Impact of rising seawater levels and subsequent flooding on microbial community function in terrestrial soils

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Impact of rising seawater levels and subsequent flooding on microbial community function in terrestrial soils

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Declaration

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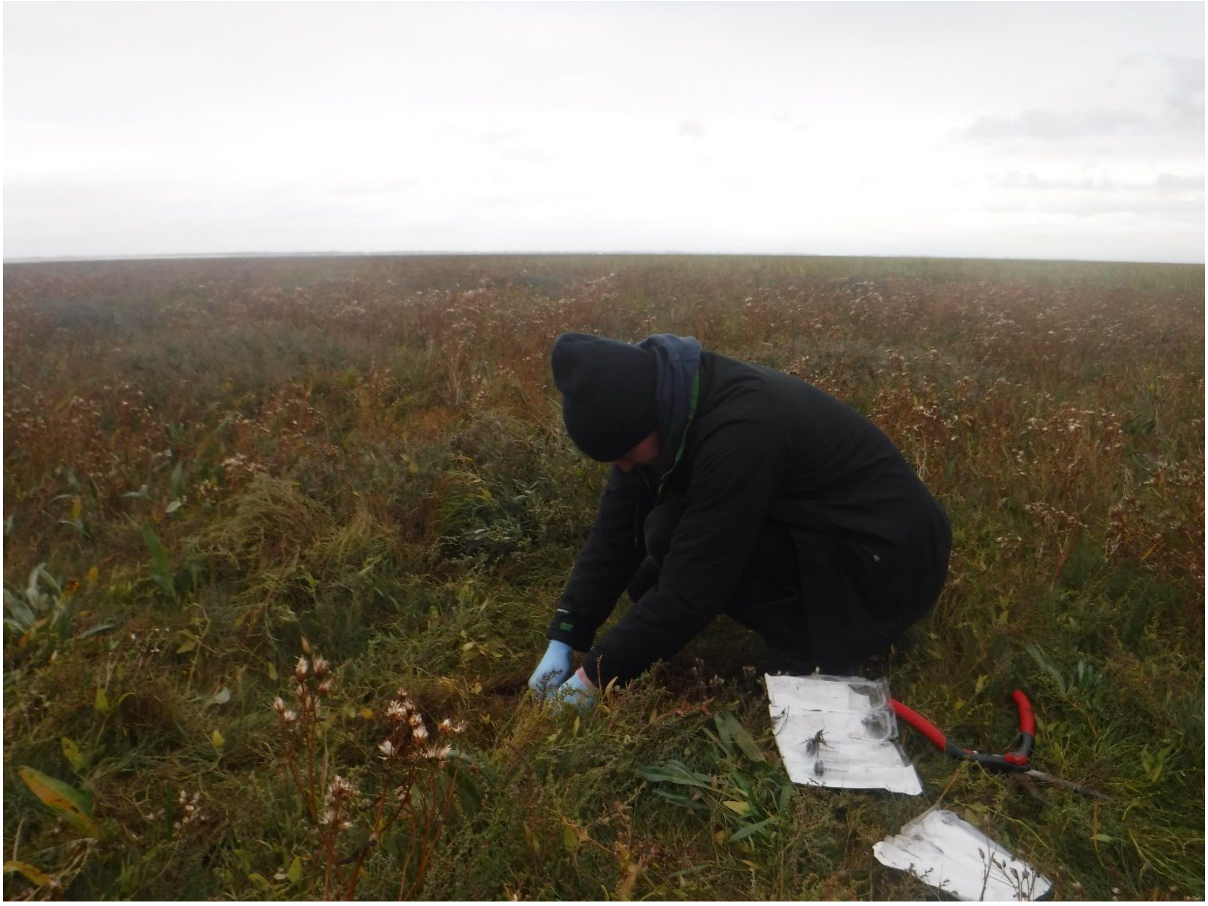


Abstract

Storm surges, flooding, and the encroaching of seawater onto agricultural land is predicted to increase with climate change fundamentally altering soil properties. It is therefore important to understand the knock-on effect seawater flooding may have on the microbial community and its functioning. This study uses a mesocosm approach to simulate the flooding of terrestrial soil and monitor 1) changes in soil environmental parameters (pH, metal concentration, conductivity), 2) microbial functioning (metabolic activity and degradative enzymes), and 3) microbial community composition (16S sequencing). The system chosen was a naturally occurring saltmarsh-terrestrial pasture gradient where three sites were sampled: Low and High elevation Saltmarsh and agricultural pastureland, incorporating a legacy of differing levels of seawater ingress and exposure. The hypotheses tested in this study were that (1) resistance of microbial community functioning and structure to not be altered during seawater flooding is a factor of pre-adaptation to the stress, and (2) this adaptation will result in communities resilient to flooding, returning to previous state prior to flooding. Soil mesocosms, established from soil taken at the sample sites, were exposed to seawater flooding for durations of 0, 1, 96- and 192-hours submersion. This equated to 72 mesocosms sampled both immediately after the flooding, and after a 14 day “recovery” period. The physicochemical properties significantly increased with seawater flooding in pasture, but not saltmarsh soils. Whilst metabolic activity decreased in all sites post flooding, the saltmarsh communities were more resilient to seawater inundation, and recovered at a faster rate to levels pre flooding. Communities from saltmarsh sites also had a higher resistance to flooding retaining enzymatic function following prolonged exposure. These results suggest that communities previously exposed to flooding have increased resistance / resilience to seawater inundation, and those that do not are significantly impacted even after short flooding durations.

1. Introduction

Microbial communities underpin processes key for life to exist on Earth (Zak et al., 2003). If these communities are damaged through stress, then functioning of key ecosystem processes can be disrupted (Unger et al., 2009). One stress that is becoming more dominant is soil inundation by seawater (Teng et al., 2014). Anthropogenic induced climate change has impacted global sea levels causing an amplification of coastal flooding events as a result of continued sea-level rise (SLR) and increased storminess (Slangen et al., 2016). A storm surge is a rise in sea level attributed by high winds from storm events pushing towards the coast. This process generates large waves that can damage and breach coastal defences along the coast; flooding land with subsequent coastal flooding cause a large amount of environmental, economic, and social consequences (Cheikh and Momen, 2020). As storm surges increase in frequency and intensity, a rise of seawater flooding events across the planet have been observed (Narayan et al., 2017; Somboonna et al., 2014). These storm surges not only cause erosion of the land, but also cause changes in the soil environment introducing high salinity and anoxic conditions under prolonged flooding events (Warrence et al., 2002; Randle-Boggis et al., 2018). It is therefore highly pertinent to understand the consequences of seawater flooding on different terrestrial soil.

1.1 Saltmarshes and coastal agricultural land

Coastal ecosystems are areas where land meets the sea that includes a diverse range of habitats (coral reefs, mangroves, saltmarshes) responsible for a range of ecosystem services consisting of natural flood defences, carbon sequestration and erosion control (Barbier et al., 2011). Saltmarshes are threatened coastal ecosystems located in the upper tidal zones between land and seawater that are subjected to cyclic variations in flooded regularly due to tidal movements. Saltmarshes are one of the largest "Blue Carbon" storage syncs on our planet. These ecosystems have high rates of productivity and anoxic sediments that impede decomposition, subsequently sequestering carbon and reducing overall atmospheric CO_2 from Anthropogenic emissions (Shepard et al., 2011).

Due to the location between land and oceans these coastal ecosystems play a key role in reducing the impacts of storm surges. As waves approach the shore, energy is lost due to vegetation obstruction and wave attenuation, naturally dissipating wave energy from storm surges (Jadhav et al., 2013). The naturally occurring saltmarsh gradient that occurs due to accretion of sediments and tidal movements creates a natural elevation which in turn protects land with the growth of vegetation, further supported by low levels of decomposition and high moisture from frequent inundation (Möller, 2006). This natural elevation created due to sedimentation leads to different elevations of saltmarsh subsequently being subjected to differing levels of tidal inundation (Li et al., 2018), which is stressed by increasing storm surges (Crosby et al., 2016).

Exposure to varying tidal inundation leads to zonation within the saltmarsh ecosystems hosting a range of unique habitats and biodiversity as a subsequent adaptation of the ecosystem, able to cope with frequent changes in salinity and osmotic pressures (Lee et al., 2016). A large amount of research has been dedicated to understanding the impacts of tidal inundation and zonation on saltmarsh vegetation within these unique ecosystems (Lee et al., 2016). Previously a study examined the community response to saltmarsh restoration and found positive responses in microbial communities potentially facilitating the regrowth of saltmarsh vegetation (Lynum et al., 2020), however the gap in understanding of the microbial community function and structure in these same zones is largely unexplored. Loss of saltmarshes can be detrimental as they play key roles in protecting low-lying farmland soils previously unexposed to seawater submersion against coastal erosion and reducing flooding impacts. Frequent inundation is common in saltmarshes, but less so in coastal agricultural land due to elevation or sea level defences (Betzold and Mohamed, 2017). Agricultural ecosystems are crucial for food and bioenergy production which are driven by microorganisms by way of nutrient cycling essential for crop productivity, regulating water quality and carbon sequestration (Power, 2010).

Salinity is an environmental stress that at high enough levels can impair metabolic functions of living organisms. Increasing salinity is represented by the electrical conductivity of soil and is key within soil ecosystems to measure amounts of salts present (K, Na, Cl in soils). Increasing salinity within soils alters physiochemical properties and changes within the soil ecosystems (Daliakopoulos et al., 2016). As salinity increases changes in water availability and an increase of ions can suppress plant growth and alter plant communities (Shrivastava and Kumar, 2015). Studies have shown the effects of salinization on agricultural soils due to irrigation, however these events do not fully saturate the soils (Glenn et al., 1998). Whereas coastal agricultural soils can be exposed to salinity and saturated due to seawater intrusion and limited knowledge is known of this intrusion on community function and structure (Weissman and Tully, 2020).

There has been an increase in focus of studies analysing salinization within wetlands and marshland ecosystems (Herbert et al., 2015) , however the focus onto terrestrial agricultural land and subsequent impact on microbial community functioning is underwhelming. The ingress of salinity as well as extended waterlogging is an increased stress factor on soil communities. Studies have shown that increased salinity will reduce biomass, activity and negatively impact community structure / function (Luo et al., 2019). Increased levels of salts present in the soils increase osmotic pressure potentially killing microbes and plants. Both plants and microbes are capable of utilizing resources and energy for defence mechanisms to prevent lysis due to osmotic pressure by production of osmolytes (Wichern et al., 2006). However, these osmolytes are energy intensive to produce and due to environmental characteristics being altered, an alteration in microbial functioning and composition can be observed. (Schimel et al., 2007)

Disruption of the environmental characteristics in soil can have knock on effects for the natural function and key ecosystem processes driven by microbial communities (Unger et al., 2009; Chaudhary et al., 2016). As microbial communities are responsible for nutrient cycling of essential elements such as carbon, nitrogen, sulphur, key for ecosystem processes (Lynum et al., 2020). Changes within soil composition and structure caused by seawater flooding will reduce the amount of organic matter, nutrient cycling, and availability of water for crop production (Bünemann et al., 2018). Flooding from storm surges affects soil properties and ecosystems fostering anoxic conditions altering above and below ground processes by altering microbial composition, activity, and functioning (Stagg et al., 2017). This could impair the ability for these ecosystems to sequester carbon as well as impact the rate of accretion in wetland ecosystems leading to a loss of saltmarsh (Chambers et al., 2016) or nutrient cycling within agricultural lands. The constant changes within selective pressures will lead to competition between communities and will eventually lead to a community that thrives and tolerates aerobic and anaerobic conditions (Randle-Boggis et al., 2018).

1.2 Perturbations and the response of microbial communities

Disturbance within communities can be attributed to changes in the overall abiotic and biotic factors directly impacting the communities or that indirectly impacts by altering the natural environment (Drenovsky et al., 2010). Disturbances have been categorised as either *pulse* short term events (seawater inundation from storm surges) or *presses* long term continuous events (CO_2 sequestration, Ocean acidification) (Shade et al., 2012). Resistance is the ability of the community function or composition, to not be altered during a disturbance. Whereas if a community is sensitive, substantial impacts of disturbances will alter community function and composition to an altered state. Resilience is the ability of the community to return to the original compositional state following a disturbance (Allison and Martiny, 2008).

Alterations in community functioning and composition can be indicators of environmental stresses and changes within ecosystems. Changes in microbial biodiversity and function has been shown to increase during environmental stresses (flooding inundation) (Galand et al., 2016). Communities are sensitive to disturbances within soil ecosystems; however, functions can be resistant to stress during changes in abiotic conditions regardless of how the composition of the community is altered (de Vries et al., 2012). Microbial communities are linked to soil quality and responsible for regulating key processes within the soil environment for example promoting plant productivity, organic material recycling and regulation of nutrient mineralisation and acting as an environmental buffer to perturbations (Heijden et al., 2008). Ecosystem functionality is classified as the rate or existence of key processes, function is highly dependent on the structure of the community that performs them (Griffiths et al., 2007). High diversity and richness allow communities to become resilient to disturbances and ability to recover quickly following perturbations (Fierer et al., 2007).

Whilst some communities are resistant and resilient to environmental disturbances, there is limited knowledge in understanding the impacts of pulse and press disturbances on the response of microbial community functioning within wetland ecosystems and subsequent flooding of coastal agricultural soils (Shade et al., 2012). Stress adapts individual components to the environmental stresses as uniquely adapted communities to salinity and anoxic conditions dominate, predicted that prolonged seawater inundation within an environment will alter the community composition and function to a uniform structure (Marshall et al., 2018). Comparing the communities from the saltmarsh sites against agricultural land, it is expected that the communities from the salt marsh sites frequently exposed to saltwater during tidal movements will be more resistant to inundations. Resulting in a more resilient community able to retain or recover to the previous composition and structure prior to inundation (Bardgett and Caruso, 2020). Data generated will allow the comparison of the habitats against each other, elucidating how differential levels of flooding has caused communities to diversify.

1.3 Flooding disturbances and changes in biogeochemical cycling

Soil health is linked to multiple abiotic and biotic factors that influence productivity of plant production and microbial communities (Heijden et al., 2008). Community activity can be limited and inhibited by changes in abiotic conditions within the environments such as salinity, pH, conductivity, and the influx of available inorganic or organic substrates (Sinsabaugh, 1994). Saltwater ingress disrupts biogeochemical processes within ecosystems as community activity and composition are altered. This alteration in activity and composition can be detrimental to soil processes involving the cycling of carbon and nitrogen, as microbial communities are responsible for key ecosystem processes. (Suddick et al., 2013; Margesin and Niklinska, 2019). For example, increased salinity will alter the soil redox potential and electron acceptors, changing how the microbes utilise resources within the environment. This increased salinity will also decrease solubility of dissolved oxygen with methanogenesis becoming the dominant reducing pathway within anaerobic conditions (Mayer and Conrad, 1990). Following the onset of anaerobic conditions, alternative terminal electron acceptors e.g., S, NO3, Mn, Fe become dominant, along with anaerobic respiration (Conrad, 2020; Weston et al., 2006) altering community function and potentially altering soil quality and carbon substrate dynamics.

An indicator of community function and soil health is extracellular enzyme activity (Dick, 1994; Dick et al., 1997). Within wetland and marshes ecosystem's microorganisms are key for organic matter decomposition and release extracellular enzymes to for hydrolysis of complex molecules within soil ecosystems for microbial and plant uptake. (Arnosti, 2003). There is conflicting knowledge concerning the impacts of salinity on microorganism derived enzymic activity. Previous research has demonstrated a decrease in extracellular activity (Xie et al., 2020), however inundation has been shown to have a more significant relationship on enzyme activity (Chambers et al., 2014). Current studies have not compared the extracellular activity of microbes from three different sites with varying degrees of previous exposure to seawater inundation and salinity.

1.4 Aims and objectives

This project aims to elucidate how seawater flooding of saltmarshes and coastal agricultural soils impacts microbial community function and structure. To investigate the ability of communities to retain function and the ability of these communities to recover following an inundation. Flooding events were conducted using plant-soil mesocosms from sites with different previous exposures to seawater, to factor legacy effects into the experiment.

Hypothesis 1 – Exposure to flooding events will cause natural microbial communities to become more distinct in terms of functioning

Hypothesis 2 – Communities from habitats frequently exposed to seawater inundation will have increased resistance and resilience of function and structure during prolonged flooding events.

Objectives

- Establish plant-soil mesocosms in controlled conditions using natural soil communities exposed to differing seawater inundation
- Monitor changes in the soils geochemical profile (metal analysis, carbon/nitrogen analysis)
- Measure community function (metabolic activity (ATP), biomass, enzyme degradation of exudates).

- Determine community structure from each site before flooding events, immediately after and during the recovery period following the seawater stress event.

2. Materials and Methods

2.1 Study Site

This study focused on an area along the Sefton Coast that has a range of unique coastal ecosystems and species, under pressure from a combination of SLR and coastal erosion (Rooney, 2010). A unique range of soft and hard engineering strategies have been adopted along this coast due to the impacts of SLR and subsequent attempts for humans to protect urban areas from flooding events (Figure 1), demonstrating the pertinence of this research.

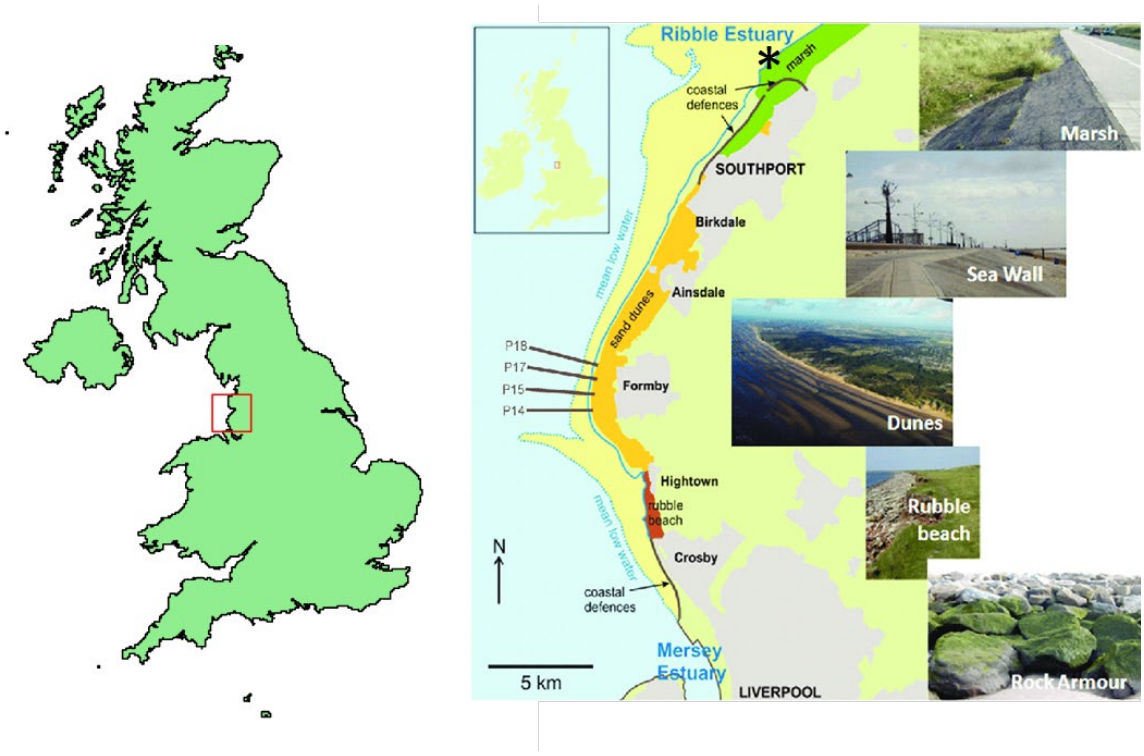


*Figure 1 Map of the UK with study site location identified, and an enlarged version of the Sefton coast with examples of the coastal locations and respective defences taken from (Souza et al., 2013). * Indicates the area of interest looking at Saltmarsh and seawall defences.*

Soil samples to be used in the mesocosms were sampled from saltmarsh and agricultural pasture sites located nearby RPSB Marshside, Southport (53° 40' 33.6"N, 2° 58' 51.60"W). The sampling sites consisted of two saltmarsh areas at different elevations and thus tidal flooding regimes (Figure 3), one at a low elevation (53° 40' 44.4"N, 2° 59' 31.2"W) that is frequently flooded (~100-200 events per year), and one at a high elevation (53° 40' 37.2"N, 2° 59' 13.2"W) that floods only ~10-20 events per year (per comms. H L Mossman.). Vegetation at the low elevation site was dominated by *Puccinellia maritima* and *Spartina anglica*, with *Festuca rubra* and *Agrostis stolonifera* dominating the high elevation site. Finally, an actively grazed terrestrial pasture site (hereafter, Pasture) was sampled (53°39' 59.98"N, 2° 59' 27.91"W). This area was located landward of the saltmarsh area behind an embankment built as sea defence. This area has not been flooded with sea water since the establishment of a sea wall in 1959 (Wisse, 1997). The saltmarsh soils at both sites consisted of a clay sediment, with a sandy loam soil at the Pasture site.

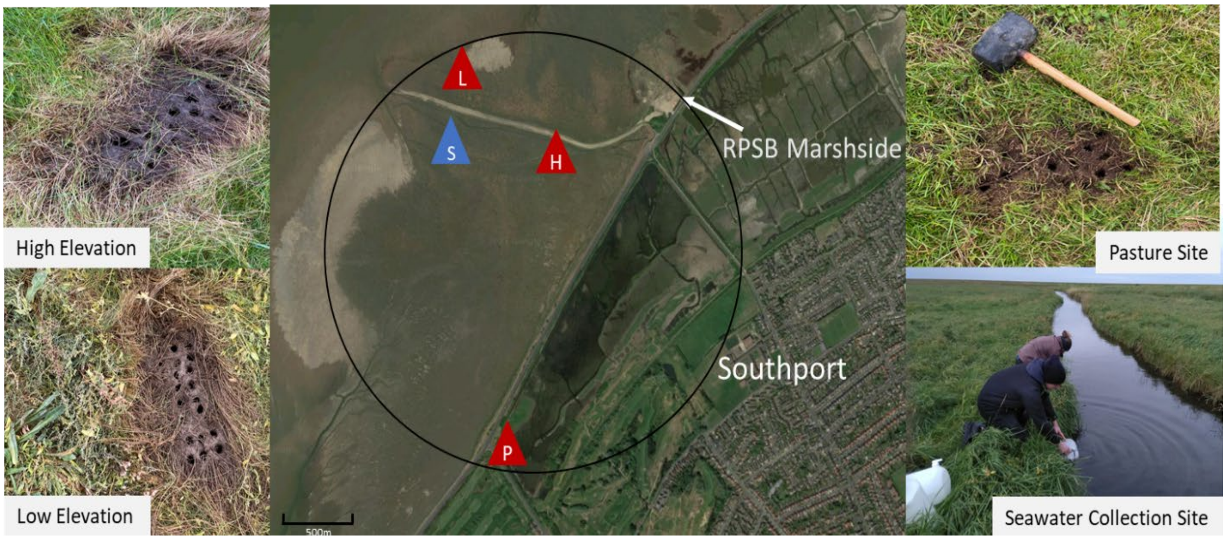


Figure 2 Enlarged image of the coast near Southport (centre) with photos of individual sample collection sites – S) Seawater collection site L) Low elevation saltmarsh site H) High elevation salt marsh site P) Agricultural pasture. The black circle represents a 1 km radius of the region sampling was conducted within.

2.2 Sampling

Soil core and seawater samples were taken on the 30th September 2020, with 20 L of seawater collected from an inlet into the saltmarsh (53° 40' 37.2"N, 2° 59' 31.2"W, Figure 2), and cores taken as described below. Prior to soil core collection, overgrown vegetation was trimmed by hand to ~1 cm. At each site, 24 cores of the top surface soil (0-5 cm depth), including any plants and roots present, were taken using a modified syringe (1.5 cm diameter), resulting in a total of 72 mesocosms. This syringe housing acted as both a miniature corer and subsequently as the mesocosm container for the duration of the experimental treatment (Figure 3).

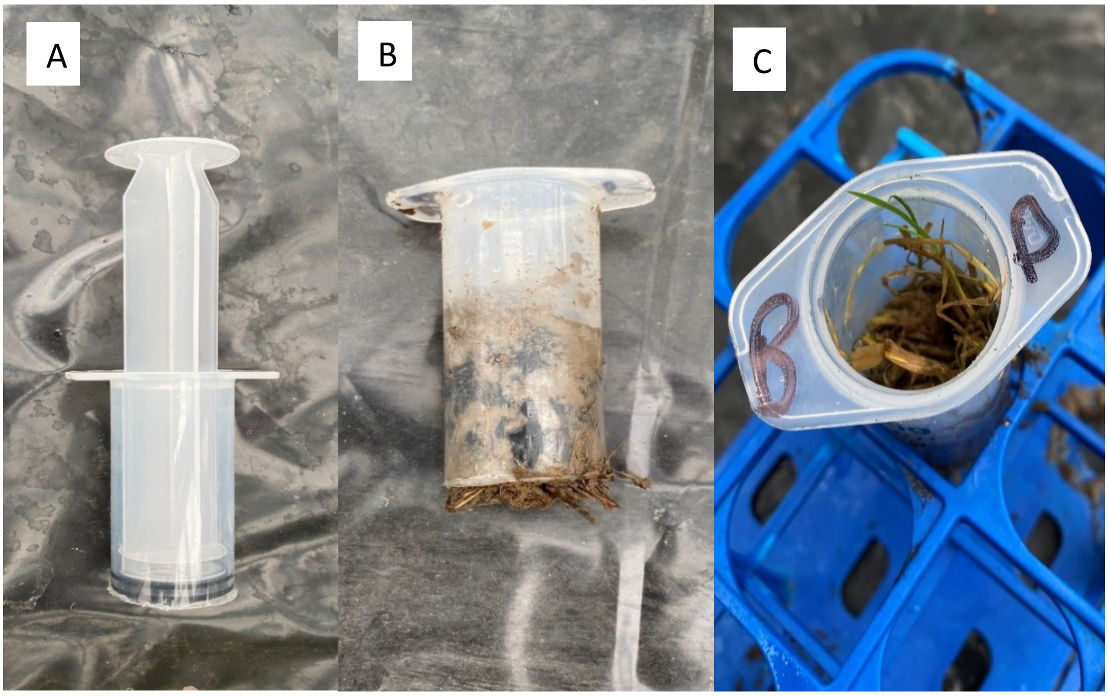


Figure 3 - A diagram of the soil core collector and subsequent mesocosm from the syringe holder. A) The modified syringe with the first 2 cm of plastic and tip removed. The plunger is used to expel the soil sample from the mesocosm. B & C) An example of one replicated mesocosm taken from the Pasture site.

2.3 Experimental Design

All samples were returned to Manchester Metropolitan University and placed into the on-site greenhouses within 24 hours. The 72 mesocosms were arranged on racks by site and treatments into plastic containers. Soil cores were acclimatised to the greenhouse conditions for four days prior to experiment initiation. This experimental design was split into mesocosms sampled at the different Sites (hereafter *Site*), and two treatment points (*State*) Flooded and Recovery (Figure 4). Each mesocosm was independently replicated in triplicate.

Flooded state: control and flooded mesocosms were subjected to no flooding and varying durations of flooding treatment, consisting of fully submerging mesocosms within the container filled with 10 L of seawater collected from the saltmarsh (Figure 2). Mesocosms were subsequently exposed to this submerging for one of four differing durations (*Flooding Duration*): 0 hours (Control), 1 hour (Flash Flood), 96 hours (4 days) and 192 hours (8 days). After flooding mesocosms were destructively sampled by placing the core into an individually labelled Ziplock bag and homogenising the samples by hand. The seawater was drained from all mesocosms from each flooding duration and half (n=36) of the mesocosms were destructively sampled immediately after their respective flooding duration. (see below).

Recovery state: The remaining mesocosms (n=36) were drained and incubated in a separate sealed “recovery” container without seawater for 14 days from the end point of their respective flooding duration. After this time, mesocosms were destructively sampled and followed the same analysis as the flooded state mesocosms (see below Figure 4). Destructive sampling was used in this experiment to avoid disrupting the soil and microbiome structure whilst processing samples for environmental and community analysis (Lombard et al., 2011).

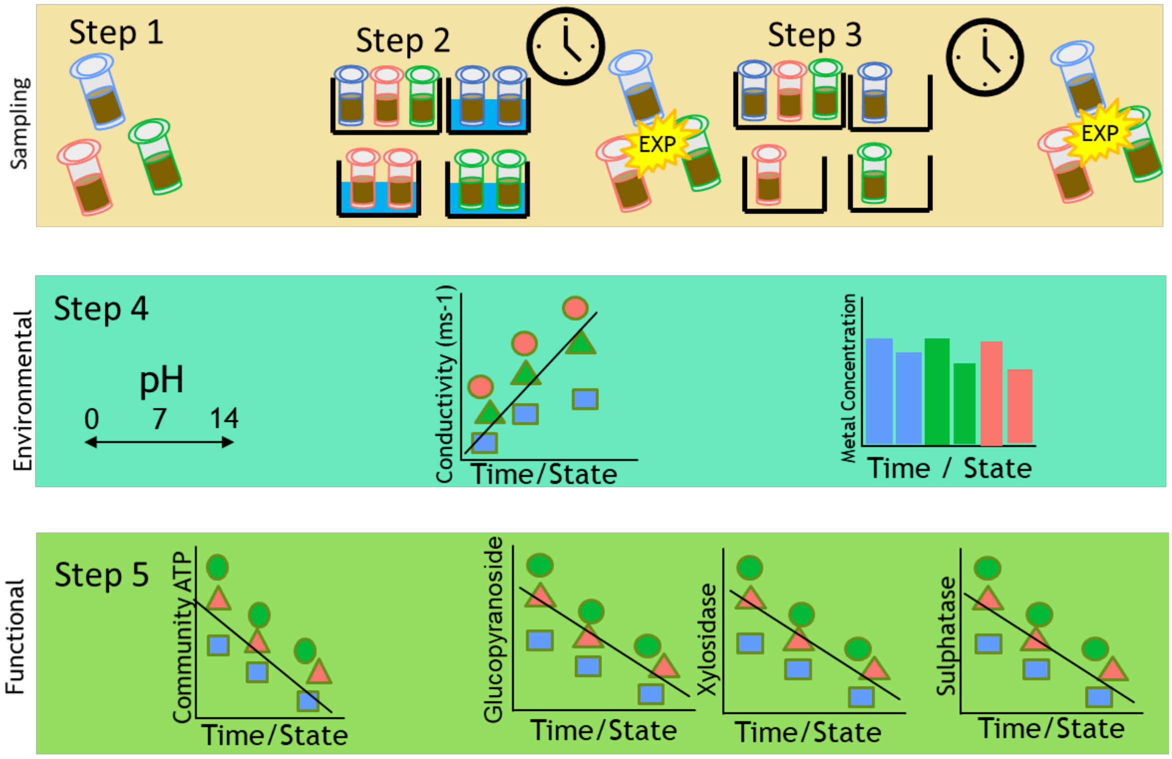


Figure 4 -Illustration of workflow - Step 1) 72 Mesocosm samples were collected from each site and transported back to the greenhouses. Step 2) Mesocosms are separated out into containers dependent on site and flooding duration. Mesocosms are then exposed to varying durations of seawater submersion (0, 1, 96 and 192 hours). Following the flooding duration, half of the samples are destructively sampled (EXP = Expired). Step 3) The remaining half of the mesocosms are incubated for 14 days following expiration of flooding duration and destructively sampled. Step 4) Following destructive sampling all soil samples from mesocosms are analysed. Geochemical measurements of soil samples are taken for pH, conductivity, and metal analysis for each mesocosm. Step 5) Community functioning was assessed for the metabolic potential, and enzyme assays for specific substrates. Step 6) (Not included) Consisted of community composition analysis using 16, 18S and ITS amplicon sequencing. At the time of completing this project, the sequencing results were unable to be included due to the global pandemic.

2.4 Environmental Measurements

As the chemical and physical properties of soil can dramatically affect microbial, and plant, communities (Griffiths et al., 2007), these data were measured throughout the experiment to give a clear picture of how the soil chemistry altered during flooding events.

2.4.1 Soil moisture and Carbon: Nitrogen ratio analysis

To understand the impacts of seawater inundation on the geochemical profile of the soil, multiple analysis of soil characteristics was performed. To enumerate the moisture content, ~1-2g of each homogenised soil sample was taken and the soil wet weight (w/w) mass measured. Samples were then placed into a drying oven set at 105°C for 24 hours (Elmholt et al., 2000), following this, dry weights of the soil were recorded, and moisture content was calculated as a percentage (wet weight-dry weight / wet weight) *100

Following the drying step, samples were then refined to a uniform consistency with a pestle and mortar. A subsample of 0.01 g was taken for the Carbon: Nitrogen analysis, Total carbon and nitrogen in the soil was enumerated through dry combustion by mass spectrometry analysis (*Elementar Vario EL analyser*) identifying the total percentage of carbon and nitrogen per sample.

2.4.2 Soil elemental metal analysis using Inductively Coupled Plasma Optical Emission Spectroscopy

Changes in elemental composition were analysed to understand how flooding impacted soil composition which can alter microbial community functioning and structure (Chu, 2018). Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) was used to identify and quantify trace elements present in the samples (Sandroni et al., 2003). Metals analysed in particular for this study consisted of heavy metals (Al, As, Co, Cr, Cu Ni, Pb, Zn,) to analyse pollution from anthropogenic activities, seawater elemental composition (Mg, Na, Ca, K, S) as well as other elements (Fe, Mn, P)

Briefly, 0.025 g of the dried soil samples were placed were dried as above and digested using microwave digestions. This was, performed by placing 0.025g of sample into a clean PTFE digestion vessel and combined with trace metal-grade nitric acid, sealed, and placed in the microwave following these steps: increase temperature to 90°C over 10 min and maintain this temperature for 5 min, increase temperature to 175°C over 10 min and then maintain at that temperature for 10 minutes. After this the contents from the digestion vessels were gravity filtered through a Fisher Scientific 110 mm filter (Whatman 540 equivalent) into a 100 ml volumetric flask and the total volume made up to 100 ml with deionised water. Initial seawater elemental composition was analysed to understand the elements introduced from seawater inundation; this was achieved by filtering 20ml of time 0 seawater sample through a Whatman syringe filter into a falcon tube in triplicate. Samples were analysed using the iCAP 6000 Series ICP spectrometer (Thermo Scientific, UK).

2.4.3 pH and conductivity

To analyse changes in the soil pH and conductivity of each mesocosm after the flooding treatment, 1 g of soil was combined with 10 ml of deionized water in a plastic universal tube to create a 1:10 dilution. The mixture was shaken for 5 minutes and left to settle for 30 minutes. Conductivity was measured first using a Jenway 4510 Conductivity probe calibrated to 1413 µS and then pH measured with a Jenway 3510 pH probe calibrated using pH 7 and pH 10 buffers.

2.5 Microbial Community Functioning

2.5.1 Enzymatic assays

Extracellular enzyme activity was measured from the mesocosms using substrate analogues with fluorescent molecule, 4-methylumbelliferone (MUB) to measure the ability of the communities to cleave from three specific substrates targeting sulfatase, xylose and cellulase (Table 1; Sinsabaugh et al., 2008)

The three substrates were incubated with 100 µl of soil suspension combined with the 3 substrates to a final concentration of 40 µm (Frossard et al., 2012). The plate was incubated for 1-hour in the dark at room temperature, prior to reading 10 µl of 0.1 M sodium hydroxide was pipetted into each well and the fluorescence (Excitation/Emission: 365 nm/445 nm) immediately measured using plate reader (Synergy HT; BioTek Swindon, UK) for 10 minutes and the maximum value recorded. Fluorescence values were converted to nM MUB after normalization across the enzymes (Rivett et al., 2016).

Table 1 - Enzymes, substrates, and respective function.

Enzyme	**Substrate**	**Function**
β-glucosidase	4-MUB-β-D- Glucopyranoside (G)	Cellulose Degradation
D-xylosidase	4-MUB-β-D-Xylopyranoside (X)	Hemicellulose degradation
Aryl-sulphatase	4-MUB-Sulphate (S)	Organic Sulphur reduction

2.5.2 Community Metabolic Potential

Total adenosine triphosphate (ATP) concentrations were measured using the Promega BacTitre Glo™ Cell viability Assay as previously described (Rivett et al, 2016). The assay was used to quantify ATP levels within the samples to gain a measurement of metabolic and functional potential within the microbial communities. (Lomakina et al., 2015) Briefly, analysis of luciferase enzyme bound to ATP which emitted a luminescent signal. This was achieved by combining 100 µl of sample, into a 96 well clear bottom plate, with 25 µl test reagent and the luminescence recorded (HT Synergy plate reader, BioTek, Swindon UK) every minute over a period of 5 minutes with the maximum value recorded. The values (nmol/g ATP) were divided by the biomass enumerated from the R2A plates of the samples (colony forming units/g) to give *per capita* ATP (Rivett et al. 2016).

2.6 Microbial enumeration and DNA extraction

2.6.1 Bacterial Enumeration

Microbial communities were detached from the soil matrix by washing the soil in Phosphate buffer solution (PBS) following the procedure described in (Mombrikotb, 2016). Briefly, 1 g of soil sample was combined with 2 ml of Phosphate buffer solution (PBS) in a sterile bijoux tube, vortexed for 5 minutes and allowed to settle until a layer of supernatant forms. The supernatant was removed for downstream processing. Viable cells from colonies were enumerated using 100 µl soil suspension using a dilution series of 10^{-1} – 10^{-8} plated onto R2A agar and Tryptone soy agar (TSA) and incubated at room temperature (20-25^{o}C) for 4 days

2.6.2 Microbial community DNA extraction

The composition of microbial communities and relative abundance of OTU’s in flooded and recovery states were analysed by next generation sequencing (NGS). The DNA extraction was performed using the Zymo Research Quick-DNA™ Faecal/Soil Microbe Miniprep Kit (ZYMO Research Irvine, CA USA), following the manufacturers protocol. For DNA extraction from the soil, samples were thawed and weighed out into lysis bead tubes (0.135 g). Seawater DNA extraction was performed to identify mixing of communities from aquatic and terrestrial ecosystems, this was conducted by filtering 250 ml of seawater through a Whatman sterile cellulose ester circle filter (0.2 µm pore 47mm) using vacuum filtration. Following this, the filters were finely cut and placed into the lysis bead tubes with 750 µl of bead buffer solution (Zymo). Using the primer sets (515F/806R) to amplify the 16S rRNA genes with Q5® High-Fidelity DNA Polymerase. The PCR cycling parameters were followed as described in the earth microbiome project (EMP) and consisted of an initial denaturation at 94 °C for 3 minutes followed by 35 cycles of 45 seconds at 94 °C, 60 seconds at 50°C and 72°C for 90 seconds finishing with a final extension time for 10 minutes at 72 °C on the PCR machine (Hain Lifescience, Thermocycler Q cycler Satellite S96, Germany).

2.7 Statistical Analysis

All statistical analyses and visualisations were performed in R (v 3.6.3; (R, 2020)) statistical environment. All data were natural log transformed to conform with the assumptions for parametric statistics. Normality of errors and homogeneity of variances were assessed visually prior to undertaking the analysis. Three-way analysis of variance (ANOVA) was conducted on all functional and environmental variables with *Site, State,* and *Flooding duration* included with all the interactions. To mitigate the order of the variables affecting the statistical analysis, three-way ANOVA with type III errors were performed utilising the “CAR” package (Fox and Weisberg, 2019). *Site* represented the location the samples were taken from (e.g., Low, High and Pasture), *State* represents the flooded or recovery period and *flooding duration* is the flooding treatment for the experiment (0, 1, 96 and 192 hours). All non-significant interactions were retained, so the model can be directly compared against all response variables from each site. To measure the relationship between *flooding duration* and environmental variables, spearman’s ranked correlation was utilised, Rho values are indicated on all graphs with an * indicating significance α=<0.05. Multiple pairwise comparisons (denoted by a p_{adj} value) were Bonferroni corrected. Principal Component Analysis (PCA) and analysis of variation (ADONIS) was performed on enzyme substrates and total metals concentration using the vegan package (Oksanen et al., 2019). PCA analysis of metals was performed to analyse the composition of metals within the seawater and each site, this is conducted to test the effects of seawater submersion on metal mobility and solubility within soil samples over the experimental flooding duration.

3. Results

3.1 Environmental characteristics

3.1.1 Soil Moisture Content

Moisture content was not significantly affected by *flooding duration* ($F_{3,48}$=7.70, p=0.969), however, significant differences were observed between sites (*Site*: $F_{2,48}$=13.35 p<0.001). The results indicated that there was an overall significant interaction between *Site* and *Time* ($F_{6,48}$ = 2.55, p = 0.032) with differences in moisture content across the 3 sites with exposure to flooding treatment (Figure 5). Overall, observed moisture content was highest within the High elevation mesocosms (**H:** mean ± standard deviation, 65.25 ± 4.95% (w/w)), significantly higher than the Pasture (**P:** 42.68 ± 6.22%) and Low sites (**L:** 48.59 ± 1.59%) (p_{adj} =0.006); there was no significant difference between Pasture and Low (P_{adj} =0.421). There was an overall significant effect of *State* ($F_{1,48}$ = 4.48, p = 0.040) with moisture content decreasing after drainage and subsequent recovery period, this can possibly be attributed to evaporation after the 14-day recovery period and no introduction of environmental moisture.

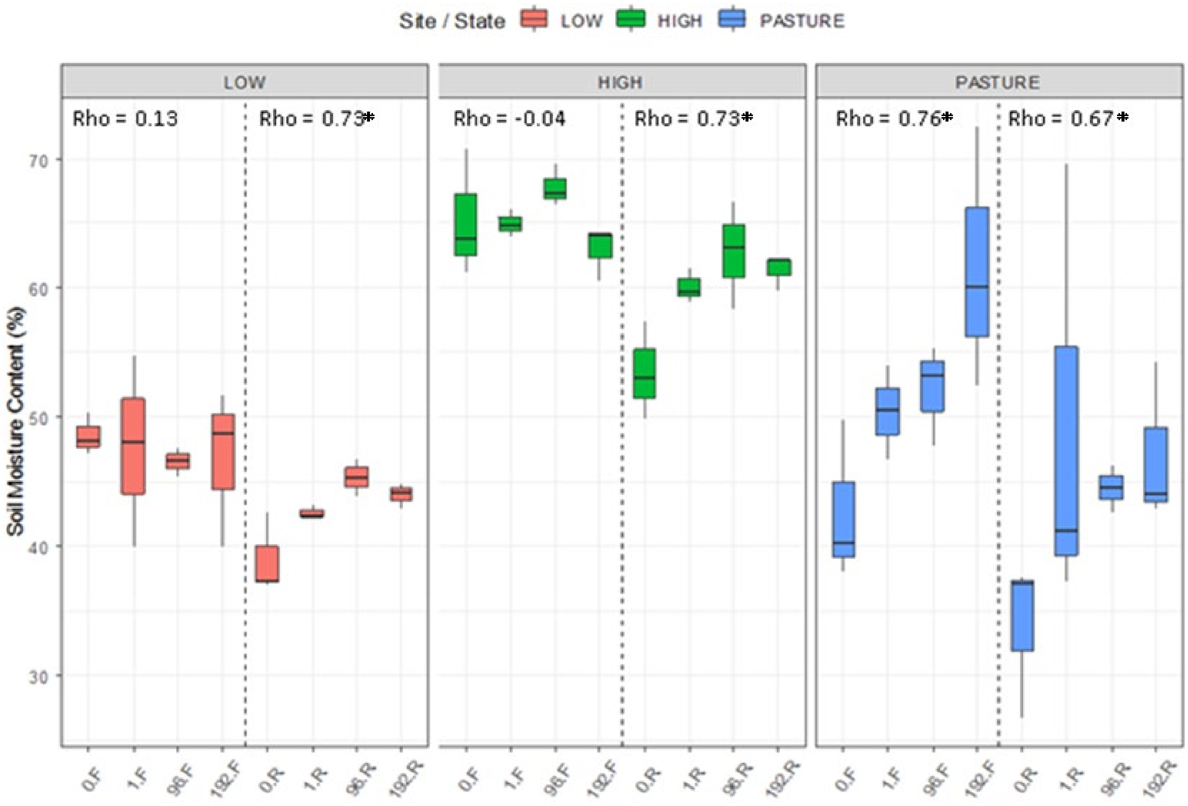


Figure 5 - Moisture content (%) of mesocosms exposed to seawater High elevation mesocosms retained a higher overall moisture content with Low elevation demonstrating a resistance to flooding duration retaining similar moisture content throughout the flooding treatment. Rho values represent spearman's correlation between pH and flooding duration for the flooded and recovery states * indicates significance (<=0.05). Box and whisker plots representing the 25% and 75% quartiles, and the solid line is the median. With the whisker representing the range of data. X-axis labels represent the Flooding duration for flooded (F) and recovery (R) states, each bar represents n=3

3.1.2 Soil pH

Overall, there was a significant effect of *Site* on pH ($F_{1,48}$ =0.989, p<0.001), with pH levels significantly different between the soils taken from the different sites (**L:** 7.76± 0.12, **H:** 7.71 ±0.19 and **P:** 7.4± 0.34; Figure 6). There was also a significant effect of *Flooding duration* ($F_{3,48}$=0.515, p<0.001) with a significant interaction between *Site* and *Flooding Duration* for the reduction of pH within the infrequently exposed mesocosms from High and Pasture sites ($F_{6,48}$ =0.101, p<0.001). Prior to flooding treatment significant differences in pH were observed between the saltmarsh site soils (**L:** 7.66 ± 0.24, **H:** 7.36 ± 0.10), and Pasture sites (**P:** 6.96 ± 0.07) (p_{adj} <0.001). pH values within the samples vary potentially due to frequent previous tidal movements and exposure to seawater (**SW:** 8.23 ± 0.05).

State had a significant impact on pH levels within the mesocosms ($F_{1,48}=0.71$, $p<0.001$) with higher values recorded within all sites after the recovery period in comparison to the samples during flooding (**L:** 7.77 ± 0.09; **H:** 7.83 ± 0.15; **P:** 7.57 ± 0.37). A significant interaction was observed between *Site* and *State* ($F_{2,48}=0.16$, $p<0.001$) with High saltmarsh and Pasture responding to changes in pH during the flooded and recovery period. Finally, the analysis found significant three-way interaction between *Site, State* and *Flooding duration* ($F_{6,48}=4.61$, $p<0.001$) with reduced degree of change to pH levels observed within the Lower saltmarsh site.

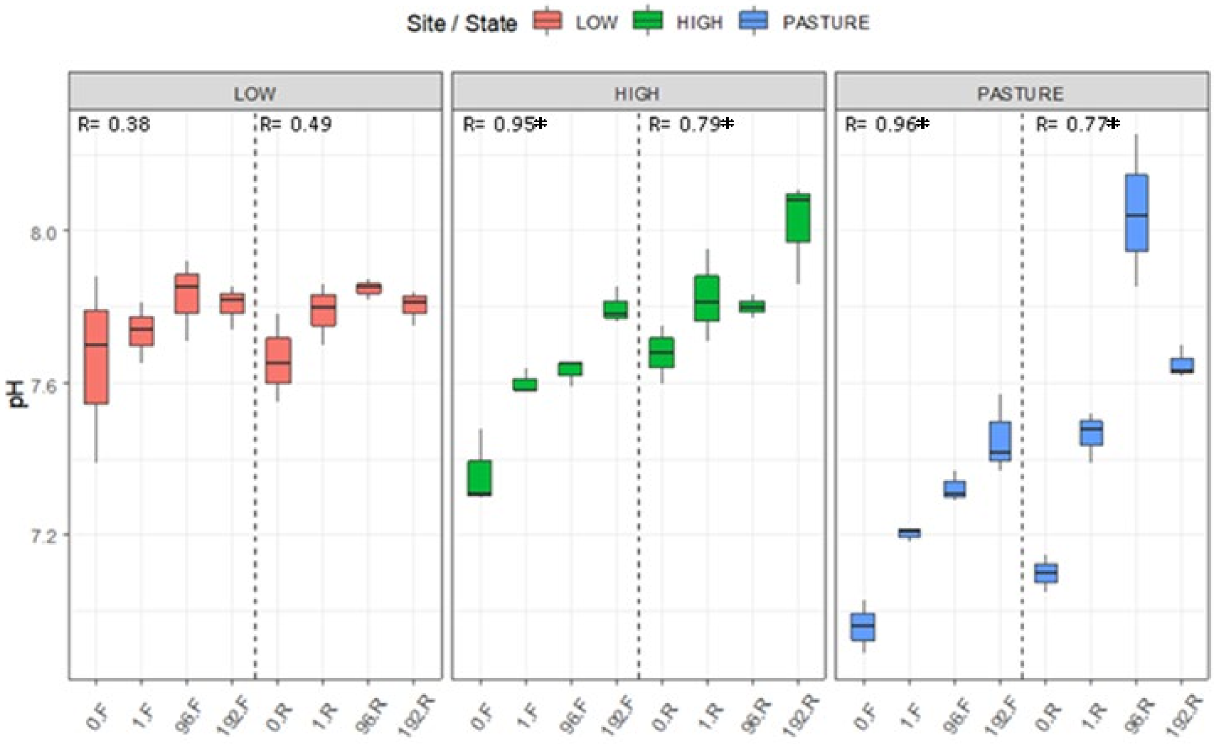


Figure 6 - *Soil pH levels rose during the flooded period with increasing duration of seawater submersion. Following drainage pH values during the 14-day recovery period increased within High and Pasture mesocosms whereas Low mesocosms followed the same pattern observed during experimental treatment. Rho values represent spearman's correlation between pH and flooding duration for the flooded and recovery states * indicates significance (<=0.05). Box and whisker plots representing the 25% and* 75% quartiles, and the solid line is the median. With the whisker representing the range of data. X-axis labels represent the Flooding duration for flooded (F) and recovery (R) states, each bar represents n=3.

3.1.3 Conductivity

Overall significant effects were observed for conductivity for *Site* ($F_{2,48}$=4.48, p<0.001) and *Flooding duration* ($F_{3,48}$=4.03, p=0.012) and a significant interaction ($F_{6,48}$=15.258, p<0.001) between the two variables (Figure 7). *Flooding Duration* had a significant effect on conductivity in all mesocosms increasing conductivity within mesocosms with extended flooding duration. Seawater was measured at (**SW:** 49.28 ± 0.13 mS/m), higher than any of the soils (**L:** 7.43 ± 0.96 mS/m; **H:** 8.84 ± 2.44 mS/m; **P:** 9.46 ± 3.44 mS/m).

State had no significant effect ($F_{1,48}$=0.07, p=0.793) on conductivity, with similar observed conductivity following the 14-day recovery period. however, there was a significant three-way interaction between *State, Site* and *Flooding duration* ($F_{6,48}$=4.53, p=0.001). Conductivity was higher within the recovery period following extended flooding duration with significantly higher levels observed within the Pasture mesocosms after 96 hours of flooding (**P:** $F_{1,48}$=5.13, p=0.028, 11.65 ± 0.51 mS/m) and 192 hours (**P:** $F_{1,48}$=18.6, p<0.001, 11.73 ± 1.07 mS/m).

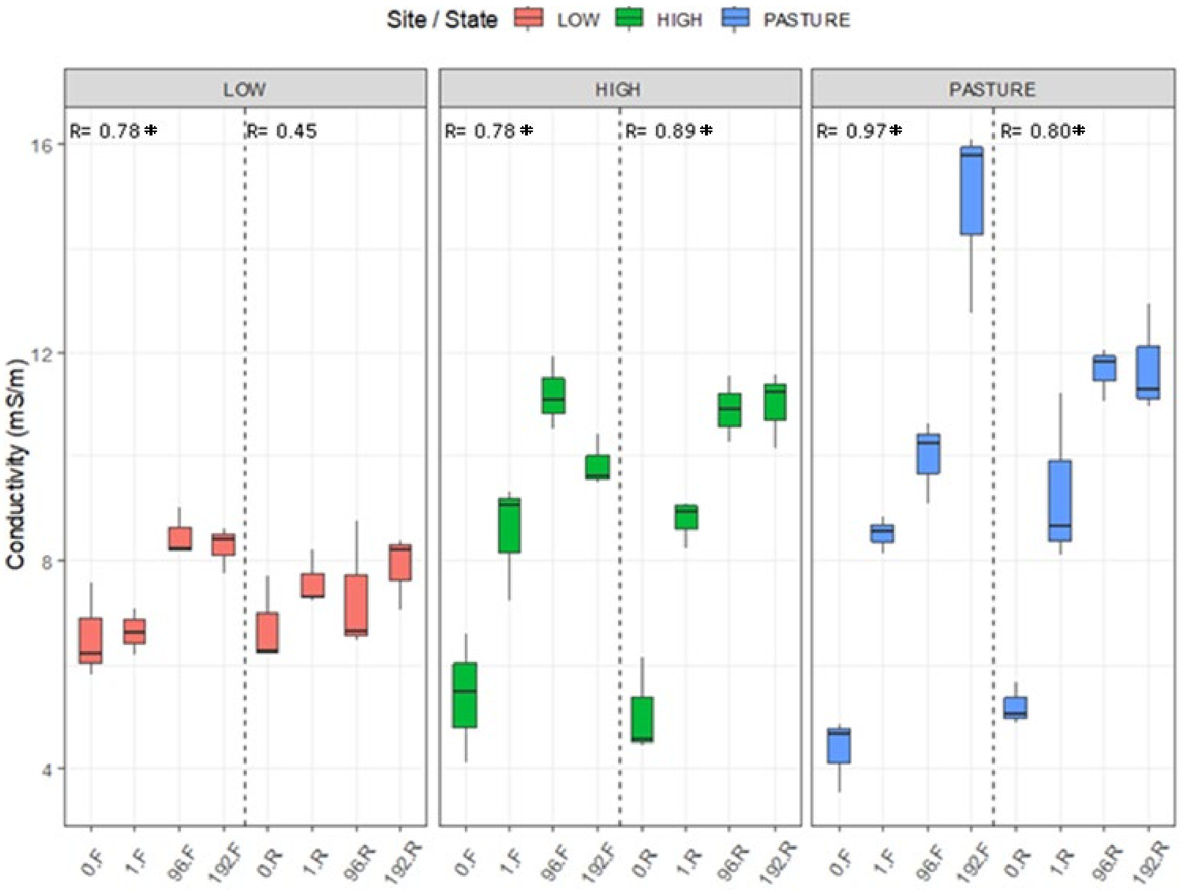


Figure 7 – Box and whisker plots representing the 25% and 75% quartiles, and the solid line is the median. With the whisker representing the range of data. X-axis labels represent the Flooding duration for flooded (F) and recovery (R) states is points are grouped by site and each bar represents n=3. Soil conductivity before and after flooding treatment (F), Conductivity increases with flooding duration in all sites with significant increases observed within the pasture and high elevation saltmarsh sites. Measured post 14-day recovery period. Elevated conductivity levels are present within each site and flooding duration point in comparison to flooded values. Exposure to seawater significantly increased conductivity within pasture site with increasing flooding duration. Rho values represent spearman's correlation between conductivity and *flooding duration* for the flooded and recovery states * indicates significance (<=0.05)

3.1.4 Carbon: Nitrogen Ratio

Overall, *Site* had a significant effect ($F_{2,65}$=24.60, p<0.001) on Carbon: Nitrogen (C: N) ratio with higher observed C:N ratios in the Low and High saltmarsh sites (Figure 8). Initially within the Saltmarsh samples C:N ratios were significantly higher prior to flooding treatment (p_{adj} =0.01) (**L:** 14.08 ± 0.47, **H:** 14.09 ± 0.44) with lower recorded C:N ratios within Pasture samples (**P:** 11.19 ± 0.23). *Flooding duration* had no significant effect on C:N ($F_{3.65}$=0.69,p=0.558) with C:N ratios varying with flooding duration. The recovery *State* had no significant effect on C:N ratio ($F_{1,48}$=1.79, p=0.186) with drainage and subsequent recovery had no impact on the C:N ratio within the soil composition.

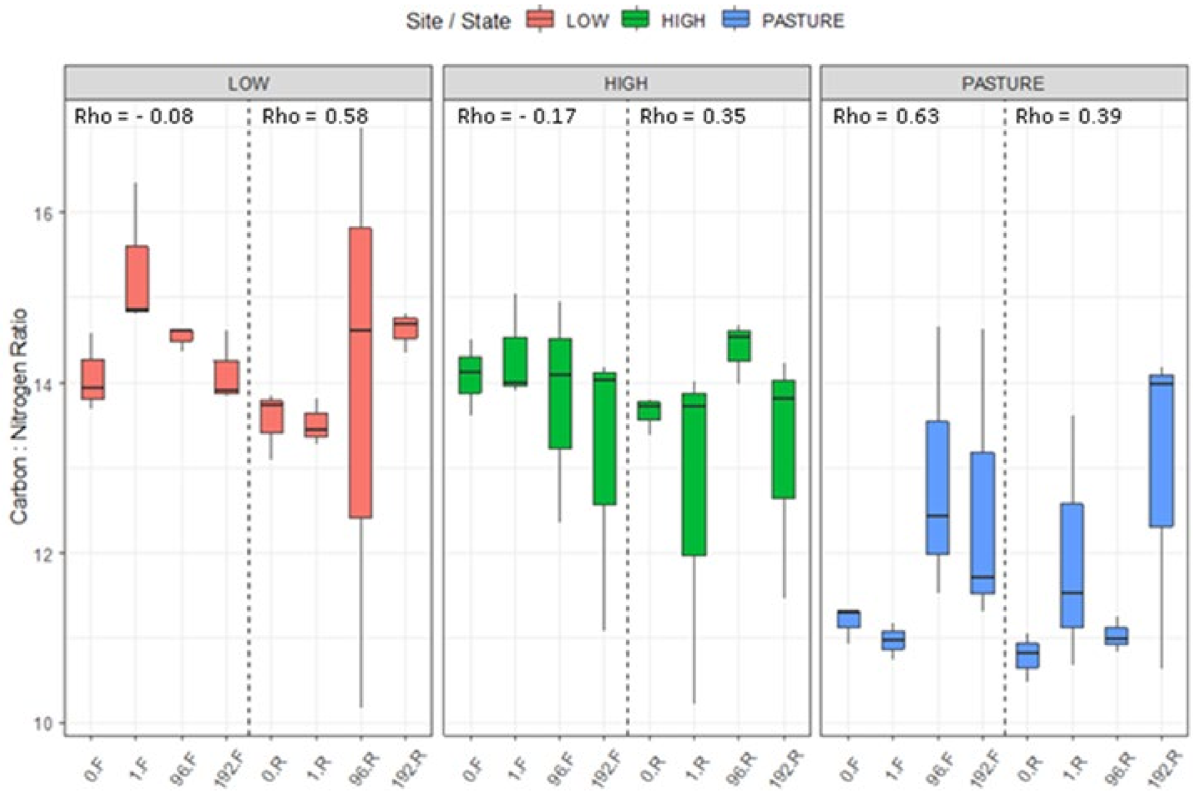


*Figure 8 - Carbon: Nitrogen ratio analysis prior and after flooding treatment (F) and post 14-day recovery (R). Carbon: Nitrogen ratio declined within the saltmarsh sites with extended flooding duration, with the C:N ratio increasing within the Pasture site during the same experimental flooding duration. Rho values represent spearman's correlation between the C:N and flooding duration for the flooded and recovery states * indicates significance (<=0.05). Box and whisker plots representing the 25% and 75% quartiles, and the solid line is the median. With the whisker representing the range of data. X-axis labels represent the Flooding duration for flooded (F) and recovery (R) states, each bar represents n=3.*

3.1.5 Total Metal Concentration

Site had a significant effect on total metal concentration within the experiment ($F_{2,65}$=617.68, p<0.001) with initial metal concentrations higher within Low and high elevation saltmarsh sites (Figure 9). Seawater composition strongly correlated with the elements Sulphur, Sodium, Magnesium, Potassium and Calcium during this experiment with other heavy metals and elements strongly correlating within the saltmarsh sites than pasture soils (Figure 10). Throughout the experiment significantly higher total metal concentrations were present in the two saltmarsh sites compared to the Pasture (P_{adj} <0.001), but with no significant difference between the High and Low saltmarsh sites (P_{adj} 0.258) (**L:** 104.75 ± 11.49 mg g^{-1}; **H:** 96.31 ± 8.87 mg g^{-1}, **P:** 26.18 ± 7.31 mg g^{-1}).

Flooding duration had a significant effect on total metal concentrations ($F_{3,48}$=5.67, P=0.002), with increasing metal concentration with increased flooding duration (Figure 10). No significant interaction between *Site* and *flooding duration* was observed ($F_{6,48}$=0.58, p=0.742), with the increasing metals increasing with flooding duration conserved across sites. There was no significant effect of *State* ($F_{1,48}$=2.72, p=0.105) suggesting that drying did not remove the metal ions once they had been bound within the pores of the soil.

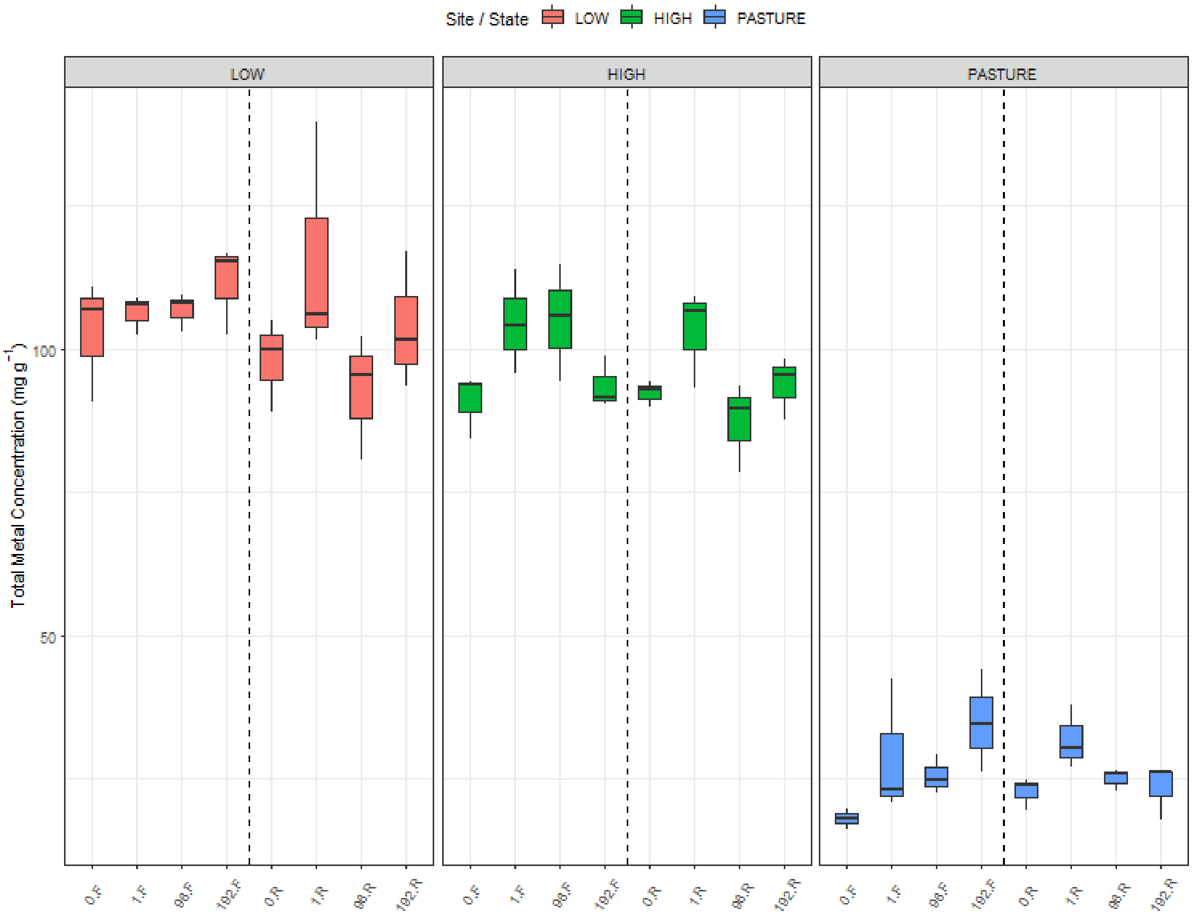


*Figure 9 - Total metal concentration for all sites before and after flooding treatment (F) and post 14-day recovery (R). Overall Metal concentrations are higher within the saltmarsh sites during flooded and recovery states. Metal concentrations vary with flooding duration slightly however levels are similar throughout the experiment. Rho values represent spearman's correlation between total metal concentration and flooding duration for the flooded and recovery states * indicates significance (<=0.05). Box and whisker plots representing the 25% and 75% quartiles, and the solid line is the median. With the whisker representing the range of data. X-axis labels represent the Flooding duration for flooded (F) and recovery (R) states, each bar represents n=3.*

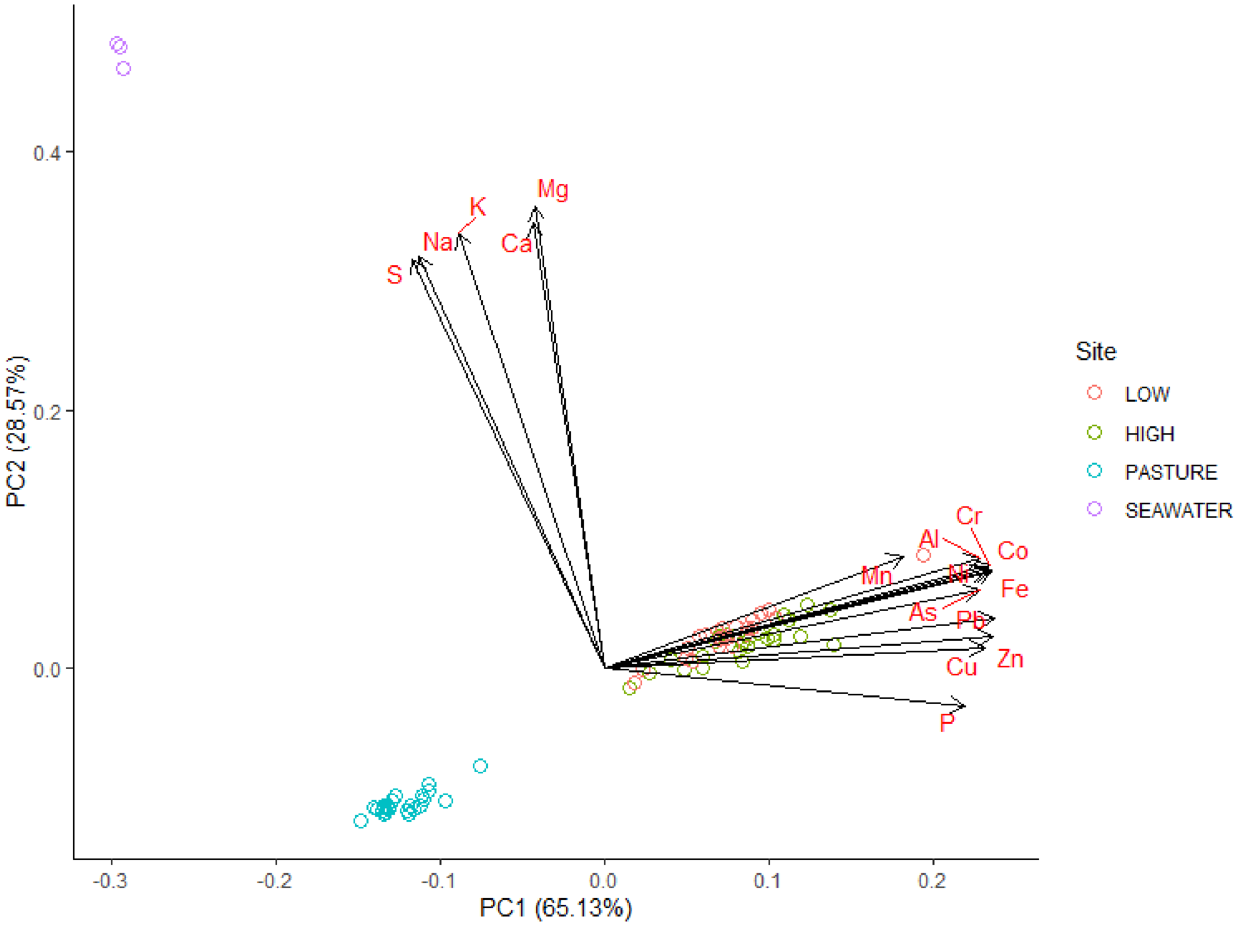


Figure 10 – Principal component analysis (PCA) of metal composition for all sites including the seawater metal composition for the total experiment. Heavy metals are more correlated towards the saltmarsh sites. Distance between points represents dissimilarity with points closer together representing similarity

The analysis then focused on understanding whether the composition of the metal ions changed over *Flooding duration.* Principal component analysis (PCA; Figure 11) indicated metal compositions were significantly clustered dependent on *Site* (ADONIS R=0.874, P<0.001). Flooding duration accounted for a large amount of variability within metal composition (R=0.031 P<0.001). Control and flash-flooding (1 hr) were closely clustered together, however, an increase in metal concentration following prolonged flooding duration with visible clusters formed for 96 and 192 hours for all sites (Figure 11). A significant interaction between *flooding duration* and *Site* was also observed (R=0.023, P=0.006). In agreement with the analysis of the total metal concentration, the recovery period (Figure 11B) had no significant effect on metal composition (R=0.001 P=0.280), with clusters due to *flooding duration* within the respective sites. This could be linked to reduced moisture content due to evaporation within the mesocosms and subsequent reduction of mobility/ solubility of elements.

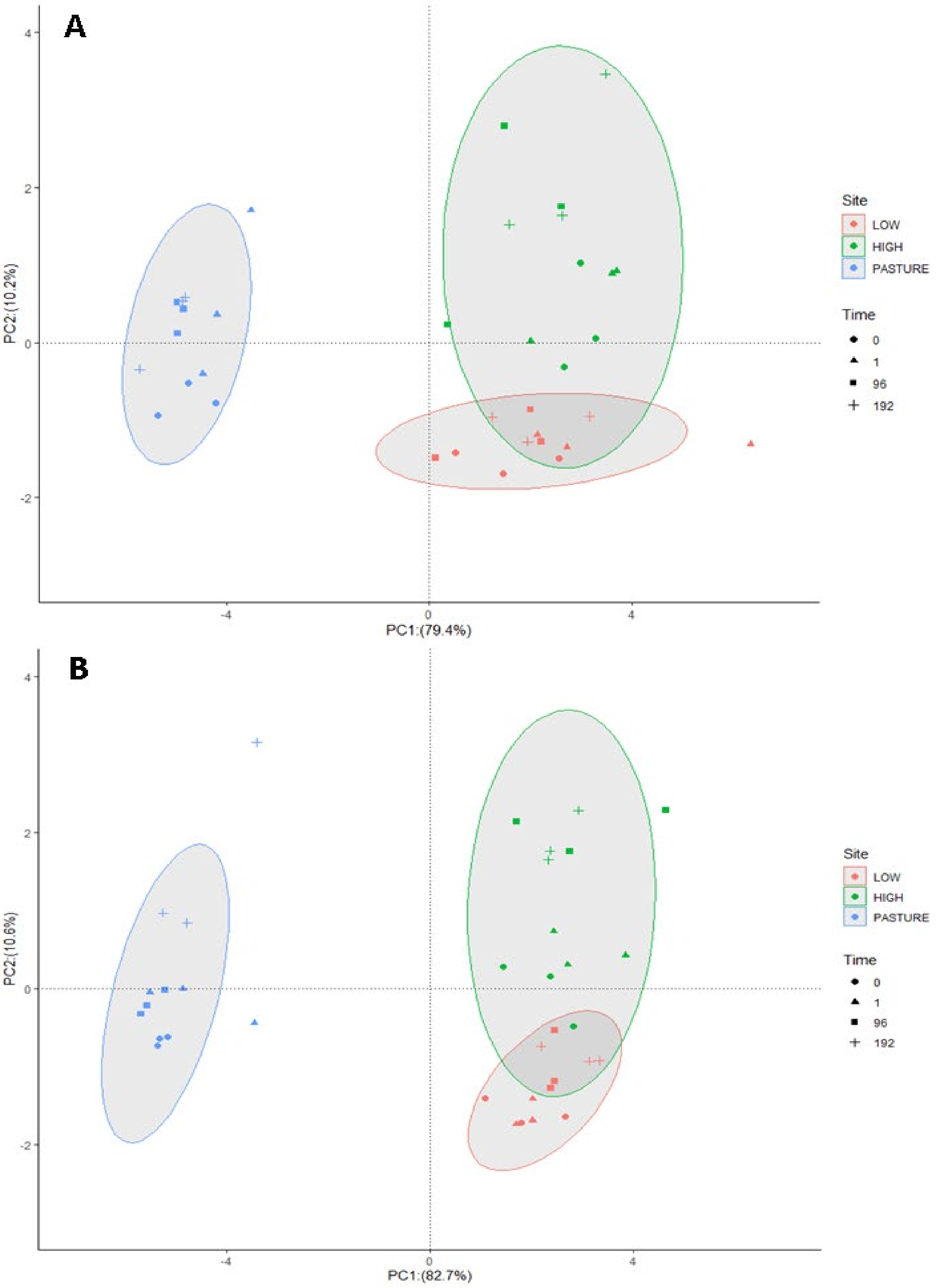


Figure 11 - Principal component analysis (PCA) of total metal concentrations for all sites before and after flooding treatment (A) and post 14-day recovery (B). Flooding duration is represented by the change in point shape with coloured ellipses representing a 95% confidence interval for all clusters. Distance between points represents dissimilarity with points closer together representing similarity

3.2 Microbial Functional Analysis

3.2.1 Bacterial enumeration

Overall a significant three-way interaction was observed between *Site, State* and *Flooding duration* ($F_{6,48}$=3.41, p=0.007) for bacterial enumeration. Neither *Site* ($F_{2,48}$=3.07,p=0.555) *State* ($F_{1,48}$=1.32, p=0.264) or *Flooding duration* ($F_{3,48}$=1.10, p=0.359) had a significant effect on bacterial load alone, however, significant relationships between *Site* and *flooding duration* were observed ($F_{6,48}$=2.85, p=0.12) as bacterial load was found to increase with extended flooding duration in all sites (Figure 12). A significant effect of *State* and *flooding duration was observed* ($F_{3,48}$=3.14, p=0.03) with bacterial numbers decreasing in the recovery period with any duration of flooding.

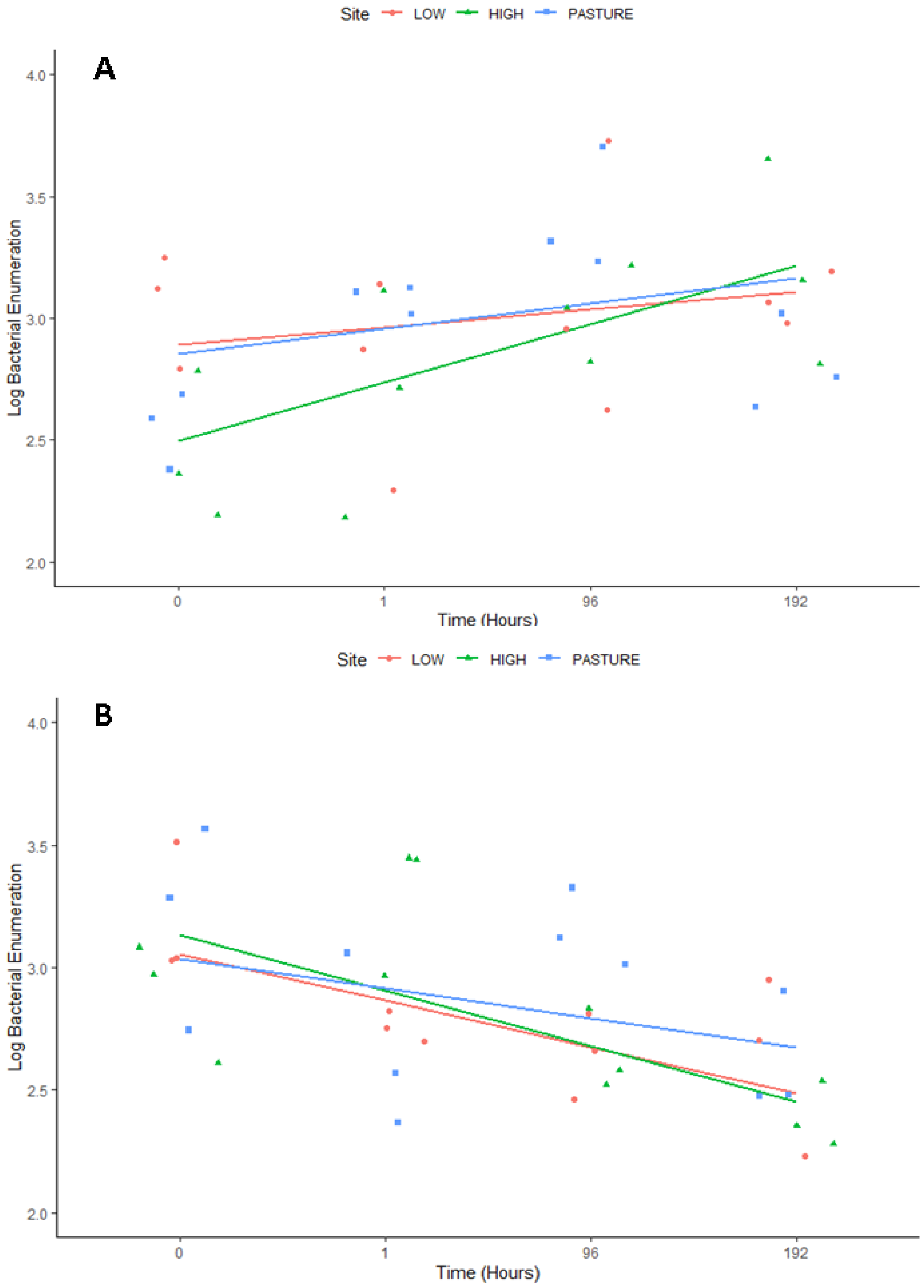


Figure 12 – log10 bacterial enumeration for all mesocosms over flooding treatment (A) with bacterial numbers increasing with flooding duration within all sites. During the 14-day recovery period (B) it was observed in all sites that they experienced a decrease of bacterial numbers with increasing flooding duration.

3.2.2 Adenosine Triphosphate metabolic potential

Microbial community activity was measured throughout the experiment to address the effects of *flooding duration* dependent on *site*. Measurements of ATP assays were used as a measure of cell metabolic activity and cell viability. All of the mesocosms reduced in nM ATP *per capita* with increased flooding, however, there was a significant interaction between *flooding duration* and *Site* ($F_{6,48}$=2.32, p=0.048, Figure 13), due to the decrease observed by the lower saltmarsh site was less (0.471 nM *ATP per capita* estimate) than the other saltmarsh site (2.28 nM *ATP per capita*) or pasture site (2.23 nM ATP *per capita*). The analysis indicated that the nM ATP *per capita* was significantly affected by *Site* ($F_{2,48}$=3.31, p=0.044) but did not differ with *flooding duration* alone ($F_{3,48}$=1.32, p=0.279) (Figure 12). Further, *State* was found to have no significant effect on nM ATP *per capita* ($F_{1,48}$=0.007, p=0.936), with the flooded measurements (2.28 ± 4.8 nM ATP *per capita*) higher than the recovery (1.48 ± 2.22 nM ATP *per capita*). There is a significant interaction between *flooding duration* and *State* ($F_{3,48}$=4.74, p<0.001) with flooding duration eliciting different responses; negative during flooding (mean decrease), and positive during recovery (mean increase). Finally, the analysis found a significant three-way interaction ($F_{6,48}$=2.40, p=0.029), once again, the lower saltmarsh site having a reduced degree of change compared with the other two sites.

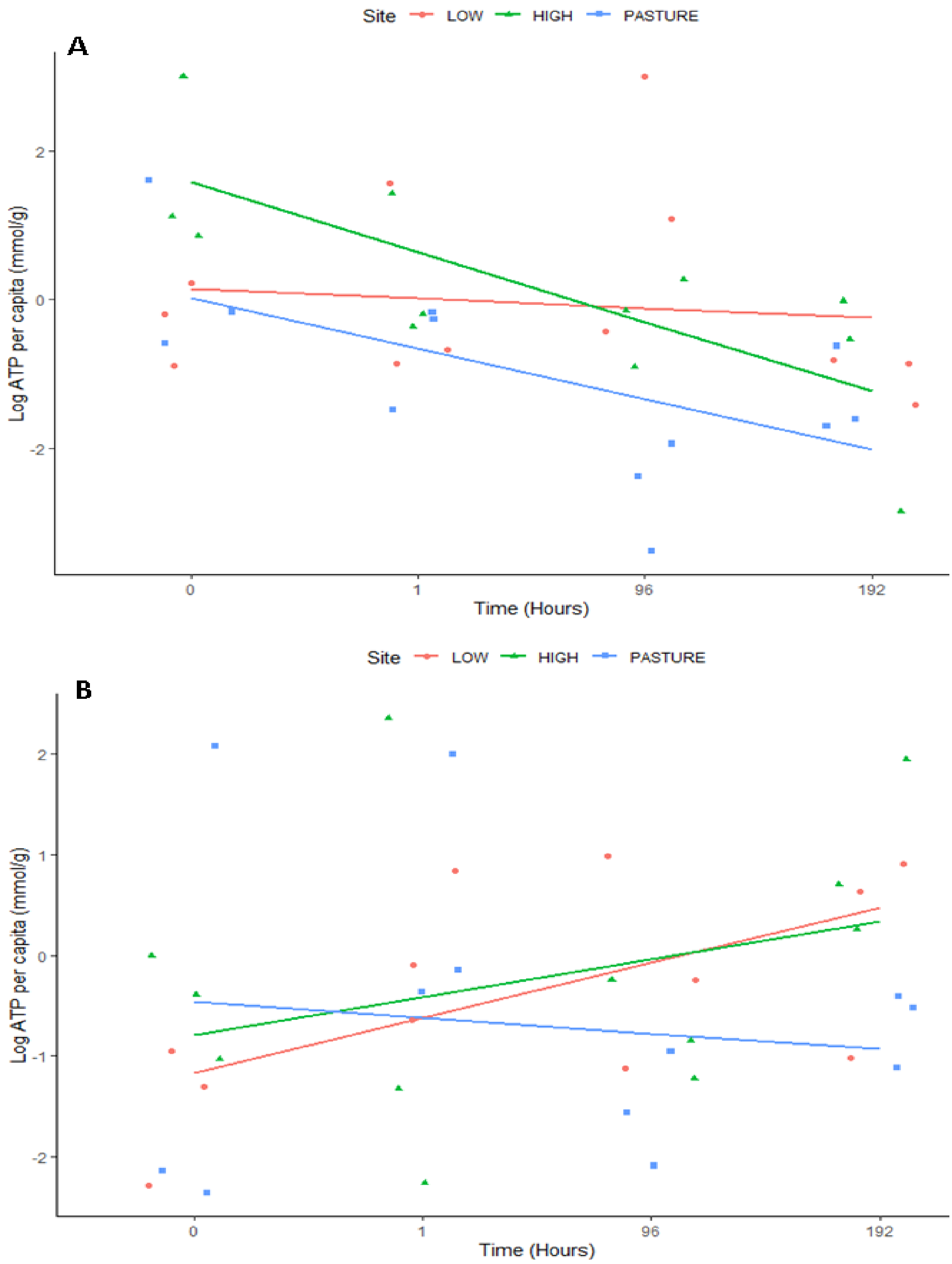


Figure 13 – The total per capita ATP for all mesocosms over flooding treatment (A) with per capita ATP decreasing with extended flooding duration with saltmarsh sites retaining higher per capita activity at the end of flooding treatment in comparison to pasture sites. Mesocosms are exposed to the subsequent 14-recovery period (B) which show the effects of extended flooding on all 3 sites following a recovery period, potential activity within the saltmarsh communities increases with flooding duration whereas *potential activity remains lower with extended flooding and subsequent recovery within pasture communities previously unexposed to seawater.*

3.2.3 Enzymatic Function

Sulfatase activity was not significantly affected by *Site* ($F_{1,48}$=1.39=0.257) or *flooding duration* ($F_{3,48}$=2.52 p=0.069). Significantly lower levels of activity were recorded in the flooded *State* ($F_{1,48}$=130.84, $p<0.001$) than those mesocosms allowed to recover (Figure 14). There was difference in response between the sites to the two *States* (*Site*State* $F_{2,48}$ =16.23, $p<0.001$), with higher sulfatase levels recorded in the pasture mesocosms compared to the two saltmarsh sites (Figure 14A). Interactive effects were observed between *State* and *Flooding duration* ($F_{3,48}$=7.23, $p<0.001$); here, the control mesocosms showed the highest activity after recovery.

In contrast, xylosidase activity was not significantly affected by the duration of flooding alone (*Flooding Duration*: $F_{3,48}$=2.73, p=0.054), however significant differences were observed in activity between *Sites* ($F_{2,48}$=71.45 $p<0.001$). The interaction between *Site* and *Flooding duration* was not significant ($F_{6,48}$=2.01, p=0.083). Overall, xylosidase activity was higher within pasture mesocosms, increasing with flooding duration. *State* has a significant impact on xylosidase activity ($F_{1,48}$=73.32, $p<0.001$) with activity levels higher in Pasture mesocosms during the recovery period.

The responses to the two states differed between the different sites (Site*State $F_{2,48}$ 26.17, $p<0.001$) with highest levels of activity recorded following recovery within control and flash flood (1 hr) mesocosms from pasture. (Figure 14B). There was also a significant interaction between *State* and *Flooding duration* ($F_{3,48}$=18.10, $p<0.001$) xylosidase activity increased in pasture mesocosms during the flooded state of the extended flooding treatments but subsequently decreased during the recovery period. A significant three-way relationship between *Site*, *State* and *Flooding duration* ($F_{12,48}$=6.14 $p<0.001$) for xylosidase activity observed.

Glucosidase activity was significantly affected by *Site* ($F_{2,48}$=6.89 p=0.042) and although there was no effect of *flooding duration* alone ($F_{3,65}$=0.761, p=0.353), there was a significant interaction between *Site and Flooding duration* ($F_{6,48}$=2.86 p=0.018). Glucosidase activity in Pasture increased more than saltmarsh sites with extended flooding duration (P_{adj}=0.004, Figure 14C). No significant effect of *State* ($F_{1,48}$=0.94 p=0.334) was observed with activity levels lower during the flooded state and activity similar with no differences observed at any time point for glucosidase activity in all sites during the recovery period (P_{adj}=1.00).

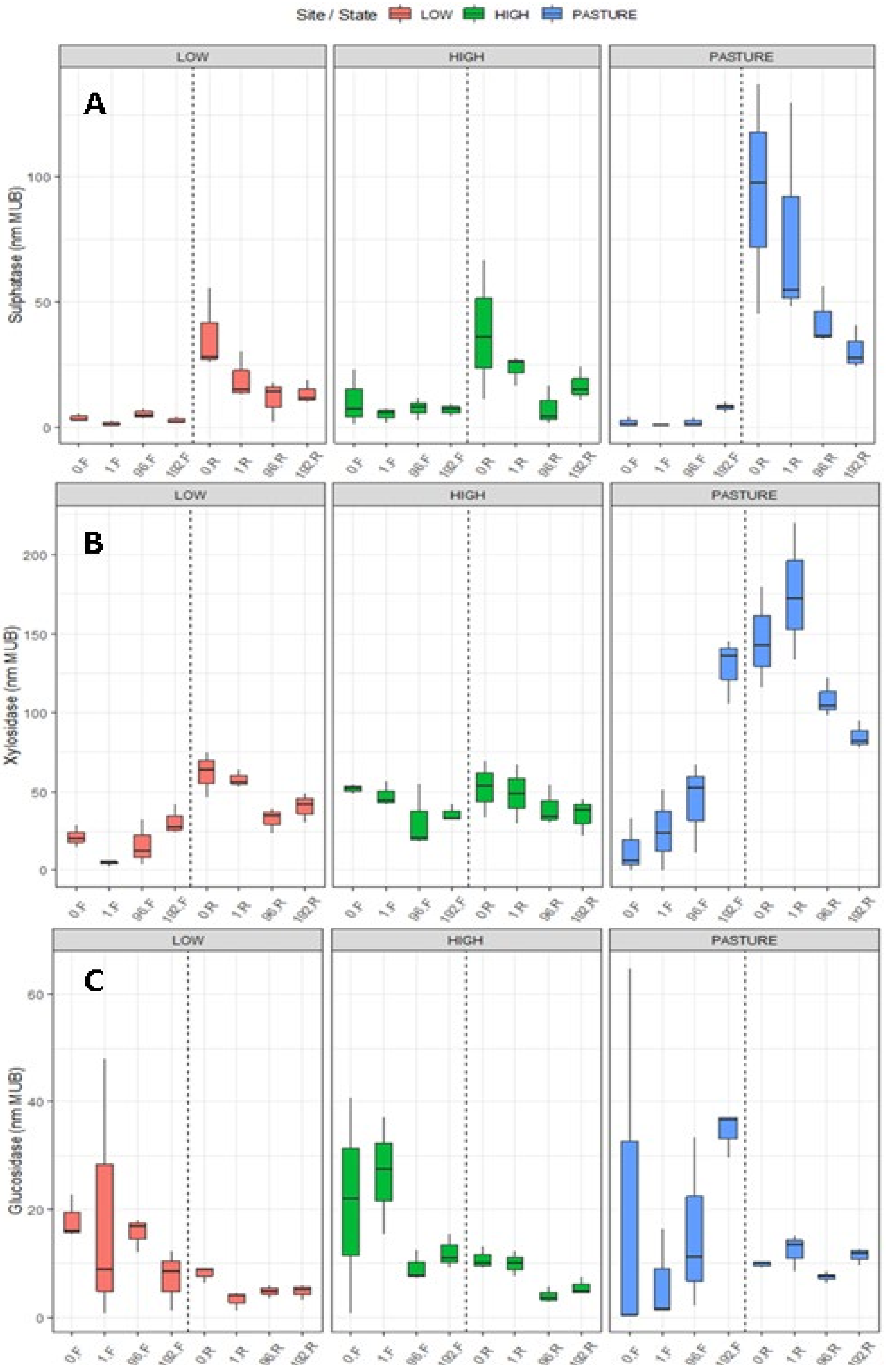


Figure 14 - The effect of differing durations of seawater flooding (0,1,96 and 192 hours) (F) and recovery period (R) on the concentration of MUB (nM) from the specific substrates, A) Sulphur by Sulphatase, B) Hemicellulose by Xylosidase and C)Cellulose by Glucosidase). Box and whisker plots representing the 25% and 75% quartiles, and the solid line is the median. With the whisker representing the range of data. X-axis labels represent the Flooding duration for flooded (F) and recovery (R) states, each bar represents n=3.

Enzymatic functionality of the communities alters between the flooded and recovery states, all three enzymes were combined to create a PCA plot (Figure 15). However, after flooding events and during the recovery periods, enzyme activity becomes similar as community function amalgamates and clusters close together, indicating a mixture of microbial community function. Its noted pasture community function during the recovery period becomes similar to that of saltmarsh sites with increasing *flooding duration.*

It’s noted that *flooding duration* has no significant effect (ADONIS R=0.0902, P=0.173) during the flooding period (A). Increasing flooding duration causes Pasture communities to converge and become similar in function with saltmarsh samples. This is observed through a grouping of both saltmarsh and Pasture communities in the centre of the PCA plot during flooding treatment. With the most extreme movement of enzyme activity after 192 hours flooding. A significant effect of *Site* is observed (ADONIS R=0.167 P=0.002), pasture sites display a larger variability of function during seawater submersion, this is represented by the larger ellipses, and spread points indicating a larger impact of seawater submersion duration on community function

Saltmarsh communities demonstrated a higher resilience to seawater inundation with community function retaining similarity under increasing exposure to seawater during the recovery period (B) compared to the flooded state (A). Exposure to seawater *flooding duration* treatment significantly altered the functionality within pasture sites after the 14-day recovery period (B) (ADONIS R=0.160 P=0.003) on community function/composition with functionality within pasture communities became similar to that of saltmarsh communities following 96- and 192-hours exposure and 14-day recovery.

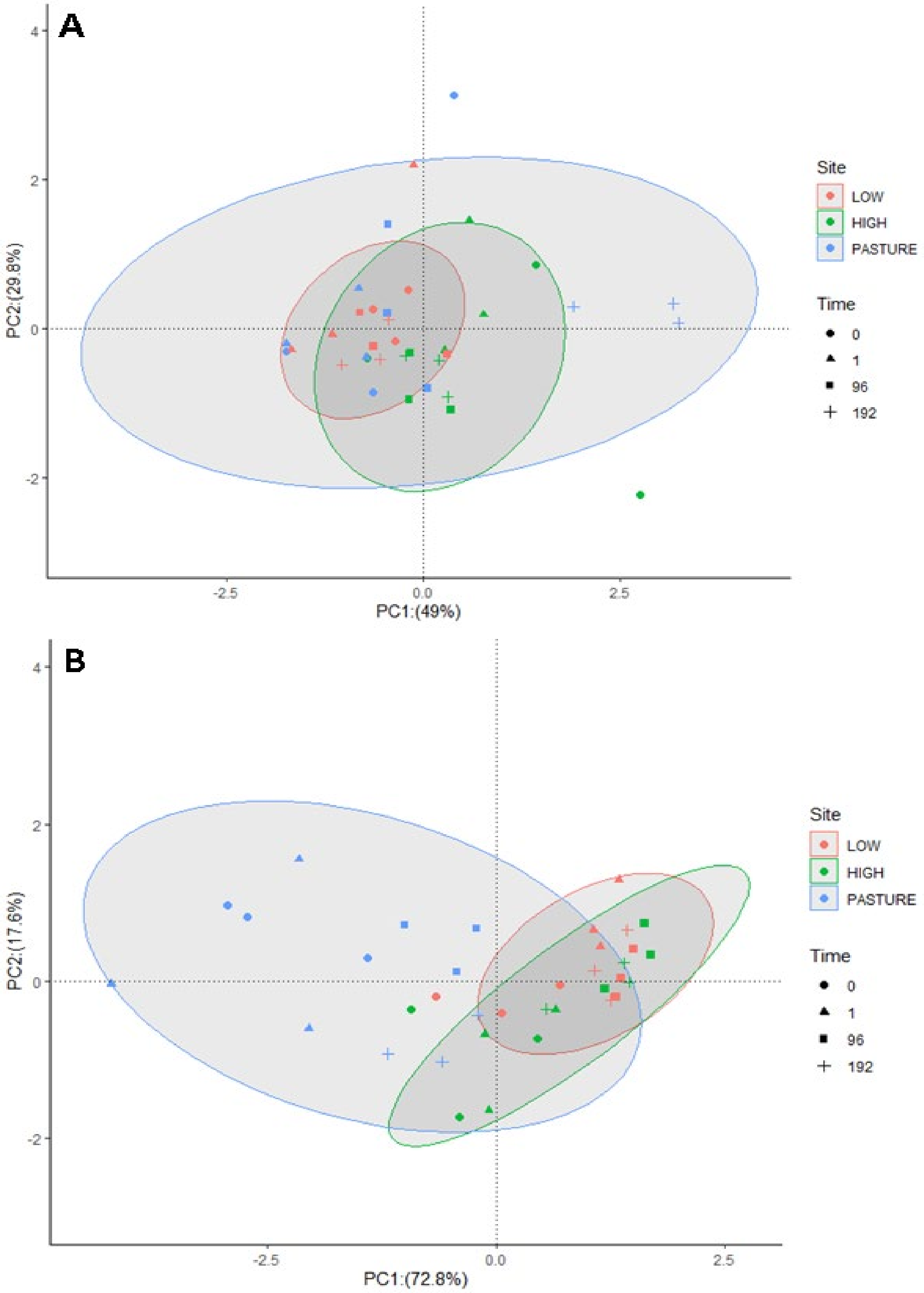


Figure 15 - Principal component analysis (PCA) plot of the 3 substrates enzyme activity (Glucosidase, Xylosidase and Sulphatase) within all the mesocosms before and after flooding treatment (A) and post 14-day recovery (B) activity was measured. Distance between points represents dissimilarity with points closer together representing similarity. Flooding duration is represented circles 0, triangles 1, squares 96 and crosses 192 hours with ellipses representing 95% confidence intervals.

Enzyme per capita activity

To understand the metabolic investment of communities in the production of degradative enzymes for resource acquisition and subsequent changes to nutrient cycling within their environment during this experiment, the enzyme *per capita* activity was calculated. No significance was observed of the *per capita* activity of glucosidase and sulphatase substrates (SI Figures 17 C,D,E,F) , however a significant interaction of flooding duration on *per capita* activity of xylosidase was observed ($F_{3,48}$=4.24 p=0.009) (SI Figures 17 A,B), with activity *per capita* significantly increasing within pasture sites (RHO=0.78,p=0.002). Xylosidase *per capita* activity was higher within High saltmarsh site (0.16 ± 0.17 nM *ATP per capita*) than pasture (0.09 ± 0.11 nM *ATP per capita*) or Low sites (0.07 ± 0.15 nM *ATP per capita*).

3.3 DNA extraction and 16S sequencing

DNA extraction and subsequent PCR has been completed, however the final results from the sequencing were unable to be included at this time point due to the global pandemic and time restrictions.

4. Discussion

This research project aimed to elucidate the effect of seawater inundation on soil characteristics, including environmental parameters, and microbial community function. Environmental parameters and microbial functioning were measured across three distinct sampling sites with varying previous exposure to seawater inundation - two saltmarsh sites previously exposed to repeated seawater inundation, but with varying frequencies, and a pasture site that had not been previously exposed to seawater. Overall, the environmental characteristics shifted in the mesocosms as flooding duration increased, Microbial community function and their respective soils composition varied in response to flooding and pasture mesocosms were shown to have more impacted in physicochemical properties and functioning within pasture communities than saltmarsh sites.

4.1 Saltwater significantly impacts environmental parameters

Of the environmental parameters measured, pH, conductivity and moisture were all significantly impacted by increased flooding duration (Figures 5,6,7). For these measures, the introduction of seawater and increasing flooding duration causes the conductivity and pH values of the soil to become more like that of the neat seawater. Whilst this was not unexpected (Nacke et al., 2017; Taylor and Krüger, 2019; Xian et al., 2019) the results suggest that even a flash flood, here mimicked by the 1-hour flooding event, can have significant changes to the soil environment. Following the drainage and recovery period; elevated physiochemical properties were retained post 14-day recovery.

Further, analysis indicated that the pasture and saltmarsh soils had varying responses to flooding. Physiochemical properties are similar throughout experimental flooding in saltmarsh soils, high alkalinity and salinity are features of coastal ecosystems due to previous tidal inundation of seawater and compositional elements. (Wang et al., 2014). Whereas in pasture site physiochemical properties (conductivity and pH) increased with flooding duration. The significant increase in pH observed within pasture mesocosms has been suggested to disrupt soil aggregate stability (Wu et al., 2017). The disruption in aggregates can be potentially damaging to organic matter within the soils impacting microbial community function and structure within the ecosystems (Wilpiszeski et al., 2019), with observed competition between plants and communities for nutrients (Ke and Miki, 2015). Due to the differences in soil type, pasture sites /

sandy loams would be more susceptible to infiltration, erosion and runoff during storm surges due to the sandy composition within urban areas (Hossain Anni et al., 2020). In this study, the changes in soil type would influence the ability of the alkaline, ion rich, seawater to access the macropores in the soil (Dongli et al., 2015) subsequently disrupting nutrients and water availability (Shrivastava and Kumar, 2015).

Soil physical properties are altered with increased salinity, disrupting the soil structure as fine particles bind together forming aggregates, altering soil porosity and drainage within the soils (Warrence et al., 2002). With increasing soil salinity, as observed within the pasture sites, this could lead to negative effect on crop productivity and yield. The lack of available water for plants to extract and domination of salts to toxic levels, potentially decreases plant yield and microbial community activity (Wilson et al., 2018). The response of microbial communities to flooding and subsequent drainage varies with soil type (Jin et al., 2013). Observed alterations within environmental characteristics strongly affect respiration and nutrient mineralization within soil environments, with observed links between decomposition and soil moisture content (Manzoni et al., 2012). The effects of a soil type able to drain water more effectively will withstand and resist prolonged water logging events. Soil type could be the reason for faster drainage and infiltration due to soil composition. Larger pores between particles are observed within sandy soils and potentially able to mitigate the waterlogging conditions more effectively as observed within Pasture sites. It was observed that moisture content in Pasture reduced during the recovery state more than when compared to saltmarsh sites. However, clay soils are compact with smaller pores retaining higher levels of moisture observed within the saltmarsh sites. Clay soils are more impacted by salinity ingress with sodium ions altering clay particles within the soils, affecting physiochemical properties (Frenkel et al., 1978).

Further evidence has suggested that clay soils retained higher levels of water following significant precipitation and recorded subsequent effects on nitrogen cycling, experiencing loss of nitrates within soils due to leaching (van Es et al., 2005). Whilst Carbon: Nitrogen (C:N) were higher in the saltmarsh sites, how this measure was influenced by seawater inundation varied. Low site subsequently decreasing with further submersion whereas C:N was similar throughout the flooding state for High. The observed C:N ratio was higher within the saltmarsh sites due to previous infrequent and frequent tidal inundation.

Increased flooding duration has shown to increase C:N within the soils, under anaerobic conditions and depleted low oxygen levels lead to carbon sequestration within soil ecosystems. Microbial activities are restricted, limiting biogeochemical cycles and reducing aerobic decomposition of organic matter (De-Campos et al., 2012). It would be expected to see potential increases of carbon sequestration and subsequently increase of C:N within pasture sites similar to levels of saltmarsh sites with repeated or prolonged submersion durations. A previous study found that higher C:N ratio and changing pH levels within soil samples disrupting community composition and structure (Wan et al., 2015).

Within this study its observed that saltmarsh sites were heavily polluted with heavy metals (Cr ,Cu, Co, Pb ,Zn SI Figures) as indicated by principal component analysis, with lower concentrations levels observed within Pasture site (SI figures 1-16). Saltmarsh ecosystems act as a natural sync accumulating metals in vegetation and sediments (Williams et al., 1994). The source of elevated levels of metals occurs due to anthropogenic activities introducing metals within the aquatic ecosystems (Gao et al., 2016). Increasing concentrations of heavy metals due to seawater inundation linked to rising sea levels and anthropogenic activities, could lead to potentially contamination of agricultural lands with heavy metals present in saltmarsh sites and marine sediment. It was observed that conductivity rose with extended flooding duration in the soils due to the influx of seawater, comprising mainly of dissolved ions of S, Na, Mg , K and Ca (Besson et al., 2014). Previous research has observed the effect of increased salinity and ionic strength (Na^+) linked to the mobility of heavy metals (Cr, Cu, Co, Pb and Zn) in soils and loamy soils (Acosta et al., 2011, Fonseca et al., 2011). A previous study conducted by Raiesi and Sadeghi (2019) had observed the negative effects of increased salinity within an experiment conducted with cadmium. Salinity subsequently increased the mobility and concentration to toxic levels, leading to decreasing enzyme activity, biomass, and respiration within microbial communities (Raiesi and Sadeghi, 2019). This also has been observed within other studies demonstrating increased occurrences of freshwater flooding altering soil properties (pH, dissolved organic matter) linked to the increased mobility of toxic elements (Ponting et al., 2021).

4.2 Flooding decreases per capita metabolic potential, but the impact is mitigated by legacy effects

Our results have demonstrated that seawater inundation causes a disturbance within the physical and chemical properties of the soil. Previous studies have indicated that this will be reflected within the microbial community composition and functioning (Neubauer, 2013), which are confirmed in this study.

Results from this study showed that prolonged seawater flooding duration and subsequent recovery have a significant effect on bacterial enumeration and community metabolic potential. With the flooding state leading to community metabolic potential reducing to similar levels between all sites, whereas a significant difference in function is observed following the cessation of flooding and 14-day recovery period. It is noted within all communities that they are stressed under anoxic, saline conditions (Hurng and Kao, 1994) with preadaptation and stress responses exudated by communities within saltmarsh sites to a lesser degree than those naïve communities.

Communities demonstrate a functional and compositional response to disturbances in abiotic and biotic conditions within soil environments (Goberna et al., 2014). This is repeated within this study with bacterial enumeration increasing with flooding duration in all sites. This is potentially due to competitive exclusion for growth being reduced as environmental parameters change and fast-growing members of the community become stressed. Thus, mitigating the main competition between common and rare species, allowing growth of communities previously inhibited (Hibbing et al., 2010; Jousset et al., 2017).

As the selection pressure of flooding is removed during the recovery period the selective advantage is also removed for those members of the communities adapted to the stressed environment. However, the reduction in observed number of bacteria to the stress events suggest that the originally dominant bacteria are still unable to return to their pre-flooded level. To understand how this affected the metabolism of these bacteria, the enumeration of bacteria was used to create a per capita response of metabolic potential observed within the mesocosms.

The soils within pasture sites were previously unexposed and un-adapted to rapid influx of salinity and anoxic conditions demonstrating the responses to flooding treatment within this study. ATP per capita significantly decreases within all sites under extended flooding duration, indicating a decline in cell viability and potential metabolic activity within all communities. Following a flash flood (1 hour) community activity within pasture site is retained in the recovery period, this suggested communities from the Pasture site were resilient to short inundations that did not drastically alter community function. However, the changes within the environmental characteristics and persistent change of the physicochemical properties of the soil has been so drastic following 1-hour inundation. The future implications on communities to contend with increased salinity and altered properties of the soils rather than the prolonged anoxic conditions could lead to shifts in community composition and function (Rath et al., 2019).

Further continued flooding demonstrated Pasture communities were susceptible to significant change in function after prolonged flooding durations of 96 and 192 hours. After this flooding duration community activity is unable to return to prior levels indicating a slower rate of recovery observed and lower metabolic potential (Colmer and Voesenek, 2009). This could indicate an altered state of community structure and function observed within Pasture site able to tolerate the influx of salinity and prolonged submersion following flooding treatment to utilize resources effectively (Hibbing et al., 2010). Whereas the results demonstrate saltmarsh community's functionality returns to previous levels prior to flooding thriving within the conditions. (Rath et al., 2019). Potentially suggesting that the aerobic microorganisms had recovered after the flooding events with no significant changes and able to cope with inundation and changes in salinity (González Macé et al., 2016).

Previous studies have shown that activity decreased under prolonged water logging and increased salinity conditions due to anoxic conditions (Wichern et al., 2006) and altered composition of microbial communities (Randle-Boggis et al., 2018). With results suggesting a shift to communities more diverse and thriving within the environmental stresses. As community composition shifts from aerobic to anaerobic respiration, this leads to depleted oxygen levels within the soils and the biochemical processes that create ATP are reduced (Wang et al., 2017; Yang et al., 2016; Wichern et al., 2006). Despite the universal decrease in metabolic potential, significant differences were observed between sites. The communities from the saltmarsh sites demonstrated a higher resilience to seawater exposure and metabolic potential recovering at a faster rate during the recovery period. Whereas communities from

Pasture sites are less resilient to seawater inundation and metabolic potential significantly reduced following extended flooding duration.

Overall, the metabolic potential following seawater flooding declined, with an altered change observed within nutrient / utilization of carbon sources within the soil organic matter. This change in function and ecosystems will have detrimental impacts on other elements present in the soils including activity of soil microorganisms (Wang et al.,2011) and nutrient availability, reduced crop yields by inhibiting growth of non-halotolerant plant species, (Zörb et al., 2019).

4.3 Extracellular enzyme activity decreases following flooding inundation

Previous studies have demonstrated the negative impacts of saline ingress and flooding duration on enzyme activity (Servais et al., 2020; Sinsabaugh et al., 2008), with inundation shown to be more disruptive than increased salinity (Chambers et al., 2016). Prolonged flooding leads to anoxic conditions and subsequently alters soils enzyme activity and readily available nutrients as well as depleting soil oxygen levels due to microbial activity (Chaudhary et al., 2016; Wright et al., 2015). Microbial activity is key for nutrient cycling through production of key enzymes responsible for hydrolysis of molecules which in turn affects primary productivity (Dregne, 1976).

Within the results, the increasing flooding duration had a significant effect on enzyme activity within all sites, extended flooding duration led to a decline of observed enzyme activity in communities within the recovery period with enzyme activity levels becoming similar across the three sites. The substrate assays demonstrate community adaptation to seawater ingress within the saltmarsh sites and the significant effects subjected on naïve community functioning. Interestingly enzyme activity within the flooded state is similar in communities from all sites following trends between saltmarsh and pasture mesocosms, however during the recovery period differences are observed within responses between sites (Figure 12 and 13). It's noted that the use of liable substrates increased with flooding duration indicating a stress response of a shift of communities utilizing the readily available carbon sources with a shift to more recalcitrant carbon sources further in the soil profile (Romero-Olivares et al., 2017).

Enzymes from soil microbial communities within this study were sensitive to both short- and long-term exposure to salinity and prolonged waterlogging, with significant effects noted within the pasture site soil samples. Reason for the decline in enzyme activity could be linked to the shift in resource use for microbial communities to produce osmolytes to prevent lysis of cells during increased negative osmotic pressure under high salinity and toxicity of heavy metals (Jogawat, 2019). Demonstrated within this study that microbes had shifted to survival mechanisms to cope with environmental stresses.

Given the constant exposure to high salinity and frequent waterlogging experienced at saltmarsh sites, observed community function is retained throughout the experimental treatment seawater exposure to naïve communities taken from Pasture demonstrated a significant impact on community function (Zhang et al., 2021). Xylosidase activity significantly increased within the Pasture communities during the flooded state, with activity increasing with extending flooding duration (figure 12), an observed shift in community composition and function indicating a community under stress from osmotic pressure and anoxic conditions Pasture communities utilizing the liable easily degradable carbon source hemicellulose (Xylosidase) for metabolic activity with competition between communities following prolonged anoxic conditions.

The results are confirmatory of a previous study conducted by (Wilson et al., 2011) and (Sjøgaard et al.,2018), who found communities from agricultural soil are altered by seawater inundation, changing composition and significantly altering activity. Communities utilized liable organic matter, and composition was significantly driven by the availability of liable organic matter in soils flooded with seawater (Sjøgaard et al., 2018). The significant increase in usage of liable substrates within the pasture communities due to stress response and mutating to adapt to the shift in environmental conditions demonstrated within this experiment. Further evidence to support a change in substrate use and decreasing metabolic activity during the recovery state could be linked to a change in community composition due to prolonged flooding events (Unger et al., 2009).

A shift in community function was observed with sulphatase activity inhibited within all sites during flooding as communities utilize easily mobile substrates and prioritise defence mechanisms to waterlogging and salinity (Randle-Boggis et al., 2018;

Sjøgaard et al., 2017) Sulphatase activity represents an increase or decrease of anaerobic respiration within the soil environment. It was shown that activity of sulphatase increases in the recovery *state* with sulphatase activity highest within the pasture mesocosms, with activity decreasing following extended flooding duration in mesocosms. Sulphur is a key macronutrient essential for growth and its other organic compounds play an important role in plants for enzyme structure, defence to biotic and abiotic stresses such as drought (Samanta et al., 2020). Sulphur deficiency within plants has been linked to reduced plant productivity and crop yield (Hawkesford, 2000). Decreasing levels of sulfatase activity observed within this study for pasture mesocosms with increasing seawater submersion altered microbial functioning leading to levels of sulphatase activity to decrease.

Previous studies have shown that the dominant anaerobic metabolic pathway in communities exposed to seawater and increased salinity is sulphate reduction (Weston et al., 2006). Sulphidation during the ingress of seawater is due to the relatively high presence of sulphide present, during flooding and low oxygen levels, sulphate reduction in the primary microbial metabolic pathway. Frequent or chronic intrusion of seawater is shown to have determinantal effects on ecosystems with the increase of hydrogen sulphide (Tully et al., 2019), the sulphur-reduced product from seawater intrusion is toxic to plants and organisms effecting plant productivity and composition with uptake of nutrients (Zhang et al., 2017).

B-Glucosidase activity is used as an indicator for soil quality with activity sensitive to changes in organic matter quality and quantity (Sánchez-Rodríguez et al., 2017; Knight and Dick, 2004). In this experiment, glucosidase activity varied during the flooded state within mesocosms. Higher levels of glucosidase activity were observed within the pasture sites, with activity increasing with flooding treatment duration. This trend was reversed with activity decreasing in both saltmarsh sites. Lower levels of glucosidase activity within saltmarsh sites can be linked to the increased concentration of heavy metals present in the sediment as shown in this experiment and previous studies confirming the toxic effects of heavy metals and increased salinity on enzyme activities (Angelovičová et al., 2014; YuanPeng Wang et al., 2007).

Heavy metal toxicity can reduce the diversity and activity of microbial communities (Chu, 2018), impacting the roles of microbial communities responsible for nutrient cycling and organic matter decomposition within the ecosystems with shifts of composition and function to tolerate heavy metal toxicity (Singh and Kalamdhad, 2011). The data showed that lower levels of heavy metals are present within the Pasture sites corresponding to the higher activity of glucosidase and overall soil decomposition and nutrient cycling more prominent within this site. However, it is noted within the recovery period the combination of flooding and increased salinity, subsequently decreased and inhibited glucosidase activity in all sites, indicating a decrease in organic matter decomposition following inundation. It has been shown that the combination of enzyme activity can be used as an indicator of pollution (Dick, 1994), especially glucosidase for the effects of heavy metal pollution within soils (Ramamoorthy, 2015).

It was hypothesised that exposure to flooding events will cause natural microbial communities to become more distinct in terms of functioning. Communities from pasture site demonstrated a larger functional diversity with a higher activity of all three enzymes in the control, and 1-hour flooding durations. However, extended flooding reduced enzyme activity indicating a possible shift in community composition and potentially altering the interactions between the bacteria, most likely the rarer abundance species (Rivett and Bell, 2018), particularly with changing environmental conditions (Rillig et al., 2016; Diez et al., 2012)

Conversely, communities from habitats frequently exposed to seawater inundation will have increased resistance to functional change during prolonged flooding events. In line with the hypothesis 2, the data suggests communities from the saltmarsh sites display preadaptation with an increased resistance to prolonged flooding duration and seawater inundation. This was observed with rapid recovery of metabolic potential and retention of community functionality by enzyme activity during flooding and after the recovery period within saltmarsh sites. Demonstrating the abilities of communities to recover following disturbances to previous states within a respective environment previously adapted to (Shade et al., 2012).

Community functioning remained similar throughout flooding and recovery periods with metabolic potential thriving following the 14-day recovery period, an indication of preadaptation to flooding conditions with increasing community activity and retained function. This was supported by a previous study that had found that communities will be resilient to pulse events, however a continuous press disturbance could alter ecosystem function and microbial diversity adversely (Mobilian et al., 2020). Community composition will alter under flooding and drainage as microbial communities are perturbed, inhibiting stabilization within the community as either aerobic or anaerobic population, with a community that tolerates either (Randle-Boggis et al., 2018), its expected with the completion of the sequencing data to identify a similar composition within the saltmarsh sites that tolerate perturbations.

Due to the Covid-19 pandemic and unforeseen circumstances, results from the Next generation sequencing were unable to be completed prior to the submission date for this thesis. However, this data will be included within future publications. The data produced from this will underpin the community dynamics to match this to the observed change in environmental and functional characteristics and identify the communities responsible for the drive-in functional changes and resilience and resistance of composition and functional redundancy (Shade et al., 2012; Allison and Martiny, 2008). From previous environmental and functional analysis conducted within this experiment, the outcome of community composition and structure would have been significantly altered in pasture sites following extended flooding duration. Saltmarsh with communities frequently exposed to perturbations able to resist the changes in anoxic conditions and rapid shifts in community function.

A previous paper had shown that periodic flooding and drainage had shaped the microbiomes within agricultural soils (Gschwend et al., 2020). This shaping of the community is expected from the sequencing data within this study displaying a similar trend to be observed with flooding significantly altering the agricultural sites as communities shift with the environmental conditions. However, we would also predict that the saltmarsh communities would be less affected by flooding due to their previous adaptation to the stress (Preece et al., 2019).

4.4 Future work

A focus onto larger scale area studies across multiple habitats, as well as emphasising the role of plant microbe interactions as previous studies have shown reduction in growth and yield of crops (Hanley et al., 2019). Further analysis on microbial functioning and structure in reclaimed saltmarshes to understand the effects of environmental shifts and potential functioning within these environments (Zhang et al., 2021).

5. Conclusion

Overall, within this experiment its noted that saltwater ingress on pasture soils significantly alters soil properties and community function in habitats previously unexposed. As storm surge flooding in areas have been shown to last for at least 7 days (Ramsey III et al., 2011), the physicochemical properties of soils will be altered to high alkalinity and pH damaging soil health.

With alterations observed within community functioning and alteration to soil health caused by seawater ingress, the results suggested a detrimental impact on key microbial ecosystem processes. This could cause knock-on effects to surrounding plants present in the affected soil, reducing crop productivity and yield. The risk to food security as climate change increases, through these storm surges, is increasing and studies like this are vital in understanding the consequences of these events.

References

Acosta, J. A., Jansen, B., Kalbitz, K., Faz, A. and Martínez-Martínez, S. (2011) 'Salinity increases mobility of heavy metals in soils.' *Chemosphere*, 85(8), 2011/11/01/, pp. 1318-1324.

Allison, S. D. and Martiny, J. B. H. (2008) 'Resistance, resilience, and redundancy in microbial communities.' *Proceedings of the National Academy of Sciences*, 105(Supplement 1) pp. 11512-11519.

Angelovičová, L., Lodenius, M., Tulisalo, E. and Fazekašová, D. (2014) 'Effect of heavy metals on soil enzyme activity at different field conditions in Middle Spis mining area (Slovakia).' *Bulletin of environmental contamination and toxicology*, 93(6) pp. 670-675.

Arnosti, C. (2003) '13 - Microbial Extracellular Enzymes and their Role in Dissolved Organic Matter Cycling.' *In* Findlay, S. E. G. and Sinsabaugh, R. L. (eds.) *Aquatic Ecosystems*. Burlington: Academic Press, pp. 315-342.

Barbier, E. B., Hacker, S. D., Kennedy, C., Koch, E. W., Stier, A. C. and Silliman, B. R. (2011) 'The value of estuarine and coastal ecosystem services.' *Ecological Monographs*, 81(2) pp. 169-193.

Bardgett, R. D. and Caruso, T. (2020) 'Soil microbial community responses to climate extremes: resistance, resilience and transitions to alternative states.' *Philosophical Transactions of the Royal Society B: Biological Sciences*, 375(1794) p. 20190112.

Besson, P., Degboe, J., Berge, B., Chavagnac, V., Fabre, S. and Berger, G. (2014) 'Calcium, Na, K and Mg Concentrations in Seawater by Inductively Coupled Plasma-Atomic Emission Spectrometry: Applications to IAPSO Seawater Reference Material, Hydrothermal Fluids and Synthetic Seawater Solutions.' *Geostandards and Geoanalytical Research*, 38(3) pp. 355-362.

Betzold, C. and Mohamed, I. (2017) 'Seawalls as a response to coastal erosion and flooding: a case study from Grande Comore, Comoros (West Indian Ocean).' *Regional Environmental Change*, 17(4), 2017/04/01, pp. 1077-1087.

Brookes, P. (1995) 'The use of microbial parameters in monitoring soil pollution by heavy metals.' *Biology and Fertility of soils*, 19(4) pp. 269-279.

Bünemann, E. K., Bongiorno, G., Bai, Z., Creamer, R. E., De Deyn, G., de Goede, R., Fleskens, L., Geissen, V., et al. (2018) 'Soil quality – A critical review.' *Soil Biology and Biochemistry*, 120, 2018/05/01/, pp. 105-125.

Chambers, L. G., Davis, S. E., Troxler, T., Boyer, J. N., Downey-Wall, A. and Scinto, L. J. (2014) 'Biogeochemical effects of simulated sea level rise on carbon loss in an Everglades mangrove peat soil.' *Hydrobiologia*, 726(1), 2014/03/01, pp. 195-211.

Chambers, L. G., Guevara, R., Boyer, J. N., Troxler, T. G. and Davis, S. E. (2016) 'Effects of Salinity and Inundation on Microbial Community Structure and Function in a Mangrove Peat Soil.' *Wetlands*, 36(2), 2016/04/01, pp. 361-371.

Chaudhary, D. R., Rathore, A. P. and Jha, B. (2016) 'Effects of seawater irrigation on soil microbial community structure and physiological function.' *International Journal of Environmental Science and Technology*, 13(9), 2016/09/01, pp. 2199-2208.

Cheikh, M. and Momen, M. (2020) 'The interacting effects of storm surge intensification and sea-level rise on coastal resiliency: A high-resolution turbulence resolving case study.' 2, 10/21,

Chu, D. (2018) 'Effects of heavy metals on soil microbial community.' *IOP Conference Series: Earth and Environmental Science*, 113, 2018/02, p. 012009.

Colmer, T. and Voesenek, L. (2009) 'Flooding tolerance: suites of plant traits in variable environments.' *Functional Plant Biology*, 36(8) pp. 665-681.

Conrad, R. (2020) 'Methane Production in Soil Environments-Anaerobic Biogeochemistry and Microbial Life between Flooding and Desiccation.' *Microorganisms*, 8(6) p. 881.

Crosby, S. C., Sax, D. F., Palmer, M. E., Booth, H. S., Deegan, L. A., Bertness, M. D. and Leslie, H. M. (2016) 'Salt marsh persistence is threatened by predicted sea-level rise.' *Estuarine, Coastal and Shelf Science*, 181, 2016/11/05/, pp. 93-99.

Daliakopoulos, I. N., Tsanis, I. K., Koutroulis, A., Kourgialas, N. N., Varouchakis, A. E., Karatzas, G. P. and Ritsema, C. J. (2016) 'The threat of soil salinity: A European scale review.' *Science of The Total Environment*, 573, 2016/12/15/, pp. 727-739.

de Vries, F. T., Manning, P., Tallowin, J. R. B., Mortimer, S. R., Pilgrim, E. S., Harrison, K. A., Hobbs, P. J., Quirk, H., et al. (2012) 'Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities.' *Ecology Letters*, 15(11), 2012/11/01, pp. 1230-1239.

De-Campos, A. B., Huang, C.-h. and Johnston, C. T. (2012) 'Biogeochemistry of terrestrial soils as influenced by short-term flooding.' *Biogeochemistry*, 111(1), 2012/11/01, pp. 239-252.

Dick, R. P. (1994) 'Soil enzyme activities as indicators of soil quality.' *Defining soil quality for a sustainable environment*, 35 pp. 107-124.

Dick, R. P., Breakwell, D. P. and Turco, R. F. (1997) 'Soil enzyme activities and biodiversity measurements as integrative microbiological indicators.' *Methods for assessing soil quality*, 49 pp. 247-271.

Diez, J. M., D'Antonio, C. M., Dukes, J. S., Grosholz, E. D., Olden, J. D., Sorte, C. J., Blumenthal, D. M., Bradley, B. A., et al. (2012) 'Will extreme climatic events facilitate biological invasions?' *Frontiers in Ecology and the Environment*, 10(5) pp. 249-257.

Dongli, S., Xuemei, G., Peng, W., Wentao, X., Yingying, L. and Yi, L. (2015) 'Comparison of soil hydraulic properties with different levels of soil salinity and sodicity.' *Arabian Journal of Geosciences*, 8(8), 2015/08/01, pp. 5351-5360.

Dregne, H. E. (1976) *Soils of arid regions.* Amsterdam: Elsevier Scientific Pub. Co.

Drenovsky, R. E., Steenwerth, K. L., Jackson, L. E. and Scow, K. M. (2010) 'Land use and climatic factors structure regional patterns in soil microbial communities.' *Global Ecology and Biogeography*, 19(1) pp. 27-39.

Elmholt, S., Munkholm, L. J., Debosz, K. and Schjønning, P. (2000) 'Biotic and abiotic binding and bonding mechanisms in soils with long-term differences in management.' *DIAS report*, 38 pp. 53-62.

Fierer, N., Breitbart, M., Nulton, J., Salamon, P., Lozupone, C., Jones, R., Robeson, M., Edwards, R. A., et al. (2007) 'Metagenomic and Small-Subunit rRNA Analyses Reveal the Genetic Diversity of Bacteria, Archaea, Fungi, and Viruses in Soil.' 2007-11-01,

Fonseca, B., Figueiredo, H., Rodrigues, J., Queiroz, A. and Tavares, T. (2011) 'Mobility of Cr, Pb, Cd, Cu and Zn in a loamy sand soil: A comparative study.' *Geoderma*, 164(3), 2011/09/15/, pp. 232-237.

Fox, J. and Weisberg, S. (2019) *An R Companion to Applied Regression.* Third edition ed.: Sage.

Frenkel, H., Goertzen, J. O. and Rhoades, J. D. (1978) 'Effects of Clay Type and Content, Exchangeable Sodium Percentage, and Electrolyte Concentration on Clay Dispersion and Soil Hydraulic Conductivity.' *Soil Science Society of America Journal*, 42(1) pp. 32-39.

Frossard, A., Gerull, L., Mutz, M. and Gessner, M. O. (2012) 'Disconnect of microbial structure and function: enzyme activities and bacterial communities in nascent stream corridors.' *The ISME Journal*, 6(3), 2012/03/01, pp. 680-691.

Galand, P. E., Lucas, S., Fagervold, S. K., Peru, E., Pruski, A. M., Vétion, G., Dupuy, C. and Guizien, K. (2016) 'Disturbance Increases Microbial Community Diversity and Production in Marine Sediments.' *Frontiers in microbiology*, 7 pp. 1950-1950.

Gao, W., Du, Y., Gao, S., Ingels, J. and Wang, D. (2016) 'Heavy metal accumulation reflecting natural sedimentary processes and anthropogenic activities in two contrasting coastal wetland ecosystems, eastern China.' *Journal of Soils and Sediments*, 16(3), 2016/03/01, pp. 1093-1108.

Glenn, E. P., Brown, J. J. and O’Leary, J. W. (1998) 'Irrigating crops with seawater.' *Scientific American*, 279(2) pp. 76-81.

Goberna, M., Navarro-Cano, J. A., Valiente-Banuet, A., García, C. and Verdú, M. (2014) 'Abiotic stress tolerance and competition-related traits underlie phylogenetic clustering in soil bacterial communities.' *Ecology Letters*, 17(10) pp. 1191-1201.

González Macé, O., Steinauer, K., Jousset, A., Eisenhauer, N. and Scheu, S. (2016) 'Flood-Induced Changes in Soil Microbial Functions as Modified by Plant Diversity.' *PloS one*, 11(11) pp. e0166349-e0166349.

Griffiths, B. S., Hallett, P. D., Kuan, H. L., Gregory, A. S., Watts, C. W. and Whitmore, A. P. (2007) 'Functional resilience of soil microbial communities depends on both soil structure and microbial community composition.' *Biology and Fertility of Soils*, 44(5), 2007-12-05, pp. 745-754.

Gschwend, F., Aregger, K., Gramlich, A., Walter, T. and Widmer, F. (2020) 'Periodic waterlogging consistently shapes agricultural soil microbiomes by promoting specific taxa.' *Applied Soil Ecology*, 155, 2020/11/01/, p. 103623.

Hanley, M. E., Hartley, F. C., Hayes, L. and Franco, M. (2019) 'Simulated seawater flooding reduces oilseed rape growth, yield and progeny performance.' *Annals of Botany*, 125(2) pp. 247-254.

Hawkesford, M. J. (2000) 'Plant responses to sulphur deficiency and the genetic manipulation of sulphate transporters to improve S-utilization efficiency.' *Journal of Experimental Botany*, 51(342) pp. 131-138.

Heijden, v. d., RD, B. and NM, v. S. (2008) 'The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems.' *Ecology letters*, 11(3), 2008 Mar,

Herbert, E. R., Boon, P., Burgin, A. J., Neubauer, S. C., Franklin, R. B., Ardón, M., Hopfensperger, K. N., Lamers, L. P. M., et al. (2015) 'A global perspective on wetland salinization: ecological consequences of a growing threat to freshwater wetlands.' *Ecosphere*, 6(10) p. art206.

Hibbing, M. E., Fuqua, C., Parsek, M. R. and Peterson, S. B. (2010) 'Bacterial competition: surviving and thriving in the microbial jungle.' *Nature reviews. Microbiology*, 8(1) pp. 15-25.

Hossain Anni, A., Cohen, S. and Praskievicz, S. (2020) 'Sensitivity of urban flood simulations to stormwater infrastructure and soil infiltration.' *Journal of Hydrology*, 588, 2020/09/01/, p. 125028.

Hurng, W. P. and Kao, C. H. (1994) 'Effect of flooding on the activities of some enzymes of activated oxygen metabolism, the levels of antioxidants, and lipid peroxidation in senescing tobacco leaves.' *Plant Growth Regulation*, 14(1), 1994/01/01, pp. 37-44.

Jadhav, R. S., Chen, Q. and Smith, J. M. (2013) 'Spectral distribution of wave energy dissipation by salt marsh vegetation.' *Coastal Engineering*, 77, 2013/07/01/, pp. 99-107.

Jin, V. L., Haney, R. L., Fay, P. A. and Polley, H. W. (2013) 'Soil type and moisture regime control microbial C and N mineralization in grassland soils more than atmospheric CO2-induced changes in litter quality.' *Soil Biology and Biochemistry*, 58, 2013/03/01/, pp. 172-180.

Jogawat, A. (2019) 'Osmolytes and their Role in Abiotic Stress Tolerance in Plants.' *In Molecular Plant Abiotic Stress*. pp. 91-104.

Jousset, A., Bienhold, C., Chatzinotas, A., Gallien, L., Gobet, A., Kurm, V., Küsel, K., Rillig, M. C., et al. (2017) 'Where less may be more: how the rare biosphere pulls ecosystems strings.' *The ISME Journal*, 11(4), 2017/04/01, pp. 853-862.

Ke, P.-J. and Miki, T. (2015) 'Incorporating the soil environment and microbial community into plant competition theory.' *Frontiers in Microbiology*, 6(1066), 2015-October-08,

Knight, T. R. and Dick, R. P. (2004) 'Differentiating microbial and stabilized β-glucosidase activity relative to soil quality.' *Soil Biology and Biochemistry*, 36(12), 2004/12/01/, pp. 2089-2096.

Lee, J. S., Kim, J.-W., Lee, S. H., Myeong, H.-H., Lee, J.-Y. and Cho, J. S. (2016) 'Zonation and soil factors of salt marsh halophyte communities.' *Journal of Ecology and Environment*, 40(1), 2016/10/24, p. 4.

Li, S.-H., Ge, Z.-M., Xie, L.-N., Chen, W., Yuan, L., Wang, D.-Q., Li, X.-Z. and Zhang, L.-Q. (2018) 'Ecophysiological response of native and exotic salt marsh vegetation to waterlogging and salinity: Implications for the effects of sea-level rise.' *Scientific reports*, 8(1) pp. 1-13.

Lomakina, G. Y., Modestova, Y. A. and Ugarova, N. N. (2015) 'Bioluminescence assay for cell viability.' *Biochemistry (Moscow)*, 80(6), 2015/06/01, pp. 701-713.

Lombard, N., Prestat, E., van Elsas, J. D. and Simonet, P. (2011) 'Soil-specific limitations for access and analysis of soil microbial communities by metagenomics.' *FEMS Microbiology Ecology*, 78(1) pp. 31-49.

Luo, M., Huang, J.-F., Zhu, W.-F. and Tong, C. (2019) 'Impacts of increasing salinity and inundation on rates and pathways of organic carbon mineralization in tidal wetlands: a review.' *Hydrobiologia*, 827(1), 2019/01/01, pp. 31-49.

Lynum, C., Bulseco, A., Dunphy, C., Osborne, S., Vineis, J. and Bowen, J. (2020a) 'Microbial Community Response to a Passive Salt Marsh Restoration.' *Estuaries and Coasts*, 43, 03/10, pp. 1-17.

Manzoni, S., Schimel, J. P. and Porporato, A. (2012) 'Responses of soil microbial communities to water stress: results from a meta-analysis.' *Ecology*, 93(4) pp. 930-938.

Margesin, R. and Niklinska, M. A. (2019) 'Editorial: Elevation Gradients: Microbial Indicators of Climate Change?' *Frontiers in Microbiology*, 10(2405), 2019-October-18,

Marshall, I. P. G., Karst, S. M., Nielsen, P. H. and Jørgensen, B. B. (2018) 'Metagenomes from deep Baltic Sea sediments reveal how past and present environmental conditions determine microbial community composition.' *Marine Genomics*, 37, 2018/02/01/, pp. 58-68.

Mayer, H. P. and Conrad, R. (1990) 'Factors influencing the population of methanogenic bacteria and the initiation of methane production upon flooding of paddy soil.' *FEMS Microbiology Ecology*, 6(2) pp. 103-111.

Mobilian, C., Wisnoski, N. I., Lennon, J. T., Alber, M., Widney, S. and Craft, C. B. (2020) 'Microbial community composition is affected by press, but not pulse, seawater intrusion.' *bioRxiv*, p. 2020.2004.2007.030791.

Möller, I. (2006) 'Quantifying saltmarsh vegetation and its effect on wave height dissipation: Results from a UK East coast saltmarsh.' *Estuarine, Coastal and Shelf Science*, 69(3-4) pp. 337-351.

Mombrikotb, S. B. (2016) 'The contribution of environmental selection on microbial community structure, function and biogeography.'

Nacke, H., Schöning, I., Schindler, M., Schrumpf, M., Daniel, R., Nicol, G. W. and Prosser, J. I. (2017) 'Links between seawater flooding, soil ammonia oxidiser communities and their response to changes in salinity.' *FEMS Microbiology Ecology*, 93(11)

Narayan, S., Beck, M. W., Wilson, P., Thomas, C. J., Guerrero, A., Shepard, C. C., Reguero, B. G., Franco, G., et al. (2017) 'The Value of Coastal Wetlands for Flood

Damage Reduction in the Northeastern USA.' *Scientific Reports*, 7(1), 2017/08/31, p. 9463.

Neubauer, S. C. (2013) 'Ecosystem Responses of a Tidal Freshwater Marsh Experiencing Saltwater Intrusion and Altered Hydrology.' *Estuaries and Coasts*, 36(3), 2013/05/01, pp. 491-507.

Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., et al. (2019) *vegan: Community Ecology Package.*

Ponting, J., Kelly, T. J., Verhoef, A., Watts, M. J. and Sizmur, T. (2021) 'The impact of increased flooding occurrence on the mobility of potentially toxic elements in floodplain soil – A review.' *Science of The Total Environment*, 754, 2021/02/01/, p. 142040.

Power, A. G. (2010) 'Ecosystem services and agriculture: tradeoffs and synergies.' *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 365(1554) pp. 2959-2971.

Preece, C., Verbruggen, E., Liu, L., Weedon, J. T. and Peñuelas, J. (2019) 'Effects of past and current drought on the composition and diversity of soil microbial communities.' *Soil Biology and Biochemistry*, 131, 2019/04/01/, pp. 28-39.

R, Core Team. (2020) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

Raiesi, F. and Sadeghi, E. (2019) 'Interactive effect of salinity and cadmium toxicity on soil microbial properties and enzyme activities.' *Ecotoxicology and Environmental Safety*, 168, 2019/01/30/, pp. 221-229.

Ramamoorthy, D. (2015) 'Assessment of heavy metal pollution and its impacts on soil physical, chemical properties and β-glucosidase activities in agricultural lands, Puducherry region.' *Pol J Environ Stud*, 25 pp. 1045-1051.

Ramsey Iii, E., Lu, Z., Suzuoki, Y., Rangoonwala, A. and Werle, D. (2011) 'Monitoring Duration and Extent of Storm-Surge and Flooding in Western Coastal Louisiana Marshes With Envisat ASAR Data.' *Selected Topics in Applied Earth Observations and Remote Sensing, IEEE Journal of*, 4, 07/01, pp. 387-399.

Randle-Boggis, R. J., Ashton, P. D. and Helgason, T. (2018) 'Increasing flooding frequency alters soil microbial communities and functions under laboratory conditions.' *MicrobiologyOpen*, 7(1) p. e00548.

Rath, K. M., Fierer, N., Murphy, D. V. and Rousk, J. (2019) 'Linking bacterial community composition to soil salinity along environmental gradients.' *The ISME journal*, 13(3) pp. 836-846.

Rath, K. M., Maheshwari, A., Rousk, J. and Bailey, M. J. (2019) 'Linking Microbial Community Structure to Trait Distributions and Functions Using Salinity as an Environmental Filter.' *mBio*, 10(4) pp. e01607-01619.

Rillig, M. C., Lehmann, A., Aguilar-Trigueros, C. A., Antonovics, J., Caruso, T., Hempel, S., Lehmann, J., Valyi, K., et al. (2016) 'Soil microbes and community coalescence.' *Pedobiologia*, 59(1), 2016/01/01/, pp. 37-40.

Rivett, D. W. and Bell, T. (2018) 'Abundance determines the functional role of bacterial phylotypes in complex communities.' *Nature microbiology*, 3(7) pp. 767-772.

Rivett, D., Scheuerl, T., Culbert, C., Mombrikotb, S., Johnstone, E., Barraclough, T. and Bell, T. (2016) 'Resource-dependent attenuation of species interactions during bacterial succession.' *The ISME Journal*, 10

Romero-Olivares, A. L., Allison, S. D. and Treseder, K. K. (2017) 'Decomposition of recalcitrant carbon under experimental warming in boreal forest.' *PLOS ONE*, 12(6) p. e0179674.

Rooney, P. (2010) Changing perspectives in coastal dune management. Vol. 14, pp. 71-73. *Journal of Coastal Conservation*: Springer.

Samanta, S., Singh, A. and Roychoudhury, A. (2020) 'Involvement of Sulfur in the Regulation of Abiotic Stress Tolerance in Plants.' *In Protective Chemical Agents in the Amelioration of Plant Abiotic Stress*. pp. 437-466.

Sánchez-Rodríguez, A. R., Hill, P. W., Chadwick, D. R. and Jones, D. L. (2017) 'Crop residues exacerbate the negative effects of extreme flooding on soil quality.' *Biology and Fertility of Soils*, 53(7), 2017-06-19, pp. 751-765.

Sandroni, V., Smith, C. M. M. and Donovan, A. (2003) 'Microwave digestion of sediment, soils and urban particulate matter for trace metal analysis.' *Talanta*, 60(4), 2003/07/04/, pp. 715-723.

Schimel, J., Balser, T. C. and Wallenstein, M. (2007) 'Microbial Stress-response Physiology and its implications for ecosystem function.' *Ecology*, 88(6) pp. 1386-1394.

Servais, S., Kominoski, J. S., Coronado-Molina, C., Bauman, L., Davis, S. E., Gaiser, E. E., Kelly, S., Madden, C., et al. (2020) 'Effects of Saltwater Pulses on Soil Microbial Enzymes and Organic Matter Breakdown in Freshwater and Brackish Coastal Wetlands.' *Estuaries and Coasts*, 43(4), 2020/06/01, pp. 814-830.

Shade, A., Peter, H., Allison, S., Baho, D., Berga, M., Buergmann, H., Huber, D., Langenheder, S., et al. (2012) 'Fundamentals of Microbial Community Resistance and Resilience.' *Frontiers in Microbiology*, 3(417), 2012-December-19,

Sheldon, A., Dalal, R., Kirchhof, G. and Menzies, N. (2017) 'The effect of salinity on plant-available water.' *Plant and Soil*, 418, 09/01, pp. 1-15.

Shepard, C. C., Crain, C. M. and Beck, M. W. (2011) 'The protective role of coastal marshes: a systematic review and meta-analysis.' *PloS one*, 6(11) 2011/11/23, pp. e27374-e27374.

Shrivastava, P. and Kumar, R. (2015) 'Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation.' *Saudi Journal of Biological Sciences*, 22(2), 2015/03/01/, pp. 123-131.

Shrivastava, P. and Kumar, R. (2015) 'Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation.' *Saudi Journal of Biological Sciences*, 22(2), 2015/03/01/, pp. 123-131.

Singh, J. and Kalamdhad, A. S. (2011) 'Effects of heavy metals on soil, plants, human health and aquatic life.' *International journal of Research in Chemistry and Environment*, 1(2) pp. 15-21.

Sinsabaugh, R. L. (1994) 'Enzymic analysis of microbial pattern and process.' *Biology and Fertility of Soils*, 17(1) pp. 69-74.

Sinsabaugh, R. L., Lauber, C. L., Weintraub, M. N., Ahmed, B., Allison, S. D., Crenshaw, C., Contosta, A. R., Cusack, D., et al. (2008) 'Stoichiometry of soil enzyme activity at global scale.' *Ecology letters*, 11(11) pp. 1252-1264.

Sjøgaard, K. S., Nordcee, D. o. B., University of Southern Denmark, Odense M, 5230, Denmark, 0000-0003-3455-1305, Treusch, A. H., Nordcee, D. o. B., University of Southern Denmark, Odense M, 5230, Denmark, Valdemarsen, T. B. and Department of Biology, U. o. S. D., Odense M, 5230, Denmark. (2017) 'Carbon degradation in agricultural soils flooded with seawater after managed coastal realignment.' *Biogeosciences*, 14(18) pp. 4375-4389.

Sjøgaard, K. S., Valdemarsen, T. B. and Treusch, A. H. (2018) 'Responses of an Agricultural Soil Microbiome to Flooding with Seawater after Managed Coastal Realignment.' *Microorganisms*, 6(1) p. 12.

Slangen, A. B. A., Church, J. A., Agosta, C., Fettweis, X., Marzeion, B. and Richter, K. (2016) 'Anthropogenic forcing dominates global mean sea-level rise since 1970.' *Nature Climate Change*, 6(7) pp. 701-705.

Somboonna, N., Wilantho, A., Jankaew, K., Assawamakin, A., Sangsrakru, D., Tangphatsornruang, S. and Tongsima, S. (2014) 'Microbial Ecology of Thailand Tsunami and Non-Tsunami Affected Terrestrials.' *PLOS ONE*, 9(4) p. e94236.

Souza, A., Brown, J., Williams, J. and Lymbery, G. (2013) 'Application of an operational storm coastal impact forecasting system.' *Journal of Operational Oceanography*, 6, 02/01, pp. 23-26.

Stagg, C. L., Schoolmaster, D. R., Krauss, K. W., Cormier, N. and Conner, W. H. (2017) 'Causal mechanisms of soil organic matter decomposition: deconstructing salinity and flooding impacts in coastal wetlands.' *Ecology*, 98(8) pp. 2003-2018.

Suddick, E. C., Whitney, P., Townsend, A. R. and Davidson, E. A. (2013) 'The role of nitrogen in climate change and the impacts of nitrogen–climate interactions in the United States: foreword to thematic issue.' *Biogeochemistry*, 114(1), 2013/07/01, pp. 1-10.

Taylor, M. and Krüger, N. (2019) 'Changes in salinity of a clay soil after a short-term salt water flood event.' *Geoderma Regional*, 19, 2019/11/01/, p. e00239.

Teng, Y., Su, J., Wang, J., Dai, N., Li, J., Song, L. and Zuo, R. (2014) 'Soil microbial community response to seawater intrusion into coastal aquifer of Donghai Island, South China.' *Environmental Earth Sciences*, 72(9), 2014-04-09, pp. 3329-3338.

Tully, K., Gedan, K., Epanchin-Niell, R., Strong, A., Bernhardt, E. S., BenDor, T., Mitchell, M., Kominoski, J., et al. (2019) 'The Invisible Flood: The Chemistry, Ecology, and Social Implications of Coastal Saltwater Intrusion.' *BioScience*, 69(5) pp. 368-378.

Unger, I. M., Kennedy, A. C. and Muzika, R.-M. (2009) 'Flooding effects on soil microbial communities.' *Applied Soil Ecology*, 42(1), 2009/05/01/, pp. 1-8.

van Es, H., Yang, C. L. and Geohring, L. (2005) 'Maize Nitrogen Response as Affected by Soil Type and Drainage Variability.' *Precision Agriculture*, 6 pp. 281-295.

Wan, X., Huang, Z., He, Z., Yu, Z., Wang, M., Davis, M. R. and Yang, Y. (2015) 'Soil C:N ratio is the major determinant of soil microbial community structure in subtropical coniferous and broadleaf forest plantations.' *Plant and Soil*, 387(1), 2015/02/01, pp. 103-116.

Wang, X., Yang, T., Lin, B. and Tang, Y. (2017) 'Effects of salinity on the performance, microbial community, and functional proteins in an aerobic granular sludge system.' *Chemosphere*, 184, 2017/10/01/, pp. 1241-1249.

Wang, Y., Shi, J., Wang, H., Lin, Q., Chen, X. and Chen, Y. (2007) 'The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity, and community composition near a copper smelter.' *Ecotoxicology and environmental safety*, 67(1) pp. 75-81.

Wang, Y., Wang, Z.-L., Feng, X., Guo, C. and Chen, Q. (2014) 'Long-Term Effect of Agricultural Reclamation on Soil Chemical Properties of a Coastal Saline Marsh in Bohai Rim, Northern China.' *PLOS ONE*, 9(4) p. e93727.

Warrence, N. J., Bauder, J. W. and Pearson, K. E. (2002) 'Basics of salinity and sodicity effects on soil physical properties.' *Departement of Land Resources and Environmental Sciences, Montana State University-Bozeman, MT*, 129

Weissman, D. S. and Tully, K. L. (2020) 'Saltwater intrusion affects nutrient concentrations in soil porewater and surface waters of coastal habitats.' *Ecosphere*, 11(2) p. e03041.

Weston, N. B., Dixon, R. E. and Joye, S. B. (2006) 'Ramifications of increased salinity in tidal freshwater sediments: Geochemistry and microbial pathways of organic matter mineralization.' *Journal of Geophysical Research: Biogeosciences*, 111(G1)

Wichern, J., Wichern, F. and Joergensen, R. G. (2006) 'Impact of salinity on soil microbial communities and the decomposition of maize in acidic soils.' *Geoderma*, 137(1), 2006/12/31/, pp. 100-108.

Williams, T. P., Bubb, J. M. and Lester, J. N. (1994) 'Metal accumulation within salt marsh environments: A review.' *Marine Pollution Bulletin*, 28(5), 1994/05/01/, pp. 277-290.

Wilpiszeski, R. L., Aufrecht, J. A., Retterer, S. T., Sullivan, M. B., Graham, D. E., Pierce, E. M., Zablocki, O. D., Palumbo, A. V., et al. (2019) 'Soil Aggregate Microbial Communities: Towards Understanding Microbiome Interactions at Biologically Relevant Scales.' *Applied and Environmental Microbiology*, 85(14) pp. e00324-00319.

Wilson, B. J., Servais, S., Charles, S. P., Davis, S. E., Gaiser, E. E., Kominoski, J. S., Richards, J. H. and Troxler, T. G. (2018) 'Declines in Plant Productivity Drive Carbon Loss from Brackish Coastal Wetland Mesocosms Exposed to Saltwater Intrusion.' *Estuaries and Coasts*, 41(8), 2018/12/01, pp. 2147-2158.

Wilson, J. S., Baldwin, D. S., Rees, G. N. and Wilson, B. P. (2011) 'The effects of short-term inundation on carbon dynamics, microbial community structure and microbial activity in floodplain soil.' *River Research and Applications*, 27(2) pp. 213-225.

Wisse, P. (1997). Southport's Sea Wall. *Coastlines*.

Wright, A. J., Ebeling, A., de Kroon, H., Roscher, C., Weigelt, A., Buchmann, N., Buchmann, T., Fischer, C., et al. (2015) 'Flooding disturbances increase resource availability and productivity but reduce stability in diverse plant communities.' *Nature Communications*, 6(1), 2015/01/20, p. 6092.

Wu, X., Wei, Y., Wang, J., Wang, D., She, L., Wang, J. and Cai, C. (2017) 'Effects of soil physicochemical properties on aggregate stability along a weathering gradient.' *CATENA*, 156, 2017/09/01/, pp. 205-215.

Xian, X., Pang, M., Zhang, J., Zhu, M., Kong, F. and Xi, M. (2019) 'Assessing the effect of potential water and salt intrusion on coastal wetland soil quality: simulation study.' *Journal of Soils and Sediments*, 19(5), 2019/05/01, pp. 2251-2264.

Xie, L., Ge, Z.-M., Li, Y., Li, S., Tan, L. and Li, X. Z. (2020) 'Effects of waterlogging and increased salinity on microbial communities and extracellular enzyme activity in native and exotic marsh vegetation soils.' *Soil Science Society of America Journal*, 84, 01/27,

Yang, J., Ma, L. a., Jiang, H., Wu, G. and Dong, H. (2016) 'Salinity shapes microbial diversity and community structure in surface sediments of the Qinghai-Tibetan Lakes.' *Scientific Reports*, 6(1), 2016/04/26, p. 25078.

Zak, D. R., Holmes, W. E., White, D. C., Peacock, A. D. and Tilman, D. (2003) 'Plant diversity, soil microbial communities, and ecosystem function: are there any links?' *Ecology*, 84(8) pp. 2042-2050.

Zhang, G., Bai, J., Tebbe, C. C., Zhao, Q., Jia, J., Wang, W., Wang, X. and Yu, L. (2021) 'Salinity controls soil microbial community structure and function in coastal estuarine wetlands.' *Environmental Microbiology*, 23(2) pp. 1020-1037.

Zhang, G., Bai, J., Tebbe, C. C., Zhao, Q., Jia, J., Wang, W., Wang, X. and Yu, L. (2021) 'Salinity controls soil microbial community structure and function in coastal estuarine wetlands.' *Environmental Microbiology*, 23(2) pp. 1020-1037.

Zhang, P., Luo, Q., Wang, R. and Xu, J. (2017) 'Hydrogen sulfide toxicity inhibits primary root growth through the ROS-NO pathway.' *Scientific Reports*, 7(1), 2017/04/13, p. 868.

Zhang, X., Liao, X., Huang, L., Shan, Q., Hu, A., Yan, D., Zhang, J. and Long, X.-E. (2021) 'Soil profile rather than reclamation time drives the mudflat soil microbial community in the wheat-maize rotation system of Nantong, China.' *Journal of Soils and Sediments*, 21(4), 2021/04/01, pp. 1672-1687.

Zörb, C., Geilfus, C.-M. and Dietz, K.-J. (2019) 'Salinity and crop yield.' *Plant Biology*, 21(S1) pp. 31-38.

Supplementary information

Total metal concentrations for each site using box and whisker plots representing the 25% and 75% quartiles, and the solid line is the median. With the whisker representing the range of data. X-axis labels represent the Flooding duration for flooded (F) and recovery (R) states, each bar represents n=3

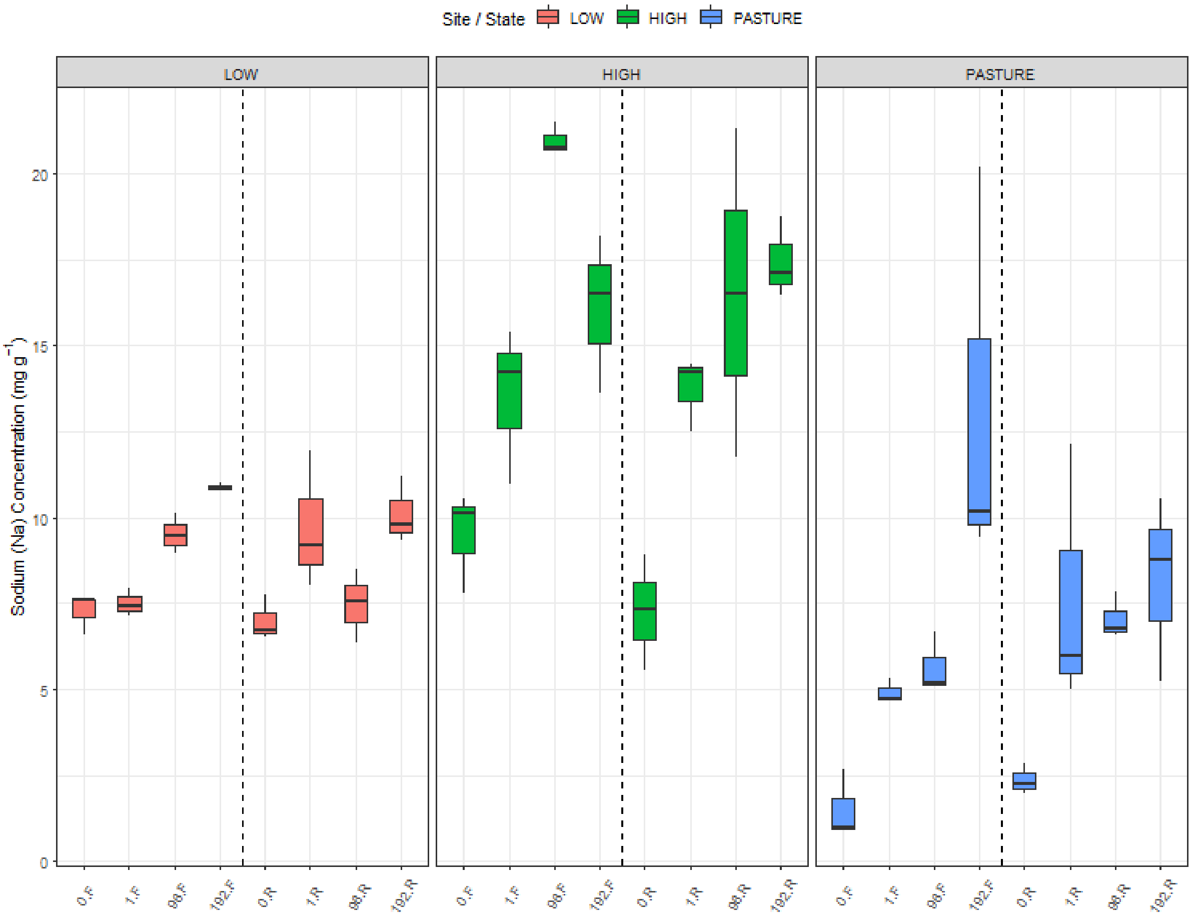


Figure SI1 - Sodium

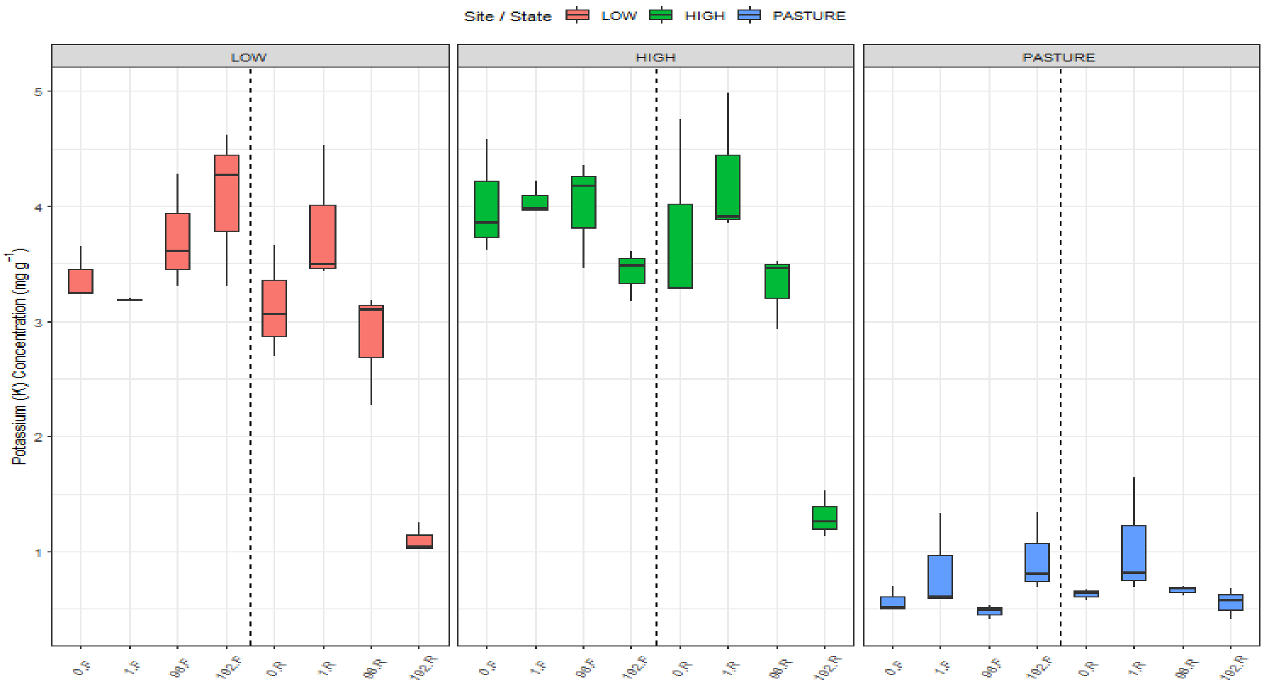


Figure SI2 - Potassium

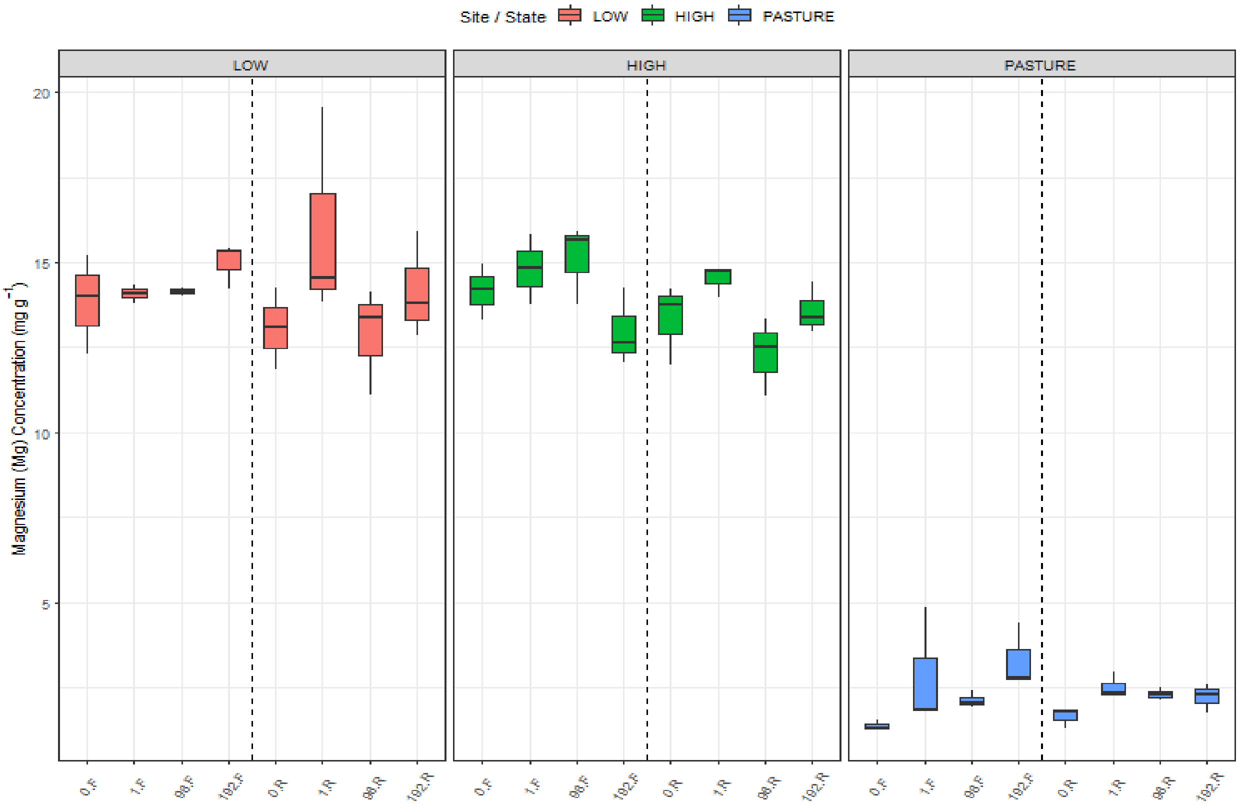
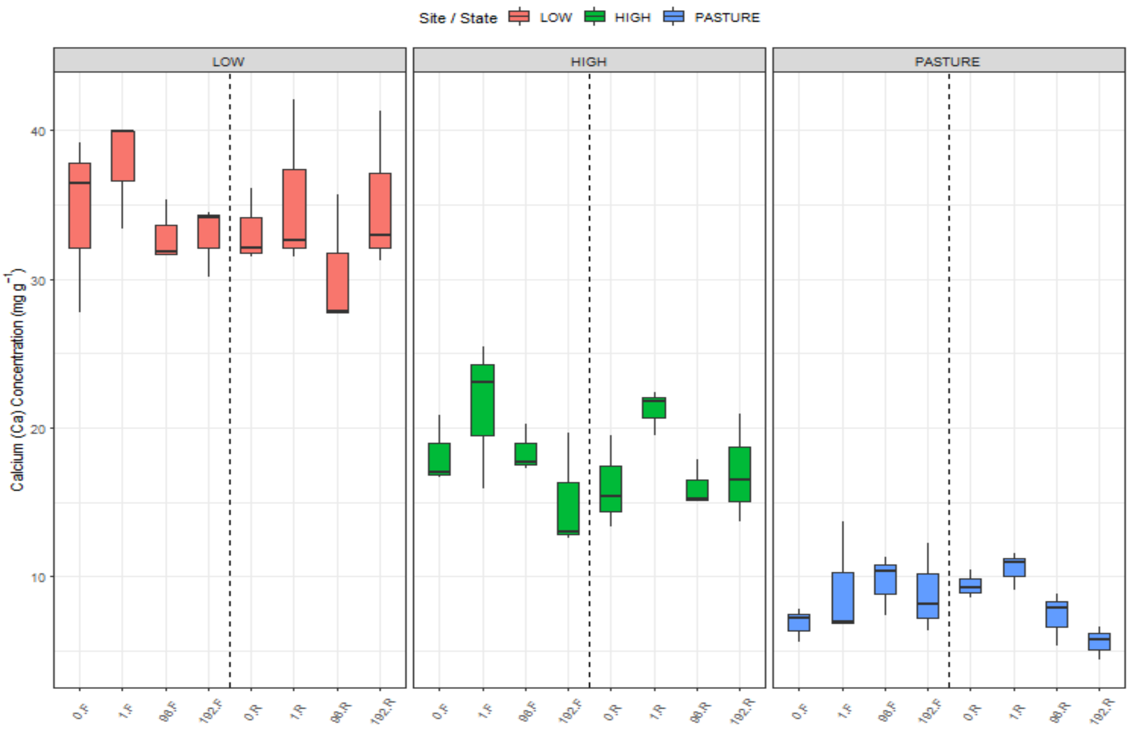


Figure SI3 - Magnesium



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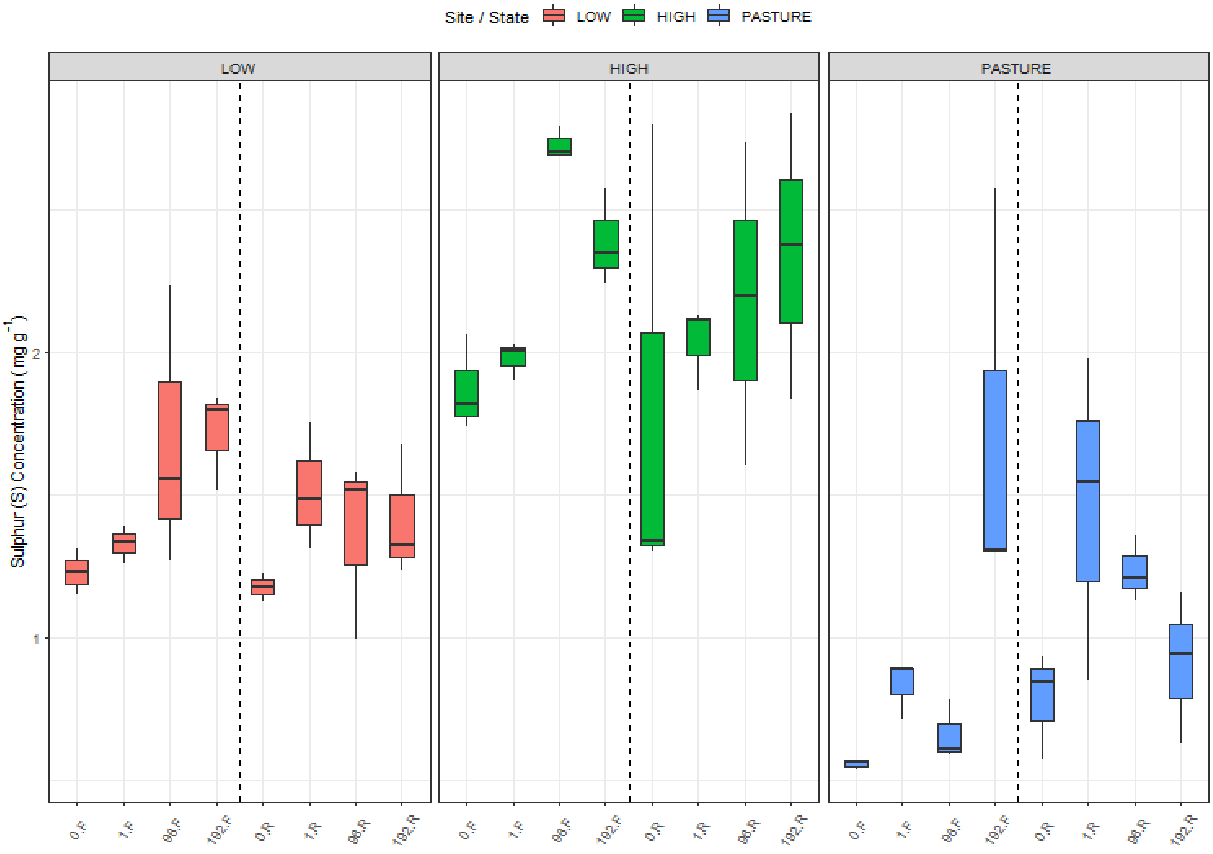


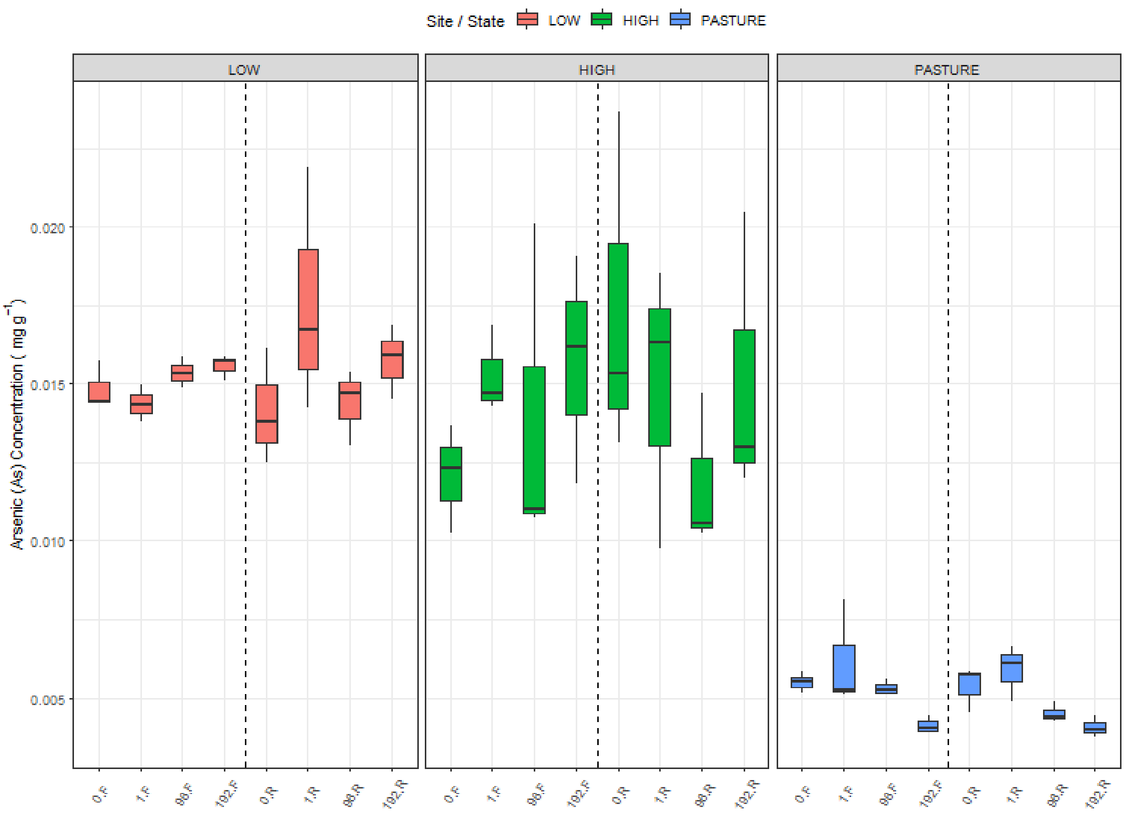


Figure SI6 - Arsenic

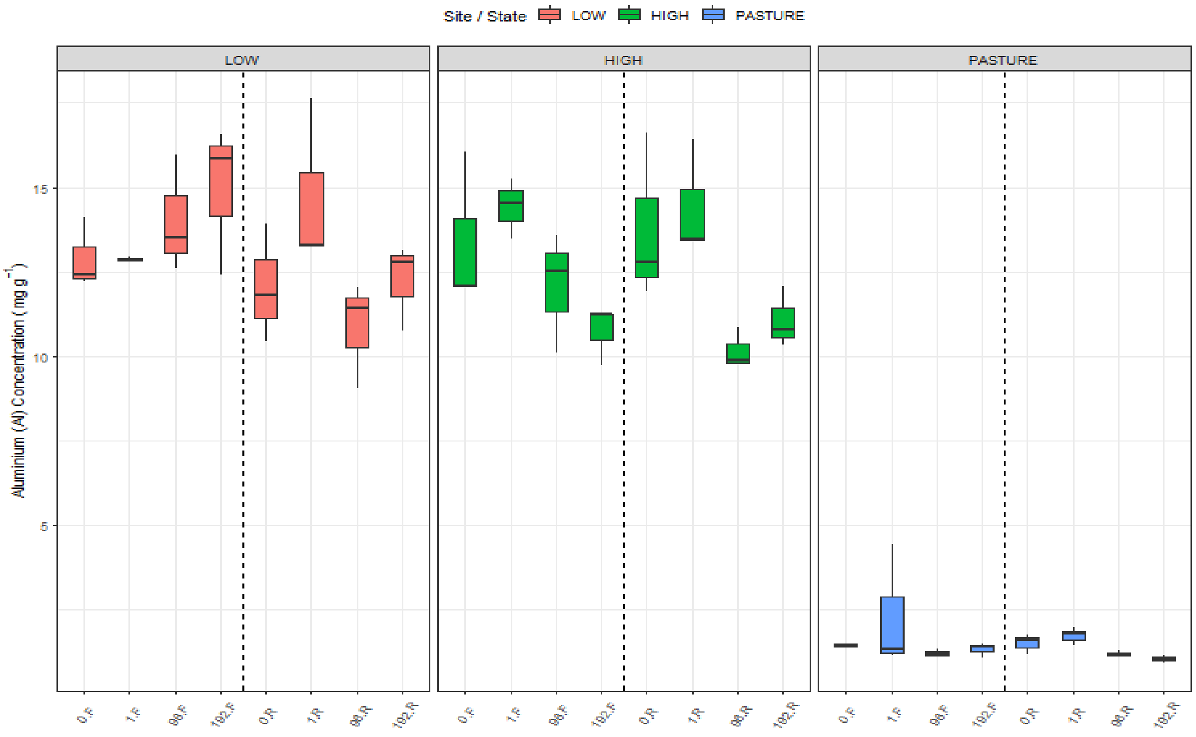


Figure SI7 – Aluminium

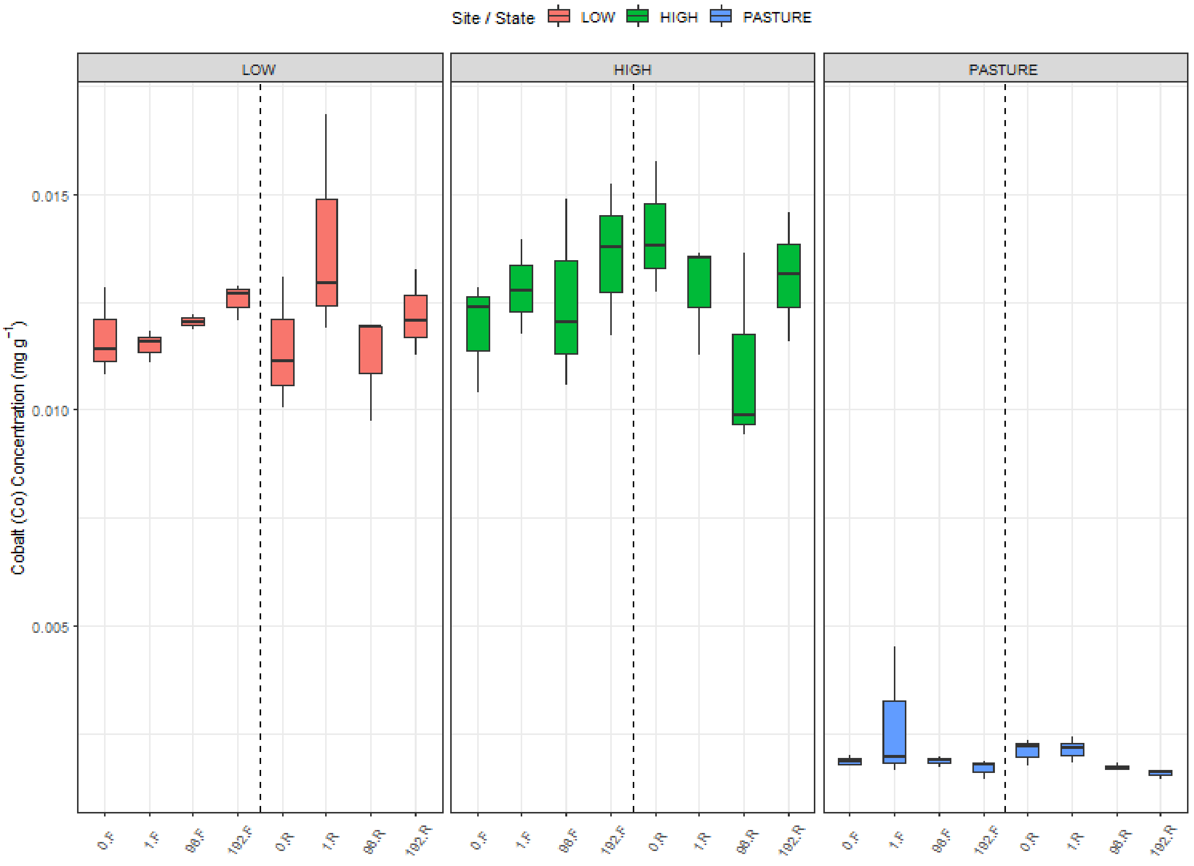


Figure SI8 – Cobalt

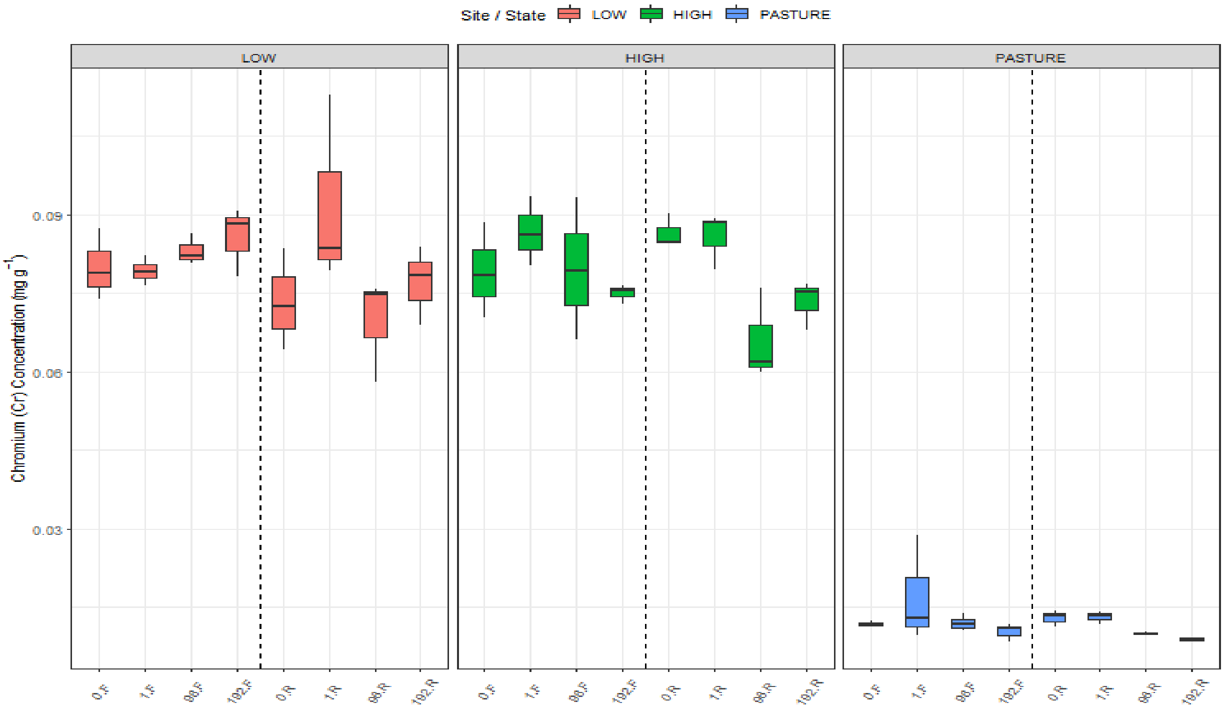


Figure SI9 – Chromium

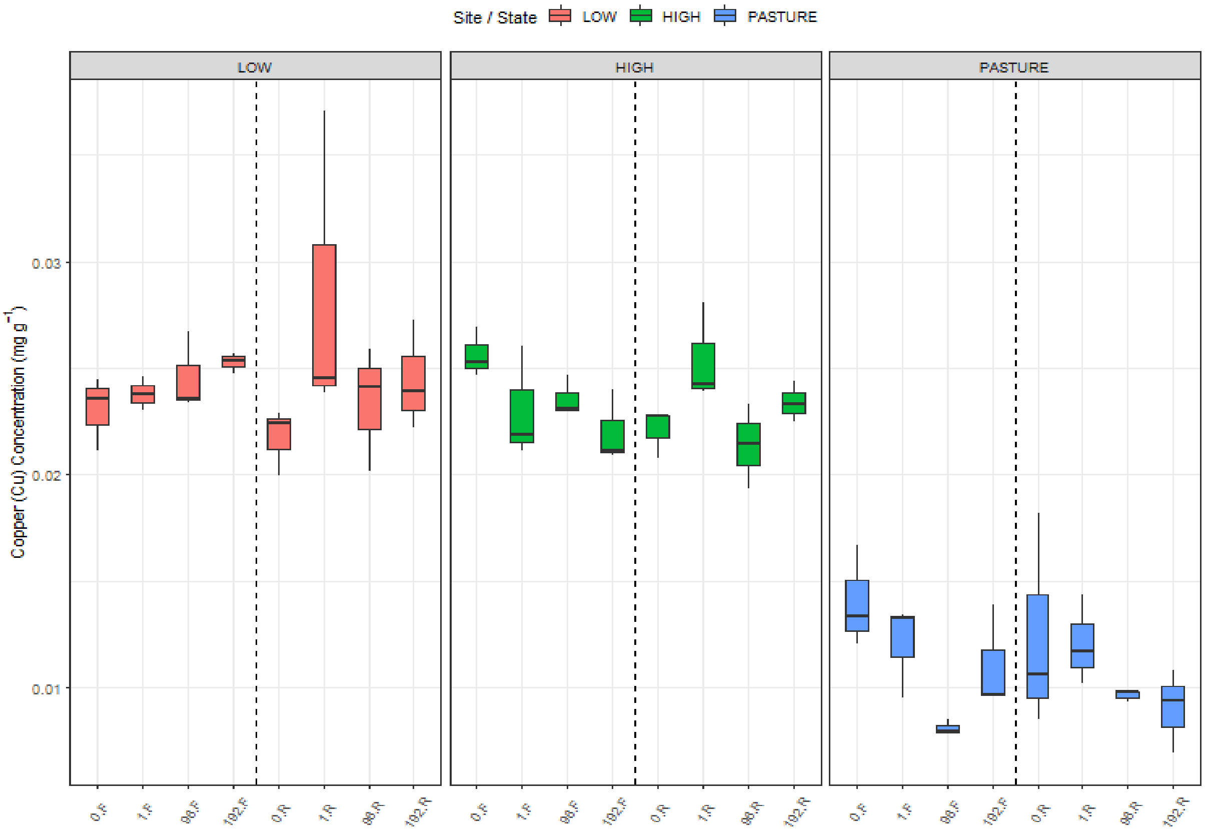


Figure SI10 – Copper

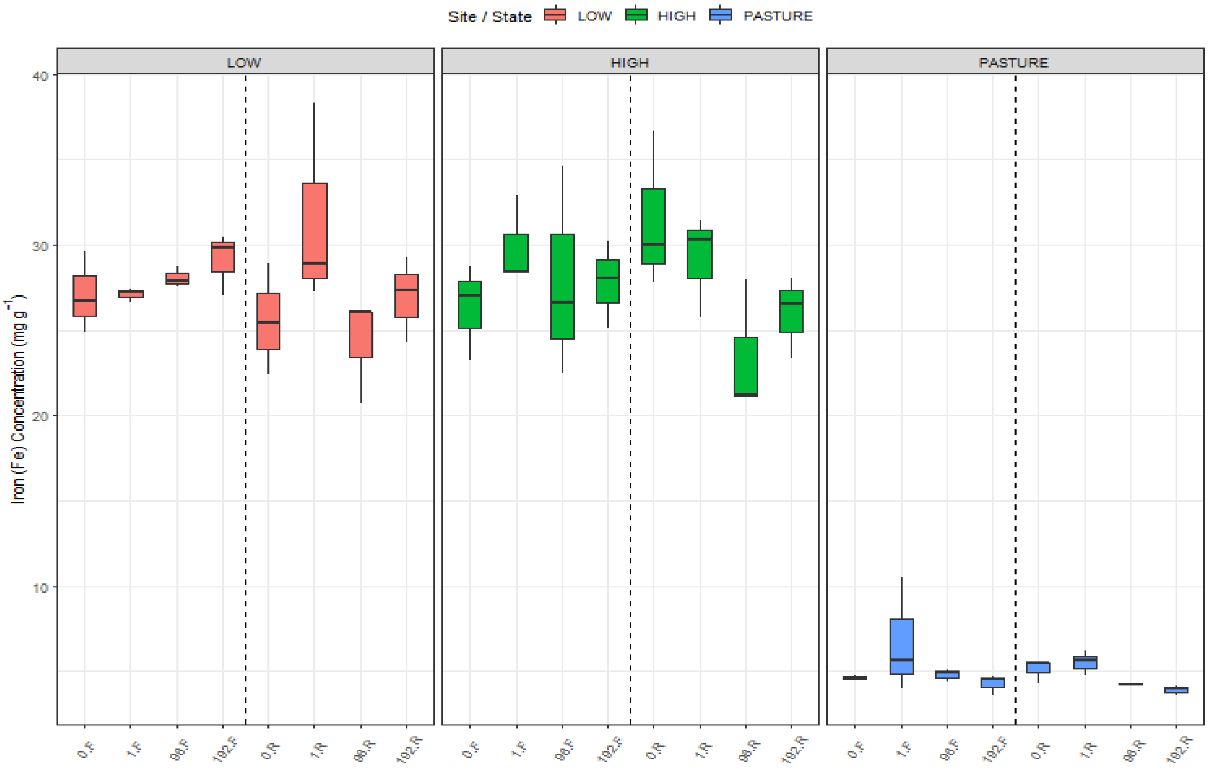
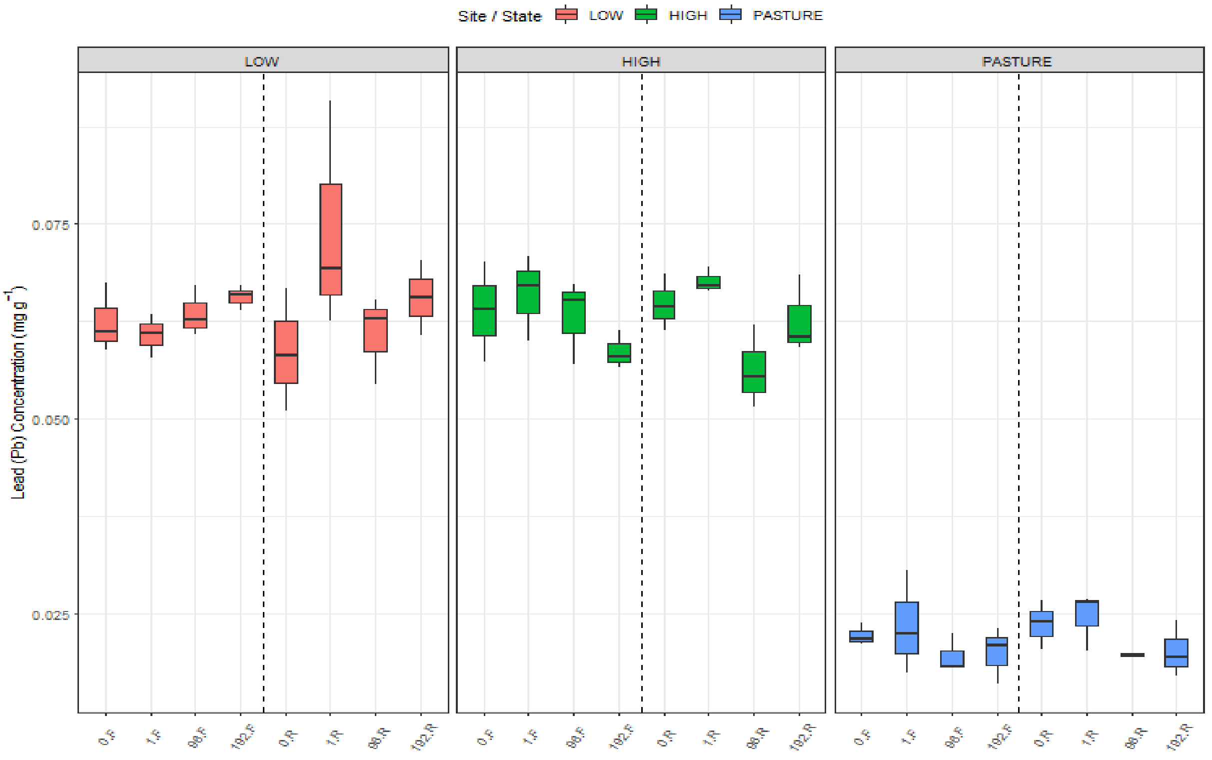
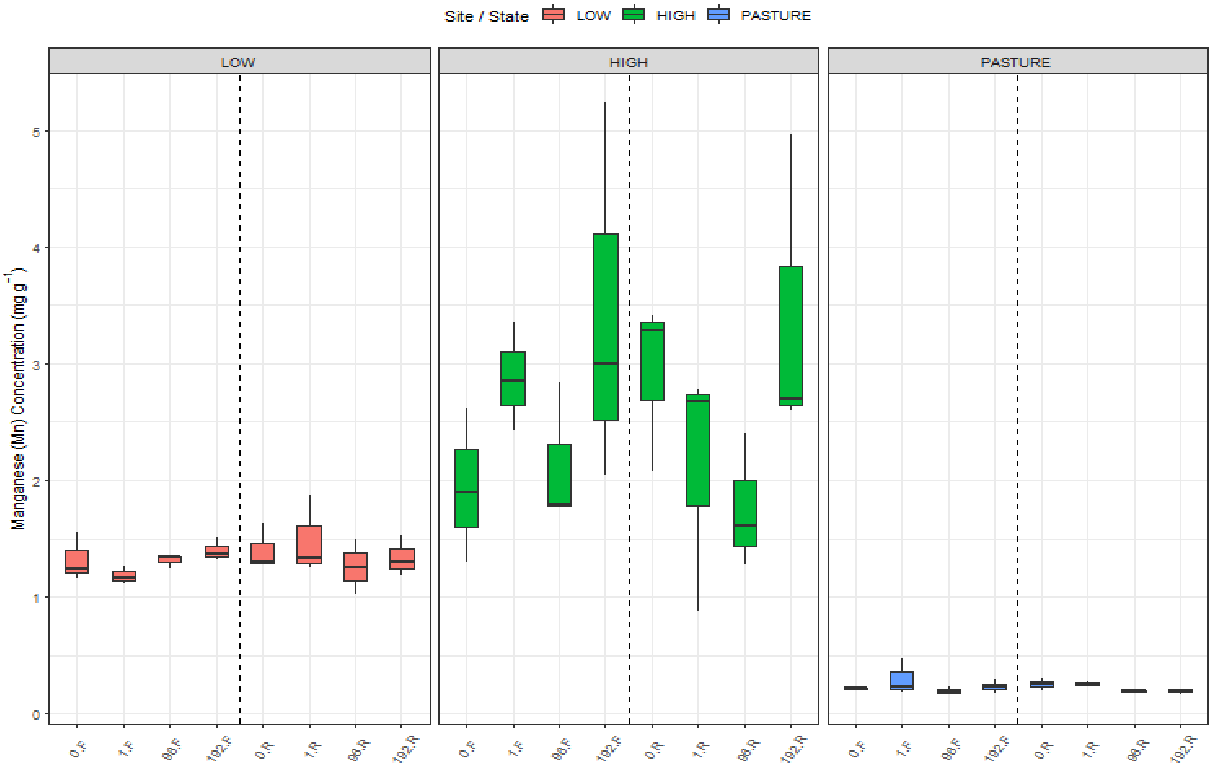


Figure SI11 – Iron


Site / State
LOW
HIGH
PASTURE
LOW
HIGH
PASTURE
Lead (Pb) Concentration (mg g^{-1})
0.075
0.050
0.025
0_F
1_F
98_F
192_F
0_R
1_R
98_R
192_R


Site / State
LOW
HIGH
PASTURE
LOW
HIGH
PASTURE
Manganese (Mn) Concentration (mg g^{-1})
5
4
3
2
1
0
0_F
1_F
98_F
192_F
0_R
1_R
98_R
192_R

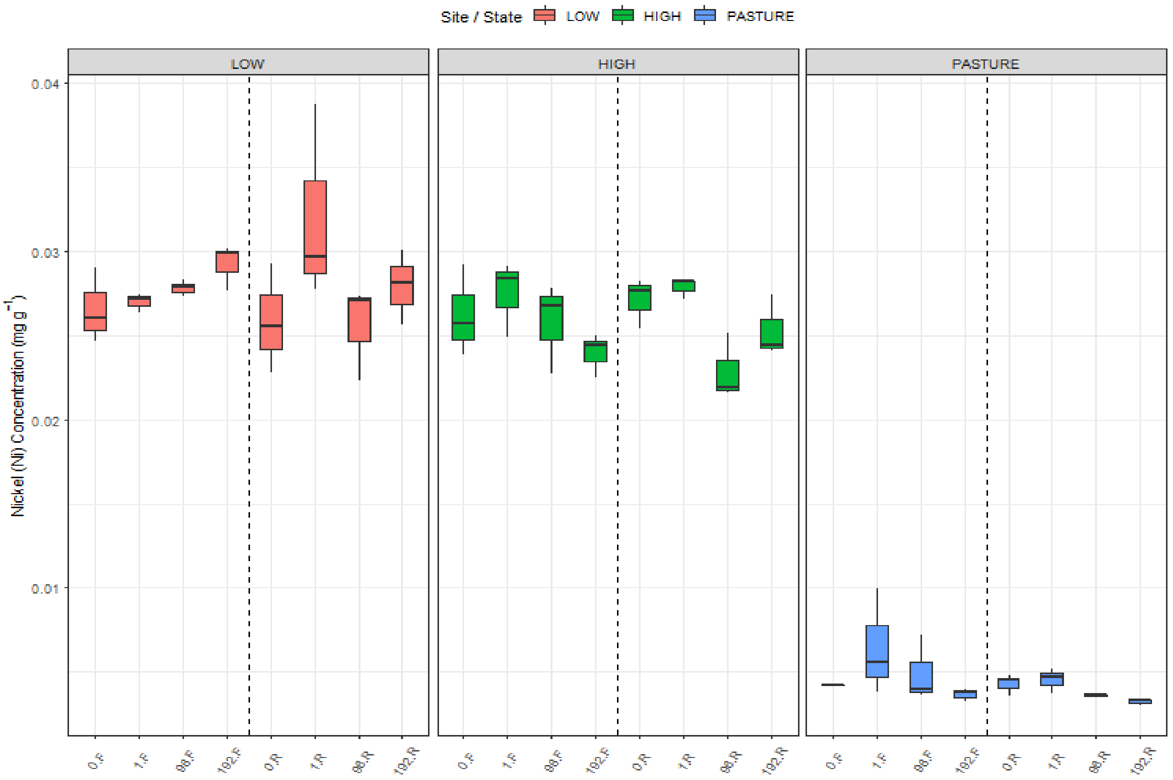
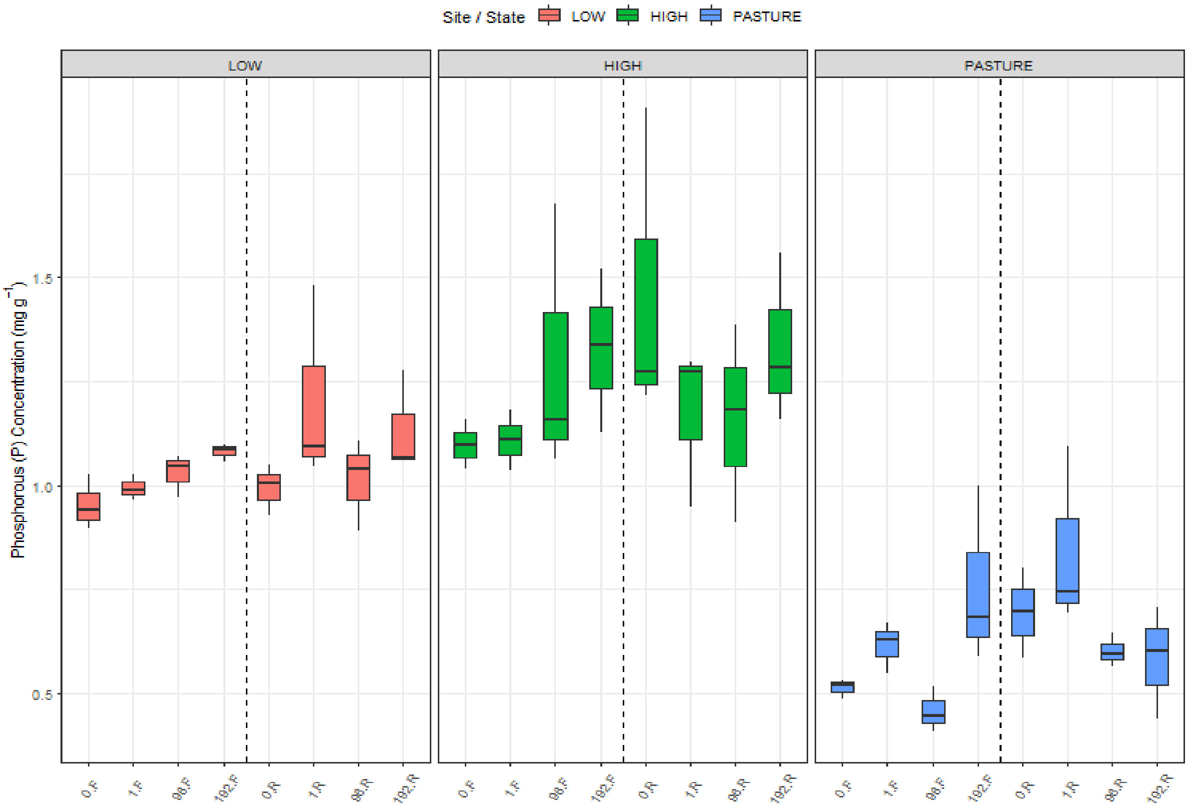
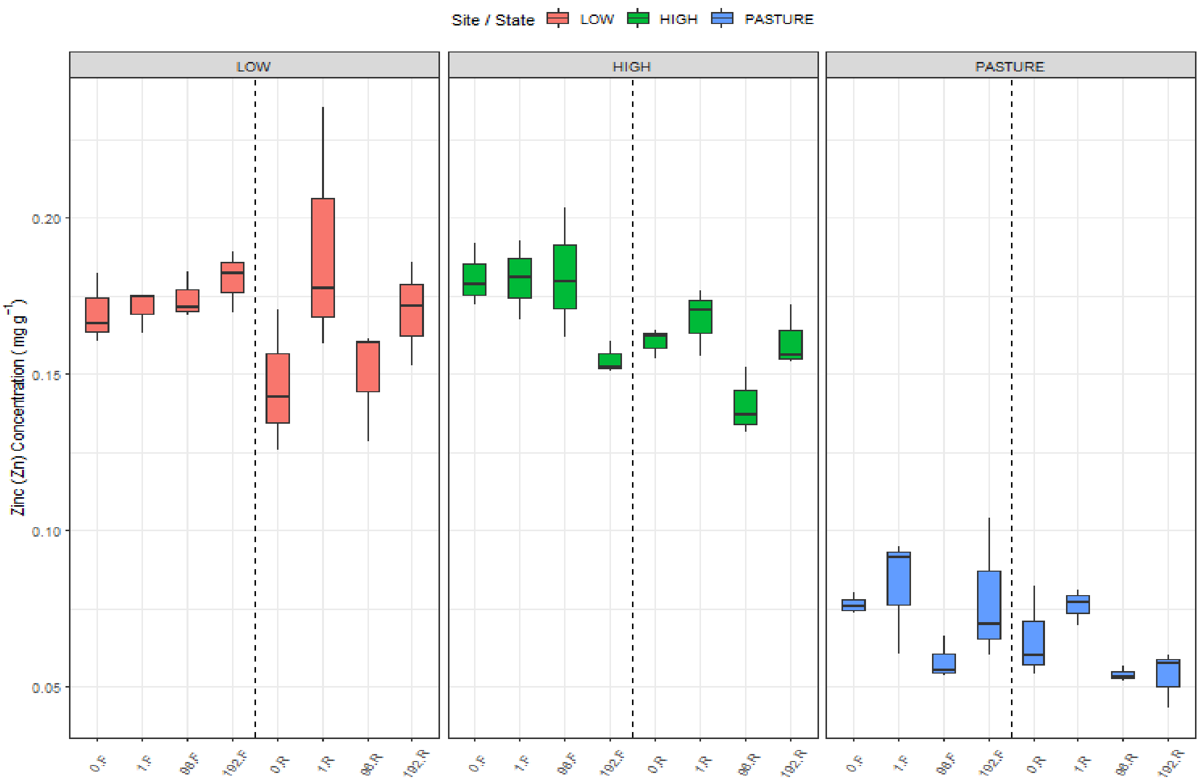
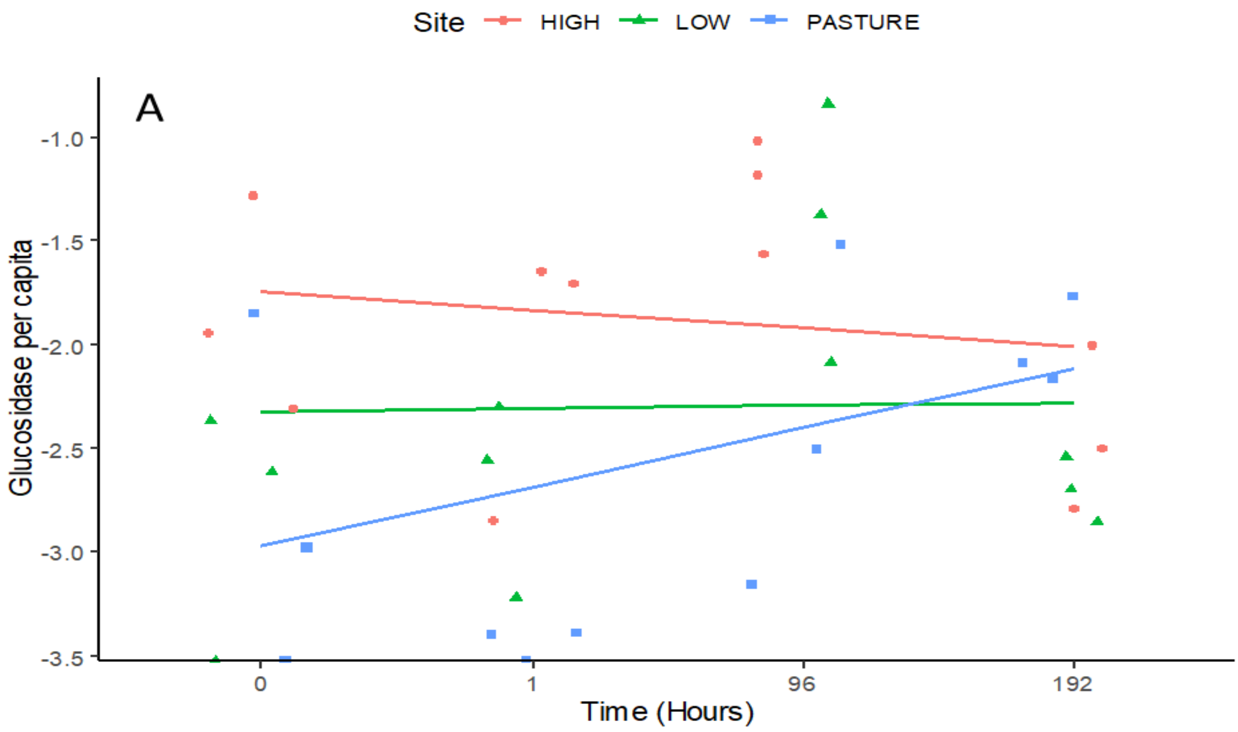


Figure SI14 - Nickel

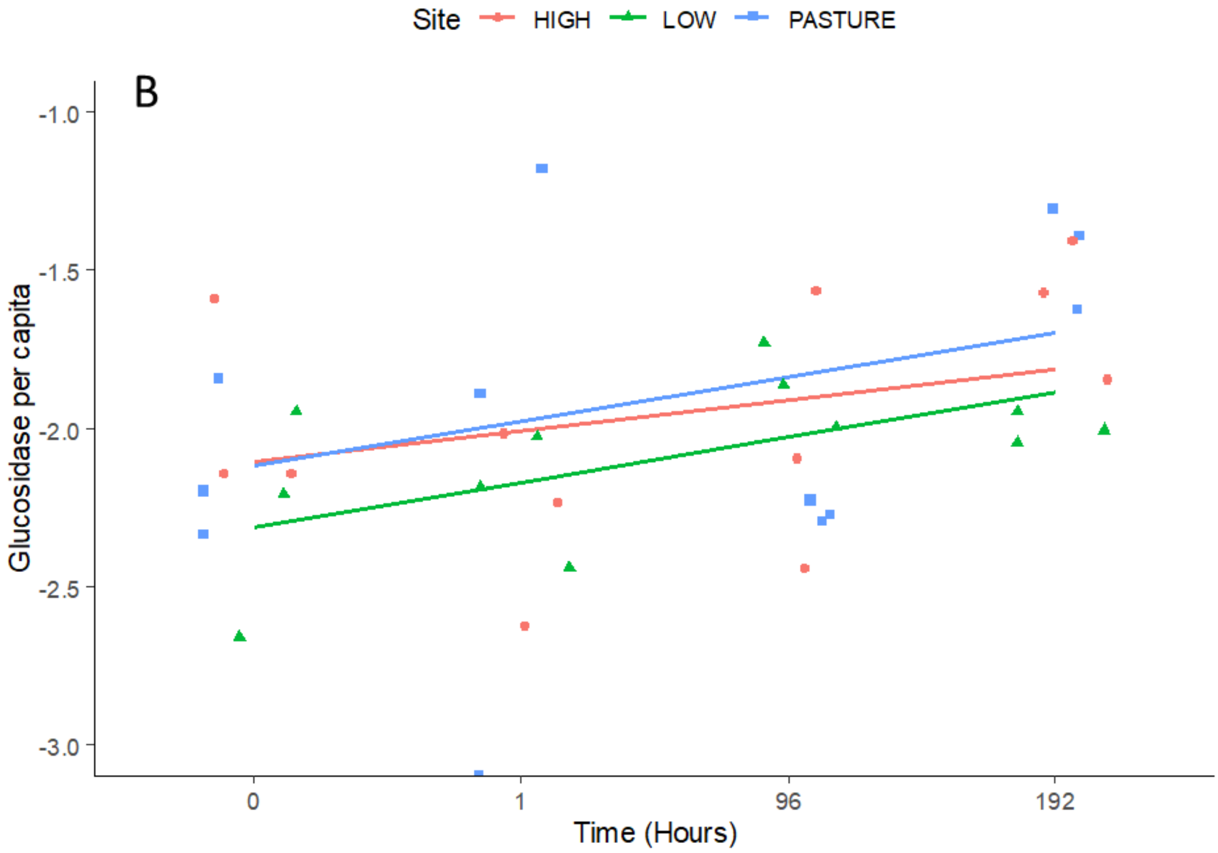




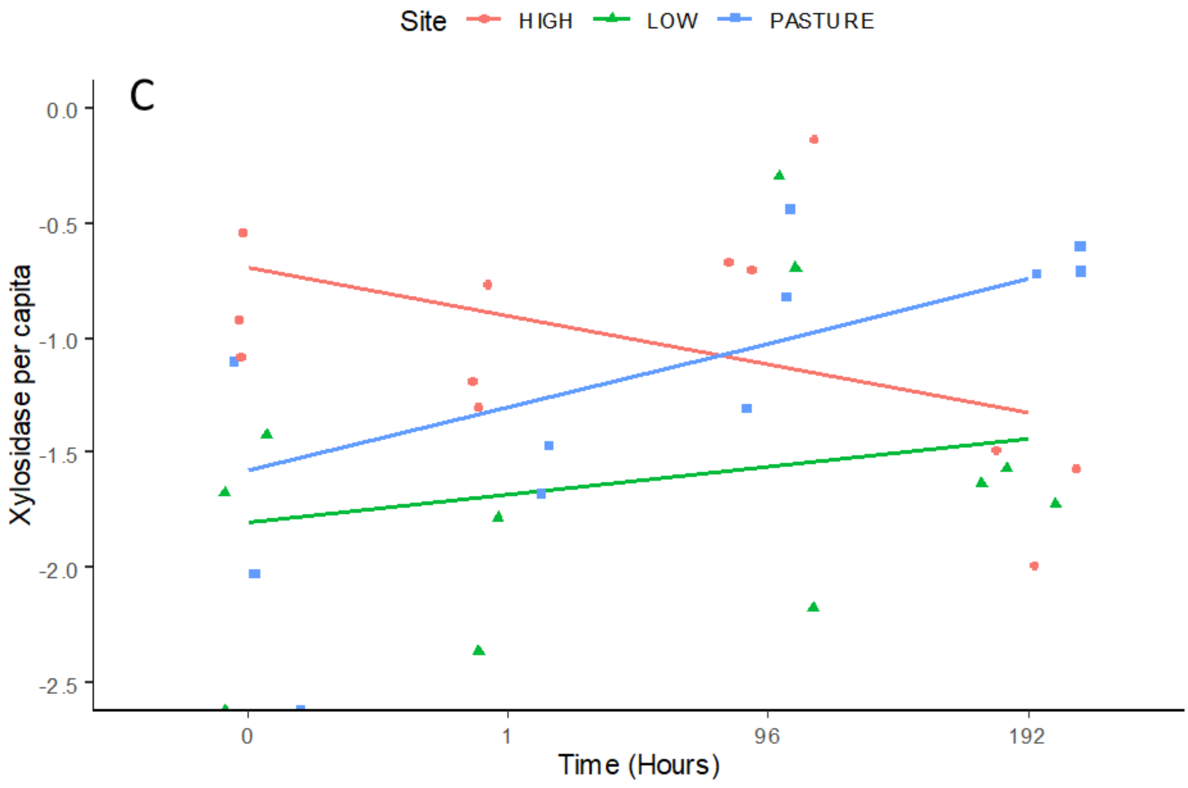
The total *per capita* Enzyme activity for substrates - Glucosidase, Xylosidase and Sulphatase in all mesocosms over flooding treatment (0,1,96 and 192 hours, A,C,E) and subsequently exposed to the 14-recovery period (B,D,F).



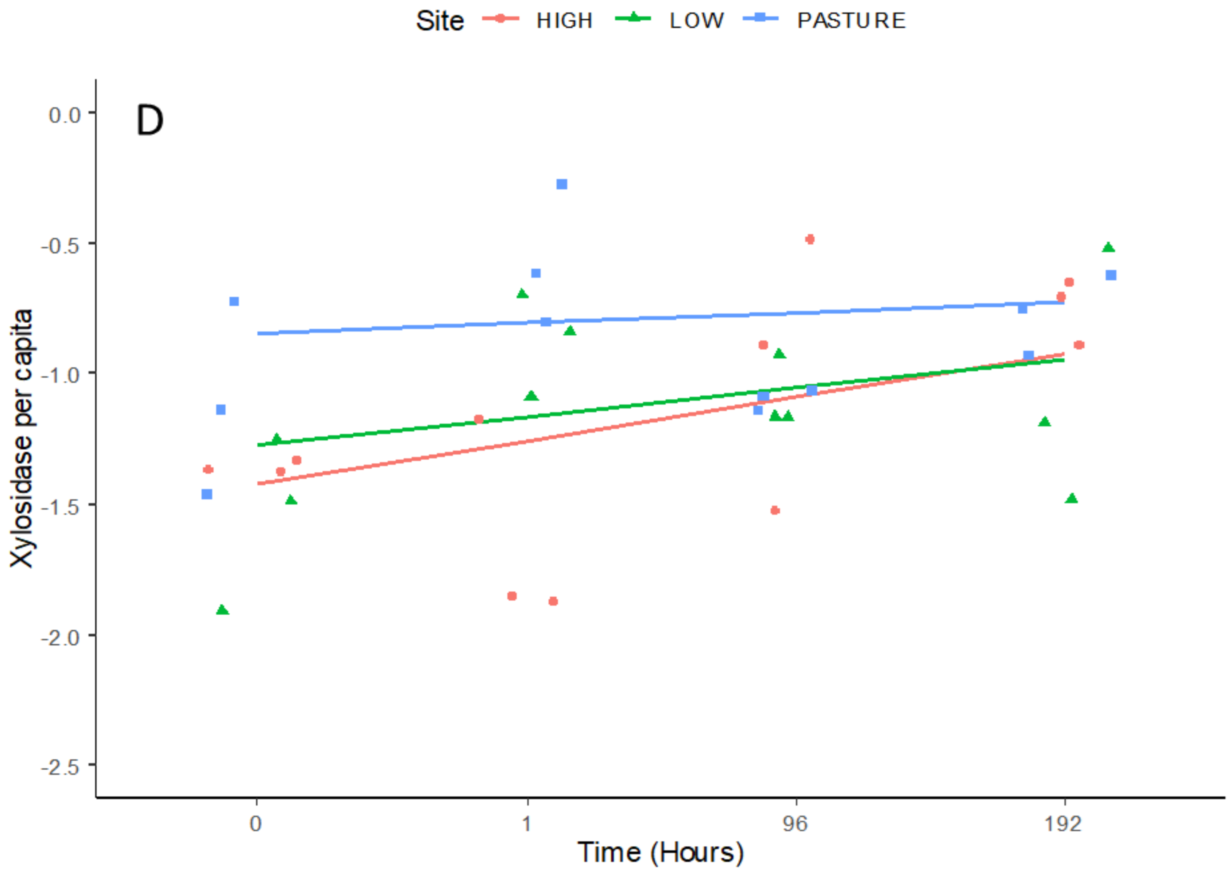
SI Figure 17A – Glucosidase per capita activity during flooding period



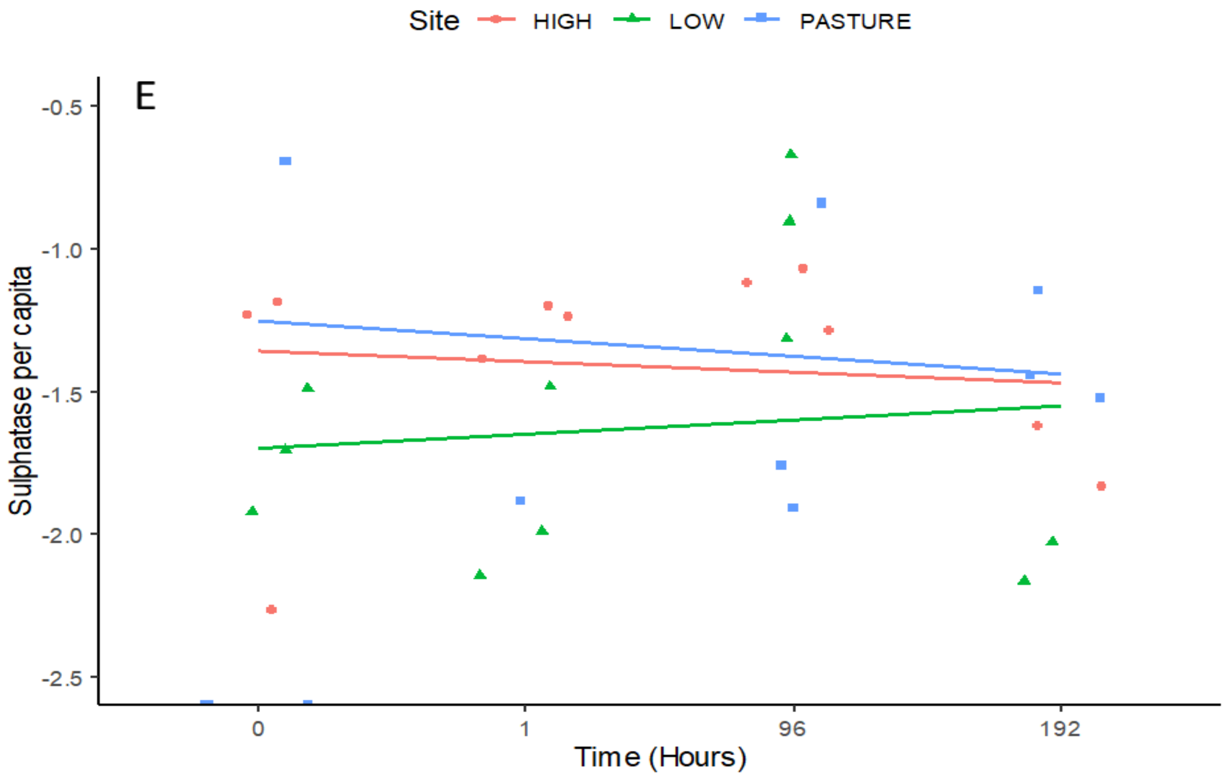
SI Figure 17B – Glucosidase per capita activity during recovery period

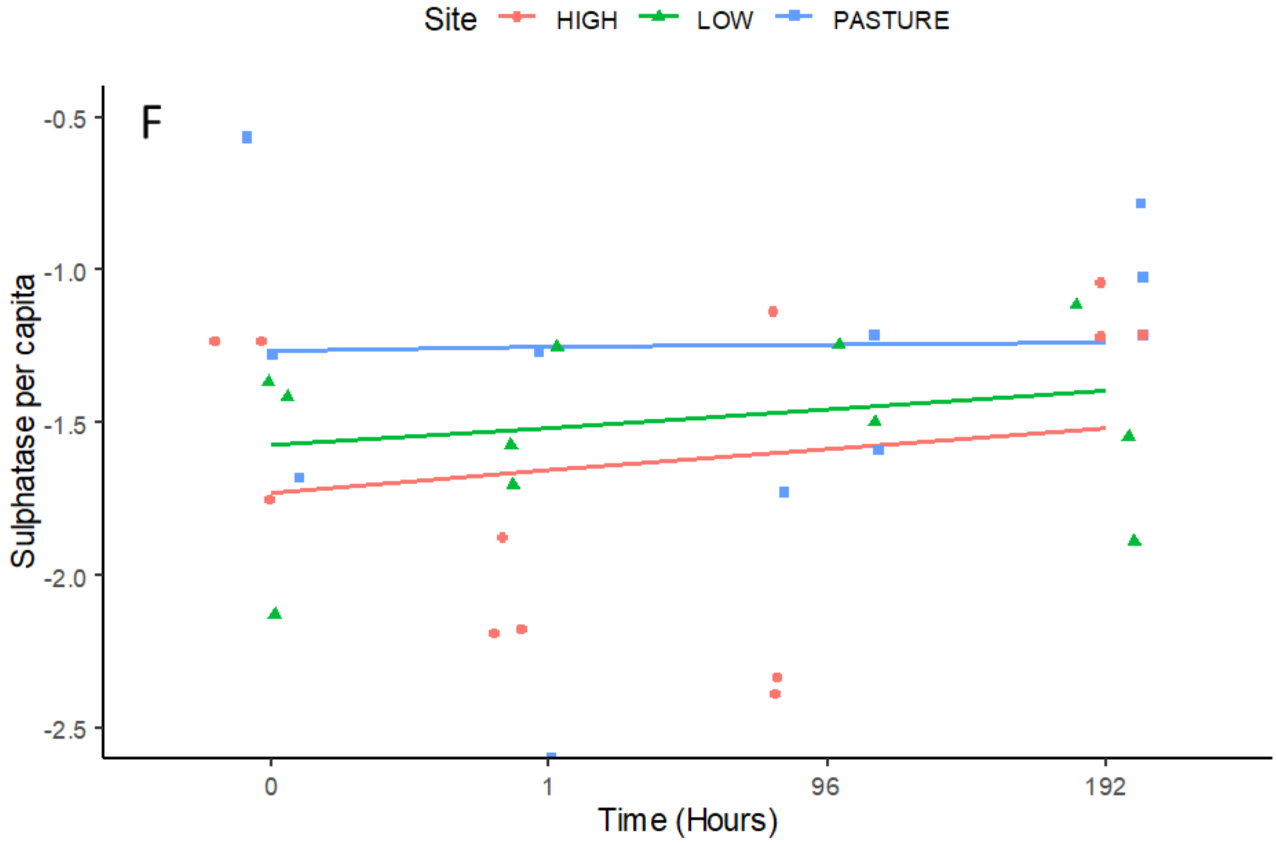


SI Figure 17C– Xylosidase per capita activity during flooding period



SI Figure 17D – Xylosidase per capita activity during Recovery period


Site
HIGH
LOW
PASTURE
E
Sulphatase per capita
-0.5
-1.0
-1.5
-2.0
-2.5
0
1
96
192
Time (Hours)


Site
HIGH
LOW
PASTURE
F
Sulphatase per capita
-0.5
-1.0
-1.5
-2.0
-2.5
0
1
96
192
Time (Hours)