The effects of shorebirds on the erodibility, properties and ecosystem functioning of intertidal muddy sediments

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Abstract

We are only beginning to understand the complex interactions between shorebirds and other organisms on intertidal mudflats. Migratory shorebirds are indicators of ecosystem functioning; their distribution and abundance can signal trends at local and global levels. Mudflats support a highly productive community of microphytobenthos (MPB) which are at the interface between sediment and the water column and influence ecosystem functioning. The MPB facilitate nutrient exchange and bind the sediment surface reducing erodibility of the sediment.

A series of manipulative field and laboratory experiments were carried out across three sites in East Anglia, UK, to determine the effects of wintering shorebird presence, density, species composition and bioturbation on MPB biomass and key ecosystem functioning parameters mediated by MPB. Shorebird presence was controlled using exclosures, shorebird density was manipulated using partial exclosures, species assemblage was compared among study sites and bioturbation was manipulated by artificially mimicking shorebird ambulatory movement. Response of chlorophyll *a* to treatments was measured *in situ* by proxy using PAM fluorometry and *ex situ* using laboratory techniques. Ecosystem function responses were sediment erodibility, measured *in situ* using a Cohesive Strength Meter, and fluxes of oxygen, nutrient and organic matter between the sediment surface and water column. Fluxes were measured *ex situ* using cores extracted from treatment plots and sampled within controlled mesocosms.

In a single site experiment shorebird presence had a significant effect on the key ecosystem functions of erodibility and fluxes of nitrate, nitrite, ammonium, phosphate and dissolved organic carbon (DOC). Within a multi-site experiment the best fitting LME model found highly significant interactions between shorebird density, species assemblage and site, with effects of density on MPB biomass varying among sites and depending on species assemblage. Principal component analysis demonstrated that nitrite flux was positively correlated with shorebird density and negatively correlated with bird assemblage scores across all three sites. The artificial manipulation experiment identified surface bioturbation by shorebirds as a process by which the observed effects described above were driven; i.e. that ‘low level’ shorebird ambulatory bioturbation significantly increased MPB biomass and erosion resistance, with significant effects on fluxes of ammonium, nitrate and DOC. These findings indicate that due to top-down effects on MPB, the primary producer on intertidal mudflats, shorebirds can be considered ecosystem engineers.

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Contents

List of Tables

List of Figures

List of Images

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Glossary and abbreviations

Muddy sediment	Non-indurated, moist, siliclastic sediments with grain sizes predominantly <0.063 mm.
Microphytobenthos (MPB)	Assemblages at the surface of marine sedimentary habitats of photosynthetic single-celled organisms including diatoms, cyanobacteria, green algae and flagellates.
Extracellular polymeric substances (EPS)	Natural polymers secreted by organisms (in this context mostly by MPB).
Biofilm	A population of microorganisms (in this context comprising MPB) bound together by an EPS matrix. This term can apply at micro and macro scales, although ‘mats’ can be a more useful term when describing visible patches (accumulations) of biofilms on a mudflat.
Infauna	Multicellular organisms inhabiting a sedimentary environment (in this context an intertidal mudflat).
Macrofauna	Infauna retained on a sieve with mesh size $\geq$0.5 mm.
Erodibility	The susceptibility of a material (in this context intertidal muddy sediment) to resuspension. The point of erosion is defined by an arbitrary but fixed amount of sediment resuspension depending on measurement device.
Stability	A frequently used term referring to erodibility, though conversely termed (ie sediment with greater stability has less erodibility).
Colloidal carbohydrate	Polymers remaining in suspension after aqueous extraction and centrifugation, used as a proxy to determine approximate EPS content of a sample.
Bioturbation	Biological reworking of soils and sediments.
Incubation	In this context, this refers to the dark or light conditions which flux cores were adapted to and subject to during sampling for oxygen, nutrient and organic matter concentration analysis.
F_o	Dark adapted minimum fluorescence yield (proxy *in situ* measurement for MPB biomass)
T_{cr}	Critical erosion threshold (measurement of sediment erodibility)

ABB	Artificial bird bioturbation
Chl *a*	Chlorophyll *a*
ANOVA	Analysis of Variance
LME	Linear mixed effects (model)
CI	Confidence interval (95%)
PCA	Principal component analysis
Cos2	Value (0-1) indicating the quality of representation of a variable on the principal component
nMDS	Non-metric multidimensional scaling
DOC	Dissolved organic carbon
TON	Total organic nitrogen

Thesis Introduction

As described in the book 'Mudflat Ecology' (Beninger 2018a):

"*the oldest known fossils of any life form, correspond to filamentous cyanobacteria colonizing coastal sediments in the form of biofilms and microbial mats*"

Indeed, the earliest known bird track fossils are from coastal sediments in the early Cretaceous, showing that interactions discovered herein may have been occurring for up to 140 million years.

1.1 Introduction

This research project employs a collaborative and innovative approach, combining classic sedimentological, proven biogeochemical, established ecological and emerging ornithological tools and techniques to dig into the muddy waters of biosedimentology from a new angle. It explores the effects of shorebirds on the microphytobenthic biomass erodibility and nutrient cycling of the valuable intertidal muddy habitat upon which they rely, considering whether effects may be driven directly or indirectly through potential effects on macrofauna.

Evidence supporting the effects of shorebirds on the erosion and properties of intertidal muddy sediments was introduced by Daborn *et al.* (1993) during a study of factors controlling erodibility on an intertidal mudflat in Canada. It was found that upon arrival of large numbers of migratory semipalmated sandpiper *Calidris pusilla*, the behaviour and grazing rate of the dominant primary consumer *Corophium volutator*, a major prey item of this sandpiper, was altered. The authors concluded that this resulted in proliferation of a primary producer (MPB biofilms) and consequently increased mean sediment erodibility (Daborn et al. 1993). This paper was the seed of the ideas for this research project.

Although the study described above relies on coincidental data without experimental manipulation to test a hypothesis relating to shorebirds, this work highlights an area of shorebird science which is potentially of great importance to our understanding of intertidal mudflat ecology and the top-down effects of shorebird activity on intertidal muddy sediments.

This thesis presents work which builds on previous research to explore how shorebird presence/absence, density, species community and ambulatory movements may cause top-down effects on mudflat characteristics. This was achieved through a series of field experiments manipulating shorebird parameters and measuring how sediment characteristics were accordingly altered. This work then goes further to investigate how these changes in sediment characteristics may translate to an overall effect of shorebirds on ecosystem functioning and ecosystem services.

In this chapter, the importance of mudflats as a habitat and resource is explored, to give context to the research. The organisms interacting within this community are discussed, including their currently known effects on sediment properties. The complex processes and interactions determining the erodibility of intertidal sediments are also examined, to set the stage for the research undertaken.

1.2 Why Shorebirds?

Shorebird populations are in drastic decline globally due to pressure on a range of habitats which they use for breeding, replenishment during migration (stopover sites) and overwintering (Koleček et al. 2021). Migratory shorebirds are most at risk, likely due to the cumulative impacts of these pressures (Koleček et al. 2021). A recent key text on shorebirds and mudflat ecology emphasises that shorebirds are 'major players' in mudflat food webs but are under-acknowledged in the mudflat ecology literature (Mathot et al. 2018). It is understood that shorebird distribution and behaviour are indicators of ecological patterns within sediments, both spatially and temporally, and the authors call for further research and investigation into the roles of shorebirds on mudflats, to enable effective mudflat conservation and restoration (Mathot et al. 2018). However, Bowgen *et al* (2015) highlight the danger in using birds as indicators of ecosystem health, due to 'masking effects' which can arise from delays between invertebrate population structures and response from more adaptable shorebird predators. This illustrates the relevance of this study; further understanding of the links between shorebirds, mudflats and infauna, both generally and in a species-specific context, is required to enable successful shorebird conservation.

1.3 Why Intertidal Muddy Sediments?

Intertidal and soft marine sediments cover approximately two thirds of the Earth's surface (Rosenberg et al. 2007). Such muddy sediments in the intertidal are known as mudflats, a sedimentary habitat consisting of mostly silts and clays deposited in low water velocity and wave energy marine or aquatic environments, such as estuaries. The sediment typically has a high organic content and is generally found upstream of the river mouth where wave action and salinity are lower (JNCC 2016).

Many modern tidal flats were formed relatively recently (during the Holocene, 10,000 YBP), by fine grained silt and clay deposits transported via river, from offshore or from deposits already present in coastal regions (Pan et al. 2018). These hydrological features are shaped predominantly by tidal currents, with wave action a secondary mechanism (Pan et al. 2018).

Working Group 106 of the Scientific Committee on Ocean Research (SCOR), which was established to increase understanding of muddy coasts, defined muddy coasts as:

> *"a sedimentary-morphodynamic type characterized primarily by fine-grained sedimentary deposits—predominantly silts and clays—within a coastal sedimentary environment. Such deposits tend to form rather flat surfaces, and are often, but not exclusively, associated with broad tidal flats"* (Wang et al. 2002)

Mud has been defined as a mixture of predominantly fine-grained clay- and silt-sized sediments, porewater and organic matter. Water tension and clay minerals with charged surfaces often lead to cohesion within the mud which is also enhanced by organic matter. Fine grains typically retain low permeability, allowing maintenance of porewater and 'wetness' during low tide when intertidal mud becomes aerially exposed (Pan et al. 2018).

The term 'mud' (or 'fine grain') refers to inorganic sediment <62.5 µm including material in the clay (0.5-3-9 µm) and silt (3.9-62.5 µm) classes. Comprehensive descriptions of the chemical compounds and subsequent bonding forces within and between intertidal muddy sediments are given in Pan *et al.* (2018). The physical attributes of a mudflat which most contribute to its formation and geomorphology have been found to be tidal range, exposure to wave action and mudflat slope (Dyer et al. 2000). Wave exposure is a function of mudflat orientation relative to the prevailing wind and maximum fetch, while slope was given as: mean tidal range/mudflat width (Pan et al. 2018). As such, these factors are important when comparing cross-site effects of tested variables, and should be considered, particularly where they differ notably.

Mudflats provide valuable habitat throughout the tidal cycle, for benthic and pelagic organisms inhabiting and feeding on them (Beninger 2018a). Terrestrial organisms including humans also benefit from their existence, through resource provision and nutrient cycling (Beninger 2018a). Mudflats were formally internationally recognised for their significant benefit to humans and biodiversity by the adoption of the Ramsar Convention on Wetlands of International Importance in 1971. Alongside salt marshes, tidal flats provide some of the most important and valuable ecosystem services for humans and other species (Foster et al. 2013), which are discussed below in Section 1.4. Principal arguments for the protection of mudflats include conservation due to them supporting internationally important numbers of waterbirds (McMullon 2008) and offering significant economic benefits by providing natural 'soft' sea defences and flood defence

(Huggett 2008), reducing the need for costly artificial ‘hard’ flood defences (van Zelst et al. 2021). Hard defences prevent natural coastal habitats from migrating landward in the face of sea level rise (a process known as coastal squeeze) (Pethick 1993), and continued erosion of tidal flats reduces wave attenuation, which puts further pressure on hard structures (Cooper et al. 2001).

The value of wetlands (including mudflats) to waterbirds has long been assessed based upon the ‘1% threshold’ (whether or not 1% or more of a species’ population uses a particular area) and is the criteria used in the designation of Ramsar sites (Taylor 2002). However, more recent alternative assessment criteria have been suggested which aim to incorporate species richness and diversity and therefore introduce assessments which consider species that may not reach the 1% threshold, but nonetheless contribute to the ecological functioning of the ecosystem (Harebottle and Underhill 2016). Therefore, as the scientific community moves towards a more comprehensive approach to wetland assessment, it would add value to better understand the interactions between particular avian species and wetland environments. For example, analysis of intertidal mudflat food webs has shown birds to be dependent upon macrofauna biomass in Brouage, France, where benthic activity dominated primary productivity and carbon transfer (Leguerrier et al. 2003). Results from this study also suggested that primary production by microphytobenthos (MPB) was the most important source of carbon in an intertidal mudflat food web.

Impacts of tidal flat loss on birds has been an area of study since at least the 1970s, with research suggesting that the level of impact of loss of foraging area is dependent upon the species involved and the area lost (Goss-Custard 1977a). The need to protect and promote intertidal mudflats at a national level also led to this habitat becoming included within the UK Biodiversity Action Plan, which states that the extent of the UK’s mudflats should be maintained ((JNCC) 2016). However, despite recognition and conservation efforts, intertidal mudflats continue to be lost due to development and sea level rise (Mazik et al. 2010, Zhang et al. 2019). Due to continual accretion and erosion of mudflats and salt marsh, coupled with a lack of mapping of these habitats, predictions of loss rates are difficult and precarious, with few published (Foster et al. 2013). However, an average loss of 230 ha of intertidal mudflats per year between 1998 and 2048 in England and Wales alone, due to coastal squeeze and sea-level rise, has been predicted (Lee 2001). Loss of intertidal mudflats is global, with loss predicted between 20% and 70% in the United States at a 2°C global warming scenario (Galbraith 2002) and loss of 93% of an important bird staging mudflat, to development, in China (Zhang et al. 2019).

1.4 Why Ecosystem Services?

Ecosystem services (ES) do not have a universal definition, but a broad and frequently cited one describes ES as “the direct and indirect contributions of ecosystems to human well-being” (Braat and De Groot 2012). A more colloquial definition is provided by the UK National Ecosystem Assessment, as “Ecosystem services are the benefits provided by ecosystems that contribute to making human life both possible and worth living” (2012). A more recent classification states that “the biosphere’s regeneration is key to the sustainability of the human enterprise” (Dasgupta 2021). The term ES has received much attention in recent decades (Bull et al. 2016), and has had widespread use as a rationale for conservation globally, although it is clear that the scientific community struggles to accept the application of this economic outlook to all areas of natural science (Bull et al. 2016). Challenges include conflict of the concept with biodiversity conservation, the fear of ‘selling out’ on nature, treating nature as a commodity and the difficulty with accuracy and subsequent vagueness (Schröter et al. 2014). Ecosystem services are intrinsically linked to ecosystem functioning (EF) (Balvanera et al. 2006), which is “the capacity of natural processes and components to provide goods and services that satisfy human needs, either directly or indirectly” (De Groot et al. 2002). For example, the functioning of the marine ecosystem provides many goods and services to humans, part of which is formed by mudflat and saltmarsh habitat, as described below.

An analysis of intertidal mudflat and saltmarsh services, classified according to the Millennium Ecosystem Assessment (2005) describes the following (Foster et al. 2013): Provisioning services include food (grazing, foraging, wildfowling and fish nurseries), biochemical products (medicinal plants) and genetic material (saltmarsh endemic plants). Supporting services include biodiversity, soil formation and nutrient cycling. Cultural services include spiritual/inspirational (though to a lesser extent than other habitats), recreational/aesthetic and educational. Regulating services include climate regulation (through uptake of CO_2 by plants/algae), hydrological regimes (as components of the water cycle), pollution control and detoxification (sediments can absorb heavy metals and organic compounds), erosion protection (biofilms reduce erodibility in these systems) and natural hazard reduction (wave energy reduction reduces erosion and flooding). Littoral and sub-littoral sediments are the largest coastal and marine sinks of nitrogen, phosphorous and carbon, with individual European marine protected areas alone contributing up to £1.1 billion in nutrient removal and climate damages (Watson et al. 2020). Recent research found that ecosystem condition is also an important element of economic value provided, which varies both spatially and temporally (Watson et al. 2022). For example, coastal and marine ecosystems reaching 'good' ecological status could increase annual service value by £376 million (Watson et al. 2022).

Although many of the ES described have been shown to be provided by saltmarshes, mudflats provide ES in their own right, and are important for saltmarsh formation and maintenance and as such mudflats contribute to maintenance of saltmarsh EF (Adnitt 2007). Evidence has been found that where sediment re-suspension occurs on mudflats it is transported and deposited onto saltmarshes, which has a positive effect on their role in flood defence (Widdows et al. 2000b). Mudflats provide additional 'shelter' to saltmarshes allowing them to persist, and in turn saltmarshes provide sediment sources and a kind of 'insurance' for mudflats against storm waves (Adnitt 2007). Therefore tidal flats and saltmarshes are interdependent coastal systems; their coastal defence provisioning is closely related, such that both are more effective when the other is also present (Adnitt 2007, Mossman et al. 2013).

Ecosystem services can be provided by habitats or individual species and groups, many of which have been shown to provide valuable services upon which humans and other biota rely, for example pollinators (Klein et al. 2007). Birds are highly mobile, and similarly provide a range of ecosystem services. Birds, including shorebirds which depend upon mudflats and saltmarshes for their survival, perform roles as invertebrate and seed dispersers (Green et al. 2008, Barros et al. 2019), although the extent and ecological importance of this role requires further research (Whelan et al. 2008, Barros et al. 2019). ES by birds have the potential to be wide ranging and prolific, with known services including control of infectious disease by scavenging vultures through removal of waste (Grilli et al. 2019, Plaza et al. 2020), pest control through predation in agricultural landscapes (Chain-Guadarrama et al. 2019), seed dispersal (Whelan 2008) and plant pollination through feeding (Anderson et al. 2016) and nutrient cycling through input of guano (Ellis 2005). These processes are likely to have non-analogous effects because many waterbirds are migratory and as such they link ecosystem processes that are greatly separated geographically and temporally (Whelan et al. 2008). Research into ES provided by birds is important due to the current loss of services and urgent need for redirection in conservation action if we are to ensure they are preserved (Girardello et al. 2019).

Some species of mudflat dwelling invertebrates are also considered ecosystem engineers, notably the common ragworm *Hediste diversicolor*, which has significant effects on biofilm and bacterial communities and sediment erodibility through its burrowing activities (see Section 1.11 below). Due to known trophic links between such macrofauna and shorebirds (Mathot et al. 2018) it was originally expected that shorebirds might cause indirect alterations to EF and ES

(Daborn et al. 1993) by affecting changes to macrofauna abundance through predation (Mendonça et al. 2007), distribution through nutrient enrichment via shorebird guano (Palomo et al. 1999) or behaviour in response to potential predation (Glassom 1992, Palomo et al. 1999), although it is acknowledged that bird-macrofauna effects can be difficult to detect (Drake and Arias 1996, Wilson Jr and Parker 1996, Hamilton et al. 2006, Cheverie et al. 2014, Touhami et al. 2017). However, recent advances in the field show that depending upon shorebird species assemblage, direct effects on MPB are equally plausible. For example, some smaller sandpiper species such as western sandpiper *Calidris mauri* (Elner et al. 2005, Kuwae et al. 2008, Beninger et al. 2011, Jardine et al. 2015, Jimenez et al. 2015, Hobson et al. 2022), dunlin *Calidris alpina* (Elner et al. 2005, Mathot et al. 2010, Kuwae et al. 2012, Drouet et al. 2015, Hobson et al. 2022) and semipalmated sandpiper *Calidris pusilla* (Quinn and Hamilton 2012), feed directly on MPB especially during and in preparation for migration. Polyunsaturated fatty acids (PUFAs) within MPB biofilms are high in energy, particularly enhancing avian performance during long-distance flight journeys (Beninger and Elner 2020), which forms current understanding of the likely benefits of biofilm consumption. These relatively recent discoveries in the field of ornithology add increased possibilities to the ways in which shorebirds could affect ES and EF on intertidal mudflats, through direct or indirect effects on MPB.

1.5 Sediment Erodibility

The literature contains a large body of work researching sediment 'stability' and 'erodibility', with these terms used interchangeably in some contexts. For example, many authors (including those on which this research has been based) refer to 'stability' when examining sediment characteristics such as erosion threshold, shear strength and shear stress (Daborn et al. 1993, Underwood and Paterson 1993, Gerdol and Hughes 1994, Thrush et al. 1996, Tolhurst et al. 2000b, Yallop et al. 2000, Tolhurst et al. 2002). Contrastingly, other researchers refer to 'erodibility' when examining shear stress (Ravens and Gschwend 1999) and the term 'erodibility' also appears to be used more within more recent publications (Grabowski et al. 2011, Zheng et al. 2013, Mathew and Winterwerp 2017), with 'erodibility' becoming explicitly differentiated from the term 'stability' when referring to critical shear stress, with stability being used as an overarching term encompassing also bed roughness (Dairain et al. 2020). The term 'stability' is also used to describe other ecological conditions on mudflats, particularly regarding communities (Austen et al. 2002). Therefore, 'erodibility' is used herein, referring to the susceptibility of sediments to erosion, given that this research investigates the threshold shear stress for sediment erosion and a semi-quantitative measure of the erosion rate.

The resistance of sediments to erosion is primarily determined by particle size, shape and density; and, for cohesive sediments, the strength of bonds and attractions between sediment particles, which dictate the ability of the sediment to withstand erosion forces such as wave action or physical disturbance from animals (Tolhurst et al. 2006b). Binding forces depend upon mineralogy, organic matter, types of ions on the surface of particles and the ionic composition of the pore water (Tolhurst et al. 2006a).

Erosion resistance of intertidal sediments allows the build-up, maintenance, and accumulation of mudflats (Beninger and Paterson 2018). This type of 'stability' is a contributing factor to erosion thresholds and rates in these environments, and therefore is an important consideration in their assessment and management, particularly from an engineering aspect (Miller et al. 1977, Pan et al. 2018). The maintenance of a sedimentary deposit provides habitat for organisms ranging from singular eukaryotic cells to micro and macro invertebrates and megafauna such as fish and shorebirds (Austen et al. 2002, Mathot et al. 2018, Moens and Beninger 2018, Van Colen 2018).

Erosion resistance of a mudflat and its susceptibility to erosion contributes to its functioning as a flood defence mechanism, which is a direct ecosystem service provided by this habitat (Spalding et al. 2014). Several other services are also provided and are discussed above in Section 1.3.

Many different factors, both physical and biotic, can affect the strength and erodibility of muddy sediments (see Sections 1.6, 1.10 and 1.11). In abiotic sediments, water content and bulk density are inversely correlated, and bulk density is positively correlated with sediment erodibility: therefore, as water content decreases the erodibility of a cohesive muddy sediment decreases (Tolhurst et al, 2000a). Accordingly, rain reduces the erosion threshold, particularly in muddier rather than sandier sediments (Tolhurst et al. 2006b).

The term 'bioturbation' is used to describe "*all transport processes carried out by animals that directly or indirectly affect sediment matrices. These processes include both particle reworking and burrow ventilation*" (Kristensen et al. 2012). Through these mechanisms, infauna can significantly affect sediment erodibility (Dairain et al. 2020). To test this experimentally, Gerdol and Hughes (1994) used an insecticide to remove *Corophium volutator* from *in situ* plots and compare these with untreated areas. They found that treated areas had significantly greater abundance of biofilms, decreased sediment erodibility and were also drier. The causes of these measured impacts of *C. volutator* were attributed by the authors to direct grazing of biofilms reducing their coverage, consumption of bacteria on grain surfaces, increased water retention of the sediment and secretion of mucopolysaccharide within their burrows by microflora and reduced compaction of the sediment allowing more pore water (Gerdol and Hughes 1994). Previous research had found the opposite effect on sediment erodibility by *C. volutator*, and considered the process to be the shallow burrows of this species acting as a barrier and reducing permeability (Meadows and Tait 1989). However, this experiment was undertaken using 'muddy sand' and therefore may not be as applicable to this research as work by Gerdol & Hughes (1994) on muddy sediment.

The findings of Gerdol & Hughes (1994) lead them to speculate that predator-prey interactions may have a top down effect upon sediment erodibility, particularly in the case of wading birds, where they cite previous work (Daborn et al. 1993). Daborn *et al.* (1993) found coincidental evidence suggesting that an ecological cascade effect decreased sediment erodibility during a study in the Bay of Fundy, Canada. It was found that with the arrival of large numbers of migratory semipalmated sandpipers *Calidris pusilla*, and for the duration of their stay, densities of their prey *C. volutator* were reduced and biofilms proliferated causing a decrease in sediment erodibility. Although this was not a manipulative experiment, it does suggest that cascade effects, such as those which this research seeks to identify, do exist within this environment and may be driven by predatory birds.

1.6 Biofilms and Extracellular Polymeric Substances (EPS)

Many areas of intertidal muddy sediment support large densities of microphytobenthos (MPB). These photosynthetic eukaryotic and prokaryotic microorganisms (mainly diatoms and cyanobacteria) grow within and upon the upper several millimetres of the sediment bed and are major components of microbial mats and transient biofilms (Chapman et al. 2010, Beninger and Paterson 2018). Evidence suggests that MPB may account for up to 50% of total primary production in an estuary (Perissinotto et al. 2002), making them a vital component of this ecosystem and particularly in intertidal mudflats (Spilmont et al. 2006). Dominant organisms in this niche are often diatoms which can form biofilms (matrices of organisms, sediment and extracellular polymeric substances (EPS) bound together) (Underwood and Paterson 1993). Biofilm aids the retention of debris, increases biocide tolerance and sequesters nutrients, also acting as a transport chain between cells at different vertical levels within it. In this arrangement, cells are able to act in a similar functional capacity to those observed in multicellular organisms (Flemming and Wingender 2010).

As discussed above, sediment erodibility is an important aspect of mudflat functioning and the ecosystem services which they provide. Laboratory (Holland et al. 1974) and field (Austen et al.

1999) studies show relationships between diatoms and sediment erodibility. The literature, summarised by Tolhurst *et al* (2002), shows consensus that the mechanism behind this effect is the excretion of EPS, which forms physical strands between sediment particles (Chenu 1993, Paterson 1995, Defarge 1997) and forms polymer bridges between clay particles, increasing the strength of bonds between them (Chenu and Guerif 1991). Tolhurst et al. (2002) also conducted laboratory studies using xanthan gum as a substitute for EPS, finding that the addition of this polymer significantly decreased the erodibility of natural sediments. The abundance and spatial distribution of MPB also affects nutrient and trophic fluxes on intertidal muddy sediments (see Section 1.13), and as such increasing our understanding of their position and role within intertidal ecosystems and trophic chains is central to assessing their contribution to ecosystem services and subsequently managing these environments (Spilmont et al. 2011).

Diatoms inhabiting estuaries are traditionally separated into two groups; the epipelon and epipsammon (Round 1965, Admiraal et al. 1984). Epipelic diatoms are free-living and highly motile, found mostly within muddy sediments, whereas epipsammic diatoms attach themselves to grains and are not motile, found most often in sandy sediments (McIntire and Moore 1977, Hamels et al. 1998). Motility is a notable selective advantage to epipelic diatoms (Round 1971), which is achieved using EPS through a suggested Adhesion/Traction model (Edgar and Pickett-Heaps 1983). Within this model EPS strands, which are adhered to the substratum and also connected to free transmembrane structures, are moved along the diatom raphe through interaction with actin bundles (Edgar and Zavortink 1983, Round et al. 1990, Underwood and Paterson 2003, Consalvey et al. 2004).

As such, this thesis is concerned primarily with epipelic diatoms, which are generally considered to have broad ecological niches, defined by temperature and light tolerances which lead to changes in seasonal abundance (Underwood et al. 1998, Underwood and Provot 2000). Over shorter timescales (hours), epipelic diatoms utilise their motility by migrating vertically within the sediment in response to tidal fluctuations (Paterson 1989). Migration between the sediment surface and depths of approximately 2mm are also related to responses to diurnal temperature and light levels (Saburova and Polikarpov 2003). Different diatom species behave differently under different environmental pressures or conditions. For example, species of stream dwelling diatom show different tolerances and responses to nutrient and disturbance gradients, depending upon their ecological guild (Passy 2007). Strong light and temperatures (e.g. in summer) can inhibit the productivity of microphytobenthos, limiting primary production on an intertidal mudflat (Saint-Béat et al. 2013). The roles of diatoms are intrinsically linked to heterotrophs such as bacteria (and to a lesser known extent within sediments fungi), through such interactions oxygen, nutrient and organic matter fluxes are mediated (Underwood et al. 2022).

1.7 Biogeochemical cycling

Mudflats provide a fundamental function, regenerating nutrients by decomposition of organic matter (Sundbäck et al. 2003). Microphytobenthos (MPB) act as primary producers on intertidal flats, due to the emersion regime resulting in available light for photosynthesis and net primary production of organic carbon (MacIntyre et al. 1996). The majority of organic matter found in mudflats however, originated from external sources, being deposited from the water column (Mann 2009). Through bioturbation, benthic fauna such as polychaete worms and molluscs mix sediments and increase oxygen levels to depths of several centimeters (Rosenberg et al. 2008).

Tidal flats vary spatially and temporally between aerobic, oxidised sediment and anaerobic, reduced sediments, with redox reactions typifying the chemical processes and biogeochemical functioning within this environment, setting it apart from other coastal habitats (Pan et al. 2018). Within fine grained sediments the high surface area to volume ratio provides unparalleled opportunities for colonisation by MPB, which have significant effects on sediment properties including aiding in transport of nutrients and other substances between the sediment and water column (Pan et al. 2018). A complete redox reaction consists of two “half reactions”, one oxidizing and one reducing, which frequently result in phase changes – for example converting solids to liquids (and vice versa) or liquids to gases. Oxidation half reactions on mudflats (loss of electrons) are primarily driven by respiration and organic decomposition by heterotrophic organisms, mainly bacteria. The availability of electrons then facilitates reduction half reactions (gain of electrons) (Pan et al. 2018). Redox reactions are driven differently in aerobic and aerobic sediments. Within the upper, aerobic, sediment layer infauna mix and ‘rework’ the sediment maintaining oxygenated porewater, where oxygen is the primary ‘electron acceptor’ facilitating redox reactions. (Pan et al. 2018). Below this are anoxic sediments where anaerobic bacteria drive reduction reactions, preferentially using up nitrate, manganese, iron, sulfate and CO_2 respectively (Richards 1965). The vertical transition between these aerobic and anaerobic layers is known as the redox potential discontinuity (RPD) layer, visually apparent due to the difference in colour – typically brown/orange above and black/green/grey below (Sturdivant et al. 2012).

As discussed above, ecosystem services are based on ecosystem functioning of a system. In this context a major function of mudflats is nutrient regeneration, which occurs through decomposition of organic matter into soluble carbon, phosphorous and nitrogen. Within the upper redox layer, aerobic bacteria ultimately convert organic matter into ammonium and phosphate and nitrate, which are released into the water column (Pan et al. 2018).

Coastal sediments are considerable compartments of microbial nitrogen cycling (Thamdrup and Dalsgaard 2008). Particulate organic nitrogen (PON) deposited onto the mudflat surface becomes converted to ammonium, which under oxic conditions (at the surface) is oxidised to nitrite and then nitrate by nitrifying bacteria, mediated by archaea (Stief 2013). Nitrite and nitrate then follow two pathways, diffusing into the water column or into deeper anoxic layers. Within anoxic layers they are reduced anaerobically by bacteria and archaea via three different pathways (Stief 2013). Nitrate and ammonium can also be assimilated at the surface by heterotrophic bacteria, archaea and microalgae (Stief 2013).

Excess fixed nitrogen in aquatic environments arising from leached agricultural runoff of nitrates from fertilisers and nitrogen deposition from fossil fuel burning contributes to eutrophication of coastal waters, leading to algal blooms, oxygen depletion and ultimately significant impacts to biodiversity and habitats (Stief 2013). Macrofauna readily consume detritus and biofilms containing fixed nitrogen, thus removing fixed nitrogen from the aquatic environment (Stief 2013).

Estuarine sediments and biofilms are central components in estuarine nutrient cycles, ultimately affecting fluxes of these nutrients between land and sea (Thornton et al. 2007, Nedwell et al. 2016). The transport chain described above facilitates influx and efflux of nutrients between the sediment and water column, benefitting organisms within the benthic and pelagic zones (Flemming and Wingender 2010). Organic compounds are recycled and remineralised within sediments, particularly in coastal marine areas where nitrogen and phosphorous loads can be very high (Correll et al. 1992, Hochard et al. 2010). Recent evidence demonstrates that species-species interactions between diatoms (autotrophs) and heterotrophs are integral to mediating fluxes of nutrients and organic matter between sediment and water column, with carbon and nutrient cycling controlled by bacteria and archaea (Underwood et al. 2022).

Aquatic ecosystems are under increasing threat from nutrient enrichment, resulting from agricultural intensification and widespread use of synthetic fertilisers (Carpenter et al. 1998) and sewage discharge (Culhane et al. 2019). Nitrogen loading into marine systems can lead to eutrophication and decline in water quality, making its source and removal pathways of high interest (Burgin and Hamilton 2007). Shallow aquatic ecosystems could contribute approximately 10% of N_2O emissions globally, up to 30-40% of which may be produced within sediments (Bakker et al. 2014).

Eutrophication can cause depleted benthic species richness and reduced ecosystem services provided by benthos, such as waste treatment and carbon sequestration (Worm et al. 2006, Smith and Schindler 2009, Caswell et al. 2018). Changes in nutrient loads can impact benthic communities with potential knock-on effects to communities which rely on them, such as shorebirds (Culhane et al. 2019). MPB mediate fluxes of NO_3^-, NO_2^-, PO_4^{3-} and NH_4^+ between the water column and sediment layers (Sundback et al. 1991, Correll et al. 1992, Feuillet-Gerard et al. 1997), contributing to this process either by direct uptake/release or by altering oxygen concentration (Sundback and Graneli 1988). The Pearson and Rosenberg theory suggests that macrofauna species richness, size and burrow structure complexity is negatively correlated with the time and/or location of contamination on a mudflat, such that these variables may increase with distance or time from the source (Pearson and Rosenberg 1978). However, consensus here is not unequivocal: resource availability and stress factors can show a ‘humpbacked’ curve, as described by the intermediate disturbance hypothesis (IDH), whereby a perceived ‘tipping point’ is observed, beyond which impacts on species richness (and often also associated attributes and services) is reduced (Connell 1978, Odum 1985, Dodson et al. 2000, Mittelbach et al. 2001, Hooper et al. 2005, Huston 2014). However, recent experimental work suggests that, in response to disturbance, intertidal soft-sediment communities do not respond according to the IDH, accentuating the need for experimental work to clarify responses within this environment (Gerwing et al. 2017).

The impacts of changes in nutrient cycling within coastal sediments are far-reaching, up to a global scale: eutrophic coastal waters may contribute up to 10% of global nitrous oxide (N_2O) emissions, a greenhouse gas (GHG) with up to 265 times greater global warming potential than carbon dioxide (CO_2) (Bongalia et al. 2017). Dissolved organic carbon (DOC) may also provide an important part of both global and coastal carbon sinks (Maher and Eyre 2010, Legge et al. 2020), making effects on DOC fluxes in this environment relevant to anthropogenic climate change effects and mitigation (McKinley et al. 2016).

Saint-Beat *et al.* (2013) studied the food web features within a key migratory and wintering bird stopover mudflat in France in the context of biogeochemical cycling. It was found that cycling and carbon retention within the system was approximately equal during the summer and winter, despite the presence of predatory birds during the winter, suggesting that shorebirds may act as to sustain biogeochemical cycling when present, maintaining balance within the system.

1.8 Shorebird Study Approach

The term “shorebirds”, as referred to herein, is generally accepted as those birds which forage on mudflats (Charadrii), as in classic studies (Goss-Custard 1977a), but in particular Scolopaci (sandpipers, jacanas, painted-snipes, seedsnipes and plains-wanderer) and Charadrii (plovers, oystercatchers, stilts and avocets, sheathbills and magellanic plover) (Christian et al. 1992, Paton et al. 2003). In England these are referred to also as waders.

However, avian communities on mudflats in the East of England also include other orders and families such as Anseriformes (ducks and geese), Ardeidae (herons, and allies), Laridae (gulls) and Corvidae (crows), which are known to be present at the mudflats studied herein (pers. obs).

Wintering species recorded within the experimental study locations include:

Waders:

- Redshank *Tringa totanus*
- Grey plover *Pluvialis squatarola*
- Ringed plover *Charadrius hiaticula*
- Dunlin *Calidris alpina*
- Knot *Calidris canutus*
- Black-tailed godwit *Limosa limosa*
- Bar-tailed godwit *Limosa lapponica*
- Avocet *Recurvirostra avosetta*
- Lapwing *Vanellus vanellus*
- Curlew *Numenius arquata*
- Oystercatcher *Haematopus ostralegus*
- Turnstone *Arenaria interpres*

Ducks and Geese:

- Shelduck *Tadorna tadorna*
- Brent goose *Branta bernicla*
- Teal *Anas crecca*
- Wigeon *Anas Penelope*

Gulls, herons and crows:

- Black-headed gull *Chroicocephalus ridibundus*
- Herring Gull *Larus argentatus*
- Little Egret *Egretta garzetta*
- Carrion Crow *Corvus corone*

Corvids are most likely to feed on carrion on the mudflat (Sazima 2020) and Little Egret generally move across the mudflat to reach creeks or pools where they hunt fish and marine invertebrates (Anton-Tello et al. 2021).

Ducks (Anatidae) at the study sites (Shelduck, Teal and Wigeon) feed in open water, on saltmarsh and mudflats where they feed on a mixture of plant matter (including seeds) and invertebrates such as molluscs (*Peringia* spp.) which are sieved from surface mud using the bill (Olney 1962, Nummi and Vaananen 2001, Viain et al. 2011).

Gulls are opportunistic, generalist predators which utilise a different feeding strategy to shorebirds, although they are known to consume a significant percentage of invertebrates (Vernon 1972, Mudge and Ferrus 1982, Curtis et al. 1985). For example, gulls remove only the siphons of bivalves (Moreira 1997) which regenerate (Hodgson 1982) reducing the impact of gulls on bivalve mortality (Leguerrier et al. 2003). Shorebirds often consume bivalves whole, consuming also a wider range and quantity of benthic invertebrates (particularly when considered as a shorebird community), resulting in a larger top-down effect of shorebirds than gulls on a mudflat, particularly where shorebirds are present in large numbers (Leguerrier et al. 2003).

This thesis examines the effect of birds as a community, either by excluding all species equally (see Chapter 3) or using pre-existing exclusion methods which have measurable effects on varying bird density.

Shorebird abundance and community assemblage fluctuate on intertidal habitats, due to factors such as prey availability (Ribeiro et al. 2004). A variety of modelling approaches have been developed depending on the area of habitat being studied and habitat heterogeneity within it. For example, simple mathematical depletion models can be used on mudflats to achieve long-term estimates of habitat use by shorebirds (Gill et al. 2001). Across larger areas computer programming can be used to model larger numbers of factors such as habitat suitability to extrapolate counts across larger areas (Martin et al. 2020). Using shorebird numbers counted when researchers were present to model estimated numbers when surveyor presence was not possible, we can gain an insight into those more abundant species, with reference to known behaviours and diets, which may contribute more greatly to any observed effects. To enable this kind of appraisal, it is necessary to review the diets and foraging strategies of shorebird species present within the study sites.

Several studies in the literature show that shorebird densities are related to different prey types and that different species also preferentially consume different prey size classes (Goss-Custard 1977a, Goss Custard 1980, Goss-Custard et al. 1991, Atkinson et al. 2001).

Many shorebird species show preferences for, and often specialise in, specific prey (Cayford 1993). However, others show the ability to adjust their prey in response to environmental changes such as invertebrate density and population structure (Bowgen et al. 2015). For example, larger birds which tend to be more specialist in their feeding behaviour, such as Eurasian curlew *Numenius arquata*, are less able to adapt to the loss of large worms. In such cases they become more dependent upon terrestrial food sources. Conversely, species such as oystercatcher *Haematopus ostralegus* were found to more readily switch their feeding from bivalves to worms when necessary, indicating a more adaptable and generalist feeding strategy (Bowgen et al. 2015). Further detail on the feeding strategies and prey of relevant shorebirds is included in Chapter 2.

Switching between prey items appears to be a common strategy among smaller shorebirds such as knot *Calidris canutus* which may be a necessary adaptation due to the variation of prey availability and abundance between sites (Quaintenne et al. 2014). Small sandpipers have more recently also been shown to feed not only upon infauna, but also graze microphytobenthos (MPB) resulting in revision of the trophic level and role of species such as western sandpiper *Calidris mauri* and dunlin *Calidris alpina* (Kuwae et al. 2008, Kuwae et al. 2012). Video surveillance and analysis, isotopic analysis of droppings and modelling were all used in this study to show that this behaviour exists and that MPB are directly targeted and consumed by small sandpipers.

Kuwae *et al.* (2012) also suggest that adaptations of small sandpipers, such as tongue spines, are for this biofilm foraging behaviour, and that this diet is suitable for their relatively small digestive organs. This discovery adds both relevance and dimension to this thesis, making it necessary to consider the potential effect(s) that this direct link between birds and MPB may have on ecosystem functioning. For example, it has been suggested that the distribution and feeding habits of Dunlin in France is dependent upon diatom distribution across the mudflats (Drouet et al, 2015). As our knowledge progresses, more evidence of small sandpipers feeding on biofilms is being recognised globally. For example Little Stints *Calidris minuta* have now also been found to consume biofilms in saltpans along the East Atlantic Flyway (Lourenço et al. 2017b). Further research is also required to fully understand the factors controlling biofilm feeding, as it has been found that western sandpipers do not shift to lower trophic levels throughout their breeding cycle; the behaviour may instead depend upon availability of invertebrate prey and energy requirement (Beninger et al. 2011). It also appears that different small sandpipers use biofilms to different extents at different times of their annual cycle; Jimenez *et al.* (2015) found that in British Columbia the Western Sandpiper foraged more extensively within the upper intertidal than Dunlin, where biofilms were more frequent, indicating a heavier reliance on this resource. The authors also emphasised the need to consider water flow and its impacts on biofilm coverage, when making coastal management decisions (Jimenez et al. 2015). Nonetheless, it has become accepted that biofilms form an important food source for small-bodied shorebirds, resulting in the emergence of technical specifications for maximising the conservation value of human created mudflats (Kuwae et al. 2021).

Other *Calidris* species, such as knot *C. canutus*, are reported to be much more specialist, feeding almost exclusively on marine molluscs (Drouet et al, 2015). Application of more modern scientific methods to this field continues to shed light on wader diet and ecosystem use, and highlights that unknowns remain regarding diet and trophic positions of wading birds: Next generation sequencing was used to examine the faeces of semipalmated sandpipers, showing that in the Upper Bay of Fundy this species is a more generalist predator than previously thought (Gerwing et al. 2016b). These kinds of environmental DNA (eDNA) analysis techniques are becoming more widely utilised in avian science in recent years, as this technology becomes more widely available (Huang et al. 2022, Correia et al. 2023a, Correia et al. 2023b). Rather than being dependent upon *Corophium volutator*, the semipalmated sandpiper also fed upon arachnids, crabs, bivalves, terrestrial and freshwater insects, ctenophores, cnidarians and fish. Another study in France aimed to understand which food web characteristics support shorebirds during their migration, by comparing times of year when birds were present and absent from the Brouage bare mudflat. It was found that due to efficient carbon cycling in the presence of birds (despite increased removal of primary producers), food resources were maintained throughout the period which birds used the site as a stopover (Saint-Béat et al. 2013).

The term bioturbation is increasingly being applied to higher vertebrates, such as rays, with emphasis on ecosystem functioning effects (Flowers et al. 2021) and this can also be applied to shorebirds, including the effect of walking across, probing and sifting the sediment surface (see Chapter 3). This is also relevant to the process by which small sandpipers are thought to consume biofilm, using tongue spines to 'scrape' MPB into their bill (Elner et al. 2005, Kuwae et al. 2008, Beninger and Elner 2020).

1.9 Ecology of Relevant Shorebirds

To facilitate comparison between sites regarding species assemblage, it is necessary to summarise the traits of species present such as foraging/prey items, searching modes and capture techniques, which could affect MPB either directly or indirectly (Daborn et al. 1993, Kuwae et al. 2012).

The relevant ecology of shorebirds, ducks and geese are described below. Emphasis and weight has been given to species present in greater numbers at study sites and which are more likely to have contributed to effects found during experiments.

1.9.1 Redshank *Tringa totanus*

Redshank range 27-29cm in length, have a wingspan of 59-66cm and mass 85-155g (Ottvall and Gunnarsson 2007). The resident subspecies here is *Tringa totanus totanus*, although *Tringa totanus robusta* also winters in the British Isles and may be represented here (Van Gils et al. 2020b). Due to the difficulty in separating these races in the field (which are usually separated through capture-mark-recapture studies, based on wing length measurements (Derrett and Smith 2001)) these subspecies are grouped for the purpose of this thesis.

Redshank inhabits coastal and inland wetlands during the breeding season, moving to predominantly coastal habitats during winter including open mudflats, salt marshes and rocky, muddy and sandy shorelines (Van Gils et al. 2020b), with birds wintering in the East of England using riverine/estuarine mudflats particularly during the day, with reports of strong winter site fidelity in north-west Europe (Burton and Armitage 2005). The nominate race migrates north from April to May, returning south from July (adults) to August -September (juveniles) (Van Gils et al. 2020b).

Redshank are traditionally considered to feed primarily upon the mud shrimp *Corophium volutator* in some locations in the UK (Goss-Custard 1977a, b), although are also known to exploit a range of food items: Evidence has been found of redshank feeding on mudflats preying upon shore crab *Carcinus maenus*, shrimps (species undefined), small cockles (species undefined), clams *Macoma balthica*, mud snails *Peringia ulvae* and ragworms *Hediste diversicolor* (Goss-Custard and Jones 1976). During this study, amphipods *Corophium* spp. and *Orchestia* spp. were consumed mostly in June, with *H. ulvae* taken predominantly between October and April. A more recent study on breeding redshank found that when utilising freshwater habitats near the coast, estuarine polychaetes remained the dominant food source with a high proportion of Diptera (fly) larvae when hatching events occurred (Ausden et al. 2003). Winter prey item dominance of *Peringia ulvae* was also shown in other studies in southern Europe (Moreira 1996, Perez-Hurtado et al. 1997). A later study in the same region, however, did not record dominance of *Peringia ulvae* during winter, instead finding prey items dominated by isopods and polychaetes (Sanchez et al. 2005). Other groups found in significant numbers during these studies included Coleoptera (beetle) and Diptera (fly) larvae and pupae. The presence of these were considered likely due to differences in management of the salt pans where these studies were undertaken; reducing the water levels of some ponds at one site enabled feeding upon Coleoptera and Diptera throughout winter, whereas these were too deep for redshank at other sites (Sanchez et al. 2005).

Other items recorded in Redshank diet include insects, spiders, fish, tadpoles and Chironomids (Ausden et al. 2003, Van Gils et al. 2020b). In the south-east, this species has been recorded kleptoparasiting dog-faced water snakes *Cerberus boddarti*, taking prey including blue-spotted mudskipper (Ooi and Eng 2013). This highlights the breadth of prey items which redshank exploit and the strategies employed, both at different times of year and during the tidal cycle (Van Gils et al. 2020b). Strategies employed at the study sites used in experiments presented in this thesis are typical of wintering foraging mode; ‘brisk’ walking and pecking, occasionally probing or jabbing the substrate (Van Gils et al. 2020b).

Trophic interactions involving redshank have been studied previously, showing that during cold weather (when their main food source, *Corophium* is scarce) birds move onto saltmarshes where the shrimp *Orchestia* remain available, albeit more deeply buried. This makes individuals more vulnerable to predation by Sparrowhawks *Accipiter nisus*, which increases flocking behaviour while feeding. This high density feeding suppresses *Orchestia* numbers, causing Redshank to separate, again increasing their vulnerability to predation (Kenworthy 2018).

1.9.2 Grey plover *Pluvialis squatarola*

Grey Plover *Pluvialis squatarola squatarola* range 27-31cm in length with wingspan 71-83cm and mass 165-395g (Serra et al. 1999, Minton and Serra 2001, Poole et al. 2020). This species is migratory, the palearctic population breeding from north-eastern European Russia across northern Siberia (Cramp and Simmons 1983), with wintering ranges from the British Isles into southern Europe and northern India (Poole et al. 2020). Flocks move to wintering grounds July to October/November and return to breeding grounds April to May (Poole et al. 2020).

Wintering habitat, including during migration, is predominantly coastal beaches and estuaries, usually on sandy or muddy substrata (Poole et al. 2020). In England, many favoured sites feature deep tidal creeks (Townsend et al. 1984), with high tide roosts on saltmarshes, beaches and islands (Poole et al. 2020).

Individual diet depends upon site location and substrate (Poole et al. 2020). Even considering mudflats alone, grey plover have a wide ranging diet, possibly due to an element of opportunism in foraging behaviour. Pienkowski (1982), however, found that thin Polychaete worms (principally *Notomastus* spp.) accounted for between 70.3 % and 78.2 % of prey taken on a

Northumberland mudflat in the UK, depending upon the area of flats observed. Between 12.5% and 16.4% of food items were unidentified. Pienkowski (1982) also recorded ragworms, lugworms *Arenicola* spp., shore crab, algae, clams *Macoma* spp., mud snails *Peringia* spp., *Littorina* spp., Diptera, sandhoppers *Talitrus* spp. and other crustaceans within the diet of grey plover. On the Wash, UK, the following species consumed by grey plover were idenfitied through dropping analysis: clam *Macoma balthica*, common cockle *Cerastoderma edule*, mud snail *Peringia ulvae*, Arctic barrel bubble *Retusa obtusa*, sea slug *Alderia modesta*, amphipod *Corophium volutator*, lugworm *Arenicola marina* and ragworm *Hediste diversicolor*, with no clear preference in any species displayed (Dit Durell and Kelly 1990). Also on The Wash, UK, found the most important prey species of grey plover were *Nereis*, *Cerastoderma*, *Carcinus* and *Macoma* (Goss Custard et al. 1977). *C. volutator* and *R. obtusa* are also included in the list of recorded grey plover prey items (Dit Durell and Kelly 1990). In south and east Africa, Madagascar and Mauritius grey plover was found to consume prey types in different ratios depending upon latitude; diet was more variable at temperate sites (Turpie and Hockey 2008). In temperate regions, grey plover was found to consume mostly small unidentified prey and polychaete worms, with crustaceans rarely eaten (Turpie and Hockey 2008). Findings in Southern Spain also reflected this, with *Hediste diversicolor* and *Cerastoderma edule* being the main prey items (Perez-Hurtado et al. 1997). Based on this wide diet, grey plover is considered a generalist, confined mostly by its foraging mode of sight, which also probably accounts also for its preference for larger prey items which are more easily seen (Poole et al. 2020).

Grey Plover foraging periods are dictated strongly by tidal regime, with birds at The Wash, UK, leaving roosts to feed 2-3 hours after high tide, and remaining until around 2-3 hours before high water, therefore feeding for around 50% of the tidal cycle (Goss-Custard et al. 1977). As such, this species is not typically a tideline follower (Recher 1966). The species forages by sight, using the typical plover ‘Stop-Run-Peck (prey seen)’ and ‘Stop-Run-Stop (no prey seen, changes vantage point)’ method (Poole et al. 2020).

1.9.3 Ringed Plover *Charadrius hiaticula hiaticula*

Ringed plover *Charadrius hiaticula hiaticula* range between 18 and 20cm in length, with 48-57cm wingspan and mass 42 to 78 grams (Wiersma et al. 2020). This species has a range of migratory distances, with some breeders possibly remaining relatively close to breeding grounds during winter. An influx of migrating birds from Iceland winter as far south as Africa (Wiersma et al. 2020). Throughout the year, ringed plover will utilise a range of coastal habitats including shingle beaches, sand and mudflats and less frequently rivers, lakes, saltmarsh, gravel pits, farmland and playing fields (Wiersma et al. 2020).

Diet is relatively broad, including small crustaceans, molluscs, polychaete worms, amphipods, insects and other invertebrates which are preyed upon by pecking and occasionally foot-trembling (intentional disturbance to cause prey to emerge) (Wiersma et al. 2020).

1.9.4 Dunlin *Calidris alpina*

A small sandpiper ranging in length from 16 to 22cm, with wingspan 33-40cm and mass 48-64g (Holmes 1966, Page 1974). The races wintering at the study locations are *C. alpina alpina* and *C. alpina schinzii* (Warnock and Gill 2020), which for the purposes of this research are not separated. The former has a breeding range from Scandanavia through Russia to Siberia, the latter breeding in Greenland and Iceland through Faroe and north British isles, to the Baltic and Scandinavia (Warnock and Gill 2020). The species typically moves north to breeding grounds during January to May, returning south July to October, into November (Warnock and Gill 2020).

Dunlin have a wide range of recorded infaunal prey items, including *M. balthica*, *C. edule*, *P. ulvae*, *R. obtusa*, *C volutator*, *H. diversicolor* and *Nephtys* spp (Dit Durell and Kelly 1990). Previously in the Severn Estuary, Worrall (1984) had recorded only C. *volutator*, *H. diversicolor* and *M. balthica*, although the authors do present evidence that dunlin rely on different prey items at different times of year, partly due to the direct relationship between temperature and burrow depth in species such as *H. diversicolor*. It is possible that these adaptations to changes in prey availability (Goss Custard et al. 1977, Pienkowski 1982, Perez-Hurtado et al. 1997, Turpie and Hockey 2008) account partly for the variation in results of studies seeking to determine prey species of shorebirds. Despite this, dunlin appear to have the widest infaunal prey diet of waders studied here (Goss-Custard et al. 1991), and have been shown using stable isotope analysis that they are adaptable, exploiting agricultural habitats outside the breeding season (Evans Ogden et al. 2005). Naturally occurring stable isotopes in biological material can be traced through food webs, leading to establishment of stable isotope analysis as a means of quantifying the relative components of an animals diet, where components are isotopically distinct (Hobson and Wassenaar 1999).

The use of more recent scientific techniques, such as stable isotope analysis, has also enabled advances in our understanding of dunlin feeding on mudlflats; scanning electron microscopy to examine the differences in tongue morphology between western sandpiper and dunlin (Elner et al. 2005). This highlighted potential differences in foraging strategy and diet breadth, leading to further investigation within the scientific community. Following this, several studies have shown that Calidrid sandpipers, including dunlin, actively feed on biofilms on the mudflat surface (Kuwae et al. 2008, Mathot et al. 2010, Beninger et al. 2011, Kuwae et al. 2012, Quinn and Hamilton 2012, Drouet et al. 2015, Jardine et al. 2015).

Dunlin is a tide follower, foraging at the tide line where sediment is softest and invertebrates nearer the surface (Brennan et al. 1985, Warnock and Gill 2020), or within depressions or runnels on mudflats for the same reasons (Mouritsen and Jensen 1992). Dunlin feed rapidly, using tactile cues, probing, jabbing and picking through substrate, often with an open bill and often running between areas (Warnock and Gill 2020). An open bill also allows taste cues and visual cues are reported, likely explaining the wide diet breadth in this species (ven Heezik et al. 1983, Evans 1986).

1.9.5 Black-tailed Godwit *Limosa limosa*

Black-tailed godwit is a larger wader ranging 36-44cm in length with wingspan 70-82cm and mass of 244-500g (Van Gils et al. 2020a). The races wintering in Europe are *L. limosa islandica* and *L. limosa limosa*, which are not separated for the purpose of this research. Black-tailed godwit breed on wet grassland, grassy marshes and raised bog and winters on intertidal mudflats, saltmarshes, sandy beaches, salt flats and inland wetlands. Race *islandica* favours estuarine habitats, while *limosa* is mainly found wintering in freshwater habitats This species migrates to wintering grounds late June to October, returning to breeding areas February to April (Van Gils et al. 2020a).

Black-tailed godwit has a more restricted diet than most other waders present at the study sites, predominantly invertebrates including insects and their larvae (particularly during breeding), annelids, molluscs, crustaceans, spiders, fish eggs and amphibian spawn (Van Gils et al. 2020a). On wintering grounds *L. limosa limosa* consumes mostly plant material (Van Gils et al. 2020a). However, other studies in Western Europe have found the dominant prey item being *Scrobicularia plana*, alongside notable amounts of *H. diversicolor* and to a lesser extent *P. ulvae* (Goss Custard et al. 1977, Goss-Custard et al. 1991, Moreira 1994). Black-tailed godwits forage using touch and sight to locate food, and has been recorded foot-trembling to disturb prey beneath the water (Van Gils et al. 2020a).

1.9.6 Bar-tailed Godwit *Limosa lapponica*

Bar-tailed godwit is a larger wader of length 37-39cm and mass 200-720g. The European race is *L.l.lapponica*, which breeds in Fennoscandia and winters south to Africa and India. Birds depart from breeding areas to wintering grounds mid-July to August and return mid-April to May. Wintering habitats include intertidal mud and sand flats and occasionally coastal marshes and meadows (McCaffery and Gill 2020).

Non-breeding diet consists mainly of invertebrates, particularly marine molluscs (*Macoma* and *Peringia*), crustaceans (*Corophium and Carcinus*), worms (*Arenicola* and *Nereis*) and occasionally small fish. This species forages mainly on coastal soft mud where it often probes into the mud using the full bill length, foraging also in shallow water, females following the tide line significantly more often than males (McCaffery and Gill 2020).

Bar-tailed godwit uses visual and tactile foraging cues, depending on whether it is feeding during the day or night (Evans 1976). A range of foraging techniques have been recorded, including 'stitching' (rapid series of probes), 'mowing' (lightly disturbing the sediment surface with bill moving side to side) (Cramp and Simmons 1983), gleaning (carefully picking) from sediment surface, taking prey aerially from above breaking waves and raking muddy sand using their feet (McCaffery and Gill 2020).

1.9.7 Knot (Red) *Calidris canutus*

A medium sized *Calidris* sandpiper, knot ranges in length from 23-25cm, with wingspan of 45-54cm and mass 85-220g. The west European wintering race is *Calidris canutus islandica* (Baker et al. 2020), which departs from breeding grounds in arctic Canada and Greenland in August to September, where they travel to staging grounds principally in the Netherlands and Germany, before most move west to coasts and estuaries of the United Kingdom, with fewer moving to France, arriving on wintering grounds from September. The majority of UK wintering birds move to staging grounds in Iceland in mid to late May, before returning to breeding grounds. Wintering habitats are coastal sand and mudflats and saltmarsh usually on estuaries (Baker et al. 2020).

Knot forage using a mix of visual and tactile cues, pecking, plowing or probing depending on prey location and visibility (Baker et al. 2020). Red knot are considered to predominantly feed upon molluscs in those areas of the world in which their diet has been studied, including *P. ulvae, M. balthica, C. edule*, and *Scrobicularia plana* (Prater 1972, Goss Custard et al. 1977, Piersma 1991, Zwarts and Blomert 1992, Dekinga and Piersma 1993). However, it is also acknowledged that during some points in their migratory cycle, knot rely heavily upon crustacean prey (Van Gils et al. 2005). In Morecambe Bay, UK, knot were also recorded feeding upon *Corophium volutator* and *H. diversicolor*. Switching between prey items appears to be a common strategy among smaller waders such as red knot which may be a necessary adaptation due to the variation of prey availability and abundance between sites (Quaintenne et al. 2014).

A study in the Tejo Estuary in Portugal into shorebird diet using stomach isotopic analysis suggests that red knot may consume biofilm alongside little stint (*Calidris minutus*), although the authors warn that a very similar isotopic signature also occurs in their macroinvertebrate prey, therefore this must be treated with caution (Lourenço et al. 2017b).

1.9.8 Avocet *Recurvirostra avosetta*

Avocet *Recurvirostra avosetta* is a distinctive medium sized wader 42-45cm in length with wingspan 77-80cm, 85-98g (Pierce et al. 2020). Widely distributed, winters from Western Europe and Africa, through middle East into South-East Asia, where wintering habitat is most often muddy intertidal flats, also sometimes lake shores, rivers and agricultural land (Pierce et al. 2020). The wide distribution makes migratory times and routes difficult to specify, but southward migration to wintering grounds occurs August to October, with return journey March to May (Pierce et al. 2020).

Avocets are largely carnivorous, taking mostly macroinvertebrates 4-15mm in length, including aquatic insects, crustaceans and worms, less frequently fish, plant matter and molluscs. In winter, diet is dominated by polychaete worms on the French Atlantic coast (Pierce et al. 2020). Foraging modes include picking or scything of the bill through mud or water, often in groups and reportedly ‘spinning’ using legs as an axis (Pierce et al. 2020).

1.9.9 Curlew *Numenius arquata*

A large wader, 50-60cm with wingspan 80-100cm and mass 410-1360g (males smaller), with a distinctive, long, down-curved bill. Subspecies *arquata* is distributed from west Europe, north to Arctic circle and east to the Ural Mountains, with wintering range from Iceland, south to UK and down to Mediterranean and north Africa/India (Van Gils et al. 2020c). Wintering habitats are predominantly muddy flats along the coast, including bays and estuaries, as well as on farmland particularly stubble fields and winter cereals or pasture, especially at high tide along the coast (Van Gils et al. 2020c).

Some British birds are resident and relatively sedentary, with the wintering population supplemented by Scandinavian migrants particularly in coastal areas. Individuals and flocks are site faithful, unless weather conditions necessitate otherwise, with southward migration June to November and northward March to April (Van Gils et al. 2020c). Diet and foraging are relatively consistent throughout the year, wide and opportunistic with evidence of some separation between males and longer billed females. Recorded dietary groups include annelids, arthropods, crustaceans, molluscs, fruit and seeds, fish, amphibians, lizards, young birds and small rodents (Van Gils et al. 2020c).

1.9.10 Oystercatcher *Haematopus ostralegus*

Oystercatcher *Haematopus ostralegus ostralegus* is a medium sized wader, 40-48cm with wingspan 76-86cm and mass 425-820g, distributed from Iceland and Scandanavia, east to Pechora and south to Mediterranean, wintering in Africa (Hockey et al. 2020). This race is migratory, with all inland breeders moving to coastal areas for the winter from the English Channel south to Iberia, joined by coastal breeders from Scandinavia. Birds arrive on wintering grounds mostly August to September, with return movement to breeding grounds late January to April (Hockey et al. 2020). The oystercatcher is a versatile wader, breeding in coastal habitats on saltmarsh, beaches, infrequently on rocky coasts and also inland alongside waterbodies, on grassland and agricultural land (Hockey et al. 2020).

On coastal soft substrates and estuaries, Oystercatchers rely on bivalves including *C. edule*, *M. balthica*, *M. arenaria* and *S. plana* and gastropods *Littorina* spp. This species is known to change its dominant prey items in response to their depth within substratum, such as between bivalves in spring to annelids in summer in the Dutch Wadden Sea (Bunskoeke et al. 1996, Ens et al. 1996). Target prey (between annelids and bivalves) also alters during the tidal cycle (DeVlas et al. 1996).

1.9.11 Turnstone *Arenaria interpres*

Turnstone *Arenaria interpres interpres* is a small shorebird, 21-26cm with wingspan 50-57cm and mass 84-190g (Nettleship 2020). Breeds in arctic regions, in Europe from Greenland and Scandinavia east through Siberia, wintering in coastal areas in western and southern Europe, down to south Africa (Cramp and Simmons 1983). Wintering destinations are coastal, although occasionally seen inland during migration, utilising almost any coastal area including estuaries and mudflats where it is often seen along mudflat fringes (Nettleship 2020). A long-distance migrant across populations, Greenland population moving to western Europe including the United Kingdom, with this southward movement occurring early August to September and birds returning to breeding grounds early to late May (Nettleship 2020).

In overwintering areas diet is highly diverse and opportunistic, including crustaceans, molluscs, annelids, echinoderms, fish, insects, spiders, carrion, human waste and bird’s eggs (Nettleship

2020). This species is an efficient forager and aggressive predator, adapting feeding method based on habitat, time of year and food availability (Nettleship 2020). Particularly during winter, turnstones forage by flipping over objects (hence the name) such as pebbles, rocks and shells to expose food items, also probing and jabbing with the bill once prey are located (Nettleship 2020).

1.9.12 Eurasian Teal *Anas crecca*

Teal *Anas crecca crecca* is a dabbling duck approximately 37cm in length, ranging 200-450g mass with wingspan 58-64cm (Snow et al. 1997). Birds breeding in Iceland, east to Russia, winter in UK and north sea regions, arriving in October to November and leaving for breeding grounds March to April (Johnson et al. 2020).

Wintering habitat includes flooded inland areas including riparian zones, also coastal marshes and muddy estuaries. Diet includes seeds of aquatic and semi-aquatic vegetation and insects, foraged for mostly within water >12cm deep (87% of time), but also just above sediments if food is available. Feeding method is dabbling, diving rarely, also probing within mud and consuming meiofauna such as nematodes at the sediment surface (Johnson et al. 2020).

1.9.13 Eurasian Wigeon *Mareca penelope*

Eurasian wigeon *Mareca penelope* is a duck 45-51cm long, 600-1000g mass with wingspan 75-86cm. Fennoscandian and Russian breeding birds migrate to western Europe, including UK, during September and October, leaving for breeding grounds in March to April (Carboneras et al. 2020).

Wintering habitat includes coastal marshes and freshwater lagoons often along estuaries, preferably sheltered. Predominantly vegetarian in diet, taking leaves, stems, roosts and seeds of aquatic and semi-aquatic vegetation, occasionally taking small invertebrates opportunistically. Most feeding is dabbling within water, with terrestrial foraging is usually within arable or grassland (Carboneras et al. 2020).

1.9.14 Shelduck *Tadorna tadorna*

The shelduck *Tadorna tadorna* is a larger duck, 568-567cm long, 562-1500g mass with wingspan 110-133cm. Populations within UK are mainly sedentary, although visitors from elsewhere are possible. Large flocks gather in costal areas outside the breeding season along the north sea within coastal mudflats and estuaries (Carboneras and Kirwan 2020).

Diet is predominantly aquatic invertebrates, particularly the mudsnail *Peringia ulvae*. Foraging modes include digging, scything and dabbling on mudflats or other exposed mud or upending within water to reach sediment and search for prey (Carboneras and Kirwan 2020).

1.9.15 Brent Goose Branta bernicla

Brent *Branta bernicla* is a small goose 55-66cm long, 850-1810g mass with wingspan 110-120cm. Arctic breeders depart in winter for north sea regions including UK, leaving for breeding grounds in April (Lewis et al. 2020).

Brents prefer intertidal mudflats which are sheltered and abundant in eelgrass and/or green algae. Eelgrass is the main food, with algae also taken. Food is taken while walking across exposed mudflats or upending in water to reach substrate (Lewis et al. 2020).

1.10 Macrofauna

Macrofauna have been defined as "*benthic invertebrates that live in or on the bottom substratum of a water body and that are retained on a sieve with a mesh size of 0.5 or 1mm after the sediment passed through*" (Van Colen 2018). However, the author acknowledges that mesh size convention varies with substratum, particularly in organically enriched mudflats, with 300 to 500µm mesh size being frequently used (Van Colen 2018).

Shorebirds are classically considered to rely heavily on macrofauna, with the majority of prey items cited within the literature falling into this classification (Mathot et al. 2018). Although, this may be due to the relative ease of recording macrofauna predation by shorebirds, compared to meiofauna (benthic invertebrates retained on a sieve mesh size $\leq$63 µm (Moens and Beninger 2018)). Indeed, recently employed techniques for examining shorebird diet, such as stable isotope analysis, are able to detect a wider range of food items including microscopic organisms such as biofilm (Kuwae et al. 2008). Nonetheless, optimal foraging theory dictates that animals will spend the least amount of energy required to fulfil their required energy intake (King and Marshall 2022), with shorebirds using visual and tactile cues to locate prey (Evans 1986, Mouritsen and Jensen 1992). Hence, it stands to reason that macrofauna continue to be a cornerstone of shorebird diets (Daggers et al. 2020).

Common ragworm *Hediste diversicolor* (previously known as *Nereis diversicolor*) is a large, burrowing polychaete worm which is abundant in intertidal mudflats and a convenient research subject, which has led to a large amount of literature being published about it (Scaps 2002). Section 1.8 refers to a large proportion of the shorebirds relevant to this thesis which consume ragworms. The interactions between the food prey of ragworm (diatoms and bacteria) have been shown to be complex: *H. diversicolor* has a wide range of prey items, including diatoms and other micro-organisms, other infauna, and phytoplankton when blooms are sufficiently high (E Costa et al. 2000). The traditional assumption is that through the removal of biofilms, *H. diversicolor* would increase sediment erodibility. *H. diversicolor* has also been suggested as a driver of the loss of saltmarsh pioneer plants, increased sediment erodibility and creek erosion (Hughes and Paramor 2004). *H. diversicolor* consumes seeds and seedlings and is a known bioturbator of sediments through burrowing, filter feeding and consumption of microphytobenthos (Widdows et al. 2009). This species filter feeds by secreting a mucous filter to trap fine particles entering the burrow, the filter consumed once enriched (Scaps 2002). It has, however, recently been found that through the increased input of nutrients into the sediment and exopolymer secretion, *H. diversicolor* can stimulate increased biofilm development (Passarelli et al. 2012). The authors went further in a subsequent publication, suggesting that through stimulation of microalgal growth (by the redistribution of nutrients), these worms can reduce the erodibility of sediment (Passarelli et al. 2014).

H. diversicolor is regarded as a keystone species, which engineers intertidal mudflats through burrowing, bio-irrigation and sediment reworking (Mermillod-Blondin and Rutger 2006) and also indicates environmental change as a limiting step in ecosystem functioning such as recycling of detritus and associated biogeochemical cycling (Moreira et al. 2006). The future of this species and its resilience in the face of climate change is now in question, due to increases in temperature exacerbating and associated ocean acidification causing a reduction in feeding rate of *H. diversicolor* (Bhuiyan et al. 2021). As *H. diversicolor* is a prey item for birds, this study has scope to investigate top-down interactions between this species and shorebirds, which could potentially contribute to the engineering activities of an invertebrate which maintains flood defence potential in coastal wetlands.

Other infauna which are known to feed on particulates and detritus, present within the study sites of this research, are *Peringia ulvae* and *Macoma balthica* (Newell 1965), with *P. ulvae* a prolific redshank prey item (Anderson 1971), which is a common species within the study sites. *H. ulvae* also graze extensively on biofilms, in a very generalist manner, with bioturbation by this species also a potential driver of biofilm control or stimulant (Hagerthey et al. 2002). It was found that under laboratory conditions, unlike some sedimentary invertebrates such as *Corophium volutator*, *H. ulvae* do not have as pronounced an effect on diatom species diversity but consume less numerous species as often as dominant ones (Hagerthey et al. 2002).

Research has been carried out in the Bay of Fundy, Canada, where winter stressors such as sub-zero temperatures, temperature variations and ice are known to be more severe than the study sites used in this thesis (Gerwing et al. 2015). The Bay of Fundy biotic communities were quantified before and after winter, and results suggested that the measured winter variables did not greatly influence the infauna, and therefore that the community exhibited resilience to the winter stressors (Gerwing et al. 2015).

Effects of macrofauna also extend to nutrient fluxes, with a study from 1999 describing these types of interaction as “long recognised” (Mortimer et al. 1999). Burrowing macrofauna physically alter oxygen spatial distribution and penetration depths within the sediment, and actively flush these burrows which also enhances solute transport (Mortimer et al. 1999). It is largely agreed within the literature that bioturbation increases sediment surface area and hence modify the redox exchange properties of the sediment (Nizzoli et al. 2007).

Eleven mudflat dwelling macrofaunal species/groups are relevant to this thesis (Table 1): *Peringia ulvae, Limecola balthica, Cerastoderma edule, Hediste diversicolor*, *Nephtys hombergii*, *Corophium volutator*, *Retusa obtusa, Mya arenaria*, *Arenicola marina*, Chironomids (*Chironomidae*) and Nematodes (*Nematoda*) although the latter two groups were not identified to species level.

Table 1 Intertidal mudflat dwelling macrofauna present at each study site (Fingringhoe winter only) as recorded during fieldwork (Chapters 3 and 6) and monitoring. (Haskoning 2015): P.ulv=Peringia ulvae, L.bal=Limecola balthica, C.edu=Cerastoderma edule, H.div=Hediste diversicolor, N.hom=Nephtys hombergii, Chir=Chironomidae, C.vol=Corophium volutator, R.obt=Retusa obtusa, M.are=Mya arenaria, A.mar=Arenicola marina, Nem=Nematodes.

Site	**Macrofauna Species**										
	P.ulv	*L.bal*	*C.edu*	*H.div*	*N.hom*	*Chir*	*C. vol*	*R.obt*	*M.are*	*A.mar*	*Nem*
Brantham	P	P	P	P	P			P		P	P
Fingringhoe	P	P	P	P		P		P			P
Trimley	P	P	P	P	P		P		P		

Interactions between macrofauna and sediment properties are highly complex, varying through space and time and dependent on local environmental conditions (Wiesebron et al. 2021). Interactions between macrofauna and their environment, relevant to this thesis, and known consequences for sedimentary and biogeochemical processes, are presented in Tables 2 and 3 below.

Table 2 Relevant ecological traits of macrofauna present at study sites (excluding nematodes which are highly diverse and most often considered meiofauna (Patrício et al. 2012)

Scientific Name	Phylum/ Class	Adult Length (mm)	Burrow Type	Burrow Depth (cm)	Feeding Strategy	Prey	Predators	Sensitivity to changes in nutrient levels ($=confidence level)	References	Comments
Peringia ulvae	Mollusca/ Gastropoda	0.3-6.0	I or J shape	≤ 3	• Surface deposit • Microbrowser	• Detritus • Periphytic microalgae	• Shelduck	Not sensitive $Moderate	(Jackson 2000, Anders et al. 2009, Hale et al. 2015)	
Limecola balthica	Mollusca/ Bivalvia	3.0 – 25.0	I or J shape	5-6	• Active suspension • Surface deposit	• Diatoms • Deposited plankton • Suspended phytoplankton • Detritus	• Shorebirds • Shrimps • Crabs • Hediste diversicolor • Retusa obtusa	Not sensitive $Moderate	(Budd and Rayment 2001, Hiddink et al. 2002, Ashley 2016, Beukema et al. 2017)	Sediment Destabiliser*
Cerastoderma edule	Mollusca/ Bivalvia	3.0-38.0	I shape	≤ 5	• Active suspension	• Phytoplankton • Zooplankton • Organic particulate matter	• Shorebirds** • Shore crab (Carcinus maenas) • Shrimp • Flatfish	Low $Moderate	(Tyler-Walters 2007, Malham et al. 2012)	

**Limecola balthica* is classed as a 'biodestabiliser' after research (Widdows et al. 2000a) found a significant relationship between sediment erodibility and *L. balthica* density.

**Known *C. edule* predators include Oystercatcher *Haematopus ostralegus*, Knot *Calidris canutus*, Grey Plover *Pluvialis squatarola* and Redshank *Tringa totanus*. Oystercatchers show a preference for cockles of at least 20mm in length (Sanchez-Salazar et al. 1987).

Table 2 Relevant ecological traits of macrofauna present at study sites (excluding nematodes which are highly diverse and most often considered meiofauna (Patrício et al. 2012)

Scientific Name	Phylum/ Class	Adult Length (mm)	Burrow Type	Burrow Depth (cm)	Feeding Strategy	Prey	Predators	Sensitivity to changes in nutrient levels ($=confidence level)	References
Hediste diversicolor	Annelida/ Polychaeta	60.0-120.0	U, Y or J shape	≤ 20***	• Carnivory • Scavenging • Filter/passive suspension • Surface deposit • Sub-surface deposit	• Other macrofauna (incl. L. balthica) • Phytoplankton and plankton • Plant debris • Bacteria Detritus, mud/sand	• Shorebirds*[4] • Fish • Shore crab (Carcinus maenas) • Shrimps	Not sensitive $Moderate	(Hiddink et al. 2002, Scaps 2002, Budd 2008)
Nephtys hombergii	Annelida/ Polychaeta	100.0-200.0	-	5-15	• Carnivory • Scavenging	• Other polychaetes	• Fish • Birds	Very low $Moderate	(Schubert and Reise 1986, Budd and Hughes 2005)

***Burrow depth shown to level off around 15cm, at which they are out of reach of Curlew (*Numenius arquata*) and Oystercatcher (*Haematopus ostralegus*).

*[4] 15 shorebird species known to feed on *H. diversicolor*, abundant food source of Avocet (*Recurvirostra avosetta*), Grey Plover (*Pluvialis squatarola*) and Curlew. Also described as 'favourite prey' of Dunlin (*Calidris alpina*) (Dierschke et al. 1999).

Table 2 Relevant ecological traits of macrofauna present at study sites (excluding nematodes which are highly diverse and most often considered meiofauna (Patrício et al. 2012).

Scientific Name	**Phylum/ Class**	**Adult Length (mm)**	**Burrow Type**	**Burrow Depth (cm)**	**Feeding Strategy**	**Prey**	**Predators**	**Sensitivity to changes in nutrient levels ($=confidence level)**	**References**
Chironomids	Chironomidae	10	-	-	• Detritus	Nematodes	• Shorebirds	-	(Sanchez et al. 2006, Ptatscheck et al. 2015)
Corophium volutator	Arthropoda/ Malacostraca	11	U-shape	-	• Suspension/ deposit dictated by tide • Epipsammic diatom browsing	• Bacteria • Diatoms • Particulate organic matter	• Dunlin • Redshank • Shelduck • Flounder • Shore crab • Common goby • Herring • Sprat • Smelt	Low $High	(Gerdol and Hughes 1994, Neal and Avant 2006) (and see within)
Retusa obtusa	Mollusca/ Gastropoda	≤ 15.00	Unknown	≤ 3.5	• Carnivory • Mobile prey	• Peringia ulvae • Limecola balthica	• Dunlin • Grey Plover • Shelduck • Fish	Unknown	(Smith 1967, Wolff et al. 1981, Dit Durell and Kelly 1990, Viain et al. 2011)

Table 2 Relevant ecological traits of macrofauna present at study sites (excluding nematodes which are highly diverse and most often considered meiofauna (Patrício et al. 2012).

Scientific Name	Phylum/ Class	Adult Length (mm)	Burrow Type	Burrow Depth (cm)	Feeding Strategy	Prey	Predators	Sensitivity to changes in nutrient levels ($=confidence level)	References	Comments
Mya arenaria	Mollusca/ Bivalvia	20 - 150	Vertical	≤ 50	• Active suspension	• Phytoplankton • Zooplankton • Diatoms • Particulates Dissolved organic matter	• Crabs • Fish	Low $Moderate	(Hunt and Mullineaux 2002, Tyler-Walters 2003)	
Arenicola marina	Annelida/ Polychaeta	15-25	U or Y - shape	20-40	• Surface deposit • Carnivory • Scavenging • Bacteria	• Diatoms • Bacteria • Meiofauna	• Fish • Grey Plover	Low	See foot notes	Substantial sediment reworking*[5] Ecosystem Engineer*[6]

A. marina references: (De Vlas 1979, Pienkowski 1982, Grossmann and Reichardt 1991, Retraubun et al. 1996, Riisgard and Banta 1998, Kristensen 2001, Volkenborn et al. 2007, Tyler-Walters 2008)

*[5] As *A marina* casts age they are 'invaded' and utilised by organisms (Reise 1981).

*[6] *A marina* maintain habitat sediment and porewater characteristics suitable for their population to persist (Volkenborn et al. 2007).

Table 3 Known effects of macrofauna present at study sites on sediment characteristics and biogeochemical cycling

Scientific Name	**Sedimentary Interactions**	**Biogeochemical Interactions**
Hediste diversicolor	• Burrows increase the sediment-water interface by up to 3 times, with material transported into sediments throughout the burrow (Scaps 2002). • Oxygenation associated with burrows may cause increased aggregations of meiofauna closer to burrows (Scaps 2002). • Increased sediment erodibility through bioturbation (de Deckere et al. 2001). • Decreased sediment erodibility through bioturbation (Meadows and Tait 1989). • Increased sediment erosion through burrowing and surface feeding (Widdows et al. 2009). • Surface adhesion increased (Passarelli et al. 2012).	• Enhanced release of CO_2 and ammonium from the sediment (Kristensen and Hansen 1999). • Increased sediment O_2 uptake and total CO_2 by 30-70%, mostly due to respiration by *H. diversicolor* (Kristensen and Anderson 1992). • Increased efflux of ammonium and silicate into the water column by up to 100 times (Davey and Watson 1995). • Increased O_2 uptake at the sediment surface (Mermillod-Blondin and Rutger 2006). • Increased nitrogen release from sediments (Mermillod-Blondin and Rutger 2006). • Increased sediment oxygen heterogeneity (Pischedda et al. 2008). • Decreased silicate and phosphate effluxes (Mortimer et al. 1999). • Increased ammonium and nitrate influxes (Mortimer et al. 1999).
Peringia ulvae	• Increased erosion rate by factor of 2 to 4 in snail presence (Andersen et al. 2002). • Increased sediment re-suspension during the biofilm exponential phase (Orvain et al. 2004).	• Efflux of silicate due to increased grazing (Janas et al. 2019). • Abundance can be significantly reduced by nutrient input (ammonium and phosphate) (O'Brien et al. 2009).
Limecola balthica	• Erodibility increases asymptomatically with population density of *L. balthica* (Willows et al. 1998). • Significant relationship between clam density and sediment erodibility (Widdows et al. 2000a). • Significant correlation between sediment erodibility and clam density (Widdows et al. 1998).	• Increases efflux of nitrate and ammonium from sediment surface (Michaud et al. 2005). • Increases efflux of ammonium and nitrite (Mortimer et al. 1999). • Increased influx of O_2 (Michaud et al. 2005).

Table 3 Known effects of macrofauna present at study sites on sediment characteristics and biogeochemical cycling

Scientific Name	**Sedimentary Interactions**	**Biogeochemical Interactions**
Cerastoderma edule	• Significant relationship between cockle biomass and sediment clearance rate (Widdows et al. 2000a). • Significant relationship between sediment erodibility and cockle density (Widdows et al. 1998). • Cockles increase sediment stability (reduce erodibility), under some hydrodynamic and sediment conditions, promoting biofilm growth (Donadi et al. 2013).	• Remove nitrogen, phosphorous and carbon from the marine environment (Carss et al. 2020). • Increased nitrogen release from sediments (Mermillod-Blondin and Rutger 2006). • Neutral effect on O_2 uptake at sediment surface (Mermillod-Blondin and Rutger 2006). • Abundance can be significantly reduced by nutrient input (fertiliser) (O'Brien et al. 2009).
Retusa obtusa	• Abundance can be significantly affected by the presence of *A marina* funnels (Brey 1991).	• Significant correlation found between abundance and total organic carbon (TOC) and total carbon (TC) (Schuckel et al. 2013). • Abundance can be significantly reduced by nutrient input (ammonium and phosphate) (O'Brien et al. 2009).
Arenicola marina	• Significant reducer of sediment stability (increases erodibility) (Volkenborn et al. 2007, Donadi et al. 2013).	• May reduce the effect of ammonium and phosphate enrichment in higher permeability sediments (O'Brien et al. 2009) • Stimulates carbon oxidation by introducing oxygen to subsurface sediment and removing metabolites (Kristensen 2001)

2 Exclosure Design and Methodological Development

2.1 Introduction

The experimental work presented in this thesis uses a multidisciplinary approach combining established sedimentological and biogeochemical measurement techniques with emerging and novel shorebird study and mudflat manipulation methods to examine the top-down effects of shorebird activity on mediated ecosystem functioning and services. This chapter justifies and details methods which are retained as common threads throughout experimental Chapters 3, 4, 5 and 6 to investigate shorebird effects on intertidal muddy sediments and ecosystem functioning (EF) and services (ES) which they provide.

2.2 Exclusion and Manipulation Methods

The method of excluding predators from areas of intertidal habitat, to compare areas where predators are present and absent, has been successfully used in previous studies (Reise 1977, Schneider 1978, Peterson 1979, Quammen 1984, Gee et al. 1985). These include exclosures on soft sediment such as mudflats, which can be successful when carefully designed (Raffaelli and Milne 1987). In more recent decades, exclosure experiments investigating predators of benthic invertebrates have become more common, although evidence of impacts upon prey density is not always detected (Rosa et al. 2007). Previous designs have been similar in shape and basic function (quadrilateral, joining four posts with material to exclude animals (Reise 1977, Schneider 1978, Peterson 1979, Quammen 1984, Gee et al. 1985). However, details such as materials, whether both sides and a roof are used, and the aperture and type of materials differ depending upon the target species for exclusion. Two different types of exclosure have been used in a single study; one to exclude both fish and birds, and one which only excluded birds (Raffaelli and Milne 1987). This was achieved by including sides where both groups were excluded and using a 'roof only' design where only birds were to be excluded. This study used string wound between four posts for one experiment, and metal caging in the second. More recently, the use of line between four posts has been employed with fishing line wound round four corner posts protruding approximately 30cm above the sediment surface (Hamilton et al. 2006). Despite the lack of 'roof', the authors report that this design "successfully excluded the majority of shorebirds", which they suggest was due to the highly visible flagging tape (Hamilton et al. 2006).

Numbers of exclosures (replicates) used in published studies varies widely; previous exclosure experiments conducted in the intertidal have used two (Raffaelli and Milne 1987), three (one control) (Gee et al. 1985), four (Quammen 1984), six (Vargas 1988), eight (Székely and Bamberger 1992, Silliman and Bertness 2002), 10 (Cheverie et al. 2014) and 20 replicates (Hamilton et al. 2006) of each treatment, with the identical number of controls except where noted. Replicate number was cited as a reason for previous failures to detect significant differences between predated and non-predated areas (Rosa et al. 2007). This study also found a significant impact of predators (nekton and shorebirds) upon *Hediste diversicolor* abundance in a study design using eight replicates of 'sites' containing two exclosure types and a control (Rosa et al. 2007).

The minimum sample size required to accurately measure benthic microalgal abundance has been experimentally tested, finding that a minimum sample number of >6 was required at the metre squared scale (Grinham 2007). The authors note that this minimum replicate number is in accordance with a previous study which found that "eight replicates would have had 80% power to detect 50% change" (Kendrick et al. 1996).

2.2.1 Exclosure Designs

The experiments presented in this thesis employed four exclosure designs, chosen depending upon the experiment aim and hypothesis. These designs are referred to as Exclosures (E) a (E_a), b (E_b), c (E_c) and d (E_d) and are described below.

Exclosure a (E_a)

This was used to test the resilience and safety of a product designed to withstand outdoor weather in terrestrial systems, however it's durability in marine systems was unknown. To test the durability and ensure that it was safe for shorebirds and other wildlife on a mudflat, avian fruit cage netting (20 mm aperture) was stretched over eight bamboo canes, positioned in a square approximately 100 cm x 100 cm, and secured to them using cable ties (Image 1).

Image 1: Exclosure *E$_a$* (n=3) on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05170 19195) between 18 February 2016 and 30 March 2016.

Exclosure b (E$_b$)

E$_b$ used a shorebird exclosure design used in existing literature (Hamilton et al. 2006), comprising four 20x 12 mm diameter bamboo canes, set out in a 100 cm x 100 cm square with a cane in each corner, extending approximately 35 cm above the sediment surface. Each side of the plot was formed by three 100 cm lengths of monofilament fishing line at 10 cm intervals, starting 5 cm above the sediment surface. Posts and lines were also marked with red and white flagging tape (Image 2).

Image 2 Exclosure *E$_b$* (n=3) on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05170 19195) between 18 February 2016 and 30 March 2016.

Exclosure c (E_c)

Once it had been established that avian fruit netting was safe to use, sufficiently durable and effective at excluding shorebirds, the material was used to design an exclosure which could withstand opening to allow repeat sampling over a period of months.

The frame of E_c was made from 20x 12 mm diameter bamboo canes, each approximately 110 cm in length (to allow for overlap), joined using cable ties. Side panel dimensions were each 30 cm (height) x 100 cm (length) and the top square was 100 cm x 100 cm. Each side rectangle and the top square were made separately, with each upright cane of the side panels extending 70 cm into the sediment to stabilise the structure. Avian exclusion netting (commonly used on agricultural fruit cages) with 20 mm aperture was fixed to each side and the top frame using heavy duty marine grade stainless steel staples, every 50 mm along each side, applied using an industrial pneumatic staple gun.

Once all sides of an exclosure were inserted into the sediment, they were cable tied together for stability and to prevent entry through the corners by birds. The top square was then positioned on top of the side panels and fixed using cable ties. This allowed removal of the top square (lid) for repeated sampling inside each exclosure. On each sampling visit, any accumulations of algae were removed from the surface of the netting (Images 3 and 5).

Image 3 Exclosure E_c (n=3) on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05170 19195) between 03 January and 03 April 2017.

Possible experimental artefacts created by this type of exclosure include changes to water flow, through disruption by canes and netting, and solar irradiance through reduction by canes and netting. The potential of these to affect results is tested and considered within Chapter 3, where effects of shading were considered similar to other manipulative studies in this type of environment. Results gathered also indicated that effects on MPB were not driven by shading (see Chapter 3).

Controls were 1m^2 plots marked using four 20x 12 mm diameter bamboo canes, set out in a square with a cane in each corner, extending approximately 35 cm above the sediment surface.

2.2.2 Density Manipulation

A different shorebird exclosure design is described in (Cheverie et al. 2014), which consists of four bamboo canes at corners of a 1 m^2 quadrat, supporting an approximately equal dimension roof of clear plastic mesh with 6mm apertures (Cheverie et al. 2014). The authors selected this to minimise effects of shading and water flow disruption (Cheverie et al. 2014) and also report some breach of this design by semipalmated sandpipers (mean percent footprint cover inside ‘exclosures’ was 4%). Evidence suggests that birds can vary widely in their feeding behaviour, both inter and intra-specifically at the population and individual levels (Norazlimi and Ramli 2015, Austin et al. 2021, Brucks et al. 2021). Therefore, use of this exclosure design was adopted at our study sites as a method of shorebird density manipulation, quantifiable through estimation of shorebird footprint cover (Robar and Hamilton 2007) (see Chapters 4 and 5).

Exclosure d (E_d)

E_d were as used by (Cheverie et al. 2014), consisting of four bamboo corner posts supporting a clear plastic mesh roof (6 mm aperture) approximately 30 cm above the sediment, with open sides on every aspect (Image 4). These significantly reduced entry by shorebirds, while allowing access to fish and invertebrates (Cheverie et al. 2014). This exclosure design has also previously been shown not to significantly affect water flow or light levels within plots (Cheverie et al. 2014).

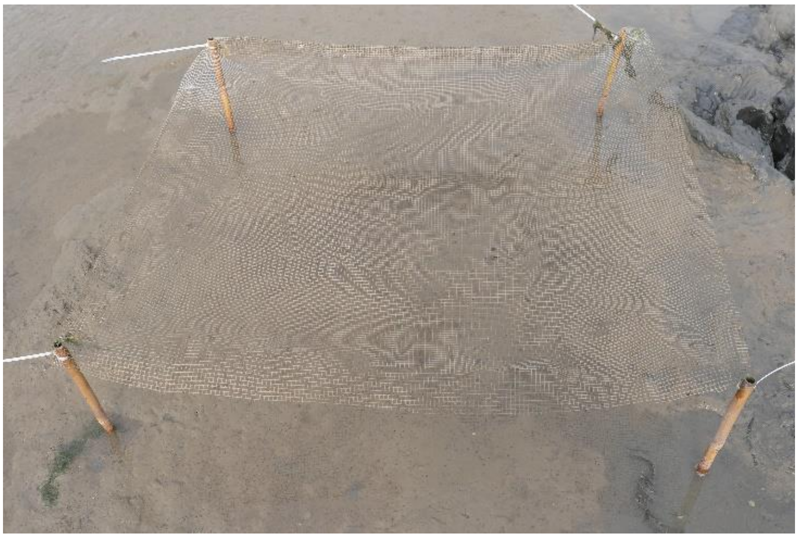


Image 4 Exclosure E_d (n=3) on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030), on the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (grid reference TM 12427 33311) and on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406) measured between 20 September 2018 and 25 April 2019 (Chapters 4 and 5).

Image 5: E_C following deployment, showing set of ten with paired controls marked by corner canes (Chapter 3).

2.3 Measurement Techniques

2.3.1 Field Measurement

2.3.1.1 Sediment Erodibility

There are numerous methods of measuring and quantifying the erodibility of sediments, which can be split into *in situ* and laboratory techniques (Tolhurst et al. 2000b). The measurements of most interest are usually shear stress (τ_{cr}) (the force required to initiate erosion) and erosion rate (Ɛ) (the amount of erosion per unit of time), and a number of *in situ* devices are used to assess these (Tolhurst et al. 2000b). *In situ* devices described in the literature include the cohesive strength meter (CSM) (Mark IV, Sediment Services, Sussex, UK), *In-Situ* Erosion Flume (ISEF) (Houwing and Van Rijn 1998), SedErode (Williamson and Ockenden 1996) and Microcosm systems (Gust 1990, Gust and Müller 1997), although SedErode and *in-situ* flumes do not appear in the recent literature. These devices were found to be comparable where τ_{cr} was measured, but that results differed by orders of magnitude where Ɛ was measured (Tolhurst et al. 2000a). Differences in measurements across devices were caused by differing amounts of time spent applying stress to the bed, size of measuring chambers, and differences in the types of pressures applied to the sediment (Tolhurst et al. 2000a). For these reasons, use of a single erosion device is recommended during a project. Due to its ease of deployment and rapid measurements a CSM was used in this thesis.

The CSM measures shear stress in Nm^{-2} (once a simple conversion has been applied to the output). The CSM applies a contained jet of water to the sediment bed, which increases in force incrementally. The re-suspended particles are measured (using turbidity as a proxy) to determine the amount of erosion at each level of stress applied to the bed. Using these steps in erosion, an erosion rate can also be calculated ($kg\ m^{-2}\ s^{-1}$) (Tolhurst et al. 2000a).

2.3.1.2 Cohesive Strength Meter (CSM)

The cohesive strength meter (CSM; Mark IV, Sediment Services, Sussex, UK) is an *in-situ* device used to measure critical erosion shear stress and suspension index of surface sediment (Tolhurst et al. 1999). The CSM subjects the sediment surface to a vertical jet of water at incrementally increasing force to measure the force at which erosion of the sediment surface occurs. The testing process is controlled by a computer, stored within a watertight container, also containing tanks, solenoids, pressure sensor, gauge, regulator and filter (Figure 1). A detachable sense head is connected to the computer by an electronic cable and water hose containing test chamber, fill tube, headlamp and sensor (Figure 2). Ancillary devices required include a high-pressure air tank (dive cylinder), high-pressure hose and holder/stand for the sense head during measurement (clamp stand).

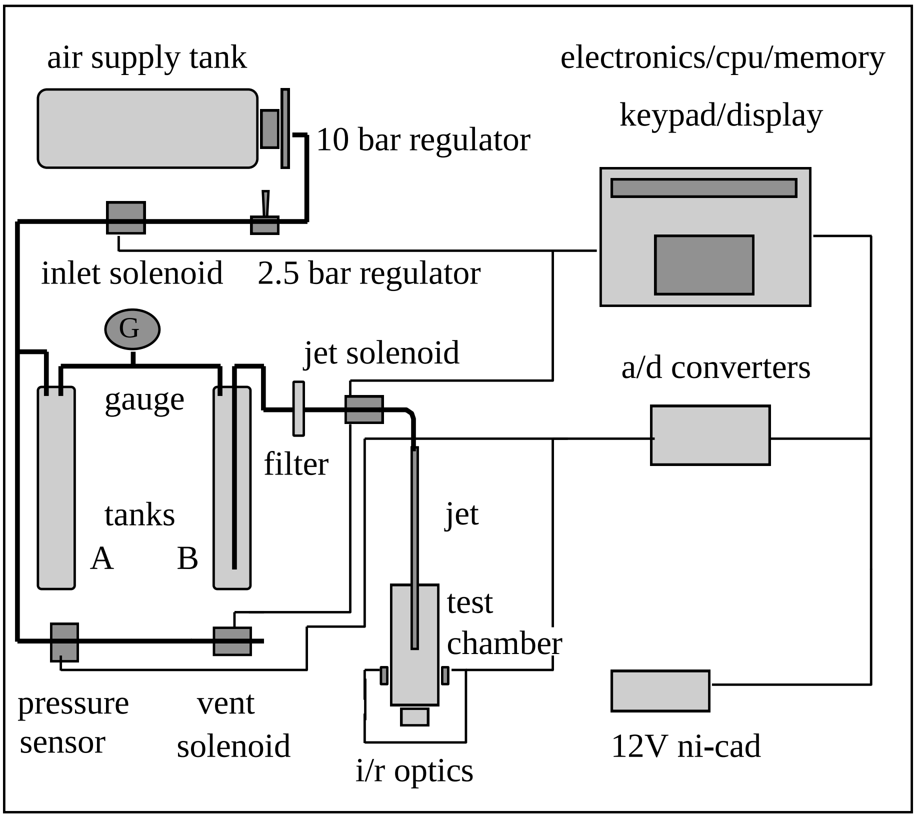


Figure 1 Electronic schematic showing the components and basic circuitry comprising the main body of the CSM, contained within a Peli-Case with external air supply tank (dive cylinder) (Tolhurst et al. 1999).

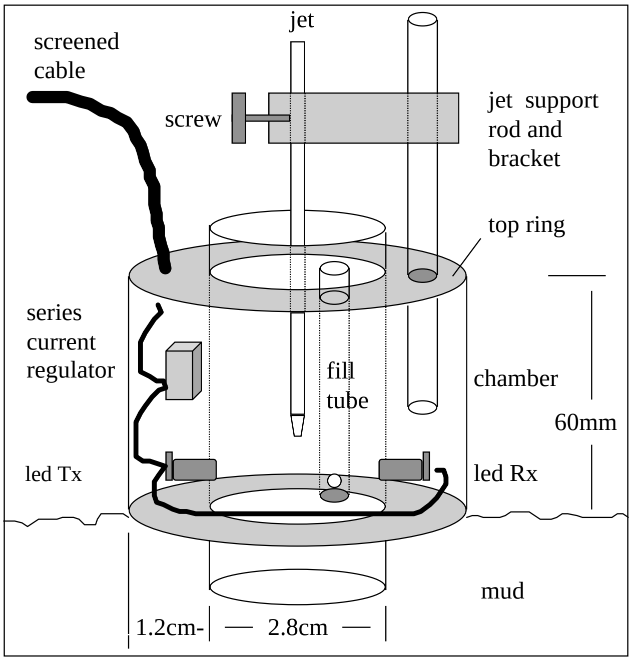


Figure 2 Electronic schematic showing the sense head, chamber and jet unit which is connected externally to the CSM unt (Fig 2.1) and pushed into the sediment (as shown), held in place by a clamp-stand (Tolhurst et al. 1999).

During setup the air tank is connected to the CSM by the high-pressure hose and the sense head is connected by the attached cable and hose. The water tank within the CSM unit is filled with ambient seawater (collected from as near to the study site as possible, with sediment settled out). The test chamber is eased carefully into undisturbed sediment containing no visible burrows (which can drain water from the test chamber), up to the lower lip (Figure 2), with the sense head held in this position by the clamp stand, itself pushed into the sediment. The test chamber is carefully filled with clear, ambient seawater. The headlamp is then switched on using the CSM unit controls, to check the initial turbidity reading (starting transmission) and ensure that the sense head filling has not disturbed surface sediment to a point which could compromise the results. Depending upon the operative’s experience of the site and the erodibility of the sediment, this threshold typically differs between study sites. For most of this work, readings below 70 were not tolerated and if this value could not be achieved the process was restarted. At this point, if the turbidity reading is appropriate, the setup is complete, and the test is ready to run.

A test is initiated via the CSM unit controls. During a test water from the CSM tank is jetted from the nozzle in the sense head onto the sediment surface at an incrementally increasing, known force. At each increment the suspension of any surface particles is measured by attenuation of an infra-red beam (the headlamp, wavelength 940nm), located 10 mm above the sediment surface, across the eroding chamber using a spectrally matched receiver on the opposite side (Tolhurst et al. 1999). The computer logs all values during the test, which are also displayed at the CSM unit controls, showing the user the headlamp on/off position and the strength of the beam across the chamber. Once the beam attenuation reaches 10% of the starting transmission the critical erosion threshold has been reached (Vardy et al. 2007) and logged and the test is complete. As insurance against the possibility of computer memory failure, the jet force at which the critical erosion threshold is reached is also manually recorded. The chamber is rinsed thoroughly before the next measurement.

The CSM has a number of pre-defined 'tests' with varying jet force increments to achieve efficient testing in a range of sediment types. During this research all tests were performed using test 'Fine 1', which is designed for fine sediments, allowing for the potential of a low erosion threshold (using low jet force initially), increasing the increments at higher pressures enabling the measurement of very stable sediments.

The application of the CSM in this research allows a critical erosion threshold τ_{cr} to be calculated (Nm^2), which is the water jet force required to cause a 10% reduction in transmission of the IR beam across the test chamber (Tolhurst et al. 2000a). Using this standardised value allows comparison of the erodibility of the mud between areas.

2.3.1.3 Pulse Amplitude Modulated (PAM) Fluorometer

Scientific studies generally quantify MPB biomass by measuring chlorophyll *a* (chl *a*) concentration at the sediment surface, which is a proxy for MPB biomass and a widely used and accepted environmental parameter in intertidal ecosystems (Bale 2005, Saint-Béat et al. 2013). PAM fluorometry provides a means of estimating chl *a* concentration, after sufficient dark adaption, by measuring the variable fluorescence yield within the top ~2 mm of sediment, as a proxy of chrorophyll abundance, predominantly diatoms (Honeywill et al. 2002). PAM measurements target the top ~2 mm of sediment because this is the depth to which motile diatoms migrate and consequently nearly comparable levels of association between chl *a* and F_o have been found up to 2 mm deep (Honeywill et al. 2002). The variable fluorescence is the difference between the dark adapted (minimum) fluorescence yield (F_o) and the maximum fluorescence yield (F_m) after a pulse of light has been applied to the sample surface (Honeywill et al. 2002). The sole use of PAM measurements, however, is still debated due to the lack of methodological standardisation. Therefore, measuring biofilm abundance requires a combination of PAM measurements, and verification through collection of sediment samples and subsequent analysis (see Spilmont et al, 2011 and references therein). The analysis of sediment is used to ground truth the chl *a* concentration in the sediment surface against the measured fluorescence.

The pulse amplitude modulated fluorometer (PAM; Diving-PAM, Walz, Effeltrich, Germany) is a device used to measure photosynthetic minimum fluorescence and maximum quantum yield of photochemical energy conversion in photosynthesis. This is achieved through application of pulse-modulated light for selective detection of chlorophyll fluorescence yield (GmbH 1998). A saturating light pulse is emitted onto the measurement area, which briefly suppresses photochemical yield (Schreiber et al. 1986), which is then measured and stored by the PAM. This 'minimum fluorescence' (F_o) can be used as a proxy for microphytobenthic biomass at the sediment surface (Honeywill et al. 2002, Eggert 2006, Jesus et al. 2006b).

To accurately measure F_o the microphytobenthos must be 'dark adapted' using custom dark adaption chambers (Jesus et al. 2006a). Partial dark adaption is a preferred method of achieving this (Jesus et al. 2006b) particularly in the intertidal where factors such as tidal regime, replicate measurement numbers and dewatering effects (Maggi et al. 2013, Orvain et al. 2014a, Fagherazzi et al. 2017) must also be considered (Booty et al. 2020). Another advantage of partial dark adaption is that a 'sampling port' can be built into the custom chamber to allow F_o measurement without removing the chamber from the sediment. Using this approach, partial dark adaption chambers are placed onto the measurement area for five minutes (Booty et al. 2020) before the PAM probe head is inserted through the sampling port against the sediment surface and a reading is taken using the measurement button on the PAM unit. A further advantage of these dark adaption chambers is that the distance between the sediment surface and the first rim of the PAM probe head is kept constant, ensuring that the distance between the fibre-optic tip and sediment surface is also constant. The chambers achieved a consistent low light sampling environment using plastic 40 mm (diameter) x 60 mm (length), cylindrical opaque dark adaption chambers with a 6mm aperture hole at the top. This also enabled *in-situ* sampling with the PAM fluorometer without removal of the chamber. This reduced the variation in light intensity during the measuring period. This ensures that comparisons between absolute values of fluorescence yields can be relied upon, as small variations in measuring distance can cause large variations in F_o measurements (Jesus et al. 2006b).

Measurement using the PAM is non-destructive, therefore these were the first measurements to be taken during all sampling fieldwork. During all field sampling events, five F_o measurements were taken within each treatment replicate on different sediment patches. As an insurance against software malfunction F_o values were recorded manually in the field.

2.3.1.4 Contact Cores (CCs)

Contact coring is a method of sampling the surface 2-3 mm of sediment using a custom metal disc core (Anderson and Black 1980, Honeywill et al. 2002). The core consists of a cup approximately 30 m volume on top, a flat base at the bottom with a circumferential 2 mm lip. When placed onto the sediment the lip extends 2 mm into the sediment surface layer. The following is carried out wearing two layers of nitrile gloves: Liquid nitrogen is poured into the top cup *in-situ*, freezing the sediment within the bottom 2 mm core lip, forming a frozen disc of sampled sediment adhered to the bottom of the core. *In-situ* freezing time varies depending upon sediment characteristics such as water content and particle size and environmental variables such as temperature and wind speed (Honeywill et al. 2002) and therefore some test samples may be required at new sites or under new conditions. Freezing time was relatively consistent across study sites, at around 60 seconds. Once frozen, the core containing the sediment disc is removed from the mudflat surface and a knife is used to ensure that the disc is flush with the bottom of the 2 mm lip by carefully scraping away excess frozen sediment. A slot in the 2 mm lip (flush with the flat base) is then used to carefully remove the frozen sediment disc from the core by inserting a thin knife. Each sediment core was then transferred to pre-labelled foils, wrapped carefully and stored in a liquid nitrogen dewar at approximately -196 °C. On return to the lab, these cores were stored in the dark at -80 °C prior to analysis.

2.3.1.5 Syringe Cores (SCs)

Syringe cores were collected using a 2 cm diameter syringe, with the base tip removed and cut surface finished to make flush and marked at 1 cm. The cut end was placed carefully against the surface of the mud, and the syringe extended to collect sediment to the 1 cm mark. Once removed from the mudflat surface, excess sediment was carefully scraped off to ensure a flush core, which was transferred to a pre-labelled plastic Ziploc bag. These samples were stored in the dark at -80 °C on return to the lab.

2.3.1.6 Measurement Configuration

Between day variation in mudflat characteristics have been shown to be of greater significance than within day variation (Tolhurst and Chapman 2005), therefore in each experiment repeated measures of F_o were made to compensate for this effect (Booty et al. 2020). MPB are key drivers of intertidal flat properties and processes (Murphy and Tolhurst 2009), so to determine when the full sampling event would be most likely to detect any effects we monitored F_o (as a proxy for MPB biomass) throughout each experimental period, as a convenient indication of treatment effects, to determine when erodibility and nutrient flux variables should be measured and to confirm that early in the experiment there were no significant procedural differences between treatments (Booty et al. 2020). To minimize the effect of varying light intensity between sampling events, sampling periods were timed to cover low tides peaking as close to midday as possible (Booty et al. 2020).

In-situ measurement locations were chosen to be representative of the heterogeneity of biofilm cover within each plot. During 'full' sampling events (when F_o measurements, erodibility measurements, contact cores and flux cores were collected) F_o measurements were taken first due to being the least destructive sampling method. Erodibility measurements were taken second, followed by contact cores, with flux cores collected last due to being the most destructive sampling technique. Due to the destructive nature of the sampling techniques, contact core and erodibility sampling locations were paired by being taken as close together as possible.

2.3.1.7 Bird Surveys (Point Counts)

To determine the level and type of use of the study area by shorebirds, monitoring began before the experimental setup to ensure current use of the study area and aid in deciding the best location for the experimental plots. Surveys were carried out using the 'look-see' methodology (Bibby et al. 2000), from a fixed location. The look-see method refers to visual observation which can be fully relied upon within open habitats such as mudflats, as opposed to physically cluttered habitats where robust bird surveys require equal, if not greater, reliance on auditory species identification (Bibby et al. 2000).

Surveys were undertaken for at least 2 hours either side of low-tide, including as much of these timeframes as possible (four hours maximum) within daylight constraints. Particular care was taken to also include visual observation of the tideline crossing the plots wherever possible. Counts of species within the surrounding visible mudflat were taken every half hour. Continual observation of the study area was made, quantifying numbers and identifying species entering presence plots throughout the surveys. Equipment included a 20−60 × 82 telescope and 10 × 42 binoculars.

2.3.1.8 Bird Density (footprint coverage)

It has been shown using video monitoring that a strong non-linear relationship exists between percent-cover of bird footprints and habitat use, using video monitoring (Robar and Hamilton 2007). Shorebird habitat use can therefore be robustly estimated using percent footprint cover (Robar and Hamilton 2007). As such, shorebird track coverage in each plot was recorded during F_o sampling as part of the multi-site investigation in 2018-2019, to monitor the level of use of the plots by shorebirds and enable determination of the effectiveness of the exclosures and the use of each plot.

Footprint percentage cover was estimated by the same researcher on each visit, to minimise the effect of surveyor bias, which would otherwise need to be taken into account (Kronenfeld and Wang 2007).

2.3.2 Laboratory Measurements

2.3.2.1 Laboratory Mesocosms

Flux cores (height = 40 cm, diameter = 8.5 cm) were carefully returned to the laboratory within an hour of leaving the site and immersed in seawater from the site, within oxygenated and temperature and light controlled indoor mesocosms (Thornton et al., 1999). Rubber bungs pushed in at the bottom of the cores were used to ensure equal headspace volume across cores. Cores were left submerged and open to settle overnight prior to sampling on the following day aligned to field site tidal cycles. Throughout headspace water sampling, Perspex lids were tightly fitted to prevent leakage. Magnetic stirrers maintained water flow over the sediment surface during all periods when samples were not being taken. Each core was sampled for sediment-water biogeochemical fluxes of oxygen, nitrate, nitrite, ammonium, phosphate and dissolved organic carbon (DOC). Headspace seawater samples were taken at the beginning and end of fixed 2 or 3 hour dark and light incubation periods. Cores were left for at least one hour to adjust to light levels prior to each incubation. Sampling was completed according to general methods described by Thornton *et al.* (1999). Flux measurements were repeated in both light and dark conditions, using 500 W halogen 'daylight' lamps to provide 'lit' conditions (500 μmol m^{-2} s^{-1} PAR) and covering mesocosms with opaque Perspex covers to provide 'dark' conditions. These incubations were timed to match periods of tidal exposure (light) and cover (dark) to align with the natural tidal rhythms of sediment organisms in the core samples.

2.3.2.2 O_2 Analysis

Water samples to determine O_2 concentrations were taken using a rubber tube, filling a glass vial containing a glass bead carefully from the bottom, to avoid creating bubbles, and fixing by addition of 0.1 ml manganese sulphate ($Mn^{+2}SO_4$) and potassium hydroxide (KOH). Samples were sealed and stored in darkness in a fridge and analysed within one week of collection.

Oxygen concentration of samples was determined on the bench following Winkler's titration method (Winkler 1888). The fixed sample was transferred to a volumetric flask. 1ml sulphuric acid (H_2SO_4) and a starch indicator were added, and the flask swirled gently by hand. Sodium thiosulphate ($Na_2S_2O_3$) was added using an automated pipette in increments of 5 µl until the solution changed to a light blue. The sodium thiosulphate was then added slowly while gently swirling the flask until the solution was first colourless. The volume of added sodium thiosulphate was recorded.

The volume of titratent was converted into µmol l^{-1} oxygen using formula 2.1 below:

$O_2 = 833.33 \times T \times (F / 4)$

Where: O_2 = volume of oxygen in µmol l^{-1}

T = volume of titratent in ml

F = F-factor (=1)

Equation 2.1: Used to calculate oxygen concentration (µmol l^{-1}) following titration using volume of oxygen, titratent volume and F-factor.

The difference in oxygen concentration at the beginning of each incubation period (dark and light) and the end of each incubation period (after either two or three hours) was then calculated for each flux core and converted into µmol m^{-2} h^{-1} using the surface area of the sediment in each core (m^2) and the time (hours) of incubation.

2.3.2.3 Nutrient Analysis – NO_3^{--}, PO_4^{3-}, NH_4^+, NO_2^-, SiO_4

Water samples for nutrient analysis (50 ml) and dissolved organic matter analysis (15 ml) were taken at the same time as the O_2 concentration samples and filtered prior to storage using Sartorius Minisart 0.2 µm syringe filters. Sample bottles were completely filled with water and sealed and stored in darkness at -80°C prior to analysis.

Samples were analysed for concentration of nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}), ammonium (NH_4^+), silicate (SiO_4), total organic nitrogen (TON) and dissolved organic carbon

(DOC), due to the ecological impacts which nitrogen and phosphorous loading have on temperate estuarine environments (Howarth et al. 2011, Regnier et al. 2013) and the significant role which our knowledge of the carbon cycle has on our understanding of climate change (Bakker et al. 2014) (Chapter 1).

Water samples were analyzed for their nutrient concentrations using a Seal AA3 segmented flow Nutrient Analyzer (SEAL Analytical Inc.), for DOC and TON using Skalar Formacs TOC model 2CA16910-02, Nitrogen detector model 2ND25900 and MCS solid module model 2MC10900, following standard operational methods for these instruments.

The difference in nutrient concentrations at the beginning of each incubation period (dark and light) and the end of each incubation period (after either two or three hours) was then calculated for each flux core and converted into µmol m^{-2} h^{-1} using the surface area of the sediment in each core (m^2) and the time (hours) of incubation.

2.3.2.4 Sediment Analysis

EPS is a mixture of proteins, proteoglycans and carbohydrates, EPS concentrations in sediments can be estimated using colloidal carbohydrate concentration as a proxy (Underwood 2003).

Prior to analysis, sediment from the syringe and contact cores was freeze dried in in the dark for 24 hours. This sediment was then analysed in the laboratory to determine the following physio-chemical variables both between and within sites:

- Modal grain size (µm)
- Chlorophyll *a* concentration (µg g^{-1})
- Total colloidal carbohydrate concentration (µg g^{-1})
- Water content (%)

Grain size and water content analyses provide important context when comparing effects between sites and controlled for natural variation within sites. Chlorophyll *a* and total colloidal carbohydrate concentrations ground truth the F_o proxy data during the full sampling events and give context to the τ_{cr} measurements, given that colloidal carbohydrates can be a biochemical predictor of sediment erodibility (Underwood and Paterson 1993). EPS is also a significant carbon source, produced in both light and dark, production rates of which are influenced by environmental factors and nutrient stoichiometry (Underwood 2022).

Chlorophyll *a* was extracted on the bench using cold methanol over 24 hours, and measured spectrophotometrically, correcting for phaeopigments (Stal et al. 1984). Approximately 100 mg of sediment from each plot was weighed into a clean centrifuge tube, and the exact weight recorded. Solvent was prepared by saturating 100% methanol with $MgCO_3$. 4 ml of solvent was added to the sediment, whirlmixed, covered in Parafilm and placed in the fridge in the dark for 24 hours. The samples were whirlmixed again after dark refrigeration and centrifuged for 10 minutes at 4500 RPM. 1 ml of each sample was placed in a new plastic cuvette and measured in a photospectrometer at 665 nm and 750 nm, to correct for turbidity. 1 ml samples of the solvent was used to prepare the blank. Each sample was acidified using one drop of 10 % HCl. After five minutes, each sample was measured again in a photospectrometer at 665 nm and 750 nm, to correct for phaeopigments.

Concentrations of Chlorophyll *a* were then calculated using equation 2.2:

$((A_n\text{-}A_a) \text{ x } V \text{ x } 1320) / (A_c \text{ x } W) = \mu g \text{ Chl } a \text{ } g^{-1}$

Where: V = Volume of solvent in ml

W = Weight of freeze dried sediment

A_n = The absorbance at 665nm before acidification minus the absorbance at 750nm before acidification.

A_a = The absorbance at 665nm after acidification minus the absorbance at 750nm after acidification.

A_c = Absorbance coefficient

Equation 2.2: Used to calculate Chlorophyll *a* concentration following chemical extraction using sample mass, solvent volume and absorbance.

Measurement of carbohydrate concentrations within sediment samples, using the Dubois Assay (Dubois 1956) is an established method of determining EPS content, although careful attention to storage and analysis methods are required if reference and comparison between studies is to be reliable (Underwood et al. 1995). Underwood also proposes and details a standard methodology for the procedure. Carbohydrate analysis has sometimes been found to be a biochemical predictor of erosion threshold (Underwood 1993).

Colloidal carbohydrates were extracted according to Underwood *et al* (1995) and quantified following the Dubois assay (Dubois 1956). Approximately 20 mg of sediment from each plot was weighed into a clean centrifuge tube. Saline solution was prepared to 35‰ (artificial seawater). 4 ml of artificial seawater was added to each sediment sample and centrifuged for 10 minutes at 4500 RPM. 1 ml of supernatant from each sample was placed in a boiling tube, with the next steps taking place under the fume hood: 0.2 ml of 5% phenol and 1 ml of concentrated H_2SO_4 were added quickly, in that order, to each sample, and placed immediately into a >80 °C water bath for 30 minutes. After cooling, 1 ml of each sample was added to a new plastic cuvette and measured in a photospectrometer at 450 nm. A standard curve made using stock solutions and repeating the above process using the same phenol solution was then used to determine the mass per sediment mass of colloidal carbohydrate in each sample. This used the equation of the straight line produced by the standard curve, taking into account the dilution factor and weight of sample.

Following the above procedures, remaining sediment from each sample was analysed to determine grain size parameters. Grain size was measured using a Malvern Mastersizer 2000 with Hydro 2000S dispersion unit. Particles between 0.01 µm and 2000 µm were measured, with outputs as percentage content for values at 81 specified intervals within this range, measured to the nearest interval. Modal particle size (µm) was then used (measured three times for each sample with the mean calculated) to represent each plot, with mean of plots used to represent each site.

2.3.2.5 Macrofaunal Extraction

Macrofauna are often a crucial link between primary producers and higher trophic levels such as shorebirds (Van Colen 2018), making them an important aspect of this research into whether top-down trophic cascades from shorebirds can influence mudflat characteristics. Macrofauna activity including feeding, burrowing and respiration can also alter mudflat characteristics such as sediment dynamics (including erodibility) and biogeochemical fluxes (Van Colen 2018), adding complexity to the system.

Following completion of biogeochemical flux sampling flux cores were sieved (500µm mesh) (Tanaka and Leite 1998, Van Colen 2018). Macrofauna were preserved in 95% ethanol and identified to species level (where possible) using a microscope where necessary. Species abundance was quantified enabling calculation of density (m^{-2}) (Tanaka and Leite 1998).

2.4 Field Testing

Observational data are valuable and remain frequently used in ecology, particularly within the field of ornithology (Rakhimberdiev et al. 2011, Lourenço et al. 2017a, Reese and Skagen 2017, Kwon et al. 2019, Linssen et al. 2019, Whitney 2020). Qualitative data were used to assess the effectiveness of exclosure materials and designs prior to each experimental period. Quantitative measurements were also made as a means of gaining insight into how sediment characteristics may be affected by shorebird manipulation.

2.4.1 Study Site - Fingringhoe Wick, Essex

Fingringhoe Wick is a nature reserve in Fingringhoe, Essex, in the East of England managed by the Essex Wildlife Trust (EWT). It is a former quarry, 48.6 ha in extent, comprising a mosaic of habitats including woodland, scrub, ponds, heathland, grassland, reedbeds, lagoons, saltmarsh and mudflats. The eastern boundary of the reserve meets the western bank of the Colne estuary (flowing north to south and entering the north-sea approximately 4 km to the south-east). Saltmarsh is present immediately south (Geedon Saltings) and north (managed realignment by EWT) of the reserve. Inland of these saltmarshes are arable fields to the north, and Geedon Creek to the south.

Fingringhoe Wick is situated on the mesotidal Colne Estuary with a range of 3.5-4.0 m and a mean fluvial flow of approximately 0.54 m^3s^{-1} (Thornton et al. 2002). Salinity along the Colne estuary is reported between 0 and 33 (Kocum 2002), and at Fingringhoe Wick was measured at approximately 31 (pers. obs.). The estuary is characterised by ebb-dominant currents, with the lowest erosion rates in the country due to a coastline sheltered by offshore banks and tidal flats. The Colne estuary is geomorphologically relatively stable, with signs of accretion in areas such as Geedon Creek, although erosion at creek entrances has been found. Intertidal mudflats show a slight net increase in elevation (DEFRA 2010). Wave height in estuaries is positively correlated with wind speed. Previous work at the site showed that during April, July and October wind induced wave height at Fingringhoe ranged between approximately 0.1 m and 1.8 m (Redzuan 2017), however at the upper shore where this study was located, wave heights on Essex open mudflats are estimated to be between 0.2 and 0.3m (Möller and Spencer 2002).

The section of mudflat associated with Fingringhoe Wick extends approximately 4.5 km from ballast Quay to the north, to Rat Island to the south where it adjoins mudflats of South Geedon Creek. Along this length, the distance between the mean low water mark and edge of the saltmarsh or seawall varies between approximately 10 m and 250 m. At the study site (grid reference TM 05060 19033) the mudflat extends approximately 250 m from the saltmarsh edge to the river channel during low tide. The study site was approximately 20 m from the salt marsh edge to allow sufficient emersion time for sampling and comprised an area of mudflat approximately 400 m^2 situated on the upper shore. The study site was set within an extensive area of estuarine mudflat, approximately 130,000m^2 of which (including the study site) could be visually surveyed for shorebird activity from a concealed fixed point (Geedon hide) (Figure 3).

The site at Fingringhoe was included within a six-site data collection effort in 2013, as part of the Natural Environment Research Council (NERC) funded Coastal Biodiversity and Ecosystem Service Sustainability (CBESS) programme.

CBESS studies showed that during winter, sediment at the Fingringhoe site is mostly silts and clays, with a very low proportion of sand (maximum 'very fine sand' content in a sample was 6.5%; coarser sand contents were all lower than this), with sediment particle size at the site predominantly < 63µm (mean 95.9 % ± 0.3 SE). Mean D_{50} = 6.9 µm ± 0.2 SE; Mean D_{16} = 1.9 µm ± 0.04 SE; D_{95} = 66.9 µm ± 13.2 SE (D_x = particle diameter representing the x% cumulative percentile) (Wood et al. 2015). Mean percentage surface sediment water content at the site is 62.3 ± 0.4 SE (Maunder and Paterson 2015). This site lies within the polyhaline section of the estuary, with salinity ranging from 18-30, depending on freshwater flow conditions, with lower salinity during winter (Nedwell et al. 2016).

CBESS research also included sampling of fauna within the Colne estuary, demonstrating that during winter fish were absent, with only Ctenophores recorded during Fyke netting (Wood et al. 2015). Macrofauna recorded during winter CBESS research included ragworm *Hediste diversicolor*, mud snail *Peringia ulvae*, Baltic clam *Macoma balthica* and nematodes across a total of 22 quadrat sites, in which three samples were taken at each (Wood et al. 2015). A year-long fyke netting fish sampling study carried out at two different locations along the estuary where our experiment was undertaken, found that fish were absent at all sampled sites during January, and absent from three out of five sites during February (Green et al. 2009). Where fish were present at two sites during February, total abundance (fish 100 m^{-2}) was approximately 2, and less than 1 during March (Green et al. 2009).

The site was chosen primarily due to the large numbers of waders congregating in the vicinity. On a typical flood tide, up to approximately 250 Dunlin *Calidris alpina*, 40 Knot *Calidris canutus*, 20 Redshank *Tringa totanus* and 10 Grey Plover *Pluvialis squatarola* were pushed into a small bay approximately 60 m x 30 m in extent. Here the birds would feed before leaving the mudflat for their high tide roosts (pers. obs.). The site forms part of the Colne Estuary Site of Special Scientific Interest (SSSI) due to supporting internationally important populations of wintering brent geese *Branta bernicla* and black-tailed godwit *Limosa limosa islandica* and nationally important populations of breeding little tern *Sterna albifrons*, alongside a high diversity of nationally scarce and decreasing habitats. The site also lies within the Colne Estuary Ramsar and Special Protection Area (SPA), designated for regularly supporting nationally and internationally significant populations of overwintering and migratory waterfowl and nationally important numbers of breeding little tern. It is also part of the Essex Estuaries Special Area of Conservation (SAC), designated for Atlantic salt meadows *Glauco-puccinellietalia maritimae*, Mediterranean and thermos-Atlantic scrubs *Sarcocornetea fruticose*, intertidal mud and sand flats, *Salicornia* and other annuals colonising mud and sand, sandbanks which are slightly covered by seawater permanently and Spartina swards *Spartinion maritimae* (Natural-England 2018). The site is referred to as ‘Fingringhoe’, with various grid references given for different areas of the mudflat used, as necessary (Chapters 3 and 4).

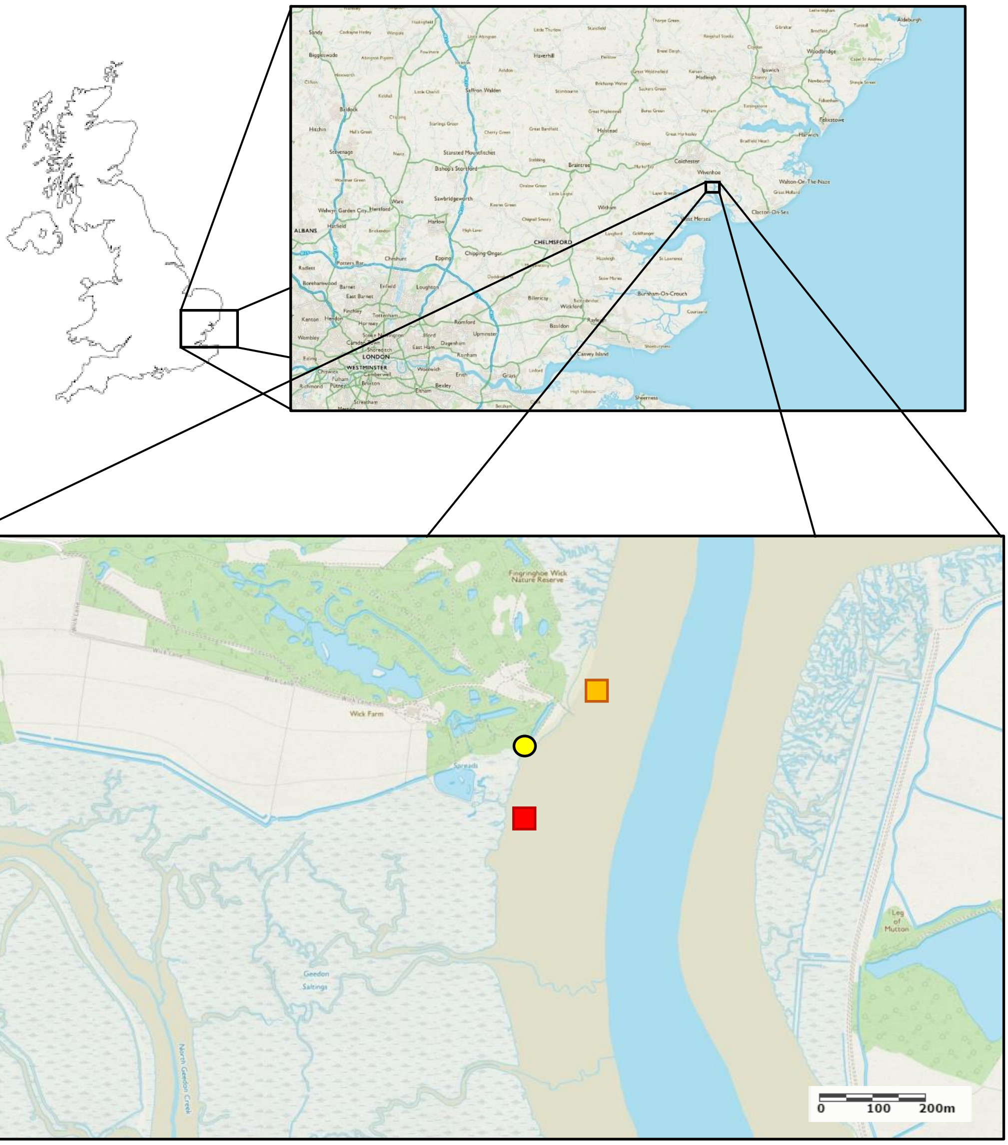


Figure 3 National, regional and local location (red square; TM 05060 19033, experimental location in 2017 and 2018-2019, orange square; TM 05170 19195, pilot study location in 2016) of the study site and Geedon Hide (yellow circle) at Fingringhoe Wick, Essex. Ordnance survey maps from OS OpenData (Ordnance Survey, 2018).

2.4.2 Exclosures

Exclosure designs E_a and E_b were deployed simultaneously to test their effectiveness at the study site. A total of nine plots (three of each and three control plots) were erected on the mudflat at the Fingringhoe site (grid reference TM 05170 19195). The experimental layout is shown in Figure 4. These were deployed on 18 February 2016, during the overwintering season for several shorebird species known to use the site for feeding (Baker et al. 2020, Poole et al. 2020, Van Gils et al. 2020b, Warnock and Gill 2020). Shorebird species and numbers within the study area were surveyed on average once weekly until the pilot was completed on 05 April 2016. Shorebird behaviour in relation to the exclosures, such as walking or foraging, was also recorded. Intense 30-minute surveying of randomly selected plots was not considered necessary due to the small amount of plots, all of which could be watched by surveyors continuously during counts. Counts were carried out for two hours before and after low tide where possible (Holt 2009), considering daylight hours. Supplementary data were collected using citizen science participation (Silvertown 2009), which consisted of information posters informing users of the observation hide (overlooking the flats and study area) about the study and enabling them to indicate whether they had seen birds inside the exclosures.

Public responses highlighted an acknowledged problem with citizen data collection; data quality (Lukyanenko et al. 2016). In total 12 responses were collected. Eight responses indicated that no birds were seen inside netted exclosures. Of the four responses reporting bird presence inside netted exclosures, one simply reported 'yes' and those reporting species within netted exclosures were of marsh harrier, little egret and penguin. Reports of marsh harrier and penguin at rest on the mudflat are clearly erroneous (marsh harriers do not rest or feed on exposed mudflats and the site lies outside the natural range of penguins) and cast doubt on the reliability of the reports in general. These species also could not have accessed the netted exclosures, which were also not damaged during the pilot study. Similarly, reports of grebes within the lined exclosures are unlikely to have been accurate; species present at the site are predominantly great crested grebe *Podiceps cristatus* and little grebe *Tachybaptus ruficollis* which do not feed on exposed mudflats but dive for fish in open water (Ulenaers and van Vessem 1994, Ceccobelli and Battisti 2010). One record of teal *Anas crecca* was reported, within a lined exclosure, which is likely to have been accurate and aided the decision not to proceed with lined exclosures.

To test the effect of each exclosure design on sediment surface fluorescence (F_o) and erosion threshold (τ_{cr}), these were measured using a PAM and CSM respectively, when the exclosures were deployed (Day 0) and after 40 days (Day 40). On each sampling day, five PAM measurements were taken in each plot, including controls. Each PAM measurement was taken following a period of five minutes complete dark adaption. Three CSM measurements were taken within each plot. All measurement locations were selected representatively of the homogeneity observed within each plot.

To evaluate the effect of each treatment on minimum fluorescence, a one-way ANOVA model was used with treatment as the fixed effect and F_o as the random effect variable. To evaluate the effect of each treatment on sediment erodibility, a one-way ANOVA model was used with treatment as the fixed effect and τ_{cr} as the random effect variable. Two separate one-way ANOVA models were used due to the unbalanced sampling design between PAM and CSM, which reflected the longer time required to initiate and execute a CSM test.

Shorebird numbers and study site area were used to calculate shorebird density. Time was factored into the analysis, to calculate the number of 'bird-days' (ha^{-1}) of activity by shorebirds. Bird-days gives a measure of the cumulative use of a site over time, based on the numbers recorded during surveys and extrapolating this based on the number of days between surveys (Gill et al. 2001).

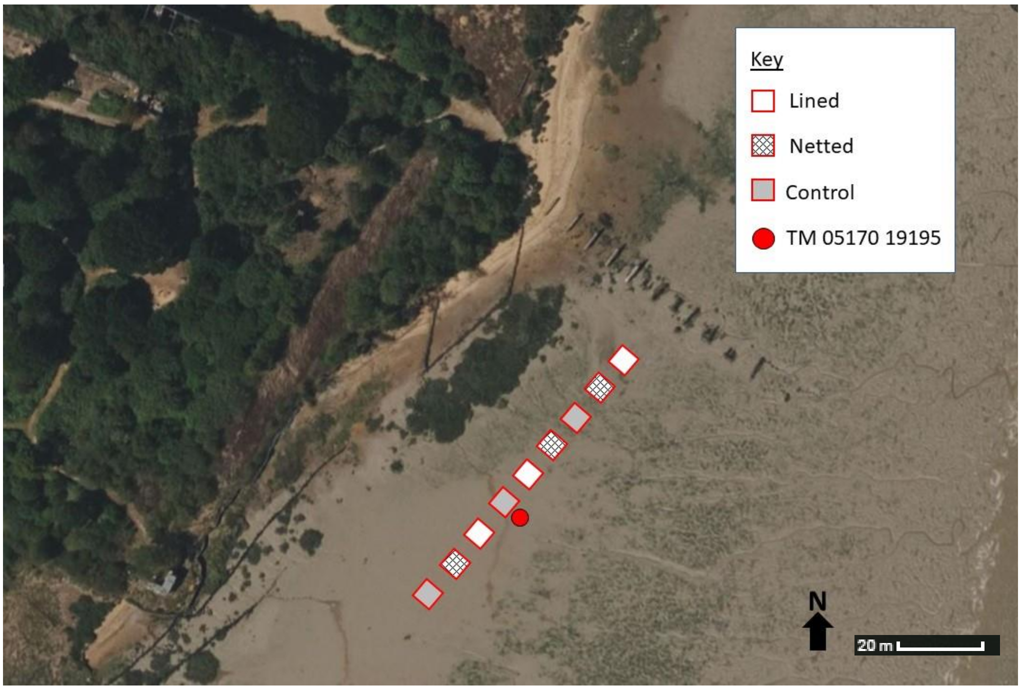


Figure 4 Experimental layout on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05170 19195) 18 February to 05 April 2016.

2.4.3 Shorebird Density Reduction - ‘roof only’ plots

During the winter of 2017-2018, shorebird exclosures previously used successfully in a similar study (Cheverie et al. 2014) (E_d) were deployed at the Fingringhoe study site and monitored to assess possible negative effects on shorebirds. This was due to concerns from the statutory regulatory body, Natural England (NE), that exclosures using a roof only may harm birds if flushed when beneath them (Charlie Williams, pers. comms.). Prior to experimental deployment, NE therefore requested trialling and monitoring of a low number of exclosures with the aim of observing predator-prey interactions at the study site, involving raptors and shorebirds.

A total of six test exclosures were deployed and monitored four times during November and December 2017; 10th, 16th and 23rd November and 17th December. During monitoring, the study area was watched using binoculars from Geedon Hide (Figure 5). The experiment in early 2017 (Chapter 3) showed the time when the majority of shorebirds interacted with exclosure and control plots was when the tide pushed foraging birds through the study area, given that the majority of species present in large numbers, such as Dunlin, are tide followers (Kelsey and Hassall 1989, Dierschke et al. 1999, Drouet et al. 2015). Therefore, surveys were undertaken from one to two hours before high tide, until the roofs had been covered by the tide. Avian predators which flush foraging shorebirds, such as Marsh Harrier *Circus aeruginosus*, Peregrine Falcon *Falco peregrinus*, Sparrowhawk *Accipiter nisus* and Merlin *Falco columbarius* were watched for within the field of view. Shorebird interactions with the exclosures were also recorded, including walking or foraging beneath them. Disturbance events were classified as those which triggered alarm calling and escape/avoidance flight (flushing) by shorebirds, particularly on the mudflat and either near to or under the exclosures.

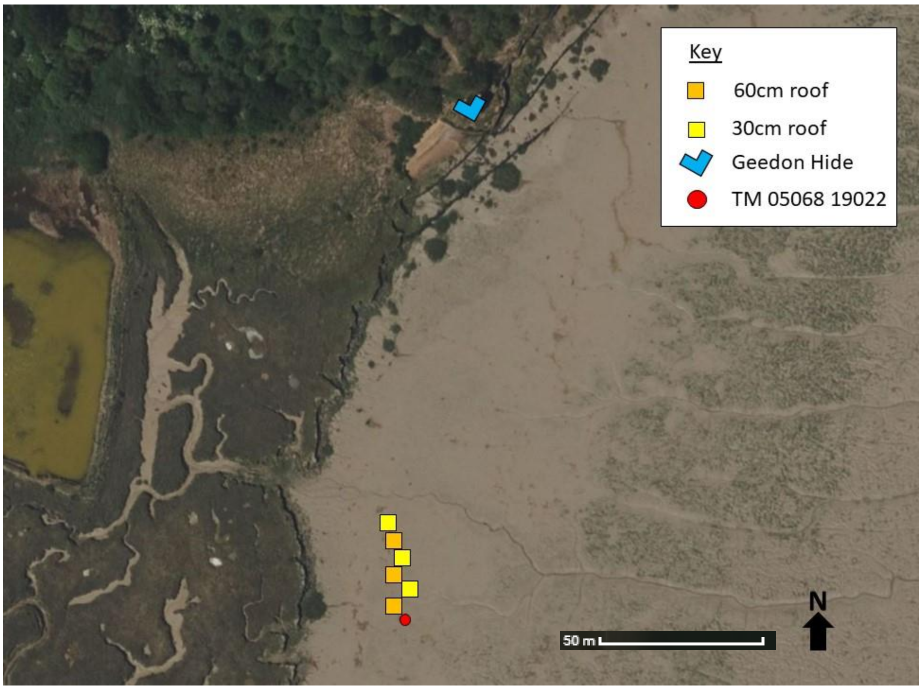


Figure 5 Experimental layout on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05068 19022) 09 November 2017 to 15 December 2017.

2.5 Results and Conclusions

2.5.1 2016 Exclosure Testing

Both chl *a* and τ_{cr} were lower in control plots than in either treatment (Table 4). This may suggest either experimental artefacts caused by the presence of the exclosures, or a possible effect of shorebirds which is not detectable (Underwood 1997). This line of questioning is explored further in Chapter 3.

Table 4 Mean measured variables of treatments deployed on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05170 19195) 18 February to 05 April 2016.

Plot No.	Treatment	Mean F_o	Mean τ_{cr}
1	E_a	134.8	0.58
2	E_b	122.6	0.41
3	Control	87.4	0.3
4	E_a	165.4	0.46
5	E_b	83.6	1.13
6	Control	127.2	0.3
7	E_a	197.0	0.53
8	E_b	128.8	0.24
9	Control	132.4	0.3

ANOVA models suggest that treatment did not significantly affect F_o (F=3.76; P=0.09) or τ_{cr} (F=0.92; P=0.4) (Table 5). This indicates that where birds were excluded entirely (netted exclosures) or deterred (lined exclosures), no significant difference in chl *a* or τ_{cr} was detected at the time of sampling compared to control plots.

Table 5 ANOVA model outputs comparing treatments deployed on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05170 19195) 18 February to 05 April 2016.

Model	Factor	Source			
		df	MS	F	P
Chlorophyll *a* ug g^{-1} = MEAN + TREAT + Plot(TREAT) + RES	Netted/Lined/Control = TREAT	2	13614.7	3.76	0.0875
	Plot (TREAT)	6	3622.9	1.6	0.1761
	Residual	36	2267	-	-
Erosion Threshold nm^{-2} = MEAN + TREAT + Plot(TREAT) + RES	Netted/Lined/Control = TREAT	2	0.2096	0.92	0.4496
	Plot (TREAT)	6	0.2287	0.66	0.68
	Residual	18	0.3472	-	-

The lack of significant treatment effect may suggest a lack of top-down effect of shorebird presence on chl *a* and τ_{cr}. It is also possible that effects were not detected due to a lack of statistical power within the experimental design, which included three replicates of each treatment (Cohen 1992).

Birds were seen accessing E_b during survey 1, and in control plots during surveys 4, 5 and 7. Shorebird tracks were present within plots 5 and 8 during F_o sampling. These results are in broad agreement with the published work which exclosure E_b was based on (Hamilton et al. 2006), where it is reported that the majority of birds avoided exclosure plots.

With the note that the effects of exclosures needs to be carefully monitored for experimental artefacts, these results suggest that use of shorebird exclosures has the potential to allow experimental testing of shorebird effects on mudflat characteristics.

Shorebird footprints were recorded in two out of three of the lined exclosures (Image 2), showing that although they may have been effective in reducing the use of these patches by shorebirds (footprint cover was higher and recorded in all control plots) they were not completely effective in shorebird exclusion.

Based on the effectiveness of the avian netting to exclude shorebirds and the robustness of this material to withstand the intertidal environment during the pilot study in 2016, it concluded that the use of avian fruit-cage netting provides a feasible method of experimentally manipulating and comparing areas of a mudflat where shorebirds are present and absent. Low replication may have led to the lack of detectable shorebird effects on chl *a* and τ_{cr}, highlighting the need to increase replication as this research progressed. This led to development of exclosure E_c, to increase robustness of the netted exclosures and allow rapid sampling (removable roofs) of a greater number of replicates.

2.5.2 2017 ‘Roof only’ Exclosure Testing

Due to the qualitative nature of this experiment, key results are described as follows:

10th November:

Species recorded foraging near to, but not under, the structures were curlew, grey plover, and redshank. One redshank recorded foraging beneath a 60 cm exclosure. Disturbance event (undetermined, possibly a person on shoreline) occurred after structures were partly flooded, when redshank were flushed from the saltmarsh edge, flying low over the water, avoiding all structures still emergent from the water line.

16th November:

Species recorded foraging near to, but not under, the structures were redshank, grey plover, and dunlin. One redshank recorded foraging beneath a 60 cm exclosure, the same bird foraged beneath a 30 cm exclosure six minutes later. No disturbance events.

23rd November:

Two out of three 60 cm exclosures were damaged and not functional, one not present. All three 30 cm exclosures were present and functional. Species recorded foraging near to, but not under, the structures were redshank and dunlin. No disturbance events.

17th December:

Species recorded foraging near to, but not under, the structures were redshank, dunlin, grey plover and turnstone. No disturbance events.

As described above, disturbance events during surveillance were infrequent, reducing ability to determine the reaction of, and potential danger to, any shorebirds present. When a disturbance event was seen, the reaction of shorebirds (distinct visual avoidance of the exclosures) suggests that redshanks can continue to avoid the exclosures if flushing is triggered. This concurs with previous research using this exclosure design, when no adverse effects on animals was reported (Cheverie et al. 2014).

The monitoring did enable confirmation that shorebirds (certainly redshank) do forage beneath both heights of exclosure (30cm and 60cm), which is also in accordance with use of this design elsewhere, where researchers report 4% footprint cover beneath exclosures (Cheverie et al. 2014). Footprint cover was not estimated during monitoring and whether this value is comparable at the site during this period is unknown. Use of this method of shorebird patch use was utilised in Chapters 4, 5 and 6, when shorebird density effects on mudflat characteristics was experimentally examined.

The testing also enabled demonstration that 30 cm exclosures are robust enough to withstand typical winter tidal velocities at the site, while 60 cm exclosures are not. Based on these observations it was concluded that larger scale deployment of E_d was unlikely to cause harm to shorebirds and that 30 cm height should be used, given that this height exclosure allows use by shorebirds (lower density than control plots) and is more likely to allow long term data collection given lower susceptibility to damage.

3 Shorebirds affect ecosystem functioning on an intertidal mudflat

This Chapter is based on a paper which has been published in a peer reviewed journal (Booty et al. 2020). The publication is included in Appendix 5, including author contributions.

3.1 Abstract

Ecosystem functioning and services have provided a rationale for conservation over the past decades. Intertidal muddy sediments, and the microphytobenthic biofilms that inhabit them, perform crucial ecosystem functions including erosion protection, nutrient cycling and carbon sequestration. It has been suggested that predation on sediment macrofauna by shorebirds may impact biofilms, and shorebirds are known to consume biofilm, potentially causing significant top-down effects on mudflat ecosystem functioning. We carried out an exclusion experiment on the Colne Estuary, Essex, to examine whether shorebird presence significantly affects sediment erodibility measured with a Cohesive Strength Meter (CSM) and microphytobenthos biomass measured using PAM fluorescence (F_o) and chlorophyll a content. We also tested for treatment effects on sediment-water nutrient fluxes [nitrate, nitrite, ammonium, phosphate and dissolved organic carbon (DOC)] during periods of both dark and light incubation. Excluding shorebirds caused statistically significant changes in regulating and provisioning ecosystem functions, including mudflat erodibility and nutrient fluxes. The presence of shorebirds lowered the sediment critical erosion threshold τ_{cr}, reduced nitrate fluxes into the sediment under illumination, lowered nitrate efflux, and reduced phosphate uptake, compared to sediments where birds were excluded. There were no significant differences in macrofauna community composition within the sediment between treatments after 45 days of bird exclusion, suggesting a direct link between shorebird presence or absence and the significant differences in biofilm-related variables. This study introduces previously unknown effects of shorebird presence on ecosystem functions within this system and highlights an area of shorebird science that could aid joint conservation and human provisioning action.

3.2 Introduction

Ecosystem functioning and ecosystem services have provided a rationale for conservation over the past decades (Cabello et al. 2012). Intertidal mudflat ecosystem functions include nutrient cycling, erosion protection and carbon sequestration, which mediate associated services (Foster et al. 2013). Intertidal flats provide natural 'soft' coastal erosion defence by reducing wave energy, lowering water velocities and thereby shear stress on the estuary bed (Spalding et al. 2014). Benthic microalgae (microphytobenthos, MPB) form complex matrices of cells, sediments and extracellular polymeric substances (EPS) (Underwood and Paterson 1993). These biofilms have a stabilizing effect on surface sediments, reducing erodibilty and aiding in the accumulation of particles and microbes (Gerbersdorf and Wieprecht 2015). Estuarine sediments and biofilms are central components in estuarine nutrient cycles, ultimately affecting fluxes of these nutrients between land and sea (Thornton et al. 2007, Nedwell et al. 2016). Organic compounds are recycled and remineralised within sediments, particularly in coastal marine areas where nitrogen and phosphorous loads can be very high (Correll et al. 1992, Hochard et al. 2010). Nitrogen loading into marine systems can lead to eutrophication and decline in water quality, making its source and removal pathways of high interest (Burgin and Hamilton 2007) and changes in nutrient loads can impact benthic communities (Culhane et al. 2019). MPB mediate fluxes of NO_3^-, NO_2^-, PO_4^{3-} and NH_4^+ between the water column and sediment layers (Sundback et al. 1991, Correll et al. 1992, Feuillet-Gerard et al. 1997), contributing to this process either by direct uptake/release or by altering oxygen concentration (Sundback and Graneli 1988). Dissolved organic carbon (DOC) may also provide an important part of both global and coastal carbon sinks (Maher and Eyre 2010, Legge et al. 2020), making effects on DOC fluxes in this environment relevant to anthropogenic climate change effects and mitigation (McKinley et al. 2016).

Mud and sand flats are essential habitats for the survival of resident and migratory overwintering shorebirds (Burton et al. 2006), which feed primarily upon infaunal and epifaunal invertebrates (Bowgen et al. 2015). Some small sandpiper species *Calidris* spp. also directly consume biofilm during, or in preparation for, migration (Kuwae et al. 2008, Jardine et al. 2015). Grazing of MPB and bioturbation by macrofauna can lead to alterations in sediment erodibility and other ecosystem functions (de Deckere et al. 2001, Hale et al. 2019). This poses questions regarding the effect of biofilm removal and bioturbation by shorebirds (Mathot et al. 2018), which may have significant knock-on effects altering ecosystem functions.

Research suggests that shorebirds could have significant direct and/or indirect effects on ecosystem function, e.g. via the impacts of foraging on macrofauna and/or biofilm or disturbance and reworking of sediment (Orvain et al. 2014b, Mathot et al. 2018). In the Bay Of Fundy (BOF), semipalmated sandpipers *Calidris pusilla* appeared to cause an ecological cascade effect by reducing densities of their mud shrimp prey *Corophium volutator*, which caused biofilm proliferation, leading to an increase in sediment stability (Daborn et al. 1993). However, subsequent research in the BOF has not indicated a trophic cascade effect, possibly due to compensatory interactions by macrofauna (Hamilton et al. 2006, Cheverie et al. 2014). Trophic webs and ecosystem functioning were compared in the Marenne-Oleron Bay, France, indicating that estuarine trophic webs including shorebirds have enhanced primary productivity through increased nutrient cycling (Saint-Béat et al. 2013). Despite evidence that estuarine shorebirds may significantly alter ecosystem functioning, the majority of shorebird research has an ornithological focus and potential top down effects on ecosystem functions such as erosion defence and nutrient cycling have not yet been experimentally tested (Mathot et al. 2018). The ecology of intertidal sediments is complex, compensatory interactions can mask effects (Hamilton et al. 2006), including trophic cascades (Fahimipour et al. 2017). Manipulative experiments are a valuable tool, to be utilized alongside ‘natural’ or ‘observational’ experiments to assess possible ecological mechanisms behind processes observed at wider spatial or temporal scales (Rogers et al. 2012).

The Colne Estuary, Essex, UK is a complex of habitats featuring many sand and mudflats, protected internationally under The Conservation of Habitats and Species Regulations 2017 (as amended), for supporting over 30,000 shorebirds. The study site within the Colne Estuary, the Fingringhoe Wick Site of Special Scientific Interest (SSSI), was a location for the six-year CBESS programme, which provides key background information on the biotic and abiotic characteristics of the site.

Changes in community composition and mudflat characteristics can be rapid, occurring over months (Sahan et al. 2007, Rosa 2008, Murphy and Tolhurst 2009) weeks (Daborn et al. 1993, Hamilton et al. 2006), days (de Deckere et al. 2001, Tolhurst et al. 2008b) and even hours (Tolhurst et al. 2006a, Tolhurst et al. 2006b). A two-month field exclusion experiment was designed and carried out, supplemented by laboratory measurements, to investigate shorebird effects on two ecosystem functions, namely erosion protection (using a measure of sediment erodibility as a proxy) and nutrient cycling (including nitrate, nitrite, ammonium, phosphate and DOC). Three hypotheses were tested: (1) surface biofilm biomass would be significantly altered in the presence of shorebirds, (2) sediment erodibility would be significantly altered in the presence of shorebirds and (3) nutrient fluxes between the sediment and water column would be significantly different between treatments (shorebird presence and absence) with flux direction and magnitude for different nutrient species increasing with greater MPB biomass.

3.3 Materials and Methods

3.3.1 Description of study site

Fieldwork was undertaken between the 20th of January and the 3rd of April 2017 on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick Essex Wildlife Trust Nature Reserve, Essex, UK (grid reference TM 05065 19030). This time period covered the peak overwintering and start of the migratory periods for shorebirds in the East of England. The study location comprised an area of mudflat approximately 400 m^2 situated on the upper shore. Observations during 2016 noted flocks of dunlin *Calidris alpina* and knot *Calidris canutus* and scattered individual redshank *Tringa totanus* and grey plover *Pluvialis squatarola* foraging at the study site on receding and incoming tides. The study location was set within a larger area of estuarine mudflat, approximately 130,000 m^2 of which could be visually surveyed for shorebird activity from a fixed point (Geedon hide).

At the study site the mudflat extends approximately 250 m from the saltmarsh edge to the river channel during low tide. The study site was approximately 20 m from the salt marsh edge to allow sufficient emersion time for sampling and comprised an area of mudflat approximately 400 m^2 situated on the upper shore. The study site was set within an extensive area of estuarine mudflat, approximately 130,000 m^2 of which (including the study site) could be visually surveyed for shorebird activity from a concealed fixed point (Geedon hide). The site is described in detail in Chapter 2, Section 2.4.1.

3.3.2 Experiment design

The manipulative experiment was set up on 20 January 2017 (day 0). The experimental layout was a randomized design of 20 spatial plots (Figure 6), each 1 m x 1 m, allocated to two treatment levels; control (shorebirds present in open un-manipulated plots) and exclosure (shorebirds absent), with n = 10 replicates of each treatment. Previous work in the estuary showed that spatial variability in biofilm abundance is greatest at the fine scale and small at the metre scale (Taylor et al. 2013, Nedwell et al. 2016), therefore a completely randomized design was employed to maximize statistical power of the experiment. Exclosures were bamboo frames, approximately 30 cm in height, covered on all sides (including the top) by opaque ‘fruit-cage’ bird exclusion netting (plastic mono-thread) with a 2 cm aperture. Exclosures prevented access to the sediment by birds but allowed access to infauna and small fish (< 2 cm width). All plots were at least three meters apart, to allow sampling from all sides and prevent plots unduly influencing each other. Exclosure and control plots were unpaired and separated by similar distances, with treatments arranged sequentially to reduce the potential for spatial bias. The exact locations of plots were selected to represent the heterogeneity within the wider mudflat. No scouring or bite marks indicating the presence of larger fish (Eggold and Motta 1992) were found within any plots during the experiment. Plots were arranged parallel to the tide line (within a minute of immersion/emersion time of one another). Plots were situated on the upper shore, where shorebirds spend most time foraging due to the longer emersion time (Granadeiro et al. 2006). Camera footage (see below) and direct observation recorded no events of birds standing on exclosures (behaviour which may otherwise have caused input of droppings into exclosure absence plots as well as control presence plots) (Schrama et al. 2013, Jauffrais et al. 2015).

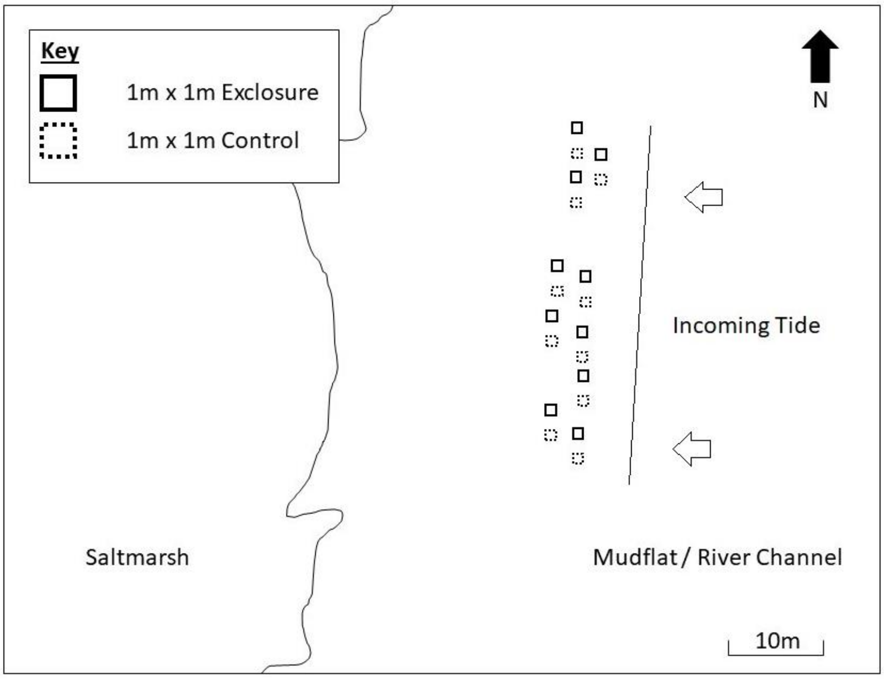


Figure 6 Experimental layout adjacent to Geedon Saltings, Essex Wildlife Trust Fingringhoe Wick, Essex, between 20 January and 03 April 2017.

3.3.3 Assessment of possible experimental artefacts

To test the effect of the exclosures on the water flow within the study area, a ‘plaster ball dissolution test’ was carried out on days 17 and 18 (Cheverie et al. 2014). No significant difference was detected between plaster dissolution rates in control plots and exclosure plots (t=-1.057; df=8; p=0.322), demonstrating that the exclosures had no significant effects on tidal water flows in the vicinity of the mudflat surface. Data are included in Appendix 1.

Exclosure shading tests were carried out after the experiment to prevent additional mudflat disturbance, during a sunny day (cloud cover < 10%), hence resulting in an estimation of shading at the higher end of the actual range during the study period. Shading effects on Photosynthetically Active Radiation (PAR) reaching the sediment surface in exclosures were small (9.9%), and of a similar level to that in other manipulative studies in this type of environment (Cheverie et al. 2014). Further information reinforcing this conclusion is given in the discussion.

A Go-Pro HERO 4 camera fitted with a Cam-Do Blink time lapse controller mounted within a Cam-Do Solar-X enclosure (Cam-Do Solutions, 2017) was deployed to monitor bird activity within the study area for four weeks (21 February 2017 to 21 March 2017). This was mounted on a vertical pole 3.5 m above the saltmarsh at grid ref: TM 05031 19032. The camera captured a still of the plots every five minutes during daylight hours. Although species identification was not possible using captured images, numbers within the field of view were used to broadly determine whether numbers of birds using the study area were consistent with those recorded during visual surveys.

Weather data were collected during the experimental period (peak wind speed (km h^{-1}), daily precipitation (hours day^{-1}) and peak temperature (°C)), and plotted against biofilm biomass (F_o) and shorebird numbers to assess potential effects of these variables on the experiment, such as extreme weather events, which can have significant effects on shorebird activity (Sutherland et al. 2012) and mudflat characteristics (Tolhurst et al. 2006b, Fagherazzi et al. 2017, Hale et al. 2019). No extreme weather events occurred during the experiment and no evidence was found of a relationship between F_o and daily precipitation (hours), peak temperature (°C) and peak wind speed (km h^{-1}) during the experiment (Fig. 2A&B), although the potential for delayed responses has not been assessed. However, all plots were subject to the same weather, and this is not considered to be a constraint to the experiment.

3.3.4 Response variables

Between day variation in mudflat characteristics have been shown to be of greater significance than within day variation (Tolhurst and Chapman 2005), therefore repeated measures of F_o were made to compensate for this effect. Table 6 shows dates and days at which sampling events took place. On 20th January 2017, immediately following plot setup, 'day 0' minimum fluoresence (F_o)

measurements were taken using a pulse amplitude modulated fluorometer (PAM, Walz, Effeltrich, Germany) to determine MPB biomass (Honeywill et al. 2002). MPB are key drivers of intertidal flat properties and processes (Murphy and Tolhurst 2009), so to determine when the full sampling event would be most likely to detect any effects we monitored F_o (as a proxy for MPB biomass) on days 3, 13 and 26, as a convenient indication of treatment effects, to determine when erodibility and nutrient flux variables should be measured and to confirm that early in the experiment there were no significant differences between treatments. F_o was also measured on day 45 to evaluate the effect of shorebird presence/absence on MPB biomass and associated properties, and on day 64 to determine if trends continued. A subset of 6 exclosure and 6 control plots were measured on day 3 for a total of 60 F_o measurements (n=5 in each of the 12 plots); subsequently all plots were measured, for a total of 100 F_o measurements (n=5 in each of the 20 plots) on days 13, 26, 45 and 64 to investigate how surface MPB biomass responded to shorebird presence/absence over time.

Table 6 Dates and numbers of days into the experiment that field sampling events occurred between 03 January 03 April 2017 on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick Essex Wildlife Trust Nature Reserve, Essex, UK (grid reference TM 05065 19030).

Date	Day	Event
03 Jan	-17	Shorebird monitoring
20 Jan	0	Experiment setup and F_o sampling
23 Jan	3	F_o sampling
27 Jan	7	Shorebird monitoring
02 Feb	13	F_o sampling
03 Feb	14	Shorebird monitoring
10 Feb	21	Shorebird monitoring
15 Feb	26	F_o sampling
17 Feb	28	Shorebird monitoring
24 Feb	35	Shorebird monitoring
06 March	45	F_o sampling, critical shear strength sampling, contact core and flux core collection.
10 March	49	Shorebird monitoring
25 March	64	F_o sampling
03 April	73	Shorebird monitoring

Due to the large number of measurements required in each plot during a tidal cycle and considering the impact of dewatering during the tidal cycle (Maggi et al. 2013, Orvain et al. 2014a, Fagherazzi et al. 2017), a 5 minute low light partial dark adaption treatment was used prior to each PAM measurement, which is a preferred method to conventional dark adaption for the measurement of minimum fluorescence as a proxy of MPB biomass (Jesus et al. 2006b). Sampling was carried out during periods of clear weather with little wind and no rain, at least one hour after the tide had exposed the sampling area to allow initial drying of plots. A consistent low light sampling environment was achieved using plastic 40 mm (diameter) x 60 mm (length), cylindrical opaque dark adaption chambers with a 6 mm aperture hole at the top. This also enabled *in-situ* sampling with the PAM fluorometer without removal of the chamber. This reduced the variation in light intensity during the measuring period. To further eliminate potential effects of varying light intensity and sediment water content during sampling events, exclosure and control plot sampling was alternated. To minimize the effect of varying light intensity and phase of vertical migration between sampling events, sampling periods were timed to cover low tides peaking as close to midday as possible.

Previous experience of the site is that variability at the metre scale is low (Redzuan 2017). Additionally, the repeated F_o sampling (described above) gives further confidence that plots were not significantly different at the beginning of the experiment. All *in situ* mudflat variables were measured on 06 March 2017, after 45 days of shorebird exclusion, to test the effect that a period of shorebird exclusion had on selected mudflat properties. Sampling included *in-situ* measurements of F_o (as described above), *in-situ* sediment critical erosion threshold (τ_{cr}) using a Cohesive Strength Meter (CSM) (three measurements within six plots of each treatment, total 36 measurements) (Tolhurst et al. 1999, Vardy et al. 2007) and contact coring for analysis of chlorophyll *a* content (three measurements within seven plots of each treatment; total 42 measurements) (Honeywill et al. 2002). Flux cores (Perspex tubes of 0.1 m diameter and approximately 0.2 m in depth) were also collected (one from each plot) for laboratory analysis of nutrients and macrofauna.

Contact cores (surface ~2 mm) were freeze dried in the dark and chlorophyll *a* extracted using cold methanol over 24 h, and measured spectrophotometrically, correcting for phaeopigments (Stal et al. 1984).

Flux cores were carefully returned to the laboratory within an hour of leaving the site and immersed in seawater from the site, within oxygenated and temperature and light controlled indoor mesocosms (Thornton et al. 1999). Rubber bungs were used to ensure equal headspace volume across cores. Cores were left submerged and open to settle overnight prior to sampling on the following day. Throughout headspace water sampling, Perspex lids were tightly fitted to prevent leakage. Magnetic stirrers maintained water flow over the sediment surface. On 07 and 08 March 2017 these were sampled for sediment-water biogeochemical fluxes of nitrate, nitrite, ammonium, phosphate and dissolved organic carbon (DOC). Headspace seawater samples were taken at the beginning and end of 2-hour dark and light incubation periods. Cores were left for at least one hour to adjust to light levels prior to each incubation. Sampling was completed according to general methods described by Thornton *et al.* (1999). Flux measurements were repeated in both light and dark conditions, using 500W halogen 'daylight' lamps to provide 'lit' conditions (500 µmol m^{-2} s^{-1} PAR) and covering mesocosms with opaque Perspex covers to provide 'dark' conditions. Water samples were analysed for their nutrient concentrations using a Seal AA3 segmented flow Nutrient Analyser (SEAL Analytical Inc.).

Individual cores used for nutrient flux measurements were subsequently sieved (500 µm mesh) to retain macrofauna. Macrofauna were preserved in 95% ethanol and identified to species level (where possible) using a microscope, quantified and densities (m^{-2}) calculated. Through data comparison with previous work at the site (Wood et al. 2015) we were confident that sufficient sampling had been undertaken to assess potential differences in community composition between shorebird presence and absence plots.

Bird surveys began on 03 January 2017 (-17 days) and were carried out at least every two weeks (Table 6 above) to monitor the level and type of use of the study area by shorebirds. Monitoring began before the experimental setup to ensure current use of the study area by shorebirds and aid in deciding the best location for the experimental plots. Surveys were carried out using the 'look-see' methodology (Bibby et al. 2000), from a fixed location (Geedon Hide; TM 05081 19170). Surveys were undertaken for at least 2 hours either side of low tide, including as much of these timeframes as possible (four hours maximum) within daylight constraints. Particular care was taken to also include visual observation of the tideline crossing the plots wherever possible. Counts of species within the surrounding visible mudflat were taken every half hour. Continual observation of the study area was made, quantifying numbers and identifying species entering presence plots throughout the surveys. Equipment included a 20-60x82 telescope and 10x42 binoculars. No birds were recorded within or on the absence plots during any of the surveys. During F_o measurements, shorebird tracks were noted within all presence plots at some point during the study, indicating use of all presence plots by shorebirds. No tracks were recorded in any absence plots at any point during the experiment.

3.3.5 Statistical analysis

To evaluate the effects of shorebird presence and time (days) on biofilm biomass throughout the experimental period, we used a linear mixed-effects model (plot nested in treatment) to analyse F_o data with plot as a random effect and time (day) and bird presence/absence as fixed effects. This model was run using nlme package (Pinheiro et al. 2017) in R version 4.0.

To evaluate the effect of shorebird presence/absence on MPB biomass and sediment erodibility, F_o (days 3, 13, 26, 45 and 64), chlorophyll *a* (from surface 2 mm) (day 45) and critical erosion threshold (day 45) data were analysed using a mixed model, two-way nested ANOVA design with (plot nested in treatment) plot as a random factor and shorebird presence/absence as a fixed factor, using the GMAV (1997) statistical package (University of Sydney, Australia). Although baseline data were not collected, ANOVA detects differences between treatments over and above variability among individual plots (Underwood 1997). To counteract the issue of multiple comparisons Bonferroni correction was used, testing each hypothesis at a confidence level of 0.01 (0.05/5).

To evaluate the effect of shorebird presence/absence on nutrient flux (day 45), nutrient data were analysed using a two-way orthogonal ANOVA design with dark/light incubation and shorebird presence/absence as fixed factors, using the GMAV (1997) statistical package (University of Sydney, Australia). Where Cochran's test was significant (ammonium and phosphate), data were normalized by rank transformation and the analysis repeated. Reversals in flux (for example an efflux from the sediment in the absence of shorebirds becoming an influx into the sediment in the presence of shorebirds) were used as an indication of changes suggesting 'ecologically significant' implications for ecosystem functioning.

To assess whether shorebird presence/absence had significantly altered macroinvertebrate community structure, day 45 taxa density was analysed using R version 3.6.1 with vegan package (Oksanen et al. 2013). Non-metric multidimensional scaling (NMDS, Bray-Curtis dissimilarity, 20 restarts) was used to visualise differences in community structure at day 45 in two dimensions (Clarke 1993). The MDS had a stress 0.037, therefore considered an adequate representation (Clarke 1993). Analysis of similarities (ANOSIM) was also performed to test quantitatively for differences in community structure between shorebird presence and absence.

To assess the potential for biases associated with the exclosures, plaster ball dissolution (days 17 and 18) and shading effect (post experiment) data were also analysed using a one-way orthogonal ANOVA, using the GMAV (1997) statistical package (University of Sydney, Australia).

To evaluate shorebird pressure on the mudflat, species count data were first converted into 'bird-days', by calculating the sum of the number of each shorebird species present on every count, multiplied by the number of days between that and the subsequent count (Gill et al. 2001, Lewis et al. 2014). This method accounts for the days birds are present and surveyors are not and allowed comparison of shorebird pressure on the wider mudflat. An example using a theoretical three-count period spanning 20 days is given in Table 7 below:

Table 7 Theoretical example of calculated bird-days during a three-count survey period spanning 20 days, adapted from Gill *et al* (2001).

Species	Date			Total bird-days
	01/01/2017	11/01/2017	20/01/2017	
Bird count (n)	100	120	80	
Days elapsed (e)	10	9	1	
Bird-days (d_b)	1000 (ne)	1080 (ne)	80 (ne)	2,160

Only species considered regular foragers on mudflats and recorded foraging on the surrounding mudflat were included in this analysis; for example lapwing *Vanellus vanellus* and golden plover *Pluvialis apricaria* were removed due to their high dependence, and almost exclusive foraging, on coastal grassland and arable fields (Mason and Macdonald 1999). Furthermore, these species were recorded roosting on the mid to low shore only during low tides, further reducing the likelihood that they contributed to any effects within the upper shore study site. To compare mudflat variables with density of species recorded in presence plots, count numbers of such species were $\log_{10}$ transformed and plotted over time with mean F_o in shorebird presence and absence.

3.4 Results

3.4.1 Microphytobenthic biomass

Results of the linear mixed effects model show a highly significant difference in F_o (measure of MPB surface chlorophyll *a*) between shorebird presence and absence, with F_o higher in the bird exclosure treatments. There were no significant effects of time (days) or interaction between treatment with time (Table 10).

F_o initially increased in shorebird presence and absence plots, increasing more rapidly in absence plots, peaking on day 26 before decreasing (Figure 7). On day 3, there was no significant difference in F_o between shorebird presence and absence plots, but on day 13 there was a significant difference. The largest difference was measured on day 26, when mean F_o in shorebird presence and absence plots was highly significantly different (Table 10).

The two subsequent sampling events (days 45 and 64) showed decreasing F_o with progressively smaller differences between presence and absence plots. Mean F_o in shorebird absence plots was still higher on day 45 but was not significantly different (Bonferroni corrected 0.01 significance level), and by day 64, F_o levels were very similar between treatments (Figure 7). There was no significant difference in chlorophyll *a* content (µg g^{-1}) in the top ~2 mm of sediment between presence and absence plots on day 45 (Figure 8).

3.4.2 Sediment Erodibility

To evaluate the effect of shorebirds on erosion protection, erosion threshold (τ_{cr}) was measured on day 45. Significantly greater erosion threshold was found in shorebird absence plots than in presence plots (Figure 8, Table 10).

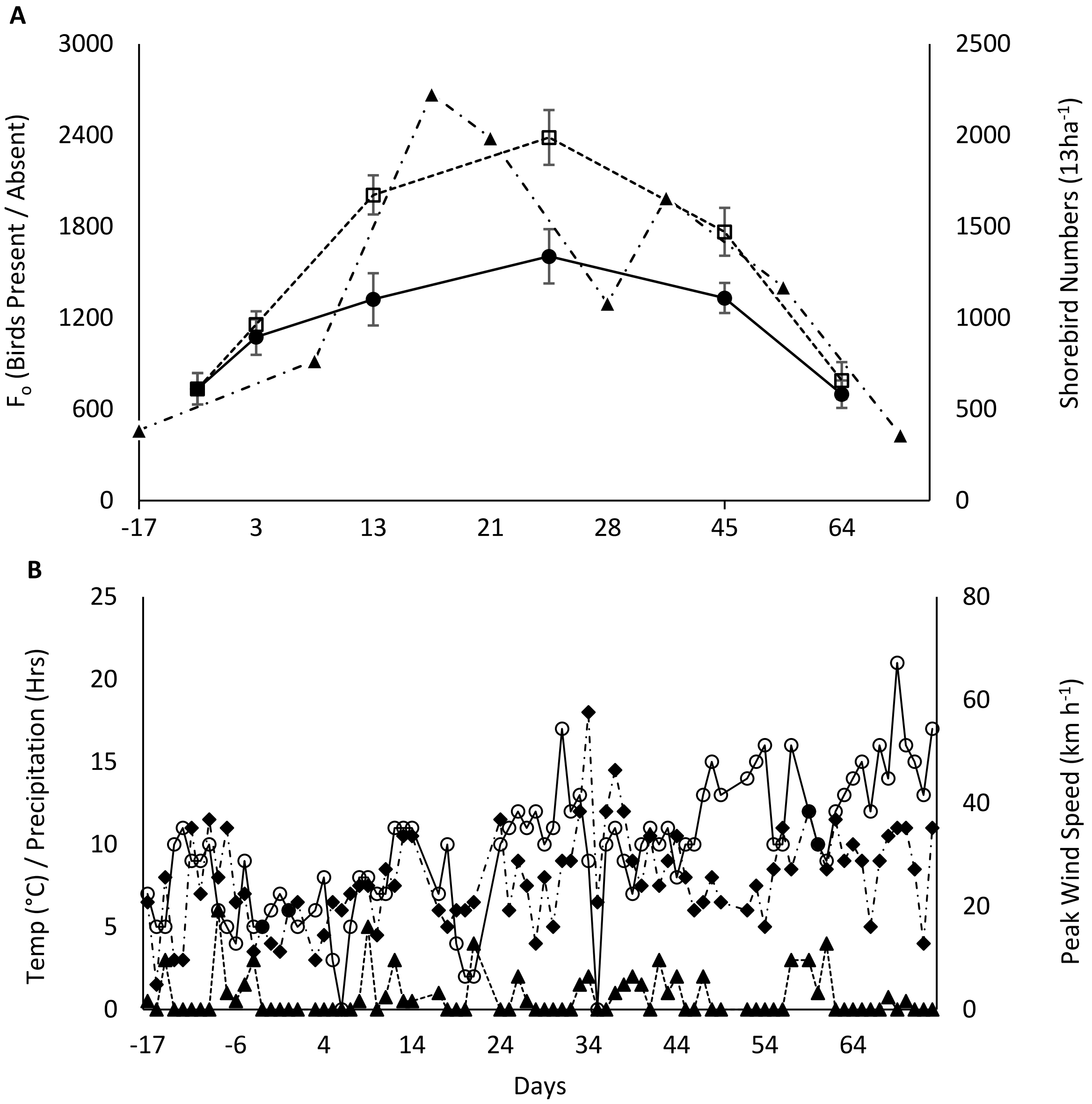


Figure 7 Time series during the experimental period: **A:** mean F$_o$ (± SE, n= 50) in shorebird presence (solid line, filled circles)/absence (dotted line, unfilled squares) plots and total peak shorebird count (multi-dash line, filled triangles). Dip in numbers on day 28 coincides with disturbance of flocks by marsh harrier **B:** weather data (per day): (peak temperature (°C): solid line with unfilled circles, peak wind speed (km h^{-1}): multi-dash with filled diamonds and precipitation (hours): dash with filled triangles).

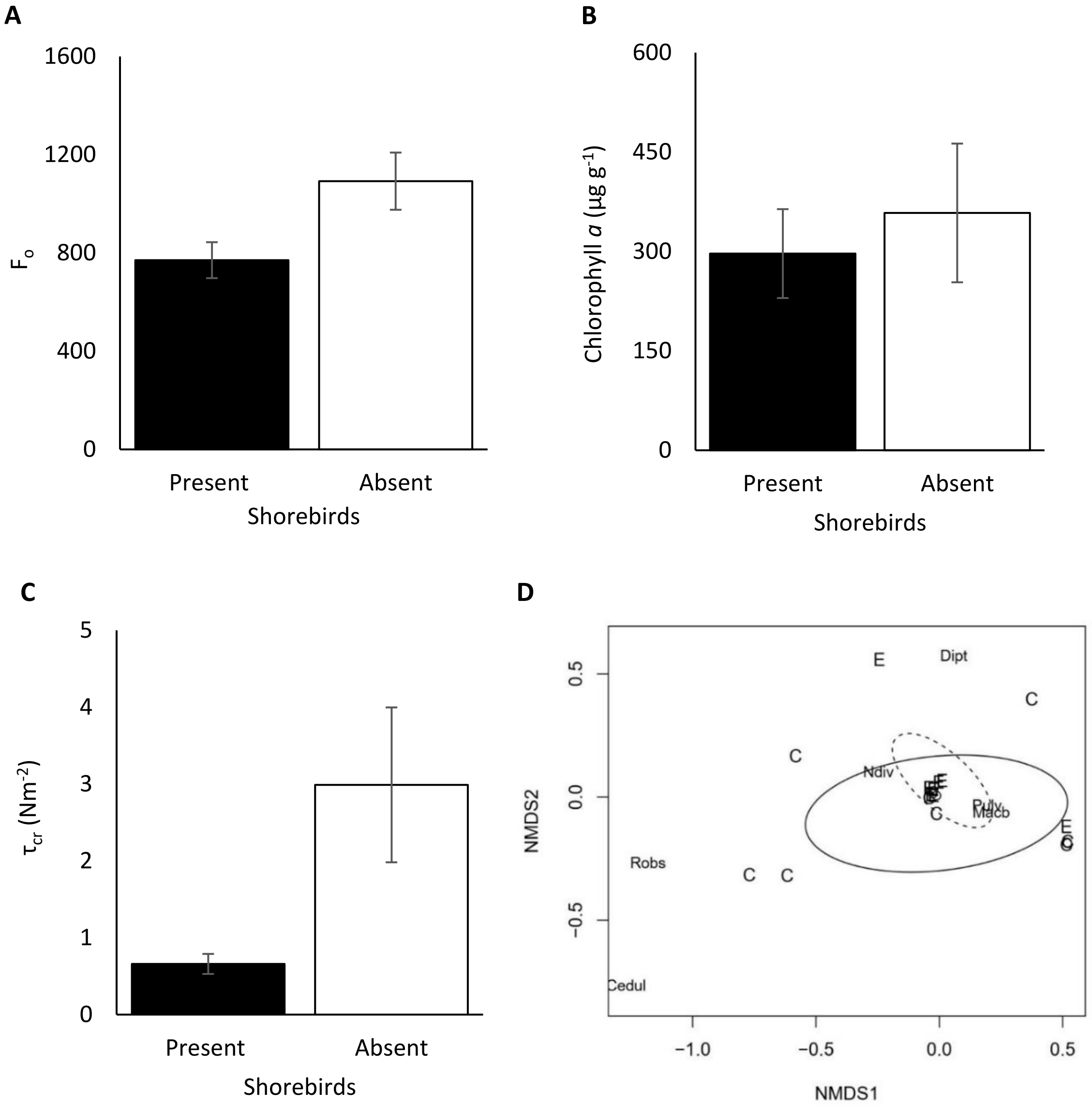


Figure 8 Measured characteristics in shorebird presence/absence on day 45 (06 March 2017): **A:** mean F_o (± SE, n = 50) **B:** mean chlorophyll *a* content in top ~2 mm ($\mu g\ g^{-1}$) (± SE, n = 14) **C:** mean erosion threshold (τ_{cr}) (± SE, n = 36) **D:** non-metric multidimensional scaling (MDS) plot depicting Bray-Curtis dissimilarity in community composition (shorebird presence = solid oval; shorebird absence = dotted oval, C=shorebird presence, E=shorebird absence, Cedul=*C.edule*, Robs=*R.obtusa*, Dipt=Chironomidae, Pulv=*P.ulvae*, Ndiv=*N.diversicolor*).

3.4.3 Macrofauna Density

To evaluate the indirect effect of shorebirds on erosion protection, nutrient cycling and carbon sequestration via changes in macrofauna density, the numbers of macrofauna were counted (from the same cores used for the nutrient measurements). Macrofauna recorded on day 45 were mud snails *P. ulvae*, Baltic clams *Macoma balthica*, midge larvae (Chironomidae), ragworms *Hediste diversicolor*, Arctic barrel-bubble *Retusa obtusa* and common cockles *Cerastoderma edule*. Mean densities (m^{-2}) in each treatment are shown in Table 8. Raw macrofauna counts revealed presence of a single specimen of *C.edule* and *R.obtusa* in only two and three plots respectively. *H. diversicolor* counts were also sparse (Table 8). On day 26 *P. ulvae* was visually noted on the mudflat surface for the first time during F_o sampling. Mud snails can compensate for the loss of higher predators on intertidal mudflats by grazing excess MPB which can mask otherwise detectable top-down effects associated with higher predators (Hamilton et al. 2006, Cheverie et al. 2014). This species was subsequently present within the study area during all F_o sampling events within presence and absence plots.

The non-metric Multi-Dimensional Scaling (nMDS) plot (Fig. 8D) indicated that macrofauna communities between treatments were not significantly dissimilar; a large overlap between community composition is indicated, although the spread of data points is larger in shorebird presence demonstrating larger variability in community composition. ANOSIM confirmed there was no significant difference in community composition between shorebird presence and absence plots (R=-0.038, P=0.623).

Table 8 Count of each macrofauna species recorded within each core extracted from the study area on Day 45 on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick Essex Wildlife Trust Nature Reserve, Essex, UK (grid reference TM 05065 19030).

Species	**Shorebird Treatment**	**Mean Count (m^{-2})**
Peringia ulvae	Present	31, 669 ± 5, 014
	Absent	30, 226 ± 3, 376
Macoma balthica	Present	1, 980 ± 366
	Absent	1, 796 ± 213
Nereis diversicolor	Present	99 ± 33
	Absent	170 ± 35
Chironomidae	Present	552 ± 347
	Absent	2, 574 ± 2, 086
Retusa obtusa	Present	42 ± 0
	Absent	0
Cerastoderma edule	Present	29 ± 18
	Absent	0

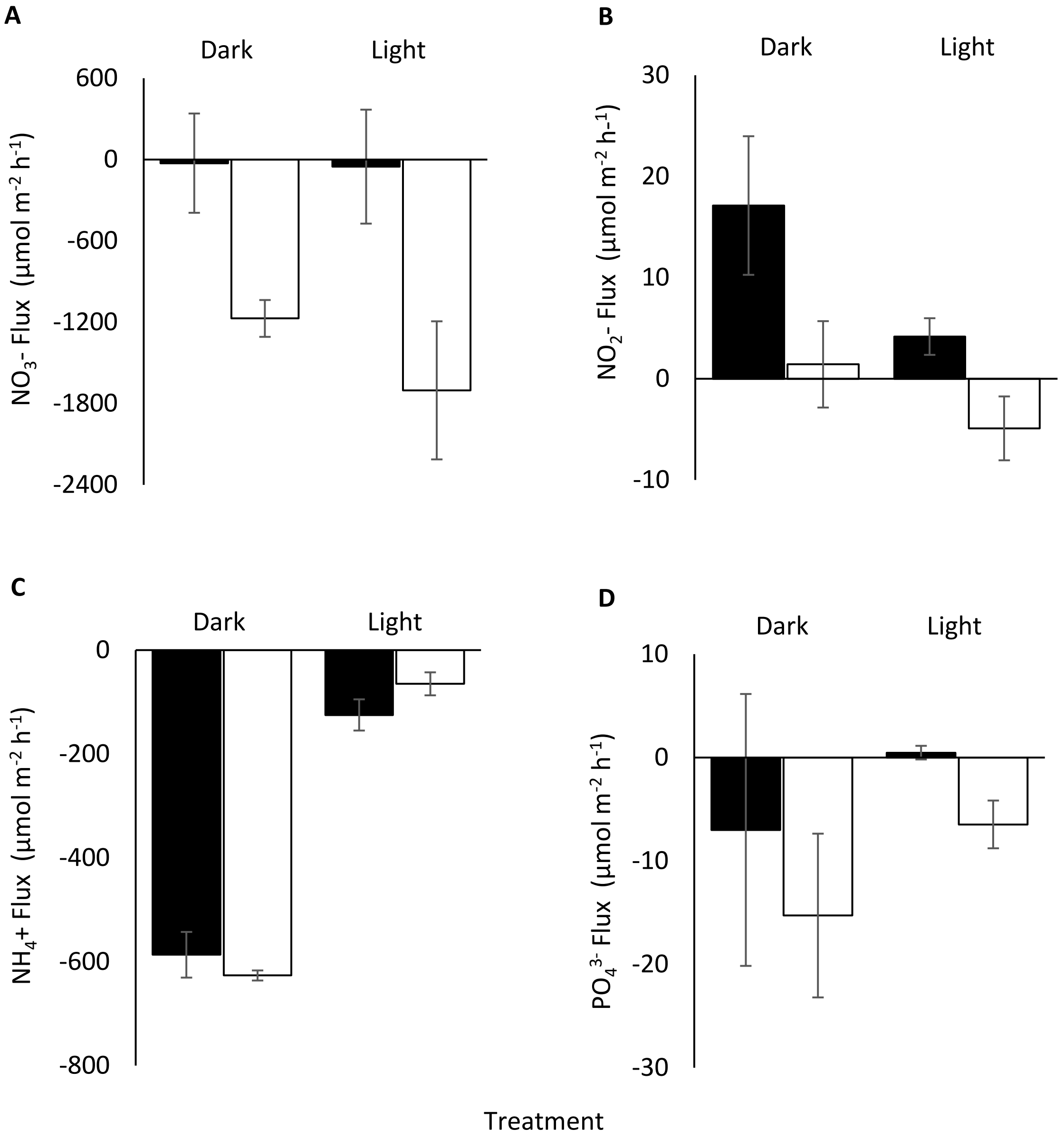
3.4.4 Sediment-water nutrient fluxes

There was significantly greater net nitrate influx into the sediment when shorebirds were absent compared to when they were present and a significantly greater net nitrite efflux from the sediment into the water column when shorebirds were present (Figure 9).

There was no significant difference in net phosphate flux between shorebird presence and absence plots. However, under lit conditions mean values changed from an influx into the sediment to a small efflux into the water column (Figure 9), which was considered ecologically significant.

There was no significant difference in net dissolved organic carbon (DOC) flux between shorebird presence and absence plots (Figure 9). However, in shorebird presence during light incubation, a large reversal in flux direction of DOC into the sediment rather than the water column was found (Figure 9).

No significant difference in ammonium flux between the sediment and water column was found (Figure 9).



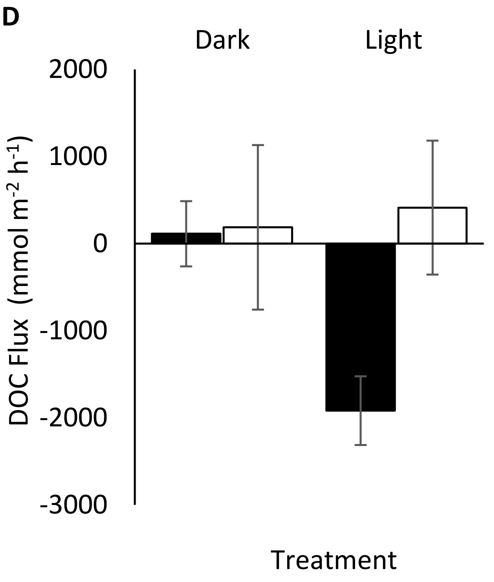


Figure 9 Sediment-water nutrient fluxes (Mean ± SE, n = 10) during light and dark incubations in cores collected from shorebird presence (filled bars) and absence (unfilled bars) plots on day 45 (06 March 2017). X-axis marks zero flux, positive values show flux out of the sediment, negative values show flux into the sediment: **A:** NO_3^- **B:** NO_2^- **C:** NH_4^+ **D:** PO_4^{3-} **E:** Dissolved Organic Carbon (DOC).

3.4.5 Bird Surveys

Over the study period, 10 shorebird species were recorded using the wider mudflat, with a total of 78,811 bird days (Table 9). Of these, three were recorded in the presence plots; *C. alpina* (84 bird-days), *T. totanus* (35 bird-days) and *P. squatarola* (28 bird-days). Camera data indicated that numbers of shorebirds using the study area were broadly consistent with those counted during surveys. Although the image quality (due to distance from the plots) made detection of individual birds difficult, flocks were noted using the plots, often as the tideline crossed them. Flocks were noted on camera footage in and around the plots between 23 February and 5 March (day before main sampling event).

The experimental plots were laid out in an area of mudflat representing approximately 0.3% of the area visually surveyed. Peak *C. alpina*, *P. squatarola* and *T. totanus* numbers within experimental plots comprised approximately 0.16%, 0.35% and 0.8% (respectively) of peak numbers within the survey area, thus within the same order of magnitude as that expected based on the areas of plots and the overall mudflat area.

Table 9 Bird days estimated for each species recorded foraging within the survey area between the 20th of January and the 3rd of April 2017 on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick Essex Wildlife Trust Nature Reserve, Essex, UK (grid reference TM 05065 19030).

Species	***Calidris alpina***	***Calidris canutus***	***Pluvialis squarola***	***Arenaria interpres***	***Tringa totanus***
Bird Days	53,853	9,363	6,358	103	3,735

Species	***Limosa limosa***	***Limosa lapponica***	***Recurvirostra avosetta***	***Numenius arquata***	***Haematopus ostralegus***
Bird Days	1,541	430	2,888	405	135

The LME model showed a highly significant difference in F_o between shorebird presence and absence. ANOVA models showed significant differences in τ_{cr} at the point of EF sampling and in F_o on days 13 and 26 between shorebird presence and absence (Table 10).

Table 10 Linear mixed-effects / ANOVA models and results for each variable and sampling time between the 20th of January and the 3rd of April 2017 on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick Essex Wildlife Trust Nature Reserve, Essex, UK (grid reference TM 05065 19030).

Variable (day if not day 45) / Model	**Factor**	**Source**			
		numDF	**denDF**	***F***	***P***
Linear Mixed Model					
F_o = MEAN x P/A x TIME x Plot(TIME)	Sampling Days = TIME	1	3	0.91	0.58
	Presence/Absence = P/A	1	85	22.2	**<0.0001**
	P/A x TIME	1	85	1.64	0.204
ANOVA Models		**df**	**MS**	***F***	***P***
Chlorophyll *a* ug g^{-1} = MEAN + P/A + Plot(P/A) + RES	Presence/Absence = P/A	1	39516	0.3	0.596
	Plots (P/A)	12	133337	2.34	0.031
	Residual	24	56973		
Erosion threshold Nm^{-2} = MEAN + P/A + Plot(P/A) + RES	Presence/Absence = P/A	1	14.4	8.44	**0.016**
	Plots (P/A)	10	1.7	3.85	**0.003**
	Residual	24	0.44		
F_o (day 3) = MEAN + P/A + Plot(P/A) + RES	Presence/Absence = P/A	1	105588	0.28	0.61
	Plots (P/A)	10	376213	0.67	0.75
	Residual	48	564759		
F_o (day 13) = MEAN + P/A + Plot(P/A) + RES	Presence/Absence = P/A	1	11777938	9.23	**0.007**
	Plots (P/A)	18	1275748	1.94	0.024
	Residual	80	658534		
F_o (day 26) = MEAN + P/A + Plot(P/A) + RES	Presence/Absence = P/A	1	15245120	8.56	**0.009**
	Plots (P/A)	18	1781747	3.04	**0.0003**
	Residual	80	564759		
F_o (day 45) = MEAN + P/A + Plot(P/A) + RES	Presence/Absence = P/A	1	4723233	4.93	0.039
	Plots (P/A)	18	957343	1.42	0.145
	Residual	80	673677		
F_o (day 64) = MEAN + P/A + Plot(P/A) + RES	Presence/Absence = P/A	1	200435	0.32	0.581
	Plots (P/A)	18	633507	3.58	**<0.00001**
	Residual	80	177121		

Table 10 Linear mixed-effects / ANOVA models and results for each variable and sampling time between the 20th of January and the 3rd of April 2017 on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick Essex Wildlife Trust Nature Reserve, Essex, UK (grid reference TM 05065 19030).

Variable (day if not day 45) / Model	**Factor**	**Source**			
		numDF	**denDF**	***F***	***P***
Linear Mixed Model					
	D/L x P/A	1	152.1	4.72	0.114
	Residual	36	32.25		0.037
Phosphate = MEAN + D/L + P/A + D/L x P/A + RES	Dark/Light = D/L	1	448.9	3.57	0.067
	Presence/Absence = P/A	1	136.9	1.09	0.304
	D/L x P/A	1	211.6	1.68	0.203
	Residual	36	125.85		
Nitrate = MEAN + D/L + P/A + D/L x P/A + RES	Dark/Light = D/L	1	773375	0.47	0.496
	Presence/Absence = P/A	1	19546821	11.95	**0.001**
	D/L x P/A	1	637317	0.39	0.536
	Residual	36	1635096		
Nitrite = MEAN + D/L + P/A + D/L x P/A + RES	Dark/Light = D/L	1	929	4.27	0.046
	Presence/Absence = P/A	1	1534	7.05	0.01
	D/L x P/A	1	110	0.50	0.483
	Residual	36	218		
Dissolved organic carbon = MEAN + D/L + P/A + D/L x P/A + RES	Dark/Light = D/L	1	8137880	1.65	0.208
	Presence/Absence = P/A	1	14457171	2.92	0.096
	D/L x P/A	1	12738121	2.58	0.117
	Residual	36	4943100		

3.5 Discussion

Excluding shorebirds caused significant changes in regulating and provisioning ecosystem functions, including mudflat erodibility, nutrient fluxes and carbon sequestration. Effects on MPB biofilm biomass and erodibility were, however, not as predicted in the hypotheses. It is suggested that these effects were driven by shorebird bioturbation of surface sediments and MPB biofilms and possible direct grazing of MPB by *C. alpina*.

3.5.1 Effects on MPB and Erodibility

Hypothesis 1 was rejected; the linear mixed-effects model showed a highly significant difference in F_o between shorebird presence and absence, with no significant interaction between other factors (Table 10). Significantly greater MPB F_o values were found in shorebird absence plots on days 13 and 26. By day 45 the difference had become less significant, to the extent of being non-significant when Bonferroni correction was applied (0.01 level) to compensate for five repeat tests. Despite this, on day 45 the difference in F_o remained visually notable in the field, which is reflected in Fig 7A. These differences between treatments occurred during a period of increased shorebird activity in the study area. Despite the decline in surveyed shorebird numbers on day 28, the 83 ha^{-1} shorebirds present at this point was notably greater than at the beginning or end of the experiment (when numbers were 30 ha^{-1} and 28 ha^{-1} respectively) (Fig. 7A). The survey visit on day 28 is also considered to be an underestimate due to the flushing of a large proportion of the foraging shorebirds on the incoming tide by a marsh harrier *Circus aeruginosus*. Differences in F_o between shorebird presence and absence on days 3 and 64 were non-significant and occurred when shorebird numbers were smaller, suggesting that the effects found may be dependent upon shorebird density (Chapter 4).

There was no significant dissimilarity in macrofauna community structure between shorebird presence and absence plots (Fig 8D). The present study recorded a greater diversity of species at the study site than during previous large scale work at the site (Wood et al. 2015), albeit the majority of infaunal species were present sporadically and in very low numbers (Table 8). This validates the macrofauna sampling effort, in that there were enough replicates to detect all species known to be present, despite likely patchiness in invertebrate distributions (Van Colen 2018). These findings differ to suggestions that a top-down ecological cascade effect driven by shorebirds can increase biofilm biomass (Daborn et al. 1993), supporting instead more recent work (Hamilton et al. 2006, Cheverie et al. 2014). These results provide strong indication that, through bioturbation and/or grazing (and/or a yet unknown pathway), shorebirds can have a significant reductive effect on the biomass of surface MPB biofilms. Thus, shorebirds can alter key ecosystem functions such as erosion protection and nutrient cycling via direct and/or indirect effects on MPB. The increase in MPB in the absence of shorebirds concurs with results reported by Hamilton *et al.* (2006), where the authors acknowledge that this finding is the opposite to that expected in the event of a trophic cascade. On day 45 bulk chlorophyll *a* content within the surface 2mm of sediments showed the same directional response as surface biofilm biomass and was also not significantly different. Bioturbation and grazing by macrofauna can significantly affect surface MPB biomass and resuspension (Grant and Daborn 1994, Hagerthey et al. 2002, Harris et al. 2015); but as macrofauna were not significantly different between shorebird presence/absence plots, and motile macrofauna could access all plots, the changes in MPB biomass are highly unlikely to have been due to macrofauna. However, the behaviour and foraging mode of macrofauna can affect MPB, which is a potential pathway responsible for the observed effects. For example, the ubiquitous *H. diversicolor* can feed as a deposit feeder, suspension feeder, herbivore or carnivore, foraging mode partly influenced by presence or absence of predators (Costa et al. 2006). Physical effects of birds upon primary producers is evident within many freshwater and marine environments (Cadee 1990, Mitchell and Perrow 1998, Nacken and Reise 2000) and physical mixing of intertidal mud has been shown to significantly reduce chlorophyll *a*, F_o and colloidal carbohydrate (Tolhurst et al. 2012). It follows that physical disturbance (bioturbation) by shorebirds, through foraging (including biofilm grazing in some species) and tracking (walking), can have a significant effect upon MPB biomass and related sediment properties. These results suggest that bioturbation by shorebirds can be a more significant driver of effects on MPB than trophic cascades. Further work is required to confirm the mechanisms by which shorebirds in this part of the world reduce MPB biomass and whether this is variable between shorebird species (Chapter 4).

Hypothesis 2 was rejected, sediment critical erosion threshold (τ_{cr}) was significantly lower when shorebirds were present than when they were absent (see Fig. 8C). This pattern is most likely to have been driven by both direct bioturbation during walking and feeding of shorebirds on the mudflat surface and, because MPB commonly significantly increase mudflat erosion threshold (Hale et al. 2019, Hope et al. 2020), indirectly by grazing decreasing the biomass of MPB. The exact mechanistic pathway(s) and their magnitude require further investigation. The erosion shear stresses exerted on intertidal mudflats by combined waves and tides are very variable, but commonly in the 0-1 Nm^{-2} range and typically below 4 Nm^{-2} (Christie and Dyer 1998, Whitehouse and Mitchener 1998). Thus, the τ_{cr} measurements suggest that erosion would occur frequently (i.e. during most tidal cycles) in the presence of shorebirds and much less frequently in the absence of shorebirds. Given the importance of sediment erodibility for many ecosystem functions (Hubas et al. 2018, Hope et al. 2020), including nutrient fluxes and erosion protection; the effect of shorebirds on erodibility demonstrates their importance as ecosystem engineers (Passarelli et al. 2014) (Chapter 7) and their significant role in ecosystem functioning.

Although F_o is widely used as a proxy for MPB biomass, this relationship varies depending upon the physiological state and taxonomic composition of MPB due to vertical migration of MPB (Serodio et al. 2001, Serodio 2004, Serodio et al. 2006, Du et al. 2018). By standardising the time of sampling within the tidal exposure period, tidal migration rhythms influencing F_o were accounted for between treatments. Though changes in the relationship between F_o and Chl *a* over time may have occurred, significant differences in F_o between treatments at each time of sampling were found. Results show the same directional response of F_o and Chl *a* to shorebird presence, suggesting an underlying relationship in this case. Actual Chl *a* concentration varies vertically within the sediment depending upon factors such as MPB migration, light intensity, water content and sediment compaction (Perkins et al. 2003, Tolhurst et al. 2003, Jesus et al. 2006a, Maggi et al. 2013) and shows temporal changes. The sampling regime was not designed to specifically focus on the F_o to Chl *a* relationship, which requires a higher level of sampling granularity.

3.5.2 Effects on Nutrient Fluxes

Hypothesis 3 was not rejected; statistically significant differences in the fluxes of nitrate, nitrite and dissolved organic carbon (DOC), were found between presence and absence treatments. Orders of magnitude changes in the scale of some fluxes were observed (nitrate ~100x, nitrite ~10x and DOC ~2000x). Despite not being formally significant, the reversal of phosphate flux into/out of the sediment is considered to be ecologically important. These results suggest that shorebirds significantly alter ecosystem functioning associated with nutrient cycling (Saint-Béat et al. 2013, Mathot et al. 2018, Hope et al. 2020) and carbon storage (Maher and Eyre 2010). Differences in the surface active MPB biomass (F_o) can explain the nutrient flux alterations by shorebirds. Photosynthesis and nutrient assimilation by MPB significantly affects nutrient flux rates, including nitrate (Dong et al. 2000) and phosphate (Sundback et al. 1991) . Further, the EPS matrix within MPB biofilms provides additional organic matter to support heterotrophic bacteria, which reduce nitrite to nitrous oxide (Dong et al. 2002). Evidence was found to suggest that the presence of shorebirds can significantly reduce nitrate uptake into intertidal sediments (Fig. 8A). The reduction of active surface MPB biofilms by shorebirds is a likely mechanism that may reduce nitrate and phosphate uptake, nitrification, coupled nitrification-denitrification, and through the reduction of extracellular organic carbon, reduce bacterial degradation rates (Thornton et al. 2007).

These findings suggest that shorebird effects on MPB can limit the drawdown of nitrate, nitrite and phosphate into sediments in an already nitrate rich estuary (Thornton et al. 2007). The observed alterations of nutrient fluxes suggest that shorebirds play a significant role in estuarine nutrient pathways, effectively controlling and engineering nutrient fluxes between the sediment and water column (Passarelli et al. 2014, Passarelli et al. 2018). Bioturbation by macrofauna is known to significantly affect nitrate and ammonia fluxes at the study site and elsewhere, through sediment reworking, ventilation and burrowing (Nizzoli et al. 2007). It is suggested that bioturbation by shorebirds (Mathot et al. 2018) is likely to have contributed to the significant effects found here.

While the measured nutrients were typically characterised by a reduction in fluxes into the sediment from shorebird presence, DOC flux into sediment from shorebird presence increased significantly in lit conditions. It is possible that through the observed reduction of MPB biomass by shorebirds, competition for nutrients may have been reduced, allowing bacteria to proliferate and increase assimilation of DOC and ammonium (Amin et al. 2012). Migratory birds can also introduce bacteria to communities (Steiniger 1969) via faecal droppings (Muller 1965) and external tissues (Muza et al. 2000), potentially further increasing these process rates. These results indicate that changes in shorebird abundance could affect wider ecosystem functioning such as carbon sequestration and coastal biogeochemistry more broadly (Nedwell et al. 2016, Hope et al. 2020).

3.5.3 Secondary Effects

Use of the mid and upper shore at low tide by *C. alpina*, despite often being a 'tide follower' (Granadeiro et al. 2006), may have been driven by the visual cues of MPB communities on the mudflats, either as a cue for the presence of invertebrate prey or to feed upon MPB directly (Hamilton et al. 2003, Drouet et al. 2015, Jimenez et al. 2015). *C. alpina* is an opportunistic feeder with a broad diet (Dierschke et al. 1999) using visual and tactile foraging cues (Drouet et al. 2015), and possibly exploited areas with high diatom biomass to maximise the breadth of feeding opportunity.

Avian guano (in particular shorebird droppings) is a potentially important source of nutrients in coastal areas (Schrama et al. 2013). It has been suggested that *C. alpina* droppings increase growth rate and biomass of the diatom species, *Entomoneis paludosa*, through increases in nitrogen and phosphorous input to the sediment (Jauffrais et al. 2015). However, the Colne estuary has very high nutrient loads (McMellor and Underwood 2014, Nedwell et al. 2016) and MPB biomass was smaller, rather than larger in shorebird presence, suggesting that nutrient enrichment of biofilms by guano was not a major mechanism at the time and in the location of this experiment. These findings reflect the complexity of the real-world scenario compared to laboratory studies (Jauffrais et al. 2015); in the present study shorebirds reduced MPB biomass on the upper shore. This indicates that the effects of bioturbation and/or grazing by shorebirds, which lead to alterations in ecosystem functioning, significantly outweigh the effects of nutrient input via guano in the study site.

Shorebirds significantly affect ecosystem functions (nutrient flux and erodibility), at least within the upper shore, in a temperate climate during late winter. However, these effects are likely to vary temporally and spatially (Underwood and Paterson 1993, Gerwing et al. 2015) depending as they do upon the abundances and functioning of other organisms present (Underwood 1994, Norazlimi and Ramli 2014). For example, it was found that shorebird effects were temporary and seasonal, restricted to an approximately one month period when shorebird density peaked at the study site (Fig 7A). This suggests that the observed phenomenon is seasonally and density dependent, reliant on sufficient density of shorebirds (which are present in larger densities during winter) to cause effects on ecosystem functioning. Similarly, compensatory grazing by the mud snail *Peringia ulvae* may have limited the temporal effect of shorebirds on MPB during this study, effectively resetting the state of the system as bird density declined (Hamilton et al. 2006, Cheverie et al. 2014). The collapse of the shorebird effect on F_o was concomitant with the emergence of large numbers of *P. ulvae*. This MPB grazer was first noticed on the mudflat surface on day 26, was noted spread across the mudflat within all plots (Table 8), and can rapidly reduce the abundance and thickness of biofilms (Sahan et al. 2007). Subsequently the difference in F_o between treatments steadily decreased, eventually becoming non-significant. On day 45, no significant difference between macrofaunal communities was evident. It is interpreted that the snails had a homogenising effect on biofilm distribution. Once the snails emerged and while birds remained, the effects of the birds became weaker. Once the birds left, continued grazing by the snails removed the residual bird effects (compensatory effect). Despite the restriction to observational evidence regarding the temporal change in numbers of *P. ulvae*, it is known that mudsnails can mask effects on MPB (Hamilton et al. 2006, Cheverie et al. 2014) and it is plausible that this occurred here, reducing the detectability of ecosystem function effect pathways.

Here it is highlighted that shorebirds play a key community role in the regulation and control of ecosystem function, through inter and intraguild interactions with macrofauna and MPB with which they are intrinsically linked (Kuwae et al. 2012, Cheverie et al. 2014). No evidence was found to suggest that macrofauna community structure differed between shorebird presence and absence, however such effects have been detected in Canada in exclusion experiments on semipalmated sandpiper *C. pusilla*, where reductions in *C. volutator* densities were found (Hamilton et al. 2006, Cheverie et al. 2014). The differences between these studies may be due to geographic or shorebird species differences, or due to the fact that *C. volutator* was not present at the study site.

Previous work using exclusion experiments has presented wide ranging and equivocal results regarding the effects of foraging shorebirds on invertebrate communities: A study in Victoria, Australia, where predatory species were predominantly red-necked stint *Calidris ruficollis*, curlew sandpiper *C. ferruginea* and sharp-tailed sandpiper *C. acuminata*, reported no measurable effect on the macroinfaunal assemblages (Morris 2003). In southern California evidence was found suggesting that dowitchers *Limondromus griseus* and *L. scolopaceus*, western sandpipers *Calidris mauri*, avocets *Recurvirostra Americana* and dunlin *Calidris alpina* do have a significant effect upon the infaunal densities during winter when bird numbers at the site peak (Quammen 1984).

It is also emphasised that differences in MPB surface biomass between treatments eventually became non-significant, despite shorebird exclosures remaining *in-situ*. It is concluded therefore that shorebirds, rather than experimental artefacts, drove the measured MPB biomass changes and subsequent effects on ecosystem functions.

3.6 Conclusions

Here, previously unknown effects of shorebirds on ecosystem functioning have been identified. Although limitations are acknowledged regarding the link between F_o measurements and actual Chl *a* content, the end effect of shorebird presence on erodibility and nutrient fluxes was found to be significant, and a large amount of existing literature indicates that MPB are highly likely to drive this effect. The removal of shorebirds significantly increased surface biofilm F_o and sediment erosion threshold. Shorebird absence was also found to affect nutrient cycling regimes and carbon sequestration on the mudflat; differences in biofilm biomass led to significant alterations in the flux of nutrients under lit conditions, including nitrate, nitrite and phosphate, all of which showed an increased flux into the sediment in the absence of shorebirds. The uptake of DOC in the light into the sediment was significantly greater in the presence of shorebirds.

The mechanism by which shorebirds reduced biofilm biomass was not experimentally tested, although the literature provides a number of possible drivers including physical disturbance (bioturbation) through tracking (walking) and foraging. Considering the presence of large numbers of *C. alpina*, which has been shown to consume MPB, it is plausible that direct consumption of biofilm may have contributed, but this is not confirmed. The lack of significant differences in macrofauna densities between treatments suggests that altered numbers of these invertebrates were not driving a change in bioturbation or grazing on the biofilms, and thus were not a significant driver of the measured effects.

The finite period of effects and community interactions between shorebirds, macrofauna and MPB reduce the clarity of the situation regarding consequences of declining shorebird species on coastal ecosystem functions. The work presented here indicates a potential shorebird density-dependent effect, resulting in stronger impacts on ecosystem function by birds during winter that may be ‘reset’ by other organisms or reduced bird densities in spring and summer. This reflects the complexity of intertidal mudflat ecosystem functions, but is a step forward in disentangling the many factors influencing them. This research indicates that shorebirds play a significant role in the ecosystem functions provided by intertidal mudflats, including erosion protection, nutrient cycling and carbon sequestration. However, further research is required, involving longer-term, larger-scale experiments, to better understand the mechanisms behind ecosystem function regulation by shorebirds.

4 Site dependent effects of shorebird density and species assemblage on sediment erodibility on intertidal mudflats

4.1 Abstract

Intertidal mudflats which provide winter-feeding grounds for shorebirds deliver provisioning, regulatory, supporting and cultural ecosystem services (ES). Recent research shows that shorebirds affect ES in this habitat including erodibility and nutrient and organic matter cycling. Populations of (particularly migratory) shorebirds are in decline globally and mudflats are one of the planets most vulnerable habitats due to loss through coastal squeeze, climate change and sea level rise. It has long been established that shorebirds rely heavily on macrofaunal communities within intertidal mudflats. Macrofauna are supported by primary producers (e.g. diatoms), forming biofilms at the sediment surface. Through production of extracellular polymeric substances (EPS) diatoms also have a binding effect on sediment particles and reduce sediment erodibility, an important regulatory ES. During recent decades, research has discovered important additional direct links between shorebirds and biofilms, including direct grazing on MPB by some smaller sandpipers, introducing new potential mechanisms for shorebird presence to significantly reduce biofilm biomass. This chapter presents a manipulation experiment designed to investigate how shorebird patch use (a proxy for shorebird density) and shorebird species assemblage may affect biofilm biomass on an intertidal mudflat using Linear Mixed Effects (LME) modelling. To accommodate geographical and biological scales, the experiment was replicated across three mudflats with different shorebird communities. High level three-way interaction between bird density, assemblage and site indicated that effects among sites were highly variable. As such, the best fitting LME model indicated variability in bird assemblage effect between sites and positive effects of bird density on MPB biomass at two out of three of the sites. The effect of shorebirds on MPB caused a significant reduction in erodibility at Fingringhoe, where previous work also suggested shorebird effects on erodibility (Chapter 3). This complex relationship between MPB biomass, shorebird density and assemblage and site (geographical location) introduces new interdependency between endangered shorebirds, their threatened wintering habitats, and the provision of ES along coastlines and estuaries.

4.2 Introduction

Intertidal mudflats provide valuable habitat for many organisms including shorebirds (Mathot et al. 2018) and provide provisioning ecosystem services which support human activities (Beninger 2018b, Beninger and Shumway 2018). Information regarding the current global extent of intertidal mudflats and the ecosystem functioning and services they provide, is lacking (Beninger and Paterson 2018), yet they remain under severe threat from coastal development, coastal squeeze and erosion (Mazik et al. 2010).

Intertidal mudflats across the globe support populations of breeding, wintering, and migrating shorebirds, many species of which are in long-term global decline (Piersma and Lindström 2004, Koleček et al. 2021). MPB biofilms are the main primary producer on mudflats (Underwood et al. 2022), formed by microbes within an extracellular polymeric substance (EPS) matrix which has a physically binding effect on sediment particles, reducing the mudflat surface erodibility and increasing the resilience of the system to erosion (Tolhurst et al. 2002, Tolhurst et al. 2003, Tolhurst et al. 2008a, Tolhurst et al. 2009, Hubas et al. 2018). By feeding on macrofauna (some of which engineer this ecosystem through their own lifestyles) and biofilms, shorebirds have become acknowledged as ecosystem engineers due to their indirect and direct effects on MPB biofilm (Daborn et al. 1993, Passarelli et al. 2014, Passarelli et al. 2018).

The research field of shorebird science related to direct interactions between shorebirds (particularly small sandpipers) and microphytobenthic (MPB) biofilm on intertidal mudflats (Mathot et al. 2018) is moving at an accelerating speed. It is accepted that a number of small sandpiper species are physically and behaviourally adapted for biofilm feeding (Hobson et al. 2022). This extends to age and sex related dietary specialisation within this foraging mode (Hall et al. 2021), resulting in proposed shorebird conservation strategies for mudflats to ensure biofilm provision (Kuwae et al. 2021). These findings raise pertinent implications regarding the ways in which shorebirds interact with the sedimentary environments upon which they rely, and how this may differ depending on bird species assemblage on an intertidal mudflat. For example, it would follow that communities containing larger proportions of smaller sandpipers would exert different top-down effects on MPB, resulting in potentially reduced biofilm coverage. This was found in the work presented in Chapter 3 (Booty et al. 2020).

Climate change is predicted to affect species distributions and community composition across the globe , the effects of which are a topic of prolific study, in attempts to predict which species will be negatively impacted (Lavergne et al. 2010). Identifying how species and communities may respond to climate change, and how this may affect ecosystem functioning and services, is a fundamental aspect of successful mitigation (Catry et al. 2011, Warren et al. 2013, Gillingham et al. 2015). Wintering migratory shorebirds are among the species at risk in this context, with evidence that wintering communities within Europe are likely to alter as climate change continues (Godet et al. 2011). It is therefore pertinent to explore how different wintering migratory shorebird communities interact with MPB on mudflats, to understand how these systems may respond to community alterations in the future.

Research indicates (Chapter 3) that shorebird presence significantly affects ecosystem functioning (EF) on an intertidal mudflat, probably through direct effects on biofilm abundance. Shorebird presence/absence EF effects extended via changes in MPB biomass to sediment erodibility and biogeochemical cycling of nitrate, nitrite, ammonium, phosphate and dissolved organic carbon (DOC). These EF are critical for maintaining coastline resilience against erosion (Spalding et al. 2014), maintaining water quality (Burgin and Hamilton 2007) and habitat provision for benthic communities (Culhane et al. 2019). As it becomes apparent that shorebirds may play a pivotal role in the 'engineering' of this system (Jones et al. 1994), this raises further questions regarding the mechanisms behind these effects, whether they may be density or species dependent, and how significant these effects may be over longer timescales.

While single site experiments which provide snapshots of data both in space and time are valuable in exploring and understanding the natural environment and species interactions, these do not necessarily extrapolate well to larger spatial scales and can succumb to undetected localised confounding factors (Fraser et al. 2013). Therefore, recognition of the need to incorporate experiments over larger spatial/temporal scales has emerged during recent decades (Borer et al. 2014).

Shorebird species assemblage (the shorebird community gathered in a given area and time) varies both spatially and temporally (Colwell 1993, Lopes et al. 2005), differences in which can be quantified using survey data within and across sites (VanDusen et al. 2012). Chapter 2 presented information on established mudflat ecology field manipulation techniques (Cheverie et al. 2014), which have been developed to manipulate shorebird patch density at a coarse level ('low' and 'high'). This manipulation is also likely to increase variability among experimental plots within a site, allowing examination of shorebird patch use effects across a greater range of bird footprint density (% cover).

Utilising these methods, a study was designed to examine how shorebird density and species assemblage may affect ecosystem functioning on an intertidal mudflat. To increase the reliability and practical application of the experiment we replicated it across three estuarine sites in the east of England on different rivers.

Estuaries along the Suffolk and Essex coastlines including the Colne Estuary, Essex and Stour and Orwell Estuaries, Suffolk, are protected under UK and EU law because they support internationally important migratory and overwintering shorebird populations. A six-to-seven-month (depending on site) field density manipulation experiment was implemented to investigate shorebird density effects on MPB biofilm biomass and the closely related ecosystem function erosion protection. Based on previous findings that shorebird presence significantly reduces MPB biomass (see Chapter 3), we tested the following hypotheses:

1. Greater shorebird density (patch use) would significantly reduce MPB biomass, i.e. MPB biomass would be greater in control plots (patches) compared to treatment plots where density is reduced.
2. Species assemblage would significantly affect biofilm biomass
3. Sediment erodibility would be significantly higher where shorebird density was ‘normal’ compared to where shorebird density was ‘low’ (erosion threshold would be lower where F_o biomass was smaller), due to the positive relationship between MPB and sediment stability.

Measuring sediment characteristics, F_o and shorebird patch use across multiple sites enabled examination of the interplay between mudflat characteristics and shorebird species assemblage and patch use in the East of England. This is becoming an important aspect of shorebird conservation, as our understanding of the role and importance of MPB biofilms in shorebird diet improves (Kuwae et al. 2021). Following on from the above hypotheses, it was also predicted that sites with larger MPB biomass would be used by more small sandpipers which rely on direct biofilm foraging (Hobson et al. 2022).

4.3 Materials and Methods

4.3.1 Study Sites

All sites were selected based upon a combination of the following attributes:

1) Landowner cooperation and permission;
2) Regular use by wintering shorebirds;
3) Accessibility and logistical considerations.

The second criterion was satisfied by selecting sites with statutory designations based on their use by internationally important overwintering shorebird assemblages. As such, all the sites are within Special Protection Area (SPA), Ramsar (site designated based upon the Ramsar Convention) and Site of Special Site of Scientific Interest (SSSI) designations for such bird assemblages. Due to the high sensitivity of shorebirds to anthropogenic disturbance and the potential impact of this on their survival (West et al. 2002), particularly during severe weather conditions (Cook et al. 2021), both landowner and statutory regulatory body permissions were required prior to commencement of fieldwork. This permission process included pilot studies to test the effects of proposed experimental structures on foraging and roosting shorebirds at Geedon Saltings (Fingringhoe Wick Essex Wildlife Trust Nature Reserve, Essex, UK (grid reference TM 05065 19030). Methods, results and interpretation of these tests are presented in Chapter 2.

4.3.2 Fingringhoe Wick, Essex

The study site used at Fingringhoe was in the same area as in Chapter 3, described fully in Chapters 2 and 3, located specifically at grid reference TM 05060 19033.

4.3.3 Brantham, Suffolk

The Stour/Orwell estuary system is meso-tidal with a 3.6 m tidal range at the convergence of the river mouths and 3.9 m at the limits of each estuary. Until 2005 the estuary system had a history of net erosion of intertidal habitats, including loss of over half of the salt marsh since the 19th century. Likely causes are cited as significant development of the estuary mouth (at the Port of Felixstowe in particular) and associated deepening of the channel. However, a revised mitigation plan was implemented in 2005 which has resulted in net gain of intertidal habitat area in the estuary system as a whole, although this has primarily occurred in the Stour estuary while the Orwell estuary continues to show a net loss of intertidal habitat area (Spearman et al. 2014).

The site at Brantham lies at the termination of Newmill Lane to the south-east of the village of Brantham within the parish of Babergh, Suffolk, in the East of England. The study area is on the northern bank of the River Stour, where intertidal mudflats terminate at an artificial seawall, beyond which is arable farmland. Wave generation within the Stour/Orwell system is locally wind driven, with wave heights in the Stour typically 0.2- 0.3 m, though reaching up to 1 m during strong westerly winds. The study site comprised an area of mudflat approximately 400 m^2 situated on the upper shore (Figure 10). Regular monitoring by Suffolk Bird Group (SBG) records wintering flocks of dunlin *Calidris alpina*, knot *Calidris canutus* and black-tailed godwit *Limosa limosa* and scattered individual redshank *Tringa totanus*, grey plover *Pluvialis squatarola*, curlew *Numenius arquata* and shelduck *Tadorna tadorna* foraging at the study site on receding and incoming tides. The study site was set within an extensive area of estuarine mudflat, approximately 150,000m^2 of which could be visually surveyed for shorebird activity from a fixed point (TM 12424 33401), concealed using a hide. The site is referred to as 'Brantham'.

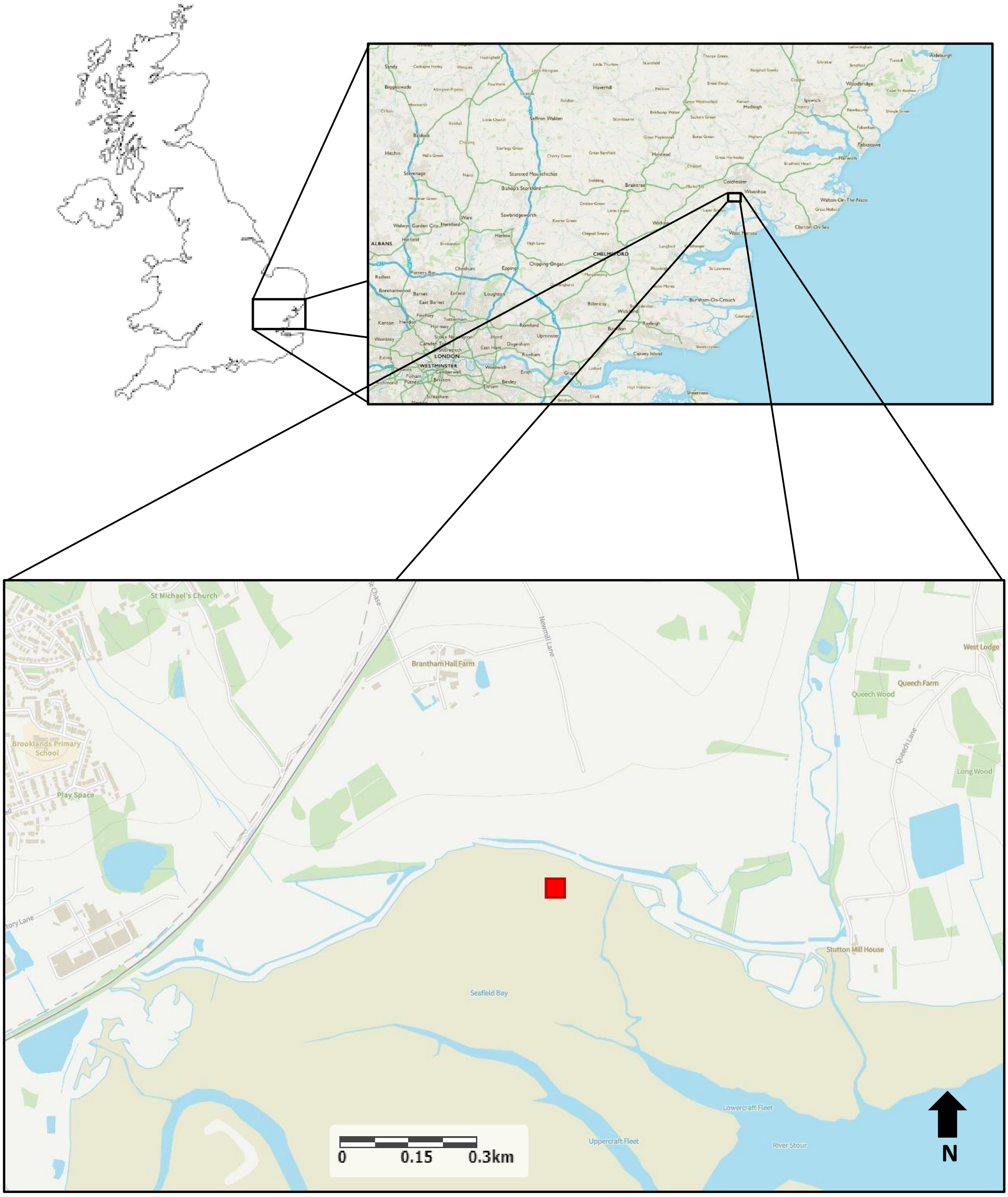


Figure 10 National, regional and local location (red square; TM 12334 33337) of the study site at Seafield Bay, Brantham, Suffolk, UK. Ordnance survey maps from OS OpenData (Ordnance Survey, 2018).

4.3.4 Trimley Marshes, Suffolk

The site is located on an intertidal mudflat at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406), on the Orwell estuary. Trimley Marshes SWT comprises a series of lagoons and associated reedbeds, grassland, woodland, scrub, dykes and intertidal mudflat which were created to compensate the loss of coastal habitats when the nearby Port of Felixstowe was created in the 1980s (Brady and Boda 2017). The reserve meets the northern bank of the Orwell estuary where shingle banks, salt marsh and intertidal mudflats are present adjacent to an artificial seawall and coastal footpath. In this area, the study site was located comprising an area of mudflat (artificially created in 1990) approximately 400 m^2 situated on the upper shore, sheltered from the incoming tide by an artificial spit (Figure 11). The study site was set within an area of estuarine mudflat, approximately 35,000 m^2 of which could be visually surveyed for shorebird activity from a fixed point (TM 25526 35386), concealed using a mobile hide.

In contrast to the Stour estuary, the Orwell is described as being well sheltered, with wind generated waves of 0.1 to 0.2 m. However, Trimley is closer to the coast than Brantham, typically subjecting this site to stronger winds than further inland.

The study area and other nearby intertidal areas were reinforced with dredged sediment as a means of mitigating the loss of intertidal habitats during expansion of Port of Felixstowe. Monitoring of benthic assemblages along the Stour and Orwell, both of which were affected by the port expansion, was carried out between 2003 and 2014. The benthic fauna recorded at the points closest to Trimley and Brantham in 2014, and macrofauna recorded at Fingringhoe in 2017 (see Chapter 3) are summarised in Table 1 in Chapter 1. The site is referred to as ‘Trimley’.

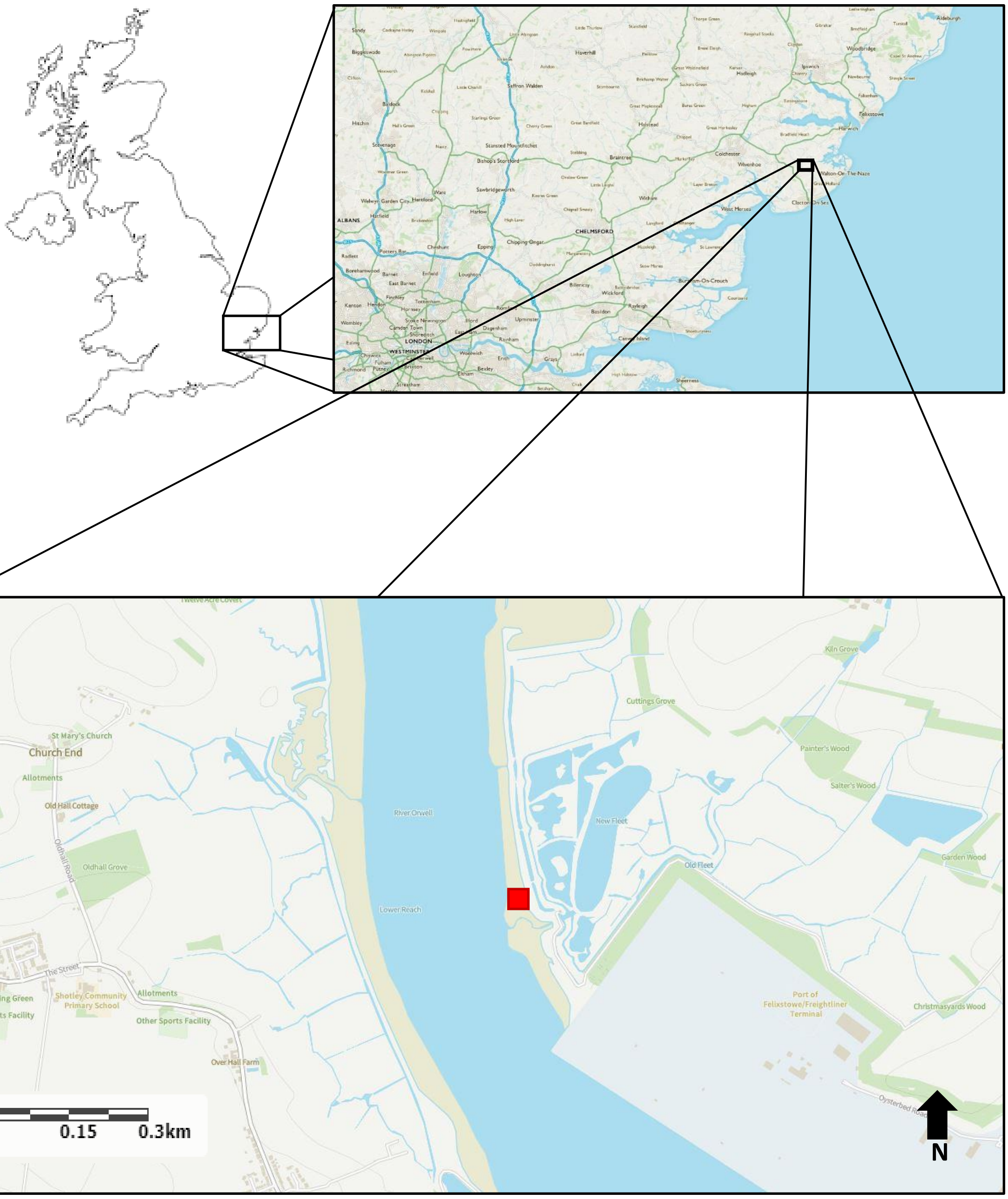


Figure 11 National, regional and local location (red square; TM 25484 35475) of the study site at Trimley Marshes, Suffolk, UK. Ordnance survey maps from OS OpenData (Ordnance Survey, 2018).

4.3.5 Experimental Setup

Fieldwork was undertaken for a total of 214 days between the 10 October 2018 and 12 April 2019 at Fingringhoe, for a total of 186 days between 05 October 2018 and 02 April 2019 at Brantham and for a total of 210 days between 20 September 2018 and 25 April 2019 at Trimley. Experimental time periods differed for logistical reasons. The experimental period covered the period for wintering migratory shorebirds in the East of England.

The experimental layout at each site was a sequential design of 20 spatial plots (Figures 12 to 14), each 1 m x 1 m, consisting of two treatment levels; control (shorebirds present 'as normal' in open un-manipulated plots) and partial exclosure (shorebird activity reduced), with n = 10 replicates of each treatment. The experiment was repeated across the three sites enabling comparison between them. At Brantham, plot location was dictated by sediment depth above a layer of clay, which prevented identical layout to that used at the other sites. Therefore, a randomised design was used at Brantham and it was ensured that the plot immersion times did not vary by more than two minutes at all sites.

All plots were at least three metres apart, to allow sampling from all sides and prevent plots unduly influencing each other (Booty et al. 2020). As described in Chapter 2, visual observations of shorebird behaviour around partial exclosures during 2018 suggested that shorebirds within the study area continued to walk and feed within <3 m of the structures, apparently unaffected by their presence. Exclosure and control plots were unpaired and separated by similar distances, with treatments interspersed to reduce the potential for spatial bias (Booty et al. 2020) (Chapter 3). The exact locations of plots were selected to represent the heterogeneity within the wider mudflat and were arranged parallel to the tide line (within a minute of immersion/emersion time of one another) (Booty et al. 2020) (Chapter 3). Plots were situated on the upper mudflat between mean tide level and mean high water neap tide, where shorebirds spend most time foraging due to the longer emersion time (Granadeiro et al. 2006). No scouring or bite marks indicating the presence of larger fish (Eggold and Motta 1992) were found within any plots during the experiment. Direct observation recorded no events of birds standing on exclosures (behaviour which may have caused an increased input of droppings into exclosure plots above that from droppings deposited during foraging) (Schrama et al. 2013, Jauffrais et al. 2015).

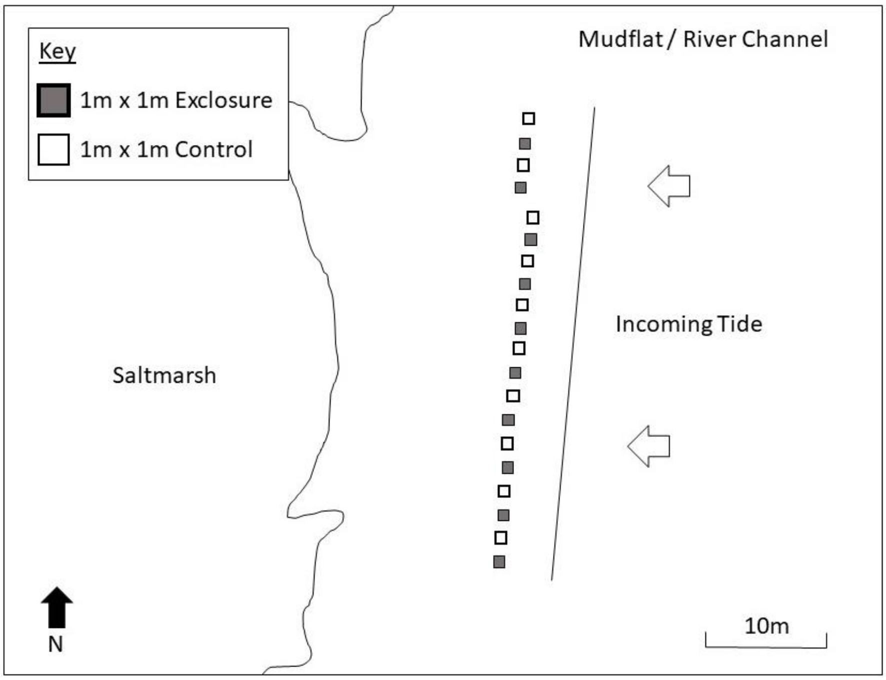


Figure 12 Experimental setup at Fingringhoe Wick, Essex, UK (TM 05060 19033) between 10 October 2018 and 12 April 2019.

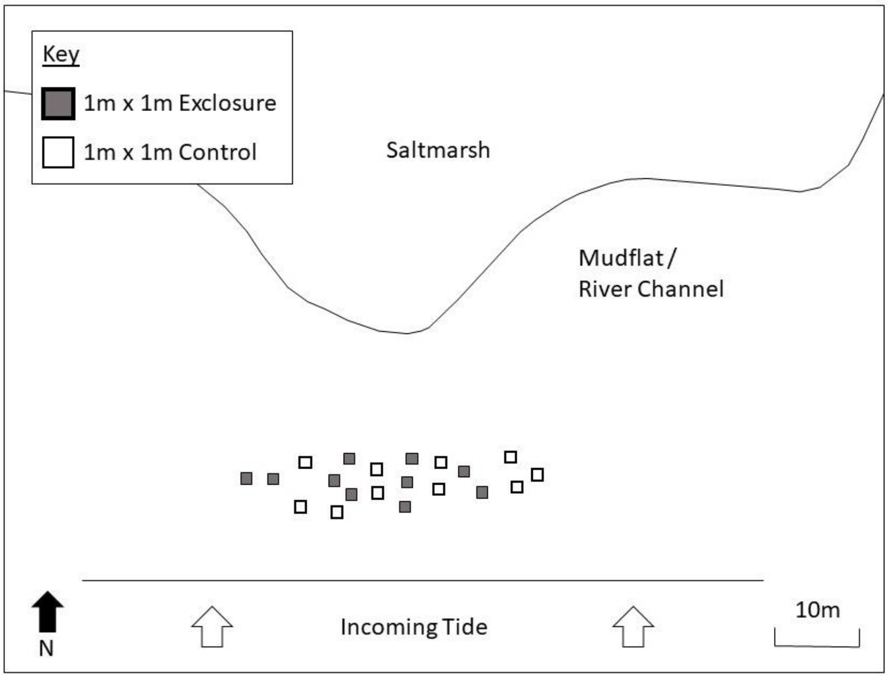


Figure 13 Experimental setup at Seafield Bay, Brantham, Suffolk, UK (TM 12334 33337) between 05 October 2018 and 02 April 2019.

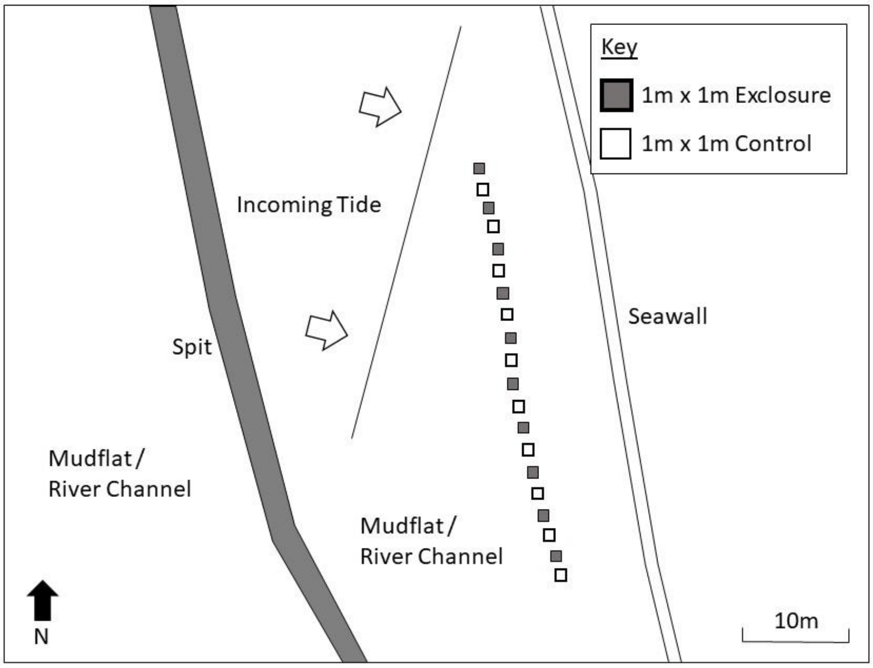


Figure 14 Experimental setup at Trimley Marshes, Suffolk, UK (TM 25484 35475) between 20 September 2018 and 25 April 2019.

4.3.6 Response Variables

Table 12 shows dates and days at which sampling events took place. Sampling events are referred to as ‘monitoring’ and ‘EF sampling’, to differentiate between events where only F_o and shorebird track coverage were measured and those when the ‘full suite’ of sampling including ecosystem functioning (EF) measures were made. Chapter 5 incorporates measurement of sediment-water oxygen, nutrient and organic matter fluxes, taken at the time of EF sampling, which are closely related to the results described here, being different data from the same experiment.

MPB are key drivers of intertidal flat properties and processes (e.g. Murphy and Tolhurst 2009), and earlier experiments (see Chapter 3) have shown effects of shorebirds on MPB. Therefore, to determine when the EF sampling events would be most likely to detect any effects of shorebirds on response variables, F_o (MPB biomass) was monitored at least every month (where possible), as a convenient indication of likely treatment effects. Between day variation in mudflat characteristics have been shown to be of greater significance than within day variation (Tolhurst and Chapman 2005), therefore repeated measures of F_o were made to compensate for this effect. Our experience of the Fingringhoe site is that variability at the meter scale is low

(Redzuan, 2017). Additionally, the repeated Fo sampling gives further confidence that plots were not significantly different at the beginning of the experiment.

During each monitoring event, minimum fluoresence (F_o) measurements were taken in all plots, using a pulse amplitude modulated fluorometer (PAM, Walz, Effeltrich, Germany) to determine baseline MPB biomass (Honeywill et al. 2002), for a total of 100 F_o measurements (n = 5 in each of the 20 plots). Use of each plot by shorebirds (% footprint cover) was also estimated visually (Robar and Hamilton 2007), to investigate how surface MPB biomass responded to shorebird density over time. Due to the large number of measurements required in each plot during a tidal cycle and considering the impact of dewatering during the tidal cycle (Maggi et al. 2013); a 5 minute low light partial dark adaption treatment was used prior to each PAM measurement, which is a preferred method to conventional dark adaption for the measurement of minimum fluorescence as a proxy of MPB biomass (Jesus et al. 2006b). Logistical, time and weather constraints reduced potential sampling days, therefore sites with fewer available background data were prioritised where these constraints meant it was necessary to at sample fewer sites. Monitoring included measurements on day 0, immediately following setup, to determine inherent spatial variability (the null hypothesis being there were no significant differences between mean values for plots allocated to different treatments).

Final sampling after 6-7 months involved collecting 2mm depth contact cores (Honeywill et al. 2002) (for chlorophyll *a*, grain size, water content and colloidal carbohydrate measurements). Erosion threshold (τ_{cr}) was measured with CSM and F_o was measured with PAM as described above. Measurement methods for response variables are detailed within Chapter 2.

In situ sediment critical erosion threshold (τ_{cr}) (sediment erodibility) was measured after six to seven months of shorebird density manipulation (Table 12), to test the effect that this period of treatment had on the ecosystem function of erosion protection. This was done using a Cohesive Strength Meter (CSM) (three measurements within ten plots of each treatment, total 60 measurements at Fingringhoe and Brantham, two measurements within ten plots of each treatment, total 40 measurements at Trimley, totalling 180 measurements overall) (Tolhurst et al., 1999; Vardy et al., 2007). Reduced measurements were made at Trimley due to the shorter emersion time at this mudflat. Contact coring for analysis of chlorophyll *a* content (two measurements within ten plots of each treatment; total 40 measurements at each site, totalling 120 measurements) (Honeywill et al., 2002) was done during the same sampling event.

Contact cores (surface ~2 mm) were freeze dried in the dark and chlorophyll *a* extracted using cold methanol over 24 h, and measured spectrophotometrically, correcting for phaeopigments (Stal et al., 1984).

Previous work suggests an absence of trophic cascade within the Fingringhoe site (Booty *et al*, 2020). To allow application of the research across multiple sites (considering limited human resources), infauna were not sieved and analysed as in previous work (see Chapter 3), although identification of species present was carried out during labwork, as live species present remained active within mesocosms during sampling and could be identified. The polychaete *A marina* was noted present in abundance during the study period at Brantham, firstly on day 70 and thereafter. This species is an ecosystem engineer which bioturbates the sediment and increases sediment oxygenation (Kristensen 2001, Reise 2012, Clarke et al. 2017). The abundance of this species at Brantham, which was not recorded at either of the other study sites, could therefore have caused significant differences in response variable measurements. To account for this during fieldwork the estimated percent coverage of each plot by *A marina* casts was recorded during fieldwork. This method was favoured over counting each cast, due to time constraints.

At Brantham one roofed treatment plot (Plot 5) had become exposed (roof removed). Therefore, during this sampling event (Day 186, ‘full sampling’) plot 5 and one plot of the other treatment were not sampled.

To enable shorebird surveys with minimal disturbance to survey subjects, and to enable accurate survey within the daylight windows available, it was necessary to undertake shorebird surveys on different days to the sampling of mudflat characteristics. To evaluate shorebird pressure on each mudflat, count data for each individual species were first converted into ‘species-days,’ by calculating the sum of the number of each shorebird species present on every count, multiplied by the number of days between that and the subsequent count (Gill et al. 2001, Lewis et al. 2014). An example of this is shown in Table 7 (Chapter 3). This approach was used to estimate the ‘species-days’ for each individual species present. This allowed comparison of shorebird pressure on the wider mudflat, including summing by species group. Species numbers at the time of mudflat characteristic sampling were modelled (Equation 4.1) using species counts during the experimental period.

$X = X_1 - (((X_1 - X_2) / (D_1 + D_2)) \times D_1)$

Where: X = estimated species count on mudflat sampling day

X_1 = most recent known individual species count value

X_2 = next known individual species count value

D_1 = days between most recent known species count and mudflat sampling

D_2 = days between next known species count and mudflat sampling

Equation 4.1: Used to model species numbers present at the time of mudflat sampling, using most recent known species count and next known species count.

An example of equation 4.1 implementation is shown in Table 11 below.

Table 11: Example working calculation results using Equation 4.1

Actual Dunlin Count (X_1)	Projected Dunlin Count (X)	Actual Dunlin Count (X_2)
06/12/2018	14/12/2018	20/12/2018
1500	929	500

$X = 1500 – (((1500-500) / (8+6)) \times 8)$

$X = 1500 – ((1000 / 14) \times 8)$

$X = 1500 – 571$

$X = 929$

Species present at each site were then grouped by order or family, including Charadriiformes (waders), waterfowl (Anatidae), gulls (Laridae), herons and allies (Ardeidae) and crows (Corvidae). Charadriformes, which comprised most species, were grouped further by existing ornithological classes by mass, adapted from Kuwae *et al.* (2021). Groups were as follows (approximate median weights taken from sources cited): smaller waders ($\leq$100g) (Warnock and Gill 2020), small-medium waders (101-300g) (Baker et al. 2020, Nettleship 2020, Pierce et al. 2020, Poole et al. 2020, Van Gils et al. 2020b) large-medium waders (301-799g) (Hockey et al. 2020, McCaffery and Gill 2020, Van Gils et al. 2020a), large waders (>800g) (Van Gils et al. 2020c).

This combination of approaches enabled assessment of bird species assemblage effects within the context of existing literature, which suggests small-bodied shorebirds (small sandpipers) have a broader range of potential top-down effects on MPB. Using this grouping places dunlin as the only smaller wader among all sites. Dunlin is the only species present in this experiment known to directly consume MPB (Mathot et al. 2010, Hobson et al. 2022).

To achieve a summary characterisation of the bird assemblage at each site for each F_o sample time, the summed species-days estimates for each bird group per site and F_o sample time were entered in a principal components analysis (PCA) performed using Canoco version 4.5 (Lepš and Šmilauer 2003). The first principal component (PC1) was the PC which accounted not only for the greatest overall variation (66%) in bird group composition across sites and sample times, but specifically also the major differences in small wader counts (dunlin). Given that smaller waders are the group implicated in direct biofilm-feeding, but that other bird groups could be impacting MPB biomass in other ways, PC1 scores were therefore selected for each sample time at each site as the summary metric for bird assemblage variation.

Table 12 Experimental setup and sampling dates during the 2018-2019 multi-site investigation on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030), on the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (grid reference TM 12427 33311) and on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve,Suffolk, UK (grid reference TM 25504 35406).

Activity	**Date / Days Since Setup**					
	Trimley		**Brantham**		**Fingringhoe**	
Setup and F_o measurement (Day 0)	20 Sept 2018	0	05 Oct 2018	0	04 Oct 2018	0
F_o and track cover measurements	17 Oct 2018	27	19 Oct 2018	14	03 Nov 2018	30
	11 Nov 2018	52 (rained off)	18 Nov 2018	44	16 Nov 2018	43
	13 Dec 2018	84	14 Dec 2018	70	30 Nov 2018	57
	10 Jan 2019	112	11 Jan 2019	98	12 Jan 2019	100
	24 Jan 2019	126	25 Jan 2019	112	NO SAMPLING	
	19 Feb 2019	152	22 Feb 2019	140		
	21 Mar 2019	182	27 March 2019 (tracks only)	173		
F_o, track cover and erosion threshold measurements and flux coring	25 Apr 2019	217	02 Apr 2019	179	26 Feb 2019	145
	26 Apr 2019	218	03 Apr 2019	180	27 Feb 2019	146
Flux core measurements	27 Apr 2019	219	04 Apr 2019	181	28 Feb 2019	147
F_o and track cover measurements	NO SAMPLING				12 Apr 2019	190

4.3.7 Statistical analysis

'Day 0' measurements are a baseline indicator of MPB mass in plots prior to experimental treatment, allowing detection of a point at which biofilm mass may return to a state prior to the arrival of wintering shorebirds, helping to discount experimental artefacts of exclosures (Booty *et al*, 2020). These were excluded from linear mixed effects (LME) models due to the presence of F_o values of '0' at Trimley on Day 0. These measurements were taken immediately after experimental setup and coincide with a time at which several species, particularly first winter individuals, are still on migration to wintering grounds . At this point track coverage was low (i.e. shorebirds had not arrived *en masse*) and no treatment effect could have taken place.

Where response variable data were not normally distributed, BoxCox (Box & Cox, 1964) transformation (ln) was applied following addition of a constant (2), to account for a single remaining F_o value of '0' within the dataset prior to running linear mixed effects models.

To test the hypotheses (1) that bird density (% footprint cover) would significantly increase MPB biomass (F_o) and (2) that bird assemblage would significantly affect MPB biomass (F_o), a linear mixed effects (LME) model was used to determine the relationship between the response variable biofilm mass proxy (F_o) and fixed effects shorebird patch use (% footprint cover), bird assemblage score and site, with a random intercept for plots and days to account for repeated measures. The model was run using glmmTMB (Brooks et al. 2017) and lmerTest (Kuznetsova et al. 2017) packages in R Version 4.2.2:

$\log F_o \sim$ Site * Assemblage * Density + (1|Site/Plot) + (1|Site/Days)

where: $\log F_o$ = biofilm biomass proxy: $\log_n (F_o+2)$

Site = experimental location (Brantham, Fingringhoe, Trimley)

Density = shorebird patch use: % footprint cover

Assemblage = modelled shorebird assemblage score at $\log F_o$ sampling time, based on individual 'species-days' summed according to size class

This model structure uses an explicit nesting design with site fitted firstly as a fixed effect and secondly as a random effect without variance assigned to it. The model includes the fixed effect of 'site' and the random effect of 'plot' within each 'site.' Without 'site/plot', for example, plot 1 from site A and plot 1 from Site B would be treated as the same, when in reality they are different.

Bird assemblages are highly variable between intertidal mudflats depending on factors such as prey availability (Bowgen et al. 2015). To test the level of collinearity between bird assemblage and site, a collinearity check was carried out using simplified main effects only model using the performance package (Lüdecke et al. 2021) in R Version 4.2.2.

The model was used to predict effects of shorebird density on MPB in two ways. The first used the whole range of modelled shorebird assemblages at all sites, to theoretically predict how assemblage would affect the relationship between shorebird density and MPB biomass. The model was then restricted to the actual shorebird assemblage range modelled at each site, enabling assessment of actual within site relationships between MPB biomass and density based on shorebird assemblages present at each site.

To test hypothesis 3, that sediment erodibility would be significantly greater where shorebird density was 'normal', compared to where shorebird density was 'low', data were firstly analysed using a mixed model, two-way nested ANOVA design (plot nested in treatment) with plot as a random factor and treatment (low and high shorebird density) as a fixed factor.

Number of plots varied between sites at the EF sampling time, due to loss of a plot at Brantham and the ability to collect more erosion threshold samples at Fingringhoe, where greater assistance was available. Therefore, ANOVA models were run separately for each site to maximise the power of the test for each site.

To facilitate interpretation of pathways which may have led to any changes in erosion threshold, the same ANOVA model was also used to test for significant differences in sediment characteristics between treatments, including Chlorophyll *a*, colloidal carbohydrates, water content and grain size.

To assess differences between sites in chlorophyll *a* and colloidal carbohydrate contents a two-way orthogonal ANOVA model was used with site as fixed factor and plot as random factor. ANOVA models were run using the GMAV (1997) statistical package (University of Sydney, Australia).

4.4 Results

4.4.1 Descriptive Comparison Between Sites

Bird species and bird numbers (estimated bird-days hectare^{-1}) recorded at each site are shown in Figure 15. Trimley had the highest species richness (17) with the lowest at Fingringhoe (10). Bird numbers were lowest at Trimley and highest at Brantham (Table 13).

Shorebird use of the sites varied during the experiment, with shorebird patch use (% footprint cover) and MPB biomass (F_o) showing coinciding peaks at all sites between these variables (Figure 16).

Table 13 Response variables mean values and standard errors (also showing separate treatment values where a significant within site difference between treatments was detected (denoted by superscript p value)) for sediment characteristics measured on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030), on the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (grid reference TM 12427 33311) and on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406) measured between 20 September 2018 and 25 April 2019. Assemblage scores are ranges of characterisations of the bird assemblage at each site during the experiment, derived from the first principal component (PC1) in principal component analysis.

Response Variable	**Site**		
	Brantham	**Fingringhoe**	**Trimley**
Mean F_o (normal bird density)	898 (±94)	350 (±33)	310 (±22.17)
Mean F_o (low bird density)	937.4 (±26)	259 (±5)	361 (±9)
Mean shorebird density (footprint coverage (%)) (normal)	34.2 (±2.68)	29.9 (±2.20)	26.83 (±2.03)
Mean shorebird density (footprint coverage (%)) (low)	6.25 (±0.4)	3.3 (±0.1)	12 (±0.5)
Total estimated shorebird-days hectare^{-1}	18,385	1,709	1,247
Dunlin -days (%)	37.4	37.4	0.7
Assemblage Score	0.0 to 1.6	-1.5 to 0.8	-1.2 to -0.4
Mean chlorophyll *a* content (µg g^{-1})	106.69 (±20.34)	79.38 (±9.74)	34.5 (±1.62)
Mean Colloidal Carbohydrates (µg g^{-1})	4767.8 (±1259.0)	1724.50 (±216.26)	1461.97 (±247.20)
Mean Erosion threshold (Tau$_{cr}$)	1.34 (±0.35)	2.28 (±0.41) (all plots)$^{*0.04}$ 2.94 (±0.69) (normal bird density) 1.63 (±0.32) (low bird density)	0.83 (±0.03)
Mean Water Content (%)	63.66 (±0.01)	63.88 (±0.004)	58.83 (±0.006)
Modal Grain Size (µm)	49.25 (±1.65)	62.70 (±2.12)	50.51 (±1.54)

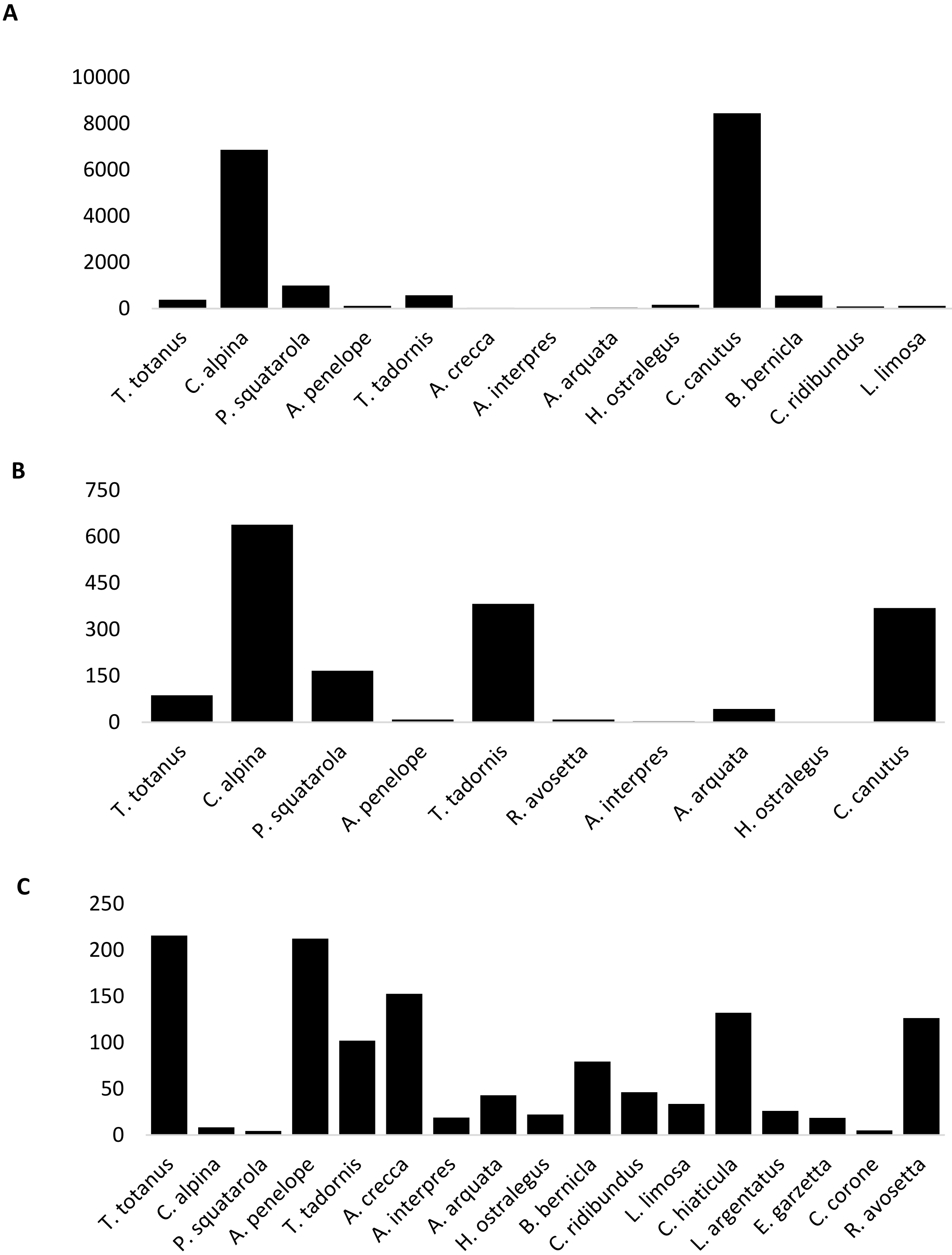


Figure 15 Total estimated bird-days (hectare^{-1}) for each species recorded at **A** the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (grid reference TM 12427 33311) between 13 October 2018 and 03 April 2019, **B** the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030). between 23 October 2018 and 02 March 2019 and **C** the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406) between 27 September 2018 and 19 April 2019.

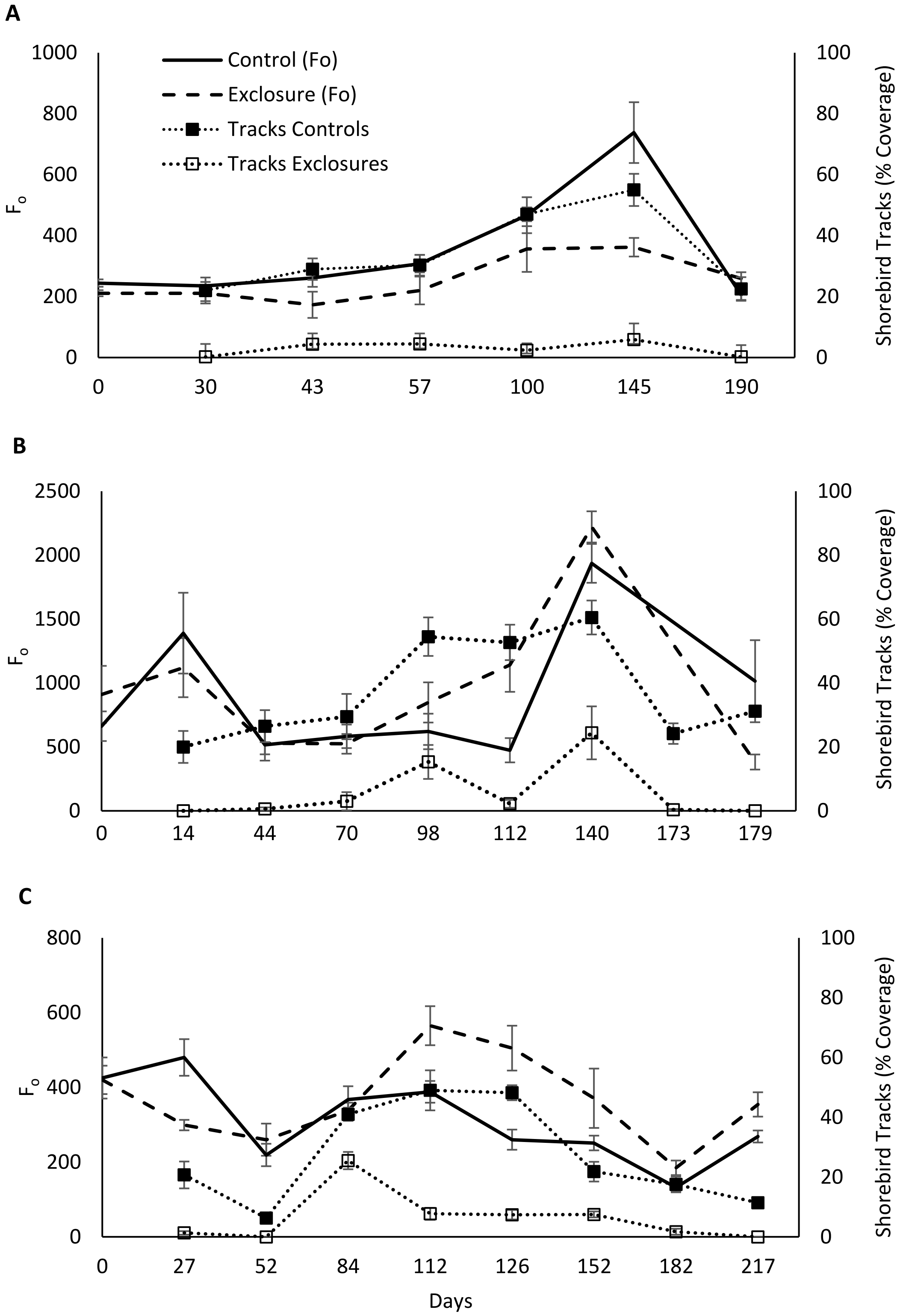


Figure 16 Microphytobenthos biomass (F_o) and shorebird footprint cover (%) measured in control and exclosure plots (n=10) **A** the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030) between 23 October 2018 and 02 March 2019, **B** the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (grid reference TM 12427 33311) between 13 October 2018 and 03 April 2019 and **C** the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406) between 27 September 2018 and 19 April 2019.

In evaluating shorebird pressure at each site, bird density on the wider mudflat (bird-days ha^{-1}), proportion of bird density accounted for by dunlin (%) and mean density (% footprints) within control plots varied similarly among sites. These variables were smallest at Trimley (1,247 bird-days ha^{-1} of which 0.7% were dunlin, 26.83 (±2.03) % footprint coverage) and largest at Brantham (18,385 bird-days ha^{-1} of which 34.7% were dunlin, 34.2 (±2.68) % footprint coverage) (Table 13). Low density treatments (roofs) varied in effectiveness with mean density (footprint cover) at the sites as follows: Fingringhoe 89%, Brantham 82% and Trimley 55% (Table 13).

Measured F_o (normal density), chl *a* (µg g^{-1}) and colloidal carbohydrates (µg g^{-1}) also had variation across the sites; smallest at Trimley (310 (±22.17), 34.5 (±1.62) µg g^{-1} and 1461.97 (±247.20) µg g^{-1} respectively) and largest at Brantham (898 (±94), 106.69 (±20.34) µg g^{-1} and 4767.8 (±1259.0) µg g^{-1} respectively (Table 13, Figure 17).

Modal grain size at Trimley and Brantham was very similar (50.51 µm (±1.54) and 49.25 µm (±1.65) respectively), with larger modal grain size at Fingringhoe 62.70 µm (±2.12), lying at the coarse end of the 'fine grained sediment' range (Table 13) (Pan et al. 2018). Sediment water content was similar across the sites, ranging from 58.8%3 (±0.006) at Trimley to 63.88% (±0.004) at Fingringhoe. ANOVA results found no significant difference between treatments at any of the sites for the measured sediment characteristic variables, chlorophyll *a* (µg g^{-1}), colloidal carbohydrates (µg g^{-1}), water content (%) and modal sediment grain size (µm) (Tables 20 to 22 in Appendix 2).

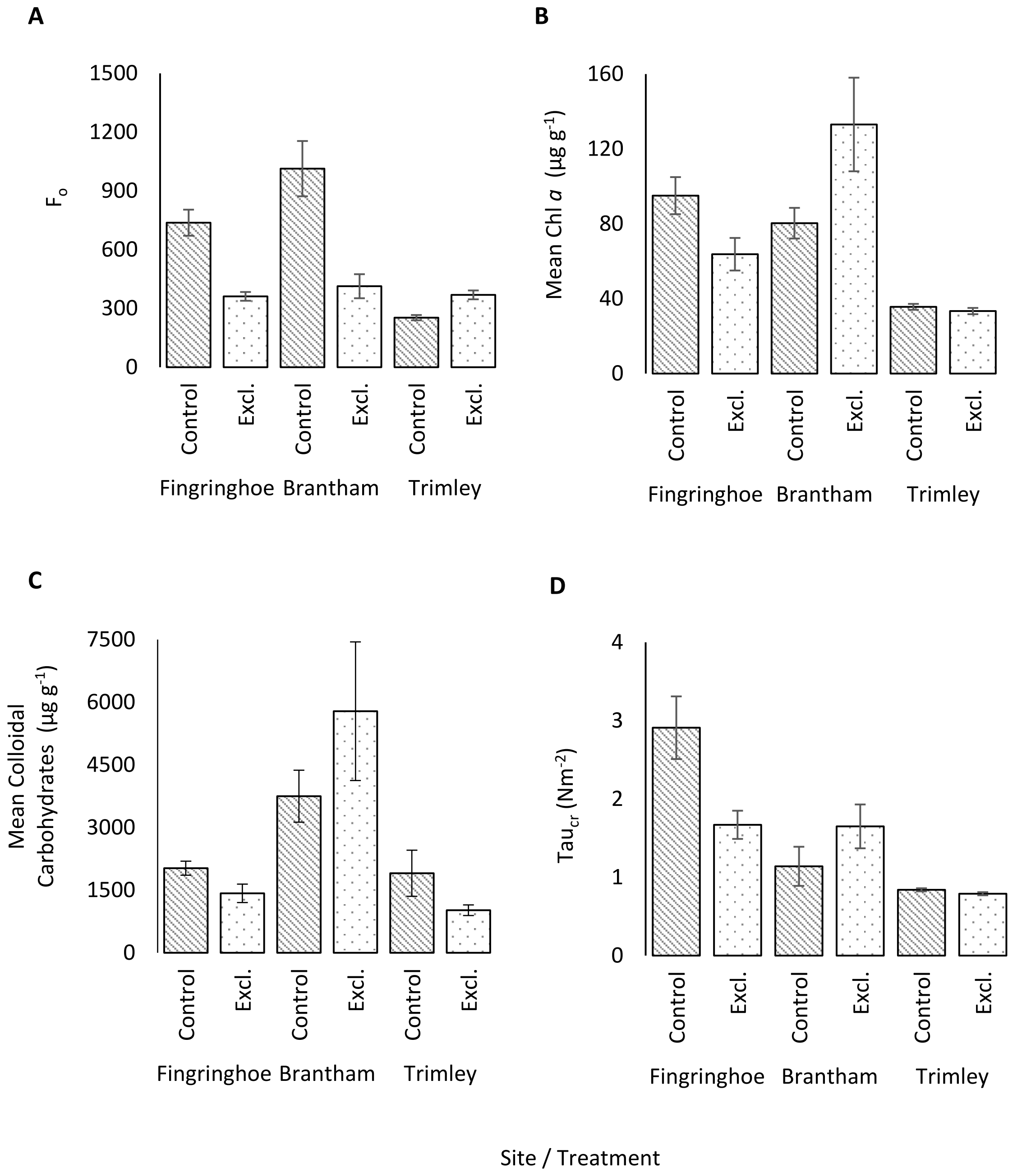


Figure 17 Mean ± SE (error bars) **A** chlorophyll fluorescence (F$_o$), **B** chlorophyll *a* concent (µg g-1), **C** colloidal carbohydrates (µg g^{-1}) and **D** erosion threshold Tau$_{cr}$ (Nm^{-2}) per shorebird treatment measured on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030), on the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (grid reference TM 12427 33311) and on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406) measured between 20 September 2018 and 25 April 2019.

4.4.2 Statistical Analysis

Bird assemblage scores determined by PCA analysis (axis 1 explaining 52% of the variance between sites) for each sampling event varied depending on the days since setup and ranged between -1.21 and -0.44 at Trimley (range = 0.77), -1.55 and 0.79 at Fingringhoe (range = 2.34) and 0.01 and 1.59 at Brantham (range = 1.58). The best fitting LME model has been used initially to predict the effect of shorebird density on MPB biomass across the full range of bird assemblage scores across the sites, although none of the bird assemblages at the sites represented the full depicted range during the experiment (Figure 18).

The best fitting LME model had significant three-way interactions between site, community (shorebird assemblage) and footprints (density) ($\chi2=20.8$, df=2, $p<0.0001$), indicating that the effect of community (shorebird assemblage) and footprints (density) on biofilm biomass varied across sites (Table 14, Figure 18). Due to these interactions the model predicts highly variable effects of bird assemblage and density on MPB biomass between sites, therefore generalisations regarding effects of bird density and assemblage on MPB should be taken with an appropriate degree of caution. Under most bird assemblage scenarios, the model predicts a positive effect of bird density on MPB biomass at Brantham (estimate=0.01 ±0.00 95% CI) and Fingringhoe (estimate=0.001 ±0.01 95% CI) and a negative effect on MPB at Trimley (estimate=-0.03 ±0.01 95% CI) (Figure 18). The effect of variation in bird assemblage on MPB biomass is predicted to be greatest at Trimley and smallest at Fingringhoe (Figure 18).

Figure 18 illustrates the model prediction at each site under a hypothetical scenario, where the full range of shorebird community scores found across the sites during the experiment are represented. This predicts that even if community assemblages more typical of small shorebirds (at the upper end of the community score range), as found at Brantham and Fingringhoe, a more negative relationship between bird density and MPB biomass would occur. This model prediction (Figure 18) emphasises the site specific nature of the bird-MPB relationship.

Table 14 Linear mixed effects (LME) model summary output (CI=95% confidence interval).

		log F_o	
Predictors	*Estimates*	*CI*	*p*
(Intercept)	6.58	6.00 – 7.15	**<0.001**
Site [Fingringhoe]	-1.25	-1.90 – -0.60	**<0.001**
Site [Trimley]	0.30	-0.94 – 1.54	0.634
Assemblage	-0.20	-0.67 – 0.27	0.399
Density	0.01	0.01 – 0.01	**<0.001**
Site [Fingringhoe] * Assemblage	0.05	-0.53 – 0.62	0.871
Site [Trimley] * Assemblage	1.40	0.18 – 2.62	**0.025**
Site [Fingringhoe] * Density	0.00	-0.00 – 0.01	0.719
Site [Trimley] * Density	-0.03	-0.04 – -0.02	**<0.001**
Assemblage * Density	-0.01	-0.01 – -0.00	**<0.001**
(Site [Fingringhoe] * Assemblage) * Density	0.01	0.01 – 0.02	**<0.001**
(Site [Trimley] * Assemblage) * Density	-0.01	-0.02 – 0.01	0.284
Random Effects			
σ^2	0.42		
$\tau_{00\ \text{Plot.Site}}$	0.04		
$\tau_{00\ \text{Site}}$	0.00		
$\tau_{00\ \text{Days.Site}}$	0.11		
$\tau_{00\ \text{Site.1}}$	0.00		
N_{Plot}	10		
N_{Site}	3		
N_{Days}	19		
Observations	1990		
Marginal R^2 / Conditional R^2	0.319 / NA		

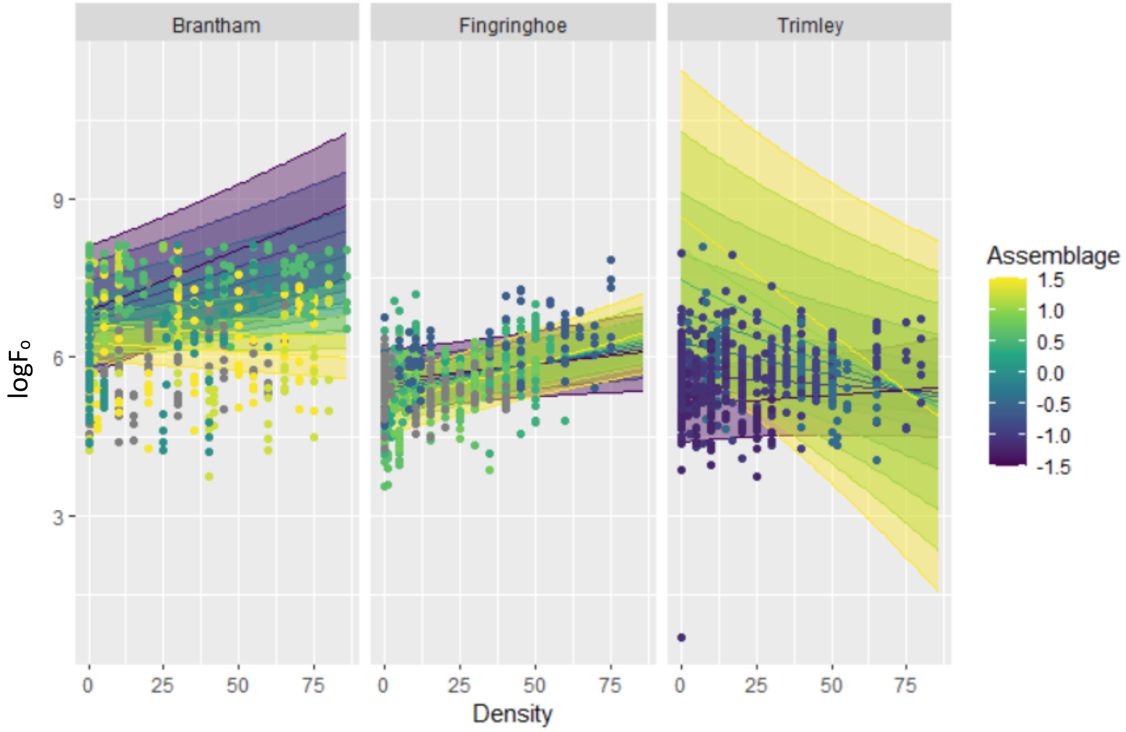


Figure 18 Plotted LME model residuals (x=shorebird density (%footprint cover), y=logF$_o$)) with points coloured by shorebird species assemblage score and ribbons showing model predictions at -1.5, -1.0, -0.5, 0.0, 0.5, 1.0 and 1.5 shorebird species assemblage scores at ‘Brantham’ (on the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (grid reference TM 12427 33311) between 13 October 2018 and 03 April 2019), ‘Fingringhoe’ (the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030) between 23 October 2018 and 02 March 2019) and ‘Trimley’ (mudflat at the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406) between 27 September 2018 and 19 April 2019).

The model was then restricted to illustrate the effects of bird density on MPB biomass using the actual assemblage scores recorded at each site (Figure 19), to assess the actual effect during the experiment at each site.

Shorebird species assemblages actually present at Trimley caused shorebird density to have a variable and predominantly negative effect on MPB biomass as shorebird density increased, although a lack of effect was present when species assemblage dropped to the ‘lowest’ score (-1.2) (Figure 19). At Trimley two dunlin were recorded with this species contributing only marginally to the modelled species assemblage scores.

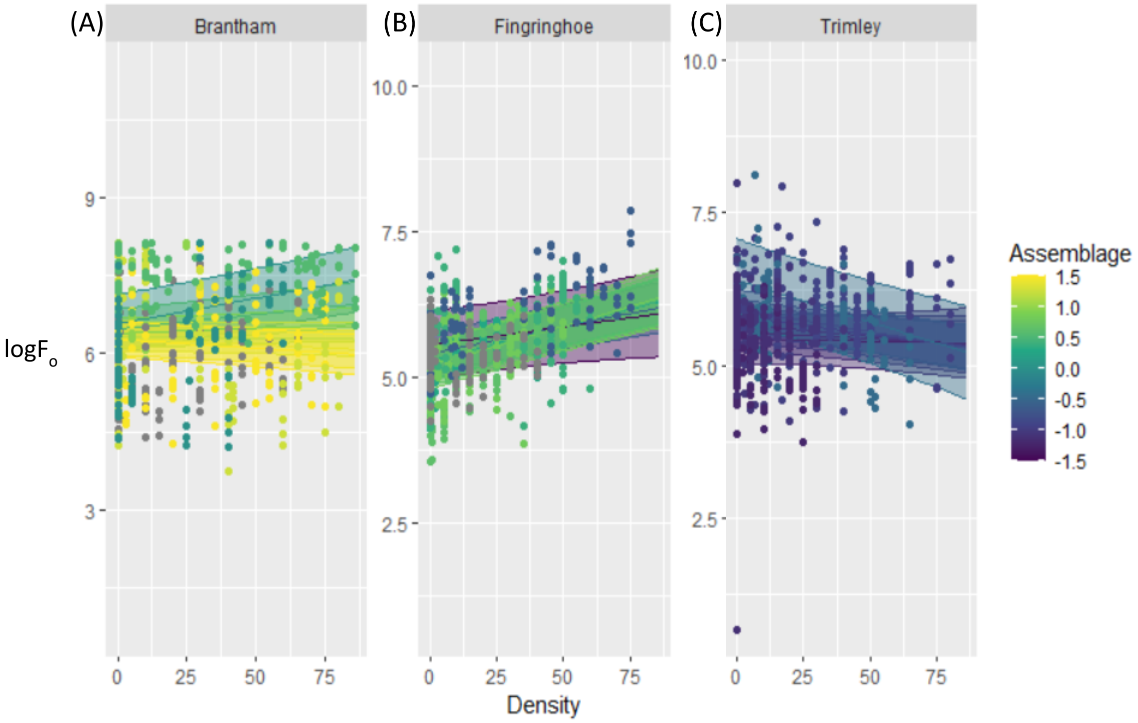


Figure 19 Plotted LME model residuals (x=shorebird density (%footprint cover), y=logF$_o$)) with points coloured by shorebird species assemblage score and ribbons showing model predictions based only on the species assemblages modelled at: (A) 'Brantham' on the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (TM 12427 33311) between 13 October 2018 and 03 April 2019 (shorebird species assemblage scores 0.0, 0.5, 1.0, 1.3, 1.5, 1.6); (B) 'Fingringhoe' on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030) between 23 October 2018 and 02 March 2019 (shorebird species assemblage scores -1.5, -0.6, 0.3, 0.4, 0.6 and 0.8) and (C) at 'Trimley' on the mudflat at the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (TM 25504 35406) between 27 September 2018 and 19 April 2019 shorebird species assemblage scores (-1.2, -1.1, -1.0, -0.9, -0.4).

At Fingringhoe the effect of shorebird species assemblage was smallest, despite the largest range in assemblage scores, with a positive effect of bird density on MPB biomass at all assemblage scores (Figure 19). Bird species assemblage scores at the larger end of the measured range at Fingringhoe (0.64 and 0.80) coincided with largest modelled dunlin counts (182 and 191 respectively).

At Brantham shorebird density had a positive effect on MPB biomass at modelled shorebird assemblage scores 0.0 and 0.5, and a marginal to negative effect on MPB biomass at assemblage scores 1.0, 1.3, 1.5, 1.6 (Figure 19). Assemblage scores at the highest end of the range (1.54, 1.59 and 1.47) coincided with the largest modelled dunlin counts (1571, 929 and 247 respectively), suggesting that at Brantham dunlin had a negative effect on MPB biomass.

There was variation in bird assemblage between sites (Figures 18 and 19), adding difficulty in separating the effects of site and shorebird species assemblage due to potential for collinearity between the predictors ‘site’ and ‘community’ (assemblage). However, the model was tested for collinearity which was indicated to be small (VIF<3).

These results indicate that where dunlin were present (at Brantham and Fingringhoe), a more positive relationship occurred between shorebird density and MPB biomass when dunlin were more prevalent within the community.

No significant difference was found in the critical shear stress needed to initiate erosion (erosion threshold) (N m^{-2}) between treatments at Brantham (F=3.32; df=1,32; p=0.2) or Trimley (F=0.08; df=1,36; p=0.8). However, a significant difference in erosion threshold between treatments was detected at Fingringhoe (F=8.45, df=1,54; P=0.04).

4.5 Discussion

A range of baseline variables among sites (Table 13) enabled investigation into how bird patch use (density), and bird species assemblage were related to biofilm biomass on an intertidal mudflat, and how this varies among sites. Variability in relationship between sites highlights an often overlooked aspect of ecological field experiments; the importance of replication across multiple sites (Fraser et al. 2020). These results differed between sites to the extent that at Brantham effects of shorebird density on MPB biomass varied from positive to marginal negative depending on shorebird species assemblage, a positive effect of density was indicated at Fingringhoe at all modelled shorebird assemblages and a negative effect was suggested at Trimley at most modelled assemblages.

Measured sediment characteristics are intrinsically linked; MPB biomass being a proxy for chl *a* content and colloidal carbohydrate, indicating likely levels of EPS secreted within the biofilm matrix (Underwood and Paterson 1993). Lack of significant differences in these physical sediment characteristics between treatments further supports previous work which used this exclosure type in Canada (Cheverie et al. 2014), demonstrating that the exclosures modified bird density but did not change other properties.

Low density treatments (roofs) varied in effectiveness, with mean density (footprint cover) reduction (%) at the sites as follows: Fingringhoe 89.%, Brantham 82% and Trimley 55%, with previous use of this exclosure design reporting 92% effectiveness (Cheverie et al. 2014). Cheverie *et al.* (2014) used this method to exclude small sandpipers (*Calidris pusilla*) at their peak migration stopover period and dunlin (small sandpiper present at the study sites) were relatively abundant at Brantham and Fingringhoe during the experimental period compared to Trimley. In comparison, dunlin accounted for approximately 0.7% of total bird-days ha^{-1} at Trimley (Table 13), indicating a possibility that, using this method, small sandpipers may be more effectively excluded from patches than other species at Trimley, such as larger sandpipers (redshank).

Smaller sandpiper (dunlin) use was greater at sites where MPB biomass, chlorophyll *a* and colloidal carbohydrate measurements were higher (Table 13). This may be partly due to sites with higher MPB biomass supporting higher numbers of macrofaunal shorebird prey (Daggers et al. 2020). However given that small sandpipers are known to directly consume MPB, their greater use of sites with higher F_o measurements follows a logical prediction that they would utilise areas with high MPB concentrations (Hobson et al. 2022).

The mudflat at Trimley is the smallest mudflat included in this experiment. It also had the lowest mean measurements of chlorophyll *a* (sites significantly different; F=9.01, p<0.001) and colloidal carbohydrate (sites significantly different; F=5.92, p<0.01) contents (Table 13). This may have contributed to differences in bird species assemblage between sites, with smaller sandpipers (dunlin) preferentially feeding at sites with greater MPB biomass. For example, requirements to maximise the value of mudflats for (particularly small-bodied biofilm feeding) shorebirds include unobstructed sightlines and a wide (usually gently sloping) tidal flat with greater available habitat (Kuwae et al. 2021), which are features not present at Trimley but characteristic of Brantham and Fingringhoe.

4.5.1 Hypothesis 1 – Greater shorebird density (patch use) would significantly reduce MPB biomass

The LME model indicates that the relationship between shorebird density and MPB biomass was highly dependent on site and, at two of the sites, shorebird species assemblage. Significant positive effects of bird density on MPB biomass were detected at Brantham, and Fingringhoe and negative effects were detected at Trimley. Due to this site variation in MPB response to bird density, hypothesis 1 is supported at Trimley, but the opposite effect was found at Fingringhoe and Brantham.

The site differences may be partly explained by intrinsic ecological and abiotic differences which exist between sites (Murphy et al. 2008, Macedo et al. 2014, Gerwing et al. 2016a, Redzuan 2017, Redzuan and Underwood 2021). Effects of bird density on MPB biomass are likely to be driven by interacting factors, including those measured (bird density and assemblage), but also unmeasured site-specific environmental conditions (see ‘potential drivers of effects’ below). This demonstrates a complex interaction between birds and MPB, with MPB being affected negatively or positively depending on site, bird density and bird assemblage.

4.5.2 Hypothesis 2 - Species assemblage would significantly affect biofilm biomass

The LME model identified a site dependent effect of species assemblage on the relationship between shorebird density and MPB biomass, supporting hypothesis 2. Shorebird assemblage was clearly different between sites (Table 13; Figures 15 and 16). The strongest effect of species assemblage on the relationship between bird density and MPB biomass was found at Trimley (Figure 18).

Although these effects were site dependent, collinearity between site and shorebird community was small. These results therefore indicate that the interactions between shorebirds and MPB are dependent on community. Wintering shorebird communities are predicted to alter as climate change continues (Godet et al. 2011, Gahbauer et al. 2022), suggesting that climate effects on shorebird community will also alter the dynamic between shorebirds and MPB on intertidal mudflats. For example, dunlin was present at Brantham and Fingringhoe, contributing 37.4% of shorebird-days at these sites and 0.7% at Trimley, indicating that the positive relationship between shorebirds and biofilm at Brantham and Fingringhoe may have been driven largely by this species. Research suggests that of the species present at the sites, dunlin are among those most susceptible to changes in winter temperature, with their abundance shifting in a north-easterly direction (Maclean et al. 2008). Given that the study sites are within the western edge of the species range (particularly in the case of *C. alpina alpina*), continued unmitigated climate change may lead to a reduction in wintering numbers and therefore a decrease in the strength of positive relationship between shorebird density and MPB biomass.

Previous work at Fingringhoe found significantly reduced biofilm biomass where shorebirds were present, upon which hypothesis 1 was based. However, that previous experiment compared shorebird presence or absence (using full exclosures) while the present design explored relationships between different densities of shorebirds. Overwintering shorebirds, particularly knot and dunlin, are likely to move between mudflats (Symonds et al. 1984, Warnock et al. 1995, Sanzenbacher and Haig 2002) and distribution of flocks and individuals within a mudflat vary depending upon food availability, distribution and substratum (Gill et al. 2001, Ribeiro et al. 2004, Norazlimi and Ramli 2014, Bowgen et al. 2015). It is plausible that differences in interannual species assemblage at Fingringhoe could have contributed to the differences in top-down shorebird effects between 2017 and 2018-2019. This is supported by the bird survey data: total estimated bird-days at Fingringhoe in 2017 were 78,811 during a three-month monitoring period (see Chapter 3) (26,270 per month) and in 2018-2019 were 22,415 during a four-month period (5,603 per month).

4.5.3 Hypothesis 3 - Sediment erodibility would be significantly greater where shorebird density was 'normal' compared to where shorebird density was 'low' (erosion threshold would be lower where Fo biomass was smaller)

Hypothesis 3 was supported only at Fingringhoe, where a significant decrease in sediment erodibility occurred where bird density was greater. This negative effect of bird density on erodibility coincides with greater F_o, colloidal carbohydrate and chlorophyll *a* content where bird density was greater (Figure 17), suggesting increased levels of these variables drove the bird density-erodibility effect at Fingringhoe (Underwood and Paterson 1993, Tolhurst et al. 2006a, Tolhurst et al. 2009, Pan et al. 2018). Increase in MPB biomass can be stimulated by biological events such as nutrient input and bioturbation, which may have driven the negative effect of birds on erodibility, as discussed in Section 4.5.4 below.

Ecosystem functioning (EF) sampling at Fingringhoe coincided with the peak in bird patch use and MPB biomass. EF sampling at Brantham and Trimley did not coincide with the peak these variables, which may have reduced the detectability of significant differences between treatments in erosion threshold (erodibility) at these sites, i.e. the sediment was more resistant to erosion where shorebird density was greater.

4.5.4 Potential Drivers of Effects

The response of MPB biomass at Brantham and Trimley was more influenced by shorebird assemblage than at Fingringhoe, with the model predicting varying strengths of effects at Brantham at Trimley as site assemblage changed (Table 14; Figure 18). Site variation is also likely to be influenced by local abiotic variables such as wind and wave action and sediment grain size. For example, research shows that environmental factors including meteorology, water quality, mud/sand content and wind/wave climate (van der Wal et al. 2010) can affect microphytobenthic (MPB) biomass: higher wind velocities can reduce MPB (van der Wal et al. 2010, Redzuan 2017) and coastal sites are more susceptible to this influence (Jacob et al. 2018). Biofilm mass and resuspension potential is also correlated with wind and wave action (Redzuan 2017). Mean MPB biomass measured at the three study sites reflect this, showing decreased mean F_o with proximity to the open sea. These factors may have contributed to the differences in measured MPB between sites.

The observed variable effect of shorebird density on MPB biomass at Brantham can be interpreted as follows: increasing MPB biomass at the sediment surface attracted shorebirds (relatively high proportions of which were dunlin)), which reduced MPB biomass either through direct feeding (Beninger and Elner 2020) or surface bioturbation (foraging and walking) (Booty et al. 2020) (see Chapter 3).

Where effects of bird density on MPB biomass were marginally negative, larger numbers of dunlin were present, which indicates the possibility of at least some direct MPB biofilm feeding. Dunlin and knot are flocking species which drove the overall bird numbers at Brantham, resulting in greater bird densities coinciding with ‘higher’ species assemblage scores and more marginal negative effects of shorebird density on MPB. This suggests that levels of bioturbation above a threshold may cause reduction in MPB biomass, similarly to known effects of some macrofauna (see Chapter 1) (Passarelli et al. 2014) and according to the intermediate disturbance hypothesis (Huxham et al. 2000) (see Chapter 6). It also indicates that where dunlin are more prevalent, MPB biomass consumption may be a driver of decreased MPB biomass.

Where effects of bird density on MPB biomass were positive, species assemblage scores and overall shorebird numbers were smaller. Where the bird bioturbation is below a threshold, it is feasible that the presence shorebirds led to proliferation of biofilms, possibly by excretion of droppings (Schrama et al. 2013, Jauffrais et al. 2015) and/or through bioturbation such as walking, creating footprints. Published research supports the former, as it has been found that shorebirds use MPBs as visual cues when foraging (Jimenez et al. 2015). It is also known that bioturbation can increase nutrient recycling and burrowing worms can alter oxygenation levels, both of which are known to increase MPB biomass (Passarelli et al. 2014, D'Hondt et al. 2018) and these pathways could also have some role in MPB stimulation via shorebird ambulation and feeding. Alternatively, other unmeasured external factors may have caused the effects found.

The above scenario (above) is particularly plausible given visual evidence of MPB accumulation within shorebird footprints (see Image 6). The image shows successional colonisation of shorebird footprints in the mudflat, which still show the foot outline earlier on in the colonisation and over time become round patches of MPB, still showing the location of the footprints but showing no defined shape. This was observed at all three sites during sampling. Other bioturbators (such as macrofauna) are predominantly considered to reduce MPB, through consumption or resuspension (Bruckner et al. 2021), but meiofauna such as nematodes can increase MPB accumulation through regeneration of nutrients (D'Hondt et al. 2018), which can significantly affect MPB proliferation and community structure (Hillebrand and Sommer 1997). It is therefore plausible that shorebird footprints expose buried nutrients to the sediment surface stimulating MPB biofilm growth.

Image 6 Photograph taken 2nd February 2017 on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030), showing shorebird footprints at various stages of MPB recruitment. Uncolonised footprints are circled yellow, more recently colonised footprints are circled orange and footprints which have been present longer can be seen as round 'blobs' of brown MPB (such as those circled red).

4.6 Conclusions

Shorebird density can significantly positively affect MPB biomass (F_o), although effects of bird density and assemblage on MPB are highly site dependent. Shorebird density and assemblage are shown to affect MPB biomass, although high level interactions between these variables and site demonstrate a complex environment where bird density and assemblage and site interact to contribute to end effects on MPB biomass.

Critical erosion threshold is found to be significantly enhanced where shorebird density is greater, at one of three sites (Fingringhoe). Erosion threshold sampling coincided with the peak in bird patch use and MPB biomass at Fingringhoe, but not at Brantham and Trimley. This may have reduced detectability of shorebird density effects on sediment erodibility and Brantham and Trimley, however this effect was found at Fingringhoe in 2017 outside the peak in bird patch use and MPB biomass. Therefore, it is most likely that the effect of shorebird density on erodibility is more influenced by site than variation in MPB within a site.

These findings indicate that the effect of shorebirds on MPB biomass is complex, effects may only be detectable where birds and MPB are present at sufficient density and specific species assemblages. Species assemblage may also affect the nature of relationship between shorebirds and MPB biomass, including whether it is positive or negative. This highlights a previously unknown effect of climate change, which previous research predicts will alter shorebird species assemblage, and therefore is also likely to alter the relationship between shorebirds and MPB biomass.

5 Shorebird density and species assemblage affect sediment-water nutrient and organic matter fluxes on intertidal mudflats

5.1 Abstract

Shorebirds are increasingly recognized as having complex bottom-up and top-down interactions between bird density, bird species assemblage, macrofauna and microphytobenthos (MPB) which influence overall ecosystem functioning. Untangling the processes which link these interactions is important given the continued global decline of migratory shorebirds and the rapid loss of coastal habitats, which support them and provide a range of ecosystem functions and services (EF and ES). Chapter 3 presents published research which found that shorebirds can significantly affect nutrient and organic matter fluxes on an intertidal mudflat. Chapter 4 presents a multi-site seven-month repeated measures manipulative field investigation into the effects of shorebird density and shorebird species assemblage on MPB biomass. Chapter 4 found that shorebird density - MPB biomass relationships are highly dependent on shorebird assemblage and site. MPB mediate nutrient exchange between the sediment surface and water column, with important effects on biogeochemical cycling within the intertidal. This chapter expands on this shorebird-MPB biomass research, to determine how bird density and bird assemblage may have affected sediment-water oxygen, nutrient and organic matter fluxes between intertidal mudflats using an *ex-situ* mesocosm experiment on sediment cores. Nutrient fluxes examined were nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), phosphate (PO_4^{3-}) and silicate (SiO_3^{2-}). Organic matter fluxes examined were dissolved organic carbon (DOC) and total organic nitrogen (TON). Nutrient and organic matter flux rates (mol m^{-2} h^{-1}) were then determined during light and dark incubation and compared between low and high bird density treatments and tested for covariance with shorebird track coverage at the time of core collection. The key finding was that at all sites, nitrite flux was positively correlated with shorebird density, regardless of inherent differences between these geographic locations. At two of the three sites total organic nitrogen (TON) correlated negatively with bird density and net nitrate correlated with bird density. Depending on site, shorebird density was found to significantly affect nutrient and organic matter fluxes, including ammonium, phosphate, nitrite, nitrate, silicate, DOC and TON, with flux responses of reduced shorebird density varying with site. These findings support those of a previous exclusion experiment at Fingringhoe, emphasising the significant role which shorebirds play in regulating ecosystem functioning and

services on intertidal mudflats. They also highlight the difference in effects among sites which Chapter 4 demonstrated are site, bird density and bird assemblage dependent.

5.2 Introduction

Many shorebirds are highly dependent on mudflats for food and refuge, particularly during winter and migration periods (Colwell 2010). Global loss of intertidal mudflats is contributing to severe decline in international shorebird numbers, as staging and wintering areas are lost to land claim and coastal squeeze (Pontee 2013), making research into conservation of these habitats of great importance.

Ecosystem functioning (EF) and services (ES) provide an established rationale for conservation of natural resources (Balvanera et al. 2006). A significant aspect of mudflat EF is biogeochemical cycling, including transfer of oxygen, nutrient and organic matter between the sediment surface and water column (Huettel et al. 2014, Passarelli et al. 2018). Ecosystem services are based on ecosystem functioning of a system. In this context a major function of mudflats is nutrient regeneration, which occurs through decomposition of organic matter into soluble carbon, phosphorous and nitrogen. Within the upper redox layer, aerobic bacteria ultimately convert organic matter into ammonium and phosphate and nitrate, which are released into the water column (Pan et al. 2018).

Microphytobenthic (MPB) biofilms at the sediment surface (see Part I) are a matrix of photosynthetic eukaryotes and prokaryotes (including diatoms, bacteria and archaea) which strongly influence the diffusion and uptake and release of oxygen, nutrient and organic matter between sediment and overlying water (Sundback and Graneli 1988, Sundback et al. 1991, Feuillet-Gerard et al. 1997, Hillebrand and Sommer 1997, Underwood et al. 1998, Passy 2007, Thornton et al. 2007, Armitage et al. 2009, Nedwell et al. 2016, Janas et al. 2019). MPB are primary producers on intertidal flats, adapted to the emersion regime resulting in available light for photosynthesis and net primary production of organic carbon (MacIntyre et al. 1996, Hubas et al. 2018).

Sediment-dwelling organisms, particularly macrofauna, provide fundamental functions, regenerating nutrients by decomposition of organic matter (Sundbäck et al. 2003), both physically and biologically (Hale et al. 2019). Organic matter comprising a mudflat can originate from external sources, being deposited from the water column (Mann 2009), or can be autochthonously produced (Sasmito et al. 2020). Through bioturbation, deposit feeders such as polychaete worms and molluscs mix sediments and increase oxygen levels to depths of several centimeters (Rosenberg et al. 2008, Van Colen 2018).

Coastal sediments are considerable compartments of microbial nitrogen cycling (Thamdrup and Dalsgaard 2008). Particulate organic nitrogen (PON) deposited onto the mudflat surface becomes converted to ammonium, which under oxic conditions (at the surface) is oxidised to nitrite and then nitrate by nitrifying bacteria, mediated by archaea (Stief 2013, Underwood et al. 2022). Nitrite and nitrate then follow two pathways, diffusing into the water column or into deeper anoxic layers. Within anoxic layers they are reduced anaerobically by bacteria and archaea, nitrate and ammonium can also be assimilated at the surface by heterotrophic bacteria, archaea and microalgae (Stief 2013, Underwood et al. 2022). Birds facilitate transport of microorganisms between locations (Steiniger 1969, Fujita and Koike 2007), suggesting a possibility that microbes, bacteria and diatoms may be transported between estuaries and mudflats by migratory or locally moving shorebirds which utilise a range of feeding sites (Evans 1976, Symonds et al. 1984). Mudflats also account for significant compartments of carbon storage on a global scale, making them of great importance to our ability to mitigate climate change (Sanders et al. 2010, Phang et al. 2015).

'Patch use' is a frequently utilised ecological measure (herein a proxy for bird density), referring to the way in which organisms utilise resources spatially, providing quantifiable and comparable measures to determine effects of variables on organism behaviour (Brown 1988, Brown et al. 1994, Brown 1999, Morris and Davidson 2000, Laundré 2010). This principle has been applied to estimating the level of shorebird habitat use on intertidal mudflats (Cheverie et al. 2014) based on experimental testing and validation of the concept (Robar and Hamilton 2007).

Previous research suggests that shorebirds can indirectly significantly affect MPB biomass (Daborn et al. 1993), with some species such as dunlin directly consuming biofilm (Elner et al. 2005, Hobson et al. 2022) and influencing ecosystem functioning, including oxygen, nutrient and organic matter flux on mudflats (Chapter 3) (Booty et al. 2020). However, this research leads to questioning of whether these effects are (a) transferable between different geographical areas and (b) dependent upon shorebird density and assemblage.

Nutrient flux data presented here were collected during the same fieldwork as presented in Chapter 4, providing an opportunity to explore this potential pathway in-depth. Chapter 4 also provides important contextual sediment characteristic data which frame the three sites studied here.

To build upon Chapter 4, after 6-7 months of field manipulation, laboratory measurements of cores from sampling plots were made to compare oxygen, nutrient and organic matter fluxes between the sediment and water column where shorebirds used mudflat patches at ‘normal’ and ‘reduced’ densities. The following hypotheses were tested:

1. There will be a significant difference in oxygen, nutrient and organic matter fluxes between the sediment and water column between ‘normal’ and ‘reduced’ bird density.
2. Shorebird density (patch use) and species assemblage are correlated with measured oxygen, nutrient and organic matter fluxes between the sediment and water column.

The above hypotheses differ in the methods used to test them. Hypothesis 1 is tested through comparison of control plots with treatment (reduced bird density) plots. The logic behind this is that a shorebird density difference ‘threshold’ may need to be reached to detect a significant difference between the treatments. This is dependent on the effectiveness of the exclosures and consistency in use between treatments. Hypothesis 2 is tested by quantifying the shorebird density within each plot and using this to test for correlation between density and oxygen, nutrient and organic matter fluxes of the plots.

5.3 Materials and Methods

The estuaries within which the study sites were located were all within complexes of habitats featuring sand and mudflats, protected internationally for supporting internationally important numbers of migratory and wintering shorebirds. Preliminary reconnaissance of the sites suggested that the sites support different wintering shorebird communities, providing an opportunity to test hypotheses across a gradient of shorebird species and use levels.

The experiments presented in Chapter 4 provide informative site measurements of sediment characteristics and bird use, including plot level grain size, water content, chlorophyll *a* concentration, F_o and colloidal carbohydrate concentration. Use of the site by birds during an extended experimental period (6-7 months leading to sampling) was also measured at the sites. These data provide important contextual data facilitating interpretation of the results among and between sites.

Chapter 4 identified no effects of shorebird density on chlorophyll *a* (µg g^{-1}), colloidal carbohydrates (µg g^{-1}), H_2O content (%) or modal sediment grain size (µm). Lowest chl *a* content was at Trimley, with similar concentrations at Fingringhoe and Brantham at the time of flux sampling. Chapter 4 does, however, emphasise variation in shorebird density among the study sites, with the lowest mean patch use at Trimley (26.83% (±2.03)) and highest at Brantham

(34.2% (±2.68)) (Table 13). The variation in mean F_o, chlorophyll *a* content and colloidal carbohydrates within control plots across the sites corresponds with shorebird use; these variables were all smallest at Trimley (mean F_o 310 (±22.17), mean chl*a* 34.5 µg g^{-1} (±1.62) and mean carbohydrates 1461.9 µg g^{-1} (±247.20)) and largest at Brantham (mean F_o 898 (±94), mean chl*a* 106.69 µg g^{-1} (±20.34) and mean carbohydrates 4767.8 µg g^{-1} (±1259.0) (Table 13).

The study sites were Fingringhoe Wick, Brantham and Trimley which are described fully in Chapter 4. The long term *in-situ* experimental design leading to this *ex-situ* experiment is described in Chapter 4, with the following methods used after manipulation (Table 13). Methods of modelling bird species numbers and estimation bird assemblage score at each site at the sampling time are described in Section 4.3.6 in Chapter 4. Only bird assemblage scores for the time of flux core collection were used for this experiment. As described in Chapter 4, low density 'roofed' plots were left in place for seven months while MPB biomass was monitored. The destructive sampling used in this experiment was carried out at the end-point of the experiment presented in Chapter 4. Measured variables are presented here for the flux collection time only, rather than for the whole experimental period.

Flux cores (Perspex tubes of 0.1 m diameter and approximately 0.2 m in depth) were collected (six from each treatment, total 12 per site, totalling 36) for laboratory analysis of nutrients on the following dates: 28 February 2019 at Fingringhoe, 04 April 2019 at Brantham and 27 April 2019 at Trimley. Flux cores were carefully returned to the laboratory within an hour of leaving the site and immersed in seawater from the site, within oxygenated and temperature and light controlled indoor mesocosms (Thornton et al. 1999). Rubber bungs were used to ensure equal headspace volume across cores. Cores were left submerged and open to settle overnight prior to sampling on the following day. Throughout headspace water sampling, Perspex lids were tightly fitted to prevent leakage. Magnetic stirrers maintained water circulation over the sediment surface. Immediately following collection (beginning within 24 hours) these were sampled for sediment-water biogeochemical fluxes of nitrate, nitrite, ammonium, phosphate and dissolved organic carbon (DOC). Headspace seawater samples were taken at the beginning and end of 2 h dark and light incubation periods. Cores were left for at least one hour to adjust to light levels prior to each incubation. Sampling was completed according to general methods described by Thornton *et al.* (1999). Flux measurements were repeated in both light and dark conditions, using 500W halogen 'daylight' lamps to provide 'lit' conditions (500 µmol m–2 s–1 PAR) and covering mesocosms with opaque Perspex covers to provide 'dark' conditions. Water samples were analyzed for their nutrient concentrations using a Seal AA3 segmented flow Nutrient Analyzer (SEAL Analytical Inc.). Net fluxes of oxygen, nutrients and organic matter variables were calculated by subtracting light incubation flux values from those taken in the dark.

Potential O_2 flux at Fingringhoe was modelled using the measured oxygen flux at the same site in 2017 (see Chapter 3). Akaike information criterion (AIC) stepwise multiple regression analysis was used to determine which of the other nutrient fluxes (total nitrogen (TN), ammonium, nitrate, nitrite, phosphate, silicate, dissolved organic carbon and F_o) was the best-fit predictor of O_2 flux (Underwood et al. 2013) under each condition (dark and light incubation). The regression analysis was run using R Version 4.0.4. Under dark conditions the analysis found a highly significant correlation between TN flux and O_2 flux ($t=-5.362$; $P=6.35\times10^{-5}$), with an R^2 value of 0.45. Under lit conditions there was a significant correlation between O_2 flux and chlorophyll fluorescence (F_o) ($t=-2.481$; $P=0.0264$). The linear relationships (best fit line equations, $y=ax\pm b$) between measured O_2 flux and these variables measured in 2017 were used to model the potential oxygen flux at the site in 2019.

This experiment was designed to focus on sampling at different sites based on the assumption that treatments would not significantly affect macrofauna distribution, as found during the experiment at Fingringhoe in 2017 (Booty et al. 2020). This approach has greater risks at Brantham and Trimley, where this effect has not been tested experimentally. However, while logic dictates that the foraging rates and densities of shorebirds would reduce prey populations (Mathot et al. 2018), detection of this effect is notoriously difficult (Sutherland et al. 2000, Meer et al. 2001, Colwell 2010, Mathot et al. 2018). Considering the above, this experiment was designed to capture shorebird effects regardless of the potential pathway, i.e. whether or not a trophic cascade via macrofauna is responsible. It is anticipated that this will provide a framework for further work into possible pathways if effects are detectable within the ‘noisy’ background of an intertidal mudflat (Beninger and Boldina 2018). As such, although results and interpretation may have an emphasis on ‘directly shorebird driven’ or ‘directly shorebird mediated’ effects, this does not discount the possibility that these may also involve indirect, trophic top-down effects involving altered behaviour by, or removal of macrofauna.

For logistical reasons the field sampling at each site could not be undertaken simultaneously. The resulting spread in measurements across the winter-spring period may have confounded between-site comparisons in non-repeat measurements of oxygen, nutrient and organic matter fluxes. Nutrient fluxes are influenced by physical and chemical parameters with the potential to vary between and within estuaries. These parameters include distance from the sea, pH conditions (Song and Müller 1999), biodegradability of organic matter and hydrodynamics

(Nedwell et al. 1993, van Raaphorst and Kloosterhuis 1994). Therefore, care must be taken when evaluating cross-site results in a comparative context. For these reasons, multi-site and single-site analyses were carried out.

Ideally, experiments compared between years are identical in design, an approach promoted to reduce the effect of interannual variation (Vaughn and Young 2010). However, experimental design was altered in favour of furthering the scope of this research, through examination of shorebird density in light of significant results relating to shorebird presence absence (Chapter 3). With inclusion of the estuarine sampling concentrations of measured variables, the use of multiple site locations in the 2019 experiments (Chapters 4 and 5) increases the general relevance of the results and processes being assessed (Thrush et al. 2000).

5.3.1 Statistical analysis

Chapter 4 detected significant three-way interactions between site, shorebird density and shorebird assemblage., indicating large variability in treatment effects between sites. Published research suggests that MPB biomass is a key driver of nutrient fluxes (Hochard et al. 2010). Therefore, in testing hypothesis 1, data from each of the three sites are analysed separately.

To test hypothesis 1 by evaluating the effect of shorebird density on nutrient flux, nutrient data were analysed using a two-way orthogonal ANOVA design with dark/light incubation and low/high shorebird density as fixed factors, using the GMAV (1997) statistical package (University of Sydney, Australia). As well as statistically significant changes in flux, reversals in flux (for example an efflux from the sediment in the absence of shorebirds becoming an influx into the sediment in the presence of shorebirds) were used as an indication of changes suggesting ‘ecologically significant’ implications for ecosystem functioning (Booty et al. 2020).

To test hypothesis 2, that oxygen, nutrient and organic matter fluxes are correlated with shorebird density and assemblage, Principal Components Analysis (PCA) using Euclidean distances was used to determine the primary pattern of covariation in measured variables. Removal of variables poorly represented by PC1 (cos2 <0.1), or which were redundant (due to covariance with light/dark incubation or net flux of the same variable) was carried out to simplify visualisation of the results (Appendix 4). Variables which were not removed are described as ‘retained’. Results were visualised using variable plots depicting cos2 values, both before and after stepwise removal, and site differences (showing sampling points grouped by site). PCA was run using dplyr (Wickham et al. 2023) and factoextra (Kassambara and Mundt 2020) packages in R Version 4.2.2.

Where PCA sampling points depicted separation between sites within the multidimensional space (suggesting differences in results between sites), PCA analyses were carried out within sites to depict covariance between measured variables.

Open access environmental monitoring data for our measured sediment-water oxygen, nutrient and organic matter fluxes were downloaded from the Environment Agency (EA) website (Environment Agency 2022) and mean estuarine sample concentrations were calculated for sampling points as near to our experiment locations as available. Sampling points used were located at (easting, northing): 608409,214662 (approximately 5.4km downstream of our Fingringhoe site), 615250,232640 (approximately 3.1km downstream of our Brantham site) and 625400,234800 (approximately 700m downstream of our Trimley site).

5.4 Results

At the time of flux core collection at each site, shorebird species assemblage was estimated by PC1 scores computed for the modelled bird assemblage (see Chapter 4). These scores summarise the variation in shorebird assemblage between sites at the time of flux core collection: Brantham = 0.01, Fingringhoe = -0.60, Trimley = -1.12 (Table 15). The assemblage at Brantham comprised small medium waders (redshank and grey plover), large medium waders (black-tailed godwit) and waterfowl (shelduck and brent geese). The assemblage at Fingringhoe comprised small waders (dunlin) and small medium waders (redshank, grey plover and knot) and at Trimley comprised waterfowl (shelduck, teal and brent geese) and large medium waders (oystercatcher) and gulls (herring gull).

Shorebird density in control plots varied among sites: smallest at Trimley (11 (±2)) and largest at Fingringhoe (55 (±6)). Estimated proportion of dunlin (the only species present reported to directly consume biofilm (Elner et al. 2005, Mathot et al. 2010, Drouet et al. 2015, Hobson et al. 2022)) at each site at the time of the flux core sampling were as follows; 0% at Trimley, 4.3% at Brantham and 20.1% at Fingringhoe. At the time of flux core collection, low density plot treatment (roof exclosures) reduced bird density (patch use) by 100% at Brantham and Trimley, and by 90% at Fingringhoe, at the time of the flux core sampling. During the long-term *in-situ* experiment the plots were subject to varying degrees of bird use as presented in Chapter 4.

Differences in mean MPB between low density (roof exclosure) and control plots were inconsistent between sites (lower under roofs at Fingringhoe and Brantham, greater under roofs at Trimley), demonstrating that the treatment effects on MPB biomass were not roof artefacts: mean MPB biomass (F_o) in both treatments showed variation among sites, smallest at Trimley

(269 (±16)) and largest at Brantham (1014 (±321) (Table 15). MPB biomass was 62% smaller in low density plots (roofs) than controls at Brantham and 51% smaller in low density plots (roofs) than controls at Fingringhoe. At Trimley mean MPB biomass was 32% greater within low density (roofed) plots.

Measured chl *a* (µg g^{-1}) and colloidal carbohydrates (µg g^{-1}) also show variation across the sites reflecting shorebird use; lowest at Trimley 34.5 (±1.62) and 1461.97 (±247.20) respectively) and highest at Brantham (898 (±94), 106.69 (±20.34) and 4767.8 (±1259.0) respectively (Table 15).

Statistically Significant differences in fluxes of ammonium (NH_4^+) (F=5.28, P=0.03) and silicate (SiO_3^{2-}) (F=7.22, P=0.014) between areas of normal and low shorebird density were detected at Trimley (Table 26 in Appendix 3). Under reduced shorebird density net flux directions of ammonium and silicate reversed from influx into the sediment, to efflux into the water column, under both dark and light incubation for ammonium and only under dark incubation for silicate (Table 16).

Table 15 Response variables mean values and standard errors (no significant within site difference between treatments detected) for sediment characteristics measured on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030) on 26 February 2019, on the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (grid reference TM 12427 33311) 02 April 2019 and on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406) 25 April 2019.

Response Variable	**Site**		
	Brantham	**Fingringhoe**	**Trimley**
Mean F_o (normal density)	1014 (±321)	738 (±100)	269 (±16)
Mean F_o (low density)	382 (±59)	362 (±31)	354 (±33)
Mean shorebird density (footprint coverage (%)) (normal)	31 (±1.8)	55 (±5.3)	11 (±2)
Mean shorebird density (footprint coverage (%)) (low)	0 (±0)	6 (±1.4)	0 (±0)
Proportion dunlin (%)	4.3	20.1	0
Community Score (determined by PCA analysis)	0.01	-0.60	-1.12
Mean chlorophyll *a* content (µg g^{-1})	106.69 (±20.34)	79.38 (±9.74)	34.5 (±1.62)
Mean Colloidal Carbohydrates (µg g^{-1})	4767.8 (±1259.0)	1724.50 (±216.26)	1461.97 (±247.20)
Mean Water Content (%)	63.66 (±0.01)	63.88 (±0.004)	58.83 (±0.006)
Modal Grain Size (µm)	49.25 (±1.65)	62.70 (±2.12)	50.51 (±1.54)

At Trimley, reduced shorebird density correlated with an ecologically significant reversal in net flux directions of nitrite, nitrate, DOC and TON, resulting in net efflux of nitrite, TON and DOC and net influx of nitrate (Table 17). During light incubation nitrite showed a net influx into the sediment under normal shorebird density and net efflux from the sediment under low shorebird density (Figure 20, Table 16).

During dark incubation at Trimley nitrate (NO_3^-) showed an efflux from the sediment under normal shorebird density and influx under low shorebird density. During light incubation dissolved organic carbon (DOC) showed an influx into the sediment under normal shorebird density and efflux under low shorebird density. During dark incubation TON showed influx into the sediment under normal shorebird density and efflux from the sediment under low shorebird density (Figure 20).

Table 16 Mean estuarine sample concentrations of nutrients located at (easting, northing): 608409,214662 (approximately 5.4km downstream of Fingringhoe site), 615250,232640 (approximately 3.1km downstream of Brantham site) and 625400,234800 (approximately 700m downstream of Trimley site) (Environment Agency, 2022) and mean flux of nutrients in 'normal' and 'low' bird density plots during dark and light incubation showing flux direction (↑=efflux from sediment; ↓=influx into sediment; bold* =significant difference between treatments; ↑↓ ='ecologically significant' difference in trend) measured on day 147 (28 February 2019) on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030), day 188 (27 April 2019) on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406) and day 181 (04 April 2019) on the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (grid reference TM 12427 33311).

			Treatment / Measured Flux (µmol L^{-1}) (*italics*= (mmol L^{-1})			
Variable	**Site**	**Mean Estuarine Conc$_n$** (µmol L^{-1}))	**Dark 'normal'**	**Light 'normal'**	**Dark 'low'**	**Light 'low'**
NH_4^+	F	8315	↑ 394(±206)	↑ 57(±30)	↑ 123(±174)	↑ 171(±76)
	B	1663	↑ 695±208)	↑ 630(±192)	↑ 1278 (±510)3	↑ 477.(±192)
	T	1441	**↓ 170(±83)***	**↓ 6.9(±43)***	**↑ 5.3(±52)***	**↑ 100(±41)***
PO_4^{3-}	F	390	↑ 38(±26)	↑ 221(±162)	↑ 51(±17)	↑ 55(±25)
	B	400	↓ 15(±8)	↓ 5(±5)	↓ 16(±8)	↑ 3(±4)
	T	305	↓ 16(±5)	↑ 6(±0.5)	↓ 12.1(±10.7)	↑ 5.0(±3.7)
NO_{2-}	F	4999	↑ 186(±187)	↑ 64(±147)	↑ 29(±234)	↑ 189(±104)
	B	1739	↑ 257(±120)	↓ 49(±60)	↓ 23 (±147)	↓ 50(±24)
	T	4347	↓ 2.6(±1.6)	↓ 0.2(±1)	↓ 8.6(±1.9)	↑ 0.8(±1.2)
NO_{3-}	F	9030	↓ 1526(±1002)	↓ 396(±310)	↓ 1006(±599)	↓ 308(±57)
	B	6128	↓ 6554(±3482)	↑ 18621(±7103)	↓ 7378(±4233)	↑ 15659(±3775)
	T	6612	↑ 25(±25)	↓ 18(±14)	↓ 15(±64)	↓ 13(±10)
SiO_3^{2-}	F	12,750	↑ 355(±63)	↓ 81(±71)	↑ 322(±223)	↓ 15(±31)
	B	6966	↑ 2491(±300)	↑ 11729(±1183)	↑ 2008(±1316)	↑ 14121(±3511)
	T	10,252	**↓ 372(±159)***	↑ 144(±51)	**↑ 174(±79)***	↑ 180(±83)

Table 17 Mean estuarine sample concentrations of oxygen and organic matter located at (easting, northing): 608409,214662 (approximately 5.4km downstream of Fingringhoe site), 615250,232640 (approximately 3.1km downstream of Brantham site) and 625400,234800 (approximately 700m downstream of Trimley site) (Environment Agency, 2022) and mean flux of oxygen and organic matter in 'normal' and 'low' bird density plots during dark and light incubation showing flux direction (↑=efflux from sediment; ↓=influx into sediment; **bold*** =significant difference between treatments; ↑↓ ='ecologically significant' difference in trend) measured on day 147 (28 February 2019) on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030), day 188 (27 April 2019) on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406) and day 181 (04 April 2019) on the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (grid reference TM 12427 33311).

			Treatment / Measured Flux (µmol L^{-1}) (*italics*= (mmol L^{-1})			
Variable	**Site**	**Mean Estuarine Conc$_n$** (µmol (*italics*=mmol) L^{-1}))	**Dark 'normal'**	**Light 'normal'**	**Dark 'low'**	**Light 'low'**
DOC	F	-	↓ *4(±11)*	↑ *199(±3)*	↓ *31(±10)*	↑ *167(±29)*
	B	-	↑ *226(±82)*	↓ *829(±66)*	↑ *311(±66)*	↓ *790(±140)*
	T	-	↓ *163(±82)*	↓ *83(±117)*	↓ *79(±213)*	↑ *91(±98)*
TON	F	*42*	↓ *0.8(±0.9)*	↓ *1.4(±0.9)*	↓ *2.0(±0.8)*	↓ *0.1(±1.2)*
	B	*28*	↑ *8(±1)*	↑ *0.4(±1.5)*	↑ *10(±1)*	↓ *1.5(±1)*
	T	*31*	↓ *0.4(±0.6)*	↓ *3.9(±0.7)*	↑ *0.5(±1.0)*	↓ *2.6(±1.0)*
O_2	F	535,625	↓ 2168(±736)	↑ 1030(±300)	↓ 618(±674)	↑ 1959(±69)
	B	561,875	↓ 542(±1402)	↓ 325(±1148)	↓ 260(±405)	↓ 1865(±762)
	T	530,000	↓ 4141(±2693)	↓ 2754(±651)	↓ 4770(±1247)	↓ 2797(±1748)

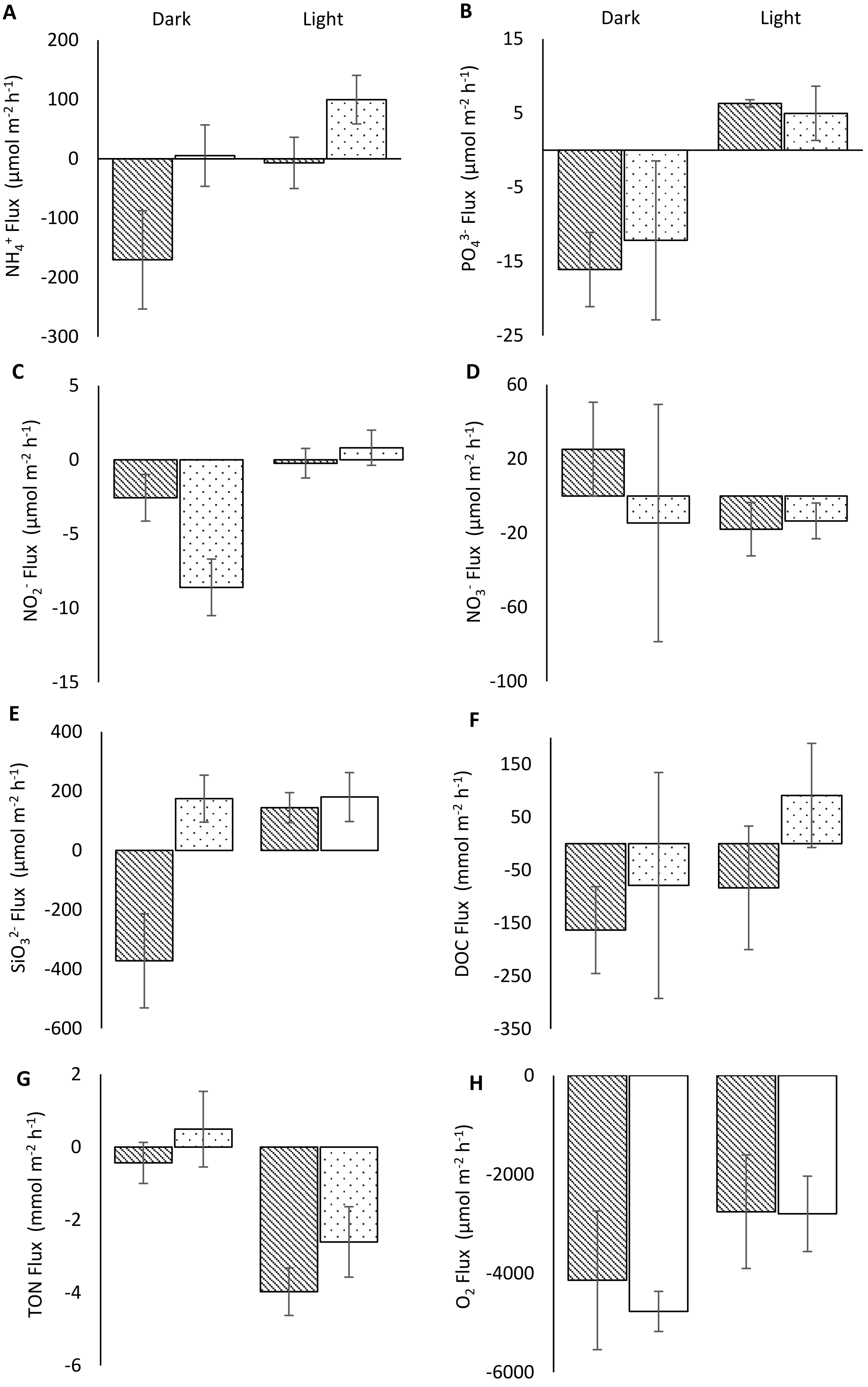


Figure 20 Sediment-water nutrient (**A** ammonium, **B** phosphate, **C** nitrite, **D** nitrate, E silicate), organic matter (F DOC, G TON) and H oxygen fluxes (Mean ± SE, n=6) during light (*right*) and dark (*left*) incubations in cores collected from shorebird 'normal density' (striped bars) and 'low density' (dotted bars) plots on day 188 (27 April 2019) on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406). X-axis marks zero flux, positive values show flux out of the sediment, negative values show flux into the sediment.

At Brantham, reduced shorebird density led to ecologically significant reversal in net flux directions of phosphate, nitrite and TON, causing efflux of phosphate and influx of nitrite and TON (Table 16). During light incubations, phosphate (PO_4^{3-}) showed net flux into the sediment under normal shorebird density and net flux out of the sediment under low shorebird density. During dark incubation nitrite (NO_2^-) showed net efflux from the sediment under normal shorebird density and net influx into the sediment under low shorebird density. During light incubation total organic nitrogen (TON) showed net efflux from the sediment under normal shorebird density and net influx into the sediment under low shorebird density (Figure 21, Table 17).

At Fingringhoe and Brantham no statistically significant differences between treatment (normal density/low density) shorebird density and any of the measured nutrient fluxes were found (Tables 23 and 24 in Appendix 3). No ecologically significant treatment effects were detected at Fingringhoe either (Figure 22, Table 24 in Appendix 3).

Tables 16 and 17 include Environment Agency data, converted to corresponding units, contextualising measured fluxes against concentrations within the overlying water column. Ambient concentrations of ammonium, nitrate, nitrite and dissolved nitrogen are notably greater at Brantham than elsewhere.

In analysing data across the three sites to test hypothesis 2, that shorebird density (patch use) and assemblage are correlated with measured oxygen, nutrient and organic matter fluxes between the sediment and water column, PCA was used. The First two components of a PCA of all measured variables explained 42% of the total variance (Figure 23). This depiction indicates covariance between bird density, F_o, chlorophyll *a*, colloidal carbohydrates, water content, oxygen and net ammonium.

PCA variable plot depicting retained measured variables (after removal) (Figure 23) explains 46.1% of variance. This depiction also indicates positive covariance between bird density and F_o, chlorophyll *a*, colloidal carbohydrates, water content, net oxygen and net ammonium and also net nitrite, and some covariance with net total organic nitrogen flux. Both PCA variable plots including data from all study sites (Figure 23) depict negative covariance between bird density and bird assemblage. No covariance is depicted between shorebird density or assemblage and net phosphate, DOC, ammonium or silicate. The PCA plot showing sampling points grouped by site (Figure 24) shows separation within the multidimensional space between the sites along component 2 axis, hence separate PCA analyses for each site were carried out.

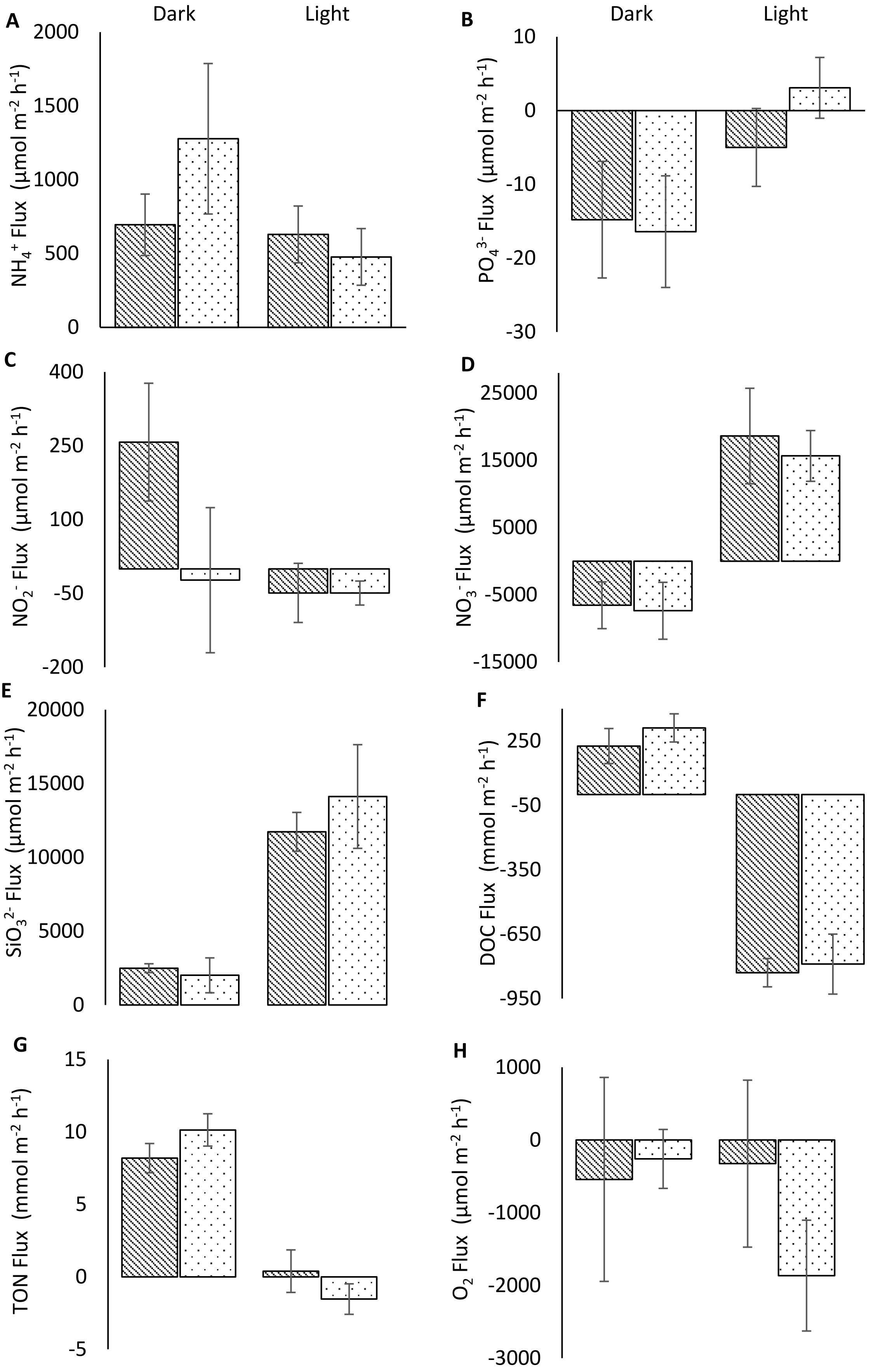


Figure 21 Sediment-water nutrient (**A** ammonium, **B** phosphate, **C** nitrite, **D** nitrate, E silicate), organic matter (F DOC, G TON) and H oxygen fluxes (Mean ± SE, n=6) during light and dark incubations in cores collected from shorebird 'normal density' (striped bars) and 'low density' (dotted bars) plots on day 181 (04 April 2019) on the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (grid reference TM 12427 33311). X-axis marks zero flux, positive values show flux out of the sediment, negative values show flux into the sediment.

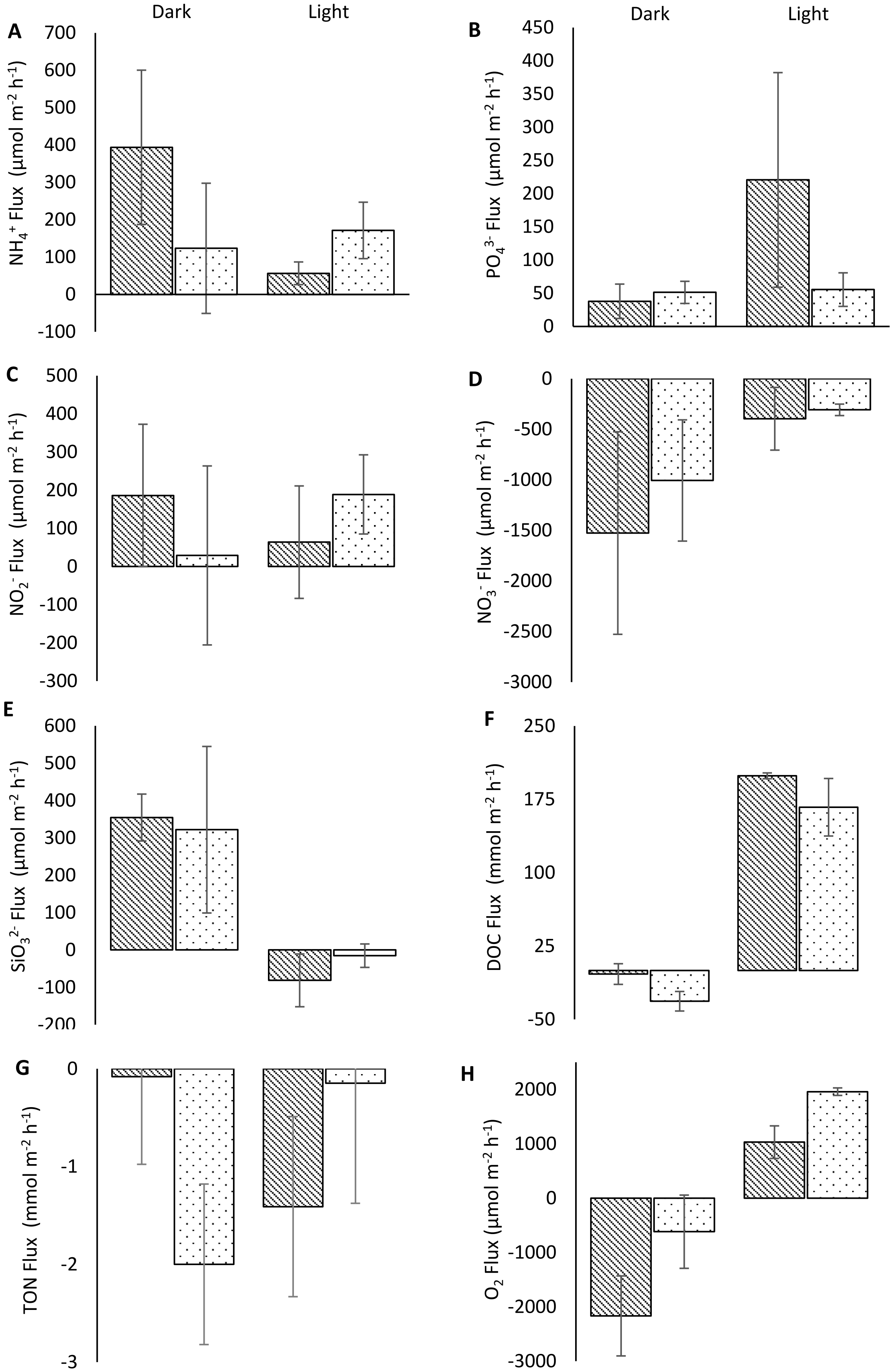


Figure 22 Sediment-water nutrient (**A** ammonium, **B** phosphate, **C** nitrite, **D** nitrate, E silicate), organic matter (F DOC, G TON) and H oxygen fluxes (Mean ± SE, n=6) during light and dark incubations in cores collected from shorebird 'normal density' (striped bars) and 'low density' (dotted bars) plots on day 147 (28 February 2019) on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030). X-axis marks zero flux, positive values show flux out of the sediment, negative values show flux into the sediment.

PCA variable plots depicting retained measured variables at Brantham (Figure 25) explain 55.9% of total variance. This depiction identifies positive covariance between bird density, F_o, water content, net phosphate flux and net nitrite flux and negative covariance between bird density and net TON and ammonium. No covariance is depicted between bird density and net DOC, oxygen, nitrate and silicate or chlorophyll *a* or colloidal carbohydrate concentrations.

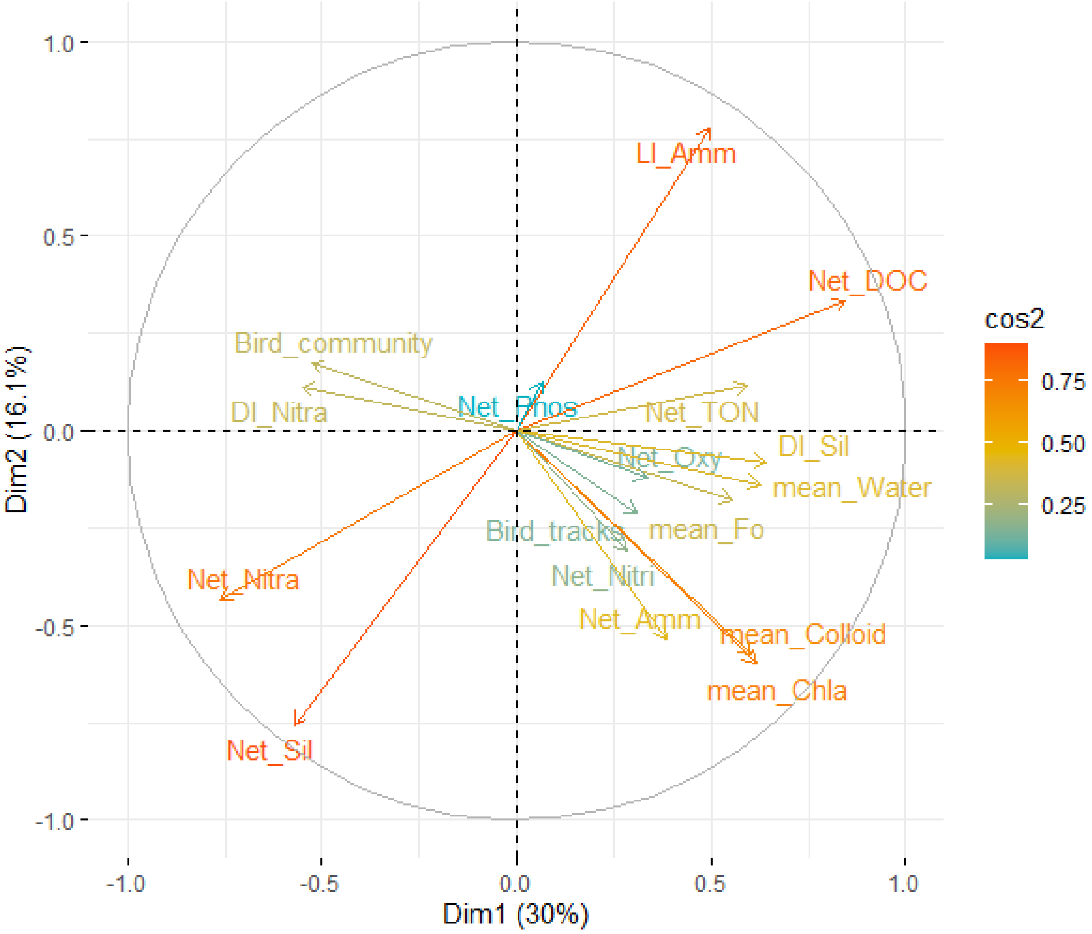


Figure 23 Principle Component Analysis (PCA) variables plot for retained measured variables (net oxygen (Oxy), nutrient (Phos=phosphate, Nitra=nitrate, Sil=silicate, Nitri=nitrite, Amm=ammonium) and organic matter (DOC=dissolved organic carbon, TON=total organic nitrogen) fluxes, mean chlorophyll *a* content (chla), mean F_o, mean colloidal carbohydrate content (colloid), mean water content (water) bird density (tracks) and assemblage (community) on day 147 (28 February 2019) on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030), day 188 (27 April 2019) on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406) and day 181 (04 April 2019) on the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (grid reference TM 12427 33311). Colour and distance of arrowhead to circle circumference both indicate cos2 value.

PCA variable plots of retained measured variables at Fingringhoe explain up to a combined 50.7% of variance (Figure 26). The depiction identifies positive covariance between bird density,

F_o, chlorophyll *a*, colloidal carbohydrates, net DOC nitrite fluxes and negative covariance between these variables and net nitrate flux. No covariance is identified between shorebird density and net ammonium, phosphate, silicate, oxygen or TON fluxes or sediment water content.

PCA variable plots depicting retained measured variables at Trimley (Fig 27) explain 48.2% of total variance. The depiction identifies positive covariance between bird density, chlorophyll *a*, water content, colloidal carbohydrates, net nitrite, nitrate and oxygen fluxes. Negative covariance between shorebird density and total organic nitrogen and net silicate fluxes is also depicted. No covariance is present between bird density and net DOC, phosphate or ammonium fluxes.

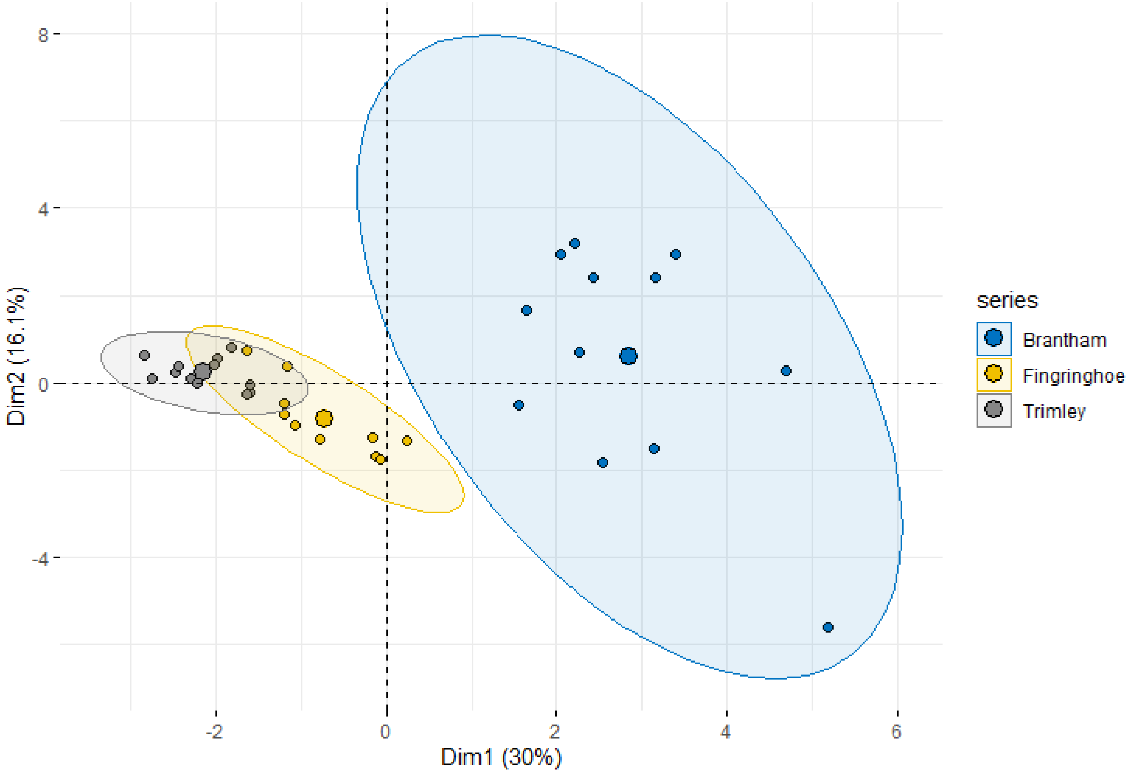


Figure 24 Principle Component Analysis (PCA) plot showing sampling points grouped by site, for all measured variables on day 147 (28 February 2019) on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030), day 188 (27 April 2019) on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406) and day 181 (04 April 2019) on the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (grid reference TM 12427 33311). Larger circles indicate centroid centre point.

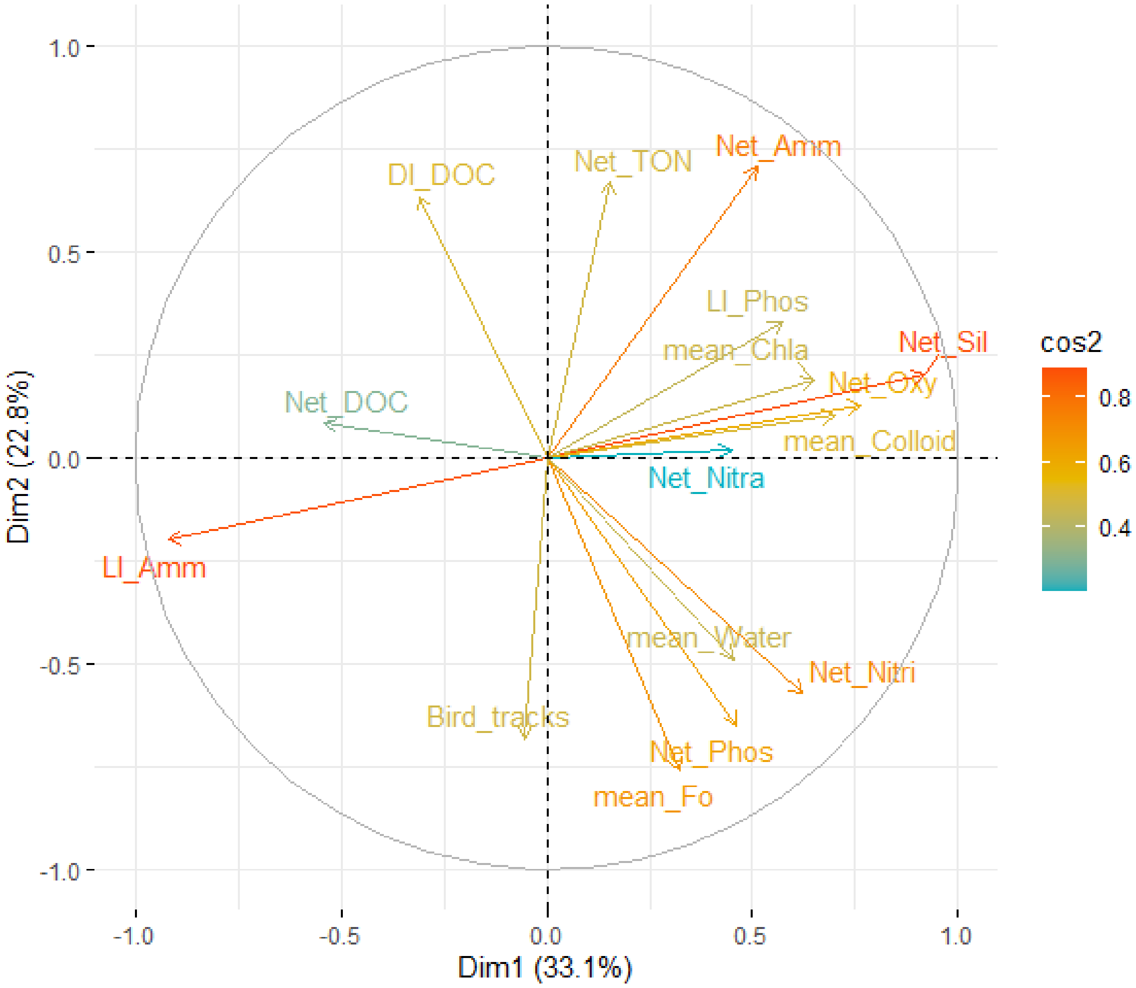


Figure 25 Principle Component Analysis (PCA) variables plot for retained measured variables on day 181 (04 April 2019) on the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (grid reference TM 12427 33311). For variable abbreviations see Figure 5.4 caption.

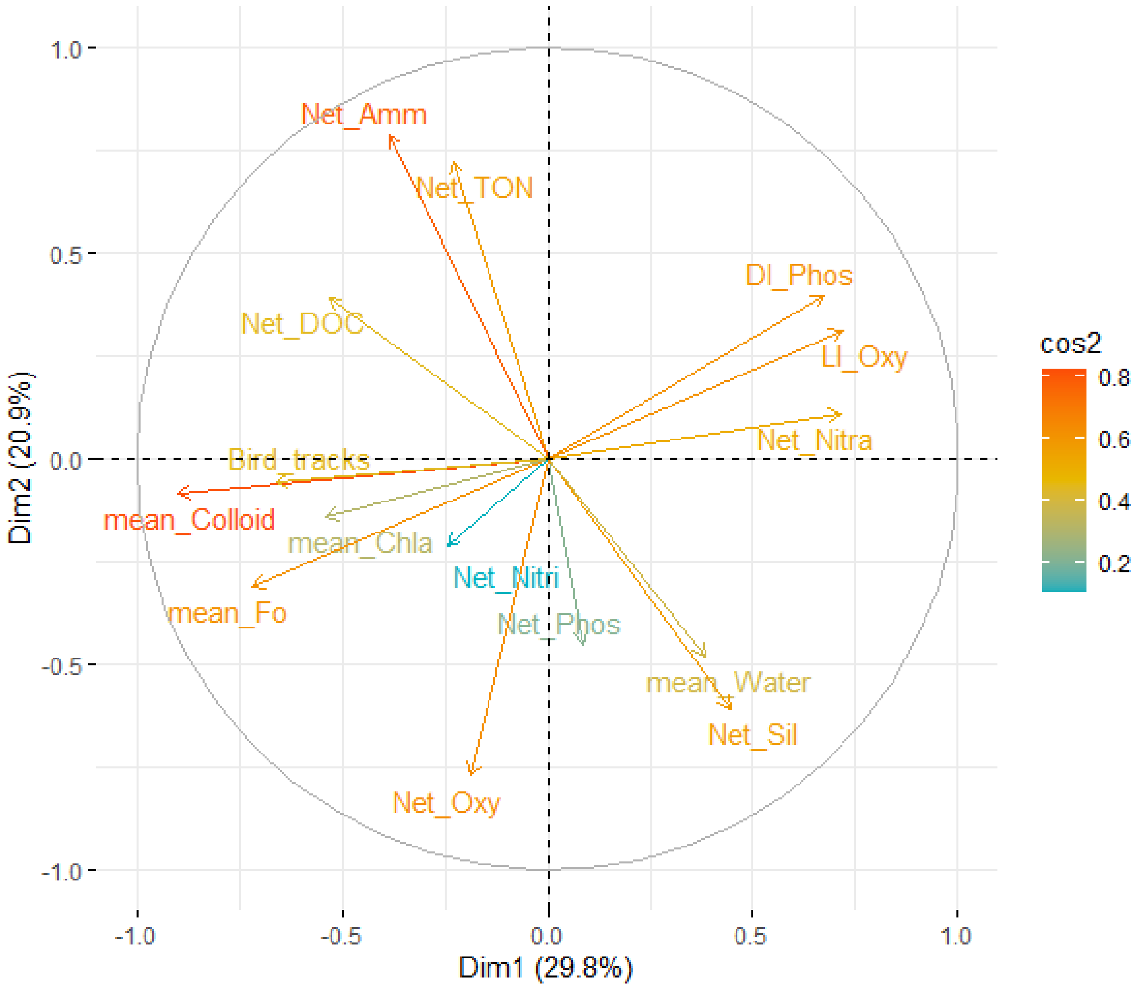


Figure 26 Principle Component Analysis (PCA) variables plot for retained measured variables on day 147 (28 February 2019) on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030). For variable abbreviations see Figure 5.4 caption.

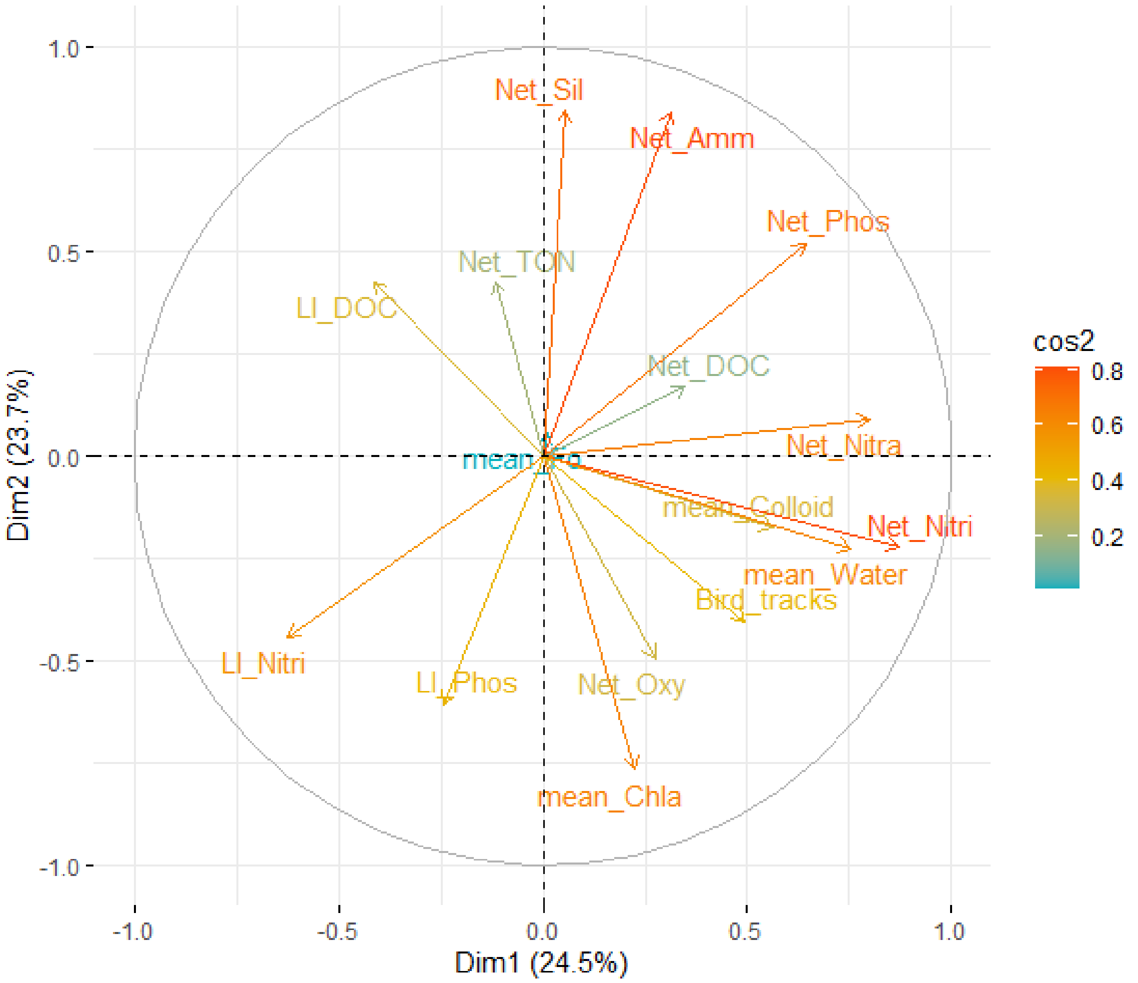


Figure 27 Principle Component Analysis (PCA) variables plots for retained measured variables on day 188 (27 April 2019) on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406). For variable abbreviations see Figure 5.4 caption.

Consistent patterns within and among sites are positive covariance between shorebird density and net nitrite flux depicted within all PCA plots, and between shorebird density and MPB biomass (F_o), chlorophyll *a* concentration, colloidal carbohydrate concentration and sediment water content, depicted in three of the four plots. Three of four PCA plots also depict no covariance between shorebird density and net fluxes of phosphate, DOC, ammonium or silicate.

One conflict between ANOVA (comparison between treatments) and PCA (correlation between bird density and fluxes) results is apparent at Trimley. Ammonium flux was significantly different between treatments but PCA only showed correlation between ammonium and shorebird density when all site data were analysed together, not within the plot for Trimley alone.

5.5 Discussion

This experiment allowed comparison of sites with a range of shorebird assemblages, unmanipulated MPB biomass, treatment efficacy (effectiveness of exclosures to manipulate bird density) and proportion of dunlin (known to directly consume MPB). This gave insight into how these site differences might have led to variation in the effect of shorebirds on ecosystem functioning and services including oxygen, nutrient and organic matter fluxes.

The manipulative treatment ('low' bird densities) was effective at reducing shorebird density. As evidenced in Chapter 4, significant differences in sediment characteristics between treatments were not detected, adding weight to previous conclusions by 'exclosure' originators that experimental artefacts are unlikely to be responsible for observed effects on measured variables (Cheverie et al. 2014).

Determination of significant differences between treatments (ANOVA) does not necessarily indicate that a linear relationship is present (covariance/correlation) between measured variables (Underwood 1997). Therefore, these methods of data analysis are considered separately with conflict between results for the same measured flux likely to indicate that either a relationship is present but not linear (e.g. ammonium), or a linear relationship is too subtle for a significant difference between treatments to be detected.

5.5.1 Hypothesis 1 - There will be a significant difference in oxygen, nutrient and organic matter fluxes between the sediment and water column between 'normal' and 'reduced' bird density

Reduction in shorebird density caused statistically significantly altered net fluxes of ammonium and silicate, which reversed from influx into the sediment, to efflux into the water column, though only at Trimley. Lower shorebird density also led to ecologically significant reversals in net flux directions of nitrite and TON (becoming efflux at Trimley and influx Brantham), nitrate and DOC (becoming influx and efflux respectively at Trimley) and phosphate (becoming efflux at Brantham), supporting hypothesis 1 for some locations/variables. Mean estuarine sample concentrations (Tables 16 and 17) suggest it is unlikely that influxes of measured variables were constrained by low concentrations in the overlying water, given that estuarine concentrations exceed hourly flux rates by at least an order of magnitude.

The only species recorded at the study sites known to directly consume biofilm (dunlin) (Elner et al. 2005) was absent from Trimley at the time of core collection, so it is highly unlikely that biofilm removal by consumption was the cause of the significant effects at Trimley on ammonium, silicate, nitrite, nitrate and DOC. If a major driver at Trimley was nitrogen enrichment through bird droppings (Thomas et al. 2022), then the reverse effect to that found on ammonium would be expected. Ammonium, silicate and nitrogen were absorbed into the sediment at Trimley where bird density was greater (Figure 20), although MPB biomass was lower and chlorophyll *a* concentration was similar to low bird density plots (Chapter 4; Figure 17). Ammonium, silicate and nitrogen were therefore potentially stimulating proliferation of nitrifying bacteria (Pan et al. 2018, Underwood et al. 2022). Mean overlying water concentrations of ammonium (14410 µmol L^{-1}), silicate (10,252 µmol L^{-1}) and dissolved nitrogen (49.25 mmol L^{-1}) were one to two orders of magnitude greater than the flux rate measured per hour, therefore a potential source of these nutrients. This suggests that the physical disturbance of the sediment, leading to bacteria proliferation, may have caused effects found at Trimley.

As described in Chapter 1 (Tables 2 and 3), macrofauna present at Trimley such as *Hediste diversicolor* (Davey and Watson 1995, Mortimer et al. 1999, Mermillod-Blondin and Rutger 2006), *Peringia ulvae* (O'Brien et al. 2009, Janas et al. 2019), *Limecola balthica* (Michaud et al. 2005) and *Cerastoderma edule* (Carss et al. 2020) can have significant effects on ammonium, silicate and nitrogen fluxes on intertidal mudflats. The effects of birds on ammonium, silicate and nitrogen fluxes may therefore be explained either by alterations in behaviour and distribution of macrofauna, or direct physical influences on the sediment through ambulation or feeding by reworking and exposing surface sediment, similar to the actions of macrofauna.

Lower shorebird density at Brantham caused reversal to influxes of nitrite and TON and efflux of phosphate, alongside lower MPB biomass (F_o). Nutrients in bird droppings, including phosphate and nitrogen (Jauffrais et al. 2015, Geizer et al. 2021) can increase primary productivity within the marine environment including MPB on mudflats (Ganning and Wulff 1969, Schrama et al. 2013, Jauffrais et al. 2015). The flux directions of total organic nitrogen and nitrites at Brantham support bird dropping input as a driver of observed effects of shorebird density, as does the greater number of birds present at this site, resulting in greater amounts of droppings.

At Fingringhoe previous investigation revealed significant differences in measured fluxes due to exclusion of shorebirds, however the present experiment suggests that reducing density of shorebirds at the same site (without excluding them completely) did not significantly affect measured fluxes (Figure 26). Reducing shorebird density at Trimley, an artificial mudflat, did cause statistically significant differences in regulating ecosystem functioning and services, including ammonium and silicate fluxes. Ecologically significant effects on nutrient and organic matter fluxes were also detected at Trimley and Brantham, but not at Fingringhoe, showing a range of effects of density on measured variables between the sites.

Chapter 4 identified high level, significant interactions between site, bird density and bird assemblage which indicate complexity in the relationship between bird community and assemblage both within and among sites. For example, a negative effect of bird density on MPB biomass was found at Trimley, but not at the other two sites except marginally at Brantham at 'lower' shorebird species assemblage scores. This complexity and variation among sites is also represented by the among site variation in nutrient and organic matter flux results, described above. Differences in bird densities and assemblages across sites are likely to have contributed to differences in nutrient and organic matter effects both temporally (at Fingringhoe) and spatially within the present experiment.

5.5.2 Hypothesis 2 - Shorebird density (patch use) and assemblage are correlated with measured oxygen, nutrient and organic matter fluxes between the sediment and water column

Hypothesis 2 is accepted based on findings that shorebird assemblage and density correlated with some nutrient and organic matter fluxes, including ammonium, nitrite, TON, phosphate (Brantham), DOC (Fingringhoe) and nitrate (Fingringhoe and Trimley). Covariance across sites was detected between bird density, MPB biomass, chlorophyll *a* concentration, colloidal carbohydrates and net oxygen flux (Figure 23).

Bird assemblage covaried negatively with bird density (Figure 23), driven by the greater shorebird density (and subsequent footprint cover) at Fingringhoe and again at Brantham, which also have differing assemblages present; assemblages at Brantham and Fingringhoe had greater proportions of knot, dunlin and black-tailed godwit (Chapter 4). These species tend to feed in flocks at greater density than typical species present at Trimley, such as redshank and grey plover (Evans 1976, Symonds et al. 1984, Ribeiro et al. 2004, Lourenco 2016, Mathot et al. 2018, Cestari et al. 2020).

Among site PCA (Figure 23) indicated strong covariance between bird density and net ammonium, reinforcing the suggestion that bird density could cause ammonium input via bird faeces.

Across and within sites, nitrite flux indicted covariance with bird density. Nitrite flux may also (like ammonium) be associated with bird dropping input stimulating diatom growth (Jauffrais et al. 2015). Estuarine nitrite formation, particularly the Colne where Fingringhoe is situated, is principally driven by denitrification within the surface layer of benthic sediment (Dong et al. 2000, Dong et al. 2002). An association between nitrite flux and bird density could also be due to surface layer bioturbation by birds, similarly to macrofauna which through their activities such as burrowing can affect biogeochemical cycling (Pischedda et al. 2008, Chen et al. 2017).

Site separation was evident among measured variables within the multivariate space, demonstrating site differences and justifying use of separate PCAs among sites. Despite elliptical overlap between Fingringhoe and Trimley, the second dimension showed reasonable separation between these sites, justifying site by site PCA (Figure 24).

Site level PCA (Figures 25 to 27) indicated that covarying nutrient and organic matter fluxes shared common themes across sites, such as covariance between bird density and net nitrite fluxes. It also highlighted differences between sites, suggesting that shorebird species assemblage and density may have been important drivers of the varying effects of shorebirds on oxygen, nutrient and organic matter fluxes on intertidal mudflats. Site level PCA suggests that all sites individually had covariance between bird density and net nitrite flux, as suggested by the multi-site PCA (see above).

At the site level, PCA suggested that net nitrogen fluxes (TON at Trimley and Brantham and nitrate at Fingringhoe) were significantly negatively correlated with bird density. Across sites, the overall trend was that greater shorebird density resulted in increased uptake (or decreased efflux) in TON (except at Fingringhoe) or nitrates, indicating that MPB were stimulating nitrogen uptake (Oakes et al. 2020). As described above, this suggests effects by birds which stimulate MPB biomass may have caused effects, such as via increased droppings, alterations in behaviour and distribution of macrofauna, or direct physical influences on the sediment by bioturbation.

Results from Brantham indicated decreased efflux of phosphate (an influx into the sediment) with increasing shorebird density and strong covariance between these variables. As discussed in the introduction (Chapter 1), it is well recognised that macrofauna play a key part in nutrient flux processes. Sediment mixing, including by macrofauna, can also have a significant enhancement effect on sediment redox (Pan et al. 2018), making it logical that shorebird activity, including walking, probing and sweeping behaviour while feeding on the mudflat surface, may have caused the measured effects in nutrient exchange (as described in Chapter 3). It is important to note, however, that the temporal length of these effects have not been tested using a single flux sampling event at each site. Assuming difference in F_o is an indicator of the likely strength of treatment effects in nutrient flux (Sundback et al. 1991), results from Chapters 3 and 4 suggest that at Fingringhoe in particular, effects would be likely to remain for a matter of weeks at the most.

For example, *Nereis* burrowing can cause sediment oxidation to a depth of up to 6 cm, and sediment reworking can maintain high redox (oxic) conditions even where the prevailing environmental redox state is low (Mortimer et al. 1999). This has the effect of considerably stimulating oxygen and ammonium fluxes between sediment and the water column, with varying effects on phosphorous and silicate between sites (Nizzoli et al. 2007). A similar affect could have been caused by shorebird bill probing, which would theoretically also result in increased spatial distribution of oxygenated column and pore water. Regarding phosphate, these findings do support a possible relationship: a study examining porewater micro-profiles and nutrient fluxes in the Humber Estuary at sampling stations with different macrofaunal communities found that phosphate efflux decreased with increasing *Hediste* density (Mortimer et al. 1999).

A possible model for the identified effect of bird density on phosphate efflux is based on research into the effect of *Hediste* density: low densities result in lower amounts of sediment reworking and oxygenation at depth, causing release of phosphate into the water column. At greater density, increased bioturbation and oxygenation at depth resulted in greater redox (oxic conditions) in the surface layers, causing increased adsorption of phosphate onto iron oxides. The result of this was to limit the available phosphate to be released from the sediment (Mortimer et al. 1999).

A similar effect is seen with the covariance between bird density and net nitrite flux at all sites. Nitrite efflux can also increase in proportion with macrofauna density (Mortimer et al. 1999), and the results from Brantham suggest a potential link with shorebird density at this site, but only assuming no significant difference in macrofauna communities between sampling plots, as found at Fingringhoe in 2017 (Booty et al. 2020) (Chapter 3). Pathways associated with nitrification and denitrification can also be mediated by microbial degradation, which results in increased ‘background noise’ and difficulty in determining the pathways involved (Pan et al. 2018).

The negative covariance between bird density and net TON at Brantham and Trimley may also be explained by shorebirds exerting similar effects to macrofauna. As with the effect of *Nereis* via burrowing, a number of taxonomic groups are referred to as ‘ecosystem engineers’. This is based on their stimulation of nitrogen cycling, and applies to macrofauna including polychaetes, crustaceans and molluscs in coastal marine sediments, particularly via burrowing (increased oxygenation and oxic-anoxic interface) (Stief 2013). Another identified potential pathway is microphytobenthos grazing, which can decrease the rate of the N-cycle by removing these facilitators (Dollhopf et al. 2005, Satoh et al. 2007, Gilbertson et al. 2012). In the case of macrofauna, it is unclear whether this leads to removal of fixed nitrogen from the system, because the fate of it in the gut of grazers is not known (Stief 2013). In the case of shorebirds, however, being mobile over greater distances leads to a logical suggestion that biofilm grazers are more likely to remove nitrogen from the system. However, this removal pathway (if present) would likely be counteracted to an extent by nutrient input via bird droppings.

5.5.3 Interannual Comparison at Fingringhoe

The results from Fingringhoe during 2017 (Chapter 3) and 2019 (present chapter) allow comparison between interannual fluxes at Fingringhoe. Measured nitrate and ammonium fluxes were within the same order of magnitude between years. Measured nitrite and phosphate fluxes were an order of magnitude (x10) higher in 2019, despite overlying water concentrations of these nutrients remaining relatively consistent between years (0.0058 mg L^{-1} and 0.05 mg L^{-1} during 2017 and 0.008 and 0.039 during 2019 respectively). Explanations for the variation in fluxes between years may include interannual variation in MPB biomass (Haro et al. 2022), increased concentrations of which can stimulate fluxes of nitrite (Oakes et al. 2020) and phosphate (Welker et al. 2002). This explanation fits the data; mean chl *a* content and F_o in control plots in 2017 was 296.8 (±67) µg g^{-1} and 1331 (±99) respectively, and in 2019 was 95.0 (±9.9) µg g^{-1} and 737.7 (±67) respectively. Nonetheless, comparison between treatments across years considers this variability and provides a valid assessment of the effect of different experimental treatments on nutrient flux.

Chapter 3 identified significant effects of shorebird presence/absence on the fluxes of nitrate, nitrite, phosphate and dissolved organic carbon (DOC) at Fingringhoe (Booty et al. 2020) . In contrast, comparison between ‘normal’ and ‘low’ bird density suggested that effects are less detectable with no significant difference detected between treatments in any measured fluxes.

This is logical because the magnitude of difference in bird density between treatments in 2017 was theoretically larger (although actual control plot bird density was not measured) than in 2018-2019. PCA demonstrated that covariance between shorebird density and some measured fluxes was detectable and driven also by shorebird density at Fingringhoe in 2019. This result agrees with the direction of effects found in Chapter 3 (net DOC, net nitrite and negative covariance with net nitrate), showing that effects on these nutrients remained present, though less detectable, when the magnitude of treatment differences was reduced. This is an important finding to emphasise, that bird density was positively correlated with DOC net flux, and reinforces previous findings at Fingringhoe, with DOC being a critical aspect of biogeochemical cycling regarding carbon sequestration and climate change (Chapter 3).

Under all treatment conditions ammonium measurements in 2017 indicated an influx into the sediment while those in 2019 indicated an efflux from the sediment (mean overlying water concentrations 0.045 mg L^{-1} during 2017 and 0.033 mg L^{-1}during 2019). The same was also true under most conditions for phosphate. Ammonium flux standard error values were greater in 2019, suggesting that such interannual variation was caused by increased variability between plots, potentially also causing the lower detectability of significant results. For example, a study in the Netherlands observed high variability in ammonium and nitrite production across distances of 1.6m (Decleyre et al. 2015). Given that mudflats are inherently heterogenous (Beninger 2018a) and sediment characteristics are known to drive benthic nutrient fluxes (Louis et al. 2021), this is a common limitation of studies conducted on intertidal mudflats (Beninger and Boldina 2018); which may be ‘solved’ by appropriately increasing the replication in future studies.

The differences between the presence/absence and the normal/low bird densities experiments, described above, suggest that shorebird density effects may be more difficult to examine than shorebird presence/absence in an *in-situ* experiment, possibly due to the habitat heterogeneity of a mudflat and the effects this has on myriad biotic and abiotic characteristics, including ecosystem functions such as erodibility (Zhu et al. 2019). Mudflat heterogeneity is complex; abiotic properties such as grain size, water content and bed roughness vary spatially (Black and Paterson 1998, Black et al. 1998, Wooldridge et al. 2018) and in turn can influence distribution of biota (Martinez et al. 2020, Brustolin et al. 2022), which can exert influence on heterogeneity (Paterson 1989, Kornman and De Deckere 1998, Widdows et al. 1998, Zhang et al. 2021), potentially resulting in feedback loops. Shorebird activity has also been shown to alter in response to small or large scale variability in mudflat characteristics (Kelsey and Hassall 1989, Ribeiro et al. 2004), adding further complexity to the situation regarding this study. Despite this heterogeneity which can mask effects, this experiment has still detected correlation between variables, albeit more concealed than within presence/absence experiments (Chapter 3).

5.5.4 Shorebird Assemblage Effects

Dunlin and knot are similar in their foraging habits, using visual and tactile cues, rapidly pecking, plowing, jabbing, picking and probing depending on prey location, visibility and substrate softness (Baker et al. 2020, Warnock and Gill 2020). In contrast, grey plovers rely mostly on sight for foraging, using the 'run-stop-peck' method, as such typically focusing on larger, more visible prey items and pecking more selectively (Poole et al. 2020). Ringed plovers forage using a similar pecking based method, also using 'foot trembling' to encourage prey to the surface (Wiersma et al. 2020). Avocet have a particularly unique foraging strategy, picking or scything the bill through mud or water, often in groups and reportedly 'spinning' using legs as an axis (Pierce et al. 2020). Redshank typical wintering foraging mode is 'brisk' walking and pecking, occasionally probing or jabbing the substrate (Van Gils et al. 2020b).

Of the waterfowl present at the study sites, which comprised a larger proportion of assemblages at Trimley; Wigeon and Brent Goose are almost exclusively vegetarian in diet, most likely to walk across the mudflat to reach saltmarsh or the upper shore edge where plants and seeds are available (Carboneras et al. 2020, Lewis et al. 2020). Teal and shelduck feed predominantly on aquatic invertebrates (Carboneras and Kirwan 2020, Johnson et al. 2020), although within European range teal spend approximately 87% of their time feeding in open water (Johnson et al. 2020). Shelduck are likely to have contributed to effects at Trimley and Fingringhoe, through digging, scything or dabbling on the mudflat (Carboneras and Kirwan 2020).

As evidenced above, the sites present a range of foraging strategies employed by shorebirds and waterfowl, with Brantham supporting mostly species which quickly probe and pick at the substrate in search of prey. Fingringhoe supports a relatively high proportion of similar activity, with the addition of digging and sweeping. In contrast, assemblages at Trimley are comprised mostly of more calculated, less frequently pecking species and scything, sweeping and digging foragers. As presented in Table 3 (Chapter 1), a range of known effects of macrofauna on nutrient and organic matter fluxes exist, which are driven by bioturbation and grazing, which can increase surface area of oxidised sediments (Kristensen 2001) and cause alterations in MPB biomass (Janas et al. 2019). Shorebirds are likely to alter MPB biomass via similar pathways.

5.6 Conclusions

The work presented demonstrates that effects of birds on biofilm mass extended to mediation of key nutrient and organic matter fluxes on intertidal mudflats. There was a positive correlation between bird density and measured fluxes, and negative correlation between shorebird assemblage and measured fluxes.

MPB are significant drivers of nutrient and organic matter fluxes (Underwood et al. 2022), and this *ex-situ* experiment has found that bird density and assemblage do affect these ecosystem functions, with variation in effects between sites. Due to the complexity of the processes involved on mudflats (Beninger and Paterson 2018) it is most likely that combinations of the possible pathways discussed, and potentially others not considered, interact to influence net nutrient and organic matter fluxes in this environment (Lurgi et al. 2020). For example, shorebird assemblage and density are likely to be interdependent, considering that different foraging and predatory avoidance behaviours (such as feeding modes and flocking) are species specific (Evans 1976, Stinson 1980, Brown 1999, Davis 2001).

This multi-site experiment has identified that nitrite flux was positively correlated with shorebird density and negatively correlated with bird assemblage scores across all three geographical locations, despite inherent environmental differences between them. At two of the three sites, total organic nitrogen (TON) correlated negatively with bird density, whilst net nitrate correlated negatively or positively with bird density, with flux directions depending on site.

Relationships between MPB and nutrient flux dynamics were not necessarily linear, with some nutrients differing significantly between shorebird density treatments but not covarying with bird density. Reduced shorebird density caused significantly altered net fluxes at two sites. Ammonium and silicate reversed to efflux from the sediment at Trimley and phosphate reversed to efflux at Brantham. Fluxes of nitrite, nitrate, and TON were reversed at both sites, with net direction in low shorebird treatments varying with site.

Although the specific mechanisms for the effects of shorebird density and assemblage have not been tested, potential pathways have been considered based on flux effects and known contributors to these. Potential pathways which fit the results include ambulatory movement by birds exposing nutrients by ‘ploughing’ the sediment surface, bill probing during feeding increasing the surface area of the sediment and porewater oxygen levels, and faecal input by shorebirds directly increasing nutrient levels such as ammonium and nitrite on the mudflat surface.

Further work is required to determine how bioturbation (either ambulatory or feeding) by shorebirds, nutrient input via droppings or shorebird species-specific interactions may cause these alterations in nutrient and organic matter flux, and drive relationships between these fluxes and shorebird density.

6 Shorebird ambulatory bioturbation increases intertidal mudflat resilience and affects supporting ecosystem services

6.1 Abstract

Intertidal mudflats play important roles in coastline protection and nutrient and organic matter cycling, supporting, and regulating ecosystem services. In previous chapters (3, 4 and 5) it was found that bioturbation may be a mechanism by which shorebirds alter microphytobenthos (MPB) biomass and associated ecosystem functions including erodibility and nutrient and organic matter exchange between sediment and the water column. Additionally, visual observation of biofilm colonisation within bird footprints led to a hypothesis that sediment surface disturbance and associated mobilisation of nutrients during shorebird ambulatory movement (bioturbation) may explain findings in previous chapters. A six-month experiment was designed to test bird bioturbation effects on ecosystem functions (EFs) erodibility and nutrient and organic matter exchange, including nitrate, nitrite, phosphate, silicate, ammonium, dissolved organic carbon and total organic nitrogen. Within exclusion cages on an intertidal mudflat, bird bioturbation was simulated artificially using 3D printed dunlin foot replicas within low bioturbation (15% track coverage) and high bioturbation (60% track coverage), alongside 0% coverage control plots. This experiment was repeated in parallel using paired plots, for two rounds of destructive sampling of ecosystem functioning related measurements (once in March and once in June). MPB biomass was monitored regularly by proxy using a PAM fluorometer. Sediment erodibility was measured *in situ* using a Cohesive Strength Meter (CSM) once in March and once in June. At these times minicores were taken to allow ground truthing of chlorophyll *a* content and measure colloidal carbohydrate content. Flux cores were also extracted from the mud and transferred to controlled indoor mesocosms where they were sampled for nutrient and organic matter exchange. To account for possible indirect effects via alterations in macrofauna assemblage, macrofauna were sieved from flux cores and identified and counted allowing comparison of assemblages between treatments. No evidence of alterations in macrofaunal assemblage between treatments was found. It is demonstrated that shorebird ambulatory bioturbation can increase MPB biomass, leading to significantly decreased sediment erodibility and significantly altering fluxes of ammonium in March to net efflux under high bioturbation, nitrate in June to net efflux under low bioturbation and dissolved organic carbon in June, increasing in efflux under high bioturbation. These findings emphasise the important role which shorebirds play in regulating and supporting ecosystem services on intertidal mudflats, indicating their potential as ecosystem engineers.

6.2 Introduction

Ecosystem functioning and ecosystem services, which are intrinsically related (Balvanera et al. 2006), have become a critical link between biodiversity, conservation and human well-being (Bull et al. 2016) in an attempt to internalise natural capital into economic models, for the benefit of mankind and the natural world (Gomez-Baggethun et al. 2010). It has been suggested that shorebirds can significantly affect ecosystem functioning (EF) on an intertidal mudflat (Daborn et al. 1993, Booty et al. 2020) (Chapter 3). Affected EF includes erodibility and fluxes of nutrients and organic matter between sediment and overlying water, illustrating the importance of interactions between birds and microphytobenthos (MPB) to the intertidal mudflat environment. Yet, significant knowledge gaps remain regarding ecosystem functioning mechanisms and responses in this habitat, particularly at a sediment and primary producer level (Guerra et al. 2020, Freschet et al. 2021).

Organisms which modify or maintain a habitat through physical changes, thus modulating resources available for other species, can be classified as ecosystem engineers (Jones et al. 1994). Numerous examples are acknowledged throughout the field of ecology including well known charismatic species such as beavers (Wright et al. 2002). Some examples specific to intertidal soft sediments are eelgrasses which can affect hydrodynamics, MPB and bacteria stabilising sediments (reducing erodibility), meiofauna which can increase sediment-water nutrient fluxes and macrofauna which can affect organic matter remineralisation and nutrient turnover (Passarelli et al. 2014).

Chapters 4 and 5 present experimental results showing that shorebird density and assemblage can be significantly related to MPB biomass, depending on site, and shorebird density and assemblage are therefore drivers affecting nutrient and organic matter fluxes. The process(es) by which these effects occur have not been experimentally tested, although it has been suggested that bird dropping input (Jauffrais et al. 2015), bioturbation (Kristensen et al. 2012) and microphytobenthos (MPB) consumption (Elner et al. 2005) may be processes contributing to these effects (see Chapters 4 and 5).

Deposit feeding macro infauna within intertidal sediments (herein macrofauna) rely heavily on MPB for core sustenance (Van Colen 2018). Macrofauna therefore significantly reduce and limit growth of MPB through grazing (Hagerthey et al. 2002, Orvain et al. 2004) and also have a reductive effect on MPB biomass through sediment reworking (Orvain et al. 2004, Morelle et al. 2021). The binding effect of the MPB ‘biofilm matrix’ enhances the stability (reduces erodibility) of the surface sediment layers (Tolhurst et al. 2002). MPB are also mediators of nutrients such as nitrogenic compounds between the sediment and water column, playing a significant role in this ecosystem functioning (Hope et al. 2020).

Numerous examples are known of macrofauna which disturb and 're-work' intertidal muddy sediments leading to alterations in sediment characteristics such as MPB mass and erodibility (Widdows et al. 1998, Willows et al. 1998, Widdows et al. 2000a), net effects most often being an increase in sediment surface erodibility (Underwood and Paterson 1993). Examples include *H. diversicolor* which can have varying effects on erodibility through bioturbation (Meadows and Tait 1989, de Deckere et al. 2001, Widdows et al. 2009, Passarelli et al. 2012), *P. ulvae* which can increase the erosion rate on mudflats by a factor of two to four (Andersen et al. 2002), and crabs which can reduce diatom biomass by up to 50%, probably due to bioturbation (Armitage and Fong 2006). It has also been postulated that sediment disturbance magnitude and frequency (Tolhurst et al. 2012) could affect infaunal communities, although research to date has found no detectable community change (Gerwing et al. 2017).

As summarised in Tables 2 and 3 (Chapter 1), macrofauna activity can have significant impacts on biogeochemical cycling, such as nutrient and organic matter fluxes, via pathways including increased oxygenation of sediments within burrows and biogenic structures (Dufour 2018), sediment reworking (bioturbation) (Xiao et al. 2022) and indirect effects through removal of MPB biomass (Janas et al. 2019).

Bioturbation impacts by birds have been recognised, with tracks included within this definition of reworking soils and sediments (Bosworth and Thibodeaux 1990, Belaústegui et al. 2018), with the effect of probing and dabbling during feeding cited as potential mechanisms for sediment disturbance. Reworking of sediment by some bird species has been examined in detail; black-headed gulls *Larus ridibundus* and Shelduck *Tadornis tadornis* rework 30% and 15% (respectively) of a mudflat annually, to a depth of 2.5 cm (Cadee 1990). Bioturbation by brent geese *Branta bernicla* and wigeon *Anas penelope* on intertidal seagrass beds has also been shown to have an engineering effect: grazing on leaves, shoots, rhizomes and roots within the upper 1 cm of upper tidal zone sediment aids persistence of seagrass by inhibiting sediment accretion (Nacken and Reise 2000, Meysman et al. 2006).

It follows that shorebird activities on intertidal mudflats, such as ambulatory movement and pecking and probing the mud in search of food, could affect MPB biomass and in turn ecosystem functioning such as sediment erodibility (Austen et al. 1999, Tolhurst et al. 2002) and nutrient and organic matter fluxes (Sundback and Graneli 1988, Austen et al. 1999, Janas et al. 2019).

Shorebird activity can theoretically affect the abundance and distribution of macrofauna, causing indirect effects on ecosystem functioning as described above, however these effects can be difficult to detect due to compensatory interactions such as competitive species filling niches, mobile species replacing predated conspecifics and age or size class preference by some shorebird species (Ferreira et al. 2005, Hamilton et al. 2006, Cheverie et al. 2014, Mathot et al. 2018).

During fieldwork between 2016 and 2019 at various intertidal mudflats in Suffolk and Essex (UK), it was noticed that shorebird footprints were colonised by microphytobenthic biofilms (Image 6). Work during 2019 at Trimley found negative effects of shorebird track density and assemblage on MPB biomass (Chapter 4). This indicates a possible link between shorebird ambulatory movement (bioturbation) across the mudflat surface and surface MPB biomass. Shorebird ambulatory movement could cause this effect through compression of sediment (referred to also as trampling) by shorebirds and other animals, which may influence diatom community composition (Van de Vijver et al. 2008). If this is occurring then such a reduction in functional diversity of diatoms could be the cause of reduction in benthic MPB biomass (Virta and Teittinen 2022).

Artificial disturbance has been used as an aid to test ecological hypotheses in a range of habitats across the globe, including forest plants in Australia (Neumann 1991) and China (Zhang and Xianfeng 2021), stream benthic macroinvertebrates in Australia (Brooks and Boulton 1991), grasslands in Tanaznia (Belsky 1986) and prairie plants and encrusting intertidal flat algae in North America (Rapp and Rabinowitz 1985; Dethier 1994).

An experiment was designed to test the hypotheses that artificial bird bioturbation (ABB) significantly:

1. increases MPB surface biomass
2. decreases mudflat erodibility
3. affects sediment-water column nutrient fluxes
4. affects mudflat macrofauna assemblage (to control for unknown effects of simulation on macrofauna behaviour and distribution)

6.3 Materials and Methods

6.3.1 Description of Study Site

The site at Trimley Marshes is located on an intertidal mudflat at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406), on the Orwell estuary. Trimley Marshes SWT comprises a series of lagoons and associated reedbeds, grassland, woodland, scrub, dykes and intertidal mudflat which were created to compensate the loss of coastal habitats when the nearby Port of Felixstowe was created in the 1980s (Atkinson et al. 2001). The reserve meets the northern bank of the Orwell estuary where shingle banks, saltmarsh and intertidal mudflats are present adjacent to an artificial seawall and coastal footpath. In this area, the study site was located comprising an area of artificially created mudflat approximately 400 m^2 situated on the upper shore, sheltered from the incoming tide by an artificial spit. Chapter 4 shows previously recorded bird species and relative abundance at the site.

The study area and other nearby intertidal areas were reinforced with dredged sediment as a means of mitigating the loss of intertidal habitats during expansion of Port of Felixstowe. Monitoring of benthic assemblages along the Stour and Orwell, both of which were impacted by the port expansion, was carried out between 2003 and 2014. During 2014, intertidal species recorded at the points closest to the study site included *Peringia ulvae, Macoma balthica, Nephtys homergiii, Corophium volutator, Cerastoderma edule* and *Mya arenaria* (Haskoning 2015).

Previous work on this mudflat has shown mean water content to be 63.88% (±0.004) and modal grain size 62.70 µm, placing it at the coarse end of the ‘mud’ spectrum (Pan et al. 2018) (Chapter 4).

6.3.2 Experiment Design

The experimental layout comprised a randomised design of 15 spatial plots, allocated to three treatment levels all with shorebirds excluded for control over shorebird bioturbation levels; control (no artificial bird bioturbation (ABB)), low manipulation (low ABB) and high manipulation (shorebirds high ABB), with n=5 replicates of each treatment. Within each plot (1-15), were two sub-plots (A and B), each 0.6 m x 0.6 m. Figure 28 shows the experimental layout at the site.

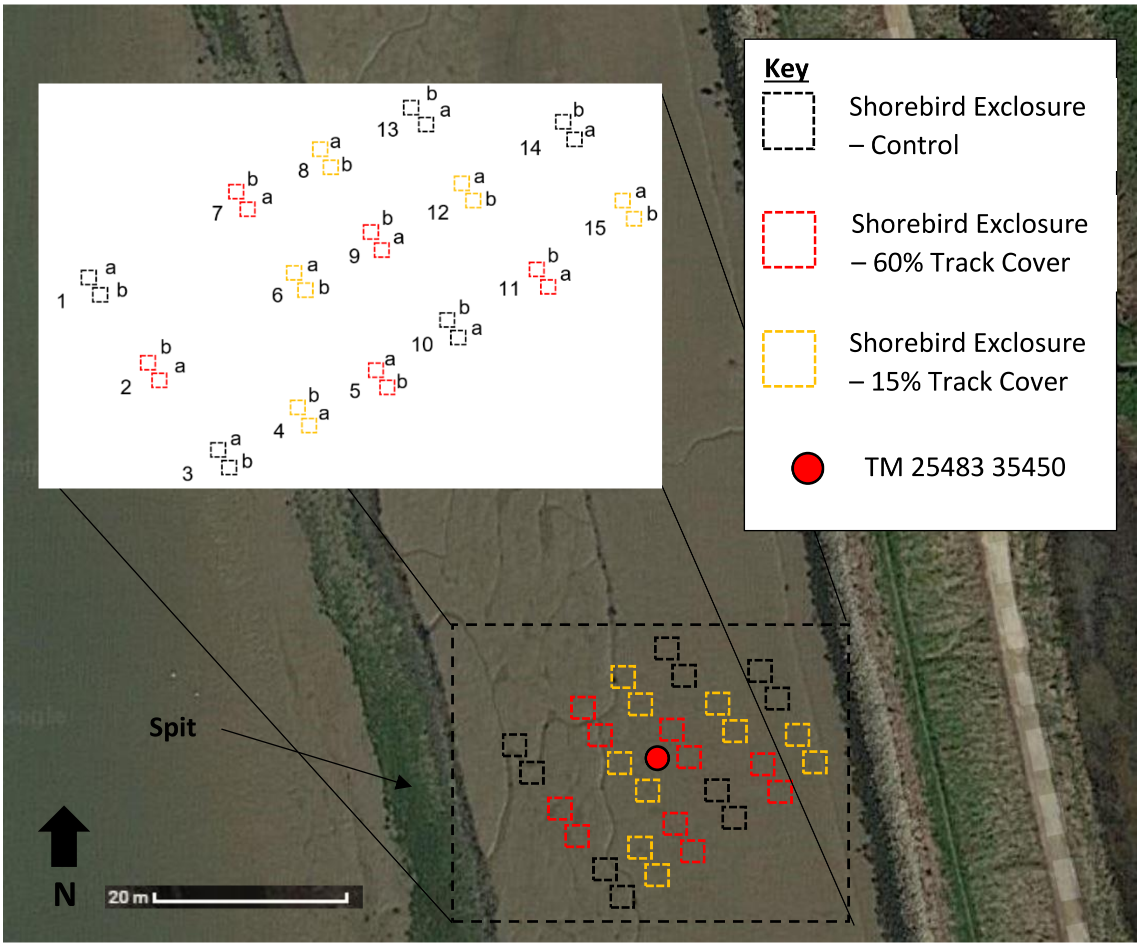


Figure 28 Indicative experimental layout on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK between 08 January and 23 June 2021. 'Track cover'=ABB.

Replicate exclosures within plots enabled two parallel experiments overlapping in time, for destructive ecosystem function (EF) sampling of 'A' plots partway through (in March) and repeated EF sampling in 'B' (June) plots. The experiment was carried out in two phases; all plots were setup on the 08 January 2021 and manipulated and sampled at least once every three days between 08 January and 09 March 2021. Following destructive sampling in March, only 'B' (June) plots were manipulated and sampled at least once every three days between 28 March and 29 June 2021 at which point June plots were destructively sampled (Table 18).

Previous work in similar estuaries showed that spatial variability in biofilm abundance is greatest at the fine scale and small at the meter scale (Murphy et al. 2008, Taylor et al. 2013, Nedwell et al. 2016, Redzuan 2017), therefore a completely randomized design was employed to maximize statistical power of the experiment. Exclosures were as used in Chapter 3 (Booty et al. 2020); bamboo frames, approximately 30 cm in height, covered on all sides (including the top) by opaque 'fruit-cage' bird exclusion netting (plastic mono-thread) with a 2 cm aperture. Exclosures prevented access to the sediment by birds but allowed access to infauna and small fish (<2 cm width). Plots were approximately five meters apart, to allow sampling from all sides and prevent plots unduly influencing each other. Sub-plots (A and B) were at least 1 m apart. Treatments and sub-plots were arranged randomly to reduce the potential for spatial bias. The exact locations of plots were selected to represent the heterogeneity within the wider mudflat. No scouring or bite marks indicating the presence of larger fish (Eggold and Motta 1992) were found within the study area during the experiment. Plots were arranged parallel to the tide line (within a minute of immersion/emersion time of one another). Plots were situated on the upper shore, where shorebirds spend most time foraging due to the longer emersion time (Granadeiro et al. 2006). 1 m x 1 m plots using this exclosure design do not significantly affect the water flow, PAR, and sediment surface characteristics (Section 3.2.3 in Chapter 3).

The experiment compared areas subject to varying levels of artificial bird bioturbation (ABB). Replica 3D printed shorebird feet were designed based on dunlin *C. alpina* foot structure, due to the relevance of this species to the study, effects on MPB during previous work probably occurring through direct biofilm grazing (see Chapter 3). Figure 29 shows a single replica with relevant dimensions.

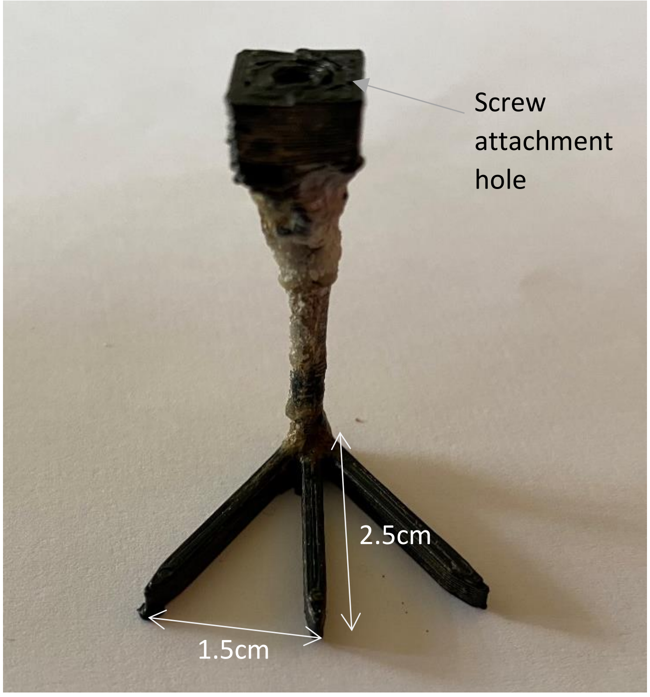


Figure 29 Replica shorebird foot used for artificial bird bioturbation on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK between 08 January and 23 June 2021.

Replica feet were designed using Autodesk Fusion 360 and 3D printed from polylactic acid (PLA) biopolymer using a Flashforge Finder. The total area covered by each foot was approximately 3.75 cm^2 ((1.5 x 2.5 / 2) x2). Six replicas were produced and attached to 0.5 mm width medium density fibreboard (MDF) 20 cm x 15 cm using screws at the top of the replica leg (Figure 29). The board with six attached feet is referred to as the 'manipulation plate'. The total weight of the plate was 150g, with six feet simulating three shorebirds. Dunlin range in weight between 48g and 64g (Warnock and Gill 2020), therefore the plate was within the weight range required to accurately simulate pressure of this shorebird on the mudflat surface.

To simulate shorebird bioturbation caused by walking across the mudflat surface, the plate was placed, feet down, onto the mudflat surface without exerting pressure onto the plate. This was done a predefined number of times depending upon the manipulation assignment of the plot (Figure 28). Coverage of tracks was spread evenly (by eye) across the plot. Overlapping artificial footprints was tolerated, as is found naturally among shorebird footprints (Robar and Hamilton 2007). Artificial footprint density was selected based on the mean track density previously recorded at Trimley under natural conditions (16.5% (±2.03)), maximum density recorded 80% (Chapter 4). To achieve low manipulation the plate was placed a total of 24 times within a plot, for a simulated track coverage of 15%. In high manipulation plots the plate was placed a total of 96 times, for a simulated track coverage of 60%. Depending upon the stability of the substratum upon which each foot was placed, feet penetrated between 0 and approximately 2mm into the surface, which is the depth range which epipelic diatoms inhabit within fine grained sediments (Tolhurst et al. 2008a). ABB was carried out at least once every three days, which was considered proportionate given the variability in patch use exhibited by shorebirds (Kelsey and Hassall 1989) and the relatively small area of the plots. Preliminary tests indicated that simulated tracks in the study area can persist for three days or longer. This may be due to the sheltered nature of the mudflat behind the spit and resulting low wave velocity (Miranda and Kobayashi 2022). Table 18 shows the experimental timeline.

Table 18 Dates of experimental setup, artificial bioturbation simulation (ABS) and sampling (including techniques) during the experiment period on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (TM 25504 35406) between 08 January 2021 and 23 June 2021.

Date	Month	Days Since Setup	Activity
08	January	0	Experiment cages setup
11	January	3	Baseline F_o measurements
11,15,18,21,24,27	January	3-19	Artificial Bird Bioturbation (ABB)
31	January	23	F_o measurements (all plots)
01,04,06,09,12,14	February	24-37	ABB
17	February	40	F_o measurements (all plots) and ABB
20,23,26	February	43-49	ABB
01,04	March	52-55	
07	March	58	F_o measurements (all plots) and ABB
10,13,16,19	March	61-70	ABB
23	March	74	F_o and shear strength measurements; flux and syringe core collection (A plots)
24	March	75	Flux sampling (A plots)
25,28	March	76-79	ABB (B plots)
31	March	82	ABB (B plots); infauna sieving (A plots)
03,06,09	April	85-91	ABB (B plots)
12	April	94	F_o measurements (B plots) and ABB (B plots)
15,18,21,23,26	April	98-108	ABB (B plots)
29	April	111	F_o measurements (B plots) and ABB (B plots)
02,05,07,10,13,16,19,21,24	May	114-136	ABB (B plots)
27	May	139	F_o measurements (B plots) and ABB (B plots)
30	May	142	ABB (B plots)
01,04,06,09,12,15	June	144-158	ABB (B plots)
16	June	159	F_o measurements (B plots)
18,21	June	161-164	ABB (B plots)
23	June	166	F_o and shear strength measurements; flux, contact and syringe core collection (B plots)
24	June	165	Nutrient flux sampling
28,29	June	167-168	Infauna sieving (B plots)

6.3.3 Response Variables

Between day variation in mudflat characteristics have been shown to be of greater significance than within day variation (Tolhurst and Chapman 2005), therefore repeated measures of F_o were made to compensate for this effect. Table 18 shows dates and days at which sampling events took place. On 11 January 2021, following plot setup, baseline minimum fluorescence (F_o) measurements were taken using a pulse amplitude modulated fluorometer (PAM, Walz, Effeltrich, Germany) to determine MPB biomass (Honeywill et al. 2002). MPB are key drivers of intertidal flat properties and processes (Murphy and Tolhurst 2009), so to determine when the full sampling events would be most likely to detect effects, F_o (as a proxy for MPB biomass) was monitored at least monthly (Table 18) as a convenient indication of treatment effects, to determine when erodibility and nutrient flux (ecosystem function – EF) variables should be measured and to confirm that early in the experiment there were no significant differences among treatments.

F_o was measured in all plots (n=5 in each of the 30 plots = total 150 measurements) until destructive sampling of sub-plots 'A' on 23 March 2021, following which F_o was measured only in sub-plots 'B' until destructive sampling in on 23 June 2021 (n=5 in each of the 15 plots = total 75 measurements), to investigate how surface MPB biomass responded to artificial bird bioturbation (ABB) over time. Immediately prior to destructive sampling all *in-situ* mudflat variables were measured to test the effect that ABB had on selected mudflat properties. To assess potential effects of weather on patterns of MPB biomass during the experimental period, peak wind speed (mph) and peak temperature (°C) data were collated from timeanddate.com.

Many measurements were required in each plot during a single tidal cycle. Considering the impact of MPB migration (Tolhurst et al. 2003), dewatering during the tidal cycle (Perkins et al. 2003, Maggi et al. 2013, Orvain et al. 2014a, Fagherazzi et al. 2017), and the effects of rain on MPB (Tolhurst et al. 2003, Tolhurst et al. 2006b, Tolhurst et al. 2008b), sampling was carried out during periods of clear weather with little wind and no rain, at least one hour after the tide had exposed the sampling area to allow initial dewatering of plots, with a 5 min low light partial dark adaption treatment used prior to each PAM measurement. This is a preferred method to conventional dark adaption for the measurement of minimum fluorescence as a proxy of MPB biomass (Jesus et al. 2006b). A consistent low light sampling environment was achieved using plastic 40 mm (diameter) × 60 mm (length), cylindrical opaque black adaption chambers with a 6 mm aperture hole at the top. This also enabled *in-situ* measurement with the PAM fluorometer without removal of the chamber and theoretically reduced the variation in light intensity during the measuring period. To further eliminate potential effects of varying light intensity and sediment water content on vertical migration of MPB between sampling events, sampling periods were timed to cover low tides peaking as close to midday as possible. Biofilm biomass (for which F_o is a proxy) varies with time (Haro et al. 2022), making it necessary to test independently for treatment effects on F_o at the point in time when the full suite of measurements were made, therefore PAM measurements and chlorophyll *a* 'ground truthing' measurements were made at the point of EF sampling (see below).

The March and June destructive sampling included *in-situ* measurements of F_o (as described above) and *in-situ* sediment critical erosion threshold (τ_{cr}) (EF variable) using a Cohesive Strength Meter (CSM) (three measurements within five plots of each treatment, total 45 measurements) (Tolhurst et al. 1999, Vardy et al. 2007). Mini cores 2 cm diameter and 1cm deep (five from each sub-plot 'A' on day 74 (March) and five from each sub-plot 'B' on day 166 (June), were collected for analysis of colloidal carbohydrates and chlorophyll *a* content. Sample locations were paired with (taken immediately adjacent to) F_o measurement locations, during each destructive sampling event. At the same time, a flux core (Perspex tubes of 0.1 m diameter and approximately 0.2 m in depth) was collected, one from each sub-plot 'A' on day 74 and one from each sub-plot 'B' on day 166, for laboratory analysis of nutrients and macrofauna.

Flux cores were carefully returned to the laboratory within an hour of leaving the site and immersed in seawater from the site, within oxygenated and temperature and light controlled indoor mesocosms (Thornton et al. 1999). Rubber bungs were used to ensure equal headspace volume across cores. Cores were left submerged and open to settle overnight prior to sampling on the following day. Throughout headspace water sampling, Perspex lids were tightly fitted to prevent leakage. Magnetic stirrers maintained water flow over the sediment surface. Headspace seawater samples were taken at the beginning and end of 3 h dark and light incubation periods. Cores were left for at least one hour to adjust to light levels prior to each incubation. Sampling was completed according to general methods described by Thornton *et al.* (1999). Flux measurements were repeated in both light and dark conditions, using 500W halogen 'daylight' lamps to provide 'lit' conditions (500 µmol m–2 s–1 PAR) and covering mesocosms with opaque Perspex covers to provide 'dark' conditions. Headspace water samples were tested for sediment-water biogeochemical fluxes of nitrate, nitrite, ammonium, phosphate, total organic nitrogen (TON) and dissolved organic carbon (DOC) across the incubation period (EF variables). Water samples were analyzed for nutrient concentrations using a Seal AA3 segmented flow Nutrient Analyzer (SEAL Analytical Inc.).

Individual cores used for nutrient flux measurements were subsequently sieved (500 µm mesh) to retain macrofauna. Macrofauna were preserved in 95% ethanol and identified to species level (where possible) using a microscope, counted and densities (m^{-2}) calculated.

6.3.4 Statistical Analysis

To evaluate the effects of ABB on MPB biomass throughout the experimental period a linear mixed-effects (LME) model was used to determine the relationship between the response variable biofilm mass proxy (F_o) and the fixed effect simulated shorebird patch use (% ABB footprint cover), with plot, days and series (parallel experiments) as random effects. This model was run using R Version 4.2.2 with package lmerTest (Kuznetsova et al. 2017):

$\log F_o$ ~ Footprints + (series|plot) + (1|series/day)

where: $\log F_o$ = biofilm biomass proxy: $\log_n (F_o)$

Footprints = % artificial bird bioturbation

In testing hypotheses 1 and 2, to evaluate the effect of ABB on MPB biomass and sediment erodibility, chlorophyll *a* content, F_o, chlorophyll *a* content, colloidal carbohydrate content (EPS proxy), and critical erosion threshold data from both destructive sampling times (March and June) were analysed using a mixed model, two-way nested ANOVA design (plot nested in treatment) with plot as a random factor and ABB cover (treatment) as a fixed factor.

In testing hypothesis 3, to evaluate the effect of ABB level on dark and light nutrient flux, nutrient data were analysed using a two-way orthogonal ANOVA design with dark/light incubation and treatment (control, low and high ABB) as fixed factors. Where Cochran's test was significant (minicore chl *a* (Hayman et al.), contact core chl *a* (June), colloidal carbohydrates (March and June), nitrate (June), nitrite (March and June), oxygen (June) and total organic nitrogen (Hayman et al.)), data were normalised by rank transformation and the analysis repeated. Reversals in flux (for example an efflux from the sediment becoming an influx into the sediment) were used as an indication of changes suggesting 'ecologically significant' implications for ecosystem functioning. To counteract the issue of multiple comparisons we used Bonferroni correction testing each hypothesis at a confidence level of 0.025 (0.05/2). Where flux graphs suggested significant differences under one incubation condition (light/dark) only, one way ANOVA (ABB treatment as fixed factor) was used to test the statistical significance of perceived differences.

To evaluate the effect of ABB level on net (light incubation flux rates subtracted from dark) oxygen, nutrient and organic matter fluxes, a one-way ANOVA design was used with dark/light incubation and treatment as fixed factors. ANOVAs were run using the GMAV (1997) statistical package (University of Sydney, Australia).

Where a significant difference in chlorophyll *a* content was found between treatments, Principal Components Analysis (PCA) using Euclidean distances was used to determine the primary pattern of covariation between chlorophyll *a* and measured net oxygen, nutrient and OM fluxes. Results were visualised using variable plots depicting cos2 values. PCA was run using dplyr (Wickham et al. 2023) and factoextra (Kassambara and Mundt 2020) packages in R Version 4.2.2.

In testing hypothesis 4, to assess whether ABB had significantly altered macroinvertebrate community structure, taxa density was analyzed using R version 4.04 with vegan package. Non-metric multidimensional scaling (nMDS, Bray-Curtis dissimilarity, 20 restarts) was used to visualize differences in community structure within and between days 74 and 166, in two dimensions (Clarke 1993). The MDS solutions had stress of 0.08, 0.07 and 0.1 respectively, and therefore are considered adequate representations (Clarke 1993). Analysis of similarities (ANOSIM) was also performed to test quantitatively for differences in community structure between treatments (control, low ABB, and high ABB) both within days and between sampling days (time).

6.4 Results

Measured variables indicating MPB biofilm abundance at the sediment surface (F$_o$, chlorophyll *a* content and colloidal carbohydrate content) and macrofauna numbers were greater during March than June (Figures 30 and 31).

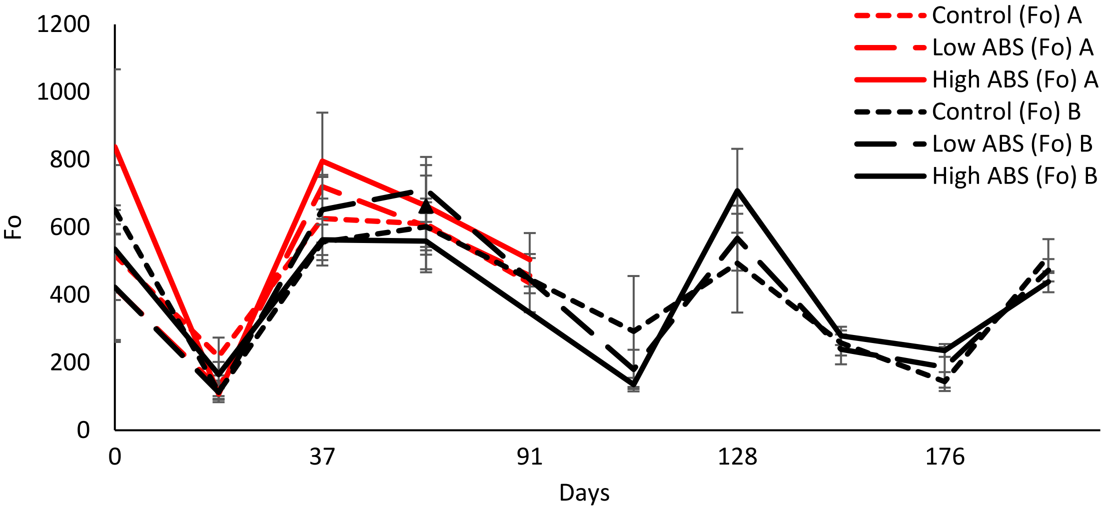


Figure 30 Microphytobenthos biomass (F$_o$) (mean ± SE, n=5) measured in control, low artificial bioturbation simulation (ABS) and high ABS plots (n=5) within two overlapping time series (A and B) on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (TM 25504 35406) between 08 January 2021 and 23 June 2021.

The variation in mean MPB biomass in all treatments followed a similar trend (Figure 30). A similar pattern of variation was not present in the weather data during the experimental period (Figure 31).

Macrofauna recorded within flux cores were chironomids (midge larvae), *Limecola balthica*, *Peringia ulvae* and *Nereis diversicolor*. Macrofauna abundance was greater in March than June. Chironomidae and *P. ulvae* were present in higher numbers during March, with numbers of all macrofauna except for chironomids dropping by June (Figure 32).

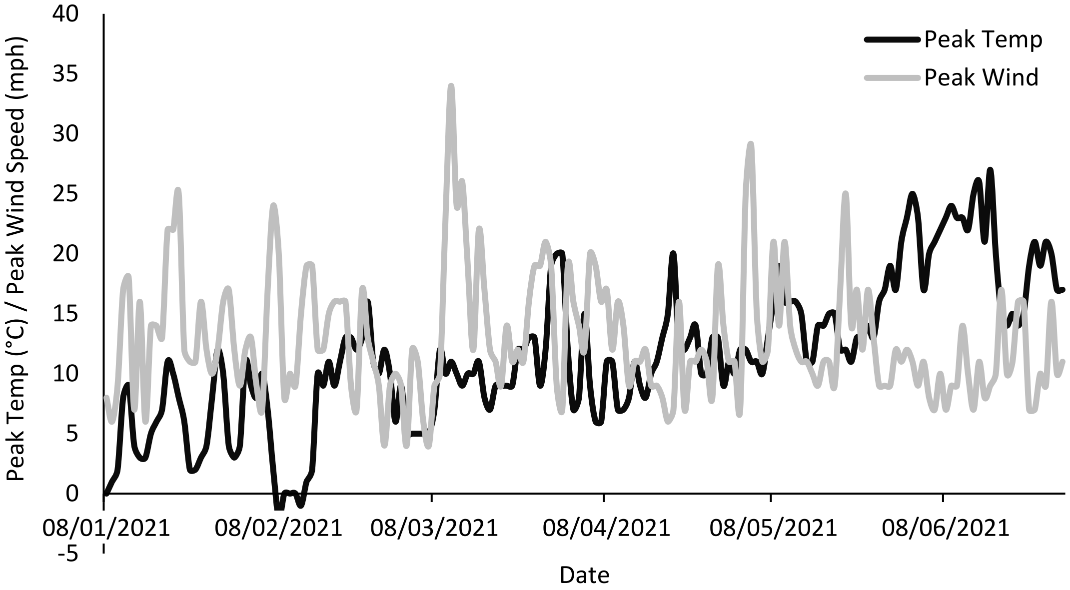


Figure 31 Weather data collated from timeanddate.com for Felixstowe Port (adjacent to the study site) during work on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (TM 25504 35406) between 08 January 2021 and 23 June 2021.

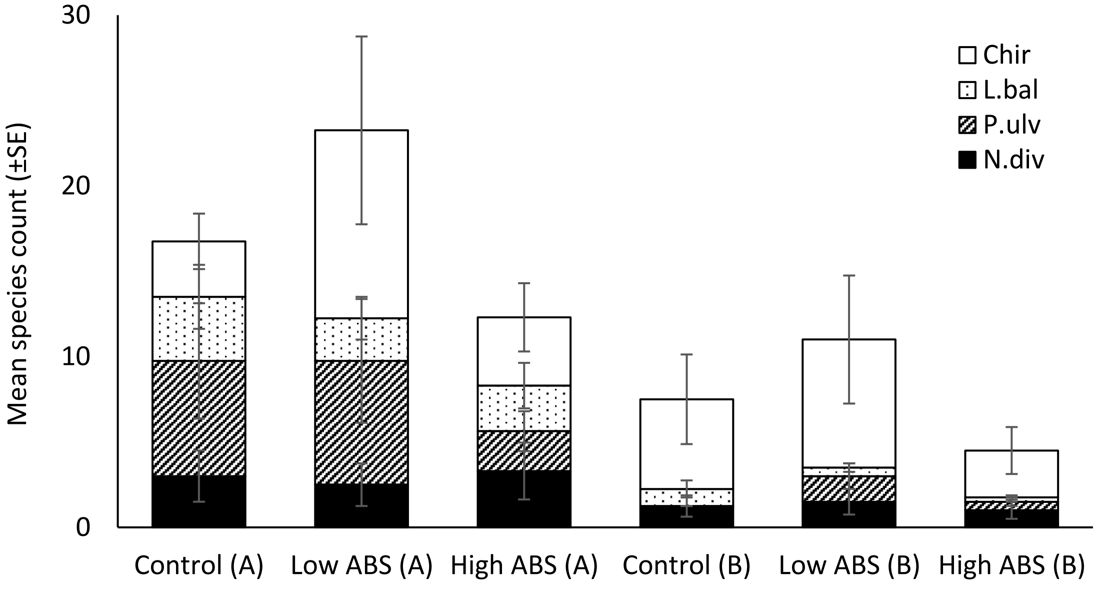


Figure 32 Macrofauna abundance (mean ± SE, n=4) measured in control, low artificial bioturbation simulation (ABS) and high ABS plots (n=5) within A plots on Day 74 (23 March 2021) and B plots on Day 166 (23 June 2021) on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (TM 25504 35406).

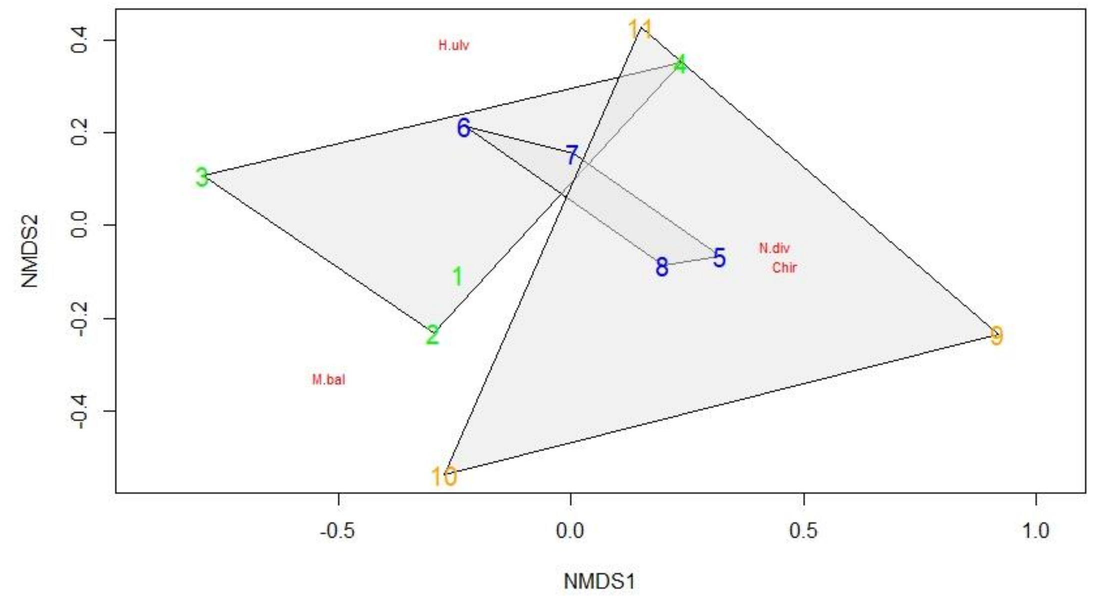


Figure 33 Non-metric multidimensional scaling (nMDS) plot depicting Bray-Curtis dissimilarity in community composition (green=control, blue=low ABS, orange=high ABS, Chir=Chironomid, H.ulv=*Peringia ulvae*, M.bal=*Macoma balthica*, N.div=*Hediste diversicolor*) in cores collected from on Day 74 (23 March 2021) on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (TM 25504 35406).

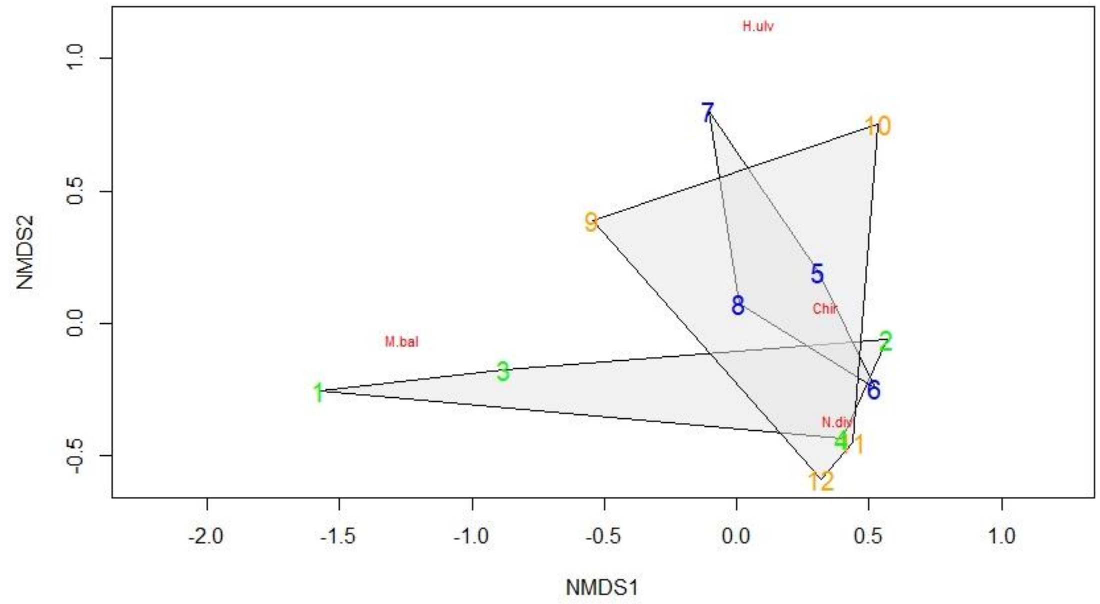


Figure 34 Non-metric multidimensional scaling (nMDS) plot depicting Bray-Curtis dissimilarity in community composition (green=control, blue=low ABS, orange=high ABS, Chir=Chironomid, H.ulv=*Peringia ulvae*, M.bal=*Macoma balthica*, N.div=*Hediste diversicolor*) in cores collected from on Day 165 (24 June 2021). on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (TM 25504 35406).

Overlap in community composition between treatments in March and June is evident (Figures 33 and 34), depicting that community composition was similar between treatments during both months of sampling. In March an association between the presence of chironomids and *H.*

diversicolor within high ABB plots is evident, and low ABB plots overlap both control and high ABB plots suggesting a low level of dissimilarity between this and both other treatments. ANOSIM results confirm that communities were not significantly different (R=0.14; p=0.13 and R=0.02; p=0.4 respectively).

Separation is evident between seasons when results are combined (Figure 35). ANOSIM results confirm that species community composition was significantly different between March and June sampling (R=0.3; p=<0.001). On day 74 the nMDS plot (Figure 33) suggests some dissimilarity (slight overlap) between controls and high ABB plots, ANOSIM confirms no significant difference in community composition among treatments (R=0.14; P=0.13).

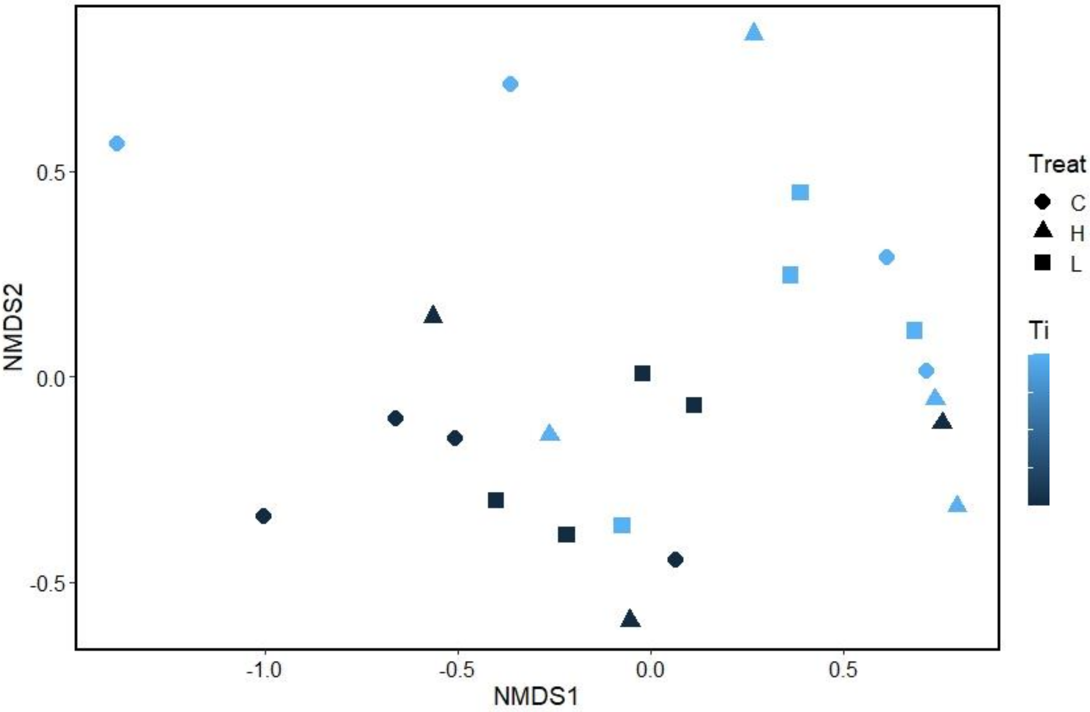


Figure 35 Non-metric multidimensional scaling (nMDS) plot depicting Bray-Curtis dissimilarity in community composition in cores collected on Days 74 (dark blue points) and 165 (light blue points) on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (TM 25504 35406).

Chlorophyll *a* content and τ_{cr} were significantly greater (F=7.5, p=0.008; F= 17.4; P=0.02, respectively) in low ABB plots than the other treatments in March (Figure 36; Table 29 in Appendix 5). In June, τ_{cr} remained greater in low ABB plots although the ANOVA model did not detect a significant effect (F=3.3; P=0.17), possibly due to the smaller difference between controls and high ABB treated plots on Day 166. No significant effects between treatments were detected for any other measured sediment variables (Table 29 in Appendix 5). In control plots chl *a* was lower than in low or high ABB plots . Where ABB is low, chl *a* peaks; where ABB is high chl *a* is reduced (Figure 36), which is also reflected in the erodibility measurements.

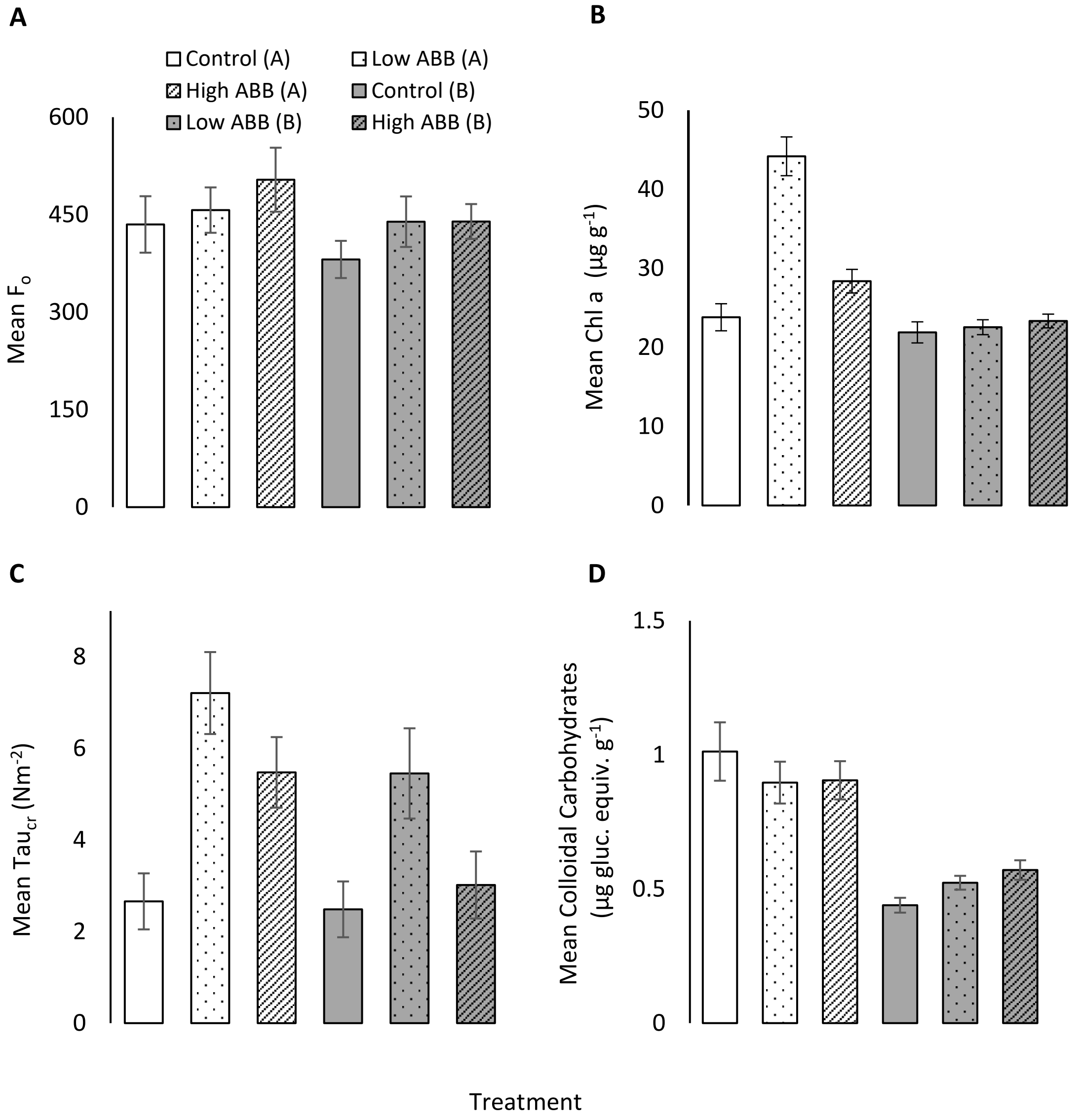


Figure 36 A Chlorophyll fluorescence (F$_o$), **B** chlorophyll *a* (Chl *a*) (µg g-1), **C** erosion threshold (τ_{cr}) (Nm^{-2}) and **D** colloidal carbohydrates (µg g^{-1}), (mean ± SE, n=5) measured using syringe cores in control, low artificial bioturbation simulation (ABS) and high ABS plots (n=5) within A plots on Day 74 (23 March 2021) and B plots on Day 166 (23 June 2021) on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (TM 25504 35406).

The best fitting LME model indicated no significant interaction between ABB (% cover) (Estimate=0.00; CI=-0.00-0.00; p=0.11) or sampling month (March and June) (estimate=-0.25; CI=-0.91-0.41; p=0.46) and MPB biomass (Table 19).

Table 7 LME model output (CI = 95% confidence interval)

		logfo	
Predictors	*Estimates*	*CI*	*p*
(Intercept)	5.83	5.30 – 6.36	**<0.001**
Series (A/B)	-0.25	-0.91 – 0.41	0.455
Treatment (low/high ABS)	0.00	-0.00 – 0.00	0.110
Random Effects			
σ^2	0.43		
$\tau_{00\ \text{plot.series}}$	0.01		
$\tau_{00\ \text{series}}$	0.00		
$\tau_{00\ \text{day.series}}$	0.35		
$\tau_{00\ \text{series.1}}$	0.00		
N_{plot}	15		
N_{series}	2		
N_{day}	10		
Observations	1050		
Marginal R^2 / Conditional R^2	0.037 / NA		

In March measured DOC fluxes show efflux from the sediment in all treatments, with no significant differences and large standard errors for most fluxes (Figure 37). High bioturbation in March caused a reversal in ammonium flux under light incubation from efflux to influx (Figure 38).

In June during dark incubation both bioturbation levels led to significantly increased efflux of DOC (F=7.89; p=0.01), efflux being greatest at low bioturbation. Low bioturbation led to a reversal in nitrate flux under dark incubation from influx to efflux (Figure 39). Low bioturbation therefore caused significant changes in fluxes of DOC (increased efflux) and nitrate (reversal to efflux). High bioturbation caused reversal in ammonium flux, to net influx. These effects were not consistent between dark/light incubation or sampling month.

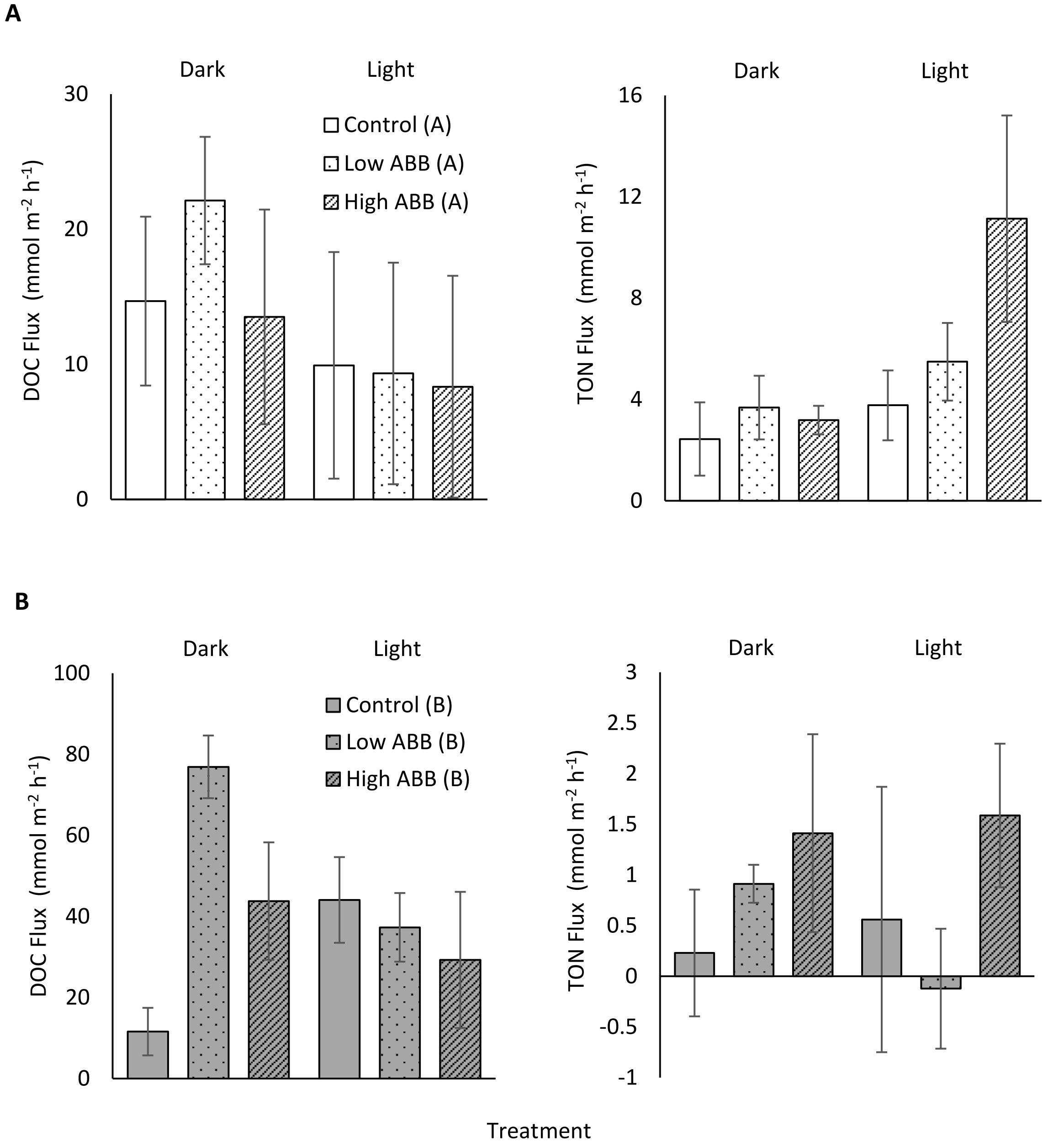


Figure 37 Organic matter (dissolved organic carbon (DOC) (*left*) and total organic nitrogen (TON) (*right*)) fluxes (mean ± SE, n=4) during dark and light incubations in cores collected from control (unfilled bars), low artificial bioturbation simulation (ABS) and high ABS plots **A** on Day 74 (23 March 2021) (white bars) and **B** on Day 165 (24 June 2021) (grey bars) on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (TM 25504 35406).

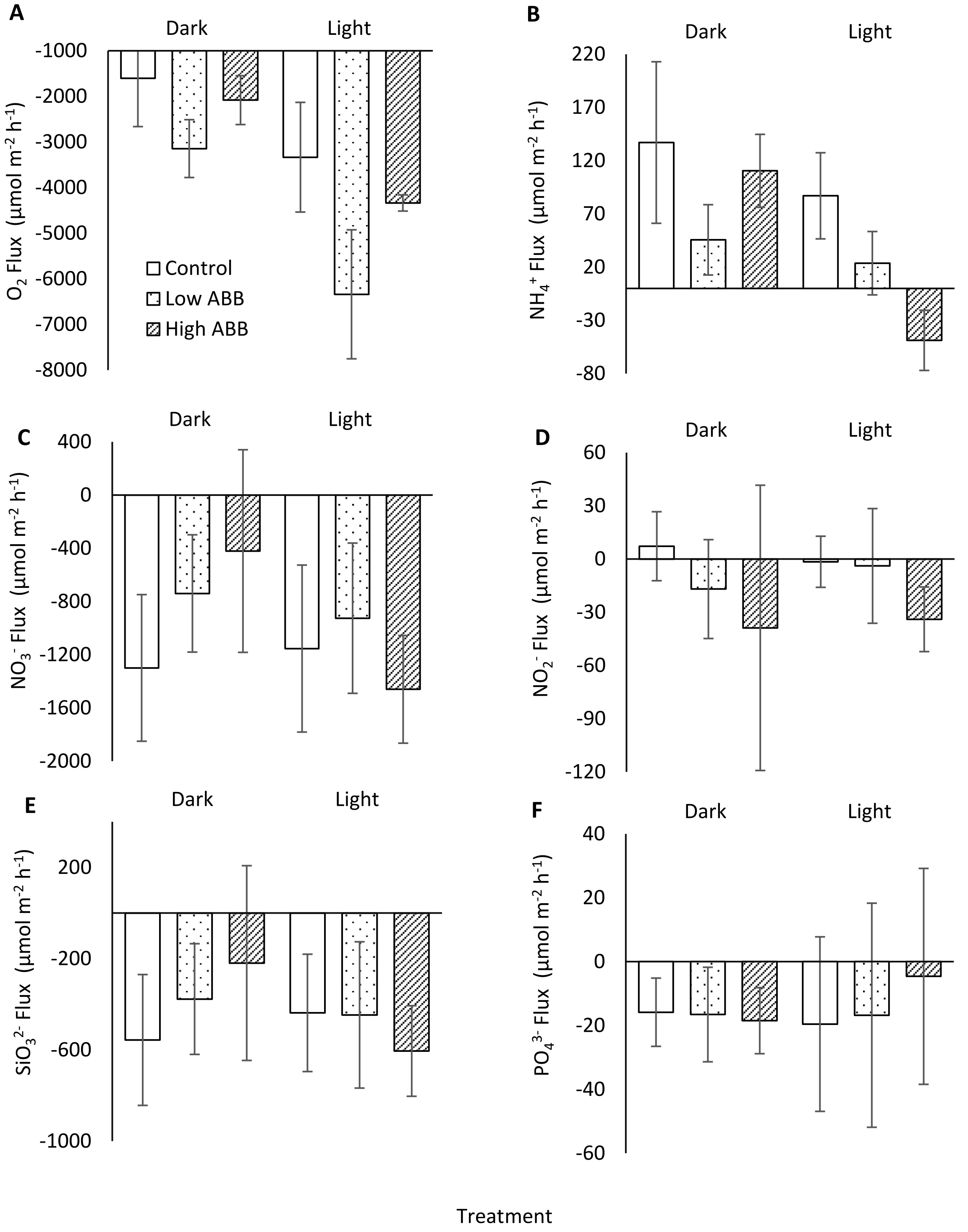


Figure 38 **A** Oxygen and nutrient (**B** ammonium, **C** nitrate, **D** nitrite, **E** phosphate and **F** silicate) fluxes (mean ± SE, n=4) during dark and light incubations in cores collected from control (unfilled bars), low artificial bioturbation simulation (ABS) and high ABS plots (referred to as 'A plots') on Day 74 (23 March 2021) on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (TM 25504 35406).

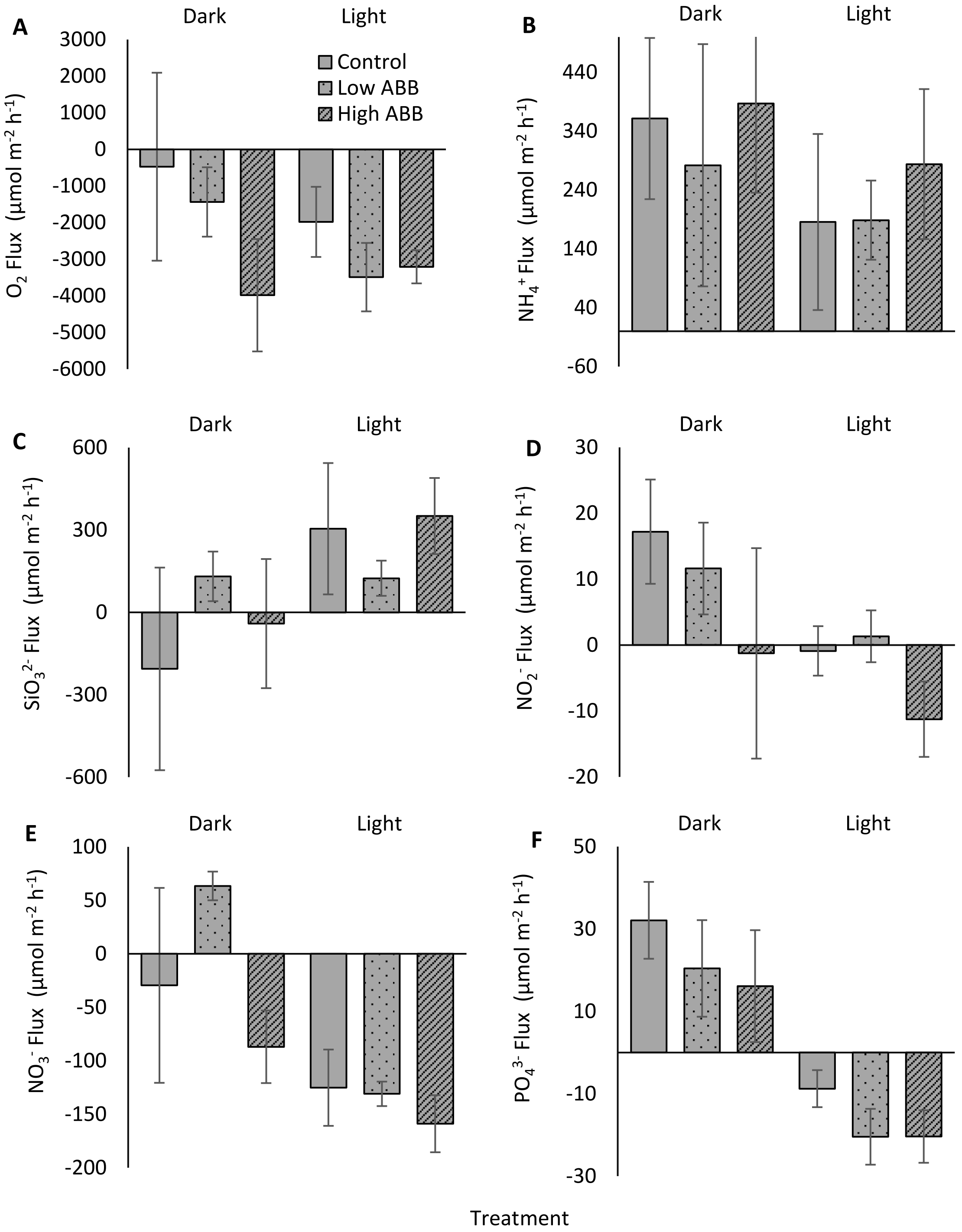


Figure 39 **A** Oxygen and nutrient (**B** ammonium, **C** silicate, **D** nitrite, **E** nitrate and **F** phosphate) fluxes (mean ± SE, n=4) during dark and light incubations in cores collected from control (unfilled bars), low artificial bioturbation simulation (ABS) and high ABS plots (referred to as 'B plots') on Day 165 (24 June 2021) on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (TM 25504 35406).

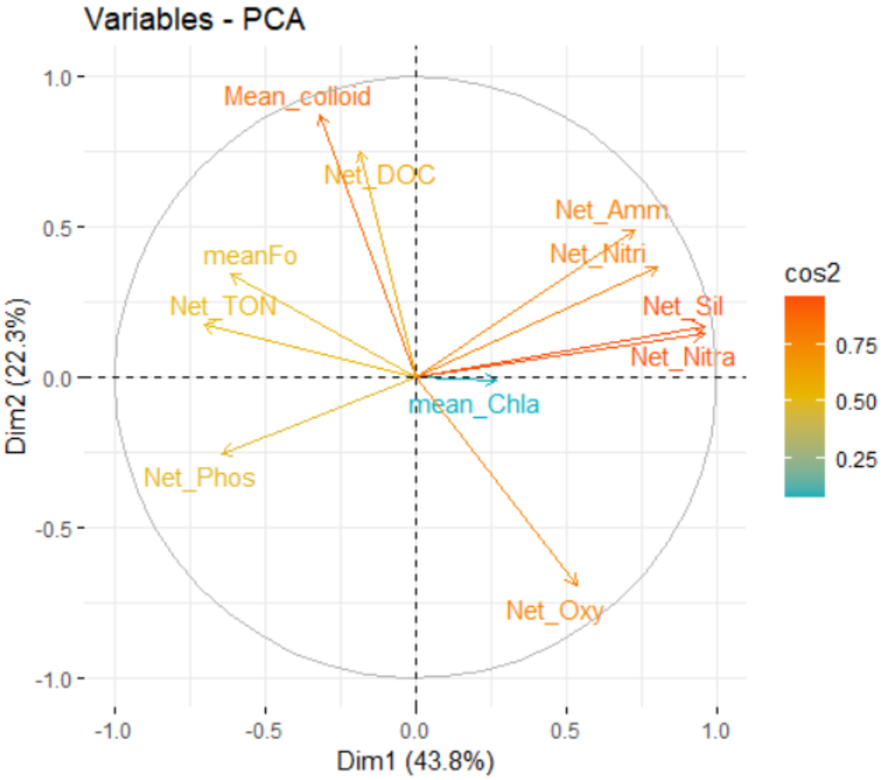


Figure 40 Principle Component Analysis (PCA) variables plot for retained variables: measured net oxygen (Oxy), nutrient (Phos=phosphate, Nitra=nitrate, Sil=silicate, Nitri=nitrite, Amm=ammonium) and organic matter (DOC=dissolved organic carbon, TON=total organic nitrogen) fluxes mean chlorophyll *a* content (chla), mean F_o and mean colloidal carbohydrate content (colloid) on 23 March 2021 on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406).

PC1 and PC2 explained 66.1% of variance between measured variables in March (Figure 40) and 50.3% in June.

In March and June ABB (tracks) had low cos2 (quality of representation on the variable plot) (cos2 = 0.1) (see Figures 40 and 41), indicating a low contribution of this variable to effects on other measured variables.

Mean MPB biomass and chlorophyll *a* did not correlate (see Figures 40 and 41) despite known associations between these variables (Eggert 2006, Jesus et al. 2006b). Mean chlorophyll *a* had a low cos2 value in March (cos2 = 0.06), indicating low contribution of chlorophyll *a* variability to flux effects in March.

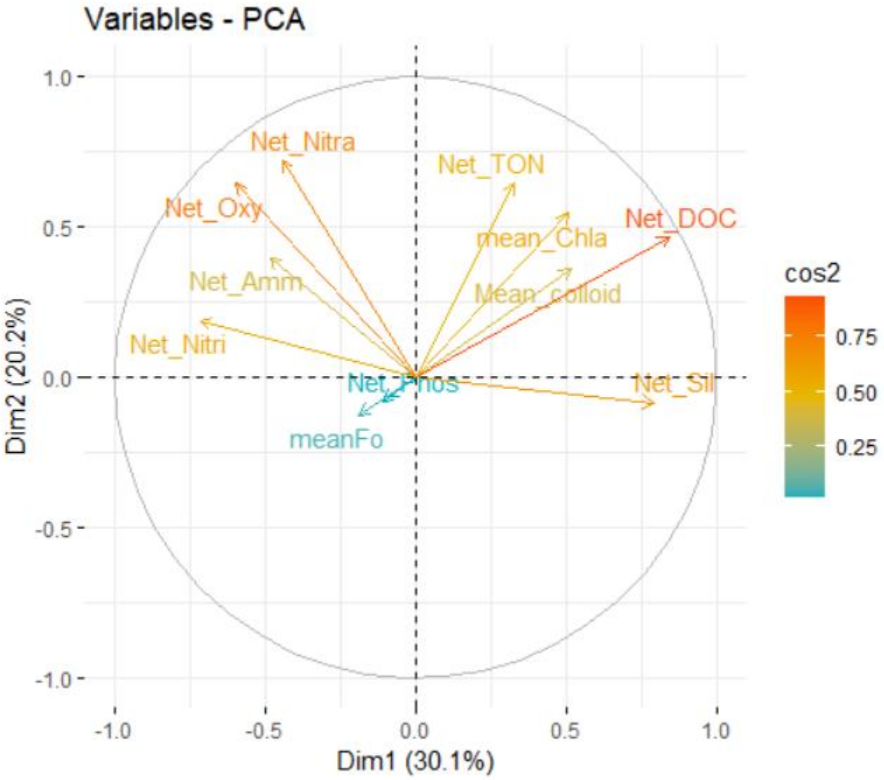


Figure 41 Principle Component Analysis (PCA) variables plot for retained variables: measured net oxygen (Oxy), nutrient (Phos=phosphate, Nitra=nitrate, Sil=silicate, Nitri=nitrite, Amm=ammonium) and organic matter (DOC=dissolved organic carbon, TON=total organic nitrogen) fluxes mean chlorophyll *a* content (chla), mean F_o and mean colloidal carbohydrate content (colloid) on 23 June 2021 on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406).

6.5 Discussion

This experiment aimed to investigate how ecosystem functioning may be affected by artificial bird bioturbation (ABB), one identified potential pathway by which this group may exert top-down effects on: 1) MPB biomass which is known to be associated with the following; 2) sediment erodibility, which contributes to shoreline erosion and flood defence services, 3) oxygen, nutrient and organic matter fluxes between the sediment and overlying water which are important aspects of biogeochemical cycling regulation services, and 4) macrofauna community assemblage (to control for possible ecological cascade effects).

The effect of ABB on the mudflat surface was compression of surface sediment up to 1-2 mm within the footprint, either creating an imprint on the existing surface layer or exposing mud by breaking the surface layer (images 7 and 8). Where the surface layer was broken, this exposed previously buried sediment and nutrients to the air and water column. Where macrofauna form biogenic structures the net effect is most often a reduction in sediment erodibility (increase in sediment stability) (Passarelli et al. 2018). ABB had a similar effect, causing increased sediment stability (i.e. a decrease in erodibility).

Image 7 Example high ABB plot immediately after simulation on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK between 08 January and 23 June 2021.

Image 8 Example low ABB plot immediately after simulation on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK between 08 January and 23 June 2021.

Where compaction occurred without breaking the surface, this would also be expected to increase sediment stability (reduce erodibility) (Tolhurst et al. 2000b) and therefore it is likely that both these mechanisms were acting in tandem during this experiment.

The underpinning process by which shorebird ambulatory bioturbation was hypothesised to significantly affect nutrient fluxes and erodibility was through changes in MPB biomass (either through physical resuspension or nutrient release stimulating growth), MPB being a key mediator of measured ecosystem functions (Chapters 3, 4 and 5). For example, Chapter 4 found significant effects of shorebird density and assemblage on MPB biomass at Trimley, indicating that this caused knock-on differences in sediment erodibility and (Chapter 5) nutrient and organic matter fluxes.

The lack of correlation between measured mean MPB biomass (F_o) and chlorophyll *a* concentration is likely to be at least partly symptomatic of the heterogenous nature of mudflats (Beninger and Paterson 2018); although PAM measurements and syringe cores were paired, they were not taken in exactly the same spot to avoid sampling where MPB at the surface had already been disturbed. Additionally syringe cores sampled to a depth of 1cm, while PAM measures fluorescence at the surface. Diatoms have the potential to migrate while PAM measurements are being taken, which can lead to differences in results and lack of correlation between these two measurement techniques (Consalvey 2005). Despite these limitations, this experiment has used both approaches, therefore acknowledging this discrepancy the results can remain valuable in assessing patterns which may be present.

6.5.1 Hypothesis 1 - ABB significantly affects MPB surface biomass

Lack of significant interaction between surface MPB and artificial bird bioturbation (ABB) (Table 19) demonstrates that ABB did not significantly affect surface MPB biomass over the time investigated (165 days), not supporting hypothesis 1. As such the site dependent significant effects of shorebird density and assemblage on MPB found in Chapter 4 were unlikely to be driven primarily by shorebird bioturbation. However, interactions between fauna and MPB can be complex, and it is noted that effects of treatment in this experiment may have been masked by the greater temporal variation in MPB biomass. *P. ulvae* was present at the study site, which has been found to compensate for interactions between shorebirds and mudflat characteristics through MPB grazing and subsequent removal of effects at the primary producer level (Cheverie et al. 2014). This species could access all plots and could have masked treatment effects by grazing on biofilm and thus decreasing MPB biomass. The complexity of interactions between macrofauna, MPB and sediment characteristics (Beninger 2018a) may therefore have impeded detection of the role which ABB plays. Nevertheless, this 'masking' effect is a natural process and simply provides a possible explanation for the way in which a mudflat community within which sufficient MPB grazers are present may respond to changes in shorebird bioturbation.

6.5.2 Hypothesis 2 – ABB significantly reduces erodibility

In March ABB significantly increased the resistance of mudflat sediments to erosion (F=17.4, p=0.02) (Figure 6.08), leading to acceptance of hypothesis 2. Effects on erodibility are likely to be driven by changes in MPB which bind sediment through EPS production (Tolhurst et al. 2002, Tolhurst et al. 2009). Also in March, chla *a* (proxy for MPB biomass) was significantly greater in ABB plots (F=7.5, p<0.01) (Figure 36) showing that ABB caused a significant effect on MPB biomass and in turn, sediment erodibility. As discussed above, this may also have been partly caused by sediment compaction, which is known to increase the stability (ie reduce the erodibility) of fine intertidal sediments (Tolhurst et al. 2000b).

Although statistically a significant treatment effect on erodibility wasn't found in June, low ABB plots increased in erosion threshold to 5.5 Nm^{-2} (±0.9) from 2.5 Nm^{-2} (±0.6) in control plots (see Figure 6.08). In practical terms, this equates to potential for low ABB plots to be less susceptible to erosion during storm events. For example, tidal shear stress under 'normal' daily tidal flow on an intertidal mudflat, accounting for wave action, is in the range 0 - 1.5 Nm^{-2} (Amos 1995, Christie and Dyer 1998) and maximum sheer stresses under storm events can be in the order of 5 Nm^{-2} (Amos 1995).

Although macrofauna often cause a reduction in MPB biomass (Orvain et al. 2004, Orvain et al. 2014b, Rakotomalala et al. 2015), MPB-macrofauna interactions are complex and some types of bioturbation have been found to cause stimulation of MPB growth through fertilisation, by recycling nutrients below the surface (Wang et al. 2010, Chennu et al. 2015). The simplest explanation for ABB leading to significantly increased chlorophyll *a* content and reduced erodibility may therefore be that ABB mixed surface sediments fertilising MPB growth.

Regardless of the mechanism behind the measured effects, these effects on biofilm (following the results for chlorophyll *a*) show some characteristics that support the Intermediate Disturbance Hypothesis (IDH) (Connell 1978). In control plots with no surface sediment disturbance (bioturbation), chl *a* is lower than in low or high ABB plots (disturbed plots). Where ABB is low, chl *a* peaks; where ABB is high chl *a* is reduced (Figure 36), which is also reflected in the erodibility measurements. High ABB are likely to cause greater erodibility through increased surface roughness, similarly to *P. ulvae* which increases sediment erodibility this way (Orvain et al. 2004, Orvain et al. 2007).

6.5.3 Hypothesis 3 – ABB significantly affects organic matter and nutrient fluxes

In March during light incubation ammonium flux changed from an efflux from the sediment to an influx into the sediment under high ABB. MPB biofilms facilitate nutrient fluxes and can have a significant effect on the balances between the sediment and water column (Sundback and Graneli 1988, Sundback et al. 1991, Thornton et al. 2007). At the point of flux sampling in March, F_o (proxy for MBP biomass) was greatest in high bioturbation plots, indicating that the effect on ammonium may have been driven by increased MPB (Figure 36) which assimilate ammonium during photosynthesis (Underwood et al. 2022).

In June during dark incubation nitrate changed from an influx to an efflux under low ABB. At the point of flux sampling in June, F_o was not greater in low bioturbation plots than high, indicating that MPB driven process did not lead to the effect on nitrate. Under dark conditions, stimulation of bacteria by sediment reworking, causing nitrification, may have led to the reversal from influx to efflux of nitrate (Cheng et al. 2020). During June dissolved organic carbon (DOC) efflux from the sediment was greatest at low ABB and lowest in control plots, with significant differences detected between treatments. Macrofauna activities on intertidal sedimentary systems can have significant effects on carbon cycling, including crab burrowing (Wang et al. 2010) and bivalve bioturbation (Ruddy et al. 1998). Resuspension of MPB, a key fixer of organic carbon, can resuspend up to 43% of MPB organic carbon (Savelli et al. 2019), however this mechanism is not supported here due to increased MPB detected within ABB plots (Figure 36). The processes of turning and mixing sediments at the mudflat surface is therefore a likely cause of the increased release of DOC where shorebird ambulatory movement is simulated. These findings lead to acceptance of hypothesis 3.

Due to the significant increase in chlorophyll *a* during March in low ABB plots ($p<0.01$) (Figure 36) PCA was used to assess covariance between this variable and measured net fluxes of oxygen, nutrients and OM. Chlorophyll *a* had a very low quality of representation (cos2) (Figure 40), indicating that this was not a primary driver of nutrient flux effects detected within the variable plot. Mean colloidal carbohydrate and mean F_o were well represented in PCA plots, but these were not significantly different between treatments and therefore unlikely to have been driven by ABB (artificial bird bioturbation).

It was therefore found that ABB may account for a proportion of effects of shorebirds on MPB and associated ecosystem functioning and services, but that other processes are likely to be equal or more significant drivers, such as nutrient enrichment via droppings, feeding (probing) activity or direct biofilm removal by grazing (Chapters 3, 4 and 5).

6.5.4 Hypothesis 4 – ABB significantly affects macrofauna assemblage

No significant effect of ABB on macrofaunal assemblage between areas of no, low and high bioturbation was detected. This demonstrates that macrofauna community was not significantly affected by ABB, not supporting hypothesis 4. This element of the experiment was designed to control for potential effects of simulated bird activity, which could have altered macrofauna activity and in turn MPB biomass. No evidence of such an effect was detected, indicating that any effects from shorebird ambulatory bioturbation are likely to be caused by direct bird-MPB interactions.

There was a significant change in macrofaunal assemblage between seasons, a temporal effect described within the literature which can result in significant alterations in ecosystem functioning (Mestdagh et al. 2020). This could partly explain the difference in effects of ABB on ecosystem functions erodibility and nutrient flux between seasons. For example, *P. ulvae* can graze up to 28% of bacteria on an intertidal mudflat (Pascal et al. 2009). This species was less prevalent in June, when nitrate was changed to an efflux (Figure 39), potentially due to denitrification by bacteria when grazing pressure on bacteria was reduced.

Similarly, *H. diversicolor* can increase the sediment-water interface area by up to three times, through burrowing, which has been found to have varying effects on nutrient fluxes (Davey and Watson 1995, Kristensen and Hansen 1999, Mortimer et al. 1999, Kristensen 2001, Mermillod-Blondin and Rutger 2006, Pischedda et al. 2008) and can stimulate ammonium flux both in and out of the sediment, depending on local conditions (Nizzoli et al. 2007). *L. balthica* increases sediment erodibility (Widdows et al. 1998, Willows et al. 1998, Widdows et al. 2000a) and is reported to increase release of ammonium from the sediment (Mortimer et al. 1999, Michaud et al. 2005). Chironomid larvae also have significant effects on fluxes of ammonium between sediment and the water column (Shang et al. 2013, Benelli et al. 2018). During March these species were more prevalent in the macrofaunal assemblage (Figure 32), in accordance with a significant alteration in ammonium flux under light incubation (Figure 38).

A consistent trend within the nMDS plots (Figures 33 and 34) is the association of *L. balthica* with control plots and dissimilarity between this species and *H. diversicolor*, suggesting an avoidance of plots where sediment is regularly disturbed, and/or avoidance of *H. diversicolor* which predates *L. balthica* (Table 2 in Chapter 1). It is most likely that both factors contribute to this effect, as *L. batlhica* is an active suspension and deposit feeder, situating itself closer to the surface in late winter and spring (de Goeij and Luttikhuizen 1998), making it susceptible to surface disturbance. Although this species can rebury itself within 17 minutes after disturbance, this is likely to increase its susceptibility to predation (Budd and Rayment 2001).

6.5.5 Limitations

Masking effects are referred to frequently within the literature regarding interactions between infauna, MPB, sediment characteristics and higher predators (Gee et al. 1985, Grilo et al. 2011, Drolet and Barbeau 2012, Pascal et al. 2013, Tanaka et al. 2013, Dissanayake et al. 2022). Research also shows that MPB (Admiraal et al. 1984, Underwood and Paterson 1993, Underwood 1994), erodibility (Andersen 2001, Orvain et al. 2014a) nutrient fluxes (Shostell and Bukaveckas 2004) and macrofauna abundance (de Deckere et al. 2002, Sahan et al. 2007) across intertidal muddy sediments vary significantly between seasons, which may have contributed to differences in MPB biomass and effects of ABB on erodibility, macrofauna assemblage and organic matter and nutrient fluxes between March and June (Matos et al. 2022).

6.6 Conclusions

Artificial bird bioturbation has enabled this experiment to build upon previous work, by investigating how the movement of shorebirds across intertidal mudflats might contribute to their effects on ecosystem functioning and services.

In March, ABB caused a significant increase in chlorophyll *a* content (indicating MPB biomass) between treatments, an effect which was not detected in June. It is likely that the lack of detectable effect in June was due to the natural decrease in MPB abundance (Haro et al. 2022), reducing the magnitude of difference between treatments. Largest mean chl *a* content occurred in low ABB treatments, followed by high ABB treatments, suggesting that shorebird ambulatory bioturbation stimulates MPB growth until a specific threshold where disturbance has a greater detrimental effect. In this regard, this finding supports the intermediate disturbance hypothesis.

The increase in MPB led to significantly decreased sediment erodibility in ABB plots, with this effect strongest in low ABB treatments. Sediment 'stability' (low erodibility) is a critical ecosystem service associated with resilience of coastlines and maintenance of valuable intertidal mudflats. This demonstrates a clear link between shorebird bioturbation on an intertidal mudflat and the resistance of this habitat to erosion, with presence of shorebirds increasing resilience of the shoreline. Erodibility may have been lowest in low ABB plots because the greater surface roughness in high ABB plots counteracted the stabilising effect of MPB.

The increase in MPB in ABB plots during March is also likely to have contributed to significant changes which were detected in the flux of ammonium between the sediment and water column in March, which changed to net efflux under high ABB. In June nitrate changed to net efflux under low ABB and dissolved organic carbon increased in efflux under high ABB, although these findings suggest flux changes in June were driven by increased surface sediment mixing.

These findings give new insights into previously discovered effects of shorebirds on these ecosystem functions and services, demonstrating that ambulatory movement by shorebirds significantly alters both resilience of mudflats to erosion and integral parts of the carbon and nitrogen cycles. Thus, shorebirds should be considered ecosystem engineers, given their effects on this habitat.

7 Discussion

The conception of this studentship was based on previous work which suggested that cascade effects of shorebirds (by altering the distribution, abundance and behaviour of MPB grazing macrofauna), led to increased production of polysaccharides by MPB where shorebirds were present (Daborn et al. 1993). Conclusions made in Chapter 3 suggest that this was not the case where and when this study took place. Further, the field of shorebird science has transformed during the time this thesis was completed. As documented in a recent review paper (Beninger and Elner 2020), smaller sandpipers are now known to directly consume MPB, particularly during migratory periods; meaning shorebird effects previously interpreted as being indirect may actually also have direct effects, depending on species present. One such smaller sandpiper, Dunlin (*Calidris alpina*), frequents East of England coastlines during winter, including the study sites in this work, in significantly different numbers. This altered the course of the subsequent experiments and shifted the focus onto direct effects of shorebirds on MPB and EF.

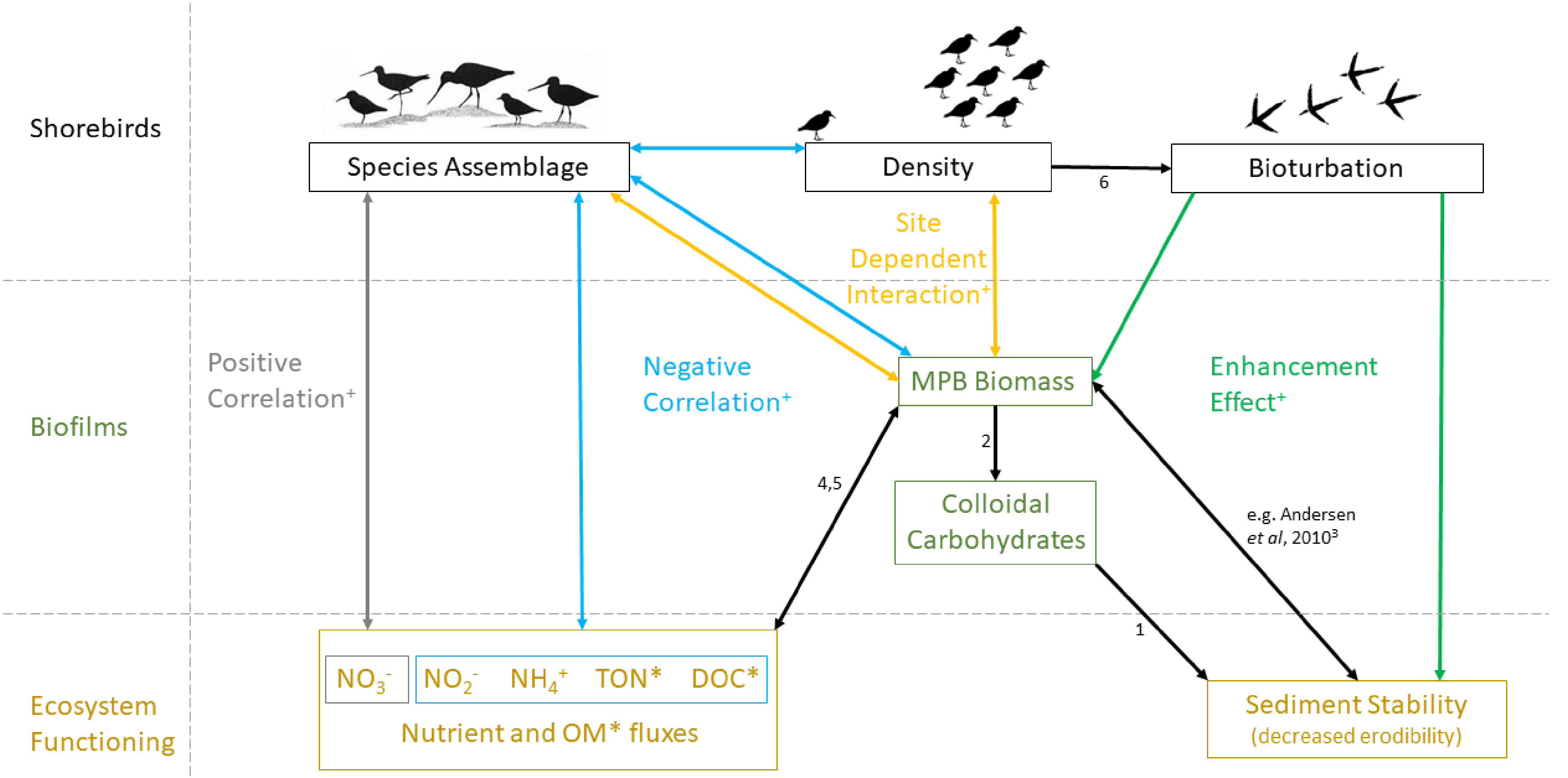
This thesis used a series of progressive and novel experiments to examine whether shorebirds had significant effects on ecosystem functioning (EF) on intertidal mudflats and provided evidence to assess how shorebird patch use, community composition and ambulatory bioturbation may affect ecosystem services (ES) including biogeochemical cycling and flood defence.

Key findings of this research on intertidal muddy sediments are summarised in Figure 42, including:

- In an exclusion experiment, shorebirds reduced microphytobenthos (MPB) biomass, reduced sediment critical erosion threshold τ_{cr}, nitrate fluxes into the sediment under illumination, nitrate efflux, and phosphate uptake (Chapter 3).
- There was a significant, positive correlation between bird assemblage/density and MPB biomass (Chapter 4).

The combined findings above demonstrate that the effects of shorebirds on biofilm mediated processes (erosion threshold and nutrient fluxes) depend on the shorebird community, which can be highly variable between years at the same site. This research suggests that relationships between shorebird community and MPB biomass were positive where shorebird communities included greater proportions of small sandpipers (dunlin), and negative where dunlin were largely absent.

- Shorebird density was positively correlated with nitrite flux and shorebird assemblage was negatively correlated with nitrite flux. To a lesser extent, total organic nitrogen (TON) correlated negatively with shorebird density and net nitrate correlated with shorebird density (direction depending on site) (Chapter 5).
- Reduced shorebird density caused ecologically significant changes in the fluxes of ammonium, silicate, nitrite, nitrate, and TON (Chapter 5).
- Simulated shorebird ambulatory movement increased MPB biomass, leading to significantly reduced sediment erodibility; and significantly altered fluxes of ammonium, nitrate and dissolved organic carbon (Chapter 6).



*OM=organic matter
*TON=total organic nitrogen
*DOC=dissolved organic carbon

+Arrowheads show effect directions

1. Yallop *et al* (2000) Microbial Ecology 39:116-127.
2. Blanchard *et al* (2000) Continental Shelf Research 20:1243-1256.
3. Andersen *et al* (2010) Coastal Shelf Science 87:197-206.
4. Thornton *et al* (2002) MEPS 27:285-300
5. Thornton *et al* (2007) MEPS 337:63-77.
6. Robar and Hamilton (2007) Waterbirds 30:116-123.

Figure 42: Summary of interactions found between shorebirds, microphytobenthos and ecosystem functioning on intertidal muddy sediments

7.1 Bird-MPB Interactions

A key theme across the findings of this thesis was the significant relationships between shorebirds (presence/density/bioturbation) and MPB biomass. The experiments presented in Chapters 3 and 6 tested for effects of shorebirds and artificial bird bioturbation, respectively, on macrofauna abundance. Results found that effects on macrofauna were not detectable, therefore an ecological cascade effect on MPB via macrofauna (Daborn et al. 1993) is not supported by this thesis.

Prey size class differs between shorebird species, which was not accounted for within the macrofauna analyses. Previous exclusion experiments on mudflats have found that infauna (specifically Corophium sp.) are significantly larger within exclosures than control plots (Raphaelli & Milne, 1987). This was likely due to prey size selectivity by shorebirds and suggests that there may have been effects on prey size class that were undetected in this thesis. However, macrofaunal community effects are notoriously difficult to detect on intertidal mudflats (Mathot et al. 2018), often resulting in ambiguity regarding mechanisms driving top-down effects (Hamilton et al. 2006, Cheverie et al. 2014). Therefore, the results of this thesis were interpreted carefully, acknowledging that a combination of factors were likely to have contributed to measured effects as is typical in mudflat ecology (Beninger and Paterson 2018).

For example, bioturbation by macrofauna can significantly affect MPB biomass (Hope et al. 2020). Chapter 6 found that bioturbation was also a direct pathway by which shorebirds could significantly increase MPB biomass, particularly during seasons when MPB biomass is greater, such as spring. The literature provides evidence supporting other possible direct effects which may have driven the findings of this thesis. This includes direct feeding on MPB by birds (Beninger and Elner 2020) and stimulation of diatom growth by dropping input (Jauffrais et al. 2015). Direct MPB feeding and increased MPB growth via droppings were indirectly manipulated during the experiments presented in Chapters 3, 4 and 5, results of which indicate that these are likely to have contributed to the significant effects on sediment erodibility and nutrient and organic matter fluxes found in this study.

This thesis has found that the relationship between birds and MPB is nuanced; for example shorebirds can decrease MPB biomass, which was likely to be due to grazing of MPB by dunlin during preparation for migration (Hobson et al. 2022) (Chapter 3). However, where shorebird density was manipulated across multiple sites, both positive and negative interactions between shorebird density and MPB (depending on site and shorebird species assemblage) were found (Chapter 4).

Overall, this thesis presents evidence that shorebird density and MPB biomass are related and depend partly on bird species assemblage (which also varies with site).

7.2 Chicken or Egg Scenario

This thesis has not sought to determine whether MPB biomass drives shorebird density, or vice versa. This creates a ‘chicken or egg’ scenario, in which either shorebird activity drives MPB biomass (depending on site and assemblage) or alternatively MPB biomass (which is variable among sites) drives shorebird patch selection (density) and species assemblage.

It is likely to be a combination of both, depending upon bird, macrofauna and MPB species assemblages. For example, only smaller sandpipers (dunlin in this case) are currently known to directly consume or use MPB as visual feeding cues (Jimenez et al. 2015, Beninger and Elner 2020), so where this species is present MPB may be the driver. Dunlin were only present consistently and in large numbers at two of the three study sites, indicating that at Trimley the effects were highly unlikely to be driven by dunlin MPB grazing. Different macrofauna communities also have varying effects on MPB biomass distribution (Morelle et al. 2021), leading to site differences in shorebird assemblage and distribution (Kuwae et al. 2021), which could indirectly affect bird density where small sandpipers are present. Conversely, regardless of species composition, bioturbation by birds (particularly ambulatory as investigated in Chapter 6) is an example of how bird density can drive MPB biomass.

7.3 Ecological interconnectedness

Nonetheless, the discovery of interactions between shorebird density and MPB were detectable across three study sites (Chapter 4) and led to significant effects on ecosystem functioning (Chapters 4 and 5). This represents a significant step forward in shorebird science. Ecological interconnectedness is a deep ecological notion describing the ‘mesh’ of all living organisms and their interactions with each other and their environment (Morton 2010). The findings of this thesis support this idea of interconnectedness; Chapter 4 demonstrates the interdependency between site (and many environmental variables which are linked with site), shorebird assemblage and shorebird density, and the effects of these variables on MPB biomass. Chapter 5 shows that bird density correlates positively and bird assemblage correlates negatively with a number of nutrient and organic matter fluxes and bird assemblage, which are mediated by MPB.

Feedback mechanisms have long been acknowledged within ecological theory (Wilson and Agnew 1992) and positive feedbacks whereby an organism benefits from modifications which it makes to its environment are being increasingly suggested (Pausas and Bond 2022). Positive feedback mechanisms can operate at population (niche) to planetary (Gaia hypothesis) scales (Pausas and Bond 2022). Shorebirds rely on MPB as the primary producer on an intertidal mudflat, either by feeding on macrofauna (which graze MPB) or directly consuming MPB (Méléder et al. 2020). In this regard, positive interactions between shorebird density and MPB biomass at two of three study sites indicate a positive feedback mechanism, whereby shorebird motile bioturbation, dropping input and other processes mediated by shorebirds cause MPB to proliferate.

Chapter 6 found that shorebird ambulatory bioturbation significantly increased sediment chlorophyll *a* content (an indicator of MPB biomass) and reduced sediment erodibility, suggesting that this process was a driver of the positive effects on MPB biomass found at two sites in Chapter 4. As discussed in Chapter 6, this was likely due to increased nutrient turnover and exposure when birds create footprints in the sediment. This process is an example of a way in which all shorebirds present at the sites contributed to interactions between birds and MPB biofilms and a positive feedback mechanism between shorebirds and MPB.

The relationships between shorebirds and MPB have considerable conservation implications. Shorebirds are a key part of their ecosystem, contributing to the maintenance of intertidal mudflat ecosystem functioning. As such, their conservation is important for the continued provision of ecosystem functioning and services associated with intertidal muddy sediments. Current literature provides key requirements of mudflat habitat with the aim that shorebird carrying capacity of MPB consuming shorebirds is maximised by optimising MPB colonisation (Kuwae et al. 2021). The findings of this thesis show that while enhancement of intertidal habitat is necessary, shorebirds also have their own role to play in maintaining the habitats on which they rely. There is a greater need to conserve shorebirds at all stages of their lifecycle (Jackson et al. 2019) because bird-MPB interactions, and therefore benefits, are two-way.

7.4 Conservation Significance

Long-distance migratory birds are in severe decline (Bairlein 2016) and combined effects of human pressure and climate change are forecast to exacerbate current rates of decline (Kubelka et al. 2022). The interactions between shorebird (the majority of which are migratory, see Chapter 1) density, assemblage, bioturbation and MPB discovered in this thesis are valuable to conservation efforts. Since the revision of the trophic structure between some shorebirds and

MPB (Kuwae et al. 2012), research in the field has increased with the aim of preserving and effectively managing important stopover and refuelling sites (Kuwae et al. 2021).

Biofilms contain polyunsaturated fatty acids (PUFAs), which are important in providing the high energy requirements of small bodied shorebirds for migration by enhancing oxidative capacity in cellular mitochondria (Maillet and Weber 2007) and increasing oxygen consumption in shorebird species such as Sanderling (Young 2019). This is now being tested in practical experiments, for example, unpublished wind tunnel experiments presented at the Western Hemisphere Shorebird Group Symposium in 2022 showed that birds fed on a PUFA-rich diet had increased flight energy efficiency, enabling them to fly longer and further on the same calories than other birds (Guglielmo, C. pers. comms).

The knowledge that a positive feedback mechanism exists between MPB and shorebirds plays an important part in their conservation, demonstrating that not only does effective management and provision of MPB benefit shorebirds, but that shorebird conservation may also aid in maintaining MPB biomass on intertidal mudflats, thereby also maintaining ecosystem functioning of this habitat. This is not to say that in the absence of shorebirds MPB would be lost from intertidal muddy sediments, particularly given the timescales over which shorebird effects on MPB have been demonstrated, and the fact that in many cases during these experiments MPB have been found to return to baseline conditions after peaks in shorebird density. Rather, this work reinforces suggestions made previously (Mathot et al. 2018) that shorebirds play an important role in the dynamic world of microphytobenthos, to the extent that knock-on effects on MPB mediated ecosystem functions have been detected.

7.5 Bird Ambulatory Bioturbation

As discussed above, there are numerous mechanisms which may have driven the interactions between birds and MPB found in this thesis. Chapter 6 explored a single type of effect of shorebirds on intertidal muddy sediments; walking (ambulatory bioturbation). Artificial bioturbation simulates only the physical aspect of the interaction between bird feet and the sediment surface; for example, the potential for introduction of bacteria or MPB from elsewhere (Muza et al. 2000, Buregyeya et al. 2014) was not been examined in this thesis.

Physical disturbance caused by birds is highly likely to play a role in interactions with MPB, and subsequently sediment erodibility and nutrient and organic matter fluxes on mudflats. The effect of sediment surface disturbance by macrofauna causing these effects is already established as summarised in Chapter 1 (Table 2).

Despite complexity caused by site specific differences (Hubas et al. 2018), effects of sediment bed roughness (Dairain et al. 2020) and resulting conflicting evidence (Table 2) it is accepted that physical reworking of surface sediments does have significant effects on MPB biomass and associated ecosystem functions including sediment erodibility and nutrient/OM fluxes (Passarelli et al. 2018). These effects extend from meiofauna to macrofauna such as fiddler crabs which burrow and produce feeding pellets (Citadin et al. 2016). It follows that similar sediment working by shorebirds would therefore have similar effects on ecosystem functioning.

Compared to controls with no bird bioturbation, low bioturbation caused significantly increased chlorophyll *a* concentration and significantly reduced sediment erodibility. This discovery begins to answer the 'chicken and egg' question raised in Section 7.2 above, suggesting that shorebird bioturbation accounts, at least partly, for the correlation with increased MPB biomass found in Chapter 4. Where bioturbation was large, only sediment erodibility was significantly reduced. This indicates that, similarly to the complexity evident with macrofauna, bioturbation by birds may increase MPB biomass to a point, beyond which MPB biomass is not enhanced and increased roughness leads to reduced sediment stability.

7.6 Shorebirds as Ecosystem Engineers

An 'ecosystem engineer' is an organism which maintains, alters or creates an environment that significantly affects other organisms (Jones et al. 1994). Birds perform these roles, including European bee-eaters (*Merops apiaster*) which bioturbate and burrow within arid terrestrial environments, creating niches utilised by other species (Casas-Crivillé and Valera 2005). Eurasian cranes (*Grus grus*) can increase plant diversity in grasslands through disturbing patches (Valkó et al. 2022) and woodpeckers (Picidae) provide sheltering and nesting resources for other animals within urban parks (Catalina-Allueva and Martín 2021).

Ecosystem engineering takes place within the intertidal, for example limpets (*Patella cochlear*) enhance the productivity of their algal 'gardens' by fertilising them with ammonium and nitrate (Plagányi and Branch 2000). Macrofauna which rework sediment and form structures such as burrows are also ecosystem engineers, through increasing oxygenated sediments and species diversity of endobenthic organisms (Passarelli et al. 2018).

The findings of this thesis support the classification of shorebirds as ecosystem engineers, by showing that through their activities they can increase or decrease MPB biomass and can have positive effects on sediment stability (reducing erodibility). This has a knock-on effect to other ecosystem functions nutrient and organic matter fluxes between the sediment and water column. The significant interaction between birds and MPB alone is sufficient to support the classification of birds as engineers. Beyond this, the decreased sediment erodibility increases the resilience of the mudflat to erosion. The relationship between shorebird density and the nitrogen cycle (nitrite, nitrate and total organic nitrogen) also further extends the reach of shorebird-MPB interactions.

The effects of an ecosystem engineer can shift from neutral or negative to positive (or vice versa) when abiotic environmental conditions become more harsh (Donadi et al. 2013). The majority of studies supporting this have considered foundation species, which have engineering effects based on their physical attributes rather than their activity (Donadi et al. 2013). Those which modify their environment due to their activities are known as allogenic ecosystem engineers, and less work has been published investigating the effect of abiotic variables on engineering effects (Donadi et al. 2013). However, work by Donadi *et al* (2013) suggests that allogenic ecosystem engineers have an increasingly significant role where environmental stress is greater. Thus, the effects of shorebirds presented here may become more pronounced, detectable, or significant as climate change progresses.

This is further supported by this thesis, which has identified a significant interaction between shorebird community and MPB biomass, to the extent that across sites with different community compositions the correlation between shorebird density and MPB biomass can be reversed from positive to negative (or vice versa). As climate change continues, the ranges of species and therefore communities is seen to be altering and projected to continue to do so, particularly in birds (Riddell et al. 2019, Cadieux et al. 2020, Kuwae et al. 2021, Riddell et al. 2021). This thesis demonstrates that as community shifts occur, for example to include greater or smaller proportions of small sandpipers, the interactions between shorebirds are also likely to be altered and potentially move from positive to negative (or vice versa) correlations between shorebird density and MPB biomass.

7.7 Limitations and Future Research

This thesis has not accounted for differences in MPB species richness or diversity among sites or plots, which are known to vary in time and space (Hubas et al. 2018). However, it has been shown elsewhere that a positive relationship exists between diatom diversity and productivity (measured using PAM fluorometry) (Koedooder et al. 2019). As such, potential differences in MPB diversity and productivity could explain some effects but are unlikely to have caused a significant limitation to the interpretation of the results. An important area for further research would be experimental testing of shorebird effects on MPB species richness and diversity, with a hypothesis that increasing shorebird diversity increases MPB species richness and diversity by influencing MPB biomass or sediment texture, both of which can be related to MPB diversity (Ribeiro et al. 2021). Finding that shorebirds alter MPB diversity (particularly if they increase competition and therefore maintain diversity) could have important implications for the maintenance of resilience of benthic mudflat communities (Virta et al. 2021).

Bioturbation by shrimps can affect bacterial communities (Li et al. 2020) which have significant roles within nutrient and organic matter recycling on intertidal mudflats (Dufour 2018, Van Colen 2018). It remains plausible therefore, that the interactions between birds and biofilms that are identified within this thesis are mediated in some way by other factors, such as bacterial communities or meiofauna.

Chlorophyll *a* measurements across the experiments tend to show greater differences within plots than treatments, which can be explained by greater variance in biofilm biomass at smaller spatial scales (Taylor et al. 1999, Paterson et al. 2000, Tolhurst et al. 2006a, Redzuan 2017). While the experiments in this thesis were sufficiently powerful to detect effects at the metre scale, it is possible that they are stronger at the cm scale and this is something which it would be beneficial to investigate further.

The multi-site investigations (see Chapters 4 and 5) results showed that while within-site data can support previous findings, between site data may fail to identify similar patterns. For example, the Chapter 4 linear mixed effects model examining shorebird density, site and bird assemblage found a significant interaction among all three sites. This variability among sites has been found elsewhere (Chapman et al. 2010) and different sites can be most influenced by variables such as algal blooms, diatom migration, macrofauna abundance and MPB biomass (Defew et al. 2002). At first glance, these findings may point to the 'reproducibility crisis', whereby reproducing experiments and finding reinforcing results is a rare phenomenon within the natural sciences (Baker 2016). However, it is more convincing in this case to consider, in Ecology at least, the possibility of a 'variability crisis' where results in field experiments are subject to such an array of interacting factors which vary spatially and temporally that properly investigating these sources of variability is unrealistic outside of large multi-year interdisciplinary projects. This theory has been tested experimentally on sandflats in New Zealand, where researchers found that 66-99% of variability in between site treatment effects could be explained by the wind-wave exposure of the sites (Thrush et al. 2000).

As discussed in Section 7.2, this thesis has begun to unravel which factors are responsible for the significant positive bird-MPB correlation found (Chapter 4), with Chapter 6 indicating that bird bioturbation is a significant driver. However, this does not appear to account for the entirety of the effects found in Chapter 4, because a significant correlation between footprint % cover (bird density) and MPB biomass was not detected in Chapter 6. It can therefore be hypothesised that other factors also contributed to the significant positive bird-MPB correlation. These may include nutrient input to the sediment via droppings (Jauffrais et al. 2015) or bioturbation associated with bird bill probing (similarly to polychaete worm burrows) (Widdows et al. 2009). These possible mechanisms could be experimentally tested by artificially spreading bird droppings (preferably shorebird droppings if sourced) or a chemical derivative, or creating artificial bill probing holes (respectively) within treatment plots and comparing them to control plots using the measurement techniques described herein. This type of approach has been used to test the effect of dunlin faeces on MPB growth (Jauffrais et al. 2015), although this was under laboratory conditions and a field experiment may yield different results due to inclusion of all relevant environmental factors. It is also plausible that attraction of shorebirds to MPB either using MPB as visual cues (Jimenez et al. 2015) or to graze (Drouet et al. 2015) plays a role in the identified bird-MPB relationship.

This work has identified that both shorebird species assemblage (Chapter 4) and ambulatory bioturbation (Chapter 6) can affect MPB biomass. This suggests a possible link between shorebird species assemblage and sediment surface bioturbation, and highlights an interesting area for further research. This could be approached using field sampling techniques similar to those used within this thesis and collecting additional data by identifying patch use to species group level. While a lack of resources exists to allow definitive identification of shorebird species based on tracks alone, using track size could be a legitimate method enabling a similar approach to the shorebird body size grouping used within chapters 4 and 5. An experimental approach such as this may begin to untangle how different shorebird size classes drive sediment surface bioturbation, and in turn the combined effects of these drivers of MPB biomass.

Ideally, future work in this area would be integrated into larger scale multidisciplinary, multi-site, long term investigations to account for the inherent differences between sites discussed above and in Chapter 4, which would be more likely to allow for widespread applicability of findings to mudflat research and conservation globally (Mathot et al. 2018). It is acknowledged that only recently have shorebirds been inducted into the ‘mud-club’ (Mathot et al. 2018) and this thesis has demonstrated the interconnected role which shorebirds play in intertidal muddy sediments. Classical mudflat study designs typically include sedimentological and macrofaunal measurements, less commonly involving diatom community composition (Beninger 2018a). Widespread integration of data collection such as shorebird footprint cover (to assess bird density within a patch), overall use of sites by shorebirds (to contextualise patch use) and shorebird community into classical mudflat study designs will be crucial to fully understand the role which shorebirds play within this environment.

Regardless of the specific pathways, this thesis has brought to light previously unknown shorebird-MPB interactions that are probably mediated through an array of mechanisms, many of which have already been identified within the literature for other groups. This interaction has knock-on implications for ecosystem functioning (EF) on intertidal mudflats, including sediment erodibility and nutrient and organic matter fluxes. These EFs are important parts of ecosystem services including flood and storm resilience of the coastline, nutrient cycling and carbon sequestration, giving wide relevance to bird-MPB interactions and helping to inform future management of intertidal muddy sedimentary habitats.

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Appendices

Appendix 1 – Chapter 3 Plaster Ball Dissolution Results

Table 20 Plaster ball dissolution results (mass (g) at zero hours and 24 hours, and percent decrease in mass during this period) as measured adjacent to Geedon Saltings, Essex Wildlife Trust Fingringhoe Wick, Essex, on 06 and 07 February 2017 (grid reference TM 05065 19030).

Plot	Treatment	Mass 0 h (g)	Mass 24 h (g)	% Decrease
1	Control	100.6	92.6	8.0
1	Exclosure	101.6	93.4	8.0
4	Control	98.9	91.2	7.8
4	Exclosure	105.6	95.6	9.5
6	Control	105.6	98.3	6.9
6	Exclosure	112.8	104.7	7.2
8	Control	102.9	95.6	7.1
8	Exclosure	98.7	92.1	6.7
10	Control	100.5	95.2	5.3
10	Exclosure	99.4	92.2	7.2

Appendix 2 – Chapter 4 ANOVA Tables

Table 21 ANOVA models and results for measured variables on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030) between 20 January and 03 April 2017.

Model	**Factor**	**Source**			
		df	**MS**	**F**	**P**
Chlorophyll *a* ug g^{-1} = MEAN + TREAT + Plot(TREAT) + RES	Roofed/Control = TREAT	1	6652	0.54	0.5
	Plot (TREAT)	2	12302	11.8	.0001
	Residual	36	1047	-	-
Carbohydrates ug g^{-1} = MEAN + TREAT + Plot(TREAT) + RES	Roofed/Control = TREAT	1	3621432	1.49	0.3
	Plot (TREAT)	2	2427732	3.59	0.037
	Residual	36	675371	-	-
Erosion threshold Nm^{-2} = MEAN + TREAT + Plot(TREAT) + RES	Roofed/Control = TREAT	1	25.5	8.45	**0.04**
	Plot (TREAT)	4	3	1.04	0.3
	Residual	54	2.9	-	-
H_2O Content % = MEAN + TREAT + Plot(TREAT) + RES	Roofed/Control = TREAT	1	2.37	0.15	0.7
	Plot (TREAT)	2	15.95	6.03	0.0055
	Residual	36	2.6	-	
Grain size um = MEAN + TREAT + Plot(TREAT) + RES	Roofed/Control = TREAT	1	4.9	0.03	0.9
	Plot (TREAT)	2	165	3.34	0.046
	Residual	36	49.6	-	-

Table 22 ANOVA models and results for measured variables on the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (grid reference TM 12427 33311) between 20 January and 03 April 2017.

Model	Factor	Source			
		df	MS	F	P
Chlorophyll *a* ug g^{-1} = MEAN + TREAT + Plot(TREAT) + RES	Roofed/Control = TREAT	1	25010	0.93	0.4
	Plot (TREAT)	2	26863	4.74	0.0158
	Residual	32	5671	-	-
Carbohydrates ug g^{-1} = MEAN + TREAT + Plot(TREAT) + RES	Roofed/Control = TREAT	1	37222550	0.38	0.6
	Plot (TREAT)	2	96993688	4.05	0.027
	Residual	32	23943091	-	-
Erosion threshold Nm^{-2} = MEAN + TREAT + Plot(TREAT) + RES	Roofed/Control = TREAT	1	1.3	3.32	0.2
	Plot (TREAT)	2	0.4	0.18	0.8
	Residual	32	2.25	-	-
H_2O Content % = MEAN + TREAT + Plot(TREAT) + RES	Roofed/Control = TREAT	1	0.0083	1.06	0.41
	Plot (TREAT)	2	0.0078	5.81	0.007
	Residual	32	0.0013	-	-
Grain size um = MEAN + TREAT + Plot(TREAT) + RES	Roofed/Control = TREAT	1	2.15	0.01	0.9
	Plot (TREAT)	2	173	7.09	0.0028
	Residual	32	24	-	-

Table 23 ANOVA models and results for measured variables on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406 between 20 January and 03 April 2017.

Model	Factor		Source			
			df	MS	F	P
Chlorophyll *a* ug g^{-1} = MEAN + TREAT + Plot(TREAT) + RES	Roofed/Control	=	1	50.4	0.4	0.6
	TREAT		2	133.2	2.8	0.076
	Plot (TREAT)		36	48.2	-	
	Residual					
Carbohydrates ug g^{-1} = MEAN + TREAT + Plot(TREAT) + RES	Roofed/Control	=	1	7836273	6.65	0.1
	TREAT		2	1179266	1.3	0.3
	Plot (TREAT)		36	906973	-	-
	Residual					
Erosion threshold Nm^{-2} = MEAN + TREAT + Plot(TREAT) + RES	Roofed/Control	=	1	0.0042	0.08	0.8
	TREAT		2	0.053	3.26	0.05
	Plot (TREAT)		36	0.017	-	-
	Residual					
H_2O Content % = MEAN + TREAT + Plot(TREAT) + RES	Roofed/Control	=	1	35.7	1.09	0.4
	TREAT		2	32.7	8.91	0.0007
	Plot (TREAT)		36	3.7	-	-
	Residual					
Grain size um = MEAN + TREAT + Plot(TREAT) + RES	Roofed/Control	=	1	24.9	2.2	0.28
	TREAT		2	11.3	0.46	0.63
	Plot (TREAT)		36	24.5	-	-
	Residual					

Appendix 3 – Chapter 5 ANOVA Tables

Table 24 ANOVA models and results for measured nutrient fluxes on day 147 (28 February 2019) on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick SSSI EWT Nature Reserve (TM 05065 19030).

Model	Factor	Source			
		df	MS	F	P
Ammonium = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	125537	0.91	0.35
	Roofed/Control = R/C	1	36363	0.26	0.61
	D/L x R/C	1	222439	1.62	0.22
	Residual	20	137580		
Nitrate = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	5	1.9	0.17
	Roofed/Control = R/C	1	0.6	0.2	0.6
	D/L x R/C	1	0.3	0.1	0.7
	Residual	20	2.5		
Nitrite = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	0.16.9	0.04	0.85
	Roofed/Control = R/C	1	10.1	2.00	0.17
	D/L x R/C	1	3.5	3.12	0.09
	Residual	20			
Phosphate = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	52410	1.10	0.30
	Roofed/Control = R/C	1	34540	0.72	0.4
	D/L x R/C	1	47845	1.00	
	Residual	20	47852		
Silicate = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	896262	8.70	**0.008**
	Roofed/Control = R/C	1	1682	0.02	0.89
	D/L x R/C	1	14620	0.14	0.71
	Residual	20	102962		
DOC = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	241292	124.	**0.0000**
	Roofed/Control = R/C	1	5389	52	0.11
	D/L x R/C	1	28	2.78	0.91
	Residual	20	1937	0.01	
TON = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	0.41	0.06	0.81
	Roofed/Control = R/C	1	0.65	0.09	0.76
	D/L x R/C	1	15.19	2.22	0.15
	Residual	20	6.85		

Table 25 ANOVA models and results for measured nutrient fluxes on day 181 (04 April 2019) on the Newmill Lane mudflat on the Stour estuary to the south-east of Brantham, Suffolk, UK (grid reference TM 12427 33311).

Model	**Factor**	**Source**			
		df	**MS**	**F**	**P**
Ammonium = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	1123666	1.72	0.20
	Roofed/Control = R/C	1	277951	0.43	0.52
	D/L x R/C	1	810792	1.24	0.28
	Residual	20	651449		
Nitrate = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	486567238	21.30	**0.000**
	Roofed/Control = R/C	1	21495265	0.13	**2**
	D/L x R/C	1	6863041	0.04	0.72
	Residual	20	163706623		0.84
Nitrite = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	165800	2.38	0.14
	Roofed/Control = R/C	1	118309	1.70	0.21
	D/L x R/C	1	117749	1.69	0.21
	Residual	20	69628		
Phosphate = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	1284	4.52	**0.04**
	Roofed/Control = R/C	1	62	0.22	0.64
	D/L x R/C	1	141	0.50	0.49
	Residual	20	284		
Silicate = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	683808581	25.45	**0.000**
	Roofed/Control = R/C	1	5468789	0.20	**1**
	D/L x R/C	1	12407833	0.46	0.66
	Residual	20	26870289		0.50
DOC = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	6971742	112.0	**0.000**
	Roofed/Control = R/C	1	23107	4	**0**
	D/L x R/C	1	2993	0.37	0.55
	Residual	20	62224	0.05	0.83
TON = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	568.76	57.65	**0.000**
	Roofed/Control = R/C	1	0.0007	0.00	**0**
	D/L x R/C	1	22.48	2.28	0.99
	Residual	20	9.90		0.15

Table 26 ANOVA models and results for measured nutrient fluxes on day 188 (27 April 2019) on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (grid reference TM 25504 35406).

Model	**Factor**	**Source**			
		df	**MS**	**F**	**P**
Ammonium = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	99627	4.41	0.049
	Roofed/Control = R/C	1	119487	5.28	**0.03**
	D/L x R/C	1	7159	0.32	0.60
	Residual	20	22610		
Nitrate = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	2641	0.30	0.59
	Roofed/Control = R/C	1	1873	0.22	0.65
	D/L x R/C	1	2943	0.34	0.57
	Residual	20	8699		
Nitrite = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	206.7	14.08	**0.001**
	Roofed/Control = R/C	1	37.44	2.55	0.13
	D/L x R/C	1	75.13	5.12	**0.04**
	Residual	20	14.68		
Phosphate = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	2343	8.84	**0.008**
	Roofed/Control = R/C	1	10.16	0.04	0.85
	D/L x R/C	1	41.60	0.16	0.70
	Residual	20	265.1		
Silicate = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	408225	5.79	**0.025**
	Roofed/Control = R/C	1	508746	7.22	**0.014**
	D/L x R/C	1	391907	5.56	**0.029**
	Residual	20			
DOC = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	93591	0.69	0.41
	Roofed/Control = R/C	1	100353	0.74	0.40
	D/L x R/C	1	12208	0.09	0.77
	Residual	20	1348730.69		
TON = MEAN + D/L + R/C + D/L x R/C + RES	Dark/Light = D/L	1	66.01	13.41	**0.002**
	Roofed/Control = R/C	1	7.887	1.60	0.220
	D/L x R/C	1	0.290	0.06	0.811
	Residual	20	4.923		

Appendix 4 – Chapter 5 PCA Variables Removed

Table 27 Removal record of variables poorly represented by PC1 (cos2 <0.1), or which were redundant (due to covariance with light/dark incubation or net flux of the same variable) during PCA analysis in production of Figure 5.4.

Removed Variable	Reason
LI_oxy	Cos2 value = 0.10
DI_oxy	Covaries with Net_oxy
DI_Amm	Covaries with Net_Amm
DI_Nitri	Covaries with Net_nitri
LI_Nitri	Cos2 value = 0.14
DI_DOC	Covaries with Net_DOC
LI_DOC	Negatively covaries with Net_DOC
DI_TON	Covaries with Net TON
LI_TON	Cos2 value = 0.13
LI_Nitra	Negatively covaries with Net_Nitra
LI_Sil	Negatively covaries with Net_Sil
LI_Phos	Negatively covaries with Net_Phos
DI_Phos	Negatively covaries with Net_Phos

Table 28 Removal record of variables poorly represented by PC1 (cos2 <0.1), or which were redundant (due to covariance with light/dark incubation or net flux of the same variable) during PCA analysis in production of Figures 5.6, 5.7 and 5.8.

Site	**Removed Variable**	**Reason**
Brantham	DI_Amm	Covariance with Net_Amm
Brantham	LI_TON	-ve covariance with Net_TON
Brantham	DI_TON	Covariance with Net_TON
Brantham	LI_Sil	-ve covariance with Net_Sil
Brantham	DI_Sil	Cos2 = 0.2
Brantham	DI_Nitra	-ve covariance with Net_Nitra
Brantham	LI_Nitra	-ve covariance with Net_Nitra
Brantham	DI_Phos	Covariance with Net_Phos
Brantham	DI_Oxy	Covariance with Net_Oxy
Brantham	LI_Oxy	-ve covariance with Net_Oxy
Brantham	LI_Nitri	-ve covariance with Net_Nitri
Brantham	DI_Nitri	Covariance with Net_Nitri
Brantham	LI_DOC	-ve covariance with Net_DOC

Table 28: Removal record of variables poorly represented by PC1 (cos2 <0.1), or which were redundant (due to covariance with light/dark incubation or net flux of the same variable) during PCA analysis in production of Figures 5.6, 5.7 and 5.8.

Site	Removed Variable	Reason
Fingringhoe	DI_Nitra	Covariance with Net_Nitra
Fingringhoe	LI_Nitra	Cos2 <0.2
Fingringhoe	DI_Sil	Covariance with Net_Sil
Fingringhoe	LI_Sil	Cos2 <0.1
Fingringhoe	DI_Amm	Covariance with Net_Amm
Fingringhoe	LI_Amm	-ve covariance with Net_Amm
Fingringhoe	DI_TON	Covariance with Net_TON
Fingringhoe	LI_TON	-ve covariance with Net_TON
Fingringhoe	LI_Phos	-ve covariance with Net_Phos
Fingringhoe	DI_Oxy	Covariance with Net_Oxy
Fingringhoe	DI_Nitri	Covariance with Net_Nitri
Fingringhoe	LI_Nitri	Covariance with Net_Nitri
Fingringhoe	DI_DOC	Covariance with DOC
Fingringhoe	LI_DOC	-ve covariance with Net_DOC
Trimley	DI_Oxy	Covariance with Net_Oxy
Trimley	LI_Oxy	-ve covariance with Net-Oxy
Trimley	DI_Nitri	Covariance with Net_Nitri
Trimley	DI_Nitra	Covariance with Net_Nitra
Trimley	LI_Nitra	Covariance with Net_Nitra
Trimley	DI_Phos	Covariance with Net_Phos
Trimley	DI_DOC	Covariance with Net_DOC
Trimley	DI_Amm	Covariance with Net_Amm
Trimley	LI_Amm	-ve covariance with Net_Amm
Trimley	DI_Sil	Covariance with Net_Sil
Trimley	LI_Sil	-ve covariance with Net_Sil
Trimley	DI_TON	Covariance with Net_TON
Trimley	LI_TON	-va covariance with Net_TON

Appendix 5 – Chapter 6 ANOVA Tables

Table 29 ANOVA model and results for each tested variable between the 8th of January and the 24th of June 2021, on Days 74 (A) and 165 (B), on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (TM 25504 35406).

ANOVA	Factor	DF	MS	F	P
F_o (March) = MEAN + TREAT + Plot(Treat) + RES	Treat	2	30608	0.2	0.8
	Plot(Treat)	12	135467	4.8	**<0.0001**
	Res	60	28048	-	-
F_o (June) = MEAN + TREAT = + Plot(Treat) + RES	Treat	2	28247	0.6	0.5
	Plot(Treat)	12	44034	2.0	0.03
	Res	60	21699	-	-
τ_{cr} (March) = MEAN + TREAT = + Plot(Treat) + RES	Treat	2	52.7	17.4	**0.022**
	Plot(Treat)	3	3.0	0.5	0.7
	Res	24	6.2	-	-
τ_{cr} (June) = MEAN + TREAT = + Plot(Treat) + RES	Treat	2	25.0	3.3	0.17
	Plot(Treat)	3	7.5	1.2	0.3
	Res	24	6.1	-	-
Syringe Colloidal (March) = MEAN + TREAT = + Plot(Treat) + RES	Treat	2	62	0.03	1.0
	Plot(Treat)	12	2104	12.9	**<0.0001**
	Res	60	163		
Syringe Colloidal (June) = MEAN + TREAT = + Plot(Treat) + RES	Treat	2	2729	2.8	0.09
	Plot(Treat)	12	970	3.2	**0.001**
	Res	60	302		
Syringe Chl *a* (March) = MEAN + TREAT = + Plot(Treat) + RES	Treat	2	7257	7.5	**0.008**
	Plot(Treat)	12	964	6.4	**<0.00001**
	Res	60	151		
Syringe Chl *a* (June) = MEAN + TREAT = + Plot(Treat) + RES	Treat	2	13.0	0.2	0.9
	Plot(Treat)	12	85.7	5.0	**0.000**
	Res	60	17.1	-	-
O_2 (March) = MEAN + D/L + TREAT + D/L x TREAT + RES	D/L	1	34353492	7.4	**0.01**
	Treat	2	10745558	2.3	0.1
	D/L x Treat	2	1102709	0.2	0.8
	Res	18	4625588		
O_2 (June) = MEAN + D/L + TREAT + D/L x TREAT + RES	D/L	1	9.4	0.2	0.7
	Treat	2	19	0.3	0.7
	D/L x Treat	2	6.1	0.1	0.9
	Res	18	60		
Net O_2 (March) = MEAN + TREAT + RES	Treat	2	11606723	1.79	0.2
	Res	6	6485902		
Net O_2 (June) = MEAN + TREAT + RES	Treat	2	9012159	0.36	0.7
	Res	6	24856156		

Table 29 ANOVA model and results for each tested variable between the 8th of January and the 24th of June 2021, on Days 74 (A) and 165 (B), on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (TM 25504 35406).

ANOVA	Factor	DF	MS	F	P
NO_3 (March) = MEAN + D/L + TREAT + D/L x TREAT + RES	D/L	1	777519	0.5	0.5
	Treat	2	331538	0.2	0.8
	D/L x Treat	2	747264	0.5	0.6
	Res	18	1571500		
NO_3 (June) = MEAN + D/L + TREAT + D/L x TREAT + RES	D/L	1	280	7.5	**0.01**
	Treat	2	55	1.5	0.3
	D/L x Treat	2	45	1.2	0.3
	Res	18	37		
Net NO_3 (March) = MEAN + TREAT + RES	Treat	2	2485574	0.47	0.6
	Res	6	5321602		
Net NO_3 (June) = MEAN + TREAT + RES	Treat	2	16863	0.67	0.5
	Res	6	25247		
NO_2 (March) = MEAN + D/L + TREAT + D/L x TREAT + RES	D/L	1	14	0.2	0.6
	Treat	2	31	0.5	0.6
	D/L x Treat	2	9.4	0.2	0.9
	Res	18	59		
NO_2 (June) = MEAN + D/L + TREAT + D/L x TREAT + RES	D/L	1	126	2.5	0.1
	Treat	2	52	1.0	0.4
	D/L x Treat	2	1.3	0.03	1.0
	Res	18	51		
Net NO_2 (March) = MEAN + TREAT + RES	Treat	2	635	0.04	0.9
	Res	6	17002		
Net NO_2 (June) = MEAN + TREAT + RES	Treat	2	84	0.11	0.9
	Res	6	790		
SiO_3^{2-} (March) = MEAN + D/L + TREAT + D/L x TREAT + RES	D/L	1	75462	0.2	0.7
	Treat	2	19315	0.05	1.0
	D/L x Treat	2	129856	0.3	0.7
	Res	18	423477		
SiO_3^{2-} (June) = MEAN + D/L + TREAT + D/L x TREAT + RES	D/L	1	534348	1.5	0.2
	Treat	2	24012	0.1	0.9
	D/L x Treat	2	146761	0.4	0.6
	Res	18	358459	-	-
Net SiO_3^{2-} (March) = MEAN + TREAT + RES	Treat	2	593860	0.52	0.6
	Res	6	1149565		
Net SiO_3^{2-} (June) = MEAN + TREAT + RES	Treat	2	293522	1.24	0.3
	Res	6	236142		

Table 29 ANOVA model and results for each tested variable between the 8th of January and the 24th of June 2021, on Days 74 (A) and 165 (B), on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (TM 25504 35406).

ANOVA	Factor	DF	MS	F	P
PO_4^{3-} (March) = MEAN + D/L + TREAT + D/L x TREAT + RES	D/L	1	66	0.02	0.9
	Treat	2	87	0.03	1.0
	D/L x Treat	2	173	0.06	0.9
	Res	18	2887		
PO_4^{3-} (June) = MEAN + D/L + TREAT + D/L x TREAT + RES	D/L	1	9319	14	**0.001**
	Treat	2	442	0.7	0.5
	D/L x Treat	2	13	0.02	0.1
	Res	18	660	-	-
Net PO_4^{3-} (March) = MEAN + TREAT + RES	Treat	2	88	0.01	0.9
	Res	6	6326		
Net PO_4^{3-} (June) = MEAN + TREAT + RES	Treat	2	25	0.01	0.9
	Res	6	2057		
NH_4 (March) = MEAN + D/L + TREAT + D/L x TREAT + RES	D/L	1	35661	3.7	0.07
	Treat	2	16792	1.7	0.2
	D/L x Treat	2	10527	1.1	0.4
	Res	18	9646		
NH_4 (June) = MEAN + D/L + TREAT + D/L x TREAT + RES	D/L	1	9967	0.1	0.8
	Treat	2	8026	0.1	0.9
	D/L x Treat	2	56173	0.4	0.7
	Res	18	143656	-	-
Net NH_4 (March) = MEAN + TREAT + RES	Treat	2	19988	0.91	0.4
	Res	6	21990		
Net NH_4 (March) = MEAN + TREAT + RES	Treat	2	8132	0.05	0.9
	Res	6	165333		

Table 29 ANOVA model and results for each tested variable between the 8th of January and the 24th of June 2021, on Days 74 (A) and 165 (B), on the mudflat on the Orwell estuary at Trimley Marshes Suffolk Wildlife Trust (SWT) Nature Reserve, Suffolk, UK (TM 25504 35406).

ANOVA	Factor	DF	MS	F	P
DOC (March) = MEAN + D/L + TREAT + D/L x TREAT + RES	D/L	1	344	1.3	0.3
	Treat	2	49	0.2	0.8
	D/L x Treat	2	41	0.2	0.9
	Res	18	264		
DOC (June) = MEAN + D/L + TREAT + D/L x TREAT + RES	D/L	1	312	0.5	0.5
	Treat	2	1809	2.6	0.1
	D/L x Treat	2	2677	3.9	<0.04
	Res	18	684	-	-
Net DOC (March) = MEAN + TREAT + RES	Treat	2	12	0.01	0.9
	Res	6	964		
Net DOC (June) = MEAN + TREAT + RES	Treat	2	5354	2.37	0.1
	Res	6	2257		
TON (March) = MEAN + D/L + TREAT + D/L x TREAT + RES	D/L	1	204	4.6	0.05
	Treat	2	41	0.9	0.4
	D/L x Treat	2	33	0.8	0.5
	Res	18	44		
TON (June) = MEAN + D/L + TREAT + D/L x TREAT + RES	D/L	1	0.2	0.1	0.8
	Treat	2	3.3	0.9	0.4
	D/L x Treat	2	1.1	0.3	0.7
	Res	18	3.5	-	-
Net TON (March) = MEAN + TREAT + RES	Treat	2	65	2.09	0.2
	Res	6	31		
Net TON (June) = MEAN + TREAT + RES	Treat	2	2.2	0.26	0.8
	Res	6	8.7		

Appendix 6 – Published Original Research Article

frontiers in Marine Science

ORIGINAL RESEARCH
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Shorebirds Affect Ecosystem Functioning on an Intertidal Mudflat

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Ecosystem functioning and services have provided a rationale for conservation over the past decades. Intertidal muddy sediments, and the microphytobenthic biofilms that inhabit them, perform crucial ecosystem functions including erosion protection, nutrient cycling and carbon sequestration. It has been suggested that predation on sediment macrofauna by shorebirds may impact biofilms, and shorebirds are known to consume biofilm, potentially causing significant top-down effects on mudflat ecosystem functioning. We carried out an exclusion experiment on the Colne Estuary, Essex, to examine whether shorebird presence significantly affects sediment erodibility measured with a Cohesive Strength Meter (CSM) and microphytobenthos biomass measured using PAM fluorescence (F_o) and chlorophyll *a* content. We also tested for treatment effects on sediment-water nutrient fluxes [nitrate, nitrite, ammonia, phosphate and dissolved organic carbon (DOC)] during periods of both dark and light incubation. Excluding shorebirds caused statistically significant changes in regulating and provisioning ecosystem functions, including mudflat erodibility and nutrient fluxes. The presence of shorebirds lowered the sediment critical erosion threshold τ_{cr}, reduced nitrate fluxes into the sediment under illumination, lowered nitrate efflux, and reduced phosphate uptake, compared to sediments where birds were excluded. There were no significant differences in macrofauna community composition within the sediment between treatments after 45 days of bird exclusion, suggesting a direct link between shorebird presence or absence and the significant differences in biofilm-related variables. This study introduces previously unknown effects of shorebird presence on ecosystem functions within this system and highlights an area of shorebird science that could aid joint conservation and human provisioning action.

Keywords: shorebirds, ecosystem function, microphytobenthos biofilm, sediment erosion, nutrient flux

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INTRODUCTION

Ecosystem functioning and ecosystem services have provided a rationale for conservation over the past decades (Cabello et al., 2012). Intertidal mudflat ecosystem functions include nutrient cycling, erosion protection and carbon sequestration, which mediate associated services (Foster et al., 2013). Intertidal flats provide natural 'soft' coastal erosion defense by reducing wave energy, lowering water velocities and thereby shear stress on the estuary bed (Spalding et al., 2014). Benthic microalgae [microphytobenthos (MPB)] form complex matrices of cells, sediments and extra polymeric substances (EPS) (Underwood and Paterson, 2003). These biofilms have a stabilizing effect on surface sediments, reducing erodibilty and aiding in the accumulation of particles and microbes (Gerbersdorf and Wieprecht, 2015). Estuarine sediments and biofilms are central components in estuarine nutrient cycles, ultimately affecting fluxes of these nutrients between land and sea (Thornton et al., 2007; Nedwell et al., 2016). Organic compounds are recycled and remineralized within sediments, particularly in coastal marine areas where nitrogen and phosphorous loads can be very high (Correll et al., 1992; Hochard et al., 2010). Nitrogen loading into marine systems can lead to eutrophication and decline in water quality, making its source and removal pathways of high interest (Burgin and Hamilton, 2007) and changes in nutrient loads can impact benthic communities (Culhane et al., 2019). MPB mediate fluxes of NO_3^-, NO_2^-, PO_4^{3-} and NH_4^+ between the water column and sediment layers (Sundback et al., 1991; Correll et al., 1992; Feuillet-Gerard et al., 1997), contributing to this process either by direct uptake/release or by altering oxygen concentration (Sundback and Graneli, 1988). Dissolved organic carbon (DOC) may also provide an important part of both global and coastal carbon sinks (Maher and Eyre, 2010; Legge et al., 2020), making effects on DOC fluxes in this environment relevant to anthropogenic climate change effects and mitigation (McKinley et al., 2016).

Mud and sand flats are essential habitats for the survival of resident and migratory overwintering shorebirds (Burton et al., 2006), which feed primarily upon infaunal and epifaunal invertebrates (Bowgen et al., 2015). Some small sandpiper species *Calidris* spp. also directly consume biofilm during, or in preparation for, migration (Kuwae et al., 2008; Jardine et al., 2015). Grazing of MPB and bioturbation by macrofauna can lead to alterations in sediment erodibility and other ecosystem functions (de Deckere et al., 2001; Hale et al., 2019). This poses questions regarding the effect of biofilm removal and bioturbation by shorebirds (Mathot et al., 2018), which may have significant knock-on effects altering ecosystem functions.

Research suggests that shorebirds could have significant direct and/or indirect effects on ecosystem function, e.g., via the impacts of foraging on macrofauna and/or biofilm or disturbance and reworking of sediment (Orvain et al., 2014b; Mathot et al., 2018). In the Bay Of Fundy (BOF), semipalmated sandpipers *Calidris pusilla* appeared to cause an ecological cascade effect by reducing densities of their mud shrimp prey *Corophium volutator*, which caused biofilm proliferation, leading to an increase in sediment stability (Daborn et al., 1993). However, subsequent research in the BOF has not indicated a trophic cascade effect, possibly due to compensatory interactions by macrofauna (Hamilton et al., 2006; Cheverie et al., 2014). Trophic webs and ecosystem functioning were compared in the Marenne-Oleron Bay, France, indicating that estuarine trophic webs including shorebirds have enhanced primary productivity through increased nutrient cycling (Saint-Beat et al., 2013). Despite evidence that estuarine shorebirds may significantly alter ecosystem functioning, the majority of shorebird research has an ornithological focus and potential top down effects on ecosystem functions such as erosion defense and nutrient cycling have not yet been experimentally tested (Mathot et al., 2018). The ecology of intertidal sediments is complex, compensatory interactions can mask effects (Hamilton et al., 2006), including trophic cascades (Fahimipour et al., 2017). Manipulative experiments are a valuable tool, to be utilized alongside 'natural' or 'observational' experiments to assess possible ecological mechanisms behind processes observed at wider spatial or temporal scales (Rogers et al., 2012).

The Colne Estuary, Essex, United Kingdom is a complex of habitats featuring many sand and mudflats, protected internationally under The Conservation of Habitats and Species (Amendment) (EU Exit) Regulations 2019, for supporting over 30,000 shorebirds. Our study site within the Colne Estuary, the Fingringhoe Wick Site of Special Scientific Interest (SSSI), was a location for the six year Coastal Biodiversity and Ecosystem Service Sustainability research program (CBESS), which provides key background information on the biotic and abiotic characteristics of the site.

Changes in community composition and mudflat characteristics can be rapid, occurring over months (Sahan et al., 2007; Rosa et al., 2008; Murphy and Tolhurst, 2009) weeks (Daborn et al., 1993; Hamilton et al., 2006), days (de Deckere et al., 2001; Tolhurst et al., 2008) and even hours (Tolhurst et al., 2006a,b). We designed and carried out a two month field exclusion experiment, supplemented by laboratory measurements, to investigate shorebird effects on two ecosystem functions, namely erosion protection (using a measure of sediment erodibility as a proxy) and nutrient cycling (including nitrate, nitrite, ammonia, phosphate and DOC). We tested three hypotheses: (1) surface biofilm biomass would be significantly altered in the presence of shorebirds, (2) sediment erodibility would be significantly altered in the presence of shorebirds and (3) nutrient fluxes between the sediment and water column would be significantly different between treatments (shorebird presence and absence) with flux direction and magnitude for different nutrient species increasing with greater MPB biomass.

MATERIALS AND METHODS

Description of Study Site

Fieldwork was undertaken between 20 January and 03 April 2017 on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick Essex Wildlife Trust Nature Reserve, Essex, United Kingdom (grid reference TM 05065 19030). This time period covered the peak overwintering and start of the migratory periods for shorebirds in the East of England. The study location comprised an area of mudflat approximately 400 m^2 situated on the upper shore. Observations during 2016 noted flocks of dunlin *Calidris alpina* and knot *Calidris canutus*, and scattered individual redshank *Tringa totanus* and gray plover *Pluvialis squatarola* foraging at the study site on receding and incoming tides. The study location was set within a larger area of estuarine mudflat, approximately 130,000 m^2 of which could be visually surveyed for shorebird activity from a fixed point (Geedon hide).

Previous CBESS studies showed that during winter, sediment at the site is mostly silts and clays, with a very low proportion of sand (maximum 'very fine sand' content in a sample was

6.5%; coarser sand contents were all lower than this), with sediment particle size at the site predominantly <63 μm (mean 95.9 % ± 0.3 SE). Mean $D_{50} = 6.9\ \mu m \pm 0.2$ SE; Mean $D_{16} = 1.9\ \mu m \pm 0.04$ SE; $D_{95} = 66.9\ \mu m \pm 13.2$ SE (D_x = particle diameter representing the x% cumulative percentile) (Wood et al., 2015). Mean percentage surface sediment water content at the site is 62.3 ± 0.4 SE (Maunder and Paterson, 2015). This site lies within the polyhaline section of the estuary, with salinity ranging from 18-30, depending on freshwater flow conditions, with lower salinity during winter (Nedwell et al., 2016).

CBESS research also included sampling of fauna within the Colne estuary, demonstrating that during winter fish were absent, with only Ctenophores recorded during Fyke netting (Wood et al., 2015). Macrofauna recorded during winter CBESS research included ragworm *Hediste diversicolor*, mud snail *Peringia ulvae*, Baltic clam *Macoma balthica* and nematodes across a total of 22 quadrat sites, in which three samples were taken at each (Wood et al., 2015). A year-long fish sampling study carried out at two different locations along the estuary where our experiment was undertaken, found that fish were absent at all sampled sites during January, and absent from three out of five sites during February (Green et al., 2009). Where fish were present at two sites during February, total abundance (fish 100 m^{-2}) was approximately 2, and less than 1 during March (Green et al., 2009).

Experiment Design

The manipulative experiment was set up on 20 January 2017 (day 0). The experimental layout was a randomized design of 20 spatial plots (**Figure 1**), each 1 m x 1 m, allocated to two treatment levels; control (shorebirds present in open un-manipulated plots) and exclosure (shorebirds absent), with $n = 10$ replicates of each treatment. Previous work in the estuary showed that spatial variability in biofilm abundance is greatest at the fine scale and small at the meter scale (Taylor et al., 2013; Nedwell et al., 2016), therefore a completely randomized design was employed to maximize statistical power of the experiment. Exclosures were bamboo frames, approximately 30 cm in height, covered on all sides (including the top) by opaque 'fruit-cage' bird exclusion netting (plastic mono-thread) with a 2 cm aperture. Exclosures prevented access to the sediment by birds, but allowed access to infauna and small fish (<2 cm width). All plots were at least three meters apart, to allow sampling from all sides and prevent plots unduly influencing each other. Exclosure and control plots were unpaired and separated by similar distances, with treatments arranged sequentially to reduce the potential for spatial bias. The exact locations of plots were selected to represent the heterogeneity within the wider mudflat. No scouring or bite marks indicating the presence of larger fish (Eggold and Motta, 1992) were found within any plots during the experiment. Plots were arranged parallel to the tide line (within a minute of immersion/emersion time of one another). Plots were situated on the upper shore, where shorebirds spend most time foraging due to the longer emersion time (Granadeiro et al., 2006). Camera footage (see below) and direct observation recorded no events of birds standing on exclosures (behavior which may otherwise have caused input of droppings into exclosure absence plots as well as control presence plots) (Schrama et al., 2013; Jauffrais et al., 2015).

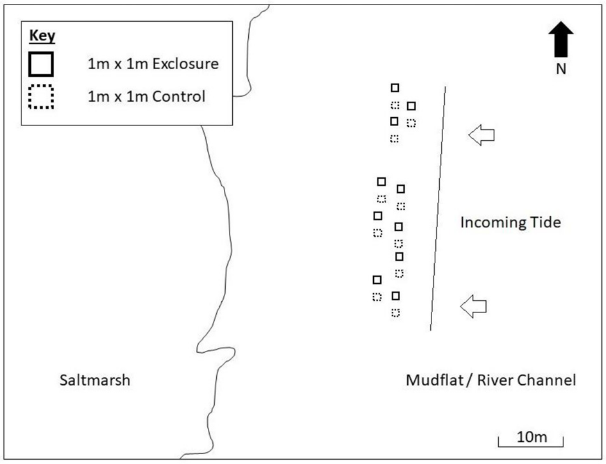

FIGURE 1 | Experimental layout adjacent to Geedon Saltings, Essex Wildlife Trust Fingringhoe Wick, Essex (grid reference TM 05065 19030).

Assessment of Possible Experimental Artifacts

To test the effect of the exclosures on the water flow within the study area, a 'plaster ball dissolution test' was carried out on days 17 and 18 (Cheverie et al., 2014). No significant difference was detected between plaster dissolution rates in control plots and exclosure plots ($t = -1.057$; $df = 8$; $p = 0.322$), demonstrating that our exclosures had no significant effects on tidal water flows in the vicinity of the mudflat surface.

Exclosure shading tests were carried out after the experiment to prevent additional mudflat disturbance, during a sunny day (cloud cover < 10%), hence resulting in an estimation of shading at the higher end of the actual range during the study period. Shading effects on Photosynthetically Active Radiation (PAR) reaching the sediment surface in exclosures were small (9.9%), and of a similar level to that in other manipulative studies in this type of environment (Cheverie et al., 2014). Further information reinforcing this conclusion is given in the discussion.

A Go-Pro HERO 4 camera fitted with a Cam-Do Blink time lapse controller mounted within a Cam-Do Solar-X enclosure (Cam-Do Solutions, 2017) was deployed to monitor bird activity within the study area for four weeks (21 February 2017 to 21 March 2017). This was mounted on a vertical pole 3.5 m above the saltmarsh at grid ref: TM 05031 19032. The camera captured a still of the plots every five minutes during daylight hours. Although species identification was not possible using captured images, numbers within the field of view were used to broadly determine whether numbers of birds using the study area were consistent with those recorded during visual surveys.

Weather data were collected during the experimental period [peak wind speed (km h^{-1}), daily precipitation (hours day^{-1}) and peak temperature (°C)], and plotted against biofilm biomass (F_o) and shorebird numbers to assess potential effects of

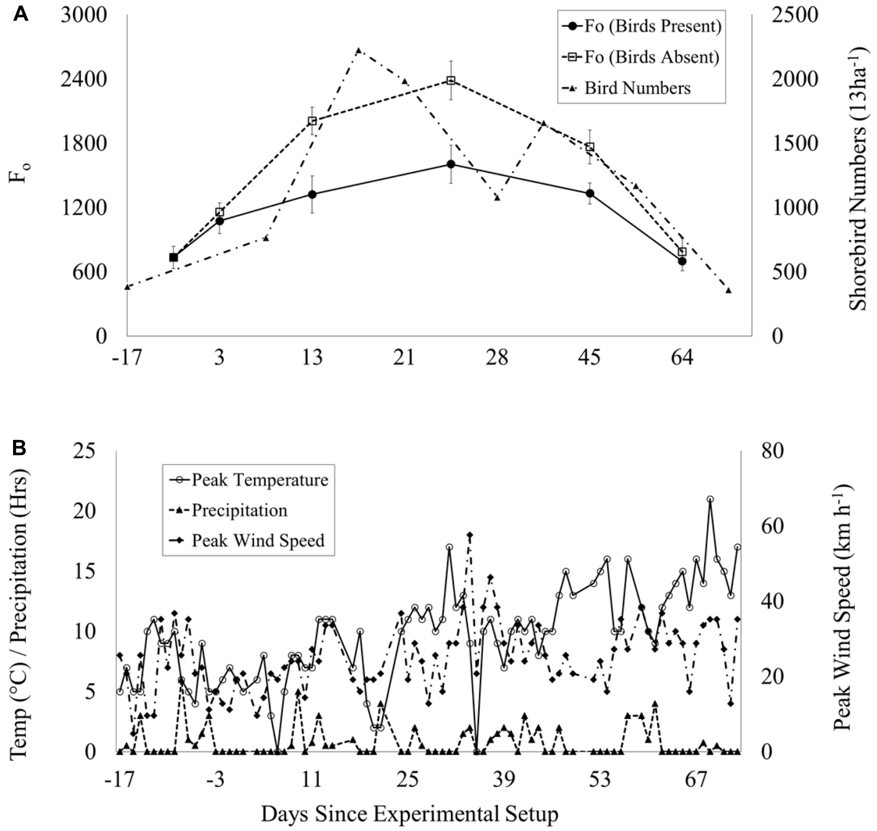

FIGURE 2 | Time series during the experimental period (03 Jan – 03 April 2017): **(A)** Mean F_o (±SE, n = 50) in shorebird presence (solid line, filled circles)/absence (dotted line, unfilled squares) plots and total peak shorebird count (multi-dash line, filled triangles). Dip in numbers on day 28 coincides with disturbance of flocks by marsh harrier. **(B)** Weather data per day: peak temperature (°C): solid line with unfilled circles, peak wind speed (km h^{-1}): multi-dash with filled diamonds and precipitation (hours): dash with filled triangles.

these variables on the experiment, such as extreme weather events, which can have significant effects on shorebird activity (Sutherland et al., 2012) and mudflat characteristics (Tolhurst et al., 2006b; Fagherazzi et al., 2017; Hale et al., 2019). No extreme weather events occurred during the experiment and no evidence was found of a relationship between F_o and daily precipitation (hours), peak temperature (°C) and peak wind speed (km h^{-1}) during the experiment (**Figures 2A,B**), although the potential for delayed responses has not been assessed. However, all plots were subject to the same weather and this is not considered to be a constraint to the experiment.

Response Variables

Between day variation in mudflat characteristics have been shown to be of greater significance than within day variation (Tolhurst and Chapman, 2005), therefore repeated measures of F_o were made to compensate for this effect. **Table 1** shows dates and days at which sampling events took place. On 20 January 2017, immediately following plot setup, 'day 0' minimum fluorescence (F_o) measurements were taken using a pulse amplitude modulated fluorometer (PAM, Walz, Effeltrich, Germany) to determine MPB biomass (Honeywill et al., 2002). MPB are key drivers of intertidal flat properties and processes (e.g., Murphy and Tolhurst, 2009), so to determine when the full sampling event would be most likely to detect any effects we monitored F_o (as a proxy for MPB biomass) on days 3, 13 and 26, as a convenient indication of treatment effects, to determine when erodibility and nutrient flux variables should be measured and to confirm that early in the experiment there were no significant differences between treatments. F_o was also measured on day 45 to evaluate the effect of shorebird presence/absence on MPB biomass and associated properties, and on day 64 to determine if trends continued. A subset of 6 exclosure and 6 control plots were measured on day 3 for a total of 60 F_o measurements (n = 5 in each of the 12 plots); subsequently all plots were measured, for a total of 100 F_o measurements (n = 5 in each of the 20 plots) on days 13, 26, 45, and 64 to investigate how surface MPB biomass responded to shorebird presence/absence over time.

Due to the large number of measurements required in each plot during a tidal cycle and considering the impact of dewatering during the tidal cycle (Maggi et al., 2013; Orvain et al., 2014a; Fagherazzi et al., 2017) a 5 min low light partial dark adaption treatment was used prior to each PAM measurement, which is a preferred method to conventional dark adaption for the measurement of minimum fluorescence as a proxy of MPB

TABLE 1 | Dates and numbers of days into the experiment that field sampling events occurred between 03 January and 03 April 2017 on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick Essex Wildlife Trust Nature Reserve, Essex, United Kingdom (grid reference TM 05065 19030).

Date	Day	Event
03 Jan	−17	Shorebird monitoring
20 Jan	0	Experiment setup and F_o sampling
23 Jan	3	F_o sampling
27 Jan	7	Shorebird monitoring
02 Feb	13	F_o sampling
03 Feb	14	Shorebird monitoring
10 Feb	21	Shorebird monitoring
15 Feb	26	F_o sampling
17 Feb	28	Shorebird monitoring
24 Feb	35	Shorebird monitoring
06 March	45	F_o sampling, critical shear strength sampling, contact core and flux core collection.
10 March	49	Shorebird monitoring
25 March	64	F_o sampling
03 April	73	Shorebird monitoring

biomass (Jesus et al., 2006b). Sampling was carried out during periods of clear weather with little wind and no rain, at least one hour after the tide had exposed the sampling area to allow initial drying of plots. A consistent low light sampling environment was achieved using plastic 40 mm (diameter) × 60 mm (length), cylindrical opaque dark adaption chambers with a 6 mm aperture hole at the top. This also enabled *in-situ* sampling with the PAM fluorometer without removal of the chamber. This reduced the variation in light intensity during the measuring period. To further eliminate potential effects of varying light intensity and sediment water content during sampling events, exclosure and control plot sampling was alternated. To minimize the effect of varying light intensity and phase of vertical migration between sampling events, sampling periods were timed to cover low tides peaking as close to midday as possible.

Our experience of the site is that variability at the meter scale is low (Redzuan, 2017). Additionally, the repeated F_o sampling (described above) gives further confidence that plots were not significantly different at the beginning of the experiment. All *in situ* mudflat variables were measured on 06 March 2017, after 45 days of shorebird exclusion, to test the effect that a period of shorebird exclusion had on selected mudflat properties. Sampling included *in-situ* measurements of F_o (as described above), *in-situ* sediment critical erosion threshold (τ_{cr}) using a Cohesive Strength Meter (CSM) (three measurements within six plots of each treatment, total 36 measurements) (Tolhurst et al., 1999; Vardy et al., 2007) and contact coring for analysis of chlorophyll *a* content (three measurements within seven plots of each treatment; total 42 measurements) (Honeywill et al., 2002). Flux cores (Perspex tubes of 0.1 m diameter and approximately 0.2 m in depth) were also collected (one from each plot) for laboratory analysis of nutrients and macrofauna.

Contact cores (surface ~2 mm) were freeze dried in the dark and chlorophyll *a* extracted using cold methanol over 24 h, and measured spectrophotometrically, correcting for phaeopigments (Stal et al., 1984).

Flux cores were carefully returned to the laboratory within an hour of leaving the site and immersed in seawater from the site, within oxygenated and temperature and light controlled indoor mesocosms (Thornton et al., 1999). Rubber bungs were used to ensure equal headspace volume across cores. Cores were left submerged and open to settle overnight prior to sampling on the following day. Throughout headspace water sampling, Perspex lids were tightly fitted to prevent leakage. Magnetic stirrers maintained water flow over the sediment surface. On 07 and 08 March 2017 these were sampled for sediment-water biogeochemical fluxes of nitrate, nitrite, ammonia, phosphate and dissolved organic carbon (DOC). Headspace seawater samples were taken at the beginning and end of 2 h dark and light incubation periods. Cores were left for at least one hour to adjust to light levels prior to each incubation. Sampling was completed according to general methods described by Thornton et al. (1999). Flux measurements were repeated in both light and dark conditions, using 500W halogen 'daylight' lamps to provide 'lit' conditions (500 μmol m^{-2} s^{-1} PAR) and covering mesocosms with opaque Perspex covers to provide 'dark' conditions. Water samples were analyzed for their nutrient concentrations using a Seal AA3 segmented flow Nutrient Analyzer (SEAL Analytical Inc.).

Individual cores used for nutrient flux measurements were subsequently sieved (500 μm mesh) to retain macrofauna. Macrofauna were preserved in 95% ethanol and identified to species level (where possible) using a microscope, quantified and densities (m^{-2}) calculated. Through data comparison with previous work at the site (Wood et al., 2015) we were confident that sufficient sampling had been undertaken to assess potential differences in community composition between shorebird presence and absence plots.

Bird surveys began on 03 January 2017 (−17 days) and were carried out at least every two weeks (see **Table 1**) to monitor the level and type of use of the study area by shorebirds. Monitoring began before the experimental setup to ensure current use of the study area by shorebirds and aid in deciding the best location for the experimental plots. Surveys were carried out using the 'look-see' methodology (Bibby et al., 2000), from a fixed location (Geedon Hide; TM 05081 19170). Surveys were undertaken for at least 2 h either side of low-tide, including as much of these timeframes as possible (four hours maximum) within daylight constraints. Particular care was taken to also include visual observation of the tideline crossing the plots wherever possible. Counts of species within the surrounding visible mudflat were taken every half hour. Continual observation of the study area was made, quantifying numbers and identifying species entering presence plots throughout the surveys. Equipment included a 20−60 × 82 telescope and 10 × 42 binoculars. No birds were recorded within or on the absence plots during any of the surveys. During F_o measurements, shorebird tracks were noted within all presence plots at some point during the study, indicating use of all presence plots by shorebirds. No tracks were recorded in any absence plots at any point during the experiment.

Statistical Analysis

To evaluate the effects of shorebird presence and time (days) on biofilm biomass throughout the experimental period, we used a linear mixed-effects model (plot nested in treatment) to analyze F_o data with plot as a random effect and time (day) and bird presence/absence as fixed effects. This model was run using NLME package in R version 4.0.

To evaluate the effect of shorebird presence/absence on MPB biomass and sediment erodibility, F_o (days 3, 13, 26, 45, and 64), chlorophyll *a* (from surface 2 mm) (day 45) and critical erosion threshold (day 45) data were analyzed using a mixed model, two-way nested ANOVA design with (plot nested in treatment) with plot as a random factor and shorebird presence/absence as a fixed factor, using the GMAV (1997) statistical package (University of Sydney, Australia). Although baseline data were not collected, ANOVA detects differences between treatments over and above variability among individual plots (Underwood, 1997). To counteract the issue of multiple comparisons we used Bonferroni correction testing each hypothesis at a confidence level of 0.01 (0.05/5).

To evaluate the effect of shorebird presence/absence on nutrient flux (day 45), nutrient data were analyzed using a two-way orthogonal ANOVA design with dark/light incubation and shorebird presence/absence as fixed factors, using the GMAV (1997) statistical package (University of Sydney, Australia). Where Cochran's test was significant (ammonium and phosphate), data were normalized by rank transformation and the analysis repeated. We also used reversals in flux (for example an efflux from the sediment in the absence of shorebirds becoming an influx into the sediment in the presence of shorebirds) as an indication of changes suggesting 'ecologically significant' implications for ecosystem functioning.

To assess whether shorebird presence/absence had significantly altered macroinvertebrate community structure, day 45 taxa density was analyzed using R version 3.6.1 with vegan package. Non-metric multidimensional scaling (NMDS, Bray-Curtis dissimilarity, 20 restarts) was used to visualize differences in community structure at day 45 in two dimensions (Clarke, 1993). The MDS had a stress 0.037, therefore considered an adequate representation (Clarke, 1993). Analysis of similarities (ANOSIM) was also performed to test quantitatively for differences in community structure between shorebird presence and absence.

To assess the potential for biases associated with the exclosures, plaster ball dissolution (days 17 and 18) and shading effect (post experiment) data were also analyzed using a one-way orthogonal ANOVA, using the GMAV (1997) statistical package (University of Sydney, Australia).

To evaluate shorebird pressure on the mudflat, species count data were first converted into 'bird-days,' by calculating the sum of the number of each shorebird species present on every count, multiplied by the number of days between that and the subsequent count (Gill et al., 2001; Lewis et al., 2014). This allowed comparison of shorebird pressure on the wider mudflat. Only species considered regular foragers on mudflats and recorded foraging on the surrounding mudflat were included in this analysis; for example lapwing *Vanellus vanellus* and golden plover *Pluvialis apricaria* were removed due to their high dependence, and almost exclusive foraging, on coastal grassland and arable fields (Mason and Macdonald, 1999). Furthermore, these species were recorded roosting on the mid to low shore only during low tides, further reducing the likelihood that they contributed to any effects within the upper shore study site. To compare mudflat variables with density of species recorded in presence plots, count numbers of such species were $\log_{10}$ transformed and plotted over time with mean F_o in shorebird presence and absence.

RESULTS

Microphytobenthic Biomass

Results of the linear mixed effects model show a highly significant difference in F_o (measure of MPB surface chlorophyll *a*) between shorebird presence and absence, with F_o higher in the bird exclosure treatments. There was no significant effects of time (days) or interaction between treatment with time (**Table 3**).

F_o initially increased in shorebird presence and absence plots, increasing more rapidly in absence plots, peaking on day 26 before decreasing (**Figure 2A**). On day 3, there was no significant difference in F_o between shorebird presence and absence plots, but on day 13 this difference had become significant. The largest difference was measured on day 26, when mean F_o in shorebird presence and absence plots was highly significantly different (**Table 3**).

The two subsequent sampling events (days 45 and 64) showed decreasing F_o with progressively smaller differences between presence and absence plots. Mean F_o in shorebird absence plots was still higher on day 45 but was not significantly different (Bonferroni corrected 0.01 significance level), and by day 64, F_o levels were very similar between treatments (**Figure 2A**). There was no significant difference in chlorophyll *a* content ($\mu g\ g^{-1}$) in the top $\sim$2 mm of sediment between presence and absence plots on day 45 (**Figure 3B**).

Sediment Erodibility

To evaluate the effect of shorebirds on erosion protection, erosion threshold (τ_{cr}) was measured on day 45. Significantly greater erosion threshold was found in shorebird absence plots than in presence plots (**Figure 3C**, **Table 3**).

Sediment-Water Nutrient Fluxes

There was significantly greater net nitrate influx into the sediment when shorebirds were absent compared to when they were present (**Figure 4A**) and a significantly greater net nitrite efflux from the sediment into the water column when shorebirds were present (Figre 4B).

There was no significant difference in net phosphate flux between shorebird presence and absence plots. However, under lit conditions mean values changed from an influx into the sediment to a small efflux into the water column (**Figure 4D**), which is considered ecologically significant.

There was no significant difference in net dissolved organic carbon (DOC) flux between shorebird presence and absence

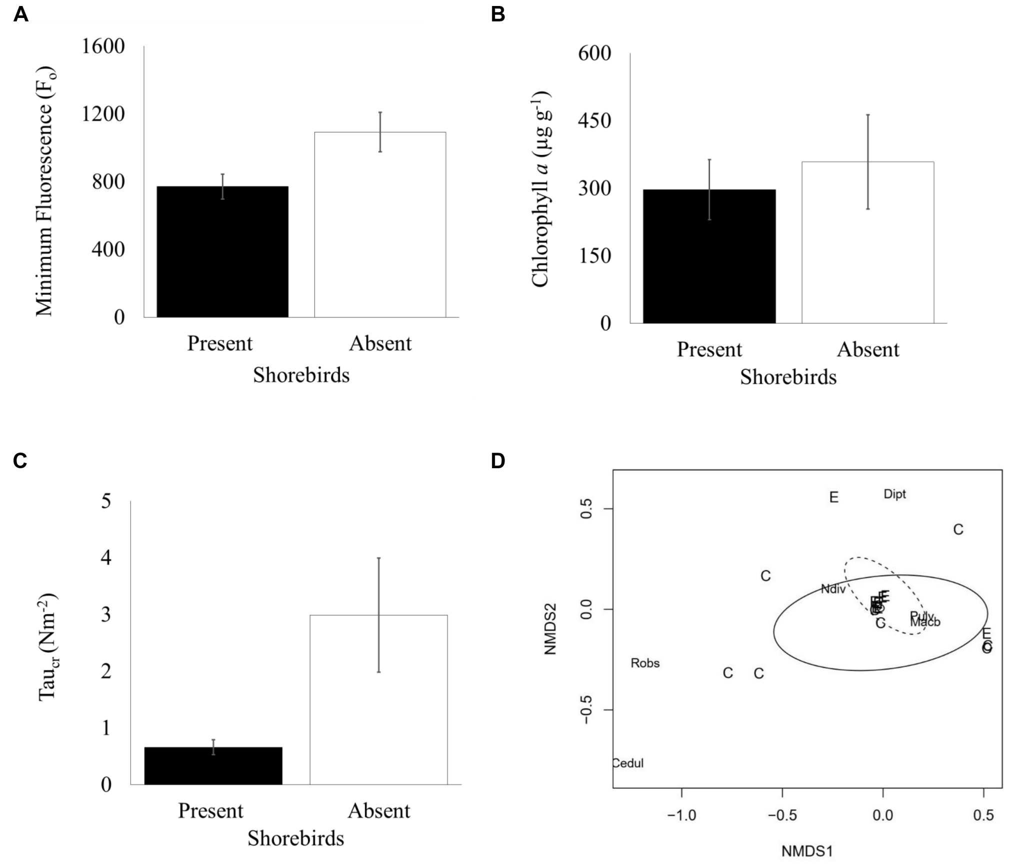

FIGURE 3 | Measured characteristics in shorebird presence/absence on day 45 (06 March 2017) **(A)** Mean F_o (±SE, *n* = 50) **(B)** Mean chlorophyll *a* content in top ~2 mm (µg g^{-1}) (±SE, *n* = 14) **(C)** Mean erosion threshold (τ_{cr}) (±SE, *n* = 36) **(D)** Non-metric multidimensional scaling (MDS) plot depicting Bray-Curtis dissimilarity in community composition (shorebird presence = solid oval; shorebird absence = dotted oval, C = shorebird presence, E = shorebird absence, Cedul = *C. edule*, Robs = *R. obtusa*, Dipt = Chironomidae, Pulv = *P. ulvae*, Ndiv = *N. diversicolor*).

plots (**Figure 4E**). However, in shorebird presence during light incubation, we found a large reversal in flux direction of DOC into the sediment rather than the water column (**Figure 4E**).

No significant difference in ammonium flux between the sediment and water column was found (**Figure 4C**).

Macrofauna Density

To evaluate the indirect effect of shorebirds on erosion protection, nutrient cycling and carbon sequestration via changes in macrofauna density, the numbers of macrofauna were counted (from the same cores used for the nutrient measurements). Macrofauna recorded on day 45 were mud snails *P. ulvae*, Baltic clams *Macoma balthica*, midge larvae (Chironomidae), ragworms *Hediste diversicolor*, Arctic barrel-bubble *Retusa obtusa* and common cockles *Cerastoderma edule*. Mean densities (m^{-2}) in each treatment are shown in **Table 4**. Raw macrofauna counts revealed presence of a single specimen of *C. edule* and *R. obtusa* in only two and three plots, respectively. *H. diversicolor* counts were also sparse (see **Table 4**). On day 26 *P. ulvae* was visually noted on the mudflat surface for the first time during F_o sampling. Mud snails can compensate for the loss of higher predators on intertidal mudflats (Hamilton et al., 2006; Cheverie et al., 2014). This species was subsequently present within the study area during all F_o sampling events, noted throughout the study site in presence and absence plots.

The non-metric Multi-Dimensional Scaling (nMDS) plot (**Figure 3D**) indicated that macrofauna communities between treatments were not significantly dissimilar; a large overlap between community composition is indicated, although the spread of data points is larger in shorebird presence demonstrating larger variability in community composition. ANOSIM confirmed there was no significant difference in community composition between shorebird presence and absence plots (R = −0.038, *P* = 0.623).

Bird Surveys

Over the study period, 10 shorebird species were recorded using the wider mudflat, with a total of 78,811 bird days (**Table 2**). Of these, three were recorded in the presence plots; *C. alpina* (84 bird-days), *T. totanus* (35 bird-days) and *P. squatarola* (28 bird-days). Camera data indicated that numbers of shorebirds using the study area were broadly consistent with those counted during surveys. Although the image quality (due to distance from the plots) made detection of individual birds difficult, flocks were

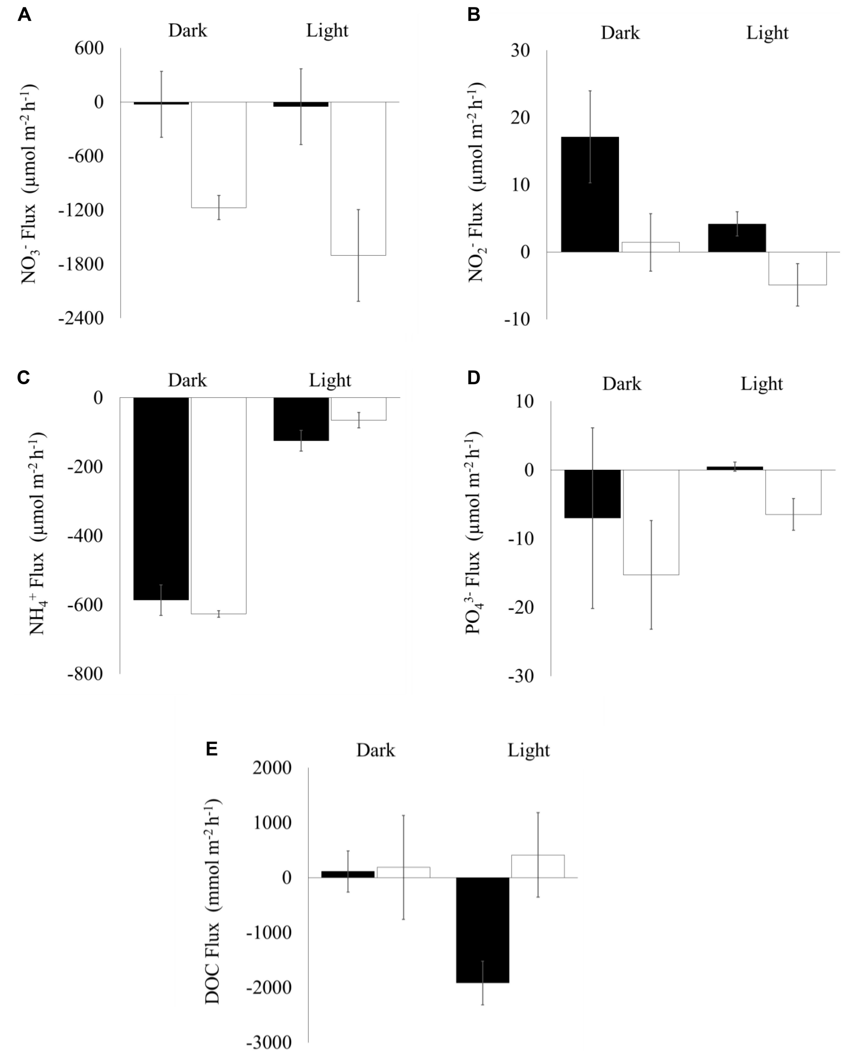

FIGURE 4 | Sediment-water nutrient fluxes (Mean ± SE, $n = 10$) during light and dark incubations in cores collected from shorebird presence (filled bars) and absence (unfilled bars) plots on day 45 (06 March 2017). X-axis marks zero flux, positive values show flux out of the sediment, negative values show flux into the sediment: **(A)** NO_3^-, **(B)** NO_2^-, **(C)** NH_4^+, **(D)** PO_4^{3-}, and **(E)** Dissolved Organic Carbon (DOC).

noted using the plots, often as the tideline crossed them. Flocks were noted on camera footage in and around the plots between 23 February and 5 March (day before main sampling event).

The experimental plots were laid out in an area of mudflat representing approximately 0.3% of the area visually surveyed. Peak *C. alpina*, *P. squatarola,* and *T. totanus* numbers within experimental plots comprised approximately 0.16%, 0.35%, and 0.8% (respectively) of peak numbers within the survey area, thus within the same order of magnitude as that expected based on the areas of plots and the overall mudflat area.

DISCUSSION

Excluding shorebirds caused significant changes in regulating and provisioning ecosystem functions, including mudflat erodibility, nutrient fluxes and carbon sequestration. Effects on MPB biofilm biomass and erodibility were, however, not as predicted in our hypotheses. We suggest that these effects were driven by shorebird bioturbation of surface sediments and MPB biofilms and possible direct grazing of MPB by *C. alpina*.

Effects on MPB and Erodibility

Hypothesis 1 was not rejected; our linear mixed-effects model showed a highly significant difference in F_o between shorebird presence and absence, with no significant interaction between other factors (see **Table 3**). Significantly greater MPB F_o values were found in shorebird absence plots on days 13 and 26. By day 45 the difference had become less significant, to the extent of being non-significant when Bonferroni correction was applied (0.01 level). Despite this, on day 45 the difference in F_o remained visually notable in the field, which is reflected in **Figure 2A**. These differences between treatments occurred during a period of increased shorebird activity in the study area. Despite the

TABLE 2 | Bird days ha-1 estimated for each species recorded foraging within the survey area between the 20th of January and the 3rd of April 2017 on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick Essex Wildlife Trust Nature Reserve, Essex, United Kingdom (grid reference TM 05065 19030).

Species	*Calidris alpina*	*Calidris canutus*	*Pluvialis squarola*	*Arenaria interpres*	*Tringa totanus*	*Limosa limosa*	*Limosa lapponica*	*Recurvirostra avosetta*	*Numenius arquata*	*Haematopus ostralegus*
Bird Days	53,853	9,363	6,358	103	3,735	1,541	430	2,888	405	135

decline in surveyed shorebird numbers on day 28, the 83 ha^{-1} shorebirds present at this point was notably greater than at the beginning or end of the experiment (when numbers were 30 and 28 ha^{-1}, respectively) (**Figure 2A**). The survey visit on day 28 is also considered to be an underestimate due to the flushing of a large proportion of the foraging shorebirds on the incoming tide by a marsh harrier *Circus aeruginosus*. Differences in F_o between shorebird presence and absence on days 3 and 64 were non-significant and occurred when shorebird numbers were smaller, suggesting that the effects found may be dependent upon shorebird density.

There was no significant dissimilarity in macrofauna community structure between shorebird presence and absence plots (**Figure 3D**). The present study recorded a greater diversity of species at the study site than during previous large scale work at the site (Wood et al., 2015), albeit the majority of infaunal species were present sporadically and in very low numbers (see **Table 4**). This validates our macrofauna sampling effort, in that we had enough replicates to detect all species known to be present, despite likely patchiness in invertebrate distributions (Van Colen, 2018). These findings differ to suggestions that a top-down ecological cascade effect driven by shorebirds can increase biofilm biomass (Daborn et al., 1993), supporting instead more recent work (Hamilton et al., 2006; Cheverie et al., 2014). Our results provide strong indication that, through bioturbation and/or grazing (and/or a yet unknown pathway), shorebirds can have a significant reductive effect on the biomass of surface MPB biofilms. Thus, shorebirds can alter key ecosystem functions such as erosion protection and nutrient cycling via direct and/or indirect effects on MPB. The increase in MPB in the absence of shorebirds concurs with results reported by Hamilton et al. (2006), where the authors acknowledge that this finding is the opposite to that expected in the event of a trophic cascade. On day 45 bulk chlorophyll *a* content within the surface 2 mm of sediments showed the same directional response as surface biofilm biomass and was also not significantly different. Bioturbation and grazing by macrofauna can significantly affect surface MPB biomass and resuspension (Grant and Daborn, 1994; Hagerthey et al., 2002; Harris et al., 2015); but as macrofauna were not significantly different between our shorebird presence/absence plots, and motile macrofauna could access all plots, the changes in MPB biomass are highly unlikely to have been due to macrofauna. Physical effects of birds upon primary producers is evident within many freshwater and marine environments (Cadee, 1990; Mitchell and Perrow, 1998; Nacken and Reise, 2000) and physical mixing of intertidal mud has been shown to significantly reduce chlorophyll *a*, $F_{o,}$ and colloidal carbohydrate (Tolhurst et al., 2012). It follows that physical disturbance (bioturbation) by shorebirds in our study location, through foraging (including biofilm grazing in some species) and tracking (walking), can have a significant effect upon MPB biomass and related sediment properties. These results suggest that bioturbation by shorebirds can be a more significant driver of effects on MPB than trophic cascades. Further work is required to confirm the mechanisms by which shorebirds in this part of the world reduce MPB biomass.

Hypothesis 2 was not rejected, sediment critical erosion threshold (τ_{cr}) was significantly smaller when shorebirds were present than when they were absent (see **Figure 3C**). This pattern is most likely to have been driven by both direct bioturbation during walking and feeding of shorebirds on the mudflat surface and, because MPB commonly significantly increase mudflat erosion threshold (Hale et al., 2019; Hope et al., 2020), indirectly by grazing decreasing the biomass of MPB. The exact mechanistic pathway(s) and their magnitude require further investigation. The erosion shear stresses exerted on intertidal mudflats by combined waves and tides are very variable, but commonly in the 0–1 Nm^{-2} range and typically below 4 Nm^{-2} (Christie and Dyer, 1998; Whitehouse and Mitchener, 1998). Thus, the τ_{cr} measurements suggest that erosion would occur frequently (i.e., during most tidal cycles) in the presence of shorebirds and much less frequently in the absence of shorebirds. Given the importance of sediment erodibility for many ecosystem functions (Hubas et al., 2018; Hope et al., 2020), including nutrient fluxes and erosion protection; the effect of shorebirds on erodibility demonstrates their importance as ecosystem engineers (Passarelli et al., 2014) and their significant role in ecosystem functioning.

Although F_o is widely used as a proxy for MPB biomass, it is important to acknowledge that this relationship varies depending upon the physiological state and taxonomic composition of MPB due to vertical migration of MPB (Serodio et al., 2001, 2006; Serodio, 2004; Du et al., 2018). By standardizing our time of sampling within the tidal exposure period, tidal migration rhythms influencing F_o were accounted for between treatments. Though changes in the relationship between F_o and Chl *a* over time may have occurred, we found significant differences in F_o between treatments at each time of sampling. Our results show the same directional response of F_o and Chl *a* to shorebird presence, suggesting an underlying relationship in this case. Actual Chl *a* concentration varies vertically within the sediment depending upon factors such as MPB migration, light intensity, water content and sediment compaction (Perkins et al., 2003; Tolhurst et al., 2003; Jesus et al., 2006a; Maggi et al., 2013) and also shows temporal changes. We did not design our sampling regime to specifically focus on the F_o to Chl *a* relationship, which requires a higher level of sampling granularity.

Effects on Nutrient Fluxes

Hypothesis 3 was not rejected; statistically significant differences in the fluxes of nitrate, nitrite and dissolved organic carbon (DOC), were found between presence and absence treatments.

TABLE 3 | Linear mixed-effects / ANOVA models and results for each variable and sampling time between the 20th of January and the 3rd of April 2017 on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick Essex Wildlife Trust Nature Reserve, Essex, United Kingdom (grid reference TM 05065 19030).

Variable (day if not day 45) / Model	**Factor**	**Source**			
		numDF	**denDF**	***F***	***P***
Linear Mixed Model					
F_o = MEAN x P/A x TIME x Plot(TIME)	Sampling Days = TIME	1	3	0.91	0.58
	Presence/Absence = P/A	1	85	22.2	<0.0001
	P/A x TIME	1	85	1.64	0.204
ANOVA Models		**df**	**MS**	***F***	***P***
Chlorophyll *a* ug g^{-1} = MEAN + P/A + Plot(P/A) + RES	Presence/Absence = P/A	1	39516	0.3	0.596
	Plots (P/A)	12	133337	2.34	0.031
	Residual	24	56973		
Erosion threshold Nm^{-2} = MEAN + P/A + Plot(P/A) + RES	Presence/Absence = P/A	1	14.4	8.44	0.016
	Plots (P/A)	10	1.7	3.85	0.003
	Residual	24	0.44		
F_o (day 3) = MEAN + P/A + Plot(P/A) + RES	Presence/Absence = P/A	1	105588	0.28	0.61
	Plots (P/A)	10	376213	0.67	0.75
	Residual	48	564759		
F_o (day 13) = MEAN + P/A + Plot(P/A) + RES	Presence/Absence = P/A	1	11777938	9.23	0.007
	Plots (P/A)	18	1275748	1.94	0.024
	Residual	80	658534		
F_o (day 26) = MEAN + P/A + Plot(P/A) + RES	Presence/Absence = P/A	1	15245120	8.56	0.009
	Plots (P/A)	18	1781747	3.04	0.0003
	Residual	80	564759		
F_o (day 45) = MEAN + P/A + Plot(P/A) + RES	Presence/Absence = P/A	1	4723233	4.93	0.039
	Plots (P/A)	18	957343	1.42	0.145
	Residual	80	673677		
F_o (day 64) = MEAN + P/A + Plot(P/A) + RES	Presence/Absence = P/A	1	200435	0.32	0.581
	Plots (P/A)	18	633507	3.58	<0.00001
	Residual	80	177121		
Ammonium = MEAN + D/L + P/A + D/L x P/A + RES	Dark/Light = D/L	1	4000	124.03	<0.0001
	Presence/Absence = P/A	1	16.9	0.52	0.47
	D/L x P/A	1	152.1	4.72	0.114
	Residual	36	32.25		0.037
Phosphate = MEAN + D/L + P/A + D/L x P/A + RES	Dark/Light = D/L	1	448.9	3.57	0.067
	Presence/Absence = P/A	1	136.9	1.09	0.304
	D/L x P/A	1	211.6	1.68	0.203
	Residual	36	125.85		
Nitrate = MEAN + D/L + P/A + D/L x P/A + RES	Dark/Light = D/L	1	773375	0.47	0.496
	Presence/Absence = P/A	1	19546821	11.95	0.001
	D/L x P/A	1	637317	0.39	0.536
	Residual	36	1635096		
Nitrite = MEAN + D/L + P/A + D/L x P/A + RES	Dark/Light = D/L	1	929	4.27	0.046
	Presence/Absence = P/A	1	1534	7.05	0.01
	D/L x P/A	1	110	0.50	0.483
	Residual	36	218		
Dissolved organic carbon = MEAN + D/L + P/A + D/L x P/A + RES	Dark/Light = D/L	1	8137880	1.65	0.208
	Presence/Absence = P/A	1	14457171	2.92	0.096
	D/L x P/A	1	12738121	2.58	0.117
	Residual	36	4943100		

Orders of magnitude changes in the scale of some fluxes were observed (nitrate ∼100x, nitrite ∼10x and DOC ∼2000x). Despite not being formally significant, the reversal of phosphate flux into/out of the sediment is considered to be ecologically significant. These results suggest that shorebirds significantly alter ecosystem functioning associated with nutrient cycling

TABLE 4 | Count of each macrofauna species recorded within each core extracted from the study area on Day 45 (06 March 2017) on the mudflat adjacent to Geedon Saltings at Fingringhoe Wick Essex Wildlife Trust Nature Reserve, Essex, United Kingdom (grid reference TM 05065 19030).

Species/Family	Treatment (shorebirds present = P, shorebirds absent = A)	Mean Count (m^{-2})
Peringia ulvae	P	$31,669 \pm 5,014$
	A	$30,226 \pm 3,376$
Macoma balthica	P	$1,980 \pm 366$
	A	$1,796 \pm 213$
Nereis diversicolor	P	99 ± 33
	A	170 ± 35
Chironomidae	P	552 ± 347
	A	$2,574 \pm 2,086$
Retusa obtusa	P	42 ± 0
	A	0
Cerastoderma edule	P	29 ± 18
	A	0

(Saint-Beat et al., 2013; Mathot et al., 2018; Hope et al., 2020) and carbon storage (Maher and Eyre, 2010). Differences in the surface active MPB biomass (F_o) can explain the nutrient flux alterations by shorebirds. Photosynthesis and nutrient assimilation by MPB significantly affects nutrient flux rates, including nitrate (Dong et al., 2000) and phosphate (Sundback et al., 1991). Further, the EPS matrix within MPB biofilms provides additional organic matter to support heterotrophic bacteria, which reduce nitrite to nitrous oxide (Dong et al., 2002). We found evidence to suggest that the presence of shorebirds can significantly reduce nitrate uptake into intertidal sediments (**Figure 3A**). The reduction of active surface MPB biofilms by shorebirds is a likely mechanism that may reduce nitrate and phosphate uptake, nitrification, coupled nitrification-denitrification, and through the reduction of extracellular organic carbon, reduce bacterial degradation rates (Thornton et al., 2007).

Our findings suggest that shorebird effects on MPB can limit the drawdown of nitrate, nitrite and phosphate into sediments in an already nitrate rich estuary (Thornton et al., 2007). The observed alterations of nutrient fluxes suggest that shorebirds play a significant role in estuarine nutrient pathways, effectively controlling and engineering nutrient fluxes between the sediment and water column (Passarelli et al., 2014, 2018). Bioturbation by macrofauna is known to significantly affect nitrate and ammonia fluxes at the study site and elsewhere, through sediment reworking, ventilation and burrowing (Nizzoli et al., 2007). We suggest that bioturbation by shorebirds (Mathot et al., 2018) is likely to have contributed to the significant effects found here.

While the measured nutrients were typically characterized by a reduction in fluxes into the sediment from shorebird presence, DOC flux into sediment from shorebird presence increased significantly in lit conditions. It is possible that through the observed reduction of MPB biomass by shorebirds, competition for nutrients may have been reduced, allowing bacteria to proliferate and increase assimilation of DOC and ammonium (Amin et al., 2012). Migratory birds can also introduce bacteria to communities (Steiniger, 1969) via fecal droppings (Muller, 1965) and external tissues (Muza et al., 2000), potentially further increasing these process rates. These results indicate that changes in shorebird abundance could affect wider ecosystem functioning such as carbon sequestration and coastal biogeochemistry more broadly (Nedwell et al., 2016; Hope et al., 2020).

Secondary Effects

Use of the mid and upper shore at low tide by *C. alpina*, despite often being a 'tide follower' (Granadeiro et al., 2006), may have been driven by the visual cues of MPB communities on the mudflats, either as a cue for the presence of invertebrate prey or to feed upon MPB directly (Hamilton et al., 2003; Drouet et al., 2015; Jimenez et al., 2015). *C. alpina* is an opportunistic feeder with a broad diet (Dierschke et al., 1999) using visual and tactile foraging cues (Drouet et al., 2015), and possibly exploited areas with high diatom biomass to maximize the breadth of feeding opportunity.

Avian guano (in particular shorebird droppings) is a potentially important source of nutrients in coastal areas (Schrama et al., 2013). It has been suggested that *C. alpina* droppings increase growth rate and biomass of the diatom species *Entomoneis paludosa* through increases in nitrogen and phosphorous input to the sediment (Jauffrais et al., 2015). However, the Colne estuary has very high nutrient loads (McMellor and Underwood, 2014; Nedwell et al., 2016) and MPB biomass was smaller, rather than larger in shorebird presence, suggesting that nutrient enrichment of biofilms by guano is not a major mechanism in this case. These findings reflect the complexity of the real-world scenario compared to laboratory studies (Jauffrais et al., 2015); in the present study shorebirds reduced MPB biomass on the upper shore. This indicates that the effects of bioturbation and/or grazing by shorebirds, which lead to alterations in ecosystem functioning, significantly outweigh the effects of nutrient input via guano in our study site.

Shorebirds significantly affect ecosystem functions (nutrient flux and erodibility), at least within the upper shore, in a temperate climate during late winter. However, these effects are likely to vary temporally and spatially (Underwood and Paterson, 1993; Gerwing et al., 2015) depending as they do upon the abundances and functioning of other organisms present (Underwood, 1994; Norazlimi and Ramli, 2014). For example, we found that shorebird effects were temporary and seasonal, restricted to an approximately one month period when shorebird density peaked at the study site (**Figure 2A**). This suggests that the observed phenomenon is seasonally and density dependent, reliant on sufficient density of shorebirds (which are present in larger densities during winter) to cause effects on ecosystem functioning. Similarly, compensatory grazing by the mud snail Peringia ulvae may have limited the temporal effect of shorebirds on MPB during this study, effectively resetting the state of the system as bird density declined (Hamilton et al., 2006; Cheverie et al., 2014). The collapse of the shorebird effect on F_o was concomitant with the emergence of large numbers of *P. ulvae*. This MPB grazer was first noticed on the mudflat surface on day 26, was noted spread across the mudflat within all plots (**Table 4**), and can rapidly reduce the abundance and thickness of biofilms (Sahan et al., 2007). Subsequently the difference in F_o between treatments steadily decreased, eventually becoming non-significant. On day 45, no significant difference between macrofaunal communities was evident. It is our interpretation that the snails had a homogenizing effect on biofilm distribution. Once the snails emerged and while birds remained, the effects of the birds became weaker. Once the birds left, continued grazing by the snails removed the residual bird effects (compensatory effect). Despite our restriction to observational evidence regarding the temporal change in numbers of *P. ulvae*, it is known that mudsnails can mask effects on MPB (Hamilton et al., 2006; Cheverie et al., 2014) and it is plausible that this occurred here, reducing the detectability of ecosystem function effect pathways. Here we highlight that shorebirds play a key community role in the regulation and control of ecosystem function, through inter and intraguild interactions with macrofauna and MPB with which they are intrinsically linked (Kuwae et al., 2012; Cheverie et al., 2014).

We found no evidence to suggest that macrofauna community structure differed between shorebird presence and absence, however, such effects have been detected in Canada in exclusion experiments on semipalmated sandpiper C. pusilla, where reductions in C. volutator densities were found (Hamilton et al., 2006; Cheverie et al., 2014). The differences between these studies may be due to geographic or shorebird species differences, or due to the fact that *C. volutator* is not present at our study site.

We also emphasize that differences in MPB surface biomass between treatments eventually became non-significant, despite shorebird exclosures remaining in-situ. We conclude therefore that shorebirds, rather than experimental artifacts, drove the measured MPB biomass changes and subsequent effects on ecosystem functions.

CONCLUSION

Here we have identified previously unknown effects of shorebirds on ecosystem functioning. Although limitations are acknowledged regarding the link between F_o measurements and actual Chl *a* content, the end effect of shorebird presence on erodibility and nutrient fluxes was found to be significant, and a large amount of existing literature indicates that MPB are highly likely to drive this effect. The removal of shorebirds significantly increased surface biofilm F_o and sediment erosion threshold. Shorebird absence was also found to affect nutrient cycling regimes and carbon sequestration on the mudflat; differences in biofilm biomass led to significant alterations in the flux of nutrients under lit conditions, including nitrate, nitrite and phosphate, all of which showed an increased flux into the sediment in the absence of shorebirds. The uptake of DOC in the light into the sediment was significantly greater in the presence of shorebirds.

The mechanism by which shorebirds reduced biofilm biomass was not experimentally tested, although the literature provides a number of possible drivers including physical disturbance (bioturbation) through tracking (walking) and foraging. Considering the presence of large numbers of *C. alpina*, which has been shown to consume MPB, it is plausible that direct consumption of biofilm may have contributed, but this is not confirmed. The lack of significant differences in macrofauna densities between treatments suggests that altered numbers of these invertebrates were not driving a change in bioturbation or grazing on the biofilms, and thus were not a significant driver of the measured effects.

The finite period of effects and community interactions between shorebirds, macrofauna and MPB reduce the clarity of the situation regarding consequences of declining shorebird species on coastal ecosystem functions. The work presented here indicates a potential shorebird density-dependent effect, resulting in stronger impacts on ecosystem function by birds during winter that may be 'reset' by other organisms or reduced bird densities in spring and summer. This reflects the complexity of intertidal mudflat ecosystem functions (Passarelli et al., 2018; Hale et al., 2019), but is a step forward in disentangling the many factors influencing them. This research indicates that shorebirds play a significant role in the ecosystem functions provided by intertidal mudflats, including erosion protection, nutrient cycling and carbon sequestration. However, further research is required, involving longer-term, larger-scale experiments, to better understand the mechanisms behind ecosystem function regulation by shorebirds.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

JB conducted the initial experimental design and background research, performed primary data analysis and interpretation, was the primary manuscript author and led field and lab work. GU was a Ph.D. supervisor and provided quality control of the experimental design, flux core methodology, data analysis and interpretation. AP performed the laboratory data analysis of contact cores (Chl *a* concentrations) and some flux core samples (dissolved organic carbon) and contributed to the manuscript. RD was a Ph.D. supervisor and provided quality control of data interpretation regarding ecological community effects. TT was the Ph.D. project originator, primary supervisor and provided quality control of the experimental design, sediment properties measurements methodologies, data analysis and interpretation. GU, RD, and TT were manuscript reviewers and contributors. All authors contributed to the article and approved the submitted version.

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