

Saltmarsh restoration: the shift from a terrestrial to a marine environment

Leda Liyue Cai

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University of York

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Abstract

Over recent decades salt marshes have been restored as a cost-effective response to coastal biodiversity loss and flood management. Previous research on established sites has demonstrated that restored marshes have significantly different sediment properties than natural marshes which appears to broadly impact ecosystem function.

A study was conducted to examine the differences in natural and realigned salt marshes in terms of plant biodiversity, sediment characteristics (bulk density, water content, pH and nutrients) and microbial communities in Colchester Essex, UK. We studied three pairs of natural and realigned salt marshes of different ages, 13, 62, 118 years since breaching of the sea wall. Furthermore, we studied monthly changes of sediment characteristics of a newly realigned site from breach to 14 months of tidal inundation. In addition to monthly sediment changes, we placed invertebrate exclusion chambers in the newly realigned marsh to examine the effect of bioturbation in changing sediment characteristics and microbial communities.

Sediment characteristics of our realigned marshes were significantly different than those of the natural marshes. In addition, natural marshes displayed higher variability and heterogeneity in nutrient and water content than our realigned marshes. Within our newly realigned salt marsh we observed that despite the different starting sediment characteristics, 14 months after inundation our realigned site was broadly similar to the natural marsh but only on the top 5cm of sediment, indicating the presence of a relic agricultural layer which can affect the hydrology and development of the system. Macro-invertebrate colonization has shown that it can influence the geochemical characteristic and microbial communities of sediment in a newly realigned salt marsh. Microbial communities' composition and abundances within a newly realigned marsh are significantly different from natural marshes 14 months post inundation.

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Author's declaration

I, Leda Liyue Cai, declare that all the material detailed in this thesis is my own work, and written by myself. This work has not previously been presented for an award at this, nor any other University. All sources are acknowledged as References.

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Chapter 1. General introduction

Saltmarshes are coastal ecosystems with herbaceous vegetation that colonize the upper coastal intertidal zone and are regularly flooded by tides. Saltmarshes occur worldwide, particularly in middle to high latitudes and are usually restricted to relatively sheltered locations. Communities of saltmarshes often exhibit zonation, typically linked to salinity and inundation time. The species composition of saltmarshes varies from site to site as well as regionally (Fischer et al., 2000). Saltmarsh environments can be highly dynamic, prone to erosion but can also rapidly colonize new sediments. Their distribution is not only influenced by, but also influences, local physical processes and geomorphology including topography and creek patterns (Adam, 1990). Recent estimates of global area of saltmarshes was 5.5Mha (Mcowen et al., 2017) with some estimates ranging from 2.2 to 40Mha (Pendleton et al., 2012).

1.1. Ecosystem services

Salt marshes provide important ecosystem services, from support of coastal and terrestrial food chains to coastal protection (Beaumont et al., 2008; Hughes and Paramor, 2004; Millenium Ecosystem Assessment, 2005). Salt marshes provide protection to coastal areas by dissipating wave and tidal energy reducing the possibility of sea walls being breached, overtopped or undermined (Möller et al., 2014, 1999; Möller and Spencer, 2002). An estimated 2000km of UK coastline is protected by saltmarshes (Doody, 1992). The cost savings salt marshes provide through natural coastal protection is between £17 and £32 billion per annum, where the maintenance cost and construction of coastal defenses is £0.3 billion per annum (Beaumont et al., 2008). Thus a decline in saltmarsh habitat is believed to have a significant economic cost in terms of requirement of artificial coastal flood defenses (Hughes and Paramor, 2004).

Vegetated coastal ecosystems, such as mangroves, salt marshes and seagrasses, play an important role in sequestering carbon (C) that would otherwise remain as atmospheric CO₂ (Chmura et al., 2003); this C has been termed “blue carbon”. Vegetated coastal ecosystems reside over rich organic sediments several meters in depth that lock up carbon due to the low oxygen conditions that inhibit decomposition of matter at depth (Pendleton et al., 2012). Carbon stores of coastal ecosystems can exceed those of terrestrial ones by several times (mangroves: 830 – 1218 Mg C ha⁻¹; savannas: 156 – 203 Mg C ha⁻¹; upland forests: 375 – 437 Mg C ha⁻¹) (Donato et al., 2012). When these ecosystems are degraded or converted the carbon in the sediment is destabilized and microbial activity is increased releasing greenhouse gases (GHG) (Pendleton et al., 2012). Salt marsh sediments globally are estimated to bury between 5 teragrams (Tg) and 87 Tg C yr⁻¹ (McLeod et al., 2011). Arriola and Cable (2017) found that high carbon burial at a Florida natural marsh was associated with low sediment accumulation in the low marsh, and low carbon burial was associated with high sediment accumulation in the high marsh. These variations in carbon burial imply that the highest carbon burial zone of that marsh occurs in the most vulnerable to erosion area.

Salt marshes can function as either sources or sinks of nitrogen, depending on their vegetation and morphology (Huang and Pant, 2009; Whiting et al., 1989). Salt marsh plants function as nutrient buffers for nitrogen and/or phosphorus, where their biomass production contributes to cycling of nutrients into estuarine systems (Lillebø et al., 2004; Sousa et al., 2010). However, marsh stability can be affected by chronic eutrophication and increased salinity. Alldred et al. (2017) found that belowground plant biomass in high-nitrogen marshes was reduced by 60-70% and enhanced by as much as 70% in high salinity salt marshes.

1.2. Nitrogen cycle in salt marshes

Nitrogen from the atmosphere (N₂) must be fixed into available (mineral) nitrogen by microbial processes in the sediment before it can be taken up by plants and used for

metabolic reactions (Francis et al., 2007). Nitrogen is an essential element of all the amino acids in plant structures. It is important in the growth and development of vital plant tissues and cells like the cell membranes and rubisco, making it essential for photosynthesis.

A major source of nitrogen in the form of $\text{NO}_3^-/\text{NO}_2^-$ and NH_4^+ into saltmarshes is bulk precipitation (Jordan et al., 1983) (Figure 1.1 A). Excess nitrogen enters salt marshes from fertilizer runoff, acid precipitation and sewage waste (Seitzinger et al., 2005). The amount of nitrogen removed or recycled by coastal wetland varies with the different types of processes as well as seasons. Nitrogen can be remineralized to NH_4^+ and recycled into the water column and sediment. It can be removed from the system as N_2 gas through the process of denitrification and it can also be immobilized by microbes, or buried as soil organic nitrogen (Velinsky et al., 2017) (Figure 1.1 B,C,F).

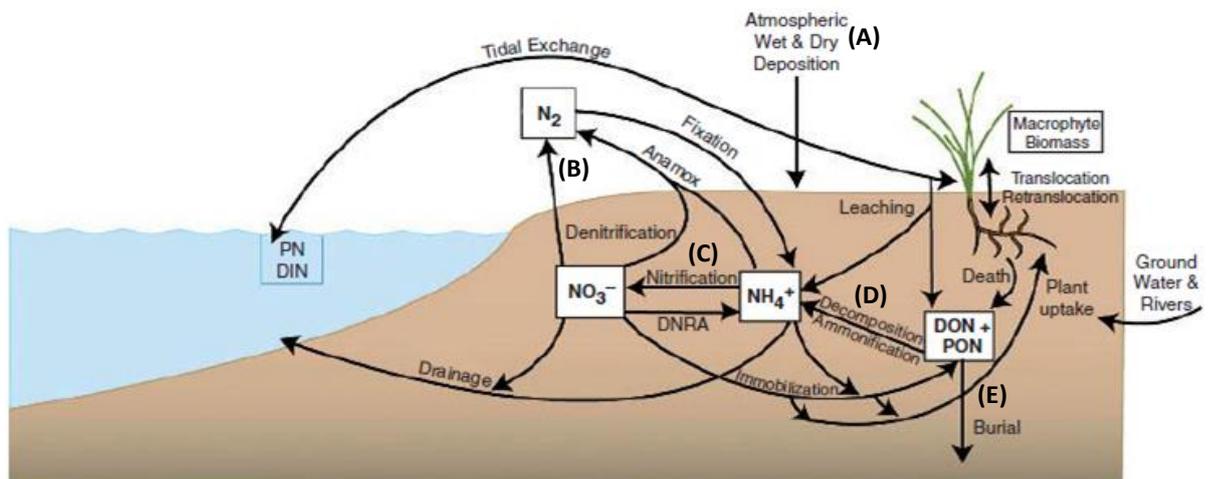


Figure 1.1. Diagram of nitrogen cycle in salt marshes. Taken from Hopkins and Giblin (2008).

Nitrogen in the form of ammonia (NH_4^+) is released in the sediment during organic matter decomposition by ammonifying bacteria and fungi in a process called ammonification (Figure 1.1 D). NH_4^+ is oxidized to nitrate and nitrite (NO_3^- , NO_2^-) by nitrifying bacteria (e.g. *Nitrosomonas*, *Nitrobacter*) and archaea in the sediment under aerobic conditions. This is a two-step process where ammonia-oxidizing archaea (AOA) and bacteria (AOB) reduce

$\text{NH}_3/\text{NH}_4^+$ to NO_2^- and then nitrate-oxidizing bacteria (NOB) oxidize NO_2^- to NO_3^- (Francis et al., 2007; Gruber and Galloway, 2008). Nitrogen loss from marine sediments is via denitrification. Denitrification, the reduction of nitrate back to nitrogen gas is an obligate anaerobic process (Figure 1.1 B). Anaerobic ammonium-oxidation (anammox) uses NO_2^- as an electron acceptor to convert nitrate to nitrogen gas (N_2) (Strous et al., 2006). The nitrate reducing potential of salt marsh sediments is relatively high (Nedwell, 1982), however the reduction process is limited by availability of nitrate.

Aziz and Nedwell (1986) examined the relative concentration of nitrate in the water column during the tidal cycle and found that nitrate concentration decreased from the water while it covered the marsh. Furthermore, when examining nitrogen fixation in vegetated and unvegetated areas of a salt marsh, they found that the greatest rates of N fixation were in mud pans populated with a layer of cyanobacteria (Vegetated marsh: $0.1 - 3.8 \text{ mmol N m}^{-2} \text{ yr}^{-1}$; creek without cyanobacteria: $2.1 \text{ mmol N m}^{-2} \text{ yr}^{-1}$, pan with cyanobacteria: $8.8 \text{ mmol N m}^{-2} \text{ yr}^{-1}$).

Nitrogen concentration in saltmarsh sediments exhibits seasonality. Denitrification in estuarine sediment and salt marshes is regulated by temperature (Kaplan et al., 1977) as well as available NO_3^- and organic carbon (Sherr and Payne, 1978). Studies have found that with an increase of NO_3^- input from rivers along with an increase in organic carbon and warmer temperatures increases denitrification rates in estuarine sediment (Jensen et al., 1988). Thompson et al. (1995) found that nitrification rates were highest in June-September when the temperature was highest and lower in December to March when the temperature dropped.

1.3. Salt marsh realignment

Over the last two decades there has been an accelerated global decline in the extent of saltmarsh systems (Millennium Ecosystem Assessment, 2005). Climate change threatens

coastal habitats by increased storm frequency and sea-level rise (IPCC, 2007). Vegetated coastal ecosystems are estimated to be declining ~ 0.5 - 3% (~8000km²) annually (Costanza et al., 1997). At this current rate 30 – 40% of tidal marshes and seagrasses will be lost in the next 100 years (IPCC, 2007). The human response to these challenges is greater installation of sea defences, which further constricts coastal shore-line habitat development and the natural expansion/development of salt marshes. These actions, in combination, have been described as coastal-squeeze (Boorman, 2003). Historically, and globally, saltmarshes have been reclaimed for either agricultural land or urban development (Adam, 1990). By their nature, these reclaimed agricultural lands started near sea level and when combined with the compaction and erosion of soil that often accompanies agricultural practice, has led to a reduction of elevation within these lands, enhancing local flood risks. Maintaining sea defenses in order to protect these low lying agricultural lands has become increasingly costly and interest is developing in converting some of these lands back into saltmarshes (Foster et al., 2013; French, 2006; Hazelden and Boorman, 2001). Managed realignment aims to restore saltmarshes by constructing new sea walls further inland and deliberately breaching existing fore-shore walls thus allowing tidal inundation of low-lying agricultural land (French, 2006).

The European Union Habitat Directive (adopted in UK legislation in 1992) maintains a no-net-loss policy that has led to the current strategy of managing coastal marsh areas and the creation of managed realigned saltmarshes. Efforts have been made in England and Wales to identify suitable areas for managed realignment, to estimate the cost of each realignment, and to monitor realignments (DEFRA, 2002; Environment Agency, 2017). The remaining saltmarsh coverage in the UK (as of 2008) is approximately 45,500 hectares, mainly in eastern England (Beaumont et al., 2008).

Andrews et al. (2006) compared the economic value of restoring marshes for flood defense which showed that they are most cost effective when viewed on a more than 25-year time

scale due to the initial costs of the restoration projects. In addition, they concluded that managed realignments are economically efficient for habitat creation, carbon sequestration and reduction of flood defense maintenance costs when viewed over a 50-year timescale. Realigned marshes may have a positive economic effect by increasing the annual sink of organic C, N and particulate phosphate in the estuary by 150%, 83% and 50% respectively (Andrews et al., 2006). Burden et al. (2013) found that it would take approximately 100 years for a restored site to have accumulated the same amount of carbon currently stored in a natural marsh. However, they have observed that the nitrogen mineralization rates of realigned marshes are similar to those of natural saltmarshes within 15 years since realignment.

1.3.1 Natural vs. realigned saltmarshes

Studies have shown that, even decades after installation, realigned saltmarshes have different plant communities and distributions than natural marshes (e.g. Garbutt & Wolters 2008, Davy et al. 2011, Mossman et al. 2012a). The species and community differences in natural versus realigned marshes may be partly explained through hydrogeological differences (Tempest et al., 2015). Previous land use of realigned sites (agriculture and drainage) results in long term changes to the subsurface sediment structure, including collapse of pore space, which leads to poor, or less, rapid marsh drainage (Spencer et al., 2008; Tempest et al., 2015, Cai et al., submitted). Furthermore, Tempest et al. (2015) found that realigned saltmarshes have two distinct sediment layers, relic and altered agricultural soil, overlain by newly deposited marine sediment. These two layers have different physical properties and are hence likely to have different hydrological characteristics in terms of water storage and movement.

Environmental data on sediment moisture, redox potential and organic matter show that restored marshes are less oxygenated with lower redox potential at lower elevations (Davy

et al., 2011). Furthermore, when comparing denitrification rates between natural and restored marshes they observed that the restored marsh denitrification rates half that of than the natural marsh. Thompson et al. (1995) hypothesized that the lower denitrification rates observed in restored marshes were due to physical limitation of the sediment. Sediment size of restored marshes was mostly coarse which provided less surface area for microbial populations, responsible for denitrification, to populate. Higher elevations in managed saltmarshes have been shown to have more oxygenated sediments that are drier and contain less organic matter relative to natural marshes of the same elevation (Mossman et al. 2012a). These sediment characteristics have substantial influence over plant community dynamics (Davy et al. 2011, Mossman et al. 2012b), which may explain the long-term trends reported by Garbutt & Wolters (2008) and Mossman et al. (2012a).

1.4. Bioturbation

Bioturbation is the mixing of sediment through biological processes from the action of infauna, epifauna, fish and mammals resulting in particle movement (Cadée, 2001). Benthic invertebrates have a significant impact on benthic sedimentary properties (Queirós et al., 2013), through their burrowing, feeding and foraging activities they influence the mixing of sediments and particulate materials as well as enhance pore water and solute advection through burrow ventilation (Volkenborn et al., 2010). Bioturbation of sediments through these activities can influence the oxygen, pH and redox gradient of sediments (Biles et al., 2002; Pischedda et al., 2008); as well as metal cycling (Teal et al., 2009); bacterial activity and composition (Gilbertson et al., 2012) and the carbon and nitrogen cycling of the system (Kristensen, 2001).

Furthermore, studies have shown that burrowing activities of invertebrates increases above ground plant production and diversity by increasing soil drainage, decreasing the amount of toxic compounds and increasing the anaerobic respiration of plant's roots (Bertness, 1985;

Montague, 1982). Daleo et al. (2007) found that burrowing activities increase the supply of nutrient to Arbuscular mycorrhizal fungi which in turn increase production of the salt marsh plant *Spartina alterniflora*, thus concluding that salt marsh productivity and success is highly depended on mutualisms between colonizing species and microorganisms.

Macrofauna community composition can also affect nutrient availability in the sediment as well as the water column. Biles et al. (2002) concluded that ammonia release into water column was influenced by infaunal community composition and is mediated by the extent of bioturbation affecting sediment particle resuspension and turnover. However, colonization of saltmarsh creeks by *Hediste diversicolor* had been found to affect the rate of erosion, by decreasing production and survival of *Salicornia* seedlings, as the plant roots would stabilize the sediments (Paramor and Hughes, 2004).

1.5. Success of salt marsh restoration

Realigned salt marshes are created in order to restore a set functionality (i.e. coastal protection, biodiversity increase, bird habitat), and success is normally measured against these parameters (Neckles et al., 2002; Strange et al., 2002). Many of the valued services provided by saltmarshes require managed realigned sites to return to a “natural” condition in order to provide the same desirable outcomes. This suggests that for a realigned site to function at the desired capacity it will need to be sufficiently similar to natural conditions at either sediment or plant biodiversity level or both. Ford et al. (2016) found that increased plant biodiversity within marshes enhances soil stability and erosion protection. However, studies have found that for realigned salt marshes to reach natural plant biodiversity it will take over 130 years (Garbutt and Wolters, 2008). In addition, restored marshes have been found to have poorer hydrology and topography compared to natural marshes (Lawrence et al., 2018; Spencer et al., 2017, 2008; Tempest et al., 2015).

Protocols have been set to assess and monitor recovery and functionality of these restored ecosystems, from above and below ground biodiversity, soil development to bird feeding and fish abundances (Neckles et al., 2002; Strange et al., 2002). For each realignment, recovery and functionality goals are set which determine success of the realignment. However, short-term recoveries and set goals may not imply long term sustainability (Zedler et al., 2001). Even when species densities within realigned marshes match those of natural marshes, functional measures often reveal a significant lag of ecological processes recovery, such as nutrient cycling, that are necessary for full functionality of a marsh.

1.6. Synthesis

Saltmarshes are important coastal ecosystems providing many ecosystem services (Beaumont et al., 2008; Hughes and Paramor, 2004; Millenium Ecosystem Assessment, 2005). Due to accelerated decline in natural salt marshes there has been an increased desire to re-create these habitats to return these services. With increased development and creation of salt marshes a greater understanding of the functionality of these new systems is required.

A limited number of studies have focused on sediment characteristics over time in realigned saltmarshes (e.g. Spencer et al. 2008, Tempest et al. 2015). Of these, most focus on the changes that have occurred several years after restoration. Spencer *et al.* (2008) and Tempest *et al.* (2015) examined the same saltmarsh 8 and 18 years post breach for changes in sediment characteristics and differences with an adjacent natural marsh; however, both studies examined the marsh at one timepoint. Changes on short-term time scales, or immediately after breach (e.g. Kadiri et al. (2011) who sampled 6 months post breach for 12 months) are often overlooked even though biotic and abiotic factors that are likely to affect sediment development and vegetation colonization (including seed availability, sediment pH, salinity, nutrient availability and anoxia (Garbutt et al. 2006, Garbutt & Wolters 2008,

Mossman et al. 2012a,b, Zhou et al. 2016)) are likely to be heavily influenced by initial conditions set within the first year of marsh re-establishment.

In the early stages of a saltmarsh realignment we can expect sediment conditions (nutrient availability, anoxia and sediment deposition) to be the most important factors affecting development of the marsh. Davy et al. (2011), Mossman et al. (2012a), Thompson et al. (1995) all found that realigned marsh sediment is more anoxic with lower nutrient concentrations, conditions which directly affect plant communities in a saltmarsh. These physical limitations of the marsh are directly related to the sediment and hydrology of the system (Spencer et al., 2017; Tempest et al., 2015; Thompson et al., 1995). Seed availability can affect the communities present in a marsh however marsh plant seeds require suitable sediment to anchor and develop (Wolters et al., 2005a).

This study aims to quantify differences between pairs of natural and realigned saltmarshes in south-east England and elucidate the mechanisms driving those differences. The following aims and hypothesis were tested:

- The aim of Chapter 2 was to examine if realigned marshes normalize (physiochemically and biologically) over time. We compared regions of varying dominant surface vegetation with physiochemical sediment characteristics between three pairs of natural and realigned saltmarshes of different ages (13, 62 and 118) in Essex, UK. We hypothesized that older realigned marshes will exhibit sediment conditions more consistent with natural marshes. The greatest difference in sediment characteristics and vegetation is predicted to be between the youngest site (13 years) and its paired natural marsh. The oldest realigned marsh (118 years) is expected to be showing more similar characteristics to natural conditions than the other realigned marshes.

- The aim of Chapter 3 was to quantify how total sediment accumulation affects early changes in sediment after flooding, and the effect of previous land use (arable and pasture) on saltmarsh realignment. We examined monthly sediment characteristic changes of a newly realigned saltmarsh in Essex UK during the first-year post inundation.
- In Chapter 4 we aimed to examine the effect of invertebrate bioturbators (organisms bigger than 250µm) in the development of sediment characteristics during the 1st year of marsh realignment between two different previous land uses (arable and pasture). We compare sediment changes in areas of invertebrate exclusion and invertebrate colonization between arable and pasture realigned fields and monitored invertebrate colonization in the newly realigned saltmarsh within the 1st year post inundation. We hypothesized that areas with invertebrate colonization and hence bioturbation would be more homogeneous across all depths than areas with no invertebrate colonization by the final sampling.
- The aim of Chapter 5 was to examine microbial communities of a newly realigned marsh within the 1st year of inundation. We compared the effect of bioturbation on microbial communities in a newly realigned salt marsh and examined the effect of previous land use (arable and pasture) as well as differences in sediment characteristic on microbial communities of the realigned salt marsh compared to paired natural marsh. We predicted that areas with invertebrate colonization would have more similar microbial communities with the natural marsh than areas of no invertebrate colonization.

Chapter 2. Differences between natural and realigned salt marshes over time, comparing plant diversity and sediment characteristics

2.1 *Introduction:*

Over the last two decades there has been an accelerated global decline in the extent of saltmarsh systems (Millenium Ecosystem Assessment, 2005) and efforts have been initiated to conserve existing marshes and to develop new coastal marsh habitats (realigned or managed saltmarshes). Saltmarshes provide important ecosystem services, from coastal protection to support for coastal and terrestrial food chains (Beaumont et al., 2008; Hughes and Paramor, 2004; Millenium Ecosystem Assessment, 2005). Saltmarshes provide protection to coastal areas by dissipating wave and tidal energy reducing the possibility of sea walls being breached, overtopped or undermined (Möller et al., 1999; Möller and Spencer, 2002). Due to the accelerated decline of natural saltmarshes more realigned marshes are being created to return these services (coastal protection, biodiversity, habitat creation, etc.).

With increased development and creation of saltmarshes, a greater understanding of the functionality of these new ecosystems is required. Many of the valued services provided by saltmarshes require managed realigned sites to return to a “natural” condition in order for these marshes to provide the same desirable outcomes. This suggests that for a realigned site to function at the desired capacity it will need to be sufficiently similar to natural conditions at either sediment or plant biodiversity level or both. The rate at which realigned saltmarshes return to a “natural” state appears to be quite slow, with evidence that it takes over 130 years (Garbutt and Wolters, 2008) for realigned marshes to match natural plant biodiversity (albeit with a different plant community composition).

The species and community differences in natural versus realigned marshes may be partly explained through hydrogeological differences (Tempest et al., 2015). Previous land use of realigned sites (agriculture and drainage) results in long term changes to the subsurface sediment structure, including collapse of pore space, which leads to poor or less rapid marsh drainage (Spencer et al., 2008; Tempest et al., 2015, Cai et al., submitted). Furthermore, Tempest et al. (2015) found that realigned salt marshes (i.e. realigned marshes created by breaching of the sea wall and allowed to regenerate naturally) have two distinct sediment layers, relic and altered agricultural soil, overlain by newly deposited marine sediment. These two layers have different physical properties and are likely to have different hydrological characteristics in terms of water storage and movement. Examining a newly realigned site during the first year of breach we found that physical characteristics of sediments (bulk density and water content) were similar to natural marshes only in the top 5cm (which consisted of newly accreted marine sediment) after 14 months post inundation, however depths below newly accreted sediments remained more dense and drier (Cai et al., submitted).

We might also predict that nutrient profiles will also vary between natural and realigned sites, and that this will be influenced by the composition of the vegetation. Nitrogen inputs from precipitation and run-off can be recycled either abiotically through chemical transformation, immobilized by microbes or recycled through uptake in vegetation.

Vegetation in a saltmarsh can enhance sediment deposition by capturing particles on leaves and stems (Mudd et al., 2010, 2009). Marsh vegetation slows the rate of water flow resulting in decreased turbulence and increased particle settling velocities, which in turn increases mineral deposition and thus increases rates of elevation (Leonard and Croft, 2006; Moller, 2006; Mudd et al., 2009). Mechanisms that increase elevation in salt marshes (mineral deposition and below ground biomass accumulation) are positively correlated with plant

biomass (Li and Yang, 2009). Thus, differences in plant vegetation of marshes could affect elevation as well as the sediment type being trapped.

Nitrogen concentration in saltmarsh sediments exhibits seasonality. Denitrification in salt marshes is regulated by temperature (Kaplan et al., 1977) as well as available NO_3^- and organic carbon (Sherr and Payne, 1978). Cartaxana et al. (1999) found a clear pattern of net nitrogen mineralization rate with season in saltmarsh sediments. They found increased inorganic nitrogen (NH_4^+ , NO_3^- , NO_2^-) availability during summer months. In addition, Cartaxana et al. (1999) observed that nitrogen mineralization rate during summer were linked to an increase in above-ground plant biomass thus indicating the importance of these parameters to net nitrogen cycling, not only in how they limit inorganic nitrogen pool capacity but also how it affects microbial and plant growth. Seasonality and temperature cannot be effectively separated, as Thompson et al. (1995) found that nitrification rates were highest in June-September when the temperature was highest and lower in December to March when the temperature dropped.

The aim of this study was to compare regions of varying dominant surface vegetation (areas with *Atriplex portulacoides*, *Limonium vulgare*, *Puccinellia maritima*, mud pans) with physiochemical sediment characteristics (bulk density, water content, sediment grain size, sediment pH and nutrients (NH_4^+ & $\text{NO}_2^-/\text{NO}_3^-$)) between three pairs of natural and realigned saltmarshes in Essex, UK. Each salt marsh pair consisted of different realignment age, including 13, 62 and 118 years since realignment. We hypothesized that older realigned marshes will exhibit sediment conditions more consistent with natural marshes. We predicted the greatest difference in sediment characteristics and vegetation diversity to be between the youngest marsh (13 years) with its natural saltmarsh pair. The oldest marsh (118 years) is expected to have the most similar characteristic in both sediment and vegetation with its natural pair.

2.2 Methods:

2.2.1 Study sites

Three pairs of natural and realigned saltmarshes were sampled in the Colne and Blackwater Estuary, in south-east England (Figure 2.1). Realigned sites examined were created over old agricultural fields by breaching of the sea wall and allowing the flooded land to regenerate to saltmarsh through ongoing seawater inundation. Reference natural marshes are located adjacent to the realigned sites studied. The youngest site, Abbots Hall (AH) ($51^{\circ}47'11.3''\text{N}$ $0^{\circ}51'38.4''\text{E}$, 13 years since breach/realignment at the time of sampling), was a managed de-embankment, meaning that the sea wall was breached through human activity. The older sites, Mersea Island (MI) ($51^{\circ}47'48.9''\text{N}$ $0^{\circ}55'18.4''\text{E}$, 62 years at the time of sampling) and Fingringhoe Range (FR) ($51^{\circ}49'46.8''\text{N}$ $0^{\circ}57'01.1''\text{E}$, 118 years) were natural de-embankments, where storm surges have broken through the sea walls historically and were never repaired. All natural and realigned site pairings were at matching elevations, within 20cm.

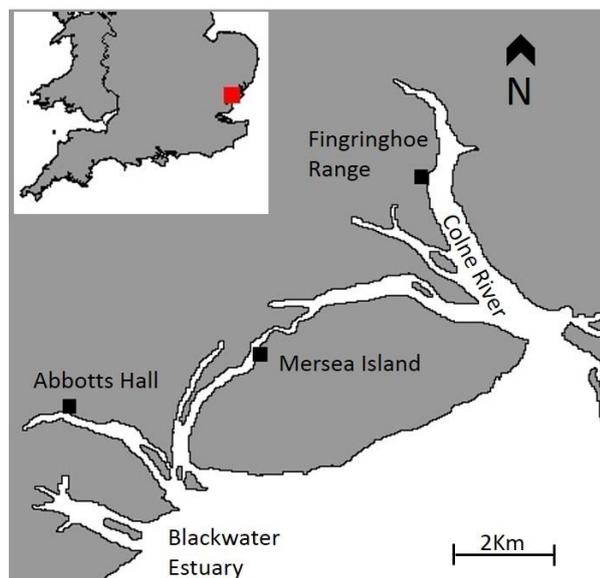


Figure 2.1. Map of the three sampling locations on the Blackwater Estuary and Colne River, UK. Distance between saltmarshes is: AH to MI 4280m, MI to FR 5100m and AH to FR 8880m.



Figure 2.2. Maps of each saltmarsh sampled marked with the locations of each natural and realigned marsh. Map of sampling sites (top left) is the same area as the map in figure 2.1 (source: google earth)

Abbotts Hall

Abbotts Hall natural and realigned saltmarshes are supplied by the Blackwater estuary. The natural marsh sampled is more exposed to wave energy than the realigned marsh (Figure 2.2). The realigned marsh is more sheltered as it is situated behind a natural marsh and the old sea wall is still present with the only inflow of water in the realigned marsh through the breach in the old sea wall. This also suggest that sediment transport to the realigned site could be limited as the creek system of the natural marsh located in front of the realignment can act as a settling tank for particles. The distance between the natural and realigned marsh at Abbotts Hall site is 350m.

Mersea Island

Mersea Island is located between the Blackwater river/estuary and the Colne river and due to its location is supplied by both (Figure 2.1). Both marshes have similar exposure to wave energy since the area has a well-developed creek system for both natural and realigned study marshes (Figure 2.2). The distance between the natural and realigned marsh which was sampled at Mersea Island is 70m.

Fingringhoe Range

The saltmarshes studied at Fingringhoe Range are supplied by the Colne river (Figure 2.1). The sampling location for the realigned marsh was further inshore than the other two marshes with a distance to the natural marsh of 1300m (Figure 2.2). The realigned site is quite well sheltered from wave energy by natural marshes located in front of it. The location of the realigned site could have affected its supply of sediment as the preceding natural marsh vegetation could have trapped the bigger heavier sediment grains.

2.2.2 *Sampling*

Three representative mid-marsh plant communities (dominated by *Atriplex portulacoides*, *Limonium vulgare*, *Puccinellia maritima*) and mud pans were sampled seasonally for plant community composition and soil characteristics. Three replicates per condition were sampled in each salt marsh in areas of either complete cover of the targeted plant species within our sampling area (0.07m²) or where that was not possible, in an area with the highest percentage of the targeted species. Plant biodiversity was determined using a 1m² quadrat centred over the sediment sampling areas (0.07m²) as well as placements 1m North, South, East and West of the sampling areas. Soil cores, (4.2 cm inner diameter and ≥20 cm length) which were used for bulk density, water content, sediment grain size, nutrients (NH₄⁺ and NO₂⁻/NO₃⁻) and pH analysis, were collected from within each sampling area. The volume of

the core at each depth was adjusted for compression during collection using the difference in depth on the inside and outside of the core before extraction; linear compression was assumed.

2.2.3 Core processing

Samples were taken from the 5-10cm and 10-15cm depths of each core. The top 5cm from each core were discarded because the dense roots made sediment separation difficult. Bulk density and water content samples were taken from the centre (2cm sub-sections) of each 5cm (5-10, 10-15 cm) depth core section. Bulk density was determined by drying the central disk at 70°C for 7 days and dividing residual weight by initial (corrected) soil volume. Percentage water content was determined from each bulk density sample through measured water loss and is shown as [%water content = (g of water/g of soil) * 100]. Subsamples from dried bulk density disks were taken for grain size analysis. Sediment grain size samples were ground to a homogenized powder and treated with hydrogen peroxide, H₂O₂ (30% concentration) to volatilize organic matter. Residual sediment was resuspended in 20ml of deionized water and stored at room temperature until analysis. Grain size analysis was performed with a Malvern Mastersizer 2000 laser particle size analyser (Malvern Panalytical, Malvern, UK).

The freshly obtained remnants of each sub-section (i.e. 5-6.5cm and 8.5-10cm) were homogenised for nutrient and pH analyses. Soil samples for nutrient analysis were prepared according to Houba et al. (1995); 3.0g of soil with 1M of KCl, shaken at 200rpm for 60 minutes, centrifuged (2000rpm for 5 minutes) and the supernatant filtered. The filtrate was then stored at -20°C until analysis. Analysis for NH₄⁺ and NO₂⁻/NO₃⁻ was performed using a Seal Analytical AutoAnalyzer3 (SEAL analytical Ltd, Southampton, UK). KCl blanks were run to correct for contamination and/or drifts in extract as well as known concentration standards (0, 0.5, 1.0, 1.5, 2.0 mg/L) to ensure the equipment was calibrated and measuring

correct concentrations. Standards were run every 10 samples. The remaining solid phase from the nutrient samples was used for pH analysis, after in-house analyses demonstrated that pH was consistent between freshly prepared samples and post-extraction samples. In post extraction samples a further 15ml of 1M KCl solution was added, the samples were placed on a rocker (60rpm) for 60 minutes and analysed using a standard pH probe (meter: Jenway 3310, probe: VWR 662-1797). Similar to nutrient analysis, standards were used to calibrate the probe before each analysis as well as every 10 samples to ensure no drifts were occurring during analysis.

2.2.4 *Statistical analysis*

Statistical analyses were conducted using the R statistical language (R Core Team, 2018) implemented in RStudio (Version 1.1.423). pH was transformed out of log scale for statistical analysis. Nutrient concentrations had 1×10^{-6} mg/Kg added to all values to avoid zero value discontinuity and were log transformed for normality distribution. Bulk density and water content were bimodally distributed therefore required no further transformations. ANOVAs were performed for all sediment analyses and Spearman's rank-order correlation was performed for comparing mean sediment grain size with bulk density. Sample depth was not significant for any parameters apart from pH and nutrients during FR realigned autumn (pH and NH_4^+) and AH realigned winter (NO_x^-) thus depth was not used as a factor during analyses (Figure 2.3).

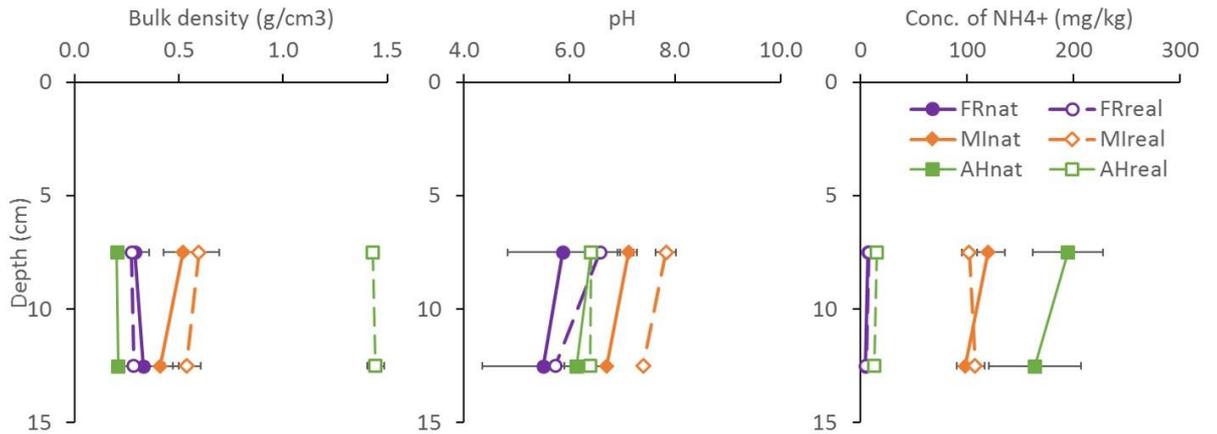


Figure 2.3. Average sediment characteristics measurements of 3 *Atriplex portulacoides* sediment cores collected from each salt marsh sampled (natural & realigned) for both depth ranges measured (5-10cm & 10-15cm) during the Spring sampling time. Error bars (\pm SE).

2.3 Results:

2.3.1 Bulk density and water content

Bulk density was significantly different between natural and realigned sites at the youngest (AH) and oldest (FR) saltmarshes (Figure 2.4). AH realigned saltmarsh sediments were significantly more dense than the paired natural marsh sediments ($F_{1,182}=2490$, $p<0.001$) and FR realigned marsh sediments were less dense than those in the paired natural marsh ($F_{1,178}=86.6$, $p<0.001$). The bulk density of natural and realigned marsh sediments was significantly different and the combined natural marsh data was significantly different from combined realigned sites data ($F_{2,544}=1203$, $p<0.001$). There was no significant difference between the MI natural and realigned marsh sediment bulk densities.

Water content showed similar, but inverse, patterns to those of bulk density in all saltmarshes (Figure 2.5). Water content of AH realigned saltmarsh sediment was significantly lower than the water content in the paired natural marsh ($F_{1,180}=2134$, $p<0.001$). FR realigned marsh water content was significantly higher than that of the paired natural marsh

($F_{1,178}=78.8$, $p<0.001$). Water content of realigned saltmarshes was significantly different from each other ($F_{2,266}=2700$, $p<0.001$) and AH natural marsh had significantly higher water content than MI natural marsh. Similar to bulk density, water content between MI natural and realigned marshes is not significantly different.

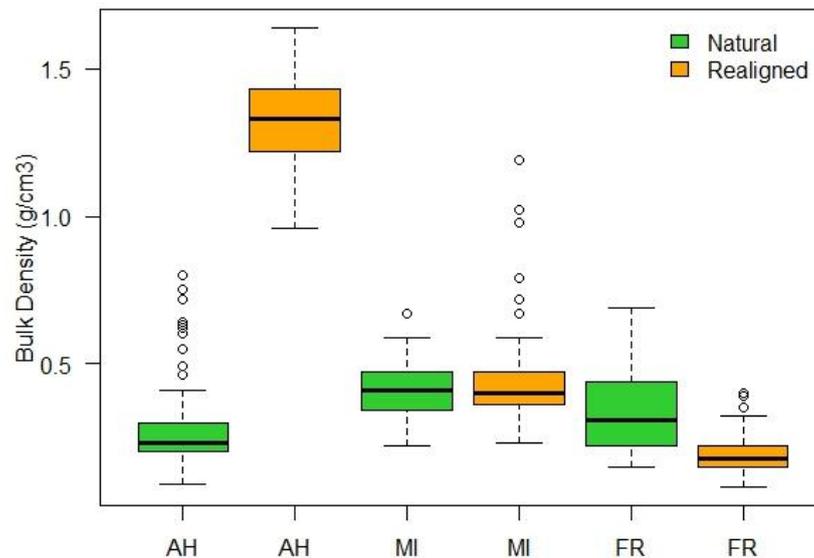


Figure 2.4. Average bulk density (g/cm^3) on all paired saltmarshes for all conditions and seasons, heavy line indicating median. AH 13 years: $n=94/90$; MI 62 years: $n=92/94$; FR 118 years: $n=93/87$

Water content in MI realigned marsh sediments varied with season ($F_{3,90}=4.77$, $p=0.003$); water content of sediments in autumn was lower than those in summer ($p=0.01$) and winter ($p<0.001$) (Table A.1). There did not appear to be any significant effect of season on bulk density or water content in any of the natural marshes.

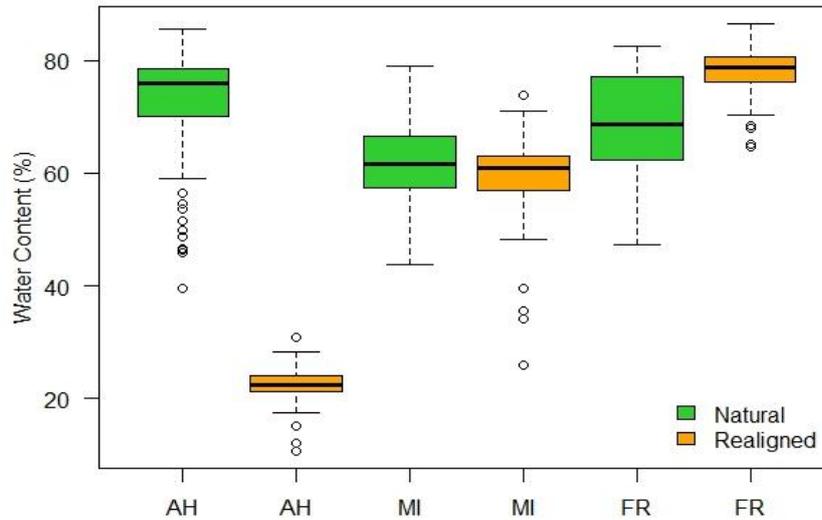


Figure 2.5. Average water content (%) on all paired saltmarshes for all conditions and seasons, heavy line indicating median. AH 13 years: n=94/90; MI 62 years: n=92/94; FR 118 years: n=93/87

Physical sediment status was correlated with dominant vegetation, primarily in that unvegetated sediments were more dense than vegetated sediments (AH: $F_{3,176}=7.07$ $p<0.001$; MI: $F_{3,178}=9.96$, $p<0.001$; FR: $F_{3,172}=9.43$ $p<0.001$). In AH natural saltmarsh the mud pan sediments were significantly more dense than sediments under vegetated conditions (*Atriplex* $p=0.037$, *Limonium* $p<0.001$, *Puccinellia* $p=0.001$). FR natural saltmarsh mud pan sediments were also more dense than FR vegetation-covered sediments (*Atriplex* $p<0.001$, *Limonium* $p<0.001$, *Puccinellia* $p<0.001$). Other significant sediment bulk density differences included; AH realigned *Limonium* sediments were significantly less dense than AH realigned *Atriplex* ($p=0.017$) and MI realigned mud was more dense than MI realigned *Limonium* ($p<0.001$) (Table A.2).

Similarly, water content in natural marshes was correlated with vegetation conditions: unvegetated sediments retained less water than vegetated sediments (AH: $F_{3,174}=12.86$ $p<0.001$; MI: $F_{3,178}=13.41$ $p<0.001$; FR: $F_{3,172}=19.3$ $p<0.001$). AH natural mud pans had significantly less percentage water than vegetated conditions (*Atriplex* $p<0.001$, *Limonium* $p<0.001$, *Puccinellia* $p<0.001$). MI natural *Limonium* had more water content than *Atriplex*

($p=0.06$), mud pans ($p=0.034$) and *Puccinellia* ($p=0.034$). FR natural mud pans had less water content than *Atriplex* ($p<0.001$), *Limonium* ($p<0.001$) and *Puccinellia* ($p<0.001$). MI realigned mud pans had less water content than *Limonium* ($p<0.001$) and FR realigned *Limonium* had more water content than *Atriplex* ($p=0.035$) (Table A.1).

2.3.2 Sediment grain size

Bulk density was significantly and negatively associated with mean grain size in all marshes (Figure 2.6). On a marsh-by-marsh basis FR exhibited the strongest, most significant negative correlation according to Spearman's rank order correlation ($p<0.001$, $r^2=-0.688$) while bulk density had less explanatory power, but retained its significance, in explaining sediment grain sizes in MI ($p=0.001$, $r^2=-0.234$), and in AH ($p<0.001$, $r^2=-0.434$) (Figure 2.6). When natural and realigned sites were compared separately we found that grain size in natural marshes was more likely to describe bulk density behaviour than in realigned sites (FRNat $p<0.001$ $r^2=-0.823$, FRReal $p<0.001$ $r^2=0.358$; AHNat $p<0.001$ $r^2=-0.500$, AHReal $p=0.037$ $r^2=0.220$;

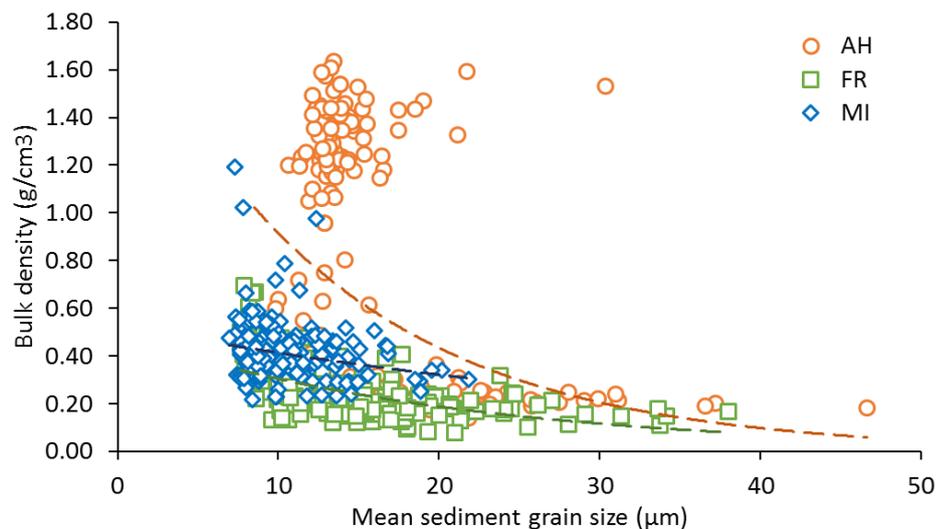


Figure 2.6. Mean grain size of sediment (μm) against bulk density (g/cm^3) at each salt marsh for all depths and seasons. FR: $p<0.001$, $r^2=-0.688$; MI: $p=0.001$, $r^2=-0.234$; AH: $p<0.001$, $r^2=-0.434$

MINat $p=0.001$ $r^2=-0.328$, MIReal $p=0.212$ $r^2=-0.130$) (Figure 2.7). Sediment grain size in MI appears to be skewed towards smaller, tightly selected grain size particles than other marshes (Table 2.1) (Figure 2.8b). In addition, it appears to be the only pair where both natural and realigned marshes have similar size distribution and sorting. AH natural marsh is less sorted and is more skewed towards finer sediment than its realigned pair (Figure 2.8a), although average grain size in the AH realigned sediments is lower than in the natural sediments. In contrast, FR realigned sediment grain size is greater than that of the natural

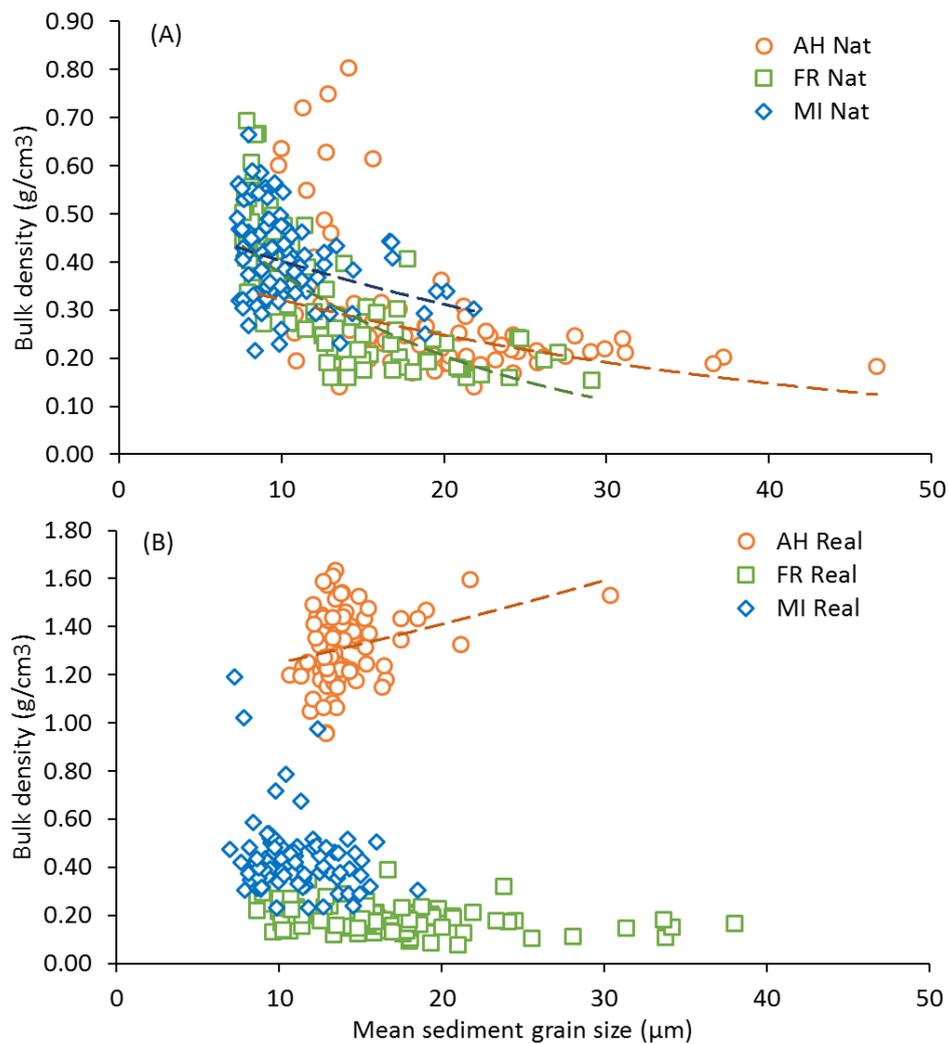


Figure 2.7. Mean sediment grain size (μm) against bulk density (g/cm^3) for each salt marsh (A) Natural and (B) Realigned for all seasons and depths. FRNat $p<0.001$ $r^2=-0.823$, FRReal $p<0.001$ $r^2=0.358$; AHNat $p<0.001$ $r^2=-0.500$, AHReal $p=0.037$ $r^2=0.220$; MINat $p=0.001$ $r^2=-0.328$, MIReal $p=0.212$ $r^2=-0.130$

site sediments, however realigned sediments are less sorted and are somewhat more skewed towards finer sediments than in FR natural (Table 2.1) (Figure 2.8c).

Table 2.1. Average sediment grain size measurements and average bulk density (BD) at each sampling depth. Sorting is grain size variation within samples (0.00-0.35=very well sorted, 0.35-0.50=well sorted, 0.50-0.71=moderately well sorted, 0.71-1.00=moderately sorted, 1.00-2.00=poorly sorted, 2.00-4.00= very poorly sorted). Skewness measures the degree to which a cumulative curve approaches symmetry (positive numbers = more coarse, negative values = finer; scale from +1 to -1). Kurtosis measures the “peakedness” in a curve (if the sample curve is better sorted in the tails than in the central portion, the curve is flat peaked or platykurtic. For normal curves = 1.00, leptokurtic curves have >1.00, and platykurtic curves have <1.00)

Sediment grain size	Abbotts Hall		Mersea Island		Fingringhoe Range	
	Natural	Realigned	Natural	Realigned	Natural	Realigned
5 – 10 cm depth						
Mean (µm)	18.2 ± 0.9	14.2 ± 0.3	10.4 ± 0.4	10.5 ± 0.3	13.3 ± 0.7	17.3 ± 1.0
Sorting (φ)	2.00 ± 0.05	1.67 ± 0.02	1.62 ± 0.04	1.65 ± 0.03	1.77 ± 0.05	2.11 ± 0.05
Skewness	-0.48 ± 0.03	0.07 ± 0.02	-0.73 ± 0.04	-0.75 ± 0.04	-0.53 ± 0.04	-0.66 ± 0.04
Kurtosis	2.60 ± 0.06	2.15 ± 0.04	3.51 ± 0.12	3.45 ± 0.09	2.97 ± 0.10	2.80 ± 0.11
BD (g/cm ³)	0.27 ± 0.02	1.34 ± 0.02	0.423 ± 0.01	0.43 ± 0.02	0.34 ± 0.02	0.18 ± 0.01
10 – 15 cm depth						
Mean (µm)	18.4 ± 1.1	14.0 ± 0.4	10.3 ± 0.5	11.2 ± 0.4	13.0 ± 0.8	17.0 ± 0.7
Sorting (φ)	1.93 ± 0.04	1.65 ± 0.02	1.62 ± 0.04	1.68 ± 0.04	1.69 ± 0.05	2.03 ± 0.05
Skewness	-0.40 ± 0.04	0.11 ± 0.02	-0.71 ± 0.05	-0.68 ± 0.05	-0.50 ± 0.04	-0.66 ± 0.03
Kurtosis	2.60 ± 0.06	2.08 ± 0.02	3.48 ± 0.12	3.27 ± 0.10	2.88 ± 0.06	2.82 ± 0.10
BD (g/cm ³)	0.29 ± 0.02	1.30 ± 0.02	0.40 ± 0.01	0.43 ± 0.02	0.34 ± 0.02	0.20 ± 0.01

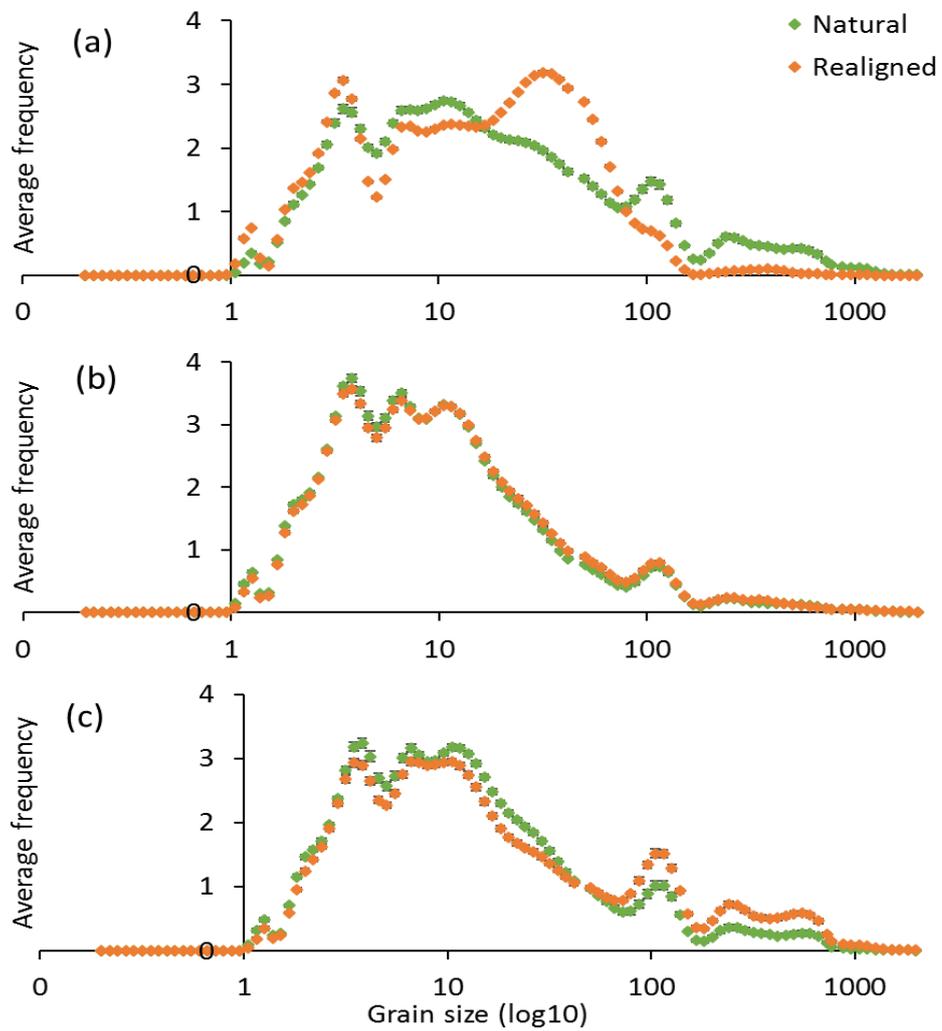


Figure 2.8. Average frequency of sediment grain size of (a) Abbots Hall, (b) Mersea Island and (c) Fingringoe Range, plotted on a log scale. Error bars \pm SE

2.3.3 Sediment pH

Realigned sites varied in sediment pH while natural sites were statistically similar. The FR realigned site pH was significantly lower than MI and AH realigned sites ($F_{2,272}=34.75$, $p<0.001$). Comparing sediment pH between natural and realigned saltmarsh pairs we found that only in FR was the natural site significantly different than the realigned site, with the realigned site having lower pH ($F_{1,183}=19.05$, $p<0.001$) (Figure 2.9). The only observed differences in sediment pH between dominant vegetation types was found between AH natural marsh mud pans and other AH vegetated conditions, where AH mud pan sediments were lower in pH ($F_{3,177}=7.584$ $p<0.001$, post-hoc: *Atriplex* $p=0.002$, *Limonium* $p=0.002$, *Puccinellia* $p=0.002$) (Table A.3). Mud pans in general showed greater variation in pH (ranging from 2.6 to 6.8). Seasonality had no apparent effect on sediment pH (Figure 2.10). Sediment pH was found to be negatively correlated with water content ($p<0.001$, $r_s=-0.23$) (Figure 2.11a), such that higher water content sediments were more acidic. When the correlation was run for each individual marsh, we found that AH and FR were also negatively correlated

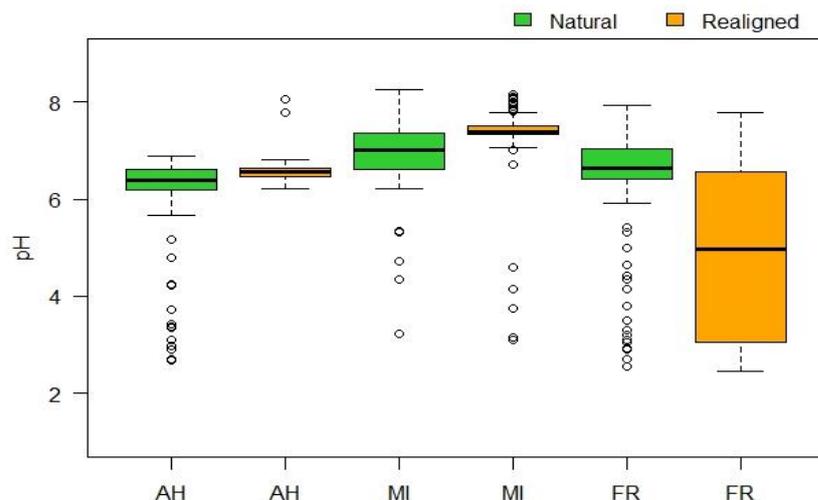


Figure 2.9. Average pH on all paired saltmarshes for all conditions and seasons. AH 13 years: $n=94/90$; MI 62 years: $n=92/94$; FR 118 years: $n=93/87$

(AH: $p=0.021$ $r_s=-0.17$; FR: $p<0.001$ $r_s=-0.25$). When natural and realigned marshes were separated, we found that only FR realigned pH was significantly correlated with water content ($p=0.008$, $r_s=-0.28$) (Figure 2.11).

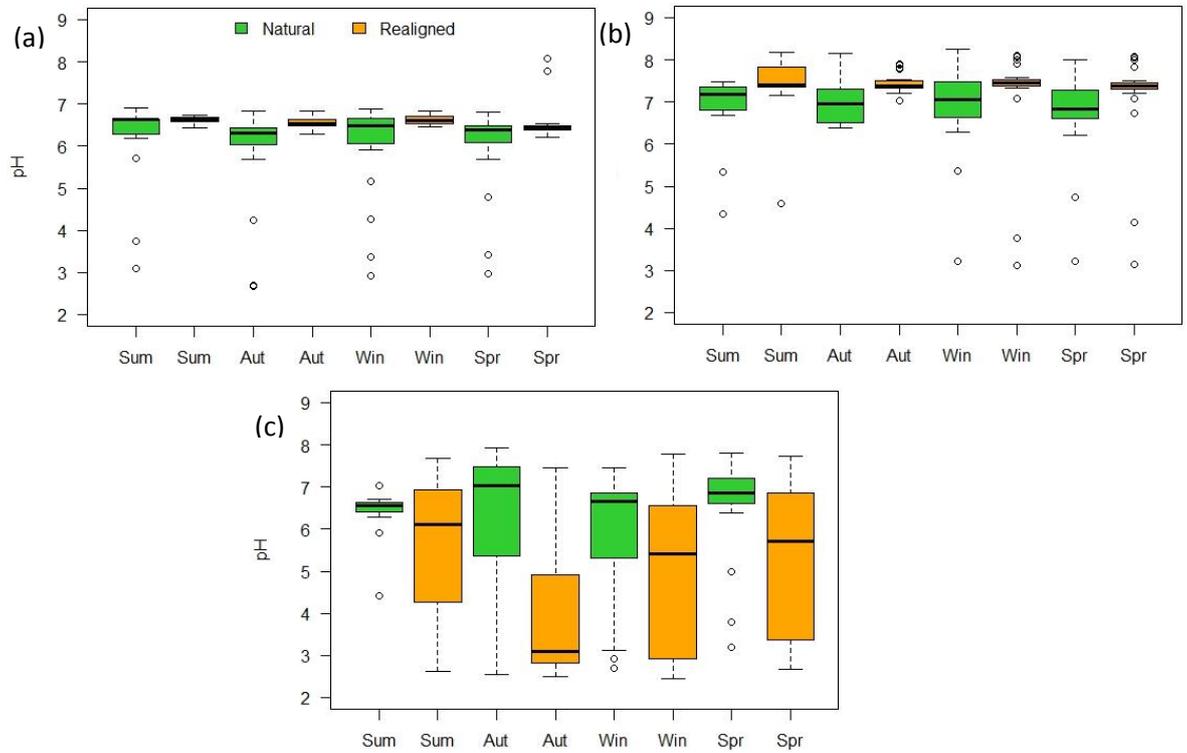


Figure 2.10. Average pH of each salt marsh (a) Abbots Hall, (b) Mersea Island, (c) Fingringhoe Range, at each season for all conditions together.

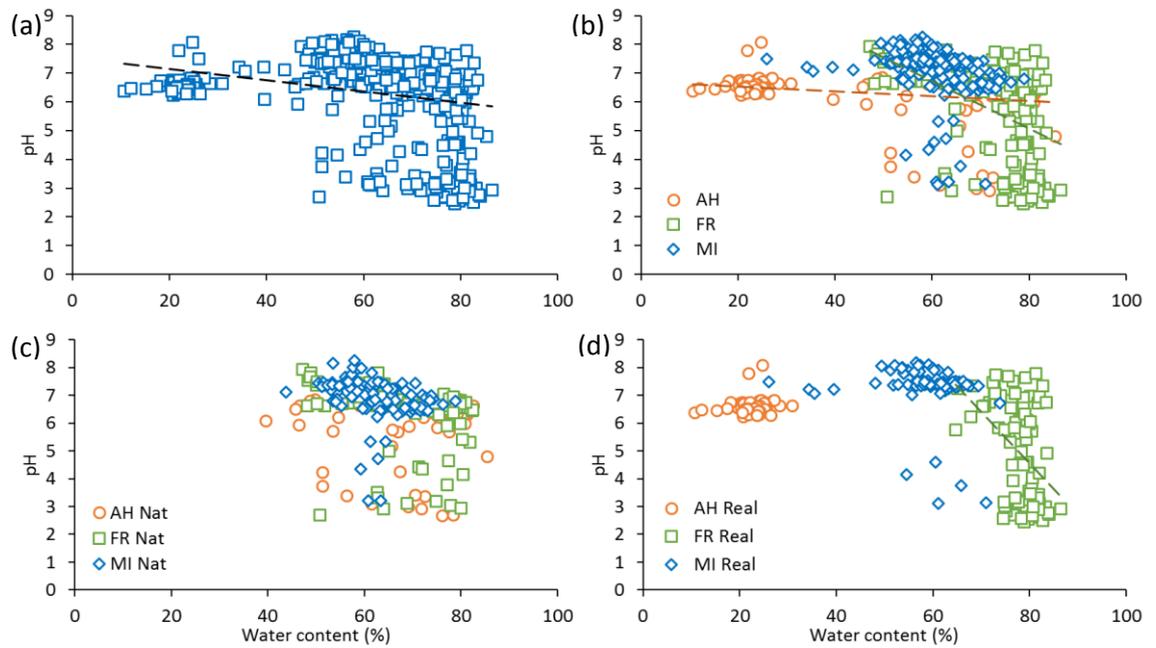


Figure 2.11. (a) pH against water content for all site and marshes. (b) pH against water content for each marsh for all seasons and depths. (c) pH against water content for all natural marshes. (d) pH against water content for all realigned marshes

2.3.4 Nutrients

Sediment nutrient content changed with management status, location, seasonality and dominant vegetation. Ammonium (NH_4^+) concentration in the sediment was significantly different between the natural marshes and realigned but also between the natural marshes themselves and between the realigned marshes ($F_{2,543}=24.36$, $p<0.001$). NH_4^+ concentration of AH natural was significantly higher than MI natural ($F_{2,543}=24.36$, $p<0.001$), and AH realigned marsh concentrations were significantly lower than those in FR and MI realigned marshes ($F_{2,543}=24.36$, $p<0.001$) (Figure 2.12). When comparing paired natural and realigned marshes only in AH natural was NH_4^+ content significantly higher than in the AH realigned marsh ($p<0.001$).

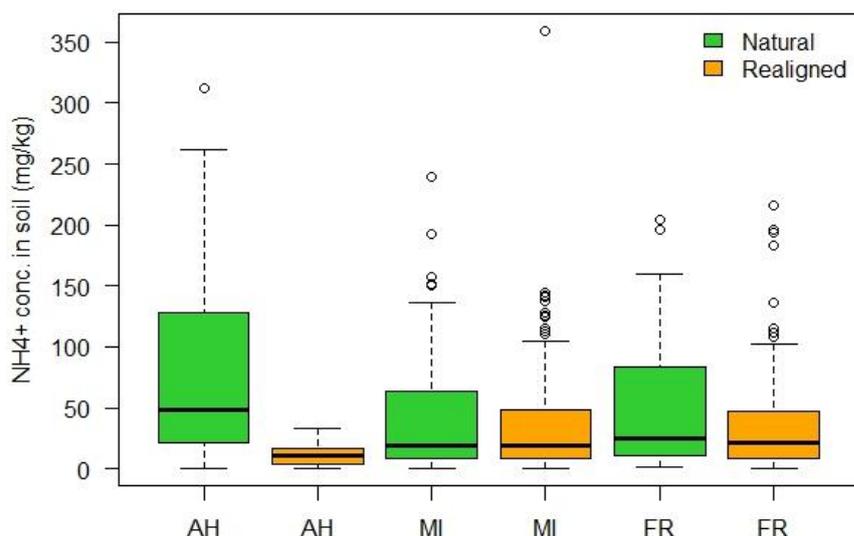


Figure 2.12. Average ammonium (NH_4^+) concentration in sediment (mg/kg) on all paired saltmarshes for all conditions and seasons. AH 13 years: $n=94/90$; MI 62 years: $n=92/94$; FR 118 years: $n=93/87$

Variation between sampling seasons was observed in all sites, both natural and realigned ($F_{6,525}=5.82$, $p<0.001$). NH_4^+ concentration in AH natural marsh in spring was significantly higher than all the other seasons ($p<0.001$) and in AH realigned marsh spring sediment NH_4^+ concentrations were significantly higher when compared to autumn and summer ($p<0.001$, $p=0.008$) (Figure 2.13). In MI saltmarsh both natural and realigned sites behaved similarly with spring NH_4^+ concentrations being significantly higher than other seasons ($p<0.001$) while autumn concentrations were also significantly higher than those of summer and winter ($p<0.001$) (Figure 2.13). FR natural and realigned marshes behaved similarly, but differently to the other marshes, with spring NH_4^+ significantly lower concentration than the other seasons ($p<0.001$). NH_4^+ concentrations in FR natural in autumn were significantly lower than summer and winter ($p<0.001$), FR realigned in autumn was significantly lower than winter ($p=0.014$) and FR natural in summer concentration was significantly higher than FR summer realigned ($p<0.001$) (Figure 2.13). Additionally, NH_4^+ concentration was positively correlated with water content ($p<0.001$, $r_s=0.36$) and weakly correlated with nitrate/nitrite concentration ($p=0.016$, $r_s=0.10$).

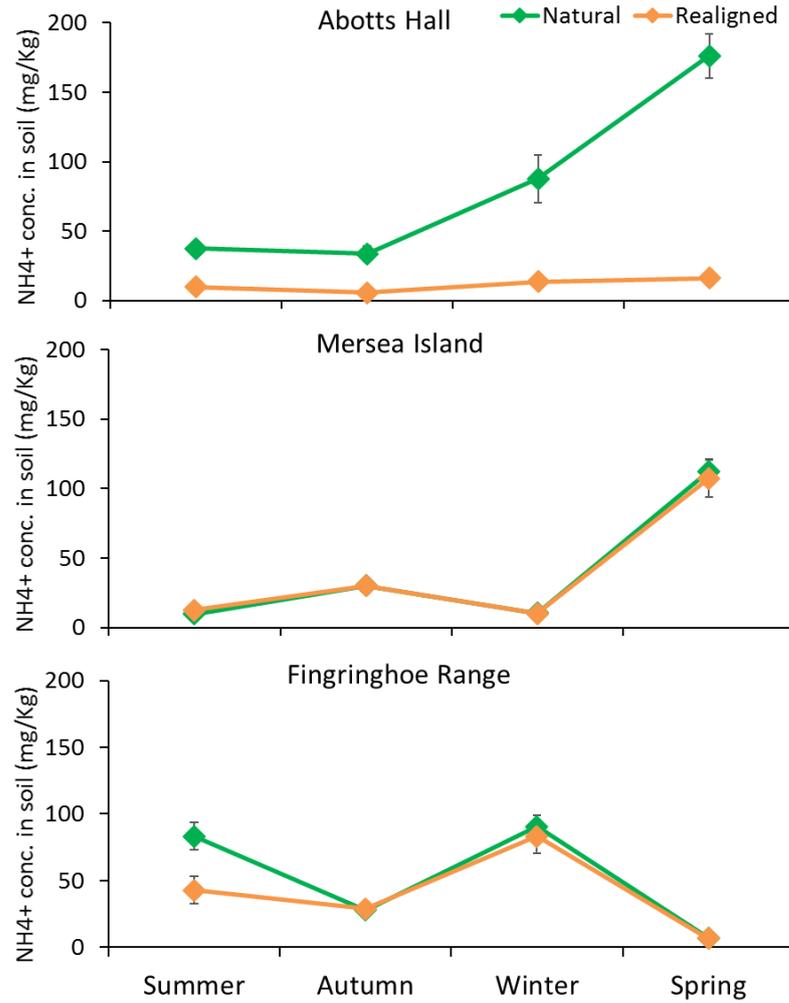


Figure 2.13. Average NH_4^+ concentration in sediment for each season for each saltmarsh and natural/realigned site. $n=24$, error bars \pm SE

Nitrate/nitrite ($\text{NO}_2^-/\text{NO}_3^-$) concentration in sediment was significantly different between AH and MI paired natural and realigned marshes ($F_{1,183}=8.18$ $p<0.001$; $F_{1,184}=12.36$ $p<0.001$) with both realigned marshes having lower concentrations than the natural marshes (Figure 2.14). $\text{NO}_2^-/\text{NO}_3^-$ concentrations did not vary with season at any site.

Surface vegetation appears to impact sediment nutrient status. Sediments under *Atriplex* and *Puccinellia* had higher concentrations of $\text{NO}_2^-/\text{NO}_3^-$ relative to other conditions. In AH natural marsh *Atriplex* and *Puccinellia* sediments NO_x concentrations were significantly higher than in *Limonium* sediments ($F_{3,91}=5.21$; $p=0.002$, $p=0.021$), and in MI natural marsh

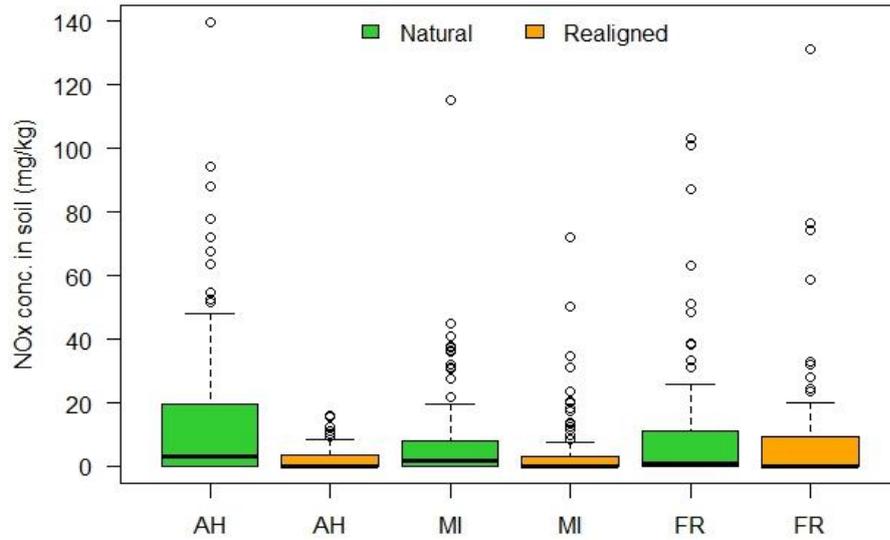


Figure 2.14. Average nitrate/nitrite ($\text{NO}_2^-/\text{NO}_3^-$) concentration in the sediment (mg/Kg) at each natural and realigned site

Atriplex and *Puccinellia* sediment NO_x was significantly higher than in mud pans ($F_{3,88}=5.39$; $p=0.021$, $p=0.001$) (Figure 2.15). No statistical significance was found between different vegetation conditions in FR, however we can see that *Atriplex* sediments had higher NO_x concentrations than other (non)vegetated sediments for both natural and realigned marshes (Figure 2.15).

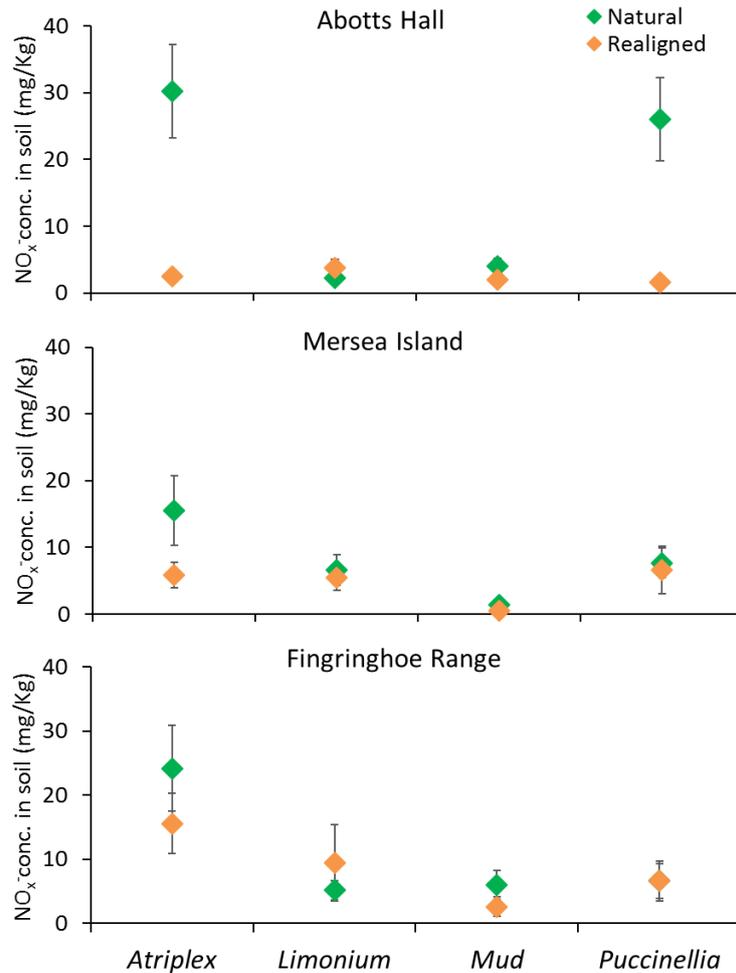


Figure 2.15. Average nitrate/nitrite ($\text{NO}_2^-/\text{NO}_3^-$) concentration in sediment at all marshes for each condition. Error bars \pm SE

2.3.5 Plant biodiversity

Plant diversity was greater for all natural marshes when compared to their paired realigned marshes. We observed significantly higher α -biodiversity index (Shannon's, accounts for both abundance and evenness of species present) in the natural marshes (Table A.4; AH $p < 0.001$, MI $p = 0.007$, FR = 0.002). Plant α -diversity in realigned marshes was variable, MI realigned had a significantly higher diversity than AH realigned ($p = 0.01$). β -diversity (Bray-Curtis, a measure of species turnover and relative abundance) also showed significant difference between paired natural and realigned marshes with lower plant β -diversity in

realigned marshes (AH $p < 0.001$, MI $p < 0.001$, FR < 0.001) (Table A.4). Variability among the natural marshes was significantly different when described by β -diversity ($p < 0.001$), and realigned marshes were also significantly different from each other ($p < 0.001$). AH natural marsh had the greatest species richness relative to MI and FR natural marshes, whereas the MI realigned marsh had the greatest species richness compared to FR and AH. AH realigned site had the least diversity, as defined by β -diversity, relative to all sites studied (Table A.4).

2.4 Discussion

Natural and realigned marshes showed similar sediment characteristics as other managed and natural marshes studied in south east England; with previous studies reporting bulk densities of natural marshes ranging from 0.5 to 0.8g/cm³, realigned bulk densities of 0.8 to 1.5g/cm³, and pH of natural marshes ranging 6.6 to 7.7 and realigned marsh pH of 7.2 to 8.2 (e.g. Kadiri et al., 2011; Spencer et al., 2008; Tempest et al., 2015). There are indications of gradual shifts in physical sediment characteristics that develop over time within the realigned sites. Abbotts Hall, the youngest realigned marsh, showed the most significant difference from its paired natural marsh for sediment characteristics measured (bulk density, water content, nutrients). The high bulk density and low water content of the realigned AH marsh is an indication of a relic agricultural sediment which is purported to remain mostly unchanged post inundation (Cai et al., submitted; Tempest et al., 2015). This unchanged relic layer in realigned salt marshes has been observed by Tempest et al. (2015) at the Orplands Farm site (Blackwater Estuary, SE England) which showed that the relic agricultural layer remained unchanged with marine sediment deposited on top post sea wall breach and inundation.

While we did not see any substantive difference between the depths that we observed, this does not reflect the relic layer in MI and FR as we did not penetrate to that depth in any of

our sampling across any of these sites. AH realigned sediment sampled even to the 10-15cm depth was visually different from any other sample collected from realigned sites. Cores collected at AH realigned marsh were comprised of red clay, whereas in MI and FR it appeared that we were sampling marine sediment deposited over the years in these sites.

Alternatively, the difference in development of our sites, especially with MI being more similar to its natural pair than FR, may be explained by relative spatial location, both to each other and within the Colne/Blackwater estuary. Marshes are regionally unique, with different wave energy, exposure and sediment delivery (Figure 2.1, 2.2) which may explain more of the observed differences than time from realignment. Lawrence et al. (2018) examined topographic variability between natural and realigned saltmarshes and found that there was no relationship between age of restoration and any topographic variables. They state that restored saltmarshes are not on a trajectory to become topographically similar to natural marshes over time, although within their sampled marshes, a few restored marshes overlapped topographically with their referenced natural marsh. Within our sampling sites we observed that MI realigned marsh was more similar to its natural pair than FR which is older. This difference can also be seen in the extensive creek formation of MI realigned compared to FR realigned (Figure 2.2).

The AH realigned site is a steeply sloped, low lying old agricultural field which is more sheltered from wave exposure than FR and MI realigned (see Figure 2.2), and this could have affected sediment deposition and inundation of the site through the years. FR is also an old agricultural field which is within a sheltered region with an extensive natural marsh between the realigned marsh and the regional coastal mudflats. In contrast, while the MI realigned marsh was more exposed and surrounded by creeks which might allow better drainage of the site, both MI natural and realigned marshes are found within a protected, bridged tributary. This effect can be seen with our sediment grain size distribution which showed MI

paired marshes being more similar but also show contrast between the other natural and realigned marshes sediment grain size sorting.

Lawrence et al. (2018) found that realigned marshes have different topography to natural marshes, they have an enhanced potential for water accumulation (higher topographic wetness index) and have lower creek densities. Realigned salt marshes are topographically more similar to the agricultural landscapes they originate from than natural marshes (Lawrence et al., 2018). This can affect the development of the marsh's vegetation and drainage which in turn can affect the sediment structure. It may be that passage of time, and time since inundation, alone does not predict return to a "natural" state. Instead location, topography, exposure and previous land use may be the determining factors in salt marsh restoration.

Furthermore, natural marshes show greater variability and heterogeneity in nutrient concentrations and sediment pore water content than realigned marshes. This was seen both through the variance observed during each sampling period between the different vegetation conditions (spatial) and also seasonal variation (temporal). Only the oldest realigned site (FR) showed greater variability in a sediment characteristic (pH; ranges from 2.5 to 7.9) relative to its paired natural marsh, while the younger sites (AH, MI) showed less variability in all other conditions compared to the reference natural marshes. The younger realigned marshes (AH, MI) showed little fluctuation in sediment characteristics over the four season sampling period. These data suggest that natural marshes are more heterogeneous than realigned marshes. When we compared nutrient concentration between the natural/realigned paired we observed that AH natural marsh had a higher concentration of NH_4^+ than realigned which could be attributed to the lower water content in the realigned marsh, facilitating nitrification of NH_4^+ to $\text{NO}_3^-/\text{NO}_2^-$, an aerobic process. In addition, we observed higher concentrations of NH_4^+ in our *Atriplex* conditions which previous studies

have shown they are associated with nitrogen fixing endophytes (Tahtamouni et al., 2016; Zahran, 1999). Heterogeneity of a natural saltmarsh in terms of nutrient concentrations in comparison with realigned saltmarshes may be explain by the differences in plant biodiversity and microbial communities which drive these processes (nitrification/ammonification).

Heterogeneity of sediment characteristics of realigned marshes and the presence of a relative dense subsurface layer could affect creek development (Hazelden and Boorman, 2001) and the topographical development of the realigned marsh (Lawrence et al., 2018), which in turn can affect the biogeochemical process of the marsh as well as plant development (Mossman et al., 2012b). Differences in plant community composition and diversity of realigned marshes can reduce their biogeochemical functions (i.e. carbon storing (Moreno-Mateos et al., 2012)) and are very likely to have knock on effects on other plant-influenced ecosystem functions such as sediment erosion (Ford et al., 2016) and wave attenuation (Möller and Spencer, 2002). Elevation is also a key determinant of vegetation colonization of restored salt marshes since saltmarsh plants have clear elevation niches (Masselink et al., 2017; Sullivan et al., 2017). In order to minimize any observed differences due to elevation zonation all sampling was performed at similar elevation (within 20cm), thus allowing us to conclude that any variances observed between natural and realigned plant biodiversity was due to other underlying factors (i.e. sediment characteristics) rather than elevation differences.

Plant biodiversity in realigned marshes was found to be lower than in natural sites. Despite our study sampling at specific plant dominant locations within consistent, set elevations (mid-marsh) our results match previous natural/realigned comparisons of saltmarsh biodiversity (e.g. Garbutt and Wolters, 2008; Mossman et al., 2012). However, α -biodiversity of our oldest realigned site (FR) was not significantly different from the youngest (AH) but

was significantly different from MI which re-emphasizes the idea that re-establishment of a marsh is complex and age of a realigned marsh (years from initial inundation) does not adequately predict return of a “natural-state” marsh. Topography (i.e. slope and creek formation) and hydrogeology of a marsh could be a more determining factor of realigned salt marsh plant development than age. In addition, changes on short-term scales, or immediately after breach are often overlooked even though biotic and abiotic factors that are likely to affect sediment development could be heavily influence by initial conditions set within the first year of salt marsh re-establishment.

Overall, our natural and realigned pairs have not behaved as predicted that the most similar pair would be the oldest. Plant biodiversity of realigned marshes was significantly lower than natural for all our sampled pairs. However, for sediment characteristics MI was the only pair that had no significant difference. The youngest site (AH) did behave as expected with the greatest variability between the pairs. With the observed differences between the FR pair we can confidently conclude that age of a realigned marsh alone cannot determine or predict the success of a marsh returning to “natural” conditions.

Chapter 3. Changes in sediment characteristics in the first year of a UK realigned salt marsh

3.1 *Introduction*

Saltmarshes are coastal habitats that provide important ecosystem services, from support for coastal and terrestrial food chains to coastal protection (Beaumont et al., 2008; Hughes and Paramor, 2004; Millenium Ecosystem Assessment, 2005). Tidal marshes are predicted to bury carbon and it is estimated that this burial of carbon in saltmarsh sediments globally is between 5 teragrams (Tg) and 87 Tg C yr⁻¹ (McLeod et al., 2011). Saltmarshes provide protection to coastal areas by dissipating wave and tidal energy, reducing the possibility of sea walls being breached, overtopped or undermined (Möller et al., 1999; Möller and Spencer, 2002). An estimated 2000km of UK coastline is protected by saltmarshes (Doody, 1992). Saltmarsh coverage in the UK (as of 2008) is approximately 45,500 hectares, mainly in eastern England (Beaumont et al., 2008)

Climate change threatens coastal habitats by increased storm frequency and sea-level rise (IPCC, 2007). The human response to these challenges is greater installation of sea defences, which further constricts coastal shore-line habitat development and the natural expansion/development of salt marshes. These actions in combination, have been described as coastal-squeeze (Boorman, 2003). Over the last two decades there has been an accelerated global decline in the extent of saltmarsh systems (Millenium Ecosystem Assessment, 2005) and efforts have been initiated to conserve existing marshes and to develop new coastal marsh habitats (realigned or managed saltmarshes). The European Union Habitat Directive (adopted in UK legislation in 1992) maintains a no-net-loss policy that has led to the current strategy of managing coastal marsh areas and the creation of managed realigned saltmarshes. Efforts have been made in England and Wales to identify suitable areas for managed realignment, to estimate the cost of each realignment, and to

monitor realignments (DEFRA, 2002; Environment Agency, 2017). Management plans for restoring or enhancing saltmarshes include: i) gathering information on the present status of the saltmarsh, ii) identifying any problems, iii) assessing the financial, environmental and social consequences of not intervening, iv) identifying suitable management option relative to efficiency and cost and v) gathering further data to monitor the effectiveness of any scheme (Adnitt et al., 2007). There are five mandatory attributes and targets for restoring salt marshes; habitat extent, physical structure (creeks and pans), vegetation zonation and structure, vegetation composition and other negative indicators (e.g. no signs of pollution). When a target is not applicable for a particular site it is excluded (Adnitt et al., 2007).

Historically, and globally, saltmarshes have been reclaimed for either agricultural land or urban development (Adam, 1990). By their nature, these reclaimed agricultural lands started near sea level and when combined with the compaction and erosion of soil that often accompanies agricultural practice, has led to a reduction of elevation within these lands to below sea level. Maintaining sea defenses in order to protect these low lying agricultural lands has become increasingly costly and protocols are being tested in converting some of these lands back into saltmarshes (Foster et al., 2013; French, 2006; Hazelden and Boorman, 2001). Managed realignment aims to restore saltmarshes by reconstructing new sea walls further inland and deliberately breaching existing fore-shore walls thus allowing tidal inundation of low-lying agricultural land (French, 2006).

Studies have shown that, even decades after installation, realigned saltmarshes have different plant communities and distributions than natural marshes (e.g. Garbutt & Wolters 2008, Davy et al. 2011, Mossman et al. 2012a). Some studies have found that previous land use of realigned sites (agriculture and drainage) results in long term changes to the subsurface sediment structure, including collapse of pore space, which leads to poor or less rapid marsh drainage (Spencer et al., 2008; Tempest et al., 2015). These changes are not

readily reversed once the land is flooded with saline water and seawater inundation may result in further downwash of fine particles into the subsurface thus further reducing porosity at depth (Macphail et al., 2010). Furthermore, Tempest et al. (2015) found that de-embarked saltmarshes (i.e. realigned marshes created by breaching of the sea wall and allowed to regenerate naturally) have two distinct sediment layers, relic and altered agricultural soil, overlain by newly deposited marine sediment. These two layers have different physical properties and are hence likely to have different hydrological characteristics in terms of water storage and movement.

Environmental data on sediment moisture, redox potential and organic matter show that restored marshes are less oxygenated with lower redox potential at lower elevations relative to sea level (Davy et al., 2011). Higher elevations in managed saltmarshes have been shown to have more oxygenated sediments that are drier and contain less organic matter relative to natural marshes of the same elevation (Mossman et al. 2012a). These sediment characteristics have substantial influence on plant community dynamics (Davy et al. 2011, Mossman et al. 2012b), which may explain the long-term trends reported by Garbutt & Wolters (2008) and Mossman *et al.* (2012a). Garbutt and Walters (2008) observed that, even after a century of re-establishment, plant communities in realigned marshes were significantly different. Realigned marshes had lower percentage cover of targeted species than natural marshes. Garbutt and Wolters (2008) concluded that realigned marshes could take up to 137 years to reach the same species richness as natural marshes however not necessarily with the same species. Mossman *et al.* (2012a) found similar results when comparing plant communities of restored versus natural (reference) marshes. In this case the restored marshes had overall similar plant community composition, but individual quadrats had more exposed bare ground than natural marshes.

We know that when we consider aboveground plant diversity natural and realigned marshes are different, however these changes are linked to, and possibly driven by, below ground processes. We therefore need to know what those processes are. Nitrogen from the atmosphere is taken up by plants as well as available organic nitrogen from the sediments and used for metabolic reactions (protein formation and chlorophyll production thus linked to photosynthetic rate of plants (Evans, 1989)). The major source of nitrogen in the form of $\text{NO}_3^-/\text{NO}_2^-$ and NH_4^+ into saltmarshes is bulk precipitation (Jordan et al., 1983). Excess nitrogen enters salt marshes from fertilizer runoff, acid precipitation and sewage waste (Seitzinger et al., 2005). The amount of nitrogen removed or recycled by coastal wetland varies with the different types of processes (i.e. nitrification/denitrification) and their rates as well as seasons. For instance, N can be remineralized to NH_4^+ and recycled into the water column and sediment. It can be removed from the system as N_2 gas through the process of denitrification and it can also be immobilized by microbes, or buried as soil organic nitrogen (Velinsky et al., 2017).

Nitrogen in the form of ammonia (NH_4^+) is released in the sediment during organic matter decomposition by ammonifying bacteria and fungi in a process called ammonification. NH_4^+ is oxidized to nitrate and nitrite (NO_3^- , NO_2^-) by nitrifying bacteria and archaea in the sediment under aerobic conditions. Nitrogen is lost from marine sediments via denitrification. Denitrification, the reduction of nitrate back to nitrogen gas, is an obligate anaerobic process. The nitrate reducing potential of salt marsh sediments is relatively high (Nedwell, 1982), however the reduction process is limited by availability of nitrate.

Nitrogen in an ecosystem can also be affected by external factors such as fertilization and animal grazing (Bazely and Jefferies, 1985; Davis et al., 2017; Ma et al., 2007). Areas where animals are grazing (e.g. pasture field) often have an increase in ammonium concentration in the sediment as well as localized "hot spots" due to animal excrements (Bazely and

Jefferies, 1985; Ma et al., 2007). These hot spots could potentially have an impact on the development of sediment and plant biodiversity of a salt marsh as nitrogen availability can affect plant biomass (Cartaxana et al., 1999), which in turn can affect sediment stabilization (Ford et al., 2016).

A limited number of studies have focused on sediment characteristics over time in realigned saltmarshes (e.g. Spencer et al. 2008, Tempest et al. 2015). Of these, most focus on the changes that have occurred several years after restoration. Spencer *et al.* (2008) and Tempest *et al.* (2015) examined the same saltmarsh 8 and 18 years post breach for changes in sediment characteristics and differences with an adjacent natural marsh. However, both studies examined the marsh at one timepoint. Changes on short-term time scales, or immediately after breach (e.g. Kadiri et al. (2011) who sampled 6 months post breach for 12 months) are often overlooked even though biotic and abiotic factors that are likely to affect sediment development and vegetation colonization (including seed availability, sediment pH, salinity, nutrient availability and anoxia (Garbutt et al. 2006, Garbutt & Wolters 2008, Mossman et al. 2012a,b, Zhou et al. 2016) are likely to be heavily influenced by initial conditions set within the first year of marsh re- establishment.

This study explores these early-stage sediment realignment processes in a south eastern UK saltmarsh established on an agricultural system. The objective of the study was to quantify how total sediment accumulation affects early changes in physical and chemical sediment status after flooding, and the effect of previous land use (arable and pasture) on newly realigned saltmarsh in Essex UK during the first-year post inundation. We hypothesized that the pasture field will have a higher load of nutrients than the arable field months after inundation due to the increased residual biomass prior to flooding.

3.2 Methods:

3.2.1 Study site

Fingringhoe Wick Nature Reserve (48.6 ha) is located in Essex, southeast England on the west border of the Colne River. The managed realignment site (22 ha) ($51^{\circ}50'25.77''\text{N}$, $0^{\circ}58'27.80''\text{E}$) was previously used for two main purposes, growing wheat and barley in rotation in the southern field, while the northern field was held as pasture for grazing by sheep. The pasture field soils were covered by a thick grass with dense root structure to 8cm in depth. The arable field was sparsely planted (every 10cm) with short wheat stubble left after the final harvest before the breach and with relatively limited root density in the top 5cm. Three locations formed the sampling areas for this study, realigned arable field (A), realigned pasture field (B) and adjacent natural marsh (C) (mud pans and creeks only). The two locations (arable, A & pasture, B) (10m x 8m) in the realigned site were selected prior to breaching to be at the same elevation. The sampled natural marsh sediments (see map for relative location) are elevated relative to the realigned marshes due to soil erosion and compaction over time in the agricultural fields. The old sea wall was doubly breached to allow tidal inundation of the site from September 2015 (Figure 3.1).

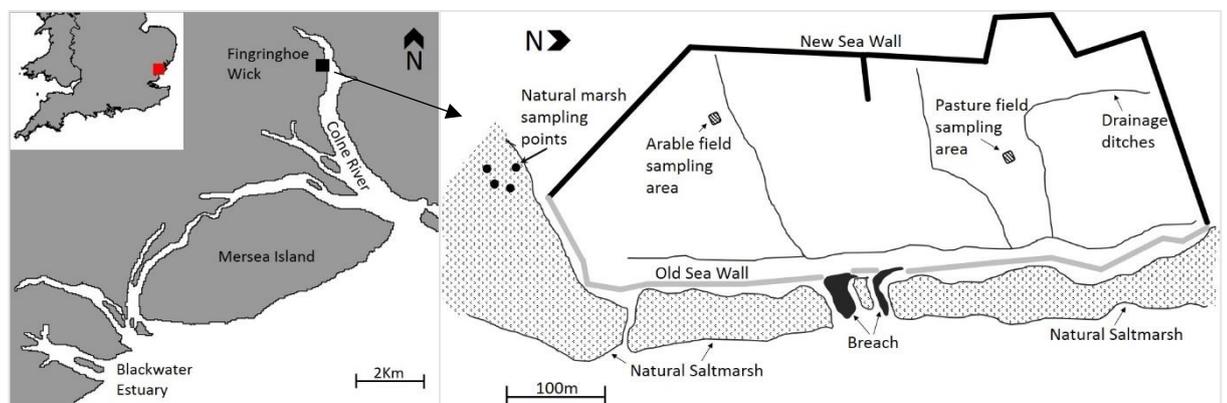


Figure 3.1. Map of location and outline of new realigned site and natural marsh sampling locations within the Colne Estuary, UK.

3.2.2 Sampling

A fixed vertical quadrat (60 cm height x 100 cm length) was placed in each field during November 2015, 2 months after breach, on the western edge of the sampling areas to measure sediment accretion rate. Rate of accretion was measured monthly thereafter and determined by the difference in the distance from the sediment surface to the top of the vertical quadrat.

Post-breach sediment samples were collected on a monthly basis from October 2015 to November 2016 (excluding December 2015). Samples were also collected prior-to-breach in March and August 2015. No significant differences were found between the two prior-to-breach months, thus both samplings were collated and used as a single time point zero reference. Four sediment cores (4.5 cm inner diameter and ≥ 20 cm depth) were collected from each location on each sampling date. Core samples were separated into 0-5, 5-10 and 10-15cm depth sections and used to determine bulk density, sediment water content, sediment nutrient concentration (NH_4^+ and $\text{NO}_2^-/\text{NO}_3^-$) and pH. The volume of the core at each depth was adjusted for compression during collection using the difference in depth on the inside and outside of the core before extraction; linear compression was assumed. Cores were stored at 4°C and processed within 72 hours from collection time.

During the sampling period, no vegetation grew within the sampling areas. *Salicornia* sp. and *Suaeda* sp. colonized higher elevation areas within the realigned site but abundance and biomass were not recorded as they were outside (>5m) our designated sampling areas.

3.2.3 Core processing

Bulk density and water content samples were taken from the centre (2cm sub-sections) of each 5cm (0-5, 5-10, 10-15 cm) section. The top and bottom of each section (e.g. 0-1.5cm and 3.5-5cm) were combined and homogenised for nutrient and pH analyses. Bulk density

was determined by drying the central disk at 70°C for 7 days and calculated by dividing remaining weight by initial (corrected) soil volume. Percent water content was determined from each bulk density sample through measured water loss and is shown as [%water content = (g of water/g of fresh soil) * 100].

Soil samples for nutrient analysis were prepared according to Houba et al. (1995); 3.0g of soil with 30mls of 1M KCl, shaken at 200rpm for 60 minutes, centrifuged (2000rpm for 5 minutes) and the supernatant filtered. The filtrate was then stored at -20°C until analysis. Analysis for NH_4^+ and $\text{NO}_2^-/\text{NO}_3^-$ was performed using a Seal Analytical AutoAnalyzer3. KCl blanks were run to correct for contamination and/or drifts in extract as well as known concentration standards to ensure the equipment was calibrated and measuring correct concentrations. Standards were run every 10 samples. The remaining solid phase from the nutrient samples was used for pH analysis, after in-house analyses demonstrated that pH was consistent between freshly prepared samples and post-extraction samples. In post extraction samples a further 15ml of 1M KCl solution was added, the samples were placed on a rocker (60rpm) for 60 minutes and analysed using a standard pH probe (meter: Jenway 3310, probe: VWR 662-1797). Similar to nutrient analysis standards were used to calibrate the probe before each analysis as well as every 10 samples to ensure no drifts were occurring during analysis.

3.2.4 *Statistical analysis*

Statistical analyses were conducted using the R statistical language implemented in RStudio (Version 1.1.423). A linear mixed effect model (lmer) (Bates et al., 2015) was used to compare the different sediment characteristics with time (months from breach) between the two realigned sites (arable, pasture) and with the natural marsh. In the mixed effect model sediment accretion was set as a random variable. pH was transformed out of log scale for statistical analysis. In addition, a time lag analysis was performed to examine whether water content and pH of previous months affected the concentration of ammonium

observed in the sediment. The analysis was performed using a linear mixed effect model (lmer) with accretion set as a random variable for each depth and each field individually; the lag analysis only examines significance up to 8 months prior to allow for sufficient temporal replicates.

3.3 Results:

Sediment accretion varied between the two realigned fields with the arable location showing more accretion than the pasture. Total sediment accretion at the last sampling (14 months post-breach, Nov 2016) was 6.4cm for the arable field and 2.2cm for the pasture (Figure 3.2).

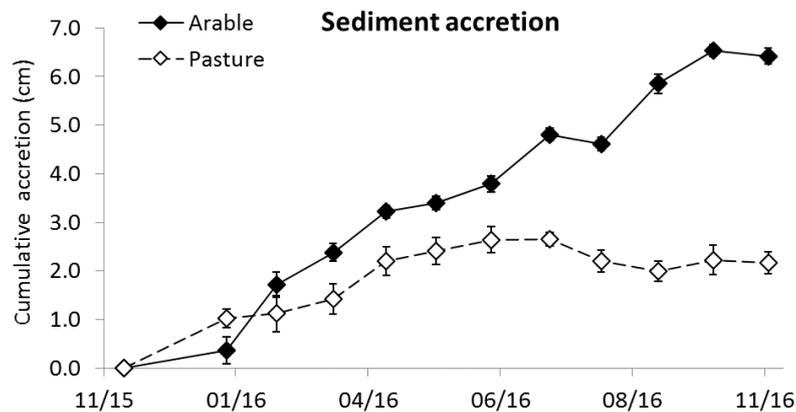


Figure 3.2. Cumulative sediment accretion in the realigned fields over time. Error bars denote standard error within 10 measurements per time point.

3.3.1 Bulk density and water content

Bulk density of the top sediments (0-5 cm) within the realigned saltmarsh decreased significantly over 14 months in the arable (from 1.15 to 0.59g/cm³; $p < 0.001$) and pasture fields (from 0.58 to 0.28g/cm³; $p < 0.001$) (Figure 3.3a). In the 5-10cm depth range the bulk density between the two fields is significantly different ($p < 0.001$), with the density of the arable field decreasing over time whereas in the pasture it remained very similar over time.

There was no significant change in bulk density within the 10-15 cm depth range in either field (Figure 3.3a).

Bulk density is significantly lower in the natural marsh but over time the top 10cm within realigned marsh sites eventually approached a similar density. However, the deeper sediments of the realigned sites (>10cm) remained significantly more dense than those in natural sediments (Figure 3.3a). Water content showed similar, but inverse, patterns to those of bulk density patterns in all three fields (Figure 3.3b). Water content of the top sediments (0-5cm) within the realigned saltmarsh increased over time in both fields (arable: from 27 to 60%, pasture: from 38 to 68%, $p<0.001$) matching natural conditions by the final sampling month. In sediments at 5-10cm depths water content was significantly different over time in both fields ($p<0.001$), increasing towards but not matching natural marsh sediment water content. In sediments at the 10-15cm depths no significant change occurred within the realigned fields which remained at ~30% throughout the sampling period; realigned fields were significantly lower in water content than the natural marsh (~60%) ($p=0.02$) (Figure 3.3b).

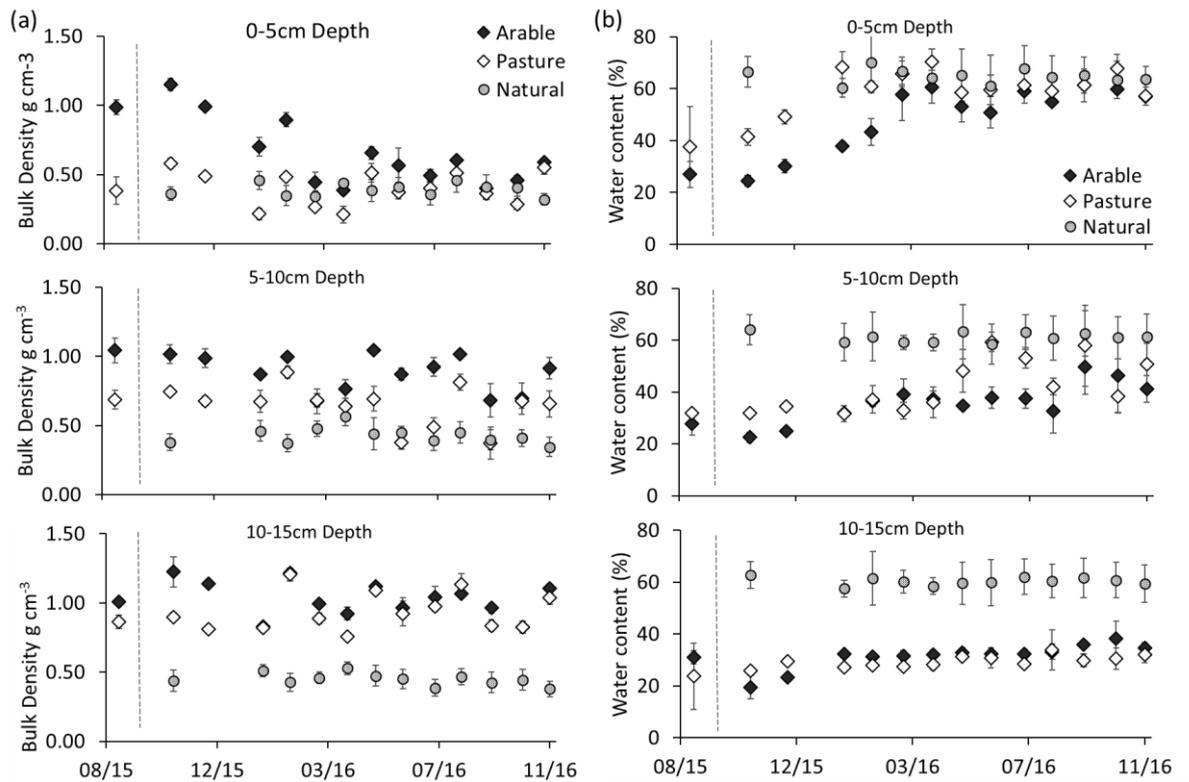


Figure 3.3. (a) Bulk density (g/cm³) and (b) Water content (%) over time at each depth in the natural and realigned fields. Breach of sea wall (dotted line) was in Sept'15. Error bars are $\pm SE$, $n=4$

3.3.2 Sediment pH

The pH of realigned saltmarsh sediments shifted from weakly acidic (between 5.8 and 6) to weakly alkaline (between 7 and 8.2) (Figure 3.4), in both fields which was significantly different over time in the 0-5cm and 5-10cm depth ranges ($p<0.001$). However, there were no significant difference in those depth ranges between the two fields as they behaved similarly over time. In the 10-15cm depth range however, there was a significant difference between the two sites over time ($p<0.001$) with the arable site remaining more acidic than the pasture.

Natural marsh sediments also transitioned from weakly acidic to weakly alkaline over this time frame, although the pH of natural marsh sediments was more variable than realigned

sediment during several sampling dates (Jan, May, June, July). pH readings for natural marsh sediments ranged between 2.95 and 8.30.

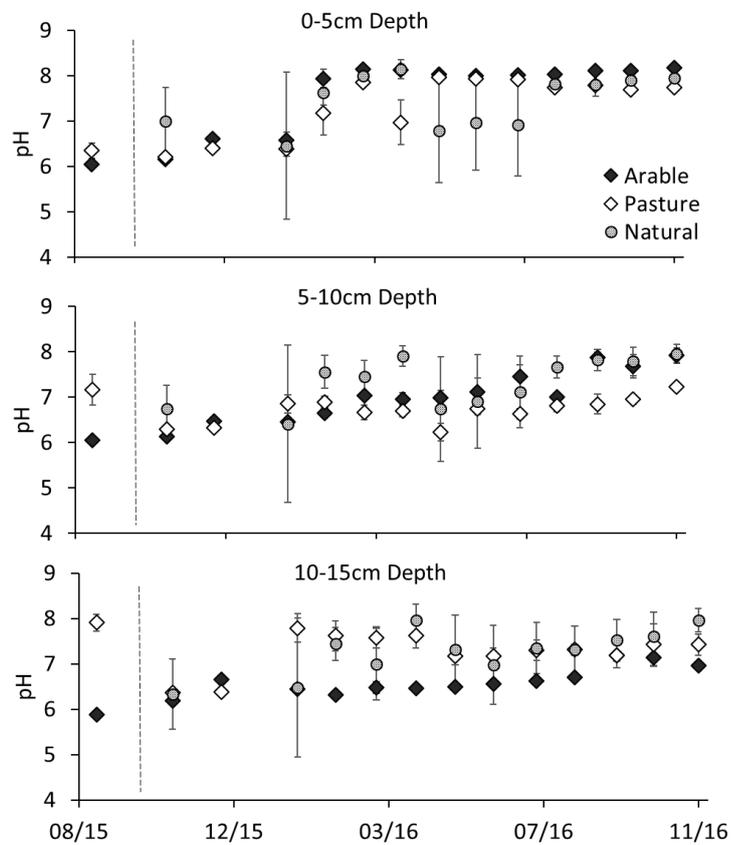


Figure 3.4. Sediment pH over time at each depth in the natural and realigned fields. Breach of sea wall (dotted line) was in Sept`15. Error bars are $\pm SE$, $n=4$

3.3.3 Nutrients

Ammonium (NH_4^+) concentrations in realigned arable sediments increased dramatically after initial inundation ($\sim 150\text{mg/Kg}$ in arable and $\sim 200\text{mg/kg}$ in pasture) at all depths and then decreased over time at all depths, with the greatest decrease ($\sim 150\text{mg/kg}$ at both fields) occurring within 2 months of the post-breach maximum (Nov to Jan) in the 5-10cm and 10-15cm depths (Figure 3.5a). In the pasture field at the 0-5cm depth however NH_4^+ concentration continued to increase for 6 months post breach before it began to decrease

(Figure 3.5a). At the 0-5cm depth, NH_4^+ was significantly different between each site ($p < 0.001$) and over time ($p < 0.001$). At all depths the pasture field had greater concentrations of ammonium than the arable field, which is likely due to the dense decomposing root mass (Jordan *et al.*, 1989). Natural marsh NH_4^+ concentrations showed similar patterns to realigned sites in fluctuations over time but often with substantially greater variation within site, especially during the May and July sampling periods with readings ranging between 30 and 1470mg/kg in May and 20 and 850mg/kg in July.

Nitrate/nitrite ($\text{NO}_3^-/\text{NO}_2^-$) concentrations in the sediment were fully depleted by the fourth month after breaching in both fields (Figure 3.5b). Only in the 5-10cm depth was there a significant difference between the fields (natural and realigned) over time ($p < 0.001$). Spikes of $\text{NO}_3^-/\text{NO}_2^-$ concentration which may have been due to bird excrement (Figure 3.5b; (Bazely and Jefferies, 1985)) (48mg/kg) was observed in one of our samples of the arable field at the 0-5cm depth with the other samples being $< 0.2\text{mg/kg}$.

3.3.4 *Lagged environmental drivers for NH_4^+ concentrations*

Change in environmental drivers may cause responses that don't occur until sometime after initial perturbation. Stockdale (2012) found that fluxes in CH_4 from a blanket bog showed delayed response to water table being lowered, soil temperature and solar radiation. Thus, we examined the potential delayed response of NH_4^+ concentrations to changes in pH, bulk density and water content at the two realigned fields. Overall ammonia concentration appeared to be driven by water content and sediment pH (more alkaline led to lower NH_4^+), however the delay period leading to maximum concentrations varied with depth and with site.

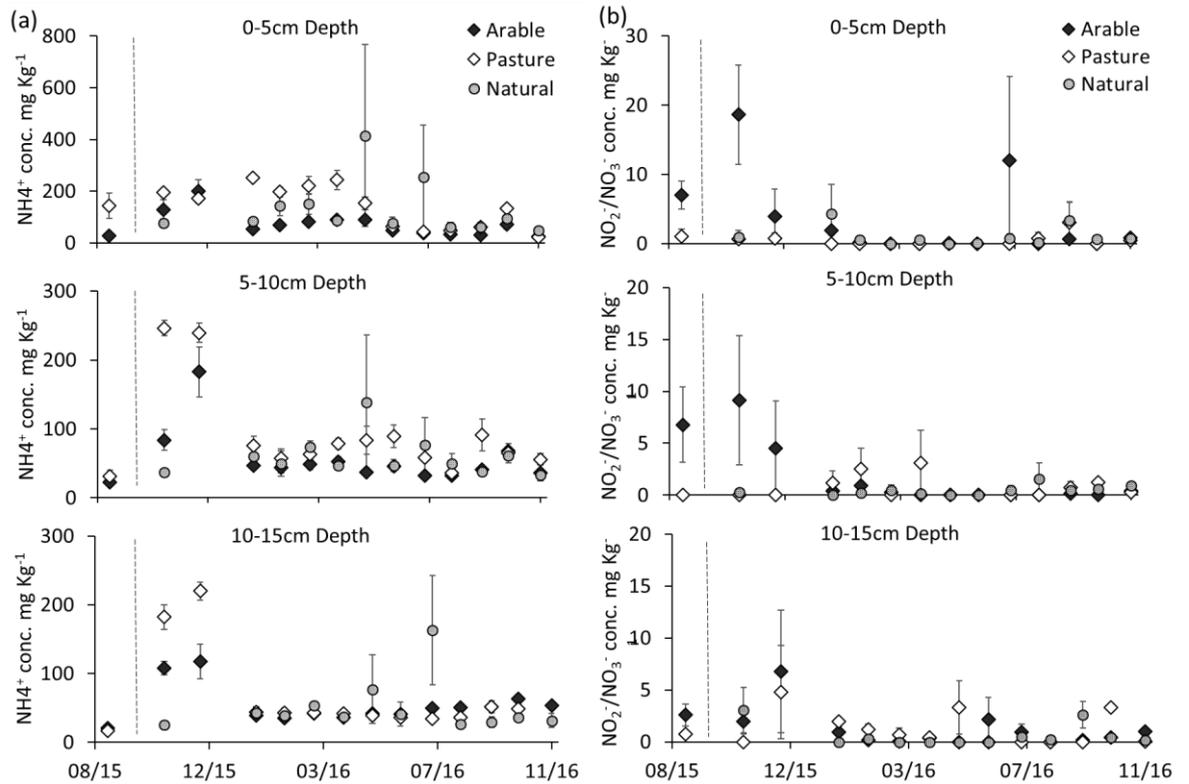


Figure 3.5. (a) Ammonium, NH_4^+ and (b) Nitrate/nitrite, $\text{NO}_2^-/\text{NO}_3^-$, concentration in sediment (mg/kg) over time at each depth in the natural and realigned fields. (Note the difference in scales for NH_4^+ and NO_x and the difference in scale in the 0-5cm depth). Breach of sea wall (dotted line) was in Sept`15. Error bars are \pm SE, n=4

For the arable field, within the 0-5cm depth range the ammonia concentrations appear to be driven by pH and water content four months prior to sampling (pH, $p=0.014$ $r^2=0.556$; $\% \text{H}_2\text{O}$, $p<0.001$ $r^2=0.469$). While still showing positive correlations with pH and water content, the 5-10cm depth ammonia content appeared to be most influenced by pH from the same sampling campaign ($p<0.001$ $r^2=0.879$) and water content 8 months prior to sampling ($p=0.045$ $r^2=0.672$). The 10-15cm depth ammonia concentrations also showed significant correlations with pH and water content, although these were most significant three months (pH; $p=0.002$ $r^2=0.583$) and one month prior to sampling ($\% \text{H}_2\text{O}$; $p<0.001$ $r^2=0.647$).

Within the pasture field, the 0-5cm depth ammonia concentration of sediments was more significantly affected by the sediment pH 3 months prior to sampling ($p<0.001$ $r^2=0.458$) and water one month prior ($p<0.001$ $r^2=0.701$). In the 5-10cm depth the ammonia concentration

was most strongly correlated best with pH of the same sampling campaign and water content from the previous month (pH, $p < 0.001$ $r^2 = 0.864$; H_2O , $p = 0.012$ $r^2 = 0.873$). The 10-15cm depth ammonia concentrations were best explained by pH and water content of the same sampling campaign (pH, $p = 0.024$ $r^2 = 0.877$; H_2O , $p = 0.002$ $r^2 = 0.919$).

When the lag analysis was run without depth separation, pH of the same sampling campaign in both fields was most significant (arable, $p < 0.001$ $r^2 = 0.610$; pasture, $p < 0.001$ $r^2 = 0.350$) (Figure 3.6). Both arable and pasture fields show two distinct time points which were significant for water content influence on sediment ammonia concentration. For the arable field, of the same sampling campaign and 8 months prior (0, $p = 0.023$ $r^2 = 0.505$; -8, $p = 0.018$ the same sampling campaign and 1 month prior (0, $p < 0.001$ $r^2 = 0.693$; -1, $p < 0.001$ $r^2 = 0.740$) (Figure 3.7).

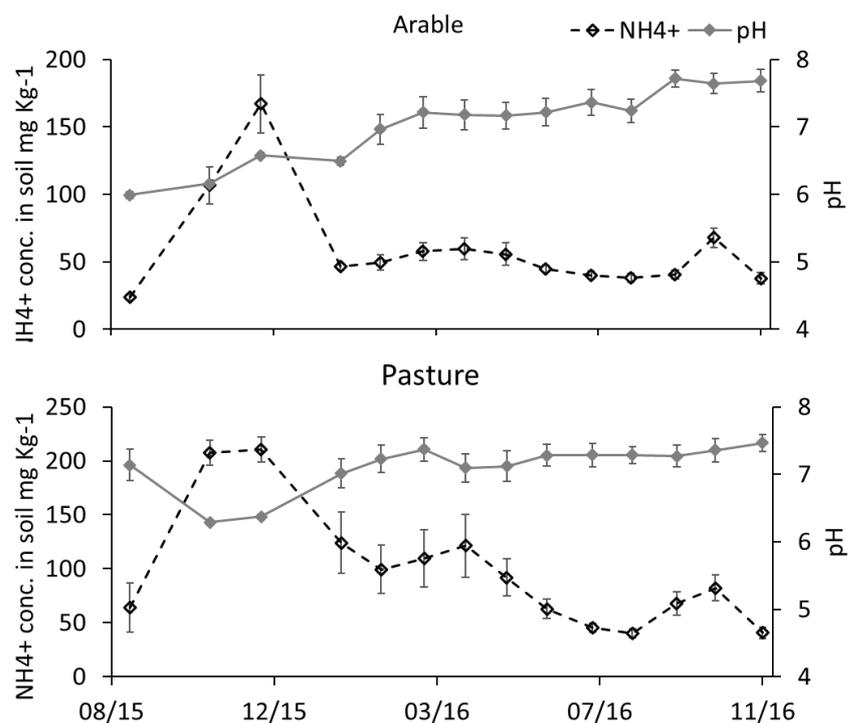


Figure 3.6. Average ammonium (NH_4^+) concentration in sediment with pH of sediment for the same sampling time point, all depths are collated together. Error bars +/- SE

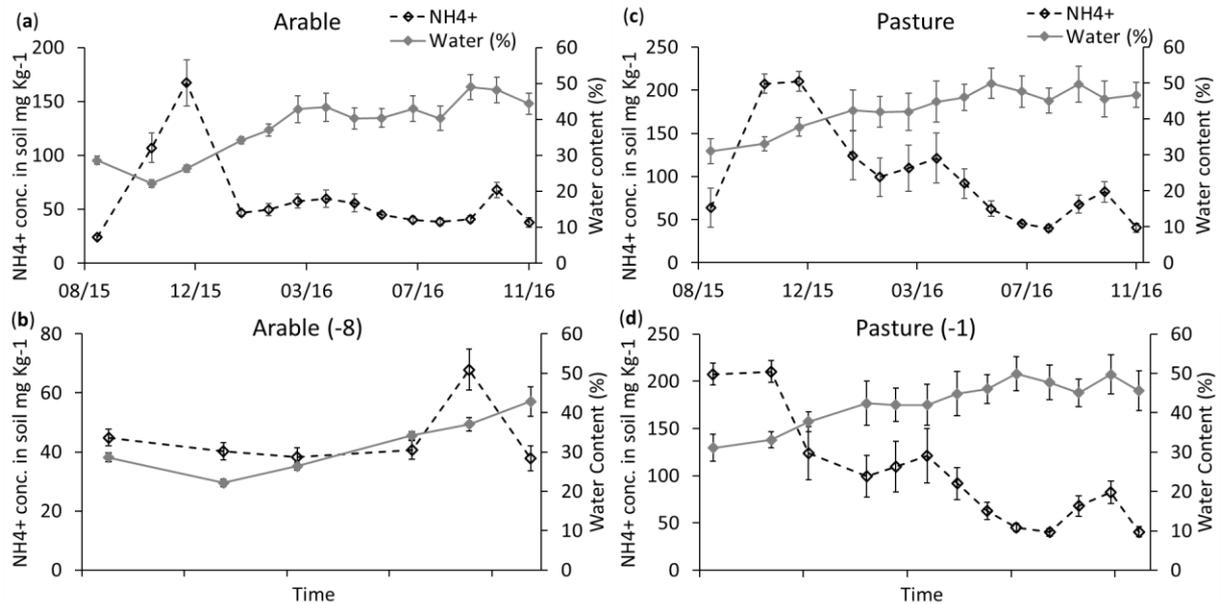


Figure 3.7. Average NH_4^+ (mg/Kg) concentration in sediment with water content (%). (a) arable field for same sampling time point, (b) arable field with water content of 8 months prior, (c) pasture field with same sampling time point, (d) pasture field with water content of 1 month prior. Error bars +/- SE

3.4 Discussion

3.4.1 Differences between arable and pasture fields

The difference between the two fields' bulk density at the start of inundation may be primarily attributed to prior use, specifically the pasture field's dense root mass which penetrates down to 8cm depth. The substantial root presence in the pasture field is likely to be the driving factor for the lower bulk density in the 0-5cm depth prior to the breach in contrast to the dense compact sediment of the arable field. Angers & Caron (1998) found that plants influence soil structure and stability, with their penetrating roots favouring fluid transport down the sediment column. Within the pasture field the extensive root structure may have allowed for more water infiltration to lower sediment depths post breach. Root mediated infiltration may explain the higher water content observed at the 0-5cm and 5-10cm depths post breach in pasture sediments.

The cores were sampled and analysed to a maximum depth of 15cm, however due to sediment accretion we did not sample the same location within the original sediment profile over time (Figure 3.8). The sediment profile changed as sediment accreted in both fields, resulting in the original sediment surface being shifted lower in depth over time (Figure 3.8). Thus, when sediments from consistent depths within the prior-use agricultural sediments are compared, we can say that the pre-breach agricultural sediments did not substantially change over time and changes observed are generally from the new sediment accumulating on site. This can more clearly be seen in our arable field, on August 2015 (prior-to-breach) bulk density within the 0-5cm depth was 0.99g/cm^3 , and by November 2016 (final sampling) we had 6.4cm of sediment accreted and bulk density of the 0-5cm depth sediments was 0.59g/cm^3 whereas the 5-10cm depth sediments retained a bulk density of 0.91g/cm^3 (Figure 3.3). The difference in accretion rates between the two sites may be explained by non-uniform erosion of the double breach (north breach less developed than south) which lead to less sediment transported to, and deposited on, the northern field (pasture). The 0-5cm depth sediments in the November 2016 arable field is representative of the marine accreted sediment and does not represent a change of the agricultural relic layer. This effect is more obvious when we look at the bulk density and water content of sediments found at lower depths (5-10cm & 10-15cm) within both fields which did not change significantly over the 14 months of inundation.

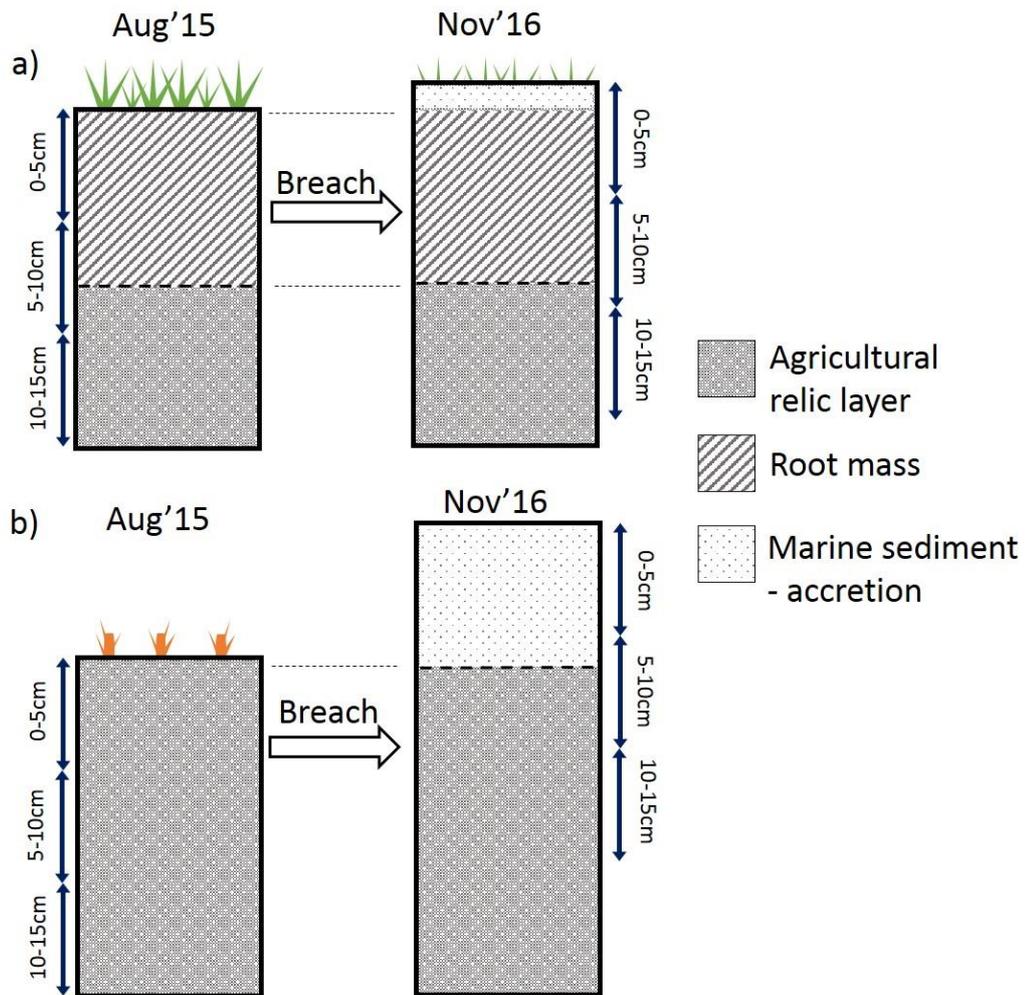


Figure 3.8. Diagram of the 15cm sediment profile collected before breach and after 14 months in each site. a) Pasture b) Arable. This shows the difference of the profile that was collected at the two time-points. Diagonal dotted lines indicate where each horizon has remained

The two fields had different starting nutrient concentrations, with the pasture field having higher NH_4^+ concentration and the arable higher $\text{NO}_2^-/\text{NO}_3^-$ concentration. These differences could be attributed to their previous land use, where the pasture field was used for grazing by sheep and their excretions increasing the available NH_4^+ within the site (Ma et al., 2007). Post breach, both fields behaved similarly with a rapid decrease of NH_4^+ concentration within the first 2 months, which could be a signal of rapid decomposition of available organic matter, and by November 2016 (final sampling) nutrient concentrations were the same for both field types. Despite the early differences in nutrient concentrations, pH of sediment

within the two fields was similar pre and post breach, with both managed sites starting slightly acidic and becoming more alkaline with saline inundation.

NH_4^+ and $\text{NO}_2^-/\text{NO}_3^-$ concentrations in sediment are driven by similar natural factors (i.e. water content and pH) and appear to have similar inundation responses. Higher concentrations of reduction compounds such as ammonia may be indicative of a lower redox potential (Velinsky et al., 2017). We observed that with inundation and higher water content (proxy over time for an anoxic environment) there was an increase in ammonium concentration in the sediment and with the formation of ammonia, pH of the sediment increased to more alkaline.

3.4.2 *Natural vs. realigned marsh*

Physical aspects of the realigned sediments, including bulk density and water content matched those of the natural marsh within 14 months post-breach in the 0-5cm depth range, however the lower depth sediments within realigned sites remained more dense and drier when compared to natural marsh sediments. Similar results were observed by Spencer et al. (2008) and Tempest et al. (2015) at Orplands Farm site (Blackwater Estuary, SE England), where an unchanged old agricultural relic layer was observed with marine sediment deposited on top years post sea wall breach and inundation.

Chemical properties of the realigned sediments, including nutrient content and pH matched those of the natural marsh within 14 months post-breach throughout the sediment profile. While the pH of realigned sites sediments became more alkaline over time, matching natural marsh sediments, natural marsh sediment pH was more variable (range = 2.95 – 8.69) when compared against the more homogeneous realigned site sediments pH (range = 5.68 – 8.49). Nutrients (particularly ammonium) behaved consistently in the realigned site sediments, in that they followed similar patterns across the field over time; but within the natural marsh

we observed spikes of very high concentrations (1470 mg/Kg) and very low (40 mg/Kg) during the same sampling time. Burden et al. (2013) also suggested that within the managed realigned saltmarsh they examined (Tollesbury, UK) nutrient mineralization rates were shifting toward natural marsh rate, however they also concluded that despite the nitrogen mineralization rates converting towards natural rates the C/N ratios remained lower than those in natural sediments thus decreasing the recovery rate of the realigned marsh.

The homogeneity of realigned sites might contribute to the differences observed in plant diversity and composition over time (Garbutt & Wolters 2008). Salt marsh plant species distribution is a balance between tolerance and competition (Gray and Benham, 1990), and within realigned sites it appears that this has not yet been achieved. This may be due to greater abundance of one pioneer species more tolerant to the hard substrate and thus not allowing other species to colonize (Garbutt and Wolters, 2008). Some plant species such as *Spartina anglica* can drastically alter the sediment hydrogeology (i.e. draining) of its surrounding thus creating waterlogged and anoxic sediments (Doody, 1984).

Density and moisture of lower sediment depths in the realigned site may also play a role since they did not change significantly, which affects the hydrology of the managed marshes. Tempest et al. (2013) found that in realigned sites the agricultural relic soils remained the same over time, constricting water movement within the sites. In our realigned sites, the top 5cm of sediment and subsequently the newly deposited marine sediment, was where all the conditions match those of the natural marsh, whereas lower depth sediments, especially the deepest depth range of 10-15cm, showed little change over time.

Through our study but also other studies have shown that realigned marshes are showing differences both between each other but also with natural referenced marshes (Garbutt and Wolters, 2008; Garbutt et al., 2006; Lawrence et al., 2018; Mossman et al., 2012b, 2012a; Sullivan et al., 2017; Tempest et al., 2015; Wolters et al., 2005b). These differences could be

attributed to physicochemical properties of the sediment; such as soil drainage (due to denser lower sediments) (Burden et al., 2013; Spencer et al., 2008; Tempest et al., 2015), nutrient cycling (Burden et al., 2013) and seed availability (Wolters et al., 2005a), and previous land use (Garbutt et al., 2006; Spencer et al., 2017).

3.4.3 *Summary*

14 months after inundation the two realigned sites were broadly similar despite their different starting points. Pasture field had more nutrients (mostly NH_4^+) than the arable field post inundation which could potentially influence development of vegetation, invertebrate and microbial communities (Chapter 4). Greater additions of N available in salt marshes can result in greater growth for below ground biomass which in turn can lead to an increased sediment capture, and increase in elevation, due to enhancement of above ground biomass and stem density (Fox et al., 2012). Furthermore, our realigned site closely matches the natural marsh conditions for nutrients and pH at all depths, but bulk density and water content only in the 0-5cm depth, where marine sediments are accreting. This unchanged agricultural layer, as seen also by Tempest et al., (2015), can affect the hydrogeology of the realigned marsh which could attribute to our observations that although the realigned site matched natural conditions by November 2016 the natural marsh remained more heterogeneous than the realigned site.

Chapter 4. Effect of bioturbators on the sediment characteristics in the first year of a realigned salt marsh

4.1 Introduction

Bioturbation is the mixing of sediment through biological processes from the action of infauna, epifauna, fish and mammals resulting in particle movement (Cadée, 2001). Studies have shown that bioturbation of soft sediments by benthic invertebrates has a significant influence on benthic sedimentary geochemical properties (Queirós et al., 2013). Through their burrowing, feeding and foraging activities benthic invertebrates influence the mixing of sediments and particulate materials as well as enhancing the pore water and solute advection during burrow ventilation (Volkenborn et al., 2010). Bioturbation of sediments through these activities can influence the oxygen, pH and redox gradient of sediments (Biles et al., 2002; Pischedda et al., 2008); as well as the metal cycling (Teal et al., 2009); bacterial activity and composition (Gilbertson et al., 2012) and the carbon and nitrogen cycling of the system (Kristensen, 2001).

Moreover, studies have shown that burrowing activities of invertebrates increase above ground plant production and diversity by increasing soil drainage, decreasing the amount of toxic compounds and increasing the anaerobic respiration of plant roots (Bertness, 1985; Montague, 1982). Daleo et al. (2007) found that burrowing activities increase the supply of nutrient to Arbuscular mycorrhizal fungi which in turn increase production of the salt marsh plant *Spartina alterniflora*, thus concluding that salt marsh productivity and success is highly dependent on mutualisms between colonizing species and microorganisms.

Macrofauna community composition can also affect nutrient availability in the sediment as well as the water column. Biles et al. (2002) concluded that ammonia release into water column was influenced by infaunal community composition and is mediated by the extent of

bioturbation effecting sediment particle resuspension and turnover. However, colonization of saltmarsh creeks by *Hediste diversicolor* can affect the rate of erosion, and decrease production and survival of *Salicornia* seedlings (Paramor and Hughes, 2004).

Saltmarshes provide important ecosystem services, including support for coastal and terrestrial food chains and coastal protection (Beaumont et al., 2008; Hughes and Paramor, 2004; Millenium Ecosystem Assessment, 2005). Saltmarshes provide protection to coastal areas by dissipating wave and tidal energy reducing the possibility of sea walls being breached, overtopped or undermined (Möller et al., 1999; Möller and Spencer, 2002). Over the last two decades there has been an accelerated global decline in the extent of saltmarsh systems (Millenium Ecosystem Assessment, 2005) and efforts have been initiated to conserve existing marshes and to develop new coastal marsh habitats (realigned or managed saltmarshes).

This study aims to examine the effect of invertebrate bioturbators (organisms bigger than 250µm) in the development of sediment characteristics during the 1st year of marsh realignment between two different previous land uses (arable and pasture). The primary objectives of the study were to (1) compare sediment changes in areas of invertebrate exclusion and invertebrate colonization between arable and pasture realigned fields and (2) monitor invertebrate colonization in the newly realigned saltmarsh within the 1st year post inundation. We hypothesized that areas with invertebrate colonization and hence bioturbation to be more homogeneous across all depths than areas with no invertebrate colonization by the final sampling.

4.2 Methods

4.2.1 Study site

Fingringhoe Wick Nature Reserve (48.6 ha) is located in Essex, southeast England on the west border of the Colne River. The managed realignment site (22 ha) ($51^{\circ}50'25.77''\text{N}$, $0^{\circ}58'27.80''\text{E}$) was previously under two contrasting land uses, an arable wheat/barley rotation in the southern field, while the northern field was held as pastureland grass. These two prior conditions within the realigned site were selected as the sample sites for this study, realigned arable field (A) and realigned pasture field (B). The two regions (10m x 8m) in the realigned site were selected prior to breaching to be at the same elevation. The old sea wall was breached at two locations to allow for tidal inundation of the site in September 2015 (Figure 4.1).

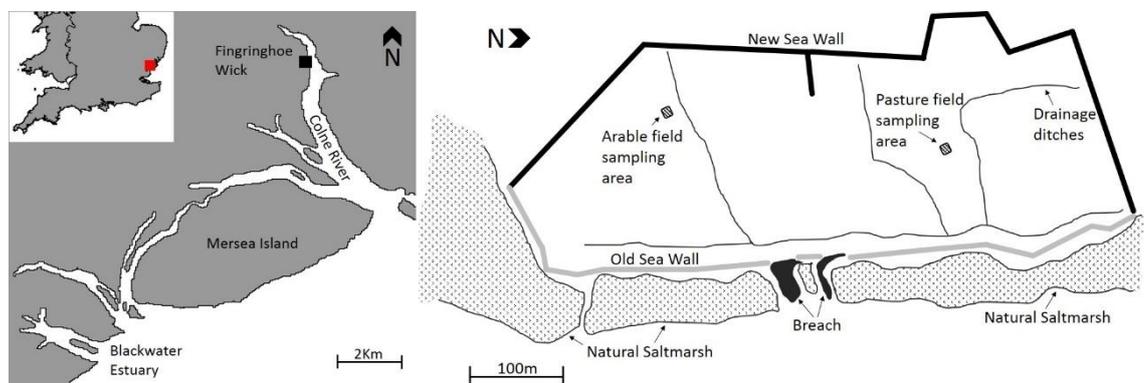


Figure 4.1. Map of location and outline of new realigned site sampling locations and sea wall breach within the Colne Estuary, UK.

4.2.2 Experimental design

64 invertebrate exclusion chambers were placed in the realigned marsh, 32 in the pasture field and 32 in the arable field. The chambers were 20cm in diameter and 30cm in height, constructed from PVC pipes with eight windows cut from their sides to allow horizontal water flow. A 250 μm aperture length mesh was placed over the windows. Half the chambers had

mesh at the top and bottom to fully exclude invertebrates (Full Exclusion chambers). The remaining chambers only had mesh at the windows, allowing free invertebrate access from the top and the bottom of the chamber (Exclusion Control chambers) (Figure 4.2). Undisturbed Control areas, without exclusion chambers, that allowed full invertebrate access were allowed to establish and develop naturally within the realigned marsh were sampled with soil cores (20cm diameter and ≥ 20 cm depth). Exclusion control chambers were used as controls that addressed effects on sediment composition and structure by chamber installation.



Figure 4.2. Invertebrate exclusion chambers, Exclusion control chamber (left) and Full exclusion chamber (right).

Preliminary sampling of the two fields in March 2015 showed discrete, but different, sediment profiles between the two fields. The pasture field had a dense root mass down to 8cm in depth thus it was crucial when placing the chambers in the site prior to breaching to maintain the sediment profile. The arable field was more homogeneous across sampling depths with only sparsely planted wheat stubble (left after harvest) and minimal root structures. For both fields, relative positioning of plant biomass, root structure, and deeper soils were maintained when installing chambers. The chambers were buried 26 days prior to

initial flooding, to a depth of 25cm in the ground with 5cm above ground to allow for sediment accretion over the sampling period (Figure 4.3). Chambers were randomly placed in a plot design within the two fields (Figure 4.4) and were sampled 4 times between placement to 14 months post breach.



Figure 4.3. Chamber placement before breaching of the sea wall. (A) shows how the top and lower sediments were separated during placement of the chambers. (B) Full exclusion chamber in the pasture field with 5cm over ground to allow for sediment deposition. (C) Full exclusion chamber in the arable field. (D) Exclusion control chamber in the pasture field.

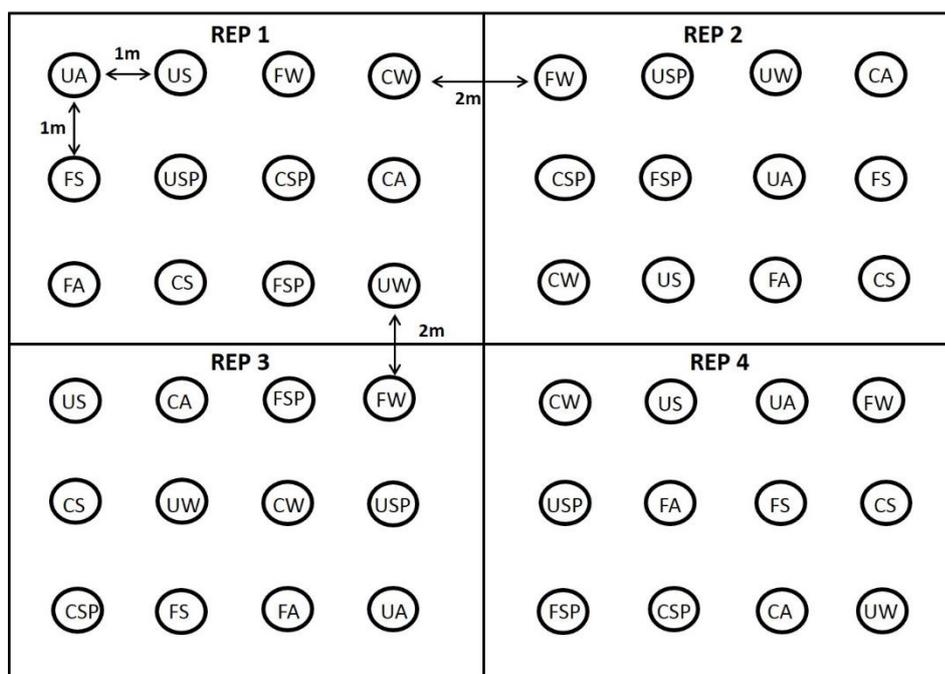


Figure 4.4. Random plot design for placing chambers in the realigned sites. First letter refers to type of chamber (U=Undisturbed control, C=Control exclusion, F=Full exclusion). Second letter refers to sampling season (W=Winter, SP=Spring, S=Summer, A=Autumn)

4.2.3 Core processing

The chambers were subdivided into 5cm intervals to provide a depth profile for the measured soil parameters and invertebrate counts. Bulk density was determined by drying and weighing disks of known volume, collected from the central section of each 5cm section. Bulk density disks were dried in an oven at 70°C for 7 days. Percent water content was determined from each bulk density sample through measured water loss and is shown as [%water content = (g of water/g of soil) * 100].

Soil samples for nutrient analysis were prepared according to Houba et al. (1995); 3.0g of soil with 30mls of 1M KCl, shaken at 200rpm for 60 minutes, centrifuged (2000rpm for 5 minutes) and the supernatant filtered. The filtrate was then stored at -20°C until analysis. Analysis for NH_4^+ and $\text{NO}_2^-/\text{NO}_3^-$ was performed using a Seal Analytical AutoAnalyzer3. KCl blanks were

run to correct for contamination and/or drifts in extract as well as known concentration standards to ensure the equipment was calibrated and measuring correct concentrations. Standards were run every 10 samples. The remaining solid phase from the nutrient samples was used for pH analysis, after in-house analyses demonstrated that pH was consistent between freshly prepared samples and post-extraction samples. In post extraction samples a further 15ml of 1M KCl solution was added, the samples were placed on a rocker (60rpm) for 60 minutes and analysed using a standard pH probe (meter: Jenway 3310, probe: VWR 662-1797). Standards were used to calibrate the probe before each analysis and between every 10 samples to ensure no drifts were occurring during analysis.

4.2.4 *Benthic macro-invertebrate collection*

Remaining sediment from each 5cm depth section was stored at 5°C until organisms were collected by sieving through a 250µm sieve mesh. Organisms were placed in 10% formalin solution and stored at 5°C until identification and counting. Organisms were identified to species level using the Hayward and Ryland (2009) identification handbook under a dissecting microscope. Only samples from February'16 (initial) and November'16 (final) were processed for invertebrate abundance. Abundance was measured as individuals per m² in 5cm depth zones.

4.2.5 *Statistical analysis*

Statistical analyses were conducted using the R statistical language implemented in RStudio (Version 1.1.423). Nested ANOVAs were used to compare the different sediment characteristics with each treatment (Half, Full exclusion chambers and Controls); depth was nested within treatment and treatment nested within field (arable/pasture). pH was transformed out of the log scale for statistical analysis. Nutrient concentrations had 1×10^{-6} mg/Kg added to all values to avoid zero value discontinuity and were log transformed to

obtain normal distribution. Due to the low species count of invertebrates (≤ 4 species total) biodiversity analyses were not performed.

4.3 *Results*

4.3.1 *Field observations*

During sampling campaigns, we observed worm burrows within the sampling area. Although these were not quantified, they were a clear indication of burrowing activity by macro-organisms within the newly realigned mash. In addition, burrowing holes with clear layers of oxygenated and anoxic sediment were observed during extraction of chambers inside as well as outside of the chambers (Figure 4.5). In addition, we observed that the full exclusion chambers retained more water on the top layer. During sampling when the top mesh was removed, the full exclusion chambers had water sitting on the top with an orange/brown tint indicating iron rich sediment and water (Figure 4.6). Furthermore, the pasture field had a layer of macro algae in it covering all chambers and undisturbed areas sampled (Figure 4.7).

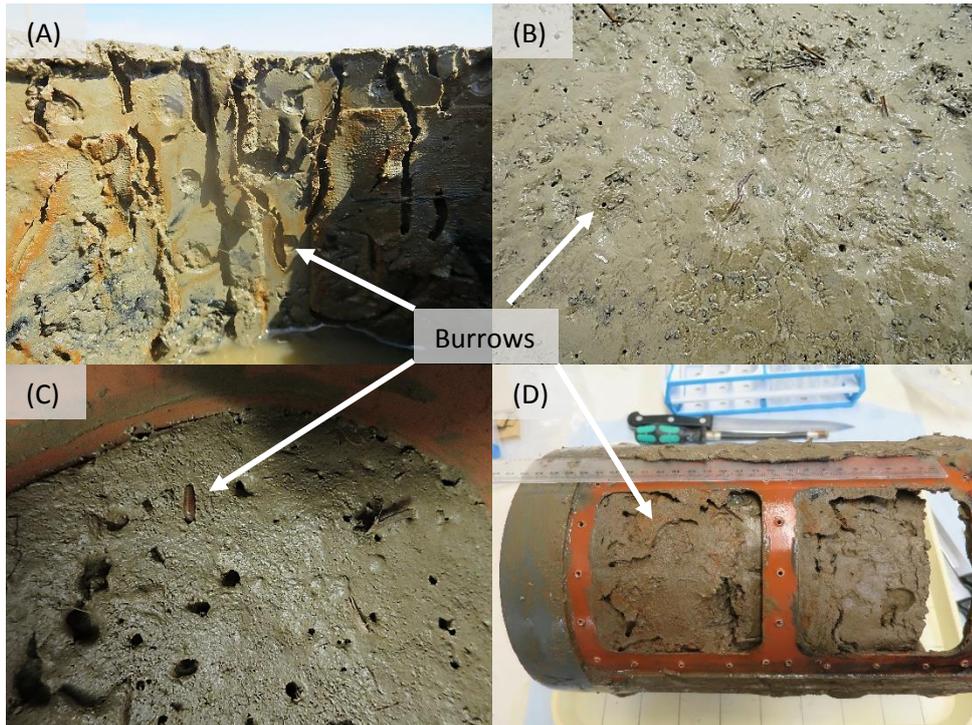


Figure 4.6. (A) image of worm burrows after chamber is being removed from the marsh. (B) Macro-organisms burrow holes visible in the realigned marsh prior to sampling. (C) worm in its burrow within a chamber sampled, image taken in the lab. (D) worm burrow also visible within an exclusion control chamber in the lab.



Figure 4.5. Full exclusion chamber with water sitting on the top and orange/brown colouration of sediment and water. Adjacent to the bamboo stick is the lid of the chamber that was removed.



Figure 4.7. Photos of undisturbed chambers in the pasture field covered in macro algae (*Ulva* sp.) and *P.ulvae* snails

4.3.2 Bulk density and water content

Bulk density in the arable field was significantly different between depths and treatments across the four months ($F_{(18,108)}=2.45$, $p=0.002$) (Figure 4.8a). Post-hoc analysis shows that undisturbed chambers are more dense at all depths over time than the other conditions (exclusion control and full), however undisturbed sediment samples had significantly lower bulk density within the 0-5cm depth zone ($p<0.001$). Exclusion control chambers have lower bulk density across all depths and all sampling months compared to undisturbed and full chambers (Figure 4.8a). Nested ANOVA for the pasture field showed no significant difference ($F_{(18,108)}=0.95$, $p=0.520$); however, there appears to be variation between the different conditions at the 5-10cm and 10-15cm depth zones with a general downwards trend (Figure 4.8b).

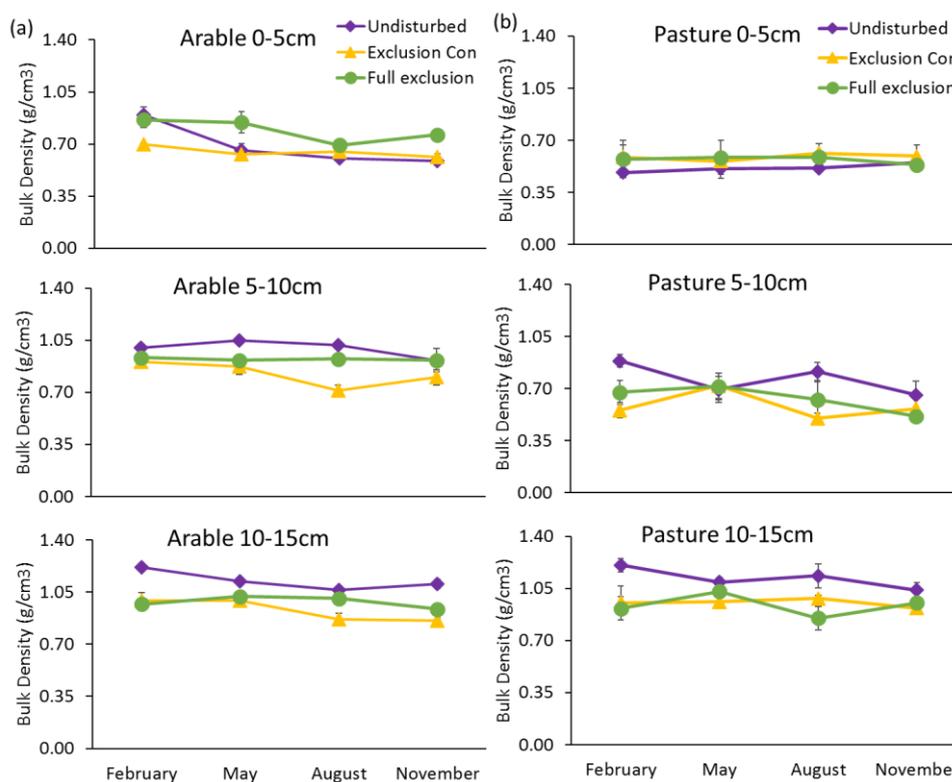


Figure 4.8. Bulk density (g/cm^3) at each depth, month and treatment for (a) Arable field and (b) Pasture field. Error bars \pm SE, $n=4$ per month per treatment

Water content in the arable field was significantly different with depth and treatment and with sampling time ($F_{(18,108)}=2.45$, $p=0.002$) (Figure 4.9a). Full chambers had less water content than exclusion control and undisturbed chambers in the 0-5cm depth zone across all sampling months ($p<0.001$). Exclusion control chambers in the arable field have consistently greater water content than other treatments ($p=0.01$). Similar to bulk density, water content in the pasture field showed no significant difference ($F_{(18,108)}=1.03$, $p=0.434$) but variations were observed in the 5-10cm and 10-15cm depth zones (Figure 4.9b).

Overall, arable and pasture fields showed similar decreases in bulk density and increases in water content over time as observed in Chapter 3 (bulk density decreased from 1.15 to $0.59\text{g}/\text{cm}^3$ and water content increased from 30 to 60% during the monthly sampling in Chapter 3 at the 0-5cm depth). With lower depths, especially 10-15cm, showing no change over time and the greatest observed changes in 0-5cm depths.

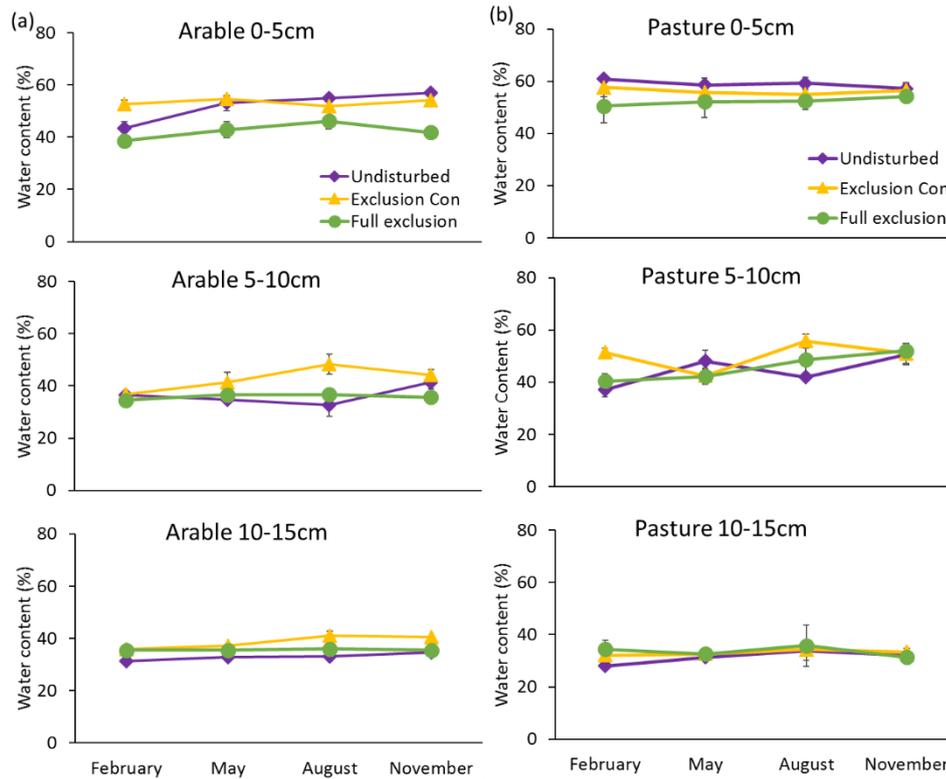


Figure 4.9. Water content (%) at each depth, month and treatment for (a) Arable field and (b) Pasture field. Error bars +/- SE, n=4 per month per treatment

4.3.3 Sediment pH

Sediment pH of the arable field was significantly different with depth and treatment across the sampling months ($F_{(18,108)}=2.30$, $p=0.004$) (Figure 4.10a). pH in the 5-10cm and 10-15cm depths of the arable field became more alkaline from February to November for all treatments with the exclusion control chambers being more alkaline than full and undisturbed chambers. Sediment pH of the pasture field was not significant different with depth or treatment across the sampling months ($F_{(18,108)}=0.80$, $p=0.692$) (Figure 4.10b).

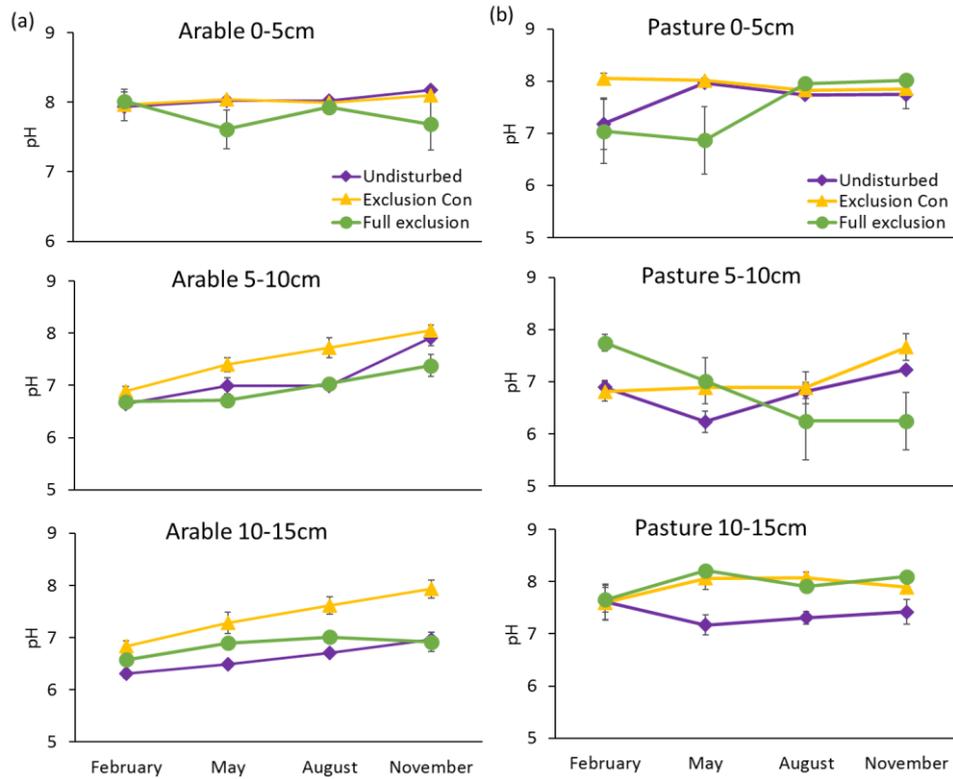


Figure 4.10. Sediment pH at each depth, month and treatment for (a) Arable field and (b) Pasture field. Error bars +/- SE, n=4 per month per treatment

4.3.4 Sediment nutrients

Ammonium (NH_4^+) concentration in the sediment of both arable and pasture fields differed significantly with depth and treatment across the sampling months (Arable: $F_{(18,108)}=2.26$ $p=0.005$, Pasture: $F_{(18,108)}=2.98$ $p<0.001$). In the arable field NH_4^+ concentration decreases within all depths for all treatments, with the greatest decrease observed in the 0-5cm zone (from 70 to 24mg/kg; $p<0.001$). In the arable field concentration of NH_4^+ increased from February to May by ~30mg/kg before it decreased by ~70mg/kg by November, leading to an overall reduction in ammonia concentrations over time (Figure 4.11a). Exclusion control and undisturbed treatments have higher ammonium concentrations than full exclusion treatments in the arable field (Undisturbed: 88 ± 13 mg/kg, Exclusion control: 125 ± 9 mg/kg, Full exclusion: 37 ± 4 mg/kg in May) and overall the pasture field sediments had higher

concentrations of ammonium than those in the arable field (arable: $\sim 70 \pm 6$ mg/kg, pasture: $\sim 160 \pm 20$ mg/kg; Figure 4.11b). NH_4^+ concentration in the pasture field sediments decreases at all depths, with the greatest decrease happening in the 0-5cm depth zone (from 197 to 22 mg/kg; $F_{(18,108)}=2.98$ $p<0.001$) (as also observed in Chapter 3). In the 5-10cm depth zone undisturbed and full exclusion treatments behave similarly whereas exclusion control chambers start at a higher concentration and by the last sampling point they have similar, low concentrations relative to other chamber types. Concentration of nitrate/nitrite ($\text{NO}_3^-/\text{NO}_2^-$) in the sediment was lower than 10 mg/kg and no significant difference was found between depth, treatment or field type (data not shown, Arable $F_{(18,108)}=1.39$ $p=0.154$, Pasture $F_{(18,108)}=0.52$ $p=0.946$).

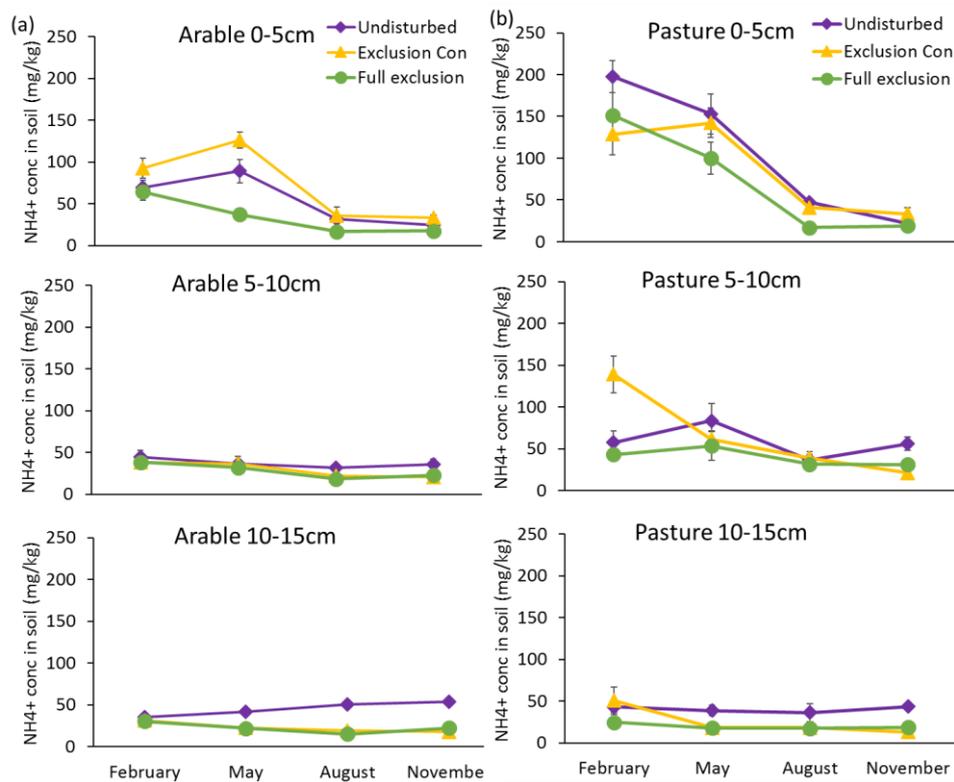


Figure 4.11. Ammonium (NH_4^+) concentration (mg/kg) in sediment at each depth, month and treatment for (a) Arable field and (b) Pasture field. Error bars \pm SE, $n=4$

4.3.5 Benthic macro-invertebrates

Four species of marine invertebrates were found in the realigned sites for February and November (Figure 4.12, Table A.5). Gastropod *Peringia ulvae* (mud snail), polychaete *Hediste diversicolor* (ragworm) and the bivalves *Limecola balthica* (Baltic tellin) and *Cerastoderma edule* (Common cockle). The greatest abundance across both fields and months was of *P.ulvae*, which was found in all conditions and depths. No biodiversity assay could be performed on the abundance of invertebrates due the low species number.

Although some invertebrates were found within our full exclusion chambers the numbers are significantly lower than those in undisturbed or exclusion control chambers. As *P.ulvae* were found only in the 0-5cm in the full exclusion chambers, the observed abundances may be due to an artefact of the extraction procedure. The pasture field has a greater abundance of *P.ulvae* ($F_{(2,138)}=5.66$ $p=0.018$), especially in exclusion control chambers ($F_{(2,69)}=5.79$ $p=0.003$; Table A.5).

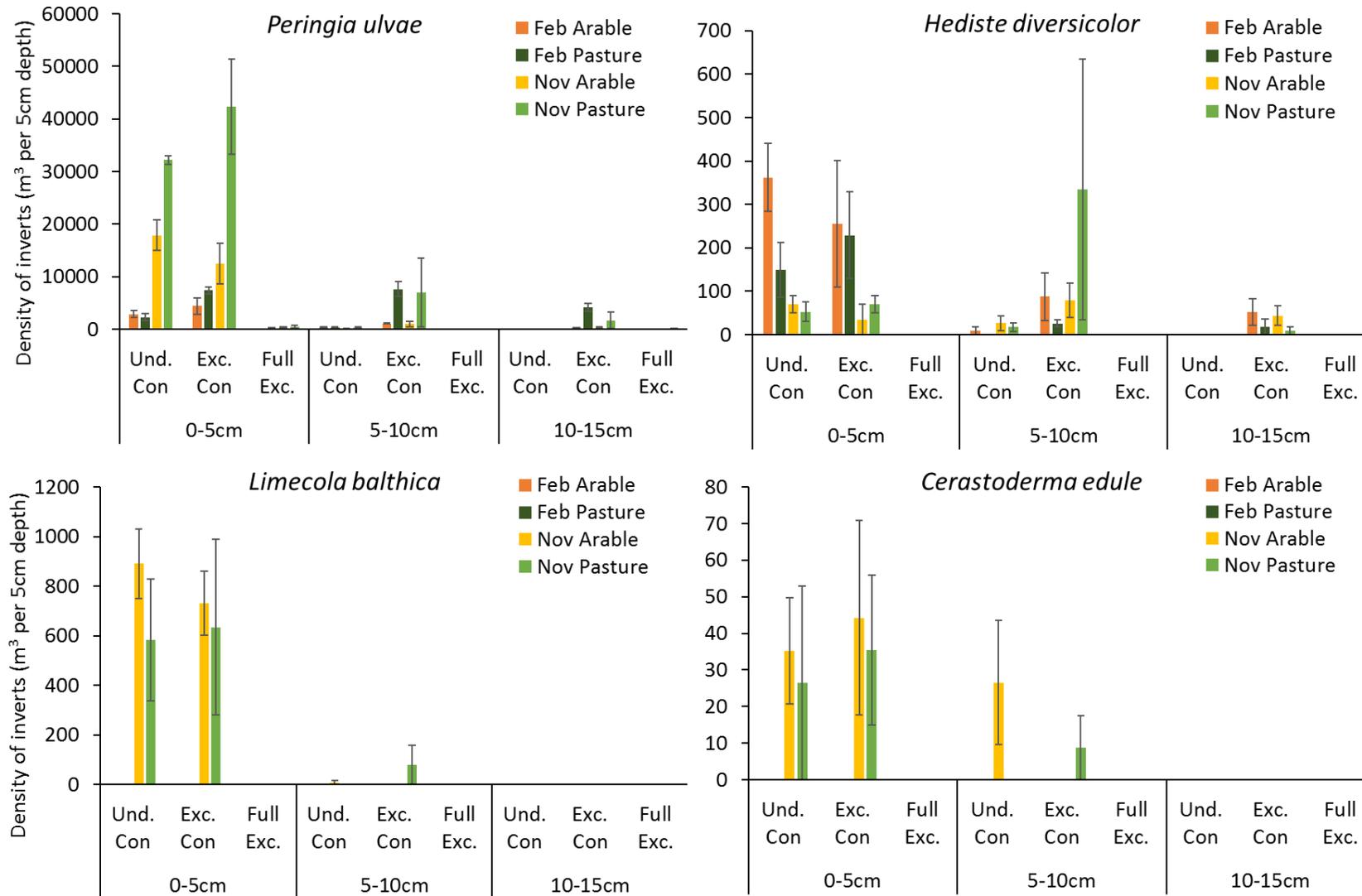


Figure 4.12 Bar chart with average density of invertebrates (m³ per 5cm depth zone). Und Con = Undisturbed Control, Exc. Con = Exclusion Control, Full Exc. = Full Exclusion. +/- = SE

1293 4.4 *Discussion*

1294 4.4.1 *Bulk density and water content*

1295 Bulk density of the realigned fields for all treatments showed the same downward trend as
1296 observed from monthly cores taken concurrently at these sites (Chapter 3). Exclusion control
1297 chambers in the arable field have a lower bulk density at all depths compared to the
1298 undisturbed and full exclusion chambers. Although our undisturbed chambers have a lower
1299 bulk density in the 0-5cm depth zone than in the lower depths (5-10cm & 10-15cm) they are
1300 more dense than the full exclusion and exclusion control chambers. In the pasture field, no
1301 change in bulk density is observed in the 0-5cm zone, however we observe fluctuation of
1302 density in the lower depths for all treatments. Similar to the arable field, undisturbed is more
1303 dense in the lower depths (5-10cm & 10-15cm) compared to full exclusion and exclusion
1304 control, with exclusion control chambers having lower density than the others.

1305 Water content in the arable and pasture fields showed an increase over time at all depths
1306 but mostly in the top 10cm. In the 0-5cm depth zone undisturbed chambers have a greater
1307 water content compared to exclusion control and full exclusion chambers especially in the
1308 arable field. In the 5-10cm depth zone however we observed that the exclusion control
1309 chambers have more water content although by November all chambers have a similar water
1310 content. Invertebrate abundance was higher in the 0-5cm depth zone thus higher water
1311 content in the exclusion control chambers in the 5-10cm depth zone could be attributed to
1312 gaps created in the sediment profile during chamber installation allowing for water
1313 penetration deeper into the sediment profile rather than bioturbation from invertebrates.

1314 When we compare the change in bulk density and water content of invertebrate exclusion
1315 and invertebrate control chambers, we can see that chambers with invertebrate colonization
1316 (invertebrate control) have lower bulk densities and higher water content, especially in the

1317 top 10 cm depth. This difference can be attributed to the biological mixing (burrowing)
1318 occurring by invertebrates over time rather than just the physical disturbance of installing
1319 the chambers prior to flooding. Both chambers were treated equally during installation with
1320 similar gaps created in the sediment profile thus the differences observed can be linked to
1321 the presence of invertebrates.

1322 4.4.2 *Sediment pH*

1323 Our arable and pasture fields behaved differently over time and with treatment. In our arable
1324 field we saw a change from a slightly acidic to a slightly alkaline sediment at all depths and
1325 conditions. In the top 5cm the control and exclusion control chambers behaved identically
1326 with the full exclusion chambers showing slight fluctuation over time. In the lower depths
1327 we see that the exclusion control chambers have a higher pH than the other treatments. In
1328 our pasture field sediment pH follows a similar trend of becoming more alkaline over time
1329 however not for all treatments and depths. pH of seawater is within the range of $7.2 \leq \text{pH}_T \leq$
1330 8.1 (Yang et al., 2014); which could account for the increasing alkalinity of the top sediment
1331 through inundation and saturation of sediment with seawater. Variations observed in the
1332 pasture field may be explained by the dense root mass which affects water movement
1333 (Angers and Caron, 1998) as was also observed in our monthly core collection in Chapter 3.
1334 pH range observed in our realigned site is similar to other studies (Ford et al., 2016; Spencer
1335 et al., 2008; Velinsky et al., 2017; Wolaver et al., 1986). Within those studies natural marshes
1336 have a pH lower than 7 whereas realigned marshes tended to have a higher pH (over 7).

1337 4.4.3 *Sediment nutrients*

1338 Ammonium (NH_4^+) concentration in both fields decreased over time for all treatments.
1339 Pasture field initially has higher concentration of NH_4^+ than arable field but by November
1340 they both have similar concentrations in the sediment. In the arable field we can see a clear

1341 effect of the treatment in the 0-5cm depth as the concentrations between the treatment
1342 from February until August are significantly different with the exclusion control chambers
1343 being higher and the lower concentration in the full exclusion chambers. In addition, in the
1344 lower sampled depth 10-15cm of the arable field the undisturbed chambers have a higher
1345 concentration of NH_4^+ which is also increasing over time. This is also observed in the pasture
1346 lower depths, however in the 0-5cm depth zone undisturbed chambers have a higher
1347 concentration than the other treatments. Nutrient concentrations in the sediment are
1348 affected by the microbial communities of the sediment, responsible for breakdown of
1349 organic matter (litter decomposition), nitrification and ammonification of available N
1350 (Francis et al., 2007; Gruber and Galloway, 2008; Nedwell, 1982; Strous et al., 2006). Overall,
1351 nutrient concentration in the realigned sites is higher in the exclusion control and
1352 undisturbed chambers than the full exclusion chambers. Biles et al. (2002) found that
1353 sediment disturbance, from bioturbation (organisms or water flow) can affect nutrient
1354 availability in the sediment as well as their released into the water column. This matches our
1355 results of higher nutrient concentration in the exclusion control and undisturbed chambers,
1356 where we have higher abundance of invertebrate colonization, than the full exclusion
1357 chambers.

1358 4.4.4 *Benthic macro-invertebrates*

1359 Macro-organisms found within our realigned sites are typical of natural and realigned
1360 saltmarshes in east England (e.g. Garbutt et al., 2006; Jackson et al., 1985) and recreated
1361 intertidal mud flats in the UK (e.g. Evans et al., 1998). Our realigned sites had similar
1362 abundances of *P. ulvae* as other studies however abundance of bivalves and ragworms was
1363 lower in our realigned site over the post-inundation period studies for the same period post-
1364 breach. Within our treatments we observed a higher abundance of organisms in our
1365 exclusion control chambers than the undisturbed areas. This might be due, not only to the

1366 open access (no top mesh) for organisms to colonize but also to the breakdown of the relic
1367 sediment layer that occurred during installation of the chambers, as well as gaps created
1368 during placing of the chambers which allowed organisms to penetrate/fall further down the
1369 sediment column to depths that otherwise would be lethal (>10cm) (Chandrasekara and Frid,
1370 1998; Huxham et al., 1995). Furthermore, Craft (2000) found that development of
1371 communities of benthic invertebrates in realigned salt marshes depended on wetland
1372 sediment formation and development. We observed that exclusion control chambers
1373 behaved differently from the undisturbed chambers, having lower bulk density in the top
1374 5cm as well as higher water content, which might have allowed the formation of more ideal
1375 sediment conditions for macro-invertebrate colonization. The development of the
1376 invertebrate community is dependent on the formation of an organic rich surface later to
1377 support the detritus feeding organisms (Craft, 2000) which can explain the increase in
1378 community numbers in our site from February to November.

1379 Benthic macro-invertebrates are an important component of salt marsh establishment and
1380 function as they are involved in sediment bioturbation (Bertness, 1985), biogeochemical
1381 cycling (Alkemade et al., 1992) and are a source of food for higher invertebrates, fishes and
1382 birds (Adam, 1990). However, in early establishment of marshes Paramor and Hughes (2004)
1383 found that ragworm, *Hediste diversicolour*, can inhibit the development of plants as it feeds
1384 on plant seedlings. Thus, macro-invertebrate colonization of realigned saltmarshes can have
1385 a diverging impact on marsh development. Invertebrates on newly realigned marshes could
1386 help develop the sediment characteristics through their burrowing activities (i.e. increasing
1387 sediment aeration/water drainage and nutrient movement) but also may inhibit through
1388 active feeding on plant seedlings and not allowing plant colonization. During our study period
1389 (14 months since breach) there was no vegetation colonization within our sampling area,
1390 thus we cannot conclude whether ragworm colonization within this realigned site has a
1391 detrimental or beneficial effect.

1392 4.4.5 *Summary*

1393 Our invertebrate exclusion experiment has shown that disturbance of the sediment can
1394 influence its geochemical characteristics in newly realigned saltmarsh. Macro-invertebrate
1395 colonization of a newly realigned site could create that disturbance (bioturbation) and
1396 influence the geochemical characteristics, as we observed by the differences in geochemical
1397 properties of invertebrate colonization chambers. In our experiment invertebrate
1398 colonization did not affect the homogeneity across all depths as both colonized and
1399 uncolonized sediments were very similar by the last sampling. Despite all experimental
1400 chambers reaching the same (or very similar) geochemical state by the end of the sampling
1401 campaign (14 months post breach) the rate of change occurring in our invertebrate
1402 colonization chambers is greater than the full exclusion chamber.

1403 In addition, that disturbance of the relic agricultural layer prior to flooding of the site can
1404 also affect those changes similarly and on occasion have greater effect, as seen in our
1405 exclusion control chambers. This active breakdown of the relic layer occurred during the
1406 installation of the exclusion chambers (especially exclusion control chambers), simulating a
1407 more active mixing of the sediment profile allowing for water movement further down the
1408 sediment post flooding. A combination of active breaking of relic layer (e.g. plowing) and
1409 colonization of invertebrates can potentially accelerate shift of geochemical characteristics
1410 of newly realigned marshes from terrestrial to marine. Disturbance of the sediment prior to
1411 flooding may also enhance plant colonization through not only more favorable sediment
1412 characteristics but also by creating a more suitable surface for saltmarsh plant seeds to
1413 anchor, increasing their chances of germinating (Garbutt et al., 2006).

1414 Chemical characteristics of sediments however are also influenced by microbial communities
1415 present. Microbes in the sediments are responsible for the breakdown of organic matter
1416 releasing readily available nutrients into the sediment for plant absorption. These

1417 communities present could be drivers for the success of plant colonization and geochemical
1418 changes observed in the sediment.

1419 Chapter 5. Microbial community changes during the first year of a
1420 newly realigned salt marsh and effect of bioturbation on them

1421 5.1 *Introduction*

1422 Salt marshes are among the most productive ecosystems on the planet and harbor diverse
1423 biological communities (Teal and Howes, 2002). It is estimated that marine sediments harbor
1424 $\sim 10^{31}$ microbial cells with a biomass rivaling those of plants making them Earth's largest
1425 microbial habitat (Whitman et al., 1998). Over the last two decades there has been an
1426 accelerated global decline in the extent of saltmarsh systems (Millenium Ecosystem
1427 Assessment, 2005) and efforts have been initiated to conserve existing marshes and to
1428 develop new coastal marsh habitats (realigned or managed saltmarshes). With increased
1429 development and creation of salt marshes a greater understanding of the functionality of
1430 these new systems is required. Easily observable biodiversity such as vegetation and animal
1431 diversity of realigned marshes has been extensively assessed (e.g. Davy et al., 2011;
1432 Environment Agency, 2017; Garbutt and Wolters, 2008; Mossman et al., 2012; Wolters et al.,
1433 2008, 2005). Realigned saltmarshes have been found to be different than natural marshes in
1434 terms of plant biodiversity, sediment characteristics and hydrogeology (e.g. Garbutt and
1435 Wolters, 2008; Lawrence et al., 2018; Spencer et al., 2008; Tempest et al., 2015; Wolters et
1436 al., 2005); where these differences can persist after years of inundation and plant
1437 colonization. Microbial communities, which affect and are affected by above ground
1438 biodiversity have been harder to measure and quantify precisely until recently (Derocles et
1439 al., 2018).

1440 Microbial communities in salt marshes are, among other processes, responsible for organic
1441 matter decomposition from plant litter, transform pollutants (Benoit et al., 2003; Lillebø et
1442 al., 1999; Smith and Hollibaugh, 1993) and affect the availability of heavy metals (Stolz and
1443 Oremland, 1999). Understanding these communities and their interactions with other taxa

1444 within salt marshes is essential for developing effective restoration and mitigation strategies
1445 for these vulnerable ecosystems, as well as maintaining genetic resources for future need.

1446 Sediment characteristics, such as pH and bulk density, have been shown to affect microbial
1447 communities composition and abundance (Li et al., 2002; Yamada et al., 2007). Bioturbation
1448 of soft sediments by benthic invertebrates can also have a significant influence on microbial
1449 communities either directly through predation (Graça et al., 2000) and indirectly through
1450 modification of benthic sedimentary geochemical properties (Queirós et al., 2013).
1451 Furthermore, Daleo et al. (2007) found that burrowing activities of crabs increase the supply
1452 of nutrients and oxygen to arbuscular mycorrhizal fungi which in turn increased biomass
1453 production by the salt marsh plant *Spartina alterniflora*. Lillebø et al. (1999) found that
1454 microbial activity was responsible for 67% of litter decomposition in salt marshes, however
1455 additions of macrofauna and meiofauna increased the degradation process.

1456 Previous land use of realigned marshes can affect the hydrogeology of the site (Lawrence et
1457 al., 2018; Spencer et al., 2008; Tempest et al., 2015) which in turn can affect sediment
1458 characteristics of a realigned marsh (Chapter 3). And these in turn could affect microbial
1459 communities of the sediment. Studies have shown that microbial communities are
1460 significantly affected by land use change (Bossio et al., 1998; Johnson et al., 2003;
1461 Steenwerth et al., 2002). Lauber et al. (2008) has also shown that land use and change not
1462 only affects community structure but also relative abundance of bacterial and fungal species
1463 within communities.

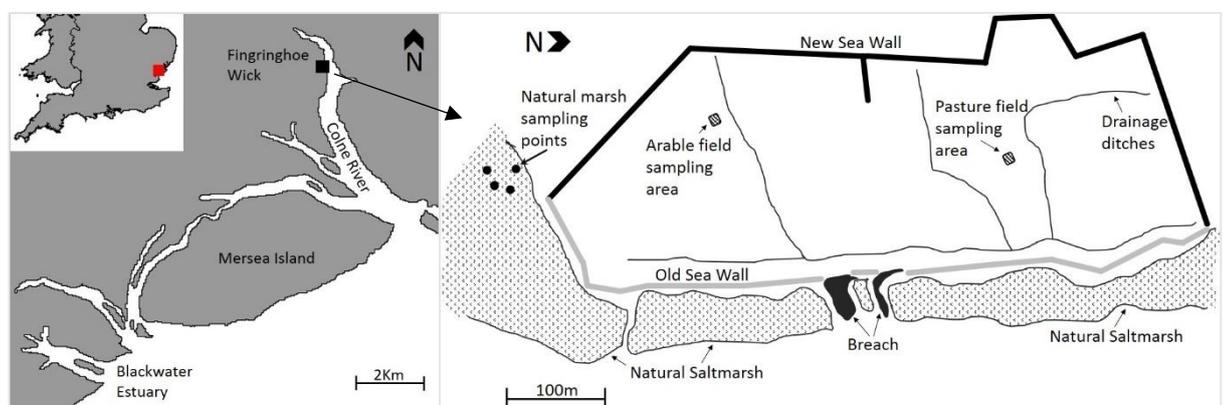
1464 Our study aims to examine microbial communities of a newly realigned marsh within the 1st
1465 year of inundation. The object of the study was to (1) compare the effect of bioturbation on
1466 microbial communities in a newly realigned salt marsh and (2) examine the effect of previous
1467 land use (arable and pasture) as well as differences in sediment characteristic on microbial
1468 communities of the realigned salt marsh. We expected to find areas with bioturbation to

1469 have more similar microbial communities with the natural marsh than areas of no
1470 bioturbation.

1471 5.2 *Methods*

1472 5.2.1 *Study site*

1473 Fingringhoe Wick Nature Reserve (48.6 ha) is located in Essex, southeast England on the west
1474 border of the Colne River. The two fields within the managed realignment site (22 ha)
1475 ($51^{\circ}50'25.77''\text{N}$, $0^{\circ}58'27.80''\text{E}$) were previously used for growing wheat and barley in rotation
1476 (southern field), and pastureland grass (northern field). Two locations within the realigned
1477 site and one local natural location, formed the sample sites for this study, realigned arable
1478 field (A), realigned pasture field (B) and the adjacent natural marsh (C). The two regions (10m
1479 x 8m) in the realigned site were selected prior to the breach to be at the same elevation, and
1480 mud pans and creeks were sampled in the natural marsh. The realigned fields are
1481 substantially lower in elevation relative to the local natural marsh but are expected to gain
1482 elevation over time until they are similar in the relative sea level elevation. The old sea wall
1483 was breached at two locations to allow for tidal inundation of the site in September 2015
1484 (Figure 5.1).



1485 *Figure 5.1. Map of location and outline of new realigned site and natural marsh sampling*
1486 *locations within the Colne Estuary, UK.*

1487 5.2.2 *Experimental design*

1488 Invertebrate exclusion chambers were placed in the two locations of the realigned site prior
1489 to the sea wall breach. There were three chamber conditions; i) Full exclusion- where there
1490 was a 250µm mesh sealing the chambers from both vertical and horizontal penetration of
1491 invertebrates but allowing water movement, ii) Exclusion control- where the mesh only
1492 covered the sides of the chambers (as a control over chamber placement) and allowed
1493 invertebrates to come in at the top and bottom, and iii) Undisturbed - regions that were left
1494 undisturbed to regenerate naturally. Chambers and undisturbed regions were sampled
1495 seasonally. Reference samples were also taken from an adjacent natural saltmarsh (mud
1496 pans and creeks) to compare sediment microbes between natural and realigned
1497 communities. Microbial sampling of realigned and natural marsh sediments was performed
1498 during the winter (February '16) and autumn (November '16) sampling campaigns for both
1499 realigned field (arable and pasture) and natural marsh sediments. The two months were
1500 chosen to represent the early beginning and end of our sampling campaign as limited funds
1501 prevented us from performing microbial analysis on all seasons sampled. Chambers were
1502 processed in 5cm intervals down to 15cm to provide a depth profile of the microbial
1503 communities. 5cm depth intervals were chosen to allow for substantial sediment for all
1504 analyses (dense roots, present in the top 5cm, hinder extraction of only sediments) as well
1505 as to maintain comparable depth profiles with the previous study (Chapters 2-3). Chambers
1506 were concurrently analysed for sediment characteristics, as described in Chapter 4.

1507 5.2.3 *DNA sampling*

1508 Sediment samples (~5g) from each chamber and depth were subsampled from the core using
1509 aseptic technique (80% ethanol cleaned knives) from the centre of the core and flash frozen
1510 in liquid nitrogen and stored in -20°C until extraction. DNA was extracted from 0.25g of

1511 sediment using DNeasy® PowerSoil® Kit (QIAGEN, Germany) following the manufacturer's
1512 protocol and stored at -20°C until PCR amplification.

1513 5.2.4 *PCR amplification and sequencing preparation*

1514 DNA samples were amplified using three sets of primers which targeted, bacteria (545FY-ill
1515 & 806rmod-ill) (Apprill et al., 2015; Parada et al., 2016), archaea (ARCH349F-ill & ARCH806R-
1516 ill) (Takai and Horikoshi, 2000) and eukaryotes (TAReuk454FWD1-ill & TAReukREV3-ill)
1517 (Stoeck et al., 2010). The "-ill" suffix of the primers indicates illumina sequencing tags were
1518 incorporated into the primer. The forward primer additionally incorporated two random
1519 bases for cluster definition. The 25µl amplification reaction for all primers consisted of 0.5µl
1520 of each forward and reverse primer (10 µM), 0.125 µl Taq Polymerase, 5 µl GoTaq G2 Flexi
1521 buffer, 2 µl MgCl₂, 0.5 µl of 10 mM dNTP mixture, 15.875 µl molecular grade H₂O and 0.5 µl
1522 DNA template. PCR reaction conditions for bacteria were initial denaturation at 94°C for 5
1523 minutes, 30 cycles consisting of denaturation at 94°C for 1 minute, annealing at 53°C for 45
1524 sec and extension at 72°C for 1 minute and final extension at 72°C for 7 minutes. Archaeal
1525 and eukaryote reactions were initiated by denaturation at 94°C for 5 minutes, followed by
1526 40 cycles of denaturation (94°C, 30 sec), annealing (53°C, 45 sec) and extension (72°C, 1 min
1527 30 sec), terminated by final extension at 72°C for 5 minutes.

1528 The size of PCR products was confirmed by agarose gel electrophoresis (Bac: 350bp, Arch:
1529 540bp, Euk: 450bp). Products were purified using Agencourt AMPure XP magnetic beads
1530 (Beckman Coulter) at a 1:0.8 DNA/PCR to bead mix, washed twice with 80% (v/v) ethanol
1531 and eluted with 20µl of molecular grade water. Cleaned PCR products were quantified using
1532 Quant-iT™ dsDNA Assay fluorescence kit (Invitrogen, Life Technologies, Carlsbad, CA, USA).
1533 PCR products were diluted to 5ng and primers from each sample were pooled together
1534 (Bac:Euk:Arch, 2:5:6 ratio) for Illumina Miseq sequencing (Illumina Inc., San Diego, CA, USA)
1535 at the University of York, UK.

1536 5.2.5 *Sequencing processing*

1537 Downstream sequence processing was carried out in PuTTY/Unix using QIIME v.1.9.1
1538 (Caporaso et al., 2010). The full pipeline for processing sequences ready for diversity analysis
1539 is detailed in Table 5.1. The databases used for the amplicons were 16S for bacteria and
1540 archaea and 18S for eukaryote from SILVA database (Glöckner et al., 2017; Quast et al., 2012;
1541 Yilmaz et al., 2014).

1542 5.2.6 *Statistical analysis*

1543 Statistical analyses were conducted using the R statistical language implemented in RStudio
1544 (Version 1.1.423). Bray-Curtis diversity index was used to compare β -diversity between the
1545 microbial communities of the natural and realigned sites and also to compare between
1546 different treatments. R packages “vegan” (Oksanen et al., 2018) and “pairwiseAdonis”
1547 (Arbizu, 2017) were used to perform diversity and Permutational Multivariate Analysis of
1548 Variance (PERMANOVA) on microbial communities. Detrended correspondence analysis
1549 (DCA) was employed to visualize and characterize ordination of each community and their
1550 relationship with environmental variables.

1551 .

1552 Table 5.1. Pipeline for down streaming of sequences and producing OTU tables using QIIME

	Action	Function	Description
1	Merge R1 & R2 files	<code>usearch -fastq_mergepairs *R1*.fastq -fastqout merged.fq -relabel @</code>	Merges R1 & R2 reads (forward and reverse reads) and puts all reads into 1 file and renames R1& R2 merged sequences with original R1 name
2	Strip off first 13 bases	<code>cutadapt -g CCAGCASCYCGGTAATTCC -a ACTTTCGTTCTTGATYRA --overlap 10 -o left-trimmed.fastq --discard-untrimmed merged.fq</code>	Trims first 13 bases from read sequence (randomised bases added to the sequences used in amplicon sequencing so that sequences can be distinguished) then separates reads by primer sequence (primer sequence used in command) #This sequence is for TarEuk primer
3	Asses read quality for filtering further on	<code>usearch -fastq_eestats2 left-trimmed.fastq -output eestats2.txt -length_cutoffs 50,550,50</code>	Assess read quality to be able to pick quality filtering stringency in next step
4	Filter reads	<code>usearch -fastq_filter left-trimmed.reads.fq -fastq_maxee 1.0 -fastq_trunclen 270 -fastaout</code>	We use maxee score from previous step and trim reads to required length. The maxEE parameter sets the maximum number of “expected errors” allowed in a read to better

		filtered_reads.fna	filter the output and speed up downstream analysis. -Bacteria trimmed to 270bp; Archaea trimmed to 390bp; Eukaryotes trimmed to 280bp
5	Rename headers in files	sed 's/^>/>barcodelabel=/' filtered_reads.fna > filtered_reads_new.fna sed 's/\./;/g' filtered_reads_new.fna >filtered_reads_new2.fna	Rename headers, stripes everything before ">" and replaces it with ">barcodelabel="
6	Merge identical reads	vsearch -derep_fulllength filtered_reads.fna -output derep.fna -sizeout	Merges identical reads to minimize effect on clustering in later steps and helps create a list of unique OTUs
7	Sort by size and remove singletons (rare samples)	usearch -sortbysize derep.fna -fastaout derep_Sort_output.fasta -minsize 2	Sorts OTUs by the size of clusters and excludes singletons. This removes very rare samples which become problematic for normalisation techniques and can skew the data.
8	Create RepSet	usearch -cluster_otus derep_Sort_output.fasta -otus otus.fna -minsize 2	Creates list of OTUs with at least 2 instances of any particular sequence

9	Remove chimeras from OTU list	Usearch8 -uchime_ref 9_otus.fna -db silva_132_97_18S.fasta -strand plus -nonchimeras RepSet_No_Chimeras.fasta	Removes chimers from OTU list using the sequencing database from SILVA. 16S database used for bacteria and archaea and 18S for eukaryotes. -uses usearch v.8
10	Re-numbers OTU clusters	python fasta_number.py RepSet_No_Chimeras.fasta OUT_ > RepSet_num.fasta	Re-numbers the OTU clusters in the RepSet with new numbers in order 1,2,3,..etc. this helps further on for sorting and BLAST
11	Adding read count on RepSet	Usearch8 -usearch_global Output_from_step_5.fasta -db RepSet_num.fasta -strand plus -id 0.97 -uc map.uc	Maps read counts onto filtered, no chimeras, RepSet file with file with renamed headers -uses usearch v.8
12	Convert map to text file	python uc2otutab.py map.uc > otu_table.txt	Converts the mapped OUT.uc file into text files so that the information can be visualised and extracted for the next step
13	Export RepSet	cp repset_num.fasta repset_num.csv	Converting the RepSet file from fasta format to a .csv file.

At this point, the RepSet and read counts files need to be manually merged in Excel

14	Assign taxonomy	<code>assign_taxonomy.py -i RepSet_num.fasta -o Tax_out -r silva_132_97_18S.fasta -t 18STax97_132.txt</code>	Assign taxonomy to 97% using SILVA db (ref fasta & taxonomy text files), onto RepSet
15	Create Excel file with RepSet sequences, OTU numbers, OTU counts for each samples and taxonomic assignment. Export the file into text		
16	Measuring alpha diversity	<code>usearch -alpha_div tax_otu_table.txt -output alpha_tax_otutab.txt</code>	Use alpha diversity scripts to calculate reads per sample. This is used to inform normalisation in the next step
17	Normalizing samples	<code>usearch -otutab_norm tax_otu_table.txt -sample_size 10000 -output norm_otu_tax_table.txt</code>	Normalizes the abundance data across samples which can remove and excess amount of data.

During assigning taxonomy some OTUs would have been “unassigned” as the databases are limited and may be unassigned sequences that need to be removed. Sequences are checked using NCBI BlastN database to assign taxonomy manually. The step 15 taxonomy file is also updated and re-imported for diversity analyses.

New taxonomy file is created and OTUs and counts are collapsed by taxon in Rstudio.

1554 5.3 *Results*

1555 Operational taxonomic unit (OTU) tables were generated from the bacteria, archaea and
1556 eukaryote amplified regions. Initial sequence reads after primer sequences were stripped
1557 (step 2, Table 5.1) were 7097571 sequences for bacteria amplicon, 3691543 sequences for
1558 archaea and 3406765 sequences for eukaryotes. Post filtering, exclusion of singletons and
1559 clustering of replicated reads, amplicon sequences totaled 450682 for bacteria, 188365 for
1560 archaea and 188615 for eukaryotes. This corresponded to 23796 OTUs for bacteria,
1561 decreasing to 23630 after removal of chimeras and to 2444 after taxonomic assignment
1562 (including collapsing of similar taxa) and removal of archaea sequences. Archaea sequences
1563 corresponded to 12603 OTUs which decreased to 12275 after chimera removals and down
1564 to 128 post taxonomic assignment and removal of bacteria sequences. Amplicon sequences
1565 for eukaryotes corresponded to 7045 OTUs, decreasing to 6920 after chimera removals and
1566 to 1093 post taxonomic assignment and collapsing of similar taxa. Unassigned sequences
1567 post-BLAST taxonomy assignment were removed and updated OTU table collapse by taxon.
1568 Final curated sequences corresponded to 1716 OTUs for bacteria, 119 OTUs for archaea and
1569 565 OTUs for eukaryotes which were used for further analysis.

1570 5.3.1 *Community diversity*

1571 A nested PERMANOVA was performed to assess differences between sites and treatments.
1572 Depth was nested within treatment and treatment nested with field. Community diversity of
1573 bacteria was found to be significantly different within our nested treatment (PERMANOVA,
1574 $F_{(9,126)}=0.05$, $p<0.001$). Natural marsh sediments had lower species richness than both
1575 realigned sites. Diversity was higher in the 0-5cm depth zone relative to the 5-10 and 10-
1576 15cm depth zones for all sites. Also, exclusion control chambers had higher bacterial diversity
1577 than undisturbed sediments and full exclusion chambers for both arable and pasture fields.

1578 Similarly, eukaryote community diversity was significantly different with depth, treatment,
1579 field and month (PERMANOVA, $F_{(9,126)}=0.06$, $p<0.001$). The top 5cm sediment depth had
1580 higher eukaryote diversity than lower depths for all fields. The natural marsh sediments had
1581 higher species richness in the top 5cm than realigned site sediments, however in the lower
1582 depths (5-10 and 10-5cm) realigned sites have a higher diversity than natural. Community
1583 abundance in the exclusion control chambers is higher than the other treatments for both
1584 arable and pasture fields.

1585 Field type (arable/pasture) was found to have no significant effect on archaea communities
1586 in realigned sediments but they varied significantly with depth, treatment and month
1587 (PERMANOVA, $F_{(9,126)}=0.47$, $p<0.001$). While archaeal communities within realigned (arable
1588 and pasture) sediments are similar, they are different from the natural marsh community
1589 (Figure 5.2). Similar to bacterial and eukaryote communities, the surface (0-5cm depth)
1590 sediments had higher diversity than lower depths sediments and the exclusion control
1591 chambers also had a higher diversity than undisturbed sediments and the full exclusion
1592 chamber sediments.

1593 Communities were collapsed to Order level (or Phylum when order was not possible (Randle-
1594 Boggis et al., 2016)) and the relative abundance of the top 10 orders/phyla were plotted
1595 relative to field and month. We observed that microbial communities and abundances within
1596 the realigned sites are different to those of the natural marsh. The archaea Nitrososphaerales,
1597 which is an ammonia oxidizing archaea (Tourna et al., 2011) is dominant in our realigned
1598 sites but almost completely absent in the natural (Figure 5.2). In addition, the order
1599 Thermoplasmata, an acidophile, is mostly dominant in our natural marsh as is the archaea
1600 Bathyarchaeia, with their communities in the realigned site increasing over time.

1601 The bacteria Chthoniobacterales were present in high abundance in our realigned sites but
1602 were completely absent in natural sediments (Figure 5.3). Chthoniobacterales is a generalist

1603 aerobic plant carbohydrate degrader whose presence in our realigned site is likely indicative
 1604 of the rich resources within the agricultural field being degraded. Thus, we might expect
 1605 Chthoniobacterales numbers to decline over time, as plant carbon resources within the
 1606 sediments decrease and salinity and anaerobic conditions of the sediment increase.
 1607 Furthermore, the top 10 orders of bacteria cover up to 50% of relative abundance, compared
 1608 to archaea (80%) and eukaryotes (60%) indicating greater diversity than non-bacterial
 1609 communities.
 1610

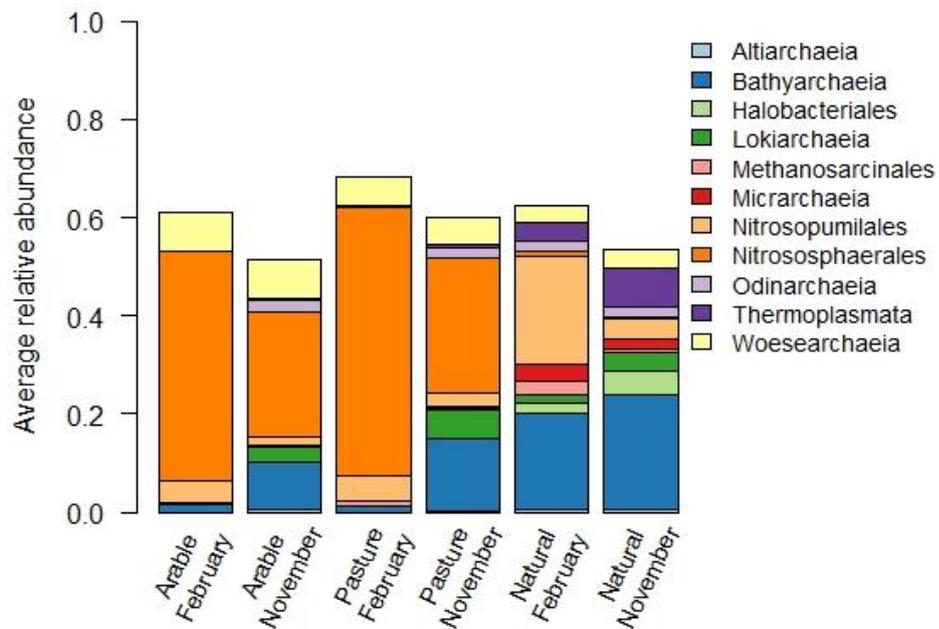


Figure 5.2. Order level archaea community comparison between sites and month. Only the 11 most abundant orders are shown.

1611 Natural marshes have a higher abundance of Dinoflagellata, mostly comprised of marine
 1612 plankton, than realigned sites even after 14 months post inundation (Figure 5.4). Pasture
 1613 field in February has the highest abundance of Charophyta, a green alga, compared to arable
 1614 and natural fields, with their abundance decreasing by November.

1615

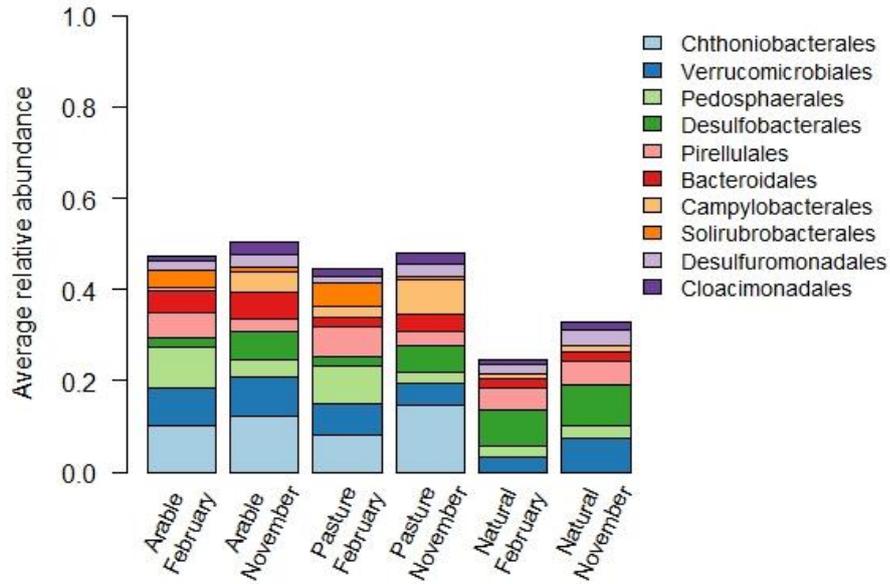


Figure 5.3. Order level bacteria community comparison between sites and month. Only the 10 most abundant orders are shown

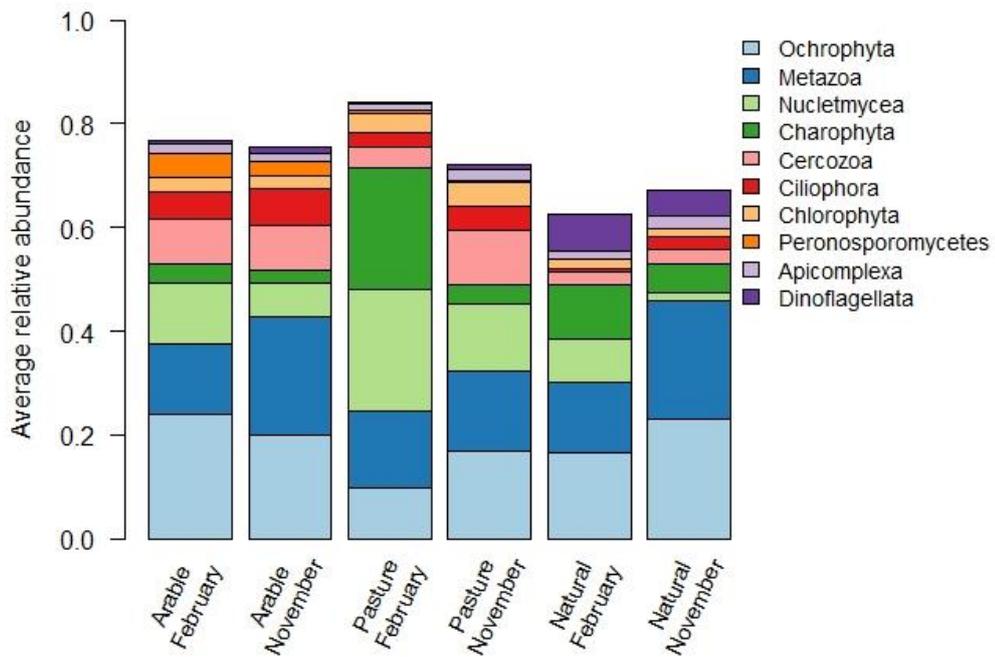


Figure 5.4. Phylum level eukaryote community comparison between sites and month. Only the 10 most abundant orders are shown

1617 5.3.2 Detrended correspondence analysis (DCA)

1618 Following DCA of communities with fitted environmental parameters we found that bulk
1619 density, water content and pH of sediment affect community abundances for bacteria
1620 ($p < 0.001$), archaea ($p < 0.001$) and eukaryotes ($p < 0.001$) in newly realigned salt marsh
1621 sediments (Figures 5.5-5.7).

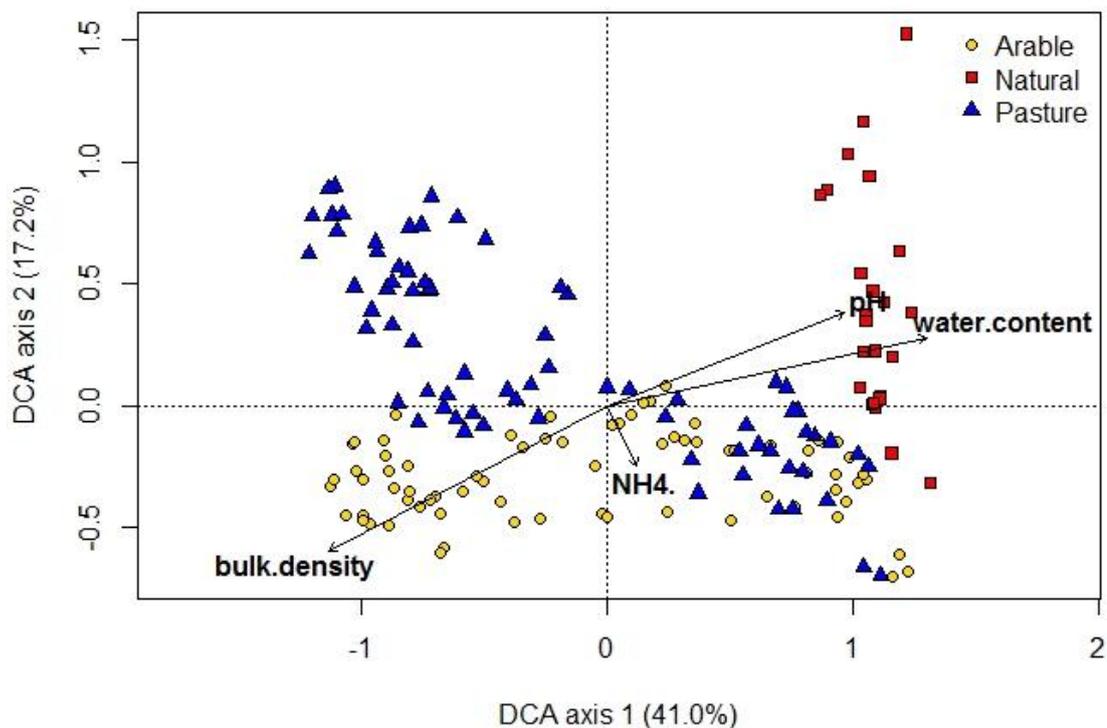


Figure 5.5. Detrended correspondence analysis (DCA) ordination plots of bacteria data. Percentage values on the axes represent variation in bacteria taxa abundance matrix explained by each axis. Arrows represent effect of each sediment variable of community

1622 Examining the DCA axis 1 scores for bacteria we observed that arable and pasture field have
1623 overlapping communities however some organisms are higher scoring in one field over the
1624 other, whereas the natural field has different composition of highest scoring organisms
1625 compared to the realigned sites. Score indicates the effect of the environmental condition
1626 on each species/community; higher score showed more effect and affect. For the archaea,
1627 Methanomicrobia and Methanobacteria are amongst the highest scoring in the arable field,

1628 whereas Thermoplasmata and Halobacteria are dominant in the pasture field. In the natural
 1629 marsh both for archaea and eukaryotes, the highest scoring organisms are a mixture of the
 1630 ones found in the realigned sites (arable and pasture). The eukaryote organisms with the
 1631 highest scores in the arable field are Rhizaria and Nuclemycea, whereas in the pasture it is
 1632 Holozoa and Alveolata.

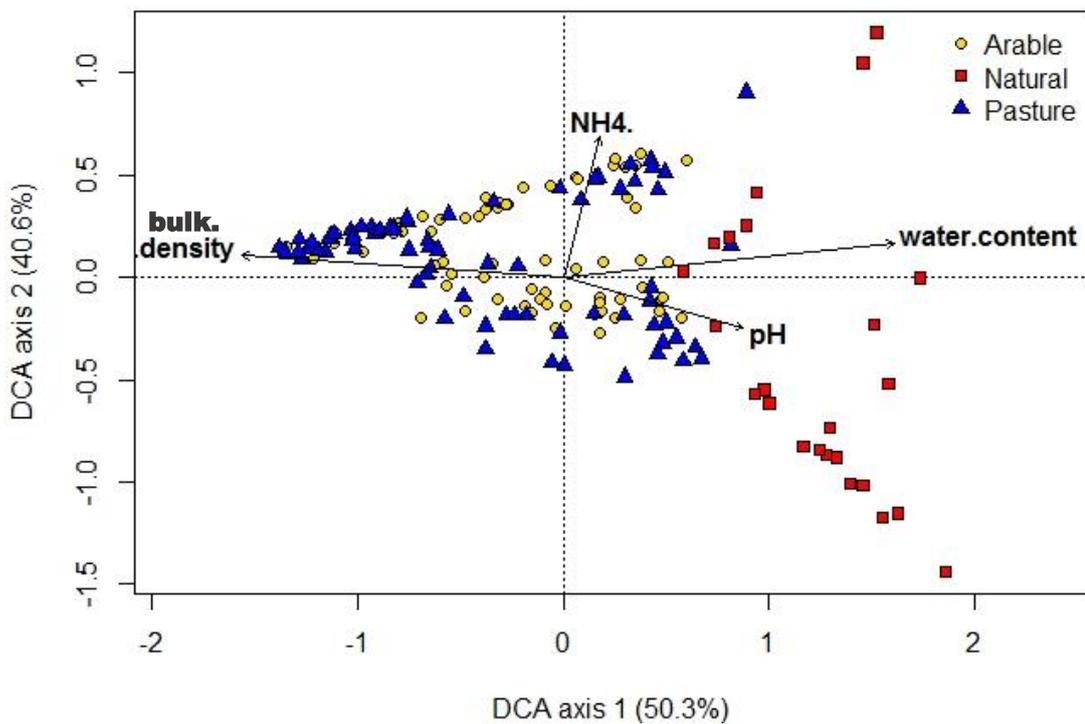


Figure 5.6. Detrended correspondence analysis (DCA) ordination plots of archaea data. Percentage values on the axes represent variation in archaea taxa abundance matrix explained by each axis. Arrows represent effect of each sediment variable of community

1633

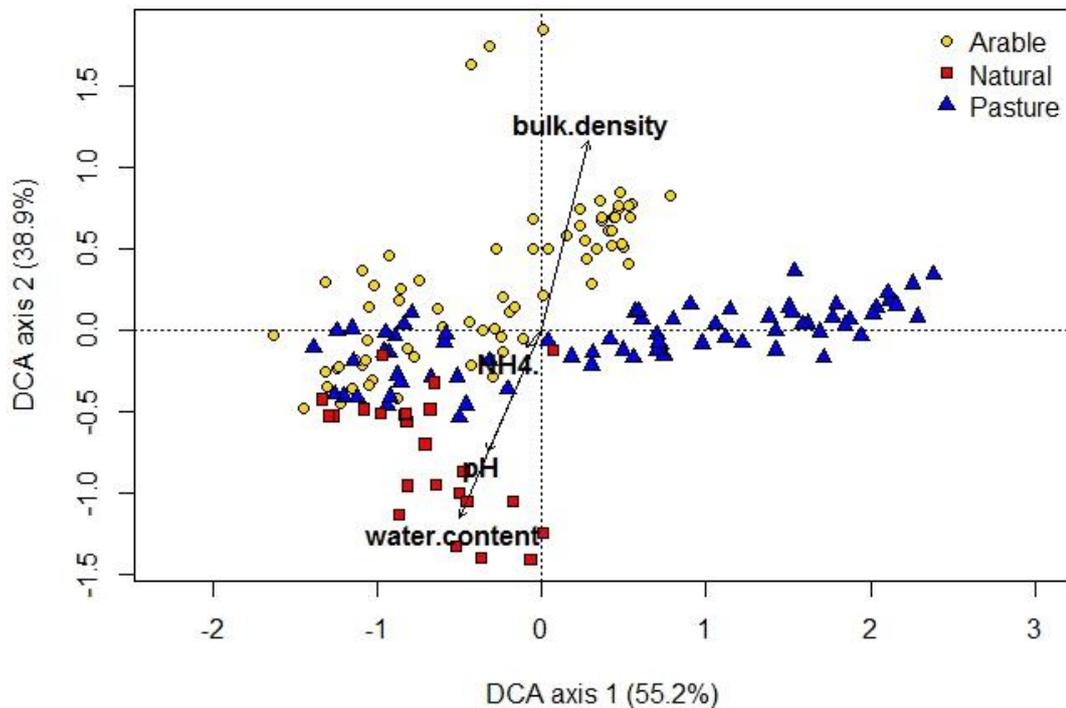


Figure 5.7. Detrended correspondence analysis (DCA) ordination plots of eukaryotes data. Percentage values on the axes represent variation in eukaryotes taxa abundance matrix explained by each axis. Arrows represent effect of each sediment variable of community

1634

1635 5.4 Discussion

1636 Microbial communities between natural and realigned sites were significantly different, as
1637 well as between the arable and pasture realigned sites (Figures 5.2-5.4). Land use has been
1638 found to affect microbial communities in terrestrial ecosystems (Fraterrigo et al., 2006;
1639 Lauber et al., 2008; Mathew et al., 2012) similar to what our study has shown. While land
1640 use and land use change are often associated with changes in microbial communities it is the
1641 changes in soil properties that drive the changes in communities. pH, water content and bulk
1642 density of the sediments were found to be significant drivers of the communities present.

1643 Similar effect of sediment characteristic on microbial communities were observed on
1644 saltmarsh sediments (Li et al., 2002).

1645 Allison and Martiny (2009) have demonstrated that environmental factors can affect the
1646 abundance and taxa of microbial communities which could be responsible for ecosystem
1647 processes and affect their resilience and resistance to disturbance. Increased soil compaction
1648 (i.e. higher bulk density) leads to decreased soil aeration and reduced nutrient availability
1649 (Jusoff, 1991). Li et al. (2002) found that higher bulk density leads to decrease of 26-39% in
1650 numbers of bacteria, fungi and actinomycetes.

1651 Bacteria community variances are correlated with water temperature, pH and dissolved in
1652 freshwater, intertidal and also marine sediments (Wang et al., 2012; Xiong et al., 2012). pH
1653 could be the best predictor for bacterial communities composition in sediments and water
1654 (Liu et al., 2015; Xiong et al., 2012). Tripathi et al. (2013) also found that pH is the dominant
1655 driver for variation in bacteria and archaeal communities in tropical soils. Numerous studies
1656 have demonstrated that pH is one of the most important factors influencing microbial
1657 communities, as pH has a direct effect on enzyme activity thus affecting physiology, growth
1658 and energy respiration (Yanagawa et al., 2013). In addition, pH could be a proxy for other
1659 changes occurring in the sediment (e.g. nutrient availability) which also influence presence
1660 and functionality of microbes (Liu et al., 2015).

1661 Bioturbation has been found to affect microbial communities by allowing predation (Graça
1662 et al., 2000), as well as changing sediment characteristics (Queirós et al., 2013). Our study
1663 has shown that bioturbation treatment and depth had an effect on community abundance,
1664 with exclusion control chambers having higher species richness than other conditions. Areas
1665 of bioturbation and greater disturbance of the sediment profile had a higher species richness
1666 than undisturbed areas. Furthermore, over time there is a community abundance shift with
1667 some orders becoming less abundant (e.g. Nucleomycea and Charophyla in realigned sites)

1668 whilst others increased (e.g. Nitrosophareles and Bathyacraheia in realigned sites) (Figures
1669 5.2-5.4).

1670 Hutchings et al. (2019) observed that iron-rich ponds had a higher percentage of bioturbation
1671 than sulfide-rich ponds in natural salt marshes. They suggest that the process of bioturbation
1672 introduces pathways for oxic water to penetrate further into otherwise anoxic sediment
1673 providing more suited redox conditions for bacterial iron reduction. In addition, they suggest
1674 that lack of bioturbation leads to more anoxic sediments where microbial sulfate reduction
1675 would become the dominant reduction pathway creating sulfide-rich ponds. Changes in the
1676 oxygenation of sediment by bioturbation creates a shift of redox conditions leading to a shift
1677 in microbial communities more suited to the changing environment.

1678 Overall, we found that our natural marsh has different organism abundances and
1679 composition compared to the realigned marsh. Even 14 months post inundation many
1680 communities are not approaching similar abundances to the natural marsh and are
1681 comprised of different communities which can affect the functionality of the system and its
1682 ability to regenerate to natural marsh condition. Furthermore, previous land use and soil
1683 management can affect long term community composition of an ecosystem and in turn its
1684 functionality (Fraterrigo et al., 2006; Mathew et al., 2012). As observed in other systems,
1685 restoration is a complex process and to understand it we need to observe them in a more
1686 holistic manner, above and below ground biodiversity needs to be taken into account to
1687 assess system functionality and recovery (Holden et al., 2019).

1688

1689 Chapter 6. General Discussion

1690 The primary aim of these studies was to investigate the differences, and their drivers,
1691 between natural and realigned salt marshes, in terms of sediment characteristics and
1692 biodiversity. To examine if realigned marshes normalize (physiochemically and biologically)
1693 over time we quantified underlying sediment characteristics with above ground biodiversity
1694 across three paired natural/realigned sites of different ages (13, 62, 118 years) since
1695 realignment in Essex UK (Chapter 2). We predicted that the oldest marsh (118 years) would
1696 be more similar both physiochemically and biologically (plant diversity) to its natural paired
1697 marsh than the younger realignments. The results of Chapter 2 demonstrated that realigned
1698 marsh sediments and biodiversity behave differently than those in natural marshes, likely
1699 due to hydrogeographic constraints. Natural marshes exhibited a heterogeneity and
1700 variability within sediments that was absent from realigned marshes. The high bulk density
1701 and low water content of the realigned AH marsh is an indication of a relic agricultural
1702 sediment which is purported to remain mostly unchanged post inundation (Cai et al.,
1703 submitted; Tempest et al., 2015). This unchanged relic layer in realigned salt marshes has
1704 been observed by Tempest et al. (2015) at the Orplands Farm site (Blackwater Estuary, SE
1705 England) which showed that the relic agricultural layer remained unchanged with marine
1706 sediment deposited on top post sea wall breach and inundation. However, we observed that
1707 the oldest marsh (FR, 118 years) was also significantly different to its natural pair, whereas
1708 Mersea Island (62 years) was matching sediment characteristics of a natural marsh. This
1709 difference in our developing sites could be explained by relative spatial location, both to each
1710 other and within the Colne/ Blackwater estuary. AH realigned marsh is a sheltered, steep
1711 sloped, low lying agricultural field which could have affected sediment deposition and
1712 inundation of the site throughout the years. In contrast, MI realigned marsh is more exposed
1713 and surrounded by creeks which allows better drainage of the site, thus facilitating a more

1714 natural development of the marsh. Topography of salt marshes can affect drainage of a
1715 marsh, sediment structure and in turn development of plant vegetation. Realigned marsh
1716 topography is different to natural, as they have an enhanced potential for water
1717 accumulation and lower creek densities (Lawrence et al., 2018).

1718 Plant biodiversity in realigned marshes was found to be lower than in natural sites. Despite
1719 our study sampling at specific plant dominant locations within consistent, set elevations
1720 (mid-marsh) our results match previous natural/realigned comparisons of saltmarsh
1721 biodiversity (e.g. Garbutt and Wolters, 2008; Mossman et al., 2012). However, α -biodiversity
1722 of our oldest realigned site (FR) was not significantly different from the youngest (AH) but
1723 was significantly different from MI which re-emphasizes the idea that re-establishment of a
1724 marsh is complex and age of a realigned marsh (years from initial inundation) does not
1725 adequately predict return of a “natural-state” marsh. Topography (i.e. slope and creek
1726 formation) and hydrogeology of a marsh could be a more determining factor of realigned
1727 salt marsh plant development than age. In addition, changes on short-term scales, or
1728 immediately after breach are often overlooked even though biotic and abiotic factors that
1729 are likely to affect sediment development could be heavily influence by initial conditions set
1730 within the first year of salt marsh re-establishment.

1731 To explore how initial conditions could affect sediment development of a realigned saltmarsh
1732 we examined the effect of total sediment deposition and previous land use on a newly
1733 realigned saltmarsh during the first-year post inundation (Chapter 3). 14 months after
1734 inundation the two realigned sites were broadly similar despite their different starting
1735 conditions. Pasture field had more nutrients (mostly NH_4^+) than the arable field post
1736 inundation which could potentially influence development of vegetation, invertebrate and
1737 microbial communities. Furthermore, our realigned site closely matches the natural marsh
1738 conditions for nutrients and pH for all depths to 20cm below the surface, but bulk density

1739 and water content only matched natural marsh sediments in the 0-5cm depth, where marine
1740 sediments had accreted during the 14 months inundation period (Figure 3.8). Although the
1741 realigned site surface sediments matched natural conditions by November 2016 the natural
1742 marsh remained more heterogeneous than the realigned site, similar to our older marshes
1743 sampled in Chapter 2.

1744 The homogeneity of realigned sites might contribute to the differences observed in plant
1745 diversity and composition over time (Garbutt & Wolters 2008). Differences between natural
1746 and realigned sediments density and moisture at lower depths in the realigned site may also
1747 play a role since they did not change significantly in the 14 months post induction, which
1748 affects the hydrology of the managed marshes. Tempest et al. (2013) found that in realigned
1749 sites the agricultural relic soils remained the same over time, constricting water movement
1750 within the sites. In our realigned sites, the top 5cm of sediment and subsequently the newly
1751 deposited marine sediment, was where all the conditions match those of the natural marsh,
1752 whereas lower depth sediments, especially the deepest depth range of 10-15cm, showed
1753 little change over time.

1754 Furthermore, we aimed to examine the effect of bioturbators (organisms bigger than 250 μ m)
1755 in the development of sediment characteristics during the 1st year of marsh realignment
1756 between two different previous land uses (arable and pasture). We compared sediment
1757 changes (physiochemical) in areas of invertebrate exclusion and invertebrate colonization
1758 between arable and pasture realigned fields and monitored invertebrate colonization in the
1759 newly realigned saltmarsh within the 1st year post inundation (Chapter 4). The realigned site
1760 was successfully colonized by macro-invertebrates within 5 months of inundation and
1761 species numbers increased by the final sampling (14 months post inundation). Organisms
1762 found within our realigned sites are typical of natural and realigned saltmarshes in east
1763 England (e.g. Garbutt et al., 2006; Jackson et al., 1985) and recreated intertidal mud flats in

1764 the UK (e.g. Evans et al., 1998). Our realigned sites had similar abundances of *P. ulvae* as other
1765 studies however abundance of bivalves and ragworms was lower in our realigned site over
1766 the post-inundation period studies. Within our treatments we observed a higher abundance
1767 of organisms in our exclusion control chambers than undisturbed areas. This might be due
1768 to the breakdown of the relic sediment layer that occurred during installation of the
1769 chambers, as well as gaps created during placing of the chambers which allowed organisms
1770 to penetrate/fall further down the sediment column to depths that otherwise would be sub-
1771 optimal (>10cm) (Chandrasekara and Frid, 1998; Huxham et al., 1995). Furthermore, Craft
1772 (2000) found that development of communities of benthic invertebrates in realigned salt
1773 marshes depended on wetland sediment formation and development. We observed that
1774 exclusion control chambers behaved differently from the undisturbed chambers, having
1775 lower bulk density in the top 5cm as well as higher water content, which might have allowed
1776 the formation of more ideal sediment conditions for macro-invertebrate colonization. The
1777 development of the invertebrate community is dependent on the formation of an organic
1778 rich surface later to support the detritus feeding organisms (Craft, 2000) which can explain
1779 the increase in community numbers in our site from February to November.

1780 Benthic macro-invertebrates are an important component of salt marsh establishment and
1781 function as they are involved in sediment bioturbation (Bertness, 1985), biogeochemical
1782 cycling (Alkemade et al., 1992) and are a source of food for higher invertebrates, fishes and
1783 birds (Adam, 1990). However, in early establishment of marshes Paramor and Hughes (2004)
1784 found that ragworm, *Hediste diversicolour*, can inhibit the development of plants as it feeds
1785 on plant seedlings. Thus, macro-invertebrate colonization of realigned saltmarshes can have
1786 a diverging impact on marsh development. Invertebrates within newly realigned marshes
1787 could help develop the sediment characteristics through their burrowing activities (i.e.
1788 increasing sediment aeration/water drainage and nutrient movement) but also may inhibit
1789 through active feeding on plant seedlings and delaying plant colonization.

1790 Our invertebrate exclusion experiment has shown that mechanical disturbance of the
1791 sediment can influence its geochemical characteristics in newly realigned saltmarsh (Chapter
1792 4). Macro-invertebrate colonization of a newly realigned site can also create disturbance
1793 (bioturbation) and influence geochemical characteristics, as we observed by the differences
1794 in geochemical properties of invertebrate colonization chambers. Mechanical disturbance of
1795 the relic agricultural layer prior to flooding of the site can also affect those changes similarly
1796 and on occasion have greater effect (faster recovery time) as seen in our exclusion control
1797 chambers. This active breakdown of the relic layer occurred during the installation of the
1798 exclusion chambers (especially exclusion control chambers), stimulating a more active
1799 mixing of the sediment profile, which allowed for deeper penetration of water within the
1800 sediment post flooding. A combination of active disruption of the relic layer (e.g. plowing)
1801 and colonization of invertebrates may accelerate the necessary shift of sediment
1802 geochemical characteristics of newly realigned marshes from terrestrial to marine.

1803 In addition to the macro-invertebrates' effect on sediment characteristics, we examined
1804 their impact on microbial communities (Chapter 5). Microbial communities between the
1805 studied natural and realigned sites were significantly different, with natural marsh having, in
1806 general, higher species abundance. Significant differences were observed as well between
1807 the arable and pasture realigned sites. pH, water content and bulk density of the sediments
1808 were found to be significant drivers of the communities present. Unsurprisingly, microbial
1809 communities appeared to be more affected in the exclusion control chambers than the full
1810 exclusion and undisturbed controls, in line with our observations of sediment characteristics.
1811 Overall, we found that the studied natural marsh microbial community had different
1812 organism abundances and composition compared to the realigned marshes. Within
1813 realigned sediments, even 14 months post inundation, many communities do not have
1814 similar abundances to natural marsh sediments and are comprised of different communities
1815 which could affect the functionality of the system and its ability to regenerate to natural

1816 marsh condition. Microbial communities are responsible for nutrient cycling in the sediment
1817 (Li et al., 2002; Lillebø et al., 1999; Yamada et al., 2007) which in turn can affect plant
1818 productivity/composition thus affecting the regeneration of a realigned marsh into a more
1819 natural community.

1820 6.1 *Conclusion*

1821 Overall, this study has shown the realigned marshes behave differently to natural marshes
1822 in respect to sediment characteristics, plant biodiversity and microbial communities. The
1823 presence of the relic agricultural sediment layer is evident in our realigned sites particularly
1824 in our youngest sites. This layer affects the hydrology of the system and consecutively the
1825 development of plant biodiversity and microbial communities similar to natural marshes.
1826 Pre-restoration land-use can affect the structure of restored salt marshes with implications
1827 for functioning and delivering of ecosystem services (Spencer et al., 2017). Hydrogeology of
1828 saltmarshes influences fluxes of nutrients in the system by affecting water movement. Water
1829 movement in sediment is linked not only to sediment compaction (bulk density) but also to
1830 vegetation composition (root structure and architecture) as well as biological bioturbation.
1831 Invertebrate presence and composition affect the rate and magnitude of biological
1832 bioturbation which in turn affects the microstructure of sediment. Changes in sediment
1833 microstructure alters both the physical space (creation of oxic or anoxic sediments) and
1834 substrate availability for microbial communities. Since microbial communities are the drivers
1835 of bulk carbon fixation, nutrient concentrations and GHG emissions, their diversity and
1836 presence can determine the development of sediment characteristics of a realigned marsh.
1837 Success of restoration is measured in the ability of the site to provide the desired ecosystem
1838 services, (i.e. biodiversity, coastal protection, habitat creation) (Strange et al., 2002). Short-
1839 term recoveries and set goals may not imply long term sustainability (Zedler et al., 2001).

1840 Even when species densities within realigned marshes match those of natural marshes,
1841 functional measures often reveal a significant lag of ecological processes recovery, such as
1842 nutrient cycling, and microbial communities (Chapter 5) that are necessary for full
1843 functionality of a marsh.

1844 Through our study (as well as other studies) we have shown that realigned marshes are
1845 showing differences both between each other but also with natural referenced marshes
1846 (Garbutt and Wolters, 2008; Garbutt et al., 2006; Lawrence et al., 2018; Mossman et al.,
1847 2012b, 2012a; Sullivan et al., 2017; Tempest et al., 2015; Wolters et al., 2005b). These
1848 differences could be attributed to physicochemical properties of the sediment; such as soil
1849 drainage (due to denser lower sediments) (Burden et al., 2013; Spencer et al., 2008; Tempest
1850 et al., 2015), nutrient cycling (Burden et al., 2013) seed availability (Wolters et al., 2005a),
1851 and previous land use (Garbutt et al., 2006; Spencer et al., 2017).

1852 Further studies of realigned sites are required to fully access how spatial variation (location
1853 and exposure), previous land use and active disturbance of the site pre-flooding, can affect
1854 the rate of marsh development and delivery of those services. Our study showed an impact
1855 of sediment disturbance prior to flooding on recovery of sediment characteristics. However,
1856 further studying of this effect is required over a longer period to fully access its influence on
1857 development and functionality of the realigned marsh.

1858

Appendix A Supplementary tables

1859

Table A. 1. Average water content (%) (\pm SE) for all saltmarshes at each condition and season

Water content (%)	Abbotts Hall		Mersea Island		Fingringhoe Range	
	Natural	Realigned	Natural	Realigned	Natural	Realigned
<i>Atriplex</i>	73.4 \pm 1.8	22.0 \pm 0.7	59.5 \pm 1.5	58.2 \pm 0.9	70.4 \pm 2.2	75.5 \pm 1.0
<i>Limonium</i>	77.9 \pm 0.4	23.2 \pm 0.4	68.1 \pm 0.9	63.4 \pm 0.5	73.4 \pm 1.4	80.5 \pm 0.4
Mud	63.0 \pm 2.2	23.0 \pm 0.6	60.5 \pm 0.7	55.8 \pm 2.3	56.6 \pm 1.3	77.7 \pm 0.9
<i>Puccinellia</i>	75.0 \pm 1.7	22.0 \pm 0.7	60.6 \pm 1.6	59.3 \pm 0.9	69.4 \pm 1.2	78.3 \pm 0.8
Summer	70.2 \pm 2.2	21.6 \pm 0.5	60.1 \pm 1.5	60.1 \pm 0.8	70.8 \pm 2.3	76.4 \pm 1.0
Autumn	72.4 \pm 2.2	23.0 \pm 0.7	60.4 \pm 1.3	55.1 \pm 1.8	63.8 \pm 2.1	79.7 \pm 0.6
Winter	73.6 \pm 1.6	23.6 \pm 0.4	64.6 \pm 1.5	62.2 \pm 0.7	68.1 \pm 1.8	77.6 \pm 0.8
Spring	73.5 \pm 1.9	22.1 \pm 0.6	62.9 \pm 1.2	59.1 \pm 1.8	68.5 \pm 2.0	77.9 \pm 0.9

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Table A. 2. Average bulk density (g/cm³) (\pm SE) for all saltmarshes at each condition and season

Bulk density (g/cm ³)	Abbotts Hall		Mersea Island		Fingringhoe Range	
	Natural	Realigned	Natural	Realigned	Natural	Realigned
<i>Atriplex</i>	0.27 \pm 0.03	1.38 \pm 0.03	0.44 \pm 0.02	0.46 \pm 0.02	0.31 \pm 0.03	0.23 \pm 0.02
<i>Limonium</i>	0.22 \pm 0.01	1.24 \pm 0.03	0.34 \pm 0.01	0.35 \pm 0.01	0.28 \pm 0.02	0.17 \pm 0.01
Mud	0.38 \pm 0.04	1.32 \pm 0.03	0.43 \pm 0.02	0.51 \pm 0.05	0.47 \pm 0.02	0.18 \pm 0.01
<i>Puccinellia</i>	0.24 \pm 0.02	1.32 \pm 0.03	0.42 \pm 0.02	0.42 \pm 0.02	0.31 \pm 0.02	0.18 \pm 0.01
Summer	0.27 \pm 0.03	1.26 \pm 0.03	0.42 \pm 0.02	0.38 \pm 0.01	0.29 \pm 0.03	0.20 \pm 0.02
Autumn	0.29 \pm 0.03	1.32 \pm 0.03	0.38 \pm 0.02	0.45 \pm 0.03	0.40 \pm 0.03	0.16 \pm 0.01
Winter	0.29 \pm 0.03	1.34 \pm 0.03	0.39 \pm 0.02	0.39 \pm 0.01	0.33 \pm 0.02	0.19 \pm 0.01
Spring	0.26 \pm 0.03	1.36 \pm 0.03	0.45 \pm 0.02	0.51 \pm 0.05	0.33 \pm 0.03	0.21 \pm 0.01

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Table A. 3. Average pH (\pm SE) for all saltmarshes at each condition and season

pH	Abbotts Hall		Mersea Island		Fingringhoe Range	
	Natural	Realigned	Natural	Realigned	Natural	Realigned
<i>Atriplex</i>	6.47 \pm 0.06	6.56 \pm 0.03	7.08 \pm 0.06	7.54 \pm 0.05	6.43 \pm 0.23	5.30 \pm 0.36
<i>Limonium</i>	6.49 \pm 0.05	6.57 \pm 0.05	6.82 \pm 0.08	7.36 \pm 0.02	6.08 \pm 0.25	5.10 \pm 0.35
Mud	4.87 \pm 0.31	6.63 \pm 0.07	6.56 \pm 0.33	6.77 \pm 0.33	6.41 \pm 0.37	4.99 \pm 0.46
<i>Puccinellia</i>	6.37 \pm 0.08	6.54 \pm 0.06	7.08 \pm 0.07	7.53 \pm 0.06	6.33 \pm 0.23	4.29 \pm 0.31
Summer	6.28 \pm 0.19	6.61 \pm 0.02	6.91 \pm 0.18	7.43 \pm 0.15	6.44 \pm 0.11	5.60 \pm 0.35
Autumn	5.93 \pm 0.23	6.54 \pm 0.03	6.99 \pm 0.11	7.44 \pm 0.04	6.27 \pm 0.34	3.91 \pm 0.32
Winter	5.96 \pm 0.25	6.63 \pm 0.03	6.92 \pm 0.20	7.19 \pm 0.24	5.95 \pm 0.32	4.83 \pm 0.42
Spring	6.01 \pm 0.20	6.53 \pm 0.09	6.75 \pm 0.20	7.13 \pm 0.23	6.59 \pm 0.22	5.24 \pm 0.34

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1873 Table A. 4. Average percentage cover of above ground biomass at each natural and realigned

1874 saltmarsh (SE)

Percentage cover (%)	Abbotts Hall		Mersea Island		Fingringhoe Range	
	Natural	Realigned	Natural	Realigned	Natural	Realigned
Bare Soil	23.6 (3.8)	4.8 (0.8)	15.7 (3.0)	10.7 (1.4)	16.7 (3.2)	14.4 (3.1)
<i>Armeria maritima</i>	9.6 (1.1)	0 (0)	0 (0)	0.2 (0.2)	2.4 (0.7)	0 (0)
<i>Aster tripolium</i>	2.0 (0.4)	0.8 (0.3)	6.8 (1.0)	3.8 (0.5)	5.0 (0.7)	2.9 (0.4)
<i>Atriplex portilacoides</i>	15.3 (2.2)	51.3 (2.3)	18.9 (1.5)	7.9 (1.0)	18.1 (1.6)	7.3 (1.0)
<i>Colchlearia anglica</i>	0.2 (0.1)	2.0 (1.0)	4.6 (0.8)	0.2 (0.1)	1.6 (0.4)	0.5 (0.2)
<i>Festuca rubra</i>	0 (0)	0.8 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Juncus spp</i>	1.4 (0.6)	0 (0)	0 (0)	0 (0)	2.6 (0.8)	0.1 (0.1)
<i>Limonium vulgare</i>	27.5 (2.8)	4.1 (0.8)	4.5 (1.3)	2.7 (0.8)	6.9 (1.4)	6.0 (0.7)
<i>Plantago maritima</i>	3.1 (1.0)	0 (0)	0.7 (0.3)	0 (0)	0.7 (0.6)	0.1 (0.1)
<i>Puccinellia maritima</i>	39.2 (2.7)	38.5 (2.7)	54.0 (2.2)	66.1 (1.8)	51.0 (3.4)	62.4 (2.7)
<i>Salicornia europaea</i>	7.3 (1.2)	0.3 (0.1)	1.6 (0.3)	0.3 (0.1)	5.8 (1.3)	1.0 (0.2)
<i>Sarcoconia perennis</i>	0.9 (0.4)	0.1 (0.1)	0.6 (0.3)	0.1 (0.1)	0 (0)	0 (0)
<i>Spartina anglica</i>	1.2 (0.4)	0 (0)	3.4 (1.2)	11.9 (1.3)	3.4 (1.1)	18.0 (1.9)
<i>Spergularia media</i>	1.6 (0.2)	2.2 (0.3)	3.1 (0.8)	0.8 (0.6)	0.1 (0.1)	0.1 (0.1)
<i>Suaeda maritima</i>	1.8 (0.4)	1.4 (0.5)	9.2 (1.1)	8.5 (1.0)	7.3 (2.4)	2.4 (0.4)
<i>Triglochin maritima</i>	0.8 (0.2)	0 (0)	0.3 (0.2)	0.4 (0.3)	2.2 (0.6)	0 (0)

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Table A. 5 . Density of invertebrates in m³ per 5cm depth zone for February and November. No organisms were detected in the arable field Full chamber for February.

“ND” = Not Detected. \pm SE

	February					November					
	Arable		Pasture			Arable			Pasture		
	Undisturbed	Exc. Control	Undisturbed	Exc. Control	Full	Undisturbed	Exc. Control	Full	Undisturbed	Exc. Control	Full
0-5cm Depth											
<i>Peringia ulvae</i>	2800 \pm 700	4400 \pm 1600	2300 \pm 700	7400 \pm 740	150 \pm 150	18000 \pm 2900	12000 \pm 3800	340 \pm 120	32000 \pm 800	42000 \pm 9100	420 \pm 300
<i>Hediste diversicolor</i>	370 \pm 80	260 \pm 150	150 \pm 60	230 \pm 100	ND	70 \pm 20	35 \pm 35	ND	50 \pm 20	70 \pm 20	ND
<i>Limecola balthica</i>	ND	ND	ND	ND	ND	890 \pm 140	730 \pm 130	ND	580 \pm 250	640 \pm 350	ND
<i>Cerastoderma edule</i>	ND	ND	ND	ND	ND	40 \pm 10	50 \pm 30	ND	27 \pm 27	36 \pm 20	ND
5-10cm Depth											
<i>Peringia ulvae</i>	260 \pm 130	1100 \pm 100	270 \pm 230	7600 \pm 1400	ND	110 \pm 10	990 \pm 570	ND	320 \pm 90	7000 \pm 6600	44 \pm 22
<i>Hediste diversicolor</i>	10 \pm 10	88 \pm 50	ND	30 \pm 10	ND	27 \pm 17	80 \pm 40	ND	20 \pm 10	340 \pm 300	ND
<i>Limecola balthica</i>	ND	ND	ND	ND	ND	10 \pm 10	ND	ND	ND	80 \pm 80	ND
<i>Cerastoderma edule</i>	ND	ND	ND	ND	ND	27 \pm 17	ND	ND	ND	10 \pm 10	ND
10-15cm Depth											
<i>Peringia ulvae</i>	27 \pm 27	260 \pm 40	ND	4100 \pm 740	ND	40 \pm 30	270 \pm 180	27 \pm 17	27 \pm 27	1700 \pm 1600	100 \pm 90
<i>Hediste diversicolor</i>	ND	50 \pm 30	ND	18 \pm 18	ND	ND	40 \pm 20	ND	ND	ND	ND
<i>Limecola balthica</i>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Cerastoderma edule</i>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

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