and nutrient translocation in coarse-textured soils compared to bacteria due to their ability grow through the greater pore sizes in coarser textured soils. However, bacteria can withstand wider ranges in moisture than fungi, and especially dominate in higher moisture conditions (Drenovsky *et al.*, 2004, Schimel *et al.*, 2007, Manzoni *et al.*, 2012). If high moisture conditions reach saturation within the soil matrix (all soil pores are filled with water) bacterial metabolic diversity and the ability of many bacteria and archaea to conduct anaerobic metabolism makes them more suitable for high moisture conditions than fungi (Unger *et al.*, 2009). Studies have shown that under higher flood frequencies there is an increased proportion of anaerobic bacteria (Pett-Ridge and Firestone, 2005, Argiroff *et al.*, 2017). Thus, it would be expected that soil bacteria communities will be more resilient to flooding compared to fungi.

Microbial communities are diverse in taxonomic and functional composition. As the relative effects of the multiple bidirectional interactions between the soil environment and microbial communities are complex, response to disturbances can be difficult to determine. There is increasing interest in microbial community response to future disturbances associated with climate change, thus my thesis will address how soil microbial communities respond to flooding in mineral soils.

1.2 *Microbial community response to disturbance*

Developing our understanding on how microbial communities respond to disturbances will help us manage soil ecosystems so that microbial community resiliency is optimized. In general, soil microbial community response to disturbances is discussed in terms of their resistance, resilience, and functional redundancy (Schimel *et al.*, 2007, Allison and Martiny, 2008, Biggs *et al.*, 2020). Where resistant microbial communities do not change in the face of disturbance; resilient microbial communities experience change due to a disturbance but will recover after the

disturbance; and functionally redundant microbial communities exhibit changes in microbial community composition in response to a disturbance with no changes in process rates (Allison and Martiny, 2008). The direction and magnitude of microbial community resistance or recovery to a disturbance has been linked with factors including species diversity (Philippot *et al.*, 2021), individual or community traits (Wallenstein and Hall, 2011), or the connectivity within community networks (de Vries *et al.*, 2018).

Higher taxonomic diversity has been associated with increased microbial community resiliency due to increased ecosystem multifunctionality (Philippot *et al.*, 2021). Studies have found that when considering the multitude of functions performed by soil microbial communities, species diversity becomes more important than considering one function at a time (Wagg *et al.*, 2019, Philippot *et al.*, 2021). The benefit of microbial community richness supporting higher ecosystem multifunctionality is termed the portfolio or insurance effect (Griffiths and Philippot, 2013, Wagg *et al.*, 2018, Wagg *et al.*, 2019, Philippot *et al.*, 2021). Thus, in the case of disturbances, the portfolio effect suggests that in systems with greater species diversity there is a greater chance that more sensitive species will be replaced with more resilient ones (Bargett and Caruso, 2020). True functional redundancy is unlikely based on the specificity of species to their habitat and resource requirements (Loreau, 2004), however with the portfolio effect, functions may be compensatory where the loss of some species are replaced by others that perform a similar function (Gonzalez and Loreau, 2009). However, whether species diversity is the most important factor determining microbial resistance or recovery to disturbance may depend on land use and abiotic soil conditions (Orwin *et al.*, 2016). Moreover, in some cases, a disturbance may select for more specialized communities, with lower diversity, but with functional traits specifically suited to the new conditions (Piton, *et al.*, 2023). Thus, microbial community

response to disturbance may depend on both community composition and the associated community traits (Wallenstein and Hall, 2011, Philippot *et al.*, 2021).

Microbial traits such as dormancy, growth rates, physiological flexibility, and those associated with the production of secondary metabolites all aid in the potential for adaptation to disturbances (Allison and Martiny, 2008, Wallenstein and Hall, 2011, de Vries and Shade, 2013, Sorensen and Shade, 2020). For example, dormancy was found to contribute to microbial community resilience and stability in response to temperature stress (Sorensen and Shade, 2020). Further, a study by Patel *et al.* (2021) found that genes coding for spore production (common mechanisms of dormancy) were found in response to both high and low soil moisture contents. As these microbial traits are expressed on an individual basis, when measurements are made at the community level, changes that reflect trait variability are observed in community composition and metabolic efficiency (Schimel *et al.*, 2007, Wallenstein and Hall, 2011).

While certain traits may support greater microbial fitness under a novel soil environment following a disturbance, energetic trade-offs can often occur between stress response and maintenance processes (Schimel *et al.*, 2007). However, trade-offs in the face of disturbance can be more complex as they can interact with inherent stress within the soil matrix. For example, enzyme production is important for nutrient acquisition for bacteria and fungi (Sinsabaugh and Shah, 2012). However, since there is no guaranteed nutrient return after releasing a protein, microorganisms likely adapt their enzyme production to maximize nutrient returns (Nunan *et al.*, 2020). In the case of disturbances that cause metabolic strains (such as reduction in oxygen), nutrient acquisition strategies may decrease while investment in oxygen-stress related traits may increase. These trade-offs and consequences for community-level fitness depend on the

disturbance pressure, what resources are available and other selective pressures are acting on the microbial community (Fierer, 2007, Malik *et al.*, 2019).

Microbial response to disturbances also depends on the type of perturbation, the timing, if there are co-occurring disturbances, and history of disturbance (DeAngelis *et al.*, 2010, Philippot *et al.*, 2021). Timing of disturbances can influence microbial response, such that the duration of a particular event may not last long enough for communities to exhibit significant shifts (DeAngelis *et al.*, 2010, Philippot *et al.*, 2021). In the case of compounding disturbances, if the baseline community has changed after one disturbance, it may be more difficult to predict how the new community will adapt (Philippot *et al.*, 2021). Finally, history of disturbance can affect the response to future events (DeAngelis *et al.*, 2010, Philippot *et al.*,2021, Evans *et al.*, 2022). Studies have found that microbial communities that have historically experienced a disturbance, i.e. flooding, have more adaptations associated with these fluctuating conditions (DeAngelis, *et al.*, 2010, Peralta *et al.*, 2013, Bargett and Caruso, 2020, Patel *et al.*, 2021). Incorporating the disturbance characteristics, such as disturbance history and duration, into microbial based soil models, in addition to microbial traits, will be important for more accurately determining how communities respond to future flood disturbances (Evans *et al.*, 2022).

Understanding how microbial communities respond to changes in the environment, such as flooding, is important as the response may have downstream effects on community persistence and nutrient cycling (Fierer, 2007, Malik *et al.*, 2019). Moisture stress is a common experience for organisms in the soil (Schimel, 2018), however, we do not clearly understand microbial response mechanisms on a community or on an individual level to flooding. Different moisture sensitivities between bacteria and fungi will likely shift their relative proportions under more anaerobic conditions, reflecting better adaptation to the new conditions (Unger *et al.*, 2009,

Argiroff *et al.*, 2017). For instance, if a community is resistant to flooding, there will likely be little changes in microbial activity during and post-flood. If a community is resilient to flooding, there will be a change in activity rates during the flood but a recovery to pre-flood rates post-flood. However, if a community experiences compensatory dynamics (Gonzalez and Loreau, 2009) resulting in functional stability, the community composition may have shifted, with no change in function, but potentially influencing responses to further disturbances or to future occurrences of flooding. Therefore, understanding how communities respond to different disturbances within different ecosystems and soil structure will help to understand functional implications of flooding.

1.3 *Soil environment as a habitat for microbial communities*

Soil microorganisms are both influenced by and influence the soil environment. Soil minerals, categorized into sand (2.0 – 0.05 mm), silt (0.05 – 0.002 mm) and clay (<0.002 mm) – sized particles come together by inter-particle forces and organic materials produced by micro-organisms to form aggregates (Krzic *et al.*, 2021). Aggregates are further classified by size, micro-aggregates < 250 µm and macro-aggregates > 250 µm, and the arrangement of aggregates in space defines soil structure (Krzic *et al.*, 2021, Hartmann and Six, 2023). Soil micro-organisms assist in the formation of aggregates through fungal hyphal enmeshment and the production of polysaccharides which cement mineral grains together (Lehmann *et al.*, 2017, Chorover, 2022). Micro-aggregates, especially within the range of 20 µm, are dominated by bacteria and bacterial byproducts such as extra-cellular polysaccharides (EPS) and microbial necromass (Lehamnn *et al.*, 2017, Totsche *et al.*, 2018). Macro-aggregates are less stable than micro-aggregates since common binding agents include plant roots and fungal hyphae which are susceptible to mechanical disturbances (Taylor and Sinsabaugh, 2015, Lehmann *et al.*, 2017,

Totsche *et al.*, 2018, Hartmann and Six, 2023). Soil aggregation and structure can also be impacted by plant rhizosphere inputs to the soil. Vezzani *et al.* (2018) suggested that plant roots contributed directly to aggregation but that root exudation stimulated microbial communities which further contributed to soil aggregation. Thus, although microbial communities contribute to aggregate binding, soil aggregate dynamics are subject to physical disturbances that can influence their disintegration and formation.

Pore space is dictated by soil structure as pores occur between aggregates, large mineral grains, (macro-pores, > 0.08 mm) or within aggregates, between small mineral grains, (micro-pores, < 0.08 mm) (Krzic *et al.*, 2021, Totsche *et al.*, 2018). Soil is further classified as a three-phase system consisting of solid materials, gas and water (Krzic *et al.*, 2021). The solid phase and porosity as described above, forms the structure through which both gas and water move. Microorganisms inhabit pore spaces and are subject to limitations placed by the arrangement of those pores. Soil pore space determines the diffusion rate of oxygen into soil and the movement of water and these factors influence the ability of bacteria and fungi to respire aerobically and the mobility of organisms and nutrients (Schimel, 2018, Fuhrmann, 2021, West and Witman, 2022). Thus, soil texture and structure can heavily influence soil microbial community response to flooding and their adaptation potential (Six *et al.*, 2006, Totsche *et al.*, 2018).

1.4 *Soil structure heterogeneity influencing microbial community resiliency and diversity*

Heterogeneity of pores and pore size distribution can influence microbial community diversity, function and inter-trophic interactions (Erktan *et al.*, 2020, Xia *et al.*, 2022). Discontinuity of resources and organisms between soil pores can create heterogeneous patchiness within the soil. This discontinuity can occur due to physical isolation and reduced soil moisture limiting mobility (Nunan *et al.*, 2020, Xia *et al.*, 2022). Reduced pore diameter can filter organism distribution by

altering access to certain areas by body size; bacteria are found within micropores and micro-aggregates whereas fungi are more common in macroaggregates (Tecon and Or, 2017, Xia *et al.*, 2022). In conditions of low soil moisture, microorganisms and especially bacteria, are limited to water films around mineral surfaces that are disconnected from each other (Bailey *et al.*, 2017, Tecon and Or, 2017). The heterogeneity of moisture-limiting movement of both organisms and substrates is common in soil and can increase species diversity (Bailey *et al.*, 2017, Portell *et al.*, 2018). Greater soil heterogeneity increases community isolation (Tecon and Or, 2017, Nunan *et al.*, 2020), thus creating conditions for speciation within each pore environment.

Soil structural heterogeneity can also be linked to microbial community resistance and resiliency when disturbances occur. In the case of flooding, hydraulic dynamics within the soil are such that water will preferentially flow through macropores (Genuchten and Pachepsky, 2014). Thus, microorganisms may be able to seek refuge within smaller pore spaces, allowing for resistance to disturbance (Griffiths and Philippot, 2013). Aggregates provide unique pore space conditions within which organisms can adapt (Fierer, 2017, Rillig *et al.*, 2017, Wanzek *et al.*, 2018, Chorover, 2022). For example, micro pores within clay microaggregates are isolated and can remain anoxic, even under dry field conditions, thus supporting species capable of anaerobic metabolism (Fig. 1.1) (Keiluweit *et al.*, 2017, Tecon and Or, 2017). Patel *et al.* (2021) found a greater number of genes that coded for motility mechanisms in soils with a history of high moisture. Thus, soil structural heterogeneity may allow for greater resiliency and redundancy in response to disturbance because of the expected higher species diversity associated with a greater number of niches (Fig. 1.1).

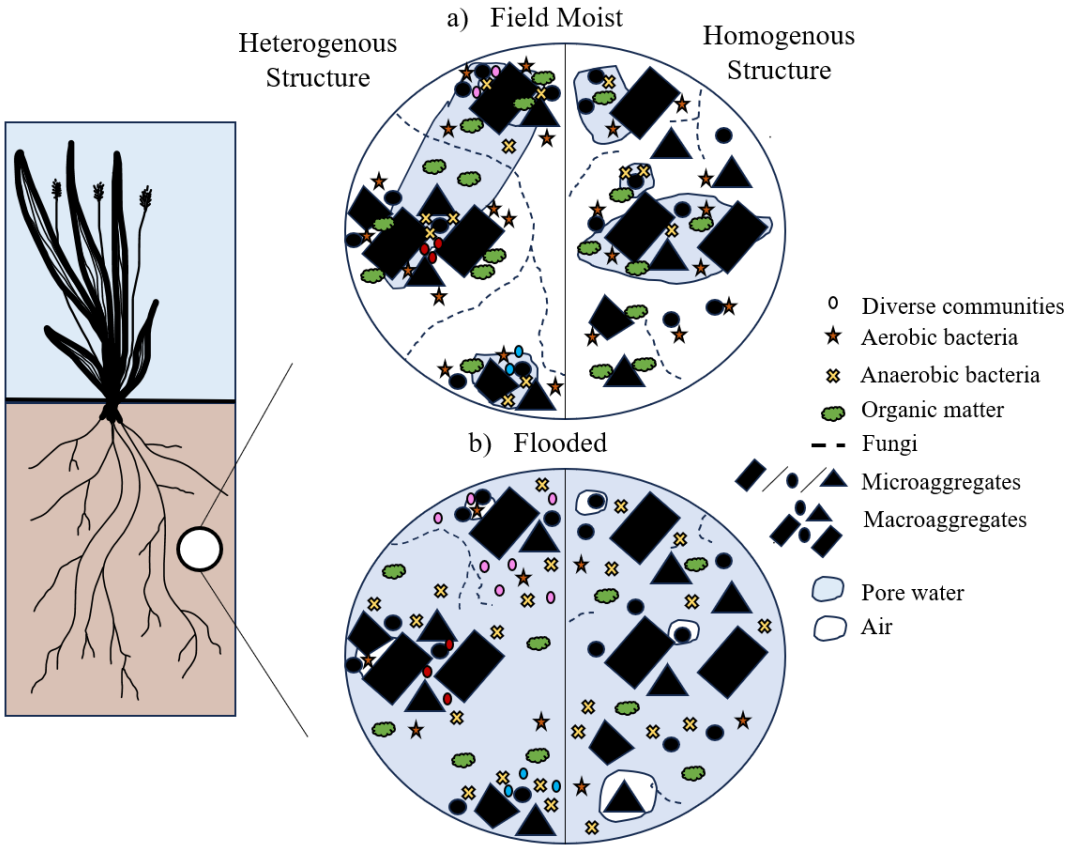

Figure 1.1 Illustration of how soil heterogeneity increases soil community diversity through increased niche space. Under field moist conditions (a) organism-organism and organism-substrate interactions are limited by moisture in both structures. Under flooded conditions (b) increases in relative abundances of adapted organisms in heterogenous structure will be more prominent than in the homogenous structure.

1.5 *Flooding as a disturbance and change in resource accessibility*

Flooding is predicted to increase in North America as climate patterns are changing (Jeong *et al.*, 2014). Flood duration and frequency are also predicted to increase, affecting areas that experience seasonal flooding and areas that have not previously experienced flooding. While some studies have found microbial compositional shifts related to flooding (Unger *et al.*, 2009, Argiroff *et al.*, 2017, Randle-Boggis *et al.*, 2017), few have looked at soil microbial community

resistance and recovery to flooding in mineral soil. What is also lacking is a comparison of how different land uses mediate the soil microbial response to flooding. As land use and microbial-mediated nutrient and carbon (C) cycling are closely linked, the compounding disturbance between intensely managed land and flooding has unknown consequences for ecosystem functions that are supported by nutrient and C supply. Thus, this area of research is important to develop as microbial community response to flood has far-reaching implications for soil C and nutrient cycling dynamics affecting soil health and plant productivity.

Flooding creates hotspots of activity due to the increased interaction between microbes and substrates (McClain *et al.*, 2003), however with prolonged flooded conditions, physical and chemical changes within the soil column require adaptation by the soil microbial community. Flooding occurs as a saturation and, or submergence of soil due to both water table rise and increased surface waters. During flooding, water fills pore spaces within the soil matrix thereby increasing connectivity and movement of organisms (Bailey *et al.*, 2017, Schimel, 2018). Experiments that look at the initial effect of soil re-wetting have found a consistent release of CO_2 known as the birch effect (Barnard *et al.*, 2020). This pulse of CO_2 reflects the hot spots of activity created through the initial movement of water increasing the interaction between organisms and substrates (Bailey *et al*., 2017, Schimel, 2018, Patel *et al.*, 2021b, McClain *et al.*, 2003). As flooding persists, anoxia can occur within the soil column and within aggregates, altering microbial metabolic requirements dictated by the redox status of the environment (Boye *et al.*, 2018). This onset of anoxia slows microbial metabolism (Keiluweit *et al.*, 2016) and can select for specialized anaerobic microbes that utilize alternative electron acceptors to oxidize organic matter (Fuhrmann, 2021). Thus, in persistently saturated wetlands, for example, organic matter cycling is slowed and results in an accumulation of C and nutrients (Boye *et al.*, 2017,

Anthony and Silver, 2020). However, with seasonal or shorter-term flooding, where chemical and physical conditions have steeper fluctuations compared to wetland systems, microbial communities may make metabolic adjustments by shifting their composition.

Microbial community composition has been found to shift in response to flooding, with studies showing decreased fungal abundances (Unger *et al.*, 2009) and increased anaerobic bacteria and archaea (Argiroff *et al.*, 2017, Randle-Boggis *et al.*, 2017). In aerated systems, the most abundant and energetically efficient terminal electron acceptor at the end of ATP production, or metabolic respiration, is oxygen. With limited oxygen conditions, some microorganisms such as facultative or obligate anaerobes, have adapted to use other elements as their terminal electron acceptor (Conrad, 2020). In flooded, anaerobic environments, both bacteria and archaea dominate as they both have taxa that are capable of anaerobic metabolism (Fuhrmann, 2021, Hartmann and Six, 2023). Different processes occur under strict anaerobic conditions performed by obligate anaerobes, but facultative anaerobes can switch between aerobic and anaerobic metabolic pathways depending on the electron acceptors available (Conrad, 2020, Fuhrmann, 2021). For example, denitrification is often carried out by facultative anaerobes across multiple phyla but include a variety of taxa within the *Gammaproteobacteria* phyla such as *Pseudomonas* and *Alphaproteobacteria* such as *Rhizobium* and *Agrobacterium* (Hartmann and Six, 2023). Denitrification can also be conducted by archaea taxa (*Euryarchaeota*) and in rare occasions by obligate aerobic fungi (*Ascomycota* and *Basidiomycota*) (Hartmann and Six, 2023). Further, methane production is mostly conducted by obligate anaerobic taxa, some examples include archaea within the phyla *Euryarchaeota* and bacterial phyla *Firmicutes* (Conrad, 2020, Hartmann and Six, 2023). Increases in *Euryarchaeota* abundances were found in response to increased flood connectivity (Argiroff *et al.*, 2017). Sulfate reducers are often obligate anaerobes

(Fuhrmann, 2021) and were also found to increase with increasing flood connectivity (Argiroff *et al.*, 2017). Importantly, the abundance and activity of facultative and obligate anaerobes carrying out iron and sulfate reduction and methanogenesis depend in part on the duration of flooding that affects the availability of the terminal electron acceptors. For example, methanogenesis is only a significant metabolic pathway under very reduced conditions (< -150 mV) such as prolonged flooding when complete anoxic conditions exist and more energetically favorable terminal electron acceptors have been exhausted (Conrad, 2020). Although activity may be maintained in flooded systems, anaerobic metabolism is much slower than aerobic metabolism (Keiluweit *et al.*, 2017, Huang *et al.*, 2020). Therefore, it is expected that aerobic CO_2 respiration decreases with prolonged flooding while nitrous oxide and methane production would increase. Thus, with changing soil redox and oxygen concentrations, community composition can shift towards an increased abundance of facultative and obligate anaerobes.

Anaerobic conditions not only lead to microbial community composition shifts which decrease reaction rates but also causes shifts in redox conditions which affect the availability and speciation of different SOM and inorganic compounds (Boye *et* al, 2017, Boye *et al.*, 2018). Changes in redox conditions can alter mineral association dynamics of both microbes and SOM (Anthony and Silver, 2020). For instance, Fe(III) reduction to Fe(II) under anaerobic conditions can release previously bound organic matter, stimulating decomposition (Hall and Silver, 2013). Proportions of nutrients such as nitrate and ammonium may change over time, where ammonium accumulates during flooding because nitrification (an aerobic process) is inhibited. At the same time, the anion nitrate, a much more mobile form of N, is more susceptible to leaching during flooding, leading to a significant loss of N from the soil system. In summary, redox conditions change which compounds are utilized and increase compound mobility, which can lead to losses.

Further, mineralization rates and nutrient uptake can decrease, thus the net effect of flooding on nutrient and C pools is quite complex.

Flooding changes both physical and chemical interactions within the soil matrix. Increased connectivity between pore spaces during flooding allows previously isolated locations to exchange nutrients, alleviating potentially nutrient limiting conditions. Microbial communities may respond by increasing abundances of anaerobic taxa, thus maintaining activity during flood (Conrad, 2020). Chemical shifts associated with redox conditions can lead to complex processes between organic matter and microbes. Thus, measuring nutrient pools in addition to community composition shifts during flood may give insight into how nutrient cycling is affected by flooding.

1.6 *Land use impacts on soil structure, nutrients and carbon*

Land use influences SOM, porosity and microbial community composition which affects nutrient cycling and further, microbial community function and diversity (Beniston *et al.*, 2014, Evans *et al.*, 2022, Patel *et al.*, 2021). The chemistry of plant inputs and proportions of C, N and P entering the nutrient and C pools may change the requirements for microbial activity, shifting microbial community structure and nutrient cycles (Fuhrmann, 2021). Land use in agriculture production can cause mechanical disturbances altering pore structure and therefore disrupting microbial habitat and access to nutrients (Samson *et al*., 2020, Six *et al.*, 2004, Six *et al.*, 2000). Changes in land use can have persistent effects on a microbial community (Kallenbach and Grandy, 2015), and it is important to understand how community composition relates to microbial functioning under different land uses. Further, the question remains if ecosystem properties with greater SOM concentrations and greater microbial activity, biomass and diversity

also increase resiliency and redundancy of the soil microbial community during and after flood events.

Higher concentrations of SOM typically correspond to higher rates of microbial heterotrophic respiration, extracellular enzyme activity (EEA) and microbial biomass (Kallenbach and Grandy, 2011, Fierer, 2017). Natural, unmanaged, ecosystems have higher concentrations of SOM, microbial biomass and microbial community activity compared to managed agricultural land (Beniston *et al.*, 2014). Natural ecosystems such as forests or grassland also have more heterogenous soil structure compared to agriculture as constant root growth contribute to both the assemblage and disintegration of aggregates (Six *et al.*, 2004, Vezzani *et al.*, 2018, Hartmann and Six, 2023). Higher concentrations of SOM also contribute to aggregation and other soil properties such as water holding capacity (Six *et al.*, 2004). Deeper roots in forests and grasslands compared to annual agriculture systems increase water distribution within the soil column as water preferentially will flow along roots and their channels. This may lead to increased functional potential of the microbial community, as anaerobic habitats are formed within moist aggregates. Natural forests and grasslands have more perennial root structures which increase root contribution to SOM and aggregation through root exudation, microbial necromass and growth compared to agriculture systems (Six *et al.*, 2004, Erktan *et al.*, 2018). Land use practices that build and retain SOM are gaining popularity to increase soil health and plant productivity, which may also increase the potential of microbial community resiliency to disturbance through increased microbial community multifunctionality (Crews and Rumsey, 2017, Wagg *et al.*, 2019).

Agricultural practices such as increasing plant diversity through crop rotations, implementing cover crops, and transitioning to more perennial or year-round plant cover often have higher

organic matter inputs, compared to conventional monocrop agriculture, that stimulate soil microbial activity (Crews and Rumsey, 2017, King and Blesh 2018). In systems with perennial roots, the constant input of nutrients and C through exudates and a stable cycle of litter inputs could lead to a more active microbial community (Rasche *et al.*, 2017). When land use changes from systems with perennial roots to fallow land, and vice versa, there is an immediate decrease, and a slow increase respectively, in total soil C and microbial biomass (Hirsch *et al.*, 2017). However, to my knowledge, there has been no direct comparison of microbial activity, such as respiration or extracellular enzyme production, among conventional agriculture, perennial agriculture, and natural systems. Therefore, it is not clear if a managed perennial agriculture system is able to truly 'mimic' processes in a natural system, compared to conventional agriculture.

In my thesis I refer to conventional agriculture as a monocrop or monocrop rotation between corn and soybean which uses practices such as tillage, inorganic fertilizers and pesticides. Due to the higher mechanical disturbance and input of inorganic fertilizers, conventional agricultural practices often select for microbes that respond quickly to often high, but variable, nutrient availability and are thus dominated by bacterial communities (Young and Ritz, 2000). Tillage breaks up and turns the soil which causes mechanical disruption with negative implications for soil aggregates and fungal hyphae. As macroaggregates are broken up by the tillage process (Six *et al.*, 2000), there is further homogenization of communities that were once isolated in those macroaggregates. Microaggregates are less sensitive to tillage perturbations and thus dominate in tilled agricultural systems (Six *et al.*, 2000). Soil aggregation and stability is increased with root growth and exudates (Erktan *et al.*, 2018), thus in annual agriculture systems aggregation is seasonally limited. Further, increased compaction and thus soil bulk density can make it more

difficult for roots to penetrate the soil, thus limiting aggregation potential. Conventional agriculture systems that disrupt soil structure over time lead to a decrease in total SOM stocks and high soil bulk density.

Across gradients of land use intensity from forests to conventional agriculture, microbial activity can vary with above and belowground litter quantity and quality (Rillig *et al*., 2015, Fanin and Bertrand, 2016, Erktan *et al.*, 2018). Litter quality impacts microbial community activity as particular enzymes may be required to target specific bonds within fresh organic matter (Sinsabaugh and Shah, 2012, Cleveland *et al.*, 2014). Litter quality is partly related to the relative proportions of C and N within plant components, such that litter with higher C:N ratios are considered 'low' quality whereas litter with lower C:N ratios are considered 'high' quality. For example, root litter with a higher proportion of C, as would be found in lignacious forest ecosystems, was slower to decompose compared to root litter with lower concentrations of C (Silver and Miya, 2001). The type of litter polymers also impacts decomposition and microbial communities. For instance, the decomposition of chemically complex polyphenol lignin compounds requires specific oxidative enzymes; thus fungi often dominate decomposition in forests as they are the main producers of oxidative enzymes (Sinsabaugh and Shah, 2012, Fanin and Bertrand, 2017). Further, decomposition of low-quality litter types was found to be dependent on starting microbial decomposer community compared to higher quality litter types (Cleveland *et al.*, 2014). Litter quality in agriculture systems depends on the crop, but common crops such as corn and wheat generally have relatively low litter quality (low N and soluble C), especially when we consider that the highest quality part of the crop is harvested and transported off the field (Córdova *et al.*, 2018). Grasslands consist of annuals and perennial non-woody forbs, grasses, and N-rich legumes, and have relatively high-quality litter compared to forests

and agriculture. Partly, because of their dense, and sometimes deep rooting structure, grasslands exhibit tighter (less 'leaky') nitrogen cycles compared to agricultural systems, minimizing losses due to denitrification and leaching (Lemaire *et al.*, 2015).

Thus, land use type not only influences physical soil characteristics such as structure but also dictates litter chemistry and the timing and fate of litter inputs. While it is difficult to separate the multiple soil characteristics changing from one land use to the next, plant communities and management are clearly important drivers affecting the quality and quantity of SOM and soil structure. It is reasonable to expect that these differences in resources and soil microhabitats will impact microbial activity, composition, and biotic interactions but, in response to flooding, it is unclear if and how soil microbial community activity and resiliency is land use-dependent.

1.7 *Soil nutrient and carbon pools*

Soil nutrient cycles, driven primarily by microbial communities, begin with plant inputs, from above and below ground biomass and root exudates. This plant biomass constitutes the dominant organic matter input into the soil (Fanin and Bertrand, 2016, Hirsch *et al*, 2017). Decomposition products have different fates in the soil and are partitioned into different SOM pools. These pools are often operationally defined by size. The largest size class is the particulate organic matter pool (> 53 μm) (Balesdent, 1996, Christensen, 2001, Contrufo *et al.*, 2019). Particulate organic matter can be bound within aggregates and can sometimes be important for aggregate nucleation (Six *et al.*, 2004, Witzgall *et al.*, 2021). The smallest operationally defined size fraction of SOM is mineral associated organic matter (MAOM, < 53 μm). Molecules within the MAOM pool are more protected from microbes as they are bound to mineral surfaces (Possinger *et al.*, 2020). The dissolved organic matter (DOM) pool consists of compounds that are dissolved or in solution within the liquid phase of the soil environment or enter it easily upon wetting. The DOM pool is

defined as being < 0.45 μm, however if a filter is not used in DOM extraction and thus includes materials > 0.45 μm, then it is referred to as the water-extractable organic matter (WEOM) pool (Kalbitz *et al.*, 2000). In my thesis I measure C and N within the WEOM pool (WEOC and WEON respectively), thus will focus on the dynamics within this fraction.

The WEOM pool is highly dynamic as it's rates of consumption and production are constantly changing. WEOM is the most accessible OM pool to microbes and can be rapidly assimilated. At the same time, microbial communities also produce WEOM via their inputs of microbial byproducts ranging from metabolites to products of cell lyses and from the process of plant litter depolymerization (Campbell, *et al.*, 2022). Upon soil wetting, WEOC and WEON concentrations are expected to increase as aggregates can become destabilized releasing physically protected OM. Redox changes can further destabilize MAOM, leading to the desorption of OM into the WEOM pool (Anthony and Silver, 2020). Thus, after soil rewetting microbial access to nutrients and C increases and results in the observed immediate increase in microbial activity (Barnard *et al.*, 2020). While WEOM is considered more physically accessible and bioavailable relative to other SOM pools and thus the primary energy source for microbial communities, other SOM pools like the particulate and mineral fraction are also critical for the maintenance of soil microbial communities. These solid phase pools of SOM, because they are not mobile like WEOM, are likely to be much more influenced by soil structure.

The degree of aggregation and the heterogeneity of the soil structure is key for the accumulation and persistence of SOM (Wolf and Lehmann, 2019). SOM pools vary in their chemical composition complexity, however, it is the ability of microbial communities to access SOM nutrient and C that determines whether decomposition occurs (Lehmann *et al.*, 2020). Wolf and Lehmann (2019) find that the long-term stability of C in soil C models depends on physical

protection and mineral sorption dynamics. This physical protection− which is strongly influenced by soil structure− can limit microbial communities from accessing organic matter, and so should be included when considering disturbances that change SOM accessibility patterns (Wolf and Lehmann, 2019). In a more heterogenous soil structure with a higher diversity of aggregate sizes and greater tortuosity, accessibility to nutrients and C is likely more limited than in a more homogenous soil structure (Nunan *et al.*, 2020). From a microbial perspective, organic matter can be both heterogenous in composition and physical placement within the soil, thus both these spatial and chemical dynamics can influence community specialization (Nunan *et al.*, 2020).

1.8 *Extracellular enzymes*

The transformation of organic polymers is dictated by oxidation and reduction (redox) reaction potential but also the enzymatic catalysis of those reactions (Fuhrmann, 2021). Extracellular enzymes are produced by macro-, meso-, micro-fauna and plants. The biological source of enzymes within the soil matrix can be difficult to distinguish, however due to the high biomass of microorganisms within the soil, I refer to the soil extracellular enzyme activity as microbially derived. Extracellular enzymes for the most part are synthesized within the cell and then released extracellularly to interact with substrates in the soil matrix (Sinsabaugh and Shah, 2012, Nunan *et al.*, 2020). Bacteria and fungi play a major role in decomposition in the soil environment in part through the production of extracellular enzymes. Extracellular enzymes target specific molecular bonds to catalyse the cleavage of compounds either hydrolytically (requiring a water molecule) or oxidatively (requiring oxygen or peroxide) (Sinsabaugh and Shah, 2012, Fuhrmann, 2021). Decomposition is a sequential process which results in the release of bioavailable monomers which can be taken up by microbes, plants and other organisms. Enzymes have an affinity for mineral attachment due to their N-containing moieties, potentially limiting interaction

with substrates (Nannipieri *et al.*, 2018). Since the production and release of enzymes can be metabolically taxing, patterns in the release of enzymes, and thus a microbial investment in obtaining resources, involves energetic trade-offs with cellular growth (Nunan *et al.*, 2020). Under limiting nutrient conditions enzyme exudation occurs to depolymerize OM and access resources (Sinsabaugh and Shah, 2012, Weintraub *et al.*, 2013). Thus, in theory, if an organism has its metabolic needs met, enzymes will not be produced. This is consistently found for the enzyme phosphatase. In the presence of available phosphorous the phosphatase activity decreases (Tresar-Cepeda, 2008, Bissett *et al.*, 2011, Sinsabaugh and Shah, 2012). Organic monomers produced by enzymatic decomposition and that are then assimilated into microbial biomass, can be temporarily immobilized within the cell or readily released as excess back into the soil environment and thus accessible to other microbes or plants (following mineralization). As microbes transform organic molecules to inorganic molecules, determining the balance between immobilization and mineralization processes can be difficult to distinguish. My thesis considers microbial biomass C and N (MBC, MBN) to help inform potential immobilization and microbial community growth, and also as a component of WEOM to determine how microbes are utilizing their resources.

Measuring soil extracellular enzyme activity is an imperfect laboratory assay procedure, and although there are strong merits to certain enzyme assays there are also limitations and complications. A common laboratory assay for determining extracellular enzyme activity rates, and the one I used in my experiments, looks at the release of substrate products via fluorescing agent in a gram of field moist soil made into a slurry with buffer (Saiya-Cork, 2002). In brief, the benefits of this method are: firstly, that it approximates *in situ* conditions by using field moist soils; secondly, by making a soil slurry the interaction between enzymes and substrate is

maximized; finally, it accounts for the enzymes that resorb back onto mineral surfaces (Nannipieri *et al.*, 2018). However, under these conditions it is important to recognize the assay represents maximum potential activity and not necessarily the *in situ* activity. This is partly because of the induced substrate saturation, and the soil slurry may release enzymes that would have otherwise been unable to perform. Activities are reported as potential activity to account for this approximation of maximum potential activity. A complicating factor is that because enzymes are released by organisms to the soil, the measured pool of enzymes contains both new enzymes but also old (or extant) enzymes (Nannipieri *et al.*, 2018, Nunan *et al.*, 2020). Extant enzymes may no longer be close to the organism that produced the enzyme or be produced in response to a particular short-term experimental treatment confounding the effects of the experiment (Taylor and Sinsabaugh, 2015, Nannipieri *et al*., 2018).

1.9 *Scaling up from aggregates to ecosystems*

Spatial and temporal scales are important when looking at microbial responses to ecosystem level disturbances, as microbial composition can change from temporal to aggregate level scales (Bargett and Putten, 2014, Tecon and Or, 2017, Upton *et al.*, 2019). Soil microbial resiliency to disturbance has been measured from species traits to ecosystem level variations in community composition (Philippot *et al.*, 2021). Therefore, it is important to approach research on disturbances at the right scale to measure the scale-appropriate response. Many assumptions about microbial processes are made when evaluating microbial community activity and diversity at ecosystem scales (Hall *et al.*, 2018). For example, when looking at the field scale, flooding effects are different than at the pore scale, where pore water dynamics influence microbial community access and interactions (Genuchten and Pachepsky, 2014). In addition, bulk soil redox conditions may not correlate with microbial community patterns as they are impacted by

microscale changes in redox (Wanzek *et al*., 2018). Larger scale, regional differences in soil texture may influence biotic and abiotic soil properties, potentially overriding microscale controls on microbial and plant community dynamics. For example, soils with a higher proportion of clay have a greater affinity for binding organic compounds including SOM and extracellular enzymes (Nannipieri *et al.*, 2018, Lehmann *et al.*, 2020). Furthermore, clay-dominated soils have higher soil moisture retention increasing the proportion of anaerobic microsites and thus increasing the diversity of microbial metabolisms within the soil matrix (Keiluweit *et al.*, 2017). Measuring microbial community dynamics across relevant scales will increase the accuracy in determining which combination of factors best correlate with microbial response to a particular disturbance.

At temporal scales (diurnal to seasonal) differences in resources and microbial limitations can occur that effect their response to flooding but also the time since flood exposure will be an important factor in estimating recovery. Seasonal variation can lead to variation in aboveground litter and root inputs related to temperature, moisture and plant senescence (Silver and Miya, 2001, Rillig *et al.*, 2015) thus leading to potential temporal shifts in microbial community activity and composition within both the bulk soil. For example, plant growth stage and time since flood was found to influence root exudation, which is an important source of C and nutrients for microorganisms in the rhizosphere (Francioli *et al.*, 2021). Thus, microbial community response to flooding was impacted by plant response to flood (Francioli *et al.*, 2021). Temporal and regional scale dynamics are important for addressing the greater impacts of microbial community response to both land use and flooding although they may not capture the fine resolution of microbial processes.

1.10 *Summary and conclusion*

With soil microbial community response to flooding becoming increasingly relevant as flooding duration and frequency are predicted to increase with climate change, understanding microbial dynamics within the context of their environment is crucial. My thesis addresses the unknowns of how the interactive effects of land use and soil structure influence microbial community response to flooding. We know that microbial communities underpin nutrient and C cycles and that they act on microaggregate scales and are limited by environmental perturbations ranging from micrometer to land scape scales. As soil microorganisms are intimately linked to the availability of nutrients and C for plants and building SOM, the unknown effects of microbial community activity and diversity due to flooding may have resounding impacts on ecosystem functioning. Starting at the ecosystem scale I will compare how microbial extracellular enzyme activity varies across a land use intensity gradient within a seasonal floodplain. Within this study I assess whether, within the context of a seasonal floodplain, land use characteristics have a greater influence on microbial activity than variability associated with regional, within-field and temporal scales. I then utilize a laboratory incubation approach to study microbial community response to flooding on a finer scale. I asses how microbial community activity and diversity response to flooding is mediated by land use, influencing starting community, substrate availability and site history, and soil structure, influencing accessibility to substrates and niche partitioning. Therefore, I hope to contribute to unknowns regarding the dynamics between microbial activity and diversity and environmental factors and how that interferes with their response to disturbances such as flooding.

Chapter 2

Floodplain land use disturbance gradients have a stronger effect on soil microbial enzyme activity than spatial and temporal variability

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2.1 Abstract

Climate change is leading to flood events with higher frequency and longer duration, especially in eastern North America. Changes in seasonal flooding that affect water saturation of soils can impact soil microbial extracellular enzyme activity (EEA) that mediates nutrient cycling of nitrogen (N), phosphorous (P) and carbon (C). Understanding controls on soil functional potential in floodplain ecosystems helps identify optimal land use practices in ecosystems with intensifying flood dynamics. Our objective in this study was to assess some of the abiotic controls on soil microbial EEA within a floodplain and determine how sensitive the relationship is between EEA and land use across spatial scales and time. We collected soils across a land use gradient, replicated four times, around the Lake Saint Pierre floodplain in Quebec, Canada. Land uses included: conventional and conservation soybean and corn cultivation, new and established managed perennial grasslands, and natural grasslands and forests. Within each land use, soils were sampled at three time periods and at three elevations representing different exposures to flood, to capture temporal and spatial variability. We found that EEAs declined with increasing

land use intensity as expected, primarily associated with soil moisture and soil organic carbon. Notably, the perennial agriculture practices had EEA and nutrient concentrations falling between those observed under annual agricultural practices and natural sites and could therefore be an appropriate compromise to converting conventional agricultural practices back to natural areas. Based on dispersion analysis, we also found that the gradient of decreasing enzyme activity with increasing land use intensity was largely conserved across spatial scales and time (MRPP, $F = 3.33$, $p<0.05$). Two exceptions were found to this conserved enzyme-land use relationship. During the peak growing season and in soils experiencing the highest flood intensity, the land use characteristics that otherwise supported high EEA seem to be overridden, as we did not observe any relationship between EEA and land use. Our results suggest that the influence of land use on supporting microbial nutrient and carbon cycling is strong across the inherent spatial and temporal variation within a heterogeneous and fragile ecosystem like floodplains, highlighting the importance of land use management across scales.

2.2 Introduction

Climate change is shifting weather patterns, increasing the incidence and duration of flooding in eastern North America (Jeong *et al.*, 2014). Yet it remains unclear how this change in soil inundation impacts soil organisms which perform important roles in soil health, especially when flooding coincides with land management practices that disrupt soil communities (de Vries *et al.*, 2013). Soil extracellular enzymes catalyze key steps in nutrient and carbon cycles that underpin ecosystem functioning and plant nutrient availability. Soil enzymes are dominantly produced by soil microorganisms (such as bacteria and fungi) and their production and activity are sensitive to changes in both moisture and disturbance (Sinsabaugh and Shah, 2012, Steinweg *et al.*, 2012, Weintraub *et al.*, 2013, Bowles *et al.*, 2014, Strickland *et al.*, 2017). Systems with intensive land

use management, such as conventional agriculture, that experience seasonal flooding may see compounding and interactive disturbance patterns, with unknown consequences to soil nutrient stocks and cycles (Ou *et al.*, 2019). Thus, to preserve the productivity and health of floodplain ecosystems, it is important to determine how land use intensity influences soil enzyme dynamics and their associated nutrient and carbon pools.

Soil microorganisms can secrete extracellular enzymes which transform specific components of organic matter including cellulose, proteins, chitin, lignin and other aromatic compounds into more bioavailable forms of carbon (C) and nutrients (Sinsabaugh and Shah, 2012). Based on the products of each enzyme reaction, extracellular enzyme activity (EEA) contributes to different nutrient and C cycles. The β-glucosidase, peroxidase and phenol oxidase enzymes each play a role in C-cycling by breaking down cellobiose (β-glucosidase) and lignin (peroxidase and phenol oxidase). Fungal and exoskeleton chitin is broken down by N-1,4-acetylglucosiaminidase releasing both C and nitrogen (N). Peptides are transformed by leucine- and tyrosine-amino peptidases predominately impacting N cycling. Phospholipids are also transformed, liberating inorganic P from organic polymers by phosphatases (Sinsabaugh and Shah, 2012). Thus, certain soluble nutrients and C compounds are a direct result of specific enzyme production, but in turn, these soluble compounds also provide energy and nutrients to produce extracellular enzymes, either limiting or enhancing enzyme production.

Understanding the constraints on EEA can give insight into soil nutrient and C cycling processes across different land uses. Land use is a well-known factor affecting soil EEAs (Bissett *et al.*, 2011, Wallenius *et al.*, 2011, Yongxing *et al.*, 2019). For instance, differences in litter availability and chemistry, dictated by the type of land use, can influence the suite of enzymes required to break specific bonds (Fanin and Betrand, 2017). In addition, we would expect higher enzyme

activity with decreasing land use intensity as a function of multiple coinciding factors, including, decreased physical disturbance, higher microbial populations and activity, and greater substrate availability fueling enzymatic production (Wallenius *et al.*, 2011, Burns *et al.*, 2013, Weintraub *et al.*, 2013, Mace *et al.*, 2016).

While the differences in land use, such as between an agricultural system and a forest, are numerous, we might expect plant cover and soil physical properties to be primary drivers on EEAs because of their impact on substrate supply to the microbial community. For example, less intensive land use types that support perennial or year-round plant cover have higher organic matter inputs that contribute to the soil substrate supply to support enzyme production and more active microbial communities (Crews and Rumsey, 2017). Unmanaged forests and grasslands ('natural' systems) typically contain more C compared to annual agriculture systems (Lugato *et al.*, 2021, Samson *et al.,* 2020). Microbial biomass is also an important organic C input and therefore land uses that support microbial activity and abundance can be expected to also increase soil carbon (Kallenbach *et al.*, 2015). This higher total soil organic matter and C further contributes to soil aggregation that promotes optimal soil structure and moisture content for EEA. Thus, soil EEA are expected to increase with decreasing land use intensity, in part because of the similar trend commonly observed for organic matter and soil C.

Land use gradients also often exhibit differences in physical disturbances that can impact soil EEA. Compared to annual agricultural systems, perennial and unmanaged ecotypes have reduced physical disturbances, like tillage, that fragment hyphae by disrupting aggregates and macropores. This can result in a bacterial-dominated microbial community in more intensively managed systems (Young and Ritz, 2000), affecting lignin-degrading enzymes that are primarily produced by fungi (Burns *et al.*, 2013, Witzgall *et al.*, 2021). Further, soil disturbances associated

with agricultural cultivation alter soil pore structure thereby influencing enzyme access to protected nutrients and C (Six *et al.*, 2000, Six *et al.*, 2004, Samson *et al*., 2020). Jackson *et al.* (2003) demonstrated a short-lived increase in soil respiration directly after tillage, and although this high CO_2 flux was not persistent, nitrogen cycling was altered due to this change in soil structure.

Within a land use intensity gradient, perennial agricultural systems are considered intermediate intensity between unmanaged forests and annual agriculture, as they do not require frequent cultivation, they have more continuous organic matter inputs, and often reduced chemical inputs. As such, perennial agriculture systems are gaining popularity as a regenerative agriculture practice (Asbjornsen *et al.*, 2013, Paustian *et al.*, 2016, Rasche *et al*., 2017, Crews and Rumsey, 2017). Yet, few studies have compared perennial agriculture to both annual agriculture and natural systems within the context of key soil health indicators, such as EEA. These comparisons are critical in determining the often-cited claim that agricultural perenniality 'mimics' natural systems more so than annual systems (Conant *et al.*, 2001). Whether these land use conditions that likely separate EEAs across a land use intensity gradient are robust enough to override the variable conditions induced by seasonal flooding remains unclear.

Independent of land use, the microbial communities' ability to access substrates and thus enzyme production, changes in response to flooding (Lieberman *et al*., 2023). Moisture influences enzyme – substrate interaction both spatially and chemically within the soil matrix. Soil moisture increases pore connectivity allowing soluble nutrients, organisms, and proteins (such as enzymes) to move within the soil matrix (Bailey *et al*., 2017). In conditions with elevated moisture, as would be expected in flooded circumstances, hotspots of activity occur as heterogeneously distributed organic matter becomes more accessible to enzymes (McClain *et al.*,

2003, Bailey *et al.*, 2017). Thus, microbial activity is thought to be more limited by lack of moisture than increased moisture activity (Steinweg *et al.*, 2012, Schimel, 2018, Barnard *et al*., 2020). However, the effects of flooding on soil EEA is not well understood, as it can lead to simultaneously higher substrate availability that may enhance EEA, while also lowering microbial activity that would limit EEA. For instance, flooding can lead to low redox conditions, abiotically increasing soluble nutrient pool concentrations available to microbes (Lieberman *et al*., 2023). We might expect these shifts in moisture and the resulting changes in redox that release substrates into the soluble phase to increase EEA (Barnard *et al*., 2020, Blankinsop and Schimel, 2018). On the other hand, in conditions of reduced oxygen availability, expected with sustained flooding, microbial activity may become limited (Boye *et al*., 2017, Huang *et al.*, 2020). If microbial metabolism is slow under reduced oxygen conditions, the ability of the community to assimilate soluble compounds or produce enzymes would be metabolically constrained (Boye *et al.*, 2017). In floodplains, moisture is highly variable in time and space and, given the pivotal role moisture is expected to have on EEA and thus nutrient and C cycling, it is important to consider whether current and historical moisture conditions minimize the expected effects of land use EEA in flooded systems.

Land use and flooding both provide sources of variability for soil enzyme dynamics. However, fundamental landscape properties, such as soil texture, and seasonal changes can also vary EEAs. Due to the complexity of enzyme dynamics in the soil environment, it is not clear whether the impacts of land use can override the heterogeneity in time and space, especially in a seasonally dynamic floodplain. Temporal changes are related to plant growth stage and senescence, weather, and time since flood. Microbial community response and recovery to flood may also shift over the growing season as the interaction with seasonal properties such as temperature and moisture

adds complexity to the flood disturbance response (Philippot *et al.*, 2021). Together, both plant growth stage and time since flood influence root exudation−an important control on substrate supply and microbial community activity (Yongxing *et al.*, 2019, Francioli *et al.*, 2021). Spatial differences in soil texture at regional scales, can result in higher or lower affinity for enzyme and substrate binding (Nannipieri *et al.*, 2018, Anthony and Silver, 2020, Lehmann *et al.*, 2020, Possinger *et al.*, 2020). Clay dominated soils, due to high proportion of micropores, increased tortuosity, and negative surface charge, are associated with higher moisture and organic matter retention (Keiluweit *et al.*, 2017, Finley *et al.*, 2021, Lehmann *et al.*, 2020), and have shown strong affinity for enzyme binding (Nannipieri *et al.*, 2018). Further, higher clay soils are more structured leading to a less heterogeneous soil environment thus increasing the probability of decomposition reactions (Nunan *et al.*, 2020). Temporal and spatial variability influence microbial community activity, however whether land use can override these properties associated with time and space is unknown in systems that experience seasonal flooding.

Floodplains are important interfaces between land and aquatic ecosystems that host a uniquely high number of ecosystem services (e.g., water management, biodiversity, hot spots of biogeochemical activity) compared to their surrounding environments (McClain *et al.*, 2003, Ding *et al.*, 2021). Yet, floodplain ecosystems are under multiple stressors, including conversion from perennial or unmanaged ecosystems to annual crops that involve more intensive farming methods (Jobin *et al.* 2014, Jobin and Brodeur, 2023). To understand how floodplain nutrient cycling and C dynamics are affected by changing land use, more research is needed that incorporates microbial activity parameters, like EEA, with land use characteristics (Baldwin and Mitchell, 2000, Moon *et al.*, 2016). The objectives of this study were to: 1) determine which soil characteristics control soil microbial EEA across a land use gradient in the Lake Saint Pierre

floodplain ecosystem and 2) determine whether site-specific controls on EEA are consistent across time and spatial scales within a land use gradient. Our hypotheses were 1) moisture will be the dominant soil control on EEA in this flooded ecosystem and 2) a decreasing trend in soil EEAs with increasing land use intensity will be consistent across time and space despite variability in soil moisture and enzyme substrate availability.

2.3 Methods

2.3.1 *Lake Saint Pierre floodplain*

Our study sites surround Lake Saint Pierre and its floodplain, located within the Saint Lawrence River, near Trois-Rivières, Québec, Canada (46.202805, -72.82804) (Fig. 2.1). The 50,000-ha lake floods near-annually, for a period of 5-9 weeks, typically starting in early April (Jean and Letourneau 2011). Spring snowmelt and precipitation flood the land surrounding Lake Saint Pierre, varying year to year based on annual snowpack and spring precipitation. The resulting floodplain covers around 28,000 ha of land, making it the largest wetland along the Saint Lawrence River (Hudon et al., 2018).

In the past several decades, the Lake Saint Pierre floodplain also experienced increased land use intensification and disturbance (increased use of external inputs− e.g. fertilizers, tillage). Most of the land in the area is used for agriculture. In the 1950's, perennial forage crops and pastures occupied 80% of the floodplain cropping systems (Jobin and Brodeur, 2023). However, by 2016, 86 % of cultivated land was in more intensive, annual crop production dominated by corn and soybean (Dauphin and Jobin 2016, Jobin and Brodeur 2023). During this same period, ca. 622 ha of natural wetlands and forests were converted to annual crop production (Jobin and Brodeur, 2023).

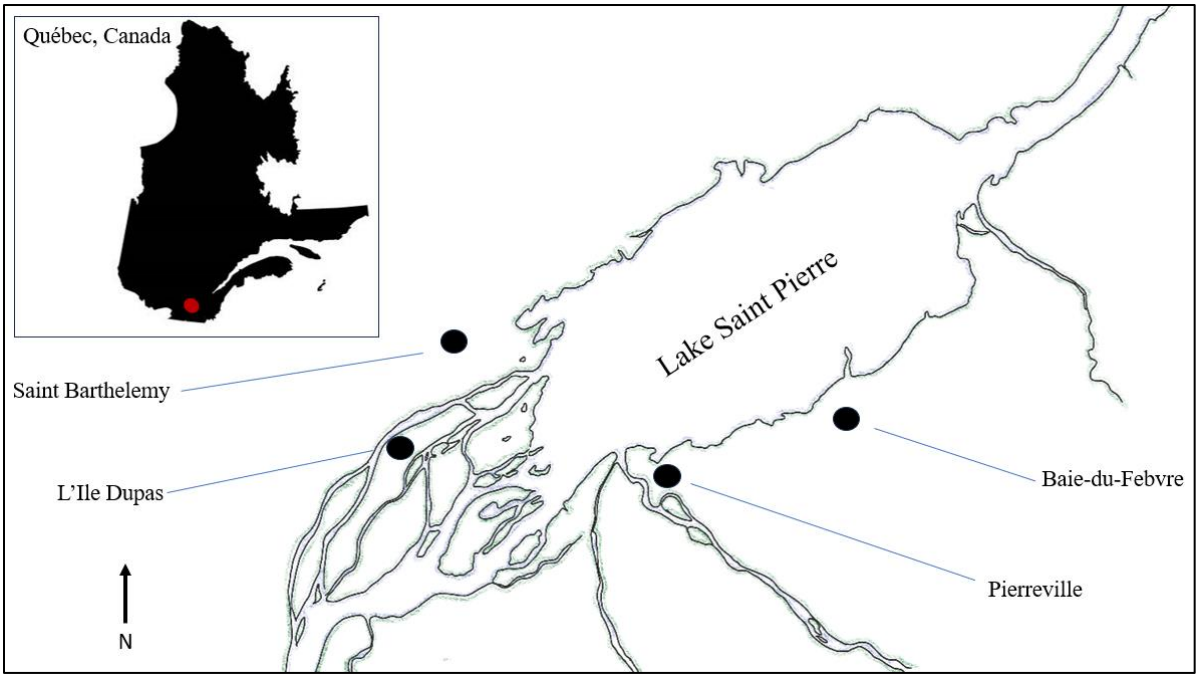

Figure 2.1. Lake Saint Pierre within the province of Québec, Canada. Four regional lake locations where each land use gradient was sampled are labeled: Saint Barthelemy, L'Ile Dupas, Baie-du-Febvre, and Pierreville.

2.3.2 *Study sites*

We studied a land use disturbance gradient at four locations located around Lake Saint Pierre within the floodplain zone. The four locations are within municipalities Baie du Febvre (Baie), Pierreville (Pier), Saint Barthelemy (Bart) and L'Ile Dupas (Dupa) (Table 2.1, Fig. 2.1). These four locations were chosen as experimental replicates for the land use gradients. While climatic and flood characteristics are relatively similar among lake locations, soil texture is highly variable (Table 2.1). Soil texture ranges from predominately clay to sandy loam. Soils in the region are largely gleysols and podzols developed over an ancient sandy river terrace overlain by alluvial deposits (Quebec Ministry of Natural Resources and Forests, and Soils of Canada (Landscape of Canada database), accessed on July 25, 2023).

Table 2.1. Characteristics of the four Lake Saint Pierre study locations including sampling coordinates, mean annual temperature (MAT), mean annual precipitation (MAP), soil type, and pH.

Land use	MAT$^{\Psi}$ (°C)	MAP$^{\Psi}$ (mm)	Soil Type*	Soil pH
Baie Du Febvre (Baie)				
Conventional Corn	5.3	924.4	Nicolet soil series poorly drained glacial till; Clay loam	6.3
Conservation Corn				6.2
New Forage				6.8
Established Forage				6.3
Wet Grassland				-
Natural Forest				-
Saint Barthelemy (Bart)				
Conventional Corn	6.3	999.7	Duaps and Berthier soil series, imperfectly drained alluvial sediment; Silt-Clay Loam	5.7
Conservation Corn				5.7
New Forage				5.8
Established Forage				-
Wet Grassland				-
Natural Forest				-
L'Ile Dupas (Dupa)				
Conventional Corn	6.3	999.7	Duaps soil series, imperfectly drained alluvial sediment; Sandy-Clay loam	5.5
Conservation Corn				5.6
Established Forage				5.7
Natural Forest				-
Pierreville (Pier)				
Conventional Corn	5.8	984.5	Comtois and Pierreville soil series, imperfectly drained alluvial sediment; Sandy Loam	5.9
Conservation Corn				5.3
Wet Grassland				-
Natural Forest				-

$^{\Psi}$ Source: Government of Canada Climate Normal from weather stations within 20 km of site locations.

*Source: Research and Development Institute for the Agri-environment, Soil Survey Database (https://www.irda.qc.ca/en/services/protection-resources/soil-health/soil-information/soil-surveys).

2.3.3 *Study site gradients*

Our experimental design represents nested spatial scales from regional (i.e. locations around the lake) to within-field spatial variability that allows us to compare variation related to flood

intensity. Samples were collected three times over a growing season to test temporal variability within a land use intensity gradient. The land use intensity gradient ranges from fields of low to high management intensity: forest, wet grassland, established and new forage, conservation and conventional agriculture (Fig. 2.2a). Our *Forest* locations are non-cultivated, non-maintained, plant communities dominated by deciduous silver maple (*Acer saccharinum* L.) and represent the lowest management intensity. The *Wet Grassland* is a non-cultivated, non-maintained, plant community consisting of perennial grasses and herbs including *Phalaris arundinacea* L., *Onoclea sensibilis, Calystegia sepium* and *Solidago rugosa* (Poulin, 2023 in prep). The *Established Forage* fields are assemblages of planted perennial grasses including: canary reed grass (*Phalaris arundinacea* L.), glyceria *spp.*, chickweed (*Stellaria media* (L.) Vill.), sedges (*Carex* spp.) and oat (*Avena sativa*), have been established for more than five years, and are maintained by mowing once to twice a year (Campeau *et al.*, 2024 in review). The *New Forage* are similar to the *Established Forage* but have been planted for less than five years. Our *Conservation Agriculture* fields are under corn (*Zea mays* L) and soybean (*Glycine max* spp.) crop rotation, planted with approximately 4 m-wide perennial buffer strips and inter-row rye grass (*Lolium multiflorum*) cover crop with corn. Perennial buffer strips consist of reed canary grass and wild species which colonized over time. The *Conventional Agriculture* fields are planted in annual crops, characterized by a corn and soybean rotation with the fields bare during the winter and represent the most intense land use. Both agricultural land uses have regular tillage and receive conventional inputs of fertilizer. All agricultural fields in this study were under corn production at the time of soil sampling. Agricultural fields are long and narrow (approximately 50 m wide, range from 1-6 ha), perpendicular to the Lake Saint Pierre shoreline.

Each field location was chosen as part of a larger study with the Pole d'Expertise for the Lake Saint Pierre project starting in 2018 (Campeau *et al.*, 2024 in review). The field selection process included conversations with farmers and landowners to obtain permission for use of their land while also trying to capture different locations around the lake. Thus, not all land uses are represented at each replicate lake location due to circumstances with landowners such that the study has an uneven sampling design (Table 2.1).

To capture the spatial gradient in flood duration and frequency within each field, we also identified within-field locations that varied in their distance from the lake and in their elevation (Fig. 2.2b). Within each field, three zones for soil sampling locations were established: close to the lake, characterized by the longest and more frequent flooding and a maximum elevation of approximately 6 m above sea level (mASL), middle elevation maximum of 7 mASL, and farthest distance and highest elevation with a maximum of 8 mASL characterized by shortest duration and less frequent flooding. Temporal changes after April flooding were captured by soil sampling in 2021 at three times: Spring (May), mid-summer (July), and Fall (November, close to freezing).

2.3.4 *Soil sampling*

Soil samples were collected in 2021, three years after the sites were established for the project (Campeau *et al.*, 2024 in review). In total, soil sampling sites were: 4 lake locations (regional-scale), each of which had 4-6 fields representing different land uses, each with 3 within-field elevation sampling zones (n= 60), plus three sampling times for a total n=180. At each sampling location, 10 soil cores were collected with a push corer (Ø= 2 cm) to a 10-cm depth and composited. Samples were kept frozen (-20 °C) until further processing. Soils were slowly thawed at 4 °C and then sieved to 4 mm and visible roots were removed. Soil moisture was

determined in the field at the time of sampling using TDR HydrosenseII (Campbell Scientific, Logan, Utah) and in the lab gravimetrically.

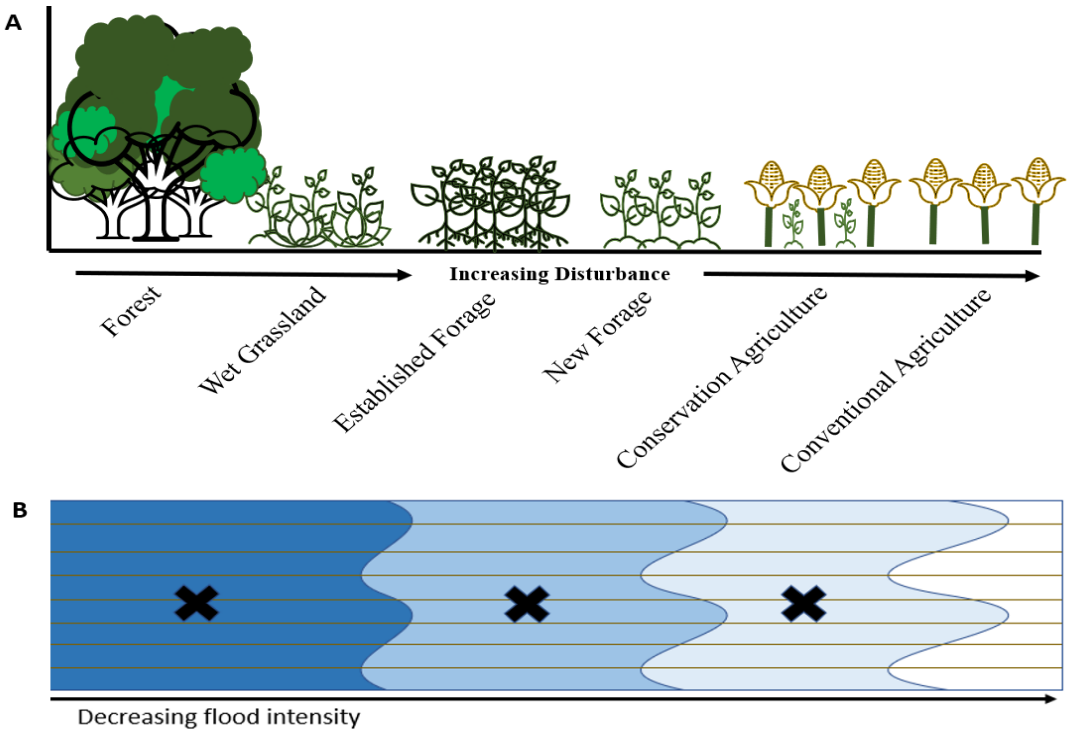

Figure 2.2. Land use disturbance gradient from least disturbed to most disturbed at each regional location around the Lake Saint Pierre shoreline (a) and simplified diagram of the sampling spatial distribution within each land use (b). Sampling positions (marked with an 'x') were determined in relation to Lake Saint Pierre shoreline where closest to the lake has a history of longer flood duration and higher flood frequency.

2.3.5 *Soil carbon and nutrient analyses*

We measured total soil C, water extractable organic C (WEOC), water extractable organic N (WEON), orthophosphate (P), nitrate (NO_3^-), and ammonium (NH_4^+) in all soil samples. To determine WEOC and TDN, 40 mL of deionized water was added to 10 g field-moist soil,

shaken on an end-to-end shaker for 20 minutes, centrifuged for 15 minutes at 8500 rpm, and then decanted, avoiding any visible particulate matter (adapted from Sun *et al*., 2015). The WEOC and WEON concentrations were measured on a TOC-N analyzer (Shimadzu Corp, Kyoto, Japan). Inorganic N was extracted with 40 ml of 2 M KCl solution added to 10 g field-moist soil, shaken for 1 h on a rotary shaker and then filtered through Whatman no. 5 (2.5 µm) filter. We quantified salt extractable soil NO_3^- and NH_4^+ colormetically at 540 nm and 660 nm respectively on a Biotek plate reader (BioTek Instruments, Winooski, VT, USA) (Doane and Horwath, 2003, Hood-Nowotny *et al.*, 2010). For orthophosphate (P), the Melich-P (III) protocol was used (Bolland *et al.*, 2003). Total soil C and N (by flash combustion) and soil texture analyses were conducted at the AgroEnviro Lab (La Pocatiere, QC) from soils collected May 2021.

2.3.6 *Soil potential extracellular enzyme activity and microbial biomass*

We determined potential extracellular enzyme activity (EEA) associated with C, N, and P cycling following previously described methods (Saiya-Cork et al., 2002). Briefly, we measured five hydrolytic enzymes that catalyze the cleavage of: cellulose (β-glucosidase, BG), chitin (N-1,4-acetylglucosiaminidase, NAG), proteins (leucine- and tyrosine- peptidase, peptidase) and phospholipids (acid phosphatase, PHOS). We also measured two oxidative enzymes involved in lignin decomposition, phenol oxidase (PHE) and peroxidase (PER). Soil slurries were made with a 50 mM sodium acetate buffer with a pH of 6.5, reflecting the average soil pH. We quantified hydrolytic potential EEA fluorometrically using black, 96-well microplates and compound-specific fluorescing substrates bound to 4-methylumbelliferone or 7-amino-4-methyl coumarin. Oxidative EEA was quantified spectrophotometrically using clear 96-well microplates and L-3,4-dihydroxyphenylalanine as a substrate. Plates were incubated in the dark at 20 °C for up to 5 hours. We report hydrolytic potential EEAs as nanomole of product produced per hour per gram

of dry soil (nmol $h^{-1}g^{-1}$), and oxidative EEA as micromole of product produced per hour per gram of dry soil (umol $h^{-1}g^{-1}$).

We determined salt-extractable microbial biomass by chloroform fumigation (Jenkinson *et al.*, 2004). For both fumigated and unfumigated samples 10 g (field-moist) of soil was massed. Fumigated samples were left for 24 hours with 1 mL of chloroform directly added to the soil. After 24 hours the fumigated samples were left open to evaporate off the chloroform for up to 4 hours. We extracted non-fumigated and fumigated samples (after 24 hours and evaporation) by adding 40 mL of 0.5 M K_2SO_4. Samples were shaken for four hours at 180 rpm and centrifuged at 8500 rpm for 20 minutes. Supernatant was then filtered with Whatman 5 filter (2.5 μm) and extracts were frozen until analyzed on Shimadzu a TOC-N analyzer (Shimadzu Corp, Kyoto, Japan).

2.3.7 *Data analyses*

Data analyses was conducted in R (R Core Team, R version 4.1.2). Land use, distance to flood (spatial variability), and time were each used as factors in this study. Linear mixed models were used to test the variance of each variable, using sample ID as repeated measures. Significance values (p values) were corrected using *posthoc_Pairwise* function in grafify R package (Shenoy, 2021) using FDR p-adjustment after each mixed model was run. Normality was tested by visually inspecting histograms and quantile plots. All variables that did not meet the assumption of normality were natural log-transformed except for phenol oxidase and peroxidase activity which were natural log+1 transformed, and inorganic N which was square root transformed. Soil moisture, phosphatase activity and total N were normally distributed and therefore were not transformed.

For enzyme correlation patterns, non-metric dimensional scaling (NMDS) (Vegan package 2.6.4, Oksanen *et al.*, 2022) ordinations were conducted using the Bray-Curtis distance matrix. Environmental variables were plotted using envfit function and a Mantel test was used to determine the significant correlations between EEAs and environmental variables. Multi-response permutation procedure (MRPP) analysis was conducted to determine the effects of each treatment: land use, distance to flood, time, and lake location. Results of MRPP are described using between-group variation (delta, significance threshold of 0.05) and within-group variation (*A*, where a value of 1 is completely homogenous). We performed a Dispersion analysis, using betadisper, to determine which scale (time or space) or land use was responsible for the most enzyme variation. Results from dispersion analysis are reported in terms of variation from centroids. Therefore, a larger average distance to a centroid refers to greater dispersion within a factor. Reported results are significant with a maximum threshold of 0.05, unless stated otherwise.

2.4 Results

2.4.1 *Effect of land use on soil moisture, nutrients, carbon pools*

Nutrient and C concentrations demonstrated gradients with increasing land use intensity, except for WEON (Table 2.2). Ammonium, WEOC, and microbial biomass C and N decreased as land use intensity increased. In contrast, melich-P and nitrate exhibited the opposite trend with relatively higher concentrations in agriculture sites (Fig. S2.1). We found that the new and established perennial forage sites had nutrient and C concentrations between the natural and agriculture sites. Concentrations of SOC and total N declined from low intensity to high intensity land use but was not significant ($p>0.05$, Fig. S2.2). Soil moisture declined from low intensity to high intensity (Fig. S2.2). All nutrient and C pools, except WEOC, were affected by time and

only WEON and soil moisture had a significant interaction between land use and time (Table 2.2). WEON was the only parameter that was influenced by distance to flood and time.

Concentrations of total SOC and total soil N were higher with decreasing land use disturbance but were similar across lake locations (Table 2.3, Table S2.1). However, soil texture only varied by lake location and not by land use. Baie had higher sand content compared to Bart, and Pier had the highest sand content of all the sites. Clay content was highest at Bart and Baie and lowest at Pier. Higher clay content was associated with higher SOC and N ($p<0.05$).

Table 2.2. Nutrient, carbon, potential extracellular enzyme activity, and microbial biomass linear mixed model results with repeated measures. Significant p-values ($p<0.05$) are in bold. Soil moisture content (SMC), Inorganic N (InorgN, nitrate plus ammonium), mehlich-P (P), water extractable organic C (WEOC), water extractable organic N (WEON), peptidase (PEP, leucine amino peptidase (LAP) plus tyrosine amino peptidase (TAP)), beta-glucosidase (BG), N-acetylglucosaminidase (NAG), phosphatase (PHOS), phenol oxidase (PHE), peroxidase (PER), microbial biomass carbon and nitrogen (MBC, MCN).

	Soil Moisture	**Nutrients and Carbon**				**Enzyme Activity**						**Microbial Community**	
Treatment	**SMC**	**Inorg N**	**P**	**WEOC**	**WEON**	**PEP**	**BG**	**NAG**	**PHOS**	**PHE**	**PER**	**MBC**	**MBN**
Land use	**<0.0001**	**0.0099**	**<0.0001**	**<0.0001**	0.28	**<0.0001**	0.056	**<0.0001**	**0.014**	**<0.0005**	**0.049**	**<0.0005**	**<0.0001**
Distance to Flood	0.35	0.50	0.12	0.17	0.15	0.56	0.50	0.94	0.39	0.987	0.32	0.71	0.93
Time	**<0.0001**	**0.0067**	**<0.0001**	0.80	**<0.0001**	**<0.001**	**<0.0001**	**<0.0001**	**<0.0001**	0.86	0.054	0.11	**<0.0001**
Land use x Distance	0.997	0.71	0.97	0.77	0.82	0.95	0.97	0.97	0.99	0.26	0.92	0.98	0.99
Land use x Time	**0.00011**	0.20	0.16	0.15	**0.008**	0.39	0.046	0.05	0.14	0.88	0.28	0.07	0.13
Distance x Time	0.97	0.046	0.80	0.29	**0.04**	0.85	0.57	0.69	0.09	**0.022**	0.13	0.93	0.70
Land use x Distance x Time	0.95	0.24	0.94	0.78	0.96	0.97	0.81	0.34	0.21	0.66	0.05	0.31	0.61

Table 2.3. Soil texture and total soil organic C and total N from samples collected May 2021. Values are averages across land uses within each lake location.

Lake Location	Sand %	Silt %	Clay %	SOC	N	C:N
Baie Du Febvre (Baie)	38 ± 13	15 ± 5	47 ± 9	4.40 ± 2.73	0.40 ± 0.20	10.8 ± 0.95
Saint Barthelemy (Bart)	22 ± 7	32 ± 4	47 ± 9	3.28 ± 0.72	0.31 ± 0.07	10.6 ± 0.35
L'Ile Dupas (Dupa)	35 ± 13	31 ± 1	34 ± 13	2.65 ± 1.85	0.26 ± 0.17	9.9 ± 0.51
Pierreville (Pier)	63 ± 11	25 ± 8	12 ± 3	2.37 ± 0.32	0.21 ± 0.03	11.3 ± 0.00

2.4.2 *Effect of land use on potential extracellular enzyme activity*

Potential EEA demonstrated a consistent pattern of decreasing activity as land use intensity increased when averaged across sampling time and distance from flood (Fig. 2.3). We saw the largest difference between the natural and agricultural sites for the lignin degrading phenol oxidase (PHE) and the smallest difference for cellulose degrading BG, 102% and 40% higher in the natural sites respectively. Potential EEA involved in N decomposition (peptidase and chitin-degrading NAG) were 77% and 78% higher in natural sites compared to agricultural sites. Phosphorus degrading enzymes (PHOS) were 47% higher in natural sites relative to agricultural sites. Land use did not significantly affect BG and the lignin-degrading enzyme, PER, however they still demonstrated a similar trend, decreasing with greater land use intensity. Pairwise post-hoc test results indicated that enzymatic activity in the perennial forage sites was consistently lower than natural sites, but higher than agriculture sites (Fig. 2.3). We found no differences between the conventional and the conservation agriculture fields for any of the enzymes except PHE, where conservation agriculture had lower activity than the conventional agriculture.

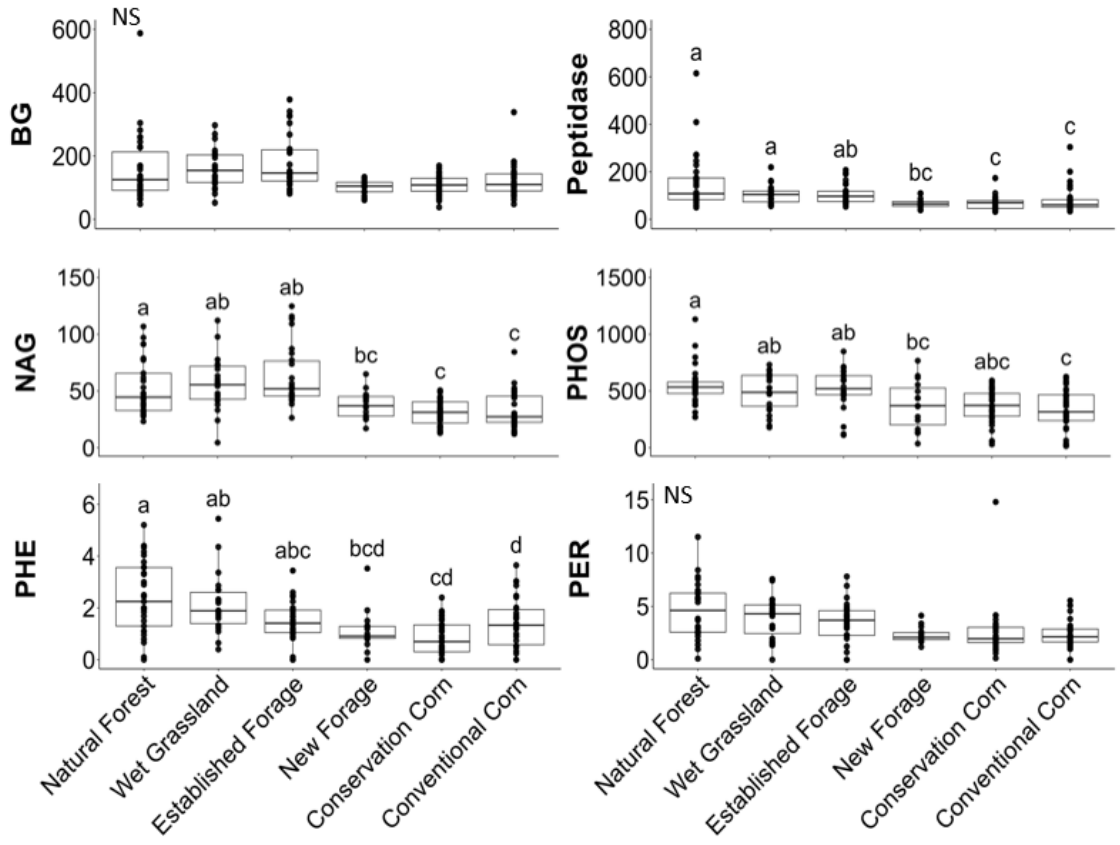

Figure 2.3. Potential extracellular enzyme activity for beta-glucosidase (BG), peptidase (PEP, leucine amino peptidase (LAP) plus tyrosine amino peptidase (TAP)), N-acetylglucosaminidase (NAG), phosphatase (PHOS), phenol oxidase (PHE), peroxidase (PER) measured in units nmol $h^{-1}g^{-1}$and umol $h^{-1}g^{-1}$ for PER and PHE, across land use treatments from low intensity (forest) to high intensity (conventional agriculture)**.** Different letters indicate significant differences (pairwise post-hoc test with FDR adjustments) among land uses, NS indicates no significant differences. The line within the boxplot indicates the median, the limits of the boxes indicate the 25^{th} and 75^{th} quartiles. The whiskers indicate 1.5 x the quartile range.

2.4.3 *Soil and land use factors associated with extracellular enzyme activities*

Results of the NMDS (Fig. 2.4) and subsequent dispersion analysis confirmed that land use correlated with observed variation in EEA. This variability in EEA by land use is primarily driven by the conventional corn treatment (average distance to centroid = 0.24) compared to the wet grassland treatment (average distance to centroid = 0.13). Vector analysis using measured environmental variables indicated that soil moisture content (SMC) and microbial biomass carbon (MBC) are correlated predominately along the second axis (associated with lower intensity land uses) compared to mehlich-P and microbial biomass C:N correlated along the first axis (associated with higher intensity land uses). Soluble nutrient pools including microbial biomass nitrogen (MBN), water extractable organic N (WEON), water extractable organic carbon (WEOC) and inorganic N (nitrate plus ammonium) were aligned with SMC and MBC and correlated with EEAs ($P < 0.1$). Pearson's correlation analysis (Fig. S2.3) illustrated positive correlations between nutrients and C pools with EEA except for phosphorous which was negatively correlated with enzyme activities. Potential EEAs were most strongly correlated with SOC (mean $r^2 = 0.50 \pm 0.15$ across enzymes), SMC (mean $r^2 = 0.53 \pm 0.11$ across enzymes), MBC (mean $r^2 = 0.59 \pm 0.10$ across enzymes), and MBN (mean $r^2 = 0.54 \pm 0.14$ across enzymes).

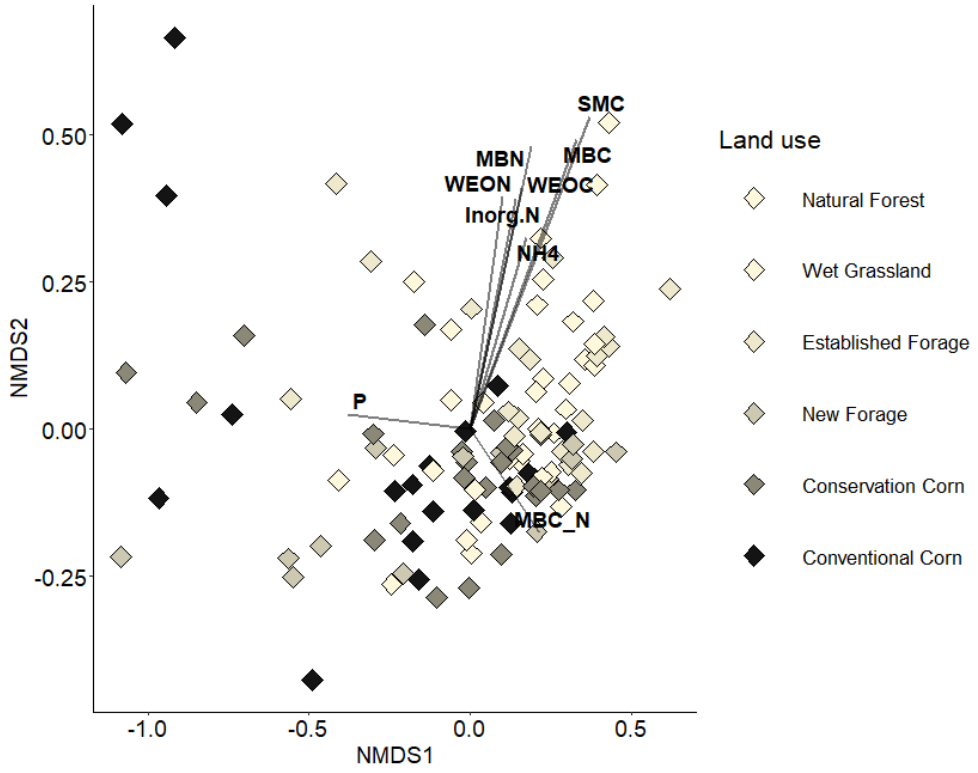

Figure 2.4. NMDS analysis of the aggregated potential activity for the seven measured enzymes using Bray-Curtis dissimilarity matrix. Colours indicate the land use gradient. Environmental vectors (soil moisture content, SMC; nitrate plus ammonium, InorgN; mehlich-P, P; water extractable organic C, WEOC; water extractable organic N, WEON; microbial biomass, MBC and MBN) that significantly align with extracellular enzyme activity matrix are shown ($p < 0.05$).

2.4.4 *Drivers of extracellular enzyme activity*

Potential EEA were significantly correlated to SMC, MBC, MBN and SOC, but most strongly correlated with SMC and SOC (Fig. S2.4). To examine the potential that MBC, SMC, and SOC are driving the observed higher EEA with decreasing disturbance, we relativized EEAs to MBC, SMC, and SOC (Fig. 2.5, S2.4). Potential EEA per unit biomass (MBC) exhibited the opposite

pattern to absolute enzyme activities, increasing as land use intensity increased, especially for BG, NAG, and peptidases (Fig. 2.5b, S2.4). Potential EEA per unit SMC and SOC only demonstrated sensitivity to land use for BG and peptidase. These results suggest that SMC and SOC, but not MBC, may be relatively strong distinguishing factors controlling EEAs within this land use gradient (Fig. 2.5c, 2.5d).

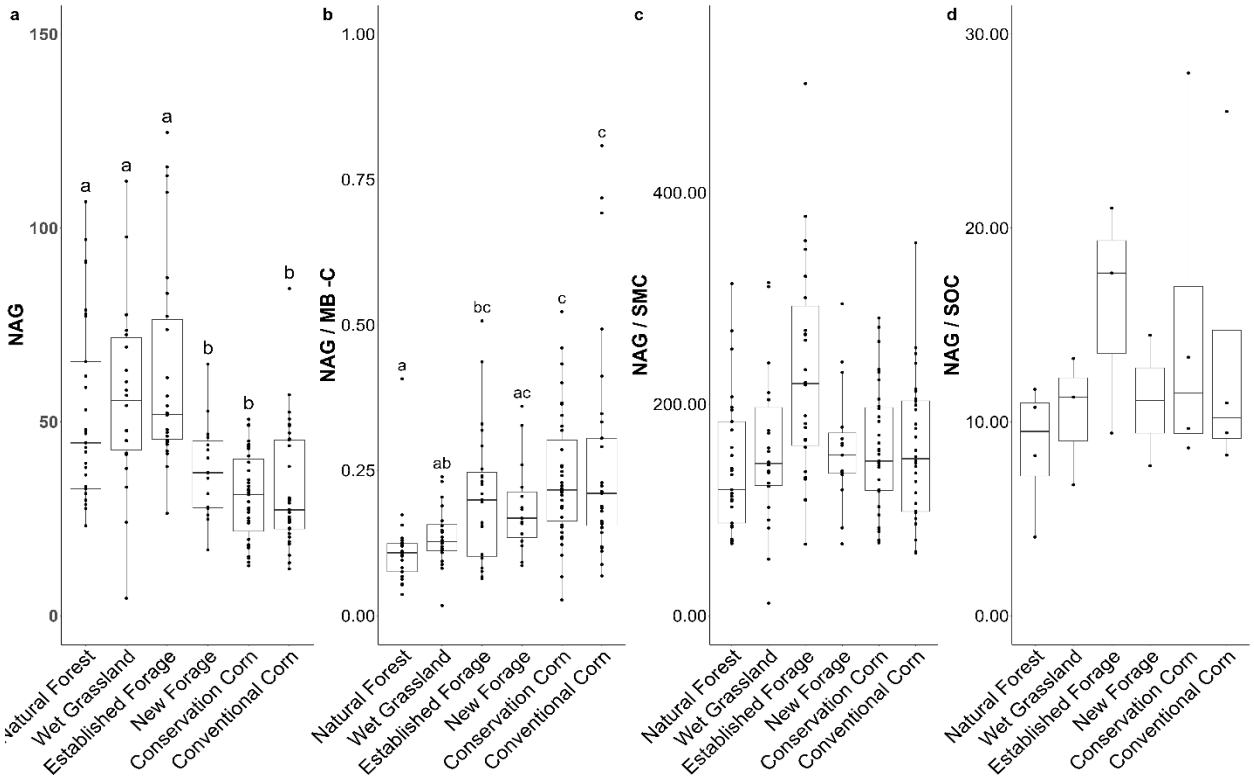

Figure 2.5. Example of how relativization of extracellular enzyme activity changes the relationship between activity and land use. N-acetyl-1,4-glucosaminidase (NAG) activity nmol $h^{-1}g^{-1}$ relativized to dry soil (a), relativized to microbial biomass (MBC) (b), relativized to soil moisture (SMC) (c), and relativized to soil organic carbon (SOC) (d). See Fig. S2.4 for data for all other measured enzymes.

2.4.5 *Enzyme activities across spatial scales and time*

We observed that the EEA pattern associated with the land use gradient was consistent across the floodplain region and time despite differences in site conditions (Fig. 2,4, 2.6). To determine how much spatial scale and time influenced enzyme variability across the land use gradient, we conducted a dispersion analysis. The most variability in EEA across land uses was accounted for by lake location (regional scale). Dispersion analysis indicated that lake location had the highest variation represented in the NMDS (Fig. 2.4) analysis ($F = 26.1$ compared to $F = 3.33$ for land use). MRPP analysis indicated that lake location had the largest within group variability ($A = 0.12$) compared to land use and distance to flood ($A = 0.069$ and 0.013 respectively). Dispersion analysis further indicated that this significance is driven by Baie which had the highest EEA variability (average distance to centroid = 0.25). These results show that the effect of regional scale properties, possibly driven by soil texture (Table 2.3) are an important consideration when using field-level replicates. Relationships with the land use gradient were enzyme specific at each lake location. For example, most enzymes (BG, NAG, PHOS, peptidase and PER) were influenced by land use at Bart compared to Dupa where only PHE varied across the land uses (Table S2.2). Thus, although the relationship between overall EEA and land use is robust across the regional scale, different enzymes are responsible for these land use effects depending on the location (Table S2.2).

Time was significant for each measured variable (Table 2) within each linear model, thus across spatial and land use variability there are significant temporal trends. Several variables were sensitive to the interactions between time and land use including: SMC, P, WEON, BG, PHOS, NAG, peptidase, MBN and MBC:N. Despite this variation in time and association with the land use gradient, when each time point was analyzed individually, land use was only significant for

most enzymes in May and November (Fig. 2.6). Enzymes did not vary significantly by land use in July, with the exception of PER activity which was driven by higher activity in the forest compared to the new forage and agriculture land uses (Table S2.3).

Linear model results did not indicate that measured EEA or soluble nutrient and C pools were sensitive to distance to flood (within-field spatial variation). However, MRPP results indicated that EEA variation across land uses is explained by within-field distance to flood (delta significance = 0.037) than time (delta significance = 0.15) (Fig. 2.4). Linear model results within each time point demonstrated that distance to flood influenced the most variation in May, associated with higher PHE activity closer to flood. This high variability closest to the flood was heavily influenced by regional lake locations (Fig. 2.6d). Indeed, when we compared EEA within locations (Fig. 2.6d-f) close to the flood zone, lake location but not land use, explained the most variability in EEA, especially within the BAIE sites. This indicates that the effect of land use on EEA is less robust at sites that experience the most intense flood duration closest to the lake. NAG was the only enzyme that maintained a significant effect of land use at the locations close to the flood zone across lake location and time. Both middle and far distances from flood maintained a significant relationship between land use and EEA but lake location was also significant (MRPP delta significance <0.01 for both). This effect of land use gradient at middle locations was mostly driven by PHE, whereas peptidase and PHE were significant at far locations.

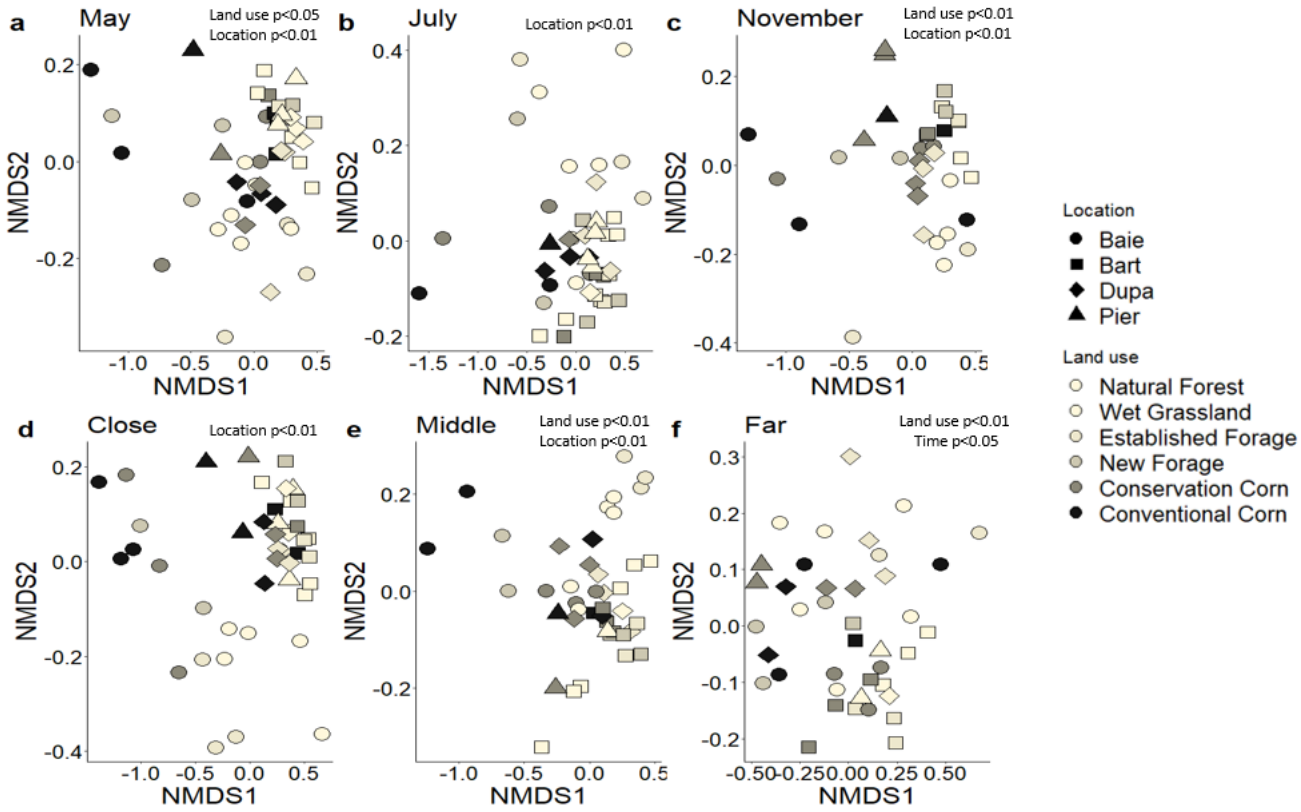

Figure 2.6. NMDS of seven extracellular enzyme activities (stress <0.2 for all) at each time point (a-c) and each distance to flood (d-f) with the legend for each land use and lake location on the right, MRPP results are indicated within each panel to indicate the significance of the land use gradient and the three scales distance to flood, time and regional location. Each time point (a-c) includes lake location, distance to flood and land use, each distance to flood point (d-f) includes time, lake location, and land use.

2.5 Discussion

Understanding controls on soil functional potential in floodplain ecosystems helps identify optimal land use practices in locations with intensifying flood dynamics. Our objective was to assess some of the abiotic relationships on soil extracellular enzyme activity (EEA) and

determine how sensitive the relationship between EEA and land use is across spatial scales and time. Soil EEA, the workhorse of nutrient cycling, is important for ecosystem functioning and yet is understudied in seasonally flooded ecosystems with mineral soils. Since soil EEA is a crucial step in organic matter decomposition (Burns *et al.*, 2013), understanding variations in activity can give insight into the production of soluble nutrients and C, which are simultaneously susceptible to ecosystem losses and important for plant nutrition and maintenance of soil communities. Further, while we would expect EEAs to decrease with increasing land use disturbance based on previous work and typical land use properties (Wallenius *et al.*, 2011, Yongxing *et al.*, 2019), floodplains are unique in that they are considerably fragile ecosystems and hot spots for biogeochemical activity that change rapidly in time and space (McClain *et al.*, 2003). Thus, exploring patterns of EEA across spatial scales and time can reveal whether this expected relationship between increasing land use intensity and EEA is maintained despite floodplain heterogeneity and the overlying disturbance of flooding. To this end, we compared EEAs across a land use gradient and found that EEAs appeared to be strongly controlled by moisture and total soil organic carbon (Objective 1), and despite high spatial and temporal variability, enzyme activities follow a consistent land use gradient trend (Objective 2).

2.5.1 *Effect of land use on enzyme activities*

We found that with increasing land use intensity, there was a decrease in EEA, soluble carbon, SOC, microbial biomass and soil moisture content (Fig. 2.3, S2.1, S2.2). This trend in EEA with land use was expected, as the higher SOC, soil moisture and larger microbial populations typically associated with the natural ecosystems would facilitate greater enzyme production (Yongxing *et al*. 2019, Karaca *et al.* 2010 and references therein). The lower C concentrations and microbial biomass under more intensive land uses that we observed suggest that there would

not only be fewer C substrates for enzymes to act on, but that there would also be reduced population sizes producing these enzymes.

We observed few differences in EEA, soil C, and nutrient concentrations between the conventional and conservation agricultural management (Fig. 2.3, S2.1). This finding was unexpected given that EEAs are thought to be early indicators of biological changes in response to different agricultural management (Bandick and Dick, 1999, Nannipieri *et al.*, 2002, Karaca *et al.*, 2010, Burns *et al.*, 2013, Borase *et al.*, 2020). Similar to our findings, Trasar-Cepeda *et al.* (2008) found that enzyme activities are not consistently affected by agricultural management since their response to different land uses depends on the enzyme measured and the land use type. As our conservation agricultural fields were only under this management system for three years, these management changes may not yet be influencing the key drivers supportive of higher enzyme activities, such as SOC. We found that SOC was a strong control on enzyme activity but was similar between the conventional and conservation agricultural fields (Fig. S2.2b) and it is well known that SOC can take several years to change under conservation management (Kallenbach and Grandy, 2014).

Notably, we found that the perennial agriculture practices showed both EEA and nutrient concentrations falling between those observed under annual agricultural practices and natural sites. We also observed that the age of the forage site impacts EEA, WEOC, MBC and MBN content, where the newly established forage sites (< 5 years) were more similar to the corn fields while the older established perennial forage sites (> 5 years) were more similar to the natural sites (Fig. 2.3, S2.1). This suggests that managed perennial plant assemblages behave more similarly to natural sites with increasing time of establishment. Perennial agriculture systems often consist of forage crops grown for livestock feed. In our study these sites are managed by

mowing one to two times per summer but not tilled, thus the plants, and importantly the root systems remain in place (Campeau *et al.*, 2024 in review). Perennial managed systems typically have higher organic C concentrations and microbial community activity compared to annual agriculture, in part because of their greater rooting depth and biomass which is more representative of natural ecosystems, especially grasslands (Crews and Rumsey, 2017). Perenniality in agriculture has become one of the prevailing pillars for achieving more sustainable agricultural systems partly based on the principle that they better mimic natural systems and are thus more supportive of soil ecosystem services (Rasche *et al*., 2017, Crews *et al*., 2018). Yet, surprisingly little research exists that directly compares perennial managed systems to both annual agriculture *and* natural systems to validate these claims. Thus, to our knowledge, our findings are some of the first to demonstrate the functional similarities between managed perennial and natural ecosystems relative to annual production systems.

2.5.2 *Soil carbon and moisture drive differences in enzyme activity*

We expected higher enzyme activities with decreasing land use disturbance and found that this was true in our floodplain system (Fig. 2.3). Further, we wanted to know which factors that correlated with land use were the biggest drivers of enzyme activity. In our study, we found that the strongest drivers of enzyme activity were soil moisture content and SOC (Fig. S2.3, S2.4). Higher soil moisture may increase enzyme activity by allowing for greater connectivity between the enzyme and the substrate within the soil matrix (Bailey *et al*., 2017, Patel *et al.*, 2021, Lieberman *et al.*, 2023). If moisture does limit enzyme activities, we would expect to see the effect of land use eliminated when we relativize enzyme activity to soil moisture. We found this to be mostly true– when enzymes were relativized to soil moisture there was only a significant effect of land use for BG. BG activity per unit soil moisture was higher in disturbed, drier land

uses. This could possibly be due to the presence of extant enzymes sorbed onto mineral surfaces (Nannipieri *et al.*, 2018). Sorbed enzymes can stay catalytically active for longer periods in the soil matrix, such that in a drier soil, higher activity per unit moisture could be due to the accumulation of enzymes and other microbial biomass products (Schimel, 2018). The influence of moisture on extracellular enzyme activities needs to be interpreted cautiously since the measurement assay creates a soil slurry and thus removes moisture limitation during the assay. However, taking assay conditions into account, Steinweg *et al.* (2012) determined that the influence of soil moisture conditions was observable in enzyme activities in soils with moisture limitation before the assay.

Enzyme activities often follow increases in SOC, where higher SOC may serve as an approximation of available energy and nutrients from organic matter that support high EEAs (Qin *et al.*, 2010, Kallenbach *et al.*, 2015). With relatively higher SOC in our natural sites (Fig. S2.2) we suspected this would drive the land use gradient trend of EEA, as a function of substrate abundance. At the same time, EEAs can also decrease with higher substrate availability where the investment in their production may be less essential (Weintraub *et al.*, 2013). This decreased investment is commonly observed with phosphatase (Saiya-Cork *et al.*, 2002, Bissett *et al.*, 2011). Phosphatase activity measured in our study followed this trend, where soil phosphorous concentrations increased, phosphatase activity decreased (Fig. 2.3, S2.1). In our study, EEAs relativized by SOC demonstrated variable responses by enzyme across the land use gradient. Other studies similarly found this variable response − Trasar-Cepeda *et al.* (2008) found that enzyme activity relativized by SOC increased with decreasing land use SOC content while Sinsabaugh *et al*. (2008) found that activities relativized to soil organic matter did not vary across ecosystems, despite a soil organic matter gradient. It is possible that higher activity per

unit SOC depends on SOC quality, not just amount (Lehmann *et al.*, 2020). Agricultural systems generally have faster nutrient cycling, where tillage and fertilization foster a soil community with faster metabolisms that cycle quickly and metabolize diverse SOC substrates (Bissett *et al.*, 2011, Rasche *et al.*, 2017). Natural systems experience less disturbance with no direct fertilization inputs, fostering slower metabolisms which cycle more slowly (Rasche *et al.*, 2017). In our study, both peptidase and BG activity per unit SOC increased with increasing land use intensity. Thus, the cycling of cellulose and peptides are potentially more efficient per unit available C in these agricultural systems, suggesting possible adaptation to faster cycling systems. The drivers of EEAs across land use gradients likely vary depending on the conditions of each system, however, our data suggest a strong relationship of both moisture and SOC, with other factors such as nutrients and microbial biomass being less related to enzyme activity.

2.5.3 *Enzyme activity spatial and temporal variability*

The element of scale is critical when considering how soils respond to disturbance, as responses likely depend on how well the perturbation scale matches the scale of the evaluated response (Solomon *et al.*, 2012, Hall *et al*., 2018, Wanzek *et al.*, 2018, Dove *et al.*, 2021). For instance, there may be sampling location-level variation that overrides even climate factors such as precipitation and temperature (Dove *et al.*, 2021). Thus, while we might expect that EEA increases with decreasing land use intensity, other disturbances− such as those associated with flooding− may weaken this land-use effect. We examined the strength of the EEA and land use gradient relationship across two spatial scales (regional lakeshore locations and distance to flood within each land use) and across time. We found that variability in enzyme activities was greatest between the regions but that the land use gradient was largely conserved across the spatial scale and seasons (Fig. 2.6).

The regional differences in soil properties around Lake Saint Pierre, especially soil texture, accounted for a large portion of EEA variation in our study. When we considered all enzymes across time and space (Fig. 2.4), lakeshore location accounted for the same amount of variation as land use ($p = 0.001$). This is a similar observation to Dove *et al.* (2021), who found sampling sites to be the strongest cause of variation, even though climate and depth were also significant factors. Thus, although the land use gradient is still robust across time and space, the regional lakeshore locations have a strong influence on EEA. Regionally, there exist differences in flood characteristics and soil type but we note that two of the largest differences are in soil texture and % SOC. Soil texture influences both water movement but also attachment of enzymes and SOC to the soil matrix (Datta *et al.*, 2017, Nannipieri *et al.*, 2018). Thus, these abiotic factors may explain the strong regional variation in observed EEAs. The Baie lakeshore region was the most distinct with most variable EEAs, likely associated with it also having the highest clay and SOC content compared to the other lake regions. Nonetheless, we also found that despite this regional variation, the relationship between EEA and the land use gradient was maintained.

Distance to flood allowed us to approximate the combined effect of flood duration and intensity, where soil sampled closer to the lake are submerged longer and more frequently (Campeau *et al.*, 2023 in prep). We found that the locations closest to Lake Saint Pierre, corresponding to the highest flood intensity, did not have a significant relationship between land use and EEA. This suggests that in the areas within the floodplain exposed to more severe flood disturbance, the influence of land use on EEA is reduced. Even though EEA did not vary by distance to flood individually, the EEA dynamics represented by NMDS analysis show that land use effects are overridden in areas of highest intensity flood disturbance (Fig. 2.6). Higher flood intensity, closer to the lake, may experience more fluctuations between saturation and dry, increasing potential

nutrient and C leaching losses that can decouple the interaction between enzymes and substrates. Argiroff *et al.* (2017) found that sites with higher intensity of flood had decreased soil organic matter and total C and N compared to sites with lower intensity flood duration. Our study had higher concentrations of water extractable C and ammonium closer to the lake but lower nitrate. Thus, we could be seeing the effect of changing substrate profiles at each distance to lake position influencing specific enzymes. Our results also demonstrated that agriculture treatments may be more sensitive to spatial variation compared to forest or grassland sites, although this was limited to only some enzymes (Fig. 2.6d-f).

When considering temporal variability, EEA was impacted by land use in May and November but not in July (Fig. 2.6). This relationship could be in part due to field conditions at the time of sampling inflating the differences between the sites. In May, the agriculture fields had not yet been seeded or fertilized and is a time when root inputs are low to non-existent. In the natural sites, continuous growth of trees and grasses contribute substrates through root inputs and litter throughout the season (Rasche *et al.*, 2017), with the highest inputs in the fall post leaf senescence. Most EEA increased in November in our study, suggesting that this higher input of litter drove activity. Similarly, Ali *et al.* (2015) found that *in situ* activities of BG and PHE varied seasonally, with the highest activity in the peak vegetative season, compared to fallow. The lack of a land use effect on EEA in July was perhaps due to the presence of crops and fertilization increasing nutrients and C within the agricultural fields and thus reducing the difference in activity between the natural and the agriculture sites.

2.6 Conclusions

This study demonstrates the strength of land use as a determinant for extracellular enzyme activity within a seasonal floodplain. Here we show a clear gradient of enzyme activity and nutrient concentrations from natural systems to perennial agriculture systems to conventional agriculture systems. Natural grasslands and forests were associated with higher enzyme activities compared to agricultural practices and this appeared to be a function of soil carbon and moisture. This consistent relationship between enzyme activity and land use suggests that when considering appropriate land use activities in areas predicted to experience increasing flood disturbance, that spatial and temporal variation is less important compared to the proposed land management. However, our data also suggests that in areas with highest flood intensity, land use characteristics supporting higher activity may be overridden by flood effects. Further research into how flooding directly affects nutrient and C concentrations and microbial activity in seasonal floodplains could help isolate the impact of the different interactions between land use and flood. We also show that when considering land management, perennial systems behave similarly to natural systems in enzyme activity, nutrients, and carbon storage, and could therefore be an appropriate compromise to converting conventional agricultural practices back to natural areas.

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Transition

In Chapter 2, I found that extracellular enzyme activities (EEA) were influenced by land use characteristics across multiple scales. Further, I found that moisture and soil organic carbon were significantly correlated with EEA. This indicates that resource and moisture availability affect microbial activity within this floodplain system. I was curious to explore the response of microbial community activity and diversity to flooding at a finer scale. Further, I wanted to determine how soil structural properties as well as land use influence microbial community response to flooding. Therefore, I conducted a laboratory incubation that allowed me to capture the specific response of microbial communities to flooding (Chapter 3) using two soil structure treatments (intact and sieved soil cores) and two land uses (conventional agriculture and natural grassland).

Chapter 3

Land use and soil structure influence soil microbial community composition and activity in response to flooding

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3.1 Abstract

Flooding is forecasted to increase in frequency and duration in temperate ecosystems, however soil microbial community response to flooding is not well understood. Microbial community activity and diversity, influenced by variability in soil conditions, are critical for ecosystem functioning and resiliency. Soil structure heterogeneity can affect microbial community composition and access to resources that might determine how well a community can recover from a flood event. The importance of soil structure in regulating microbial flood responses might be strengthened or weakened by land use characteristics such as soil resource abundances, starting communities, or disturbance history. We conducted a laboratory incubation to determine if soil heterogeneity supports greater microbial resiliency to experimental flooding, and how land use modulates this response to flooding. We sampled intact cores from two land uses, agriculture and grassland, from a floodplain around Lake Saint Pierre, Quebec Canada. We kept one set of intact soil cores as our heterogenous treatment and for our homogenous treatment, we sieved one

set of intact cores using a 4 mm sieve. Cores of both structures within each land use were subjected to flood treatment for three weeks and included unflooded controls. Our results demonstrated that soil heterogeneity increased microbial functional (CO_2 respiration and enzymes) recovery to flood in the agricultural soils, but there was no effect of soil structure on functional recovery in the grassland. The agriculture soils had higher species richness and diversity compared to the grassland ($p<0.05$), which may account for greater overall recovery post-flood. Bacterial and fungal community abundances recovered post-flood in both agriculture and grassland land uses ($p <0.05$), however, the extent of recovery was structure-dependent in the agricultural soil, with full recovery only occurring in the intact soil. Soil structure affected community composition in both land uses, where the grassland compositional shifts occurred throughout the flood event, demonstrating compositional plasticity in response to flood. Community composition shifted with flood but not with time in the agriculture soil and thus the post-flood functional recovery in the agriculture soil was likely was from the same community composition during the flood. Thus, we found that with increased flooding microbial community recovery differed by land use and was partly determined by soil heterogeneity in the agriculture land use. Agricultural practices such as reduced tillage can increase soil heterogeneity, thereby promoting more resilient microbial communities to flood.

3.2 Introduction

Soil microbial communities perform essential ecosystem functions from primary decomposition to nutrient cycling. Thus, their activity and community composition shifts during and after environmental disturbances likely have strong effects on ecosystem recovery (Allison and Martiny, 2008). Flooding is a major disturbance to the soil microbial community habitat that impacts the functioning and composition of microbial communities (Unger *et al.*, 2009, Peralta *et*

al., 2014). For instance, flooding decreases soil oxygen availability, slowing or shifting microbial metabolism, but it also increases microbial access to soluble soil nutrients and carbon (Bailey *et al.*, 2017, Boye *et al.*, 2018). These changes in nutrients and oxygen with flooding, and the corresponding microbial responses, are intimately linked to soil structure which influences diffusion of solutes and gas, water movement, and the connectivity of microbial communities with resources and oxygen (Rillig *et al.*, 2017, Wanzek *et al.*, 2018). Soil structure also regulates microbial community assembly, dispersal, and speciation such that a more heterogenous soil environment has been linked to increasing species diversity (Nunan *et al.*, 2020). Thus, how soil structural heterogeneity contributes to microbial community compositional and functional response to flooding is unclear. While we might expect microbial communities to be sensitive to short-term flood events, differences in soil structure may regulate the degree and direction of microbial responses and their ability to recover post-flood. With increased flooding predicted for mineral soil ecosystems in eastern North America, it will be important to understand how soil structure mediates soil microbial diversity and activity responses to short-term flood events.

In the context of disturbances, soil microbial communities may exhibit taxonomic or functional resiliency (recovering after the disturbance), may be relatively insensitive to the disturbances (e.g. resistant), or may not recover (Schimel *et al.*, 2007, Allison and Martiny, 2008, Biggs *et al.*, 2020). The ability of a microbial community to resist or recover from a disturbance has often been linked to several mechanisms including microbial community taxonomic diversity, a community's trait profile, or the taxonomic connectivity of the community (Wallenstein and Hall, 2011, Philippot et al., 2021, de Vries *et al.*, 2018). For instance, higher microbial community richness has been shown to be associated with increased ecosystem function (Wagg *et al.*, 2014, Wagg *et al.*, 2019) demonstrating the 'portfolio effect' (Orwin *et al.*, 2016, Delgado-

Baquerizo *et al.*, 2017). The portfolio of potential functions increases with increasing diversity, which may support compensatory dynamics (Gonzalez and Loreau, 2009) allowing for maintained function in the face of disturbance. Communities with certain traits such as those related to dormancy or rapid growth have also demonstrated higher potential for disturbance recovery (de Vries and Shade, 2013, Sorensen and Shade, 2020). Microbial community intra-kingdom co-occurrences have been used to compare potential microbial community stability, where, for example, the strength of connections among taxa was linked to higher fungal stability to drought (de Vries et al. 2018). Microbial compositional shifts have been observed in response to flooding (Unger *et al.*, 2009), but whether these compositional shifts are related to maintained function during the flood or recovery post-flood is not clear. Whether a microbial community exhibits one or some of the above mechanisms that could contribute to relative stability during flooding or recovery, will likely be ecosystem-specific, depending on factors including niche space and access to nutrients and C.

Soil niche space for microorganisms largely occurs in soil pore spaces that vary in their size and connectivity, which dictates microbial environmental conditions, access to nutrients and carbon, and biotic interactions (Bailey *et al.*, 2012, Keiluweit *et al.*, 2017, Erktan *et al.*, 2020). Soil pore structure, influenced by the arrangement of macro- and micro-aggregates, is highly variable within a soil and across ecosystems. Mechanical disturbances associated with agriculture such as tillage have a pronounced effect on soil structure and pore space, breaking apart macro-aggregates, homogenizing the aggregate structure to be dominated by microaggregates (Six *et al.*, 2000). We expect that this homogenizing effect of tillage, or other sources of physical mixing (West and Whitman, 2022), impacts the microbial community's ability to recover or resist flooding in multiple, perhaps conflicting, ways. First, a less spatially structured environment

(*i.e.*, more homogenous) could reduce taxonomic diversity and thus functional redundancy and community resilience or recovery to flooding (Philippot *et al.*, 2021). For instance, studies have determined differing diversity and functional potential within different aggregate size classes (Bailey *et al.*, 2012, Hartmann and Six, 2023), suggesting more niche space with a diversity of aggregates. The abundances of anaerobic sites fostering bacterial and archaeal communities adapted to sub-oxic conditions may also be higher in spatially structured heterogenous soils (Keiluweit *et al.*, 2017). Indeed, the high spatial heterogeneity of soil and abundance of microenvironments is the primary hypothesis explaining soil's high level of biodiversity (Nunan et al., 2020). Second, greater soil heterogeneity has been shown to increase microbial community network stability (Wang et al., 2023). Due to patchiness of resources, and micro-environments that are supported through increased soil heterogeneity (Bailey *et al.*, 2017, Portell *et al.*, 2018), microbial network complexity has been found to increase, which can be further correlated with network stability (Wang *et al.*, 2023). Agricultural tillage practices were found to decrease stability between fungal taxonomic abundances, however, did not affect bacterial communities (Wagg *et al.*, 2018), thus stability of microbial networks may be kingdom dependent. Models of microbial communities suggest that despite high microbial diversity within aggregates, microorganisms only interact with a few other species within a given location (Raynaud and Nunan, 2014), and further, optimized synergistic interactions between individuals, required a minimum separation distance, supported through complex soil structures (Kim *et al.*, 2008). Thus, heterogeneity of soil structure can stabilize microbial community networks through higher network complexity and mediation of organism interactions.

On the other hand, more homogenized soils may have higher connectivity between pores and fewer, isolated microsites, especially when water is present. This can increase opportunities for

microorganisms to encounter substrates (Bailey *et al.*, 2017) and thus help communities to maintain their function under suboptimal conditions. This is seen in rewetting experiments as a flush of CO_2 is released, indicating increased access to nutrients and carbon (Schimel, 2018, Barnard *et al.*, 2020). Soil saturation with flooding is expected to alter microbial access to soil C and nutrients (Boye *et al.*, 2018, Anthony and Silver, 2020). However, the ability of the microbial community to benefit from resource enrichment will likely be ecosystem dependent where variation might exist in soil structure or the degree to which a community is initially nutrient- or C-limited.

Land use can influence soil C and nutrient quality and quantity and alter soil structure thus impacting microbial activity and habitat dynamics, with unclear implications for flood response (Six *et al.*, 2000, Six *et al.*, 2004, Fuhrmann, 2021). Soil organic matter (SOM) concentration and composition are influenced by plant characteristics such as richness, diversity, and perenniality (Fanin and Bertrand, 2016, Hirsch *et al*, 2017, Crews and Rumsey, 2017). Natural ecosystems, dominated by perennial plant assemblages, typically have higher concentrations of SOM (Beniston *et al.*, 2014) that might help buffer microbial communities against disturbances like flooding. In addition to higher microbial resource availability and associated greater microbial biomass, natural ecosystems may be more likely to exhibit a portfolio effect and thus recovery to flood since they likely have more heterogenous soil habitats compared to agricultural ecosystems which homogenize soil structure with tillage practices (Six *et al.*, 2000). Alternatively, under conventional agriculture systems, microbial communities are more likely adapted to fluctuating soil structure and nutrient and C availability such that their recovery or resiliency to flood may be relatively high. It is unclear whether ecosystems that regularly experience disturbances and fluctuating resource concentrations respond better (e.g., greater

recovery) to a flood disturbance compared to ecosystems, with little disturbance and higher resource availability.

Microbial community responses to disturbances are interactive and complex, and resiliency to flooding as a disturbance is not well characterized. Microbial community resiliency may depend on factors influencing their diversity and habitat, such as land use and soil structure heterogeneity. To our knowledge, factors related to land use and soil structure have not been studied in combination with microbial community response to flooding. To address this, we used a laboratory incubation to examine how soil structure impacts soil microbial community response to flooding and how the mediating effect of soil structure vary under different land uses. Our objectives were to: 1) determine if soil heterogeneity supports greater microbial functional or compositional resiliency or recovery to experimental flooding, and 2) determine if responses to flooding within agriculture and grassland differ.

3.3 Methods

3.3.1 *Study design*

To compare the microbial response to flooding of different land uses we sampled soils from both a conventional agriculture site in a corn-soybean rotation (maintained by tillage) and a natural wet grassland established for more than five years (unmaintained). Both sites are located near Saint Barthelemy, Quebec, Canada (46°11'27.2"N 73°07'11.0"W) and are a part of the Saint Pierre Lake floodplain. Both land uses experience similar spring flood duration and have a similar silt-clay loam soil texture (Table 3.1). The study is a three-factor fully crossed design where each factor has two levels as follows: 1) land use, which includes agriculture or wet

grassland; 2) soil structure, which compares sieved or intact soils; and 3) flooding, which compares flooded and field moist soils.

Table 3.1. Soil data for two land uses used in lab incubation that include elevation of sampling location, soil texture, pH, and moisture (%) determined using TDR Hydrosense (III).

Location	Elevation m	Texture			pH	Moisture %
		Sand %	Silt %	Clay %		
Agriculture	5.7	35.1	30.6	34.3	5.75	40
Grassland	5.8	28.1	28.2	43.8	5.67	46

3.3.2 *Soil core collection and pre-incubation processing*

Soils were sampled in late October 2022 using an intact soil corer with a 15 by 4 cm plastic sleeve (AMS Soil core sampler, 5/8" thread, 6" length) (AMS, USA) to a depth of 15 cm. Prior to sampling, plastic sleeves were sprayed with 100% ethanol and air-dried to ensure no cross-contamination between cores. We collected six replicates per sampling point per treatment for a total of 180 soil cores, 90 from each land use. Soil cores were taken approximately 30 cm apart to minimize compaction and differences in soil properties.

Samples were immediately put in coolers on ice and transported to the lab where they were kept at 4 °C until further processing (maximum one week). Due to inherent differences in surface plant material between the agriculture and grassland sites, all cores were standardized by removing the top one to two cm of soil and debris. Pre-treatment soil core gravimetric water content (GWC) was 29% and 74% for the agriculture and grassland site, respectively. We raised the agriculture GWC up to 55% by adding deionized water to the top of the core to remove a possible influence of different starting moisture contents.

3.3.3 *Incubation treatments*

To determine how soil heterogeneity impacts microbial response to flooding, we homogenized half of the cores from each land use (n=45 for both agriculture and grassland) by sieving to 4 mm. Sieved soils were then repacked into their original plastic sleeves. Soils were not compacted but the cores were tapped to settle the soil particles. If roots were encountered, they were also repacked to mimic the biological conditions of the intact cores as much as possible.

After sieving, all cores (sieved and un-sieved) were pre-incubated for one week to allow the soil microbial community to stabilize after the disturbance of sampling and sieving and to utilize any newly released bioavailable soil C. During the pre-incubation, all cores were capped on the bottom and covered with parafilm on the top to allow for gas exchange and kept in the incubator at 14 °C, the average spring temperature of the region (Government of Canada, Canadian Climate Normals;1981 – 2010) to represent the conditions of spring flood.

After the one-week pre-incubation, half of the soil cores were flooded (Fig. 3.1). Prior to flooding, fine mesh was attached to the bottom of all cores to allow for water exchange with minimal soil loss. Two septa were also inserted and sealed using silicone sealant at two depths in each core (3.5 and 7 cm from the core bottom). These septa allowed us to insert a microsensor (Unisense, Denmark) to measure redox conditions throughout the flood event (Fig. S3.1). To flood the soil, half of the cores were uncapped and placed in deionized water for 24 hours to absorb water by capillary action through the bottom mesh. All cores were then capped on the bottom and sealed with silicone sealant. Additional water was added by syringe injection and directly to the core surface. This was done until the cores reached their pre-determined 100% water-holding capacity. However, the agriculture intact cores only reached approximately 80% WHC, potentially due to their high bulk density. Flooding was maintained with a ~1-cm layer of

water above the soil surface of each flooded core. Cores were weighed every three to four days to maintain water weight and flooding conditions, non-flooded cores were maintained at field moist conditions. A summary of the treatments and sampling times are shown in Figure 3.1.

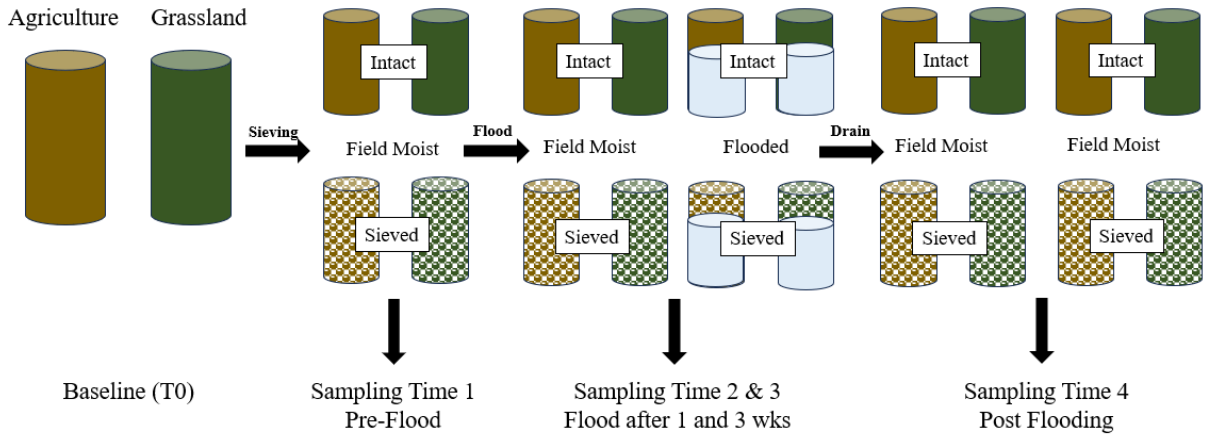

Figure 3.1. Treatment and sampling design for the incubation. Six replicates for each treatment per time point were sampled, for a total of n = 12 (T0), n= 24 (T1), and n= 48 (T2, T3 and T4).

3.3.4 *Incubation sample design*

Soil cores were destructively sampled at six time points with six replicates for each treatment at each time point (Fig. 3.1). Cores were destructively harvested directly after field sampling (T0) to obtain baseline differences between the two land uses (n = 12). Cores were harvested after the pre-incubation (T1) to determine pre-flood conditions in both structural treatments (n= 24). Three destructive sampling events occurred during the flood, after one and three weeks (T2, T3, n= 48 for each). The fourth and final sampling occurred after the cores had reached field moist conditions post-flood (T4, n= 48).

3.3.5 *Soil analyses*

3.3.5.1 Extracellular enzymatic potential

We determined potential enzyme activity for four hydrolytic enzymes associated with C and N cycling following previously describes methods (Saiya-Cork *et al.*, 2002). We determined activity for betaglucosidase (BG), N-1,4-acetylglucosaminidase (NAG), L-tyrosine peptidase (TAP), and L-leucine peptidase (LAP) (Saiya -Cork *et al.*, 2002). Soil slurries were made with 50mM sodium acetate buffer, pH of 5.7, reflecting the average soil pH. We quantified potential hydrolytic enzyme activity fluorometrically using black 96-well microplates and compound-specific fluorescing substrates bound to 4-methylumbelliferone (MUB) or 7-amino-4-methyl coumarin (MC). Hydrolytic enzyme activity is reported as nanomole of product produced per hour per gram of dry soil (nmol $h^{-1}g^{-1}$).

3.3.5.2 Soil carbon and nutrient pools

From each time point, we analyzed water-extractable organic C (WEOC) and N (WEON), total soil C and N, nitrate (NO_3^-), and ammonium (NH_4^+). To determine WEOC and WEON, 40 mL of deionized water was added to 10 g (field moist) soil, shaken on an end-to-end shaker at 180 oscillations per minute for 20 m, centrifuged for 15 m at 8500 rpm, and then decanted, avoiding any visible particulate matter. Extracts were frozen at -20 °C until they were run on a TOC-N analyzer (Shimadzu Corp, Kyoto, Japan). Total soil C and N were determined on pulverized dry samples on a flash combustion ECS 4010 Elemental Analyzer (Costech, Valencia, CA, USA).

Inorganic nitrogen (NO_3^- and NH_4^+) were determined from the same 0.5 M K_2SO_4 extracts used for unfumigated microbial biomass C and N. Soil NO_3^- and NH_4^+ were spectrophotometrically determined (Doane and Horwáth, 2003, Hood-Nowotny *et al.*, 2010, Kandeler and Gerber, 1988)

at 540 nm for NO_3^- and 660 nm for NH_4^+ on a Biotek plate reader (BioTek Instruments, Winooski, VT, USA).

3.3.6. *DNA extraction and quantification*

We extracted DNA from 5 replicate soil subsamples for the following time points: pre-flood (T1), flood after 1 week (T2), flood after 3 weeks (T3) and post flood (T4). Subsamples were immediately frozen at -20 °C after destructive sampling and then prior to extraction were slowly thawed at 4 °C. DNA was extracted using MP Biomedicals FastDNA SPIN Kit soil (MP Biomedicals, Irvine, CA, USA). The following amendments were made to the standard protocol: 1) after adding the sodium phosphate and MT buffers, samples were incubated at room temperature for 5 m, 2) the centrifuge time for the first centrifugation step was 15 m, 3) DNA was eluted using 50 °C PCR water and before centrifuging, the samples were incubated at room temperature for 5 m. Nucleic acids were immediately frozen at -20 °C until extracted DNA could be quantified. We quantified extracted DNA, following a slow thaw, using Qubit 3.0 Fluorometer (Qubit 1X DNA Broad range protocol, Thermo Fisher Scientific, Waltham, USA). Samples were then diluted to a working solution of 10 ng μl^{-1}, then submitted to Genome Quebec (Montreal, QC) for library generation and sequencing.

3.3.7 *Bacterial and fungal marker gene abundances*

The abundance of bacterial and fungal marker genes in soils were quantified with qPCR on purified DNA (diluted to 0.5 ng μL^{-1}). The bacterial community abundance (16S rRNA gene) was assessed using primers and conditions from Fierer et al. (2005): forward Eub338 (5′-ACTCCTACGGGAGGCAGCAG-3′) and reverse Eub518 (5′-ATTACCGCGGCTGCTGG-3). Fungal community abundance (28S rRNA gene) was assessed with primers and conditions detailed in White et al. (1990): cTW13 (5′-CGTCTTGAAACACGGACC-3′) and TW14 (5′-

GCTATCCTGAGGGAAACTTC-3′). Each 10 µL PCR reaction for 16S rRNA and 28S rRNA contained: 5 µL of PowerTrack^{TM} SYBR Green Master Mix for qPCR (Thermo Fisher Scientific, Waltham, USA), 0.25 µL of each forward and reverse primers (250 nM), 2 µL of template DNA (2 ng µL^{-}), and PCR-grade water. Thermocycling conditions are as follows for both 16S and 28S rRNA genes: denaturation occurred at 95 °C for 5 m, this was followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, elongation at 72 °C for 30 s, and then melting curve was produced from 60 °C - 95 °C which took 20 m, for a total duration of 2 h and 5 m.

3.3.8 *Microbial biomass carbon and nitrogen*

For each time point, we determined salt-extractable microbial biomass C and N using the chloroform fumigation method (Jenkinson *et al.*, 2004). We fumigated 10 g (field-moist) soil for 24 h with 2 mL of chloroform directly added to the soil. After 24 h, fumigated samples were left uncapped for 4 h to evaporate off the chloroform. We extracted fumigated samples along with another 10 g of unfumigated soil (10 g) with 40 mL of 0.5 M K_2SO_4. Samples were then shaken for 4 h at 180 rpm and centrifuged at 8500 rpm for 20 m and supernatant was filtered with Whatman 5 filter (2.5 µm). Extracts were frozen until analyzed on Shimadzu a TOC-N analyzer (Shimadzu Corp, Kyoto, Japan). Microbial biomass C and N was calculated as the difference in total C or N between fumigated and unfumigated samples, and without an extraction coefficient to avoid potential differences in extraction efficiencies between the land uses.

3.3.9 *Microbial community library preparation and sequencing*

Bacterial and fungal communities were characterized by amplifying and sequencing the bacterial V4-V5 region of the 16S ribosomal RNA gene with the primer pair 515F-Y (5′-GTGYCAGCMGCCGCGGTAA−3′)/926R (5′-CCGYCAATTYMTTTRAGTTT−3′) (Parada et

al., 2016) and the fungal ITS2 region with the primer pair ITS9 (5′-GAACGCAGCRAAIIGYGA−3′) (Menkis et al., 2012)/ITS4 (5′-TCCTCCGCTTATTGATATGC−3′) (White *et al.*, 1990). NextSeq sequencing used staggered tagged primers for both forward and reverse primers (Table S3.1). Targeted PCR occurred and was performed in a 25 µl reaction mix composed of 19.35 µl of UltraPure™ DNase/RNase-Free distilled water (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), 0.2 mM of dNTP mix (10 mM_NEB), 1X buffer with 18 mM of $MgCl_2$ (Roche, Basel, Switzerland), 5% DMSO (Roche, Basel, Switzerland), 0.6 µM of each primer, 0.02 U/µl of Roche FastStart High Fi 5U/µl (Roche, Basel, Switzerland), and 1 µl of DNA extract. For 16S rRNA (bacteria) community, thermocycling conditions were as follows: initial denaturation step at 94 °C for 2 m, 26 cycles at 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s, and a final elongation step at 72 °C for 7 m. For ITS region (fungi), thermocycling conditions were as follows: initial denaturation step at 96 °C for 15 m, 33 cycles at 96 °C for 30 s, 52 °C for 30 s, and 72 °C for 60 s, and a final elongation step at 72 °C for 10 m. Indexation of PCR products occurred by adding Dual-indexes (Integrated DNA Technologies) to each sample and Illumina adapters required for DNA to bind to flowcell. Verification of barcode incorporation for each sample was conducted using 2% agarose gel. Quantification of each amplicon was conducted using Quant-iT^{TM} PicoGreen$^{®}$ dsRNA Assay kit (Life Technologies) and were pooled using equimolar (ng) concentrations. Library was cleaned using sparQ PureMag Beads (Quantabio, Beverly, MA, USA). Library was then quantified using Kapa Illumina GA with revised primers-SYBR Fast Universal kit (Kapa Biosystems, Millipore Sigma, Burlington, MA, USA). Average fragment size was determined using LapChip GX (Perkin Elmer, Shelton, CT, USA) instrument. Sequencing was performed on an Illumina NextSeq platform at Genome Quebec (Montreal, Quebec, Canada).

3.3.10 *Bioinformatic processing*

Sequencing resulted initially 8,817,121 and 8,253,132 high quality reads for the 16S rRNA gene ITS2 region, respectively. We further trimmed and filtered the sequences using nf-core (Ewels *et al.*, 2020) and DADA2 pipelines (Callahan *et al.*, 2016). Due to the binning of quality scores in Illumina NextSeq, the error learning step (ErrF/R) in DADA2 pipeline was adjusted to maximize the identification of individual ASV's (code adjustment in supplemental material, Table S3.2). The Silva (v.138.1, Quast *et al.*, 2013) database was used for the 16S rRNA taxonomic identification and the UNITE (v. 8.3, Kõljalg *et al.*, 2020, Abarenkov *et al.*, 2023) database was used for the ITS taxonomic identification. Bioinformatic processing resulted in 6,819,994 and 6,451,813 high-quality sequences for the 16S rRNA gene ITS2 region, respectively. Sequences were grouped into ASV's for a final number of 33,521 and 3,829 16S rRNA gene and ITS2 region ASV's respectively.

3.3.11 *Computerized tomography imaging*

One soil core from each land use was preserved for computerized tomography (CT) imaging to verify pore and aggregate structural differences between the sieved and intact treatments. An unflooded intact and sieved core were subsampled using a sheet metal 2 cm by 3 cm mould and imaging was conducted on the subsamples on either the same or next day. Soil core imaging was conducted using Skyscan1174 micro-CT scanner (SkyScan N.V., Bruker AXS, Kontich, Belgium). We used the following specifications to obtain a 9 mm by 9 mm image of the inner core of each subsample to minimize edge effects. A 0.5 mm filter was added to ensure that only high energy x-rays were entering the sample due to the density of the material. The interior of the cores were scanned with an image pixel resolution of 10024 x 8.9, exposure of 3200 s, at an angle increment of 0.5° with a frame average of 2 (each CT number was computed from 2

'replicates'). Source voltage was 50 kV and source current was 800 mA. To account for the potential that connected air-filled voxels ('pores') could be considered functionally one pseudo-pore, we analyzed the center of connected pseudo-pores. CT images were then processed as described below to compare differences in pore connectivity, degree of heterogeneity, and aggregation among land uses and structural treatments.

3.3.12 *Statistical analyses*

Data was processed in R (R core team, 4.1.2, 2021). Analysis of variance analysis (ANOVA) was used to compare interactive treatment effects of WEOC, WEON, MBC, MBN, NO_3^-, NH_4^+, CO_2, diversity metrics, and qPCR. After visual inspections of normality CO_2, NO_3^-, NH_4^+ data were natural log transformed to fit the assumption of a normal distribution. Non-parametric Kruskal-Wallis test was used for WEON only when comparing variances across land uses. Community abundances from qPCR are presented and analyzed after logarithmic transformation.

The Phyloseq package (McMurdie and Holmes, 2013) was used to process 16S rRNA and ITS sequence data. Rarefaction curves were produced (Fig. S3.2), confirming that sequencing was successful within each sample. Sequences for both 16S rRNA gene and ITS2 region were rarefied to even sampling depth before performing any statistical analysis. After rarefying to even sampling effort for the 16S rRNA gene sequence data, 2,812 ASVs were removed as they were no longer present in samples after random subsampling. After rarefying to an even sampling effort for the ITS data, 93 ASVs were removed as they were no longer present in samples after random subsampling. All 16S rRNA gene and ITS analyses were conducted with this rarefied data. 16S rRNA gene sequencing captures both bacteria and archaea. Only 0.68% of 16S rRNA gene sequence data was identified within the kingdom Archaea, due to their low prevalence we refer to 16S rRNA gene sequence results being 'bacterial'. Archaea were not

filtered out based on their functional abilities in low oxygen conditions potentially associated with the flood treatment.

To determine differential abundances within each treatment, a Kruskal-Wallis test was used on relative abundances of each ASV. Network analyses were conducted using the Spiec-easi R package (Kurtz *et al.*, 2015), inference was determined using 'mb' neighbourhood selection method and a lambda ratio of 0.001. Graphical model established using the Stability Approach to Regularized Selection (StARS). For the 16S rRNA data ASV's with a minimum of 10 counts and were present in >50% of samples were kept for a total of 232 ASVs. For the ITS data, due to low number of ASV's (approximately 30) present in>50% of samples, a minimum of 10 counts in >10% of samples were kept for a total of 244 ASV's. To analyze connections within each network we used the degree of each taxa which indicates how connected a particular individual is in the network. This degree should be interpreted as associations with other taxa, not physically connected.

For CT scanning imaging, a Diggle's randomization testing was conducted (Dutilleul, 2011, Diggle, 2014) to determine the degree of aggregation and heterogeneity between pore spaces. First, voxels selected to be representative of pores had a pseudo-CT number ranging from 600-900, depending on the soil structure. Nearest-neighbour distances between points (pseudo pore voxels and pseudo pore centroids) were calculated in 3-D cylindrical space (Fig. S3.3 and S3.4). One thousand partial realizations of a completely random point process over the cylindrical domain, with 1000 points (pseudo-pore voxels) vs. 5000 points (pseudo-pore centroids), were simulated. To perform the randomization testing procedure at an approximate 5% significance level, the generated distribution of nearest-neighbor distances for a given value of the cumulative frequency provided the lower (2.5%) and upper (97.5%) envelopes that we used as the

acceptance region. Pseudo-pore centroids were calculated using MATLAB R2023b (MathWorks Inc.) *centroid* function.

3.4 Results

3.4.1 *Results of CT imaging*

Differences in soil structure heterogeneity were observed after accounting for connected pores, rather than individual units of air space within the two structure treatments of this study (Fig. 3.2, S3.3, S3.4). When air-filled points were considered one pseudo pore for both land uses, intact cores show higher aggregation whereas the sieved cores show weaker aggregation (Fig. S3.4). Thus, when considering connected air-filled points to be one pseudo-pore we see that sieving had an overall homogenizing effect on air-filled space by increasing randomness within both the agriculture and grassland soils.

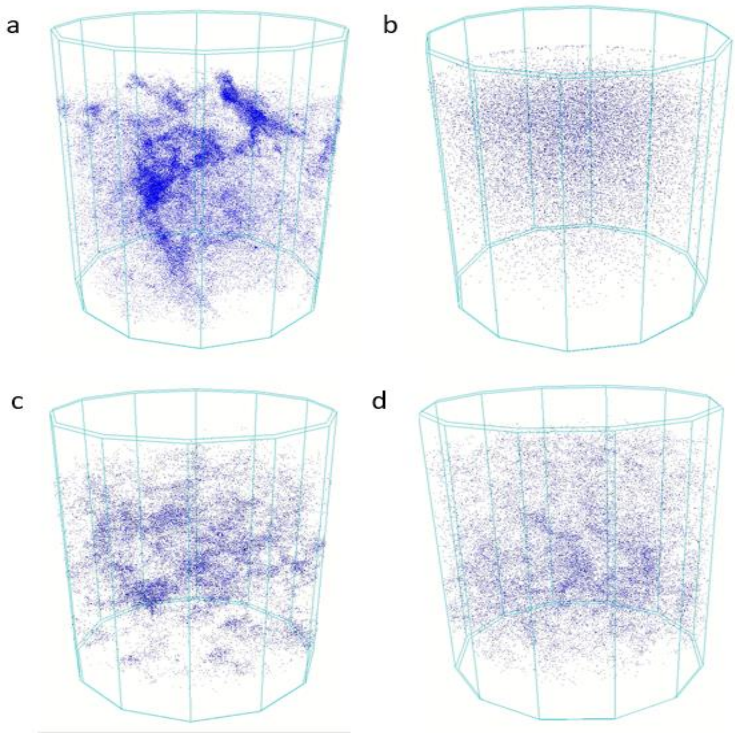

Figure 3.2. Micro CT scans of the one-cm core of each structural treatment for the grassland intact (a) and sieved (c) and agriculture intact (b) and sieved (d). The blue colouring denotes the voxels (image units) which are considered "pseudo-pore" space.

3.4.2 *Soil moisture, redox and pH*

The soil flooding was effective, with flooded soils having on average 1.3 times higher water-filled pore space (WFPS) compared to unflooded, field-moist soils ($F = 359.7$, $p < 0.001$). On average, flooded soils were at 81% WFPS while unflooded soils were at 60% WFPS (Table S3.3). During the flood event, we observed that soil redox was lower in the flooded intact cores (253 mV and 214 mV for agriculture and grassland) than in the flooded sieved cores (308 mV and 276 mv for agriculture and grassland) (Table S3.4), however this was not statistically

different. Soil pH only differed within the grassland by flood, where flooding increased pH from an average of 5.7 in unflooded to 6 in flooded cores (Table S3.5).

3.4.3 *Soil nutrient and C response to flooding: N and C pool concentrations*

Initial total C and N were higher in the grassland (5.06% and 0.46% respectively) compared to agriculture soils (2.02% and 0.18% respectively) ($p < 0.05$) (Table S3.6). Post-flood, there was no effect of flooding or structural treatment on total soil C or N in either land use.

Soluble N and C pools varied in their response to flood, however, soil structure did not influence response to flooding for most of the pools we measured, except for WEON and nitrate. WEOC concentrations varied by land use ($F = 185.4$), structure ($F = 10.3$), flood ($F = 118.9$), and time ($F = 6.2$) (Table S3.7, S3.8). Overall WEOC concentrations were 41% higher in the grassland compared to agriculture and higher in flooded soils compared to unflooded soils. Compared to unflooded soil, WEOC increased with flooding in both agriculture and grassland by 23% and 75% respectively. Moreover, WEOC concentrations increased compared to pre-flood and remained high post-flood (Fig. S3.5 a,b). Soil structure only affected WEOC in the grassland averaged across flood and time treatments, where sieved soil had 10% higher WEOC compared to intact soil. We did not observe an interactive effect between soil structure and flooding in either the grassland or agriculture.

WEON concentrations varied by land use (Chi-sq. = 125.3) and flood (Chi-sq. = 22.0). WEON concentrations were 76% higher in the grassland compared to the agricultural land use. Compared to unflooded soils, flooding decreased WEON concentrations by 63% and 28% in the grassland and agriculture respectively. While there was no overall effect of soil structure on WEON, some interactions between structure and flood or time were observed. Within the

unflooded grassland, intact, but not sieved, soil led to an increase in WEON over the course of the incubation (Fig. S3.5 c,d). Notably, in the agricultural soil, only the intact soil (and not the sieved) WEON fully recovered post-flood, with similar concentrations to pre-flood conditions (Fig. S3.5 c,d).

Nitrate concentrations varied by land use ($F = 422.8$), flood ($F = 147.6$), and time ($F = 14.2$). Nitrate was 82% higher in the grassland compared to agriculture and flooding decreased nitrate concentrations by 71% and 48% in the grassland and agricultural soils respectively. Soil structure was only significant in the agricultural soil, where the intact soil had 29% more nitrate than the sieved soil, when averaged across time and flood treatments. However, we also observed significant interactions between structure and flood or time for the agricultural soil. Nitrate concentrations decreased more so in intact soil (by 57%) compared to sieved soil (39%) during the flood. Both intact and sieved flooded soils recovered post-flood back to concentrations similar to pre-flood conditions in the agriculture soils (Fig. S3.5 f).

Ammonium concentrations varied by land use ($F = 153.3$) and flood ($F = 18.6$). Ammonium concentrations were 62% higher in the grassland compared to the agriculture soil. In the grassland, flood increased ammonium concentrations by 48%, relative to unflooded soil. In the agriculture soil, we only observed an effect of flood on ammonium with the sieved soils, declining during the early period of the flood (1 week) and peaking later in the flood (3 weeks). In the sieved agriculture soils, ammonium fully recovered, where pre- and post-flood ammonium were within the same concentration range (Fig. S3.5 h).

3.4.4 *Microbial functional response to flooding: respiration and enzyme activity*

Soil respiration was consistently more than 3 times higher in the grassland compared to agricultural soils, regardless of flooding or structural treatments. Following flooding, respiration decreased by 73% and 78% in both grassland and agriculture samples, respectively (Fig. 3.3). In the grassland, CO_2 was similar between the two soil structures throughout the incubation and did not increase post-flood. However, we did observe an effect of structure in the agricultural soils. In the agricultural soils, respiration was initially 47% higher in the sieved soil pre-flood, compared to the intact soil. Notably, despite initially higher respiration for sieved soil, the intact soil recovered in respiration post-flood to similar pre-flood CO_2 rates, while the sieved soil did not recover.

Unflooded, field-moist intact cores behaved differently between the two land uses: in the grassland, respiration dropped immediately with no change over the incubation period while in the agriculture soil, respiration only decreased towards the end of the incubation (post-flood period). We also observed a structural effect in agricultural unflooded soil but not in the grasslands, such that intact agricultural soils had higher respiration compared to the sieved soil (Fig. S3.7).

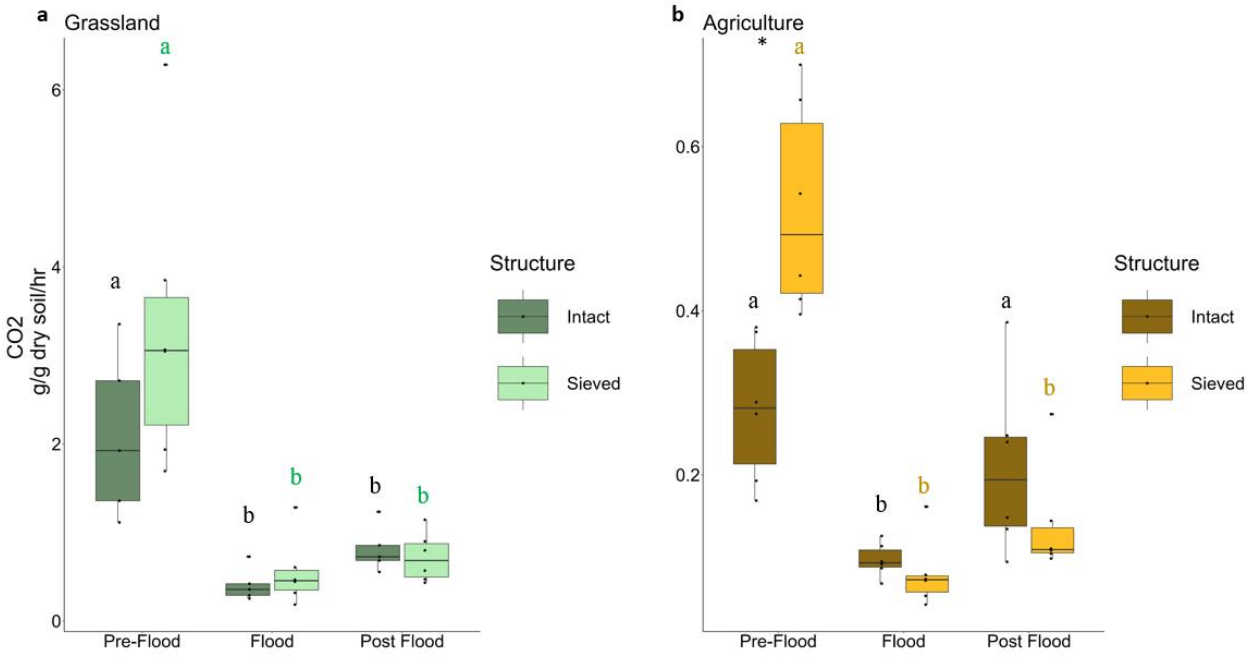

Figure 3.3. Soil CO_2 respiration in flooded soil with sieving and without (intact) for grassland (a) and agriculture (b) soil. Respiration is presented as g CO_2 per gram dried soil per hour. Respiration data collected every 2-3 days during the incubation was grouped into three flood periods: Pre-flood, Flood, and Post-Flood. The significance letters denote differences across time within each structure treatment and the * indicates a significance between the structure treatments. The line within the boxplot indicates the median, the limits of the boxes indicate the 25th and 75th quartiles. The whiskers indicate 1.5 times the quartile range.

We observed a strong influence of soil structure on potential extracellular enzyme activity (EEA) moderating the response to flooding (Fig. S3.8). Intact soil generally exhibited higher EEAs with less of a decrease throughout the incubation. Between the two land uses, grasslands consistently had higher EEAs relative to agricultural soils. We also observed that the temporal trends in EEAs changed depending on the flood treatment, particularly for BG and NAG. For instance, while

EEA was generally highest at the start of the incubation, unflooded soil EEAs generally declined sooner during the incubation period, compared to flooded soil (Fig. S3.8, S3.9, Table S3.7, S3.9).

We saw the largest treatment effects for BG activities. BG activity varied by land use ($F = 111.2$), structure ($F = 18.6$), and time ($F = 16.8$). BG activity was 28% higher in the grassland compared to the agriculture. Intact soil had 11% and 13% more activity compared to the sieved soil in the grassland and agriculture land uses respectively. For both land uses, BG activity was highest at the start of the incubation (pre-flood), but depending on soil structure, declined over time. For example, in both land uses, the sieved soil BG activity declined over time while the intact soil maintains a relatively high BG activity, similar to pre-flood conditions. Moreover, in the flood-treated soils, BG activity is 28% (grassland) and 24% (agriculture) higher in the intact than the sieved soil. Further, there is no variation of BG activity over time in the intact grassland cores.

NAG activities varied by land use ($F = 19.3$), structure ($F = 6.6$), flood ($F = 9.4$), and time ($F = 8.5$). Between land uses, the grassland had 20% higher activity than the agricultural soil. Within the grassland, soil structure only significantly interacted with flood, where intact flooded cores had 25% higher activity than unflooded intact cores ($p < 0.1$), and there was no flood effect in sieved cores. In agriculture, regardless of flood treatment, the intact soil had 22% higher activity compared to sieved cores. We observed a moderating effect of soil structure on NAG activity response to flood only for the agricultural soil and not for the grassland; flooding only increased NAG activity in intact cores (by 32% compared to unflooded). Further, intact flooded cores had 35% higher NAG activity than sieved flooded cores. NAG activity in the sieved flooded cores recovered post-flood to within variation of pre-flood activity.

Peptidase (LAP plus TAP) varied by land use (F = 341.5), flood (F = 6.4), and time (F = 3.2). Structure was not a source of variation in peptidase activity in either land use. Peptidase activity in the grassland was 42% higher than in the agriculture. Overall flooding increased peptidase activity by 9% in the grassland and 10% in the agriculture cores.

3.4.5 *Fungal and bacterial community size and biomass*

We observed overall more bacterial and fungal taxonomic marker gene copy numbers in agricultural soil compared to grassland when measured by quantification of 16S rRNA and 28S rRNA gene copy number, respectively (Fig. 3.4). However, only in the agricultural soils did we observe an influence of soil structure on both fungal and bacterial population response to flooding.

The bacterial community size as measured by qPCR varied by land use (F = 2830), structure (F = 67.6), flood (F = 356), and time (F = 78.6). Agriculture had 50% higher gene copy numbers compared to the grassland. In the grassland, flooding decreased copy numbers by 27% averaged for both sieved and intact, and post-flood, copy numbers recovered to within pre-flood values regardless of soil structure (Fig. 3.4 a). In the agriculture, intact soils had 15% higher bacterial copy number compared to sieved. Flooding decreased copy numbers by 18% averaged for both intact and sieved cores compared to unflooded cores (Fig. 3.4 b). Bacterial abundance recovery depended on soil structure for the agricultural soil. Post-flood, sieved soil recovered to within 19% of pre-flood bacterial copy numbers, yet still lower than pre-flood values. However, the intact soil fully recovered post-flood to within 2% of pre-flood copy numbers.

Fungal community size was influenced by land use (F = 2761), structure (F = 77.4), flood (F = 344.4), and time (F = 70.1). The agriculture soils had 50% more fungal gene copy numbers

compared to the grassland (Fig 3.4 c,d). In the grassland, flooding decreased fungal community by 27%, averaged for both sieved and intact relative to unflooded treatment, but in flood-treated soils, gene copy numbers recovered post-flood to similar pre-flood levels, regardless of soil structure. In agriculture, intact soils had 16% higher fungal copy numbers compared to the sieved. Flooding decreased copy numbers by 18% averaged for both sieved and intact structures relative to unflooded soils. Unlike the grassland, recovery of fungal community size in agricultural soil depended on soil structure. Post-flood, sieved soil recovered to within 15% of pre-flood copy numbers, but in the intact soil, post-flood copy numbers recovered to within 2% ($p>0.05$) of pre-flood copy numbers, thus indicating greater recovery in the intact soil compared to the sieved.

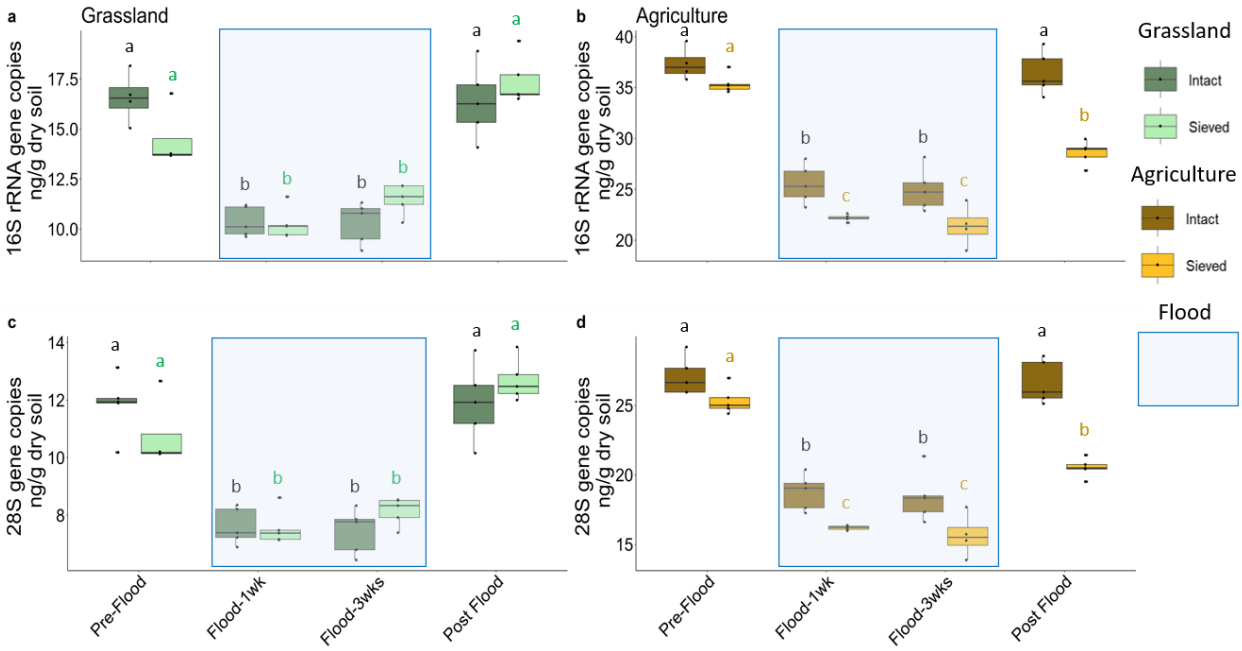

Figure 3.4. Microbial fungal (c,d) and bacterial (a,b) populations of grassland (a,c) and agricultural soils (b,d) based on qPCR of 16S rRNA and 28S gene copies across two soil structural treatments (n=4) in flooded soils. Letters denote significant differences over time

within each structure treatment (tukeyHSD). The line within the boxplot indicates the median, the limits of the boxes indicate the 25th and 75th quartiles. The whiskers indicate 1.5 times the quartile range. Gene copies are relativized by grams of dry soil.

Microbial biomass carbon (MBC) varied by land use (F = 2215.6), structure (F = 4.4), flood (F = 4.9), and time (F = 42.4) (Fig. S3.11, Table S3.9). The grassland had 4 times the MBC than the agricultural soil. In the grassland, MBC concentrations varied over time with different responses depending on structure. In the grassland, flood-treated sieved soil MBC concentration declined with flooding but recovered post-flood, whereas MBC concentrations in the intact flooded soil did not change during the flood period and increased post-flood. In the agriculture, intact soil had 9% higher MBC concentrations compared to the sieved soil, averaged over flood treatment and time. Compared to pre-flood, MBC concentrations during the flood period increased in the intact soil but remained the same for the sieved soil. Throughout the incubation, MBC concentrations were highest post-flood for both sieved and intact soils.

Microbial biomass nitrogen (MBN) varied by land use (F = 2648), structure (F = 30.5), flood (F = 10.5) and time (F = 6.4). MBN concentrations were 78% higher in the grassland compared to the agriculture. In the grassland, intact soil had 9% higher concentrations than the sieved soils and there was no change in MBN over time or between flood treatments. In the agriculture soil, intact soil had 19% higher MBN concentrations compared to the sieved soils. Flood-treated agriculture soils led to an 11% increase in MBN concentrations, compared to unflooded soil. We only saw an MBN response to the flood event in the sieved soil, where MBN decreased after 3 weeks of flooding but fully recovered post-flood.

3.4.6 *Bacterial and fungal community composition*

Microbial community alpha diversity (species richness and Shannon diversity) varied between land uses, soil structure, and between fungal and bacterial communities (Table S3.9, S3.10). Within the bacterial community, Shannon diversity was 3.6% higher in the agriculture (6.82) compared to grassland (6.57), total richness was 14% higher in the agriculture (1689) compared to grassland (1446), and inverse Simpson (evenness) was 33% higher in the agriculture (493) compared to the grassland (330) (Table S3.9, S3.11). The effect of soil structure was only significant within the agriculture land use, where the agriculture intact soil had higher diversity compared to the sieved soil by 1%, 5%, and 13% for Shannon, richness, and inverse Simpson respectively. For the grassland, there were no differences in diversity metrics by structure, flood, or time.

Within the fungal community, alpha diversity varied only between land uses (Table S3.10, S3.11). Shannon was 17% higher in agriculture (3.67) compared to grassland (3.06), richness was 23% higher in the agriculture (148) compared to grassland (114), and inverse Simpson (evenness) was 52% higher in the agriculture (19) compared to the grassland (9.12). While there was no main effect of soil structure, in the agriculture soils, sieved soil had 11% higher Shannon diversity compared to intact soil.

Beta diversity (Bray-Curtis dissimilarity matrix) of the bacterial community was distinct between the two land uses (PERMANOA: $F = 49$, $p=0.001$) and there was an overall effect of structure across land uses (PERMANOVA: $F = 2.3$, $p = 0.047$). When comparing within each land use, only the grassland bacterial community shifted in composition upon flooding. However, in the agricultural soil, even though community compositions between pre-flood and during the flood were similar, communities were distinct between pre- and post-flood, suggesting subtle

community shifts during the flood event. Beta diversity of grassland flooded bacterial community sequences varied by soil structure (PERMANOVA: $F = 1.7$, $p < 0.01$), and time (PERMANOVA: $F = 1.4$, $p < 0.01$) (Fig. 3.5). Variation in bacterial grassland communities during the flood period was more associated with WEOC and soil moisture whereas the post-flood community structure was associated with microbial biomass C and N (Fig. 3.5 a). Mantel test results indicated that measured soil N and C pools were significantly associated with the grassland bacterial community experiencing flooding (Mantel test: $r = 0.1743$, $p < 0.05$). To determine which time points were significant from each other, PERMANOVA was used to compare between times. Grassland bacterial composition changed with both soil structures between pre-flood and flood after 1 week ($F = 1.4$, $p < 0.05$) and did not change from flooding between 1 and 3 weeks, and finally changed again post-flood ($F = 1.6$, $p < 0.01$). However, only in intact cores was there a significant difference between pre- and post-flood community composition ($F = 1.5$, $p < 0.01$).

Agriculture beta diversity only varied by structure (PERMANOVA: $F = 1.8$, $p < 0.01$). The agriculture bacterial community during the flood period was associated with MBC, MBN, and WEOC concentrations. Further, the Mantel test indicated that measured environmental variables were correlated with agricultural bacterial community composition (Mantel test: $r = 0.15$, $p < 0.05$). However, when comparing bacterial community composition differences within each time point, communities were only different between pre-flood and post-flood, regardless of soil structure (PERMANOVA: $F = 1.4$, $p < 0.01$).

Beta diversity (Bray-Curtis dissimilarity matrix) of the fungal community was distinct between the two land uses (PERMANOA: $F = 104.8$, $p = 0.001$) and there was an overall effect of structure across land uses (PERMANOVA: $F = 2.8$, $p = 0.02$). In the flood-treated soil, fungal beta

diversity only varied by structure and only in the grassland, along the first axis (PERMANOVA: $F = 3.2$, $p < 0.01$), with no strong associations with any of the soil N and C pools (Fig. 3.5 c). The fungal community in the agriculture soil only associated with WEOC which was more strongly correlated with intact core community (Fig. 3.5 d). Mantel test indicated that measured environmental variables together did not significantly explain fungal community composition in either land use.

In the unflooded soil within each land use, soil structure was significant (PERMANOVA: $p < 0.05$) for both the fungal and bacterial community composition and structure, time and their interaction were significant for bacterial communities in the grassland (Fig. S3.13).

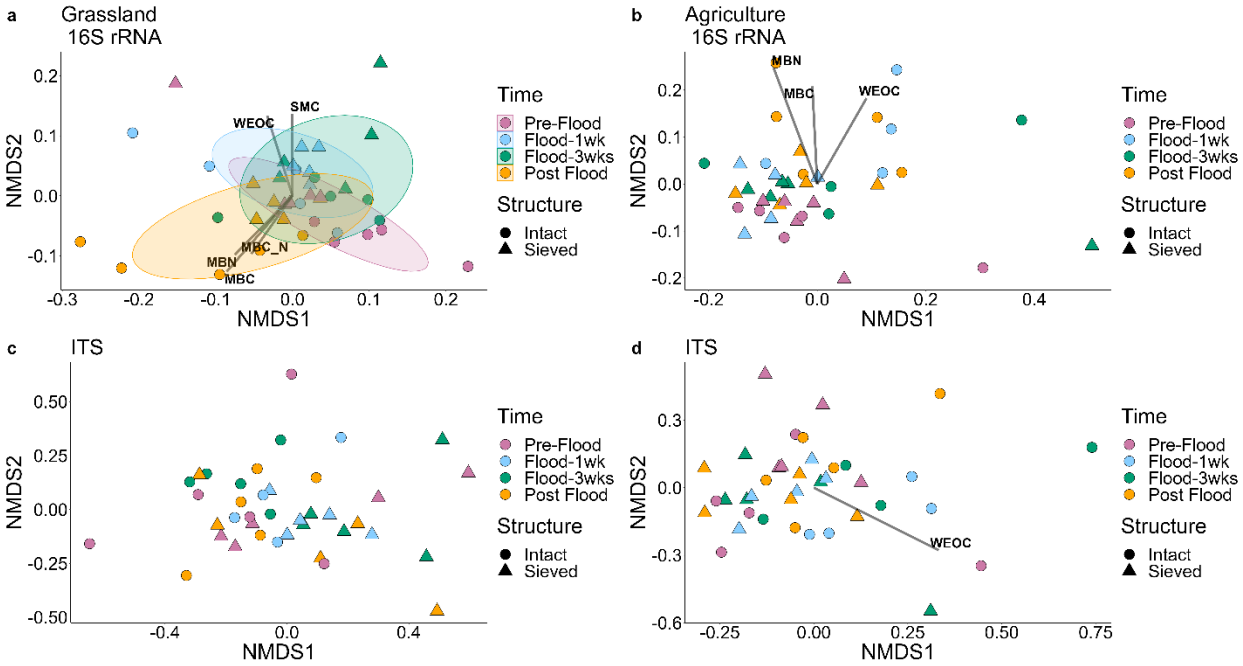

Figure 3.5. NMDS of soil 16S rRNA (a,b) and ITS (c,d) ASVs in grasslands (a,c) and agriculture (b,d) over the course of a flood event and two soil structures: with sieving and without sieving (intact). Stress NMDS for all plots were < 0.2. Time is indicated by colour and ellipses when significant differences occur within time (based on PERMANOVA), soil structure is denoted by

circles and triangles for intact and sieved soil. Environmental parameters that significantly associated with axis 1 or 2 are shown by vectors, with the following abbreviations: WEOC = water extractable organic carbon, SMC = soil moisture content, MBC = microbial biomass carbon, MBN = microbial biomass nitrogen, TAP = tyrosine amino peptidase and NAG = N-1,4-acetylglucosaminidase.

3.4.7 *Pairwise comparisons of phyla relative abundances across treatments*

In both the bacterial and fungal communities, relative abundance at the phyla level responded to the structural treatments within both the agriculture and grassland soils (Table 3.2). The most abundant bacteria phyla that increased in the intact relative to the sieved soil was *Chloroflexi* in the agriculture soil and *Planctomycetota* in the grassland. *Acidobacteria* and *Actinobacteria* had the highest abundances associated with sieved compared to intact soil in the agriculture and grassland respectively (Table 3.2). Bacterial phyla within the grassland, and not agriculture, responded to the flooding treatment (Table 3.2). The most abundant phyla that increased with flood was *Proteobacteria* and in the unflooded soil, *Acidobacteria* was the most abundant. Within the fungal communities, structure (but not flooding) affected two phyla in both grassland and agriculture soil (Table 3.2), with *Mortierellomycota* being more abundant in the sieved soil.

Table 3.2. Differential abundance highlighting the main phyla that varied by treatment for bacterial and fungal sequence data. Treatment indicates the overall treatment whereas the dominant treatment column refers to which treatment the indicated phyla had higher abundance in, and greater abundance column indicates the percentage difference between treatment levels. Shading indicates the phyla with the greatest abundance within each treatment.

Kingdom	Land use	Phylum	Treatment	% Greater abundance	Dominant treatment
Bacteria	Agriculture	Acidobacteriota	Structure	9.6	Sieved
Bacteria	Agriculture	Bdellovibrionota	Structure	21.4	Intact
Bacteria	Agriculture	Chloroflexi	Structure	11.3	Intact
Bacteria	Agriculture	Desulfobacterota	Structure	18.9	Intact
Bacteria	Agriculture	Elusimicrobiota	Structure	34.3	Intact
Bacteria	Agriculture	Fibrobacterota	Structure	37.3	Intact
Bacteria	Agriculture	Firmicutes	Structure	22.4	Sieved
Bacteria	Agriculture	Latescibacterota	Structure	16.7	Sieved
Bacteria	Agriculture	Methylomirabilota	Structure	22.6	Sieved
Bacteria	Agriculture	Myxococcota	Structure	18.2	Intact
Bacteria	Agriculture	NB1-j	Structure	42.2	Sieved
Bacteria	Agriculture	Patescibacteria	Structure	36.3	Intact
Bacteria	Agriculture	Planctomycetota	Structure	6.2	Intact
Bacteria	Agriculture	Spirochaetota	Structure	46.3	Intact
Bacteria	Agriculture	Unclassified	Structure	28.2	Intact
Archaea	Agriculture	Nanoarchaeota	Structure	52.3	Intact
Bacteria	Grassland	Acidobacteriota	Flood	6.4	Unflooded
Bacteria	Grassland	Dependentiae	Flood	45.1	Unflooded
Bacteria	Grassland	Desulfobacterota	Flood	25.4	Flood
Bacteria	Grassland	Fibrobacterota	Flood	43.0	Flood
Bacteria	Grassland	Firmicutes	Flood	32.9	Flood

Bacteria	Grassland	Halobacterota	Flood	44.6	Flood
Bacteria	Grassland	Methylomirabilota	Flood	14.7	Unflooded
Bacteria	Grassland	NB1-j	Flood	20.5	Unflooded
Bacteria	Grassland	Proteobacteria	Flood	5.7	Flood
Bacteria	Grassland	Actinobacteriota	Structure	8.1	Sieved
Bacteria	Grassland	Bdellovibrionota	Structure	27.2	Intact
Bacteria	Grassland	Fibrobacterota	Structure	49.7	Intact
Bacteria	Grassland	Patescibacteria	Structure	30.9	Sieved
Bacteria	Grassland	Planctomycetota	Structure	11.5	Intact
Bacteria	Grassland	Unclassified	Structure	33.3	Intact
Fungi	Agriculture	Unclassified	Structure	24.6	Intact
Fungi	Agriculture	Mortierellomycota	Structure	5.8	Sieved
Fungi	Grassland	Unclassified	Structure	79.6	Intact
Fungi	Grassland	Mortierellomycota	Structure	35.3	Sieved

3.4.8 *Community networks*

We estimated the number of connections of each ASV through co-occurrence network analyses that combined the sequenced archaeal, bacterial, and fungal communities. The networks with higher degrees (number of individual connections) have more associations with each other. When all ASVs were analyzed together, the degree of connectedness varied by kingdom (F = 112.4) and structure (F = 4.5) (Fig. 3.6 a). Land use was significant through interactions with kingdom (F = 18.5), structure (F = 15.) and flood (F = 49.3). Across kingdoms, bacteria and archaea had 7% more connections than fungi. Bacteria had 11% and 5% more connections than fungi in the grassland and agriculture respectively. Overall, intact soil had 3% more connections than sieved. Within the agriculture, intact soils had 6% more connections than sieved. When comparing between land uses, the agriculture soil had 2% more connections within the fungal kingdom

compared to the grassland. Agriculture intact and sieved soils had 2% and 1% more connections than the grassland intact and sieved soils respectively. In addition, agriculture unflooded soils had 6% more connections compared to grassland unflooded soils. In the agriculture, unflooded soils had 5% more connections than flooded soils. Grassland flooded soils had 8% more connections compared to agriculture flooded soils, and grassland flooded soils had 9% more connections compared to grassland unflooded soils.

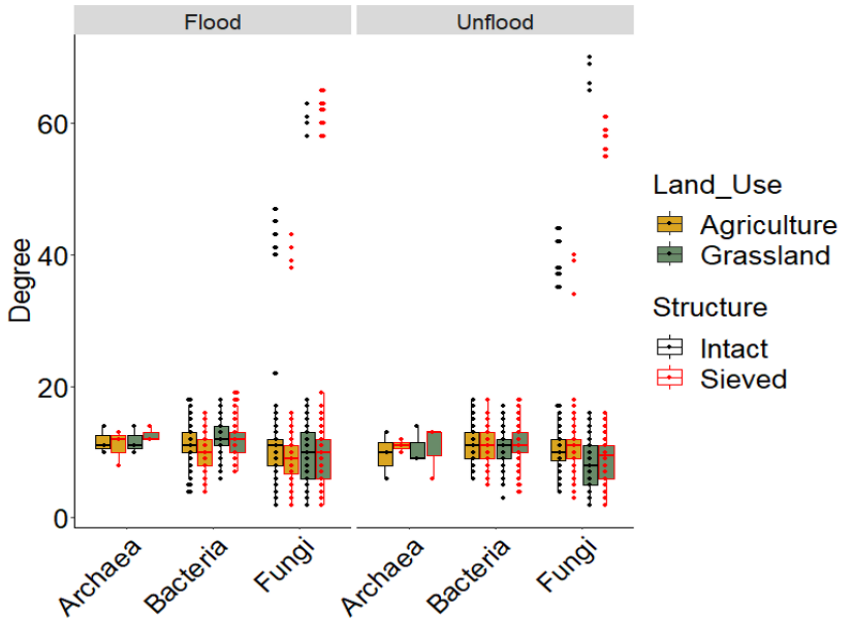

Figure 3.6. Co-occurrence network degree (metric of number of connections of ASVs) for archaeal, bacterial and fungal kingdoms across flood-treated and unflooded soils for two land uses (agriculture and grassland) and two soil structures (intact and sieved).

3.5 Discussion

Our objective was to determine how soil structural heterogeneity influences microbial community resiliency in response to flooding and how this response is further mediated by land

use. Microbial community dynamics under flooding in mineral soils are not well studied but is an important area of research as flooding frequency and duration is predicted to increase with unknown consequences to ecosystem functioning (Yin *et al.*, 2019, Jia *et al.*, 2020). In response to environmental disturbances several factors influence microbial community functional and compositional responses, including: the starting community (Steenworth *et al.*, 2005); resource availability (de Vries *et al.*, 2012); history and timing of the disturbance (DeAngelis *et al.*, 2010, Philippot *et al.*, 2021); and compounding disturbances (Peralta *et al.*, 2014, Philippot *et al.*, 2021). Soil structural heterogeneity, which influences microbial access to nutrients and microbial competition dynamics, may also impact microbial response to disturbance by fostering more diverse and resilient communities under more heterogenous environments (Keiluweit *et al.*, 2017, Rillig *et al.*, 2017, Hartmann and Six, 2023). In our study we examined how microbial communities from land uses with two distinct starting microbial communities, disturbance histories, and resources respond to flood and how this is mediated by differences in soil heterogeneity. We hypothesized that: 1) soil heterogeneity supports a microbial community with greater functional or compositional resiliency, or recovery, to experimental flooding, and 2) the influence of soil structure mediating the response to experimental flooding, will differ between an agriculture or grassland ecosystem.

3.5.1 *Microbial community recovery from flooding*

We found that microbial community recovery to flooding was dependant on both land use and soil structure (Fig. 3.3, 3.4). Microbial communities under the agriculture land use exhibited quicker functional recovery to flood compared to the grassland. This recovery was further mediated by soil structure where the more heterogenous soil had a higher degree of recovery compared to the homogenous soil within the agriculture. We observed that recovery post-flood

occurred for bacterial and fungal abundances and some functional responses in these agricultural soils. For instance, we found that respiration, a measure of microbial function, in flooded intact and sieved soil recovered to 81% and 50% of the pre-flood respiration rates, respectively (Fig. 3.3) and the chitin targeting enzyme, NAG, activity recovered post-flood in the heterogenous cores (Fig. S3.8). While we would not expect respiration rates to return completely to pre-flood conditions due to decreasing available C over the incubation period, the 80% recovery for the intact agricultural soil suggests that the community is rapidly responding to drier conditions post-flood. Fungal and bacterial community abundances also demonstrated recovery, where the intact soil had a higher degree of recovery compared to the sieved, but only in the agriculture land use. Functional and community abundance recovery was associated with greater bacterial and fungal species diversity in the agriculture soil. Quicker recovery may be due to a greater selection of species that respond more rapidly to optimal conditions, supported by the portfolio effect (Griffiths and Philippot, 2013, Wagg *et al.*, 2018, Wagg *et al.*, 2019, Bargett and Caruso, 2020). Beta diversity in the agriculture soil exhibited differences between pre- and post-flood microbial communities, but no difference between 3-week flood and post flood communities. This implies that the functional recovery from the flood that we primarily observed for the agricultural soil was carried out by a similar community that was sustained during the flood, but that there were some subtle changes compared to pre-flood communities.

Microbial communities under the grassland did not exhibit the same soil structure-dependant functional recovery to flooding as the agriculture soil, but bacterial and fungal abundances did recover post-flood (Fig. 3.4) and there were structural differences in community composition recovery from flood. Grassland microbial community respiration, and enzyme activities that decreased over time, did not recover to within the variation of pre-flood levels. The grassland

soil moisture was higher pre-flood and during flood compared to the agriculture soil (Table S3.7, S3.8), and therefore post-flood the soil did not dry down to the same extent as in the agricultural soil. Thus, it is likely that a higher proportion of anaerobic pore space remained in the grassland soils post-flood, resulting in the persistence of lower metabolic rates which required more time to recover post-flood (Keiluweit *et al.*, 2017, Fuhrmann, 2021). It also plausible that some anaerobic respiration was also occurring that our CO_2 did not capture, although low abundances of methanogens and denitrifiers do not support this.

The different community compositional responses throughout the flood event did not appear to be resource-dependent, as there were little changes in substrate concentrations after the onset of flood (Fig. S3.5). Moreover, while grasslands had higher soil C and N (total and soluble) compared to the agricultural soils, we would expect this higher grassland substrate availability to contribute to a relatively greater grassland functional recovery which we did not observe. Thus, we suspect that the diverging levels of recovery between the grassland and agricultural soil is related more to the initial differences between the microbial communities of the different land uses.

The grassland had lower species richness, diversity, and bacterial and fungal abundances compared to the agriculture. In our study, the grassland did not experience the same degree of recurrent soil disturbance, associated with tillage, as the agriculture soil. Thus, elevated species richness in the agriculture soil may be related to more frequent soil disruptions, whereas the grassland experiences more stable environmental conditions, which can sometimes result in lower microbial diversity (Peralta *et al.*, 2014). The shifts in beta diversity with time indicated that grassland microbial community composition responded to flood. Community composition shifted with flood onset and after dry down, however, in the sieved cores, communities pre- and

post-flood were similar whereas this was not the case for the intact cores (Fig. 3.5). Shifts in bacterial community composition associated with soil structure were not reflected in species richness or Shannon diversity, however it may be possible that the difference in recovery between the two soil structures is related to the initial effect of sieving on the grassland microbial communities.

Compositional shifts in the grassland bacterial community were associated with slower recovery of respiration to flood, suggesting that the flood disturbance applied a selection pressure shifting the community members to have different functions better suited for flood conditions (Ho *et al.*, 2017). Further, the history of flooding and general high moisture conditions in this grassland have likely applied selection pressure to these communities over time resulting in local adaptation to flooded conditions (Hawkes and Keitt, 2015). The decrease in both activity and community abundances during flood, possibly demonstrated that flooded conditions stimulated dormancy and resuscitation within different groups (Sorensen and Shade, 2020, Patel *et al.*, 2021). As respiration began to increase post flood, it is possible that the slow recovery is due to the resuscitation of taxa as dormancy was the prevalent stress response during flood.

As we observed bacterial community composition shifting with flood onset in the grassland, it is possible that flooding could be selecting for phyla that are more adapted to excess substrate induced with flood. We found that abundances of *Gammaproteobacteria* and *Acidobacteriota* increased in flooded and unflooded soils respectively. Many *Acidobacteriota* perform well under lower nutrient conditions, with higher substrate use efficiency, sometimes referred to as oligotrophic metabolic strategies (Fierer *et al.*, 2007, Ho *et al.*, 2017). In contrast, some *Gammaproteobacteria* phyla respond to high nutrient environments, exhibiting a more copiotrophic metabolic strategy (Ho *et al.*, 2017). Thus, perhaps copiotroph abundance

responded to more accessible substrates (such as WEOC and ammonium), but as soil redox conditions shifted with declining oxygen, function was inhibited such that we observed low respiration and no change in nutrient concentrations with flood. Furthermore, post-flood shifts in community composition to a community similar to pre-flood is associated with recovery of bacteria abundances in the homogenous cores. However, communities within the heterogenous cores had a different post-flood community composition compared to pre-flood suggesting that recovery of bacterial abundances may be due to a more variable and more slowly adapting community. Future research linking functional gene or physiological trait data with community composition will help to illuminate the microbial justification of community shifts with flooding.

Interestingly, co-occurrence network analysis determined that the grassland community had a greater number of highly associated individual ASVs during the flood period compared to the agriculture flood communities (Fig. 3.6). Highly connected networks have also been associated with lower stability due to a greater likelihood that a perturbation resonates throughout the network (de Vries *et al.*, 2018). However, other studies have demonstrated that ecosystem multifunctionality is higher in communities with higher linkage densities (links per ASVs) (Wagg *et al.*, 2019). Although functional recovery may be slower in the grassland, we know that the microbial community did recover to within pre-flood community abundances. Thus, perhaps the increased interactions between ASVs during the flood led to greater shifts in community composition.

Our study demonstrated that the agriculture land use with higher species diversity exhibited more functional recovery to flood. However, here we are comparing microbial recovery in the sense of a 'return' to pre-flood conditions, but some level of microbial community resistance or stability may also be occurring that we have not captured in our data analysis. Thus, if we were to

compare the magnitude of changes between the flooded and unflooded soil, grasslands could have relatively greater stability (smaller or no change). Further, while the agricultural communities are functionally recovering more so than the grasslands, we are seeing compositional recovery occurring in homogenous grassland cores. Therefore, the mechanism of recovery appears to be ecosystem dependent.

3.5.2 *Microbial community response to flooding was mediated by soil structural heterogeneity*

Flooding was expected to increase microbial nutrient and C availability by alleviating substrate and microbe mobility limitations (Bailey *et al.*, 2017, Schimel, 2018, West and Whitman, 2022). We found that flooding increased nutrient concentrations compared to the unflooded cores, however, the soil structure had little effect on substrate concentration response to flood in both land uses, except for WEON and nitrate concentrations in the agriculture land use. The increased connectivity between soil pores that we expected with flooding may have obscured any differences in resource concentrations between soil structures. Differences in soil heterogeneity can impact microbial access to resources before, during, and after a flood and thus influence how microbial communities respond to flooding. However, because we saw minimal change in substrates by soil heterogeneity, our results suggest that the modulating effect of soil structure on microbial community flood response is not necessarily resource-dependant.

We hypothesize that the higher degree of recovery we observed in the more heterogeneous, intact soil (especially in agriculture) for some of the function and community responses is due more to effects on biotic interactions than resource access. For instance, disintegrating macroaggregate structure and 'releasing' their previously isolated microbial communities may change interactions within the newly associating pool of soil organisms (Rillig *et al*., 2017), possibly increasing both synergistic and antagonistic interactions (Kim *et al.*, 2008). Thus, while nutrient

limitations are lifted during flood, interspecies interaction may be the dominant cause of structure-dependent shifts in community composition and function with flooding in our study.

Soil microbial dynamics measured at an ecosystem scale may contrast processes that occur at the more microbially relevant soil aggregate scale (Upton *et al.*, 2019). Thus, even if a natural system is undisturbed, compared to an intensely managed agriculture system, the soil structure is more heterogenous, and micro scale disturbances or fluctuations may override the larger scale stability of an undisturbed natural ecosystem. We expected that in a homogenized environment there would be increased access to nutrients but that in a more heterogenous environment, the microbial community would have higher diversity, such that each soil structure could support processes that facilitate flood resiliency and recovery (West and Whitman, 2022). However, because our grassland already had relatively high resource availability, we expected microbial resiliency and recovery in response to flood to be highest with higher soil heterogeneity in the natural grassland ecosystem. This study shows that the grassland community composition was responsive to flood, shifting over time, however this was not associated with functional recovery. Thus, aggregate scale selection pressures from higher moisture and resource availability may result in community composition shifts in response to flood favoring taxa which exhibit slower metabolisms and growth, yet better adaptations with flooded conditions.

3.6 Conclusions

We found that microbial community response to flooding was modulated by land use and soil structure. Microbial communities within the agriculture land use displayed functional recovery from flood to a greater extent than the grassland, likely moderated by the higher microbial

diversity found in the agriculture land use. However, this recovery in the agricultural soil was primarily limited to the intact, more heterogenous soils, suggesting that recovery to flood is dependent on soil structure. Ecosystems with higher degrees of disturbance may have higher diversity due to the more frequently changing niche space and opportunities for colonizing species. Microbial communities within the grassland land use demonstrated greater shifts in community composition over time, with lower functional recovery post-flood. Thus, the grassland ecosystem may be experiencing shifts in taxa more adapted for the new conditions, at the expense of quickly recovering overall metabolic process rates.

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General Discussion

The two chapters presented in this thesis sought to identify abiotic and biotic soil characteristics impacting soil microbial activity and community composition within a seasonal floodplain across scales, and under a more controlled environment to more specifically understand microbial community response to experimental flooding. At the field-level scale, I determined the relationship between soil abiotic characteristics and extracellular enzyme activity (EEA) and the sensitivity of EEA to land use intensity across time and spatial scales (Chapter 2). Using a laboratory incubation, I determined how soil heterogeneity and land use influenced microbial community resiliency and recovery to experimental flooding (Chapter 3). I found that EEAs were sensitive to land use across spatial and temporal scales related to soil moisture and soil organic carbon. Further, I found that microbial community response to flood was land use dependent with differing sensitivities to soil structure. A notable outcome of doing this research was that the literature on flooding in mineral soils, especially non-paddy soils, is often conducted at a larger ecosystem scale, and when finer resolution is incorporated to understand microbial community response to flooding there is a paucity of information regarding both microbial responses and how these responses are measured. In this section, I will discuss some aspects of microbial ecology that are missing from our scientific discussion on microbial communities in flooded systems.

In my research for Chapter 3, I observed microbial functional and compositional responses to flooding and recovery post-flood which were mediated by both structure and land use. In the agriculture soil, I saw functional recovery associated with higher species diversity, whereas in the grassland I observed bacterial community compositional shifts with flooding and over the course of the incubation. Studies looking at microbial trait-based response to disturbance often

highlight the importance of connecting microbial community compositions, which control traits, to the ecosystem processes (Wallenstein and Hall, 2011, Hall *et al.*, 2018). However, microbial community traits related to perseverance through flood disturbance are not well characterized. Understanding trade-offs in microbial response to flooding will require further investigation into physical and metabolic traits that improve community function during flooding and that facilitate recovery.

Studies focussed on microbial traits associated with drought response have found traits related to osmolyte production, stress compounds and sporulation genes (Schimel *et al.*, 2007, Griffiths and Philippot, 2013, Schimel, 2018, Patel *et al.*, 2021). Patel *et al.* (2021) found sporulation genes in both flooded and drought samples suggesting that spore production (associated with dormancy) is an adaptation to both low and high moisture conditions. Dormancy, however, does not explain persistent microbial respiration and activity during flood which was observed in my experiment. Physical adaptations for flood may include long-term metabolic trade-offs compared to short-term defenses to stress. For example, gram-positive bacteria are more resilient to moisture fluctuations and although they may have lower metabolic stress in response to rewetting, it is metabolically more expensive to maintain their larger cell wall (Schimel *et al.*, 2007). As microorganisms can largely be limited in their mobility within the soil matrix (Bailey *et al.*, 2017, Schimel, 2018, Lehmann *et al.*, 2020), traits associated with motility were found to increase with flooding or be consistently present in soils with high moisture conditions, thus demonstrating a physical adaptation to flood (Patel *et al.*, 2021). Although Patel *et al.* (2021) identified microbial traits associated with fluctuations in soil moisture, the connection between traits and community composition is missing.

Metabolic adaptations such as the ability to use alternate terminal electron acceptors will also assist communities in remaining active during floods, where declining oxygen concentrations results in reduced redox conditions (Boye *et al.*, 2018). Comparing sites with different moisture histories, genes coding for anaerobic respiration were found in sites with high moisture and greater moisture fluctuation history (Patel *et al.*, 2021). If and at which point microbial community composition shifts from being dominated by organisms with physical adaptations to flood to being dominated by anaerobic bacteria is not clear.

In ecosystems with high microbial diversity there is increased likelihood that beneficial traits are present thus allowing the microbial community to harness functional redundancy promoting resiliency and recovery to flood (Philippot *et al.*, 2021). In my study, the agriculture soils exhibited faster functional recovery to flood compared to the grassland, especially in the heterogenous intact soil cores. Recovery in the agriculture microbial community post-flood was associated with greater bacterial and fungal species diversity. In contrast, the grassland bacterial community shifted with time thus showing that functional redundancy was less of a factor than increasing organism abundance with potentially optimal traits in response to flood. In the grassland, community compositional shifts over time were only observed in the bacterial community, not with the fungal community. As previous studies have shown (Unger *et al.*, 2009) bacterial and fungal communities respond differently to flood, with fungal markers often decreasing.

Selecting for beneficial microbial traits under flood stress may influence the allocation of resources to the production of extracellular enzymes. The soil environment poses many stressors not related to ecosystem-level disturbances such as flood, thus enzyme production is constantly a trade-off between using resources for maintenance and growth or nutrient acquisition

(Sinsabaugh and Shah, 2012, Malik *et al.*, 2020, Nunan *et al*., 2020). As metabolic requirements become more expensive due to disturbance stress response, the production of extracellular enzymes may become even more metabolically unfeasible, and we may observe trade-offs in enzyme production under high-stress environments. In Chapter 2, I found that land use mediated enzyme activity across spatial and temporal scales, indicating that site characteristics were important determinants of enzyme activity. However, within the sites that experienced the greatest effect of flood, enzyme activity became disconnected from land use. This may suggest that previous mechanisms determining enzyme production become more variable with increasing flood intensity. Interpretation of soil enzyme activity is difficult, as they are controlled on the organism, trait-level but are typically measured at the bulk soil scale and at their maximum potential. Thus, under flooded conditions the unknown implications for nutrient cycling as organisms respond to environmental stress requires further research.

Broader Implications

Land use influences microbial dynamics across many scales and disturbances. Thus, as increases in flooding duration and frequency are predicted, land management will play an important role in microbial community function. Across the ecosystem scale, I found that increasing land use intensity decreases EEA. Thus, promoting land management practices that increase SOC stocks, and moisture retention will likely have a positive influence on microbial community size and activity. Areas exposed to higher flood intensities may reduce the effects of land use, as I saw in Chapter 2 for field locations closer to the lakeshore. However, less intense land uses were still associated with higher enzyme activity in May, which was the closest sampling time to flood. Thus, timing and scale of a disturbance is important for evaluating a response (Philippot *et al.*, 2023). Further, I found that although species diversity is important for recovery in some systems

this may be a trade-off with compositional flexibility which seems to increase long-term resiliency in natural systems.

Future Directions

The effects of flooding on ecosystem functioning deserve greater attention as incidence of these flood events are becoming more likely. Flood frequency may not only be relevant in floodplain ecosystems, but also areas that have not experienced flooding before, or rarely experience flooding. Thus, studies comparing how land use influences microbial community response to flooding in both floodplains and soils that have not previously experienced flood will be an important addition to this research area. Continuing to study microbial response to disturbances at microbially relevant scales in tandem with ecosystem-level scales will help to connect interactions and their implications at the small- to the large scale. Further, connecting microbial functional response to traits involved in microbial flood perseverance will also help the interpretation of community-level shifts in function and composition with flooding.

General Conclusions

This thesis demonstrates how land use is a strong modulator of microbial activity and response to flooding. In the second chapter, I found that more natural sites, associated with higher moisture and carbon resources, compared to agricultural land uses, were associated with higher extracellular enzyme activity. However, my research also suggests that at sites exposed to greater flood intensity, the influence of land use is overridden by the effects of flood. Further, I observed that over time, perennial agriculture systems begin to function similarly to natural ecosystems when compared to conventional agriculture systems.

In the third chapter, I found that agriculture land use displayed functional recovery from flood but primarily in intact, more heterogenous soils. Grassland microbial communities did not exhibit functional recovery to the same degree as the agriculture, however community composition varied with flood and time with no differences associated with soil structure. Thus, different microbial responses to flood are occurring within each land use. Species diversity is likely driving the functional recovery in the agriculture, which is further associated with greater soil structure heterogeneity in the intact cores. Within the grassland, microbial community composition shifts in response to changing conditions, potentially demonstrate a trade-off between increasing abundances of species that are more adapted to the new conditions and metabolic recovery.

As flooding intensity and frequency are already increasing around the world, understanding microbial community responses to this disturbance is becoming increasingly critical to predicting key ecosystem functions like nutrient, C, and N cycling. While research on soil responses to drought is abundant, flood impacts on mineral soils have remained understudied. These two

research chapters demonstrate the large impacts that flood events and systems exposed to regular flooding have on soil and microbial dynamics and that flood-induced changes to microbial-mediated nutrient and C cycling are ecosystem- and soil structure- dependent.

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Supplemental Material
Chapter 2

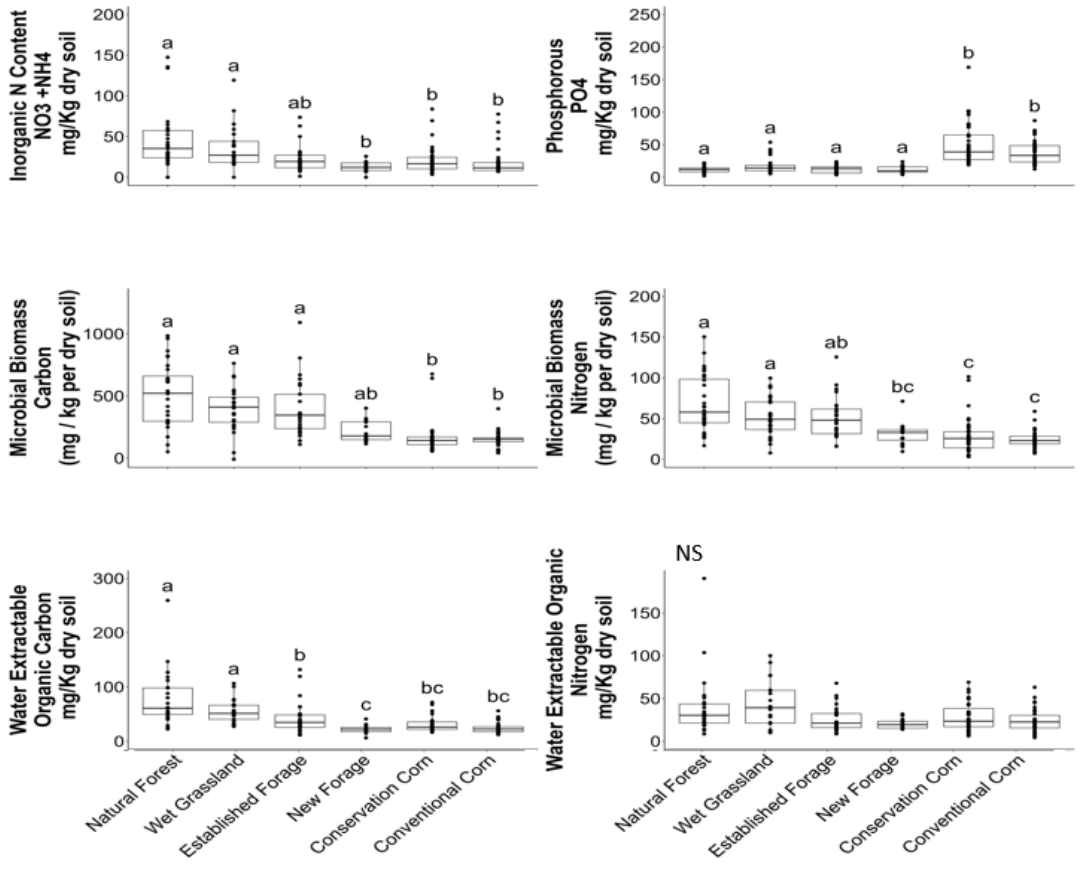

Figure S2.1. Nutrient and carbon (C) concentrations across land use treatments from low intensity (forest) to high intensity (conventional agriculture)**.** Different letters indicate significant differences (pairwise post-hoc test with FDR adjustments) among land uses, NS indicates no significant differences. The line within the boxplot indicates the median, the limits of the boxes indicate the 25th and 75th quartiles. The whiskers indicate 1.5 x the quartile range.

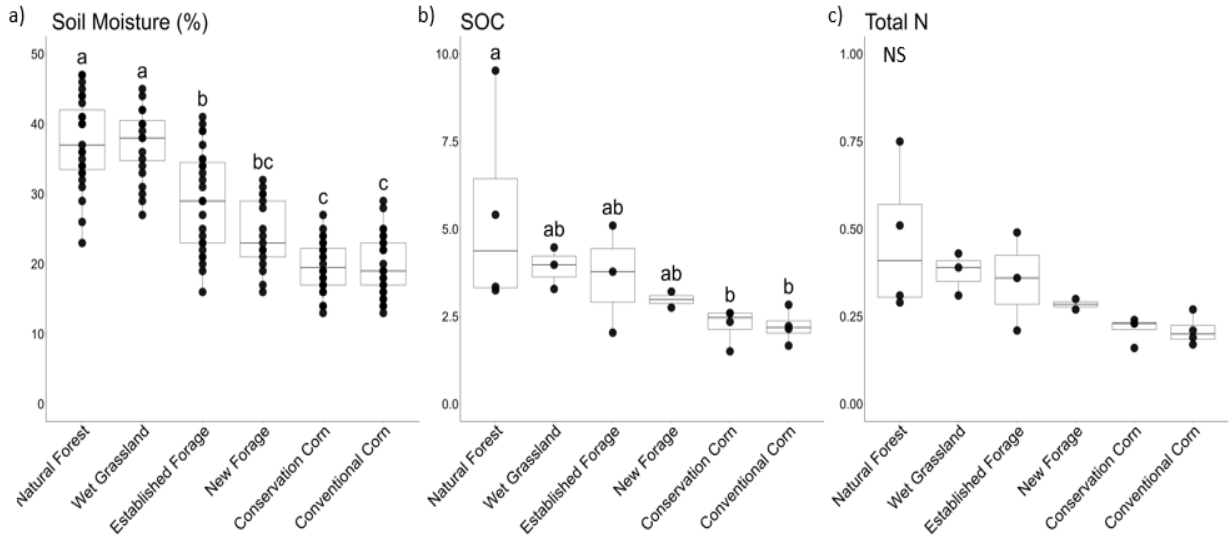

Figure S2.2. Soil moisture (%), soil organic carbon (SOC) (%) and total soil nitrogen (TN) (%) across the land use intensity gradient. Letters denote pairwise comparisons from linear model results. Linear model demonstrated that soil moisture ($p<0.05$) and SOC ($p<0.05$) varied by land use, but not TN ($p<0.1$). The line within the boxplot indicates the median, the limits of the boxes indicate the 25th and 75th quartiles. The whiskers indicate 1.5 x the quartile range.

Table S2.1. Mean and standard deviation of soil organic carbon (SOC) and soil total nitrogen (TN) across land use gradient. Samples for SOC and TN were only obtained after the May sampling point 2021. Samples were analyzed at the AgroEnviro Lab (La Pocatiere, QC).

Land use	SOC %	TN %
Conventional Corn	2.22 ± 0.48	0.21 ± 0.04
Conservation Corn	2.26 ± 0.52	0.22 ± 0.04
New Prairie	2.98 ± 0.32	0.29 ± 0.02
Old Prairie	3.64 ± 1.53	0.35 ± 0.14
Wet Prairie	3.91 ± 0.60	0.38 ± 0.06
Natural Forest	5.38 ± 2.94	0.47 ± 0.21

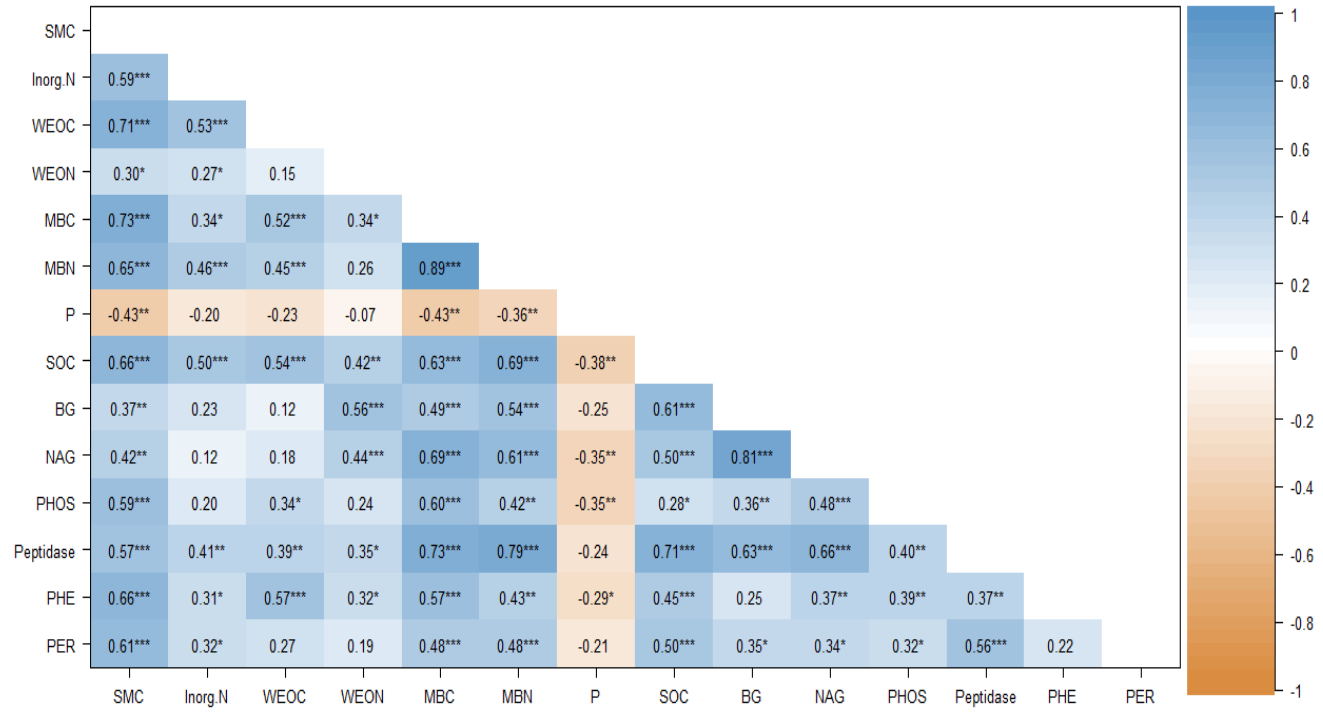

Figure S2.3. Pearson correlation plot between environmental variables and extracellular enzyme activities (EEA). Environmental variables in this figure include: soil moisture content (SMC), inorganic nitrogen (N) (nitrate plus ammonium), water extractable organic carbon (WEOC), water extractable organic nitrogen (WEON), microbial biomass C and N (MBC, MBN), melich-phosphorous (P), and total soil organic carbon (SOC). EEA are: beta-glucosidase (BG), N-acetylglucosaminidase (NAG), phosphatase (PHOS), peptidase (PEP, leucine amino peptidase (LAP) plus tyrosine amino peptidase (TAP)), phenol oxidase (PHE), and peroxidase (PER). Positive correlations are blue whereas negative correlations are orange. Correlation coefficients are shown as numbers within the boxes and the addition of an asterisk signifies significant correlations with a threshold value of 0.05.

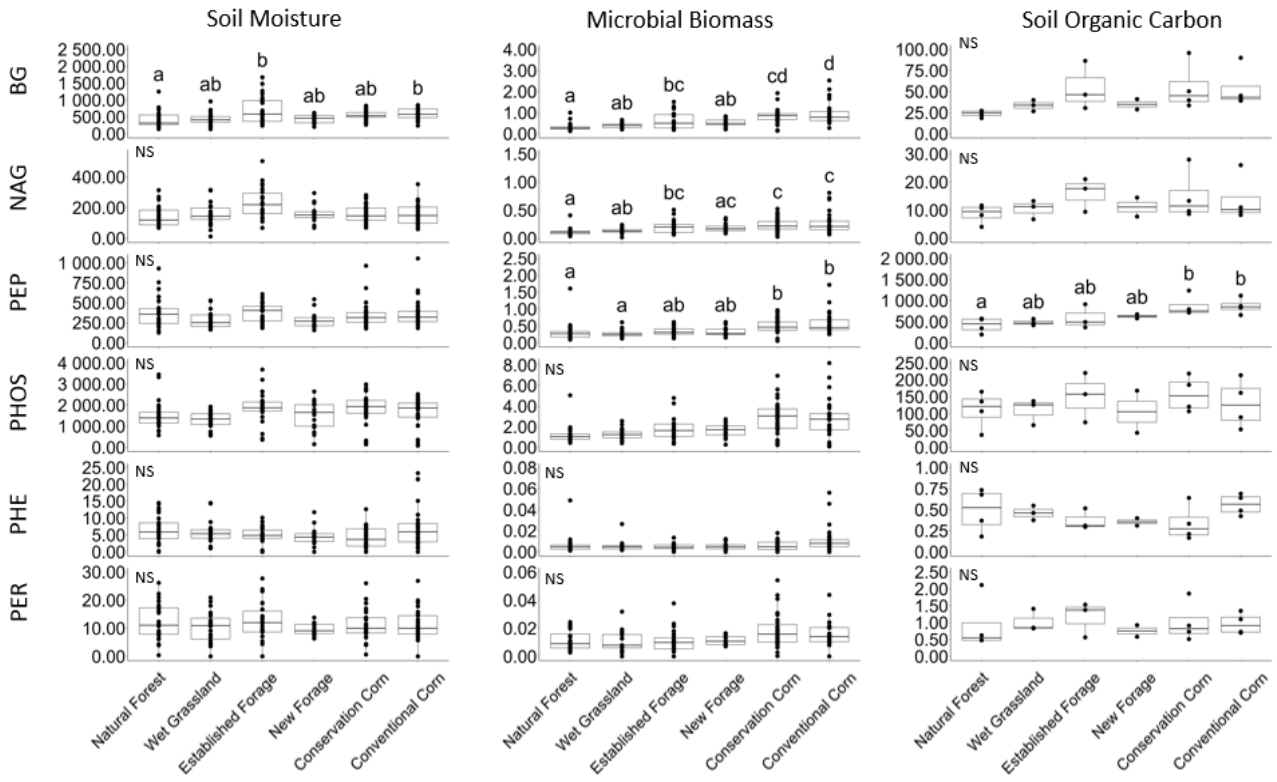

Figure S2.4. Potential extracellular enzyme activity for beta-glucosidase (BG), peptidase (PEP, leucine amino peptidase (LAP) plus tyrosine amino peptidase (TAP)), N-acetylglucosaminidase (NAG), phosphatase (PHOS), phenol oxidase (PHE), peroxidase (PER) measured in units nmol $h^{-1}g^{-1}$and umol $h^{-1}g^{-1}$ for PER and PHE, across land use treatments from low intensity (forest) to high intensity (conventional agriculture) relativized by soil moisture, microbial biomass carbon, and total soil organic carbon. Different letters indicate significant differences (pairwise post-hoc test with FDR adjustments) among land uses, NS indicates no significant differences. The line within the boxplot indicates the median, the limits of the boxes indicate the 25th and 75th quartiles. The whiskers indicate 1.5 x the quartile range.

Table S2.2. Effect of land use on potential extracellular enzyme activity linear mixed model results with repeated measures. Significant p-values ($p<0.05$) are in bold. Beta-glucosidase (BG), N-acetylglucosaminidase (NAG), phosphatase (PHOS), peptidase (PEP, leucine amino peptidase (LAP) plus tyrosine amino peptidase (TAP)), phenol oxidase (PHE), and peroxidase (PER).

Lake Location	**Extracellular Enzyme Activity Across land use**					
	BG	NAG	PHOS	Peptidase	PHE	PER
Baie	**0.038**	**0.0013**	0.25	**0.011**	0.15	0.24
Bart	**0.0037**	**0.00087**	**0.048**	**0.0082**	0.066	**0.0079**
Dupa	0.38	0.76	0.054	0.42	**0.022**	0.8
Pier	0.64	**0.036**	**0.019**	0.074	**0.021**	**0.04**

Table S2.3. Potential extracellular enzyme activity and standard deviation for beta-glucosidase (BG), peptidase (PEP, leucine amino peptidase (LAP) plus tyrosine amino peptidase (TAP)), N-acetylglucosaminidase (NAG), phosphatase (PHOS), phenol oxidase (PHE), peroxidase (PER) in units nmol $h^{-1}g^{-1}$and umol $h^{-1}g^{-1}$ for PER and PHE, across land use treatments from low intensity (forest) to high intensity (conventional agriculture)**.** Where there is no value for standard deviation only one replicate was viable for analysis.

Land use	Distance to Flood	Time	BG	Peptidase	NAG	PHOS	PHE	PER
Conventional Corn	Close	May	95.00 ± 37	91.80 ± 75	25.08 ± 15	252.78 ± 195	0.93 ± 0.61	2.68 ± 1.64
Conventional Corn	Middle	May	117.73 ± 39	65.30 ± 19	31.43 ± 17	290.68 ± 198	1.45 ± 0.24	1.97 ± 0.15
Conventional Corn	Far	May	125.95 ± 24	56.38 ± 11	27.15 ± 8	299.30 ± 121	1.33 ± 0.31	1.87 ± 0.59
Conservation Corn	Close	May	111.20 ± 26	75.55 ± 26	31.55 ± 8	286.15 ± 176	1.85 ± 0.78	1.95 ± 0.92
Conservation Corn	Middle	May	117.20 ± 31	85.45 ± 61	30.93 ± 15	383.10 ± 106	0.55 ± 0.39	2.18 ± 0.83
Conservation Corn	Far	May	111.18 ± 24	73.75 ± 29	28.20 ± 7	357.05 ± 90	0.70 ± 0.84	1.80 ± 0.48
New Forage	Close	May	73.70	39.50	17.00	36.40	1.50	2.30
New Forage	Middle	May	109.65 ± 11	50.40 ± 16	31.45 ± 8	345.35 ± 296	0.95 ± 0.07	2.15 ± 1.34
New Forage	Far	May	105.20 ± 27	82.55 ± 38	37.15 ± 8	305.70 ± 91	0.90 ± 0.42	2.05 ± 0.07
Established Forage	Close	May	145.83 ± 24	142.60 ± 56	57.93 ± 25	461.77 ± 266	1.87 ± 0.81	4.87 ± 2.54
Established Forage	Middle	May	228.40 ± 138	88.45 ± 36	76.70 ± 46	496.70 ± 9	0.90 ± 1.27	3.75 ± 0.92
Established Forage	Far	May	191.03 ± 88	79.83 ± 13	47.57 ± 24	430.83 ± 67	0.97 ± 0.90	2.17 ± 1.56
Wet Grassland	Close	May	146.10 ± 43	123.17 ± 84	48.50 ± 25	434.90 ± 223	2.20 ± 0.56	4.37 ± 1.07
Wet Grassland	Middle	May	116.93 ± 58	104.50 ± 18	30.40 ± 22	405.13 ± 162	1.30 ± 0.90	4.10 ± 1.10
Wet Grassland	Far	May	117.65 ± 28	73.95 ± 14	41.60 ± 5	365.30 ± 174	1.80 ± 0.14	3.00 ± 2.12
Natural Forest	Close	May	107.20 ± 42	119.78 ± 77	36.43 ± 5	437.88 ± 141	3.15 ± 1.53	4.08 ± 2.24
Natural Forest	Middle	May	134.53 ± 78	138.75 ± 88	46.00 ± 14	504.88 ± 89	1.90 ± 0.58	4.18 ± 2.07
Natural Forest	Far	May	125.20 ± 5	115.63 ± 51	35.37 ± 11	480.00 ± 147	1.50 ± 1.64	4.87 ± 3.12
Conventional Corn	Close	July	102.94 ± 42	72.40 ± 39	30.24 ± 15	315.45 ± 235	1.38 ± 1.67	1.61 ± 1.98
Conventional Corn	Middle	July	94.37 ± 16	61.44 ± 16	30.10 ± 15	399.88 ± 142	1.83 ± 0.84	2.44 ± 0.58
Conventional Corn	Far	July	88.22 ± 2	58.10 ± 30	23.15 ± 4	292.24 ± 24	2.21 ± 1.28	2.16 ± 0.75
Conservation Corn	Close	July	84.40 ± 43	65.19 ± 19	23.37 ± 13	304.88 ± 260	1.11 ± 0.94	1.17 ± 1.28
Conservation Corn	Middle	July	104.13 ± 12	49.18 ± 16	38.00 ± 14	411.77 ± 131	0.78 ± 0.40	2.02 ± 0.17

Conservation Corn	Far	July	77.98 ± 23	60.01 ± 5	21.66 ± 7	362.23 ± 161	0.34 ± 0.48	2.27 ± 0.87
New Forage	Close	July	82.82 ± 31	55.00 ± 17	30.20 ± 7	313.16 ± 260	2.22 ± 1.85	2.35 ± 0.41
New Forage	Middle	July	133.60	65.85	44.07	766.44	-	3.40
New Forage	Far	July	98.51 ± 45	63.10 ± 3	40.42 ± 17	356.74 ± 118	1.09 ± 1.15	2.04 ± 0.40
Established Forage	Close	July	115.39 ± 5	99.35 ± 18	50.71 ± 2	414.75 ± 282	1.90 ± 0.56	4.57 ± 0.85
Established Forage	Middle	July	183.31 ± 127	90.55 ± 51	72.07 ± 46	633.33 ± 41	1.55 ± 0.85	1.56 ± 2.70
Established Forage	Far	July	211.58 ± 130	91.30 ± 8	71.40 ± 39	645.74 ± 184	1.32 ± 0.30	1.40 ± 1.49
Wet Grassland	Close	July	157.66 ± 44	89.79 ± 26	61.43 ± 7	462.11 ± 257	1.06 ± 0.36	4.13 ± 3.83
Wet Grassland	Middle	July	140.51 ± 60	72.80 ± 23	54.03 ± 16	517.09 ± 127	3.13 ± 2.19	3.09 ± 2.22
Wet Grassland	Far	July	135.19 ± 48	111.68 ± 45	50.22 ± 15	478.87 ± 141	3.86 ± 0.70	4.12 ± 1.09
Natural Forest	Close	July	217.58 ± 248	137.32 ± 85	62.32 ± 26	558.07 ± 66	1.96 ± 2.02	4.15 ± 2.74
Natural Forest	Middle	July	121.30 ± 76	108.10 ± 28	50.91 ± 27	485.81 ± 197	2.15 ± 1.42	5.70 ± 1.89
Natural Forest	Far	July	160.06 ± 98	114.46 ± 51	46.15 ± 14	552.99 ± 136	2.54 ± 1.60	5.41 ± 2.37
Conventional Corn	Close	November	126.55 ± 43	90.14 ± 38	41.58 ± 19	339.23 ± 256	0.79 ± 0.64	3.33 ± 1.19
Conventional Corn	Middle	November	139.75 ± 34	75.40 ± 12	31.93 ± 9	363.99 ± 227	1.88 ± 1.04	1.82 ± 1.41
Conventional Corn	Far	November	214.30 ± 108	96.11 ± 56	52.43 ± 29	538.19 ± 119	1.41 ± 1.51	4.22 ± 1.87
Conservation Corn	Close	November	112.23 ± 41	68.05 ± 19	32.47 ± 13	353.28 ± 227	0.45 ± 0.64	3.60 ± 0.67
Conservation Corn	Middle	November	118.73 ± 30	63.81 ± 19	33.06 ± 14	430.54 ± 83	1.23 ± 0.74	2.48 ± 1.48
Conservation Corn	Far	November	136.27 ± 24	60.42 ± 20	36.13 ± 6	429.06 ± 143	0.82 ± 0.54	2.22 ± 0.57
New Forage	Close	November	87.53	87.13	40.70	615.69	1.31	4.14
New Forage	Middle	November	110.06 ± 9	64.95 ± 15	37.29 ± 14	397.73 ± 333	0.83 ± 0.02	2.23 ± 0.28
New Forage	Far	November	113.52 ± 4	91.69 ± 25	55.47 ± 13	399.27 ± 50	1.06 ± 0.30	1.70 ± 0.01
Established Forage	Close	November	148.01 ± 5	129.16 ± 49	61.98 ± 15	454.67 ± 299	1.49 ± 0.66	4.42 ± 2.33
Established Forage	Middle	November	223.82 ± 134	151.32 ± 72	71.89 ± 36	590.81 ± 120	2.20 ± 1.08	3.72 ± 1.29
Established Forage	Far	November	212.21 ± 97	96.38 ± 15	59.43 ± 22	555.36 ± 111	0.90 ± 0.80	4.67 ± 1.00
Wet Grassland	Close	November	269.12	161.69	77.61	648.66	1.88	4.27
Wet Grassland	Middle	November	236.12 ± 27	130.78 ± 3	94.87 ± 24	608.16 ± 174	2.49 ± 0.29	3.48 ± 3.00
Wet Grassland	Far	November	207.71 ± 88	116.44 ± 8	79.03 ± 26	617.32 ± 115	2.00 ± 0.29	4.48 ± 2.86
Natural Forest	Close	November	260.04	409.24	91.07	534.42	0.96	11.51
Natural Forest	Middle	November	281.45	272.25	96.99	536.40	3.56	3.12
Natural Forest	Far	November	195.05 ± 58	150.17 ± 61	61.69 ± 33	796.00 ± 270	3.44 ± 0.83	1.66 ± 1.37

Chapter 3

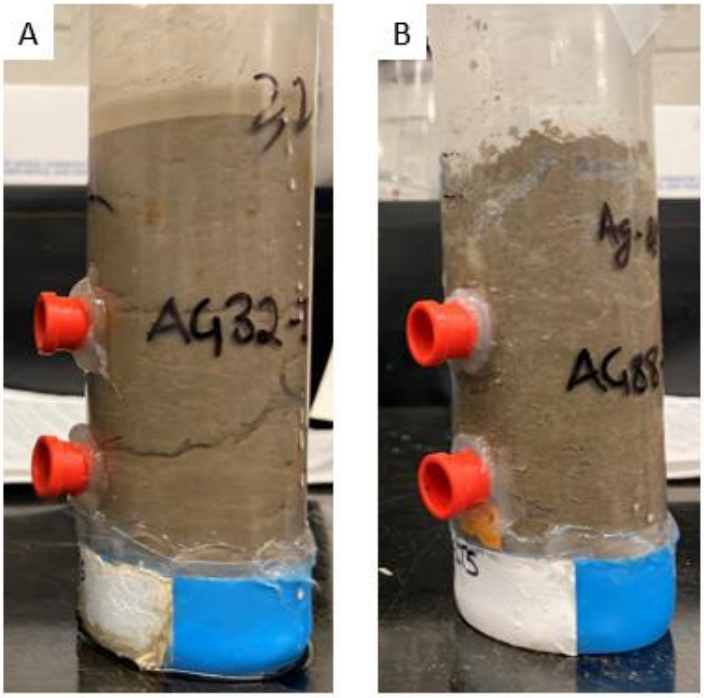

Figure S3.1. Incubation design with intact cores showing position of septa in a) flooded and b) unflooded cores.

Ampliseq with Nextseq or Novaseq reads

References

- From the github of dada2: Binned quality scores and their effect on (non-decreasing) trans rates https://github.com/benjjneb/dada2/issues/1307#issuecomment-957680971

Consequences of using dada2 on NovaSeq data https://github.com/benjjneb/dada2/issues/791

- From illumina https://www.illumina.com/content/dam/illumina-marketing/documents/products/appnotes/novaseq-hiseq-q30-app-note-770-2017-010.pdf

Description of the issue

Novaseq and Nextseq sequencing technology have a different way of calculating quality scores (Q-scores) for base calling. Q-scores are now binned into 4 groups:

| Q-scores | bins | score codes | |----------|------|-------------| |0-2 | 2 | # | |3-14 | 12 | * | |15-30 | 23 | 5 | |31-40 | 37 | C |

Error estimation and quality filtering steps in dada2 are affected by this and a workaround is proposed to overcome this caveat.

For quality filtering, nf-core/ampliseq parameters can be easily changed to obtain the desired results, but for error estimation, a modification to a script must be made.

Modification procedure

1- The file dada2_err.nf is replaced with the one provided here

2- Two R packages must be installed in a singularity container:

- The pipeline has to be run once in order to get the singularity environment called `depot.galaxyproject.org-singularity-bioconductor-dada2-1.22.0--r41h399db7b_0.img`
- Then open a terminnal into the following folder: `nf-core/work/singularity`
- Activate the singularity container: `singularity run depot.galaxyproject.org-singularity-bioconductor-dada2-1.22.0--r41h399db7b_0.img`
- In the container, start R and install dplyr and magrittr packages. Answer yes when R asks to install packages in a local library, and yes again to the next question. Install the packages with these commands. `install.packages("dplyr")` `install.packages("magrittr")`

Table S3.1. Staggered primer sequences including adaptors for both bacterial (16S rRNA) and fungal (ITS region) amplicons for both forward (ends in F) and reverse (ends in R) primers.

Amplicon Sequence	Primer ID	Primer Sequence
16S rRNA	515FP1-TruSeqF	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGCCAGCMGCCGCGGTAA
	515FP2-TruSeqF	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGTGCCAGCMGCCGCGGTAA
	515FP3-TruSeqF	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACGTGCCAGCMGCCGCGGTAA
	515FP4-TruSeqF	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAAGTGCCAGCMGCCGCGGTAA
	806RP1-TruSeqR	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGACTACHVGGGTWTCTAAT
	806RP2-TruSeqR	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGACTACHVGGGTWTCTAAT
	806RP3-TruSeqR	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACGGACTACHVGGGTWTCTAAT
	806RP4-TruSeqR	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATGGACTACHVGGGTWTCTAAT
ITS region	ITS1FP1-TruSeqF	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTGGTCATTTAGAGGAAGTAA
	ITS1FP2-TruSeqF	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTTGGTCATTTAGAGGAAGTAA
	ITS1FP3-TruSeqF	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACCTTGGTCATTTAGAGGAAGTAA
	ITS1FP4-TruSeqF	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAACTTGGTCATTTAGAGGAAGTAA
	58A2RP1-TruSeqR	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGCGTTCTTCATCGAT
	58A2RP2-TruSeqR	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTGCGTTCTTCATCGAT
	58A2RP3-TruSeqR	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGCGTTCTTCATCGAT
	58A2RP4-TruSeqR	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATCTGCGTTCTTCATCGAT

Table S3.2. Results of filtering steps from bioinformatics processing through DADA2 for each sample. The values at each step are the number of remaining sequences.

	ITS						16S					
Sample ID	DADA2_input	filtered	denoisedF	denoisedR	merged	nonchim	DADA2_input	filtered	denoisedF	denoisedR	merged	nonchim
S1_GR164_S_T1	62494	50068	49834	49910	49466	49028	36926	32953	31860	31782	28884	28650
S10_GR127_IFL_T4	67072	53100	52977	52815	52139	51595	58680	51747	49890	49703	45097	45003
S100_GR177_I_T2	47569	36547	36346	36436	36062	35891	55079	48865	47717	47790	44273	43989
S101_GR103_SFL_T2	57328	45268	45164	45171	44892	44617	61001	53656	52255	52307	48614	48433
S102_AG55_S_T4	54333	42529	42303	42244	41643	41246	81925	72823	70667	70896	64500	64258
S103_GR113_I_T2	49563	40982	40869	40913	40558	40319	61561	53945	52474	52295	48175	47929
S104_AG222_I_T1	64487	52287	52050	52054	51386	50963	71646	62890	61115	61153	55439	55401
S105_GR183_S_T4	55338	43436	43288	43346	42987	42752	64651	55469	54103	54140	50418	50229
S106_AG97_S_T2	64143	52653	52408	52425	51911	51407	64785	57166	55581	55437	50502	50326
S107_AG32_IFL_T5	61289	45982	45914	45871	45545	45227	53200	47265	45709	45647	41001	40838
S108_GR223_SFL_T5	57318	46422	46340	46306	46095	45603	65551	57392	55828	55814	51256	51054
S109_AG100_SFL_T2	44531	36177	35929	35904	35526	35367	69987	61392	59696	59681	54036	53881
S11_GR106_S_T1	61411	49377	49199	49161	48387	48068	68838	60649	59065	58894	54348	54124
S110_GR182_IFL_T5	60377	46510	46277	46261	45735	45356	62925	55583	54212	54195	50191	49923
S111_GR157_S_T4	63682	51014	50822	50776	50230	49992	56439	49168	47929	47923	44159	44046
S112_AG57_SFL_T4	67759	53858	53697	53662	53243	52879	68712	60837	59020	58963	53176	53032
S113_AG96_I_T5	52532	42149	41939	41949	41507	40966	63173	55498	54000	54088	49384	49206
S114_AG213_I_T4	36719	30059	30010	30028	29770	29413	62908	55510	53682	53482	47881	47783
S115_AG211_I_T2	55908	44196	43887	43940	43475	42829	70545	62517	60377	60100	53300	53151
S116_AG65_IFL_T5	58470	45351	45054	45172	44654	44394	27130	23909	22099	22289	18292	18277
S117_GR208_SFL_T2	72298	61477	61319	61309	60739	60009	69982	61990	60483	60468	56057	55904
S118_GR177_S_T5	42571	32646	32554	32555	32193	32034	46258	40833	39699	39537	36396	36292
S119_GR139_SFL_T4	58535	48663	48564	48538	48200	47777	58748	52203	50822	50723	46781	46648
S12_GR135_I_T5	57688	44415	44132	44215	43472	43102	65561	57276	55752	55638	51166	50918
S120_AG45_I_T5	51303	39501	39269	39267	38887	38404	49653	44140	42153	42119	36398	36213
S121_GR152_I_T4	68433	57668	57548	57515	56994	56538	66769	58871	57304	57138	52586	52343

S122_AG80_SFL_T4	61575	49278	49103	49109	48753	48323	72004	63925	62275	62155	56807	56382
S123_GR173_SFL_T2	57452	49046	48935	48882	48562	48202	47785	42752	41552	41657	38260	38228
S124_AG26_IFL_T4	65422	54263	54019	54049	53229	52379	77199	68199	66002	65874	59065	58828
S125_AG84_SFL_T4	53549	42384	42182	42167	41831	41496	74810	65562	63765	63493	57800	57654
S126_GR154_I_T2	57100	45315	45192	45222	44856	44613	61697	54544	53083	53215	49037	48811
S127_GR146_SFL_T5	59887	49629	49518	49526	49366	48973	73249	63818	62276	61999	57556	57314
S128_GR128_SFL_T5	50224	41568	41526	41503	41330	41031	70278	61420	60111	60188	55888	55712
S129_AG60_S_T5	67682	54263	54052	54068	53630	53235	68463	60288	58683	58686	53252	53151
S13_GR175_IFL_T5	51328	40496	40271	40341	39751	39517	68013	59985	58582	58481	54457	54246
S130_AG231_SFL_T4	55051	44039	43816	43834	43252	42676	70213	61432	59597	59686	54064	53905
S131a_AG73_IFL_T5	66674	51831	51543	51535	51032	50744	71695	63112	60665	60646	53688	53389
S131b_AG73_IFL_T5	47460	36942	36942	36935	35952	35952	48908	43004	41402	41495	37064	36934
S132_AG99_IFL_T4	47092	38312	38073	38046	37849	37485	65969	57824	55857	55564	49616	49458
S133_AG19_SFL_T5	57027	44377	44232	44211	43657	43417	62029	54818	52944	53044	47752	47564
S134_GR105_IFL_T2	57206	45233	45077	45052	44651	44412	46318	40619	39227	39433	35819	35656
S135_AG59_SFL_T5	67566	55296	55067	55081	54456	54126	54164	47388	45487	45742	40551	40456
S136a_AG205_SFL_T5	53825	42081	41832	41911	41489	41209	58169	51384	49298	49660	44061	43830
S136b_AG205_SFL_T5	45653	35948	35948	35947	32076	32076	60264	52913	51030	50841	44928	44866
S137_AG214_SFL_T4	53193	42448	42110	42152	41816	41473	70583	62033	60338	60460	54893	54634
S138_AG25_SFL_T2	65293	53127	52865	52874	52412	52118	52323	46578	45150	45067	41417	41299
S139_GR136_SFL_T2	55619	46525	46452	46405	46207	45397	61305	54110	52460	52000	46388	46152
S14_AG21_S_T1	63765	51798	51681	51662	51424	51035	60586	53272	51797	51961	48139	47905
S140_GR147_IFL_T4	53978	44819	44666	44657	44334	43991	67019	59278	57402	57259	51631	51477
S15_AG35_S_T1	62356	49470	49280	49172	48579	48280	56090	49410	47483	47526	41738	41601
S16_AG34_IFL_T2	68101	55378	55119	55104	54435	53753	54463	48101	46730	46751	42921	42751
S17_GR101_S_T5	51901	37437	37262	37242	36649	36307	63308	56814	55259	55225	50090	49985
S18_AG91_S_T4	55066	44162	43945	43955	43409	43101	71014	62775	61357	61051	56538	56351
S19_GR186_S_T1	67309	55813	55648	55703	55153	54686	46699	41254	39732	39591	34887	34752
S2_AG215_S_T1	52494	43001	42756	42801	42174	41665	29302	25882	24604	24683	21787	21641
S20_GR120_I_T5	64335	50693	50604	50485	49964	49721	62362	55478	53862	53616	48142	48010
S21_AG39_I_T1	61419	49349	49155	49126	48789	48114	66936	58831	57215	57031	52418	52119
S22_GR224_S_T2	57489	46121	45964	45988	45655	45291	62422	54430	52925	52607	48480	48298

S23_GR137_I_T2	55449	43839	43742	43720	43390	43263	64287	56172	54735	54762	50733	50555
S24_GR190_I_T4	64451	49813	49732	49713	49226	41237	58083	51052	49452	49543	45161	45064
S25_AG77_S_T4	55246	42051	41875	41891	41731	41438	75138	66188	64002	63927	57229	57133
S26_AG78_I_T5	56612	44441	44213	44275	43585	43114	65779	57709	56041	55865	51399	51214
S27_GR108_IFL_T5	66144	50722	50597	50617	49928	49614	61644	54483	53254	53225	49240	48951
S28_GR161_IFL_T5	52689	40878	40822	40762	40000	39804	57637	50470	48572	48594	43358	43107
S29_AG16_I_T1	50031	39914	39640	39661	39376	39006	59417	52994	51760	51716	47416	47210
S3_AG13_S_T5	56744	45541	45329	45414	44988	44574	69436	61075	59117	59286	53290	53139
S30_AG76_S_T2	61609	50183	49948	49927	49432	49034	53200	46880	45008	45138	39768	39666
S31_AG43_IFL_T4	50964	41683	41462	41421	40847	40452	53971	47323	45231	45234	39605	39461
S32_AG58_IFL_T2	61957	46933	46715	46679	46382	45296	64105	56060	54023	53985	48034	47885
S33_AG225_S_T5	67990	54775	54561	54529	54051	53250	30166	26604	25722	25626	23154	23062
S34_GR199_SFL_T4	61240	50415	50325	50339	50111	49422	64453	57439	55150	55370	49360	49213
S35_AG7_S_T4	56494	44114	43938	43853	43317	43010	52937	47501	45949	45949	41470	41365
S36_AG95_S_T2	65708	52980	52744	52690	52364	51904	66371	58173	56746	56784	52510	52334
S37_GR130_IFL_T2	50544	38999	38848	38832	38456	37766	58178	50981	49314	49117	44188	44135
S38_AG81_SFL_T2	66272	53670	53443	53437	52881	52448	71386	62761	61557	61425	57888	57759
S39_GR216_SFL_T4	65076	55475	55267	55335	54650	53832	67834	59899	57735	57829	51307	51056
S4_AG61_IFL_T2	58399	47843	47581	47566	47200	47076	69420	61459	59902	59872	55264	55073
S40_GR193_IFL_T4	64747	53615	53453	53546	53018	52726	63912	55943	53688	53856	47916	47771
S41_AG31_I_T4	59960	47466	47182	47214	46830	46294	72409	63986	62400	62394	56744	56592
S42_AG54_I_T1	53573	43243	43088	42992	42435	41989	61790	53955	52466	52431	47447	47151
S43_AG24_I_T5	60503	47505	47254	47157	46398	45963	62819	55023	53014	53094	47036	46905
S44_AG33_S_T2	54875	43011	42773	42749	42345	41910	65463	57282	55826	55924	51988	51651
S45_GR123_I_T2	49957	40884	40757	40796	40467	39765	63788	55908	54531	54629	50610	50397
S46_GR169_I_T1	51518	40690	40542	40549	40279	39759	66955	58579	56975	56798	52175	52070
S47_GR162_I_T5	64636	51477	51403	51384	50890	50359	56764	49659	48406	48370	44799	44726
S48_GR110_S_T4	61356	49719	49469	49603	48902	48518	55420	48554	46721	46571	40796	40628
S49_AG94_IFL_T5	61450	50176	49947	49907	49230	48547	63474	55851	54150	54053	49192	49003
S5_GR102_I_T5	56938	43328	43250	43228	42995	42751	40212	35346	34243	34109	30974	30847
S50_GR163_IFL_T5	60189	46821	46703	46726	46316	45852	63040	55505	53653	53673	48300	48228
S51_AG53_I_T1	57299	42675	42539	42496	42105	41858	72173	63251	61907	61804	57585	57269

S52_GR142_S_T5	55914	45126	45014	44993	44732	44299	72554	63964	62375	62264	57407	57140
S53_GR141_I_T5	65367	51524	51445	51390	51092	50718	56394	49750	47968	47945	42926	42711
S54_AG1_I_T5	57837	46333	46124	46114	45421	44987	70968	62635	61087	60995	56070	55856
S55_GR218_SFL_T4	54176	44218	44127	44153	43885	43573	63866	56169	54838	54833	50861	50701
S56_GR107_S_T4	51177	38388	38234	38186	37923	37567	65983	58060	56411	56253	51348	51079
S57_GR116_I_T1	51103	40603	40503	40416	40180	39660	59250	52039	50639	50729	47031	46878
S58_GR220_I_T1	52514	42560	42433	42444	42149	41775	72861	63938	62096	62099	55764	55477
S59_AG63_I_T2	57670	47441	47290	47288	47024	46499	59924	53080	51693	51769	47748	47621
S6_GR196_S_T5	63152	52960	52842	52829	52542	52018	67107	59024	56949	56775	50397	50209
S60_AG79_SFL_T5	54890	43449	43263	43220	42620	42242	68326	60035	58006	57734	51693	51463
S61_AG93_IFL_T5	54515	43825	43568	43564	43054	42504	55499	49228	47620	47423	42255	42119
S62_AG68_IFL_T4	63402	51580	51282	51289	50848	50201	61862	54232	52868	52692	48315	48082
S63_GR114_I_T5	60807	48273	48186	48166	47566	46960	62036	54466	53195	52881	49270	49094
S64_GR144_SFL_T5	48788	41854	41721	41806	41545	41456	60847	53696	51897	52036	46655	46496
S65_AG49_S_T4	53582	43071	42887	42938	42579	40828	68644	60664	58785	58836	53035	52835
S66_AG17_I_T4	68666	57718	57448	57458	56918	56615	69016	61259	59925	59938	55737	55638
S67_GR174_S_T5	59326	48717	48529	48597	48273	47700	64737	56627	54886	54453	48982	48798
S68_AG44_SFL_T5	55265	43807	43553	43578	43154	42563	54338	47786	45953	45958	41080	40953
S69_AG36_SFL_T2	61639	48955	48666	48684	48155	47224	66848	58601	57152	57026	52453	52223
S7_GR140_S_T4	60511	46054	46006	45999	45941	45849	72597	62830	61591	61607	57983	57726
S70_GR195_I_T1	54323	45414	45294	45358	45015	44694	40239	35343	33773	33867	29612	29518
S71_AG71_S_T5	63183	48777	48578	48599	47818	47330	67943	59772	58409	58298	54048	53795
S72_GR172_S_T1	51557	42575	42476	42498	42257	41975	72529	63369	61970	61710	57189	57060
S73_GR197_IFL_T2	65650	56678	56514	56517	55525	54071	68749	60223	58897	59091	55283	55035
S74_GR219_IFL_T4	61712	50659	50452	50392	49976	49521	74484	65265	63702	63568	58895	58689
S75_GR185_I_T4	64628	51046	50886	50891	50441	50007	60394	52704	51475	51252	47617	47423
S76_GR159_I_T4	57799	47243	47162	47004	46334	45932	73693	64825	63112	63345	58864	58629
S77_GR118_I_T4	58573	43971	43888	43888	43413	43253	58771	51441	49781	49901	45621	45489
S78_GR198_S_T2	57013	44461	44382	44292	44047	43743	68915	60556	59013	58737	54071	53903
S79_GR181_S_T2	62196	51525	51362	51386	50991	50462	53897	47616	45893	45898	40807	40682
S8_AG27_IFL_T4	31077	23883	23819	23820	23704	23648	72727	64442	62479	62319	56287	56043
S80_AG50_S_T1	66840	49797	49586	49495	48807	48501	71104	62529	60650	60583	54852	54664

S81_AG86_I_T2	63673	50485	50232	50262	49661	49338	77662	68053	66001	66084	59567	59458
S82_AG40_I_T4	57511	46182	45920	45949	45431	45074	52627	46353	44696	44720	39996	39807
S83_AG28_IFL_T2	50607	39757	39599	39526	39126	38616	58421	50875	49191	48963	43737	43601
S84_AG47_IFL_T2	57104	47657	47462	47410	46833	46480	67630	59351	58042	58084	53782	53599
S85_GR165_SFL_T4	61472	49610	49408	49470	48944	48375	76796	67965	66540	66460	62060	61867
S86_GR132_S_T2	56839	45716	45580	45522	45170	44851	72270	63509	62109	61837	57237	56993
S87_GR176_S_T2	65665	53878	53661	53777	53313	53049	70105	61592	60293	60086	56037	55700
S88_GR207_I_T1	57377	47740	47651	47606	47245	46853	55187	48594	46989	46768	42180	42027
S89_GR133_S_T1	61798	49350	49246	49194	48800	48254	69357	60793	59060	59044	53455	53208
S9_AG18_I_T2	67661	53815	53545	53566	53064	52538	59616	52442	50882	50801	46082	45877
S90_AG203_S_T2	53767	41747	41576	41531	41177	40869	57417	50842	48918	49048	43658	43496
S91_AG85_S_T4	67329	54408	54114	54060	53569	52669	61020	53612	51604	51622	45672	45479
S92_AG64_S_T2	62741	49403	49062	49119	48613	47933	65497	57403	55603	55501	49902	49771
S93_AG41_S_T1	63550	49388	49330	49242	49043	48797	55921	49247	47448	47411	42014	41911
S94_AG52_SFL_T2	51064	42253	42034	42028	41436	41308	81137	71532	69426	69298	62803	62606
S95_AG37_I_T4	55292	45722	45605	45578	45395	45011	69441	61104	59211	59096	53904	53648
S96_GR206_IFL_T4	54744	44629	44426	44441	44055	43795	66435	58890	57554	57559	53747	53541
S97_GR184_SFL_T2	64230	52619	52476	52459	52087	51635	36536	31827	30564	30727	27628	27489
S98_GR179_IFL_T2	54035	43296	43169	43226	42791	42527	57011	50111	48733	48462	44250	44057
S99_GR151_IFL_T2	53959	43934	43666	43756	43331	43061						
SStandard1	47888	40672	40663	40469	39282	39282						
SStandard2	42201	37066	37066	37061	36545	35818						
SStandard3	46974	41451	41320	41330	39926	39471						
Standard1b							19847	16234	16181	16223	16166	16166
Standard2b							9034	8019	8018	8017	8009	8009
Standard3b							4228	3695	3695	3695	3694	3694
B2_NegCon2							33	30	27	27	27	27
B3_NegCon3							122	110	109	108	108	108
B4_NegCon4							253	119	115	117	115	115
B7_NegCon7							42	17	15	15	15	15

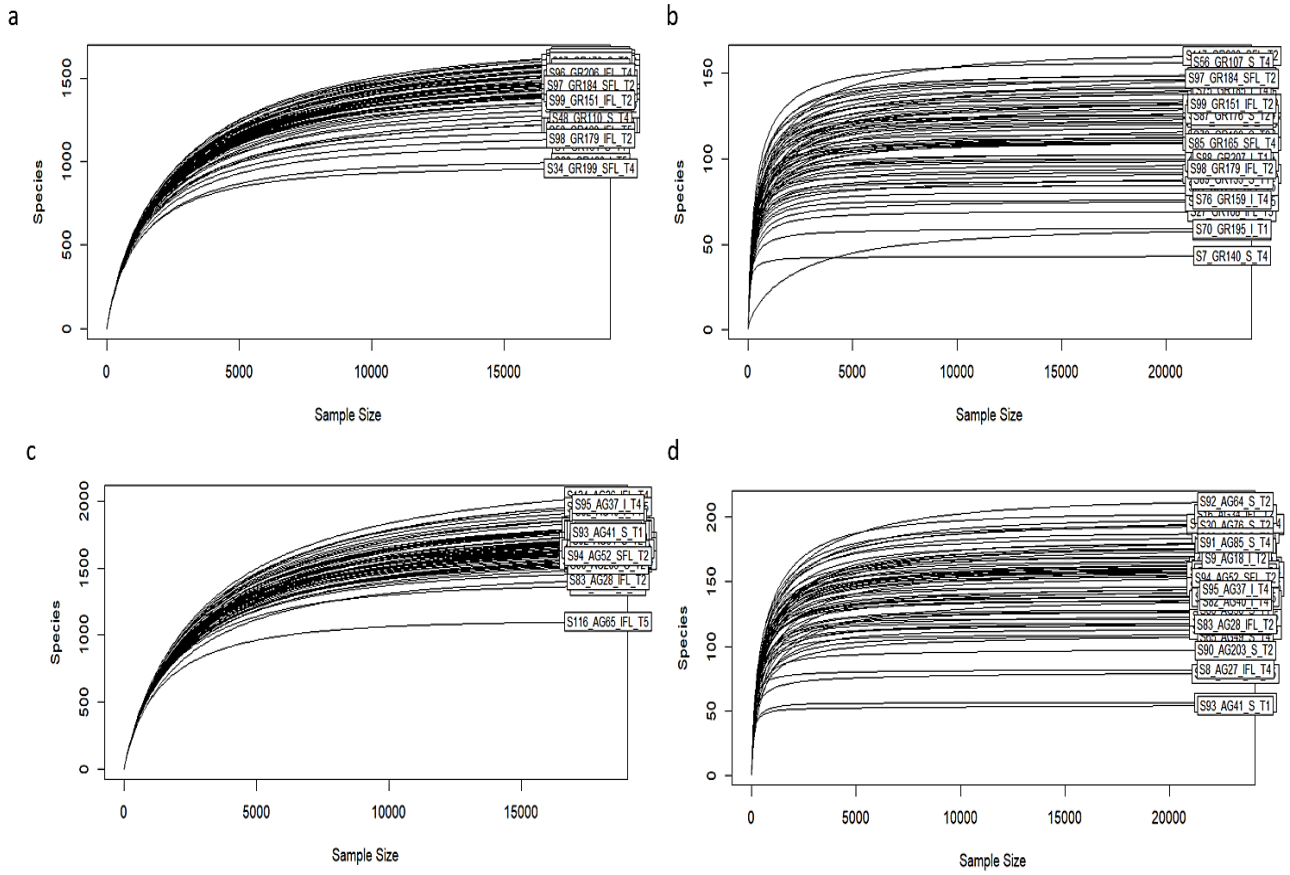

Figure S3.2. Rarefaction curves produced in R (R core team, 4.1.2, 2021) using vegan package *rarecurve* function for grassland samples for 16S rRNA (a) and ITS (b) and agriculture samples for 16S rRNA (c) and ITS region (d).

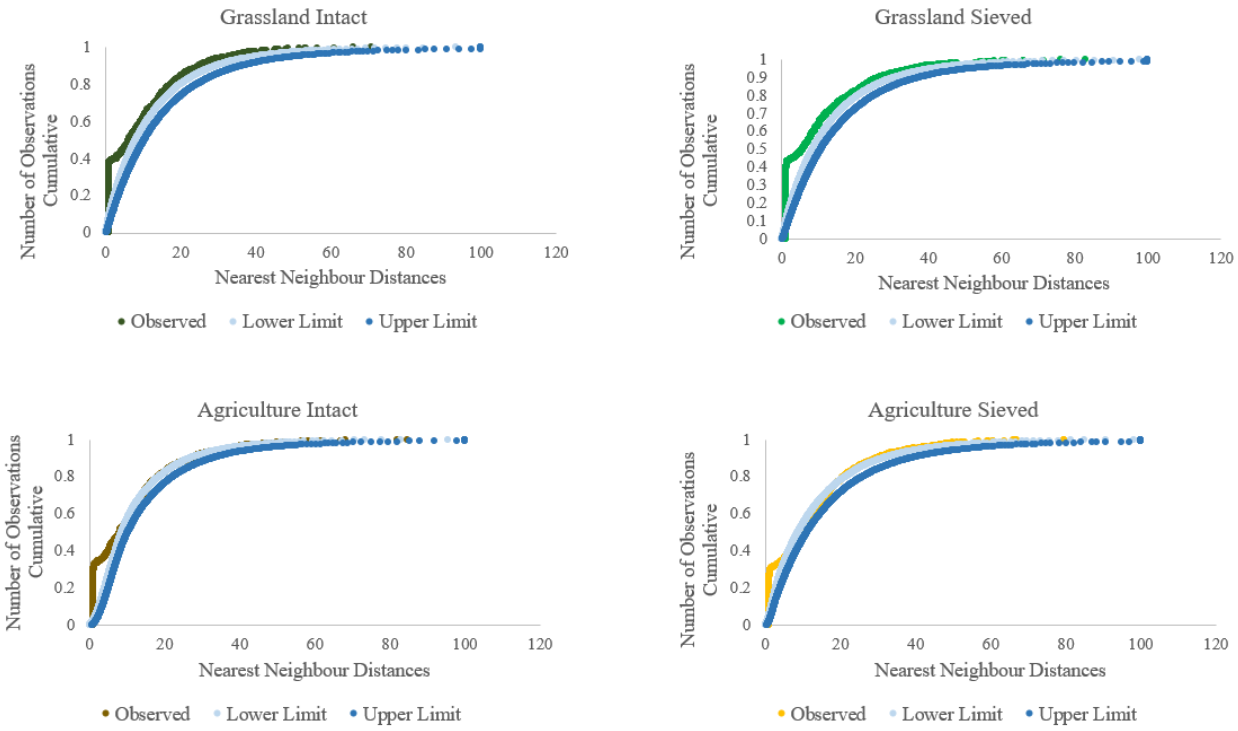

Figure S3.3. Results of statistical analysis conducted on 3-D spatial coordinates of 1000 voxels identified as 'pseudo-pore voxels' based on their CT numbers. Statistical analysis determined the frequency of each nearest-neighbour distance observed for each of the 1000 voxels. Cumulative observations for each distance are shown as the proportion of voxels at a certain distance out of the total.

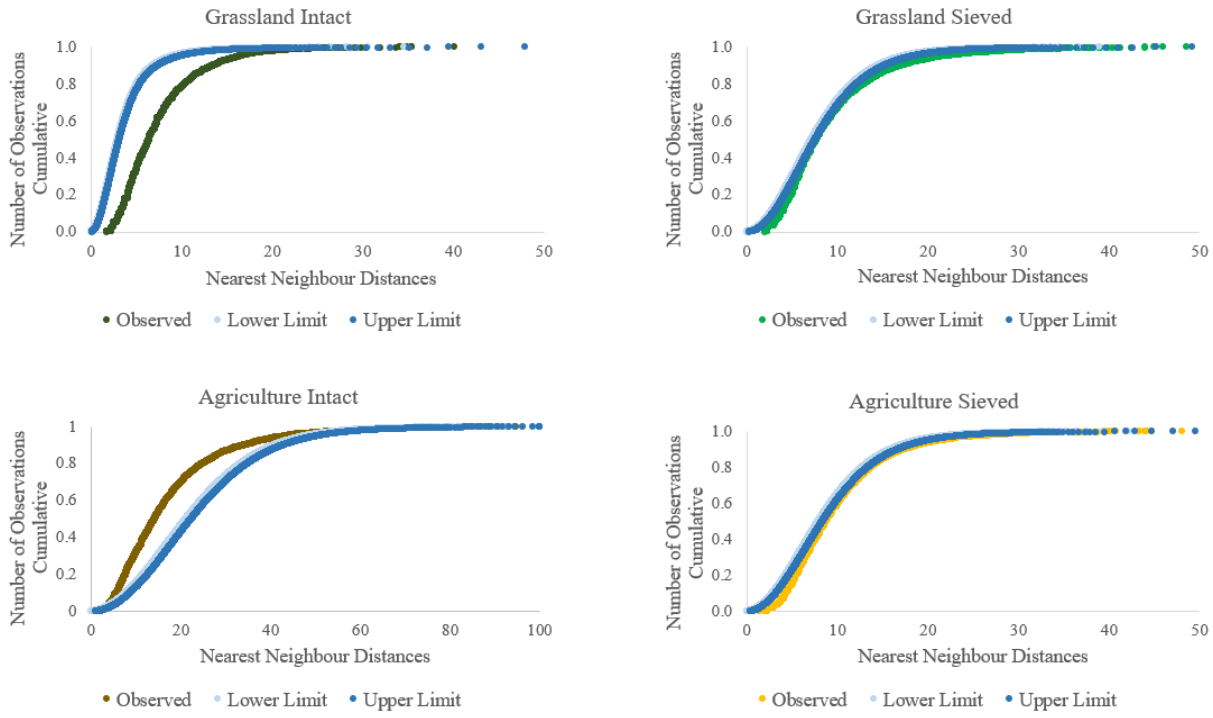

Figure S3.4. Results of statistical analysis conducted on 3-D spatial coordinates of 5000 centroids of pseudo-pores identified from 'pseudo-pore voxels' based on their CT numbers. Statistical analysis determined the frequency of each nearest-neighbour distance observed for each of the 5000 centroids. Cumulative observations for each distance are shown as the proportion of voxels at a certain distance out of the total.

Figure S3.3, S3.4 show the effectiveness of changing soil structure by sieving. The pattern of air-filled voxels (blue image units, "pseudo-pores") became closer to within the upper and lower limits, indicating increased randomness, as distances between the pores increased (Fig. S3.3, S3.4). We see the proportion of touching individual pseudo-pores increased by 5.3% and decreased by 10% from intact to sieved in the grassland and agriculture cores respectively (Fig. S3.3). This suggests that in grasslands there is increased and in agriculture there is decreased

aggregation and connectivity from intact to sieved treatments. The images illustrate the pore space in the grassland intact core (Figure 3.2a) is heterogenous with larger pore spaces and the pore space in the agriculture intact core (Figure 3.2b) is more homogenous with smaller pores. The sieving treatment re-distributed the pore space in both grassland and agriculture cores. In the grassland sieved core (Figure 3.2c) pore space appears more homogenously distributed without one large, connected space in the middle. In the agriculture sieved core (Figure 3.2d), the pore space appears more homogenously distributed throughout the core, not just concentrated near the top, as in the intact. Statistically, the degree of aggregation is approximated by the proximity of pore spaces, thus, the grassland intact cores are more aggregated than agriculture intact cores (38% vs 30% pores touching) (Figure S3.2). Further, the intact sieved core exhibits the lowest degree of aggregation (27% pores touching) and the grassland sieved cores have a similar degree of aggregation to the intact (40% pores touching).

The second statistical analysis looks at pore centroids, and thus represents individual pores themselves (Fig. S3.4), this analysis further emphasizes the difference sieving makes in the degree of aggregation of each soil. The grassland intact core has strong aggregation and sieved has a weak degree of aggregation. In the agriculture, the intact core has strong aggregation, and the sieved sample has weak aggregation.

Table S3.3. Average soil water-filled pore space for incubation (± is standard deviation) for both agriculture and grassland land uses. Water-filled pore space was calculated using an average density of 1.6 g/cm^3 for each sample. Times during flood are flood week 1 after 1 week of flooding and flood week 3 after 3 weeks of flooding.

Land Use	Structure	Flood	Time	Water-Filled Pore Space
Agriculture	Intact	UnFlood	Baseline	54.30 ± 9.54
Agriculture	Intact	UnFlood	Pre-flood	58.79 ± 3.30
Agriculture	Intact	Flood	Flood 1 wk	72.34 ± 2.39
Agriculture	Intact	UnFlood	Flood 1 wk	62.44 ± 3.81
Agriculture	Intact	Flood	Flood 3 wk	75.01 ± 3.13
Agriculture	Intact	UnFlood	Flood 3 wk	66.20 ± 4.02
Agriculture	Intact	Flood	Post-flood	55.17 ± 2.10
Agriculture	Intact	UnFlood	Post-flood	59.67 ± 2.61
Agriculture	Sieved	UnFlood	Pre-flood	51.57 ± 3.36
Agriculture	Sieved	Flood	Flood 1 wk	71.49 ± 1.70
Agriculture	Sieved	UnFlood	Flood 1 wk	66.68 ± 5.44
Agriculture	Sieved	Flood	Flood 3 wk	84.60 ± 10.27
Agriculture	Sieved	UnFlood	Flood 3 wk	67.06 ± 4.21
Agriculture	Sieved	Flood	Post-flood	64.57 ± 5.33
Agriculture	Sieved	UnFlood	Post-flood	60.76 ± 5.41
Grassland	Intact	UnFlood	Baseline	54.95 ± 6.08
Grassland	Intact	UnFlood	Pre-flood	61.16 ± 4.26
Grassland	Intact	Flood	Flood 1 wk	78.42 ± 1.41
Grassland	Intact	UnFlood	Flood 1 wk	59.41 ± 4.53
Grassland	Intact	Flood	Flood 3 wk	88.17 ± 4.28
Grassland	Intact	UnFlood	Flood 3 wk	62.05 ± 2.65
Grassland	Intact	Flood	Post-flood	56.64 ± 3.97
Grassland	Intact	UnFlood	Post-flood	58.28 ± 4.05
Grassland	Sieved	UnFlood	Pre-flood	56.83 ± 4.91
Grassland	Sieved	Flood	Flood 1 wk	76.95 ± 2.34
Grassland	Sieved	UnFlood	Flood 1 wk	60.26 ± 3.02
Grassland	Sieved	Flood	Flood 3 wk	81.17 ± 8.53
Grassland	Sieved	UnFlood	Flood 3 wk	55.36 ± 4.58
Grassland	Sieved	Flood	Post-flood	50.78 ± 11.13
Grassland	Sieved	UnFlood	Post-flood	54.36 ± 5.78

Table S3.4. Soil redox (mV) for flooded soil cores within each structure and land use. Redox was measured after day 6 flooding, day 15 flooding and after 21 days of flooding. Due to technical difficulties n=1 for each land use and structure treatment.

		Redox (mV)		
Land use	**Structure**	**Flood day 6**	**Flood day 15**	**Flood day 21**
Agriculture	Sieved	358	300	268
Agriculture	Intact	160	-	-
Agriculture	Intact	drained	291	308
Grassland	Sieved	310	313	204
Grassland	Intact	268	154	220

Table S3.5. Average soil bulk density and pH for the different land uses, soil structure, and flooding treatments and two different times (Pre-flood and at 3 weeks after flood treatment) and the standard deviation (±).

Land use	**Structure**	**Flood**	**Time**	**pH**	**Bulk Density**
Agriculture	Intact	UnFlood	Pre-flood	5.92 ± 0.12	1.06 ± 0.07
Agriculture	Sieved	UnFlood	Pre-flood	5.92 ± 0.01	0.97 ± 0.01
Grassland	Intact	UnFlood	Pre-flood	5.79 ± 0.23	0.62 ± 0.01
Grassland	Sieved	UnFlood	Pre-flood	5.70 ± 0.05	0.56 ± 0.06
Agriculture	Intact	Flood	Flood 3 wk	6.12 ± 0.18	0.98 ± 0.07
Agriculture	Intact	UnFlood	Flood 3 wk	5.94 ± 0.15	1.06 ± 0.06
Agriculture	Sieved	Flood	Flood 3 wk	5.96 ± 0.27	0.96 ± 0.11
Agriculture	Sieved	UnFlood	Flood 3 wk	6.02 ± 0.06	0.98 ± 0.07
Grassland	Intact	Flood	Flood 3 wk	6.09 ± 0.09	0.60 ± 0.06
Grassland	Intact	UnFlood	Flood 3 wk	5.52 ± 0.08	0.65 ± 0.06
Grassland	Sieved	Flood	Flood 3 wk	5.96 ± 0.01	0.60 ± 0.06
Grassland	Sieved	UnFlood	Flood 3 wk	5.64 ± 0.18	0.64 ± 0.05

Table S3.6. Average total soil C (%) and N (%) and the standard deviation (±) measured at two time points pre- and post-flood.

Land Use	Structure	Flood	Time	N %	C %
Agriculture	Intact	UnFlood	Pre-flood	0.19 ± 0.02	2.07 ± 0.18
Agriculture	Intact	Flood	Post flood	0.19 ± 0.02	2.06 ± 0.14
Agriculture	Intact	UnFlood	Post flood	0.18 ± 0.02	2.03 ± 0.11
Agriculture	Sieved	UnFlood	Pre-flood	0.18 ± 0.01	1.99 ± 0.09
Agriculture	Sieved	Flood	Post flood	0.18 ± 0.01	1.98 ± 0.10
Agriculture	Sieved	UnFlood	Post flood	0.18 ± 0.01	2.01 ± 0.14
Grassland	Intact	UnFlood	Pre-flood	0.46 ± 0.05	4.89 ± 0.60
Grassland	Intact	Flood	Post flood	0.45 ± 0.06	5.07 ± 0.77
Grassland	Intact	UnFlood	Post flood	0.46 ± 0.04	4.89 ± 0.48
Grassland	Sieved	UnFlood	Pre-flood	0.48 ± 0.03	5.43 ± 0.34
Grassland	Sieved	Flood	Post flood	0.46 ± 0.02	5.05 ± 0.28
Grassland	Sieved	UnFlood	Post flood	0.44 ± 0.05	4.77 ± 0.48

Table S3.7. ANOVA results table for soil nutrients, C, moisture, microbial biomass and enzyme activities. Differences between land uses were calculated across all treatments, other treatments were compared within each land use (grassland and agriculture). A Kruskal Wallis test was used for WEON only when comparing across land uses. SMC = soil moisture content, WEOC = water extractable organic carbon, WEON = water extractable organic nitrogen, NO_3^- = nitrate, NH_4^+ = ammonium, MBC = microbial biomass carbon, MBN = microbial biomass nitrogen, peptidase = leucine amino peptidase plus tyrosine amino peptidase, NAG = N-acetyl-glucosaminidase, BG = beta-glucosidase. Significant p values are bolded with a significance threshold of 0.05.

ANOVA Results	**SMC**	**WEOC**	**WEON**	**NO_3^-**	**NH_4^+**	**MBC**	**MBN**	**Peptidase**	**NAG**	**BG**
Land use	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Grassland										
Structure	0.87	**0.03**	0.80	0.49	0.7148	0.38	**0.005**	0.94	0.81	**0.02**
Flood	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.48	0.42	**0.046**	0.52	0.97
Time	**<0.001**	**0.04**	**<0.001**	**0.0002**	0.65	**<0.001**	0.10	0.074	**<0.001**	**<0.001**
Structure*Flood	0.21	0.43	**0.008**	0.24	0.35	**0.04**	0.11	0.75	**0.008**	**0.001**
Structure*Time	0.07	0.17	**0.004**	0.77	0.47	0.62	0.85	0.78	0.14	0.55
Flood*Time	**<0.001**	**0.02**	**0.0004**	0.81	0.69	**0.01**	0.12	0.19	0.57	0.19
Structure*Flood*Time	0.85	0.33	**0.0001**	0.62	**0.02**	**0.04**	0.08	0.22	0.15	0.83
Agriculture										
Structure	**<0.001**	0.70	0.41	**0.006**	0.06	**0.006**	**<0.001**	0.39	**0.0009**	**0.006**
Flood	**<0.001**	**0.01**	**<0.001**	**<0.001**	0.82	0.27	**0.03**	**0.01**	**0.001**	0.26
Time	**<0.001**	**0.004**	**0.02**	**0.0005**	0.06	**<0.001**	**0.003**	0.06	**0.049**	**<0.001**
Structure*Flood	0.52	0.52	0.08	**0.01**	0.53	0.26	0.1	0.18	**0.039**	**0.03**
Structure*Time	**0.001**	0.42	**0.003**	**0.006**	0.50	0.20	0.85	0.66	0.67	0.71
Flood*Time	**<0.001**	0.39	0.91	0.42	**0.03**	**0.001**	**<0.001**	0.99	0.073	0.67
Structure*Flood*Time	0.09	0.73	0.43	0.062	0.99	0.36	0.32	0.72	0.6	0.67

Table S3.8. Mean soil nutrients, C and moisture content throughout incubation (± standard deviation). SMC = soil moisture content, WEOC = water extractable organic carbon, WEON = water extractable organic nitrogen, NO_3^- = nitrate, NH_4^+ = ammonium.

Land Use	Time	Structure	Flood	SMC	WEOC	WEON	NO_3^-	NH_4^+
Agriculture	Preflood	Intact	Unflood	25 ± 0.02	9.27 ± 1.27	4.37 ± 1.27	3.80 ± 1.84	0.55 ± 0.27
Agriculture	Preflood	Sieved	Unflood	26 ± 0.01	8.26 ± 2.73	10.28 ± 12.16	9.36 ± 14.00	0.46 ± 0.38
Agriculture	Flood 1 wk	Intact	Flood	32 ± 0.02	16.97 ± 1.88	2.66 ± 0.63	1.22 ± 0.77	0.71 ± 0.24
Agriculture	Flood 1 wk	Intact	Unflood	27 ± 0.01	14.25 ± 2.78	4.93 ± 1.44	4.25 ± 1.53	0.97 ± 0.40
Agriculture	Flood 1 wk	Sieved	Flood	35 ± 0.01	15.88 ± 3.60	2.66 ± 0.51	1.34 ± 0.59	0.24 ± 0.35
Agriculture	Flood 1 wk	Sieved	Unflood	32 ± 0.01	13.16 ± 4.91	2.59 ± 0.70	1.28 ± 0.49	0.51 ± 0.73
Agriculture	Flood 3 wk	Intact	Flood	33 ± 0.02	13.61 ± 2.69	2.15 ± 0.66	1.27+/-0.46	1.34 ± 1.18
Agriculture	Flood 3 wk	Intact	Unflood	27 ± 0.02	11.99 ± 2.24	4.06 ± 0.87	3.36 ± 1.03	0.71 ± 0.47
Agriculture	Flood 3 wk	Sieved	Flood	36 ± 0.02	14.32 ±.70	3.36 ± 1.01	1.83 ± 0.62	1.33 ± 0.90
Agriculture	Flood 3 wk	Sieved	Unflood	30 ± 0.02	14.03 ± 3.29	4.45 ± 0.91	3.16 ± 1.45	1.17 ± 1.37
Agriculture	Post Flood	Intact	Flood	25 ± 0.01	12.41 ± 2.25	3.53 ± 1.10	2.85 ± 1.04	0.79 ± 0.33
Agriculture	Post Flood	Intact	UnFlood	25 ± 0.01	13.55 ± 2.64	6.83 ± 3.75	5.39 ± 3.41	1.27 ± 0.52
Agriculture	Post Flood	Sieved	Flood	29 ± 0.02	11.87 ± 5.02	3.51 ± 0.96	1.74 ± 0.67	1.27 ± 1.48
Agriculture	Post Flood	Sieved	UnFlood	29 ± 0.02	12.39 ± 3.70	4.26 ± 2.25	2.92 ± 1.64	1.06 ± 1.06
Grassland	Preflood	Intact	Unflood	43 ± 0.02	13.97 ± 2.66	13.41 ± 8.72	9.13 ± 5.61	1.29 ± 0.62
Grassland	Preflood	Sieved	Unflood	45 ± 0.03	16.52 ± 4.39	14.00 ± 4.50	12.44 ± 5.12	1.68 ± 0.52
Grassland	Flood 1 wk	Intact	Flood	53 ± 0.03	30.03 ± 7.80	8.70 ± 5.20	5.15 ± 5.09	4.07 ± 4.06
Grassland	Flood 1 wk	Intact	UnFlood	42 ± 0.03	17.15 ± 6.17	20.90 ± 6.99	22.98 ± 11.18	1.29 ± 0.59
Grassland	Flood 1 wk	Sieved	Flood	54 ± 0.02	29.74 ± 5.58	7.95 ± 1.47	5.67 ± 2.84	4.04 ± 3.59
Grassland	Flood 1 wk	Sieved	UnFlood	45 ± 0.02	20.25 ± 4.66	16.49 ± 8.83	14.92 ± 7.19	1.46 ± 0.3
Grassland	Flood 3 wk	Intact	Flood	53 ± 0.04	28.49 ± 5.31	9.07 ± 4.86	6.68 ± 5.28	4.64 ± 6.27
Grassland	Flood 3 wk	Intact	UnFlood	42 ± 0.03	17.49 ± 4.35	24.75 ± 12.03	24.51 ± 10.62	1.49 ± 0.38
Grassland	Flood 3 wk	Sieved	Flood	51 ± 0.02	34.47 ± 4.82	9.26 ± 3.76	6.82 ± 4.26	6.91 ± 7.53
Grassland	Flood 3 wk	Sieved	UnFlood	40 ± 0.03	14.89 ± 6.94	21.02 ± 7.99	21.07 ± 10.17	1.02 ± 0.32
Grassland	Post Flood	Intact	Flood	42 ± 0.03	23.54 ± 3.78	9.79 ± 5.10	7.59 ± 4.92	2.98 ± 1.89
Grassland	Post Flood	Intact	UnFlood	41 ± 0.02	14.98 ± 3.45	45.66 ± 17.88	49.78 ± 25.51	1.90 ± 1.02
Grassland	Post Flood	Sieved	Flood	41 ± 0.02	25.92 ± 8.68	11.29 ± 6.13	9.27 ± 7.27	2.18 ± 0.52
Grassland	Post Flood	Sieved	UnFlood	40 ± 0.02	15.17 ± 6.98	19.84 ± 12.60	28.81 ± 9.07	1.31 ± 0.58

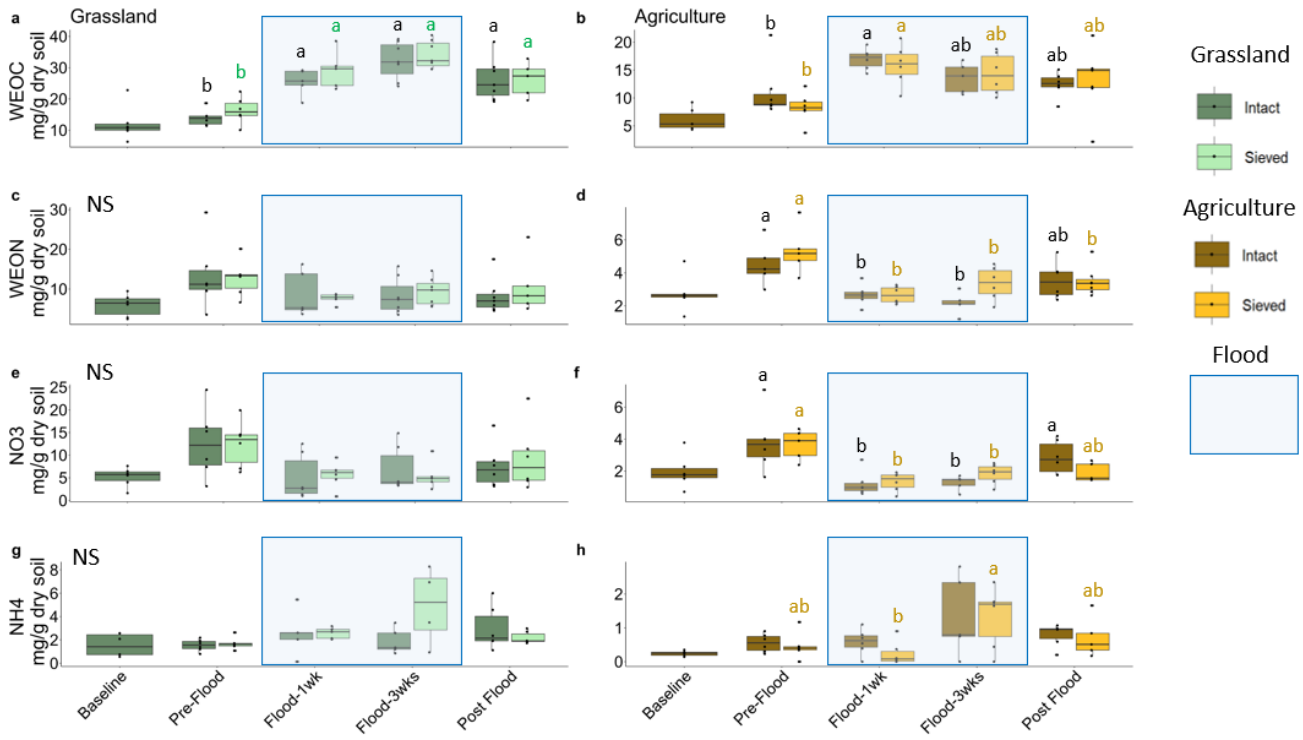

Figure S3.5. Soil water-extractable organic C and N (WEOC and WEON), soil nitrate (NO_3^-), and ammonium (NH_4^+) of flooded cores of each structure treatment varying with time within each land use, grassland (a, c, e, g) and agriculture (b, d, f, h). Letters denote significant differences over time within each structure treatment (tukeyHSD), NS signifies no significant result from tukeyHSD. Flood period for each panel is depicted by the blue box, and intact structure is in dark green (grassland) and dark orange (agriculture) and sieved structure is in light green (grassland) and yellow (agriculture). The line within the boxplot indicates the median, the limits of the boxes indicate the 25th and 75th quartiles. The whiskers indicate 1.5 times the quartile range.

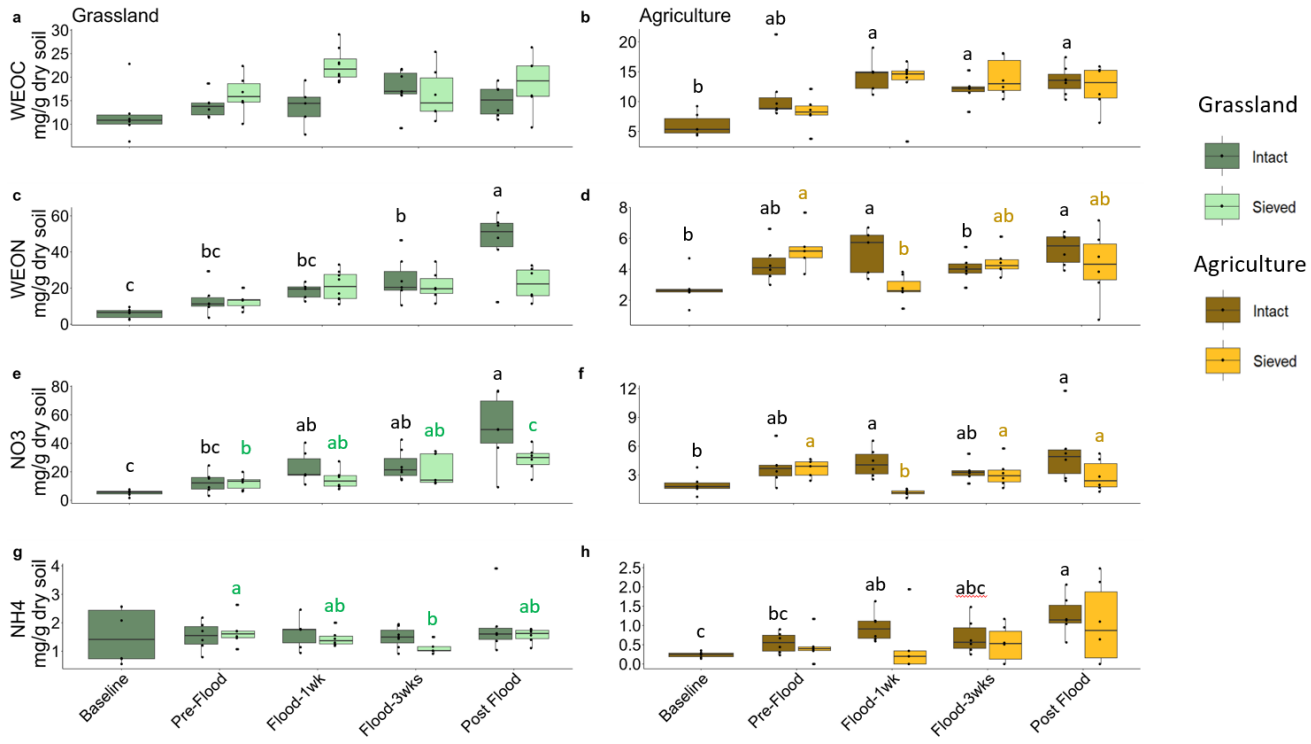

Figure S3.6. Soil water-extractable organic C and N (WEOC and WEON), soil nitrate (NO_3^-), and ammonium (NH_4^+) of unflooded cores of each structure treatment varying with time within each land use, grassland (a, c, e, g) and agriculture (b, d, f, h). Letters denote significant differences over time within each structure treatment (tukeyHSD), NS signifies no significant result from tukeyHSD. Intact structure is in dark green (grassland) and dark orange (agriculture) and sieved structure is in light green (grassland) and yellow (agriculture). The line within the boxplot indicates the median, the limits of the boxes indicate the 25th and 75th quartiles. The whiskers indicate 1.5 times the quartile range.

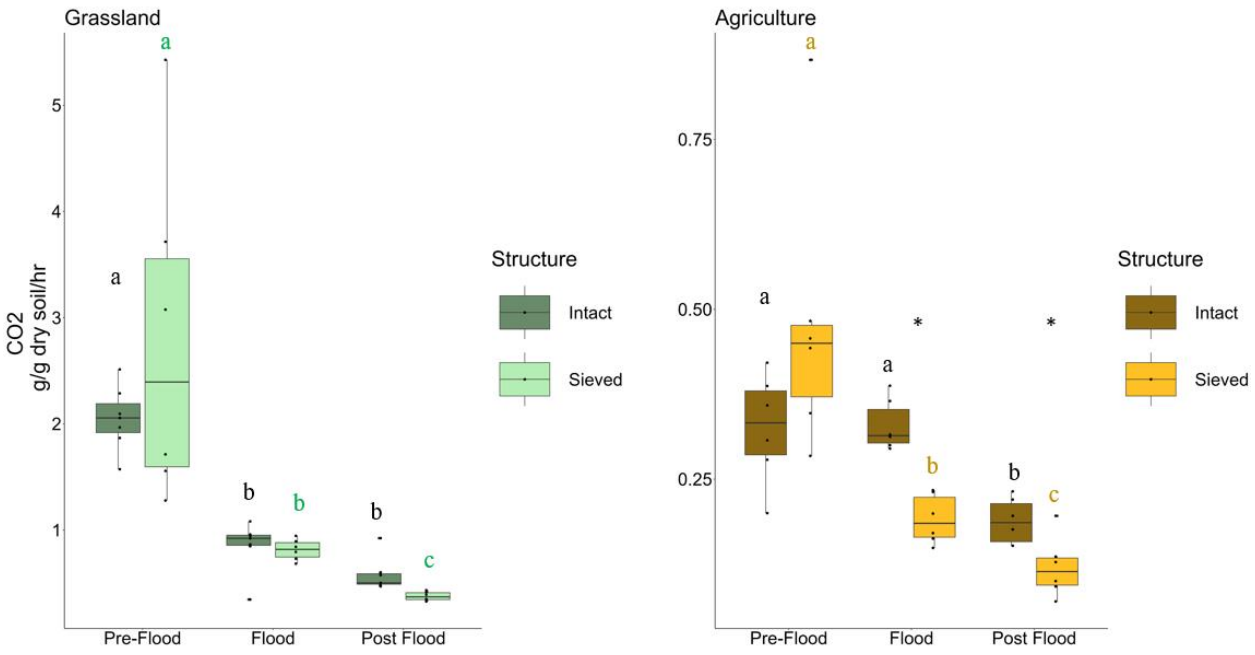

Figure S3.7. Soil CO_2 respiration in unflooded soil with sieving and without (intact) for grassland (a) and agriculture (b) soil. Respiration is presented as g CO_2 per gram dried soil per hour. Respiration data collected every 2-3 days during the incubation was grouped into three flood periods: Pre-flood, Flood, and Post-Flood. The significance letters denote differences across time within each structure treatment and the * indicates a significance between the structure treatments. The line within the boxplot indicates the median, the limits of the boxes indicate the 25th and 75th quartiles. The whiskers indicate 1.5 times the quartile range.

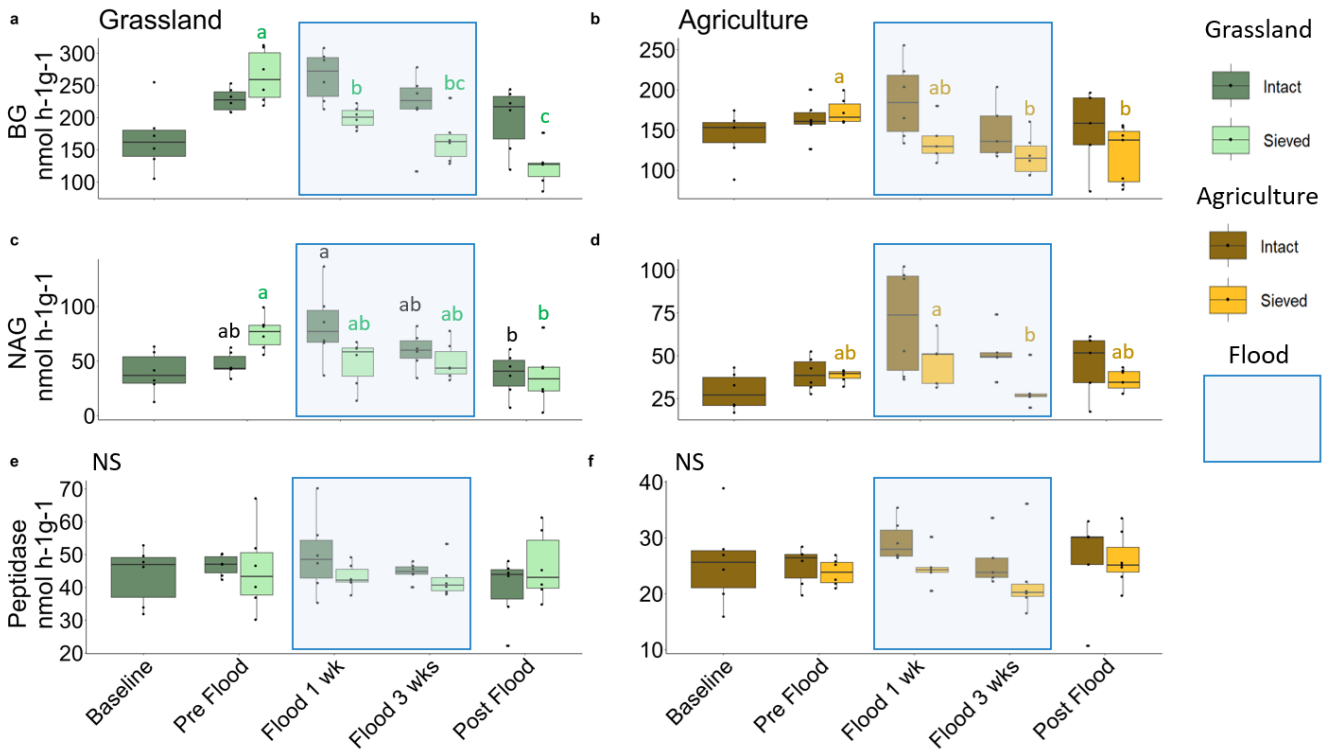

Figure S3.8. Beta-glucosidase (BG), N-1,4-acetylglucosaminidase (NAG), and peptidase (leucine amino peptidase (LAP) plus tyrosine amino peptidase (TAP) activity expressed as nmol $h^{-1}g^{-1}$ of flooded cores of each structure treatment varying with time within each land use, grassland (a, c, e, g) and agriculture (b, d, f, h). Letters denote significant differences over time within each structure treatment (tukeyHSD), NS signifies no significant result from tukeyHSD. Flood period for each panel is depicted by the blue box, and intact structure is in dark green (grassland) and dark orange (agriculture) and sieved structure is in light green (grassland) and yellow (agriculture). The line within the boxplot indicates the median, the limits of the boxes indicate the 25th and 75th quartiles. The whiskers indicate 1.5 times the quartile range.

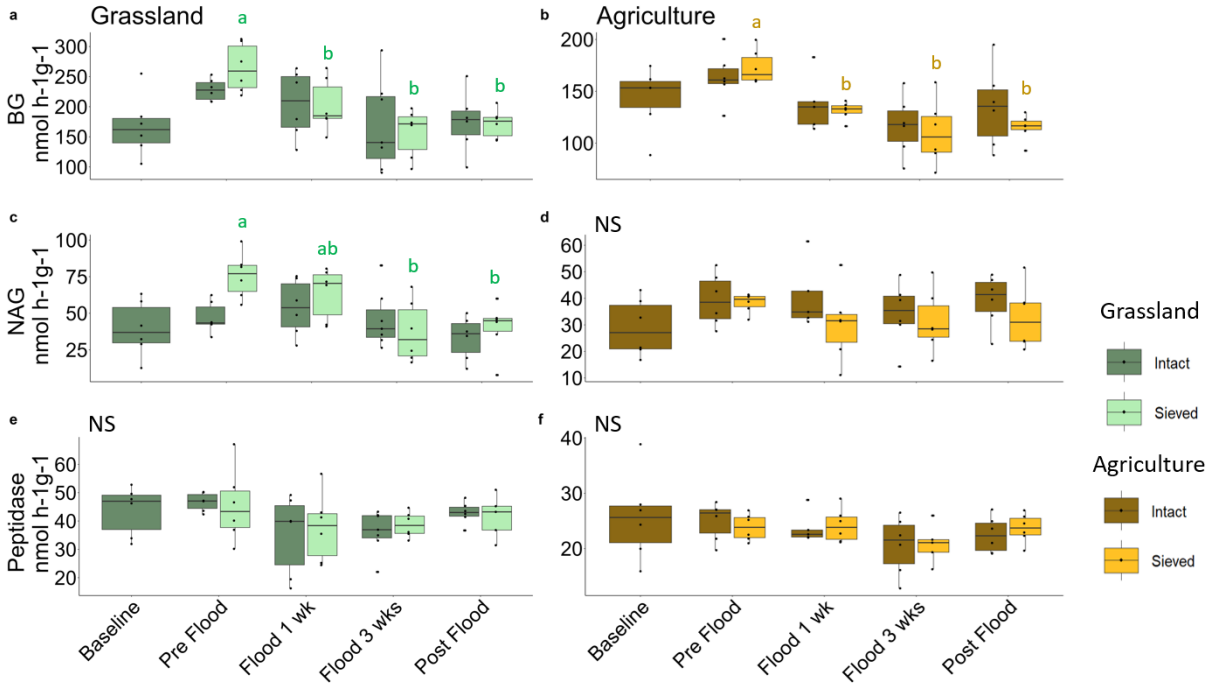

Fig. S3.9. Beta-glucosidase (BG), N-1,4-acetylglucosaminidase (NAG), and Peptidase (leucine amino peptidase (LAP) plus tyrosine amino peptidase (TAP) activity expressed as nmol $h^{-1}g^{-1}$ of unflooded cores of each structure treatment varying with time within each land use, grassland a, c, e, g and agriculture b, d, f, h. Letters denote significant differences over time within each structure treatment (tukeyHSD), NS signifies no significant result from tukeyHSD. Intact structure is in dark green (grassland) and dark orange (agriculture) and sieved structure is in light green (grassland) and yellow (agriculture). The line within the boxplot indicates the median, the limits of the boxes indicate the 25th and 75th quartiles. The whiskers indicate 1.5 times the quartile range.

Table S3.9. Mean microbial biomass C and N (MBC, MBN) and enzyme activities LAP = leucine amino peptidase, TAP = tyrosine amino peptidase, NAG = N-acetylglucosaminidase, BG = beta-glucosidase across treatments with standard deviation (±).

Land Use	Time	Structure	Flood	MBC	MBN	LAP	TAP	NAG	BG
Agriculture	Preflood	Intact	Unflood	185.64 ± 33.18	26.91 ± 5.11	16.94 ± 2.14	8.04 ± 1.35	39.42 ± 9.77	163.38 ± 24.24
Agriculture	Preflood	Sieved	Unflood	161.83 ± 22.76	22.10 ± 4.52	16.29 ± 2.00	7.56 ± 0.77	38.28 ± 3.62	173.10 ± 16.55
Agriculture	Flood 1 wk	Intact	Flood	167.66 ± 45.48	25.57 ± 8.01	19.26 ± 2.66	10.17 ± 1.68	70.12 ± 31.20	187.33 ± 48.13
Agriculture	Flood 1 wk	Intact	UnFlood	207.65 ± 28.92	33.72 ± 4.93	15.91 ± 1.32	7.85 ± 1.58	47.02 ± 14.77	137.96 ± 27.41
Agriculture	Flood 1 wk	Sieved	Flood	132.18 ± 34.59	19.80 ± 5.69	17.24 ± 2.11	7.45 ± 1.42	40.58 ± 12.49	136.62 ± 27.19
Agriculture	Flood 1 wk	Sieved	UnFlood	163.60 ± 65.45	23.70 ± 10.40	16.48 ± 1.65	7.72 ± 1.78	30.36 ± 13.96	131.30 ± 8.55
Agriculture	Flood 3 wk	Intact	Flood	235.96 ± 35.44	23.92 ± 7.84	17.02 ± 2.04	8.77 ± 2.87	51.78 ± 14.23	149.37 ± 36.18
Agriculture	Flood 3 wk	Intact	UnFlood	251.94 ± 26.62	26.20 ± 4.69	13.91 ± 4.22	6.66 ± 1.14	34.22 ± 11.92	116.83 ± 28.74
Agriculture	Flood 3 wk	Sieved	Flood	175.67 ± 19.55	13.66 ± 4.32	15.49 ± 5.96	6.95 ± 1.04	29.64 ± 10.66	118.82 ± 25.58
Agriculture	Flood 3 wk	Sieved	UnFlood	246.54 ± 24.40	24.75 ± 4.62	17.88 ± 8.26	6.51 ± 1.10	31.27 ± 11.82	110.11 ± 31.30
Agriculture	Post Flood	Intact	Flood	405.44 ± 41.79	32.56 ± 4.53	17.75 ± 5.22	7.90 ± 2.92	44.12 ± 16.67	149.02 ± 44.79
Agriculture	Post Flood	Intact	UnFlood	361.64 ± 36.74	25.87 ± 3.92	15.24 ± 2.25	7.25 ± 1.33	39.16 ± 9.71	134.73 ± 38.84
Agriculture	Post Flood	Sieved	Flood	399.13 ± 39.44	26.26 ± 2.71	20.19 ± 6.43	7.47 ± 2.34	37.84 ± 12.81	126.89 ± 56.49
Agriculture	Post Flood	Sieved	UnFlood	374.70 ± 49.25	24.03 ± 3.84	16.10 ± 1.42	7.58 ± 1.37	32.74 ± 11.96	115.04 ± 12.56
Grassland	Preflood	Intact	Unflood	869.37 ± 97.54	112.16 ± 9.45	36.09 ± 3.06	9.62 ± 0.96	53.53 ± 17.52	230.79 ± 20.19
Grassland	Preflood	Sieved	Unflood	1002.59 ± 197.69	121.84 ± 24.10	34.82 ± 9.87	10.64 ± 3.18	75.74 ± 15.63	264.47 ± 40.76
Grassland	Flood 1 wk	Intact	Flood	883.32 ± 153.51	123.90 ± 19.39	39.17 ± 10.40	10.80 ± 1.94	82.36 ± 33.93	264.41 ± 39.20
Grassland	Flood 1 wk	Intact	UnFlood	830.19 ± 131.60	115.58 ± 15.50	27.93 ± 10.97	7.38 ± 3.15	53.80 ± 19.16	204.38 ± 55.68
Grassland	Flood 1 wk	Sieved	Flood	628.47 ± 183.09	91.41 ± 21.50	35.23 ± 4.67	9.41 ± 0.68	45.28 ± 20.43	202.03 ± 19.43
Grassland	Flood 1 wk	Sieved	UnFlood	955.19 ± 227.54	138.38 ± 49.97	29.12 ± 9.06	8.56 ± 3.19	63.71 ± 17.63	201.80 ± 44.39
Grassland	Flood 3 wk	Intact	Flood	880.43 ± 168.01	107.33 ± 17.40	32.18 ± 6.83	10.78 ± 2.31	53.21 ± 13.20	202.77 ± 58.01
Grassland	Flood 3 wk	Intact	UnFlood	961.00 ± 126.02	115.01 ± 19.12	28.51 ± 6.07	7.79 ± 2.63	45.75 ± 19.52	169.43 ± 75.15
Grassland	Flood 3 wk	Sieved	Flood	825.53 ± 159.50	94.48 ± 25.64	32.94 ± 4.63	9.52 ± 1.26	49.62 ± 17.38	165.68 ± 36.94
Grassland	Flood 3 wk	Sieved	UnFlood	906.36 ± 153.74	106.65 ± 19.64	30.01 ± 3.93	8.69 ± 0.85	37.48 ± 21.15	156.41 ± 40.77
Grassland	Post Flood	Intact	Flood	1214.43 ± 198.67	125.90 ± 18.62	31.34 ± 7.46	8.34 ± 2.55	37.75 ± 19.60	197.64 ± 50.39

Grassland	Post Flood	Intact	UnFlood	1119.38 ± 80.21	121.92 ± 7.71	34.66 ± 3.50	8.27 ± 1.04	33.09 ± 14.66	174.91 ± 50.68
Grassland	Post Flood	Sieved	Flood	1185.12 ± 120.37	118.56 ± 9.58	35.59 ± 7.25	10.91 ± 3.77	36.38 ± 26.65	124.90 ± 30.88
Grassland	Post Flood	Sieved	UnFlood	1042.68 ± 120.06	106.33 ± 11.76	104.40 ± 178.23	16.06 ± 15.25	39.99 ± 17.60	171.72 ± 23.97

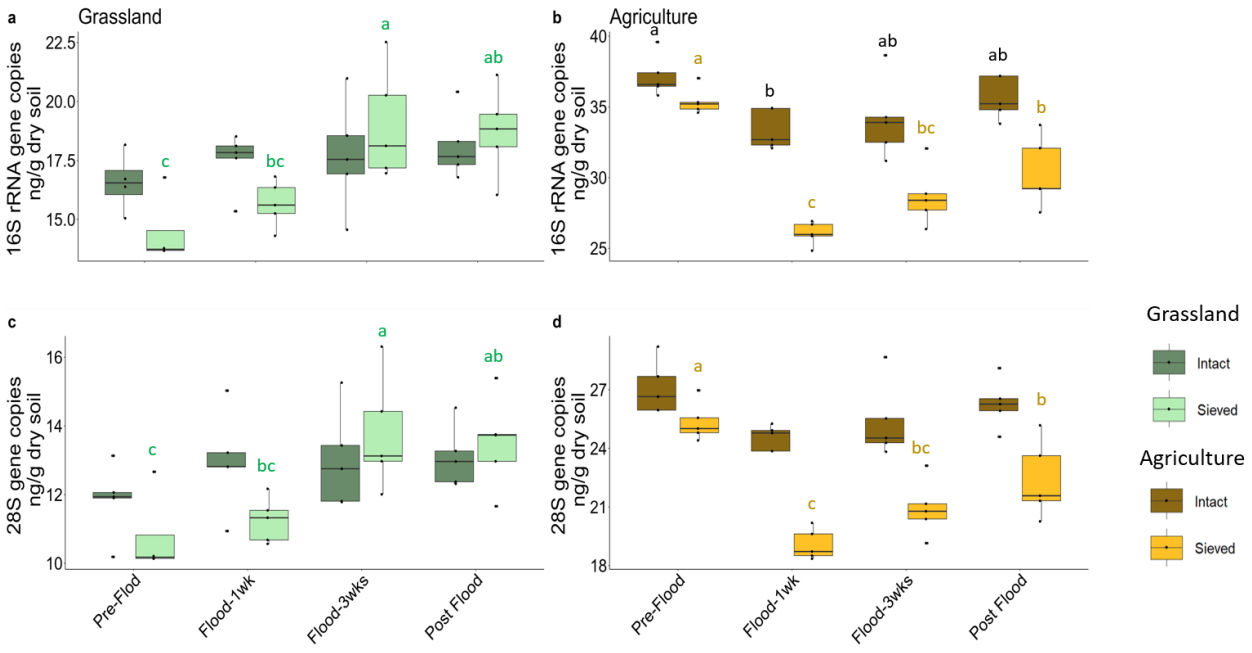

Figure S3.10. Microbial fungal (c,d) and bacterial (a,b) populations of grassland (a,c) and agricultural soils (b,d) based on qPCR of 16S rRNA and 28S gene copies across two soil structural treatments (n=4), in unflooded soils. Letters denote significant differences over time within each structure treatment (tukeyHSD). The line within the boxplot indicates the median, the limits of the boxes indicate the 25th and 75th quartiles. The whiskers indicate 1.5 times the quartile range. Gene copies are relativized by grams of dry soil.

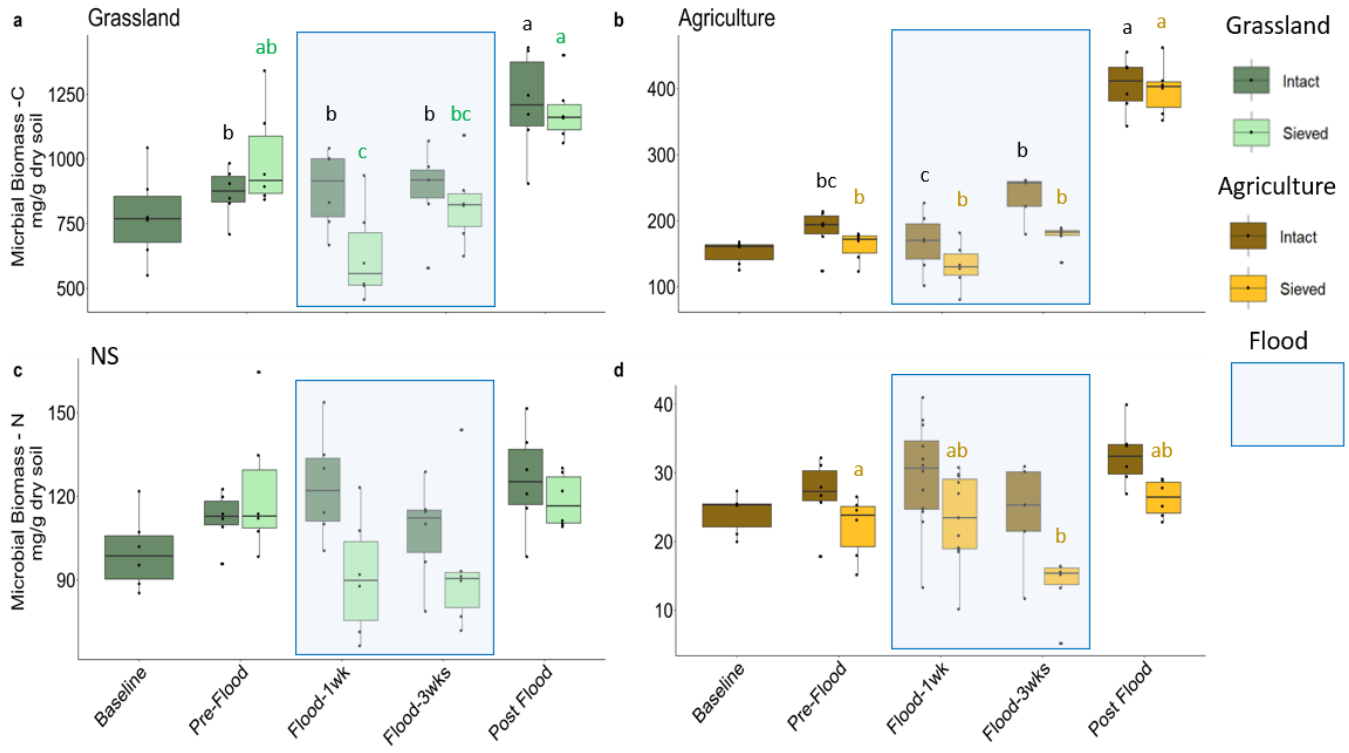

Figure S3.11. Microbial biomass C and N (MBC, MBN) in flooded cores of each structure treatment varying with time, within each land use: grassland a, c, e, g and agriculture b, d, f, h. Letters denote significant differences over time within each structure treatment (tukeyHSD), NS signifies no significant result from tukeyHSD. Flood period for MBC and MBN is depicted by the blue box, and intact structure is in dark green (grassland) and dark orange (agriculture) and sieved structure is in light green (grassland) and yellow (agriculture). The line within the boxplot indicates the median, the limits of the boxes indicate the 25th and 75th quartiles. The whiskers indicate 1.5 times the quartile range.

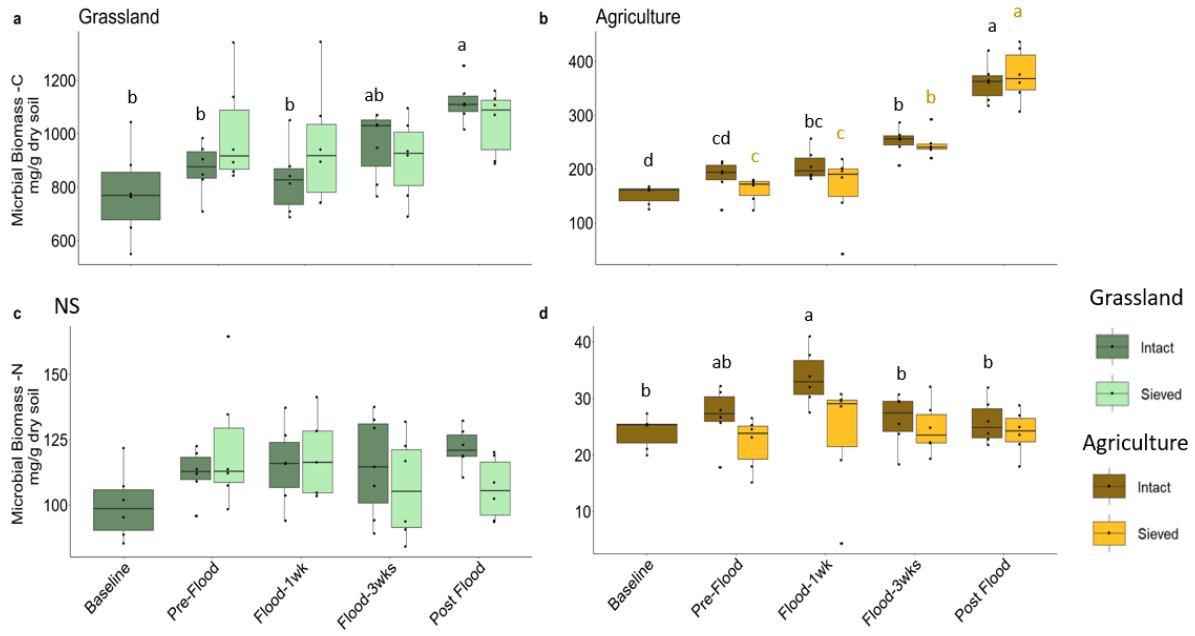

Figure S3.12. Microbial biomass C and N (MBC, MBN) in unflooded cores of each structure treatment varying with time, within each land use: grassland a, c, e, g and agriculture b, d, f, h. Letters denote significant differences over time within each structure treatment (tukeyHSD), NS signifies no significant result from tukeyHSD. Intact structure is in dark green (grassland) and dark orange (agriculture) and sieved structure is in light green (grassland) and yellow (agriculture). The line within the boxplot indicates the median, the limits of the boxes indicate the 25th and 75th quartiles. The whiskers indicate 1.5 times the quartile range.

Table S3.10. Richness (observed ASV's), Shannon diversity and inverse Simpson (evenness) of 16S rRNA for both agriculture and grassland land uses. Means and standard deviation (±).

Diversity Metric	Structure	Flood	Time	Measure ± SD Grassland	Measure ± SD Agriculture
Richness	Intact	Flood	Flood 1 wk	1386.53 ±1.85	1668.10 ±0.89
Richness	Intact	Flood	Flood 3 wks	1502.99 ±0.45	1738.68 ±1.35
Richness	Intact	Flood	Post-flood	1470.41 ±1.41	1606.45 ±2.41
Richness	Intact	UnFlood	Pre-flood	1494.37 ±0.44	1704.66 ±0.96
Richness	Intact	UnFlood	Flood 1 wk	1461.21 ±0.41	1830.63 ±0.78
fRichness	Intact	UnFlood	Flood 3 wks	1534.65 ±0.45	1875.56 ±0.60
Richness	Intact	UnFlood	Post-flood	1408.95 ±2.17	1683.97 ±1.36
Richness	Sieved	Flood	Flood 1 wk	1397.08 ±0.99	1617.42 ±1.20
Richness	Sieved	Flood	Flood 3 wks	1366.72 ±2.09	1703.29 ±0.35
Richness	Sieved	Flood	Post-flood	1490.07 ±1.33	1661.72 ±0.54
Richness	Sieved	UnFlood	Pre-flood	1407.65 ±1.69	1642.73 ±1.59
Richness	Sieved	UnFlood	Flood 1 wk	1499.63 ±1.00	1627.15 ±1.23
Richness	Sieved	UnFlood	Flood 3 wks	1393.22 ±0.50	1714.97 ±1.03
Richness	Sieved	UnFlood	Post-flood	1423.74 ±0.68	1569.01 ±1.62
Shannon	Intact	Flood	Flood 1 wk	6.54 ±0.00	6.82 ±0.00
Shannon	Intact	Flood	Flood 3 wks	6.60 ±0.00	6.87 ±0.00
Shannon	Intact	Flood	Post-flood	6.61 ±0.00	6.81 ±0.00
Shannon	Intact	UnFlood	Pre-flood	6.59 ±0.00	6.79 ±0.00
Shannon	Intact	UnFlood	Flood 1 wk	6.61 ±0.00	6.94 ±0.00
Shannon	Intact	UnFlood	Flood 3 wks	6.61 ±0.00	6.94 ±0.00
Shannon	Intact	UnFlood	Post-flood	6.54 ±0.00	6.84 ±0.00
Shannon	Sieved	Flood	Flood 1 wk	6.56 ±0.00	6.75 ±0.00
Shannon	Sieved	Flood	Flood 3 wks	6.53 ±0.00	6.78 ±0.00
Shannon	Sieved	Flood	Post-flood	6.59 ±0.00	6.80 ±0.00
Shannon	Sieved	UnFlood	Pre-flood	6.52 ±0.00	6.76 ±0.00
Shannon	Sieved	UnFlood	Flood 1 wk	6.58 ±0.00	6.76 ±0.00
Shannon	Sieved	UnFlood	Flood 3 wks	6.52 ±0.00	6.82 ±0.00
Shannon	Sieved	UnFlood	Post-flood	6.57 ±0.00	6.76 ±0.00
Inverse Simpson	Intact	Flood	Flood 1 wk	323.15 ±0.79	525.66 ±1.44
Inverse Simpson	Intact	Flood	Flood 3 wks	337.04 ±0.95	526.29 ±1.30
Inverse Simpson	Intact	Flood	Post-flood	332.59 ±0.87	532.24 ±1.76
Inverse Simpson	Intact	UnFlood	Pre-flood	333.73 ±0.81	446.26 ±0.76
Inverse Simpson	Intact	UnFlood	Flood 1 wk	351.04 ±0.59	595.05 ±0.62
Inverse Simpson	Intact	UnFlood	Flood 3 wks	337.96 ±0.51	531.93 ±0.72
Inverse Simpson	Intact	UnFlood	Post-flood	311.05 ±1.02	541.34 ±0.68
Inverse Simpson	Sieved	Flood	Flood 1 wk	351.19 ±0.95	433.39 ±0.89
Inverse Simpson	Sieved	Flood	Flood 3 wks	336.36 ±1.01	433.00 ±1.00
Inverse Simpson	Sieved	Flood	Post-flood	330.11 ±0.56	477.37 ±1.03

Inverse Simpson	Sieved	UnFlood	Pre-flood	319.60 ±0.98	459.47 ±1.13
Inverse Simpson	Sieved	UnFlood	Flood 1 wk	324.90 ±1.19	452.53 ±0.99
Inverse Simpson	Sieved	UnFlood	Flood 3 wks	308.13 ±1.43	482.63 ±1.28
Inverse Simpson	Sieved	UnFlood	Post-flood	327.34 ±0.86	469.78 ±0.72

Table S3.11. Richness (observed ASV's), Shannon diversity and inverse Simpson (evenness) of ITS region for both agriculture and grassland land uses. Means and standard deviation (±).

Diversity Metric	Structure	Flood	Time	Measure ± SD Grassland	Measure ± SD Agriculture
Richness	Intact	Flood	Flood 1 wk	121.36 ±0.38	155.95 ±0.25
Richness	Intact	Flood	Flood 3 wks	121.85 ±0.23	131.22 ±0.28
Richness	Intact	Flood	Post-flood	113.93 ±0.28	136.11 ±0.31
Richness	Intact	UnFlood	Pre-flood	107.12 ±0.19	149.17 ±0.30
Richness	Intact	UnFlood	Flood 1 wk	96.50 ±0.25	148.98 ±0.18
Richness	Intact	UnFlood	Flood 3 wks	103.11 ±0.37	136.95 ±0.56
Richness	Intact	UnFlood	Post-flood	115.27 ±0.38	156.55 ±0.25
Richness	Sieved	Flood	Flood 1 wk	136.13 ±0.24	159.15 ±0.17
Richness	Sieved	Flood	Flood 3 wks	113.80 ±0.24	156.92 ±0.27
Richness	Sieved	Flood	Post-flood	95.38 ±0.36	141.55 ±0.22
Richness	Sieved	UnFlood	Pre-flood	116.06 ±0.22	118.91 ±0.20
Richness	Sieved	UnFlood	Flood 1 wk	123.93 ±0.07	176.10 ±0.25
Richness	Sieved	UnFlood	Flood 3 wks	109.83 ±0.35	158.83 ±0.58
Richness	Sieved	UnFlood	Post-flood	121.32 ±0.28	140.35 ±0.23
Shannon	Intact	Flood	Flood 1 wk	3.09 ±0.00	3.68 ±0.00
Shannon	Intact	Flood	Flood 3 wks	3.09 ±0.00	3.50 ±0.00
Shannon	Intact	Flood	Post-flood	3.16 ±0.00	3.64 ±0.00
Shannon	Intact	UnFlood	Pre-flood	2.97 ±0.00	3.62 ±0.00
Shannon	Intact	UnFlood	Flood 1 wk	3.15 ±0.00	3.66 ±0.00
Shannon	Intact	UnFlood	Flood 3 wks	2.78 ±0.00	3.39 ±0.00
Shannon	Intact	UnFlood	Post-flood	3.04 ±0.00	3.64 ±0.00
Shannon	Sieved	Flood	Flood 1 wk	2.85 ±0.00	3.83 ±0.00
Shannon	Sieved	Flood	Flood 3 wks	3.00 ±0.00	3.72 ±0.00
Shannon	Sieved	Flood	Post-flood	2.99 ±0.00	3.74 ±
Shannon	Sieved	UnFlood	Pre-flood	3.01 ±0.00	3.78 ±0.00
Shannon	Sieved	UnFlood	Flood 1 wk	3.12 ±0.00	3.79 ±0.00
Shannon	Sieved	UnFlood	Flood 3 wks	3.08 ±0.00	3.69 ±0.00
Shannon	Sieved	UnFlood	Post-flood	3.05 ±0.00	3.67 ±0.00
Inverse Simpson	Intact	Flood	Flood 1 wk	9.98 ±0.08	18.72 ±0.06
Inverse Simpson	Intact	Flood	Flood 3 wks	8.62 ±0.02	17.33 ±0.10
Inverse Simpson	Intact	Flood	Post-flood	9.34 ±0.04	17.99 ±0.07
Inverse Simpson	Intact	UnFlood	Pre-flood	8.44 ±0.04	16.74 ±0.06

Inverse Simpson	Intact	UnFlood	Flood 1 wk	10.94 ±0.02	19.87 ±0.05
Inverse Simpson	Intact	UnFlood	Flood 3 wks	8.53 ±0.06	16.61 ±0.11
Inverse Simpson	Intact	UnFlood	Post-flood	8.39 ±0.03	17.71 ±0.03
Inverse Simpson	Sieved	Flood	Flood 1 wk	7.20 ±0.01	20.27 ±0.06
Inverse Simpson	Sieved	Flood	Flood 3 wks	9.13 ±0.04	17.89 ±0.07
Inverse Simpson	Sieved	Flood	Post-flood	9.38 ±0.06	21.06 ±0.06
Inverse Simpson	Sieved	UnFlood	Pre-flood	8.14 ±0.03	24.74 ±0.05
Inverse Simpson	Sieved	UnFlood	Flood 1 wk	9.77 ±0.04	19.34 ±0.07
Inverse Simpson	Sieved	UnFlood	Flood 3 wks	10.86 ±0.08	18.42 ±0.05
Inverse Simpson	Sieved	UnFlood	Post-flood	9.02 ±0.03	18.90 ±0.07

Table S3.12. ANOVA results for microbial community abundances, and diversity metrics. Land use mean comparisons were made across land uses, structural treatments, and time (top line). Additional ANOVAs were conducted within each land use (grassland and agriculture). Significant p values are in bold.

Variables	Community abundances		Bacteria Diversity metrics			Fungal Diversity metrics		
	16S rRNA	28S rRNA	Richness	Evenness	Shannon	Richness	Evenness	Shannon
Land use	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Grassland								
Structure	0.61	0.82	0.24	0.65	0.15	0.37	0.90	0.24
Flood	**<0.001**	**<0.001**	0.62	0.36	0.90	0.37	0.71	0.51
Time	**<0.001**	**<0.001**	0.99	0.78	0.94	0.73	0.77	0.71
Structure*Flood	0.09	0.09	0.90	0.24	0.73	0.18	0.52	0.38
Structure*Time	**0.04**	0.07	0.23	0.82	0.36	0.33	0.48	0.97
Flood*Time	**<0.001**	**<0.001**	0.24	0.80	0.32	0.11	0.56	0.57
Structure*Flood*Time	0.6	0.43	0.98	0.31	0.66	0.92	0.96	0.75
Agriculture								
Structure	**<0.001**	**<0.001**	**0.02**	**0.0003**	**0.0007**	0.49	0.067	**0.024**
Flood	**<0.001**	**<0.001**	0.26	0.61	0.33	0.86	0.89	0.67
Time	**<0.001**	**<0.001**	**0.046**	0.20	0.13	0.15	0.37	0.29
Structure*Flood	0.9	0.86	0.08	0.64	0.33	0.49	0.71	0.99
Structure*Time	**0.005**	**0.01**	0.57	0.21	0.33	0.17	0.26	0.74
Flood*Time	**<0.001**	**<0.001**	0.54	0.67	0.50	0.96	0.90	0.97
Structure*Flood*Time	**0.018**	**0.01**	0.97	0.62	0.83	0.54	0.84	0.91

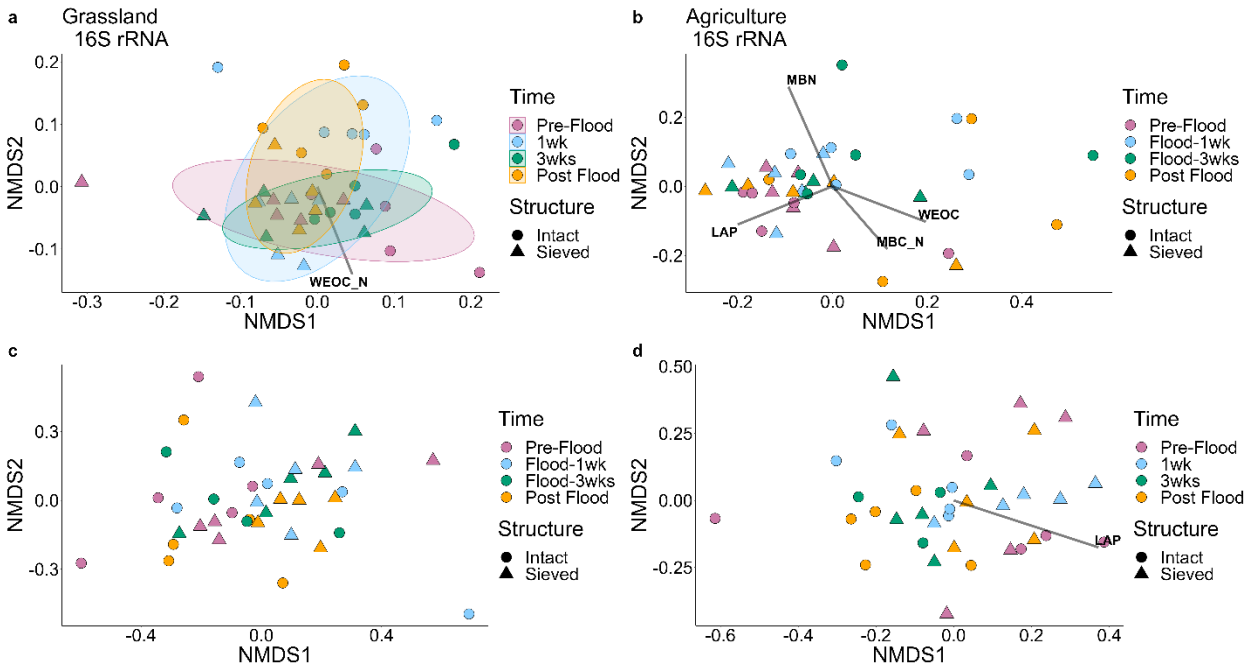

Figure S3.13. NMDS (Bray-Curtis) of soil 16S rRNA (a,b) and ITS (c,d) ASVs in grasslands (a,c) and agriculture (b,d) over the course of the incubation in unflooded soils and two soil structures: with sieving and without sieving (intact). Stress NMDS for all plots were < 0.2. Time is indicated by colour and ellipses when significant differences occur within time (based on PERMANOVA), soil structure is denoted by circles and triangles for intact and sieved soil. Environmental parameters that significantly associated with axis 1 or 2 are shown by vectors, with the following abbreviations: WEOC = water extractable organic carbon, WEOC_N = water extraction C:N ratio, MBC_N= microbial biomass C:N ratio, MBN = microbial biomass nitrogen and LAP = leucine amino peptidase.

Table S3.13. Baseline mean soil moisture, nutrient, carbon, microbial biomass and extracellular enzyme activity for the incubation. Soil moisture content (SMC, %), water extractable organic, carbon (WEOC, mg g^{-1} dry soil), water extractable organic nitrogen (WEON, mg g^{-1} dry soil), nitrate (NO_3^-, mg g^{-1} dry soil), ammonium (NH_4^+, mg g^{-1} dry soil), microbial biomass carbon (MBC, mg/g dry soil), microbial biomass nitrogen (kg g^{-1} dry soil), leucine amino peptidase (LAP, nmol $h^{-1}g^{-1}$), tyrosine amino peptidase (TAP, nmol h^{-1} g^{-1}), N-1,4-acetylglucosaminidase (NAG, nmol $h^{-1}g^{-1}$), β-glucosidase (BG, nmol $h^{-1}g^{-1}$). The * indicates a significant ($p<0.05$) difference by land use based on one-way ANOVA.

Treatments			Moisture and Nutrients					Microbial Biomass and Enzyme Activity					
Time	**Land Use**	**Structure**	**SMC**	**WEOC**	**WEON**	**NO_3^-**	**NH_4^+**	**MBC**	**MBN**	**LAP**	**TAP**	**NAG**	**BG**
Baseline	Agriculture	Baseline	23 ± 0.05	6.09 ± 1.96	2.76 ± 1.09	1.98 ± 1.02	0.24 ± 0.08	152.46 ± 17.77	24.10 ± 2.89	17.23 ± 6.75	8.44 ± 3.82	28.96 ± 10.82	143.18 ± 30.81
Baseline	Grassland	Baseline	*43 ± 0.04	*12.05 ± 5.35	7.32 ± 5.66	*5.19 ± 2.11	*1.54 ± 0.96	*777.25 ± 173.54	*100.06 ± 13.39	*35.81 ± 8.06	7.88 ± 1.56	39.46 ± 18.98	167.22 ± 51.11