The impact of future environmental change on benthic ecosystem functioning and Carbon cycling in UK shelf seas

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The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

Copyright Declaration

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Abstract

Marine sediments are considered to be one of the largest carbon reservoirs in the world and play an important role in climate regulation. It is essential that there is a better understanding of the distribution, sources, lability, and vulnerability to degradation of carbon within shelf sea sediments. This will aid the improved management of carbon storage as an ecosystem service, especially considering predicted future environmental change, such as ocean warming. Therefore, it is also essential that a better understanding is gained on how intact benthic communities will respond to predicted environmental changes (e.g. temperature rises). Research was undertaken using both an observational approach using a spatially distributed dataset and using a manipulative experiment looking in detail at benthic ecosystem function.

This thesis demonstrates that temperature influences biodiversity and ecosystem function (BEF) relationships, especially the uptake and processing of organic carbon entering the benthic environment. This research indicated that temperature rises resulted in a higher sediment community oxygen uptake, higher amount of bioirrigation and more organic carbon was respired releasing more carbon dioxide. The results also indicate that it is possible that the functional structure of benthic communities could shift in the future due to temperature increases.

This research also demonstrated that the distribution, lability, and vulnerability of carbon within shelf sediments can be complex over relatively small spatial scales. This research indicates that in the northern North Sea more organic carbon exists in sediments found further offshore compared to sediments found closer to the coastline. However, an amino acid based degradation index indicates that this organic carbon found further offshore is more degraded in comparison to sediment found nearer the coastline. This suggests that shelf sediments found nearer the coastline are potentially more vulnerable to degradation than sediments found further away from the coastline. This information can inform more detailed and targeted management strategies for shelf sea sediments which regard to carbon storage.

This research examines the impact that future ocean warming could have on the functioning, specifically carbon cycling and storage, of marine benthic environments. This thesis also highlights the need to better understand the composition and reactivity of carbon in marine sediments to better inform management of the marine environment, and better predict the impact that future environmental change could have on marine sedimentary carbon storage.

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1. Introduction

1.1. Background

This thesis uses multidisciplinary techniques and manipulative laboratory experiments to understand the effects of ocean warming on ecosystem functioning, with particular regard to carbon (C) cycling, processing and storage in muddy marine systems.

This thesis also aims to address the current limited understanding of organic carbon composition and reactivity in shelf sea sediments.

1.1.1. Why shelf seas?

Shelf seas are regions between the land and open ocean where the water depth is less than 200 m (Kröger et al., 2018). Shelf environments make up <10% of the global oceans, however they are much more productive than open ocean environments and are therefore considered to be the most valuable biome on the planet through provision of a variety of ecosystem goods and services (Levin et al., 2001; Kröger et al., 2018; Luisetti et al., 2019). Services provided to human populations by shelf systems include biodiversity, nutrient cycling, carbon cycling and storage, recreation, renewable energy sources and support 90% of global fisheries (Kröger et al., 2018). However, despite their known importance, these environments are under significant stress from human activities such as, nutrient inputs, overfishing and climate change (Halpern et al., 2008; Rabalais et al., 2009; Hall et al., 2013; Kröger et al., 2018; Kenny et al., 2018). However, human activities occurring in shelf sea environments provide human populations with economic benefits, with the most widespread human impact being bottom trawling (Engel and Kvitek, 1998; Hinz et al., 2009; Kröger et al., 2018; Kenny et al., 2018; Smeaton and Austin, 2022).

Shelf seas are considered to be carbon sinks with a net annual uptake of CO_2 from the atmosphere in Northwest European shelf seas, absorbing 15-40 million tonnes of carbon per year from the atmosphere (Thomas et al., 2004; Wakelin et al., 2012; Kröger et al., 2018). In addition, shelf sea sediments are also capable of capturing and storing carbon over geological timescales, play an important role in the global carbon cycle and climate regulation (Hedges and Keil, 1995; Atwood et al., 2020; Smeaton and Austin, 2022). The

removal of carbon from the climate system and its storage in sedimentary habitats has been termed Blue Carbon (Gao et al., 2016; Kröger et al., 2018). Over recent years concerns have grown relating to the impact that anthropogenic activities could have on the storage of Blue carbon in marine sediments, with the primary concern being the release of CO_2 from marine sediments which are then release into the water column and atmosphere (Bauer, W.J. Cai, et al., 2013; Smeaton and Austin, 2022). Human activities which are of primary concern, in relation to disturbance of sediments and remineralization of organic carbon, are bottom trawling gears (Kröger et al., 2018; Smeaton and Austin, 2022). Such gears remobilize surface sediments where the most labile and bioavailable OM is located (Oberle et al., 2016; Smeaton and Austin, 2022). Within European waters approximately 30% to 85% of the continental shelf is impacted by trawling activities, indicating that it is likely that a large number of potential carbon stores in marine sedimentary environments could be exposed to disturbance (Eigaard et al., 2017; Smeaton and Austin, 2022).

The quality or reactivity of OM entering marine sediments determines the vulnerability of OC to disturbances (natural and/or anthropogenic), which in turn determines that role which the sediments play in carbon storage and climate regulation (Smeaton and Austin, 2022). It has been estimated that of the OC which enters the marine sedimentary system and accumulates, approximately 90% has already been degraded to some extent because of processing which has occurred within the marine water column or processing which has occurred within terrestrial and/or fluvial systems (Middelburg, 2019). More labile carbon entering marine sediments is considered to be more reactive and bioavailable and is therefore more easily remineralized (Arndt et al., 2013; Smeaton and Austin, 2022). In contrast more refractory carbon is considered to be more resistant to degradation processes meaning that the likelihood that the OC will be lost through remineralization processes is lower (Smeaton and Austin, 2022).

Smeaton et al. (2021) observed that the quantity and density of OC in surficial sediments within the UK Exclusive Economic Zone varied significantly, and that fjords and estuaries are hotspots for carbon burial and storage (Bianchi et al., 2020). Smeaton & Austin (2022) used the carbon reactivity index (CRI) to determine the quality and reactivity of OM

and associated OC of marine sediments across the UK EEZ. The authors observed a significant gradient in the CRI value across the EEZ, with values ranging from 0.31 to 0.94, low CRI values indicate high reactivity (labile) OM whereas higher CRI values indicate lower reactivity (refractory) OM. It was observed that the highest reactivity OM was found in sediments at the boundary between land and ocean, and the quality, quantity, and reactivity of OC within sediments decreased moving away from land (Smeaton and Austin, 2022). The authors also observed that the change from more labile OM to more refractory OM occurred over relatively short distances from the land, with the highest reactivity occurring within 5 km from land masses. In their analysis Smeaton & Austin (2022) observed that low CRI values were exclusively observed in inshore locations where the bottom water oxygen concentration was low. This is because hypoxic conditions slow the degradation of OC and therefore preserve the labile OM within that location (Arndt et al., 2013; Jessen et al., 2017; Smeaton and Austin, 2022). Excluding sediments which were influenced by hypoxia the authors noted that the CRI of inshore sediments varied between 0.45 and 0.9. It has been suggested that inshore sediments experience high input levels from the terrestrial and marine environment, in addition to high burial rates which mean that large quantities of labile organic matter can be buried and stored within inshore sediments without undergoing significant additional degradation (Smith et al., 2015; Bianchi et al., 2020; Smeaton and Austin, 2022).

Smeaton & Austin (2022) observed that higher CRI values, >0.75, within the outer most reaches of the inshore zone and were therefore dominated by more refractory OM. Organic matter located in coastal and offshore sediments is primarily derived from marine sources, which is known to be significantly more labile and reactive than terrestrially derived OM (Smeaton and Austin, 2022). Compared to inshore sediments continental shelf seas are characterized by well oxygenated waters, comparatively low sedimentation rates, longer OM transport times, higher rates of biological consumption and processing and natural cycles of sediment resuspension (Bao et al., 2018; Bröder et al., 2018; Coughlan et al., 2021; Smeaton and Austin, 2022), all of which enhance degradation of OM within the sedimentary environment. These processes occur naturally within the marine environment

and result in sediments located further offshore failing to retain labile OC, meaning that the OM store within these sediments has a lower reactivity compared to that of more inshore sediments (Smeaton and Austin, 2022).

Sediment type has also been observed to influence the reactivity of OM present within sedimentary environments. Smeaton & Austin (2022) observed that sediments with a CRI value <0.75 were exclusively muddy and that coarser sediments characterized by more refractory OM had a higher CRI value which was not impacted by water depth or proximity to land. These trends could be explained by two processes. Either OM entering coarser sediments was of lower quality to start with, or OM entering these systems is rapidly processed and degraded through natural deposition and resuspension processes.

In addition to natural processes and conditions (bottom oxygen concentration, sedimentation rate, biological consumption, and hydrodynamics) the processing and degradation of OM within marine sediments is impacted by anthropogenic activities (Smeaton and Austin, 2022). Bottom trawling activities are thought to be of significant risk to sedimentary carbon stores within shelf sediment environments (Smeaton and Austin, 2022). However, despite the known disturbances occurring to the seafloor environment, only ~4% of the global seafloor is protected though marine protected areas (MPAs) and only 2.7% is protected from bottom disturbances (Sala et al., 2021; Smeaton and Austin, 2022). The importance of understanding the impact of anthropogenic activities which disturb marine sedimentary environments is important as such activities are thought to increase the risk of CO_2 release into the water column and potentially the atmosphere and therefore risk exacerbating the predicted impacts of global environmental change, including temperature rises (Smeaton and Austin, 2022). This is of particular interest within UK shelf seas, which have a long history of bottom trawling activities and is considered to be one of the most heavily trawled seabeds in the world (Smeaton and Austin, 2022). Current bottom trawling activity is primarily undertaken in muddy sediments more than 5km from shore (Smeaton and Austin, 2022). Therefore, based on the observations by Smeaton & Austin (2022) it is likely that the risk of any remineralization of carbon within these sediments is relatively low as the authors research indicates that the reactivity of OM and carbon within

these sediments is relatively low and is more refractory as the more labile carbon has already been processed and remineralized in comparison to sediments located nearer land. Therefore, it is possible that it could be concluded that the offshore sediments where more bottom trawling activities occur are more stable and resilient to disturbance activities and therefore disturbance activities may not significantly contribute to rises in CO_2 release due to remineralization processes.

It is essential that more work is undertaken to better understand the distribution, composition and reactivity of OM entering marine shelf environments to better characterize carbon storage environments within these systems. Identifying locations where carbon is stored is essential to protect and manage these locations providing essential goods and services to the human population. It is also essential that these locations are characterized and identified in order for modelling activities to better predict the impacts of predicted environmental change (e.g. ocean warming) on this resource.

1.1.2. Estuaries

Shelf sea environments include intertidal and estuarine environments, which are transitional environments between terrestrial, freshwater, and marine systems (Levin et al., 2001). Estuarine environments interact with a variety of adjoining systems, including groundwaters, sediments, shallow coastal environments, and the atmosphere, meaning that changes that they experience have the potential to have a wider impact and are likely to be impacted by their adjoining systems (Jickells, 1998). Estuarine environments are systems which are considered to be stressed as they experience a wide variety and frequent fluctuations in their conditions, for example: temperature, water depth, salinity and dissolved oxygen content (Levin et al., 2001). As a result of these variable conditions these systems are characterized by a relatively low species richness but are known to be one of the most productive systems on a global scale (Levin et al., 2001). The productivity of these systems results in them providing a variety of ecosystem goods and services to the human population (Levin et al., 2001). Services include nutrient cycling, fisheries resources, habitats and food for migratory and resident species, recreational activities, and C storage (Jickells, 1998; Levin et al., 2001; Kröger et al., 2018).

Due to their importance in OM production, biogeochemical cycling and their already naturally stressed nature, estuarine and shelf sea environments are at particular risk from future environmental change, such as ocean warming. Therefore, due to the sensitivity of these environments it is essential that we fully understand how future environmental change is likely to impact them as thoroughly as possible.

The UK shelf sea environment covers 1.6 million km^2, ~50% of the North-West European shelf, and has an average depth of 80m (Kröger et al., 2018). It has been estimated that UK shelf seas are worth in the region of £47 billion (Kröger et al., 2018; Walport, 2018). However, UK shelf seas are almost entirely impacted by human activities (Kröger et al., 2018).

1.1.3. Why study ocean warming?

Over the decades it has been well established that temperature plays a key role in ecosystem health and function through driving the metabolism of individual organisms, interactions within and between trophic levels and through the distribution of species on a global level (Brown et al., 2004; Fussmann et al., 2014; Salo et al., 2020). Therefore, global warming as a result of anthropogenic activities will impact the health and functioning of the world's ecosystems, including the marine environment (Salo et al., 2020). It has been estimated that atmospheric and sea surface temperatures will continue to rise into the future, with sea surface temperatures expected to increase between 2°C and 4.5°C, with the most likely value around 3°C (Meehl et al., 2007; Godbold and Solan, 2013).

1.2. The global carbon cycle

The global carbon cycle (Figure 1.1) consists of four components, marine, atmospheric, terrestrial, and geological, with the first three being considered active surface reservoirs and the latter being a sub-surface geological loop (Post et al., 1990; Cole et al., 2007; Shepherd, 2009). The three surface reservoirs are interconnected via processes, such as photosynthesis and respiration, by which carbon is chemically transformed (Post et al., 1990; Cole et al., 2007; Shepherd, 2009).

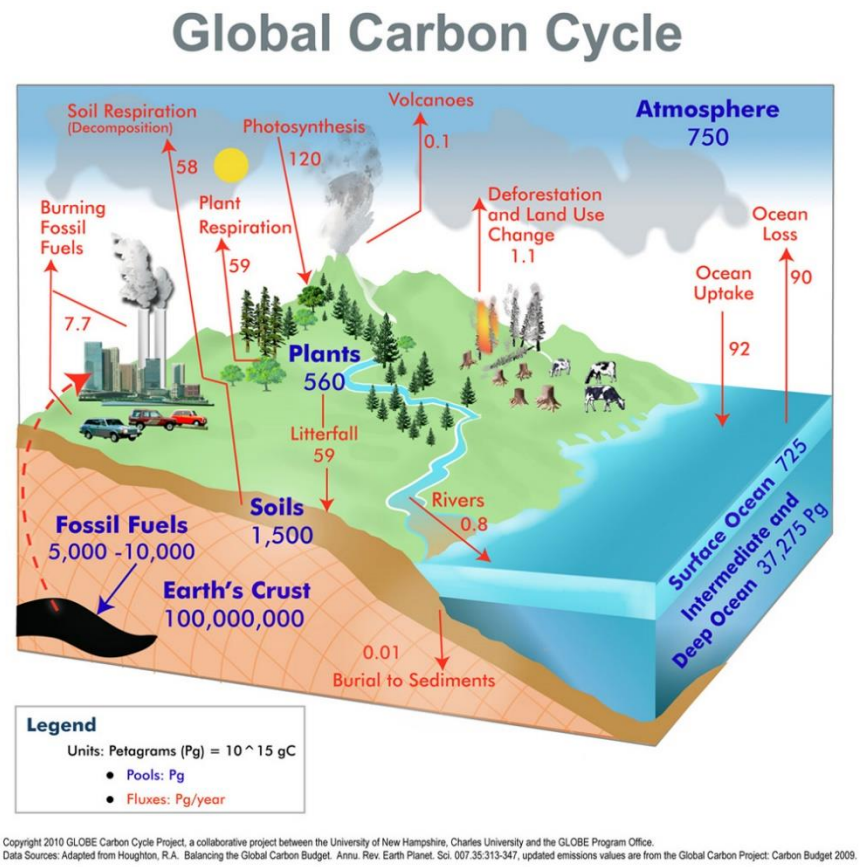

Figure 1.1: A schematic of the global carbon cycle, indicating key carbon pools (blue) and fluxes (red), in Pg/year (NASA GLOBE Program https://airs.jpl.nasa.gov/resources/155/global-carbon-cycle/).

The marine carbon reservoir is considered to be the largest of the surface reactive reservoirs, with carbon being stored in three forms: dissolved inorganic carbon (DIC), dissolved organic carbon (DOC) and particulate organic carbon (POC) (Post et al., 1990; Gruber, 2004). The marine environment also plays an important role in determining the concentration of atmospheric CO_2 through three pumps: physical pump (mixing and circulation), chemical pump (buffering) and biological pump (respiration, photosynthesis, bioturbation etc.) (Riebesell et al., 2009; Henson et al., 2011; Passow and Carlson, 2012). The biological pump involves the transport of products, including dead organisms and faecal pellets, through the water column to the sea floor (De La Rocha and Passow, 2007; Riebesell et al., 2009; Henson et al., 2011; Passow and Carlson, 2012; Keil, 2017). Once these products reach the seafloor, they are subject to a series of complex interactions and processes, known as sedimentary carbon cycling. The majority of organic carbon (OC) is remineralized

and/or decomposed by bacterial activities in the water column and only a small portion of the carbon is buried in the sedimentary record (De La Rocha and Passow, 2007; Keil, 2017). Of all buried organic matter (OM), 80% organic is buried in estuarine and shelf sea environments (Berner, 1982), which is an essential way in which carbon is removed from the active surface pools and into the geological reservoir (Keil, 2017; Kröger et al., 2018). The proportion of deposited OM that is eventually buried in the sedimentary environment is known as the burial efficiency. Burial efficiency is dependent on a balance between decay and preservation processes in a specific location. Decay and preservation processes can be influenced by factors such as temperature, sedimentation rate and oxygen concentration which are important to consider in climate change research (Burdige, 2007; Arndt et al., 2013).

It is generally accepted that anthropogenic activities, such as burning fossil fuels and land use changes, are altering the global carbon cycle (Guinotte and Fabry, 2008; Diaz and Rosenberg, 2008; McLeod et al., 2011). Factors which are thought to influence the marine carbon cycle include changes in surface ocean temperatures, ocean alkalinity, oxygen concentrations and ocean circulation systems (Feely et al., 2009; Doney et al., 2012; Pitacco et al., 2018). However, there has been little work to understand how anthropogenic climate change could influence marine benthic communities and their role in the carbon cycle. It is challenging to understand and observe how intact benthic communities will respond to climate change, but it is known that factors (e.g. temperature or physical disturbance) which disturb a system can result in the release of CO_2 from the sediments back into the water column and potentially the atmosphere (Keil, 2017; Smeaton and Austin, 2022).

1.3. Ocean Warming

The role that temperature plays in the natural world has been studied for several decades, in terms of both the terrestrial and marine environments (Sanz-lázaro et al., 2011; Wernberg et al., 2012). Temperature is known to be a driver of species distributions and activity over a wide range of scales ranging from the community to species level (Clarke and Gaston, 2006; Tittensor et al., 2010; Wernberg et al., 2012) and physiological processes (Helmuth et al., 2006).

Temperature manipulation experiments have been undertaken across a wide range of marine habitats and species, ranging from benthic species to macrophytes and zooplankton, and have generally shown an increase in mortality and respiration rates, reductions in growth, metabolic suppression, and behavioural changes (Anestis et al., 2007; Isla et al., 2008; Massa et al., 2009). Anestis et al. (2007), for example, studied the impact of temperature rises on the Mediterranean mussels. The authors results indicated that temperature increases result in the Mediterranean mussel expressing a stress response, as they spend more time with their valves closed, increased expression of heat shock proteins, and mortality. Isla et al. (2008) indicate that copepods in the Western Baltic Sea demonstrated increase respiration rates and ingestion of organic matter increased at higher temperatures, in addition to increased mortality rates. Temperature has also been seen to impact plants, such as the seagrass species *Zotera noltii* which have been observed to demonstrate a decrease in photosynthetic capacity and increase in mortality under increased temperatures, as demonstrated by Massa et al. (2009), potentially indicating a decrease in seagrass habitats under future warming scenarios. However, it has also been shown that temperature changes don't influence species in the same way, and that an organism's ability to cope with temperature changes varies across the globe, and the ability to respond to a changing environment dictates the success or failure of the population over time (Peck et al., 2004). For example, Antarctic species are thought to be more sensitive to temperature changes than species located in other regions of the planet (Peck et al., 2004). Peck et al. (2004) observed that the Antarctic bivalve *Laternula elliptica* and the limpet *Nacella concina* suffered a 50% failure in essential biological activities when exposed to temperatures of 2-3°C and complete loss of the functions at a temperature of 5°C, and death after a few days of being incubated at 9-10°C. The authors also observed that the Antarctic scallop, *Adamussium colbecki*, was more sensitive than the species mentioned previously, and lost its ability to swim when temperatures reached 2°C. In general Antarctic species appear to be poor at adapting to surviving temperature changes and are more sensitive to such changes compared to temperate environments (Convey and Peck, 2019).

The complexity of temperature impacts on species distributions and abundances has also been demonstrated through long-term (10 year) in situ observations using a BACI (before-after, control-impact) design undertaken by Schiel et al. (2004). The study explored the impact of a 3°C temperature rise due to the thermal outfall of a power station over a 2 km stretch of Californian rocky coastline. The authors did not observe the expected changes resulting from an increase in temperature, where warm water species replace cold water species. Instead, it appeared that the overall trend was that the effects of temperature increases cascaded through the system, where there were impacts to key species within the community, especially kelps and red algae. The overall trend of temperature increases appeared to result in a decrease in abundance of algae, in comparison to increased abundances of grazing species. More specifically the authors observed that of the species whose abundances were impacted by temperature 38% demonstrated an increase in abundance and 49% demonstrated a decrease. It was also observed that 54% of algal species, such as *Mastocarpus papollatus*, declined by at least 50% due to warming, whereas the abundance of intertidal grazers, such as *Tegula funebralis*, increased in abundance (Schiel et al., 2004). Work undertaken by Bamber & Spencer (1984) also demonstrated that higher temperatures could potentially have a positive impact on some macrofaunal groups. The authors observed that the presence of a power station thermal outfall on the Medway estuary resulted in higher densities of opportunistic benthic species, such as species of Tubificoides. Results from these studies indicate that the impact of even a single environmental change, such as temperature, is complex and not uniform across all species within an ecosystem and can give some species a competitive advantage over others. Therefore, changes in community structure could potentially alter the character of community and ecosystem function.

The ability of organisms to cope with stress depends on their ability to adapt and survive environmental changes through behavioural and physiological plasticity, which can change over the course of an individual's lifetime depending on the environmental context it experiences and contribute to population survival and evolution over time (Scapini et al., 2019). The intensity of a response to environmental change, whether positive or negative

can vary depending on the geographic origin of the species in question and its life history, for example temperature rises influence life history traits including organisms growth, population structure and reproduction (Marques et al., 2003; Scapini et al., 2019; Reed et al., 2020). Long-term trends have been observed in species found within sandy beach communities around the coast of South America (Ortega et al., 2012; Celentano & Defeo, 2016; Ortega et al., 2016; Scapini et al., 2019). For example, the Mesodesma genus originates from Antarctica and is associated with cold water environment in both the pacific and Atlantic (Ortega et al., 2012). *Mesodesma mactroides* experienced lower population abundance, recruitment, and adult survival at higher temperatures (Ortega et al., 2016; Scapini et al., 2019). Another Mesodesma species, *Mesodesma donacium*, found in the Pacific experienced negative impacts of the El Ninõ Southern Oscillation in Peru and northern Chile and changed the community structure, but a positive response in Southern Chile at the southern edge of the species distribution where abundances of the species increased (Ortega et al., 2012). In comparison *Emerita brasiliensis*, a species of crab, has an affinity with warmer, more tropical temperatures (Celentano & Defeo, 2016). The authors note that La Ninã events along the coast of Uruguay are characterized by more tropical waters, and that as a result this had a positive impact on the abundance of *Emerita brasiliensis*, including an increase in population abundance and the reproduction and recruitment period was extended because of such events. In addition, Celentano & Defeo (2016) state that an increase in growth rate, reproduction and recruitment periods was observed from temperate waters to more tropical waters along the Atlantic coast of South America. This information could suggest that species which exist at the edge of their thermal tolerances could demonstrate either a positive or negative response to temperature changes depending on their affinity to colder or warmer water conditions, with species which are better adapted to warmer conditions exhibiting a positive response to warming at the lower end of their thermal tolerance, whereas species better adapted to cooler conditions demonstrating a negative response to warming at the top end of their thermal tolerance (Scapini et al., 2019).

Significant research has been undertaken over recent decades to determine the impact of environmental stressors (temperature, pH, oxygen, eutrophication) on marine benthic community structure and functioning. However, there are still very few studies which use intact communities (benthic and microbial) to determine the impacts of environmental change on the functioning of the marine benthic ecosystem as a whole. Therefore, fully understanding how single stressors, such as temperature, impact ecosystem structure and function is the first essential step which needs to be undertaken, before multiple stressor experiments can be undertaken on intact communities. A full understanding of how temperature influences ecosystem structure and function is also an essential step in forecasting the impacts of climate change on marine systems, especially as temperature impacts on the marine environment are not equivalent across all habitats, systems, and species.

1.4. Biodiversity and ecosystem function (BEF) relationships

Macrofauna living within and on marine sediments play a central role in processing OM within the marine environment and influence the exchange of particulate and dissolved nutrients, including carbon, across the sediment-water interface (Ehrnsten et al., 2019). Individuals and communities do this through ingesting OM, growth, respiration and excretion (Herman et al., 1999; Josefson and Rasmussen, 2000; Middelburg, 2018; Ehrnsten et al., 2019) and through sediment reworking activities such as bioturbation and bioirrigation (Woulds et al., 2009; Ehrnsten et al., 2019).

1.4.1. Species composition

The distribution, composition and biomass of macrofauna within natural systems is known to be patchy (Kraan et al., 2009; Godbold et al., 2011) due to variability in environmental factors including, but not limited to sediment type and OM inputs, which are known to be some of the key controls driving faunal distributions within the marine environment (Kelaher et al., 2003; Godbold et al., 2011). Macrofaunal distribution, abundance and biomass has been linked to the amount and quality of OM entering the system (Dauwe et al., 1998; Ingles et al., 2009; Leduc et al., 2020). Submarine canyons, for example, are thought to transfer OM from the coastal ocean to the deep oceans which

could be an important food source for organisms living within deep sea sediments and can therefore act as a hotspot for benthic biomass and activity (Leduc et al., 2020). For example, Ingles et al. (2009) observed that the amount and quality of OM available for direct consumption was higher within the Nazaré Canyon than surrounding slopes, which was positively correlated with nematode abundance and biomass. It has also been observed that canyons can show higher meio and macrofaunal abundances and biomass compared to the surrounding slopes (Leduc et al., 2020; Vetter & Dayton, 1998). For example, Vetter & Dayton (1998 & 1999) observed that infaunal density and biomass were higher within Scripps and La Jolla Submarine Canyons compared with the surrounding slopes. An experimental approach undertaken by Kelaher et al. (2003) also demonstrated that the amount of organic matter within an intertidal mud flat environment could alter the distribution of the foraging snail species *Ilyanassa obsolete*. The authors observed that areas which had been experimentally enriched with detritus resulted in the creation of locations with higher densities of foraging snails, and that as most of the snails observed in such areas were predominantly adult it was suggested that they had actively foraged and moved into areas with enhanced detritus and therefore food supply. Observations have also suggested that macrofaunal biomass decreases with increasing water depth (Rowe et al., 1974; Briones et al., 2008; Miatta and Snelgrove, 2021a), which could be explained by negative relationships between water depth and quality and quantity of OM entering sedimentary systems, where deeper environments receive a lower quality and quantity OM in comparison to shallower systems (Miatta and Snelgrove, 2021a). For example, Briones et al. (2008) observed that the highest biomass and richness values were observed at the head of a canyon head and the lowest at its mouth.

Distribution, quality, and quantity of OM also influences the vertical distribution of macrofauna within the sediment column (Dauwe et al., 1998). For example, Dauwe et al. (1998) observed that large amounts of higher quality OM were present within the German Bight and most organisms present were found within the top 2cm of the sediment column and primarily consumed the fresh OM entering the environment. In comparison, the Skagerrak hosted smaller organisms which lived deeper within the sediment column (up to

20cm in depth), most likely due to the sediments containing more refractory carbon which is less bioavailable (Dauwe et al., 1998). Finally, it has been noted that highly dynamic locations which have coarse sediments, such as in the Broad Fourteens, have a low macrofaunal diversity (Dauwe et al., 1998).

Understanding the structure of macrofaunal benthic communities is important for understanding the impacts that the community has on ecosystem functioning, defined here as processes occurring within the system (Biles et al., 2002) such as carbon remineralization and nutrient fluxes, as it is known that such processes are linked to faunal activities. Therefore, it is essential that in addition to understanding the community structure, which describes "who" is present, it is essential that functional diversity is considered to understand ecosystem functioning more fully.

1.4.2. Biological traits

It is becoming more widely accepted that only understanding the structural composition of benthic ecosystems is not sufficient in understanding the role of infaunal communities in mediating ecosystem functioning, especially in relation to effects of anthropogenic impacts to benthic function (Bolam and Eggleton, 2014). Therefore, understanding how communities function is essential, especially when considering the impact of anthropogenic pressures on ecosystem function and processes and will form the basis of ecosystem based approaches for management of the marine system (Bolam and Eggleton, 2014).

Broadly speaking biological traits comprise of any morphological, physiological or phenological characteristic of a species (de Juan et al., 2022). For example, life history traits such as life span and reproduction and behavioural traits such as mobility, feeding ecology (de Juan et al., 2022). However, traits can be further divided into effect and response trait categories (Hadj-Hammou et al., 2021; de Juan et al., 2022). Some traits expressed by a species respond to environmental gradients and disturbance, known as response traits, and are directly linked to the survival of a community under changing environmental conditions (Hadj-Hammou et al., 2021; de Juan et al., 2022). For example, taxa which are capable of

burrowing deeper into the sediment column are more likely to be capable of avoiding and surviving disturbances at the sediment-water interface (Bolam et al., 2016). Whereas, other traits, known as effect traits, play a key role in ecosystem functioning processes (Bolam et al., 2016; Hadj-Hammou et al., 2021; de Juan et al., 2022). For example, species mobility and sediment reworking type could influence the penetration of oxygen into the sediment column through bioturbation (Bolam et al., 2016; de Juan et al., 2022).

Biological traits analysis (BTA) is a method by which ecological functioning can be described using various life history, morphological and behavioural characteristics, including factors such as body size, feeding mechanism, bioturbation type, morphology, longevity, position in sediment and living habit (Bremner et al., 2006; Bremner, 2008; Bolam and Eggleton, 2014; Chapman et al., 2018). BTA is a useful method through which to look at the functionality of a community as traits can be shared by organisms that are taxonomically different and it can be applied over a large variety of geographic ranges (Bremner et al., 2006). BTA incorporates both abundance/biomass data and a wide range of biological characteristics of species into a single analysis (Bremner et al., 2003), using a multivariate ordination approach, such as fuzzy principal component analysis, to describe patterns in biological traits over entire assemblages (Bremner et al., 2003; Bremner et al., 2006; Bolam et al., 2017). Selection of traits to use within the analysis is an important step as a wide variety of traits are available for describing ecological functioning, however not all traits are equally important, useful or relevant in each study (Bremner et al., 2006). Constraints on trait selection exist in the form of information availability (Bremner et al., 2006; Bolam et al., 2017; Degen et al., 2018). Biological traits can be further divided into effect and response traits, which describe how a species responds to environmental factors, including disturbances.

As the threat of climate change becomes more apparent, management of the marine environment is required to be pro-active, and it is essential that managers of the marine environment and policy makers are able to prepare response strategies for various future scenarios (Bremner, 2008). Marine conservation and management of the marine environment is starting to move towards focusing more on ecosystem functioning

(Bremner, 2008; Miatta et al., 2021). For example, biological traits have been used to determine how the functional composition of different sedimentary environments varies (Bolam et al., 2017). Bolam et al. (2017) determined that assemblages located in deep, muddy sediments demonstrated a higher proportion of downward conveyors and surface deposit feeders, whereas coarser sediments demonstrated a dominance of more sessile individuals, upward conveyors, and suspension feeders. In comparison coarser sediments in shallow water environments were dominated by diffusive mixers, burrowers, scavengers, and predators (Bolam et al., 2017). The results from this study indicate that shallow water assemblages are more likely to be less sensitive to the impacts of bottom trawling, due to the dominance of traits such as scavengers. It has been suggested that BTA could be used to inform functional sensitivity assessments which could aid the management of marine sedimentary environments. In addition to understanding the current functional composition of benthic communities, BTA can be used to determine the impact of anthropogenic activities on community functional composition. For example, this approach has been used to determine the impact of a dredge disposal site in NE England on benthic assemblages (Bolam et al., 2016). The authors determined that there was a reduced number of species and invertebrate density in addition to functional composition within the disposal site in comparison to locations which were sampled outside from the impact area, most likely a result of the smothering effect of disposal activities.

1.4.3. Community impacts on ecosystem function

Biodiversity-Ecosystem functioning (BEF) research is a useful approach to understand how ecosystems could respond to an environmental change (Snelgrove et al., 2014). As previously indicated the species present and the activities/functions which they undertake influence the functioning (e.g., OM processing and nutrient fluxes) of marine systems (Solan et al., 2003). The most common approach to understanding BEF relationships is through the use of relatively small-scale microcosm experiments, which manipulate the community present (e.g., species richness, evenness and diversity) in each core and measure the response to ecosystem functioning (e.g. nutrient fluxes). For example, macrofaunal bioturbation activities have been recognised as an important biological activity which

controls ecosystem functioning (Kristensen et al., 2012; Gilbert et al., 2021) and can fundamentally change sediment biogeochemistry (Solan et al., 2020). It has been observed that both species richness and evenness have influenced nutrient fluxes in the marine environment (Ieno et al., 2006; Bulling et al., 2010; Dolbeth et al., 2019). For example, Dolbeth et al. (2019) observed that more evenly distributed benthic communities resulted in increased particle reworking activities and increased nutrient release. Leno et al (2006) and Bulling et al. (2010) both noted that an increase in species richness resulted in an increase in ammonium and phosphate release. Although both Leno et al. (2006) and Dolbeth et al. (2019) noted that in addition to species richness and or/evenness, the identity and density of species was also important in driving the observed changes in ecosystem functioning, indicating that species-specific traits are important in determining ecosystem functioning in marine benthic systems. This was also observed by Raffaelli et al. (2003), who undertook experiments where species richness was manipulated and concentrations of nutrients in overlying waters was measured. The experimental tanks were allocated to different species richness treatments from zero, where no macrofauna were introduced, to monoculture tanks up to ten species per tank. The authors note that in single species treatments, such as *Corophium volutator* and *Hediste diversicolor*, a high concentration on ammonium was measured. Whereas, in other single species treatments, such as the bivalves *Cerastoderma edule* and *Mytilus edulis*, lower concentrations of ammonium were measured which could be accounted for their ability to remove suspended particles from within the water column. The authors observed that the sum of ammonium measured by each species in isolation did not equate to the observations in multi-species tanks. The authors therefore determined that functional trait richness rather than species richness was important in driving ecosystem functions.

As indicated by Raffaelli et al. (2003) functional traits play an important role in ecosystem functioning of the marine benthic system. Laboratory based experiments have been undertaken which aim to understand how community composition influences sediment reworking and ventilation activities (Solan, Cardinale, et al., 2004). Understanding how macrofauna manipulate their surrounding environment is important because it is

considered one of the most important factors controlling benthic ecosystem functioning, including oxygen penetration into the sediment column, OM processing and nutrient fluxes (Solan, Cardinale, et al., 2004; Solan, Wigham, et al., 2004; van de Velde and Meysman, 2016). Bioturbation describes the activities of organisms which live within marine sediments and cause disruption to the sediment column through burrowing activities or through ingestion of sediment particles (Biles et al., 2002; Meysman et al., 2006; Bertics et al., 2010; Kristensen et al., 2012; Queirós et al., 2013). These processes also result in the ventilation of the sediment column, known as bioirrigation, where water with a higher concentration of oxygen is transported into sediments which are oxygen depleted (Pischedda et al., 2008; de Moura Queirós et al., 2011; Kristensen et al., 2012). There are four broad categories of bioturbation activities which have been described by Kristensen et al. (2012), biodiffusors, Upward/Downward conveyors and regenerators. Each of these four categories interact with the sediment in different ways, including penetrating into the sediment column to different depths, are developed/constructed at different rates and are different sizes (Pischedda et al., 2008; Queirós et al., 2013). The rate of bioturbating activities undertaken by benthic macrofauna is dependent on several factors including abundance and biomass, size of individuals and sediment reworking method (Kristensen et al., 2012).

As previously outlined, bioturbation activities manipulate the sedimentary environment and cycling in several ways, which often occur concurrently (van de Velde and Meysman, 2016). Primary activities include faunal feeding, digestion, and defecation activities, modifying the structure of the sediment column and transporting solutes and OM within the sediment column (Meysman et al., 2006; Middelburg and Levin, 2009). An exclusion experiment undertaken on intertidal sandflats in the Wadden Sea indicated that exclusion of the lug worm (*Arenicola marina*) resulted in increased amounts of fine OM clogging porewaters within the sandy sediment and sediment permeability decreased (Volkenborn, Polerecky, et al., 2007; Volkenborn, Hedtkamp, et al., 2007). In addition, the authors noted that the oxygen penetration depth decreased and there was an accumulation of ammonium, phosphate, and sulphide within porewaters of the sediments. This study indicates that the presence of *Arenicola marina* results in a system which is primarily driven

by advection processes and if this species is removed the system becomes more dependent of diffusive processes and demonstrates how the presence or absence of a single species can alter the entire sedimentary environment. The authors suggested that if *Arenicola marina* was not present in this location an expansion of mudflats would be observed and a reduction in the extent of sandflat environments.

Understanding the impact of macrofaunal activities, especially oxygen penetration depth, on the sedimentary environment is important as they have a significant impact on the chemical properties of the sediment column and are linked to the benthic microbial community (Meysman et al., 2006; Volkenborn, Polerecky, et al., 2007; Tarhan et al., 2021). For example, it has been observed that sediment surface microbial communities are distinct from sub-surface communities (Bertics and Ziebis, 2009). Betrics et al. (2009) aimed to determine how bioturbation activities undertaken by the ghost shrimp (*Neotrypaea californiensis*) and the fiddler crab (*Uca crenulate*) influenced the structure of the benthic microbial community. These two species undertake two different sediment reworking methods and have different lifestyles, where the ghost shrimp permanently lives in deep burrows and supplies the burrows with oxygen rich water through bioirrigation activities (Bertics and Ziebis, 2009). In contrast, the authors described the fiddler crab as constructing simple "J" shaped burrows which it does not permanently inhabit and which it does not actively ventilate. The results indicated that oxygen was the most important factor in driving the presence and abundance of different microbial taxa and that microbial communities found within macrofaunal burrows demonstrated a similarity to the composition of surficial sediments (Bertics and Ziebis, 2009).

It has been demonstrated that sediment reworking and ventilation activities play an important role in determining the ecosystem function of marine benthic communities. The challenge comes in measuring sediment reworking and ventilation activities. Several different methods are used to measure sediment reworking and ventilation activities. The most common method used is the use of luminophores and sediment profile imaging, which can be undertaken in situ or ex situ (Solan, Wigham, et al., 2004). Luminophores are particles, usually sand, which fluoresce under ultraviolet light (Solan, Wigham, et al., 2004).

This approach enables the redistribution of particles within the sediment column to be measured over time, without disturbing the sediment cores. Bioirrigation can also be measured using an inert tracer, sodium bromide, where negative values indicate that bioirrigation has occurred (Hale et al., 2014). However, over recent years indexes have been developed which can estimate the bioturbation and bioirrigation potential of communities, based on functional traits associated with sediment reworking and ventilation activities in addition to abundance and biomass of the species (Queirós et al., 2013; Wrede et al., 2018; Wrede et al., 2019). This is particularly useful when sediment reworking, and ventilation activities have not been directly measured.

In addition to understanding BEF relationships under current conditions, studies have been undertaken to determine the impact of environmental changes on BEF relationships, such as ocean acidification and warming. For example, increases in temperature are known to drive physiological processes and can result in behaviour changes of organisms (Verdelhos et al., 2015; Salo et al., 2020; Dolbeth et al., 2021). Dolbeth et al. (2021) undertook and experiment on intact sediment cores over a 24-day period and observed that increased temperatures resulted in increased bioturbation and nutrient release, compared to control temperatures. However, in comparison an experiment undertaken over an 18-month period on a single species (*Alitta virens*) indicated that the depth of particle reworking shallowed over a 12-month period (Godbold and Solan, 2013).

The impact of temperature on ecosystem functioning can be observed through examining different climatic zones across the globe. For example, oxygen fluxes have been observed to be lower in polar regions, such as the Beaufort Sea (0.5-11.5 mmol m^{-2} d^{-1}; Link et al., 2013) and Fran Strait (0.5 – 5.1 mmol m^{-2} d^{-1}; Hoffmann et al., 2018), compared to tropical regions, such as in French Polynesia (5.52 – 42.72 mmol m^{-2} d^{-1}; Grenz et al., 2021) and New Caledonia (35.04 – 71.28 mmol m^{-2} d^{-1}; Boucher et al., 1994). This could suggest that higher temperatures could result in a higher sediment community oxygen demand. Bourgeois et al. (2017) undertook a synthesis which studied organic matter remineralization within Arctic marine sediments. Work undertaken by the authors indicates there is a large amount of variability in the sediment oxygen demand within the Arctic, with the lowest

demands being almost zero in the Laptev Sea at 3500m water depth (0.07 mmol m^{-2} d^{-1}; Boetius & Damm, 1998) but occasionally reported much higher rates such as 45.62 mmol m^{-2} d^{-1} in the northern Bering and Chuckchi seas (Grebmeier & McRoy, 1989). However, such high rates appear to be unusual in Arctic areas, with the mean rate appearing to be ~10 mmol O_2 m^{-2} d^{-1} in water depths between 0-200m, and decreasing with water depth (Bourgeois et al., 2017). It has been suggested that the decrease in sediment oxygen demand with increasing water depth (Link et al., 2013; Bourgeois et al., 2017) could be linked to decreases in food availability with increasing water depth (Bourgeois et al., 2017). This demonstrates that multiple environmental variables, in addition to temperature, have a role in driving ecosystem functioning.

1.5. Organic matter: Distribution, preservation, processing, and composition

Due to the importance of OM in a variety of processes it is essential to fully understand factors which influence the sources, distribution, and preservation of OM within shelf sea environments in the UK. A significant amount of research has been undertaken to understand the factors which control OM distribution and preservation in the marine environment. However, many factors which are outlined are interconnected and are likely to vary between different locations.

1.5.1. Sedimentation rate

It is thought that degradation of OM in marine sediments is controlled by input from the water column (Arndt et al., 2013). This means organic degradation is dependent on processes occurring from within the water column, such as photosynthesis, and export from the terrestrial environment and chemoautotrophy (Middelburg, 2011; Arndt et al., 2013).

Organic matter is transported through the water column vertically, through gravitational settling, and laterally, through water circulation and via erosion and deposition cycles at the sea floor as a result of strong bottom water currents (Sanchez-Vidal et al., 2008; Arndt et al., 2013). As OM is transported through the water column it is being degraded and therefore becomes increasingly more refractory (Wakeham et al., 1997; Arndt et al., 2013). This combination of gravitational settling through the water column and

processing as it falls means that the transport efficiency of OM is key in determining the quality and lability of OM reaching the sedimentary environment in marine ecosystems (Arndt et al., 2013). Transport efficiency is heterogeneous on a global scale and is dependent on a variety of interconnected processes, meaning that understanding how much OM reached the seafloor is difficult, but essential when modelling impacts of OM on biogeochemical cycling (Arndt et al., 2013).

1.5.2. Organic matter source and composition

OM source and composition play an important role in determining the preservation of OM within the Marine environment. OM found in marine sediments is made from a variety of different compounds with different rates of degradability (Emerson and Hedges, 1988; Stedmon and Markager, 2005; Arndt et al., 2013; Burd et al., 2016). Identifying all these individual compounds is complex and there are still gaps in our knowledge relating to OM composition, with the majority of OM remaining molecularly uncharacterized (Burdige, 2007; Arndt et al., 2013; Burd et al., 2016; Middelburg, 2018).

It Is recognized that different compounds have varying levels of degradability, for example proteins, pigments and fatty acids are particularly labile whereas molecules such as cellulose and lignin are less labile as most marine organisms haven't developed the ability to digest these molecules, meaning that in general marine OM is more labile than that derived from the terrestrial environment (Hedges et al., 1988; Lee et al., 2000; Arndt et al., 2013). OM derived from the terrestrial environment is not only less labile in comparison to that derived from the marine environment, but it has already undergone some degree of processing within the terrestrial and fluvial systems before it reaches the marine sedimentary environment (Arndt et al., 2013).

Different compounds can also be used to determine the degradation state of OM within sediments. For example, amino acids have been used to create a degradation index (Dauwe and Middelburg, 1998). For example, the degradation index has been used to characterize the state of sediments within the Changjiang Estuary (Wang et al., 2018). The authors demonstrated, using the degradation index, that sediments which contained more

terrestrially derived OM were more refractory and that OM which was sourced from within the marine environment was more labile. The authors also noted that fine grained and hypoxic sediments were also more labile, based on the degradation index. This also demonstrates the interconnection between different factors known to drive distribution and preservation of OM within the marine environment (oxygen and sediment size).

1.5.3. Temperature

Temperature is an important factor known to influence the metabolism and rate of biogeochemical processes occurring in marine sediments (Sanz-lázaro et al., 2011; Arndt et al., 2013; Sanz-Lázaro et al., 2015; Malinverno and Martinez, 2015). It has been observed that temperature increases in a temperate environment have resulted in an increased rate of OM decomposition (Crill and Martens, 1987; Middleburg et al., 1996; Arndt et al., 2013). However, in sediments which are intrinsically cold, such as arctic sediments, rates of OM decomposition were not significantly different to rates observed in tropical environments (Arnosti et al., 1998; Glud et al., 1998; Arndt et al., 2013). These observations suggest that bacterial communities within sedimentary habitats are physiologically adapted to the temperature regime of the area (Arndt et al., 2013). Therefore, it is likely that the impacts of temperature changes will not be uniform on a global scale but will depend on conditions on smaller scales (Arndt et al., 2013).

Overall, it is clear that the influence which temperature has on organic degradation is complex and still not fully understood (Arndt et al., 2013). It has been observed that the dependence of OM degradation on temperature is reliant on microbial physiology, reaction pathways, timescales, magnitude of change, location, and composition of the OM (Arndt et al., 2013).

1.5.4. Sorptive protection

The sediment particle size plays an important role in driving sedimentary OC content and has been observed to inversely related to organic carbon content in sedimentary habitats (Mayer, 1994). Fine grained sediments, such as muds and silts, have a high surface area to volume ratio and have been observed to have higher concentrations of OC, due to

similar hydrodynamic properties of OM and fine-grained sediment particles and due to the sorption of OC on to sediment particle surfaces (Mayer, 1994; Hedges and Keil, 1995). OM can adsorb onto the surface of sediment particles in a thin layer just one molecule thick, and any OM residing in small cracks on particles is particularly protected from interactions with bacterial enzymes (Mayer, 1994; Ransom et al., 1997; Arndt et al., 2013). It is likely that spatial variability in OM preservation is due to a combination of factors in addition to sorptive preservation, such as oxygen (Hedges and Keil, 1995; Ransom et al., 1998).

1.5.5. Oxygen

It is generally accepted that oxygen concentration plays an important role in the preservation and accumulation of OM in marine sediments. In general, the absence of oxygen (anoxic) or low oxygen (hypoxic) concentrations promote the preservation and accumulation of OM within marine sediments (Canfield, 1989; Paropkari et al., 1992; White et al., 2019). This has been observed in a number of locations across the planet, from the Black Sea, Arabian sea and the Baltic Sea (Glenn and Arthur, 1985; Cowie et al., 1999; Levin et al., 2000; White et al., 2019). It has also been suggested that it is not only the concentration of oxygen which drives the preservation of OM, but the exposure time to oxic conditions, before entering an anoxic or hypoxic environment (Cowie et al., 1995; White et al., 2019). The exposure time of sediment to oxic environments is dependent on the sedimentation rate, water depth and the oxygen penetration depth within the sediment column (Hartnett et al., 1998; Hedges et al., 1999).

1.6. Measuring organic matter processing

Determining how OM is being processed in sediments depends on the question being asked, and there are a variety of tracers available which are capable of tracing carbon processing and sediment reworking in the benthic environment, including luminophores (coloured sand) and stable isotopes (Solan, Wigham, et al., 2004; Woulds et al., 2009).

1.6.1. Stable isotopes

Isotopes are elements which have the same atomic number but different masses. Stable isotopes do not undergo radioactive decay and can therefore be used as a tracer in

experiments. Carbon has two stable isotopes, the first with an atomic mass of 12 and the other with an atomic mass of 13. ^{12}C is more abundant in the natural environment than ^{13}C. Therefore, the ^{13}C and be used as a tracer in carbon processing and ecosystem functioning studies. When a stable isotope is used as a tracer in environmental studies an isotopic signature can be calculated, which measures the ratio of ^{13}C:^{12}C, with the value being reported as parts per thousand (ppt – ‰). The ^{13}C:^{12}C ratio is defined against a standard, Pee Dee Belemnite (PDB) for carbon which as a ^{13}C:^{12}C ratio of 0.0112372 (Middelburg et al., 2000; White et al., 2019).

Tracking stable isotopes through a system is a powerful tool in ecological and biogeochemical studies. However, abundances of these isotopes are relatively low in the natural environment, therefore isotopically enriched compounds can be used as a tracer in benthic systems. Compounds which are used to be isotopically enriched should replicate the natural environment as much as possible, for example labelling algae for photosynthetically active environments (shelf seas) and bicarbonate for chemosynthetically active environments (Woulds et al., 2009; White et al., 2019; Woulds et al., 2019; Woulds et al., 2020). These isotopically labelled compounds are often used in incubation experiments and can be traced through different carbon pools including sediment and fauna. A variety of benthic processes, including respiration, assimilation and feeding guilds, can be quantified using isotope tracing methods.

1.6.2. Microcosm experiments

Microcosm experiments are a useful tool and a popular approach used to determine the role of fauna in ecosystem functioning, including the processing of organic matter under natural and perturbed environmental conditions. This is because environmental factors, including but not limited to temperature, oxygen concentration and pH, can be easily controlled making the experimental set-up relatively simple to replicate. Experimental approaches using microcosms are also very flexible both in terms of the community that can be studied and environmental factor(s) to be controlled. For example, previous studies have used microcosms to study single species (Godbold et al., 2013; Wholgemuth et al., 2017), constructed communities (Wholgemuth et al., 2016, Wholgemuth et al., 2017) and

intact communities (Godbold et al., 2017, White et al., 2019, Solan et al., 2020). The design of microcosm experiments, use of single, constructed, or intact communities, depends on the question being put forward and each approach has its own set of merits and constraints.

Experimental designs which use single species or constructed communities could be considered as non-representative of the natural environment. However, this kind of approach is widely used and is useful because studying simplified communities allows the rigorous testing of specific ecological theories (Benton et al., 2007) such as predator-prey dynamics (Beier et al., 2004), has contributed to a mechanistic understanding of BEF relationships and they can be easily replicated with minimal variability between replicates. The use of relatively simple constructed communities or single species experiments are an ideal approach to separate biotic effects from the influence of a complex environment. However, the question has been raised on whether biotic effects observed in constructed communities have any significance in the real world (Benton et al., 2007), where heterogeneity of the natural environment may influence ecosystem functioning (Strong et al., 2015). For example, it has been demonstrated that the structure of benthic communities plays a role in the scale and pattern of processing of organic matter (Woulds et al., 2007), suggesting that the use intact benthic communities may provide a more realistic approach to understanding OM processing by benthic communities and provide more detailed information on OM processing by a community at a site-specific location. Therefore, the use of intact benthic communities in laboratory-based microcosm experiments may better represent the complexities of natural communities, provide information which more accurately represents how natural communities respond to environmental perturbations, such as temperature rises, and influence ecosystem functioning. This is because benthic groups, both faunal and microbial, are not preferentially selected or excluded. However, the variability between replicates that comes along with the use of natural, intact communities can be challenging, and can limit the interpretations placed on the experimental results.

However, representing the natural variability of benthic communities within laboratory experiments is just one factor within an infinitely complex system. Laboratory based

microcosm experiments, whether using single species, a constructed community, or an intact community, will always be highly controlled environments (Wernberg et al., 2012) which cannot entirely replicate the variability present within the natural environment. For example, species, populations and individuals within natural environments constantly experience a changing environment where there are fluctuations of physico-chemical and biological influences both predictably (e.g., through changes in tidal cycles) and randomly (Wernberg et al., 2012). In addition, biological communities are connected over a range of spatio-temporal scales which go beyond the confines of a closed system, such as a microcosm, and it has been observed that species interactions differ when samples are incubated under highly controlled conditions compared to conditions which are more similar to conditions experienced in the natural environment (Skelly, 2002; Van Doorslaer et al., 2010; Wernberg et al., 2012). Therefore, there needs to be a compromise between the control and replication of laboratory-based studies and the realism of field studies, and results must be used with caution when extrapolating experimental findings to natural systems (Yvon-Durocher et al., 2011). However, this does not detract from the value which such laboratory-based approaches can provide in untangling the complexities of natural systems and isolate and predict how global change scenarios, such as temperature increases, may affect ecosystem level processes (Yvon-Durocher et al., 2011).

1.7. Thesis Aims and Objectives

This thesis used a combination of interdisciplinary techniques and manipulative laboratory experiments to investigate two primary objectives relating to carbon processing and characterization in marine shelf sediments.

Objective 1: Investigate the impact of temperature on biodiversity and ecosystem functioning in intertidal muddy sediments at Blackness (Firth of Forth, Scotland), with a particular focus on carbon processing by the benthic ecosystem.

Objective 2: Characterize UK shelf sea sedimentary carbon storage within the northern North Sea.

1.7.1. Objective 1 – Manipulative laboratory experiment (Blackness)

The aim of this study was to undertake manipulative experiments using natural, intact communities that would measure the impacts of temperature on community structure, function, and carbon processing capabilities. The reasoning behind using intact benthic communities in manipulative experiments was to provide an improved understanding of how environmental change, specifically temperature, would impact biodiversity ecosystem function relationships, specifically carbon processing, compared to constructed or single species communities. In this study intact community is defined as the undisturbed faunal and microbial communities collected directly from the study site location (Blackness).

Aim 1.1: To investigate the effect of temperature on benthic macrofaunal community structure and functional structure (Chapter 2).

Aim 1.2: To investigate the effect of temperature on ecosystem functioning, specifically benthic community oxygen consumption, bioturbation, bioirrigation and nutrient fluxes (Chapter 3).

Aim 1.3: To investigate the effect of temperature on carbon processing by the benthic community at Blackness (Chapter 4).

1.7.2. Objective 2 – Characterizing marine sedimentary carbon (northern North Sea)

In addition to examining the impacts of environmental changes (temperature) on the processing of OC within UK shelf sea sediments, it is essential that carbon within shelf sediments is better characterized, to improve management and protection of ecosystem goods and services provided by shelf sediment environments. This was achieved using multiple biogeochemical markers. This work is described in Chapter 5.

Aim 2.1: To characterize predominant organic matter (OM) source (terrestrial or marine) within sediments of the case study region, in relation to distance from the coastline.

Aim 2.2: To characterize the degradation state, and therefore reactivity, of OM present within surface sediments of the case study region, in relation to distance from the coastline.

2. Characterizing ecological and functional diversity of intertidal macrofauna and their response to ocean warming: A case study of a cohesive benthic environment, Blackness (Firth of Forth, Scotland).

2.1. Introduction

The industrial revolution, usually defined as the mid-1800s (Mitchell, 1989), resulted in the increase of atmospheric greenhouse gasses, including carbon dioxide (Farshchi et al., 2020). As a result, the temperature of the earth's surface has increased by ~0.8°C since the beginning of the industrial revolution (Pachauri et al., 2014; Farshchi et al., 2020). The role that the production of greenhouse gases plays in driving environmental change, including ocean warming, is well accepted and is influencing ecosystems on a global scale, at an unprecedented rate (Jokiel and Brown, 2004; Brierley and Kingsford, 2009; Gingold et al., 2013; Antão et al., 2020).

The shallow nature of coastal oceans, especially intertidal and estuarine environments, puts them at particular risk from ocean warming (Gingold et al., 2013). Temperature is one of the most important factors controlling distribution and behaviour of marine benthic communities and warming as a result of global environmental change is causing changes to marine systems from polar to tropical regions (Byrne, 2011). The temperature of intertidal environments tends to closely track air temperature (Harrison and Phizacklea, 1985) and they experience significant variations over tidal and diurnal cycles (Lee et al., 2017). Taxa living in shallow subtidal and intertidal systems could already be living at the limit of their thermal tolerances (Gingold et al., 2013; Lee et al., 2017). Therefore, it is possible that under predicted environmental change scenarios, extremes in temperature could result in ecosystems experiencing a shift in diversity and community structure, which could also result in changes in the way that the ecosystem functions and the services (e.g., nutrient cycling and carbon cycling) that it provides humanity (Benton et al., 2007; Gingold et al., 2013; Gaylord et al., 2015; Lee et al., 2017).

Understanding how environmental stressors impact benthic communities is essential to aid the development of policies to protect and mitigate against environmental

change. The physiological processes and behaviour of benthic communities, specifically the macrofauna (individuals greater than 500µm), influence ecosystem functioning and fluxes across the sediment-water interface (Nielsen et al., 2004; Michaud et al., 2006; Van Colen et al., 2012; Norkko et al., 2015; Pratt et al., 2015; Mestdagh et al., 2020). Behaviours including bioturbation, reworking of the sediment particles, bioirrigation, and ventilation of the sediment all influence nutrient, oxygen, and carbon dynamics (Aller, 1994; Lohrer et al., 2004; Kristensen et al., 2012; Pascal et al., 2019) by increasing the exchanges between the sediment and water column. These processes subsequently influence microbial communities that drive biogeochemical processes, including the mineralization of organic matter (Kristensen and Hansen, 1999; Foshtomi et al., 2015; Mestdagh et al., 2020). The activities undertaken by faunal communities depend on environmental factors such as temperature and biotic factors such as the identities of the taxa present and the concomitant functional diversity (Needham et al., 2011; Godbold et al., 2011; Mestdagh et al., 2018; Mestdagh et al., 2020; Cassidy et al., 2020).

Both observational and experimental studies have been undertaken to help determine the impact of temperature on biodiversity-ecosystem function relationships. It has been observed that temperature increase has resulted in increased sediment community oxygen consumption (Mestdagh et al., 2020) faunal abundances (Farshchi et al., 2020), and increased metabolism by benthic communities, potentially resulting in increased growth and reproduction rates (Farshchi et al., 2020; Mestdagh et al., 2020). The response of taxa to changes in temperature is varied. For example, experimental warming of *Alitta virens* in defaunated cores, showed that long-term exposure to elevated temperatures resulted in increased growth (Godbold and Solan, 2013). It has also been observed that increased abundances of opportunistic species, such as Tubificoides sp., have been observed at locations where temperature is raised due to the presence of power plant effluent, whereas taxa within the Hydrobiidae family, which are grazers (Hagerthey et al., 2002), appear to be negatively impacted by temperature increases and demonstrate a decreased abundance in locations impacted by power plant effluent outfalls (Farshchi et al., 2020).

Understanding how temperature influences community structure will help determine how ocean warming could influence the rate of biological processes (e.g. bioturbation and bioirrigation), which drive ecosystem functioning, in the future (Kristensen et al., 2012; Mestdagh et al., 2020). However, standard taxa diversity measures do not encapsulate the role or importance that each taxon plays in ecosystem functioning (Braeckman et al., 2014; Belley and Snelgrove, 2016; Mestdagh et al., 2020). Therefore, a better understanding how ocean warming influences biodiversity-ecosystem function (BEF) relationships can be gained by better understanding the functional composition of faunal communities and how functional traits influence processes (Mestdagh et al., 2020).

Previous experimental studies focusing on marine benthic ecosystem structure and functioning have been limited to either single-species scenarios, or of a limited number of species (Biles et al., 2002; Ieno et al., 2006; Bulling et al., 2008; Bulling et al., 2010; Hicks et al., 2011; Godbold and Solan, 2013; Wrede et al., 2018). Studies which focus on whole benthic communities, including the benthic faunal and microbial communities (White et al., 2019), are relatively rare in comparison and are frequently undertaken as in situ studies rather than *ex situ* incubation experiments (Whomersley et al., 2010). However, a number of studies have incubated intact benthic communities *ex situ* for short periods of time (Woulds et al., 2009; Woulds et al., 2007, 2019, 2020). These studies aim to determine the carbon processing capabilities of different sites along naturally occurring gradients, such as oxygen concentrations along the Arabian Sea oxygen minimum zone (Woulds et al., 2007) or comparing chemosynthetic environments to background/control locations in the Southern Ocean (Woulds et al., 2019, 2020). The most common way of determining the impact of a stressor on a community is by focussing on the species level, through the use of measures such as species richness, and evenness (Miatta et al., 2021). By focussing on the species level there is the potential that roles of some species are underrepresented and does not consider how the system as a whole and its functioning, respond to environmental stressors (Miatta et al., 2021). This study aims to examine the influence of increasing water temperature on the structure and function of benthic macrofaunal communities using experimental mesocosm approach. This study will improve our understanding of how ocean

warming influences the structure of benthic macrofaunal communities through the use of traditional biodiversity measures (e.g. abundance and biomass) and through biological traits analysis (BTA).

2.2. Methods

2.2.1. Study site description and characterization

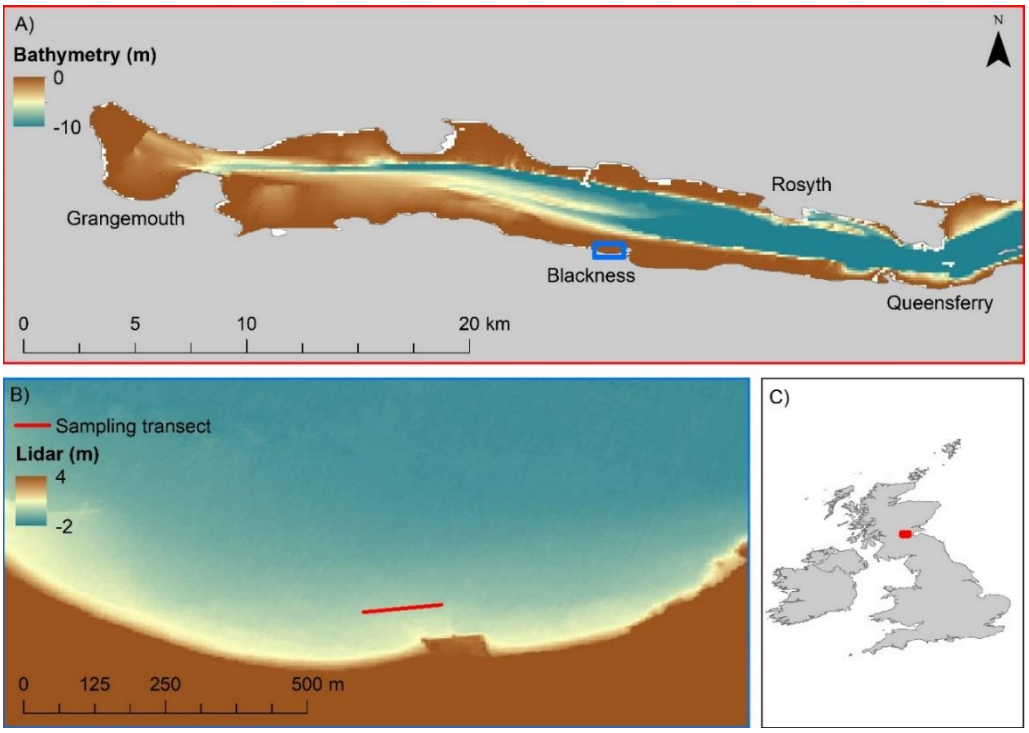

Figure 2.2: Maps demonstrating A) The location of the study site (Blackness) indicated by the blue indicator, B) Location of the sampling transect at Blackness, and C) the location of the study site, indicated by the red indicator, in relation to the wider region of the UK.

The Firth of Forth is located on the East coast of Scotland (Figure 2.1) and stretches from Stirling to the open sea. The Forth estuary is used by a number of different industries (e.g., shipping and sewage disposal) and provides a number of ecosystem goods and services (e.g. recreation and nature conservation; Emmylou et al., 2013). Salinity in this location varies seasonally, with values between 29 and 33 ppt in the summer and 24 and 33 ppt in the winter (Mathieson and Berry, 1997) and the sediment surface temperatures have been observed to be around 14°C at high tide and reach 25°C at low tide in the summer months and are below 5°C in the winter (Harrison and Phizacklea, 1985).

Sampling within this region was carried out at Blackness (Figure 2.1), which is located within the lower Forth Estuary, Scotland, and hosts a large intertidal mudflat environment (Bolam et al., 2002). Being more than 8km downstream from the industrial inputs of Grangemouth this site is considered to be relatively unpolluted and representative of intertidal estuarine environments along the coastline of the UK (McLusky et al., 1983; Bolam et al., 2002). Five sediment types, based on the Folk classification system (Long, 2006), were observed within the study location (Figure 2.2). The two dominant sediment types observed were "(g)M – slightly gravelly mud" and "gM – Gravelly mud. This site has been relatively well studied in terms of benthic infaunal communities, with the most prominent species including *Macoma balthica*, Tubificoides sp., nematodes and *Pygospio elegans* (personal observation).

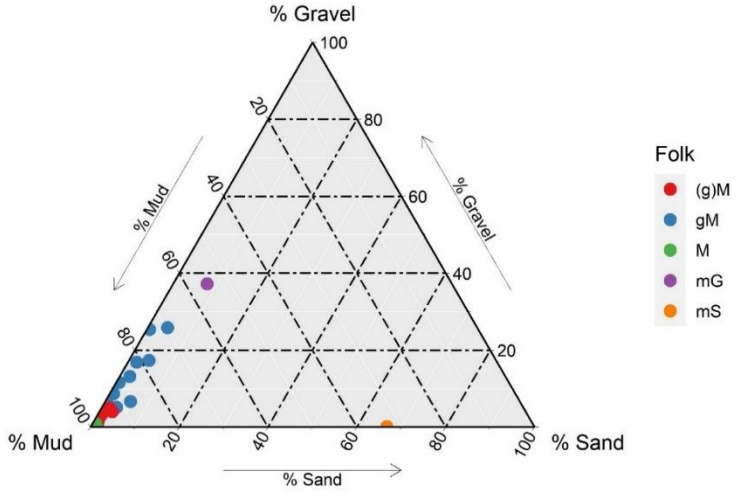

Figure 2.3: Triplot showing the composition (percentage mud, sand and gravel) of the mudflats at Blackness. (g)M = slightly gravelly mud, gM = gravelly mud, M = mud, mG = muddy gravel and mS = muddy sand

2.2.2. Sample collection & Experimental procedure

In April 2019 eighteen stations were sampled from within the intertidal zone at Blackness. Samples were collected along a transect 100 m from and parallel to the high tide mark (Figure 2.1B). Acrylic push cores, 14 cm in diameter (Figure 2.3), were used to collect intact sediment cores to a depth of at least 10 cm.

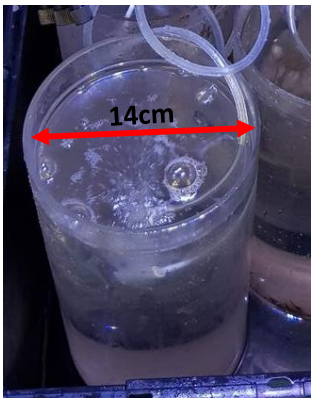

Figure 2.4: Handheld acrylic push cores used to collect sediment samples for incubation experiments.

All cores were transported to The Lyell Centre (Herriot Watt University, Edinburgh) and placed within randomly assigned water baths, set up outlined in Figure 2.4, within controlled temperature (CT) rooms. Three temperature scenarios were studied in this incubation experiment and six cores were assigned to each scenario. The incubation temperatures, based on IPCC predictions by 2100 (IPCC, 2007), were a control temperature (14 °C) which was the mean temperature for the study site during the sampling season, control +3°C (17 °C) and maximum observed temperature (21 °C) at the study site location. One CT room was set at the ambient temperature, two sets of water baths were set up in this room. One set of the water baths were used to hold the six microcosms assigned to the ambient temperature conditions and the second was used to hold the six microcosms assigned to the ambient +3°C conditions, achieved using a Grant T100 heater. The second CT room was set at 21°C, where a water bath was set up to contain the six microcosms assigned to the maximum observed temperature for the study site. It was decided to use water baths within the CT rooms as they would keep the temperature of the microcosms more stable. Water baths were filled with filtered local seawater, which was continuously aerated during the incubation period by bubbling with air. Water bath water was continuously replaced with clean, oxygenated seawater to prevent the build-up of toxic metabolites, such as ammonium and sulphide. The flow rate of new water was sufficiently slow to prevent the re-suspension of sediment in the cores and to prevent changes in the temperature. Cores were acclimated to the temperature treatments for 48 hours before measurements of benthic process rates commenced.

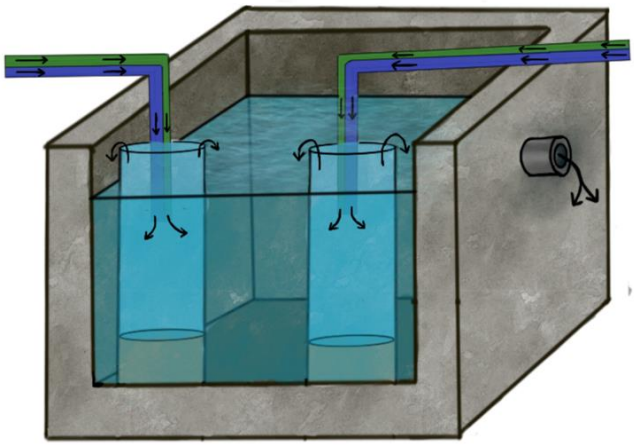

Figure 2.5: Experimental set up for core incubation experiment. The image demonstrates a schematic of the experimental set up, with air supplies inserted (green) and tubes slowly replenishing the water column (blue). To prevent the waterbath overflowing a tube was attached to a port where water would flow into a drain.

During the experiment sediment community oxygen consumption (SCOC) was periodically measured. During the final 24 hours of the incubation period isotopically labelled algal detritus was added to each mesocosm to trace the biological carbon processing. Full details on the methods and results of these aspects of the experiment are provided in Chapters 3 and 4.

2.2.3. Faunal sample processing

At the end of the two-week incubation period the cores' top water was siphoned off, sediment was extruded from the cores. The extruded sediment column was subsampled for analysis of phospholipid fatty acids (see Chapter 5). Once all required subsamples were taken the remaining sediment was preserved using 4% formaldehyde solution and transported to the University of Leeds (School of Geography) for processing and analysis of the faunal community.

The preserved sediment samples from each core were sieved over a 500µm mesh to extract all the macrofauna present within each sample, and then washed to remove any remaining traces of formalin. Macrofauna retained on the sieve were then extracted under a dissecting microscope and identified to the lowest possible taxonomic level (Hayward and Ryland, 1990b; Hayward and Ryland, 1990a; Pleijel, 1993; Petersen, 1998; Worsfold, 2003;

Southward and Campbell, 2006; Worsfold, 2009; Jirkov, 2009; Radashevsky, 2012; Dnestrovskaya and Jirkov, 2013; Caramujo, 2015). Only intact fauna were counted as an individual, intact in this case is defined as individuals where the head of the individual was present. Sections of macrofauna where the head was missing did not contribute to the abundance of that species but did contribute to the biomass. If it was not possible to identify the species from the fragments available (where the head of the species was missing) they were classified as macrofaunal fragments. Macrofaunal fragments did not contribute to the total abundance but did contribute to the total biomass. Individuals in each taxon were counted and weighed, after blotting, to the nearest 0.0001 g. Of the six cores incubated only five were analysed due to time constraints resulting from the impacts of COVID-19.

Biodiversity measures calculated included species abundance and biomass, total abundance and biomass, species richness, species evenness and diversity. Abundance and biomass measures were standardized to the number of individuals per square meter and grams per square meter of sediment. After all species had been identified counted and weighed, they were assigned functional traits. A full outline of how traits were assigned to species can be found in Chapter 3.

2.2.4. Biodiversity

Five metrics were used to describe complementary aspects of taxonomic diversity within the samples incubated in this experiment and were used to determine whether temperature drives a change in community structure over the course of the experiment. In addition to standard measures of diversity (total abundance, total biomass, and species richness), taxonomic diversity was characterized by the Simpson diversity index (Equation 2.1) and Pielou's evenness index (Equation 2.2 & Equation 2.3).

Simpson's diversity index was used because it down-weights the role of rare species (Hill, 1973), which may not have been adequately sampled. This index measures the probability that two individuals selected from a sample, at random, are the same species. Pielou's index is a measurer of evenness, which include information on the relative

abundance of each species. Measures of evenness compare the relative abundance of species within a sample.

$$D = 1 - \sum_{i=1}^{S} p_i^2$$

Equation 2.1: Simpson's diversity index, where S = species richness of the assemblage, pi = relative biomass of species i.

$$H' = -\sum_{i=1}^{S} pi \; log_e \; pi$$

Equation 2.2: Shannon_Wiener index, where S = species richness, pi = proportion of the ith species in the population (Shannon and Weaver, 1949).

$$J' = H'/H'max$$

Equation 2.3 : Pielou's index, where H' = the Shannon-Wiener Index, H' max = log2S and S = species richness (Pielou, 1966).

2.2.5. Functional diversity

To describe the traits composition of the samples six traits were selected: taxa size, feeding method, mobility, reworking type, burrowing type and irrigation depth. These describe the life history, morphological and behavioural characteristics of each taxon within the samples (Table 3.1). Traits were selected to best reflect the role of taxa in carbon processing and cycling, however there is no standardized approach to trait selection, and it is often restricted by the limited availability of biological information available for benthic taxa (Bremner, 2008; Bolam and Eggleton, 2014).

Each of the selected traits were subdivided into categories which were chosen to encompass the range of possible values for all the taxa present, in total 32 categories were identified (Table 3.1). Information relating to all 8 traits were required for all the 28 taxa (to at least genus level) identified across all the samples. Biological traits information was collected from various sources including, published journal papers and scientific institution websites (e.g., Biological Traits Information Catalogue; MarLIN, 2006). Where information was not available for a trait the most closely related taxon was used, instead of assigning a value of zero (Bolam and Eggleton, 2014). This approach is consistent with other

researchers adopting the 'Best Professional Judgement' approach (Bolam and Eggleton, 2014). See Appendices 1 & 2 for a full outline of how traits were assigned to species and associated supporting literature and databases.

When undertaking biological traits analysis, a single trait is rarely assigned to a taxon (Bolam and Eggleton, 2014). This is because the majority of taxa express a number of trait categories to varying degrees, depending on the given conditions and resources available to them at the time (Bolam and Eggleton, 2014; Bolam et al., 2017). Therefore, a fuzzy-coding approach is used based on the extent to which a taxon displays a given trait category (Bolam and Eggleton, 2014; Bolam et al., 2017). Fuzzy-coding allows a taxon to express trait categories to varying degrees and removes the forced assignment of taxa to a single category, which leads to inaccurate interpretation of ecological taxa profiles (Bolam and Eggleton, 2014).

Traits were coded to a scoring range of 0-3 (Appendix 2), where 0 = no affinity to the trait category and 3 = a high association to the given category (Bolam and Eggleton, 2014; Bolam et al., 2017). Once all taxa had been coded to create a "taxon x trait" matrix, it was weighted by taxon biomass, which had been transformed using log10 (biomass+1) (Bremner et al., 2006). The data were weighted by biomass as this factor better describes the role of an individual plays within important ecological processes and distribution of resources compared to abundance (Cesar and Frid, 2009; Bolam and Eggleton, 2014). The biomass weighted "taxon x trait" matrix was used to derive a "station x trait" matrix based on biomass using the infaunal data (Bolam and Eggleton, 2014). This matrix was brought into the statistical program 'R'(version 4.0.1) and processed using the package "ade4" (Dray and Dufour, 2007) to undertake the biological traits analysis. Prior to analysis, the biomass weighted "station x trait" matrix was standardized using Hellinger's method so that the total for each "station x trait" summed to 1 (Bolam and Eggleton, 2014).

Table 2.1: Description of traits and categories used in the biological traits analysis.

Trait	Category	Description
Size (MARLIN, 2006; Bolam and Eggleton, 2014)	Extra small (<1 cm) Small (1-2 cm) Small-medium (3-10 cm) Medium (11-20 cm)	Maximum size the taxon reported to reach
Feeding method (F_i) (MARLIN, 2006)	Predator/Scavenger	Taxon actively predates other animals, or that feeds upon dead animals
	Deposit feeder	Consume particulate material from the benthic environment
	Suspension feeder	Removal of particulate food from the water column.
	Omnivore	Taxon feeds on a mixture of plant and animal material
Mobility (M_i) (Queirós et al., 2013)	Fixed tube Limited movement Free movement	Four levels of increasing activity scored on a categorical scale. Lowest level of activity being in a fixed tube and the highest level being free movement through the sediment matrix (Queirós et al., 2013).
Reworking type (R_i) (Queirós et al., 2013)	Surficial modifier Upward/downward conveyor Biodiffusor Regenerator	Five levels of increasing impact on sediment mixing (surficial modifier to Regenerator), scored on a categorical scale (Queirós et al., 2013).
Burrow type (Bt_i) (Wrede et al., 2018)	Epifauna & internal irrigation Open irrigation Blind end burrow	Describes taxon burrow shape. Epifauna/internal irrigation describes the actions of taxa living at the sediment-water interface and the actions of taxa who have a siphon. Open irrigation describes taxa who live in burrows with at least two openings at the sediment surface, and blind ended burrows, or I-shaped burrows, have a single opening at the sediment surface (Kristensen et al., 2012).
Irrigation depth (Id_i) (Wrede et al., 2018)	0-2 cm 2-5 cm 5-10 cm >10 cm	Describes the depth to which a taxon ventilates the sediment

Functional diversity metrics were calculated using the 'FD' package (Laliberte and Legendre, 2010) in 'R' on Hellinger transformed data. The values calculated included: Functional evenness (FEve), functional divergence (FDiv) and community weighted trait means (CWM). Functional Evenness is a representation of the evenness of abundance/biomass distribution in a functional trait space (Llanos et al., 2020). Functional divergence represents how abundance/biomass is distributed along functional traits axis (Llanos et al., 2020). The community weighted mean of each trait, weighted by abundance/biomass, can be used to represent functional composition, and determine whether changes in trait composition and expression occur due to stressors being applied to the community (Laliberte and Legendre, 2010; D'Alessandro et al., 2020).

Community weighted mean (CWM) was calculated for each trait. The CWM is the mean of trait values in the community, weighted by the relative biomass of the taxon carrying each value (Díaz et al., 2007). The CWM represents the expected functional value of a random community sample and can be used to analyse changes in trait composition and expression (Díaz et al., 2007; D'Alessandro et al., 2020).

Biological traits analysis was undertaken excluding *Cerastoderma edule* and Carcinus sp. This is due to the extremely patchy distribution of these taxa across experimental cores, and the much larger size of the species in question causing results to be disproportionately skewed.

2.2.6. Statistical analysis

Normality of the data was determined using Shapiro's normality test and then, if necessary, the data were transformed to meet parametric test assumptions. If parametric assumptions could not be met by transforming the data, then non-parametric tests were used. To determine whether there is a significant difference in response variable between the three temperature treatments a one-way ANOVA (analysis of variance) followed by a Tukey HSD *post hoc* test the significance level of 0.05 was performed, using 'R'.

2.3. Results

2.3.1. Community composition

Within the cores incubated in this experiment 32 different taxa were identified. Of the species present within the experimental cores, 20 were identified as being Annelids, 4 were bivalves, 2 were gastropod, 2 were arthropods, 1 was a Nemertea, 1 was a Nematoda, 1 was a Copepoda and 1 was an Echinodermata. Annelids were the dominant type of taxon present within the samples and was the most diverse phylum.

Differences in macrofaunal community structure in each temperature treatment are shown using MDS plots (Figure 2.5 A &B). The nMDS plots indicate that there in an overlap between the three temperature scenarios, although cores incubated at 17°C and 21°C appear to be more similar in terms of both abundance and biomass. However, there was not a distinct separation in the macrofaunal community structure between the three temperature scenarios based on either abundance (PERMANOVA: df = 2, F = 1.0241 and p = 0.391; Figure 3.1A) or biomass (PERMANOVA: df = 2, F = 0.8641 and p = 0.633; Figure 3.1B). SIMPER analysis indicated that >50% of the dissimilarity in assemblage composition between each of the temperature scenario combinations (14°C and 17°C = 51.07%; 14°C and 21°C = 58.19%; 17°C and 21°C = 61.27%) was associated with just two taxa, Nematoda and Tubificoides, when based on abundance. In each case Nematoda accounted for the largest portion of dissimilarity between each of the temperature scenario combinations. Approximately 50% of the dissimilarity in assemblage composition between each of the temperature scenario combinations (14°C and 17°C = 50.68%; 14°C and 21°C = 48.62%; 17°C and 21°C = 57.05%) was associated with two of three taxa, when based on biomass. The dissimilarity between cores incubated at 14°C and 17°C was attributed to *Macoma balthica* (27.67%) and *Cerastoderma edule* (23.01%). The dissimilarity between cores incubated at 14°C and 21°C was attributed to *Macoma balthica* (25.03%) and Tubificoides (23.59%) and the dissimilarity between cores incubated at 17°C and 21°C was attributed to Tubificoides (33.05%) and *Macoma balthica* (24%). The SIMPER results indicate that based on abundance Nematoda and Tubificoides are most important, whereas bivalves and Tubificoides are most important in terms of biomass. With *Macoma balthica* and

Tubificoides being particularly important when considering the impact of incubating cores at 21°C.

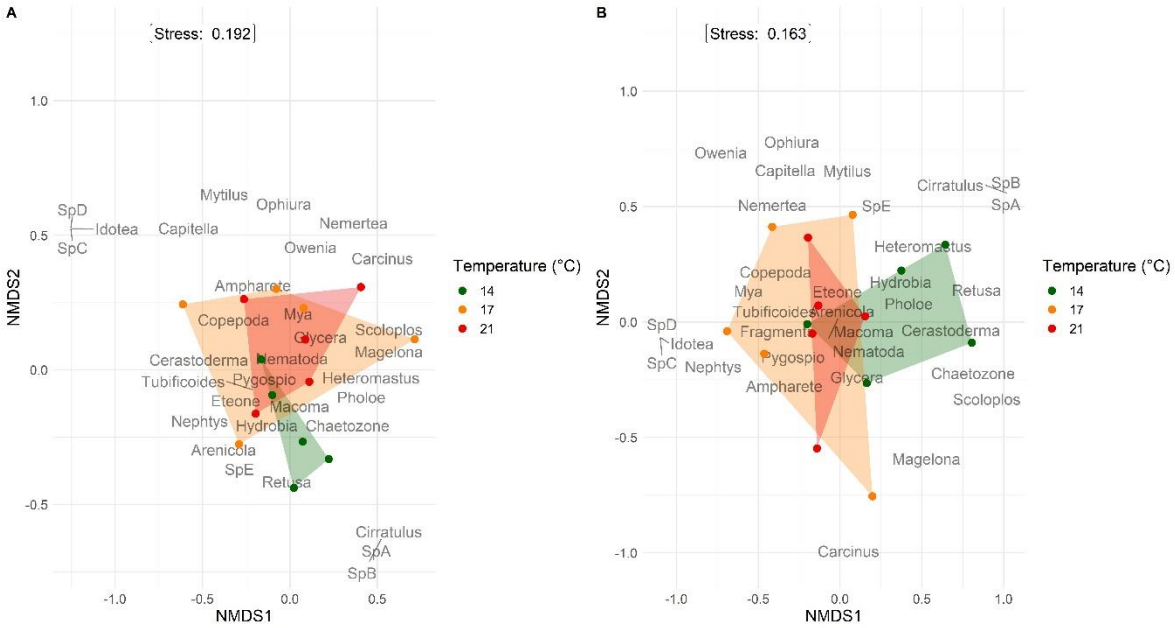

Figure 2.6: nMDS plots indicating the difference in community structure between the three temperature scenarios based on A) Abundance (root transformed) and B) Biomass (untransformed).

2.3.1.1. Abundance

The most abundant taxon present in cores incubated at 14°C was Nematoda which had a mean abundance of 79,400 ± 24,219 individuals m^{-2}, followed by Tubificoides sp. (5440 ± 1760 individuals m^{-2}), *Pygospio elegans* (1293 ± 466 individuals m^{-2}), *Macoma balthica* (1280 ± 395 individuals m^{-2}), *Hydrobia ulvae* (533 ± 401 individuals m^{-2}), *Eteone flava* (267 ± 42 individuals m^{-2}), *Retusa obtusa* (147 ± 39 individuals m^{-2}), *Heteromastus filiformis* (107 ± 54 individuals m^{-2}) and *Glycera alba* (67 ± 30 individuals m^{-2}). The abundance of none of the dominant taxa was significantly impacted by temperature increases. Mean abundance for each of the dominant taxa incubated under each of the three temperature scenarios can be seen in Figure 2.6 A-I.

The abundance of nematodes and *Eteone flava* appears to be slightly lower at 17°C (49,827 ± 19,702 m^{-2} and 2137 ± 83 m^{-2} respectively) and higher in cores incubated at 21°C (127,107 ± 43,009 m^{-2} and 333 ± 110 m^{-2} respectively), although an ANOVA indicates that the observed differences in nematode and *Eteone flava* abundance between the three

temperature scenarios is not statistically significant (Nematodes: df = 2, $df_{residuals}$ = 12, F = 1.615 and p = 0.239; *Eteone flava*: df = 2, $df_{residuals}$ = 12, F = 0.526 and p = 0.604). The homogeneity of variance was also tested for each of the species using Levene's test (Nematodes: df = 2, F = 0.7498 and p = 0.4934 and *Eteone flava*: df = 2, F = 1.8095 and P = 0.604), indicating that there is not a difference in variance between the three temperatures.

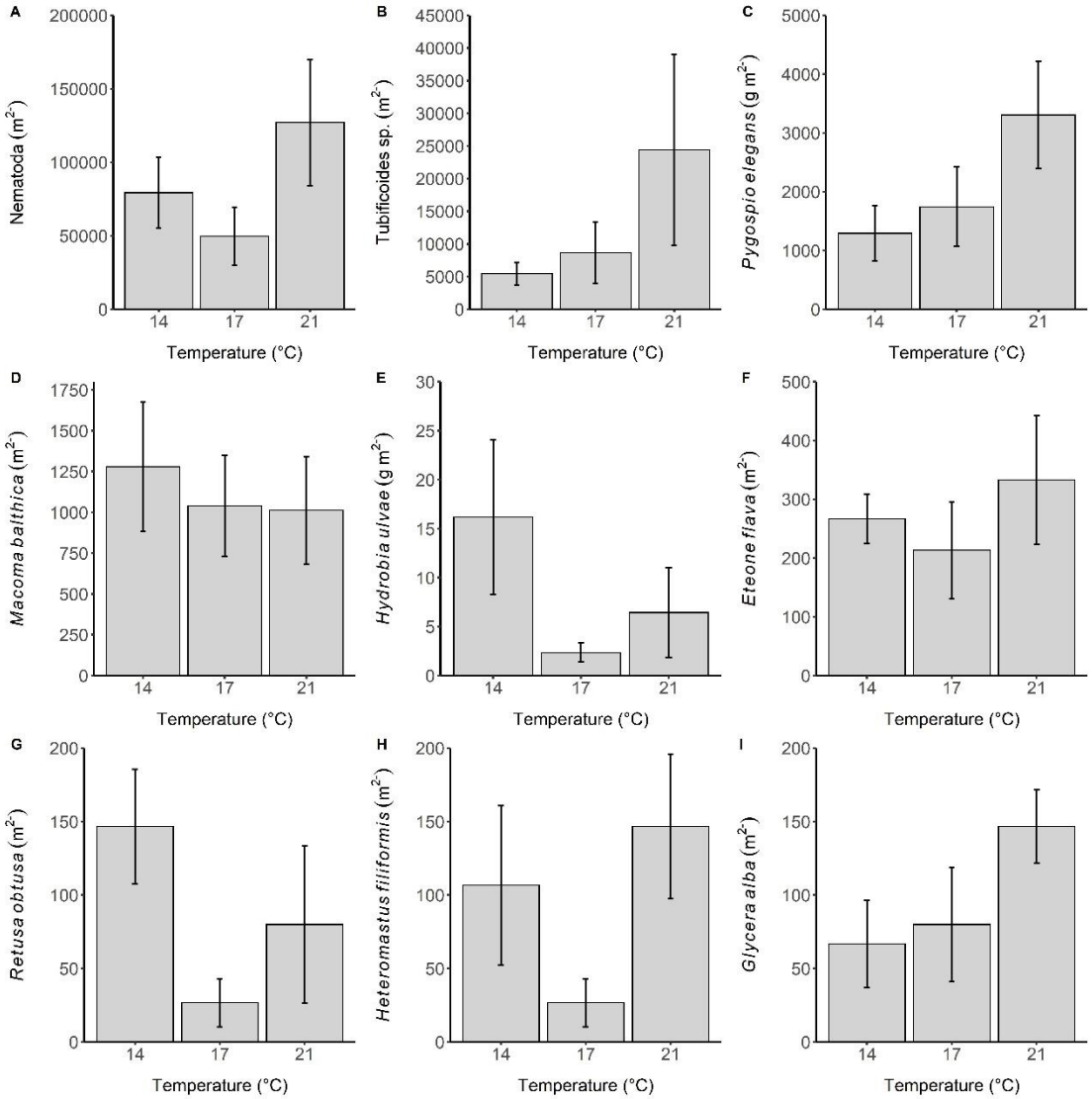

Figure 2.7: Mean abundance (number of a given taxa for each square metre of sediment) of dominant taxa within the study site at Blackness, for each temperature scenario (14°C, 17°C and 21°C). Error bars represent the standard error of the mean.

The data indicate that the abundance of Tubificoides sp., *Pygospio elegans* and *Glycera alba* is slightly higher in cores incubated at 17°C and 21°C (Tubificoides = 8,640 ±

4,682 m^{-2} and 24,440 ± 14,616 m^{-2} respectively, *Pygospio elegans* = 1,747 ± 680 m^{-2} and 3307 ± 907 m^{-2} respectively, and *Glycera alba* = 80 ± 39 m^{-2} and 147 ± 25 m^{-2} respectively). However, an ANOVA indicated that the apparent increase in Tubificoides sp. (df = 2, $df_{residuals}$ = 12, F = 1.301 and p = 0.308), *Pygospio elegans* (df = 2, $df_{residuals}$ = 12, F = 1.59 and p = 0.244) and *Glycera alba* (df = 2, $df_{residuals}$ = 12, F = 1.824 and p = 0.203) abundance was not statistically significant. The homogeneity of variance was also tested for each of the species using Levene's test (Tubificoides sp.: df = 2, F = 1.5161 and p = 0.2588 and *Pygospio elegans*: df = 2, F = 0.2567 and P = 0.7778 and *Glycera alba:* df = 2, F = 0.6 and P = 0.5645), indicating that there is not a difference in variance between the three temperatures.

The abundance of *Macoma balthica, Hydrobia ulvae* and *Retusa obtusa* appears to be lower in cores incubated at 17°C and 21°C (*Macoma balthica* = 1,040 ± 309 m^{-2} and 1013 ± 328 m^{-2} respectively, *Hydrobia ulvae* = 1337 ± 47 m^{-2} and 387 ± 191 m^{-2} respectively and *Retusa obtusa* = 27 ± 16 m^{-2} and 80 ± 5 m^{-2} respectively). However, despite the observed decrease in abundance an ANOVA indicated that there was not a statistically significant difference in the abundance of *Macoma balthica* between the three temperature scenarios (df = 2. $df_{residuals}$ = 12, F = 0.18 and p = 0.837). The homogeneity of variance was also tested for each of the *Macoma balthica* data using Levene's test (df = 2, F = 0.0199 and p = 0.9803), indicating that there is not a difference in variance between the three temperature scenarios. A Kruskal-Wallis test also indicated that there was not a significant difference in the abundance of *Hydrobia ulvae* and *Retusa obtusa* between the three temperature scenarios (*Hydrobia ulvae:* df = 2, chi-squared = 0.71544 and p-value = 0.6993; *Retusa obtusa:* df = 2, chi-squared = 4.6339 and p-value = 0.09857).

Finally, *Heteromastus filiformis* did not demonstrate a clear trend in abundance in relation to incubation temperature, the abundance of this species appeared to be lower in cores incubated at 17°C relative to cores incubated at 14°C (27 ± 16 m^{-2}), but higher in cores incubated at 21°C (147 ± 49 m^{-2}). A Kruskall-Wallis indicated that there was not a statistically significant difference in the abundance of *Heteromastus filiformis* between the three temperature scenarios (df = 2, chi-squared = 2.9702 and p = 0.227).

2.3.1.2. Species Biomass

The most dominant taxon in cores incubated at 14°C in terms of biomass was *Macoma balthica* (32.06 ± 19.45 g m^{-2}), followed by *Glycera alba* (2.71 ± 1.53 g m^{-2}), *Hydrobia ulvae* (2.02 ± 1.23 g m^{-2}), Tubificoides sp. (1.69 ± 0.64 g m^{-2}), Nematoda (1.39 ± 0.43 g m^{-2}), *Retusa obtusa* (0.98 ± 0.78 g m^{-2}), *Eteone flava* (0.47 ± 0.20 g m^{-2}), *Pygospio elegans* (0.441 ± 0.144 g m^{-2}) and *Heteromastus filiformis* (0.413 ± 0.265 g m^{-2}). The biomass of none of the most dominant taxa were significantly impacted by ocean warming. However, despite no statistical significance being observed, similar trends were observed in biomass (Figure 2.8 A-I) as in abundance for each of the dominant taxa.

The biomass of nematodes and *Pygospio elegans* did not appear to differ between cores incubated at 14°C and 17°C (Nematodes: 17°C = 1.02 ± 0.44 g m^{-2} and *Pygospio elegans:* 17°C = 0.744 ± 0.308 g m^{-2}). But the observed biomass for these two taxa appeared to be higher in cores incubated at 21°C (Nematodes = 2.33 ± 0.88 g m^{-2} and *Pygospio elegans* = 1.750 ± 0.469 g m^{-2}). However, despite the apparent changes in biomass of these two taxa, an ANOVA indicated that the biomass of nematodes (df = 2, $df_{residuals}$ = 12, F = 2.754 and p = 0.104) and *Pygospio elegans* (df = 2, $df_{residuals}$ = 12, F = 2.098 and p = 0.165) were not significantly impacted by temperature. The homogeneity of variance was also tested for each of the species using Levene's test (Nematoda: df = 2, F = 0.148 and p = 0.864 and *Pygospio elegans*: df = 2, F = 1.3538 and P = 0.295), indicating that there is not a difference in variance between the three temperatures.

The biomass of Tubificoides sp. Is higher in cores Incubated at both 17°C and 21°C (13.38 ± 7.99 g m^{-2} and 29.88 ± 17.90 g m^{-2} respectively), relative to those incubated at 14°C. However, despite this observed increase in biomass a Kruskall-Wallis test indicated that there is not a statistically significant difference (df = 2, chi-squared = 0.615 and p = 0.7353) between the three temperature scenarios.

The biomass at 17°C and 21°C for *Macoma balthica* (19.58 ± 11.17 g $m^{-2}$and 15.62 ± 8.233 g m^{-2} respectively), *Hydrobia ulvae* (0.30 ± 0.13 g m^{-2} and 1.11 ± 0.534 g m^{-2} respectively), *Retusa obtusa* (0.02 ± 0.02 g m^{-2} and 0.02 ± 0.02 g m^{-2} respectively) and *Eteone*

flava (0.375 ± 0.300 g m^{-2} and 0.29 ± 0.15 g m^{-2} respectively) appears to be lower than in cores incubated at 14°C. However, despite the apparent decreases in biomass an ANOVA on log_{10} transformed *Macoma balthica* (p = 0.418) data and square root transformed *Hydrobia ulva* (p = 0.331) and square root transformed *Eteone flava* (p = 0.353, F = 1.137, df = 2 and $df_{residuals}$ = 12) data indicated that there was not a significant difference in the biomass of these three taxa between the three temperature scenarios examined in this experiment. Data analysed using an ANOVA were also tested for homogeneity of variance using Levene's Test (*Macoma balthica*: df = 2, F = 0.698 and p = 0.5167; *Hydrobia ulvae*: df = 2, F = 3.8716 and p = 0.05042; *Eteone flava*: df = 2, F = 0.0883 and p = 0.9161), indicating that there is not a difference in variance between the three temperatures. A Kruskal-Wallis test also indicated that there was not a significant difference in the biomass of *Retusa obtusa* between the three temperature scenarios.

There was not a clear trend in biomass in relation to temperature for *Glycera alba* and *Heteromastus filiformis*. The biomass of these two species is lower at 17°C (1.31 ± 0.61 g $m^{-2}$and 0.061 ± 0.061 g m^{-2} respectively) than those in cores incubated at 14°C. The biomass of individuals incubated in cores at 21°C (4.00 ± 1.088 g m^{-2} and 0.354 ± 0.242 g m^{-2} respectively) was higher, in comparison to those incubated at either 14°C or 17°C. However, an ANOVA indicated that there was not a significant difference (p = 0.210) in the biomass of *Glycera alba* between the three incubation temperatures. Data analysed using an ANOVA were also tested for homogeneity of variance using Levene's Test (df = 2, $df_{residuals}$ = 12, F = 0.8496 and p = 0.4518), indicating that there is not a difference in variance between the three temperatures. A Kruskal-Wallis test indicated that there was also not a significant difference (df = 2, chi-squared = 1.896 and p = 0.388) in the biomass of *Heteromastus filiformis* between the three temperature scenarios.

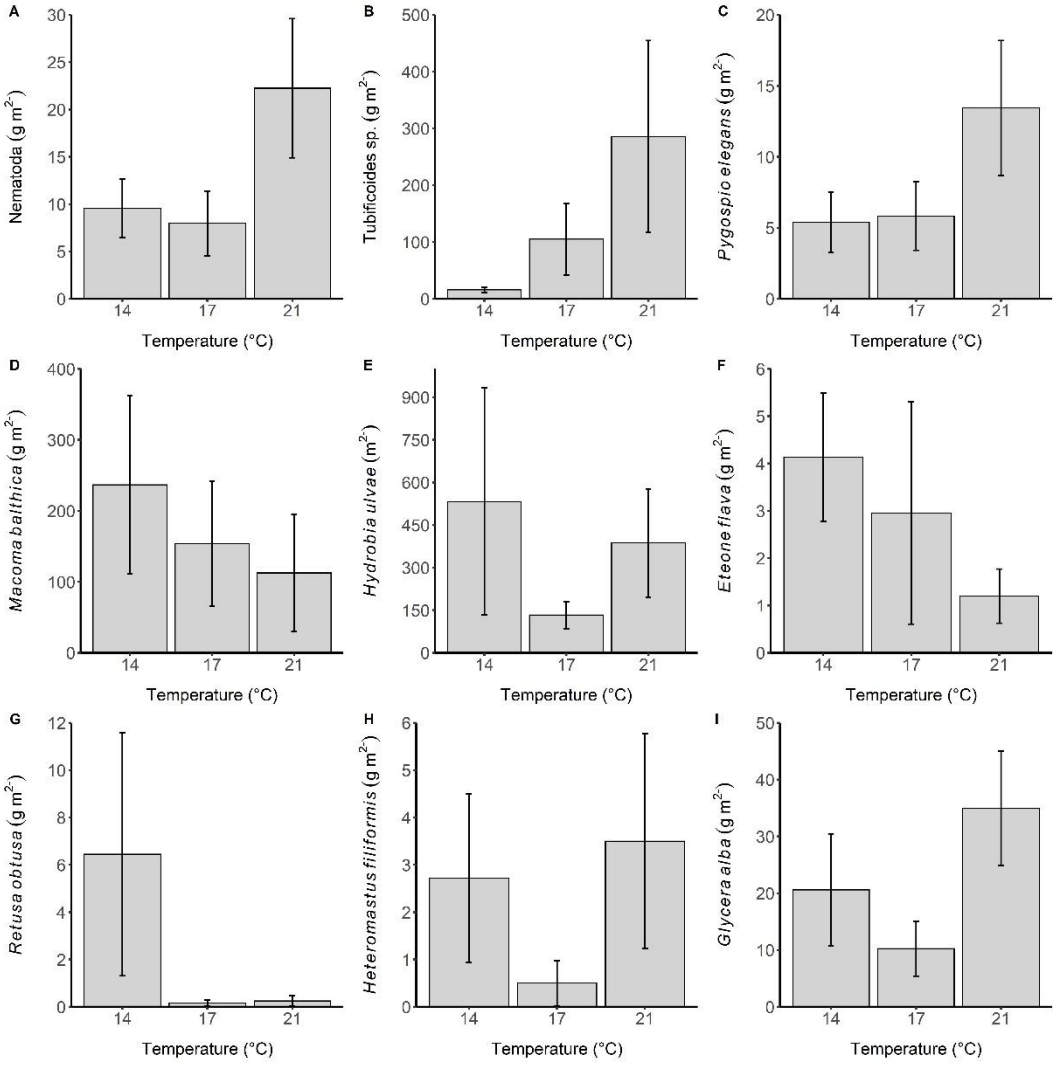

Figure 2.8: Mean biomass (mass of a given taxa in grams for every square metre of sediment) of dominant taxa within the study site at Blackness, for each temperature scenario (14°C, 17°C and 21°C). Error bars represent the standard error of the mean.

2.3.2. Community biodiversity measures

The mean total abundance (Figure 2.8 A) of cores incubated at 21°C (157,640 ±52,221 individuals m^{-2}) was 76% higher than that of cores incubated at 14°C (89,453 ± 25,208 individuals m^{-2}). There appeared to be a slightly lower mean total abundance in cores incubated at 17C (62,440 ± 20,560 individuals m^{-2}). An ANOVA revealed that there is not a significant difference (df = 2, $df_{residuals}$ = 12, F = 1.908 and p = 0.191) in abundance between the three temperature scenarios, despite the apparent difference seen in Figure 3.4A. Data analysed using an ANOVA were also tested for homogeneity of variance using

Levene's Test (df = 2, $df_{residuals}$ = 12, F = 1.3236 and p = 0.3024), indicating that there is not a difference in variance between the three temperatures.

The mean biomass (Figure 2.8 B) of cores incubated at 21°C (37.66 ± 6.66 g m^{-2}) was 25% higher than the mean biomass of cores incubated at 14°C (30.16 ± 9.03 g m^{-2}). The mean total biomass of cores incubated at 17°C (29.55 ± 5.19 g m^{-2}) did not appear to be different to that of cores incubated at 14°C. However, an ANOVA test revealed that there was not a statistically significant difference (df = 2, $df_{residuals}$ = 12, F = 0.401 and p = 0.678) in the biomass between the three temperature scenarios. Data analysed using an ANOVA were also tested for homogeneity of variance using Levene's Test (df = 2, $df_{residuals}$ = 12, F = 0.2475 and p = 0.7847), indicating that there is not a difference in variance between the three temperatures.

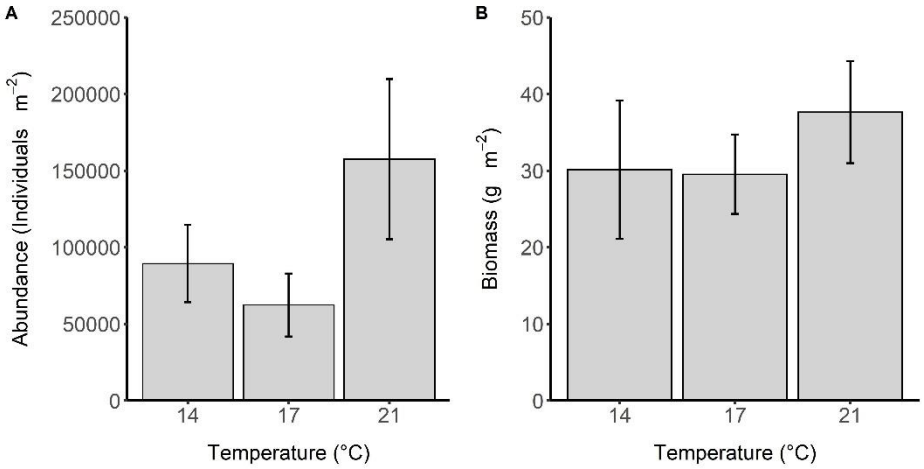

Figure 2.9: A) Mean abundance (m^{-2}) of benthic fauna identified in cores; B) Mean biomass (g m^{-2}) of benthic fauna identified in experimental cores. Error bars represent the standard error of the mean.

Measures of diversity (Figure 2.9 A-C) were calculated for each of the temperature scenarios, species richness (14°C = 12.6 ± 0.87; 17°C = 13.4 ± 1.66; 21°C = 13.2 ± 0.80), Pielou's evenness index (14°C = 0.798 ± 0.002; 17°C = 0.771 ± 0.001; 21°C = 0.795 ± 0.003) and Simpson's diversity index (14°C = 0.804 ± 0.022; 17°C = 0.814 ± 0.045; 21°C = 0.830 ± 0.011). However, an ANOVA undertaken on Species richness (SR; df = 2, $df_{residuals}$ = 12, F = 0.125 and p = 0.0.884), Pielou's evenness (df = 2, $df_{residuals}$ = 12, F = 0.12 and p = 0.888) and Simpson's diversity (df = 2, $df_{residuals}$ = 12, F = 0.194 and p = 0.826) indices, weighted by biomass, did not demonstrate a significantly significant difference between the three

temperature scenarios within this experiment (p = 0.88; 0.83 and 0.57 respectively). Pielou's evenness data were transformed to meet the assumptions of ANOVA (transformation = *Pielou/(1- Pielou)*). Data analysed using an ANOVA were also tested for homogeneity of variance using Levene's Test (SR: df = 2, $df_{residuals}$ = 12, F = 1.1852 and p = 0.3391; Pielou's: df = 2, $df_{residuals}$ = 12, F = 0.2832 and p = 0.7582; Simpson's: df = 2, $df_{residuals}$ = 12, F = 0.7247 and p = 0.5045), indicating that there is not a difference in variance between the three temperatures for each of the three measures.

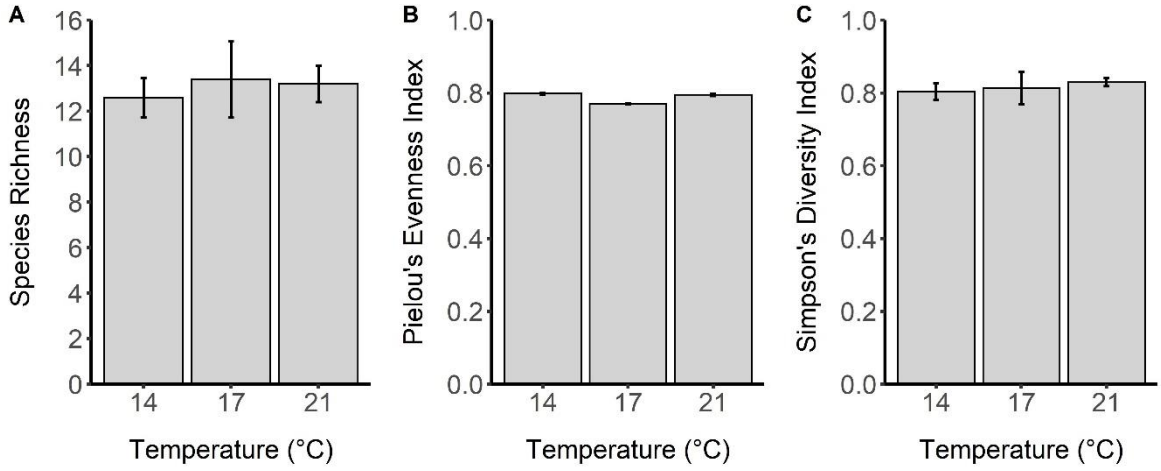

Figure 2.10: A) Species Richness; B) Pielou's Evenness index; C) Simpson's Diversity Index, for cores incubated at 14°C, 17°C and 21°C. Error bars represent the standard error of the mean.

2.3.3. Functional diversity

An f- PCA (Figure 2.10) indicates that axes 1 and 2 account of 88.3% (82% and 6.3% respectively) of the variation in functional trait composition of cores incubated at 14°C, 17°C and 21°C. The f-PCA indicates that there is a large amount of variability in the functional composition of communities within temperature treatments.

Cores incubated at 14°C appear to be predominantly dominated by taxa who are small (Si_S) and are surficial modifiers (R_i_SM) with limited mobility (M_i_LM) who undertake irrigation at the sediment surface or internally (Bt_i_EpiInt). On the other hand, cores incubated at 21°C appear to be dominated by taxa who are small-medium in size (Si_SM; i.e. larger than taxa in cores incubated at 14°C), who are biodiffusors (R_i_Bdif) who are predominantly predators/scavengers (F_i_P_Sc) which irrigate the sediment via blind-ended burrows (Bt_i_BEB) and up to 2-5cm in depth (Id_i_2.5cm).

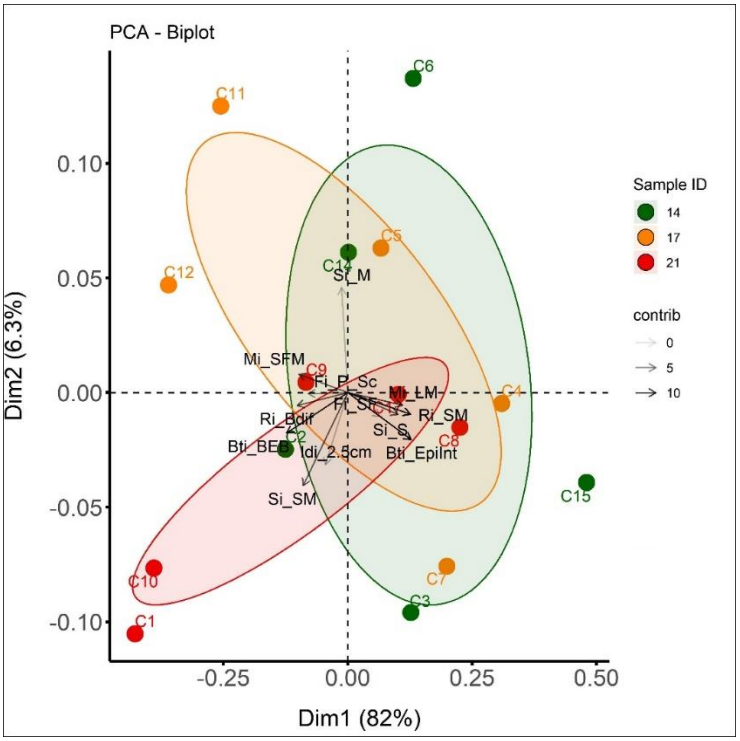

Figure 2.11: f-PCA plot demonstrating the functional composition of cores incubated under the three experimental temperature scenarios (14°C, 17°C and 21°C). clusters represent confidence at the 95% level

2.3.3.1. Community weighted mean of functional traits

The community weighted mean of trait modalities demonstrates how the trait composition of cores was impacted by increased temperature over the experimental duration. Most of the trait modalities present within the sampled community did not appear to be significantly impacted by ocean warming. The exception being the mobility type trait modality of "fixed tubes". However, despite most trait modalities showing statistical insignificance in relation to ocean warming scenarios, trends in the data were observed.

Size – All three temperature scenarios were dominated by fauna which on average grow to a size between 1cm and 10cm. Less of the biomass was dominated by taxa which are <1cm and >10cm (Figure 2.11 A).

The CWM of taxa classified as being extra small (<1cm) was on average lower in cores incubated at 17°C (0.29 ± 0.06) and higher in cores incubated at 21°C (0.41 ± 0.07)

compared to cores incubated at 14°C (0.40 ± 0.10). An ANOVA indicated that there was not a significant difference between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.698 and p = 0.517). Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.415 and p = 0.6695).

The CWM of taxa classified as being small (1cm – 2cm) was on average lower in cores incubated at 21°C (0.97 ± 0.21) compared to cores incubated at 14°C and 17°C (1.24 ± 0.29 and 1.22 ± 0.25 respectively), although an ANOVA indicated that there was not a significant difference between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.372 and p = 0.697). Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.093 and p = 0.9118).

The CWM of taxa classified as being small/medium (3cm-10cm) was on average higher in cores incubated at 21°C (1.46 ± 0.30), compared to cores incubated at 14°C and 17°C (1.22 ± 0.41 and 1.16 ± 0.17 respectively). However, and ANOVA indicated that there was not a significant difference between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.277 and p = 0.763). Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 1.3794 and p = 0.2889).

Finally, CWM of taxa classified as being medium (>10cm) in size was on average higher in cores incubated at 17°C (0.50 ± 0.16) and lower in cores incubated at 21°C (0.24 ± 0.06), compared to those incubated at 14°C (0.30 ± 0.14). An ANOVA on square-root transformed data indicated that there was not a significant difference between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 1.086 and p = 0.369). Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.5542 and p = 0.5885). Despite trends being observed in the data, there is a large amount of variation between cores within each temperature scenario.

Feeding Type – Five feeding type modalities were observed within the benthic community at Blackness (Figure 2.11 B). At 14°C the dominant feeding type by the benthic communities, weighted by biomass, was 'deposit feeders' followed by 'suspension feeders', 'predators/scavengers', 'herbivores' and finally 'omnivores. The role of omnivores will not be discussed in this study as the trait modality was only observed once in a single core.

Under ocean warming scenarios (17°C and 21°C) CWM of taxa classified as deposit feeders remained the dominant feeding trait modality and does not appear to demonstrate a clear trend with ocean warming (Figure 3.5B). Although on average cores incubated at 17°C (1.34 ± 0.08) had a slightly higher CWM and cores incubated at 21°C (1.17 ± 0.13) were slightly lower in comparison to cores incubated at 14°C (1.26 ± 0.24). However, an ANOVA indicated that there was not a significant difference between the three temperature scenarios (df = 2, $df_{residuals}$, F = 0.273 and p = 0.766). Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 1.8317 and p = 0.2022).

The dominance of the suspension feeder modality is lower in cores incubated at 17°C (0.92 ± 0.15) and 21°C (0.86 ± 0.16), in comparison to cores incubated at 14°C (1.09 ± 0.18). However, an ANOVA indicated that there was not a significant difference (df = 2, $df_{residuals}$ = 12, F = 0.567 and p = 0.581) between the three temperatures. Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.0348 and p = 0.9659).

The dominance of the predator/scavenger modality is higher in cores incubated at 17°C (1.10 ± 0.13) and 21°C (1.12 ± 0.18) in comparison to cores incubated under ambient temperatures (14°C; 0.68 ± 0.15). However, an ANOVA indicated that, despite the observed increases at elevated temperatures, there was not a significant difference (df = 2, $df_{residuals}$ = 12, F = 2.124 and p = 0.162) between the three scenarios, most likely due to variability between cores within a treatment. Levene's test was used to test the data for homogeneity

of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.286 and p = 0.7562).

The CWM of the herbivore feeding modality does not appear to vary between the three temperature scenarios within this study (14°C = 0.04 ± 0.02; 17°C = 0.03 ± 0.02 and 21°C = 0.04 ± 0.01). This was supported through an ANOVA, which demonstrated that there was not a statistically significant difference (df = 2, $df_{residuals}$ = 12, F = 0.539 and p = 0.597) in the herbivore modality between the three temperature scenarios. Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.0146 and p = 0.9855).

Mobility – Three mobility trait modalities were observed within the benthic community at Blackness (Figure 2.11 C). The dominant modality at 14°C was taxa with 'limited movement', followed by taxa with 'free movement' and finally taxa which exist in 'fixed tubes'. Under ocean warming scenarios (17°C and 21°C) the biomass is no longer dominated by a single mobility trait modality.

The CWM of taxa with 'free movement' was higher in cores incubated at 17°C (1.22 ± 0.20) and 21°C (1.32 ± 0.20), in comparison to cores incubated under ambient temperature conditions (14°C; 0.89 ± 0.19). An ANOVA indicated that there was not a significant difference between the three temperatures (df = 2, $df_{residuals}$ = 12, F = 0.683 and p = 0.523). Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.2035 and p = 0.8186).

The CWM of taxa with 'limited movement' was lower in cores incubated at 17°C (1.55 ± 0.20) and 21°C (1.48 ± 0.22), in comparison to cores incubated under ambient temperature scenarios (14°C; 2.01 ± 0.18). However, despite the observed increase in this modality under elevated temperature scenarios, an ANOVA indicated that there was not a significant difference (df = 2, $df_{residuals}$ = 12, F = 1.175 and p = 0.342) between the three temperature scenarios. Levene's test was used to test the data for homogeneity of variance

and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.1981 and p = 0.8229).

Taxa which exist within 'fixed tubes' occupy less of the community biomass. However, on average the CWM of cores incubated at 17°C (0.25 ± 0.06) and 21°C (0.20 ± 0.03) was higher in comparison to cores incubated at 14°C (0.10 ± 0.02). An ANOVA indicated that a significant difference does exist (df = 2, $df_{residuals}$ = 12, F = 3.788 and p = 0.0.053) between the three temperatures. Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 2.9749 and p = 0.08928). A Tukey HSD test was undertaken and indicated that the CWM of taxa in 'fixed tubes' is significantly higher in cores incubated at 17°C in comparison to cores incubated at 14°C (p = 0.05). A significant difference was not detected in the CWM between cores incubated at 14°C and 21°C or between cores incubated at 17°C and 21°C (p = 0.18 and 0.72 respectively).

Reworking Type – Three sediment reworking modalities were identified in the communities at Blackness (Figure 2.11 D). At 14°C the dominant sediment reworking modality was 'surficial modifiers', followed by 'Biodiffusors' and finally 'Upward/Downward conveyors'.

Under ocean warming scenarios (17°C and 21°C) 'Surficial modifiers' remain the dominant sediment reworking trait modality, with a CWM of 1.48 ± 0.27 and 1.47 ± 0.22 respectively, although to a lesser extent when compared to cores incubated at 14°C (CWM = 2.02 ± 0.24). A Kruskal-Wallis test indicated that there was a marginally insignificant difference (p = 0.09) between the three temperature scenarios.

The CWM of taxa classified as undertaking the sediment reworking modality 'biodiffusor' is higher in cores incubated at 17°C and 21°C (1.10 ± 0.20 and 1.14 ± 0.21 respectively) in comparison to cores incubated at 14°C (0.57 ± 0.13). An ANOVA indicated that there was a marginally insignificant (df = 2, $df_{residuals}$ = 12, F = 3.076 and p = 0.0835) difference between the three temperature scenarios. Levene's test was used to test the

data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.1516 and p = 0.8609).

Finally, the CWM of taxa which exhibit the sediment reworking trait modality 'Upward/Downward conveyors' does not appear to be impacted by ocean warming (14°C = 0.40 ± 0.12, 17°C = 0.41 ± 0.13 and 21°C = 0.38 ± 0.06). Despite the observed trends in the biomass of sediment reworking trait modalities observed, they were not statistically significant (Kruskall-Wallis; p = 0.9704).

Burrow type- Three burrow type modalities, reflecting how the sediment column is ventilated by the benthic community, were observed in the benthic community of Blackness (Figure 3.6E). At 14°C the biomass of the benthic community was dominated by taxa which ventilated the sediment as 'Epifauna/internal irrigation' (described in Table 3.1), followed by 'Blind ended burrows' and finally 'open irrigation'.

Under ocean warming scenarios (17°C and 21°C) no single trait modality is dominant, which differs from cores incubated at 14°C. The CWM of biomass classed under the 'epifauna/internal irrigation' modality is lower under both ocean warming scenarios (17°C = 1.46 ± 0.25 and 21°C = 1.55 ± 0.21) in comparison to cores incubated under ambient (14°C; 2.06 ± 0.23) conditions. However, and ANOVA indicated that there was not a significant difference (df = 2, $df_{residuals}$ = 12, F = 1.976 and p = 0.181) between the three temperature scenarios. Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.067 and p = 0.9355).

The CWM of taxa classified within the 'Blind ended burrows' modalities is higher in cores which have been incubated at 17°C (1.33 ± 0.25) and 21°C (1.41 ± 0.22), in comparison to cores incubated at 14°C (0.88 ± 0.23). However, despite the observed trend an ANOVA indicated that there was not a significant difference (df = 2, $df_{residuals}$ = 12, F = 1.482 and p = 0.266) between the three temperature scenarios. Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.0852 and p = 0.9189).

Finally, CWM of taxa classified as undertaking 'open irrigation' did not demonstrate a clear trend with temperature, although this is the rarest of the three burrow type modalities. A higher biomass of taxa were observed to exhibit this trait in cores incubated at 17°C (0.25 ± 0.09) in comparison to cores incubated at either 14°C (0.09 ± 0.05) or 21°C (0.05 ± 0.03). An ANOVA indicated that there was a marginally insignificant difference (df = 2, $df_{residuals}$ = 12, F = 3.301 and p = 0.072) between the three temperature scenarios. Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 2.0998 and p = 0.1652).

Irrigation depth – The cores used in this experiment had a sediment depth of 10cm, and this is also reflected in the irrigation depth trait (Figure 2.11 F). However, trends can be observed within each of the irrigation depth modalities between ocean warming scenarios.

The CWM of taxa which are only capable of irrigating the surface sediments (0-2cm) was lower in cores incubated at 17°C (1.54 ± 0.06) and 21°C (1.35 ± 0.19), in comparison to cores incubated under ambient temperatures (14°C; 1.64 ± 0.17). The taxa which occupy this space are more frequently exposed to the stressor. However, an ANOVA indicated that there was not a significant difference (df = 2, $df_{residuals}$ = 12, F = 0.922 and p = 0.424) between the three temperature scenarios. Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 1.1131 and p = 0.3602).

The CWM of taxa which are capable of irrigating deeper into the sediment column, to a depth between 2cm and 5cm, is higher in cores incubated at 17°C (1.46 ± 0.06) and 21°C (1.58 ± 0.17), compared to cores incubated at 14°C (1.31 ± 0.19). However, an ANOVA demonstrated that there was not a significant difference (df = 2, $df_{residuals}$ = 12, F = 0.814 and p = 0.466) between the three temperature scenarios. Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 1.9727 and p = 0.1817).

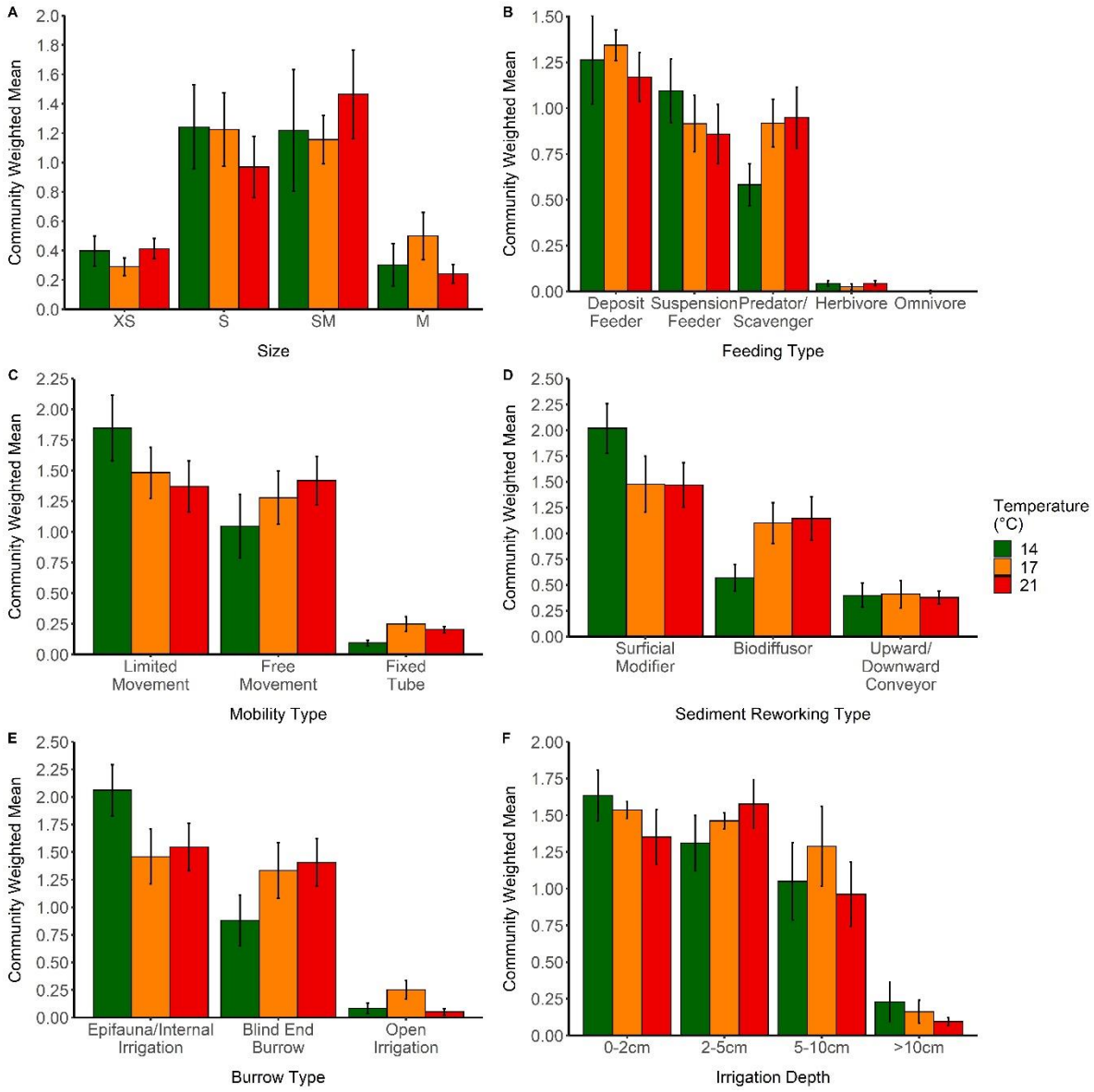

Figure 2.12: Bar charts showing the CWM for traits, averaged for each temperature. A) Size; B) Feeding type; C) Mobility type; D) Sediment reworking method; E) Burrow type and F) Irrigation depth. Error bars represent the standard error.

There is not a clear trend with temperature for fauna which can irrigate the sediment deeper than 5cm, which is demonstrated through the results from an ANOVA which demonstrate that there was not a significant difference (df = 2, $df_{residuals}$ = 12, F = 0.447 and p = 0.65) between the three temperature scenarios. However, the data collected indicate that on average the biomass of taxa which exhibit this modality is higher in cores incubated at 17°C (1.29 ± 0.27), compared to those incubated at 14°C (1.05 ± 0.26) or 21°C

(0.96 ± 0.22). Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.0822 and p = 0.9216).

Some taxa present within this study have been classified as being capable of ventilating the sediment column deeper than 10cm. The data collected in this study indicates that the biomass of these taxa decreased with temperature rises (14°C = 0.23 ± 0.13, 17°C = 0.16 ± 0.08 and 21°C = 0.10 ± 0.03), although an ANOVA indicated that there was not a significant difference (df = 2, $df_{residuals}$ = 12, F = 0.534 and p = 0.60) between the three temperature scenarios. Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.5939 and p = 0.5676).

2.3.4. Measures of functional diversity

It was determined that neither of the measures of functional diversity (functional evenness and divergence) considered were significantly impacted by ocean warming. However, despite statistical insignificance some trends were observed in the data.

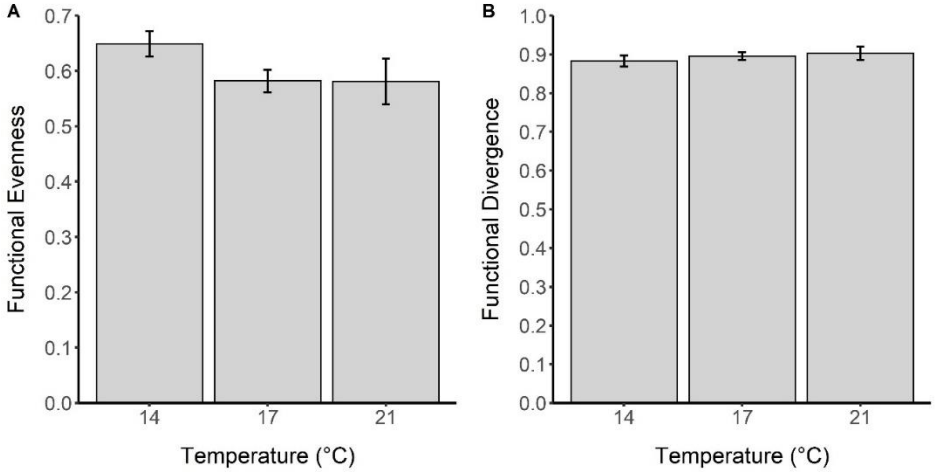

Figure 2.13: Measurers of functional diversity calculated on Hellinger transformed traits, weighted by biomass, data. A) Mean Functional richness; B) Mean functional evenness; C) Mean functional diversion and D) Mean functional dispersion. Where error bars represent ± standard error.

The mean FEve decreases under ocean warming scenarios (14°C = 0.649 ± 0.023; 17°C = 0.582 ± 0.020 and 21°C = 0.581 ± 0.041; Figure 2.12). The small decrease in FEve

under ocean warming scenarios could indicate that warming under the duration of the experiment does begin to impact the structure of functional traits of the blackness community. An ANOVA undertaken on $1/\log_{10}$ transformed FEve data indicated that there was not a significant difference in FEve between cores incubated at each of the temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 2.419 and p = 0.131). Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.3709 and p = 0.6978).

Functional Divergence (FDiv) does not appear to be impacted by temperature in this experiment (14°C = 0.883 ± 0.014; 17°C = 0.896 ± 0.010 and 21°C = 0.902 ± 0.017), and an ANOVA indicated that there was not a statistically significant difference between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.479 and p = 0.631; Figure 2.12 B). Levene's test was used to test the data for homogeneity of variance and indicated that there was not a different in variance between the three temperature scenarios (df = 2, $df_{residuals}$ = 12, F = 0.3517 and p = 0.7105).

2.4. Discussion

The impact of ocean warming on the marine environment has been studied for many decades across the various oceanographic disciplines, including marine ecology, biogeochemistry and physical oceanography, and a range of spatial scales from global down to site specific scales. This research paper focuses on the impact of ocean warming on biodiversity and functional diversity of intact benthic cores collected from an intertidal location, using an experimental mesocosm approach.

Whilst experimental setups do not necessarily allow for a full assessment of how the structure and function of the Blackness benthic community responds to ocean warming, the use of experiments and model systems is important in improving our understanding of processes that influence complex systems (Benton et al., 2007), such as intertidal mudflats at Blackness. They also have value in indicating the relative abilities of different taxa to withstand temperature stress, and the resultant changes in functional diversity. Therefore, developing a robust understanding of processes occurring through the use of controlled

experiments is essential for the improvement and refinement of mathematical and computational models, which are often used in the formation of policy and management scenarios (Benton et al., 2007). The results obtained in this experiment offer an insight to improve our understanding regards the way ocean warming may influence ecosystem functioning and carbon processing, of which faunal communities are a major player. Therefore, the first factor that needs to be understood is the structure and functional potential of the community in question, as this is the primary driver of how an ecosystem functions, which will be discussed in detail within chapters 5 and 6.

2.4.1. Biodiversity

The taxa observed within experimental cores were typical of intertidal mudflat communities, and typical for the study site (Bolam et al., 2002), with the dominant taxa being; Nematoda, Tubificoides sp., *Macoma balthica, Hydrobia ulvae, Eteone flava, Retusa obtusa, Heteromastus filiformis* and *Glycera alba.* The numerically dominant species present within samples collected were nematodes, Tubificoides sp. and *Pygospio elegans*. However, due to their small size they do not individually contribute greatly to the total biomass present within sample. Although less abundant, species such as *Macoma balthica* and the polychaete *Glycera alba* dominated the biomass at Blackness. This was also observed by Bolam et al. (2002) who sampled sediments slightly further downstream at blackness and at other intertidal temperate mudflat environments, such as in the Dutch Wadden Sea (Beukema, 1976). The different taxa identified within the experimental cores all responded differently to the three temperature scenarios. The use of an intact community makes it difficult to pinpoint the precise reasons why the taxa responded in the ways that they did, without directly measuring the impact on each taxon individually.

2.4.2. Abundance & Biomass

The data collected from the incubation experiment indicate that both total abundance and total biomass increase at 21°C, although not significantly. It is important to note that as a closed system was used over a short time period, benthic communities were not necessarily able to respond to the increase in temperature in the same way that they would in the natural environment, for example avoidance through migration. However, the

benthic community and individuals within the community will have still experienced and responded to the change in temperature, and due to the timescale of the experiment responses are likely to reflect the impact of heatwaves on benthic communities. The increase in abundance and biomass could be due to a combination of factors influencing the various taxa differently within the community, including: 1) some taxa may have a higher tolerance to the stressor (temperature), 2) change in food supply, and 3) the stressor induces reproduction. Another factor which needs to be considered is how temperature impacts meiofaunal taxa, such as nematodes. This experiment only considered macrofaunal communities (i.e., taxa which were retained on a 500µm sieve). Nematoda, typically considered to be part of the meiofaunal community, which were retained on the 500µm sieve were also included in the analysis. This taxon is considered to play an important role in ecosystem function and carbon processing in the marine benthic environment (Gingold et al., 2013; Schratzberger et al., 2019; Liao et al., 2020). However, despite the observed trends, it is also important to note that due to the use of intact benthic communities in this study, it is possible that natural variability and patchiness of intertidal mudflats could have resulted in differences in abundance and biomass of the benthic community and that apparent differences observed could be a random effect. To determine whether this is the case it would be beneficial to undertake the experiment on a significantly larger number of cores.

Stressor tolerance: Tubificoides sp. have been observed to be the dominant taxa in an area impacted by a thermal discharge in the Medway estuary (Bamber and Spencer, 1984), demonstrating that temperature increases do not negatively impact abundance of this taxon. This result is also reflected within this study which showed a higher abundance and biomass in cores incubated at 21°C, compared to cores incubated under ambient conditions (14°C).

The abundance and biomass of *Macoma balthica* in this experiment both decreased under ocean warming scenarios. The general consensus is that *Macoma balthica* are a species which is sensitive to temperature, meaning that the upper, lower and optimal temperatures occur over a relatively narrow range (Beukema et al., 2009). This species may

therefore be a good indicator of warming impacts to benthic macrofauna communities. In Europe, this species has been observed as far south as the Iberian Peninsula (Ashley, 2016), however, this does not mean that this species in general is capable of surviving/tolerating temperatures observed at this location. For example, a study undertaken by Wilson (1981) indicated that the lethal temperature for *Macoma balthica* in Dublin Bay was 37.5°C in the summer and 27.5°C in the winter. Although this taxon may be capable of surviving the temperatures that they were exposed to in this experiment it is likely they started to demonstrate negative side effects which could have contributed to the decrease in abundance and biomass under ocean warming scenarios. It is also important to consider that life histories of populations at a certain location are important in determining their tolerance to change and their survival. For example, populations of *Macoma balthica* along the Iberian coast will have a different life history to populations found along the Firth of Forth. It is also possible that life histories of populations will vary within a relatively small country, like the UK. Therefore, this could mean that the impacts of ocean warming will not be uniform across the country, and it is possible that different management strategies will need to be produced for different regions of the UK.

Food supply: The species *Glycera alba* has been classified as being a predator/scavenger in this study. Both abundance and biomass of this species increases under ocean warming scenarios. It is possible that this could be due to this species benefitting from the decrease in abundance/biomass of other taxa within the community and/or benefitting from increased food supply provided by less tolerant taxa or individuals who did not survive exposure to increased temperature (Nielsen and Gosselin, 2011).

It is thought that higher temperatures will result in a higher metabolic rate and therefore higher respiration rate (Hummel et al., 2000). Therefore, if this occurs an increase in food supply will be required to maintain the increase in metabolic rate of individuals and communities under ocean warming scenarios (Hummel et al., 2000). The biomass of some taxa decreased under ocean warming scenarios, therefore it is possible that during the experiment food became a limiting factor for some taxa, such as *M. balthica*. If food availability becomes a limiting factor, then it is possible that taxa will stop building or even

maintaining mass and will deplete their energy reserves to maintain the increased metabolic rate due to increased temperature (Hummel et al., 2000). However, this experiment was a closed system, where additional food was not added during the experimental period. Therefore, if food did become a limiting factor during the experiment, it is possible that in the natural environment the decreases in biomass observed in some species may not occur or may not be as pronounced. However, Jansen et al. (2007) noted that the population of *M. balthica* has decreased along the Bay of Biscay over the past 40-50 years due to temperature rises. The authors attributed this decline to the population experiencing short-term but frequent exposure to temperatures >30°C in estuaries, which increased respiration rates of the taxon and ultimately resulted in starvation (Jansen et al., 2007).

Reproduction: Oligochaetes tend to be a fairly dominant taxon in coastal areas enriched in organic matter and are often described as an opportunistic taxon which are well adapted to rapid environmental fluctuations and stress, such as that brought about by ocean warming (Bagheri and McLusky, 1982; Giere, 2006). It has been observed that populations of *Tubificoides benedii* in the Forth estuary do not demonstrate a clear reproductive seasonality (Bagheri and McLusky, 1982), suggesting that this taxon is capable of reproducing all year round. Temperature increases have also been seen to trigger the onset of reproduction in *Tubifex costatus* which are in the same sub-family (Tubificinae) as Tubificoides sp. (Birtwell and Artgur, 1980). These observations suggest that it is possible that the temperature increases undertaken in this experiment could explain the observed increase in abundance and biomass of oligochaetes. In addition, what appeared to be oligochaete eggs were observed in samples incubated at both 17°C and 21°C, but not at 14°C, further suggesting that the increase in temperature, initiated reproduction in this taxon. Increased temperature has also been seen to increase the rate of reproduction in spionid species. For example, *Pygospio elegans* has demonstrated a relationship in timing of reproduction and temperature (Ashley, 2016). This spionid is capable of reproducing sexually and asexually, with some populations being more inclined to undertake asexual reproduction than others (Gibson and Harvey, 2000). Gibson & Harvey (2000) observed that

temperature did not affect the reproduction strategy but could have impacted the timing of reproduction. This suggests that ocean warming could alter the start of reproduction in this species, or it could result in a longer reproduction season, resulting an increased abundance and biomass of this species.

Over the past two to three decades, it has been observed that the populations of *M. balthica* have decreased in the Wadden Sea (Beukema et al., 2009). Temperature has been shown to be one of the primary drivers of reproduction in marine species (Hummel et al., 2000). Spawning of *M. balthica* has been shown to occur between 10°C and 12°C, and it has been indicated that ocean warming has a negative impact on recruitment of this taxon (Hummel et al., 2000; Beukema et al., 2009).

Nematoda: As previously outlined nematodes are typically considered to be part of the meiofaunal community due to this group being interstitial (Aller and Aller, 1992; Schratzberger et al., 2019), although in this study those which were retained on a 500µm sieve were included in and considered part of the macrofaunal community. Meiofauna, including nematodes, are known to undertake a range of biological activities, which impact the environment in which they live (Schratzberger and Ingels, 2018). For example, they can move on and within the sediment matrix and therefore undertake sediment particle reworking, ingestion, defecation, and excretion of metabolic waste (Schratzberger and Ingels, 2018). They also play an important role in food-webs by influencing the structure and function of microbial communities (Schratzberger and Ingels, 2018). Lee et al. (2017) undertook an experiment over a 90-day period and observed that an increase in temperature from 15°C to 19°C, which is similar range to the ambient and ambient +3°C scenario used in this experiment (14°C and 17°C), did not affect meiofaunal abundance. In the study undertaken by Lee et al. (2017) nematodes contributed to approximately 55% of the meiofaunal community. The observations made by Lee et al. (2017) are consistent with the results presented here, which demonstrated that an increase in water column temperature from 14°C to 17°C did not affect the abundance or biomass of nematoda incubated in the experimental cores. However, in cores incubated at 21°C the abundance and biomass of nematoda did increase. It has been observed that under experimental

conditions nematodes which have been incubated at increased temperatures exhibit shorter lifecycles (Stroustrup et al., 2016; Lee et al., 2017). Therefore, it is possible that an increase in temperature to 21°C could be resulting in a shorter life-cycle for nematodes at this study location, and thus reproduction could be occurring at a faster rate in comparison to cores incubated at the two lower temperatures. However, it is important to note that these observations were made for nematoda which remained on a 500µm sieve and are therefore not necessarily representative of the entire nematoda community. The nematodes were also not identified beyond the Phylum level, therefore the functional roles of nematoda present have not been taken into account.

Understanding how ocean warming impacts on nematodes is essential, because as they feed, they consume microbes from the sediment (Schratzberger and Ingels, 2018). Therefore, it is possible that the increase in abundance and biomass of nematodes under increased temperatures observed in this experiment could impact the structure and function of microbial communities in the experimental cores (Gingold et al., 2013). The presence of nematodes and other meiofaunal taxa can stimulate bacterial growth as they mechanically break down organic detritus in the sediment matrix (Schratzberger and Ingels, 2018). This action undertaken by nematodes and the meiofaunal communities makes the detritus more accessible to microbial processes (Schratzberger and Ingels, 2018). Previous studies (Alkemade et al., 1992; Coull, 1999; Nascimento et al., 2012) have indicated that the presence of meiofaunal taxa, including nematodes, enhances the mineralization process of organic matter, due to their relationship with microbial communities and function. However, there is a lack of research into the effects of meiofauna-microbial interactions in benthic systems and the overall impact is therefore largely poorly understood. However, this interaction is beyond the scope of this thesis.

2.4.3. Functional Diversity

Examination of abundance and biomass alone does not provide insight into the complex functions occurring within the sediment. Functional diversity considers the composition of trait modalities present under each of the temperature scenarios, represented as the community weighted mean (CWM) and the how the composition of

these modalities influences the measures of functional diversity at the community level (functional evenness, functional divergence).

2.4.3.1. Community Weighted Mean

The community weighted mean was used to determine changes in trait composition and expression between different environmental scenarios, in this case between temperature scenarios (Díaz et al., 2007; D'Alessandro et al., 2020). These results are based on traits data which have been weighted by biomass.

Size: At 14°C the dominant taxon size is the modality classed as "Small", which is fauna which grow to a maximum of 1-2cm, and the modality classed as "Small-Medium", which is fauna which can grow to a maximum of 3-10cm. The size modalities of "Extra small", fauna <1cm, and "Medium", fauna >10cm, are less dominant. This is most likely due to fauna classed as "Medium" being relatively rare and therefore accounting for less of the total biomass of the community, and fauna classified as "Extra Small" having a low biomass due to their size, despite having a high abundance within the community (e.g. nematodes). Temperature does not appear to have impacted the CWM of the "Extra Small" and "Medium" modalities.

An Increase from ambient to ambient +3°C (14°C to 17°C) does not appear to Impact the CWM of the "Small" or "Small-Medium" size modalities, but an increase in temperature to 21°C results in a decrease in the CWM of the "Small" modality but an increase in the "Small-Medium" modality. Prolonged exposure to temperatures of 21°C could result in the community at the firth of Forth favouring taxa which are larger in size. This change in body size could have important implications to the benthic community, as the surface area to volume ratio of an individual has been linked to oxygen uptake in low oxygen environments, such as oxygen minimum zones (OMZs), where smaller fauna can facilitate respiration by enhancing surface area to volume ratios (Rhoads and Morse, 1971; Levin, 2003). Under ocean warming scenarios it is expected that the concentration of oxygen within the water column will decrease due to a reduction in oxygen solubility and reduced ventilation (Schmidtko et al., 2017). Based on this assumption it would be expected that smaller bodied

organisms would be more successful. However, based on the results from this study this does not appear to be the case.

However, the term "size" is a relatively broad term, which does not encapsulate the varying body types of different taxa. For example, in this study both *Tubificoides sp.*, *Glycera alba* and *Cerastoderma edule* have been classified as expressing the size modality of "Small-Medium", which is the maximum size these taxa have been reported to reach. However, these three taxa are anatomically very different, despite falling into the same category for this trait. For example, *Tubificoides sp.* has a thin, round, elongated body compared to *Glycera alba, also an annelid,* which is less round (more oval) and has parapoda protruding from each segment. *Cerastoderma edule (*Bivalvia*)*, despite falling within a different phylum to both *Tubificoides sp.* and *Glycera alba* is also classified as falling within the "Small-Medium" modality. However, the anatomy of this taxa is completely different to that of the annelid worms. These three taxa despite all falling within the same modality are all anatomically very different. Therefore, when considering the size of a taxon in functional trait experiments, it would most likely be beneficial to include a body shape trait, to better distinguish between fauna which are morphologically different.

Feeding type: The results suggest that the most dominant trait modality under all three temperature scenarios is that of deposit feeders. The CWM of the deposit feeding modality does not show a clear trend with temperature as it marginally increases at 17°C but decreases at 21°C. Whereas the prevalence of the predator/scavenger modality increases when cores are exposed to temperatures of 17°C and 21°C, and the suspension feeder modality decreases under both ocean warming scenarios.

It has already been stated by Bamber & Spencer (1984) that Tubificoides sp. was the dominant species in an area affected by thermal discharge in the Medway estuary due to their opportunistic nature. It is possible that opportunistic taxa are successful under stressful conditions due to their feeding mechanism. In the natural environment food availability is known to be a driving factor in the success of an organism, including scavengers and predators in the marine benthic environment (Nielsen and Gosselin, 2011).

In the case of scavengers, food availability is driven by the availability of food sources and the rate at which these taxa die and become available, whereas food availability to predators depends on the availability of prey (Nielsen and Gosselin, 2011). However, it is common for taxa which are predominantly a scavenger to also perform as a predator, and vice versa, if the conditions require the change in trait expression (Brewer and Konar, 2005; Nielsen and Gosselin, 2011). This is why predators and scavengers have been considered under a single modality, as it is not known which method of feeding they will have been undertaking at the time of the experiment, or whether the method remains the same throughout the experiment. In this study it is possible that at 21°C some taxa (e.g., the suspension feeding modality) become stressed, which could mean that they become easier for predators/scavengers to catch. This was observed by Nielsen & Gosselin (2011), where the mussel species *Mytilus trossulus* was more vulnerable to predation by *Lirabuccinium dirum* under low salinity levels, in comparison to healthy mussels under ambient conditions. It is also possible that increased mortality of less temperature tolerant taxa occurred which would have also increased food availability to taxa expressing the predator/scavenger modality, resulting an increase in biomass. However, conditions which are stressful enough to kill prey/food sources could also potentially harm predators/scavengers (Nielsen and Gosselin, 2011). Therefore, for this modality to be successful under stressful conditions they must have adaptations to be capable of tolerating the stressor to exploit the availability of food (Nielsen and Gosselin, 2011). For example, *Glycera alba,* which has been classified as a predator/scavenger in this study (Appendix 2), is known to have a relatively low energy requirement and can remain inactive in their burrows whilst they wait for prey to move into their territory (Fauchald and Jumars, 1979; McLusky and Berry, 1997). This low energy requirement could benefit the species under relatively short periods of stress, such as those experienced during this study. The response of communities exposed to ocean warming scenarios over longer time scales and any subsequent changes in feeding types wasn't assessed in this experiment but remains an important area of research.

Taxa mobility & Sediment reworking: Under ambient conditions the dominant type of mobility is that of "limited movement", followed by "free moving" taxa and finally those

who live in a “fixed tube”. Under ocean warming scenarios mobility traits are no longer dominated by a single modality but are dominated relatively equally by both “Limited movement” and “Free movement”, as the CWM of “Limited movement” decreased under warming scenarios and “Free movement” increased. The “Free movement” modality is the highest activity modality where fauna move freely through the sediment matrix or freely using a burrow system (Queirós et al., 2013). Fauna which undertake limited movement are less active and tend to remain within a burrow, for example bivalves such as *Macoma balthica* (Queirós et al., 2013). It is possible that the biomass of taxa who fall within the “Limited movement” decreases slightly under ocean warming scenarios as they could predominantly exist within the top few cm of the sediment column, where it is more likely that the effects of temperature change in the water column could be experienced. Due to having limited movement these taxa may not have able to avoid the stressor (temperature increase), by migrating deeper into the sediment column, as effectively as taxa which fall in the “Free movement” modality and could easily move through the sediment matrix/burrow system to avoid the effects of the temperature change in the water column. However, it has also been previously observed that different life-stages of a taxon may behave differently and migrate within the sediment column differently (Kennedy and Mihursky, 1971). For example, Lammens (1967) studied a population of *Macoma balthica* and found that younger individuals lived within the first 3.5cm of the sediment column, whilst adults live deeper within the sediment column (~7cm). It has also been observed that as a result to younger individuals living shallower within the sediment column that they also spawn earlier than adult individuals due to earlier warming of surficial sediments and reaching the critical temperature (Lammens, 1967; Kennedy and Mihursky, 1971).

The ability of a taxon to avoid the effects of a stressor will enhance the probability that it will survive the event, and those which cannot do this effectively may not be capable of surviving under future environmental conditions.

Sediment reworking trait modalities appear to be impacted by ocean warming scenarios, and demonstrate a similar trend observed within the mobility trait. The dominant sediment reworking modality, weighted by biomass, under all three temperatures is that of

"Surficial modifiers" (e.g., *Macoma balthica*) followed by "Biodiffusors" (e.g. Tubificoides sp.) and finally "Upward/Downward conveyors" (e.g. *Arenicola marina*). The dominance of surficial modifiers decreases under ocean warming scenarios, whereas the dominance of biodiffusors increases. Surficial modifiers tend to be found in the first few millimetres of the sediment column, at the water column-sediment interface, meaning that they are directly exposed to the environmental stressor, whereas biodiffusors move freely within the sediment column. The sediment column should buffer against the effects of ocean warming, meaning that fauna which can move freely within the sediment should be relatively unimpacted by the direct influence of temperature.

Burrow type & Irrigation depth: Burrow type is linked to how the sediment is ventilated by the infaunal community, and therefore impacted by ocean warming. The dominance of blind-ended-burrows increases with ocean warming. Whereas the dominance of fauna which undertake either epifaunal or internal irrigation decreases with ocean warming. Blind ended burrows are those which only have one end open to the water column (e.g., *Glycera alba* and Tubificoides sp.), rather than two or more (e.g., *Arenicola marina*), and in the case of this study site is most likely ventilated bidirectionally and irrigation occurs through diffusion between the burrow wall-water interface (Kristensen et al., 2012). Fauna which fall within the "Epifauna/internal irrigation" are predominantly bivalves and gastropods (e.g. *Macoma balthica* and *Hydrobia ulvae*), which live within the first few mm – cm of sediment, and are therefore exposed to the conditions of the water column. Whereas taxa which fall into the "Blind-ended-burrows" modality live deeper within the sediment column, which to an extent, buffers against the conditions in the water column. This is also demonstrated in the irrigation depth trait modalities, where under ocean warming scenarios the CWM of the "0-1cm" modality decreases and the CWM of the "2-5cm" modality increases.

2.4.4. Measures of functional diversity

Functional evenness & divergence: Functional evenness (FEve) represents how evenly the biomass of the community is distributed within the functional space (Llanos et al., 2020). In this study the FEve decreases under both ocean warming scenarios (17°C and

21°C), suggesting the functional structure of communities at Blackness have started to demonstrate a shift due to temperature increases, although not significantly. A higher FEve indicates a more regular filling of the trait space, whereas a lower FEve suggests that parts of the functional space are becoming less populated, whilst other parts are becoming more populated (Mouchet et al., 2010; Rivadeneira and Nielsen, 2017). The functional evenness could be lower under ocean warming scenarios due to the increase/decrease in biomass of certain taxa, and therefore the traits which they express. For example, the biomass of Tubificoides sp. increases under ocean warming scenarios and *Macoma balthica* decreases and account for a large portion of the community biomass. Although functional evenness indicates how biomass is distributed among the traits/trait modalities present, there is no indication on which traits/modalities are becoming less populated and which are becoming more populated.

The observations in this study are not statistically significant, meaning that although a slight difference has been observed, it is unlikely that the functioning of the system has changed over the duration of this experiment. This is supported by the functional divergence (FDiv), which does not change under warmer temperature scenarios. High FDiv values indicate that there is a high degree of niche differentiation and therefore low resource competition and increased levels of ecosystem function due to a more efficient use of available resources (Mason et al., 2005). As the FDiv does not change with temperature in this study it could be suggested that based on functional traits, the resource use and ecosystem function do not change with temperature. However, this metric does not consider the effects of temperature on metabolic rates of fauna. The impact of temperature on ecosystem functioning will be examined in detail within chapters 4 and 5.

2.4.5. Limitations & Future work

This experiment used a relatively small number of replicates per temperature scenario, and as natural benthic communities were used in the experiment this resulted in a high level of within treatment variation. To improve this in the future it would be beneficial to repeat the study with a significantly higher number of replicates per treatment.

The use of microcosm experiments, although commonly used to study the impact of environmental change in a controlled manner, do have their limitations. Microcosms are closed systems and do not replicate the complexities of the natural environment, and the taxa cannot behave completely naturally. For example, it is possible that some taxa which were negatively impacted by temperature increases in this experiment may be able to avoid the stressor more successfully in the natural environment (e.g., moving to deeper waters).

The use of biological traits in conjunction with traditional measures of biodiversity provide us with a more complete picture of how ocean warming could potentially impact the structure of benthic communities in the future. Traditional biodiversity measures indicate *who* is present, whereas the use of functional traits give an idea of *what* the community is doing and how they influence their environment. However, there are still large knowledge gaps when it comes to understanding the life histories and functional traits of many benthic infaunal species (Munari, 2013; Degen et al., 2018). Not only do life histories need to be taken into account, but it is also known that marine benthic fauna are capable of expressing functional trait plasticity, meaning that the trait they express under ambient conditions may not necessarily be expressed when they are under stress from an environmental variable, such as ocean warming. Further complexities are introduced when considering that the life histories and trait expression of a single species can differ between study site locations (Wohlgemuth et al., 2017).

Nematoda and meiofaunal communities are briefly mentioned in this study, however, it is essential that an in depth and complete study is undertaken, including species identification, and assigning functional traits, to fully understand how these taxa are influenced by ocean warming and other environmental variables.

2.4.6. Conclusions

Temperature increases have the potential to result in changes in intertidal mudflat macrofaunal community structure and function. This experiment indicates that the effects of temperature rises could be complex, with some species being positively impacted whilst others are negatively impacted. Overall community trends indicate that there was not a

significant difference in species richness, diversity, and evenness between the three temperature scenarios. However, the total abundance and biomass appeared to increase with temperature rises. The abundances and biomasses of different taxa responded to temperature changes in different ways. For example, the abundance and biomass of *Macoma balthica* appeared to be lower at higher temperatures whereas the abundance and biomass of taxa such as Tubificoides sp. and *Pygospio elegans* were higher at higher temperatures. Temperature not only appears to result in a shift in community structure, but also functional structure. It appears that higher temperatures appear to favour taxa which wall under the predator/Scavenger feeding type and those which can move freely through the sediment column but is detrimental to taxa which exist on or within the first few millimetres of the sediment surface. However, caution must be taken when making conclusions on the impact of single-stressor experiments, as in the natural world multiple stressors/environmental conditions act on an ecosystem simultaneously.

3. The impact of ocean warming on marine sediment ecosystem functioning: a case study of intertidal muddy sediments (Firth of Forth, Scotland)

3.1. Introduction

Shelf sediments cover a relatively small area of the ocean (approximately 7%) yet despite this they are known to play an important role in ocean productivity, biogeochemical cycling and, accumulating and burying organic matter (Hicks *et al.*, 2017; Silburn *et al.*, 2017; Neumann *et al.*, 2021). Many of the key functions of shelf sediments, such as organic matter remineralisation, nutrient supply to the water column, and C storage, are biologically mediated by the activities of macro, meio and microfauna (Neumann *et al.*, 2021). Therefore, it is essential that there is an understanding of how future environmental change will alter ecosystem function and associated biogeochemical processes within the system.

As a result of anthropogenic activities (e.g., burning fossil fuels) since the industrial revolution, atmospheric temperature has been steadily increasing (Findlay *et al.*, 2010; Schratzberger and Somerfield, 2020). Shallow coastal oceans and intertidal environments are most likely to be among the first habitats to feel the effects of temperature increases due to global environmental change (Vinagre et al., 2018). This is because their shallow depth means that they have a lower thermal inertia in comparison to open oceans, and their thermal regime is driven by both hydrodynamic and atmospheric conditions (Vinagre et al., 2018). It is expected that atmospheric and ocean temperatures will continue to increase into the future, with sea surface temperatures increasing by between 2°C and 4.5°C, with the most likely value of 3°C (Meehl et al., 2007; Godbold and Solan, 2013). Temperature is known to be one of the fundamental factors affecting all ecosystems and spatial scales, from the metabolism of individual organisms to global patterns of biodiversity (Salo, Mattila and Eklöf, 2020).

This study specifically focusses on the impact of temperature rises, as a result of ocean warming due to climate change, on biodiversity-ecosystem function (BEF) relationships of an intertidal mudflat system. Macrofauna play an important role in benthic biogeochemical processes, in less permeable sedimentary environments (i.e., sediments

which have a higher mud content), due to the impact that their sediment reworking activities (bioturbation and bioirrigation) have on the sediment matrix (Neumann et al., 2021). These sediment reworking activities can enhance biogeochemical processes within the sediment matrix and result in an increased rate of oxygen consumption by the benthic community (Neumann et al., 2021). Previous studies have demonstrated that the use of sediment reworking indices, bioturbation and bioirrigation potential (BP_c and IP_c), are a useful way of estimating the role of macrofaunal communities in biogeochemical processes (Neumann et al., 2021). These indices have also been correlated with a variety of other measures of ecosystem functioning, including sediment oxygen consumption and nutrient flux across the sediment-water interface (Neumann et al., 2021).

The role of biodiversity in driving ecosystem function Is relatively well understood. For example, increased temperatures result in an increase in organism metabolism and feeding rate (Salo, Mattila and Eklöf, 2020). Organisms within communities interact in various ways, meaning that if the metabolism and/or feeding rate of a single taxon changes then this could result in a cascade of modifications throughout all levels of biological organization and modify the functioning of the system in question (Salo, Mattila and Eklöf, 2020). For example, it has been observed that warming has resulted in increased consumer metabolism and feeding rates (O'Connor, 2009; Vucic-Pestic et al., 2011) increasing top-down control (Kratina et al., 2012; Carr and Bruno, 2013) and cause a shift in food web structure.

Mesocosm experiments have demonstrated that there is a relationship between biodiversity and ecosystem functioning. For example, it has been observed that using constructed communities an increase in species richness influenced nutrient generation, where the concentration of ammonium and phosphate increased with species richness (Ieno et al., 2006). However, the authors also observed that species identity and density drove their observations, indicating that the role of species-specific bioturbation traits was important. Relationships between organisms and ecosystem functions are also influenced by environmental context (Godbold and Solan, 2009; Godbold et al., 2011), and are therefore likely to vary spatially and temporarily (Ieno et al., 2006). By extension alterations

in the natural environment due to global environmental change (e.g., ocean warming) will likely alter a taxon's behaviour and alter processes that drive ecosystem functioning, including biogeochemical cycling of macronutrients (Godbold and Calosi, 2013). However, the majority of experiments are undertaken in mesocosms containing a single species or a pre-selected combination of only a few species (Michaud et al., 2006; Ieno et al., 2006; Michaud et al., 2010; Godbold and Solan, 2013), and the natural environment is significantly more complex and heterogeneous than these simplified experimental environments suggest.

The aim of this study Is to determine the Impact of ocean warming on several benthic ecosystem functions, using intact cores collected from Blackness, Firth of Forth (Scotland). As intact cores are being used in this experiment both benthic faunal and microbial communities are considered. Ecosystem functions which will be studied include the sediment community oxygen consumption (SCOC), nutrient fluxes and the bioturbation and bioirrigation potential of the benthic community.

3.2. Methods

3.2.1. Study sites and sample collection

Sediment samples were collected from an intertidal mudflat at Blackness (Firth of Forth, Scotland) in April 2019. A full description of the study site and sampling strategy is outlined in Chapter 2.

3.2.2. Experimental design

Cores were transported to The Lyell Centre (Herriot Watt University) and placed within randomly assigned controlled temperature (CT) environments. The temperature environments set up include a Control temperature (14°C), Control +3°C (17°C) and Maximum observed temperature (21°C) at the study location. Temperature treatments were maintained through a combination of CT rooms and submerged water heaters. A full outline of the experimental design can be found in Chapter 2.

3.2.3. Oxygen measurements

Rates of respiration for each temperature scenario were measured using a benthic incubation chamber set-up. The chamber set-up consisted of watertight acrylic tubes which had a mean capacity of 4.3L of water in addition to the sediment sample. Oxygen optodes connected to a FireSting O_2 fibreoptic oxygen meter (both from Pyro Science GmbH, Germany) were used to continuously measure the oxygen concentration (µmol l^{-1}) in the water column of each chamber, during capped periods. The optodes were calibrated for each of the temperature scenarios (14°C, 17°C and 21°C) by a two-point calibration (0% and 100%). The 0% calibration point was achieved by bubbling nitrogen gas through seawater and the 100% calibration point by saturating seawater with oxygen by bubbling air through it. Each individual respiration chamber housed a motor driven stirrer. The respirometry equipment set up can be seen in Figure 3.1. Total oxygen uptake (TOU) by the benthic community was measured every other day over a four-hour period for 14 days. During periods where TOU was being measured all light was excluded from the cores, air stones were removed, and water replacement stopped. Each core was then capped so that they were airtight, and all air pockets removed, optodes were inserted into the cores through a port in the lid and measurements started. All cores were continuously stirred during the capped period.

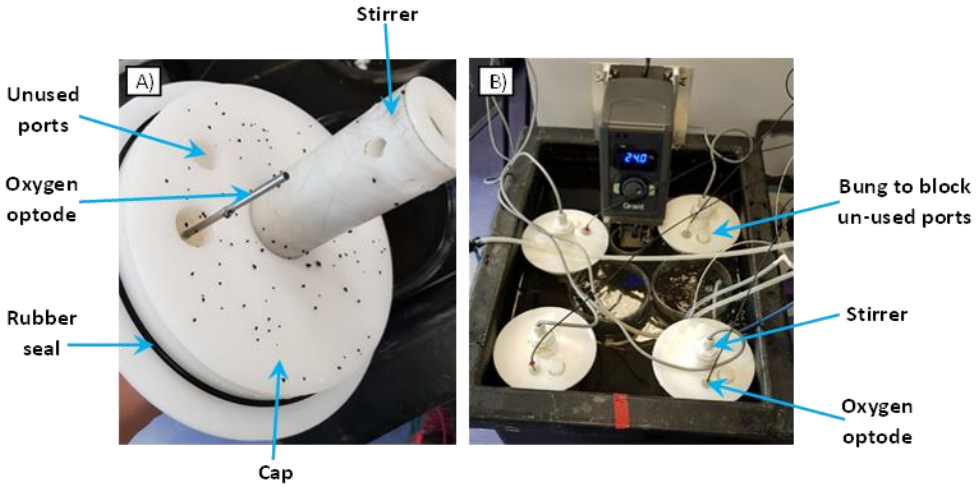

Figure 3.1: Oxygen optode set -up, A) Demonstrates the oxygen optaode and stirrer which is within the core, B) Demonstrates the external set-up

Five respirometry measurements were collected for each temperature scenario over the course of the experiment. The first four measurements were made before any organic detritus was added to the cores, and the fifth measurement was made after ^{13}C labelled algae were added. The first respirometry measurement was undertaken 48 hours after cores had been introduced to their new temperature environment. This delay in respirometry measurements was to reduce potential effects associated with initial stress responses of the organisms to temperature change.

Once oxygen uptake data had been collected for each core, rate of uptake by the benthic community was calculated (Equation 3.1).

$$TOU = \frac{V}{A} \times \frac{\delta[O_2]}{\delta T}$$

Equation 3.1: Total oxygen consumption (TOU), where V = volume of water, A = area of enclosed water multiplied by the rate of oxygen consumption, where [O2] = Oxygen concentration and T = time (Glud, 2008; Hicks et al., 2017).

At the end of the two-week experiment porewater oxygen profiles were acquired in all sediment cores using an oxygen microprofiler (50µM tip, Unisense A.S., Denmark) to determine the concentration of oxygen down the sediment profile. The microprofiler was also calibrated using the same two-point calibration method outlined in the respirometry section. The microprofiler entered the sediment column in 1mm intervals until the concentration of oxygen reached zero, this is known as the oxygen penetration depth (OPD).

Profiles were converted to excel workbooks within the Unisense profiling software (v3.4.000). Each profile was then plotted and the point at which the probe entered the sediment was identified, through a change in slope of the measured oxygen profile and set to 0cm. This process enables the standardization of all profiles and makes them comparable. An average oxygen concentration was calculated for each depth measurement under each temperature treatment.

The data collected from using oxygen optodes and microprofilers allowed further categorization of the total oxygen uptake by the benthic community, into the diffusive oxygen uptake (DOU; Equation 3.2) and Faunal oxygen uptake (FOU; Equation 3.3).

$$DOU = D_0 \times \frac{\delta[O_2]}{\delta Z}$$

Equation 3.2: Diffusive oxygen uptake (DOU) rate (known as Fick's first law of diffusion), where D0 = oxygen molecular diffusion coefficient (based on temperature and salinity), [O2] = concentration of oxygen at Z = position in diffuse boundary layer (DBL) (Glud, 2008; Hicks et al., 2017).

$$FOU = SCOC - DOU$$

Equation 3.3: Faunal oxygen uptake is the difference between SCOC and DOU

3.2.4. Measures of functional processes

Macrofaunal sediment reworking activities (ventilation and sediment reworking) were determined using three methods: bioirrigation using the inert tracer sodium bromide (NaBr), bioirrigation potential (Wrede et al., 2018; Wrede et al., 2019) and bioturbation potential (Queirós et al., 2013). Sodium bromide (NaBr) was dissolved in seawater [Br^-] and added to each microcosm to an approximate concentration of 10mM. Once the tracer had been added to each core, they were left for ~1 hour before the first sample was collected. Water samples (15ml) were taken using a plastic syringe at 0, 4 and 8 hours on the final day of the experiment, filtered (0.45µm nylon syringe filters) and immediately frozen at -80°C. The samples were diluted by factor 50 and then analysed at the University of Leeds (School of Geography) using liquid chromatography (Dionex, ThermoScientific ICS 500; column = AS19 (2x250) mm + AG19 (2x50) mm). Negative changes in [Br^-] indicate an increase in levels of bioirrigation activities being undertaken by the macrofaunal community.

Community bioturbation potential (BP_c) was calculated using the species composition within each core following methods outlined by Solan et al. (2004) and Queirós et al. 2013). To calculate the BP_c each taxon present was scored based on their mobility (M_i) and sediment reworking (R_i) and relating them to taxon abundance (A_i) and biomass (B_i):

$$BP_c = \sum_{i=1}^{n} \sqrt{\frac{B_i}{A_i}} \times A_i \times M_i \times R_i$$

In addition to community bioturbation potential the community bioirrigation potential (IP_c) was calculated as outlined by Wrede et al. (2019 & 2018). As with the BP_c the IP_c is related to faunal functional traits which have been related to ventilation activities of the benthic macrofauna community. Each taxon present was scored based on their burrow type (BT_i), feeding type (FT_i) and irrigation depth (ID_i) and relating them to taxon abundance and biomass (A_i and B_i):

$$IP_c = \sum_{i=1}^{n} \sqrt{\frac{B_i}{A_i}} \times A_i \times BT_i \times FT_i \times ID_i$$

A full outline of how mobility, sediment reworking, feeding type, burrow type and irrigation depth categories were assigned to fauna identified within these experimental cores can be seen in Chapter 3 and Appendices 1 & 2 of this thesis.

In addition to sediment reworking activities, nutrient fluxes between the sediment and water columns were measured. Core top water samples were collected at the start and end of respiration measurements for nutrient analysis. Nutrients analysed were ammonium (NH_3^+), nitrate, nitrite and phosphate. Water samples were collected using a plastic syringe, filtered using 0.45µm nylon syringe filters, and stored in plastic sample tubes and frozen at -80°C until analysed at the University of Leeds (School of Geography).

Nutrient samples were defrosted in batches using cold tap water, on the morning of analysis. An auto diluter diluted the samples by factor 6 using low nutrient sea water (Sal 35, *Soil*). Nutrients were analysed using a continuous flow auto analyser and spectrophotometric detection (Skalar SAN ++ continuous flow auto analyser). Samples were analysed in randomized batches and 10% of within batch and between batch duplicates were included. The quality of the analysis was determined by measuring between batch sample and reference standard (Sigma Aldrich QC3179 diluted in *Osil* seawater) replicates (NH_4 = 12.49 ± 0.80, NO_2 = 1.56 ± 0.12, NO_2 + NO_3 = 8.87 ± 0.15, PO_4 = 1.40 ± 0.07). Certified

reference material (WW1b, diluted in salinity 35) were run alongside samples (NH_4 = 0.94 ± 0.0.03, NO_2 = 0.003 ± 0.002, NO_2 + NO_3 = 4.50 ± 0.36, PO_4 = 0.49 ± 0.01).

3.2.5. Statistical analysis

The data were checked for normality using Shapiro's normality test, and if necessary, the data were log transformed to meet the assumptions of parametric statistical tests. A significance level of 0.05 was chosen as the threshold for statistical significance. Differences in response variables between the three temperature treatments were analysed using a one-way ANOVA (analysis of variance) followed by a Tukey HSD *post hoc* test at a significance threshold of 0.05. Levene's test was used to determine whether homogeneity of variance assumptions were met prior to analysis.

3.3. Results

3.3.1. Oxygen Penetration Depth

The mean oxygen penetration depth (OPD) for cores incubated at 14°C and 17°C was 2.52 ± 0.30 and 2.60mm ± 0.28 respectively, and the mean OPD for cores incubated at 21°C was 2.44mm ± 0.07 (Figure 4.2A). An ANOVA indicated that the OPD was not significantly impacted by temperature (df = 2, $df_{residuals}$ = 13, F = 0.154 and p = 0.859). The homogeneity of variance was also tested for each of the species using Levene's test (df = 2, $df_{residuals}$ = 13, F = 2.6176 and p = 0.1108), indicating that there is not a difference in variance between the three temperatures. Overall, the oxygen microprofiles demonstrate that oxygen is consumed within the first 2.60mm of the sediment column (Figure 3.2 A & C).

3.3.2. Sediment-water interface oxygen concentration

The concentration of oxygen at the surface-water interface (SWI) decreased with temperature increase, with the mean oxygen concentration for cores incubated at 14°C being 186.20 ± 15.06 µmol L^{-1}, 17°C being 166.73 ± 5.97 µmol L^{-1} and at 21°C 132.91 ± 9.00 µmol L^{-1}. An ANOVA indicated that the concentration of oxygen at the SWI was significantly different (Figure 3.2 B & C) between the three temperature treatments (df = 2, $df_{residuals}$ = 13, F = 5.565 and p = 0.0179), with a Tukey HSD test indicating that the difference exists between cores incubated at 14°C and 21°C (p = 0.01) but not between cores incubated at

14°C and 17°C or 17°C and 21°C (p = 0.47 and 0.15 respectively). The homogeneity of variance was also tested for each of the species using Levene's test (df = 2, $df_{residuals}$ = 13, F = 1.754 and p = 0.2116), indicating that there is not a difference in variance between the three temperatures.

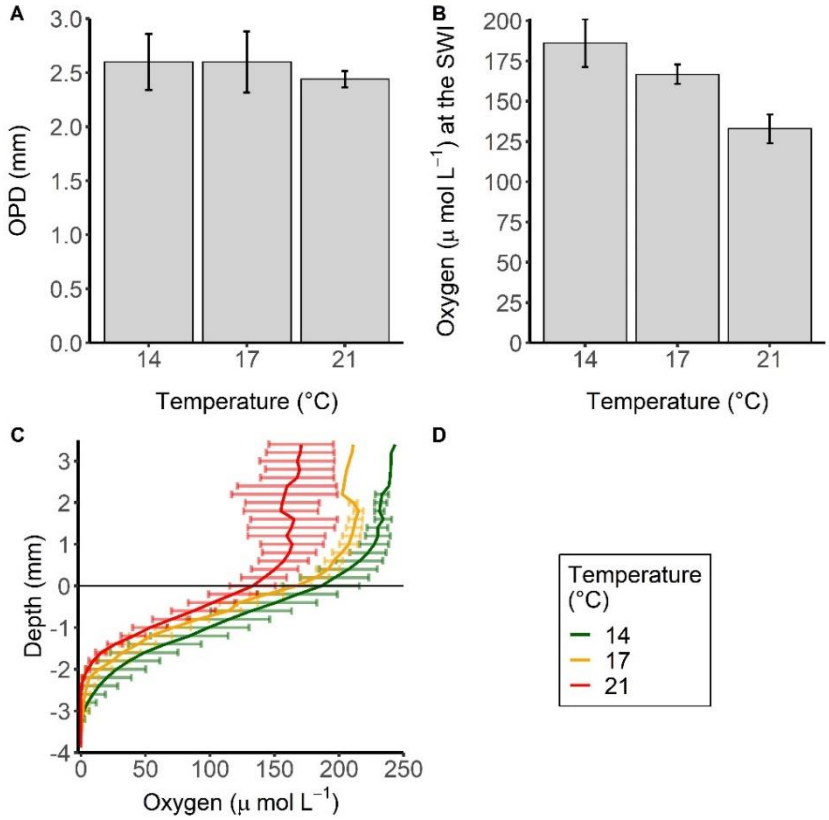

Figure 3.2: Oxygen dynamics within cores incubated under three different temperature treatments. A) Average oxygen penetration depth (OPD; ±S.E) for each temperature treatment; B) Oxygen concentration at the surface-water interface (SWI; ±S.E) for each of the three temperature treatments; C) Mean down core oxygen concentrations (±S.E) for each of the temperature treatments. 0 represents the SWI, values above the SWI represent oxygen concentration in the water column and values below the SWI represent the oxygen concentration within the sediment column.

3.3.3. Benthic oxygen consumption

Total oxygen uptake: The highest TOU (Figure 3.3A) was observed in cores incubated at 21°C (34.56 ± 3.35 mmol O_2 m^{-2} d^{-1}, SE, n = 5), followed by cores incubated at 17°C (21.42 ± 1.93 mmol O_2 m^{-2} d^{-1}, SE, n =5) and 14°C (20.80 ± 1.09 mmol O_2 m^{-2} d^{-1}, SE, n =6).

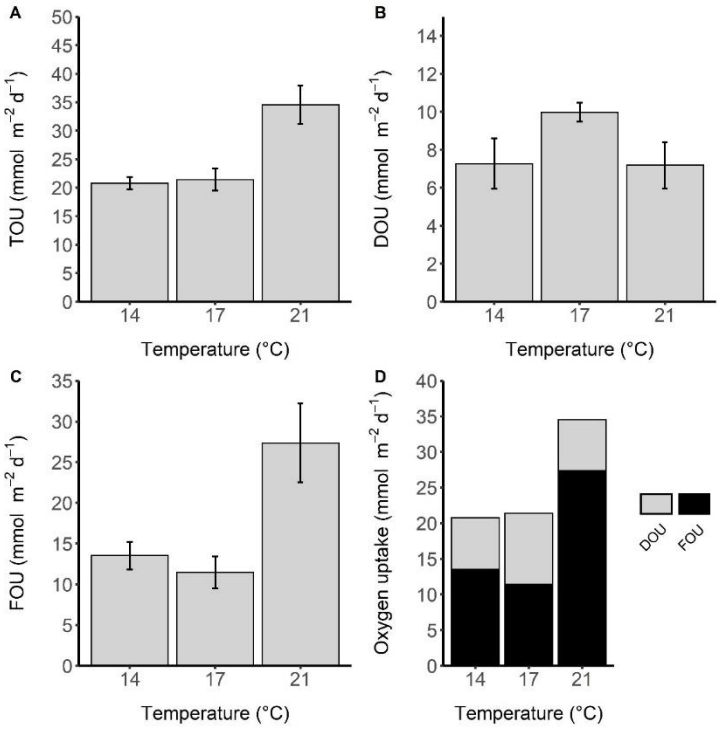

Figure 3.3: Oxygen uptake by the sediment community: A) Mean total oxygen uptake (TOU) for each temperature (±SE), B) Mean diffusive oxygen uptake (DOU) for each temperature (±SE), C) Mean faunal oxygen uptake (FOU) for each temperature (±SE) and D) Stacked bar chart demonstrating the relative contribution of DOU and FOU to the TOU for the different temperature scenarios. DOU data were root transformed to meet the assumptions of ANOVA.

An ANOVA Indicated that there was a significant difference In TOU exists between the three temperature treatments (df = 2, $df_{residuals}$ = 13, F = 12.13 and p = <0.001). A Tukey HSD test indicated that a significant difference exists between cores incubated at 14°C and 21°C and between cores incubated at 17°C and 21°C (p = 0.002 and 0.003 respectively). A significant difference in TOU does not exist between cores incubated at 14°C and 17°C (p = 0.98). The homogeneity of variance was also tested for each of the species using Levene's test (df = 2, $df_{residuals}$ = 13, F = 1.154 and p = 0.3457), indicating that there is not a difference in variance between the three temperatures.

Diffusive oxygen uptake: The average DOU (Figure 4.3B) for cores incubated at 14°C was 7.27 ± 1.32 mmol O_2 m^{-2} d^{-1} (n = 6), 17°C was 9.97 ± 0.49 mmol O_2 m^{-2} d^{-1} (n = 5) and at 21°C was 7.19 ± 1.22 mmol O_2 m^{-2} d^{-1} (n = 5). An ANOVA undertaken on root transformed data indicated that a significant difference does not exist in DOU between the three

temperature treatments (df = 2, $df_{residuals}$ = 13, F = 2.265 and p = 0.143). The homogeneity of variance was also tested for each of the species using Levene's test (df = 2, $df_{residuals}$ = 13, F = 0.782 and p = 0.4779), indicating that there is not a difference in variance between the three temperatures.

Faunal oxygen uptake: The highest FOU (Figure 4.3C) was observed in cores incubated at 21°C (27.37 ± 4.85 mmol O_2 m^{-2} d^{-1}, n = 5), followed by 14°C and 17°C (13.53 ± 1.67 mmol O_2 m^{-2} d^{-1}, n = 6; 11.45 ± 1.95 mmol O_2 m^{-2} d^{-1}, n = 6). An ANOVA indicated that a significant difference in FOU was present between the three temperature treatments (df = 2, $df_{residuals}$ = 13, F = 12.58 and p = <0.001). A Tukey HSD test indicated that significant differences exist between cores incubated at 14°C and 21°C (p = 0.003) and between cores incubated at 17°C and 21°C (p = 0.001). However, there was not a significant difference in FOU between cores incubated at 14°C and 17°C (p = 0.810). The homogeneity of variance was also tested for each of the species using Levene's test (df = 2, $df_{residuals}$ = 13, F = 0.9566 and p = 0.4097), indicating that there is not a difference in variance between the three temperatures.

Total oxygen uptake consists of faunally mediated oxygen uptake and diffusive oxygen uptake. Figure 4.3D demonstrates the relative contribution of FOU and DOU to the TOU. The results suggest that at 21°C FOU contributes much more than DOU to the TOU. At 14°C and 17°C the contribution of FOU and DOU is more equal.

3.3.4. Bioturbation and Bioirrigation Potential

Data collected on the bioturbation and bioirrigation potentials and the bioirrigation rate of cores incubated under the three different temperature scenarios indicate that the benthic community increases their sediment reworking and ventilation activities under higher temperature scenarios. The average bioturbation potential (BP_c) for cores incubated at 14°C was 185.9 ± 24.6. The BP_c was higher in cores incubated at both 17°C and 21°C (222.5 ± 42.2 and 302.5 ± 51.4 respectively; Figure 3.4A). However, an ANOVA undertaken on root transformed BP_c data indicates that there is not a statistically significant difference (df = 2, $df_{residuals}$ = 11, F = 2.095 and p = 0.169) in the BP_c between the three temperature

scenarios. The homogeneity of variance was also tested for each of the species using Levene's test (df = 2, $df_{residuals}$ = 11, F = 0.4736 and p = 0.6349), indicating that there is not a difference in variance between the three temperatures. The same trend was observed in the bioirrigation potential, where the mean IP_c was 299.4 ± 61.1 at 14°C and was higher in cores incubated at 17°C and 21°C (381.3 ± 152.4 and 722.4 ± 305.0 respectively; Figure 3.4B). However, an ANOVA on log_{10} transformed data indicated that there was not a statistically significant difference (df = 2, $df_{residuals}$ = 11, F = 0.707 and p = 0.514) in IP_c between the three temperature scenarios. The homogeneity of variance was also tested for each of the species using Levene's test (df = 2, $df_{residuals}$ = 11, F = 2.9848 and p = 0.09214), indicating that there is not a difference in variance between the three temperatures.

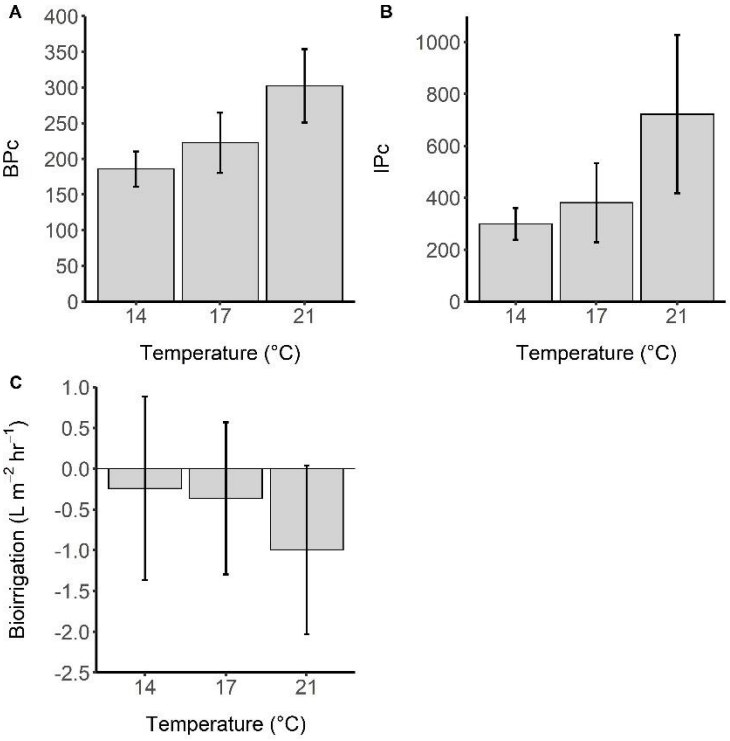

Figure 3.4: A) Bioturbation potential and, B) Bioirrigation potential; for experimental cores incubated at 14C, 17C and 21C, based on abundance, biomass and functional traits of the macrofaunal community, and C) The bioirrigation rate determined through the use of the inert tracer sodium bromide.

The bioirrigation rate, measured using the inert tracer sodium bromide, indicates that cores incubated at 14°C demonstrate the lowest bioirrigation rate (-0.241 ± 1.125 L m^{-}

^{2} hr^{-1}). The rate of bioirrigation is higher in cores incubated at 17°C (-0.363 ± 0.933 L m^{-2} hr^{-1}) and 21°C (-0.993 ± 1.035 L m^{-2} hr$^{-1;}$ Figure 4.4C). However, an ANOVA indicated that there was not a statistically significant difference (df = 2, $df_{residuals}$ = 15, F = 0.093 and p = 0.912) in the bioirrigation rate between the three temperature scenarios. The homogeneity of variance was also tested for each of the species using Levene's test (df = 2, $df_{residuals}$ = 15, F = 0.2892 and p = 0.753), indicating that there is not a difference in variance between the three temperatures.

3.3.5. Nutrient fluxes

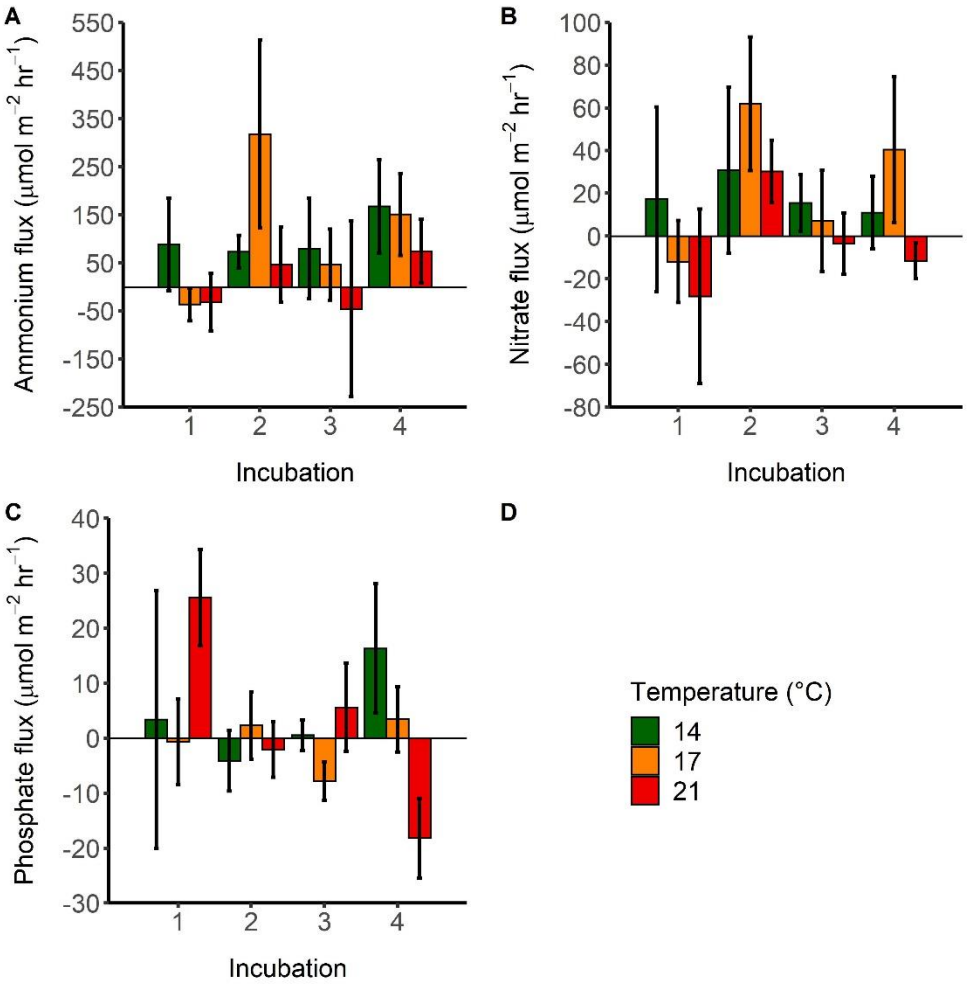

Figure 3.5: Bar charts demonstrating the fluxes of nutrients into and out of the sediment under the three experimental sediment scenarios, A) Ammonium, B) Nitrate and, C) Phosphate. Error bars represent ± standard error.

The flux of ammonium, nitrate and phosphate, into and out of the sediment were determined for each core at several timepoints throughout the incubation experiment. The results demonstrate that there was a large amount of variability between cores within temperature scenarios for all three nutrients measured (Figure 3.5A-C). This was most likely a result from using intact sediment cores in this experiment.

Ammonium

At the beginning of the experiment there was a net ammonium efflux (Figure 4.5A) from the sediment into the water column in cores incubated at 14°C (88.22 ± 96.20 µmol m^{-2} hr^{-1}). There is a net ammonium flux from the water column into the sediment for cores incubated at 17°C and 21°C (-36.56 ± 34.30 µmol m^{-2} hr^{-1} and -32.29 ± 28.22 µmol m^{-2} hr^{-1} respectively). A Kruskal-Wallis test indicated that there was not a statistically significant difference ($p = 0.63$) in the ammonium flux between the three temperature scenarios. By the end of the experiment there was a net efflux of ammonium from the sediment into the water column under all three temperature scenarios. There was a larger efflux of ammonium in cores incubated at 14°C (167.51 ± 96.87 µmol m^{-2} hr^{-1}) and 17°C (150.60 ± 85.17 µmol m^{-2} hr^{-1}) in comparison to cores incubated at 21°C (73.96 ± 65.98 µmol m^{-2} hr^{-1}). However, an ANOVA indicated that there was not a statistically significant difference ($df = 2$, $df_{residuals} = 15$, $F = 0.355$ and $p = 0.707$) in the efflux of ammonium from the sediment into the water column between the three temperature scenarios. The homogeneity of variance was also tested for each of the species using Levene's test ($df = 2$, $df_{residuals} = 15$, $F = 0.642$ and $p = 0.5401$), indicating that there is not a difference in variance between the three temperatures.

Nitrate

At the beginning of the experiment there was a net efflux of nitrate (Figure 4.5B) from the sediment into the water column for cores incubated at 14°C (17.23 ± 43.19 µmol m^{-2} hr^{-1}). Whereas there was a net flux of nitrate into the sediment, from the water column, for cores incubated at both 17°C (-11.93 ± 19.24 µmol m^{-2} hr^{-1}) and 21°C (-28.22 ± 40.88 µmol m^{-2} hr^{-1}). However, an ANOVA indicated that there was not a significant difference (df

= 2, $df_{residuals}$ = 15, F = 0.407 and p = 0.673) in the flux of nitrate between the three temperature scenarios. The homogeneity of variance was also tested for each of the species using Levene's test (df = 2, $df_{residuals}$ = 15, F = 1.2041 and p = 0.3274), indicating that there is not a difference in variance between the three temperatures. By the end of the experiment the efflux of nitrate from the sediment into the water column remained at a similar rate for cores incubated at 14°C (10.96 ± 16.93 µmol m^{-2} hr^{-1}). There was a net efflux of nitrate from the sediment into the water column for cores incubated at 17°C (40.45 ± 34.15 µmol m^{-2} hr^{-1}) by the end of the incubation period. Finally, cores incubated at 21°C (-11.49 ± 8.21 µmol m^{-2} hr^{-1}) still demonstrated a net flux of nitrate from the water column into the sediment, however, the flux is at a lower rate compared to that at the beginning of the experiment. An ANOVA indicated that there was not a significant difference (df = 2, $df_{residuals}$ = 15, F = 1.942 and p = 0.178; ^3 transformation) in the flux of nitrate between the three temperatures at the end of the experiment. The homogeneity of variance was also tested for each of the species using Levene's test (df = 2, $df_{residuals}$ = 15, F = 2.7816 and p = 0.09386), indicating that there is not a difference in variance between the three temperatures.

Phosphate

At the beginning of the experiment there was a marginal net efflux of phosphate (Figure 4.5C) from the sediment into the water column in cores incubated at 14°C (3.39 ± 23.45 µmol m^{-2} hr^{-1}) and a marginal flux of phosphate from the water column into the sediment for cores incubated at 17°C (-0.66 ± 7.75 µmol m^{-2} hr^{-1}). Cores incubated at 21°C (25.57 ± 8.74 µmol m^{-2} hr^{-1}) demonstrated a much larger net efflux of phosphate from the sediment into the water column. However, an ANOVA indicated that there was not a significant difference (df = 2, $df_{residuals}$ = 15, F = 2.325 and p = 0.132; ^2 transformation) in phosphate flux between the three temperature scenarios at the start of the experiment. The homogeneity of variance was also tested for each of the species using Levene's test (df = 2, $df_{residuals}$ = 15, F = 2.068 and p = 0.161), indicating that there is not a difference in variance between the three temperatures. By the end of the experiment cores incubated at 14°C (16.30 ± 11.77 µmol m^{-2} hr^{-1}) and 17°C (3.43 ± 5.93 µmol m^{-2} hr^{-1}) demonstrated a net efflux of phosphate from the sediment into the water column. Whereas cores incubated

at 21°C (-18.17 ± 7.24 µmol m^{-2} hr^{-1}) demonstrate a large net flux of phosphate from the water column into the sediment. However, a Kruskal-Wallis test indicated there was a marginally insignificant difference ($p = 0.055$) in the phosphate flux between the three temperature scenarios. Mann-Whitney U tests were undertaken to indicate where the difference exists. The Mann-Whitney U tests between cores incubated at 14°C and 17°C and between 17°C and 21°C indicates that there was not a significant difference ($p = 0.394$ and 0.132 respectively) in the phosphate flux between the cores incubated at these temperatures at the end of the experiment. However, it indicated that there was a significant difference ($p = 0.026$) in phosphate flux between cores incubated at 14°C and 21°C at the end of the experiment.

3.4. Discussion

Temperature is known to be an important factor which drives physiological processes and can result in behavioural changes (Ouellette et al., 2004; Verdelhos et al., 2015; Dolbeth et al., 2021). However, the impact of temperature on interactions between species and communities and the environment and their role in ecosystem functioning can be unpredictable (Dolbeth et al., 2021). For example, Dolbeth et al. (2021) observed a slight increase in bioturbation and nutrient release at higher temperatures compared to control temperatures over a 24-day experiment on intact benthic communities, although their results were inconclusive. In comparison, in a long-term (18 months) incubation experiment of *Alitta virens* undertaken by Godbold & Solan (2013) indicated that the maximum bioturbation depth of cores incubated under higher temperatures became shallower over the first 12 months of the incubation, and the observed seasonal increase in bioturbation depth present under ambient temperatures was not observed under increased temperature scenarios. Maire et al. (2007) also investigated the impact of temperature (summertime (20°C) vs. wintertime (10°C) temperatures) and food availability on sediment reworking by *Abra ovata*, a deposit feeding bivalve, collected from Lapalme lagoon (NW Mediterranean) using luminophore tracing experiments. The authors determined that in the summer both temperature and food availability impacted sediment reworking activities by the bivalve. Whereas, in the winter just temperature, and not food availability, controlled

sediment reworking activities (Maire et al., 2007). Ouellette et al. (2004) determined that the intensity of sediment reworking activities by *Neanthes virens*, a gallery biodiffusor, was lower at colder temperatures than under warmer conditions. Sediment reworking activities have further implications for the processing of organic matter and any changes in bioturbation intensities could have a knock-on effect on organic matter degradation and therefore the structure of the ecosystem (Ouellette et al., 2004). In addition, such activities are also important for the transport of oxygen deeper into the sediment column and reducing the build-up of metabolites (Kendzierska et al. 2020).

3.4.1. Total oxygen uptake (TOU)

Clear trends in marine benthic oxygen dynamics linked to temperature were observed. From the experimental results the TOU by benthic communities was related to temperature increases, with cores incubated at 21°C demonstrating significantly higher rates of TOU during the experimental timeframe, compared to those incubated at 17°C and 14°C.

The TOU by sediment communities Incubated at 14°C and 17°C were not significantly different from each other. This could be a result of a variety of different factors relating to both the functioning of the sediment communities and to the experimental design. It is likely that increasing the temperature by 3°C (form 14°C to 17°C) is within the natural annual variation of the study site, meaning that the life histories of the benthic community are adapted to this variation and therefore have the capability to adapt to the increase. This likely to be the case for estuarine fauna, which are well adapted to temperature variability (Dolbeth et al., 2021). However, the experiment was only run over a short time period, meaning that it is possible that the experimental cores were not exposed to the temperature increase for long enough for the communities to exhibit a response. Therefore, it would be beneficial for the experiment to be re-run over a longer time period, to determine whether the benthic community at this study location are adapted to prolonged exposure to a temperature increase of 3°C, or whether the community are capable of compensating for a period of time, but prolonged exposure results in a change in ecosystem functioning and oxygen consumption. Long-term

experiments which are capable to taking into account seasonal variability are essential to produce data which will help us reliably predict the impacts of environmental perturbations on ecosystem functions and to better inform assessments of long-term environmental changes (Godbold and Solan, 2013).

Higher TOU in cores incubated at 21°C meanes that the benthic community consumed oxygen at a higher rate, in comparison to cores incubated at lower temperatures (14°C and 17°C). Higher oxygen consumption at 21°C could be due to several factors. For example, it is known that increased temperatures result in faster rates of reactions, including metabolic functions within individuals living within the sediment column (Neumann et al., 2021). Increased metabolic rates mean that more oxygen and energy is required for the individuals and community to maintain function (Li et al., 2015). Another reason why oxygen consumption could increase at 21°C is stress. When under stress it has been observed that taxa exhibit a heat shock response, meaning that they increase production of heat shock proteins (HSPs), which can therefore be used as an indicator of stress responses in organisms (Chen et al., 2018). HSPs are proteins which have been damaged through environmental stressors, including but not limited to temperature (Lesser and Kruse, 2004), and an organisms ability to maintain production of HSPs is a common defence strategy against environmental stressors (Li et al., 2015). However, the production of heat shock proteins requires a lot of energy (Li et al., 2015) which could contribute to observed respiration rates at higher temperatures. If more energy is being used to produce HSPs, then it is also a possibility that less energy will be used for growth and reproduction by benthic taxa (Li et al., 2015).

The TOU considers the oxygen uptake of the entire benthic community. To gain a better insight into how different fractions of the community respond to temperature increase, the faunal oxygen uptake (FOU) and Diffusive oxygen uptake (DOU) were calculated. These two variables give an insight into how the respiration rate of the benthic macrofaunal community and microbial community respond to temperature increases.

The DOU represents the diffusion of oxygen from the water column, at the diffusive boundary layer (DBL), into the surface sediments. The DOU is often used as a proxy for microbial respiration, in the absence of any influence from faunal reworking activities (Hicks et al., 2017; Kiesel et al., 2020). The DOU is not significantly impacted by ocean warming over the duration of this experiment. This suggests that exposure to increased temperatures, over the duration of this experiment, does not impact the baseline functioning of microbial communities.

The FOU rate (TOU-DOU) indicates the relative role of benthic faunal communities to the TOU through respiration and stimulation of microbial activity through sediment reworking and ventilation activities (Glud, 2008; Hicks et al., 2017). The FOU rate demonstrates the same trends as the TOU rate, suggesting that changes in macrofaunal activities is the primary driver of the temperature response observed in the SCOC data. It has been previously indicated that the presence of macrofaunal within sediments can increase the total oxygen consumption by as much as 38% (Moodley et al., 1998) and different taxa can have varying oxygen demands (Kendzierska et al., 2020). In addition, higher densities of benthic fauna frequently result in a higher oxygen demand and therefore higher flux of oxygen into the sediment (Urban-Malinga et al., 2013; Kendzierska et al., 2020).

These results suggest that under the experimental timescale of 14 days benthic faunal community and their activities are impacted more by temperature increases compared to baseline microbial activities. Increased temperature is known to be a key factor driving reaction rates and organism behaviour (Verdelhos et al., 2015; Dolbeth et al., 2021). However, different taxa do not respond to changes in temperature in a uniform way. Some species have been observed to tolerate, or even benefit, from increased temperatures, such as the oligochaete Tubificoides sp. (Bamber and Spencer, 1984). Therefore, it is possible that an increase in temperature could result in increased respiration of this taxon due to functioning at a higher rate, which could also translate into increased sediment reworking activities and therefore microbial stimulation. Whereas other taxa have been observed to be less tolerant to temperature rises due to having a narrower optimal temperature range, such as the bivalve *Macoma balthica* (Beukema et al., 2009), and an

increase in temperature could be due to a stress response from the taxon. A full description on how temperature increases impact ecology and functional diversity can be found in Chapter 3.

Oxygen consumption and carbon dioxide production are closely coupled processes (Graneli, 1979), suggesting that the observed increase in the oxygen consumption by faunal communities under increased temperatures could lead to an increase in carbon dioxide release into the water column and potentially the atmosphere. Coastal sediments are considered to be carbon sinks (Glud et al., 1998), however increased carbon dioxide concentrations due to remineralization of OM, due to increased temperatures, could mean that a decrease in carbon storage will occur in UK shelf sea environments (Legge et al., 2020).

The temperature of intertidal and shallow subtidal sites tracks the air temperature closely (Harrison and Phizacklea, 1985). Therefore, although the temperature of the study site reached 21°C infrequently and only for short periods of time, there is the potential that in the future these temperatures at this site could become more frequent and be sustained for longer periods of time, due to increased frequency of extreme events such as heatwaves (Dolbeth et al., 2021).

The use of oxygen optodes and porewater microprofilers can provide important and high-resolution data which helps to understand how environmental change could impact benthic communities. However, these data alone do not indicate why oxygen consumption of the benthic community increases, whether it is a result of increased activity or due to stress.

3.4.2. Bioturbation and Bioirrigation potential

Macrofauna living within and on the sediment are considered to be ecosystem engineers due to their capability to significantly alter the sediment physically and chemically through burrowing and irrigation activities (Laverock et al., 2011). The burrowing and ventilation activities alter the physicochemical properties within the sediment due to the introduction of fresh oxygenated water into the sediment (Laverock et al., 2011). The

introduction of oxygenated water into burrows effectively extends (Laverock et al., 2011). Oxic sediment is particularly important to nitrification and denitrification reactions, therefore the extension of the oxic surface due to burrowing activities will play an important role in biogeochemical cycling and breakdown of organic matter (Laverock et al., 2011; Dolbeth et al., 2021).

Based on the BP_c, IP_c and $\Delta[Br^-]$ results from this experiment suggest that bioturbation and bioirrigation activities of the benthic community at Blackness could increase with temperature rises. Increases in bioturbation and bioirrigation activities have been observed in other temperature manipulation experiments. For example, Dolbeth et al. (2021) observed an increase in bioturbation during short-term heatwaves. However, under longer term heat waves bioturbation remained at a similar level to their control temperature (Dolbeth et al., 2021). Increases in bioturbation and bioirrigation potentials under increased temperatures could increase the oxygen supply to the sediment column and stimulate microbial respiration activities (Volkenborn, Hedtkamp, et al., 2007; Braeckman et al., 2010). However, community response to environmental changes, such as temperature rises, are also influenced by density dependent factors and species interactions, which can make it difficult to detect patterns in response variables when considering intact benthic communities rather than constructed communities (Dolbeth et al., 2021). The increase in the BP_c, IP_c and $\Delta[Br^-]$ with temperature rises along with the observed oxygen consumption suggests that the community as a whole in this experiment was demonstrating increased activity as a result of temperature rises, and therefore not necessarily demonstrating a stress response. Stress responses to temperature increases could be expressed as a decrease in activity (Dolbeth et al., 2021). However, not all species present in the experimental cores will necessarily respond to the temperature increase in the same way, as some may respond negatively and express a stress response to temperature increase, whereas others may respond positively.

Bioturbation and bioirrigation activities have been linked to oxygenating the sediment column (Volkenborn, Polerecky, et al., 2007; Bouchet et al., 2009; Birchenough et al., 2012; Pascal et al., 2019). However, when cores are incubated at higher temperatures

the concentration of oxygen in the water column is lower than that of cores incubated under ambient temperatures. Bioturbation and bioirrigation potentials (BP_c and IP_c) are calculated using a combination of biodiversity measures including abundance, biomass and relevant functional traits. Results from this experiment suggest ocean warming could cause a shift in the community composition (Chapter 3), resulting in an increase in both bioturbation and bioirrigation potential.

The BP_c and IP_c are not quantitatively measured rates of sedimentary reworking activities by the faunal community, meaning that they do not directly consider the effect of temperature on the reworking and ventilation activities of the faunal community. The use of the inert tracer, sodium bromide (NaBr), enabled the quantitative measurement of water flux from the water column into the sediment. The $\Delta[Br^-]$ results also indicated that bioirrigation increased with temperature rises, especially at 21°C. However, the NaBr tracing method resulted in a large amount of variability between cores within a treatment, and occasionally indicated higher water column concentrations that at the start of the experiment. This is clearly a limitation of the method, which has been observed previously, for example by Godbold & Solan (2013). Despite this source of uncertainty and variability, the method remains a useful and low-cost way to quantitatively determine bioirrigation rates, as long as the high degree of variability is considered.

Research into the bioturbating activities of individual species has been undertaken for several years and is relatively well understood (Dolbeth *et al.*, 2021). However, species behaviours within a natural community may differ to those observed in single species or constructed community experiments, due to the effect that the habitat and other environmental variables, such as temperature, have on organism behaviour (Dolbeth *et al.*, 2021). It is also possible that behaviours that species express under ambient conditions may not be the same that they express when under stress or adapting to environmental change, for example under ocean warming scenarios.

3.4.3. Nutrient fluxes

Due to their predominantly anoxic nature marine sediments are important habitats for nitrogen cycling (Laverock et al., 2011). In oxic conditions OM enters the sediment environment and is remineralized to ammonium ($NH4^+$; Laverock et al., 2011). Nitrification occurs within oxic sediments, which tends to only be the first few cm (Laverock et al., 2011). In anoxic sediments nitrate is converted into N_2 or N_2O (Laverock et al., 2011). Therefore, the oxic/anoxic interface plays an important role in nitrogen cycling of marine sediments. As outlined in the previous section bioturbation and bioirrigation activities undertaken by macrofaunal communities effectively extend the oxic-anoxic interface deeper into the sediment, and therefore also extend the (Laverock et al., 2011)

The results from this experiment indicate that at the beginning of the experiment cores incubated at 14°C demonstrate a mean efflux of ammonium from the sediment into the water column. Whereas cores incubated at both 17°C and 21°C demonstrate a mean flux of ammonium into the sediment from the water column. The same trend can be seen for nitrate fluxes at the beginning of the experiment. This could suggest that under increased temperatures, ammonium and nitrate could be needed in the processes of denitrification and anammox, in anoxic parts of the sediment column. Whereas under ambient conditions ammonium and nitrate were produced in excess within the sediment via ammonification and nitrification processes, in oxic sediments.

The presence of macrofauna and their activities have been identified as playing and important role in benthic fluxes, with macrofauna explaining up to 41% of the variation in benthic fluxes (Miatta & Snelgrove, 2021b; Wyness et al., 2021). For example, Miatta & Snelgrove (2021b) determined that the density of scaphopods, which are predominantly considered upward and downward conveyors, explained 15% of the variability in benthic nutrient fluxes within their study area. Upward and downward conveyors can relocate sediment vertically between the sediment surface and deeper within the sediment column (Kristensen et al., 2012; Miatta & Snelgrove, 2021b). Miatta & Snelgrove (2021) observed an increase in the efflux of nitrate with higher numbers of taxa present, suggesting that with a higher species richness is likely to result in higher levels of sediment reworking activities

and therefore lead to higher levels of ecosystem functioning, as observed in other studies (Ieno et al., 2006; Solan et al., 2008; Belley & Snelgrove, 2017). Therefore, based on bioturbation, and bioirrigation potentials and $\Delta[Br^-]$ data collected from this experiment it would be expected that the efflux of ammonium and nitrate from the experiment would increase under warmer temperatures. However, this was not the case in this experiment. Therefore, despite the observed increase in bioirrigation activities under warmer temperature scenarios, additional nitrate and ammonium may be required to fuel processes such as denitrification and anammox within the sediment column, potentially indicating that the sediment has become more anoxic. However, benthic systems are extremely heterogeneous environments over relatively small spatial scales, despite appearing visually homogeneous, which makes it difficult to make conclusions and extrapolate results to larger spatial scales (Miatta & Snelgrove, 2021b).

3.5. Limitations and future work

The experiment was only run for two weeks; therefore, the results are only representative of short-term impacts of temperature rises or heat waves. To understand the impact of long-term temperature rises on benthic ecosystem function experiments need to be run for a period of months rather than weeks. The impact of heatwaves and increased heatwave frequency on benthic ecosystem function is also important to understand as this is likely to occur within systems such as the study site.

Sodium bromide was added to the cores at the same time as fresh isotopically labelled algae. Therefore, it is possible that the $\Delta[Br^-]$ values represent bioirrigation as a response to the addition of fresh food after a period where no food was added. Also, bioirrigation does not occur uniformly over time, meaning that there may be periods where very little irrigation activities are occurring and others where there is a large amount of activity. It would be beneficial to add the NaBr to the cores at the beginning of the experiment and measure the change in concentration over a period of days throughout the experiment.

Bioturbation was determined using the bioturbation potential index, which does not directly measure whether the rate of bioturbation changes under different temperature scenarios. This is because the BP_c is determined using biodiversity and functional trait metrics. It would be beneficial to determine whether the rate changes, for example a quantitative bioturbation rate could be determined using $\delta^{13}C$ concentration within the sediment as isotopically enriched algae is mixed by the benthic community within the sediment column. Another widely used method which determines the impact of environmental stressors on bioturbation is the use of luminophores. Luminophores can be used to track sediment reworking down the sediment column over time, by photographing the cores under a *uv* black light and analysing the distribution of the florescent luminophore particles using 'ImageJ' software. The benefit of using luminophores over bulk $\delta^{13}C$ concentrations in sediments is that they are a low-cost method, which has been shown to be an accurate way of determining bioturbation in marine sediments under environmental change.

3.6. Conclusions

Over the relatively short time scale of this experiment benthic oxygen consumption did not increase under a temperature rise of 3°C above ambient (14°C to 17°C). This is most likely due to this temperature rise being within the normal range for the site in question. However, benthic oxygen consumption increased when cores were incubated at 21°C.

Bioturbation and bioirrigation potentials both appear to increase under the influence of temperature rises. This would fit with the observation that the benthic community oxygen up take also increases with temperature rise, and the observed increase in bioirrigation observed using the bromide tracer.

Nutrient fluxes show a more complex and variable trend not only between the three temperature treatments in this experiment, but also within treatments and within individual cores throughout the duration of the experiment.

4. Characterizing carbon processing in coastal sediments in response to ocean warming scenarios: A case study of an intertidal estuarine environment, Blackness (Firth of Forth, Scotland)

4.1. Introduction

Intertidal sediments are known to be important locations for carbon processing (Riekenberg et al., 2018). In these locations organic carbon (OC) is produced, remineralized and transformed, before being transported on to offshore shelf sediments (Bauer, W.-J. Cai, et al., 2013; Riekenberg et al., 2018). Intertidal sediments receive large amounts of carbon from both terrestrial and marine sources (Bouillon and Boschker, 2005; Luisetti et al., 2020). However, one of the least well understood aspects of carbon cycling and processing in a changing world is that of the role of benthic communities (Woulds et al., 2007; Woulds et al., 2009; White et al., 2019).

Benthic faunal communities play an important role in OC cycling through interacting with and manipulating marine sediments via burrowing and ventilating, ingestion during deposit feeding, and stimulation of microbial metabolism, which influence OC remineralization and burial (Woulds et al., 2007; Woulds et al., 2009; Mäkelä et al., 2018). These processes have been studied using microcosm experiments, which often examined single taxa, or a small number of taxa and processes (Michaud et al., 2006; Ieno et al., 2006; Michaud et al., 2010; Godbold and Solan, 2013). Whilst such approaches have their place in understanding mechanisms underpinning processes such as OM processing, and are replicable, they lack ecological realism (Benton et al., 2007; Drake and Kramer, 2012; Santos et al., 2018). To better understand the effects of fauna on OC processing and introduce a higher level of ecological realism to experimental studies, intact benthic communities (both faunal and microbial) should be considered.

Stable isotope pulse-chase experiments are a useful tool to enable quantification of carbon processing and uptake by benthic communities (Middelburg et al., 2000; Oakes et al., 2010; Bauer, W.-J. Cai, et al., 2013; Miyatake et al., 2014; Nordström et al., 2014; Riekenberg et al., 2018). This method has frequently been utilized to better understand the

impact of environmental variables, frequently along natural gradients such as oxygen gradients within the Pakistan and Indian continental margins of the Arabian Sea (Woulds et al., 2007; Woulds et al., 2009; White et al., 2019) and hydrothermal vent and background sites within the Southern Ocean (Woulds et al., 2019; Woulds et al., 2020). However, there are very few examples using this approach to determine the impact of temperature on carbon processing (Hunter et al., 2019) and using this approach in a temperature manipulation experiment.

Temperature is an important factor known to regulate the rate of biogeochemical processes, with the overarching trend being rates increase with temperature increases (Moodley et al., 2005; Mäkelä et al., 2018). However, the natural environment is complex and the degradation of OC in marine benthic systems occurs through the activities and interactions of microbial, meiofaunal and macrofaunal communities (Kristensen and Andersen, 1992; Snelgrove, 1997; Bianchi et al., 2021). Increased temperatures, such as those predicted to occur due to global environmental change, could result in benthic communities experiencing thermal stress and therefore cause a change in rates of faunally mediated processes and functions and change the balance in carbon entering different pools within the benthic environment (Thomas, 2003; Welsh, 2003). In temperate environments it has been observed that the rate of OC degradation increases seasonally with temperature increases (Arndt et al., 2013). However, it has also been observed that OM degradation rates in sediments which are permanently cold, such as in the Arctic, are not inherently slower than in temperate sediments (Arnosti et al., 1998). This suggests that there could be some level of adaptation in the benthic communities which allows them to function effectively in permanently cold conditions (Arnosti et al., 1998). Environments which are more variable, whether seasonally or because of diurnal cycles, such as estuaries, will likely have communities which are adapted and considered resilient to a wide range of environmental fluctuations, including temperatures (Elliott and Quintino, 2007).

This study aims to understand how temperature increases influence the processing of OC by intact benthic communities. To achieve this, this study will use ^{13}C enriched OC to trace the pathway followed by fresh OC when it enters the system, and how temperature

increases effect this. The amount of ^{13}C entering the microbial and faunal community biomass will be measured under each temperature scenario, and how much of the ^{13}C enriched OM is respired will also be measured.

4.2. Methods

4.2.1. Study sites and sample collection

Sediment samples were collected from an intertidal mudflat at Blackness (Firth of Forth, Scotland) in April 2019. A full description of the study site and sampling strategy is outlined in Chapter 2.

4.2.2. Experimental design

Cores were transported to The Lyell Centre (Herriot Watt University) and placed within randomly assigned controlled temperature (CT) environments. In summary ^{13}C pulse-chase experiments were undertaken on 18 intact (faunally and microbially intact) sediment cores incubated at three different temperatures (14°C, 17°C and 21°C; 6 cores per treatment). The macrofaunal community of the experimental cores were comprised of various polychaetes, oligochaetes, bivalves, and gastropods, as well as Nematoda and other meiofaunal taxa (full description of the community found in Chapter 2). The cores were incubated using control temperature rooms and thermostatically controlled water baths.

4.2.3. Isotope Pulse-Chase Experiment

Freeze dried *Phaeodactylum tricornutum* cells were used as an isotopically labelled food source which was added to each core at the end of the experiment. One litre of water was removed from each core, then 77.4 mg of Algae labelled with the stable isotope ^{13}C (22.48 at% ^{13}C) was added to each core, at a dose of 424.74 mg C m^{-2}. The cores were left for an hour once the algae had been added, to allow all algae to settle on top the sediment surface before any samples for dissolved inorganic carbon could be taken.

The algae were labelled following the method outlined by Sweetman et al., (2019). In brief, the phytoplankton culture was grown in an algal medium in artificial seawater and labelled with ^{13}C by replacing half of the ^{12}C bicarbonate within the growth medium with $Na^{13}CO_3$. The algae were harvested using a 0.45µm filter, rinsed with unlabelled algal

medium to remove excess isotopically enriched bicarbonate. The algae were there centrifuged and washed with unlabelled algal medium five times, frozen and freeze dried.

4.2.4. Dissolved inorganic carbon

Dissolved inorganic carbon (DIC) samples were collected at six time points during the last 24 hours of the experiment, after the ^{13}C labelled algae had been added. Cores were capped, three times, to prevent gas exchange. Water samples were taken at the start and end of each capped period, which lasted approximately 2 hours, using a plastic syringe, filtered (0.45µm nylon syringe filters) and then transferred into a 12ml exetainer. Each sample was then poisoned using 20µl of mercuric chloride.

DIC samples were sent to Lancaster where they were analysed using isotope mass spectrometry (Isoprime100 IRMS coupled with an Isoprime Multiflow inlet). DIC vials were placed on the IRMS, and a needle injected 20µl of the headspace (CO_2 evolved from acidifying the sample). The injected gas travelled through a drying tube to remove moisture and then a GC column to separate other gases from the CO_2. The CO_2 then flowed into the IRMS which analysed masses 44 and 45 for the $^{12}CO_2$ and $^{13}CO_2$.

The IRMS calculated a raw ratio of $^{13}C/^{12}C$, which was normalized to international standards: LSVEC ($\delta^{13}C$ = -46.59 ± 0.049), NBS 18 ($\delta^{13}C$ = -5.03 ± 0.11), CO1 ($\delta^{13}C$ = 2.51 ± 0.042) and LEC ($CaCO_3$), which were run throughout the analysis run.

4.2.5. Phospholipid fatty acids

At the end of the experiment once oxygen profiles had been collected, the water was removed, and sediment extruded from the microcosm. The sediment column was sliced at 0-1 cm, 1-5 cm and 5-10 cm horizons. Each horizon was homogenized and subsampled for phospholipid fatty acid (PLFA) analysis using 50ml centrifuge tubes. The remaining sediment from each horizon was combined and preserved for macrofaunal analysis.

Phospholipid fatty acids (PLFAs) in each of the sediment horizons for each core were analysed by GC/MS (ThermoScientific ISQ; column = VF23-ms 60m x 0.32mm id x 0.15DF) and GC/C/IRMS (ThermoScientific Delta V Plus; column = VF23-ms 60m x 0.32mm id x

0.15DF) in the School of Chemistry (University of Bristol) following a support in kind grant from the National Environment Isotope Facility (NEIF, formally LSMSF).

A total lipid extraction was undertaken using ~2g of each sediment sample using the Bligh-Dyer method (Bligh and Dyer, 1959). The total lipid extraction was fractionated into simple lipid, glycoid lipid and phospholipid fractions as described by Frostegård et al. (1991) and Dickson et al. (2009). In this study only the phospholipid fraction was analysed. Acid catalysed methylation of the phospholipid fraction produced phospholipid fatty acid methyl esters which were analysed using GC/MS to identify compounds present in the samples. After analysis using GC/MS samples were analysed using GC/C/IRMS to obtain the $\delta^{13}C$ for each compound identified in the sample. The internal standard used was C19n alkane and the isotope standard used was a mixture of six FAMEs which had a known ^{13}C value and were examined between samples. The inherent instrument precision was ± 0.3‰.

4.2.6. Bulk enriched ^{13}C macrofauna analysis

Macrofauna were extracted from sediment cores using a 500µm sieve and processed as outlined in section 2.2.3. Macrofauna present were subsampled for isotopic analysis and any hard bodied fauna, such as gastropods and bivalves, were removed from their shells. Where possible macrofauna samples were tailored to yield between 0.15 mg and 0.20 mg of carbon, which equated to approximately 5.51-10.02 mg macrofaunal wet weight. The aim was to acquire ^{13}C uptake for individual species, however there were not always sufficient individuals per species to yield the target mass of carbon. When this was the case similar taxa were pooled. For example, polychaetes would be combined with other polychaetes. Fauna were placed in pre-weighed ultra clean tin capsules, air dried at 45°C, re-weighed, sealed, and sent to the Scottish Enterprise Technology Park (East Kilbride, Scotland where they were analysed under the National Environment Isotope Facility (NEIF, formally LSMSF).

Sample analysis was undertaken using continuous flow isotope mass spectrometry using an Elementar (Hanau, Germany) Pyrocube elemental analyser (EA) interfaced with a Thermo Fisher Scientific (Bremen, Germany) Delta XP stable isotope ration mass

spectrometer (IRMS). Samples were analysed alongside several standards including gelatine from Fluka (GEL = -20.13 ± 0.19), ^{13}C enriched lab standards (E, F, G and H) which were made by adding different quantities of 99% 13C enriched alanine to natural abundance samples with a known $\delta^{13}C$ and two internal reference materials (USGS40 with a precision of -26.22 ± 0.29).

4.2.7. Statistical analyses

The data were checked for normality using Shapiro's normality test, and if necessary, the data were transformed to meet the assumptions of parametric statistical tests. A significance level of 0.05 was chosen as the threshold for statistical significance. Differences in response variables between the three temperature treatments were analysed using a one-way ANOVA (analysis of variance) followed by a Tukey HSD *post hoc* test at a significance threshold of 0.05. Levene's test was used to determine whether homogeneity of variance assumptions were met prior to analysis.

4.3. Results

Results indicate that at the end of the experiment most of the added carbon remained unprocessed under all three temperature scenarios (14°C = 89%, 17°C = 87% and 21°C = 78%). The total amount of carbon processed ('processed' referring to the sum of uptake into microbial and macrofaunal biomass, plus total respired C) increased with temperature rises (14°C = 44.92 ± 4.77 mg C m^{-2}, 17°C = 53.83 ± 4.25 mg C m^{-2} and 21°C = 92.31 ± 33.48 mg C m^{-2}: Figure 4.1).

Of the processed carbon the majority was remineralized to CO_2 (14°C = 82.64%, 17°C = 78.30% and 21°C = 84.12%). The remaining processed carbon entered the faunal pool (14°C = 12.64%, 17°C = 14.41% and 21°C = 10.16%) or the microbial pool (14°C = 4.72%, 17°C = 7.22% and 21°C = 5.72%).

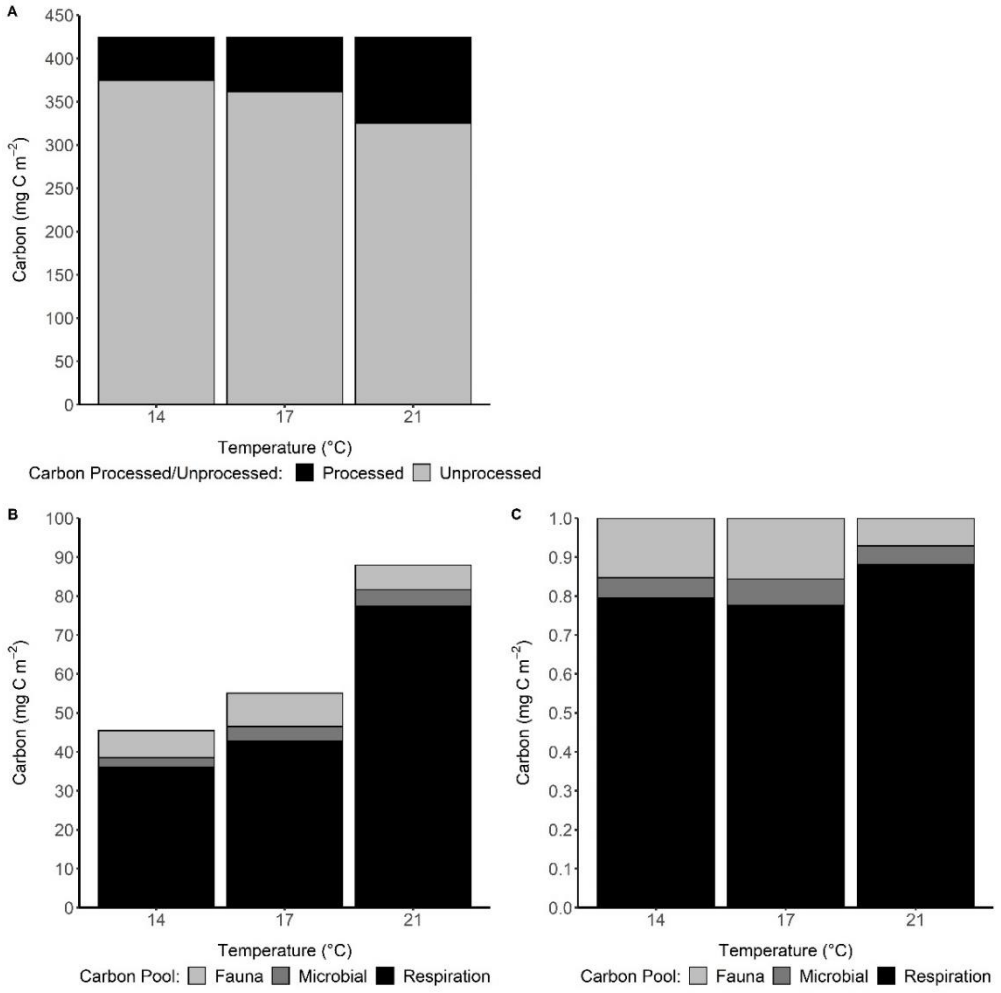

Figure 4.1: Stacked barcharts representing A) How much of the added carbon was processed by the benthic environment, B) How much of the processed carbon entered different carbon pools of the benthic environment and C) The proportion of the processed carbon entering each of the three benthic carbon pools.

4.3.1. Respiration

The amount of carbon respired by benthic communities (Figure 4.2) was lowest in cores incubated at 14°C (36.171 ± 0.468 mg DIC m^{-2}) and increased slightly in cores incubated at 17°C (42.748 ± 2.038 mg DIC m^{-2}). The amount of carbon respired at 21°C (77.466 ± 28.261 mg DIC m^{-2}) was much larger compared to levels at 14°C and 17°C. The same trends were observed in the benthic oxygen consumption rate, discussed in Chapter 4. An ANOVA undertaken on 1/square-root transformed data indicates that there was a significant difference ($p = 0.023$) in the amount of carbon respired between the three

temperature scenarios. A Tukey HSD post—hoc test indicates that there was not a significant difference in the amount of carbon respired by the benthic community between cores incubated at 14°C and 17°C (p = 0.525) or between cores incubated at 17°C and 21°C (p = 0.152). However, a significant difference was present between cores incubated at 14°C and 21°C (p = 0.019).

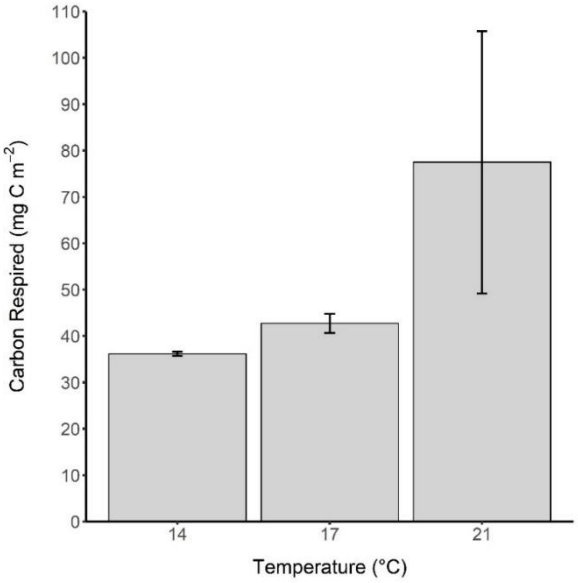

Figure 4.2: Bar chart representing carbon remineralized as CO_2 under the three experimental temperature scenarios. Error bars represent ± standard error.

4.3.2. Bacterial carbon Uptake

Total bacterial uptake of carbon (Figure 4.3 A) increased as temperature increased, with bacterial communities incubated in cores at 14°C consuming the lowest amount of carbon (2.36 ± 0.32 mg C m^{-2}). The amount of carbon consumed by the bacterial community at 17°C increased to 3.75 ± 1.14 mg C m^{-2}, and there was a further, but smaller, increase to 4.22 ± 1.09 mg C m^{-2} at 21°C. However, an ANOVA test indicated that there was not a significant difference (p = 0.363) in the bacterial carbon uptake between the three temperature scenarios.

Bacterial uptake of carbon was highest within the surface sediment (0-1 cm) under all three temperature scenarios (Figure 4.3 B). Less carbon was consumed by bacterial communities in the deeper horizons (1-5cm and 5-10 cm). Within the 1-5cm horizon the bacterial carbon uptake was at a similar level for all three temperature scenarios (14°C =

0.020 ± 0.003 µg C g^{-1} sediment, 17°C = 0.016 ± 0.002 µg C g^{-1} sediment and 21°C = 0.022 ± 0.011 µg C g^{-1} sediment). An ANOVA test undertaken on log_{10} transformed data indicates that there was not a statistically significant difference in bacterial carbon uptake (p = 0.70). Bacterial C uptake within the deepest sediment horizon (5-10 cm) was slightly lower at 14°C (0.007 ± 0.001 µg C g^{-1} sediment) and increased slightly at 17°C and 21°C (0.015 ± 0.009 µg C g^{-1} sediment and 0.017 ± 0.006 µg C g^{-1} sediment respectively). An ANOVA undertaken on root transformed data indicates that there is not a significant difference in the bacterial carbon uptake within the 5-10cm horizon (p = 0.64).

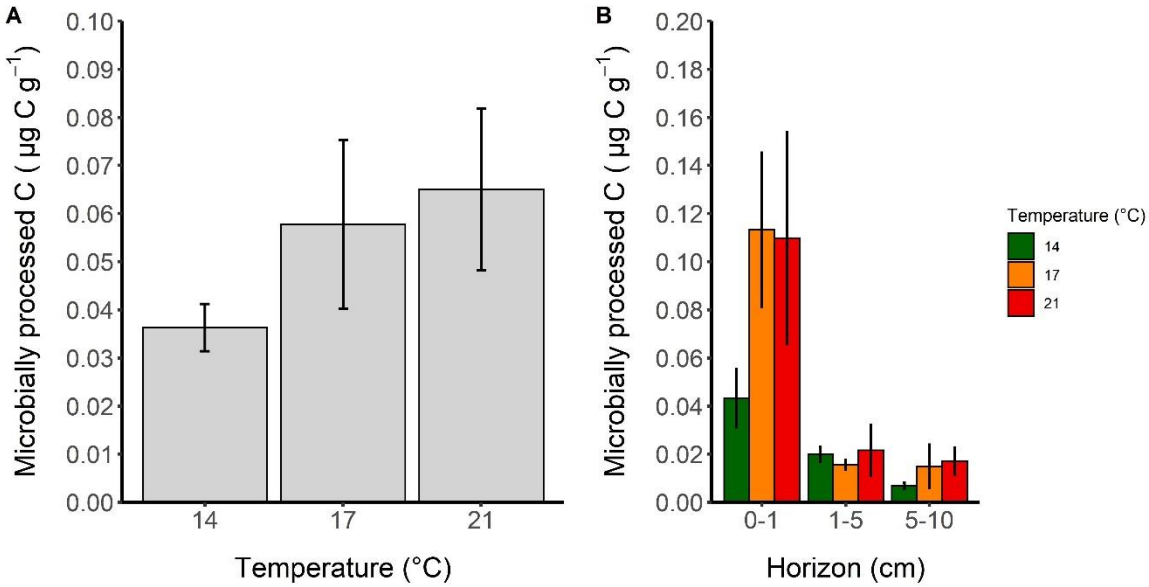

Figure 4.3: Bacterial carbon uptake for each of the three temperature scenarios, A) Total bacterial carbon uptake (mg C m^{-2}) and B) Bacterial carbon uptake for down core horizons (0-1 cm, 1-5 cm and 5-10 cm). Error bars represent ± standard error.

4.3.3. Macrofaunal Carbon Uptake

4.3.3.1. Total faunal carbon uptake

When considering the entire macrofaunal community present within each core at each temperature scenario, it appeared that the faunal uptake of carbon (Figure 5.4A) increased between cores incubated at 14°C and 17°C (6.94 ± 4.41 mg C m^{-2} and 8.30 ± 2.89 mg C m^{-2} respectively: Figure 4.4A). At 21°C the total faunal C uptake decreased (6.27 ± 1.47 mg C m^{-2}) to a similar level as cores incubated at 14°C. However, an ANOVA undertaken on

square root transformed data indicated that there was not a statistically significant difference ($p = 0.807$) in the total faunal C uptake between the three temperature scenarios, and there appeared to be a large amount of variation within each of the three temperature scenarios.

Normalizing the total carbon uptake by biomass demonstrates a similar trend to the total faunal carbon uptake (Figure 5.4B), where more carbon was consumed relative to biomass in cores incubated at 17°C (9.20 ± 3.28 mg C mg^{-1} biomass) compared to cores incubated at 14°C (5.52 ± 1.38 mg C mg^{-1} biomass). However, normalizing the total carbon uptake by biomass indicated that less carbon was consumed relative to biomass at 21°C (7.28 ± 1.74 mg C mg^{-1} biomass) compared to cores incubated at both 14°C and 17°C. However, despite the observed trend there was a large amount of variability within temperature scenarios, resulting in no statistically significant difference in biomass normalized total carbon uptake (ANOVA, $p = 0.541$).

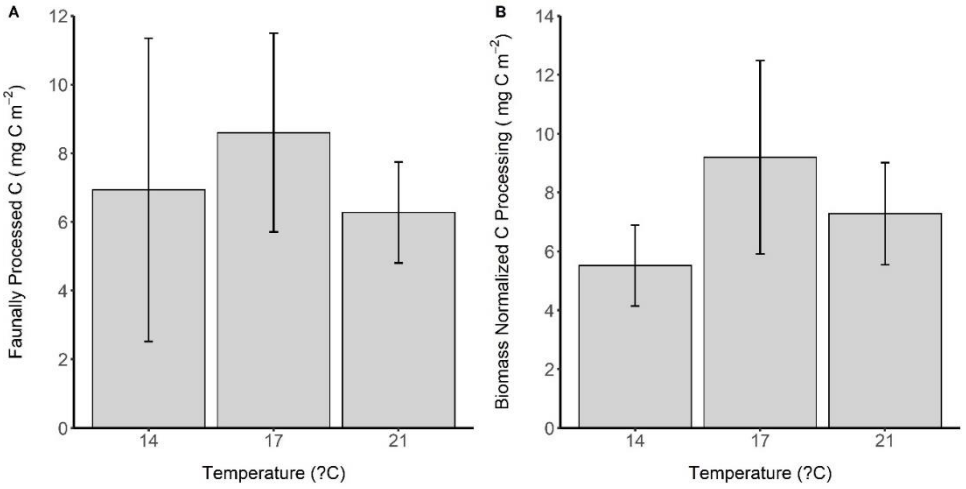

Figure 4.4: Bar charts demonstrating the impact of temperature on total faunal carbon uptake. A) the mean amount of carbon consumed by the entire benthic faunal community present in the cores incubated during the experiment; B) Total faunal carbon uptake normalized by biomass. Error bars represent ± standard error

4.3.3.2. Taxon specific carbon uptake

It was possible to determine which phyla were responsible for most of the faunal carbon uptake, and how temperature influenced carbon processing by different phyla and taxa (Figure 4.5A). For example, at 14°C, 73% of the faunal carbon uptake was processed by

bivalves. The proportion of carbon processed by this phylum decreased when cores were incubated at warmer temperatures. Where feasible it was also possible to determine the role of individual taxa in the processing of carbon (Figure 5.5B). However, for a variety of reasons it was not possible to determine the contribution to carbon uptake by all taxa present within each of the experimental cores. For example, some taxa had low biomasses or low abundances meaning that any carbon processing that they undertook may not have been detectable in the analytical process. Therefore, some taxa are reported within a pooled category such as mixed polychaetes or mixed fauna. Where it was possible to determine the carbon processing activities of individual taxa then these values are reported. However, due to the number of observations of some taxa it was not possible to undertake reliable statistical analyses at this level.

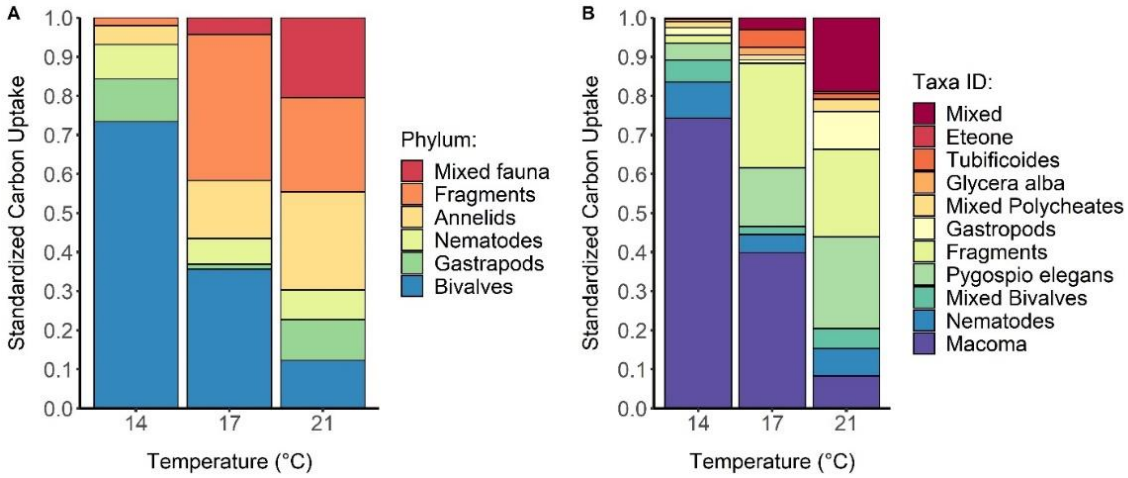

Figure 4.5: stacked bar charts representing the proportion of carbon processed by faunal groups, A) Phyla and B) Lowest possible taxonomic level

Annelids: Carbon processing by annelids was lowest in cores incubated at 14°C (0.42 ± 0.15 mg C m^{-2}), accounting for 5% of the macrofaunal carbon uptake (Figure 4.6). The amount of carbon consumed by annelids was higher when incubated at higher temperature scenarios with the phylum accounting for 15% of the carbon processing at 17°C (1.52 ± 0.55 mg C m^{-2}) and 25% at 21°C (1.71 ± 0.93 mg C m^{-2}). However, despite the observed trend an ANOVA indicated that there was not a significant difference ($p = 0.329$) in carbon uptake by annelids between the three temperature scenarios.

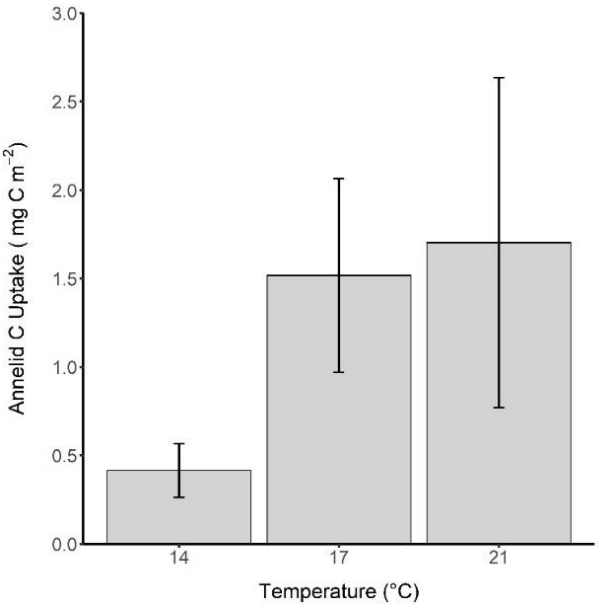

Figure 4.6: Bar charts demonstrating the carbon uptake by annelids under the three temperature scenarios (14°C, 17°C and 21°C). Error bars represent ± standard error.

The Annelida phylum was the most diverse phylum present within the Blackness community and where possible carbon uptake was obtained at a species level. However, there were not always enough individuals present within a core or treatment to be able to undertake statistical analyses on the data. The species which had sufficient individuals included; *Pygospio elegans*, *Glycera alba*, Tubificoides sp. and *Eteone flava*. Of the annelid species which had sufficient observations to analyse for species specific carbon uptake, *Pygospio elegans* processed the most carbon at 14°C (0.353 ± 0.167 mg C m^{-2}), accounting for 4% of the faunally mediated carbon uptake at 14°C (Figure 4.7A). The carbon uptake by this species was higher when incubated at warmer temperatures (17°C = 2.127 ± 0.342 mg C m^{-2} and 21°C = 1.719 ± 1.136 mg C m^{-2}), accounting for 15% and 23% of the faunally mediated carbon at 17°C and 21°C respectively. There were not sufficient measurements of carbon uptake by *Pygospio elegans* at 17°C to include the temperature in statistical analyses. However, a t-test indicated that there was not a significant difference in carbon uptake by *Pygospio elegans* between cores incubated at 14°C and 21°C.

Glycera alba processed the second largest amount of carbon (Figure 4.7B) by the Annelida phylum at 14°C (0.058 ± 0.055 mg C m^{-2}), accounting for just 0.7% of the faunally mediated carbon uptake. The amount of carbon consumed by this species was higher in cores incubated at 17°C (0.270 ± 0.267 mg C m^{-2}), accounting for 2% of the faunally mediated carbon uptake at 17°C. The amount of carbon processed by *Glycera alba* at 21°C (0.012 ± 0.007 mg C m^{-2}), accounting for 0.2% of the faunally mediated carbon uptake at 21°C. This was lower than that of individuals incubated in cores at 17°C, but higher than that of cores incubated at 14°C. However, there were not sufficient observations to undertake statistical analyses to determine whether temperature has a significant impact on carbon uptake by *Glycera alba*.

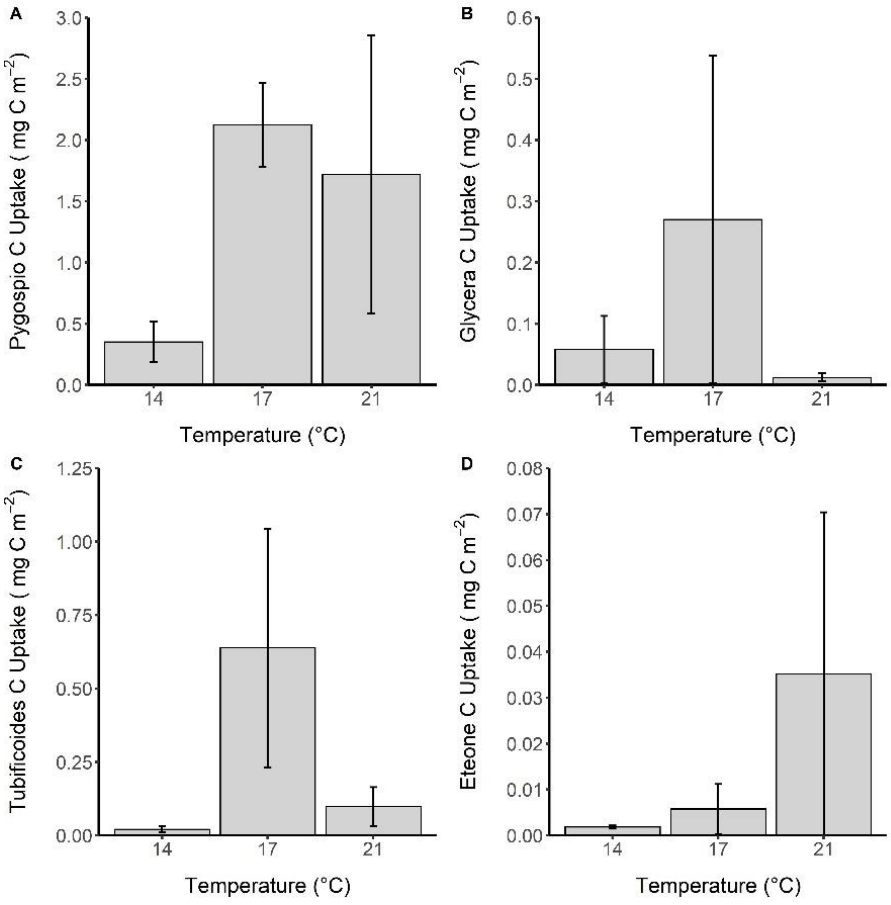

Figure 4.7: Carbon uptake by annelid species under three different temperature scenarios (14˚C, 17˚C and 21˚C), A) Carbon uptake by Pygospio elegans, B) Uptake by Glycera alba, C) Uptake by Tubificoides and, D) Uptake by Eteone flava. Error bars represent ± standard error.

Tubificoides processed the third largest amount of carbon (Figure 4.7C) by the Annelida phylum at 14°C (0.021 ± 0.010 mg C m^{-2}), accounting for 0.3% of the faunally mediated carbon uptake at 14°C. The amount of carbon processed at 17°C was higher (0.639 ± 0.406 mg C m^{-2}), accounting for 4% of the faunally mediated carbon uptake at 17°C. The amount of carbon processed by Tubificoides was lower in cores incubated at 21°C (0.099 ± 0.066 mg C m^{-2}) in comparison to those at 17°C, but slightly higher than that of cores incubated at 14°C. There were sufficient measurements of C uptake by Tubificoides within each of the temperature scenarios (14°C = observed in 3 cores; 17°C = observed in 3 cores and 21°C = observed in 4 cores). An ANOVA indicated that there was not a significant difference ($p = 0.165$) in carbon processing by Tubificoides between the three temperature scenarios.

Eteone flava contributed the least amount to carbon processing (Figure 4.7D) by the annelid phylum. At 14°C *Eteone flava* consumed 0.002 ± 0.0003 mg C m^{-2}, accounting for just 0.02% of the faunally mediated carbon processing. The amount of carbon processed by this species was higher in cores incubated at warmer temperatures (17°C = 0.006 ± 0.005 mg C m^{-2} and 21°C = 0.035 ± 0.035 mg C m^{-2}), accounting for 0.04% and 0.5% of the faunally mediated carbon processing respectively. There were not sufficient observations of carbon uptake by *Eteone flava* to undertake statistical analyses.

Bivalves: Carbon uptake by bivalves (Figure 4.8) was highest in cores incubated at 14°C, accounting for 73% of the faunally mediated carbon processing (6.29 ± 5.05 mg C m^{-2}) and decreased with temperature increases accounting for 36% at 17°C (3.64 ± 3.09mg C m^{-2}) and 12% at 21°C (0.84 ± 0.42 mg C m^{-2}). However, despite the observed trend an ANOVA on log10 transformed data indicated that there was not a statistically significant difference ($p = 0.337$) in the carbon uptake by bivalves between the three temperature scenarios, most likely due to the variability within treatments.

The primary bivalve species observed in the experimental cores was *Macoma balthica* and was responsible for almost all the carbon uptake by the phyla. *Macoma balthica* incubated in cores at 14°C consumed the highest amount of carbon (6.06 ± 5.13

mg C m^{-2}), accounting for 74% of the faunally mediated carbon uptake. The amount of carbon processed by this species was lower in cores incubated under warmer temperatures (17°C = 5.671 ± 5.141 mg C m^{-2} and 21°C = 0.614 ± 0.316 mg C m^{-2}), accounting for 40% of the faunally mediated carbon uptake at 17°C and 8% at 21°C.

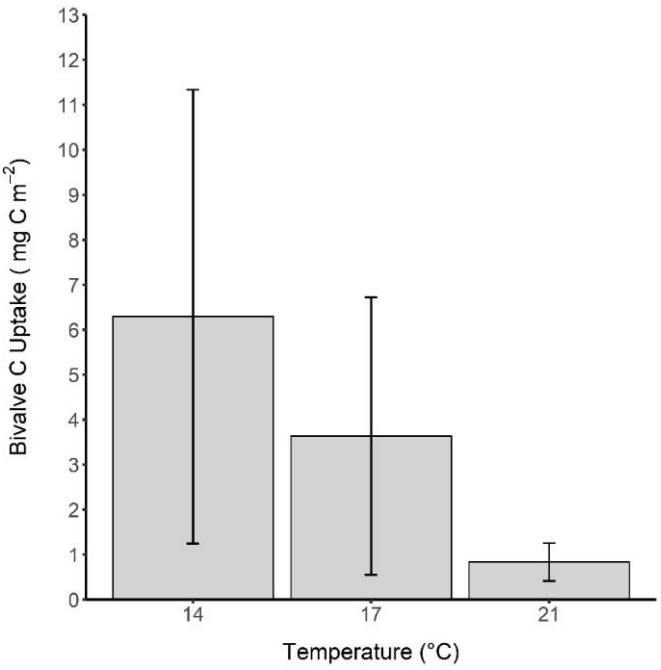

Figure 4.8: Bar charts demonstrating the carbon uptake by bivalves under the tree temperature scenarios (14°C, 17°C and 21°C). Error bars represent ± standard error.

Gastropods: The presence of gastropods appeared to be patchy across the experimental cores, and due to their small size and mass it was not always possible to measure carbon processing by this phylum on its own. Of the five cores which were analysed at each temperature scenario for faunal carbon uptake it was possible to determine the C content of gastropods within three cores incubated at 14°C, one core at 17°C and 4 cores at 21°C. As there was only a single measurement for this taxon at 17°C this temperature was excluded from statistical analyses.

The total carbon uptake by gastropods was highest in cores incubated at 14°C (0.935 ± 0.713 mg C m^{-2}), which accounted for ~11% of the faunally mediated carbon uptake (Figure 4.9). The carbon uptake by gastropods was slightly lower in cores incubated at 21°C

(0.709 ± 0.565 mg C m^{-2}), accounting for 10% of the faunally mediated carbon uptake at 21°C. A t-test on square-root transformed data indicated that there was not a statistically significant difference in the carbon processing by gastropods between cores incubated at 14°C and 21°C.

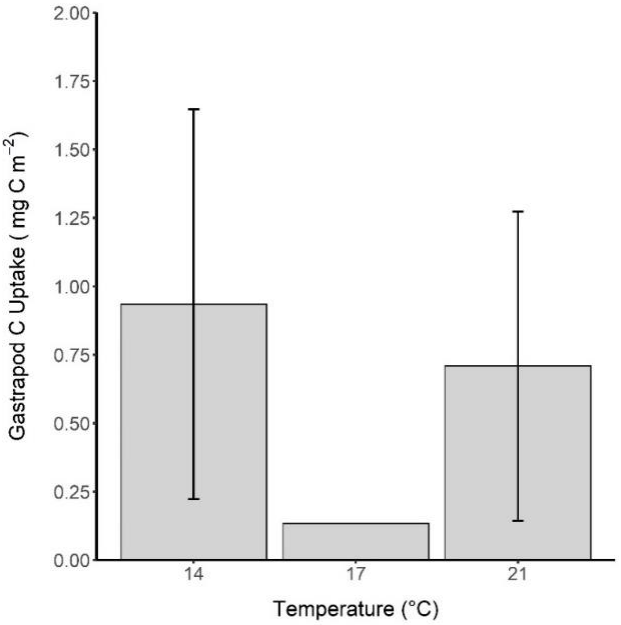

Figure 4.9: Bar charts demonstrating the carbon uptake by gastropods under the tree temperature scenarios (14°C, 17°C and 21°C). Error bars represent ± standard error.

There were two species of gastropod identified within the experimental cores incubated in this experiment, *Hydrobia ulvae* and *Retusa obtusa*. These two species were analysed for carbon uptake together due to their small size and relatively low biomass and abundance within the experimental cores. *Nematodes:* Total carbon uptake by nematodes (Figure 4.10) was highest in cores incubated at 14°C, accounting for 9% of the faunally mediated C uptake (0.758 ± 0.436 mg C m^{-2}) and decreased in cores incubated at 17°C which accounted for 7% (0.668 ± 0.662 mg C m^{-2}) and a further slight decrease in cores incubated at 21°C which accounted for 8% (0.512 ± 292 mg C m^{-2}). However, there was a large amount of variability within temperature treatments and an ANOVA on log10 transformed data indicated that there was not a significant difference ($p = 0.878$) in the total carbon uptake by nematodes between the three temperature scenarios.

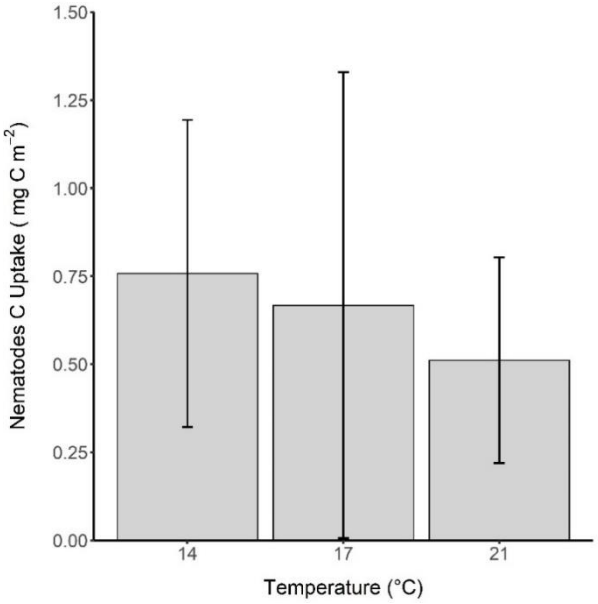

Figure 4.10: Bar charts demonstrating the carbon uptake by Nematodes under the tree temperature scenarios (14°C, 17°C and 21°C).

4.4. Discussion

This study aimed to examine the effects of temperature on biological processing of organic carbon in intertidal estuarine sediments. Warmer temperatures resulted in an overall increase in carbon processing by the benthic community. This experiment measured three carbon pools within intertidal estuarine sediments: remineralization and efflux as CO_2, benthic macrofaunal uptake and microbial uptake. Temperature increase resulted in; 1) Increased amount of organic carbon processed by the benthic community, 2) Increased loss of organic carbon from the sediment via DIC efflux, 3) Decreased uptake by the benthic macrofaunal community and 4) Increased uptake by the benthic microbial community. These results indicate that there is the potential for intertidal sediments to process carbon differently in the future because of ocean warming. The most likely result of ocean warming in the future is a reduction in carbon retention within the sediments of intertidal estuarine environments.

4.4.1. Carbon remineralization

Across all three temperature treatments in this experiment, remineralization to DIC was the only 'loss' pathway measured for organic carbon. As this experiment was

undertaken excluding light, production of DIC is representative of total respiration by benthic faunal and microbial communities, with no consumption through photosynthesis. An increased production of DIC, indicating respiration, by the benthic community could represent an increased demand for OM by the benthic community, to maintain physiological and metabolic activities (Jones et al., 2021). There was a slight increase, although not significantly, in DIC produced in cores incubated at 17°C, compared to cores incubated at 14°C. A significantly larger production of DIC was observed in cores incubated at 21°C compared to cores incubated at 14°C. The same trend was observed in the sediment community oxygen consumption (SCOC) rate, outlined in Chapter 4. It is known that increased temperature increases metabolic process rates, including respiration (Anderson et al., 2017; Włodarska-Kowalczuk et al., 2019; Jones et al., 2021). These data mean that, at the community level, there is higher oxygen consumption and higher remineralization of carbon to DIC by the benthic community at 21°C relative to lower temperatures (14°C and 17°C), suggesting that there is a higher metabolic demand and therefore higher OM requirement by the benthic community at higher temperatures in order to maintain metabolic activities of the benthic community, known as aerobic scope which includes activities including movement, feeding, digestion, growth and reproduction (Rubalcaba et al., 2020; Jones et al., 2021). Aerobic scope is expected to decrease at warmer temperatures and/or increased activities, due to the oxygen supply to the system no longer being capable of meeting the demand required by the benthic community meaning that the metabolic rates of the community and individuals reaches their limits (Rubalcaba et al., 2020). Therefore, it is possible that the aerobic scope of communities decreased in this experiment, meaning that less energy was put into metabolic activities at warmer temperatures. This could be due to a stress response because of increased oxygen demand at higher temperatures but a lower oxygen concentration within the water column due to decreased solubility of gases at higher temperatures (see chapter 5). Most of the carbon processed by macrofauna is used to maintain respiration, therefore if more carbon is required by benthic fauna to maintain respiration, then it is possible that under warming scenarios that there will be a decline in other processes such as growth (Iguchi and Ikeda,

1995; Heilmayer et al., 2004; Anderson et al., 2017). The data could also suggest that there could be higher levels of carbon retention at lower temperatures and large temperature rises could result in the alteration of carbon processing within the benthic environment, resulting in carbon stores becoming depleted due to consumption of OM by the benthic community (Jones et al., 2021).

In general, estuarine environments as a whole (i.e., water column and sediments together), such as the study site at Blackness (Firth of Forth, Scotland), are CO_2 sources under current environmental conditions (Abril and Borges, 2005; Cai, 2011). Based on the data produced in this experiment, this characteristic of these environments is likely to intensify. It is predicted that under future environmental change scenarios that there will be an increased frequency and intensity of heatwave events, meaning that increased production of CO_2 will be produced during these events.

This study determines DIC production at the benthic community level, however, despite the observed community level trends, there is likely to be large amounts of variability and complexity in oxygen demand and OM remineralization within different taxonomic groups within the community (Jones et al., 2021). For example, it has been suggested that the age, life-stage, size and temperature (Włodarska-Kowalczuk et al., 2019) can determine the respiration rate of individuals. For example, juveniles require more OM and a higher oxygen demand due to higher growth rates compared to adult counterparts (Jones et al., 2021). It is also possible that there is variability in temperature tolerance between different species within the community, such as lower metabolic requirements, which could impact the carbon processing and remineralization by these species under different temperature scenarios (Jones et al., 2021). All these factors could mean that the metabolic response of benthic faunal communities to temperature rises could vary throughout the year depending on the community structure.

Community DIC production not only includes DIC production by the faunal community, but also includes DIC production by the benthic microbial community. It was not possible to disentangle respiration undertaken by faunal and microbial communities in

this experiment. However, as previously mentioned it has been observed that oxygen consumption and metabolic demand, and food requirements go hand in hand. Therefore, it may be possible to infer the impact of temperature on microbial respiration through using the microbial carbon processing data, discussed in the next section.

Overall, the results from this experiment indicate that a 3°C increase in temperature is unlikely to significantly impact benthic production of DIC and, therefore, release of additional CO_2 into the water column and potentially atmosphere. However, exposure to much higher temperatures, such as those likely to be experienced during more frequent, intense, and extended heatwave events, is likely to significantly increase the production of DIC by the benthic community. It is therefore possible that this additional production of DIC could eventually be released back into the atmosphere.

4.4.2. Microbial carbon uptake.

The absolute amount (mg C m^{-2}) of carbon entering the microbial pool increased with temperature. It is possible that increased temperatures resulted in a decreased oxygen concentration within the experimental cores. Previous studies have shown that microbial carbon uptake is higher under reduced oxygen concentrations (White et al., 2019). However, when considering the percentage of the total amount of processed carbon entering the pool it appears that a slightly higher percentage of processed carbon is consumed by the microbial community in cores incubated at 17°C. However, the percentage of entering the microbial pool does not appear to vary much between cores incubated at 14°C and 21°C. Changes in carbon processing by the microbial community could have several explanations, including 1) Increases in sediment reworking activities by faunal communities, 2) a decrease in competition with and/or grazing pressure from the macrofaunal community and 3) the impact of temperature on microbial growth efficiency.

The observed Increase In absolute microbial carbon uptake with temperature rises could be a result of increased faunal sediment reworking activities, such as bioturbation and bioirrigation, which are known to stimulate microbial activities through introducing fresh oxygenated water into the sediment column and extending the depth of the oxic layer and

can increase the diversity of the microbial community (Solan, Wigham, et al., 2004; Laverock et al., 2011; Kristensen et al., 2012). Bioturbation and bioirrigation potentials were calculated for cores incubated in this experiment and the use of an inert tracer (NaBr) was used to measure bioirrigation rate (see Chapter 5). The results from these measurements indicated that both bioturbation and bioirrigation potential increased under increased temperatures, and the use of NaBr indicated that the rate of bioirrigation also increased with temperature. Therefore, based on these results in combination with an observed increase in carbon processed by the microbial community it could be a reasonable conclusion that microbial carbon processing was higher in cores incubated at higher temperatures due to the impact of macrofaunal sediment reworking activities. This would be particularly true of microbial communities living deeper within the sediment column. This is because these communities would have been entirely dependent on sediment reworking activities of macrofauna to gain access to the added organic matter (Van Nugteren et al., 2009).

The absolute carbon processing by the microbial community could have also increased due to a decrease in competition and/or pressure with macrofaunal communities. For example, deposit feeders, such as *Macoma balthica*, consume sediment and its contents including microbes (Lopez and Levinton, 1987; Törnroos et al., 2015) and it has been observed that high densities of *Macoma balthica* are positively correlated with high microbial biomass (Tunnicliffe and Risk, 1977). When *Macoma balthica* are expressing their deposit feeding trait modality they use their siphon to consume the topmost layer of sediment and consume microbes present (Tunnicliffe and Risk, 1977). In this experiment the abundance and biomass of *Macoma balthica* was lower in cores incubated at higher temperatures, which could indicate that there is less pressure being exerted on the microbial community by this species. It has been suggested by Hunter et al. (2013) that macrofaunal activities such as grazing and microhabitat destruction may inhibit microbial organic matter processing activities. Therefore, reductions in biomass or abundance of macrofauna which exert pressure on microbial communities, for example *Macoma balthica*, may result in the observed increase in microbial carbon uptake at 21°C in this experiment.

This is also supported by the fact that carbon processing by *Macoma balthica* was observed to be lower in cores incubated at higher temperatures.

Bacteria play an important role in driving whether OC is respired or converted into biomass and therefore retained within the food-web and the carbon demand by the bacteria (Muscarella et al., 2020). It is possible that the increase in absolute carbon processing by the bacterial community could indicate that more of the consumed carbon is assimilated into bacterial biomass suggesting that the bacterial growth efficiency (BGE) was higher in cores incubated at warmer temperatures (Muscarella et al., 2020). As the bacterial respiration was not measured separately in this experiment, the bacterial growth efficiency can only be approximated as bacterial C uptake divided by the sum of bacterial C uptake and faunal community respiration (Woulds et al., 2016). Using this method to approximate the BGE indicates that the BGE was higher at 17°C compared to cores incubated at either 14°C or 21°C, and therefore more processed carbon was turned into bacterial biomass at 17C in comparison to cores incubated at 14°C and 21°C. However, the total benthic community respiration rate is not representative of microbial respiration. The diffusive oxygen consumption was also approximated in this experiment (see Chapter 5 for full details). The diffusive oxygen uptake has been used as a proxy for microbial respiration (Hicks et al., 2017). When the diffusive oxygen uptake is used instead of community respiration rate the BGE remains steady in cores incubated at 14°C and 17°C, but increases in cores incubated at 21°C, suggesting that the amount of carbon which is assimilated into microbial biomass was not impacted by a temperature increase of 3°C, but did increase when exposed to much higher temperatures, which would fit with the observed increase in absolute carbon processing by the microbial community at 21°C. However, due to the uncertainties in calculating the BGE with available data the trends in BGE can only be viewed as an approximation. To obtain accurate bacterial growth efficiency data the bacterial respiration would have to be determined in isolation, but this in itself would introduce uncertainties due to the important role that microbial communities play in stimulating the microbial community in natural environment.

4.4.3. Faunal carbon uptake

Uptake of added carbon by the faunal community in isotope tracer experiments is frequently patchy (Woulds et al., 2020). Patchiness in intertidal benthic communities is often a result of localized differences in sedimentary environment, such as, carbon content, grain size and nutrients (Godbold et al., 2011).

Faunal uptake of added carbon appeared to be greatest at 17°C and there appeared to be a slight decrease in uptake at 21°C (Figure 4.4A & B). However, variation between replicate cores limits the conclusions which can be made regarding faunal carbon uptake between the three temperature scenarios. Normalizing the carbon uptake by biomass provides an indication of the impact of temperature on carbon uptake, by removing the potentially confounding effect of biomass differences. There was a similar trend in biomass specific carbon uptake at 14°C and 21°C to the total faunal carbon uptake. However, there appeared to be a much larger decrease in the biomass specific carbon uptake at 21°C, compared to the total faunal uptake at this temperature. This could suggest that decreased carbon uptake by fauna is, at least partially, due to the impacts of temperature. An increase in faunal carbon uptake in cores at 17°C along with minimal changes in DIC production at this temperature could indicate that benthic fauna were not experiencing thermal stress as a result of a 3°C increase above ambient temperatures. In comparison, a decrease in carbon incorporation into biomass in cores incubated at 21°C in conjunction with the observed increase in DIC production by the benthic community could be an indication that benthic fauna were experiencing stress at higher temperatures (21°C). It is possible that fauna were using less of the consumed carbon to incorporate into biomass and a larger fraction to maintain metabolic processes, such as respiration (Iguchi and Ikeda, 1995; Heilmayer et al., 2004; Anderson et al., 2017). This could be the case for bivalves within the benthic community at Blackness, which demonstrated a decrease in carbon uptake in cores incubated at 17°C and 21°C relative to those incubated at 14°C. In comparison annelids demonstrated an increase in carbon uptake at higher temperatures (17°C and 21°C) in comparison to those incubated in cores at 14°C, indicating that this phylum was potentially capable of maintaining metabolic activities at higher temperatures and still incorporate

carbon into biomass. It has been suggested by Rubalcaba et al. (2020) that an increase in temperature results in an increase oxygen demand and a decrease in aerobic scope (metabolic activities including feeding, reproduction, and growth). This impact is thought to have a greater impact on larger fauna, meaning that they experience a larger decrease in aerobic scope (i.e. experience a greater impact on metabolic activities such as growth). Certain species are more sensitive to changes in temperature, such as *Macoma balthica* which were the dominant bivalve species present within cores collected from Blackness and have a narrower optimal temperature range than other species (Beukema et al., 2009). This means that their ability to effectively function could be impacted much faster than other taxa within the community which have a much larger optimal temperature range. Other phyla, such as annelids, appear to increase their carbon processing capabilities in cores incubated at higher temperatures. However, this phylum (annelids) is much more diverse than that of bivalves within this study site. Therefore, this introduces complexities when unpicking the impacts of temperature on carbon uptake by this phylum. Other phyla present within the Blackness benthic community (nematodes and gastropods) did not appear to demonstrate a trend in carbon uptake with temperature, suggesting that these phyla may not necessarily experience a stress response at higher temperatures.

Not only was there variability in the carbon uptake by different phyla, but there was variability within a phylum. It was possible to obtain carbon uptake data for a small number of annelid species individually, although due to the relatively low number of observations the data must be used with caution when making conclusions. For example, the carbon uptake by *Pygospio elegans* and *Eteone flava* increased with temperature, whereas the carbon uptake by Tubificoides sp. and *Glycera alba* was highest at 17°C and was lower at 21°C. Therefore, it is possible that although there was not a clear trend in the uptake of carbon by the faunal community, it is possible that the way in which the carbon is distributed within the pool changes with temperature, with response to temperature being taxon specific, even within phyla. These differences in carbon uptake by different taxa could be related to the functional traits of species within the groups, and/or competition between different taxa.

Overall, the faunal carbon uptake data indicates that there is variability in carbon processing activities between different phyla within the benthic faunal community and within different phyla. Therefore, more work needs to be undertaken to better understand the impact of carbon processing by different phyla and taxa within the benthic faunal community.

4.5. Conclusions

In conclusion, of the carbon processed by the benthic community, the majority was remineralized to DIC, through the process of respiration. More carbon was remineralized to DIC at 21°C in comparison to cores incubated at 14°C and 21°C.

Microbial uptake of carbon also increased with temperature which could be linked to increase faunal sediment reworking activities at higher temperatures and a slight increase in the bacterial growth efficiency at higher temperatures. It is also possible that microbial carbon uptake could have increased due to a decrease in competition and grazing pressure by the macrofaunal community.

Finally, the absolute faunal carbon uptake did not demonstrate a clear trend with temperature, although a reduction in faunal C uptake at the higher temperature seems likely. It is also likely that the distribution of carbon within the faunal pool could change under warmer temperature scenarios.

5. Characterizing sedimentary organic carbon in UK shelf seas: A case study of the northern North Sea

5.1. Introduction

Marine sediments are considered to be one of the largest carbon reservoirs on the planet and play a key role in regulating climate change and in biogeochemical cycling (Hunt et al., 2020; Atwood et al., 2020; LaRowe et al., 2020; Carneiro et al., 2021). Organic carbon which enters the marine sedimentary system, and is subsequently buried within sediments, and is capable of remaining there for thousands of years (Atwood et al., 2020). However, exploitation of the marine environment and disturbance of the sedimentary environment by humans (e.g. trawling) has increased, making carbon in these environments vulnerable to being remineralized (Atwood et al., 2020; Luisetti et al., 2020; Carneiro et al., 2021). When sediment is disturbed, it is mixed and resuspended, exposing stored carbon to oxygen, causing it to be remineralized to carbon dioxide (CO_2) which can then be released into the atmosphere (Atwood et al., 2020; Luisetti et al., 2020). It has been suggested that in shelf seas, areas where the water depth is <200m, CO_2 could be released back into the atmosphere within a year of a disturbance event (Luisetti et al., 2020). Although the existence of this C store in marine sediments is well established, there is still a lack of information on the sources and fluxes of carbon in shelf sediments, as well as the quantity and reactivity or degradation state of the carbon stored (Luisetti et al., 2020). This arises from the fact that marine sedimentary organic C is largely uncharacterizable (Middelburg, 2018), which has long proved a barrier to understanding the factors driving C preservation and storage in 'blue carbon' habitats and developing effective management of these environments.

Understanding shelf sea C stocks, specifically in shelf sea sediments, is strategically important for the UK, as this understanding will facilitate effective, targeted, and informed management and preservation of these systems (Hunter et al., 2020), especially considering future environmental change. The marine environment and climate are interconnected

through the global C cycle, where the marine environment is capable of being both a source and sink of carbon (Bauer, W.J. Cai, et al., 2013; Atwood et al., 2020).

Over the past ~5 years research into quantifying and better understanding carbon stocks and fluxes of carbon in marine sedimentary environments has increased. For example, Diesing et al. (2017) used random forest modelling and a large particulate organic carbon (POC) dataset held by Cefas to estimate that surface sediments on the NW European shelf contain 476 Mt of C. However, this estimate has large uncertainties arising from a lack of a physical mechanism based (as opposed to a statistical) method for predicting POC concentration in any given location. Uncertainties in quantifying and characterizing marine sedimentary carbon need to be reduced to improve assessments of the marine benthic environment in carbon stock assessments, and to thus facilitate their protection (Hunt et al., 2020).

Carbon stored within marine sediments in coastal environments is heterogeneous and sourced from a variety of different environments, including the marine environment, terrestrial environment and fluvial environments (Carneiro et al., 2021). Accumulation of OC in sediments is traditionally thought to be driven by a number of factors including: 1) an association with sediments which have a higher mud content (Diesing et al., 2017), due to mechanisms such as sorption of OM to mineral surfaces leading to higher concentrations of OM in fine-grained sediments which have a larger surface area (Mayer, 1994; Diesing et al., 2017), 2) Sedimentation and accumulation rates (Mayer, 1994; Carneiro et al., 2021), 3) Oxygen concentration (Glud, 2008; Diesing et al., 2017) and 4) Temperature (Diesing et al., 2017; Luisetti et al., 2019). Work by Diesing et al. (2017) used a statistical approach developed through machine learning to produce a particulate organic carbon model for the UK shelf seas. This model predicted areas on the shelf where organic carbon is most likely to accumulate, based on sediment mud content, annual average bottom temperature, eastings, distance to shoreline, sediment gravel content and peak orbital velocity of the water column (Diesing et al., 2017). The modelling study by Diesing et al. (2017) indicated that the mud content of sediments was the most important factor in determining the organic carbon content within surface sediments. However, the work by the authors does

not characterize the carbon present on the shelf beyond defining it as organic, and their work does not provide mechanistic explanations for the relationships observed with environmental factors. Further understanding of organic C stocks on the UK shelf now requires insight into the sources, transport and alteration of organic C which result in the observed C accumulation, which are needed to inform future management decisions for UK shelf seas. For example, the question of terrestrially derived OM (TOM) in the marine environment has fascinated scientists for several decades, and better understanding its role within the marine environment is essential to better understand the carbon cycle (Hedges et al., 1997). Approximately 0.9 Pg C yr-1 is estimated to enter the ocean from terrestrial environments (Bianchi, 2011; Painter et al., 2018). However, despite TOM being predicted to enter the marine environment, there is limited evidence that it significantly contributes to the marine carbon pool (Hedges et al., 1997; Painter et al., 2018). Painter et al. (2018) collected surface water samples in the summer of 2016 and used fluorophores to detect TOM in the North Sea. The authors determined the presence of fluorophore 2 within their samples which is generally considered to be indicative of terrestrial humic substances (Coble, 1996; Painting et al., 2018) and determined that its presence was stable at distances up to 200km from the coastline and then its concentration decreased. This indicates that TOM is present within the surface waters of the marine coastal region within the North Sea and detectable up to ~200km from the coastline. However, the question remains on how much, if any, TOM reaches the sedimentary environment within the North Sea.

This study aims to better characterize OC sources in northern North Sea sediments, through determining the contribution of more terrestrially derived organic carbon and marine derived organic carbon. Secondly, it seeks to better characterize the degradation state of organic carbon in northern North Sea sediments. In the North Sea it is well established that currents carry water, and OC, anti-clockwise from the Northwest along the Eastern coast of the UK and Northern Europe coastline to the main burial area in the deep Norwegian trench (Luisetti et al., 2020). Thirdly, the study is designed to characterize spatial factors driving organic matter characteristics. Finally, this study seeks to investigate the

aforementioned factors on a finer spatial resolution than previous work, to characterise the extent of smaller scale heterogeneity.

5.2. Methods

5.2.1. Study site location

The Northern North Sea is a sub-section of the North Sea which stretches from the north-east coast of Scotland to Flamborough Head in Yorkshire. The survey (CEND2018) undertaken on *RV Cefas Endeavour* took place South of the Scottish border (Figure 5.2).

Sediment samples collected during the CEND2018 survey indicate that the dominant sediment type within the sampling region was muddy sand (mS), as demonstrated in Figure 5.1. The figure also demonstrates that were also three observations of slightly gravelly muddy sand ((g)mS) and an observation of sand (S).

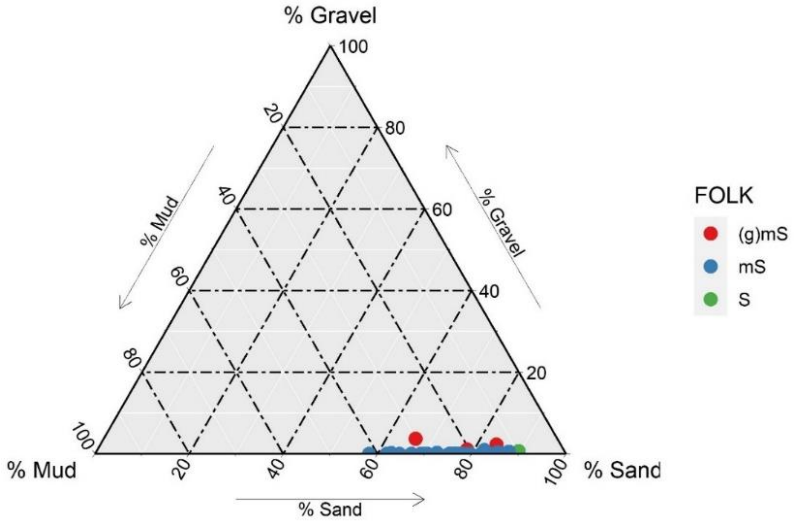

Figure 5.1: Triplot showing the composition (percentage mud, sand and gravel) of sediments within the northern North Sea study region, collectd and analysed by Cefas during the CEND2018 survey. Folk classes (Long, 2006) present include (g)mS – Slightly gravelly muddy sand, mS = Muddy sand and S = Sand.

5.2.2. Sampling methods

Sample locations were selected to represent a range of distances from the shore, as this was a key determining factor in the modelling work of Diesing et al. (2017) and was also expected to influence the source of organic matter stored in the sediment. Sampling

locations were also constrained by other survey objectives, resulting in three groups of samples: two along-shore transects, one close to shore (~50km from the coastline) and the other further offshore (~250km from the coastline; Figure 5.2), and a third group of samples forming an transitional transect connecting the two other groups. Data has not been presented for samples collected from the transitional transect as they were only used to help characterize and calculate the degradation index of the sediment within the study area.

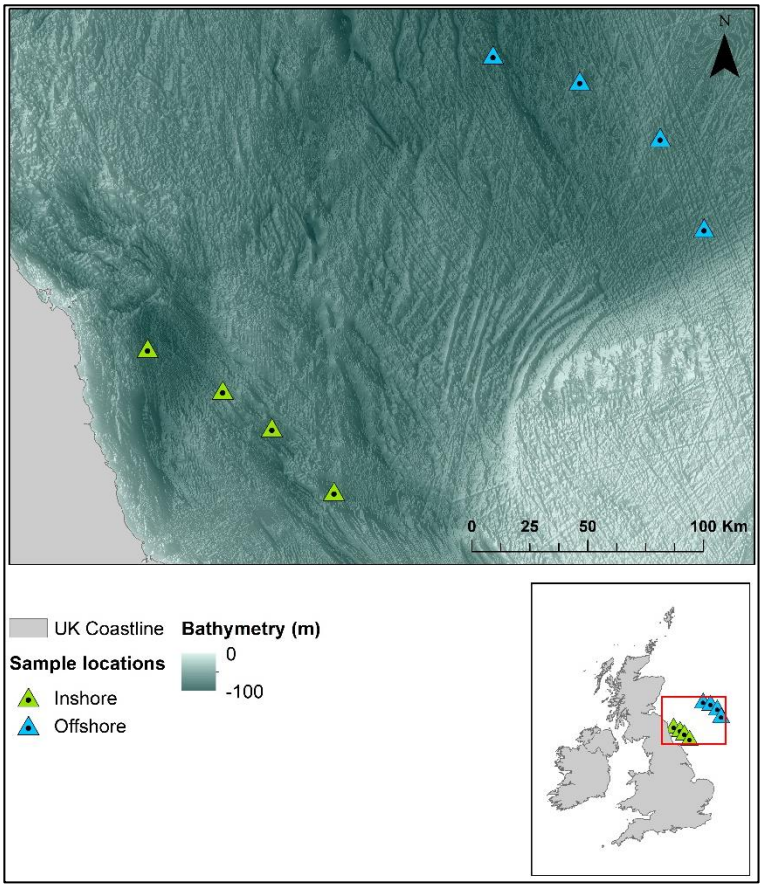

Figure 5.2: Day grab sample locations collected onboard RV Cefas Endeavour (Survey code: CEND2018).

Sediment samples were collected from the northern North Sea sampling area using a day grab onboard the RV Cefas Endeavour (Cruise code: CEND2018), during a multidisciplinary survey in December 2018. A day grab was used as the preferred NIOZ corer and Shipek grab were not successful in collecting samples due to weather conditions. Due to this, samples were only taken from the Day Grab if the sediment surface had not been visibly compromised. Three sub-cores were collected from each successful Day grab using

60ml (26ml internal diameter) plastic syringes. Each sub-core was sliced at 1cm intervals. Samples from one of the syringes were frozen at -80°C for amino acid analysis. Samples from the remaining syringes were frozen at -20°C for TOC/TN ratios and the natural abundance of $\delta^{13}C$ and $\delta^{15}N$.

5.2.3. TOC/TN and bulk $\delta^{13}C$ / $\delta^{15}N$

Samples were freeze dried to completely remove any water and were then ground into a fine powder. Samples for TN and bulk $\delta^{15}N$ analysis required no further preparation, so subsamples were weighed into tin capsules ready for analysis. Samples for TOC and $\delta^{13}C$ needed to be decarbonized, to remove inorganic carbon from the samples, using 10% HCl. For the decarbonization process each sediment sample was saturated in 10% HCl and left to soak overnight for all inorganic carbon to be dissolved from the sample. Once all the inorganic carbon had been removed each sample was centrifuged and the acid disposed of. Each sample was then washed and centrifuged in deionized water and the pH measured. The process was complete once a neutral pH was achieved. The decarbonized samples were then dried at 30°C to remove all moisture. The temperature had to be high enough to evaporate the water but low enough that organic carbon was not burned off. Once the drying process was complete the samples were again ground into a fine powder and weighed into tin capsules ready for analysis.

The samples for TOC and TN analysis were analysed using an Elamentar vario MICRO cube with sulfanilic acid and reference material B2152 (Soil standard low organic content) used for the calibration of the instrument. The data output from the analysis gave the TOC and TN values as a percentage, with a precision of ±0.07389% and ±0.00897% respectively. The percentage TOC and TN were converted into mg/g of sediment and the TOC/TN ratio was calculated by dividing the TOC by the TN.

Samples prepared for $\delta^{13}C$ and $\delta^{15}N$ were analysed by Iso-Analytical. At Iso-Analytical sediment samples were prepped for carbon and nitrogen isotope analysis by weighing 50 mg aliquots of sediment into tin capsules (8 x 5 mm) and then sealed. The

carbon isotope analysis was undertaken using a Europa scientific Elemental Analyser – Isotope Ratio Mass Spectrometry (EA-IRMS).

Reference material used for $d^{13}C$ analysis of the sediment samples was IA-R001 (wheat flour, $\delta^{13}Cv_{\text{-PDB}}$ = -11.64‰ with a precision of ± 0.02‰). For quality control IA-R001, IR-R005 (beet sugar, $\delta^{13}Cv_{\text{-PDB}}$ = -26.03, with a precision of -26.03 ± 0.01‰) and IA-R006 (cane sugar $\delta^{13}Cv_{\text{-PDB}}$ = -11.64, with a precision of ± 0.03‰) were analysed during batch processing of the samples.

Reference material used for nitrogen isotope analysis of the sediment samples was IA-R001 (wheat flour, $\delta^{15}N_{AIR}$ = 2.55‰, with a precision of 2.58 ± 0.04‰). For quality control IA-R001, IA-R045 (ammonium sulphate, $\delta^{15}N_{AIR}$ = -4.71, with a precision of ± 0.04‰) and IA-R046 (ammonium sulphate, δ^{15}NAIR = 22.04, with a precision of ± 0.04‰) were used during the batch process of sediment samples.

Reference materials for ^{13}C and ^{15}N are calibrated against IAEA-CH-6 (sucrose, $\delta^{13}C_{v\text{-PDB}}$ = -10.449) and IAEA-N-1 (ammonium sulphate, $\delta^{15}N_{AIR}$ – 0.4). This is an inter-lab standard distributed by the International Atomic Energy Agency, Vienna.

5.2.4. Amino acids

Once sediment samples were freeze dried, amino acid extraction and analysis was undertaken in the Organic Geochemistry facility, within the School of Geosciences at the University of Edinburgh, using the method outlined in Cowie & Hedges (1992), but a brief description is provided below.

Amino acids were measured using high-pressure liquid chromatography (HPLC; Agilent Infinity 1260 with a 1260 Spectra Fluorescence detector with an Agilent Infinitylab Poroshell 4.6 x 100mm, 2.7 micron column) which used charge matched recovery standards (Cowie and Hedges, 1992). Aqueous hydrolysis was undertaken in 6 N HCl in an oxygen free environment, for 70 minutes at 150°C. The hydrolysis mixture was dried, dissolved in purified water and amino acids present converted to florescent o-phthaldialdehyde derivatives (Cowie and Hedges, 1992). Amino acid data were used to calculate the

degradation index (DI) of the samples using the following equation, as outlined by Dauwe & Middleburg (1999):

$$DI = \sum_i \left(\frac{var_i - AVG\ var_i}{STD\ var_i} \right) \times fac.coef_i$$

Where:

var_i = Mole percentage of amino acid *i*

AVG var_i = Mean of amino acid *i*

STD var_i = Standard deviation of amino acid *i*

$fac.coef_i$ = factor coefficient (first axis of PCA) of amino acid *i*

The DI was calculated in two ways for the data in this study. The first method calculated DI based only on samples collected in this study. In order to do this a principal component analysis (PCA) was undertaken using mole percentage data for each amino acid at each sample location from the CEND2018 survey, and the first axis was used as the factor coefficient value for each of the amino acids, the AVG var_i and STD var_i are the mean and standard deviation of the mole percentage values for each of the amino acids across the sampling locations.

The second method for calculating the DI of the samples collected during the CEND2018 survey used factor coefficient values, mean and standard deviation values for each amino acid from Dauwe & Middleburg (1998). This enabled the DI of samples collected during CEND2018 to be compared to the DI of North Sea samples analysed in Dauwe & Middleburg (1998). This made it possible to check that DI calculated using PCA for this study operated in the same direction as that published by Dauwe and Middelburg (1998) and allowed direct comparison between the sample set in this study and their previous work.

5.2.5. Environmental variables

Environmental variables have been determined through a variety of methods for each of the sample locations, including direct measurement to determine the particle size

of the sediment at sampling locations. The particle size analysis (PSA) samples were collected and processed by scientists at the Centre for Environment Fisheries and Aquaculture Science (Cefas). PSA samples were processed by Cefas following the recommended methodology outlined by the National Marine Biological Analytical Quality Control (NMBAQC) scheme using a combination of laser diffraction and sieving (Mason, 2016). Other environmental variables were determined using modelled GIS layers for the area (Cefas THREDDS server – https://cefasbfmdata.cefas.co.uk/thredds/catalog/catalog.html).

5.2.6. Statistical analyses

Statistical analyses were undertaken using the "R" statistical programming environment (version 4.0.1) and R studio (2022.02.2, build 485). Prior to statistical analysis the data were checked for normality using Shapiro's normality test, and if necessary, the data were transformed to meet the assumptions of parametric statistical tests. The two transects (inshore and offshore) in this study were compared using a t-test where Shapiro's normality test indicated that data were normally distributed. A significance level of 0.05 was used as the threshold for statistical significance.

A PCA was used to determine whether the composition of amino acids was different between samples collected along the inshore and offshore transects. Further t-tests or Mann-Whitney U tests were undertaken to determine whether there was a significant difference in PC1 and PC2 values between the two transects.

5.3. Results

Results are presented and described in the form of mean ± standard error for each of the inshore and offshore groups of samples, with statistical analysis of similarity and difference. This approach was guided by the findings of Diesing et al. (2017) that distance to shore was a key variable driving sediment POC. This approach was also most effective in identifying patterns in the data.

5.3.1. Modelled environmental variables

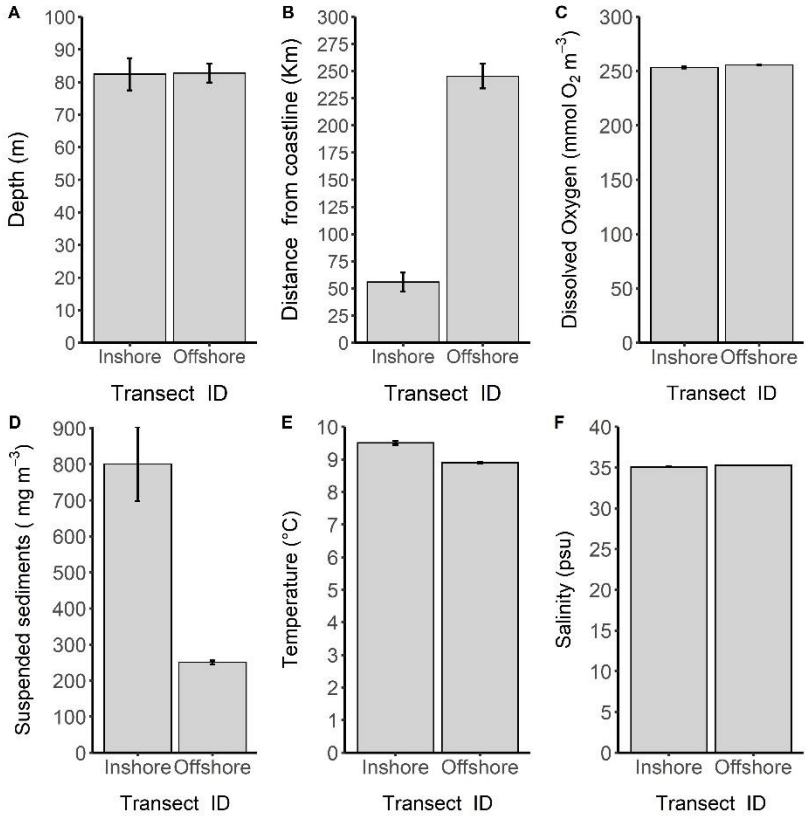

Figure 5.3: Bar charts demonstrating the differenced in modelled environmental variables between inshore and offshore transects undertaken during CEND2018. Error bars represent ± standard error.

A t-test indicated that there was not a significant difference (p = 0.958) in water depth between the two transects (inshore = -82.43 ± 4.95m and offshore = -82.75 ± 2.91m; Figure 5.3 A). The remaining environmental variables appeared to demonstrate a difference between the inshore and offshore transects. Bottom water oxygen concentration (Figure 5.3 C) was significantly higher (t-test; p = 0.037) along the offshore transect (255.79 ± 0.39 mmol O_2 m^{-3}) than the inshore transect (253.39 ± 0.73 mmol O_2 m^{-3}). Salinity (Figure 5.3 F) was also significantly higher (t-test; p= <0.011) along the offshore transect (35.27 ± 0.001 psu) in comparison to the inshore transect (35.08 ± 0.03 psu), although there was only 0.19 psu between the mean salinity of inshore and offshore transects. The bottom water temperature (Figure 5.3 E) of sampling locations along the offshore transect (8.91 ± 0.03°C) was significantly (t-test; p = <0.001) lower than that of samples collected along the inshore transect (9.51 ± 0.06°C). A t-test on log_{10} transformed suspended sediment (Figure 5.3 D)

data indicated that sampling locations along the offshore transect received a significantly ($p = 0.002$) lower concentration of suspended sediments (250.92 ± 5.12 mg m^{-3}) compared to sampling locations along the inshore transect (801.12 ± 102.62 mg m^{-3}).

5.3.2. TOC and TN

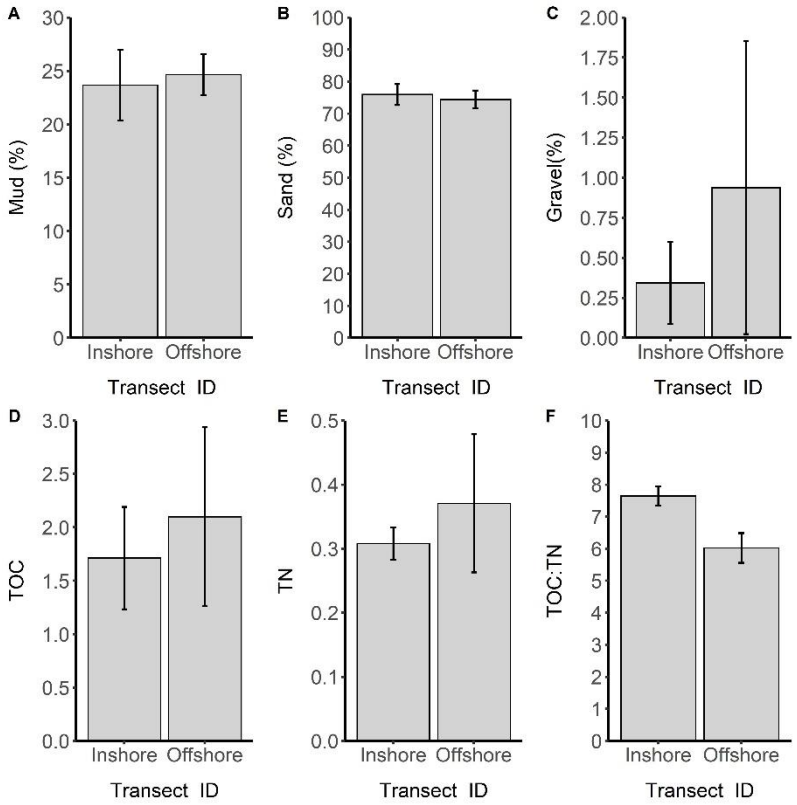

Figure 5.4: Bar charts demonstrating the variation in sedimentary properties between inshore and offshore transects, a) Percent mud content, B) Percent sand content, C) Percent gravel content, D) TOC, E) TN and, F) TOC:TN. Error bars represent ± standard error.

A t-test indicated that there was not a significant difference in the % mud (inshore = 23.67 ± 3.32% and offshore = 24.64 ± 1.92%) and % sand (inshore = 75.99 ± 3.27% and offshore = 74.42 ± 2.81%) content of sediments (Figure 5.4 A & B) collected along the offshore and inshore transects ($p = 0.812$ and 0.729 respectively). A Mann-Whitney U test indicated that there was also not a significant difference ($p = 0.882$) in the % gravel (Figure 5.4 C) between inshore (0.34 ± 0.25%) and offshore (0.94 ± 0.91%) sediments. However, offshore sediment samples appear to have a slightly higher TOC (2.10 ± 0.84 mg g^{-1}) and TN (0.37 ± 0.12 mg g^{-1}) content compared to inshore samples (1.71 ± 0.48 mg g^{-1} and 0.31 ±

0.03 mg g^{-1} respectively; Figure 5.4 D & E), although not significantly (p = 0.706 and 0.607 respectively). The TOC:TN ratio (Figure 5.4 F) indicates that there is a significant difference (t-test; p = 0.032) between the two transects, with samples collected along the offshore transect having a lower TOC:TN (6.02 ± 0.46) ratio than samples collected along the inshore transect (7.64 ± 0.30; Figure 6.3).

5.3.3. Bulk isotopes

Samples collected along the inshore transect had a higher mean $\delta^{13}C$ value (-21.91 ± 0.06) than samples collected along the offshore transect (-22.17 ± 0.08), although there was not a statistically significant difference (t-test, p = 0.052), though the p-value indicates that the difference was only marginally insignificant. However, the mean $\delta^{13}C$ for samples collected along the inshore transect was only 0.26 units higher than the mean $\delta^{13}C$ of samples collected along the offshore transect (Figure 5.5A).

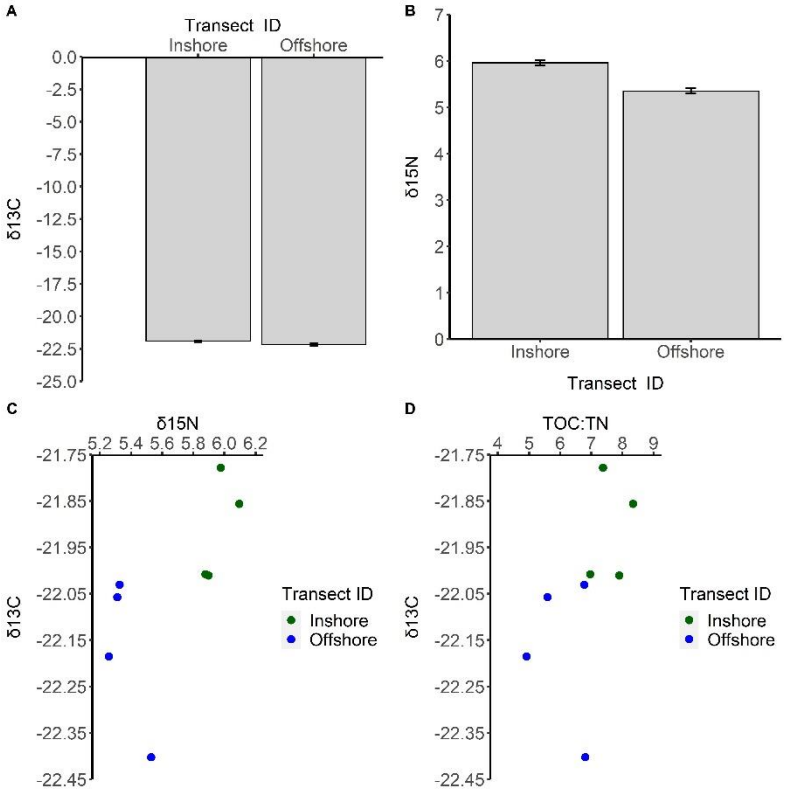

Figure 5.5: Bar charts demonstrating the difference in sedimentary bulk isotopic composition between inshore and offshore sediments during CEND2018, A) $\delta^{13}C$, B) $\delta^{15}N$ and, C) a scatter plot demonstrating the relationship between $\delta^{13}C$ and $\delta^{15}N$ between inshore and offshore transects and D) a scatter plot indicating the relationship between the TOC:TN ratio and $\delta^{13}C$.

A t-test indicated that samples collected along the inshore transect had a significantly higher ($p = <0.001$) mean $\delta^{15}N$ value (5.96 ± 0.05) than samples collected along the offshore transect (5.36 ± 0.06). Although, the mean $d^{15}N$ value of samples collected along the inshore transect was only 0.6 units higher than the mean of the offshore samples (Figure 5.5 B).

A scatter plot indicated that samples collected along the inshore transect had a higher TOC:TN ratio in addition to higher $\delta^{13}C$ values. Whereas samples collected along the offshore transect had a lower TOC:TN ratio and lower $\delta^{13}C$ values (Figure 5.5 D).

5.3.4. Amino acids

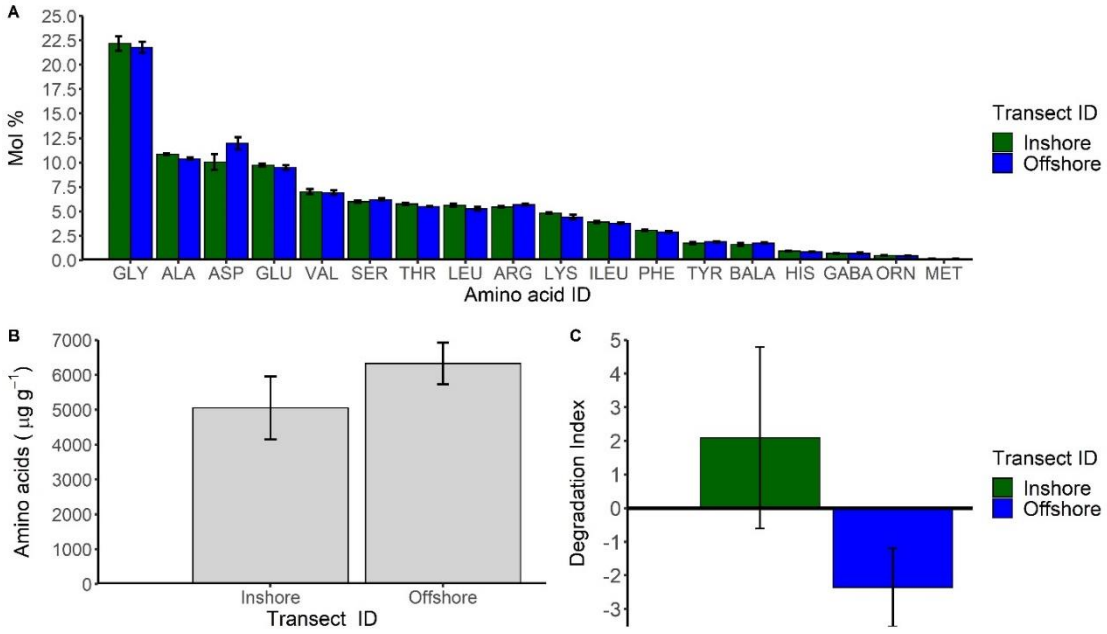

Figure 5.6: A) Mean amino acids present for cores collected along inshore and offshore transects collected during CEND2018, B) Bar charts describing the Total amino acid composition (µg g^{-1}) and, C) Degradation index of samples collected along inshore and offshore transects during CEND2018.

60% of the amino acid composition, of both the inshore and offshore transects, was dominated by glycine, alanine, aspartic acid, glutamic acid and valine (Figure 5.6A). The PCA (Figure 5.7) indicated that a total of 62.26% of the variation could be explained by axis1 and axis 2, with the first axis being responsible for 39.74% of the variation and axis 2 being responsible for 22.51%. Samples collected along the inshore transect appear to have a

positive axis 1 value, whereas samples collected along the offshore transect have a negative value. However, a t-test indicated that there was not a statistically significant difference in axis 1 values between the two transects (t = 1.0625, df = 3.853, p-value = 0.35). There does not appear to be any clear groupings based on axis 2, and a Mann-Whitney test indicated that there was also not a statistically significant difference in axis 2 values between the two transects (W = 12, p-value = 0.3429). Overall, the PCA results indicate that the composition of amino acids for the two transects are not too dissimilar.

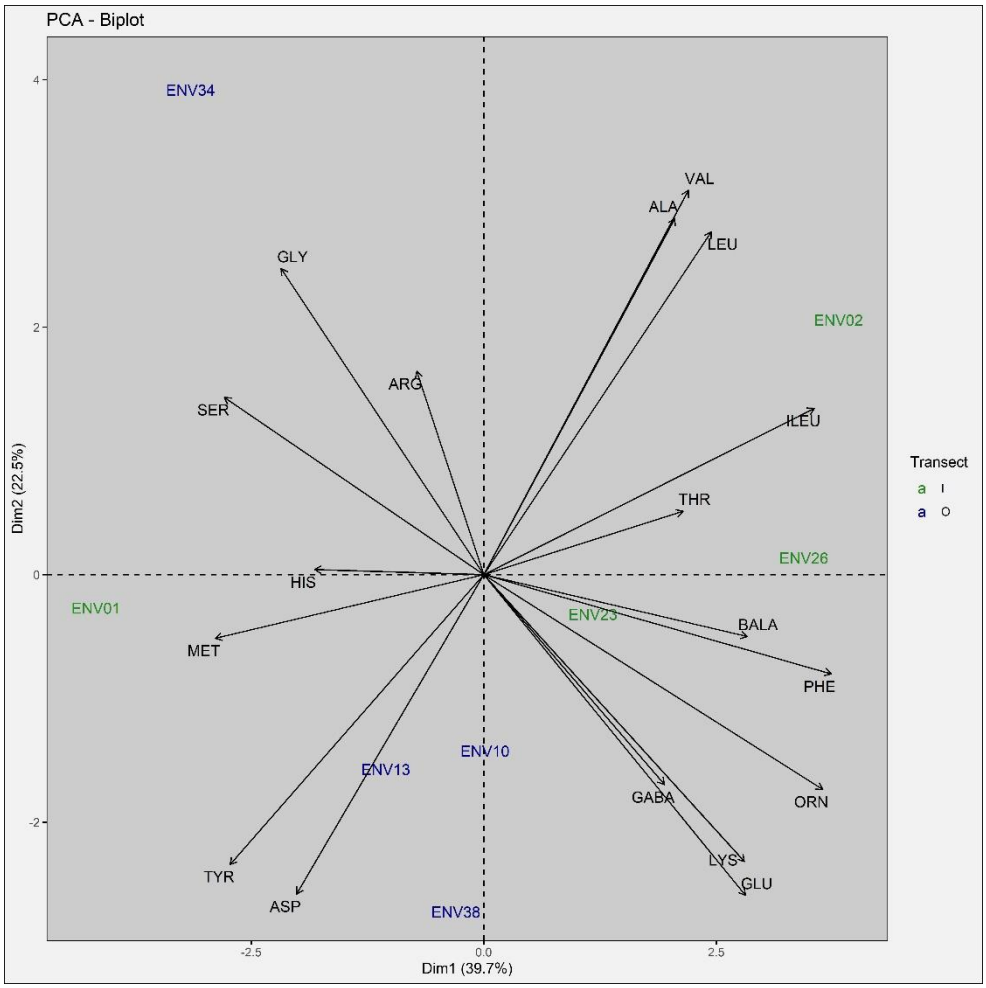

Figure 5.7: A PCA plot demonstrating the amino acid composition between samples collected along the inshore and offshore transects during CEND2018.

The total amino acids present in the sediment was on average higher in samples collected along the offshore transect, in comparison to the inshore transect (6329.92 ± 596.31 µg g^{-1} and 5054.78 ± 900.87 µg g^{-1} respectively; Figure 5.6 B). However, a t-test on

data transformed to the power of 3 indicated that the observed difference was not statistically significant (p = 0.344).

5.3.5. Degradation Index

The first axis of the PCA (Figure 5.8) explains 24.6% of the total variation and has positive coefficients for Glutamic acid (GLU), Histidine (HIS), Arginine (ARG), β-alanine (BALA), Alanine (ALA), Valine (VAL), Phenylalanine (PHE), Isoleucine (ILEU), Leucine (LEU) and Lysine (LYS), and negative coefficients for Aspartic Acid (ASP), Serine (SER), Threonine (THR), Glycine (GLY), ɣ-amino butyric acid (GABA), Tyrosine (TYR), Methionine (MET) and Ornithine (ORN).

The second axis of the PCA (Figure 5.8) explains a further 20.5% of the total variation and has positive coefficients for HIS, SER, THR, GLY, BALA, ALA, GABA, TYR, PHE, ILEU, ORN and LYS, and negative coefficients for ASP, GLU, ARG, MET, VAL and LEU.

Axis 1 has been interpreted to represent the organic matter degradation, so the scores on this axis can be considered as degradation state indicators. Samples which were collected along the inshore transect appear to fall along the positive side of the first axis, whereas samples collected along the offshore transect appear to fall on the left (negative) side of the first axis. Of the samples collected along the offshore transect the first horizon (0-1cm) of sample cores also has a less negative, or even positive (e.g. ENV10 and ENV38), axis 1 value. Whilst deeper horizons (2-3cm and 5-6cm) have a more negative axis 1 value.

The degradation index (DI, Figure 5.6 C) indicates that samples collected along the inshore transect (2.09 ± 2.70) is higher than that of samples collected along the offshore transect (-2.36 ± 1.16). Although a t-test indicated that the observed difference was not significant (p = 0.203).

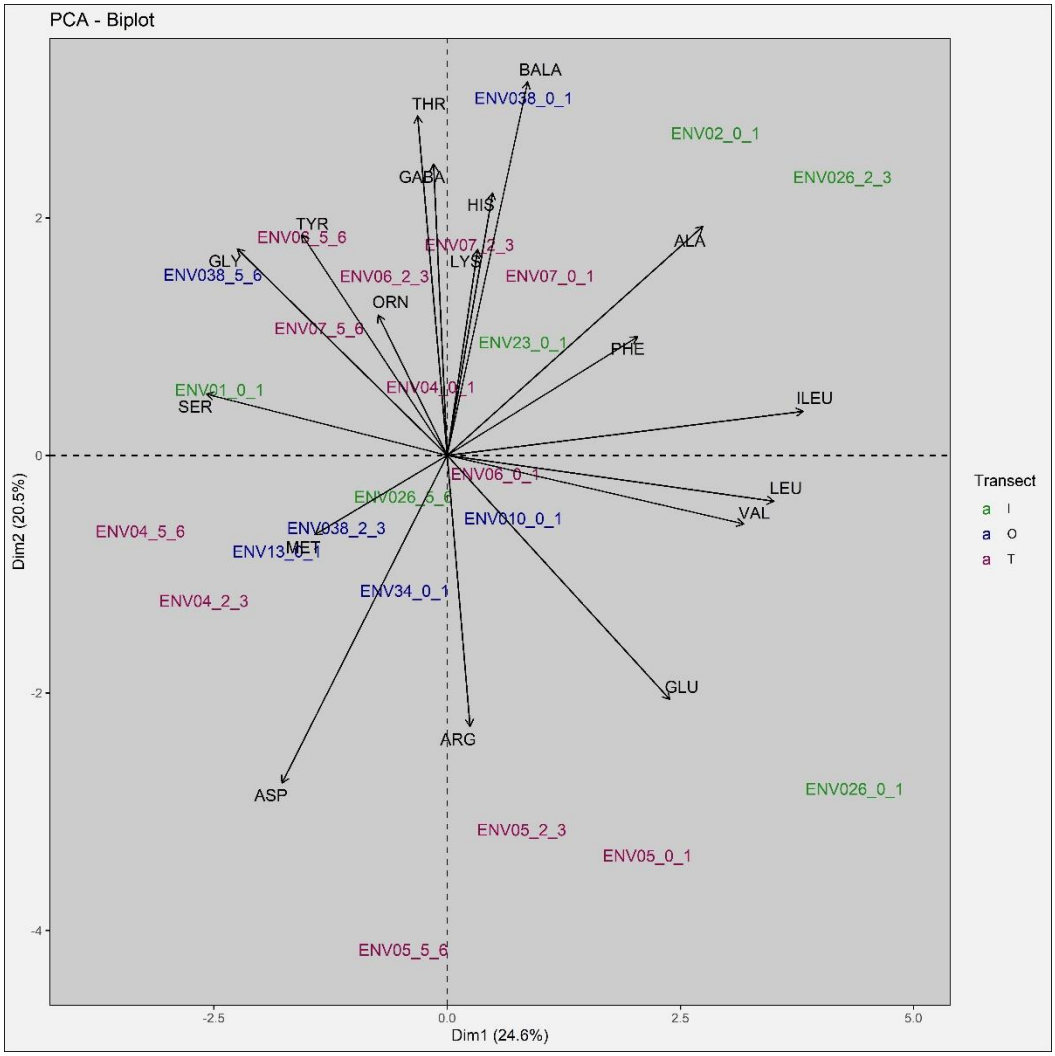

Figure 5.8: PCA biplot of sediment horizons (0-1cm, 2-3cm and 5-6cm) and amino acids identified within sediment samples.

Applying the factor coefficient, mean and standard deviation of amino acids calculated by Dauwe & Middleburg (1998) enables comparison in DI between samples collected during CEND2018 and other samples collected in the North Sea (Dauwe & Middleburg, 1998; Figure 5.9 A & B).

Figure 5.9 indicates that samples collected during CEND2018 have a DI similar to samples collected from Skagerrak, Friesian Front and German bight 2 by Dauwe & Middleburg (1998). Dauwe and Middleburg (1998) suggest that these samples are more degraded than samples with a positive DI (Bhgat A & B, Broad fourteens, and the German Bight 1). Therefore, based on this samples collected along the inshore transect, which have

a higher DI, are less degraded in comparison to samples along the offshore transect, which have a lower DI and are therefore more degraded.

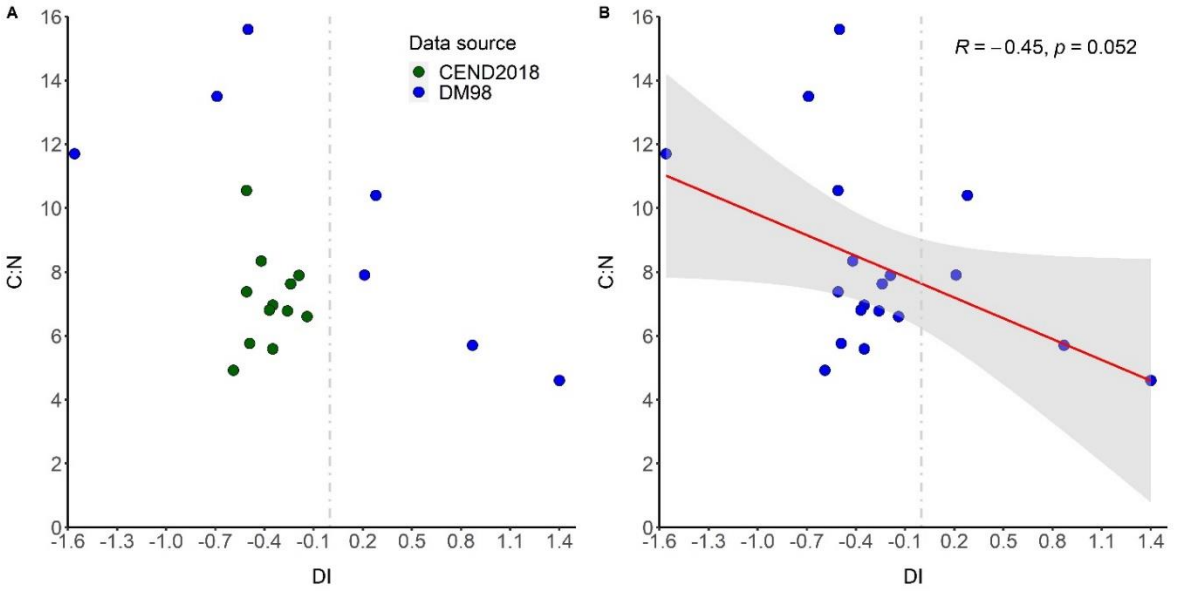

Figure 5. 9: Scatter plots demonstrating A) The DI of samples collected during CEND2018 in relation to the DI of other samples in the North Sea (DM98) as determine by Dauwe and Middleburg (1998), against C:N ratio and B) The relationship between DI and TOC:TN

5.4. Discussion

5.4.1. Environmental characteristics

Sampling locations along the inshore and offshore transects are found at similar depths with similar sediment grainsize compositions. However, differences in some physical characteristics were observed between inshore and offshore sampling locations, with sampling locations along the inshore transect generally having lower dissolved oxygen (DO) and salinity and higher levels of suspended sediments and temperature in comparison to sampling locations along the offshore transect. The lower DO observed inshore is consistent with higher temperatures, which are known to reduce the solubility of gases in the ocean, and lower salinity is consistent with distance from the coastline and therefore proximity to fresh water sources. However, despite being statistically significant, differences in these parameters between inshore and offshore sampling locations were relatively small, except for the modelled suspended sediment concentration. A significantly higher amount of suspended sediments was modelled for the sampling locations along the inshore transect

in comparison to the offshore transect. This higher amount of suspended sediments could be due to higher levels of productivity nearer the coastline, or due to water circulation and sediment reworking patterns (Luisetti et al., 2020). For example, the primary current pattern in the North Sea which transports carbon runs from the NW side of the basin along the coast of the UK and the northern European coastline and into the main carbon burial location for the basin, the Norwegian trench (Dauwe and Middelburg, 1998; Luisetti et al., 2020). However, the resolution of the modelled environmental variables is fairly coarse, meaning that they are not necessarily capable of capturing the fine scale variability frequently observed in the natural environment, especially with the size of the region of interest in this study. The results from this study do not follow the same trends as that outlined by Diesing et al. (2017). For example, Diesing et al. (2017) indicates that one of the most important predictors of organic carbon at the regional scale was the mud content of sediments. However, all samples collected during the CEND2018 survey which were used in this study had similar sedimentary compositions, despite also demonstrating variability in OM composition. This indicates that there is smaller scale heterogeneity within the marine sedimentary environment, which is not necessarily detected in larger scale, regional models. It is also possible that the time of year that samples were collected (December 2018) could have had an impact on the results, especially the degradation index calculated. This is because it is likely that OM which has entered the benthic environment, through phytoplankton blooms, has already been degraded. For example, studies which have analysed the surface waters of the North Sea and Celtic Sea have observed that concentrations of Chlorophyll-a are highest in the spring and autumn and lowest in the summer and winter due to the timing of phytoplankton blooms (Suratman et al., 2009; Carr et al., 2019), indicating that the supply of OM to the sedimentary environment will also vary seasonally. Suratman et al. (2009) also noted that during the winter there was more resuspended particulate organic matter within the water column, possibly due to increased mixing in the winter and therefore further processing of sedimentary OM. Boon & Duineveld (1998) undertook a study which aimed to understand the relationship between Chlorophyll-a supply to sediments in the southern and central North Sea and the response

of benthic communities. The authors found a similar trend in the sedimentary environment to that observed by Suratman et al. (2009) and Carr et al. (2019) in the water column. Boon & Duineveld (1998) found that the flux of Chlorophyll-a was highest in the spring and lowest in the winter and the oxygen demand by the benthic community was highest in mid-late summer. The authors concluded that fresh, labile carbon builds up in the spring, but is degraded by late summer. Therefore, it would be beneficial to undertake sampling within different seasons to gain a more complete understanding of benthic carbon dynamics throughout the year and provide the best possible advice in order to manage the resource effectively.

5.4.2. Organic Matter Source

Organic matter source was predominantly determined using the TOC/TN ratio and bulk sediment isotopes ($\delta^{13}C$ and $\delta^{15}N$). Generally, marine OM has a TOC/TN value between 4 and 10, and terrestrial plants have a value ≥20 (Meyers, 1994; Szymczak-Żyła and Lubecki, 2022). For bulk sediment stable isotopes $\delta^{13}C$ values between -19‰ and -22‰ are typical for marine organic matter and terrestrial C3 plants typically have values between -26‰ and -28‰ (Szymczak-Żyła and Lubecki, 2022). This information indicates that all organic C in CEND2018 sediment samples was primarily sourced from the marine environment, and not from the terrestrial environment. However, despite this there were differences between the inshore and offshore transects.

The TOC/TN ratio of samples collected along the Inshore transect was significantly higher than that of samples collected along the offshore transect, and the mean $\delta^{13}C$ was marginally higher than that of the offshore transect. The slightly lower $\delta^{13}C$ values observed along the offshore transect suggest that there was some terrestrially derived OM present within the offshore sediment samples, which is not what would be expected based on the proximity of the inshore transect to the coastline. If this is the case, then one explanation could be that terrestrial OM enters the system (e.g. fluvial/estuarine outputs and coastal erosion), is deposited onto the seafloor near the coastline, but is resuspended via hydrodynamic processes and/or anthropogenic activities (Smeaton and Austin, 2022) and then re-deposited further offshore where it may be more likely to be preserved. It is also

possible that any terrestrial OM present is preferentially preserved (Burdige, 2005; Huguet et al., 2008; Smeaton and Austin, 2022) to some extent along the offshore transect. However, it has been observed that there is overlap between bulk sediment properties (TOC/TN, $\delta^{13}C$ and $\delta^{15}N$) used to help identify OM source (Bianchi and Canuel, 2011), meaning that these methods alone are not capable of reliably indicating OM source. Therefore, without a more complete breakdown of the chemical composition of organic matter which could specifically identify terrestrially derived compounds, such as the use of lignin phenols, it is not possible to determine whether the observed differences in bulk sediment properties are due to the presence of more terrestrial OM along the offshore transect. Therefore, despite the observed slightly more negative $\delta^{13}C$ values along the offshore transect, the values fall within the accepted ranges of marine derived OM, and it must be concluded that the dominant source of organic matter along both transects is marine in origin.

It Is possible that the lower TOC/TN ratio observed in sediments collected along the offshore transect, compared to sediments collected along the inshore transect, could be due to a higher concentration of amino acids present in the samples, which are a key source of organic Nitrogen (Bianchi and Canuel, 2011). Vascular plants which are typically found in the terrestrial environment contain compounds rich in carbon such as cellulose, whereas marine algae have higher protein concentrations, which are rich in nitrogen (Carneiro et al., 2021). It has been observed that macroalgae, for example, generally has a TOC/TN ratio between 4 and 8 (Carneiro et al., 2021). Therefore TOC/TN ratios are also consistent with a dominantly marine source for sedimentary OM.

5.4.3. Degradation State

Offshore sediments had a higher concentration of amino acids compared to samples collected along the inshore transect, which could be linked to their higher concentration of TN, as well as higher TOC. Based on this information it appears that more OM is stored within offshore sediments. However, this information does not provide an insight into the quality of OM, this can be achieved through using degradation index.

The TOC/TN ratio and amino acid based degradation Index (DI) show that the difference in organic matter concentrations were accompanied by a difference in degradation state. It has been suggested that selective degradation of more labile OM alters the TOC/TN ratio (Kim et al., 2006; Lamb et al., 2006). For example, as previously mentioned higher plants have a higher TOC/TN ratio than marine OM (Meyers, 1994; Kim et al., 2006; Szymczak-Żyła and Lubecki, 2022). However, it has also been observed that the TOC/TN ratio of soil OM in the terrestrial environment, meaning terrestrial OM which has undergone degradation, can have a TOC/TN ratio similar to that of marine derived OM (Kim et al., 2006), suggesting that as OM is processed and becomes more degraded the TOC/TN ratio decreases. The DI for inshore samples was higher than that of samples collected along the offshore transect, indicating that these samples were less degraded in comparison to samples collected along the offshore transect. This suggests that despite more OM being present along the offshore transect, it is more degraded compared to sediment collected along the inshore transect which receives fresher/more labile OM.

The results from the amino acid-based degradation index support the suggestion in the previous section, that fresher, more labile OM could be entering the sediment in locations nearer the coastline through rivers, estuaries and marine primary production settling through the column (Smeaton and Austin, 2022). However, some of this labile OM will be degraded, resuspended and redeposited further offshore through biological, hydrodynamic and anthropogenic sediment reworking activities (Luisetti et al., 2020). This could be one explanation for results obtained in this study, which observed more, but more degraded OM in samples collected along the offshore transect. Smeaton & Austin (2022) also observed a higher amount of OM with a lower DI offshore, which could indicate that sediments further away from the coastline could be carbon stores for carbon which has been processed nearer shore, resuspended, and redeposited. However, the more degraded nature of sediments located along the offshore transect means that these sediments are more stable and any further decomposition of the OM within them will occur at a slower rate. Whereas, sediments along the inshore transect are more labile and therefore reactive,

suggesting that management of this resource should be focussed on locations closer to the coastline.

The higher OC content of the offshore sediments could have also been Influenced by the mean bottom temperature of the transect locations. Temperature is known to influence metabolic activities of marine fauna (Salo et al., 2020). The mean bottom temperature along the inshore transect was marginally higher than that of the offshore transect. This may increase the processing of OM closer to the coastline as it enters the coastal environment and slows the process of further degradation of OM which is deposited further offshore.

Samples collected during CEND2018 were collected from a relatively small spatial area within the northern North Sea. However, by applying the PC1, mean and standard deviation values calculated by Dauwe & Middleburg to CEND2018 amino acid data it was possible to understand the degradation state of OM within the CEND2018 study area relative to areas sampled by Dauwe & Middleburg (1998) within the wider North Sea. The results indicated that the sediment in the Northern North Sea study area was slightly less degraded than samples collected and analysed by Dauwe & Middleburg (1998) from Skagerrak (-1.561), the German Bight 2 (-0.502) and the Friesian Front (-0.689). These locations have been identified as deposition areas within the North Sea (Dauwe and Middelburg, 1998), meaning that these are locations where sediment is redeposited after being processed and resuspended elsewhere. In comparison samples collected during CEND2018 were more degraded than samples collected by Dauwe & Middleburg (1998) from Bhgat-B (1.397), Broad Fourteens (0.873), the German Bight 1 (0.276) and Bhgat-A (0.208). These sites have been described as production areas by Dauwe & Middleburg (1998), indicating that these are locations where fresher OM enters the marine system and has not yet undergone processing within the sedimentary environment. Based on this, the samples collected during CEND2018 fall in the middle of the DI spectrum indicated by Dauwe & Middleburg (1998) for the North Sea, although on the more degraded end of the spectrum. On the scale of the North Sea the results could indicate that the CEND2018 sampling area could fall within a deposition area within the North Sea. However, when

considering the scale of the CEND2018 study region there appears to be fine-scale variation which is not as apparent at the larger, regional scale of the North Sea. Understanding this fine-scale variability is essential when developing management priorities.

5.4.4. Importance of understanding carbon distribution and lability in coastal shelf seas and recommendations

There has been a growing focus on understanding carbon stocks within coastal shelf seas, due to the important role shelf seas play in processing and storing organic carbon (Luisetti et al., 2020). Anthropogenic activities, such as bottom trawling, undertaken in regions which contain significant carbon stocks could result in the release of carbon, in the form of CO_2, which has been left undisturbed for long periods of time (decades-centuries) and could eventually reach the atmosphere (Luisetti et al., 2020; Smeaton and Austin, 2022). Therefore, being capable of identifying regions within shelf sea environments which are most likely to be carbon stores is essential for the effective management of this resource. However, currently marine systems which sequester carbon are less well managed in comparison to terrestrial systems (Luisetti et al., 2020). Therefore, the need to understand the distribution of organic carbon within the marine environment has been identified, and studies have been undertaken to better understand the distribution of organic carbon. However, many studies have primarily focussed on the use of bulk organic carbon measures, for example Diesing et al. (2017) who spatially predicted the distribution of particulate organic carbon in surface sediments. To effectively manage carbon stocks within shelf sea environments there needs to be a better understanding of carbon source and degradation. This means that future research programmes undertaken by both universities and publicly funded bodies, need to shift their focus away from simply collecting TOC/POC data, which are a standard method of characterizing the seafloor.

Understanding carbon source and degradation, which can be achieved through using a combination of measures including TOC/TN, $\delta^{13}C$, $\delta^{15}N$ and amino acid-based degradation index, can help our understanding of carbon store dynamics and resilience and help target locations which should be prioritized for protection and/or management. The use of a variety of different analytical techniques can provide varying levels of information

on OM source and reactivity (Figure 6.9), in comparison to using a single approach (Bianchi and Canuel, 2011).

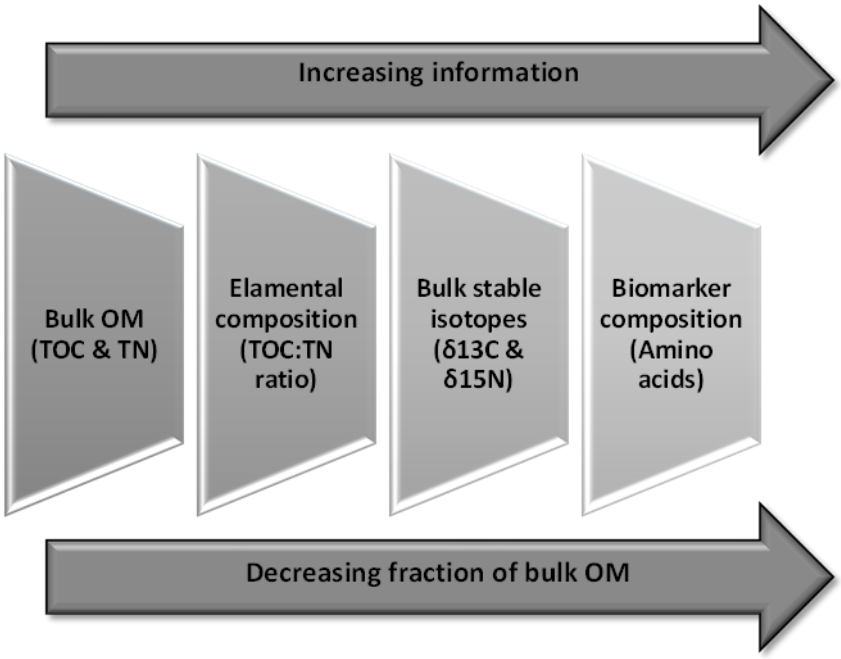

Figure 5.10: A diagram indicating the the amount of information provided by different OM characterization techniques and the proportion of OM that each of the methods represent. Adapted from Bianchi & Canuel (2011).

Regional scale OC distribution modes, such as that produced by Diesing et all. (2017), are important tools in identifying locations which should be targeted for higher resolution sample collection campaigns. Targeted sampling campaigns should be undertaken using an integrated approach, meaning that samples should be collected to thoroughly characterize the environment. For example, determining sediment particle size analysis (PSA), benthic macrofaunal composition which can help characterize how the sediment is reworked by the faunal community, sediment profile imagery which can indicate the depth of the sediment oxic layer, TOC, TN, $\delta^{13}C$, $\delta^{15}N$, amino acid composition, as well as high resolution acoustic data (bathymetry and backscatter) and video transects. It would also be important to create hydrodynamic model outputs at sufficient resolution for these small, targeted areas. Targeted surveys are essential as the marine environment is extremely heterogeneous over relatively small spatial scales, and this heterogeneity cannot be detected using models

created at the regional scale. For example, models created for the regional scale are most likely to be driven by large scale processes, such as distance from coastline, bottom temperature, and peak wave orbital velocity, as identified by Diesing et al. (2017). Finer scale models are more likely to be primarily driven by smaller scale bottom topography and sedimentary characteristics. Being able to produce spatial predictions of carbon which can characterize the distribution of carbon characteristics, such as OM source and degradation, within shelf seas sediments will aid the management of these systems. For example, through the production of marine protected areas (MPAs) which are designated based on the capabilities of locations to store carbon.

5.5. Summary

A variety of different methods have been used in this study to characterize the sediments within a region of the northern North Sea. Results indicate that the OM present within the study region is predominantly sourced from the marine environment, based on the TOC/TN and bulk isotope data. The data also indicate that based on the TOC more organic carbon is found further away from the coastline. However, the use of an amino acid based degradation index suggests that this carbon further away from the coastline is more degraded, and therefore potentially less vulnerable to further degradation in comparison to organic matter found closer to the coastline. Finally, better understanding the distribution, sources, lability, and vulnerability to degradation of carbon within shelf sea sediments will better inform policy and management strategies for shelf sea sediments with regard to sedimentary carbon stocks.

6. Discussion

To address the identified research gaps, this thesis used multidisciplinary techniques and manipulative laboratory experiments to understand the effects of ocean warming on ecosystem functioning, with particular regard to carbon I cycling, processing and storage in muddy marine systems. This thesis also aimed to address the current limited understanding of organic carbon composition and reactivity in shelf sea sediments.

To address the identified research gaps, this thesis investigated two primary objectives relating to carbon processi9ng and characterization in marine shelf sediments;

- Objective 1: To investigate the impact of temperature on biodiversity and ecosystem functioning in intertidal muddy sediments at Blackness (Firth of Forth, Scotland), with a focus on carbon processing (Chapters 2, 3 & 4).
- Objective 2: To characterize UK shelf sea sedimentary carbon within the northern North Sea (Chapter 5).

6.1. Examining Objective 1 – Benthic Ecosystem Functioning

Multiple factors are known to influence ecosystem functioning of marine sedimentary ecosystems, including environmental properties, species present and functional traits they possess (Figure 6.1). It is well established that temperature plays a key role in ecosystem health and functioning through driving the metabolism of individuals present, interactions between trophic levels, and distribution of species on a global level (Salo et al., 2020). The impact of temperature on biodiversity and ecosystem functioning relationships in intertidal muddy sediments from Blackness were examined using manipulative laboratory experiments described in chapters 2, 3 & 4. This study used intact sediment communities to determine the impact of temperature on community structure (taxon and traits), sediment reworking activities, ecosystem function (oxygen consumption and nutrient fluxes) and carbon processing capability.

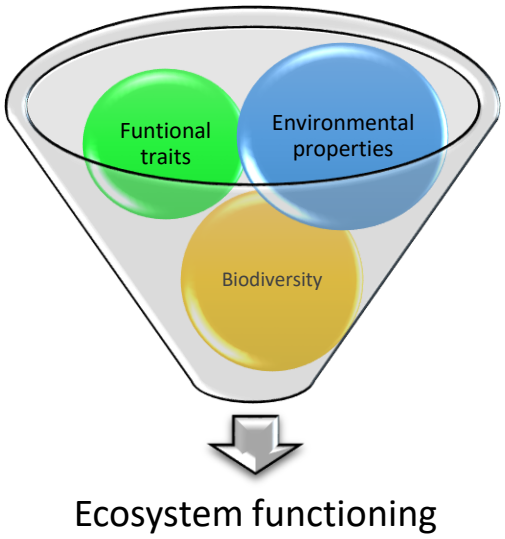

Figure 6.1: Factors which contribute to the understanding of ecosystem functioning

6.1.1. Benthic invertebrate community response to temperature

In Chapter 2 the overall trends in measures of taxon diversity indicate that total invertebrate abundance and biomass were not impacted by an increase of 3°C from ambient. However, an increase was observed in cores incubated at 21°C. Variability in abundance and biomass was also observed between taxa within a temperature treatment. This indicates that temperature does not uniformly impact the abundance and biomass of all benthic macrofauna in a uniform way. This thesis has identified several different explanations of why abundance and biomass of taxa may respond differently to temperature changes, including: 1) tolerance to the stressor; 2) change in food supply; and 3) stressor induced reproduction, indicating that the impact of temperature increases on community structure could be extremely complex. The use of intact benthic communities, within this study was able to provide a representation of how natural communities may respond to environmental change at a broader level, enabling more realistic change scenarios and responses to be established than more simplified experimentally manipulated conditions. The factors outlined all involve interactions between species, however due to the use of intact communities it was not possible to examine interactions in detail. However, as outlined in Chapter 3 the observed trends in total abundance and

biomass associated with temperature rises are likely attributed to a combination of these factors.

Understanding community composition and the interactions between species are the most important factors which drive many sediment biogeochemical properties (Ólafsson and Persson, 1986; Emmerson et al., 2001; Bolam et al., 2002). Therefore, it would be recommended that future work aims to better characterize the interactions between species within an intact community. Such information will be important in predicting the impacts of environmental change, including community change and stressor impacts on ecosystem functioning. However

In addition to traditional biodiversity measures (abundance and biomass) which indicate community composition, the use of functional traits provided information on how a community and individuals within the community may influence ecosystem functioning. Understanding the impact of environmental change on different functional traits, and their modalities, is important as it provides information on how the functioning of the system could be altered in the future (Bremner et al., 2006). The data reported in Chapter 3, although not significant over the time scales of this experiment, indicate that future temperature changes could result in a shift in functional trait composition of benthic communities in muddy intertidal sediments. The response of functional traits to temperature appeared to be more variable in comparison to that observed by community abundance and biomass. For example, surficial modifiers appear to be negatively impacted by temperature increases to 17°C and 21°C whereas biodiffussors appear to be positively impacted. The variability in trait response to temperature is most likely due to species-specific responses, and biomass weighted functional trait data being at a finer scale in comparison to community level biodiversity measures.

The use of functional traits to determine community response to environmental stressors is particularly useful when it is not necessarily feasible to measure ecosystem functioning at the rate or scale required. For example, Solan et al. 2004) developed a bioturbation classification of European marine benthic macrofauna and Wrede et al. (2018

& 2019) developed a classification for sediment ventilation activities. Such indices have been correlated with ecosystem functions including nitrogen fluxes (Wrede et al., 2018; Wrede et al., 2019). Therefore, such proxies may be useful in the absence of direct measurements of ecosystem functioning. Such indices only require the collection, identification, and measurement (abundance and biomass) of taxa present, which are routinely collected during offshore surveys. However, it has been noted that a species and/or individual does not necessarily express just one trait. The trait expressed by an individual can change, known as trait plasticity, when they are exposed to a stressor (Törnroos et al., 2015), although this information is not as well understood. Therefore, it is essential that more work is undertaken to better understand how a species trait expression changes in response to a stressor, to gain a better insight into the impacts of environmental change on ecosystem functioning. To achieve this, further experiments will need to be undertaken which focus on individual species, or a small number of species in a constructed community. Although this is contrary to the approach used in this thesis, these types of experiments are required to gain the fine scale knowledge which is required to feed into larger scale experiments at the community or site scale and to feed into the development and fine tuning of models.

6.1.2. Temperature influence on biodiversity and ecosystem functioning

Sediment reworking activities (Chapter 3), by marine benthic macrofaunal communities, are related to ecosystem functioning and are dependent on abundance and biomass in addition to trait expression (Queirós et al., 2013; Wrede et al., 2018; Wrede et al., 2019). Therefore, interpreting the higher abundance and biomass observed in cores incubated at higher temperatures alone, would indicate that sediment reworking activities and therefore ecosystem functioning rates also increase at higher temperatures.

Benthic macrofaunal oxygen uptake measurements demonstrated the same trend observed for benthic macrofaunal abundance and biomass. There was not a change in oxygen demand with an increase in temperature of 3°C above ambient (17°C), but there was an increase in cores incubated at 21°C, which supports the initial statement that faunal abundance and biomass are related to ecosystem functioning. However, in addition to

faunal abundance and biomass influencing measures of ecosystem functioning, such as oxygen uptake (Bolam et al., 2002), temperature also impacts the metabolic process rates (Salo et al., 2020) and demands of taxa present within benthic communities. Therefore, following future environmental change it is likely that under increased temperature scenarios, total biomass and abundance will increase, resulting in increased oxygen requirements by the community. This is in addition to the likely increased oxygen demand required to maintain the increased metabolic demands of the faunal community due to increased temperatures. Increased metabolic demands at higher temperatures by benthic macrofaunal communities, as indicated by faunal oxygen uptake, would also suggest that there will be an increased food demand (Brockington and Clarke, 2001; Clarke and Fraser, 2004), which could have a knock-on effect for sedimentary carbon storage.

Increases in oxygen demand could also be an indication of increases in macrofaunal sediment reworking activities. Proxies for sediment reworking activities, bioturbation and bioirrigation potentials (BP_c & IP_c), were calculated to act as indicators for the impact of temperature on sediment reworking and ventilation activities. Both these indices increased under both temperature manipulations explored in this experiment (17°C and 21°C). These data suggest that under increased temperature scenarios more sediment reworking and irrigation will occur. Sediment reworking activities play an important role in distributing and processing organic matter throughout the sediment column (Dauwe et al., 1998; Solan, Wigham, et al., 2004; Birchenough et al., 2012) and therefore carbon cycling and storage. Therefore, this increase in sediment reworking undertaken by the benthic macrofaunal community could explain the observed increased in oxygen demand. Direct measurement of bioirrigation, and by extension, sediment reworking activity was obtained using sodium bromide as a tracer. The sodium bromide results indicated that the rate of bioirrigation by the benthic community increased both in cores incubated at 17°C and 21°C, confirming the results obtained by calculating the IP_c.

The Impact of temperature on measures of ecosystem function as represented by bioturbation and bioirrigation, appears to differ to trends observed on the impact of temperature on total abundance and biomass from Chapter 2. As previously outlined the

abundance, biomass, and oxygen uptake of the macrofaunal community did not appear to be impacted by a temperature increase of 3°C (14°C to 17°C). In contrast, the sediment reworking activities undertaken by the community did appear to increase both in cores incubated at 17°C and 21°C. This indicates that although there was not an overall change in abundance, biomass or oxygen uptake in cores incubated at 17°C, there could have been a slight shift in the composition and or distribution of sediment reworking traits within the community.

The results provided In Chapters 2 & 3 on the Impacts of biomass, abundance, and ecosystem function (oxygen consumption and sediment reworking) indicate that the higher temperatures predicted to occur in the future will result in increases in the factors outlined (abundance, biomass, oxygen consumption and sediment reworking activities). This suggests that under future ocean warming scenarios the amount of carbon processed by the faunal community will increase. Although, information on abundance, biomass, and measures of ecosystem functioning (oxygen uptake and sediment reworking activities) do not give an indication of which pools OM enters once it has been processed.

6.1.3. Carbon processing by sedimentary communities.

To further explore how increasing ocean temperatures under warming scenarios will influence carbon processing, an isotope pulse-chase experiment outlined in Chapter 4 was conducted. The results indicate that the total amount of carbon processed by the benthic community increased with temperature, which is consistent with the observed increases in biodiversity and ecosystem functioning measures. However, the isotope tracing results give a further insight into where processed organic carbon will end up under warming climate.

The Isotope tracing experiment Indicates that the carbon is incorporated into macrofaunal biomass does not clearly change with temperature increases. An increase, although not significant, in biomass normalised faunal uptake was observed in cores incubated above ambient temperatures (17°C and 21°C). However, there was a large amount of variability within temperature treatments. Therefore, given the observed increase in total carbon processed by the benthic community this indicates that organic

carbon entering the benthic system is not necessarily being processed and retained by the macrofaunal community. Instead, carbon entering the benthic system is primarily being processed and remineralized to DIC (CO_2), as discussed in Chapter 4. There was also an increase, although not significant, in carbon retained in microbial biomass at higher temperatures, which could indicate that the bacterial growth efficiency is higher at increased temperatures, although this was not directly measured. Increases in bacterial growth efficiencies under higher temperature scenarios could indicate that microbial communities could increase their biomass, possibly through cell division or growth, under future environmental change scenarios. However, the relative role of the microbial community in carbon processing in this experiment is comparatively small when considering the role that remineralization to DIC plays. Increased remineralization of carbon to DIC at higher temperatures is also supported by the probable increased metabolic demands by the macrofaunal community (Chapter 4), due to observed increases in oxygen demand by the faunal community and higher levels of sediment reworking activities, in addition to the observed increase in total abundance and biomass (Chapter 3). Therefore, under higher temperature scenarios it is likely that more carbon entering marine sediments could be remineralized to DIC and therefore reduce the amount of carbon available to be stored within the sedimentary environment.

Overall, the isotope pulse-chase experiment indicates that under future warming scenarios it is likely that intertidal muddy sediments could produce higher amounts of CO_2 than they do today. This could suggest that it is possible that the source/sink dynamics of coastal sediments could shift in the future. Even if sediments remained a carbon sink under future environmental change scenarios, the results from this experiment indicate that sedimentary communities are likely to be under increased stress in the future, as indicated by increased respiration and remineralization of carbon. It is possible that a proportion of carbon dioxide produced could re-enter the atmosphere and further contribution to atmospheric temperature rises. In addition, it is important to consider that environmental stressors do not occur on their own, multiple stressors occur and influence the natural environment is different ways, such as synergistic, antagonistic, and additive stressor

interactions. For example, it has been observed that significant interactions exist between temperature and pCO_2 rather than purely additive effects (Bulling et al., 2010; Kroeker et al., 2013). Interactions between stressors introduces additional complexities when attempting to predict the impacts of future environmental change on ecosystem functioning, however research such as this begins to elucidate the direction of change in these benthic environments.

6.1.4. Experimental limitations & recommendations

This objective was examined using an experimental approach which aimed to take a step towards better replicating natural variability using intact sediment cores. However, the use of microcosms in environmental experiments have several limitations that are well characterized. Experimental approaches are not capable of truly representing the variability present within the natural environment (Skelly and Kiesecker, 2001). For example, microcosm experiments do not allow faunal communities to entirely express natural behaviours, especially as they cannot laterally migrate to avoid environmental stressors. For example, it has been suggested that reworking behaviour undertaken by macrofauna could be altered as a result of wall effects and the geometry of the microcosm, with species such as *Hediste diversicolor* observed to preferentially burrow along the walls of microcosms (Davey, 1994; Lindqvist et al., 2013). In addition to microcosms potentially influencing macrofaunal behaviour, they also exclude higher, more mobile trophic levels from experiments and do not consider impacts which could be occurring within the water column. Environmental change will also likely impact these aspects of the marine environment which will further influence the benthic macrofaunal and microbial environment. Despite these limitations, laboratory-based microcosm experiments play an important role in informing scientists on how environmental changes could impact ecosystem functioning and community dynamics (Benton et al., 2007). However, it needs to be acknowledged that laboratory-based experiments cannot fully replicate the complexities of the natural environment. Such factors which were not taken into account include natural temperature variability experienced by the site on a day-to-day basis, changes in water column depth, day-night cycles and the role of other benthic communities

including benthic micro-phyto benthos. The micro-phyto benthos could potentially be an additional carbon pool which has not been considered in this experiment. However, as cores in this experiment were incubated in the dark such communities will not have been undertaking photosynthesis, although they could have been undertaking some level of respiration, at least initially at the start of the experiment.

A further limitation of this experiment was the short incubation time scale used. Experiments undertaken over short time scales represent the short-term impacts of environmental change as they may not necessarily detect the long-term impacts of environmental change (Godbold and Solan, 2013). Therefore, in future studies it would be recommended that incubations are undertaken over a period of months rather than weeks, to gain a better understanding of the impacts of long-term exposure to temperature rises. However, the relatively short incubation length used in this experiment ensured the survival of cores, especially as cores were not being fed during the timescale of the experiment.

There was a large amount of variability in response variables within temperature scenarios, most likely due to the level of natural spatial and temporal variability present within the study site location. Therefore, a further recommendation would be to incubate a larger number of cores to take the heterogeneity of intertidal muddy sediments into account.

A final limitation for this experiment would be that the results from this small-scale study are not necessarily transferrable to the scale of the entire mudflat at Blackness, and that they are not transferrable between different study site locations, due to the heterogeneity of benthic environments within a single location (Kraan et al., 2009; Godbold et al., 2011). It has also been suggested that traits expressed by species can vary depending on location and therefore ecosystem functioning can vary between different sites (Wohlgemuth et al., 2017). Instead, they can be used as an indication of how future environmental change could impact benthic systems. However, to scale-up the results to entire sites, coordinated sampling and experimental programs need to be developed which consider different locations around the UK shelf, which would ideally include both intertidal

and offshore locations and a full range of sediment types, although this would require a large amount of space, money, time and coordinated collaborations between several different institutes (Benton et al., 2007).

6.2. Examining Objective 2 – Characterizing carbon in UK shelf sediments

In addition to understanding how environmental changes, such as temperature increases, could impact the processing of organic carbon within UK shelf sea sediments, it is important to better characterize the organic carbon present and its storage within the system. Better characterization of carbon over larger spatial scales will allow improved management and protection of this service provided by shelf sediment environments.

The characterization of UK shelf sea sedimentary carbon within the northern North Sea was examined in Chapter 5. This study collected offshore sediment samples from the northern North Sea which were analysed for TOC, TN, $\delta^{13}C$, $\delta^{15}N$ and sedimentary bulk amino acids, to better characterize the carbon present in terms of source and degradation.

6.2.1. Characterizing OM

Understanding OM sources, reactivity and fate within marine shelf sea sediments is essential for understanding the biogeochemical processes, such as carbon storage, occurring within the system, although this is challenging due to the temporal and spatial variability of OM distributed throughout the marine sedimentary environment (Smeaton and Austin, 2022). In this thesis the focus was on better characterizing carbon within marine surface sediments. A variety of different analytical techniques can be used to characterize organic matter sources and reactivity (Figure 5.10), which provide varying levels of information on OM source (Bianchi and Canuel, 2011).

Bulk OM methods (e.g., TOC and TN) provide the most basic indicators of OM, particularly TOC as approximately 50% of OM is comprised of carbon (Bianchi and Canuel, 2011). Combining bulk OM analyses with additional elemental compositions (e.g., TOC:TN ratio) provides a basic understanding of OM source (Meyers, 1997; Bianchi and Canuel, 2011) and therefore an assumption on the reactivity of the OM present as terrestrial OM is thought to generally be less labile and/or bioavailable in comparison to organic matter

derived in the marine environment (Smeaton and Austin, 2022). The use of bulk stable isotopes can be used as a complementary analysis to TOC:TN ratios to better characterize the source of OM in marine sediments (Bianchi and Canuel, 2011). However, due to the complexity of OM present within coastal systems it is common for there to be overlap in the isotopic signatures of different sources (Bianchi and Canuel, 2011). Therefore, the use of specific biomarkers (e.g., amino acids and lignin phenols) can provide more detailed and specific information (Bianchi and Canuel, 2011). It was planned that lignin phenol information would be obtained for samples collected within the northern North Sea study site. However, due to time constraints the data was not received in time to incorporate into this thesis. The use of lignin phenols would provide a specific signature of terrestrially derived OM.

Amino acids are considered to be a useful indicator of organic matter degradation (Dauwe and Middelburg, 1998; Wang et al., 2018). Dauwe & Middleburg (1998) developed the Degradation Index (DI) based on the amino acid composition of marine sediments, which indicated that more negative DI values indicated the presence of more degraded OM and more positive values indicated the presence of fresher and more labile OM. However, there is an outstanding question relating to the likelihood that OM will be stored or remineralized based on its DI. Better understanding the degradation state and age of OM present within marine sediments, and its ability to be stored or its vulnerability to further decay, will better inform policy makers and the development of management plans for protecting carbon within shelf sea sedimentary environments. In addition to understanding the degradation state of organic matter in marine sediments to inform management, it would be beneficial to better understand the age of carbon being stored within the sedimentary environment, and therefore gain an understanding of how long the carbon has been stored.

The use of multiple analytical techniques can provide more detailed information on OM source and degradation. However, in order for these data to be useful in the development of management plans for the benthic environment and inform policy makers

and decision makers they need to be presented in a way which is clear and informative to the end user (e.g. in the form of spatial predictive models).

6.2.2. Applications of data

The use of spatial predicative modelling techniques are useful tools to effectively communicate information on the distribution of many different factors, including species (Bergström et al., 2013; Bucas et al., 2013; Downie et al., 2013), habitats (Brown et al., 2011; Calvert et al., 2015; Brown et al., 2017; Ware and Downie, 2020) and carbon density and accumulation rates (Diesing et al., 2017; Diesing et al., 2021; Mitchell et al., 2021) within the marine environment. Spatial predictive modelling techniques use machine learning to determine the relationship between a set of spatial environmental predictor variables (e.g. bathymetry, mud content of sediment etc.) and observations/samples (e.g. TOC:TN ratio and the degradation index), the model created is then applied to predict the spatial distribution of the response variable at unsampled locations within the extent of the predictor variables (Diesing et al., 2017). This method was undertaken by Diesing et al. (2017) who predicted the distribution of particulate organic carbon (POC) using random forest modelling within the NW European continental shelf. The model created by the authors selected mud content of the sediment, annual average water column bottom temperature, eastings, distance to shoreline, gravel content of the sediment and peak wave orbital velocity as the most important variables driving the distribution of POC within the regional scale of the NW European continental shelf.

Regional scale models such as the one produced by Diesing et al. (2017) are useful in providing a large-scale view of the distribution of variables which characterize carbon within marine sediments. However, the marine environment is diverse, and this heterogeneity cannot necessarily be detected in large scale regional models, meaning that when considering the development of site-specific management plans and monitoring strategies finer spatial scales need to be considered (Elith and Leathwick, 2009; Ware and Downie, 2020). This is because a balance needs to exist between protecting the marine environment and the ecosystem goods and services it provides and allowing key stakeholders (e.g. the fishing industry) to use the environment to support their livelihood

and provide additional goods and services to the human population. Therefore, to achieve this, an understanding of the small-scale heterogeneity needs to be obtained.

This thesis collected data from within a small spatial area in the northern North Sea and characterized it using Bulk TOC, TN, TOC:TN ratio, stable isotopes ($\delta^{13}C$ & $\delta^{15}N$) and amino acids. These data in addition to environmental predictor variables (source outlined in Chapter 6) were used to produce an example of the kind of outputs which could be achieved if sufficient sampling campaigns and data were collected to better characterize the sediment carbon present within UK shelf seas. The two examples produced (Figure 7.3) are spatial distributions for the TOC:TN ratio and the degradation index, both were produced using a random forest approach. It is important to note that these two outputs are only being used as a demonstration of what could be possible given the collection of fit for purpose data. The number of samples collected in this study mean that it is not possible to produce statistically robust models and therefore, currently, cannot be used to advise on the management and protection of carbon within the study area. To achieve this, a targeted survey would need to be undertaken which collects sufficient samples across environmental gradients within the study area. Sufficient samples need to be collected to create models using training and test datasets and to undertake validation of models created.

The model selection process Indicated that the more Important variables for determining the distribution of the TOC:TN ratio were mean bottom wave velocity, L-S factor, and degree of closed depressions. The L-S factor is derived from the bathymetry and represents the slope length vs. the slope gradient and has been used in the terrestrial environment to predict probability of landslides and avalanches occurring (Aktas and San, 2019; Lucchese et al., 2021; Akay, 2021). However, in the context of the marine environment the L-S factor can give an indication of sediment stability. For example, locations which have a high L-S factor will have a long slope length and steep gradient, meaning that it is more likely that the sediment will be unstable and prone to movement when disturbed by physical factors such as wave action or trawling activities. Therefore, locations which have a high L-S factor will be areas where sediment, and therefore carbon, will not accumulate and locations where OC present could be further remineralized through

microbial activities. Closed depressions represent the degree to which a pixel in a raster is surrounded by higher ground and can be used as an indicator of a depositional environment, and therefore locations where sediment and OC will be likely to accumulate.

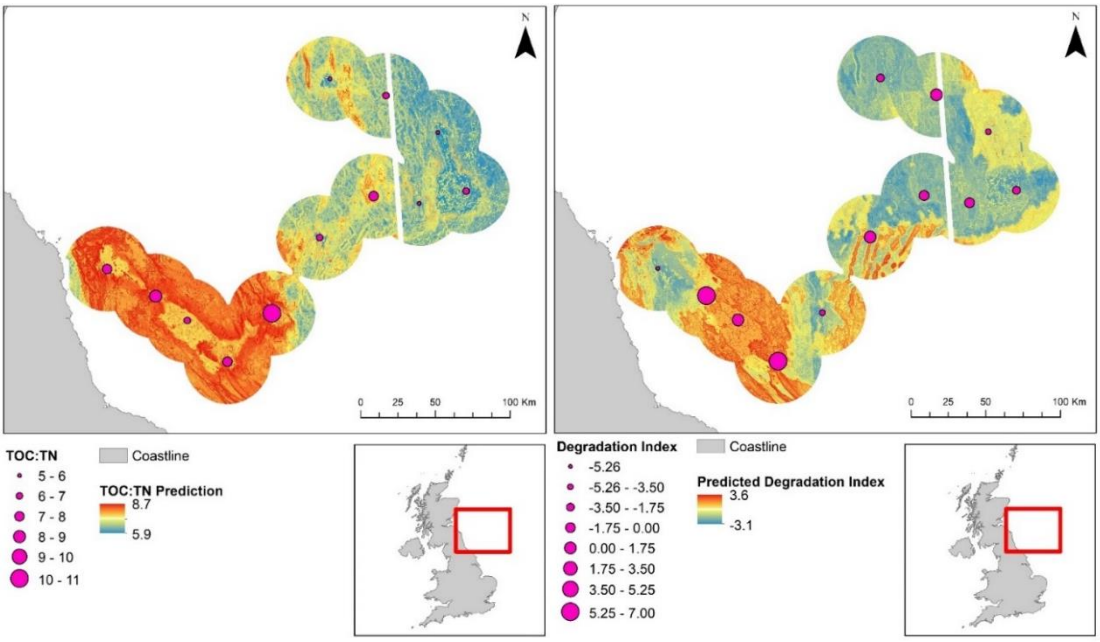

Figure 6.2: Examples of how data collected within this study could be applied to aid management of the marine sedimentary environment. Spatial predictions of the TOC/TN ratio and degradation index within the northern North Sea have been produced using a random forest modelling technique within "R" (version 4.0.1) and R studio (2022.02.2, build 485) using the randomForest package. Models were produced using point-based response variables (TOC/TN and DI) and environmental raster layers. The source of environmental raster layers used are outlined in Chapter 5.

6.2.3. Future work & Advice

Future work should focus on the more in-depth characterization of marine sedimentary carbon to better understand its sources and degradation state. This information would better inform policy makers when developing and justifying the development of additional MPAs to protect environments which store carbon or amend management plans for existing MPAs to incorporate the preservation of carbon storage environments. To achieve this the best approach would be to use regional scale spatial predictions of organic carbon distribution, such as that produced by Diesing et al. (2017), to target locations for high resolution data collection campaigns. Data collected from such survey campaigns would feed into the development of spatial predictions of organic carbon

within the selected locations. To be able to create statistically robust spatial predictions and determine the performance of models created, sufficient samples need to be collected. Future survey campaigns should not only consider the characterization of organic carbon on the seafloor but should also consider variables which may be contributing to the sedimentary environment and carbon processing, meaning that a holistic or integrated approach to survey planning is required. For example, the sediment composition and faunal communities present on and within the sediment and the activities which they undertake should be considered. Therefore, it would be advisable to use grab samples to collect PSA (particle size analysis) and macrofaunal samples and undertake video tows to characterize the communities which live on the sediment surface and the wider seafloor environment. These data would enable the production of fine scale resolution models of % Sand, % Mud and % Gravel within selected sites. Characterization of the sedimentary macrofaunal community could potentially enable the spatial characterization of sediment reworking activities (bioturbation and bioirrigation) using sediment reworking indices such as the bioturbation and bioirrigation potentials (Queirós et al., 2013; Wrede et al., 2018; Wrede et al., 2019). These activities have been shown to influence the processing of carbon within the sedimentary environment, as outlined in objective 1 (Chapters 4, 5 & 6).

During the CEND2018 survey (December 2018) on RV Cefas Endeavour, the weather meant that it was not possible to use the NIOZ corer, and instead a day grab was employed. The day grab was sufficient to collect surface sediment samples up to ~10cm in depth. Therefore, this method is only capable of characterizing the surface sediments which are still relatively fresh. It would be beneficial to collect samples which penetrate deeper into the sediment column using a corer. This information would provide additional information on how the organic carbon entering the sedimentary environment is preserved over time. Sediment samples collected using a corer should be sliced and each slice analysed for TOC, TN, stable isotopes and amino acids, in addition to other biomarker techniques such as lignin phenols. This information may be capable if indicating whether less labile OM, such as that derived from the terrestrial environment, is preferentially preserved over OM derived from the marine environment.

In addition to collecting sufficient physical sediment samples, it is also essential to consider the resolution of environmental predictor variables which will be used in the development of future models, and which environmental factors are suitable in driving carbon characteristics. Many environmental variables, particularly hydrodynamic variables, tend to be modelled at relatively coarse resolution which is predominantly due to the computing power required to model these variables at higher resolutions (Mertes and Jetz, 2018). This presents issues when predicting the distribution of a variable within a small area, such as that of an MPA and, therefore it would be recommended where feasible to produce higher resolution hydrodynamic models for specific sampling and modelling locations.

In addition to modelling and understanding the source of OM and degradation of OM present within UK shelf sediments, the accumulation and sedimentation rates of carbon also need to be better understood and potentially modelled, as these variables contribute to the preservation of OC on the seafloor. An improved understanding of these processes within UK shelf seas and their spatial representation would further improve the outputs produced by a variety of different models, including those produced using machine learning techniques and diagenetic models such as OMEXDIA (Soetaert et al., 1996), which can also help predict the impact that warming has on carbon processing and storage in the future.

7. References

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8. Appendices

8.1. Appendix 1

An outline of literature used to assign functional traits to species present within samples collected from Blackness intertidal mudflats (Firth of Forth, Scotland).

Species Name	Databases	References
Ampharete	MarLIN, 2006; Polytraits Team, 2013	Fauchald and Jumars, 1979; Rouse and Pleijel, 2006; Marine ecological surveys limited, 2008; Wrede et al., 2019
Ampharete acutifrons	MarLIN, 2006; Palomares and Pauly, 2022	(López-Jamar, González and Mejuto, 1987; Marine ecological surveys limited, 2008
Ampharete falcata	MarLIN, 2006	(Marine ecological surveys limited, 2008
Arenicola marina	MarLIN, 2006; Polytraits Team, 2013	(Goodrich, 1909; Fauvel, 1927; Newell, 1948; Beukema and De Vlas, 1979; Boon and Haverkamp, 1979; Reise, 1979; Fauchald and Jumars, 1979; Grossmann and Reichardt, 1991; Plante and Mayer, 1996; Plante, Mayer and King, 1996; Retraubun, Dawson and Evans, 1996; Hyslop and Davies, 1999; Österling and Pihl, 2001; Kristensen, 2001; Williams et al., 2004; Kristensen et al., 2012

Species Name	Databases	References
Capitella capitata	MarLIN, 2006	(Fauchald and Jumars, 1979; Rouse and Pleijel, 2006; Queirós et al., 2013; Wrede et al., 2019
Carcinus maenas	MarLIN, 2006	(Smiths, 1907; Crothers, 1968; Klein Breteler, 1975; Sanchez-Salazar, Griffiths and Seed, 1987; Scott-Fordsmand and Depledge, 1993; Thresher et al., 2000; Kuris, Torchin and Lafferty, 2002; Walton et al., 2002; Wrede et al., 2019
Cerastoderma edule	MarLIN, 2006	Iglesias et al., 1996; Queirós et al., 2013
Chaetozone	Polytraits Team, 2013	Marine ecological surveys limited, 2008; Queirós et al., 2013
Cirratulus cirratulus	Polytraits Team, 2013	Queirós et al., 2013
Eteone flava	MarLIN, 2006; Polytraits Team, 2013	Marine ecological surveys limited, 2008; Queirós et al., 2013; van der Wal et al., 2017
Glycera alba	MarLIN, 2006; Polytraits Team, 2013	Blackstock, 1980; Josefson, 1987; Arvanitidis et al., 1999; Marine ecological surveys limited, 2008; Queirós et al., 2013; Wrede et al., 2019
Harpacticoida		Dahms and Qian, 2004; Cnudde, 2013; Melic, 2015; Ma and Johnson, 2017

Species Name	Databases	References
Heteromastus filiformis	MarLIN, 2006; Polytraits Team, 2013; Annelida.net – A guide to information on annelids, and current worm research and researchers. http://www.annelida.net	Wolff, 1973; Cadee, 1979; Fauchald and Jumars, 1979; Zarkanellas and Kattoulas, 1982; Josefson, 1987; Arvanitidis et al., 1999; Österling and Pihl, 2001; Hertweck, Wehrmann and Liebezeit, 2007; Quintana, Tang and Kristensen, 2007; Marine ecological surveys limited, 2008; Can, Kevrekidis and Cihangir, 2009; Queirós et al., 2013
Hydrobia ulvae	MarLIN, 2006	Newell, 1962; Queirós et al., 2013
Idotea pelagica	MarLIN, 2006	Queirós et al., 2013
Macoma balthica	MarLIN, 2006	Queirós et al., 2013; van der Wal et al., 2017; Wrede et al., 2019
Magelona	Polytraits Team, 2013	Fauchald and Jumars, 1979; Marine ecological surveys limited, 2008; Queirós et al., 2013; Mortimer and Mackie, 2014; van der Wal et al., 2017
Mya arenaria	MarLIN, 2006	Marine ecological surveys limited, 2008; Queirós et al., 2013
Mya truncata	MarLIN, 2006	Marine ecological surveys limited, 2008; Queirós et al., 2013
Mytilus edulis	MarLIN, 2006	Queirós et al., 2013; Wrede et al., 2019
Nematoda		Traunspurger, Bergtold and Goedkoop, 1997; Schratzberger, 2012; Queirós et

Species Name	Databases	References
		al., 2013; Schratzberger and Ingels, 2018; Schratzberger et al., 2019
Nemertea		Queirós et al., 2013; van der Wal et al., 2017; Wrede et al., 2019
Nephtys hombergii	MarLIN, 2006; Polytraits Team, 2013	Fauvel, 1927; Jones J D, 1955; Olive et al., 1981; Davey and George, 1986; Pedersen, 1991; S. F. Rainer, 1991; Sebastian F. Rainer, 1991; Arndt and Schiedek, 1997; Desroy, Retiere and Thiebaut, 1998; Mazik and Elliott, 2000; Österling and Pihl, 2001; Desroy and Retière, 2003; Williams et al., 2004; Marine ecological surveys limited, 2008; Bouchet et al., 2009; Queirós et al., 2013; Wrede et al., 2019
Ophiura	MarLIN, 2006	Queirós et al., 2013; Wrede et al., 2019
Owenia fusiformis	MarLIN, 2006	Queirós et al., 2013; Wrede et al., 2019
Pholoe spp.	MarLIN, 2006; Polytraits Team, 2013	Marine ecological surveys limited, 2008; Queirós et al., 2013; Wrede et al., 2019
Pygospio elegans	MarLIN, 2006; Polytraits Team, 2013	Queirós et al., 2013; van der Wal et al., 2017
Retusa obtusa		Smith, 1967; Emmerson and Raffaelli, 2004; Queirós et al., 2013

Species Name	Databases	References
Scoloplos armiger	MarLIN, 2006; Polytraits Team, 2013	Fauvel, 1927; Anderson, 1959; Reise, 1978, 1979; Fauchald and Jumars, 1979; Volckaert, 1987; Dauvin, 1998; Arvanitidis et al., 1999; Österling and Pihl, 2001; Kruse, 2003; Williams et al., 2004; Kruse, Strasser and Thiermann, 2004; Volkenborn and Reise, 2006; Marine ecological surveys limited, 2008; Queirós et al., 2013; Wrede et al., 2019
Tubificoides spp.	MarLIN, 2006	Queirós et al., 2013; van der Wal et al., 2017

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8.2. Appendix 2

A table outlining how traits and their modalities were assigned to taxa present within cores collected from Blackness in 2019. 0 = no affinity and 3 = high affinity.

Category	Size			
	XS	S	SM	M
Ampharete	0	3	2	0
Arenicola	0	0	2	3
Capitella	0	1	2	0
Carcinus	0	0	3	0
Cerastoderma	0	0	3	0
Chaetozone	0	3	0	0
Cirratulus	0	0	0	3
Harpacticoida	3	0	0	0
Eteone	0	0	1	2
Glycera	0	0	3	0
Heteromastus	0	0	0	3
Hydrobia	3	0	0	0
Idotea	1	3	0	0
Macoma	0	3	0	0
Magelona	0	0	2	1
Mya	0	0	3	0
Nematoda	3	0	0	0
Nemertea	0	0	2	2
Nephtys	0	0	0	3
Ophiura	0	3	0	0
Owenia	0	0	3	0
Pholoe	0	3	0	0
Pygospio	0	3	0	0
Retusa	0	3	0	0
Scoloplos	0	0	0	3
Tubificoides	0	0	3	0

Category	Feeding type				
	Predator/Scavenger	Deposit feeder	Suspension feeder	Herbivore	Omnivore
Ampharete	0	2	1	0	0
Arenicola	0	3	0	0	0
Capitella	0	2	0	0	0
Carcinus	2	0	0	0	3
Cerastoderma	0	0	2	0	0
Chaetozone	0	2	0	0	0
Cirratulus	0	2	0	0	0
Harpacticoida	0	1	0	2	0
Eteone	2	0	0	0	0
Glycera	2	0	0	0	0
Heteromastus	0	2	0	2	0
Hydrobia	0	2	0	0	0
Idotea	0	0	0	0	3
Macoma	0	2	2	0	0
Magelona	0	1	2	0	0
Mya	0	0	2	0	0
Nematoda	2	2	0	0	0
Nemertea	3	0	0	0	0
Nephtys	2	0	0	0	0
Ophiura	0	2	2	0	0
Owenia	0	2	2	0	0
Pholoe	3	0	0	0	0
Pygospio	0	2	2	0	0
Retusa	3	0	0	0	0
Scoloplos	0	2	0	0	0
Tubificoides	2	1	0	0	0

Category	Mobility type			
	Fixed Tube	Limited Movement	Slow, free movement	Free movement
Ampharete	0	0	3	0
Arenicola	0	3	0	0
Capitella	0	3	0	0
Carcinus	0	0	0	3
Cerastoderma	0	3	0	0
Chaetozone	0	3	0	0
Cirratulus	0	3	0	0
Harpacticoida	0	0	0	3
Eteone	0	0	3	0
Glycera	0	0	3	0
Heteromastus	0	3	0	0
Hydrobia	0	0	3	0
Idotea	0	0	3	0
Macoma	0	3	0	0
Magelona	0	2	0	0
Mya	0	3	0	0
Nematoda	0	3	0	0
Nemertea	0	0	3	0
Nephtys	0	0	3	0
Ophiura	0	3	0	0
Owenia	3	1	0	0
Pholoe	0	3	0	0
Pygospio	3	0	0	0
Retusa	0	0	3	0
Scoloplos	0	0	3	0
Tubificoides	0	0	3	0

Category	Reworking type				
	Epifauna	Surficial modifier	Upward/Downward Conveyor	Biodiffusor	Regenerator
Ampharete	0	0	3	0	0
Arenicola	0	0	3	0	0
Capitella	0	0	3	0	0
Carcinus	0	0	0	0	3
Cerastoderma	0	3	0	0	0
Chaetozone	0	3	0	0	0
Cirratulus	0	3	0	0	0
Harpacticoida	2	2	0	0	0
Eteone	0	0	0	3	0
Glycera	0	0	0	3	0
Heteromastus	0	0	3	0	0
Hydrobia	0	3	0	0	0
Idotea	0	3	0	0	0
Macoma	0	3	0	0	0
Magelona	0	2	0	0	0
Mya	0	3	0	0	0
Nematoda	0	3	0	0	0
Nemertea	0	0	0	3	0
Nephtys	0	0	0	3	0
Ophiura	0	3	0	0	0
Owenia	0	3	0	0	0
Pholoe	0	3	0	0	0
Pygospio	0	0	3	0	0
Retusa	0	3	0	0	0
Scoloplos	0	0	0	3	0
Tubificoides	0	0	0	3	0

Category	Burrow type			Irrigation depth (cm)			
	Epifauna/internal irrigation	Open Irrigation	Blind-ended burrows	0-2	2-5	5-10	>10
Ampharete	0	0	3	3	0	0	0
Arenicola	0	3	1	0	0	2	3
Capitella	0	0	3	3	0	0	0
Carcinus	3	0	0	3	0	0	0
Cerastoderma	3	0	0	1	2	0	0
Chaetozone	0	0	3	3	0	0	0
Cirratulus	3	0	0	3	0	0	0
Harpacticoida	3	0	0	3	0	0	0
Eteone	3	0	0	3	0	0	0
Glycera	0	0	3	0	2	1	0
Heteromastus	0	0	3	0	0	0	3
Hydrobia	3	0	0	3	0	0	0
Idotea	3	0	0	3	0	0	0
Macoma	3	0	0	2	2	3	0
Magelona	0	0	3	3	0	0	0
Mya	3	0	0	2	1	0	0
Nematoda	3	0	0	3	0	0	0
Nemertea	0	3	0	0	3	0	0
Nephtys	0	0	3	3	0	0	0
Ophiura	3	0	0	3	0	0	0
Owenia	0	0	3	3	0	0	0
Pholoe	3	0	0	3	0	0	0
Pygospio	0	0	3	1	1	3	0
Retusa	3	0	0	3	0	0	0
Scoloplos	0	0	3	0	0	0	3
Tubificoides	0	0	3	0	3	0	0