

Understanding the drivers of carbon sequestration in Scottish seagrass

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A thesis submitted in partial fulfilment of the requirements of Edinburgh Napier
University, for the award of Doctor of Philosophy

Signed Declaration

The work in this thesis has not been submitted for any other degree or professional qualification and is the result of my own independent work.

Details on all published data is also listed in Appendix 1

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Dani Whitlock

Date 10/06/2021

Abstract

Seagrass meadows are found in coastal environments worldwide and over the last decade they have been celebrated as powerful natural carbon sinks. Whilst the global importance of seagrasses to carbon sequestration and storage is accepted in general, there is wide variability in the data between sites and species, and the drivers of this variation are poorly understood. In the UK, seagrass meadows occur in estuaries, sea lochs and lagoons in settings with a diverse range of environmental and physical conditions. Few published studies have examined seagrass carbon in the UK; those that have, show large differences in carbon stocks between sites, which are not simply explained by the presence or absence of seagrass. Hence, there is a need to understand the reasons for variation in carbon stocks between seagrass sites to inform fundamental science and policy. This thesis explores three potential drivers of variation in carbon storage: decomposition, sediment characteristics and rates of burial. Effects of seagrass on decomposition were experimentally examined using a standardised material, tea litter, and the relationships between decomposition rate, sediment microbial communities and physical properties, such as particle size, were examined. Surprisingly, there was no evidence for slowed decomposition under seagrass compared with bare mudflat areas, rather seagrass habitats showed statistically significant enhanced rates of decomposition, at least for shallow (15 cm) depths. This may be linked to changes in the microbial fauna associated with seagrass.

Sediments within seagrass meadows were compared to adjacent bare mudflat areas to investigate the influence of seagrasses on sediment characteristics. Overall, sediment stability, explored through the use of a cohesive strength meter and shear vane, is lower in mudflat areas than in seagrass meadows. Sediment grain size analysis found no statistically significant evidence to support the hypothesis that seagrass meadows enhanced finer grained particle deposition where it is present, however the influence of *Z.noltii* on associated sediment grain size was more variable. Differences in carbon storage may be explained by long term enhancement of carbon burial within seagrass beds due to enhanced rates of sedimentation. The

utility of radionuclide dating (using anthropogenic radionuclide markers ^{137}Cs and ^{241}Am) as a method to detect sedimentation rates within intertidal seagrass beds was tested. However due to sediment mixing within the intertidal zone it was not a reliable method for sediment dating at the field site.

The most widely adopted method used to measure carbon stocks in marine sediments is Loss on Ignition (LOI). However, this method as applied in marine and coastal sediments has not been standardised, which has led to large discrepancies in the combustion temperatures used across studies (375-550°C), and consequent difficulty in comparing between studies. Therefore, this thesis also conducted an experimental validation of LOI to explore the effects of combustion at a range of temperatures that have been used regularly in marine and coastal sediment studies. The use of a stepped combustion approach helped differentiate between organic and inorganic losses. Although 550°C is the most commonly used temperature across sediment LOI research, the main findings identified that its use may lead to an overestimate of organic matter (OM) within samples, whilst the use of 450°C is likely to lead to an underestimation, as the largest volume of CO_2 is combusted at 500°C.

Overall, this thesis explored eleven sites (ten intertidal meadows and one subtidal) across Scotland. Carbon stocks were quantified for all eleven sites, including nine where no previous records are available. West coast intertidal seagrass organic carbon (OC) stocks ranged between 29.86 ± 0.77 - 120.03 ± 13.72 Mg C/ha, whilst the subtidal *Z. marina* OC stock was 36.96 ± 0.84 Mg C/ha. These values were within a similar range of previous OC stocks data for Scottish seagrass meadows. However, the additional sites did not support previous research findings and seagrasses did not appear to significantly enhance the mudflat's carbon sequestration. Nonetheless 50 cm profile depths indicated higher carbon stocks within seagrass meadows when compared to adjacent bare mudflat areas in Firth of Clyde meadows. The expected correlation between seagrass meadows and increased carbon stocks was not consistent across sites. In this study higher OC stocks were found in mudflat areas in five of the eleven sites investigated. The study provides insight into potential drivers of carbon sequestration and the role seagrasses play in carbon storage.

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Abbreviations

| Abbreviation | Description |
|-------------------|---|
| BEA | Beaully Firth |
| CAR | Firth of Clyde (Cardross) |
| CROM | Cromarty Firth |
| CUT | Dornoch Firth (Cuthill) |
| EDE | Eden Estuary |
| EYN | Eynhallow |
| FOR | Firth of Forth |
| HUNT | Hunterston Sands |
| KIRK | Kirkcudbright |
| MON | Montrose Basin |
| ROCK | Rockcliffe |
| RYA | Loch Ryan |
| SOL | Solway Firth |
| SOU | Southannan Sands |
| TAY | Firth of Tay |
| d.p. | Decimal place |
| ha | Hectare |
| kPa | Kilopascal |
| Mg | Megagram |
| Mt | Million tonnes |
| Nm ⁻² | Newton/square meter |
| OTU | Operational Taxonomic Unit |
| Pg | Petagrams |
| SVS | Shear vane-derived strength |
| Tg | Terragram |
| ALAGEL | Alanine-gelatine solution spiked with ¹³ C-alanine |
| C | Carbon |
| CaCO ₃ | Calcium carbonate |
| CET | Critical erosion threshold |
| CHN | carbon hydrogen nitrogen |
| CO ₂ | Carbon dioxide |
| CSM | Cohesive strength meter |
| DBD | Dry bulk density |
| FCS | Fluorescence correlation spectroscopy |
| FeCO ₃ | Siderite |
| GEL | Gelatin solution |
| GLYGEL | Glycine-gelatine solution |
| HCl | Hydrochloric acid |
| IC | Inorganic carbon |
| IRMS | Isotope Ratio Mass Spectrometer |
| k | Decomposition rate |
| LOI | Loss on ignition |

| | |
|-------------------|---|
| MgCO ₃ | Magnesite |
| NGS | Next generation sequencing |
| OC | Organic carbon |
| OM | Organic matter |
| OTUs | operational taxonomic units |
| POM | Particulate organic matter |
| PSA | Particle size analysis |
| PVC (corer) | Polyvinyl chloride |
| TC | Total carbon |
| TG-DTA | Thermogravimetric-differential thermal analyses |
| TN | Total nitrogen |
| TOC | Total organic carbon |
| TOM | Total organic matter |
| WOS | Web of Science |
| EEZ | Exclusive Economic Zone |
| IFLI | Inner Forth Landscape Initiative |
| MCA | Marine Consultation Area |
| MPAs | Marine protected areas |
| NSA | National Scenic Area |
| RSPB | Royal Society for the Protection of Birds |
| SAC | Special Area of Conservation |
| SBCF | Scottish Blue Carbon Forum |
| SPA | Special Protection Area |
| SWT | Scottish Wildlife Trust |
| UNEP | United Nations Environmental Programme |

Chapter 1 Introduction

1.1 The importance and status of coastal habitats²

Coastal habitats, including mangroves, saltmarshes, seagrass meadows and mudflats, are where terrestrial and marine environments meet and transfer materials. The coastal zone is a unique landscape influenced by both marine and terrestrial features, and the coastal habitats within it, help support an array of ecosystem goods and services (Barbier et al., 2011; Economidou, 2021; Figure 1-1). The role of coastal habitats is estimated to be of high value when compared to other marine and terrestrial habitats (Costanza et al., 1997; de Groot et al., 2012; Martínez et al., 2007). Costanza et al. (1997) estimated the total annual value of mangroves forests per hectare to be US\$19,580 ha, ~8.5 times higher than all other tropical and temperate forests. However the areal extent of mangroves is ~0.7%, of all total tropical forests (Giri et al., 2011). These coastal vegetated habitats cover <0.5% of the sea bed (Nellemann et al., 2009) and yet provide some of the most important ecosystem services.

1.1.1 Ecosystem services

Vegetated coastal habitats are often described as 'biogeomorphic' because they have the ability to modify the physical landscape of the coast (James et al., 2021; Reijers et al., 2020), due to their structural complexity, for instance, seagrass meadows (Fonseca and Cahalan, 1992; Zhu et al., 2021) and saltmarsh grasses are able to attenuate tidal flow (Stark et al., 2015). Thus vegetated coastal habitats can have a strong influence on sediment deposition and erosion thresholds (Carr et al., 2018; Méndez et al., 1999). The reduced velocity of tides and winds hitting coastal habitats lowers the risk of flooding and damage to coastal communities from storm surges (Badola and Hussain, 2005; Cunha et al., 2021; Neumann et al., 2015). Also by reducing tidal flow, vegetated coastal habitats trap organic matter (OM; Gillis et al., 2014), nutrients (Kumara et al., 2010) and anthropogenic pollutants (Jordan et

² Additional published review:

Huxham, M., Whitlock, D., Githaiga, M., Dencer-Brown, A., 2018. Carbon in the coastal seascape: How interactions between mangrove forests, seagrass meadows and tidal marshes influence carbon storage. *Curr. For. Reports* 4, 101–110. <https://doi.org/10.1007/s40725-018-0077-4>

al., 2003; Lamb et al., 2017). Excess nutrients and anthropogenic pollutants can have wide lasting effects on biodiversity, but vegetated coastal habitats play a key role of regulating and cycling these inputs (Hussain and Badola, 2008). Lamb et al (2017) found a 50% reduction in the relative abundance of potential bacteria pathogens, when using 16S ribosomal RNA gene from meadows across four Indonesian islands compared with sites where seagrass was absent. Hussain and Badola (2008) identified that agriculturists in the Bhitarkanika Conservation Area (India) valued nutrient retention within mangrove forests as the second highest secondary function of the habitat, with fish production as highest. Further to this, the carbon sequestration potential within coastal vegetated habitats has been highlighted over the last decade (McLeod et al., 2011).

Due to their stability, vegetated coastal habitats are highly productive habitats that supports dense and diverse communities of epibenthic and epifaunal species, for some, or all life stages (Lee et al., 2014; Maxwell et al., 2017), particularly when compared with adjacent unvegetated areas (Beck et al., 2001). Their varied above ground structures and complex root systems help support a plethora of residential and migratory species. Lefcheck et al. (2019) conducted a meta-analysis of 1600 peer-reviewed studies which highlighted the importance of nearshore structured habitats, including vegetated habitats, for juvenile density, growth and survival. Vegetated habitats showed the highest promotion of density and growth of juvenile populations than other structured habitats (Lefcheck et al., 2019). In addition to the provisional service, vegetated habitats provide for fish and invertebrates, they are commercially important if harvestable species utilise these habitats (Heck et al., 2003; Unsworth et al., 2019). In the UK, nine species of commercially important fish species are known to use *Zostera marina* meadows as a juvenile habitat (Bertelli and Unsworth, 2014) and therefore the conservation management of these habitats could help support fisheries.

| | | | |
|--|--|-----------------------------------|---|
| Primary production – critical role as food | Canopy structure – environment for habitat, refuge and nursery for lots of species | Epiphyte and epifaunal substratum | Nutrient and contamination filtration |
| Sediment trapping | Sediment stability | Oxygen production | Organic matter production, exportation and accumulation |
| Wave and current dampening | Seed production and vegetation expansion | Carbon sequestration | Self sustaining ecosystem |

Figure 1-1 Important ecosystem services vegetated coastal habitats provide.

1.1.2 Pressures on coastal habitats

Since the onset of industrialisation there have been human induced imbalances to the natural carbon cycle causing the greenhouse effect. Coastal areas have seen particularly intense anthropogenic activity and change, with population density significantly higher than non-coastal areas (Small and Nicholls, 2003). In Europe alone over 70 million people live on the coastline and globally the UN estimates 40% of the world's population live within 1 km of the coast (Martínez et al., 2007; Ondiviela et al., 2014). The combination of population and economic growth has created a strain on the provision of these ecological services. For example, eutrophication and overexploitation of fisheries, including physical disturbances due to dredging and trawling, are widespread (Cullen-Unsworth and Unsworth, 2013; McLeod et al., 2011). In 2018, an estimated 179 million tonnes of seafood was extracted from the marine environment for aquaculture production, human consumption as well as non-food uses (FAO, 2020). The average annual rise in seafood consumption is 3.1% (compared with annual world population growth of 1.6% between 1961-2017; FAO, 2020) and there are major concerns about the direct and indirect (such as extraction of feed for aquaculture) impacts this is having.

A further pressure, land-use change has resulted in large losses and degradation of intertidal habitats worldwide (Lovelock and Reef, 2020; Waycott et al., 2009). Saltmarsh loss has been largely attributed to land reclamation for agricultural practices (Almeida et al., 2014; Hobbs

and Shennan, 1986), whilst mangroves have been relied upon for food, timber and fuel (Alongi, 2002). Pendleton et al. (2012) estimated that 0.15-1.02 Pg CO₂ yr⁻¹ has been lost across vegetated habitats (mangrove forests, seagrass meadows and salt/tidal marshes) directly due to land use change; with 0.05-0.33 Pg of CO₂ yr⁻¹ being released from seagrass meadows based on an annual area loss between 0.4-2.6%. In addition to the anthropogenic pressures extensive losses to seagrass meadows have occurred due to outbreaks of 'wasting disease' in the early 1930s (Green and Short, 2003). With sea level increasing by 3.3 mm per year and warming by an average of 1.02°C (GISTEMP, 2021) it is unclear how these coastal ecosystems will continue to provide essential services that are critical for climate adaption and resilience, when they are also vulnerable to climate change.

Despite widespread knowledge of the main threats to coastal and marine habitats, there is limited understanding of the impacts of these threats at local scales and in specific sites. Extensive long-term mapping and monitoring of coastal and marine habitats is limited, but in recent years due to rapid advancements in technology, increasing affordability and innovative application, aerial imagery, satellite imagery, drones and high-definition (HD) scanners are being more frequently used to monitor and map these habitats (Fletcher et al., 2009; Uhrin and Townsend, 2016; Yang et al., 2020). Data covering larger temporal and spatial scales would help identify appropriate conservation status for areas and identify the most disturbed habitats (Godet et al., 2009). Waycott et al. (2009) estimated that seagrass meadows have declined at a rate of ~7% per annum since the 1990s, which if true would make seagrasses amongst the most rapidly declining of all global habitats. Other estimates of global decline are more modest (for example Pendleton et al. (2012) suggested 0.4-2.6% yr⁻¹) but all estimates show alarming losses. The growing pressures of sea-level rise and rising sea surface temperatures, combined with intensifying human use and direct impact, are predicted to increase the risk of physiological stress and loss of suitable substrate for seagrass and other coastal ecosystems (Cullen-Unsworth et al., 2014; Davis et al., 2016; Short and Neckles, 1999).

1.2 Blue carbon

The ocean is a critical component of the global carbon cycle and its potential to act as a carbon sink is recognised globally (Duarte et al., 2005b; Nellemann et al., 2009).

Approximately 86% of all organic carbon that has been buried in the ocean during the Holocene is preserved in coastal sediments (Berner, 1982; Burdige, 2005; Hedges, 1992). Vegetated coastal ecosystems -mangroves, salt marshes and seagrass meadows, the so-called blue carbon habitats (Grimsditch et al., 2013; Nellemann et al., 2009) - are amongst the most highly efficient natural carbon sinks, with exceptional carbon densities and rates of carbon burial (Fourqurean et al., 2012; Hedges and Keil, 1995; McLeod et al., 2011). The important role that coastal ecosystems play in both the marine and global carbon cycles is due in part to the anaerobic nature of coastal sediment (Zonneveld et al., 2010). The perpetually saturated state of the substrate creates reduced oxygen conditions, this decreases rates of microbial decomposition and consequently creates optimal conditions for OM accumulation (Kearns et al., 2016). Coastal vegetated habitats typically also show higher sedimentation rates than unvegetated coastlines, which also contribute to carbon storage by helping to bury carbon and by bringing carbon sources associated with the sediment (Potouroglou et al., 2017). In addition, blue carbon habitats are often highly productive, generating large amounts of autochthonous carbon that may become permanently buried in sediment (Figure 1-2).



Figure 1-2 Blue carbon infographic (Permission to use for non-commercial purposes was granted by the Artist (Campbell, n.d.))

Unlike terrestrial habitats, such as rainforests, blue carbon habitats do not become saturated in carbon due to their vertical accretion and have greater rates of carbon burial because they are situated in fast sediment accumulation environments and calm tidal flows (Chmura, 2013;

Duarte et al., 2005b; McKee et al., 2007; McLeod et al., 2011). Nelleman et al. (2009) estimated that these unique ecosystems could mitigate the effects of up to 50% of the CO₂ emissions from the global transport sector. The importance of mitigating climate change through marine ecosystem management to enhance carbon sequestration is recognised at an international level and is now receiving increasing policy attention (Burrows et al., 2017; Garrard and Beaumont, 2014; McLeod et al., 2011; Nellemann et al., 2009; O'Sullivan et al., 2011; Sifleet et al., 2011). However, the capacity of these habitats to sequester and store carbon varies widely depending on region, species and other site-specific characteristics.

Seagrass meadows are estimated to cover the greatest area of the three blue carbon habitats, with estimates for total global coverage ranging from 300 000 km² to 600 000 km² (Duarte et al., 2005). Using a combination of organic carbon content measures from 946 seagrass meadows across the world, Fourqurean et al. (2012) estimated that the seagrass organic carbon pool (within the top 1 m of sediments) is between 4200-8400 Tg C worldwide (0.014 Tg C/km²). Comparatively, Houghton, (2007) estimated within the top 1m of terrestrial soils, over an area 250 times larger (150 000 000 km²), contained 1 500 000 to 2 000 000 Tg C (0.01-0.013 Tg C/km²).

Despite the acknowledgement of the potential of these blue carbon habitats to mitigate against climate change, in addition to other ecosystem services, they are still poorly protected through legislation and international conventions and agreements (Brisman, 2011; Williams, 1996). Marine protected areas (MPAs) are a driving force in management and protection of important coastal and marine ecosystems. They are defined as areas where certain activities and uses are prohibited or restricted for the purpose of protecting and managing habitats, conserved for economic resources, species and habitat protection and biodiversity conservation. Approximately 7.68% of the ocean now lies within MPAs and coverage has rapidly grown in the last 14 years (Figure 1-3; UNEP-WCMC, 2019). Within known distributions of the three blue carbon habitats, only 43% of mangroves, 32% of saltmarshes and 26% of seagrasses lie within MPAs (UNEP, 2020).

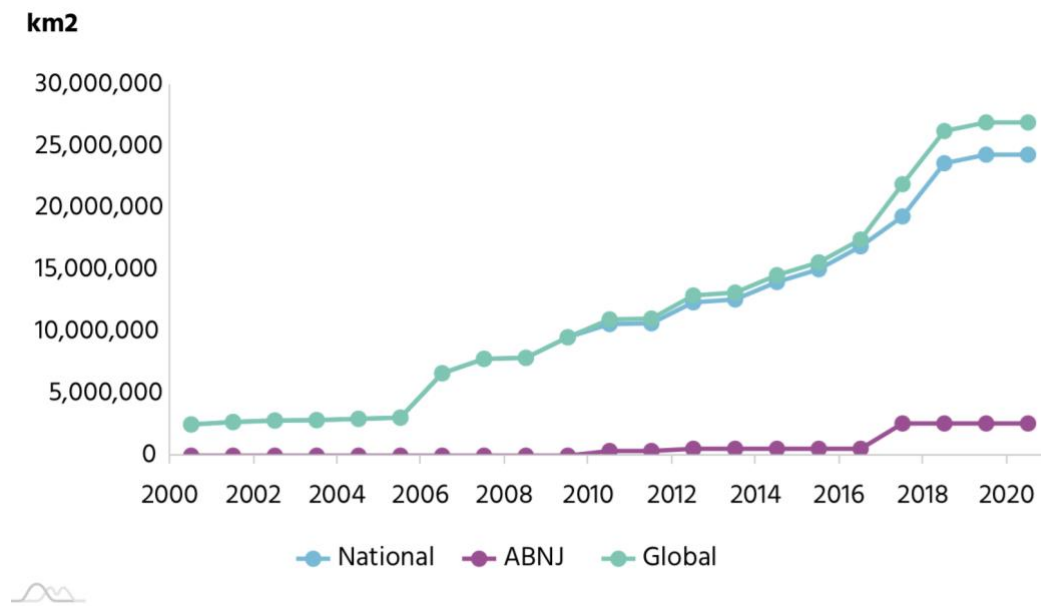


Figure 1-3 Growth in marine protected area coverage (km²) over the last 20 years, where 'National' represents coastal waters extending out to the limits of the Exclusive Economic Zone (EEZ), 'ABNJ' represents marina areas beyond national jurisdiction of any one country and no one country is solely responsible for management (UNEP-WCMC, 2019).

1.2.1 Blue carbon in a Scottish context

With 83% of the total territorial area of Scotland comprising of sea, utilising and enhancing coastal ecosystems and the marine environment to offset CO₂ emissions is a plausible approach to help meet greenhouse gas emission targets. Burrows et al. (2017) estimated that there are 18 million tonnes (Mt) of organic carbon in the top 10 cm of marine sediments and a further 1738 Mt of inorganic carbon stored in shell material within Scottish seas. Marine Scotland, with partners Nature Scot, the Lyell centre and seven Scottish Universities, launched the Scottish Blue Carbon Forum (SBCF) in 2018 to measure the ability of Scotland's marine environment to store CO₂ and mitigate against the effects of climate change; hence there is growing policy focus within Scotland on the contribution of blue carbon towards Scotland's goals of net-zero carbon emissions by 2045.

1.3 Seagrass meadows

Seagrasses are marine flowering plants that form extensive meadows in shallow intertidal and sub-tidal waters. Seagrass meadows are widely distributed all over the world, except Antarctica (Short et al., 2007). Seagrasses cover ~1% of the ocean, and account for 15% of all buried organic oceanic carbon (Laffoley and Grimsditch, 2009). The genus *Zostera*, meaning eelgrass, has the most widespread distribution of any seagrass and includes 16

species (Green and Short, 2003; Guiry and Guiry, 2019). Whilst seagrasses are in the spotlight due to their unique ability to naturally sequester carbon from both its own vegetation and surrounding environments it provides a plethora of other benefits from supporting biodiversity to nutrient cycling and even coastal protection (Figure 1-1)

1.3.1 Scottish seagrasses

Scotland's shallow marine intertidal and subtidal coasts host approximately 20% of all north Europe's seagrass meadows (Green and Short, 2003). The two species of *Zostera* found in Scotland are common eelgrass (*Zostera marina* L.) and its intertidal morphotype, narrow-leaved eelgrass (*Zostera marina* var. *angustifolia*); and dwarf eelgrass (*Zostera noltii*). *Z. noltii* is found extensively across the east coast of Scotland, which is dominated by large estuarine habitats, it is also found in large beds in the Solway Firth, Firth of Clyde and Loch Ryan on the west coast. *Z. marina* whilst predominately a subtidal species found extensively across the west coast of Scotland and Orkney is also found in the lower intertidal range of some estuaries including Solway Firth, Tayport, Beaully and Montrose Basin.

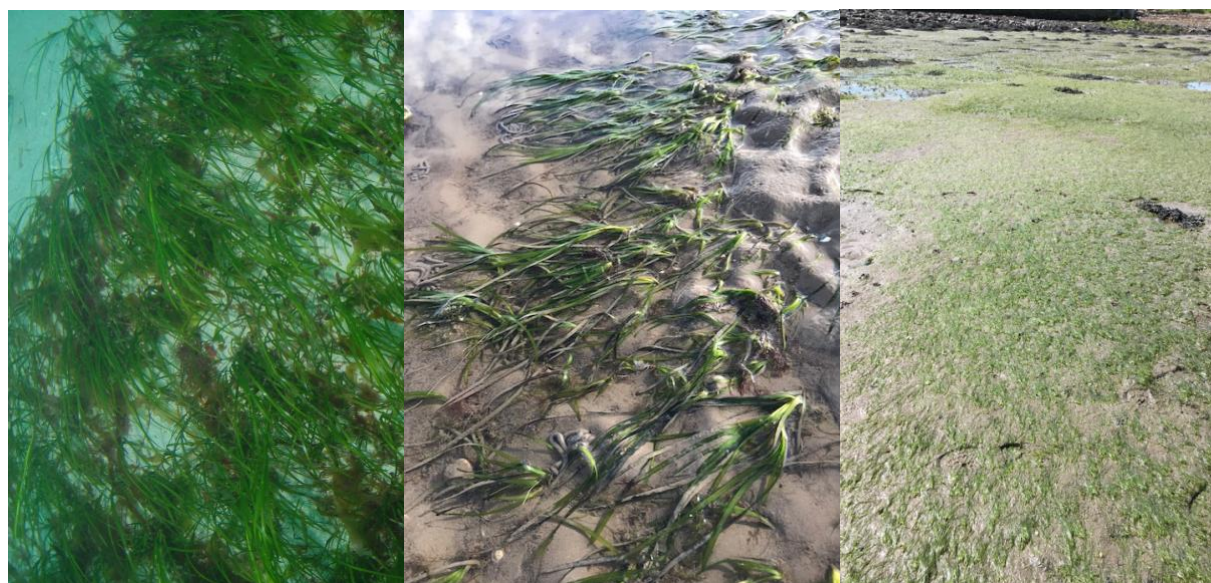


Figure 1-4 *Zostera marina* (subtidal), *Zostera marina* (intertidal) and *Zostera noltii* (left to right)

Seagrass beds are believed to be the dominant blue carbon habitat in Scotland, although considerable uncertainty about true spatial extent of seagrass beds remains (Burrows et al., 2017). Potouroglou (2016) conducted a carbon assessment of intertidal seagrass meadows across the east coast of Scotland and predicted that within the upper 50 cm of seagrass sediments ~91 200 Mg of organic carbon (OC) is stored across all Scottish seagrass beds

(including subtidal meadows). The small number of studies on seagrass within the UK have reported high variability in OC stocks between sites, regardless of species (Green et al., 2018; Lima et al., 2020; Potouroglou, 2016). Green et al. (2018) found subtidal *Z.marina* OC stocks across sites in England were between ~98-140 Mg C/ha however at one site, Drakes Island, had a significantly higher standing OC stock (380 Mg C/ha) even though all sites are found in the south of England. Similarly, Potouroglou (2016) found intertidal seagrass within two estuaries to be much more carbon rich than in other east coast areas. Seagrasses occur in a range of different exposed and sheltered environments, with differing geomorphological and sedimentary characteristics, as well as living in intertidal and subtidal zones. Therefore, there are a wide range of interacting physical, chemical and biological factors that potentially control carbon sequestration within and surrounding seagrass meadows; increasing our understanding of which of these factors are important, and how they might interact, will aid effective management of these coastal habitats and help understand their potential role in carbon policies.

1.4 Research aims

There are large variations in carbon densities across Scottish seagrass meadows and in the differences found in carbon concentrations between vegetated and associated un-vegetated areas within the same estuary. There is therefore a need to understand the key influencing factors which determine these differences as well as implications of this science for policy and management. This thesis has the following broad research aims.

- To increase our understanding of stocks and variability of carbon in a range of Scottish seagrass meadows and to explore how the presence of seagrass affects carbon concentration compared with appropriate un-vegetated areas of mudflat.
- To explore how carbon decomposition, post-deposition, is influenced by variations in microbial communities and physical properties.
- To determine the age (sedimentation rates), resilience (labile or refractory) and origin (through $\delta^{13}\text{C}$ isotopes) of accumulated carbon within seagrass meadows.
- Propose management, protection and restoration measures for Scottish seagrass habitats.

1.4.1 Research Questions

- I. Why are there such large differences in the impacts of seagrass on carbon density between sites in Scotland?
- II. How long is carbon stored for in seagrass sediments and what determines storage times? What does this tell us about persistence of seagrass patches?
- III. What is a defensible range of carbon storage in seagrass sediments?

1.4.2 Study sites

This PhD focuses on understanding six main seagrass meadows; three east coast meadows; Drum Sands, Firth of Forth (FOR), Eden estuary (EDE), Tayport, Firth of Tay (TAY), and three west coast meadows; Auchencairn bay, Solway Firth (SOL), Loch Ryan (RYA) and Cardross, Firth of Clyde (CAR). Two of those sites (FOR and SOL) are used across all main data chapters, whilst EDE, TAY, CAR and RYA are excluded from chapter 5. Four additional west coast intertidal sites include: Hunterston Sands (HUNT), Southannan Sands (SOU), Kirkcudbright (KIRK) and Rockcliffe (ROCK) and one subtidal site (EYN) also feature in multiple experiments (excluding chapter 4 and 5). Finally, four additional east coast sites are included in the grain size work; Montrose Basin (MON), Cromarty, Cromarty Firth (CROM), Beaulay, Beaulay Firth (BEA) and Cuthill, Dornoch Firth (CUT) cores (Figure 1-5).

All samples used for organic matter/organic carbon stocks analyses were analysed within two weeks of sediment core extraction to reduce temporal confounding. Although due to volume of cores collected the samples were taken across a range of months and years that will have had an influence on the data (Table 1-1). All cores were collected using polyvinyl chloride (PVC) cores except SOL, ROCK cores collected in 2016, SOL combined core and the cores collected in 2014. The cores collected in 2014 and 2016 were collected by a previous PhD student using a Russian peat corer. The SOL combined core was also collected using a Russian peat corer. A detailed description on sampling method can be found in Chapter 3.

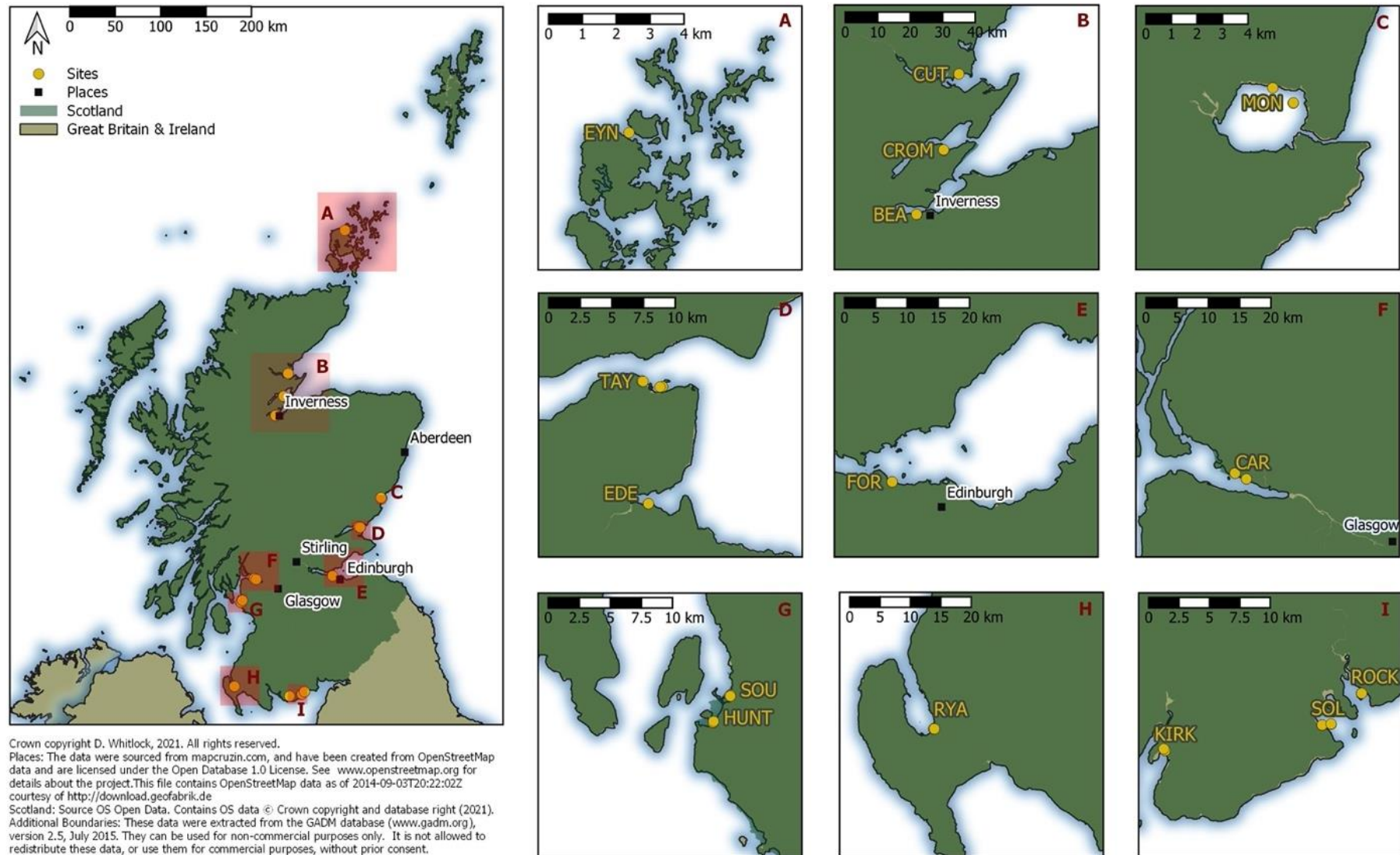


Figure 1-5 Map of all sites used across the thesis

Table 1-1 Summary of all site sample cores used across thesis and when they were sampled

| Site | Month | Year | Total seagrass cores | Total mudflat cores | Species |
|--------------|------------|------|----------------------|---------------------|------------------------------|
| FOR | July | 2019 | 3 | 3 | <i>Z.noltii</i> |
| EDE | July | 2019 | 3 | 3 | <i>Z.noltii</i> |
| TAY | July | 2019 | 3 | 3 | <i>Z.noltii</i> |
| SOL | Sept | 2016 | 1 | 1 | <i>Z.noltii and Z.marina</i> |
| SOL combined | Jan | 2017 | 1 | 1 | <i>Z.marina</i> |
| SOL deep | July | 2018 | 3 | 3 | <i>Z.marina</i> |
| RYA | June | 2018 | 3 | 3 | <i>Z.noltii</i> |
| CAR | Aug | 2018 | 4 | 3 | <i>Z.noltii</i> |
| HUNT | June | 2018 | 3 | 3 | <i>Z.noltii</i> |
| SOU | June | 2018 | 3 | 3 | <i>Z.noltii</i> |
| KIRK | July | 2018 | 3 | 3 | <i>Z.noltii</i> |
| ROCK | Sept | 2016 | 2 | 2 | <i>Z.noltii and Z.marina</i> |
| EYN | June | 2017 | 3 | 3 | <i>Z.marina</i> |
| TAY | June -Sept | 2014 | 1 | 1 | <i>Z.marina</i> |
| MON | | | 1 | 1 | <i>Z.noltii</i> |
| CROM | | | 1 | 1 | <i>Z.noltii</i> |
| BEA | | | 1 | 2 | <i>Z.noltii and Z.marina</i> |
| CUT | | | 1 | 1 | <i>Z.noltii and Z.marina</i> |

1.4.2.1 Firth of Forth – Drum Sands

The Firth of Forth is an SSSI situated in the south east of Scotland stretching from Alloa to Dunbar. The drainage basin cover is as far north as Ben Lomond from the River Forth and to the west of Stirlingshire the River Teith. The firth is host to a range of important coastal habitats underlain by Carboniferous strata. The Inner Forth to the west of the Forth bridges has been heavily altered and reclaimed in the past for both agriculture and industrial purposes, as much as 50% is believed to have been reclaimed in the last 200 years (Nature Scot, 2011). Over the last 15 years there has been a drive to restore intertidal and coastal habitats, with large areas of the Inner Forth being bought by the Scottish Wildlife Trust (SWT) and the Royal Society for the Protection of Birds (RSPB) and the launch of the Inner Forth Landscape Initiative (IFLI) in 2014 which encompasses 202 km² of the firth from river

to the inter-tidal zone (Stirling to Rosyth). In the outer firth, to the east of the Forth bridges, the mudflats are sandier, supporting many feeding birds, and rocky shorelines are more dominant.

The seagrass meadow used in this study is based at Drum Sands, just west of Crammond beach in a sheltered bay. Drum Sands is home to a large patchy seagrass meadow. This monospecific seagrass habitat of *Z.noltii* has been extensively researched for multiple projects making it an excellent choice for not only a decomposition study but also analysis of microbial makeup of the habitat.

1.4.2.2 Firth of Tay & Eden Estuary – Tayport and Eden and Montrose Basin

These two estuaries are key habitats within a complex geomorphological area. The River Tay has undergone minimal human modification though it is very exposed to tidal influences and as a result has a complex pattern of erosion and deposition (Bates et al., 2004). Tayport bay is at the mouth of the firth with a manmade promenade surrounding its south and southwest sides. The bay is composed of a large expanse of sand and shingle home to a vast seagrass bed, primarily composed of *Z.noltii* with *Z.marina* present in smaller patches. The sample area is in the centre of the bay, away from the manmade promenade. The smaller Eden Estuary lies close to the Tay but is very different; it is sheltered from tidal influences, and as a result is home to a unique host of habitats including saltmarshes, reedbeds and seagrass beds creating optimal conditions for an abundance of important plant and animal communities (Bates et al., 2004; JNCC, 2001). Eden estuary is a Site of Special Scientific Interest (SSSI), meaning it is an offence to damage its protected natural features. It is also designated as a Special Protection Area (SPA) for supporting populations of migratory species and a Ramsar wetland area. The sample area lies within the Edenside flats, one of the most sheltered areas of the bay and a patchy *Z.noltii* meadow runs along the upper intertidal mudflat. Montrose basin (MON) is a large enclosed intertidal estuarine basin on the mouth of the River Esk. Both *Z.marina* and *Z.noltii* are found here.

1.4.2.3 Firth of Clyde – Cardross , Hunterston Sands and Southannan Sands

The entrance to the firth is an impressive 26 miles (42 km) wide. Its catchment area stretches as far east as Crianlarich and its main river, the River Clyde, flows through the

densest city in Scotland, Glasgow (McIntyre et al., 2012). All three sites are underlain by Carboniferous strata. Hunterston Sands is situated next to a decommissioned coal power station and is only separated from Southannan Sands by a quay wall. The main seagrass meadow chosen for this study is situated on a mudflat in Cardross. The area surrounding this site is industrial with a train station within 10-15 m of the mudflat, the mudflat is shallow supporting a small patchy *Z.noltii* meadow. During the high summer tides, algae accumulates across the mudflat in thick matts. The presence of summer algae could have a negative effect on the seagrass bed, by essentially smothering seagrass fronds, however there is no long-term monitoring in place to know of the impacts, if any.

1.4.2.4 Solway Firth – Auchencairn, Rockcliffe and Kirkcudbright

The Solway Firth is the third largest estuary in the UK. Due to the unique make-up of this estuary, a large part of the inner estuary is a Special Area of Conservation (SAC), whilst the main study site is outside of this area; it lies within a National Scenic Area (NSA) of East Stewartry Coast (Taylor, 2009). Sample sites are found within the two Bays; Auchencairn Bay and Balcary Bay secluded from the main firth, the main freshwater source the bay is exposed to is from the River Uss within the neighbouring bay as well as freshwater from a collection of small burns. There is a single-track country road running alongside the west of the bay. Both the seagrass species *Z.noltii* and *Z.marina* are present here, with *Z.marina* fronds often fully submerged even during low tides in pools of water.

1.4.2.5 Loch Ryan

Loch Ryan is a shallow sea loch, with its mouth width the average depth between 2-5 metres. A main road follows the majority of Loch Ryan shoreline. Loch Ryan is a Marine Consultation Area (MCA) due to the high quality and sensitive marine environment, including the presence of the native oyster (*Ostrea edulis*) beds. Loch Ryan is subject to fast tides, but the site area chosen for this study is situated deep into the bay and therefore relatively sheltered. This site has been surrounded by large scale human activity, Cairnryan ferry terminal and a disused ferry terminal is in Stranraer (discontinued use in 2011) resulting in the loch's main channel being regularly dredged. Further to this, there have been on-going issues with the loch's water quality due to poorly treated wastewater being discharged into the loch by Scottish water (BBC, 2010). Despite the high level of activity at

Loch Ryan, no long-term monitoring of the seagrass beds' size and health has been in place to know if there has been any impact. Local anglers have said since the 1990's the seagrass bed here has been gradually diminishing but it remains a large continuous bed. Both *Z.noltii* and *Z.marina* are found in the loch.

1.4.2.6 Orkney – Eynhallow

Eynhallow is a small uninhabited island off the north coast of Orkney mainland, which is surrounded by strong tidal surges. The subtidal *Z.marina* meadow is one of the few seagrass meadows found around the coast of Orkney which is not situated on a maerl bed. The meadow has a mean spring tidal between 2.1-3 m.

Table 1-2 Main site characteristics summary³

| Site | Salinity (‰) | Average pH ¹ | Sediment classification | Average surface temp (°C) | Min surface temp (°C) | Max surface temp (°C) | Average Rainfall (mm) | Tidal range (m) | Percentage Cover of seagrass across mudflat area (%) | Meadow formation | Seagrass species |
|------|--------------|-------------------------|-------------------------|---------------------------|-----------------------|-----------------------|-----------------------|-----------------|--|-------------------------|-------------------------------------|
| EDE | 27 | 7.68 ± 0.19 | muddy sand | 9.57 | -0.7 | 21.7 | 56.37 | 0.29 – 7.13 | 33.5±23.85 | Large patchy meadow | <i>Z.noltii</i> |
| FOR | 29.3 | 7.50 ± 0.11 | sandy mud | 9.57 | -0.7 | 21.7 | 56.37 | 4.1 – 5 | 30.25±29.97 | large patch meadow | <i>Z.noltii</i> |
| TAY | 30.22 | 7.84 ± 0.16 | Muddy sand | 9.57 | -0.7 | 21.7 | 56.37 | 0.29 – 7.13 | 40.5±39.13 | large continuous meadow | <i>Z.noltii</i> and <i>Z.marina</i> |
| CAR | 19.33 | 7.69 ± 0.13 | muddy sand | 10.28 | 0.1 | 21.6 | 101.22 | -0.37 – 6.01 | 10.35±17.22 | scarce patchy meadow | <i>Z.noltii</i> |
| RYA | 15.83 | 7.77 ± 0.17 | Sand | 9.36 | 0.8 | 18 | 112.23 | 0.07 – 4.1 | 66.3±29.30 | Dense continuous meadow | <i>Z.noltii</i> |
| SOL | 29.22 | 7.88 ± 0.22 | sandy mud | 8.29 | -2.2 | 20.7 | 155.61 | -0.49 -9.97 | 60.6±29.74 | large continuous meadow | <i>Z.noltii</i> and <i>Z.marina</i> |

³ Methods section for this information can be found in Appendix 3

1.4.3 Thesis Structure

The thesis is structured as individual chapters, each containing relevant methods, therefore detailed relevant methods are repeated where relevant.

Chapter 2

A critical review and preliminary methods test for the application of loss on ignition (LOI) in coastal and marine studies, further to this OM origin is explored.

This chapter includes the following experiments:

- Stepped LOI, with incremental combustion of samples at temperatures 450°C, 500°C and 550°C.
- Testing combustion temperature suitability using a stepped combustion which traps CO₂ released during combustion.
- The use of sediment $\delta^{13}\text{C}$ values to determine OM sources across sites.

Chapter 3

This chapter provides information on OC stocks across twelve seagrass sites and compares stocks to bare mudflat areas. Further to this sedimentary OM was characterised based on combustible lability of OM. The following objectives were addressed;

- 1) To assess the impact of seagrass meadows on carbon density when compared to adjacent mudflat areas.
- 2) To compare carbon storage between sites.
- 3) To characterise sedimentary OM using different combustion temperatures to infer OC persistence within seagrass sediments.

Chapter 4

This chapter presents work with a collaborative global wetland study exploring decomposition patterns of tea litter degradation. Tea litter was buried across six intertidal sites and monitored over a three-year period. The aim of this study was to infer how OM is typically degraded and stored within seagrass meadows and if decomposition varies between seagrass and bare mudflat habitats within close proximity. Two tea materials were used; rooibos tea (refractory litter) and green tea (labile litter). Further to this, a one-year study explored the potential influence of depth on decomposition of tea material across two sites.

Chapter 5

This chapter describes the microbial communities present within two intertidal sites; FOR and SOL, across both seagrass and bare mudflat areas. In addition to this, the study describes microbial communities associated with rooibos tea litter after 3 months of burial to see how these microbes function and influence decomposition rates.

Chapter 6

This chapter explores if differences in sediment physical properties, including sediment grain size, are found between seagrass and bare mudflat areas within intertidal sites. The chapter focuses on sediment erodibility, exploring surface and shallow subsurface stability. Further to this the chapter explores the feasibility of determining sedimentation rates at SOL using anthropogenic radioisotopes.

Chapter 7

This chapter brings together all research from preceding chapters to discuss the wider context of the research presented and potential future application of the research, as well as highlighting key findings.

Chapter 2 A critical review of the loss on ignition method for the oxidation of organic matter

Abstract

This study assessed current literature and its use of the Loss on ignition (LOI) method in research. Coastal and marine habitats, particularly coastal wetlands, are recognised for their ability to sequester extensive amounts of carbon within sediments. For this reason there is mounting interest in managing, protecting and restoring blue carbon ecosystems at local and national levels to aid climate change mitigation policies (McLeod et al., 2011). The most widespread method for quantifying total organic matter (OM) is LOI because it is inexpensive, easy to perform and no specialised equipment or training is required, yet there is no standardised application of this method in coastal and marine sediment research, which increases potential error across studies and makes them difficult to compare.

This chapter considers how LOI has been and is used in studies of sedimentary carbon and records the range of approaches that are typically used. Stepped combustion trials were used to explore the likely effects of different temperatures of combustion on estimates of carbon content. This study recommends the continued application of LOI for determination of OM, however standard methods need to be determined. This study recommends 500°C for 6 hours as a suitable combustion temperature and sample exposure duration for application of the method in Scottish coastal and marine sediments. The results from the preliminary stepped combustion test confirms LOI at a combustion temperature of 450°C could lead to an underestimation in OM by ~38%.

2.1 Introduction

Deepening our understanding of the roles of coastal ecosystems in the mitigation of climate change relies on improved knowledge on the dynamics of carbon capture, burial and release of organic matter (OM) within coastal and marine sediments (Howard et al., 2017; McLeod et al., 2011). The unique geographical position of coastal ecosystems means that these habitats receive both allochthonous (e.g. terrestrial derived plant detritus, man-made compounds transported through runoff and atmospheric deposition) and autochthonous (e.g. phytoplankton debris and other biogenic particulate matter from detritus) OM inputs; both sources can contribute to long term carbon storage.

Coastal sediments are important sites of OM burial and remineralisation (Canfield, 1994; Middelburg, 2019a). There are two main reasons for this: Firstly, sedimentation rates are generally higher in coastal habitats compared with other marine ecosystems; this encourages increased burial efficiency of OM. Fast accumulation rates, in turn, reduce oxygen penetration within sediments creating low oxygen environments which reduce OM decomposition supporting OM preservation (Borum et al., 2007; Hartnett et al., 1998). Secondly, the refractory nature of the OM; due to these coastal habitats being at the interface of both marine and terrestrial environments, they receive a supply of terrestrial OM that is widely accepted to be less efficiently remineralised than marine OM within coastal environments (Burdige, 1991). These two factors apply particularly to vegetated coastal habitats, such as seagrass meadows, which are also sites of intense primary productivity and therefore of autochthonous carbon. This explains why coastal, vegetated habitats have received such attention for their carbon burial efficiency (Duarte et al., 2013).

2.1.1 Influence of particle size on organic matter

Sediments with higher proportions of finer sized particles ($<63\mu\text{m}$) accrue more OM than those made up of coarser, sand-sized particles, because the small size of the particles, particularly when in colloidal suspension, means that a larger external surface area is exposed to processes such as adsorption, catalysis, and microbial colonisation and subsequent biofilm formation (Chattopadhyay and Chattopadhyay, 2003; Dang and Lovell, 2016). Vegetated coastal ecosystems promote deposition of suspended particulate OM, which comprise predominantly of colloids. For example, as a wave propagates through a

seagrass bed, energy is lost causing a reduction in wave height (John et al., 2016). The ability to absorb the forces of waves causes suspended particle fallout and hence seagrass beds are widely known to be important for assisting sediment accumulation (Potouroglou et al., 2017). The deposition of these finer sized particles contributes towards sediment stability and positively influences OM within vegetated coastal ecosystems (Dahl et al., 2016; Miyajima et al., 2017; Röhr et al., 2016).

2.1.2 Determination of organic matter

Many methods exist for the determination of OM in sediment, the three main ones are: the sequential weight loss on ignition (LOI), Thermogravimetric-differential thermal analyses (TG-DTA) and the measurement of total organic carbon (TOC) estimated by CHN analyser. The LOI approach is a method that crudely estimates OM and carbonate content, where sequential weight loss is observed during the ignition of samples at a range of temperatures (Ball, 1964; Dean, 1974). To determine OM, a sample is oxidised to carbon dioxide (CO₂) and ash by heat. The weight loss from this reaction is recorded at every temperature step to produce percentage values for OM content. The TG-DTA is similar to the LOI technique as the sample is heated at incremental temperatures, however it provides more detailed information on the thermal reactions characterising the various OM structures, including the amount and properties of clay structural water, organic species and carbonates. Using a CHN analyser to determine TOC is the most accurate method and measures total carbon (TC) and inorganic carbon (IC) as well as total nitrogen (TN). However, it is the most expensive technique, which makes it beyond the scope of many projects; even well-funded research must often limit replicates below desirable numbers if using CHN. Further to this, marine sediments are often carbonate-rich (CaCO₃ >30%) and OC-poor (<1%). Therefore methodologies such as CHN which rely on acid digestion for carbonate rich sediments, prior to OC analysis, can often result in errors linked to incomplete combustion which has led to the CHN method being subject to further scrutiny (Phillips et al., 2011).

2.1.3 Current application of loss on ignition technique in literature

The LOI method has been progressively updated since its inception in 1950 and is the most widely used technique for determining OM because it is inexpensive, quick and easily repeated. It has also been used as a metric for quantifying OC when using a conversion

factor (Frangipane et al., 2009; Nelson and Sommers, 1996; Tinsley, 1950) specific to the ecosystem under investigation and where possible, relevant for the particular site to reduce error in OC estimates. Literature suggests that in most coastal and marine sediments 40-60% of OM is made up of OC (e.g. Fourqurean et al., 2014; Łukawska-Matuszewska et al., 2014; Nelson and Sommers, 1996; Wright et al., 2008).

Despite the long use and wide adoption of LOI for OM and OC determination, there is no standardised protocol adopted for sediments within or across research disciplines. Standardisation of the method is essential if results across different scales and ecosystems are to be compared (Bisutti et al., 2004; Heiri et al., 2001). The temperature used and the duration of combustion are the two variables that differ most widely between studies. Variation in sample position in the furnace, crucible material variances and volatile salt losses may also influence results, however these are often deemed of negligible importance (Dean, 1974; Frangipane et al., 2009; Heiri et al., 2001).

2.1.4 Clay water content

The influence that clay water content has on determining OM via LOI depends on the composition and proportion of colloid sized particles within the sediment sample (Mook and Hoskin, 1982). The clay-humus complex within sediments has the ability to adsorb high volumes of water (Chattopadhyay and Chattopadhyay, 2003), and sediments that contain a high fraction of these colloids could result in an overestimation of OM content via LOI because dehydroxylation of clay minerals occurs at $>400^{\circ}\text{C}$ (Ball, 1964; Eleftheriou, 2013; Wang et al., 2012).

2.1.5 Composition of sediment carbonates

One of the largest errors associated with the LOI approach is centred around the thermal instability of carbonate compounds when determining OM content (Bisutti et al., 2007; Hirota and Szyper, 1975; Howard and Howard, 1990). The most common carbonate minerals present in sediments include: calcite (CaCO_3), aragonite (CaCO_3), dolomite ($\text{CaMg}(\text{CO}_3)_2$), magnesite (MgCO_3) and siderite (FeCO_3). Some of these carbonates begin to decompose via combustion at similar temperatures to OM compounds, particularly MgCO_3 (Figure 2-2; Bisutti et al., 2007). In the early adoption of combustion methods to determine

OM, samples were acidified to remove IC before combustion. This was generally done using hydrochloric acid (HCl) at varying concentrations both via vaporous and aqueous acidification methods, however HCl is known to cause partial loss of OC, as well as other OM components including clays (Bisutti et al., 2004; Dean, 1974; Komada et al., 2008). Ball (1974) found that adding a known volume of 0.5 N HCl to sediments leached other components (e.g. clays) in addition to carbonates. Therefore, if IC removal prior to combustion is required, it needs to be determined if additional components being leached are negligible. It is more reliable to avoid acidification and alteration of OM by using the combustion technique to measure both OM and IC.

Interestingly, Dean (1974) and Heiri et al. (2001) both recommend the use of 550°C as an appropriate combustion temperature for lake sediments and are cited in many coastal and marine papers that use 550°C to combust OC. In Dean (1974) lake sediments analysed either contained low (6% calcium carbonate) or no carbonates in the samples, whilst the Heiri et al. (2001) study tested three sediment types, one of which had a high proportion of calcium carbonate. It is known that calcium carbonates are amongst the most thermally stable carbonates with research determining that temperature derived decomposition begins between 600-800°C (Dean, 1974).

Bisutti et al. (2007) combusted a mixture of soil types, natural materials and synthetic substances, as well as synthetic mixtures of organic and inorganic compounds to explain carbonate behaviour. Decomposition of carbonate minerals including calcite and dolomite generally did not occur at temperatures <600°C; although magnesite begins to degrade at 400°C, the loss may be marginal (Figure 2-1). To combat concerns over thermal instability of carbonates, particularly magnesite, a correction factor could be used for sediments with high concentrations of carbonates. Bisutti et al. (2007) concluded that 500°C would remove all OM, even though approximately 10% of magnesite was lost between 400-500°C.

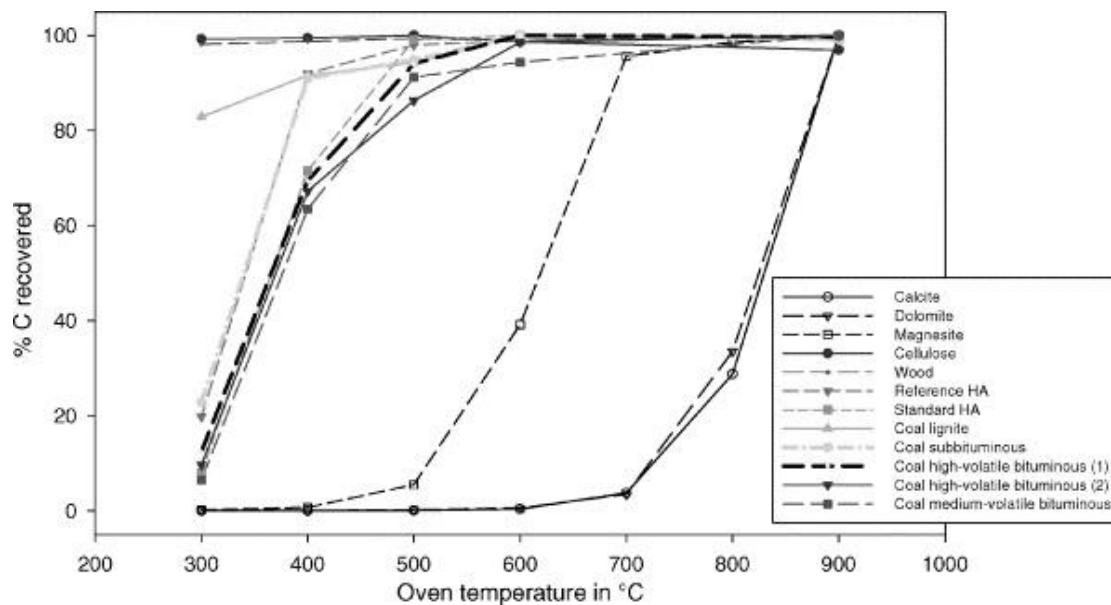


Figure 2-1 Combustion and operational decomposition temperatures of different organic and inorganic carbon compounds (Bisutti et al., 2007).

2.1.6 From a blue carbon perspective

The increased emphasis on determining carbon stocks in blue carbon habitats led to the launch of the Blue Carbon Initiative manual, which is freely available online and proposes methods to use in both the field and laboratory to assess carbon stocks globally (Fourqurean et al., 2014). The manual mentions LOI as a possible option for determining OC by using the relationship found so frequently in literature between OM and OC, but the manual falls short of standardising the method. It recommends 450°C as the appropriate combustion temperature, however it does not clearly state sample size or combustion duration. Instead the manual advises a combustion time of ‘4-8 hours’ and cites Heiri et al. (2001) to justify the method, despite this source never proposing 450°C as a suitable temperature. The wide range in recommended combustion duration could result in further incomparable datasets. This lack of consistency could explain why research in this field that uses LOI continues to deviate not only in combustion periods but also in temperature, even after this attempt at standardisation in 2014. These examples include research from the manual’s co-authors (e.g. Campbell et al., 2014; Comeaux et al., 2012; DelVecchia et al., 2014; Elmore et al., 2016; Ensign and Currin, 2017; Green et al., 2018; Samper-Villarreal et al., 2016).

2.1.6.1 Scottish blue carbon

The Scottish Blue Carbon Forum (SBCF) was launched in 2018 with the aim to further understanding of Scottish blue carbon stocks and their relevance to policy. Given the wide range of biogeomorphological settings and the many researchers investigating blue carbon, it is crucial to have a standardised method for quantification of carbon in these marine ecosystems to aid comparative understanding. To handle the potentially large variation in sediment types across Scotland, particularly where sediments contain high fractions of either, or both, clay content and carbonates, this field would benefit from a definitive checklist to clarify if a sediment meets the conditions required for successful application of LOI or if other methods are most appropriate. A standard protocol could eliminate the wasteful application of expensive alternative techniques that quantify OC and IC, which are frequently relied upon because there is a lack of confidence around the suitable temperature and duration of combustion in LOI techniques. This would also help focus future SBCF funding towards other critical areas, such as stability of carbon and determining sources of carbon.

2.1.7 Chapter aim and objectives

The aim of this chapter was to explore the application of LOI as a technique to quantify OC in coastal and marine sediments. This was addressed through the following objectives:

- I. Conducting a review of relevant literature to document the range of approaches, particularly regarding temperature and time of combustion, that are reported in studies using LOI.
- II. Using empirical laboratory tests to determine what temperature is required to achieve complete oxidation of OM in coastal and marine sediments.
- III. Exploring the implications of using 450°C, 500°C and 550°C as standard temperatures for OC loss in LOI.
- IV. Exploring the effects of granulometry in determining any discrepancy in LOI results between 450-500°C.

Further to this, the chapter briefly explores the potential variation in sources of OM based on preliminary research undertaken thanks to SUERC and University of Glasgow undergraduate students.

2.2 Methods

2.2.1 Sediment samples

Sediment samples were taken across seven intertidal (EDE, FOR, TAY, CAR, HUNT, RYA, and SOL) and one subtidal location (EYN). The sediment cores were retrieved using polyvinyl chloride (PVC) corers. All samples were collected as single cores across sites, except two habitat cores from the SOL site were sampled differently with five cores being extracted within the same plots and then centimetre increments were combined (Table 2-1). Full methodology for core sampling can be found in Chapter 3. All sample location coordinates can be found in Appendix 2.

Sediment cores were collected between Autumn 2016 – Summer 2019 using polyvinyl chloride (PVC) corers. Full methodology for core sampling can be found in Chapter 3. All samples were collected as single cores across sites, except two cores (one control, one seagrass) from the SOL site were sampled differently with five cores being extracted within the same plots and then centimetre increments were combined (Table 2-1).

Table 2-1 Summary of sediment samples used across the different analyses in this chapter.

| Sample name | Cores | LOI | Grain size | Stepped combustion | $\delta^{13}\text{C}$ determination |
|-------------|-------|-----|------------|--------------------|-------------------------------------|
| EDE | 2 | x | x | x | |
| FOR | 2 | x | x | x | |
| TAY | 4 | x | x | x | |
| CAR | 2 | x | x | | |
| HUNT | 2 | x | x | | |
| RYA | 2 | x | x | | |
| SOL | 4 | x | x | x | x |
| EYN | 2 | x | x | x | |

2.2.2 Literature review

A review of the literature was conducted to examine the range of conditions typically used in LOI applied to coastal sediments. Whilst the search for literature was as comprehensive

as possible, the purpose of this was not to produce a complete list of all studies that have used the method; such a list is likely to be very large and is difficult to compile given the routine application of LOI and how it is often a relatively minor part of the methods in studies exploring a wide range of ecological, geological and sedimentary phenomena. Rather the purpose was to find a wide and representative selection of literature to explore how the LOI technique was and is used and reported. The review was conducted using ISI Web of Science (WOS) and all other relevant papers known by myself to include LOI, but not identified by the WOS search, were also included. Four different searches were conducted, using a range of combinations of search terms such as 'sediment', 'LOI', 'carbon' and 'combustion temperature' (Table 2-2). All abstracts and methods sections were investigated to determine if a paper was relevant. Finally, the references cited in the most relevant papers were also scrutinised to find relevant missing studies. Methodology information from all relevant literature – particularly the temperatures and times used – was recorded.

2.2.3 Stepped combustion with loss on ignition

All samples were from sediment cores, which were sliced into 1 cm increments and air dried in aluminium trays. Then subsamples were oven dried at 60°C for 48 hours (GENLAB OV200 SFD), or until a constant weight was reached. To prevent humidity in samples before weighing, samples were cooled to room temperature in a desiccator before being weighed. (All samples were weighed to 4 d.p). Approximately 2 g aliquots of dried sediment were transferred into ceramic crucibles. These samples were then placed in a muffle furnace and ignited at 450°C as advised by Fourqurean et al. (2014). After cooling and weighing, the residual sample was then burned at 500°C (to have directly comparable results to another Scottish sediment study (Potouroglou, 2016; Wang et al., 2011)) and then again at 550°C (as this is the most widely used temperature for OM combustion; Figure 2-2). Hence, a stepped combustion approach was taken, with samples combusted for six hours at each temperature. For each sample LOI was calculated as:

LOI =

$$\frac{\text{initial dry weight of sample (g)} - \text{weight of remaining sample after burning at all relevant temperatures (g)}}{\text{Initial dry weight of sample (g)}} \times 100$$

2.2.4 Particle size analysis

In order to explore whether granulometry played a role in determining the most suitable temperature for the application of LOI, particle size analysis (PSA) was performed using a laser diffraction particle sizing analyser (Beckman Coulter LS230, Software version 3.39).

A total of 96 sediment samples were analysed via this approach and three replicates were studied for each sample. Samples were first sieved to remove any large (>2mm) or unnecessary particles, such as rocks, shells and plastic material, then ground with a rubber pestle and mortar to break up any agglomeration. To provide a non-biased representation of the whole sediment sample a riffle box⁴ was used to reduce the volume of the sample, without removing an unfair distribution of any particles. Each representative sample was then dispersed using 6ml of Calgon (35 g of sodium hexametaphosphate, 7 g of sodium carbonate and 1 L of distilled water) and 35 ml of distilled water to ensure proper wetting and reduce the risk of further agglomeration. The dispersed samples were also sonicated in an ultrasonic bath for 3 minutes prior to being mixed (using a magnetic stirring bar), then pipetted into the coulter instrument. The sediment size scale used was adopted from Gradistat program version 8 (Blott and Pye, 2001), where sand size particles <2 mm-63 µm, silt sized particles <63-2 µm and clay sized particles are <2 µm. Across all sediments clay sized particles were of the smallest proportion and silt and clay particles were defined as fine grain sized particles throughout.

2.2.5 Stepped combustion for stable isotopes and carbonates analyses

2.2.5.1 Sediment material

To determine a suitable temperature for oxidation of OM a stepped combustion approach was applied. A preliminary sediment sample, which contained a high proportion of OC was used to test the method. Thereafter it was intended that a range of sediment samples across a varied particle size spectrum (Figure 2-5) from sites across intertidal and subtidal sediment cores would help address the influence of grain size on the suitability of using OM determined by LOI to determine OC content in Scottish sediments. Sediment samples (0.15 ± 0.005) were weighed (g) to 4 d.p into clear quartz inserts and placed into a

⁴ Riffle boxes are used to divide material, in this instance sediment, into two equal homogenous samples for testing.

precleaned quartz combustion tube containing copper oxide then sealed. The samples were then subjected to a seven (during the preliminary test) or six (finalised temperature stages) stepped combustion with temperature increasing incrementally through a thermocouple. Final combustion temperatures included; 250°C, 350°C 450°C, 500°C, 550°C and 750°C. During the preliminary trial of the method combustion temperatures did not include 350°C instead 150°C and 400°C were measured. However, literature indicated that 350°C is often the operational temperature when samples lose all structural water (Frangipane et al., 2009) and 400°C was not commonly used in LOI literature (Figure 2-2). The released CO₂ from the combustion was collected at each temperature point and the gas analysed for $\delta^{13}\text{C}$ isotopic signature using a VG SIRA 11 Isotope Ratio Mass Spectrometer (IRMS) to interrogate the correct temperature for release of total organic matter (TOM) from the bulk sediment and the cut-off point where IC is released. It is also important to note that temperature readings were taken as the CO₂ vials are collected and therefore exact temperature readings at the time of extraction are noted, even though the sample would have been exposed to the correct temperature for the entire stepped duration.

2.2.5.2 Shell material

The validation of this method included analysis of pure shell material (CaCO₃). The shell sample underwent the same stepped combustion technique (as described above) however an additional mechanical clean was required to remove any detrital material on the shell surface. The shell was placed in 100 ml of water in an ultrasonic bath for 10 minutes, then rinsed with ultrapure water. Then 1 M HCl was added to the surface to remove any additional CaCO₃ contamination before being rinsed with ultrapure water and oven dried (Dunbar et al., 2016).

Further to this, 0.1014 g of this pre-cleaned shell material was weighed into a separate hydrolysis unit and treated with 1 M HCl at room temperature (20°C). The CO₂ liberated from the hydrolysis of this shell material was collected and measured to provide a baseline $\delta^{13}\text{C}$ isotopic value for IC within this sediment type.

2.2.6 $\delta^{13}\text{C}$ isotopic signature determination

In addition to the CO_2 IRMS from the stepped combustion test described above, additional sediment samples which were used to compare temperatures 450°C, 500°C and 550°C (in the LOI study) were analysed by elemental analyser – isotope ratio mass spectrometry (EA-IRMS) to measure the ratio of stable carbon isotopes, $^{12}\text{C}/^{13}\text{C}$ ($\delta^{13}\text{C}$) within the sediment.

2.2.7 Statistical analysis

Paired t-tests were used to determine if there were statistically significant differences between cumulative amounts of OM recorded at combustion temperatures 450°C, 500°C and 550°C, this was conducted using Microsoft Excel (version 16.5). All data were tested for normality and homogeneity of variance before running statistics. All figures were produced in R studio using the package ggplot2 (Wickham, 2016).

2.3 Results

2.3.1 Literature review

Out of a total of 1097 papers initially found in the literature search, 319 were deemed relevant and were analysed for details of LOI methods. Sixty-seven of these 319 papers stated they used a LOI method based on protocols from Dean (1974) and/or Heiri et al. (2001), hence these two papers exert a particularly strong influence on the literature. Across all literature Heiri et al. (2001) was the most cited article (2182 citations⁵), and they advised a combustion temperature of 550°C. However, many of the papers that did cite these two studies varied in either or both combustion temperature and duration from the procedures used in the cited papers or did not state what temperature or duration of combustion the study chose to use. Overall, the Heiri et al. (2001) advised combustion temperature of 550°C was used in 49% of papers explored in this review (Figure 2-2).

Table 2-2 Overview of search terms used in Web of Science and results summary.

| Search terms | Search results | Explicitly mentioned LOI in abstract or methods section | Irrelevant studies | Unable to access |
|---|----------------|---|--------------------|------------------|
| TS=((Loi OR loss on ignition OR ignition loss) AND sediment* AND carbon*) Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=1900-2019 | 312 | 179 | 129 | 4 |
| TS=((combustion temperature) AND sediment* AND carbon*) Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=1900-2019 | 207 | 10 (6 new) | 198 | N/A |
| TS=((combustion method) AND sediment* AND carbon*) Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=1900-2019 | 251 | 8 (5 new) | 241 | 2 |
| TS=((Loi OR loss on ignition OR ignition loss) AND sediment* AND organic matter*) Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=1900-2019 | 327 | 175 (102 new) | 129 | 23 |
| Own literature search (not previously found through searches via WOS) | N/A | 27 | N/A | N/A |
| Total unique papers explored | | | | 319 |

⁵ This is based on statistics from a Web of Science search (www.webofknowledge)

After 550°C, the second most common temperature category was ‘did not report’ at 17%, followed by 500°C stated in 11% of studies. Where stated, temperatures ranged between 375-700°C (e.g. Hong et al., 2017; Roner et al., 2016; Wang et al., 2011; Wilding, 2006). However the largest variation occurred in combustion duration, which ranged from 20 minutes at 550°C in a study combusting lake sediments (Zhang et al., 1993) to 24 hours at 550°C (Lehtoranta et al., 2004). Despite the criticism the LOI method receives, due to the errors previously mentioned, reported use of LOI continues to rise yearly, to both determine OM and to use OM to infer OC content; assuming all literature that cites these papers apply the LOI method and do not simply discuss its application. Over a 16-year period the citation of papers that apply LOI has increased from 50 citations in 2002 to 700 in 2018, with the top 10 papers cited 398 times in 2018 (Figure 2-3).

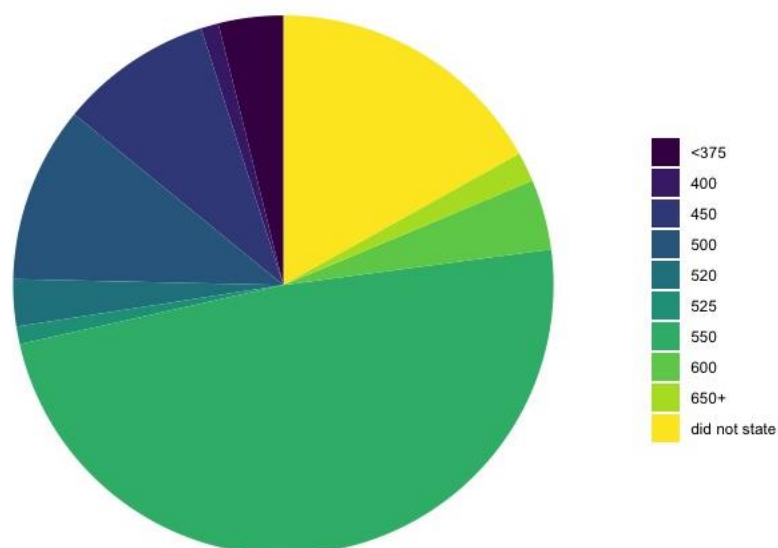


Figure 2-2 Hierarchy chart of the variation in combustion temperatures (°C) across 319 research studies.

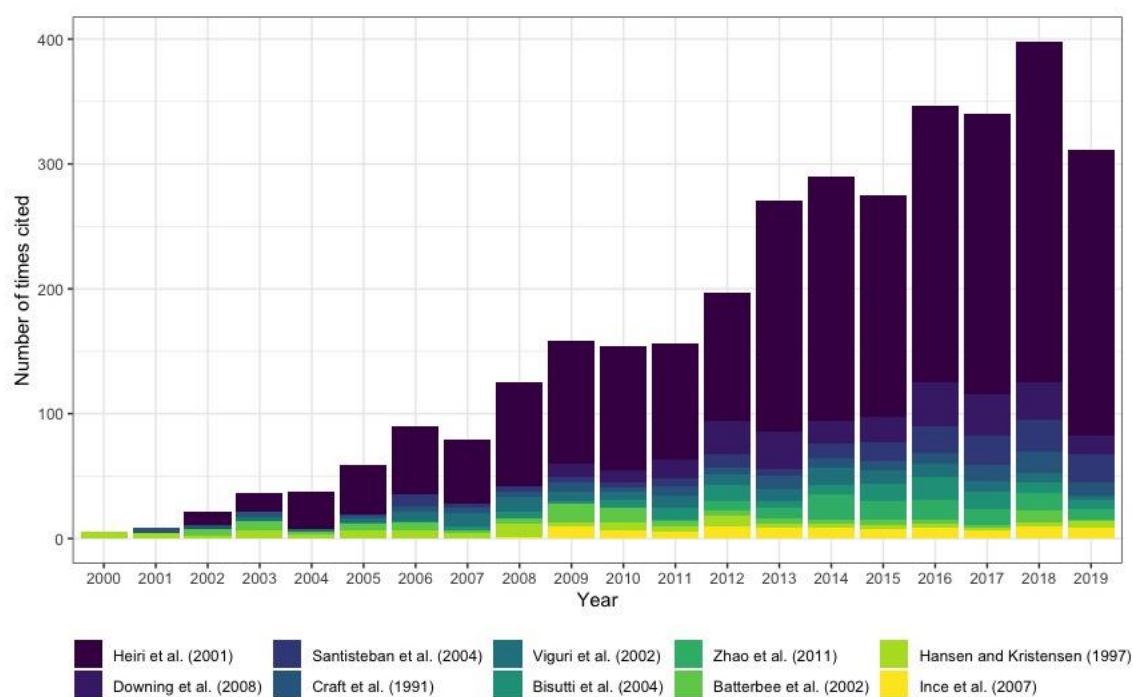


Figure 2-3 Number of times the top 10 cited papers for the application of LOI determination were cited per year over the last 20 years.

2.3.2 Loss on ignition

Testing whether OM significantly differs between combustion temperatures included all cores except the combined SOL core, as it was sampled differently. Paired t-tests found highly significant differences in cumulative proportions of OM between 450°C-500°C (df = 227, t-value = -18.17, $p < 0.001$), 500-550°C (df = 44, t-value = -10.81, $p < 0.001$) and 450°C-550°C (df = 44, t-value = -15.88, $p < 0.001$), showing that samples continued to lose weight as they were heated at higher temperatures. The mean differences in cumulative percentage weight loss (g) moving from 450°C-500°C was $0.373 \pm 0.24\%$, from 500°C-550°C $0.259 \pm 0.16\%$ and from 450°C-550°C $0.55 \pm 0.23\%$.

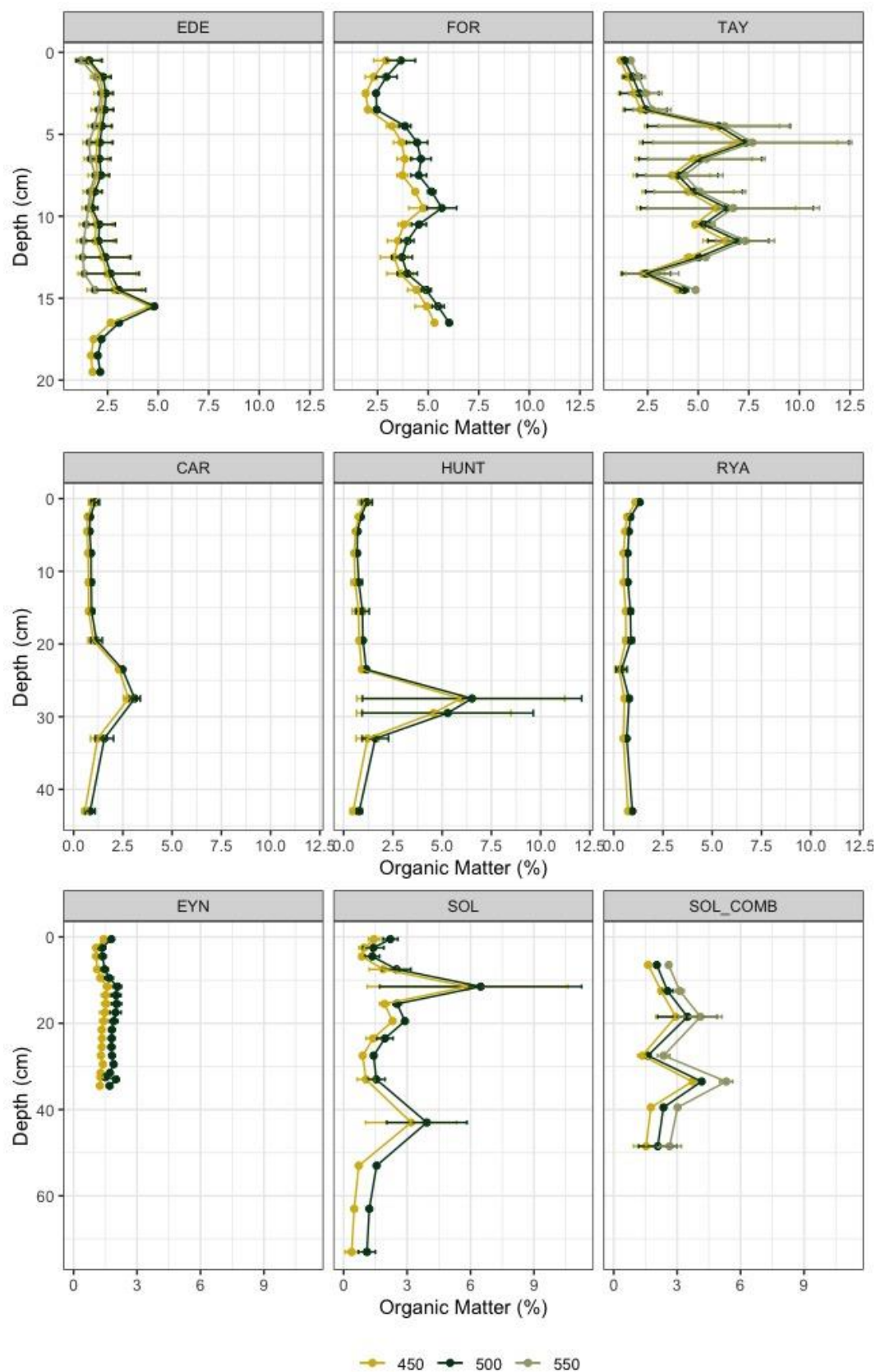


Figure 2-4 Line graph representing mean organic matter and associated standard error across sites over a range of combustion temperatures (450°C, 500°C and 550°C). Where (A) 20 cm depth sediment core (EDE, FOR and TAY), (B) 50 cm depth sediment core samples (CAR, HUNT, RYA), (C) subtidal sediment core (EYN), (D) SOL deep sediment core (E) SOL combined sediment core,

2.3.3 Stepped combustion

2.3.3.1 Particle size analysis

The sediment samples ranged from very coarse sand to fine silt reflecting a high spatial heterogeneity across sites (size scale adopted from Gradistat program version 8; Blott and Pye, 2001). No silt or clay particles were found in the three EYN sediment samples, whilst the largest proportion of fine grain sized particles were found in FOR sediment samples (maximum fine grain sized particles present: 80.68%). Across all samples there was a low proportion of clay sized particles for all sites with silt and sand fractions dominant (Figure 2-5). For the stepped combustion analysis eight samples across the full range of sediment grain profiles were selected. These included 0%, 8.5%, 13%, 22.6%, 39.2%, 61.5% and 80.2% of finer grain sized particles (<63 μm) contained within the sediment⁶.

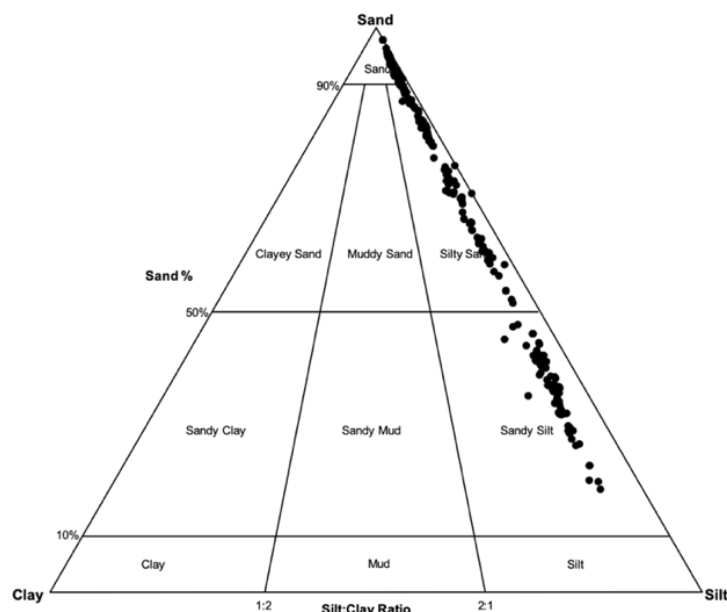


Figure 2-5 sediment texture analysis for all sediment samples, according to size scale adopted in the GRADISTAT version 8 program (Blott and Pye, 2001)

2.3.3.2 Preliminary stepped combustion method test

A sample rich in OM from a SOL sediment core associated with seagrass (depth 37 cm) was used to validate the method. Within the stepped sediment sample, all temperatures resulted in CO₂ release, though the combustion of the shell material did not release CO₂ at its lowest temperature of 150°C (Figure 2-6A). Combustion temperatures up to and including 250°C are representative of the labile proportion of OM content, which represents

⁶ Only the preliminary test of this method has been included in this thesis due to delays in sample analysis at the laboratory facilities (pandemic related restrictions). In future, these results will form part of a report and potential publication.

2.7% of TC in the sample. This labile OM had a more terrestrial $\delta^{13}\text{C}$ signature of -26‰ (Figure 2-6C), within the sediment sample. The largest cumulative volume of CO_2 released for a single combustion temperature, within the sediment sample, occurred at 500°C. The average $\delta^{13}\text{C}$ signature across the stepped stages up to and including 500°C matched the $\delta^{13}\text{C}$ signature for total OC combusted via the single-step method and was -23.3‰. This $\delta^{13}\text{C}$ signature suggests that the sediment is composed of a mix of marine and terrestrial OC. The matching $\delta^{13}\text{C}$ signature confirms the high likelihood that all OC was combusted within the subsample at a 500°C. Therefore, the application of a 450°C LOI combustion temperature for a sediment subsample, in this instance, would underestimate TOC by as much as 37.68%. Despite similar $\delta^{13}\text{C}$ signatures for the two combustion steps, the 500°C combustion released an additional CO_2 volume of 2.95 ml, compared to 450°C. The difference in TOC estimates between 500°C and 550°C is much smaller, however the higher combustion temperature could overestimate TOC by 5.49%. The 550°C combustion indicated a more marine $\delta^{13}\text{C}$ signature and was more enriched (-18.8‰), which suggests an increase in marine and/or seagrass material over terrestrial OM. IC made up 29.8% of TC within the sediment sample, assuming the small volume of CO_2 released at 550°C is more inorganic based on its increased marine $\delta^{13}\text{C}$ signature. The final combustion at 750°C has a $\delta^{13}\text{C}$ signature that is consistent with the shell material and therefore inorganic (Figure 2-6C). Overall, this test validated, alongside the acid washed 850°C combustion, that 95.4% of TC was recovered using the stepped combustion approach temperature of 500°C (Table 2-3). As expected, the shell sample lost a much smaller volume of CO_2 at each combustion stage below 750°C, with the highest CO_2 release (1.12 ml CO_2) occurring at the highest temperature (Figure 2-6B). Due to the low CO_2 volumes collected for the shell material only two $\delta^{13}\text{C}$ signatures were measured (450°C and 750°C combustion stages). Both $\delta^{13}\text{C}$ signatures for the shell material values were less than -5‰ which confirms that the material was inorganic in origin. Table 2-3 provides a breakdown of proportions of carbon across different cumulative stages as well as TOC and TC across the single and stepped combustion test for the sediment sample.

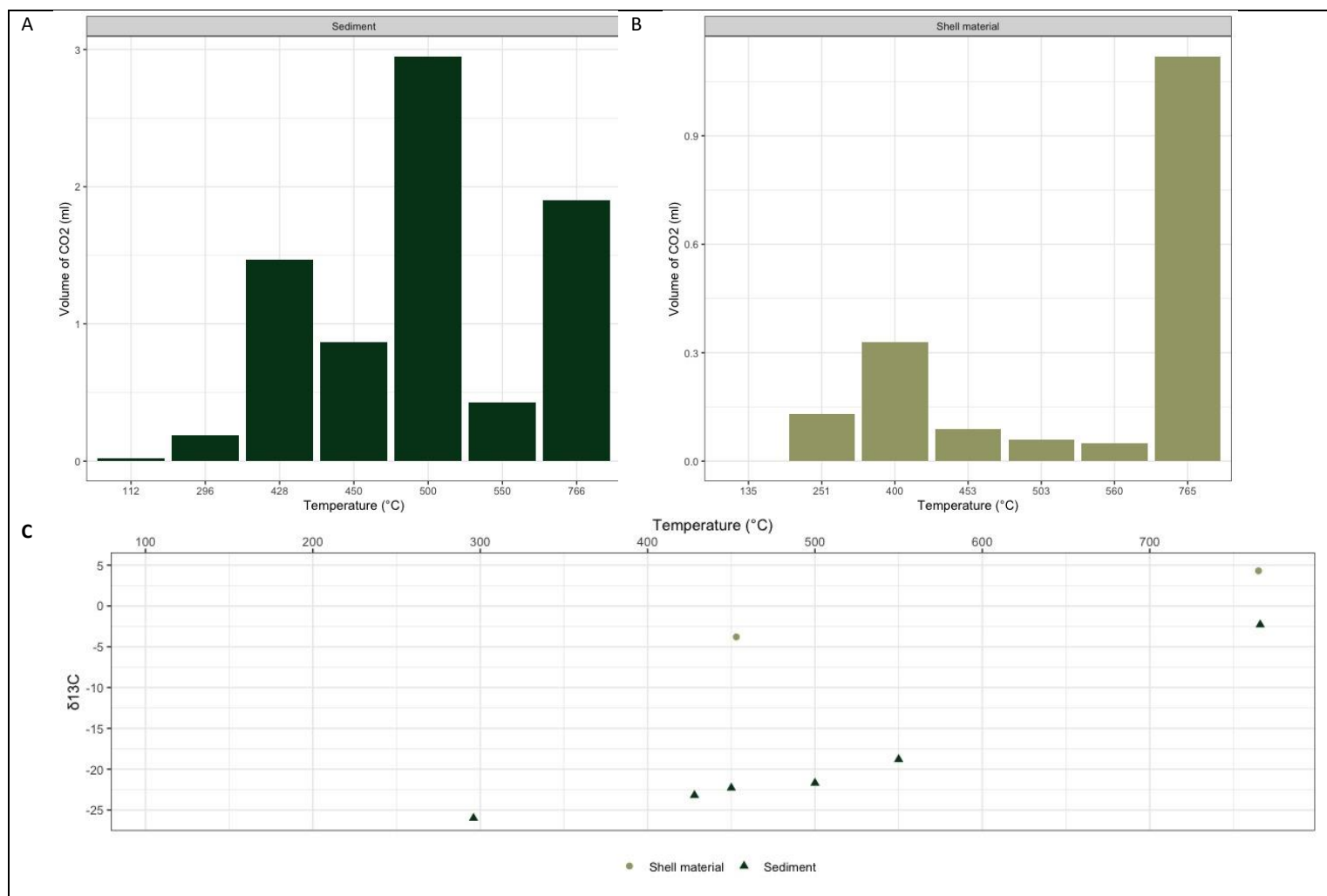


Figure 2-6 Stepped combustion method validation bar charts of (A) a sediment sample (0.1522 g) and (B) a fragment of shell (0.1014 g) from SOL. A and B show the volume of CO₂ released during stepped combustion at seven temperatures of 150°C, 250°C, 400°C, 450°C, 500°C, 550°C and 750°C. (C) Scatter graph of δ¹³C signatures across the stepped combustion temperatures for both material types; shell and sediment.

Table 2-3 Summary tables of stepped and single stepped combustion of the OM-rich sediment sample from SOL.

| Combustion method | Sediment summary | Carbon type | Volume CO ₂ (ml) | Total C (%) | δ ¹³ C value |
|--------------------|--|--|-----------------------------|--------------|-------------------------|
| Single step | Sediment acid washed (weight 0.0529 g) | total organic carbon | 2.91 | | -23.3 |
| Stepped combustion | Bulk sediment (weight 0.1522 g) | cumulative CO ₂ volume to 250°C (labile organic) | 0.21 | 2.68 | -26 |
| | | cumulative CO ₂ volume 250-500°C (refractory organic) | 5.29 | 67.56 | -22.4 |
| | | total organic carbon | 5.5 | 70.24 | -23.3 |
| | | inorganic C 500-750°C | 2.33 | 29.76 | -10.55 |
| | | total carbon | 7.83 | | |

| | |
|---|-------|
| Total volume CO ₂ released from OC/g (single step) | 55.01 |
| Total volume CO ₂ released from OC/g (stepped combustion) | 36.14 |
| % OC recovered on a per g basis using the stepped combustion technique | 65.69 |
| % Inorganic C recovered on a per g basis using the stepped combustion technique | 29.76 |
| % Total recovery | 95.45 |

2.4 Discussion

The literature review of the application of LOI in studies of coastal sediments shows that, often, little consideration is given to the temperature or duration of combustion applied to samples. Despite literature identifying the importance of sediment characteristics when choosing a suitable temperature (Frangipane et al., 2009; Sutherland, 1998), there was little evidence to suggest that most authors considered this. There was large variation in combustion temperatures and durations, with many studies referencing methods they do not then follow nor explain why they have deviated from the combustion temperature or duration. For example, ten papers which did not use a combustion temperature of 550°C still referenced research which recommended 550°C without explanation for the temperature actually used. There is no standardisation, so it makes it difficult to make reliable data comparisons within blue carbon research.

2.4.1 Loss on ignition and preliminary stepped combustion test

Exploring the influence of temperature on OM content across a range of sediments in Scotland highlighted significant differences between the combustion temperatures and the need to adopt a uniform method for the application of LOI. Testing the stepped combustion method further confirmed a much greater volume of CO₂ released from the sediment sample at 500°C (Figure 2-6A). Arguably, using cautious temperatures below 500°C will fail to combust all OM within a sediment sample, leading to gross underestimations; in this instance the application of a 450°C combustion would have underestimated sediment OC by as much as ~37.7%. Whilst caution should be taken with combustion of clay-rich samples, Craft et al. (1991) found that, during the combustion of clay-rich estuarine marsh sediments for 8 hours at 450°C, structural water loss accounted for 2% of the ignition, concluding that water loss was negligible. When dealing with large sample sizes, if possible, a range of subsamples should undergo both LOI and TG-DTA to determine if clay structural water loss is negligible. For example TG-DTA of OM from lagoon sediments showed that at 350°C samples began to lose clay structural water and total extraction of clay structural water occurred at 567°C (Frangipane et al., 2009). Frangipane et al. (2009) recommended a correction factor be used in samples with

high clay content, to combat the loss of structural water without jeopardising the application of a higher temperature for removal of refractory OM. However, within the current study, no site contained a high clay content, further testing could determine a potential suitable correction factor for clay-rich Scottish sediments.

Although the majority of samples found OM to vary across combustion temperatures, some sites, including TAY showed less variability between combustion temperatures, this could be explained by the natural variability in the types of OM content within the samples. Ultimately, the preliminary test confidently rules out the use of 450°C as a suitable combustion temperature for coastal sediment samples. With increased temperature, the $\delta^{13}\text{C}$ signature of the sediment sample also became more enriched, the CO_2 released during combustion at 550°C had a more marine signature (-18.8‰) than all lower temperatures suggesting any remaining OC was also very refractory in nature.

Magnesite combusts at temperatures exceeding 400°C (Bisutti et al, 2007), however marine and coastal literature including Luczak et al. (1997) and Wang et al. (2011) recommend the combustion of marine and coastal sediments at higher temperatures. Luczak et al. (1997) recommends a combustion of 500°C for 6 hours, specifically because there was no significant change in CaCO_3 under those combustion conditions. The combustion temperature of 500°C has been used for numerous coastal habitats including seagrass meadows, saltmarsh and sea lochs but duration ranges from 2 to 16 hours (Kristensen et al., 1995; Loh et al., 2002; Luczak et al., 1997; Sousa et al., 2017; Wang et al., 2011). Wang et al. (2011) went further, suggesting different combustion temperatures for marine (550°C) and coastal sediments (500°C), both for 12 hours, stating the lower temperature for coastal sediments takes into consideration the variation in OM sources.

Combustion of the shell material showed minimal CO_2 release before the 750°C combustion. The detectable CO_2 was released from the shell material from 250°C, with the largest, low temperature, volume released at 400°C (0.33 ml CO_2). The CO_2 release could not have been magnesite, which burns at temperatures as low as

400°C, or carbonates, therefore it is likely that the small amount of CO₂ released at low temperatures was due to remnants of OM that were not effectively removed from the shell with the hydrochloric acid prior to combustion. The amount of CO₂ released was small and no $\delta^{13}\text{C}$ signature was determined. However, the $\delta^{13}\text{C}$ signature determined at the next combustion temperature (500°C) had a more inorganic signature, with a value of -3.8‰, but was still more depleted than the inorganic $\delta^{13}\text{C}$ signature of 4.3‰ measured for the shell material when combusted at 750°C. Therefore, for Scottish coastal and marine sediments, concerns over the possibility of inorganic matter content being combusted at temperatures above 400°C are likely to be misplaced if a combustion temperature of 500°C is used. If inorganic matter is lost, it is likely to be negligible based on Bisutti et al. (2007) findings (Figure 2-1). Despite the higher temperature of 550°C being the most used extensively across all habitats, the justification of this higher temperature for combustion is largely based on just two papers: Dean (1974) and Heiri et al. (2001). Both papers tested the method on lake sediments where carbonate material would not have been a concern, as it is in coastal and marine sediments. Further stepped combustion work could provide, with more certainty, evidence for the application of either 500°C or 550°C as the most appropriate temperature to completely remove OM across differently grain sized proportioned samples. However, to provide a method that can cover a range of sediment types, without removing all water or combusting inorganic matter, 500°C is likely to be the most appropriate temperature to use, with potential application of conversion factors for samples with known high clay content or from high magnesite areas. The largest proportion of studies recommended an exposure time of 4-8 hours or no more than 7 hours with little change in weight exceeding after this time (Campbell et al., 2015; Fourqurean et al., 2014; Luczak et al., 1997), although Wang et al. (2011), did note continued weight loss during after combustion periods of 12 and 15 hours for marine sediments. Heiri et al. (2001), found lake sediments samples would continue to see negligible weight loss at a slow rate for up to 64 hours, but linked this to loss of volatile salts, structural water of clay minerals or metal oxides, concluding 4 hours to be a suitable exposure time. Therefore, this study advises that the LOI approach, particularly for intertidal sediments across Scotland, should involve a default combination of 500°C

for 6 hours. This combustion temperature and duration should avoid over-or-underestimation of OM content across the largest range of sediment samples.

This study was also able to address that structural water losses, which can be detected at temperatures as low as 100°C (Dankers and Laane, 1983), would unlikely be an issue in Scottish sediments due to the preliminary combustion method test. This study confirmed that these structural water losses, at least below 150°C, should not prohibit the use of LOI to determine OM because the percentage of TC released is <0.25%, based on the volume of CO₂ released (Figure 2-6A). Thus, confirming that either 60°C or 105°C, which are the most used temperatures to dry sediments for dry bulk density (DBD) determination, would be suitable, as neither is likely to influence structural water loss from clay minerals, and losses would be negligible. However, drying temperatures for DBD would also need to be standardised to make datasets comparable, if the samples were then to be used for determining OM content by LOI.

2.5 Conclusions

Despite the application of LOI facing criticism, due to differences during combustion associated with (1) loss of CO₂ from carbonates and (2) loss of structural water from clay minerals, the method is being increasingly used in research (Figure 2-3). LOI is the simplest, most accessible and rapid method used to calculate OM content and determine OC content. The method is often the only option to estimate OC stocks data, particularly in less affluent countries with limited laboratory resources and therefore should not be overlooked for more advanced techniques. Even when more expensive techniques can be used, the availability of a cheap and simple approach will generally allow larger number of samples to be processed, which is important given the often large variability between and within sites. Increasing the precision of estimates should not be at the expense of reducing their representativeness for sites or habitats as a whole. The review of the literature highlights the current inconsistencies with the method and this needs to be urgently addressed; a simple starting point is to encourage better reporting of important methodological details in the literature. Method validation of LOI is important to ensure that the most accurate description of OC stocks can be produced across habitats associated with sediments. It is evident that, taking into consideration the heterogeneous nature of sediments and the complexities of structural water and carbonate combustibility, that there is not a standardised approach that will apply in all habitats. The best approach is for researchers to validate an appropriate temperature and duration that is fitted to their site and study, using stepped combustion and C/N analysis of appropriate subsamples to confirm accuracy. If this is not possible, as advised by Heiri et al. (2001), studies have a responsibility to clearly state combustion temperature, duration and sample size, as these parameters will help produce more easily comparable datasets. The current work identifies a combustion temperature of 500°C for 6 hours as a default approach that is likely to remove all OC across a range of coastal and marine sediments with minimal error for most samples. Although final tests across sediments with high clay contents need to be conducted to ensure suitable correction factors can be applied where clay content may result in misleading OM data. Further, this study suggests that OM in intertidal and subtidal

mudflat and seagrass areas across Scotland is predominately derived from allochthonous sources, with the most dominant source, in all instances, except RYA, being terrestrial in nature.

Chapter 3 Scottish seagrass carbon Inventory

Abstract⁷

Seagrass meadows provide a plethora of ecosystem services. Their prominence as a blue carbon habitat has made their global status rise in the last decade. This study builds on the knowledge of organic carbon (OC) stocks across the East coast of Scotland's intertidal seagrass meadows and introduces information on eight additional study sites: seven west coast intertidal meadows, one east coast intertidal meadow, and one subtidal meadow in Orkney, as well as building on OC stocks information at two east coast sites. This is a descriptive chapter providing a carbon inventory that can be directly compared with other Scottish estimates of OC stocks. This study distinguishes between types of organic matter (OM) and inorganic matter, exploring lability from a thermal/mechanical perspective using different combustion temperatures. The study also explored sources of OM within seagrass sediments, finding sediment $\delta^{13}\text{C}$ isotopic signature across sites to share similar values, however the proportion of OM sources varied greatly across sites. The study supports the theory that seagrasses are important location for inwelling of allochthonous derived OM.

Similar to other Scottish research, the implied positive relationship between carbon sequestration and seagrass meadows is highly variable. Overall, the meadows chosen for this study do not significantly enhance carbon accumulation when compared to adjacent bare mudflat areas. This study highlights the importance of understanding site-specific differences to ensure management practices which aim to promote and enhance carbon are suitable. Further research of sedimentary and environmental variables need to be explored to help explain OC variation across and within sites.

⁷ Data from within this chapter is published in the following peer reviewed article:

Potouroglou, M., Whitlock, D., Milatovic, L., MacKinnon, G., Kennedy, H., Diele, K., Huxham, M., 2021. The sediment carbon stocks of intertidal seagrass meadows in Scotland. *Estuar. Coast. Shelf Sci.* 258, 107442. <https://doi.org/10.1016/j.ecss.2021.107442>

3.1 Introduction

Marine sediments are the largest sink for organic carbon (OC) on Earth (Hedges and Keil, 1995). Organic carbon and calcium carbonate (CaCO_3) reactions are the most dominant biogeochemical processes in the oceanic carbon cycle (Raven and Falkowski, 1999) with the majority of preserved OC associated with mineral matrices through sorption of both fine minerals (clays) and metal oxides (particularly iron oxides: Fe-OC complexes). Globally, approximately 0.7 Pg C per annum of inorganic carbon (IC) and OC is transported from terrestrial to marine ecosystems (Raven and Falkowski, 1999). Less than 0.5% of the total production of OC in marine habitats is estimated to be stored long-term in marine sediments (Hedges and Keil, 1995). In contrast, a third of all CaCO_3 production escapes dissolution and is stored in oceanic sediments (Lerman and Mackenzie, 2005; Milliman and Droxler, 1996).

3.1.1 Carbon pools

Within coastal environments carbon exchange contributes significantly to the trophic status of the sediment-water interface due to benthic oxygen demand and organic matter burial (McGlathery et al., 2001). Coastal areas typically differ in hydrology and chemistry due to anthropogenic stresses such as proximity to urbanisation, industrialisation and agricultural runoff, which all control volume and quality of water, as well as the quality of allochthonous OM introduced to the coastal environment. Therefore, it is important to distinguish whether a coastal environment is a carbon source, a sink or both (if the site is subject to both inwelling and outwelling) to understand the permanence of the various types of carbon within these dynamic habitats.

The role of marine macrophytes as carbon sinks was initially studied 40 years ago by Smith (1981), but in the last decade OC accumulation in coastal seascapes has received an increasing level of research attention. The recent interest has produced OC sediment depth profiles beneath vegetated coastal habitats, usually extracted at one time point only. In seagrass research there has been a focus on species that contribute more to C deposits, including *Posidonia* spp., *Thalassodendron* and *Enhalus acoroides* (Macreadie et al., 2014; Mateo et al., 2006). Often, studies do not

specify the types of carbon present, the carbon content of surrounding habitats within the seascape and carbon accumulation rates. By quantifying sediment cores for OC in isolation it cannot be determined if a blue carbon habitat OC stock is accumulating, stable or declining (Jennerjahn, 2020). In order to produce accurate sediment stocks it is important to determine the types and proportions of carbon present, accumulation rates and sources of carbon present (Jennerjahn, 2020; Macreadie et al., 2014); for example ~17-30% of all sedimentary carbon is comprised of OC (Lerman and Mackenzie, 2005; Milliman and Droxler, 1996). Labile OC is the most readily available material for benthic metabolism, and often sediment OM is dominated by the labile fraction (Middelburg, 2019b). Therefore, determining the type of OC present and/or the sources of OC can help infer the permanence of the overall OC stock and prevent overestimation of a habitat's long-term OC stocks. Overall, the interest in quantifying carbon stocks has focused on the retention and persistence of OC, as there is a net gain in carbon after all reactions are considered. Approximately 25% of surface marine sediments are comprised of CaCO_3 , and carbonates play an important role in the global carbon cycle (Balch, 2005; Macreadie et al., 2019; Saderne et al., 2019). The production of carbonates uses carbon dioxide (CO_2) which releases 0.6 mol of CO_2 per mol of CaCO_3 , which would offset OC stored (Smith, 2013). As both OC and IC occur within marine sediments, any consideration of a marine habitat as a carbon sink should consider labile and refractory OC as well as IC.

3.1.2 Tracing organic matter sources

It is important to determine the role coastal ecosystems play in the carbon cycle, as coastal sediments can be important sources of organic carbon (OC) and other nutrients, act as sedimentary sinks for OC or act as both a source and a sink (Gonneea et al., 2004; Hiraishi et al., 2014; Huxham et al., 2018; McLeod et al., 2011). Understanding the balance of sedimentary carbon cycling across coastal ecosystems and the relative importance of the various available sources of OM between and within coastal ecosystems could help elucidate the variability in intertidal mudflats' retention of OC (Burdige, 2005) and aid future management strategies.

Conventional methods used to distinguish between sources of organic materials that contribute to sediment OM often include the measurement of the ratio of stable carbon (C) isotopes ($^{12}\text{C}/^{13}\text{C}$ ($\delta^{13}\text{C}$)), nitrogen (N) isotopes ($\delta^{15}\text{N}$), and C/N ratios. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C/N ratios are properties of the sediment that are based on natural OM and are therefore natural tracers for assessing both origin and movement of C and N in the ecosystem (Middelburg and Nieuwenhuize, 1998; Mitchell et al., 2003; Raymond and Bauer, 2001). In particular the $\delta^{13}\text{C}$ signature allows identification of its parent vegetation (by estimating proportions of C_3 and C_4 terrestrial plants and marine material) and is often studied across sediment depth profiles to identify changes in source and OC burial rates over time (Meyers and Teranes, 2002). The variation of $\delta^{15}\text{N}$ is induced by microbial assimilation, mineralisation, denitrification and decomposition (Craine et al., 2015). However, when applying such methods to dynamic coastal ecosystems the heterogeneity of OM composition and source contribution makes it difficult to understand the evolution of the environment and associated carbon burial (Fry et al., 1977).

Whilst there is limited overlap in OM isotopic signatures across marine and terrestrial sources, estuaries receive large inputs of nutrients and OM from both, creating biogeochemical hotspots which encourage estuarine primary production assimilating biogenic elements from multiple sources by consumers including plankton and polychaetes (Figure 3-1; Alling et al., 2008; Nicastro et al., 2017). The quality of organic material within a coastal ecosystem can also vary depending on how the OM was transported (active or passive) and utilised. The utilisation of OM by primary consumers means it is altered to such a degree that only a fraction of the OM will resemble the composition of the biological precursors when deposited into the sediment bed. OM deposited relatively quickly and/or following relatively short distances of active transportation from its source will be of a higher quality because it is exposed to fewer cycles of deposition – rest and reaction – remobilisation before settling in a sediment bed. Whereas long transport distances result in OM movement through multiple environments and therefore it is assumed the OM has been utilised during transportation (Kuwae and Hori, 2018). For instance, soil-

derived OM from an upland agriculture field could be transported via erosion to a watershed. As the OM passes through river and estuarine zones, before being deposited in a coastal ecosystem, the soil-derived OM would be exposed to numerous cycles of oxidation and remineralisation which alters its OM properties. OM mixing within estuarine environments of marine, riverine and terrestrial sources makes it difficult to distinguish end member OC and N isotopic signatures that reside in coastal sediments because they are modified during biogeochemical processes, which results in uncertainty when estimating the sources and fate of OC and N (Figure 3-1; Alling et al., 2008; Gonnee et al., 2004; Leorri et al., 2018).

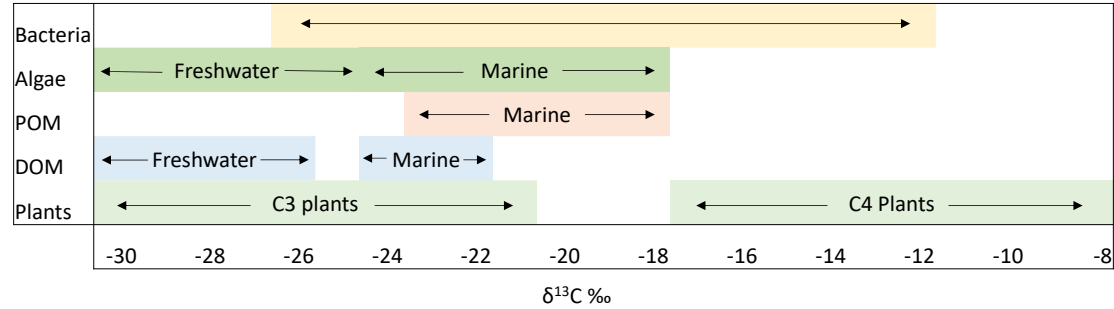


Figure 3-1 δ¹³C signature ranges of marine and terrestrial sources of OM (adapted from Goñi and Thomas, 2000; Leorri et al., 2018), where POM represents particulate organic matter and DOM represents dissolved organic matter.

3.1.3 Blue carbon

Marine macrophytes, such as seagrasses, contribute to the carbon cycle through biomass production, OM burial, oxidation and CaCO₃ production (Fourqurean et al., 2012; Lima et al., 2020). The drive to audit blue carbon resources has focused on three main blue carbon habitats; mangrove forests, salt marshes and seagrass meadows, with OC being the main component of interest (Duarte et al., 2005b; Fourqurean et al., 2014; McLeod et al., 2011). The importance of aboveground biomass to the carbon cycle depends on the vegetation type and species, with some mangrove forests supporting very substantial aboveground stocks but with most blue carbon sites distinguished by high carbon intensities in the sediment. For example, seagrass species such as *Zostera noltii* accumulate only minor stocks, with very short fronds and wilting during winter. Therefore, any aboveground biomass is unlikely to accumulate within the seagrass meadow and is more likely to be exported by tides to other areas of the coastal seascape, or out to the ocean if it is not

degraded due to its labile nature (Duarte and Krause-Jensen, 2017; Mateo et al., 2006). In contrast, sedimentary stocks of OC may be very large, and much of this in seagrass is attributed to their ability to efficiently capture transported allochthonous carbon (Chmura, 2013; Kelleway et al., 2017). Despite seagrasses' relatively small ocean distribution (area of ~0.1% of the ocean floor) they contribute ~10% of global marine OC burial annually (Costanza et al., 1997; Duarte and Cebrián, 1996; Kennedy et al., 2010). Based on the most recent global collation of data across all seagrass species, ~4.2-8.4 Pg C is stored in the top metre of seagrass sediment (Fourqurean et al., 2012).

3.1.4 Seagrass organic carbon stocks from a UK perspective

In the UK, there are two species of seagrass, *Zostera marina* and *Zostera noltii*, both eelgrasses. There are no complete estimates of total coverage of seagrass meadows in UK waters. Estimates range from a modest 15.9 km² for Scottish waters (Burrows et al., 2014) to 50-100 km² for UK waters (Garrard and Beaumont, 2014) with the most recent UK wide estimation of ~84.9 km² (Green et al., 2021). Three studies have determined OC stocks of meadows around the UK using the sequential loss-on-ignition (LOI) techniques to combust sediment samples from seagrass meadows (Table 3-1). Potouroglou (2016) found that intertidal seagrass sediment OC stocks ranged between 15.92±3.5-107.62±21.9 Mg C/ha (50 cm depth) across seven estuaries in Scotland whereas the mean OC stocks of intertidal meadows in the south of England was 56.3±30.7 Mg C/ha for a 50 cm depth integrated OC stock (Lima et al., 2020). Another south of England subtidal study of *Z.marina* meadows found sediment OC ranged between ~49-190 Mg C/ha for a 50 cm depth-integrated OC stock (Green et al., 2018). Despite all three UK studies using the LOI method, they all combusted samples at differing temperatures and combustion periods making it difficult to compare the three datasets, let alone datasets across the North Atlantic and beyond. Comparisons across datasets is important to confidently produce carbon stocks, identify the importance of the stocks across different spatial scales and aid effective management decisions.

Table 3-1 UK studies using loss on ignition technique to derive OC stocks.

| Study | Location | Seagrass type | Combustion temp (°C) | Period of combustion (hours) | Equation used for %OC | Equation R ² | Estimated OC stocks (Mg C/ha) |
|---------------------|----------|--|----------------------|------------------------------|---|-------------------------|--|
| Potouroglou (2016) | Scotland | Intertidal <i>Z.noltii</i> and <i>Z.marina</i> | 500 | 6 | $(0.41 * \%LOI) - 0.13$ | 0.59 | 15.92±3.52 107.62±21.90 (integrated depth of 50cm) |
| Green et al. (2018) | England | Subtidal <i>Z.marina</i> | 550 | 2 | $(0.3708 * \%LOI) + 0.3732$ | 0.38 | 29.40±0.65 – 114.02±21.45 (integrated depth of 30cm) |
| Lima et al. (2020) | England | Intertidal <i>Z.noltii</i> and <i>Z.marina</i> | 450 | 24 | $\%LOI < 0.2 \ \%OC = (0.40 * \%LOI) - 0.21;$ $\%LOI > 0.2 \ \%OC = (0.43 * \%LOI) - 0.33$ | 0.96 | 33.80±18.40 OC stock to a (integrated depth of 30cm) |

3.1.5 Chapter aim and objectives

This chapter provides information on OC stocks within seagrass meadows across Scotland and aims to provide estimates of carbon density for nine new sites, one of which is subtidal, as well as building on information for two sites that have been previously investigated. The objectives include: 1) to assess the impact of seagrass meadows on carbon density when compared to adjacent bare mudflat areas; 2) to compare carbon storage between sites; and 3) to characterise sedimentary OM using different combustion temperatures to infer OC persistence within seagrass sediments. The following hypotheses are tested in this chapter:

H_{0a} =There is no difference in carbon density between seagrass meadows and adjacent bare mudflat areas

H_{0b} =There is no difference in carbon density between seagrass meadows at different sites

H_{0c} =There is no difference in labile carbon between seagrass and adjacent bare mudflats

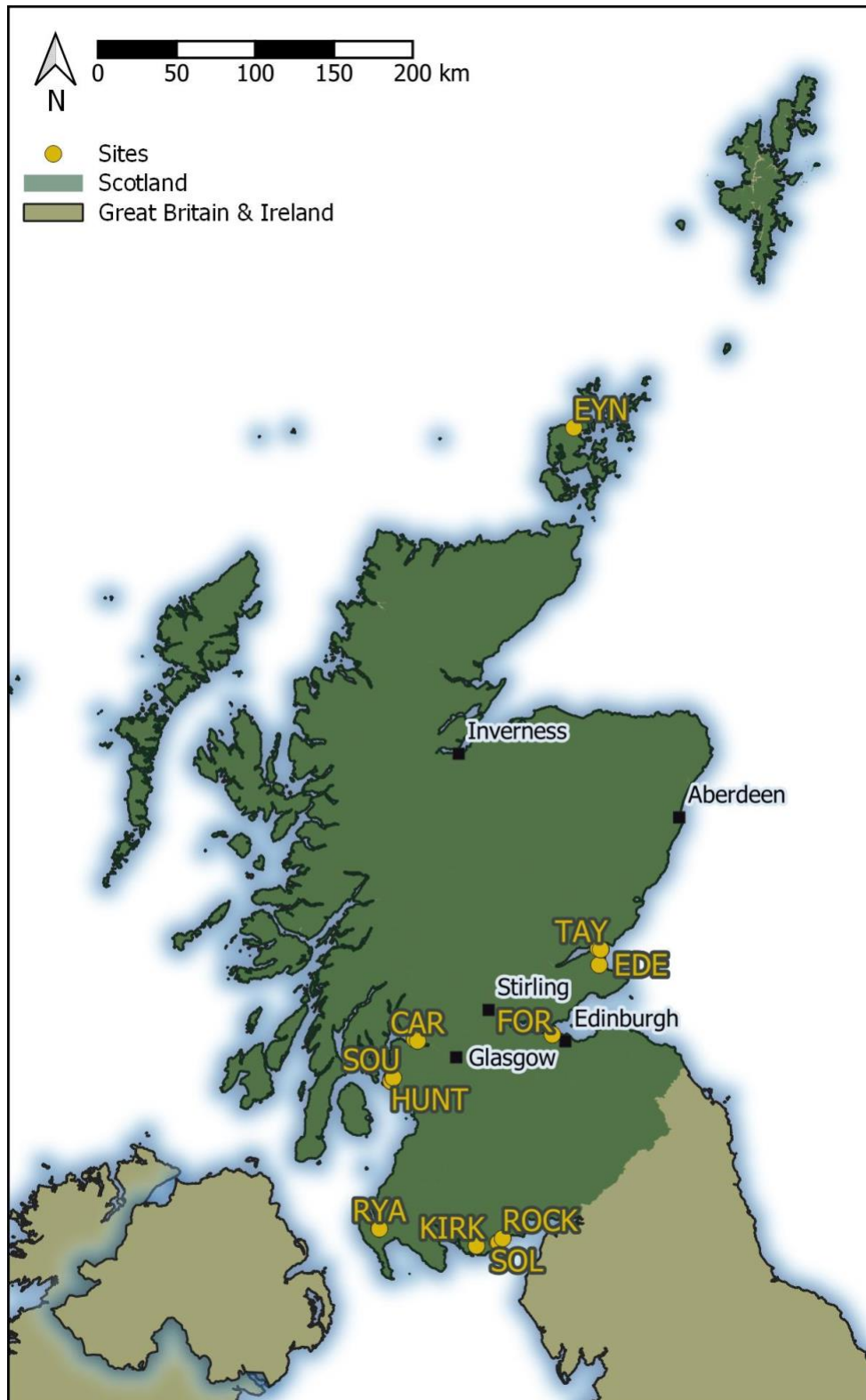
3.2 Methodology

3.2.1 Study area

The study was conducted across eleven Scottish sites; ten intertidal and one subtidal site (Figure 3-2). Three of the sites are situated on the east coast of Scotland: Firth of Forth (FOR), Eden Estuary (EDE) and Firth of Tay (TAY); five on the west coast of Scotland: Firth of Clyde (CAR, HUNT and SOU), Loch Ryan (RYA) and Solway Firth (KIRK, ROCK and SOL); and one site off the mainland of Orkney, Eynhallow (EYN). All intertidal sites were host to the shallow intertidal species of eelgrass, *Z.noltii* with the intertidal variation of *Z.marina* found at SOL and ROCK, whilst EYN was the only subtidal eelgrass *Z.marina* site in this study. Plots and habitats are referred to as either *Z.noltii* associated sediments or *Z.marina* associated sediments throughout the study, with habitat types (seagrass or mudflat) clearly stated. Table 3-2 provides a summary of what samples were used for each test.

Table 3-2 Number of cores associated with each statistical test across each site and habitat type.

| Coast | Site | Associated seagrass | Dry Bulk Density | | Organic Matter | | Organic Carbon | | Carbon Density | |
|-------|------|---------------------|------------------|----------|----------------|----------|----------------|----------|----------------|----------|
| | | | Mudflat | Seagrass | Mudflat | Seagrass | Mudflat | Seagrass | Mudflat | Seagrass |
| East | EDE | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | FOR | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | TAY | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| West | CAR | <i>Z.noltii</i> | 3 | 3 | 3 | 4 | 3 | 4 | 3 | 3 |
| | HUNT | | 1 | 1 | 3 | 3 | 3 | 3 | 1 | 1 |
| | SOU | | 1 | 1 | 3 | 3 | 3 | 3 | 1 | 1 |
| | KIRK | | 1 | 1 | 3 | 3 | 3 | 3 | 1 | 1 |
| | ROCK | | 1 | 1 | 3 | 3 | 3 | 3 | 1 | 1 |
| | | <i>Z.marina</i> | 1 | 1 | 3 | 3 | 3 | 3 | 1 | 1 |
| | SOL | <i>Z.noltii</i> | 1 | 1 | 3 | 3 | 3 | 3 | 1 | 1 |
| | | <i>Z.marina</i> | 2 | 2 | 3 | 3 | 3 | 3 | 2 | 2 |
| | RYA | <i>Z.noltii</i> | 1 | 1 | 3 | 3 | 3 | 3 | 1 | 1 |
| North | EDE | <i>Z.marina</i> | 1 | 1 | 3 | 3 | 3 | 3 | 1 | 1 |



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Scotland: Source OS Open Data. Contains OS data © Crown copyright and database right (2021).
 Additional Boundaries: These data were extracted from the GADM database (www.gadm.org), version 2.5, July 2015. They can be used for non-commercial purposes only. It is not allowed to redistribute these data, or use them for commercial purposes, without prior consent.

Figure 3-2 Map of all study sites.

3.2.2 Sediment sampling

3.2.2.1 Intertidal sediment sampling

All intertidal samples were collected during low tide to safely access each mudflat.

Sediment cores were collected in plots including one seagrass core from the centre of a 1 m² quadrat and one bare adjacent mudflat core. Quadrats were placed in the middle of habitat patches. All plots were a minimum of 2 m² in size to avoid edge effects which include reduced density of seagrass fronds towards the edge of a habitat patch. The seagrass sample location was chosen first and then the nearest bare mudflat habitat patch within 3m at all sites (except Loch Ryan, where this was not possible due to the nature of the seagrass meadow). Three plots were chosen randomly for each site, where possible. Sediment cores were collected using 50 cm or 1 m long PVC pipes (internal diameter 53 mm). The bottom edges of the cores were filed down to create a sharp edge to improve ease of cutting through roots and rhizomes. The pipes were pushed into the sediment by hand until refusal. If the pipe was not submerged to a minimum of 40 cm a weighted hammer was used to reach 50 cm, if possible. At three sites; EDE, FOR and TAY, sediment cores were collected to a depth of 20 cm, these were additional cores collected for the global wetland study and have been included in this chapter because globally shallow cores are regularly used to determine carbon stocks. To remove the PVC cores, rubber stoppers were placed on the top of the corers to create suction when lifting the core out of the ground. The cores were then labelled, wrapped in clingfilm and transported to the laboratory upright to prevent further disturbance. Sample measurements were repeatedly taken of the distance between the top of the soil corer and the sediment surface, before extraction, after extraction, after transportation and before removal from the core. These measurements ensured an accurate compaction factor (when applicable) was applied for each individual core.

3.2.2.2 Subtidal sediment sampling

All subtidal samples were collected by Heriot Watt University scientific divers. The same PVC pipes (50 cm) used for intertidal sampling were used. Cores were pushed into the sediment by hand until refusal (approximately 40 cm), no additional force was applied, therefore all cores collected were shallower than intertidal cores.

Stoppers were placed on the top of the cores and gently extracted from the seafloor and then immediately sealed with a stopper before being placed upright in a mesh bag. Due to conditions during the dive, core depth measurements before and after extraction were not taken, however corers were marked at 10 cm intervals to provide rough estimates of the depth that was reached in situ. Once on the boat the distance between the top of the PVC pipes and the sediment surface were accurately measured. The cores were then wrapped with cling film for transportation to the laboratory upright to reduce sample disturbance. Unfortunately, potential compaction occurring in these sediment cores could not be noted accurately due to the unavoidable limitations of the extraction method.

3.2.3 Dry bulk density

Each core was sliced into 1 cm increments and air dried in aluminium trays. An aliquot of 5 cm³ for each increment was separated and placed in a pre-weighed tray. The tray and sample therein was weighed before being dried at 60°C for 48 hours, or until a constant weight was reached (Fourqurean et al., 2014). Once dried, the sample was re-weighed to calculate dry bulk density (DBD). The often-used temperature of 105°C for DBD determination in soil science was not used due to discrepancies linked to clay structural water loss which can occur when sediment is heated at >100°C (Dankers and Laane, 1983), as discussed in Chapter 2. Sample DBD was calculated using the following equation:

$$\text{DBD (g cm}^{-3}\text{)} = \frac{\text{dried sediment sample weight (g)}}{\text{initial wet sample volume (cm}^3\text{)}}$$

3.2.4 Carbon density and lability

3.2.4.1 Organic matter determination by loss on Ignition

As described in Chapter 2, dried sediment samples were ashed in ceramic crucibles to determine OM content by loss on Ignition (LOI). The following temperatures were used; 250°C for labile OM, 500°C for refractory OM (both for six hours), chosen to coincide with temperatures used in a previous Scottish seagrass meadows study (Potouroglou, 2016). Samples were then ashed at 950°C for two hours to determine IC. The following equations were used for this stepped combustion.

$$\text{labile OM (\%)} = \frac{\text{initial dry sediment weight (g)} - \text{weight of sediment after ignition at 250°C (g)}}{\text{initial dry sediment weight of sample before temperature step (g)}} \times 100$$

$$\text{Refractory OM (\%)} = \frac{\text{initial dry sediment weight at 250°C step (g)} - \text{weight of sediment after ignition at 500°C (g)}}{\text{initial dry sediment weight of sample before temperature step (g)}} \times 100$$

$$\text{Total OM (\%)} = \frac{\text{initial dry weight of sample before all temperature steps (g)} - \text{weight of remaining sample after burning at 500°C (g)}}{\text{Initial dry weight of sample before temperature step (g)}} \times 100$$

$$\text{Inorganic matter} = \frac{\text{initial dry sediment weight at 500°C step (g)} - \text{weight of sediment after ignition at 950°C (g)}}{\text{initial dry sediment weight before temperature step (g)}} \times 100$$

3.2.4.2 Determination of organic carbon density

To determine the percentage of OC content (%) by using LOI a regression equation needs to be applied. Most recently Potouroglou (2016) created a Scotland specific equation, despite the weaker relationship strength of this equation when compared to global equations (Table 3-3). The blue carbon manual (Fourqurean et al., 2014) advises to use a local or regional equation where possible and therefore the Scotland specific equation ($\text{OC (\%)} = (0.41 \times \text{OM(\%)}) - 0.13$) has been used throughout this study. Organic carbon content was then used to determine carbon density at each increment before being summed to provide carbon density for each core depth. The compaction factor was derived from the measurements taken before and after extraction, in addition to the measurement taken before the samples were removed for sample preparation.

Table 3-3 Summary of equations available to convert OM content (%) to OC content (%) across Scottish seagrass meadows.

| Equation | Relationship Strength (R ²) | Relationship between LOI (%) and OC (%) | Source |
|----------|---|---|--------------------------|
| Global 1 | 0.87 | OC (%) = (0.4 × OM) – 0.21 | Fourqurean et al. (2012) |
| Global 2 | 0.96 | OC (%) = (0.43 × OM) – 0.33 | Fourqurean et al. (2012) |
| Scotland | 0.59 | OC (%) = (0.41 × OM) – 0.13 | Potouroglou, (2016) |

The following equation was used to convert OC to carbon density:

$$\text{Carbon density (g C cm}^3\text{)} = \left(\text{dry bulk density (cm}^{-3}\text{)} \times \frac{\text{organic carbon (\%)}}{100} \right) \times \text{compaction factor}$$

3.2.5 $\delta^{13}\text{C}$ isotopic signature determination

In addition to the sediment $\delta^{13}\text{C}$ isotopic analysis conducted in chapter 2, four biomass samples of collected seagrass material were also analysed. Approximately 5-15 mg of sediment sample or 1.5 – 2 mg of biomass (weight used dependant on % OM content of sample) was accurately weighed into 3 x 5 mm tin capsules and loaded into the autosampler of an Elementar (Hanau, Germany) Pyrocube elemental analyser. OC in the samples were converted to CO₂ and measured for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ respectively on a Thermo-Fisher-Scientific (Bremen, Germany) Delta XP Plus IRMS. In-house reference materials used were GEL (gelatin solution), ALAGEL (alanine-gelatine solution spiked with ¹³C-alanine), and GLYGEL (glycine-gelatine solution), each dried for 2 hours at 70°C and interspersed throughout the samples. In addition four USGS 40 glutamic acid standards (Coplen et al., 2006; Qi et al., 2003) were used as independent checks of accuracy. All samples were processed and analysed at National Environmental Isotope Facility – Stable Isotopes Laboratory (NEIF-SI, SUERC).

To estimate the contribution of different OM sources to sediments across sites the Isosource 1.3.1 mixing model software was used (Phillips and Gregg, 2003). Four seagrass samples alongside literature derived isotopic signatures were run in the model.

3.2.6 Statistics

All analyses were conducted in R Studio. All figures were produced using the package ggplot2 (Wickham, 2016). Initially one-way ANOVA models were used (CAR package; Fox and Weisberg, 2021) to determine if there were differences between seagrass species and their associated mudflat habitats, if no statistically significant difference was found further analyses included both seagrass species. Nested ANOVA models were used to statistically test differences across and within sites and habitat types for sedimentary OM (%), OC (%) and OC density. using the lme4 package (lmer function) using a nested ANOVA (Bates et al., 2015), where depth is nested within sample site. The nested models were also run through a post-hoc comparison of means via the glht function in the multcomp package (Hothorn et al., 2008). Prior to analysis all data were tested for normality and homogeneity of variance before running statistics. Unless stated otherwise all mean values are represented with associated standard deviations.

3.3 Results

3.3.1 Dry bulk density and organic matter

The mean DBD (g cm^3) in *Z.noltii* sediments ranged from 1.02 ± 0.05 (TAY) to 1.55 ± 0.09 g cm^3 (RYA), whilst across the mudflat habitat type there was a larger range in mean DBD values from 0.85 ± 0.18 (TAY) to 1.63 ± 0.1 g cm^3 (SOU; Table 3-4). East coast sites shared a similar range in DBD values (Figure 3-3). Within *Z.marina* meadows in both seagrass and mudflat habitats, EYN had the lowest mean DBD values (1.21 ± 0.08 and 1.11 ± 0.09 g cm^3 , respectively), and SOL had the highest mean DBD values (1.33 ± 0.06 and 1.35 ± 0.06 g cm^3 , respectively; Table 3-4). A one-way ANOVA identified there was no statistical difference between DBD means across seagrass species and associated mudflat habitats ($\text{df} = 3$, $F = 2.01$, $p = 0.111$). Therefore, both seagrass species were analysed together in a nested ANOVA. There was a statistically significant interaction term between sites and habitat type ($\text{df} = 10$, $F = 7.09$, $p < 0.001$). seven of the eleven sites had within-site differences between DBD values, with only FOR, HUNT, RYA and SOL sharing similar DBD values regardless of habitat type.

Table 3-4 Summary of mean dry bulk density (DBD), organic matter (OM), organic carbon (OC) and OC stock from the 11 seagrass meadows and adjacent mudflat areas surveyed.

| | | | DBD (g cm ³) | | OM (%) | | OC (%) | | Organic carbon stock 50cm (Mg C/ha) | |
|------|--------------|---------------------|--------------------------|-------|--------|-------|--------|-------|-------------------------------------|----------------|
| Site | Habitat type | Associated seagrass | μ | σ | μ | σ | μ | σ | Σ | σ |
| EDE* | Mudflat | <i>Z. noltii</i> | 0.976 | 0.113 | 2.881 | 0.781 | 1.051 | 0.320 | 19.883 | 0.174 |
| | Seagrass | | 1.050 | 0.048 | 1.532 | 0.319 | 0.498 | 0.131 | 10.455 | 0.121 |
| FOR* | Mudflat | | 1.039 | 0.055 | 4.128 | 1.184 | 1.562 | 0.485 | 32.445 | 0.464 |
| | Seagrass | | 1.049 | 0.090 | 4.289 | 1.016 | 1.628 | 0.417 | 33.833 | 0.341 |
| TAY* | Mudflat | | 0.847 | 0.180 | 5.909 | 3.434 | 2.293 | 1.408 | 35.769 | 0.704 |
| | Seagrass | | 1.015 | 0.049 | 2.793 | 1.509 | 1.015 | 0.619 | 20.507 | 0.535 |
| CAR | Mudflat | | 1.466 | 0.121 | 1.563 | 0.652 | 0.511 | 0.267 | 31.405 | 0.410 |
| | Seagrass | | 1.419 | 0.119 | 2.038 | 0.838 | 0.705 | 0.343 | 47.189 | 1.357 |
| HUNT | Mudflat | | 1.552 | 0.087 | 0.910 | 0.247 | 0.243 | 0.101 | 11.692 | 0.198 |
| | Seagrass | | 1.518 | 0.317 | 1.835 | 2.269 | 0.623 | 0.930 | 40.010 | 2.025 |
| SOU | Mudflat | | 1.632 | 0.102 | 0.851 | 0.266 | 0.219 | 0.109 | 21.250 | 0.633 |
| | Seagrass | | 1.537 | 0.091 | 1.060 | 0.304 | 0.304 | 0.125 | 29.859 | 0.766 |
| KIRK | Mudflat | | 1.432 | 0.044 | 2.053 | 0.488 | 0.712 | 0.200 | 50.066 | 1.913 |
| | Seagrass | | 1.348 | 0.054 | 2.155 | 0.476 | 0.753 | 0.195 | 58.288 | 2.154 |
| ROCK | Mudflat | | 1.568 | 0.052 | 2.189 | 0.377 | 0.768 | 0.155 | 61.883 | 1.760 |
| | Seagrass | | 1.379 | 0.048 | 1.730 | 0.322 | 0.579 | 0.132 | 39.587 | 1.116 |
| SOL | Mudflat | | 1.448 | 0.134 | 3.081 | 0.663 | 1.133 | 0.272 | 75.687 | 1.638 |
| | Seagrass | | 1.434 | 0.130 | 2.583 | 0.615 | 0.929 | 0.252 | 67.791 | 2.091 |
| RYA | Mudflat | | 1.578 | 0.105 | 1.016 | 0.405 | 0.287 | 0.166 | 32.400 | 1.434 |
| | Seagrass | | 1.547 | 0.091 | 0.986 | 0.305 | 0.274 | 0.125 | 32.071 | 1.519 |
| ROCK | Mudflat | <i>Z. marina</i> | 1.514 | 0.062 | 1.916 | 0.404 | 0.656 | 0.165 | 51.837 | 1.559 |
| | Seagrass | | 1.522 | 0.080 | 1.745 | 0.271 | 0.585 | 0.111 | 45.566 | 1.328 |
| SOL | Mudflat | | 1.349 | 0.065 | 2.475 | 1.202 | 0.885 | 0.493 | 69.887 (73.801) | 1.633 (3.293) |
| | Seagrass | | 1.390 | 0.073 | 2.688 | 3.056 | 0.972 | 1.253 | 120.033 (76.033) | 13.715 (2.151) |
| EYN | Mudflat | | 1.112 | 0.088 | 1.699 | 0.270 | 0.567 | 0.111 | 29.530 | 0.848 |
| | Seagrass | | 1.214 | 0.080 | 1.999 | 0.296 | 0.690 | 0.121 | 36.950 | 0.836 |

* Total stock figures in green are down core profiles to a 20 cm depth. Numbers within brackets are the carbon stocks for the 75 cm depth core profile

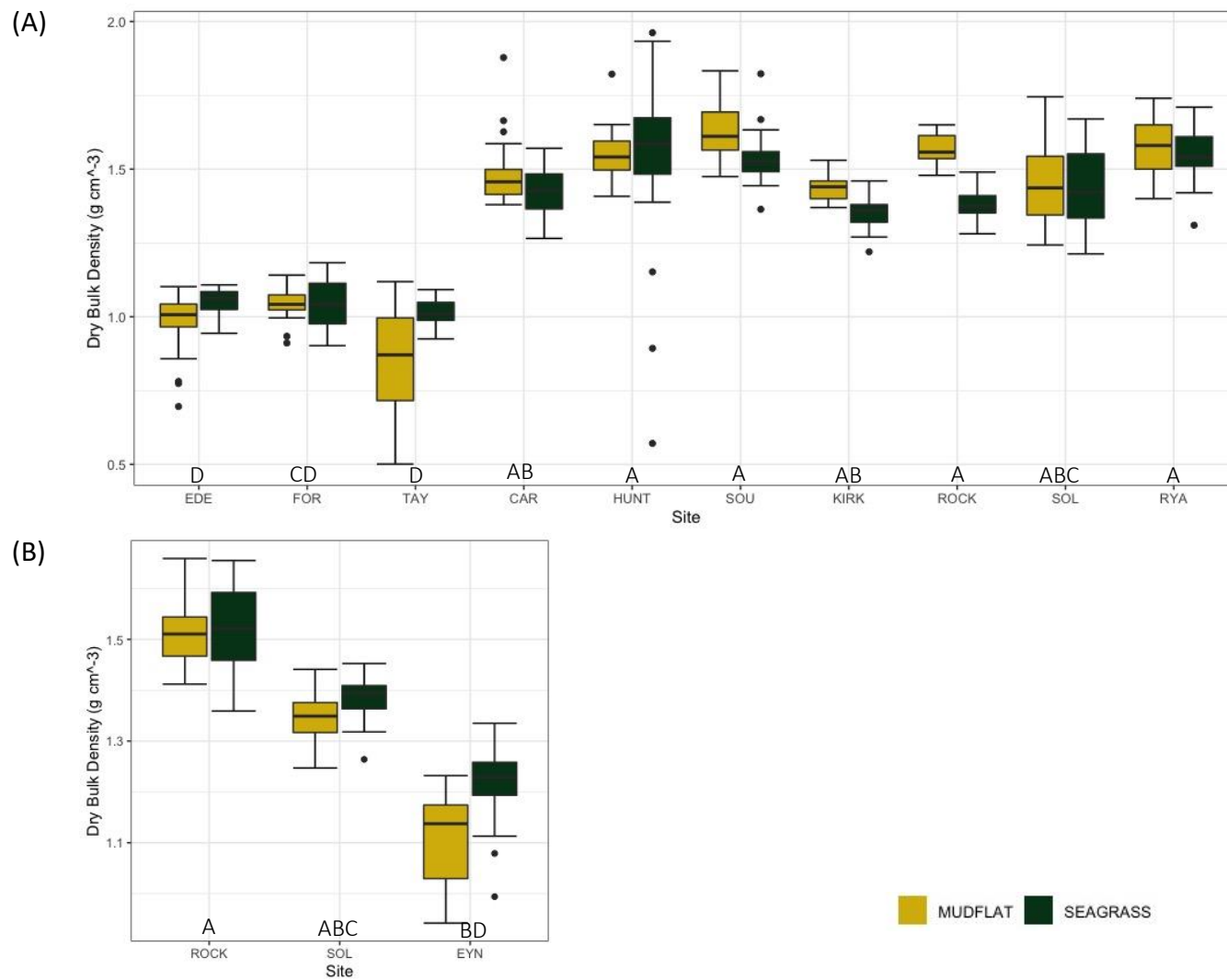


Figure 3-3 Dry bulk density (g cm^{-3}) per site and habitat type within (A) *Z. noltii* (B) *Z. marina* meadows. Model used (DBDmodel= lmer(DBD~SITE+HABITAT TYPE+SITE*HABITAT TYPE+(1|SITE/cm_increment),data=carbon_allsites,REML=TRUE). Letters represent post-hoc Tukey's test between all sites, regardless of seagrass species.

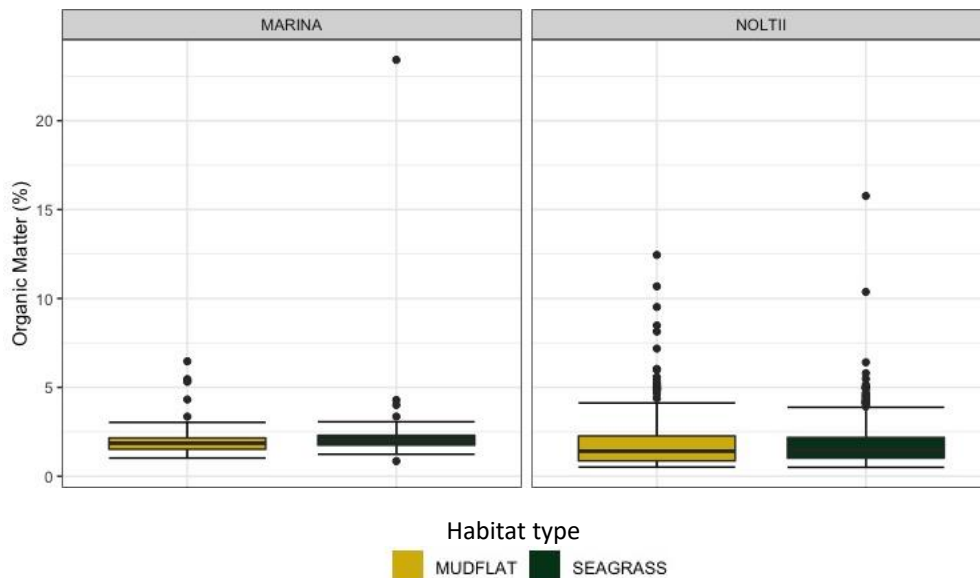


Figure 3-4 Organic matter (%) of habitat types (mudflat and seagrass) associated with *Z.marina* and *Z.noltii* meadows.

Organic matter content (%) within *Z.noltii* associated sediments ranged from 0.50-15.77%, with an average of $1.81 \pm 1.29\%$ and a median of 1.48% within seagrass cores, whereas within mudflat cores a smaller range of OM content values were recorded (0.51-12.45%) but the average ($1.83 \pm 1.49\%$) and median value (1.41%) were similar. With a higher median value $1.90 \pm 0.24\%$. *Zostera marina* meadows had a greater proportion of samples within a much closer range, fewer outliers and higher average and median OM content than samples associated with *Z.noltii* areas, but it is important to note that *Z.marina* was found at fewer sites (Figure 3-4). *Z.marina* mudflat samples the average OM content was $2.02 \pm 0.85\%$ with a median of 1.86%. The maximum OM content in mudflat samples was 6.47%, whilst the largest OM content value in the seagrass samples was 23.42% at SOL. The average OM content within *Z.marina* seagrass samples was $2.22 \pm 1.93\%$ with a median of 2.01%). Despite Fig-3-4 showing sediment associated with *Z.marina* and *Z.noltii* meadows appears to have similar OM values regardless of habitat type, a one-way ANOVA determined there were statistically significant differences in OM content between the seagrass species ($df = 3$, $F = 3.49$, $p = 0.02$), therefore nested analysis was carried out on species separately. For OM associated with *Z.noltii* meadows and mudflats there was a significant two-way interaction between site and treatment ($df = 9$, $F = 17.65$, $p < 0.001$). However, there was no statistical difference for interaction

term ($df = 2$, $F = 0.43$, $p = 0.65$) or individual factors in OM content for *Z.marina* associated habitats.

Figure 3-5 shows for *Z.marina* associated sites, regardless of habitat type, there is not a relationship between OM and DBD ($R^2 = 0.011$ and 0.0107 in seagrass and mudflat samples, respectively). However in *Z.noltii* associated plots there is a weak negative relationship for both habitat types ($R^2 = 0.309$ and 0.5616 in seagrass and mudflat samples, respectively). This relationship emerging between OM and DBD follows expected trends. Analysis of the data suggests more sample points are required to determine, with confidence, if the weak negative relationship within *Z.noltii* meadows and mudflat plots is important, given neither regression analysis explained $> 56\%$ of the variability. Further to this, when looking at the relationship between OM content and DBD at a site level only HUNT, EDE and TAY show significant difference from other sites because OM content, in general, is low amongst the sites chosen in this study (Figure 3-6) and are likely to be the main sites creating the negative relationship between OM and DBD, where decreased DBD values support greater OM content (Figure 3-5).

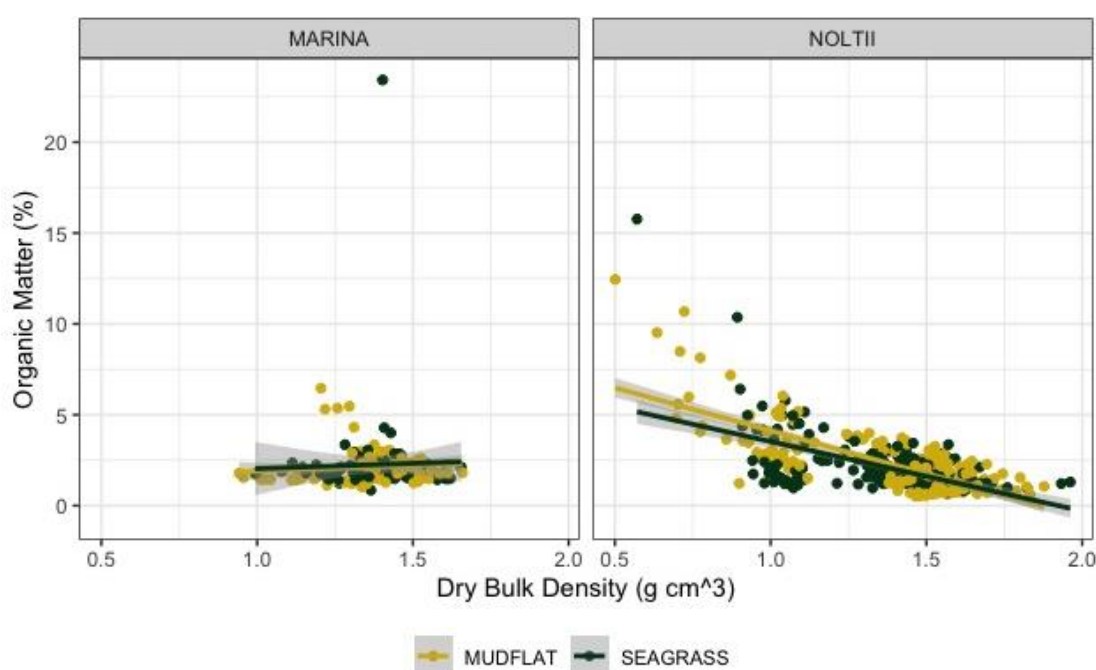


Figure 3-5 Linear regression relationship between DBD ($g\ cm^3$) and OM (%) for both species of seagrass and mudflat areas. Lines have been fitted for each habitat type using 'geom_smooth (formula = $y \sim x$, method = "lm")

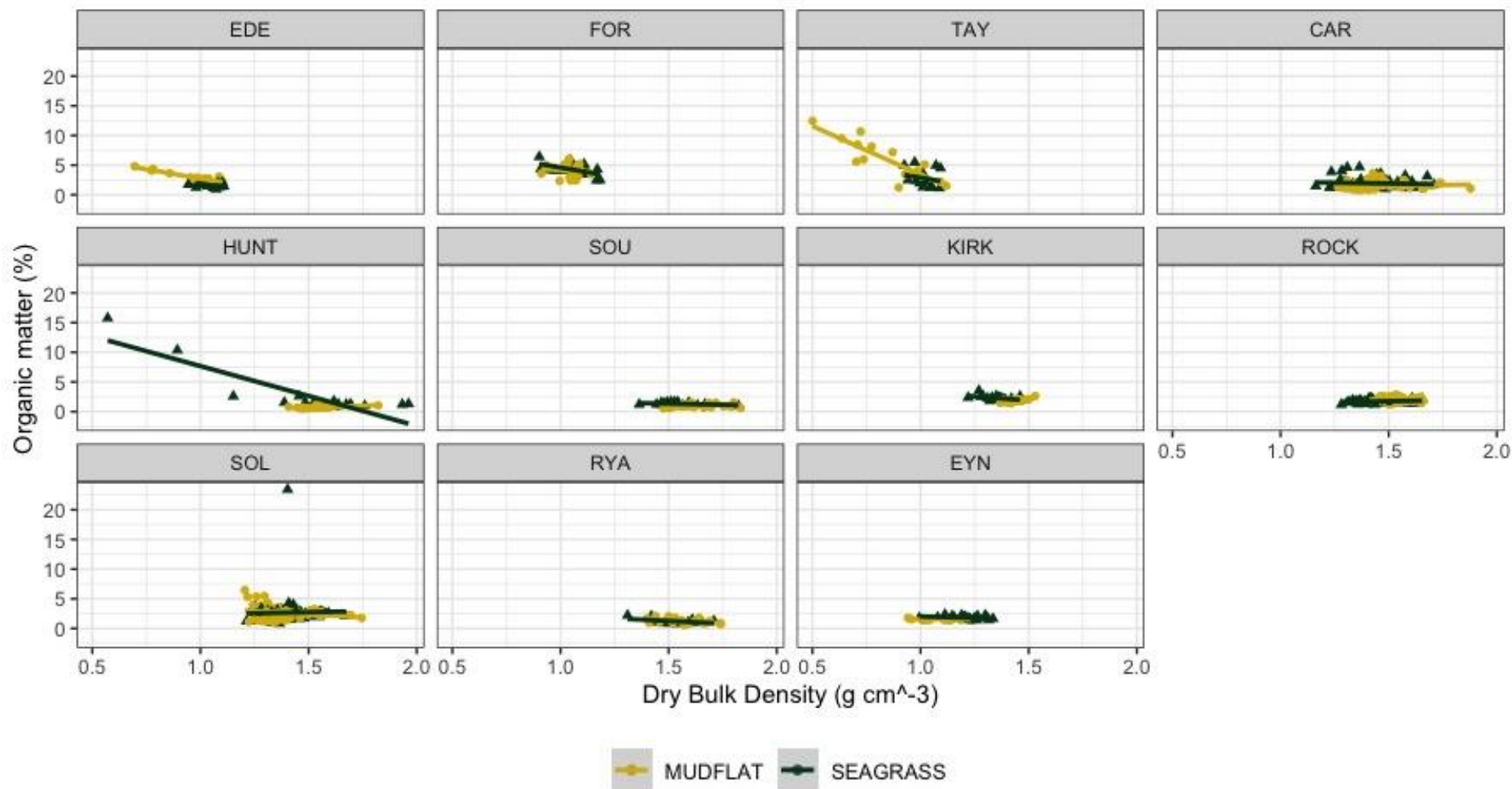


Figure 3-6 Linear regression relationship between DBD (g cm³) and OM (%) for each site. Fitted using 'geom_smooth (formula = y ~ x, method = "lm")'.

3.3.2 Organic carbon content

Average OC content within the core subsamples ranged between 0.08-6.33% in seagrass samples and from 0.08-4.97% in mudflat samples across *Z.noltii* meadows (Figure 3-7). The largest range in OC content is seen across the three east coast sites (EDE, FOR, TAY) and HUNT. Figure 3-8A shows that the largest distinction in habitats is seen at EDE, TAY and HUNT. Exploring this variation in *Z.noltii* sites, the TAY mudflat core highlights large down-core variation in OC content at each depth increment, whereas the seagrass core is less varied and begins to increase after 15 cm depth. At HUNT there is a spike in OC content down-core, however the large uncertainty associated with the spike should be noted (Figure 3-8A). The average OC content across all *Z.marina* meadows was similar across habitat types within sites (Figure 3-7B). Organic carbon content within *Z.marina* associated sediments ranged between 0.29-2.52% in mudflat samples and 0.22-9.47% in seagrass samples. The largest value found was within a seagrass core at SOL at 37.5 cm (Figure 3-8B).

A one-way ANOVA confirmed mean OC content was significantly different across seagrass species ($df = 3$, $F = 3.489$, $p = 0.015$), therefore species were analysed in two separate nested ANOVA models. Statistical analysis on OC (%) identified a two-way interaction between site and habitat type for *Z.noltii* associated sites ($df = 9$, $F = 17.65$, $p < 0.001$). Despite this strong interaction term, there are only three sites (EDE, TAY and HUNT) where within-site differences are statistically significant. In contrast, when testing *Z.marina* associated samples, no statistically significant interaction terms were found between site and habitat type OC content ($df = 2$, $F = 0.43$, $p = 0.65$) and neither factor; site ($df = 2$, $F = 0.85$, $p = 1$) and habitat type ($df = 1$, $F = 0.34$, $p = 0.56$) had significantly different OC content values (Figure 3-7B).

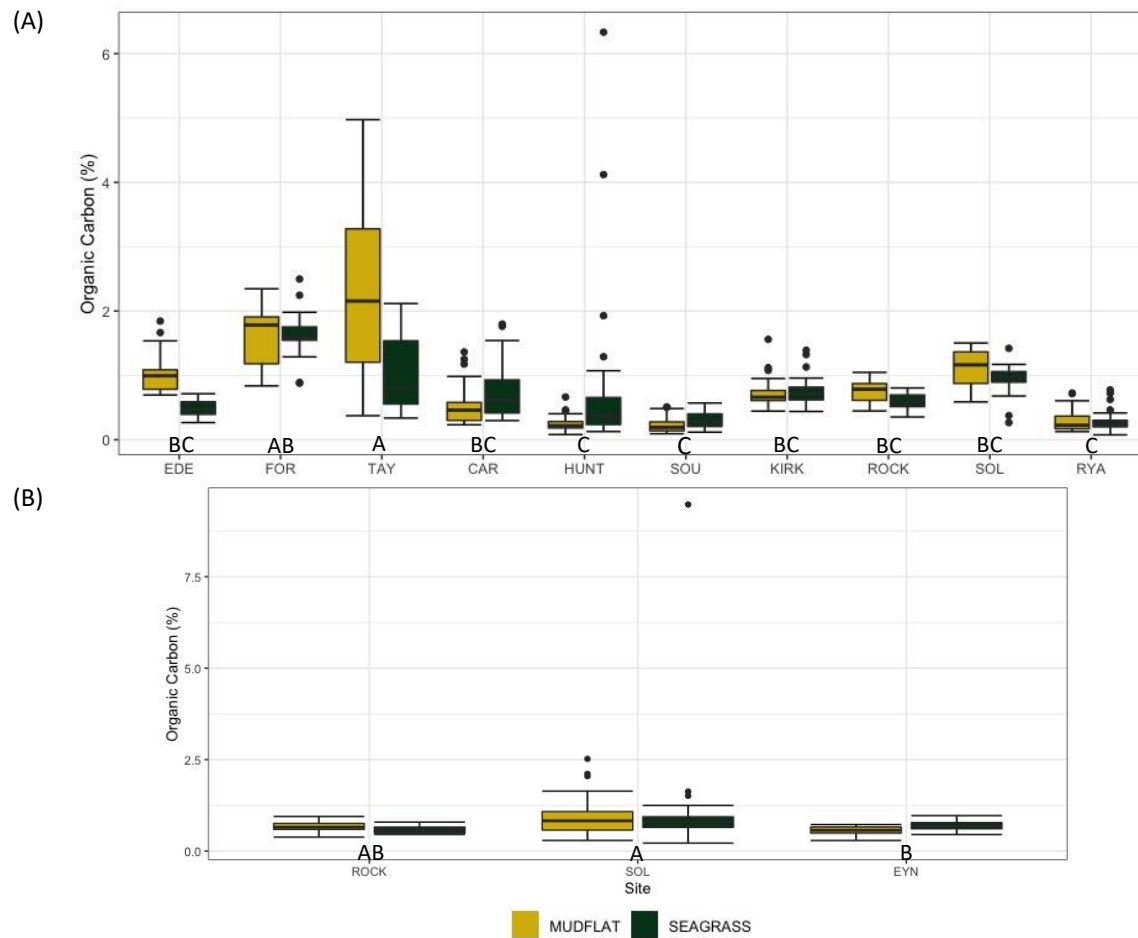
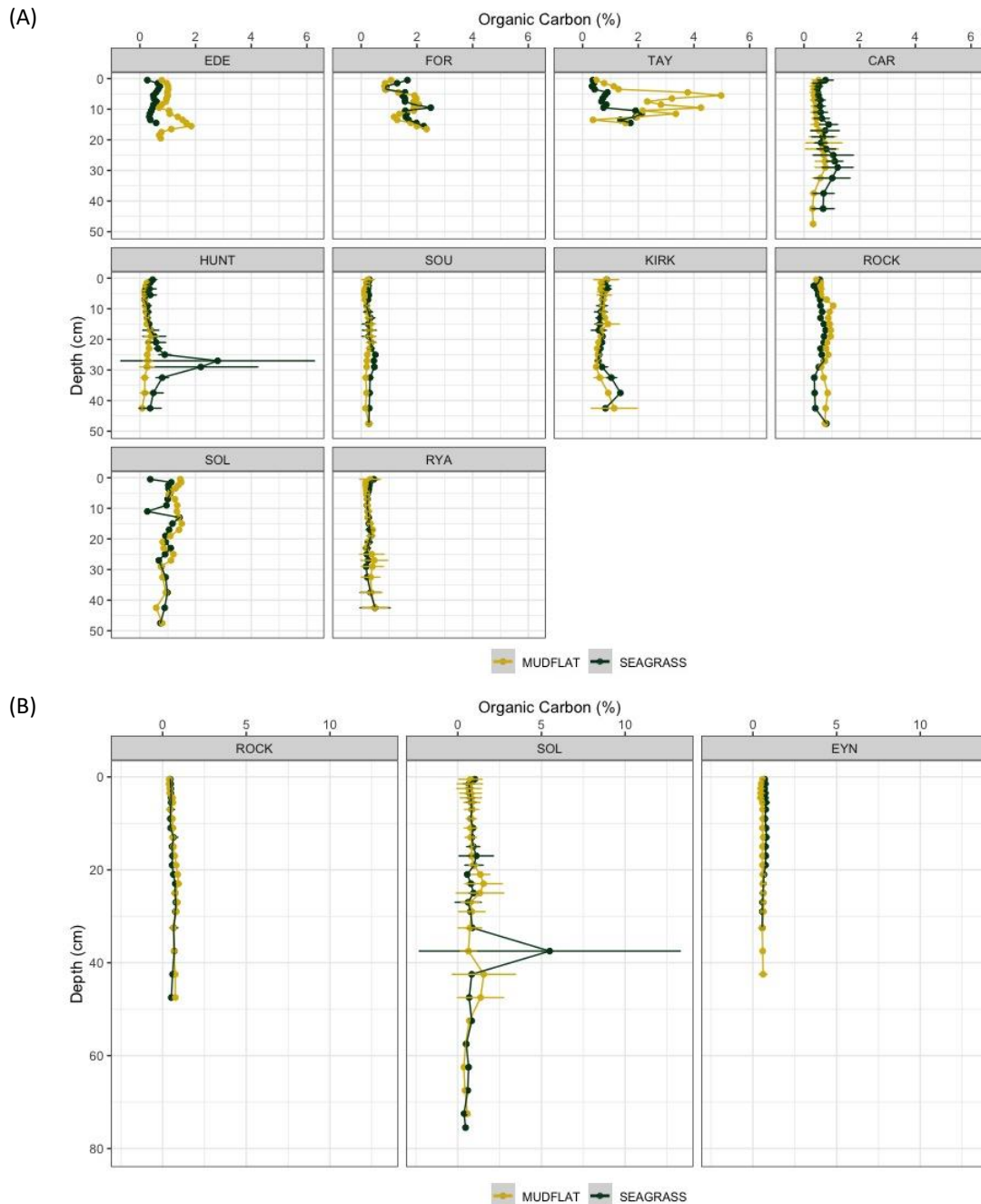


Figure 3-7 Boxplot of organic carbon content (%) across (A) ten *Z. noltii* sites and (B) three *Z. marina* sites. Model used (OCmodel= lmer(OC~SITE+HABITAT TYPE+SITE*HABITAT TYPE +(1|SITE/cm_increment), data=carbon_allsites,REML=TRUE). Letters represent post-hoc Tukey's test preformed for individual seagrass species datasets.



3.3.2.1 Variation in organic carbon content across coasts

Across *Z. marina* cores, regardless of habitat, OC content is similar across the full depth profile (Figure 3-9B). However, within the subtidal seagrass habitat there was a slight negative relationship found between OC content and depth, where over 38% of the data was explained ($R^2 = 0.381$). Within *Z. noltii* meadows, particularly across the west coast, OC content did not follow any trend across the sediment depth profile with linear trendlines explaining <1% of data in mudflat sediments and 2% of

data in seagrass sediments (Figure 3-9A). Only the shallower east coast seagrass habitats imply there could be a positive relationship between OC content in seagrass cores with depth, but only 17% of these data is explained ($R^2 = 0.172$) with even less of the mudflat core data from east coast sites explained by a linear trendline ($R^2 = 0.004$). Therefore, there is no evidence to suggest sediment depth has a significant influence on OC content within these Scottish intertidal seagrass meadows or mudflat areas. However further exploration of the relationship between subtidal seagrasses and OC content at depth should be explored.

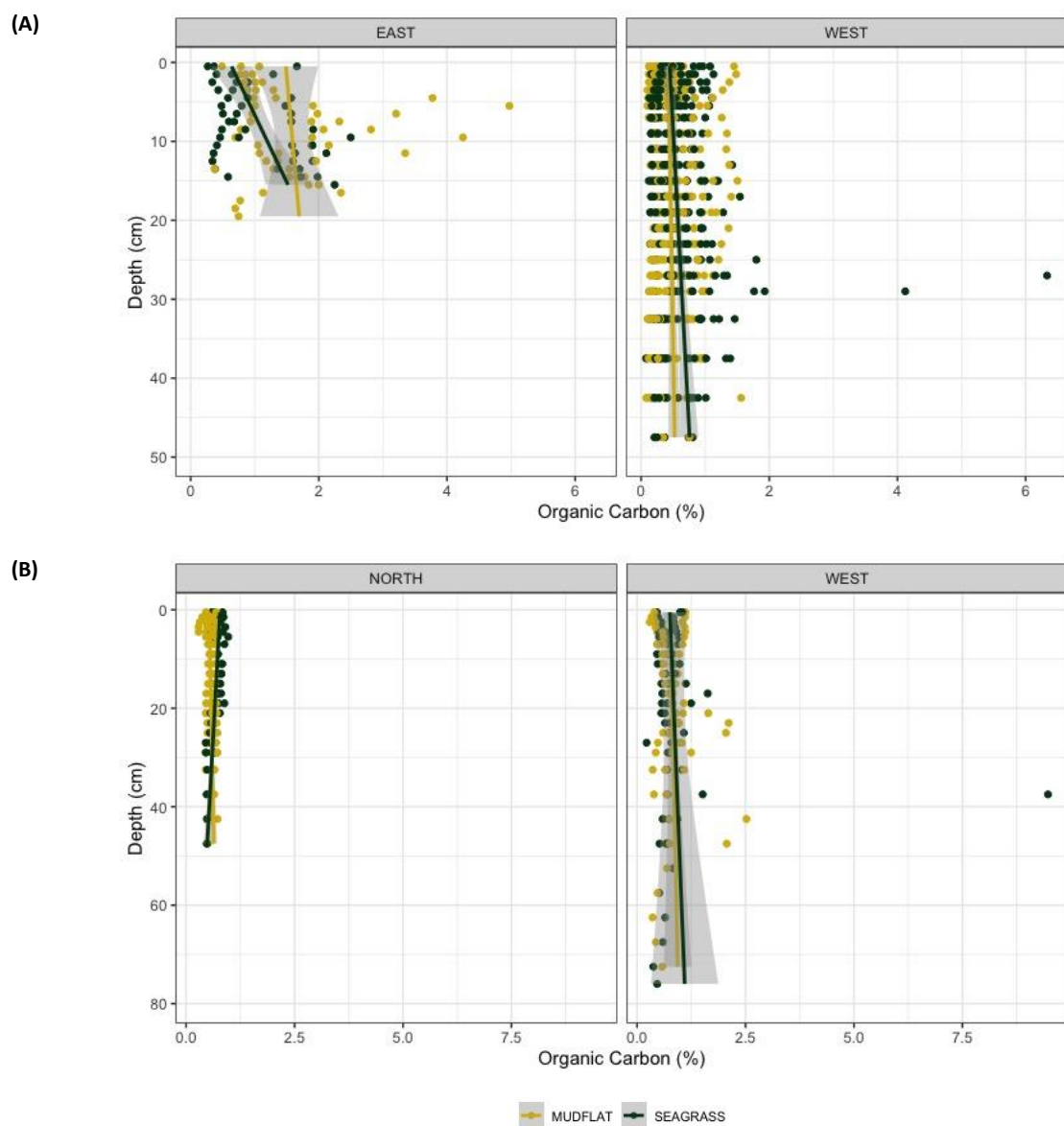


Figure 3-9 Regression analysis of average OC content (%) against depth for *Z. noltii* (A) and *Z. marina* (B) associated sediments across coasts. Fitted using 'geom_smooth (formula = $y \sim x$, method = "lm")'.

3.3.3 Carbon density

A one-way ANOVA found no difference between carbon density (Mg C/ha) across seagrass species, and associated mudflats ($df = 3$, $F = 1.50$, $p = 0.213$). Therefore, the nested model included both species. Both site ($df = 10$, $F = 0.53$, $p = 0.867$) and habitat type ($df = 1$, $F < 0.00$, $p = 0.954$) as single factors were not statistically different, however the two-way interaction term was significantly different ($df = 10$, $F = 3.40$, $p < 0.001$). Within-site differences were only found at EDE, TAY, CAR and HUNT. Overall combined factors drove statistically significant differences between carbon density and *Z.noltii* associated samples. Whereas carbon density of *Z.marina* associated samples, firstly were from a much smaller sample size and there was less variation between sites and habitat types, with no within-site differences being found for any *Z.marina* associated samples.

3.3.3.1 Organic carbon stocks for *Z.noltii* meadows

Total sediment OC stocks confirm the statistical result, stocks vary across sites and habitat types within sediment associated with *Z.noltii* meadows (Figure 3-10; Table 3-4). The largest total OC stocks both for seagrass and mudflat areas were found at SOL (67.79 ± 2.09 and 75.69 ± 1.64 Mg C/ha, respectively). The largest difference in OC stocks between habitat types, for *Z.noltii* areas, was found at HUNT, where seagrass meadows enhanced carbon storage by a factor of 3.42, compared to mudflat habitats. CAR, HUNT and SOU are all located within the Firth of Clyde and within each meadow, *Z.noltii* seagrass enhanced OC stocks compared to mudflat habitats. The lowest OC stocks found within seagrass meadows was at EDE (10.45 ± 0.12 Mg C/ha) where OC stocks were almost double in the adjacent mudflat area (19.88 ± 0.17 Mg C/ha) at a 20 cm depth profile, whilst the lowest OC stocks within seagrass meadows (50 cm depth profile) was at SOU (29.86 ± 0.77 Mg C/ha). Four sites (EDE, TAY, ROCK and SOL) had higher OC stocks for mudflats than *Z.noltii* meadows, four sites had higher OC stocks in seagrass than mudflats samples and at two sites there was no difference between seagrass and mudflat habitats (Figure 3-10). Overall, the range of OC stocks across the seven *Z.noltii* areas (50 cm depth profile) is 29.86 ± 0.77 – 67.79 ± 2.09 Mg C/ha with a median OC stocks value of 40.01 ± 13.85 Mg C/ha for

sediment associated with seagrass. Whereas in mudflat areas associated with *Z.noltii* (50cm depth profile) the range of OC stocks was 11.69 ± 0.2 - 75.69 ± 1.64 Mg C/ha with a median OC stock value of 32.40 ± 22.86 Mg C/ha.

3.3.3.2 Organic carbon stocks for *Zostera marina* meadows

Total organic carbon stocks associated with *Z.marina* meadow cores show habitat type does differ, but is less varied than the OC stocks associated with *Z.noltii* meadows (Figure 3-10), though no within-site differences between habitat types were significant. The largest difference in habitat type is seen in the 50cm seagrass stock (120.03 ± 13.72 Mg C/ha). The SOL stock was over 1.7 times higher than the adjacent mudflat (69.89 ± 1.63 Mg C/ha). The standard deviation is also very high for the SOL seagrass OC stock, resultant of carbon density being predominantly concentrated within one 5 cm section of the depth profile (Figure 3-8B). However, there is no statistical difference between habitats found in the deeper OC stock (75 cm depth profile) at SOL (76.03 ± 2.15 Mg C/ha for seagrass and 73.8 ± 3.29 Mg C/ha for mudflat). Overall, focusing on the OC stocks within the 50 cm depth profile cores, the median OC stocks within *Z.marina* associated sediments (45.57 ± 45.68 Mg C/ha) was lower than adjacent mudflat areas (51.84 ± 20.22 Mg C/ha), however, due to the much higher OC stock found within one *Z.marina* bed at SOL there is large error associated with the values.

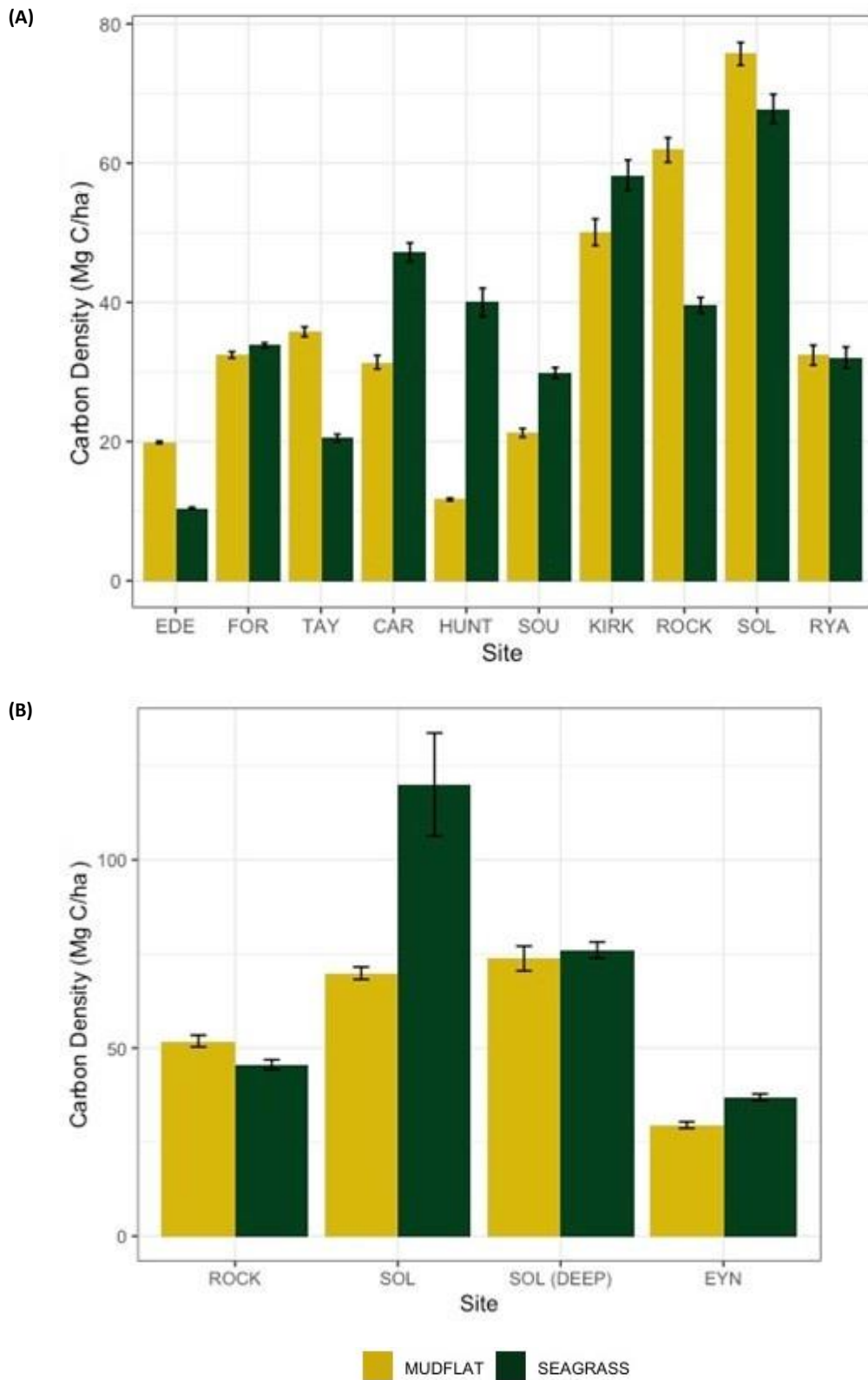


Figure 3-10 Total carbon density within cores across sites and habitat types depth for *Z. noltii* (A) and *Z. marina* (B). Values are representative of 50 cm depth profiles, except the three east coast values (EDE, FOR, TAY) which represent 20 cm depth profiles, and SOL (DEEP) representing 75cm depth profiles. Error bars represent standard deviation.

3.3.4 Carbon lability

For this section of the study only eight sites were explored, six *Z.noltii* associated meadows (EDE, FOR, TAY, CAR HUNT and RYA) and two *Z.marina* associated meadows (SOL and EYN). The lowest mean labile matter content (%) was found in the two *Z.marina* associated sediments, regardless of habitat type (Table 3-5). The highest mean proportion of labile content was found in EDE regardless of habitat type ($31.71 \pm 6.01\%$ within seagrass samples and $30 \pm 5.32\%$ within mudflat samples). Across all *Z.noltii* sites, average labile matter content was generally similar within sites, but seagrass samples consistently returned higher values (Figure 3-11). The HUNT site was exceptional in this regard, demonstrating much higher labile content in seagrass sediment ($17.49 \pm 7.52\%$) compared with the respective mudflat samples ($9.21 \pm 3.56\%$). There was a different pattern for the refractory component; FOR contained the highest average proportion of refractory content; $49.34 \pm 2.41\%$ for seagrass and $56.31 \pm 6.28\%$ for mudflat habitats and the lowest inorganic content for seagrass ($29.29 \pm 2.92\%$) and mudflat habitats ($25.12 \pm 5.01\%$). Core samples from EYN, the subtidal *Z.marina* site, contained the lowest refractory content and highest inorganic content, regardless of habitat, which was expected as it is much coarser with a very low proportion of silt or clay particles ($<2\%$; Chapter 6). Further to this, FOR, TAY and CAR all contained a higher average proportion of refractory content within mudflat samples than seagrass samples. Statistical analysis showed there was a significant two-way interaction term between site and habitat type when testing the differences between the proportion of labile OM within a sample (Table 3-6).

Table 3-5 Summary of all sites and habitats mean and standard deviation of labile, refractory and inorganic matter (%) composition.

| Site | Habitat type | Associated seagrass | Mean labile (%) | | Mean refractory (%) | | Mean inorganic (%) | |
|------|--------------|---------------------|-----------------|----------|---------------------|----------|--------------------|----------|
| | | | μ | σ | μ | σ | μ | σ |
| EDE | Mudflat | <i>Z.noltii</i> | 30.00 | 5.32 | 29.48 | 1.60 | 40.51 | 1.60 |
| | Seagrass | | 31.71 | 6.01 | 37.70 | 6.28 | 30.59 | 6.28 |
| FOR | Mudflat | | 18.57 | 2.52 | 56.31 | 6.28 | 25.12 | 5.01 |
| | Seagrass | | 21.37 | 2.48 | 49.34 | 2.41 | 29.29 | 2.92 |
| TAY | Mudflat | | 20.98 | 6.14 | 44.10 | 7.80 | 34.92 | 11.85 |
| | Seagrass | | 18.61 | 4.96 | 28.44 | 3.29 | 52.95 | 6.10 |
| CAR | Mudflat | | 14.90 | 5.66 | 42.59 | 13.09 | 42.51 | 12.52 |
| | Seagrass | | 16.06 | 5.99 | 37.66 | 13.37 | 46.27 | 16.11 |
| HUNT | Mudflat | | 9.21 | 3.56 | 37.00 | 9.15 | 53.79 | 11.06 |
| | Seagrass | | 17.49 | 7.52 | 41.56 | 10.50 | 40.95 | 15.62 |
| RYA | Mudflat | | 11.77 | 3.57 | 30.59 | 8.30 | 57.64 | 11.46 |
| | Seagrass | | 12.52 | 3.99 | 30.99 | 7.27 | 56.49 | 11.12 |
| SOL | Mudflat | <i>Z.marina</i> | 4.01 | 1.48 | 26.44 | 10.28 | 69.55 | 11.25 |
| | Seagrass | | 3.42 | 1.88 | 32.38 | 13.75 | 64.19 | 13.45 |
| EYN | Mudflat | | 1.90 | 0.30 | 3.69 | 0.30 | 94.41 | 0.23 |
| | Seagrass | | 1.64 | 0.67 | 3.44 | 0.38 | 94.93 | 1.02 |

Exploring down-core profile trends, there is a gradual decline in labile material at most sites, with the exception of mudflat habitats for HUNT and RYA (Figure 3-12). Across EDE and EYN down-core refractory matter proportions do not vary, regardless of habitat. Within FOR cores the refractory proportion varies down profile, however, it remains consistently high within both habitat types (Figure 3-12). Refractory matter in the downcore profile of both habitats is consistent until 23.5 and 27.5 cm increments, where both habitats see an increase in refractory matter before rapidly depleting at the lower depth profile (33 and 43 cm). Within the HUNT cores, there is a notable drop in refractory matter at a profile depth of 15.5 cm for both habitats. Deeper in the profile, both habitats have a higher proportion of refractory material, but the seagrass core contains a larger proportion of refractory matter at depth than the mudflat habitat.

Table 3-6 Statistical summary of lability data. Model used (labilemodel= lmer(OM~SITE+HABITAT TYPE+SITE*HABITAT TYPE+(1|SITE/cm_increment),data=LabileOM,REML=TRUE).

| | Sum Sq | Mean Sq | NumDF | DenDF | F value | Pr(>F) |
|---------------------|--------|---------|-------|-------|---------|--------|
| Site | 555.5 | 79.4 | 7 | 135.1 | 13.30 | <0.001 |
| Habitat type | 92.6 | 92.6 | 1 | 99.1 | 15.52 | <0.001 |
| Site * Habitat type | 450.1 | 64.3 | 7 | 99.2 | 10.77 | <0.001 |

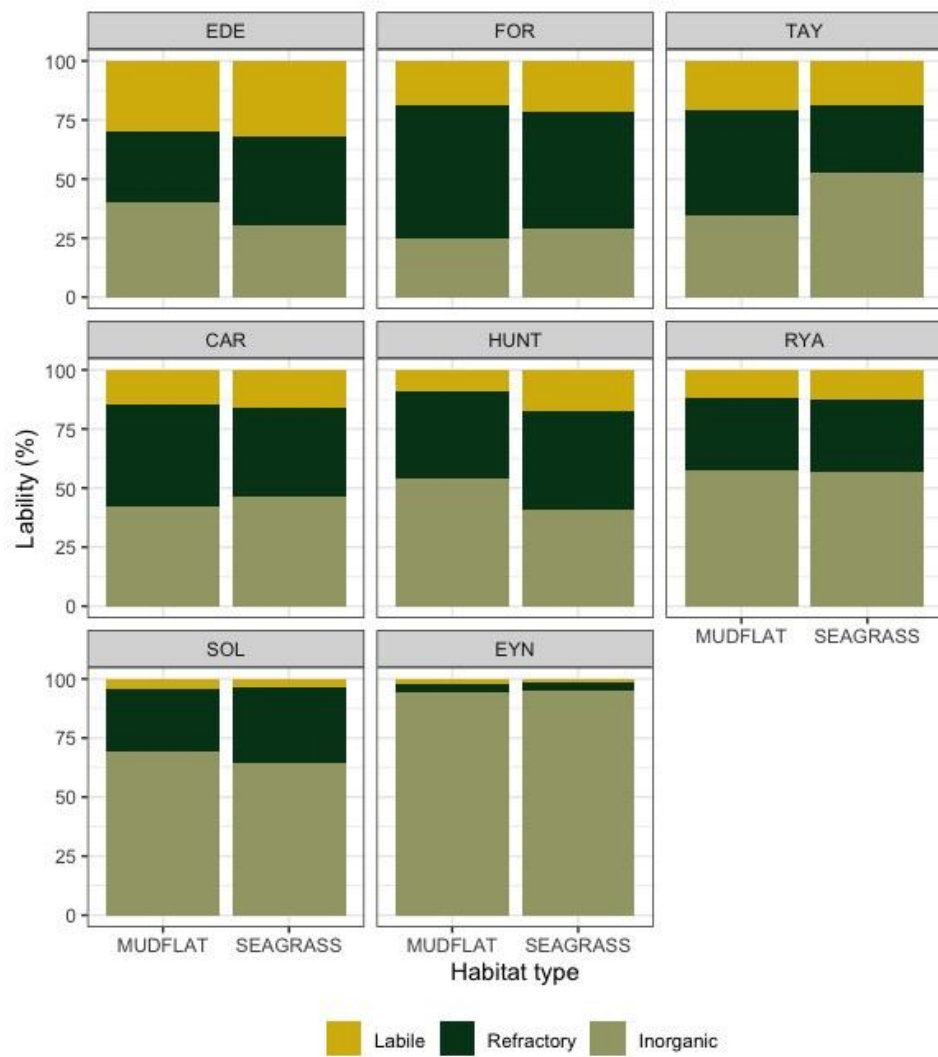
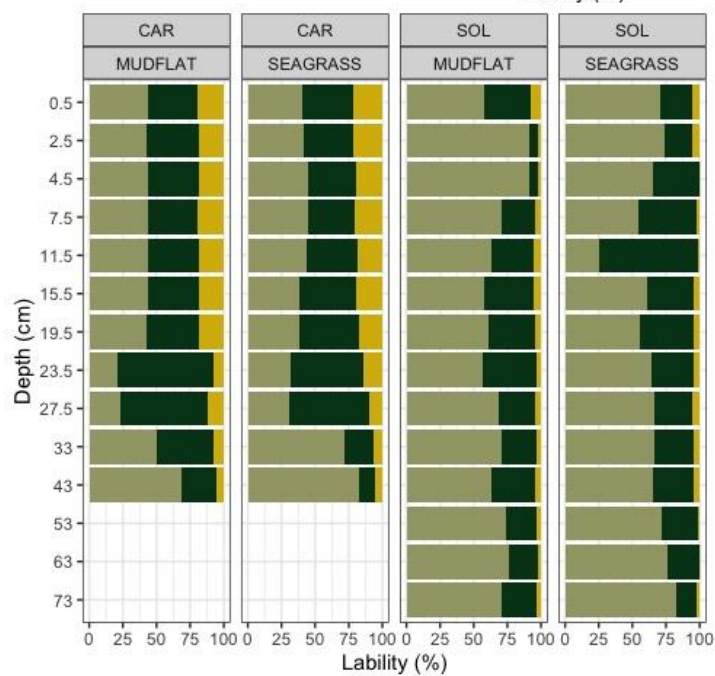
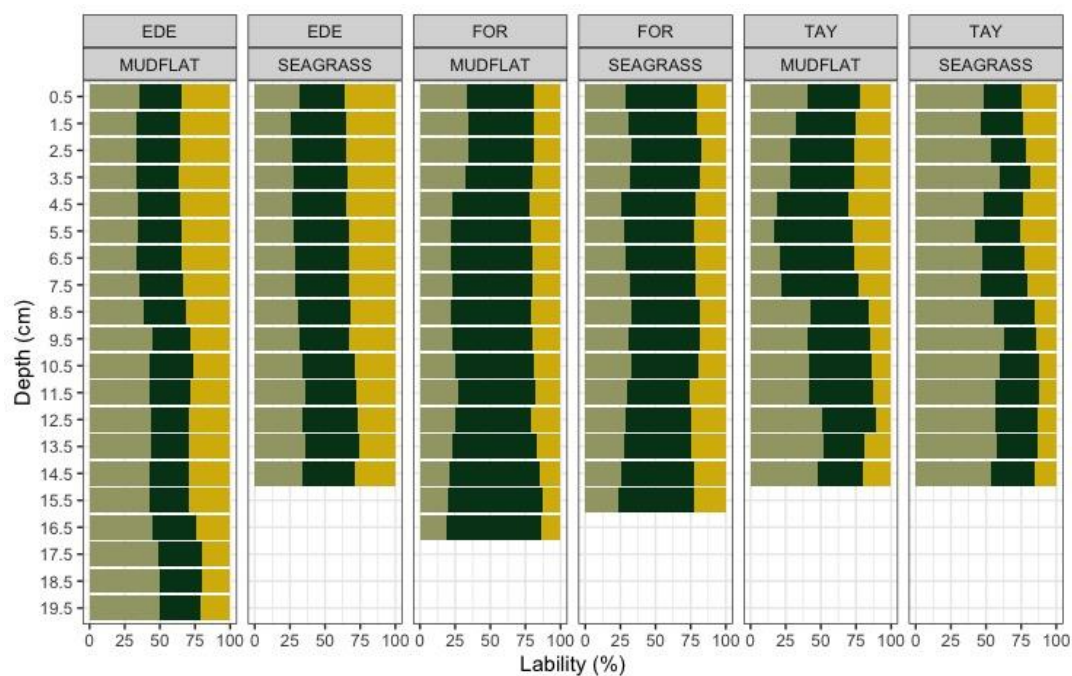


Figure 3-11 Summary of mean combusted matter (%) stacked by labile, refractory and inorganic matter, split by site and habitat type.



■ Labile
 ■ Refractory
 ■ Inorganic

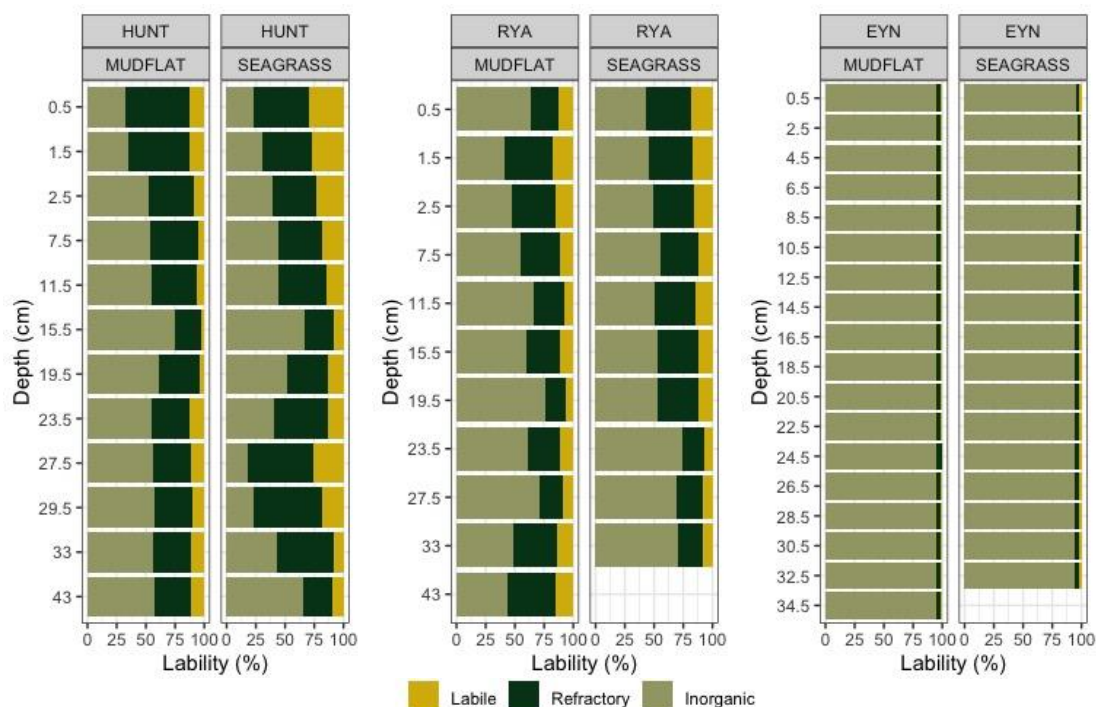


Figure 3-12 down core profile of sediment organic and inorganic matter. Where all sites are *Z.noltii* associated meadows, except SOL and EYN which are *Z.marina* associated meadows.

3.3.5 $\delta^{13}\text{C}$ isotopic signature determination

All sites shared similar $\delta^{13}\text{C}$ signatures, the most depleted range were found at EYN (-29.80--25.27‰) but a singular much less enriched signature of -36.22 ‰ was found for one CAR sample at a 28.5 cm depth, which could perhaps be explained by the carboniferous strata. Statistically there was no significant difference in sediment $\delta^{13}\text{C}$ values between sites (Table 3-7).

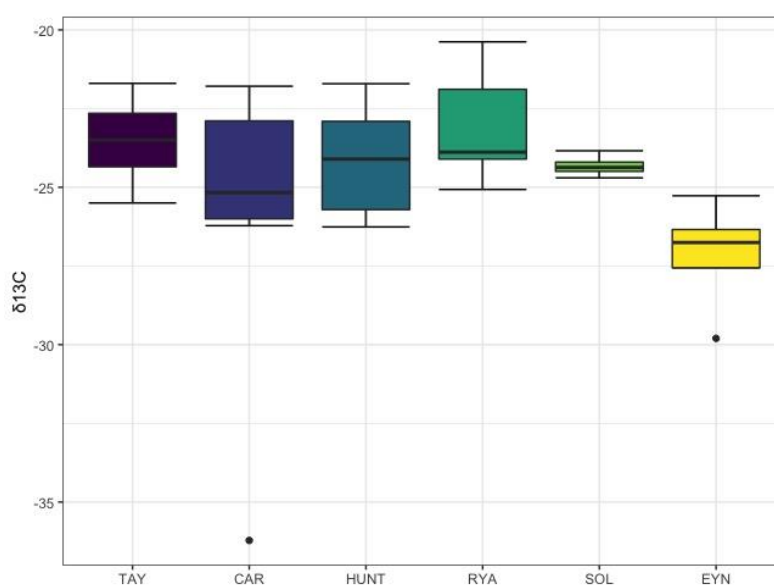


Figure 3-13 Average sediment $\delta^{13}\text{C}$ signatures across six sites.

With limited $\delta^{13}\text{C}$ values it is difficult to determine patterns in $\delta^{13}\text{C}$ with depth, nonetheless most sites sharing similar signatures across depth (Figure 3-14). At CAR and HUNT $\delta^{13}\text{C}$ signatures depleted at depth, but this pattern was not seen across all sites. At TAY $\delta^{13}\text{C}$ signatures initially deplete with depth before becoming more enriched at 40 cm depth. As sediment cores at all sites were taken from both seagrass meadows and bare mudflat areas $\delta^{13}\text{C}$ signatures for seagrass fronds (both *Z.noltii* and *Z.marina*) were also analysed across three sites; RYA, SOL and EYN (Table 3-8). The seagrass materials ranged from -16.27 (RYA) to -11.25‰ (EYN), which were much higher than sediment samples which ranged between from -36.22 (CAR) to -20.38‰ (RYA; Figure 3-13)

Table 3-7 Statistical summary of $\delta^{13}\text{C}$ compared across sites. Model=lm(d13C~SITE, data=D13CR_XTRA).

| | Df | Sum Sq | Mean Sq | F value | p value |
|-----------|----|--------|---------|---------|---------|
| Site | 5 | 66.62 | 13.32 | 2.16 | 0.08 |
| Residuals | 32 | 197.21 | 6.16 | | |

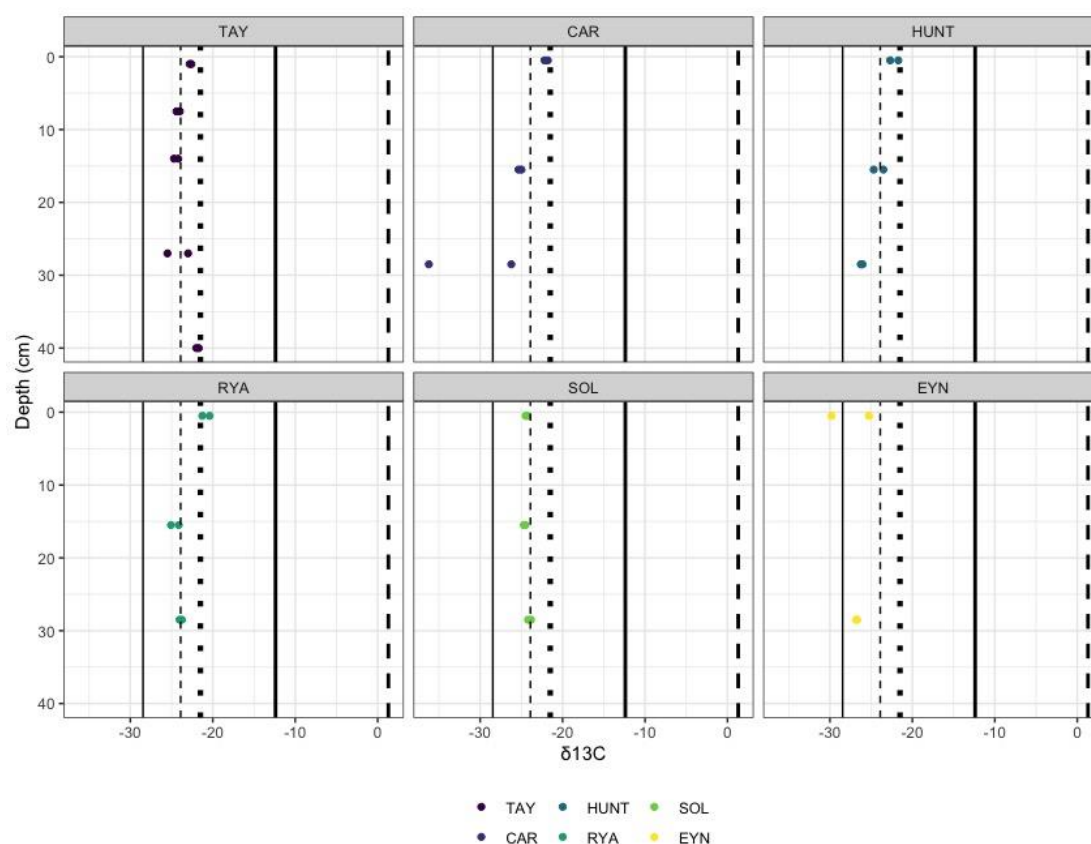


Figure 3-14 downcore profiles of $\delta^{13}\text{C}$ signatures across sites, regardless of habitat type. End member isotope values are represented as follows: thin line = terrestrial signature; thin dashed lines = phytoplankton signature; dotted line = zooplankton signature; thick line = average seagrass signature and; thick dashed line = carbonate shell signature.

Table 3-8 Summary table of $\delta^{13}\text{C}$ signature of sediment and seagrass material and the difference seagrass $\delta^{13}\text{C}$ signature and sediment $\delta^{13}\text{C}$ signature in meadows of different seagrass species.

| Site | Associated seagrass | Sediment $\delta^{13}\text{C}$ (‰) | | | | Seagrass $\delta^{13}\text{C}$ (‰) | | $\Delta^{13}\text{C}$ seagrass - sediment |
|------|-------------------------------------|------------------------------------|--------|------|------|------------------------------------|---|---|
| | | Count | mean | SD | SE | Count | mean | |
| TAY | <i>Z.noltii</i> | 10 | -23.49 | 1.25 | 0.77 | - | -12.39 | 11.1 |
| CAR | <i>Z.noltii</i> | 6 | -26.13 | 5.26 | 4.21 | - | | 13.74 |
| HUNT | <i>Z.noltii</i> | 7 | -24.25 | 1.69 | 1.26 | - | | 11.86 |
| RYA | <i>Z.noltii</i> | 6 | -23.1 | 1.84 | 1.47 | 1 | -16.27 | 6.84 |
| SOL | <i>Z.noltii</i> and <i>Z.marina</i> | 6 | -24.33 | 0.3 | 0.24 | 1 | -11.57 (<i>Z.noltii</i>) and -12.39 (<i>Z.marina</i>) | 12.35 |
| EYN | <i>Z.noltii</i> | 4 | -27.15 | 1.9 | 1.87 | 1 | -11.25 | 15.9 |

Data was modelled, using the IsoSource software package (Phillips and Gregg, 2003), to estimate the likely proportion of seagrass derived OM in samples. Literature derived values were used for zooplankton ($-21.5 \pm 0.5\text{‰}$), phytoplankton ($-23.9 \pm 1.4\text{‰}$) figures derived for the North East Atlantic shelf (Magozzi et al., 2017) and the global terrestrial average $\delta^{13}\text{C}$ signature ($-28.46 \pm 2.52\text{‰}$; Diefendorf et al., 2010). Further to this, the hydrolysis $\delta^{13}\text{C}$ signature for shell material (Figure 2-6) was used (1.3‰). All other figures were taken from Table 3-9, and calculations assumed that seagrass leaf material is a representative tracer for seagrass-derived carbon. The largest terrestrial derived contribution to OM content was at EYN, the subtidal site ($83.8 \pm 0.05\%$), whilst the lowest terrestrial contribution was found at TAY ($42.1 \pm 0.17\%$). The largest seagrass derived contribution to OM was at RYA, this was still $<15\%$ ($11.3 \pm 0.09\%$). The second highest contribution of OM across all sites was phytoplankton (Table 3-10).

Table 3-9 The proportion (%) contribution of likely OM sources to the sediments across sites. Mean values represent percentage alongside standard deviations.

| Reference material | Zooplankton | | Phytoplankton | | Terrestrial | | Seagrass | | Shell material | |
|--------------------|-------------|----------|---------------|----------|-------------|----------|----------|----------|----------------|----------|
| Site | μ | σ | μ | σ | μ | σ | μ | σ | μ | σ |
| TAY | 18.4 | 0.15 | 27.8 | 0.22 | 42.1 | 0.17 | 7.7 | 0.06 | 4 | 0.03 |
| CAR | 8.9 | 0.07 | 13.9 | 0.11 | 71.9 | 0.09 | 3.5 | 0.03 | 1.7 | 0.02 |
| HUNT | 15.6 | 0.13 | 24.2 | 0.19 | 50.4 | 0.15 | 6.5 | 0.05 | 3.3 | 0.03 |
| RYA | 20 | 0.16 | 28.6 | 0.22 | 35.8 | 0.16 | 11.3 | 0.09 | 4.3 | 0.04 |
| SOL | 15.3 | 0.12 | 23.6 | 0.19 | 51.4 | 0.14 | 6.4 | 0.05 | 3.2 | 0.03 |
| EYN | 5.2 | 0.04 | 8.2 | 0.07 | 83.8 | 0.05 | 1.8 | 0.02 | 0.9 | 0.01 |

3.4 Discussion

Prior research exploring OC stocks in Scottish seagrass focused on the intertidal east coast meadows; this study provides estimates for OC stocks from west coast intertidal seagrass meadows and the first OC stock estimates for a Scottish subtidal seagrass meadow. Similar to other studies, results highlight that there is considerable variation in both carbon storage across sites as well as the influence of seagrass meadows on this carbon store, regardless of species (Fourqurean et al., 2012; Röhr et al., 2018). Overall, there was no consistent differences in carbon density between seagrass and mudflat habitats.

3.4.1 Dry bulk density and organic matter content

There was a significant interaction term of site and habitat on DBD, however there was no difference between seagrass species; median DBD of $1.39 \pm 0.02 \text{ g/cm}^3$ in *Z.noltii* and $1.4 \pm 0.02 \text{ g/cm}^3$ in *Z.marina* seagrass sediments. At west coast sites, mudflat samples had higher DBD values than seagrass sediments. This could be linked to the absence of a root structure and lower levels of sediment accretion typical of unvegetated mudflats (Potouroglou et al., 2017). However, removing site as a factor, there is no real difference between habitats due to the variation in DBD values across sites. The DBD values were relatively large, which could indicate high compaction and or a high proportion of sand particles representing the more inorganic nature of the sediment – most evident within the west coast sites (Figure 3-3). Our median DBD seagrass values are higher than the global median DBD for seagrass meadows of $1.03 \pm 0.02 \text{ g/cm}^3$ (Fourqurean et al., 2012). However, this study's DBD results are similar to a European study of *Z.marina* meadows in Finland and Denmark where DBD average was 1.35 ± 0.01 and $1.25 \pm 0.02 \text{ g/cm}^3$, respectively (Röhr et al., 2016), or the previous Scottish intertidal seagrass study of the east coast where the mean DBD was $1.26 \pm 0.02 \text{ g/cm}^3$ (Potouroglou, 2016).

The mean OM content for seagrass habitats were $1.81 \pm 1.29\%$ (*Z.noltii*) and $2.22 \pm 1.93\%$ (*Z.marina*). The mudflat habitat mean for *Z.noltii* associated sites was similar to the seagrass habitat of $1.83 \pm 1.49\%$, whereas the *Z.marina* associated mudflat habitat had a lower OM content of $2.02 \pm 0.85\%$. Overall there is a

statistically significant difference between habitats for *Z.noltii* associated sites. However, only four of the ten sites (EDE, TAY, CAR and HUNT) have within-site differences and two of the sites contained higher OM content in mudflat habitats. It was expected that seagrass meadows would contain a higher OM content than mudflats due to meadow characteristics that support carbon storage (Ricart et al., 2015), including: high primary production (Mateo et al., 2006); the vegetation's ability to reduce wave movement (Fonseca and Cahalan, 1992), which in turn promotes sediment accretion; and the anaerobic environment created due to limited oxygen which reduces microbial decomposition (Mateo and Romero, 1996). Other research supports this theory, for example, Duarte et al. (2005) found that sediments were significantly enriched in OM regardless of seagrass type; on average containing 1.7 times more OM than unvegetated sediments.

This study's average seagrass OM content ($1.81 \pm 0.13\%$ (SE) for *Z.noltii* and $2.21 \pm 0.33\%$ (SE) for *Z.marina*) is in contrast to the east coast study by Potouroglou (2016) which found a higher average OM content ($2.58 \pm 0.08\%$ (SE)). The lower median OM content for mudflat habitats, associated with *Z.marina* sites, was not significantly different between habitats. Moreover, our *Z.marina* OM content is considerably lower than subtidal *Z.marina* meadows in England where average OM content was $10.78 \pm 3.54\%$ (Green et al., 2018). However this study is more similar to Nordic countries, including Finland and Denmark, where *Z.marina* meadows had an average OM content of $1.4 \pm 0.3\%$ and $3.9 \pm 1.5\%$, respectively (Röhr et al., 2016).

The higher median DBD value may have contributed to the weak negative correlation between DBD and OM content found, regardless of habitat (Figure 3-4). Sediment bulk density has been found to have an inverse relationship with increased OC concentrations in aquatic and flooded sediments, therefore a stronger negative correlation between DBD and OM was expected (Avnimelech et al., 2001). Nonetheless combining these results with further sediment characteristic information including particle size analysis may help identify and potentially target sites where carbon is significantly enhanced, as the proportion of finer grain sized

particles has been shown, alongside salinity and water depth, to explain OC stocks and their variation in temperate *Z. marina* meadows size (Röhr et al., 2018).

3.4.2 Organic carbon stocks and comparable datasets

The range of intertidal OC stocks (50 cm profile depth) for *Z. noltii* meadows was 29.86 ± 0.77 - 67.79 ± 2.09 Mg C/ha (mean OC stock of 37.96 ± 16.91 Mg C/ha) whilst the range for *Z. marina* was 45.57 ± 1.33 - 120.03 ± 13.72 Mg C/ha (mean OC stock of 67.52 ± 20.53 Mg C/ha). The SOL *Z. marina* core had a concentrated layer of high OC content that did not exist across the entire depth profile, however it influenced the larger *Z. marina* mean value and contributed to the high standard deviation. The mean intertidal OC stock, regardless of *Zostera* species is 45.43 ± 28.12 Mg C/ha and the median is lower at 39.80 ± 8.12 Mg C/ha. The range and average OC stocks found for west coast meadows is smaller than the east coast study, where OC stocks range from 15.93 ± 3.52 to 107.62 ± 21.90 Mg C/ha and the average OC stock is 57 Mg C/ha (50 cm depth profile; Potouroglou et al., 2021). The mean Scottish east coast stocks reported by Potouroglou et al. (2021) is similar to values from English intertidal meadows where a mean OC stock of 56.3 ± 30.7 Mg C/ha was determined (50 cm depth profile: Lima et al., 2020). However, it is important to note that the English study used a lower combustion temperature (450°C; Table 3-1) and therefore, it is expected that their mean OC stock would be higher than if samples had been combusted at 500°C.

The subtidal *Z. marina* meadow carbon stock was lower than its intertidal variation with an OC stock of 36.96 ± 0.84 Mg C/ha (50 cm depth profile). This value is lower than subtidal seagrass meadows found on the South east coast of England, which had an average range of 46.12-104.49 Mg C/ha⁸ for *Z. marina* OC stocks (Green et al., 2018). Again, it is important to note that Green et al. (2018) used a higher combustion temperature (550°C) than this study. If both studies had applied the same combustion temperature our subtidal OC stock would have likely been within the lower range of OC stocks found for English subtidal meadows, regardless of local

⁸ based on extrapolation of the average 30 cm depth from the stock to produce a 50 cm stock average and dividing the average 100 cm depth stock to produce a 50 cm depth profile.

regression equations being applied to convert OM content to OC content (Figure 3-1). Further to this, intertidal *Z.marina* meadows within this study were found in very small patches, rarely exceeding 2 m² in area. Whilst the subtidal *Z.marina* meadow contained larger patches the overall bed was patchy and small, whereas the English study had a mix of very patchy to dense meadows (Green et al., 2018).

3.4.2.1 Comparison between 50 cm depth profile organic carbon stocks and deeper organic carbon stocks

The most recent global collation of data estimated ~4.2-8.4 Pg C is stored in the top metre of seagrass sediments, taken from median estimates across multiple seagrass species meadows (Fourqurean et al., 2012). However, 22% of data used to develop this global average were from the Mediterranean, where *Posidonia oceanica* is dominant. This species has a unique ability to sequester carbon because it has a complex vertical rhizome and unlike any other seagrass species has long (up to 25 cm) and thick woody sheaths (Larkum et al., 2007).

At SOL 1 m cores were sought in the hopes of making the results in this dataset directly comparable to global datasets. The most recent global OC stocks dataset explores stocks to an integrated depth of 1 m. This is problematic because the largest proportion of sediment data used in Fourqurean et al. (2012) global study was from ≤30 cm depth cores. These cores were then extrapolated to 1 m depth stocks by applying a multiplication factor to their results (Fourqurean et al., 2012; Röhr et al., 2018). It is expected that this method of extrapolation will lead to an overestimate in OC stocks, because it assumes uniformity of OC storage. As down-core profiles are unlikely to receive new OC inputs below bioturbation depths, and labile OC would experience leaching and microbial consumption (Emerson and Hedges, 1988) – therefore OC would either plateau after a certain depth or continue to experience a slow decline (Bedulli et al., 2020; Serrano et al., 2020). Supporting this, Bidulli et al. (2020) found across 24 sediment core profiles of *Posidonia/amphibolis* spp. and *Halophila* spp. found a decline in OC content below a 20cm depth profile.

Collecting both 50 cm and 1 m cores would have provided an indication of whether data can be reliably extrapolated from 50 cm to 1 m for temperate *Zostera* seagrass meadow. However, due to the compact nature of the sediments, sampling beyond 40-50 cm was unfeasible across most intertidal seagrass meadows and mudflats without causing significant disturbance to the habitats through the use of machinery. Due to difficulty penetrating deeper sediments and increasing core weight at depth (which triggers sediment collapse within cores; suction failure), manpower alone is not a practical option when sampling at these sites. The deepest core extracted within this study was 76 cm and therefore we were unable to produce accurate OC stocks of a seagrass meadow to the advised 1 m (Fourqurean et al., 2014). However, in order to retrieve those cores, the surrounding mudflat was severely disturbed. The problematic nature of obtaining deep cores, could explain the limited number of 1 m cores across seagrass meadow carbon stocks data.

Serrano et al. (2012) found that an average of 32% of total OM was found in the top 50 cm of a 4.75 m *P.oceanica* sediment core, which thereafter becomes increasingly dominated by inorganic fractions with total OM content concentration expected to decrease exponentially at a rate of 0.0005 y^{-1} . The exponential decrease was derived from a 4.75 m core with the total OM values being produced for 1 cm increments down core (Serrano et al., 2012). Whilst Serrano et al. (2012) followed expected trends, Green et al. (2018) conversely, found that extrapolation temperate subtidal *Z.marina* cores <40 cm in depth to 1 m led to underestimated stocks by more the 40%; however the study did not collect cores deeper than 1 m to identify if this was a short-term trend, and therefore not representative sediments deeper than 1 m. In this study, comparing the two core depths at SOL, the shorter seagrass core contained ~1.58 times more OM than that of the deeper core (Table 3-4). This is an example of the variability in carbon across the site, despite uniform sampling, as generally a higher or similar OC stock may be expected for the deeper core as they were sampled within close proximity to one another. However, the down-core proportion of inorganic matter in the deep SOL core followed expected trends; the proportion of inorganic matter increased, and labile matter decreased, with depth across several Scottish seagrass meadows (Figure 3-12).

Extrapolating carbon data without extensive sampling of core profiles that exceed 50 cm in depth could produce misleading bias on the patterns of carbon storage at depth within seagrass meadows. Open access of published raw data would enable studies, regardless of depth profiles, to compare data across different depth profiles to produce global comparisons without the need to extrapolate findings to 1 m. This would reduce, in general, overestimated OC stocks particularly in geographical regions where limited OC stocks data is available. Although the most conservative figures are used to determine seagrass OC storage, values for North Atlantic OC stocks may still be an overestimation due to Mediterranean and Tropical data sets skewing results (Fourqurean et al., 2012; Lavery et al., 2013).

In the absence of a suitable alternative approach, there is a reliance upon extrapolation to compare this study to data from sites outside the UK. Rohr et al. (2018) predicted, from extrapolated 25 cm depth profile cores, that average OC stocks within Eastern Atlantic meadows (predominantly *Zostera* genus meadows) was 55.4 Mg C/ha for a 1 m depth profile. Similarly the North Atlantic region average was 48.70 ± 14.50 Mg C/ha in a global study (Fourqurean et al., 2012), which is lower than this study, where average OC stocks for intertidal seagrass meadows across the west coast of Scotland is 90.86 Mg C/ha when extrapolated to 1 m. Further to this, within our subtidal meadow, the average OC stock would be 75.92 Mg C/ha if extrapolated to 1 m, which is much lower than the mean OC stock of 208.98 ± 11.67 Mg C/ha found for subtidal *Z. marina* meadows in England. Overall, the carbon storage capacity of the region explored in this study, and the variation found across sites, is entirely expected, based on UK OC stocks data from the three UK studies, although much lower than the global range of 115.5-829.2 Mg C/ha.

3.4.3 Comparison between habitat types

As previously stated, there was a statistically significant difference in OC content between habitat types for *Z. noltii* associated cores, this was likely to have been driven by a few sites where differences were large. However there was no significant differences found for OC content between *Z. marina* associated cores. All seagrass

cores were sampled in the centre of their respective patches (areas where seagrass coverage is $\sim 2 \text{ m}^2$) within the meadows to avoid edge effects, with mudflat areas being chosen if they were more than $\sim 2 \text{ m}^2$ in size. However, it is important to note, that west coast meadows, in particular, vary seasonally, with meadows being denser in the summer growing months and sparser over winter. Water channel scars were visibly different across all meadows annually, with disturbance activities, such as storm events, having an evidently disruptive impact on seagrass meadows. This was particularly noticeable for the *Z.noltii* species that have characteristically shallow roots despite extensive rhizomes (mean depths of 4.6 cm; Duarte et al., 1998). Therefore, the varied within-site influence of seagrass meadows on OC storage could be explained by the integrity of the mudflat habitats (i.e. are the bare mudflat areas permanent within the sites?) chosen within sites as well as meadow composition and spatial arrangement. Most of the meadows are scarce and patchy, creating a mosaic of smaller meadows and mudflat habitats rather than large areas of seagrass meadows (Boström et al., 2011). For example, at RYA where little difference was found between the OC stocks within habitats could be due to the lack of permanent mudflat areas within the meadow. To identify permanent mudflat areas would require long-term monitoring before setting up sampling, which was not possible due to the timeframe of this study. To combat this issue, future work would need to focus on high-volume large-scale sampling across individual sites to determine if the variability within site is habitat specific or due to the dynamic nature of intertidal habitats.

Overall, within-site differences between habitats were minimal. Sites in which large differences were found between habitat types contradicted results from Potouroglou's (2016) east coast study. Seagrass sediment shallow core data did not show enhanced carbon stocks when compared to mudflat cores (Figure 3-10), even in sites where research had previously noted a statistical difference. At TAY, Potouroglou (2016) found that OM content within *Z.noltii* meadows displayed significantly higher proportions down to 40 cm depths, with huge variance noted within the OC stock ($67.8 \pm 63.25 \text{ Mg C/ha}$), compared to the 20 cm depth cores collected for this study that indicated that there were higher OC stocks in the

mudflat samples. These results highlight the variability in carbon storage across intertidal seagrass meadows, where continual vertical and horizontal sediment mixing through bioturbation will disturb with OC stocks across a depth profile (Middelburg, 2019a). Further to this, it is important to note total stocks for sites could only be produced at a single plot, for most sites, due to limited DBD data.

3.4.4 Lability section

Global OC stocks do not differentiate between labile and refractory organic carbon. Therefore, OC stocks do not represent permanent stocks. Intertidal environments are turbid environments where surface sediments are continuously exposed to short term changes in moisture, salinity, oxygen, carbon and nitrogen which could all influence decomposition of OM (Abril et al., 2010). This study investigated the mechanical lability of eight seagrass meadows. On average, there was a higher proportion of labile matter within east coast meadows ($37.34 \pm 10.36\%$) than west coast meadows ($26.81 \pm 7.45\%$), when exploring the composition of OM. Similar average labile content ($32.48 \pm 6.40\%$) was found for the subtidal north coast meadow, however the intertidal variation of *Z.marina* found on the west coast had a much lower average labile content of $12.55 \pm 6.43\%$. The higher percentage of refractory OM content found in the intertidal *Z.marina* bed (SOL), indicates that the seagrass meadow's OC stock is likely to be a more accurate representation of permanent OC stocks than other sites, where labile OM is much higher. As the SOL site has such a small component of labile matter with the OC stock, the overall OC stock is more stable and less vulnerable to decomposition and labile OC losses due to physical environmental processes which could reintroduce oxygenation down the sediment profile and therefore stimulate microbial colonisation of more readily available labile material.

Whilst average labile matter content is $>25\%$ across all but one site (SOL), it is important to note that because the labile matter is present across the full depth profiles of all eight sites, the data infers that unless deliberate oxygenation or damage of the seagrass or mudflat sediment substrate took place, the mechanically labile stocks are unlikely to see large losses of this less stable matter (Moodley et al.,

2005). The average trend of labile OM content reduction ranges from $0.29 \pm 6.31\%$ (SOL, mudflat habitat) to $1.80 \pm 7.57\%$ (CAR, seagrass meadow) per centimetre based on plots where a reduction in labile matter occurred at depth. There is a large variation in the range of labile OM content and its change with respects to depth, illustrating the complexity of site-specific factors, such as bioturbation and water percolation, that may influence labile carbon burial and loss.

Some of the exceptionally high OM content peaks observed could be due to natural artifacts in the sediments, as all but three of the eight sites explored (EYN, SOL and RYA) are underlain by Carboniferous strata. Similarly, five sites are situated within the midland valley, which was the epicentre of the hydrocarbon industry from the mid-19th century (Hallett et al., 1985). The FOR study site is situated between two large Coalfields; Central Coalfield and the Midlothian-fife Coalfield, with HUNT situated within the Ayrshire Coalfield (Browne et al., 1999). These seagrass meadows are naturally within the most organically rich locations of central Scotland and this explains the high refractory matter found at both FOR and HUNT (Figure 3-11). HUNT also shows the significantly enhanced increase in carbon at depth within seagrass meadows over the mudflat habitat, which supports research that the presence of seagrass naturally helps encourage a depository environment.

All sites contained a high proportion of inorganic matter, with >40% of sediments in west coast meadows composed of inorganic matter (mean inorganic matter ranged between 40.95 ± 15.62 - $64.19 \pm 13.45\%$; Table 3-5). The subtidal cores contained a much higher mean inorganic matter content of $94.93 \pm 1.02\%$. A limitation within this study is that whilst inorganic matter is recorded for eight seagrass meadows, there was no additional laboratory analysis conducted to determine IC and produce a suitable regression to help build an IC stock inventory for Scottish seagrass meadows. Mazarrasa et al. (2015) reported that concentrations of sediment IC within seagrass meadows were five times greater than OC content. The North Atlantic IC stocks regions (mean IC stocks of 204.9 ± 35.4 (SE) Mg IC/ha) were significantly lower than other biogeographic regions and the average IC stock across all data (654 ± 24 SE Mg IC/ha). Similar to Fourqurean et al. (2012) there was a

geographic bias with the largest sample populations from Australia and the Mediterranean regions (Mazarrasa et al., 2015). Furthermore, similar to OC stocks, the *Zostera* genera had the lowest average IC stocks when compared to six other genera, which overall could denote the seagrass genera as less influential at enhancing carbon storage.

3.4.5 Tracing organic matter sources

Scottish coastal sediments share similar $\delta^{13}\text{C}$ values within sites (Figure 3-13), regardless of their association to a particular habitat. This was expected, due to how close in proximity core collection was, particularly across intertidal habitats. EYN sediment $\delta^{13}\text{C}$ values were the most significantly different from other sites, with the most depleted mean sediment substrate $\delta^{13}\text{C}$ signature, of $-27.15 \pm 1.87\text{‰}$ and the enriched $\delta^{13}\text{C}$ signature of associated seagrass litter (-11.25‰). All seagrass $\delta^{13}\text{C}$ signatures were consistent with reported values for both *Z.noltii* and *Z.marina* seagrass material (Hemminga and Mateo, 1996; Kennedy et al., 2010; Röhr et al., 2018). However, sediment signatures appeared less enriched in ^{13}C than literature for similar habitats, the mean sediment signature across all sites was $-23.40 \pm 4.28\text{‰}$, lower than *Z.marina* sediments in South England (mean value -17.3‰ ; Green, 2020) and surface sediments in both Finland (ranging from -22.8 to -18.9‰) and Denmark (-17.6 – -13.5‰ ; Röhr et al., 2016). Further to this, global sediments associated with both *Z.noltii* and *Z.marina* meadows were more enriched in ^{13}C with a mean $\delta^{13}\text{C}$ value of -18.4‰ (Kennedy et al., 2010).

The isotope mixing calculations revealed that the average proportion of terrestrial derived OM across sites varied greatly ranging from 35.8% (RYA) to 83.8% (EYN). Seagrass derived OM was very low, despite the proximity of the samples to seagrass meadows, with half the sediments coming from in-situ seagrass plots and the other half from adjacent bare mudflat areas (1.8–11.3%). Global studies found a much greater seagrass derived OM contribution for sediments associated with seagrass. Kennedy et al. (2010) found that seagrass-derived OM contributed 51% of the OC in the surface sedimentary OM pool. Similar to the calculations of Kennedy et al. (2010), this study does not take into consideration seasonal variation of changes in

$\delta^{13}\text{C}$ signatures. Phyto- and Zoo-plankton contribution to the OM pools of sediments were much higher than seagrass contributions across all sites, contributing as high as 48.6% to the OM pool at RYA. Seagrass material does share similar signature values to other studies for each species (Choi et al., 2017; Fry et al., 1977; Kennedy et al., 2010; Zanden and Rasmussen, 1999). Although, the model findings should be used with caution due to a large proportion of $\delta^{13}\text{C}$ values being derived from literature, this study supports other literature findings identifying intertidal habitats as important locations for inwelling of OM from a variety of sources, which can then become trapped within fast accumulating sediments (Huxham et al., 2018; Loh et al., 2008).

3.5 Conclusion

This chapter provides descriptive information on OC stores within intertidal seagrass meadows and adjacent bare mudflat areas on the west coast of Scotland, as well as a subtidal seagrass meadow off mainland Orkney Island. This adds to prior knowledge of intertidal seagrass meadows which had previously focused on east coast sites, and confirms that there is large variation in OC stores across sites, as well as the affect seagrass has on carbon storage. Overall, west coast seagrass meadows, where present, do not significantly enhance carbon storage. Results demonstrate the need to assess additional site characteristics, such as OM decomposition (Chapter 4), microbial communities (Chapter 5) sediment stability and grain size analysis (chapter 6) to determine if there are specific driving factors controlling OC stocks in seagrass meadows.

Chapter 4 Decomposition of organic material in intertidal seagrass meadows and mudflat sediments in Scotland

Abstract

Seagrass meadows are known for their ability to trap and store carbon. The processes that enable the long-term storage of organic matter (OM), and thus carbon, remain only partially understood. Understanding the decomposition process in seagrass meadows could help to understand long-term storage of OM, since slower decomposition within hypoxic seagrass sediments is suggested to be one of the leading mechanisms that could lead to enabling carbon storage. This study used a standardised, widely established method of burying tea litter for understanding decomposition. Six intertidal seagrass (*Zostera noltii*) meadows across Scotland, three from both the east and west coasts, were used in the study, with two types of tea litter: green tea (which is relatively labile, and was used only in seagrass habitats) and rooibos (which is relatively refractory and was used in both seagrass and mudflat habitats). Decomposition of rooibos was recorded at 15 and 25 cm depths.

Unexpectedly, seagrass meadows consistently showed faster rates of rooibos tea litter decomposition than adjacent bare mudflats at 15 cm depth; decomposition at 25 cm was slower within seagrass plots but not significantly so. Decomposition rates of this refractory tea differed between sites; an explanatory variable for site differences could be temperature, with warmer west coast sites having the fastest decomposition rates. Labile litter decomposed at similar rates across all sites, and decomposition rates within the first 90 days were faster than other published studies in this initial stage of decomposition. Further site characteristics need to be explored to understand typical OM storage and decomposition within seagrass meadows.

4.1 Introduction

Seagrass meadows are highly productive habitats with a large capacity for both the production and accumulation of organic matter (OM). The fate of seagrass leaf litter is generally consumption through herbivory, outwelling to the sea, in-situ decomposition or burial and storage in sediment within the seagrass meadows (Duarte and Cebrián, 1996; Mateo et al., 2006). Seagrass sediment contains an average of 166 Mg C/ha, although this global mean is based on a dataset with large geographical gaps and a wide range of values, 115.5-829.2 Mg C/ha (Fourqurean et al., 2012). Kennedy et al. (2010) estimated, on average, that 50% of the OM stored in seagrass meadow sediments originates from the primary production of seagrasses and seagrass epiphytes. The rest of the stored OM in seagrass sediments was attributed to allochthonous sources, which highlights the efficiency of this habitat in capturing organic material, but again, there was a very wide range of values in their data. Hence there is strong evidence for the importance of seagrass beds as carbon stores on a global scale, but much site-to-site variation and large gaps in our understanding of what drives this. One mechanism is differences in decomposition rates between sites and seagrass species. Despite the potential influence of seagrass and other source litter quality on carbon accumulation and variation, this remains little-studied within seagrass ecosystems (Lavery et al., 2013; Mateo et al., 2006; Serrano et al., 2016). The temporary or permanent storage of carbon in seagrass sediments depends on a balance between the input and output of OM, with the latter occurring mostly as oxidation within the sediment. There is therefore a need to better understand what determines the breakdown of organic material in seagrass sediments.

There are three main phases of litter decomposition which are all affected by litter quality (Godshalk and Wetzel, 1978). The first stage is the passive leaching of soluble compounds (labile material, including phenolics, cellulose carbohydrates and amino acids). The latter two stages involve microbes actively breaking down more refractory compounds, often those high in lignin, over a longer period; slower

growing K- strategists and oligotrophs typically dominate these stages (Berg and McClaugherty, 2014; Cebrian et al., 1998; Valiela et al., 1985).

The structural carbohydrate base of all plant material includes three main polymers; cellulose, hemicellulose and lignin. Therefore, the ratio of structural carbohydrates within seagrass leaf and root material can influence its susceptibility to decomposition and carbon accumulation within sediments. Unlike other marine macrophytes, seagrass leaves, roots and rhizomes are all relatively lignin-rich, mirroring the composition of terrestrial plants, where lignin is relied upon for structural rigidity (Klap et al., 2000). The amount of lignin found within seagrasses varies due to climatic conditions, species and tissue type, with roots typically containing the most lignin because they are woodier than rhizomes and leaves (Trevathan-Tackett et al., 2017). Overall, Trevathan-Tackett et al. (2017) found that tropical seagrass plant tissues (*Enhalus* and *Thalassia*) were more refractory when compared to temperate and Mediterranean seagrasses (*Posidoniaceae*, *Zosteraceae* and *Halophila*), with the exception of rhizome tissues. Since refractory carbon is most likely to enter long-term storage in the sediment, understanding the distribution of lignin between plant tissues, species and sites is likely to help with predicting carbon storage. Further to this, with a large proportion of refractory carbon in seagrass meadows being allochthonous, understanding the breakdown of organic material regardless of origin, will aid in the understanding of variations in the ability of seagrass meadows to trap and store carbon.

4.1.1 TeaComposition

The use of litterbags for plant decay experiments both in-situ and in the laboratory is a long established method (Bourguès et al., 1996; Enríquez et al., 1993; Wieder and Lang, 1982). However, a standardised method was missing until 2013, raising problems in comparing between sites and species. Keuskamp et al. (2013) established an approach which used a standardised litter material to explore decomposition, with the aim of allowing comparisons between different habitats. The approach uses commercially available tea bags to mimic leaf litter, thus resulting in a readily available, affordable, less environmentally inobtrusive and uniform,

quality-controlled approach that can be replicated across different scales and multiple ecosystems. Rooibos and green tea are used as a metric for refractory and labile litter fractions of a plant, respectively. The rooibos tea decomposes at a lower rate over a longer time, whereas the more labile, tea breaks down at a faster rate over a shorter period of time. The decomposition of the tea litter material has been rapidly adopted since 2013 with two notable global initiatives being launched; the first a terrestrial study 'TeaComposition' in 2016 comprising of 570 sites across nine terrestrial biomes (Djukic et al., 2018). Then in 2017 a global wetland initiative 'TeaComposition H₂O' was set up with over 300 sites, including coastal, freshwater wetlands and aquatic ecosystems. These two global datasets will help to create a global long-term directory of the drivers of decomposition at different stages and across a multitude of important biomes. The current study adopts the TeaComposition H₂O methodology (as well as pursuing some additional objectives) and will contribute to the global findings of that work.

4.1.2 Study aim and objectives

This chapter aims to explore whether rates of decomposition of standard substrates are different in seagrass compared with un-vegetated reference habitat (mudflat) and, whether decomposition is affected by factors such as sediment depth and litter type. If differences are found, these may help to infer how OM is typically degraded and stored within seagrass meadows and how this promotes enhanced carbon storage. The work will also contribute to a global comparison of decomposition in aquatic habitats.

These aims were addressed through the following objectives:

- 1) To measure decomposition rates in seagrass meadows across six intertidal locations within Scotland
- 2) To test for differences in decomposition between seagrass and reference un-vegetated (mudflat habitat) plots.
- 3) To test for differences in decomposition between two different depths of burial.

- 4) To test for differences in decomposition between two different types of standard litter.

Chapter 5 will explore microbial communities associated with early stages (3 months) of decomposition of tea litter across two seagrass meadows: FOR and SOL. The following hypotheses were formulated and explored to address these objectives:

H_{0a} = Decomposition rates do not differ between intertidal *Zostera noltii* seagrass meadows across multiple sites

H_{0b} = Decomposition rates do not differ between intertidal *Zostera noltii* patches and adjacent bare patches at the same site

H_{0c} = Decomposition rates do not differ between material types being broken down

H_{0d} = Decomposition rates do not differ at different sediment depths

4.2 Methods

4.2.1 Study area

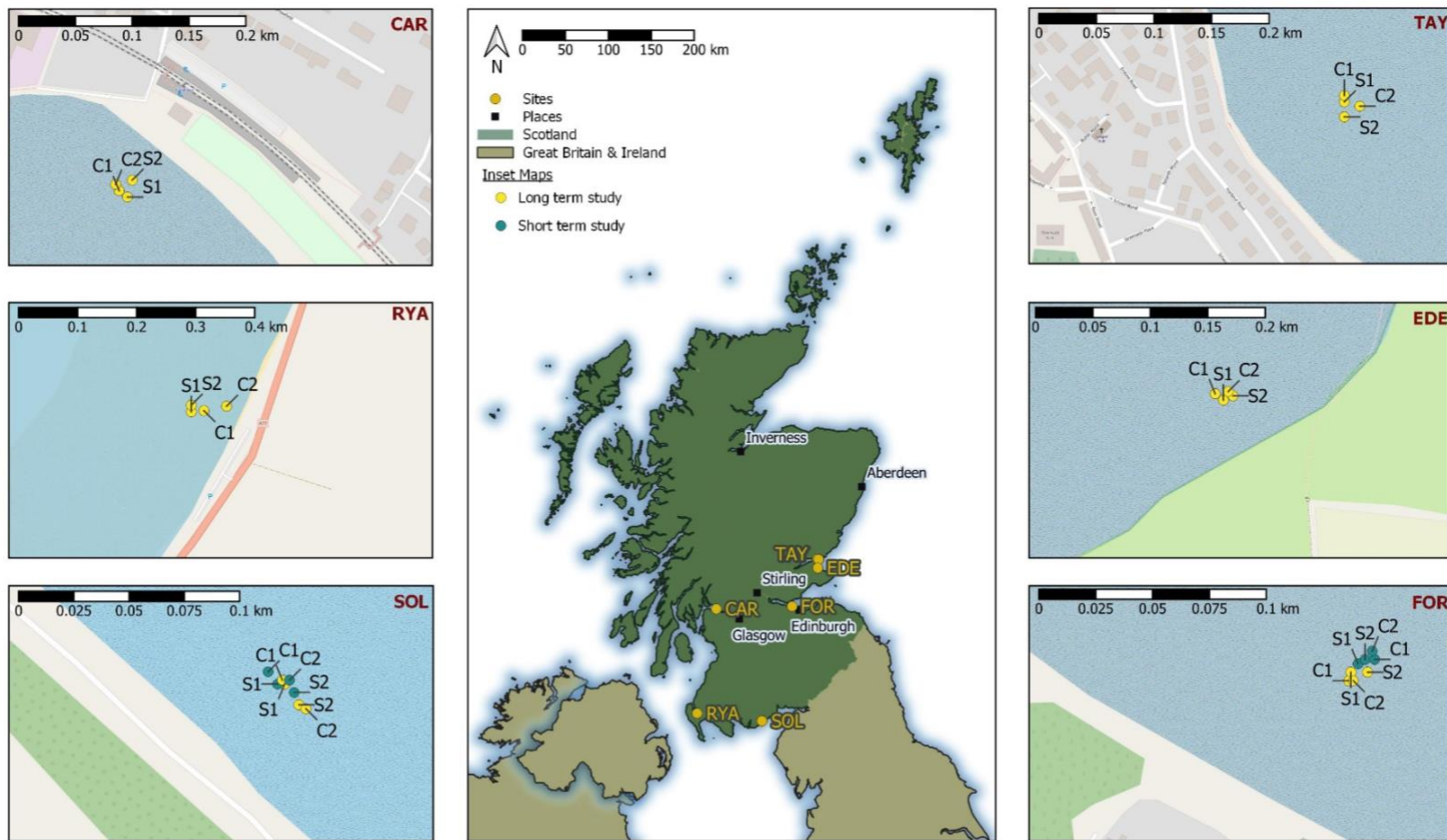
The study was conducted at six intertidal *Zostera noltii* sites: three east coast meadows; FOR (Drum Sands, Firth of Forth), EDE (Eden Estuary), TAY (Tayport, Firth of Tay), and three west coast meadows; SOL (Auchencairn, Solway Firth), RYA (Loch Ryan) and CAR (Cardross, Firth of Clyde; Figure 4-1).

4.2.2 Experimental design

This chapter includes the results from an experiment conducted as part of the global wetland initiative. Throughout this chapter, this will be referred to as the ‘long term study’, which followed the TeaComposition H₂O proposed experimental design and method adapted from Keuskamp et al. (2013) and Djukic et al. (2018). The study’s protocol was set by the terrestrial TeaComposition team to ensure data can be compared across the global dataset (Djukic et al., 2018). Two varieties of tea, rooibos and green tea (approximately 2 g and weighed to 4.d.p), were buried 15 cm below the sediment surface at six sites (Figure 4-1) and retrieved across five time points (Figure 4-2). However, two of these sites (FOR and SOL) were also chosen for a microbial study (Chapter 5) and therefore had a slightly different plot design (Figure 4-3).

Table 4-1: Tea bag specification.

| Tea bag | EAN Number | Ingredients |
|-------------|-----------------|---|
| Green tea | 8 722700 055525 | Tea (89%), flavouring (9.3%), rose petals (1%) |
| Rooibos tea | 8 722700 188438 | Rooibos sub-African (93 %), arôme hibiscus (1%) |



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Scotland: Source OS Open Data. Contains OS data © Crown copyright and database right (2021).

Additional Boundaries: These data were extracted from the GADM database (www.gadm.org), version 2.5, July 2015. They can be used for non-commercial purposes only. It is not allowed to redistribute these data, or use them for commercial purposes, without prior consent.

OSM tiles: © OpenStreetMap contributors. The map tiles in the "standard style" at www.openstreetmap.org are a Produced Work by the OpenStreetMap Foundation using OpenStreetMap data under the Open Database License. Base map and data from OpenStreetMap and OpenStreetMap Foundation

Figure 4-1 Map of all locations used across both TeaComposition studies and insert maps of seagrass (S1 and S2) and mudflat (C1 and C2) habitats at a site level.

A second study, referred to as the ‘short term study’, explores decomposition over a one-year period. The characteristics of this were similar to the long term study but only rooibos tea was buried, using two burial depths of 15 cm and 25 cm. There were six retrieval time points to capture information from the earlier stages of decomposition (Figure 4-4). This study used two of the sites used in the long term study; FOR and SOL.

Table 4-2: Summary of key details from the two studies.

| Study | Short Term | Long Term |
|--|-----------------|----------------------------|
| Length | 1 year | 3 years* |
| No. Sites | 2 | 6 |
| Tea Varieties | Rooibos | Rooibos and Green |
| Burial Depths | 15 cm and 25 cm | 15 cm |
| No. Retrievals | 6 | 5 |
| Total number of rooibos teabags per plot (excluding teabags buried for microbial analysis) | 12 | 48 |
| Total number of green teabags per plot (excluding teabags buried for microbial analysis) | | 10 (seagrass habitat only) |

* The final retrieval time, due to COVID-19 restrictions, was 38 months.

All tea litter used in both experiments were characterised by Keuskamp et al. (2013) and each litterbag is made of a nylon mesh (0.25 mm in size), the size excludes larger fauna from consuming or disturbing the tea litter as only microorganisms and mesofauna can enter the bags (Setälä et al., 1996).

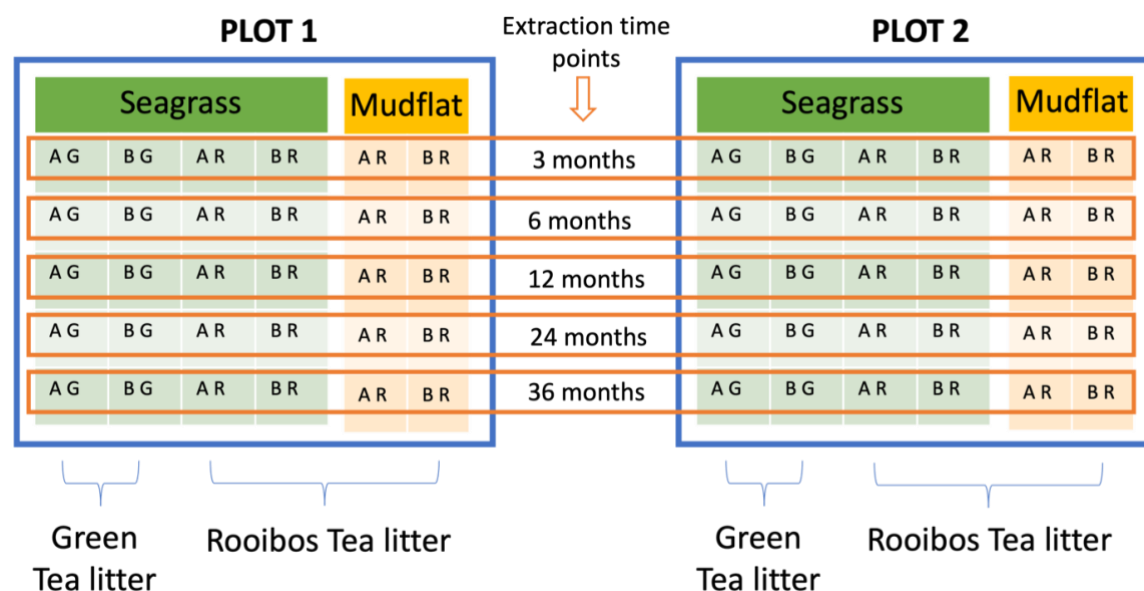
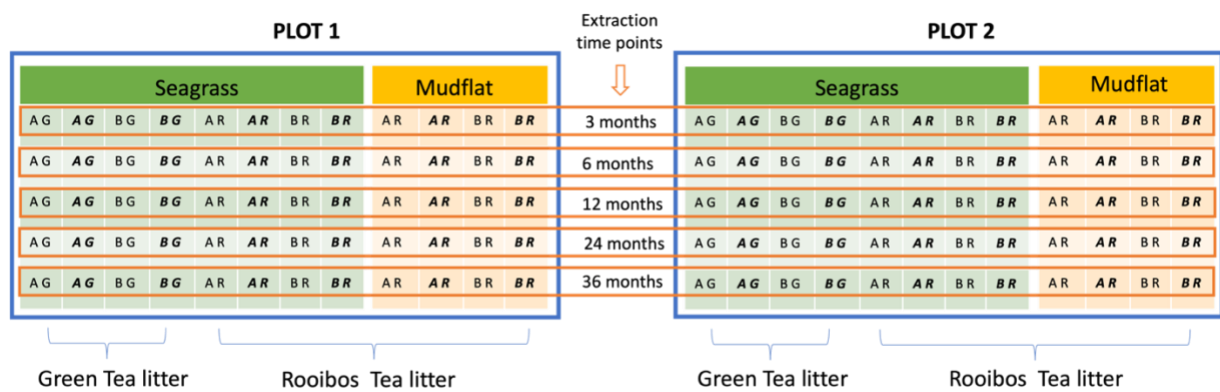


Figure 4-2 Long term study plot set up at EDE, TAY, RYA and CAR, where ‘A’ and ‘B’ represent replicate tea litter and ‘G’ and ‘R’ represent type of tea litter buried, ‘Green’ and ‘Rooibos’ respectively.



**All plot codes in bold and italic font represent tea litter samples being analysed for microbial analysis – see chapter 5*

Figure 4-3 Long term study plot set up at FOR and SOL, where ‘A’ and ‘B’ represent replicate tea litter and ‘G’ and ‘R’ represent type of tea litter buried, ‘Green’ and ‘Rooibos’ respectively.

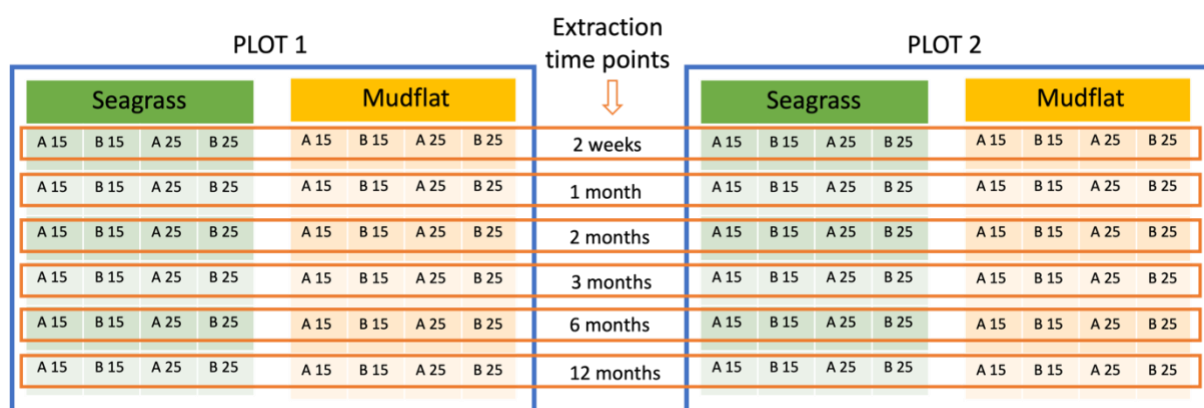


Figure 4-4 Short term study plot set up at FOR and SOL, where ‘A’ and ‘B’ represent replicate tea litter and ‘15’ and ‘25’ represent the tea litter burial depth, ‘15 cm’ and ‘25 cm’ below the sediment surface, respectively.

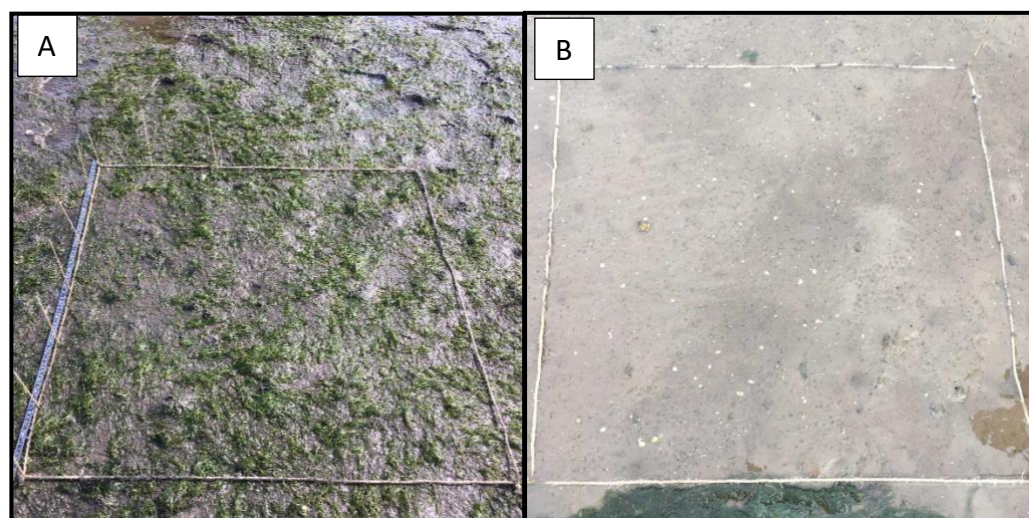


Figure 4-5 (A) An example of seagrass meadow within a plot. (B) An example of mudflat habitat within a plot.

4.2.2.1 Teabag protocol

Green and rooibos tea was dried at 70°C for 48 hours then labelled and weighed on a 4-decimal place (d.p) scale before being sealed in individual sample bags to ensure minimal sample loss during transition. At each site, two plots were chosen. Within each plot, two 1 m² quadrats were created, each containing one seagrass habitat and an adjacent bare mudflat habitat. For ease of retrieval, teabags were connected via stringed pegs in the field. 15 cm PVC cores were pushed into the ground and gently lifted to keep the natural sediment stratigraphy unchanged when placing the teabags at the desired depth. The pegs were pushed below the appropriate depth profile, with care taken to ensure the teabag sat at either 15 cm or 25 cm, then the core was placed directly on top of the teabag. A HOBO pendant temperature logger (UA-001-64) was also placed 15 cm below the sediment surface at each site, set to log temperature every two hours over the course of the study. The GPS locations of each plot was recorded, and discrete site markers were then left at the site to help locate the plots at future time points.

Samples were retrieved at extraction time points by using the PVC cores to remove the sediment above them, keeping plot disturbance to a minimal. Teabag depth was also checked to monitor plot disturbance. Once retrieved, the samples were placed in individual sample bags and transported to the laboratory. In the laboratory, teabags were checked carefully for damage and cleaned using highly purified water to wash away any finer grain sized particles that may have been attached to the tea litter material, before being dried at 70°C for 48 hours. Where root in-growth occurred, tweezers were used to remove them by hand. The tea was removed from the teabag before being weighed on tared-out KimWipes on a 4 d.p scale.

4.2.3 Decomposition rate

The litter mass remaining across both locations at each retrieval time was determined using the following equation:

$$\text{Remaining tea litter mass (\%)} = (W_t/W_i) \times 100$$

Where W_t Dry litter mass after burial duration (g)
 W_i Initial dry litter mass (g).

Whilst remaining mass of tea litter could be used to explore differences in the data, using a decomposition rate (k) is more robust as it integrates across time. Previous studies exploring decomposition have used the exponential decay model to determine the decomposition rate (adapted by Olson, 1963):

$$W_t/W_i = e^{-kt}$$

Where k Decomposition constant
 t Time period (months)

To modify the exponential decay model into a linear form time period was converted using the natural log. Decomposition constants for each plot * habitat type combination were produced by plotting remaining tea litter masses (y) for each timepoint (x) of each replicate litter bag within each plot. The gradient from each logarithmic equation created to fit the data was used as the decomposition constant for each plot * habitat type combination, and these values of k were subsequently used as replicates in statistical analyses.

$$\text{Remaining litter mass (\%)} = k \ln(t) + c$$

Where k Decomposition constant
 \ln Natural logarithm
 t Time period
 c value of y where the line crosses the $x = 1$ axis

Decomposition constants were also produced for every timepoint to provide an illustration of the changes in decomposition across sites and over different burial points for the long term study (Figure 4-6). Statistical analysis was originally intended to be carried out after 38 months of litter burial, to prevent running tests on different subsets of the same data. However, due to loss of samples across two sites, CAR and TAY, additional statistical analysis was conducted on k values produced after 24 months of litter burial. This was done to include all sites and have a more complete set of results for statistical analysis despite the associated inflation of type 1 error. Similarly, statistical analysis was only conducted on the 12-month data for the short term study.

4.2.4 Statistical analysis

All statistics were carried out using R studio, applying a confidence level of 95% (unless stated otherwise). All data were checked for normality and mixed model ANOVAs (lmer from the lme4 package; Bates et al., 2015) were used to analyse the similarities between sites, habitat types, depths and plots, as well as the interactions between these factors. Where there were significant differences, post-hoc tests were conducted using the glht function in the multcomp package (Hothorn et al., 2008). To analyse differences in temperatures across sites, a factorial repeated measures ANOVA model (lme from the nlme package; Pinheiro et al., 2021) was applied to compare daily mean temperature across the duration of the study. A post-hoc tests using the package emmeans assessed the significance of differences between pairs of group means (Searle et al., 1980).

4.3 Results

4.3.1 Long term study

Across the five timepoints, decomposition rates of rooibos tea varied among sites and habitat types, with remaining mass ranging from 60.2-79.5% after 3 months (Table 4-3). Initial within-site differences are found between habitat types at EDE and CAR (Figure 4-6A). These within-site differences are more evident after 12 months of burial but persist across all burial periods. Within mudflat plots rooibos tea litter, buried for a 24-month period, had the largest variation (~20%) in remaining weight masses (Table 4-3). CAR saw the largest loss in tea litter with an average of $45.31 \pm 1.49\%$ remaining mass and EDE had the slowest rates of decomposition with an average remaining mass of $60.33 \pm 3.44\%$. Overall, regardless of burial duration, rooibos tea litter buried at 15 cm depth within seagrass plots decomposed at a faster rate than litter buried in mudflat plots. CAR is the only site where, after 24 months of litter burial, the range of decomposition rates were similar for both habitat types. After 38 months, RYA had lower decomposition rates for seagrass meadows, but the decomposition rates were over a large range and one full mudflat plot was not retrieved at this site. CAR also consistently has the fastest decomposition rates in seagrass plots, whereas EDE has the slowest decomposition rates.

Statistical analysis of decomposition rates of rooibos tea litter found no significant 2-way interaction term (site * habitat type) present after a 24 and 38-month burial period (Table 4-4). After 24 months there are statistically significant differences among both sites ($df = 5$, $F = 17.7$, $p < 0.001$) and habitat types ($df = 1$, $F = 18.4$, $p < 0.001$). However, there was no significant difference between habitat types after a 38-month burial period ($df = 1$, $F = 2.4$, $p = 0.261$), although data was missing from two of the sites (Table 4-4).

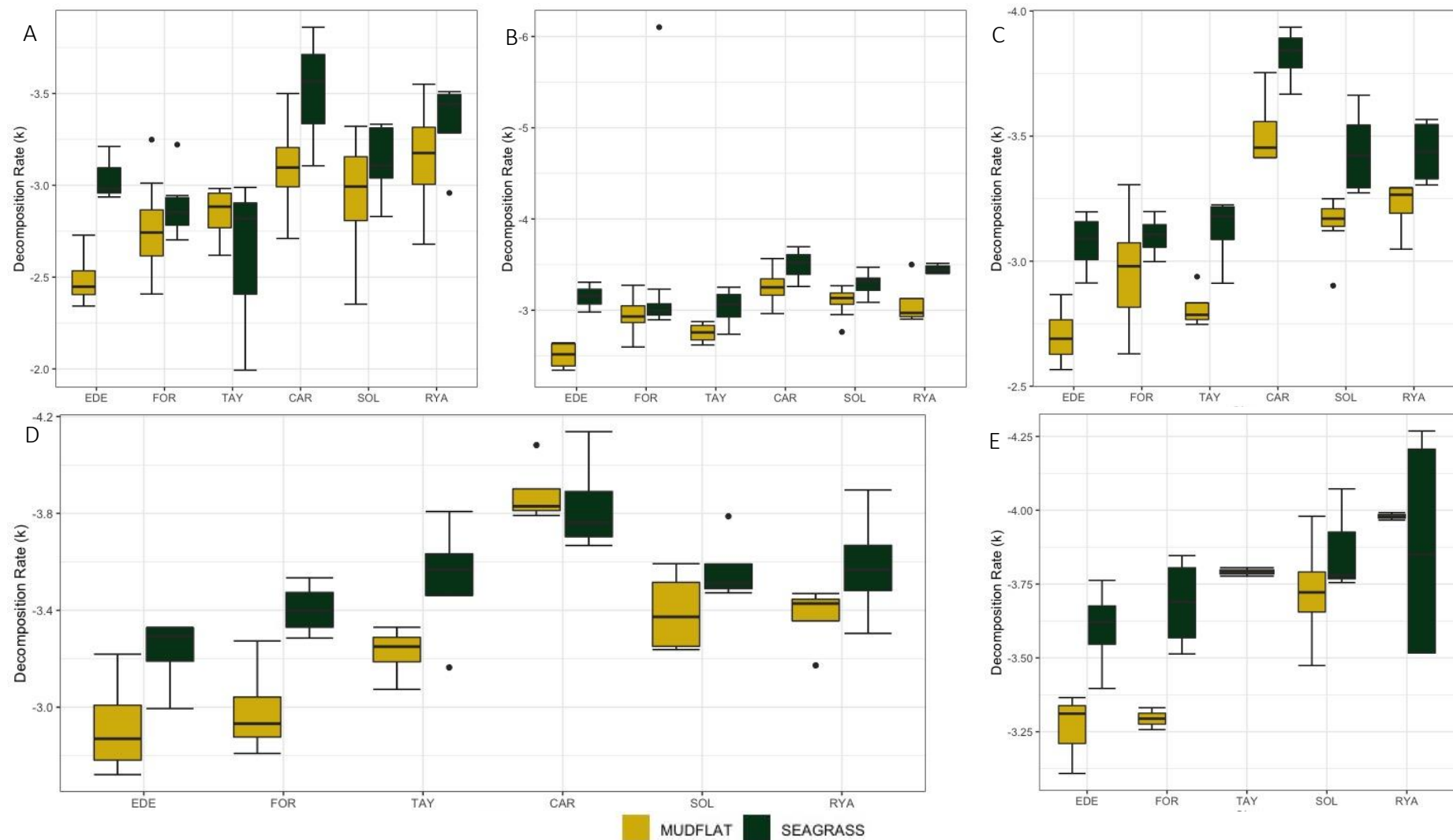


Figure 4-6 Boxplot of decomposition rates (k) over time for rooibos tea litter across all sites after burial at 15 cm depth within mudflat (Yellow) and seagrass (Green) habitats for (A) 3 months (B) 6 months (C) 12 months (D) 24 months (E) 38 months.

Table 4-3 Summary of remaining litter mass after each litter retrieval time point for habitat type and tea material, where weights are in grams (g).

| Habitat type | Tea material | Time (months) | Count | Min weight | Mean weight | Max weight | Median weight | St dev | Standard Error |
|--------------|--------------|---------------|-------|------------|-------------|------------|---------------|--------|----------------|
| Seagrass | Green | 3 | 22 | 8.869 | 17.233 | 24.66 | 16.274 | 4.01 | 1.676 |
| Seagrass | Green | 6 | 21 | 8.72 | 13.756 | 22.023 | 13.494 | 2.854 | 1.221 |
| Seagrass | Green | 12 | 21 | 7.508 | 13.121 | 22.648 | 13.021 | 4.194 | 1.794 |
| Seagrass | Green | 24 | 22 | 5.566 | 9.131 | 12.071 | 9.163 | 1.969 | 0.823 |
| Seagrass | Green | 38 | 15 | 4.141 | 6.171 | 11.822 | 5.499 | 2.018 | 1.021 |
| Mudflat | Rooibos | 3 | 24 | 63.397 | 70.667 | 75.852 | 71.113 | 3.646 | 1.459 |
| Mudflat | Rooibos | 6 | 21 | 60.213 | 66.816 | 74.106 | 66.18 | 4.052 | 1.733 |
| Mudflat | Rooibos | 12 | 23 | 53.323 | 62.755 | 70.545 | 63.932 | 5.209 | 2.129 |
| Mudflat | Rooibos | 24 | 23 | 43.381 | 54.534 | 63.488 | 54.196 | 6.191 | 2.53 |
| Mudflat | Rooibos | 38 | 13 | 35.16 | 43.869 | 52.916 | 43.653 | 5.462 | 2.969 |
| Seagrass | Rooibos | 3 | 21 | 60.197 | 68.114 | 79.452 | 68.734 | 4.032 | 1.724 |
| Seagrass | Rooibos | 6 | 20 | 57.702 | 62.843 | 66.707 | 63.054 | 2.711 | 1.188 |
| Seagrass | Rooibos | 12 | 24 | 49.61 | 59.959 | 65.933 | 61.272 | 4.656 | 1.863 |
| Seagrass | Rooibos | 24 | 20 | 44.498 | 52.462 | 62.106 | 52.36 | 4.657 | 2.041 |
| Seagrass | Rooibos | 38 | 17 | 32.235 | 41.901 | 51.658 | 44.311 | 5.823 | 2.768 |

Table 4-4 Long term study - statistical test summary. Model = lmer(K ~ SITE + HABITAT TYPE + SITE * HABITAT TYPE + (1|PLOT), data=Rooibos(X months), REML=TRUE).

| Time (months) | Factor | sum Sq | Mean Sq | NumDF | DenDF | F value | p value |
|---------------|---------------------|--------|---------|-------|-------|---------|---------|
| 24 months | Site | 2.967 | 0.593 | 5 | 36 | 17.701 | <0.001 |
| | Habitat type | 0.616 | 0.616 | 1 | 36 | 18.381 | <0.001 |
| | Site * Habitat type | 0.257 | 0.051 | 5 | 36 | 1.533 | 0.204 |
| 38 months | Site | 1.094 | 0.273 | 4 | 18 | 6.376 | 0.002 |
| | Habitat type | 0.103 | 0.103 | 1 | 2 | 2.395 | 0.261 |
| | Site * Habitat type | 0.229 | 0.076 | 3 | 18 | 1.783 | 0.187 |

Investigating within-site differences between means after three months of burial, only EDE and CAR habitat types were dissimilar. After six months, within-site differences between means appear for RYA and remain for EDE. After 12 months of litter decomposition, prominent differences between mean decomposition rates for habitats within sites are significant at EDE, TAY, SOL and RYA. The 24 months burial time point is the final full dataset retrieved, Figure 4-6D shows the clear within-site differences found for all east coast sites. However, across the West Coast, within-site mean decomposition rates are not significantly

dissimilar. After 38 months, despite being unable to retrieve all tea litter from all locations, the significant difference between decomposition rates were found for EDE and FOR.

It is clear from the data that the null hypothesis of no significant difference between decomposition rates across sites (H_{0a}) is not accepted. Further to this, the Tukey post-hoc test revealed the following groupings after 24 months: (a) EDE-FOR-TAY; (b) RYA, SOL, TAY; (c) CAR. The null hypothesis of no difference in decomposition between habitat types (H_{0b}) was also rejected, as there was a significantly faster rate of decomposition in seagrass compared with mudflat plots.

In contrast with decomposition of rooibos litter, the green tea litter showed similar rates of decomposition between sites at all time points, with no significant site differences at the end of the experiment after 24 months ($df = 5$, $F = 1.2$, $p = 0.364$; Figure 4-7), or after 38 months which excluded two sites, due to tea litter loss ($df = 3$, $F = 0.95$, $p = 0.482$). Only after the first extraction point (three months) were differences found between sites. After 24 months decomposition rates were within a tight cluster (mean = -7.582 ± 0.064) at all sites except SOL. There was a large range of decomposition rates of labile material at SOL (-9.456 -- -6.393).

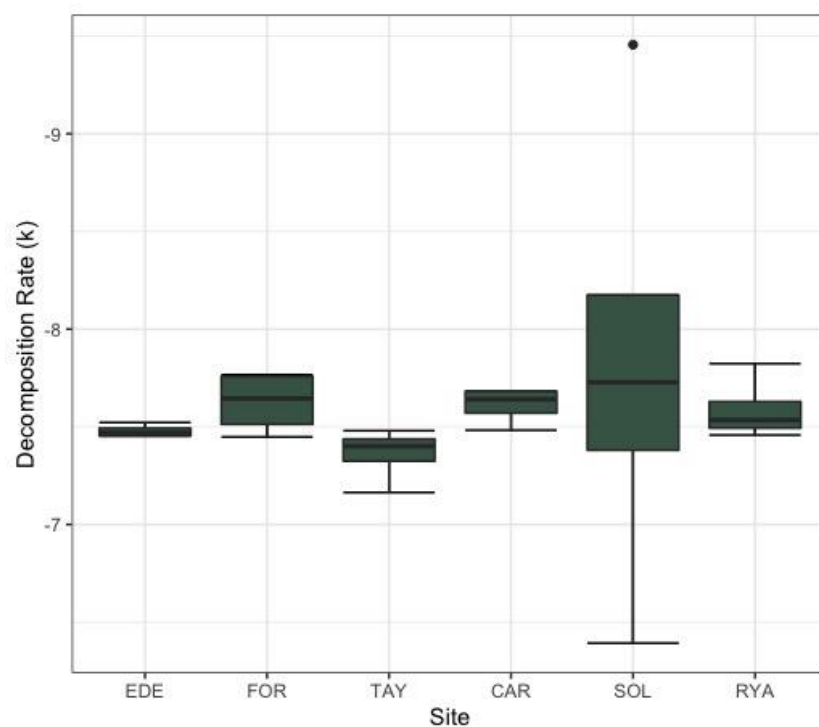


Figure 4-7 Boxplot of green tea litter decomposition at 15 cm depth across seagrass meadows at all sites after 24 months of burial. Model = $\text{lmer}(K \sim \text{SITE} + (1|\text{PLOT}), \text{data}=\text{Green}(\text{X months}), \text{REML}=\text{TRUE})$.

Rooibos, the more complex refractory litter, decomposes at a slower rate than labile litter (DF = 1, F = 1913.6, $p < 0.001$; Figure 4-8A). Both litter types showed rapid initial declines in mass before plateauing. After three months, mass loss for rooibos tea litter was greater than for green tea litter (Figure 4-8B). The mass of the labile tea after three months was reduced to an average of 17.3 ± 3.3 remaining mass (%) compared with $69.4 \pm 3.1\%$ for the refractory tea litter.

Average daily sediment temperatures across each month, was significantly different across study sites each month (DF = 5, F = 3.8, $p = 0.002$). Every site, every month had a different mean temperature throughout the study long term study. When exploring post hoc analysis, all sites were dissimilar from each other except two pairings, CAR and SOL, and TAY and FOR. Overall, the west coast sites had the highest mean temperatures throughout the study period (Figure 4-9).

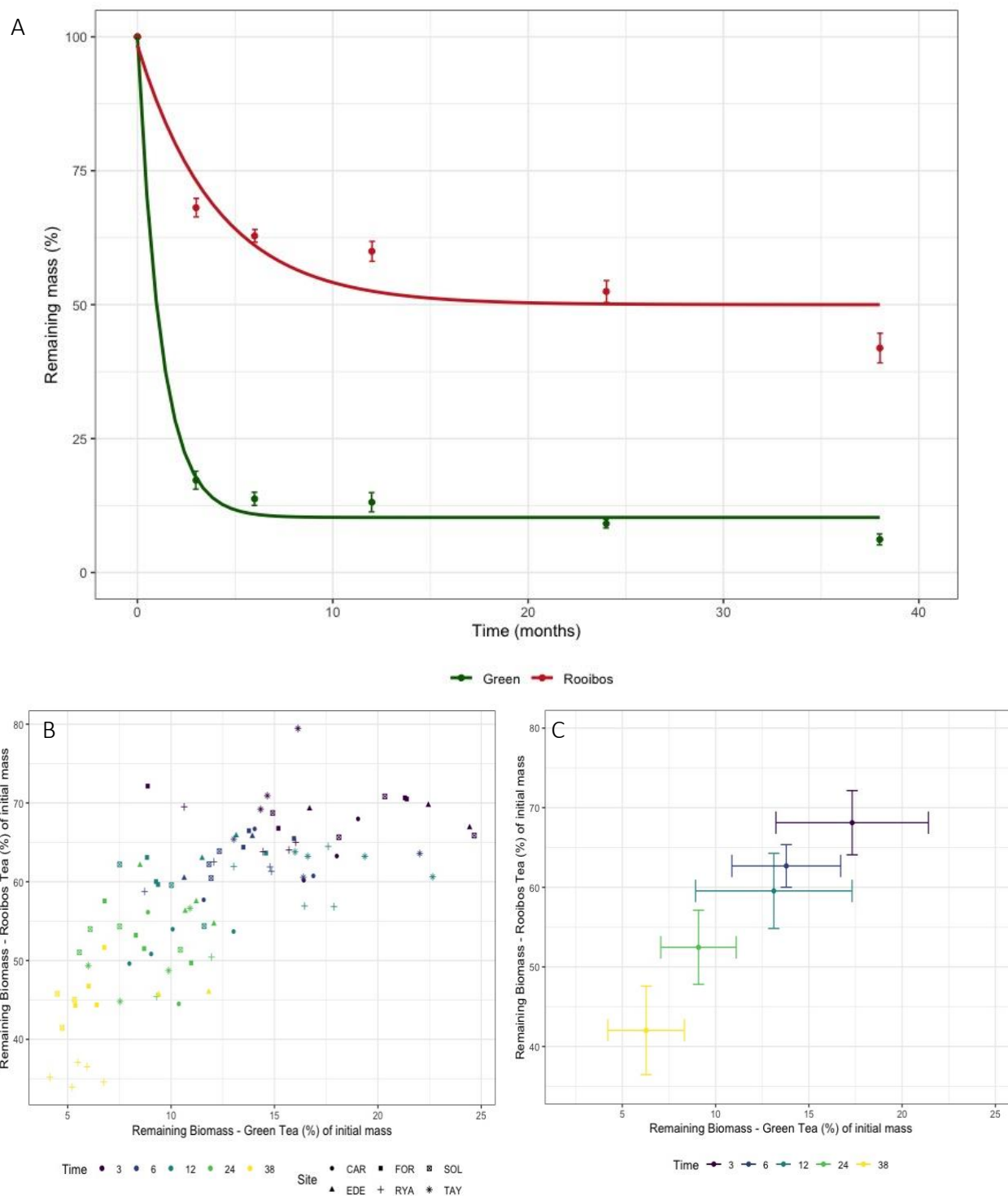


Figure 4-8 (A) Scatterplot of mean values of remaining tea litter mass (%) over time (months) within seagrass areas, plotted with confidence intervals. Logarithmic curves fitted where $y = -7.44\ln(x) + 30.265$ and $R^2 = 0.992$ (green tea litter) and where $y = -3.896\ln(x) + 66.148$ and $R^2 = 0.9118$ (rooibos tea litter). Model = lmer($K \sim \text{TEA_MATERIAL} + \text{SITE} + \text{TEA_MATERIAL} * \text{SITE} + (1|\text{PLOT})$), data=K_38, REML=TRUE). **(B)** Remaining mass (%) of tea litter after 38 months of incubation in seagrass meadows, where point shape = site and point colour = time tea litter was buried (months) and **(C)** displays means and standard deviations for the data at each time point.

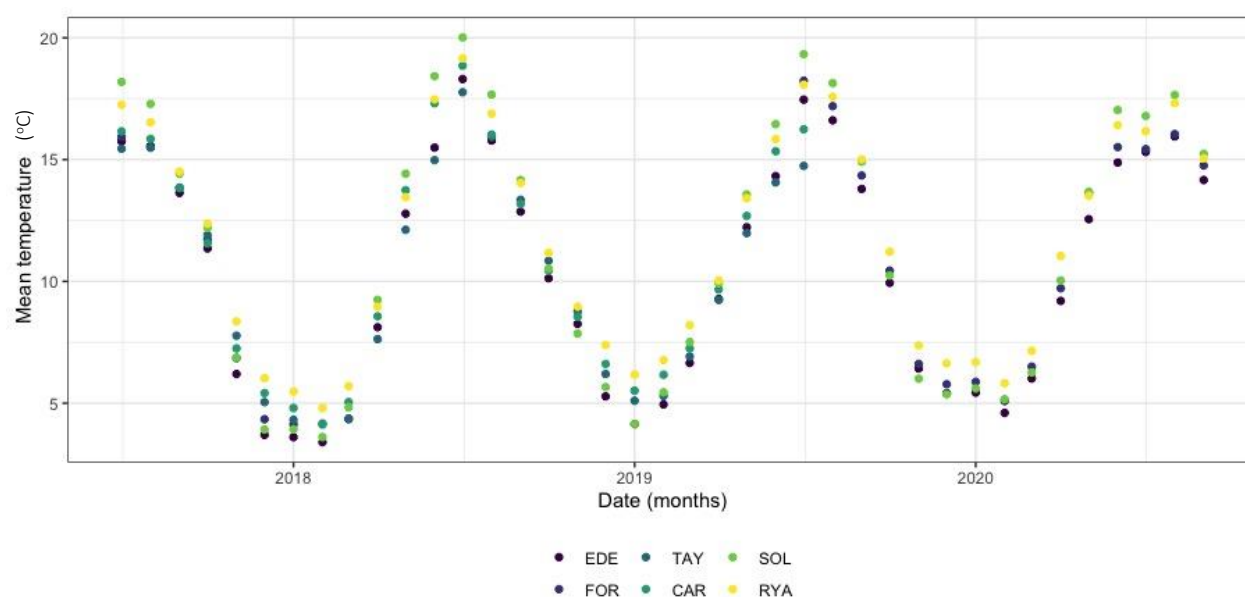


Figure 4-9 Average monthly temperatures (°C) between July 2017 and September 2020 across all study sites. Model = lmer (TEMP~ SOM * SITE, random = ~1|FULL_DATE, data=TEMPS).

4.3.2 Short term study

After the final burial period (12 months) the greatest mean loss of litter material was in litter buried to a 15 cm depth within seagrass areas (remaining mass = $57.57 \pm 1.73\%$), with SOL seagrass meadows showing higher decomposition rates than those at the FOR site (Figure 4-10). However, initial litter masses (after two weeks of burial) were similar across both sites, depths and habitat types, with small variation amongst the litter material. There was a slightly higher range in average remaining mass values across litter buried to a depth of 25 cm (80.26 ± 0.32 - $86.89 \pm 7.01\%$) than 15 cm (77.93 ± 1.69 - $85.75 \pm 9.17\%$). This pattern of large variance in average remaining mass values continued across each time point with a slightly higher range being found for litter buried at 25 cm.

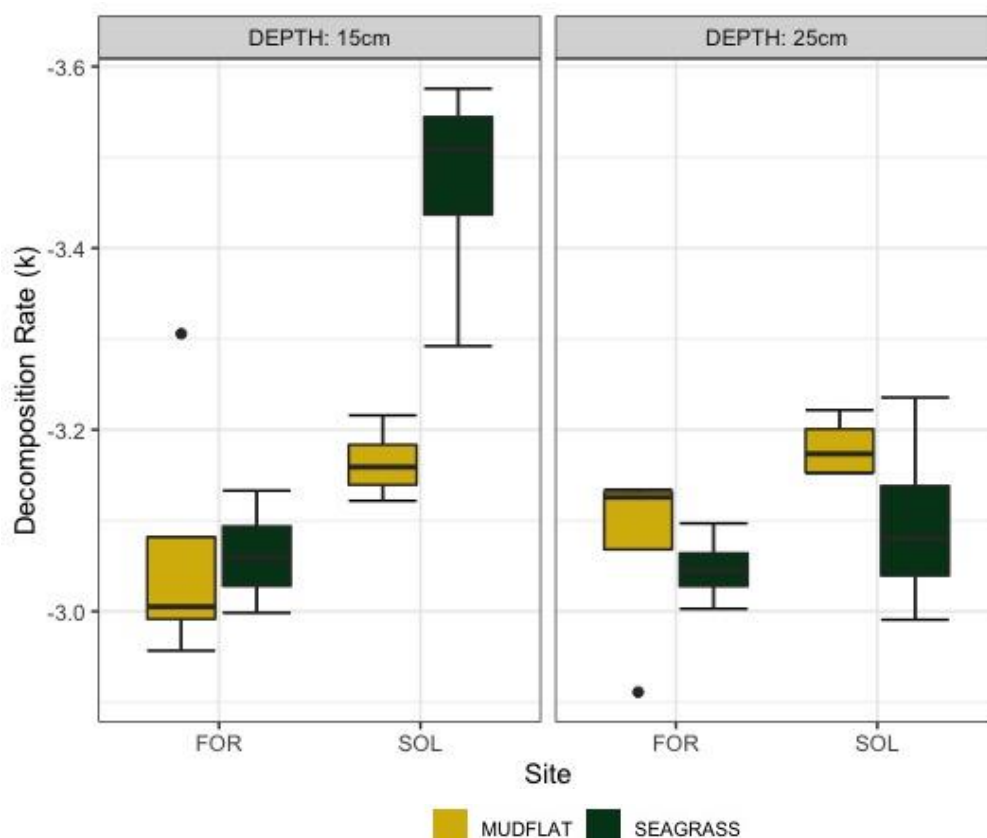


Figure 4-10 Average decomposition rates of buried rooibos tea litter, separated by burial depths (15 cm and 25 cm), location and habitat type in the short term study (12 months).

Within the short term study, as all samples were retrieved, statistics were only carried out on the final decomposition rates (12 months), which were generated for each sample column per plot (Figure 4-4). The mixed model ANOVA found statistically significant two- and three-way interaction terms between site and depth, depth and habitat type and site, depth and habitat type (Table 4-5). All significant interaction terms include burial depth as an key factor influencing decomposition. Decomposition at FOR is less influenced by depth in the early stages of decomposition than SOL where there is a sharp contrast between decomposition rates between seagrass meadows. The fastest decomposition rate was recorded for tea litter buried at a 15cm depth in SOL, faster than tea litter buried in the mudflat area, a difference that was not found at 25 cm. Intriguingly rates of decomposition at 25 cm were slower within seagrass compared to mudflat areas, although this difference was not significant.

Table 4-5 Short term study after 12 months - statistical test summary. Model = lmer(K ~ SITE + HABITAT TYPE + DEPTH + SITE * HABITAT TYPE * DEPTH + (1|PLOT), data = SHORT TERM STUDY, REML = TRUE).

| | Sum sq | Mean sq | Num DF | DenDF | F value | Pr(>F) |
|----------------------------|--------|---------|--------|-------|---------|--------|
| Site | 0.218 | 0.218 | 1 | 24 | 24.404 | <0.001 |
| Depth | 0.068 | 0.068 | 1 | 24 | 7.561 | 0.011 |
| Habitat type | 0.018 | 0.018 | 1 | 24 | 2.058 | 0.164 |
| Site * Depth | 0.061 | 0.061 | 1 | 24 | 6.817 | 0.015 |
| Site * Habitat type | 0.033 | 0.033 | 1 | 24 | 3.696 | 0.066 |
| Depth * Habitat type | 0.085 | 0.085 | 1 | 24 | 9.505 | 0.005 |
| Site *Depth * Habitat type | 0.068 | 0.068 | 1 | 24 | 7.650 | 0.011 |

Table 4-6 Decomposition rates ($\text{g g}^{-1}/\text{month}$) summary for short term study after 12 months of tea burial.

| Site | Habitat type | Burial depth (cm) | Count | Decomposition rate (k) | | | | | |
|------|--------------|-------------------|-------|------------------------|--------|--------|--------|--------------------|----------------|
| | | | | Min | Mean | Max | Median | Standard deviation | Standard error |
| FOR | Seagrass | 15 | 4 | -3.133 | -3.062 | -2.998 | -3.059 | 0.058 | 0.057 |
| FOR | Mudflat | 15 | 4 | -3.306 | -3.068 | -2.957 | -3.005 | 0.160 | 0.157 |
| SOL | Seagrass | 15 | 4 | -3.576 | -3.472 | -3.292 | -3.510 | 0.125 | 0.123 |
| SOL | Mudflat | 15 | 4 | -3.216 | -3.164 | -3.122 | -3.159 | 0.040 | 0.040 |
| FOR | Seagrass | 25 | 4 | -3.097 | -3.047 | -3.003 | -3.044 | 0.039 | 0.038 |
| FOR | Mudflat | 25 | 4 | -3.134 | -3.074 | -2.911 | -3.126 | 0.109 | 0.107 |
| SOL | Seagrass | 25 | 4 | -3.236 | -3.097 | -2.991 | -3.081 | 0.104 | 0.102 |
| SOL | Mudflat | 25 | 4 | -3.222 | -3.180 | -3.152 | -3.173 | 0.034 | 0.033 |

4.4 Discussion

Tea litter buried at 15 cm depth in seagrass meadows had significantly faster decomposition rates than litter buried in bare mudflat habitats. This was unexpected as seagrass meadows and other coastal and marine habitats are acknowledged as OM accumulation hotspots (Fourqurean et al., 2012). A leading purported driver of storage is the reduced rate of decomposition in the organic rich sediments beneath seagrass meadows. Coastal habitat sediments often become hypoxic or anoxic only a few centimetres below the sediment surface, with vegetated sediment generally having a shallower redox layer than bare sediment. It was therefore expected that seagrass meadows would have slower decomposition rates than mudflat areas, but these results showed the opposite. The strength and consistency of this difference suggests that it is statistically valid and is not a type 1 error. Explaining it is difficult; the following three mechanisms are hypothetical and further work would be needed to establish whether they applied in this instance. First, there is some evidence of enhanced bacterial activity and enhanced rates of decay of fresh materials in seagrass compared with mudflat habitats. For example, Honkoop et al. (2008) report a higher ratio of decayed to fresh chlorophyll *a* pigments in seagrass compared with bare sediment. This could result from differences in the nature of the litter that accumulated in these areas, or in differences in decay following accumulation, or both. Enhanced rates of decay within the rhizosphere, particularly in relatively sandy sediments in which oxygenated water can percolate, could result from oxygen leakage from roots.

Second, the results may be an artefact of introducing unusual organic materials. The major advantage of using the TeaComposition methodology was one of consistency between sites and studies. The major potential disadvantage is of experimental artefacts related to the use of novel and possibly unrepresentative substrates. Although, Keuskamp et al. (2013) and three other studies all found tea litter to be biochemically representative of local litter chemistries as it is sensitive to ecosystem specific differences whilst also following climatic trends at a global scale. Didion et al. (2016) found differences between local (*Fagus sylvatica* and *Pinus nigra*, native to Austrian Alpine forests) litter and the use of a standardised litter (green and rooibos tea material) for decomposition. However, the study noted the standardised litter had similar responses to changes in temperature and precipitation,

concluding it was a suitable method for understanding decomposition within that environment (Didion et al., 2016). Duddigan et al. (2020) approached the chemistry of tea litter decomposition differently to Keuskamp et al. (2013), focusing solely on the solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy, with cross-polarization within tea litter, as a more representative analysis of OM characteristics and compared this to three litter decomposition studies: Baldock et al. (1997), Bonanomi et al. (2013) and He et al. (2016). Duddigan et al. (2020) found that both carbon/nitrogen ratios and lignin peaks mirrored assumptions made by Keuskamp et al. (2013) confirming tea litter degradation was typical of other plant litters across a 91-day period. All the studies mentioned above only examined the breakdown of terrestrial plant litter. Trevathan-Tackett et al. (2020) looked at the chemical characterisation of the breakdown of seagrass leaf litter (*Zostera muelleri*) and found it was most similar to green tea litter, sharing the same percentage of lignocellulose and near similar carbon proportions. Across three Irish *Zostera* meadows (two *Z.marina* and one *Z.noltii*) seasonal proximate constituent data varied across sites and parts of the plant (blades, rhizome and roots; Table 4-7). Although average insoluble carbohydrates within the rhizome of *Z.marina* were most similar to that of green tea litter at one location, *Z.marina* blades had the highest insoluble carbohydrate range between 39.7-48.3% (Dawes and Guiry, 1992). Conversely, *Z.noltii* proximate constituents of carbohydrates were found to be much lower than both green and rooibos tea litters, this may have been linked to a smaller dataset spanning over fewer timepoints (Dawes and Guiry, 1992). Despite the tea litters being less similar to *Z.nolitii* litter material, much OM stored within seagrass meadows is allochthonous in nature and therefore the use of tea litter which is terrestrial in origin, is likely to represent a large proportion of OM degradation in seagrass meadows based on studies which validate the similarity between native litter and tea litter chemical composition (Baldock et al., 1997; Didion et al., 2016; Duddigan et al., 2020; He et al., 2016; Keuskamp et al., 2013).

Table 4-7 Average proximate constituents adopted from Trevathan-Tackett et al.,(2020) based on values for tea litter chemical analyses from Keuskamp et al. (2013) and ¹³C-NMR from Duddigan et al. (2020). With proximate analyses from Dawes and Guiry (1992) to describe *Z.marina* and *Z.noltii* information and Nicastro et al. (2012) and NMR from Trevathan-Tackett et al. (2017) to describe *Z.muelleri*.

| | | | <i>Z. marina</i> | | | <i>Z. noltii</i> | | | <i>Z. muelleri</i> |
|---------------------|------------------|--------------------|------------------|----------------|-------------|------------------|----------------|-------------|--------------------|
| Carbon quality | Green tea litter | Rooibos tea litter | Leaf litter | Rhizome litter | Root litter | Leaf litter | Rhizome litter | Root litter | leaf litter |
| Lignocellulose | 44% | 73% | 39.40% | 44.90% | 30% | 35.50% | 26.90% | 27.90% | 44% |
| Water soluble | 49% | 21% | 17.50% | 40.10% | 15.70% | 11.90% | 15.90% | 23.80% | - |
| ¹³ C-NMR | Alkyl carbon | 23% | 15.20% | - | - | - | - | - | 17% |
| | O-Alkyl carbon | 57% | 71% | - | - | - | - | - | 57.10% |
| | Aromatic carbon | 14.60% | 10.20% | - | - | - | - | - | 14.60% |
| | Carbonyl carbon | 5.40% | 3.20% | - | - | - | - | - | 10.20% |

Third, and related to both the other two mechanisms, is the possibility of microbial priming enhancing degradation within seagrass meadows. Kuzyakov et al. (2000) defined the priming effect as “strong short-term changes in turnover of soil organic matter caused by comparatively moderate treatments of soil”. The introduction of a new nutrient input, such as tea litter, to any sediment substrate could stimulate changes to microbial community size, structure and activity. The quality of OM material and its readily available nutrients is likely to control the strength of the priming effect. Further to this, Fontaine et al. (2003) noted the mechanisms of the priming effect are more complex than initially hypothesised, arguing the need to consider the concept of nutritional competition. This could help support the faster decomposition rates occurring in seagrass meadows. If there was already higher competition for nutrients and overall, a higher biomass of microbes competing for these resources, the addition of a new nutrient source would lead to fast colonisation from specialised microorganisms that can utilise new nutrient inputs. However, the significant differences in decomposition between habitat types are found across all timepoints, beyond any short-term priming effects across the entire experiment duration.

The earliest stages of decomposition are highly dynamic, with large variance in remaining mass throughout both studies. In the long term study, after three months, green tea litter had lost between 75-91% of its original mass (Figure 4-8). This rapid decomposition of green tea litter is much faster than other studies in temperate regions using the tea litter method. A study on terrestrial biomes found that tea litter in temperate regions after three months

retained approximately 40% of its original mass (Djukic et al., 2018). Similarly, a Scottish study of intertidal mudflats and estuarine marshes which buried tea litter at a depth of 2-5 cm, found after 100 days, a higher mean mass of green tea litter remained (~30-40%) at all six sites, regardless of habitat type, in this study (Marley et al., 2019). The marsh and intertidal mudflats within the Marley et al. (2019) study are less than 40 miles from FOR, therefore despite different brands of green tea being used in each study, it was expected they would have similar rates of decomposition.

Comparable to patterns seen with green tea litter decomposition, the results from the rooibos tea litter samples supported faster decomposition than tea litter buried for the same incubation period, when compared to terrestrial temperate biome experiments (Djukic et al., 2018). However, unlike the green tea litter decomposition, when comparing initial weight losses of rooibos tea litter from a similar habitat, comparable litter masses remained (Marley et al., 2019). Local tea litter had been used by Marley et al. (2019), however the chemical composition when investigated was comparable. These stark differences in decomposition, for similar green tea litter, emphasise the importance of understanding localised environmental variables that promote initial labile decomposition as well as habitat type. Whereas the initial breakdown of more refractory litter is slower and more similar within the same climate region regardless of habitat.

Interestingly, green tea litter decomposition rates, regardless of burial time, remained similar across all sites, with the Tukey post-hoc test only revealing different groupings in the first three months where EDE was dissimilar to RYA and TAY. The continuously similar decomposition rates, regardless of time point, is perhaps linked to the most significant mass losses, associated with leached labile material, which incurred within the first 14 days of burial (Duddigan et al., 2020; Keuskamp et al., 2013). The very rapid mass loss of green tea was therefore expected; however, it was far greater than other studies in this field. Both Duddigan et al. (2020) and Keuskamp et al. (2013) tested laboratory incubations of terrestrial soil. Other studies have confirmed that leaf litter submerged in saturated soils and sediments, accelerate leaching and therefore breakdown of litter, notably plant litter with high total nitrogen and phosphorus, forming the fundamental base media for fast microbial colonisation (Glazebrook and Robertson, 1999; Nakajima et al., 2006; Ping et al.,

2017). Despite disturbance being inevitable in terrestrial experiments, the initial experimental set up of an intertidal study would have created greater disturbance. Air exposure would have less of an impact on most terrestrial soils, where natural oxygenation down to a 15 cm depth is more probable than a compact intertidal sediment substrate. Further to this, the UK has two tides daily, therefore all sites were exposed to tidal flushing which is likely to have caused further sediment disturbance. Tidal flushing after the experiments were set up may have led to quick establishment of the leaching of labile compounds accelerating the loss rate of the water-soluble fraction of the litter (Kirwan et al., 2013). The samples hereon would have been saturated, whilst terrestrial samples would have been regularly naturally drained, which could explain the high losses found when compared to terrestrial research in particular.

Differences among refractory material breakdown across sites could be explained by temperature (Figure 4-9). Climate is widely known as a main driver of OM decomposition, particularly during the initial decomposition phases, with less of an influence over longer timescales (Djukic et al., 2018; Marley et al., 2019; Seelen et al., 2019). Mean monthly temperatures indicated the west coast sites consistently had higher mean daily temperatures than the east coast sites. Regardless of time point, west coast sites have the fastest rates of decomposition, this is even noted between the short term study at 15 cm depth where overall the SOL site had faster decomposition rates than at FOR.

4.4.1 Organic matter degradation and inferred relationship with organic carbon

It is important to note using OM as a proxy for organic carbon (OC) degradation in seagrass meadows may be misleading, because the decomposition pathways of different components of OM may vary widely, particularly between marine and terrestrial sources (Calvert, 1987; Mackenzie et al., 2004). Tea litter, whilst not identical to local terrestrial litter found in sediments, will share similar compounds, however terrestrial OM only makes up $51.4 \pm 0.14\%$ of OM found within seagrass sediments at the SOL site (Table 2-6). Further to this, OM would only make up $\sim 50\%$ of OC within litter. Keuskamp et al. (2013) determined that the tea litter comprised a mean total carbon (%) of 49.06 ± 0.11 and 50.51 ± 0.29 for green and rooibos tea, respectively. The breakdown of OM could cause uniform degradation of OC, however this is unlikely given that microbial assimilation will

differ based on litter material, decomposition phase, as well as microbial community composition. Marley et al. (2019) tested the degradation of OC concentration within the tea litter over time and did not find a direct link between tea litter mass loss and OC loss in estuarine and saltmarsh habitats across Scotland. A study on the rhizosphere microbiome of seagrass species, including *Z.noltii*, in the North-eastern Atlantic, found that the microbiome was not only controlled by plant metabolism, but also by anoxic decomposition pathways, since the bacterial community largely comprised of sulphur cycling species (Cúcio et al., 2016). The study also found that the composition of the microbiome present did not differ significantly between seagrass species, but did do so between geographical regions, supporting a cautious approach to assuming the degradation of OC is uniform with decomposition of OM (Cúcio et al., 2016). Further to this, irrespective of the potentially higher biological activity present in seagrass meadows compared to bare mudflats, there is large variation in the influence of seagrass meadows on OM (Chapter 3), therefore the consistently faster decomposition rates of litter in seagrass meadows over mudflat areas was unexpected.

The short term study highlighted a site specific significant difference of burial depth on the decomposition rates of refractory litter (Figure 4-10), which was dissimilar to general trends found across a widespread terrestrial study (Craine et al., 2010). However, the change in sediment compaction, biogeochemistry (carbon, nitrogen and phosphorus and sulphur) and physical properties (particle size), change the sediment stratigraphy and therefore a significant difference was expected across both sites, not just at SOL (Lu et al., 2011; Łukawska-Matuszewska et al., 2014). Interestingly, although not statistically significant, median *k* rates were higher in seagrass habitats at both sites at a burial depth of 15 cm and higher in mudflat habitats at a burial depth of 25 cm (Table 4-6). The change in sediment stratigraphy after 15 cm depth could explain this difference, which could infer the importance of sediment accumulation in order to preserve OM within seagrass meadows further down the sediment profile. This is based on lability profiles in chapter 3, supporting the decrease in labile OM content with depth. Exploration of decomposition rates across multiple burial depths within seagrass meadows may help to directly understand how secure labile matter is at deeper burial depths. Depth could be an important factor in determining OM accumulation and protection in seagrass meadows. For example, it is

possible that seagrass beds will accumulate OM, even if surface sediments associated with seagrasses have fast initial rates of labile litter decomposition, provided that degradation rates are significantly slowed at deeper depths (the pattern that these results imply).

As previously mentioned, the reason for heightened interest of understanding decomposition processes in blue carbon habitats is to identify patterns of OC movement and potential storage. It is important to consider the implications of the biomass losses across the different phases of decomposition for the different types of material. Labile organic material within a seagrass meadow is rapidly leached across all six meadows (Figure 4-8). Seagrass leaf litter is more labile than its woodier counterparts (roots and rhizomes), regardless, it is one of many sources of sediment organic matter within intertidal habitats. Therefore, whilst the use of tea litter can act as a metric for decomposition of organic components within seagrass meadows, it should still be used with caution.

4.5 Conclusion

Overall, the litter buried at 15 cm depth in sediments associated with seagrass decomposed at a faster rate than in comparable adjacent bare mudflat areas. This was an unexpected result that challenges the general assumption that seagrass meadows are environments where slower decomposition always occurs. The differences between decomposition rates across sites could be explained by temperature, as faster rates of decomposition occurred in warmer water west coast sediments. In addition to this, exploration of the role of depth across multiple depth profiles, may help to pinpoint if there is a critical depth where rates of decomposition are faster in mudflat habitats over seagrass meadows. This study highlighted a change in decomposition rates between habitats with depth; mudflat areas had higher rates of decomposition than seagrass meadows at a depth profile of 25 cm (although this difference was not significant). There are many potential drivers of carbon release and storage within complex intertidal seagrass meadows and depth, as well as sediment characteristics including chemical, physical and biological properties, need to be explored with scrutiny to help determine the long-term OM storage potential of seagrass meadows across Scotland.

The drive to understand decomposition in blue carbon habitats is linked to the need to understand what determines decay rates of labile and recalcitrant carbon compounds in general. The use of tea litter provides a method of directly comparing potential decomposition patterns across many habitats. However, these rates of decomposition do not directly describe OC within seagrass meadows. The use of tea litter will help infer the rate of OM degradation in seagrass meadows and other blue carbon habitats, however fresh, allochthonous plant litter is only one source of OM accumulating within seagrass meadows. Further experiments would ideally use organic matter that closely resembles the material that accumulates naturally at the experimental site; such work would be challenging but could help remove the danger of artefactual results caused by the introduction of novel material.

Chapter 5 The microbial communities associated with *Zostera noltii* sediments and adjacent bare mudflats in Scotland, UK

Abstract

Seagrass meadows across Scotland play an important role in the functioning of coastal ecosystems. They host an array of important animals, stabilise sediments, store carbon, provide protection from coastal erosion and recycle nutrients. All of these functions involve microbial communities, and some may critically depend on how seagrass habitats modify or support microbial activity, but little is known about the microbial communities associated with seagrasses and how these differ from surrounding bare mudflat areas. Here we present the results of a study on the microbial communities associated with the rhizospheres of two *Zostera noltii* meadows, using mudflats as a comparative habitat, as well as microbial communities associated with early stages of decomposition in the two habitats. The latter was explored using The Teabag Index Method and identified microbial communities associated with the decomposition of refractory tea litter (rooibos tea). Next generation sequencing was used on the isolated gDNA from sediment and tea litter samples to amplify and target the 16S genes. The results suggest microbial communities at depth (15 cm) do not vary between habitat types. Colonisation of microbial communities on tea litter varied across sites, with the key difference in decomposition rates most likely linked to the types of microbes associated with initial decomposition of litter substrates, being more efficient colonisers at one site over the other.

5.1 Introduction

Seagrasses grow in the low intertidal and shallow subtidal zones of all continents except Antarctica. Scotland is home to 20% of all Northern Europe's seagrass meadows, with two species, *Zostera noltii* and *Zostera marina*, established across the country. In Scotland seagrasses are found in a diverse range of sedimentary and hydrological conditions, from soft cohesive muds to sandy muds, within sea lochs, estuaries and even open shallow seas.

Along with mangroves and salt marshes, seagrasses are one of three 'blue carbon' vegetated marine habitats that are known to trap and accumulate organic carbon, although the rates of sequestration and carbon stocks involved vary widely between sites. Although there is large variability in organic matter (OM) accumulation in Scottish intertidal seagrasses, along the east coast, where present, seagrasses have been shown to naturally enhance OM when compared to adjacent bare mudflats (Potouroglou, 2016). This enhanced sediment carbon density may arise from one, or both, of two possible processes: enhanced accumulation (from autochthonous or allochthonous sources) or reduced decomposition. Enhanced accumulation of sediment, which incorporates particulate OM, has been demonstrated in numerous studies, including in Scotland (Potouroglou et al., 2017). The influence of seagrass on decomposition processes, particularly at depths beyond 5 cm, are less well known. Most literature assumes that decomposition will be slower beneath seagrass beds than in comparable unvegetated areas. This is because of the relative hypoxia often found in seagrass sediments (Hemminga, 1998) which is itself associated with, and often caused by, higher OM concentrations. In addition, the presence of seagrass may inhibit the activities of oxygenating macrofaunal ecosystem engineers, such as polychaete worms. For example, Eklöf et al. (2015) found that lugworm dominated areas had a negative impact on seagrass growth, whilst seagrass dominated areas competitively excluded lugworms. In some areas, dominance by either seagrass or bioturbators may represent different stable states. For example, Githaiga et al. (2019) showed how seagrass removal can lead to rapid losses of sedimentary carbon driven by the colonisation of the area by large bioturbating fauna.

It is possible that seagrass will stimulate rather than reduce decomposition. Seagrasses, like terrestrial plants, release 30-40% of their photosynthetically fixed carbon as root exudates (Badri and Vivanco, 2009; Lynch and Whipps, 1990). Root exudation within the seagrass rhizosphere is an important process which transports carbon to the roots and eventually from the roots to the sediment. Whilst this should add to the total carbon store in the sediment, it may also stimulate microbial activity through a process called 'microbial priming', that can result in more refractory carbon being decomposed (Canfield, 1994; Turnewitsch et al., 2007; van Nugteren et al., 2009). In addition to carbon leakage, oxygen is also transported from the surface to the roots and can leak into the rhizosphere, directly oxygenating contiguous sediment and potentially enhancing decomposition rates (Hemminga, 1998). It is evident that the influence of seagrass on sedimentary microbial communities, and the subsequent influences on decomposition processes are complex and likely to be dependent on a range of environmental factors, including sediment depth, grain size and ambient nutrient concentrations; much remains to be discovered about how these factors may interact.

The acceleration of new technologies in microbial science including next-generation sequencing, (meta)genomics, metabolomics, (meta)transcriptomics and (meta)proteomics, has led to a rapid growth in understanding terrestrial microbial communities (Chaparro et al., 2014). Important sediment processes such as biogeochemical cycling, sediment functional stability and substrate turnover, are influenced by the microbial communities within a habitat, for example, microphytobenthos (photosynthetic microbiota) are known for their stabilising effects (Hope et al., 2020). However, there is much less literature on microbial communities in marine environments, particularly the composition, activity and functions between bacterial communities in seagrass meadows (Cúcio et al., 2016; López et al., 1995). López et al. (1995) compared bacterial abundance between seagrass meadows and adjacent bare mudflats and noted a higher abundance of bacteria present in seagrass sediments, citing nutrient availability as the reason for the variability between the two neighbouring habitats. More recently rhizosphere microbial communities of seagrass meadows were explored by Cúcio et al. (2016). They found sedimentary bacterial communities differed between seagrass rhizospheres and the sediment of surrounding mudflats at small spatial scales and further learned of microbial community hosts

(rhizosphere, rhizoplane and endophytic compartments) with distinct microbial communities. However at larger spatial scales, plant species, sediment type, nor percentage of available OM, even if shared, were influencing factors of bacterial community assemblage within seagrass rhizospheres (Cúcio et al., 2016). Instead Cúcio et al. (2016) noted rhizosphere microbial communities were dominated by bacteria involved in sulphur related processes, with the most abundant being from the genus *Desulfococcus* which are important hydrocarbon degraders (Miralles et al., 2007). Further to this, 2% of the core taxa present belonged to the phylum *acidobacteria* which are important in carbon cycling in marine environments because they degrade complex sugars (Ward et al., 2009).

With limited knowledge of microbial communities found within seagrass meadows, especially within the UK, the role that the microbiome plays in establishing distinct carbon sequestration and storage functions within seagrass habitats is unclear. Recording and understanding any differences between microbial communities among seagrass meadows and between seagrass and bare mudflat habitats is the first step in determining the effects the microbiome might have. Decomposition rates in seagrass sediments and in contiguous bare habitats have also been explored using experimental manipulations with tea bags (see Chapter 4). Using rooibos tea produced from the leaves and woody stems of *Aspalathus linearis* as a manufactured representative of dead plant litter, provides a standard source of litter quality for comparison between sites. Examining the microbial communities present on these experimental sources of litter, in seagrass and bare mudflat habitats, should help explain the results of this decomposition experiment. The microbial results from this work are therefore also presented here.

5.1.1 Study aim

The main aim of this chapter is to describe the microbial communities present within two intertidal Scottish seagrass rhizospheres and adjacent bare mudflat habitats. In addition, microbial communities associated with the tea decomposition experiment (chapter 4) in the two habitats (seagrass and mudflat) are also compared. If differences are found, these may help to infer how these microbes function and influence decomposition rates.

The following hypotheses were formulated and explored to address these objectives:

H_{0a}= Microbial communities do not differ between seagrass and mudflat habitats.

H_{0b}= Microbial communities do not differ between seagrass meadows across multiple locations.

H_{0c}= There is no relationship between recorded rates of decomposition and microbial abundance or diversity.

H_{0d}= Microbial communities do not differ between those sampled within bulk sediment and those sampled within tea material.

5.2 Methods

5.2.1 Experimental set up

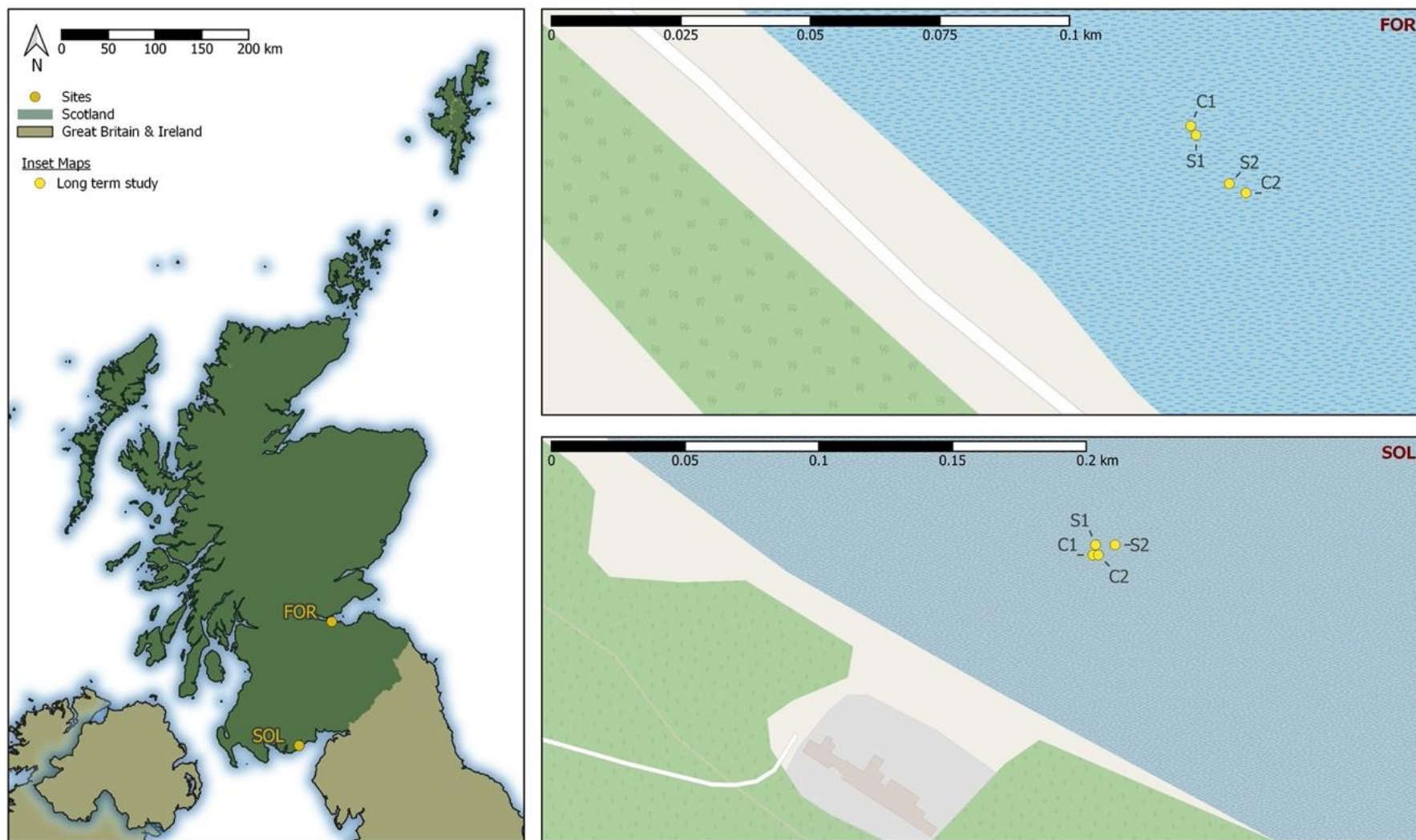
This study explores sediment and rooibos tea litter samples taken from seagrass meadows and bare mudflat areas after three months of burial (Table 5-1). One site on the East Coast, Drum Sands, located in the Firth of Forth (FOR) and the other, Auchencairn Bay, located on the South-West Coast in the Solway Firth (SOL; Figure 5-1). All samples were prepared for burial according to the global initiative protocol described in Chapter 4.

Table 5-1 Summary of key points for microbial study.

| | |
|---|------------------------------|
| Burial duration | 3 months (July-October 2017) |
| No of sites | 2 |
| No of habitat types | 2 (seagrass and mudflat) |
| Tea variety | Rooibos |
| Burial depth | 15 cm |
| Total number of sediment substrate samples at each site | 8 (4 in each habitat type) |
| Total number of teabags at each site | 8 (4 in each habitat type) |

5.2.2 Bacterial community analysis

Tea and sediment samples from *Z.noltii* seagrass meadows were collected on 9 October 2017. At each site the samples were retrieved from a 15 cm depth within two plots, each containing two habitat types within 1km of the shoreline. Sterilised equipment was used to collect sediment samples which were then transported on ice in a sealed cool box to the laboratory. All samples were stored at -20°C awaiting processing. Further to this, tea litter (operational controls) that had not been buried were also analysed to determine the microbial community of the tea samples before introduction to the two habitats within each plot.



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Scotland: Source OS Open Data. Contains OS data © Crown copyright and database right (2021).

Additional Boundaries: These data were extracted from the GADM database (www.gadm.org), version 2.5, July 2015. They can be used for non-commercial purposes only. It is not allowed to redistribute these data, or use them for commercial purposes, without prior consent.

OSM tiles: © OpenStreetMap contributors. The map tiles in the "standard style" at www.openstreetmap.org are a Produced Work by the OpenStreetMap Foundation using OpenStreetMap data under the Open Database License. Base map and data from OpenStreetMap and OpenStreetMap Foundation

Figure 5-1 Map of study sites; FOR and SOL and insert maps of individual habitats, where S1 and S2 represent seagrass and C1 and C2 represent mudflats, respective of the plot numbers.

The DNeasy® PowerSoil Kit (Qiagen) and the manufacturer's protocol was followed to isolate microbial gDNA from the sediment samples (DNeasy PowerSoil Kit handbook 05/2017). All DNA extracts were kept at -20°C until preparation for sequencing. The primer pair used was CO1f (AGT TTG ATC CTG GCT CAG) and CO2R (TAC CTT GTT ACG ACT T) to amplify and target the 16S gene. The conditions to produce the PCR product were: 1 cycle at 94°C for 180 seconds (s), 35 cycles at 94°C for 45 s, 50°C for 60s, 72°C for 90 s and 1 cycle at 72°C for 600 s, before being held at 4°C - this was conducted using a thermocycler. This generated 498 bp for gDNA sequencing.

All PCR products were resolved on a 1.2% agarose gel at 100 V for 45 minutes to identify the best dilution for the NGS work (1 in 10 µl dilution). Samples were then sent to Nu-OMICS DNA sequencing facility at Northumbria University for external laboratory analysis using Illumina massively parallel sequencing.

5.2.3 Bioinformatics

Data preparation for analysis was performed using USEARCH v9 (Edgar and Flyvbjerg, 2015). The data from the NGS analysis was provided in "R1" – forward and "R2" – reverse format and underwent the following data transformation: (a) merged; (b) filtered for errors; (c) unique DNA sequences were then identified; (d) clustered into groups; (e) renamed to match the operational taxonomic units (OTUs) from the reference database (Wang et al., 2007), based on a 97% threshold of similarity, linking each sequence to a reference sequence. OTUs classified as chloroplasts or mitochondria were discarded from the dataset and all OTUs that had an abundance of one (singles) were removed. The data could then be used for alpha and beta diversity metrics. The R studio heatmaply package (Galili et al., 2018) was also used to determine relative abundance of the top 500 OTUs across all environmental conditions.

5.2.4 Statistical analysis

Mixed model ANOVAs were used to statistically test differences in alpha and Shannon's diversity in habitat types and substrate types within and across sites. All analyses were conducted in R Studio using the lme4 package using a mixed effects model with the lmer function (Bates et al., 2015). All data were tested for normality and homogeneity of variance

before running statistics. Venny diagrams were produced for OTUs across all sample types using Venny version 2.1 (Oliveros, 2007-2015). XLSTAT (Addinsoft, 2019) was used to visualise the principal coordinate analysis (PCoA). All other figures were produced using the package ggplot2 (Wickham, 2016).

5.3 Results

5.3.1 Alpha and Shannon's diversity

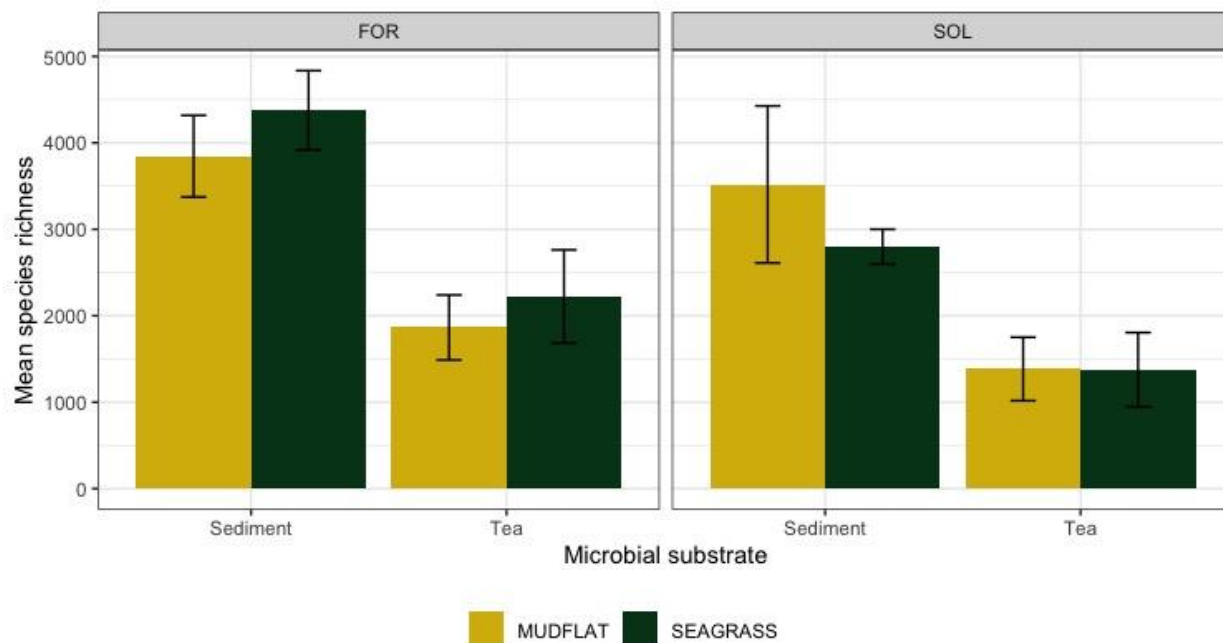


Figure 5-2 Column chart of species richness for all sample types, error bars represent confidence intervals.

Statistical analysis of bacterial alpha diversity found there to be no significant three-way interaction between habitat type, substrate and site, however a statistically significant interaction term was found between habitat type and site ($df = 1$, $F = 5.69$, $p = 0.026$).

Bacterial alpha diversity was higher in all sediment samples when compared to tea samples, regardless of site or habitat type ($df = 1$, $F = 128.38$, $p < 0.001$; Figure 5-2). Overall, there was higher species richness at FOR than SOL, regardless of habitat type and substrate type. The highest species richness was found within seagrass sediments at FOR. Although there was higher species richness within seagrass sediments than mudflat sediments at FOR the opposite effect was found at SOL.

Table 5-2 statistical summary of alpha diversity data. model used; Model = lmer(alpha~SITE+HABITAT_TYPE+SUBSTRATE+SITE*HABITAT_TYPE*SUBSTRATE+ (1|plot), data = Species_Richness, REML =TRUE).

| | Sum Sq | Mean Sq | NumDF | DenDF | F value | p value |
|---------------------------------|----------|----------|-------|-------|---------|---------|
| Site | 5228570 | 5228570 | 1 | 22 | 22.70 | <0.001 |
| habitat type | 4569 | 4569 | 1 | 2 | 0.02 | 0.901 |
| Substrate | 29577663 | 29577663 | 1 | 22 | 128.38 | <0.001 |
| Site * habitat type | 1311795 | 1311795 | 1 | 22 | 5.69 | 0.026 |
| Site * substrate | 165744 | 165744 | 1 | 22 | 0.72 | 0.405 |
| Habitat type * substrate | 145665 | 145665 | 1 | 22 | 0.63 | 0.435 |
| Site * habitat type * substrate | 388742 | 388742 | 1 | 22 | 1.69 | 0.207 |

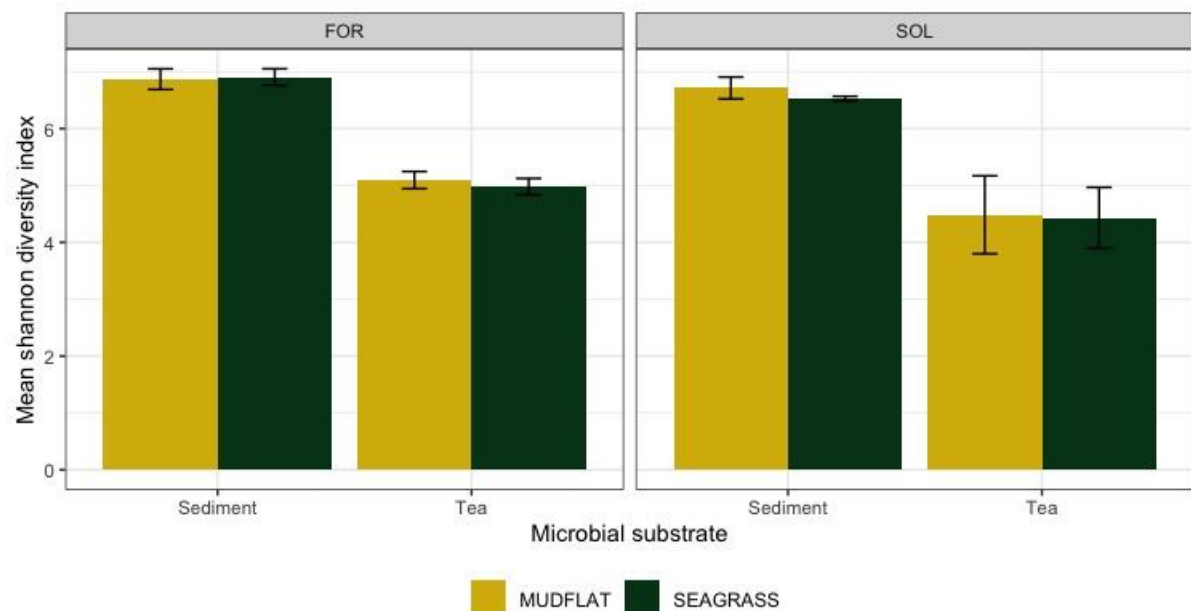


Figure 5-3 Column chart of Shannon's diversity (log e) for all sample types. Statistical model used; Model = lmer(SHANNON~SITE+HABITAT_TYPE+SUBSTRATE+SITE*HABITAT_TYPE*SUBSTRATE+ (1|plot), data = Shannon_div, REML =TRUE).

The bacteria community composition across all samples was highly diverse with all Shannon's index values exceeding 4 (Figure 5-3). There were no statistically significant interaction terms between sample conditions, and no significant difference between habitat types (df = 1, F = 0.43, p = 0.516), based on a confidence level of 95%. The highest diversity was found across all sediment samples, regardless of habitat type, and there was a significant difference in Shannon's diversity between substrates (df = 1, F =277.31, p<0.001). Microbial community diversity was higher at FOR than corresponding habitats at SOL,

similar to richness ($df = 1$, $F = 12.33$, $p = 0.002$). However, there was little variation between the replicate samples for each sample condition, the largest variation in diversity occurred between tea samples buried in mudflat habitats.

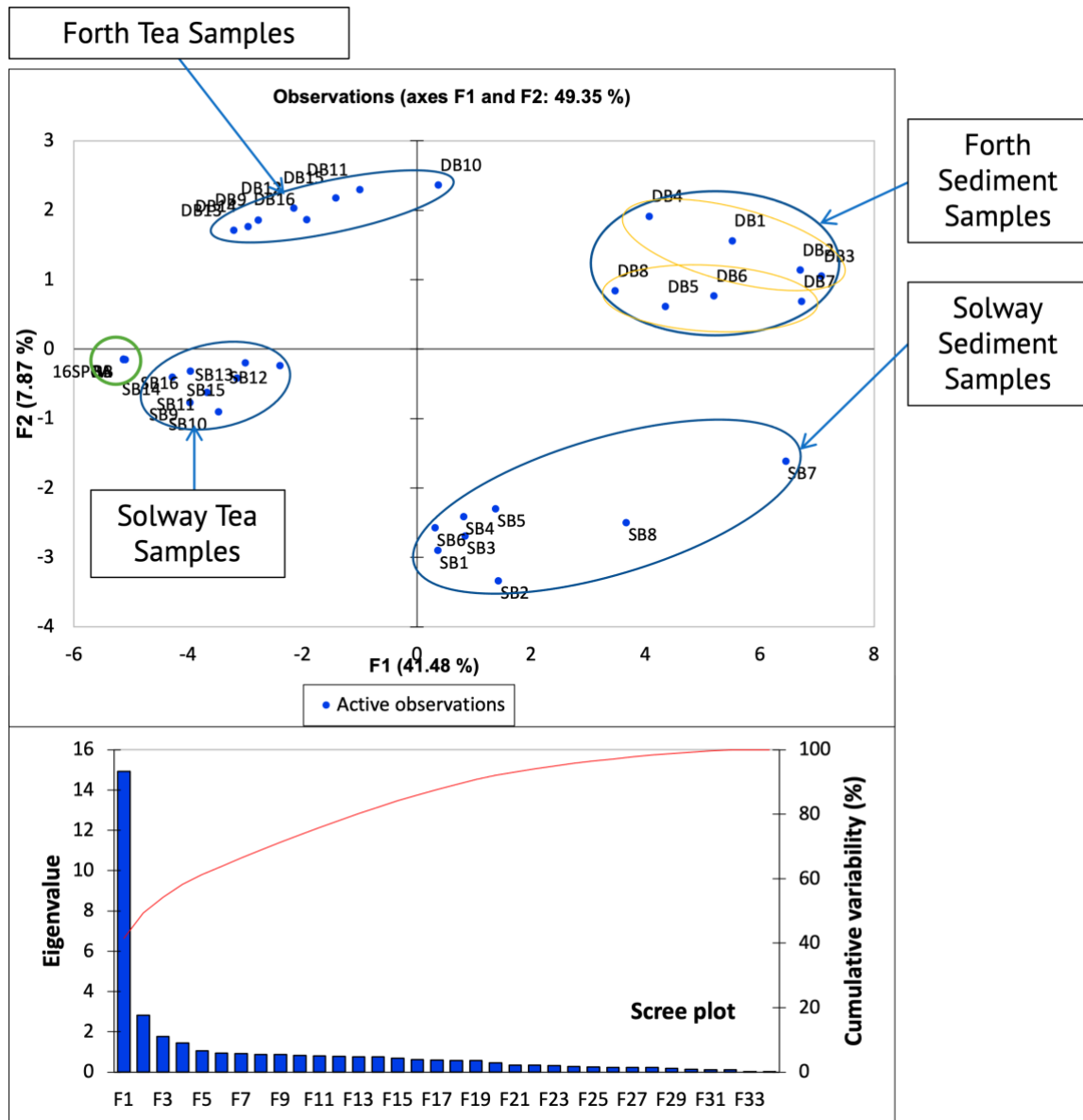


Figure 5-4 Principle coordinate analysis using Euclidean distance (and scree plot) to determine dissimilarity between all sites, habitat types and sample substrates (ellipses represent distinct clustering) where percentage of community variance explained by each axis is indicated in parentheses ($F1 = 41.48\%$ and $F2 = 7.87\%$). The green ellipses show all operational control (16S positive, 16S negative and tea) communities.

The principal coordinate analysis (PCoA) plot for bacteria communities shows there are large dissimilarities between sites and substrate types. The tea samples that were not buried within either site are also shown in Figure 5-4 (green ellipsis). The microbial

communities of tea buried at SOL was more similar to the tea not subjected to environmental conditions than bulk sediment at SOL, regardless of habitat type. The SOL tea material ellipsis shows that the tea litter samples, regardless of habitat type, are more similar and clustered than microbial communities associated with tea litter at FOR (Figure 5-4). Most of the SOL site sediment samples are tightly clustered together suggesting highly similar microbial communities across the samples, although SB7 and SB8 are outwith the main cluster. Interestingly, the ellipsis containing the sediment samples from FOR could be split between habitats further (yellow ellipses) with DB1-4 representing sediment substrate within the seagrass rhizosphere and DB5-8 representing the adjacent sediment substrate which was sampled within the mudflat habitats. However, this split between habitat types is small with DB2, DB3 and DB7 more similar than the visual ellipses splits between habitat types within FOR plots (Figure 5-4).

5.3.2 Operational taxonomic units

A total number of 3,130,006 sequences that comprised of 372,796 unique sequences, and 265,742 singletons were detected from NGS. The unique sequences were then clustered into 16,569 OTUs, the summary of OTUs found within each sample condition is presented in Table 5-3. The smallest number of OTUs were found in the operational controls (16s Negative and Positive and Tea operational controls) and the largest number of OTUs were associated with mudflat sediments. Regardless of site or habitat type, all tea litter had a lower number of OTUs than the adjacent sediments.

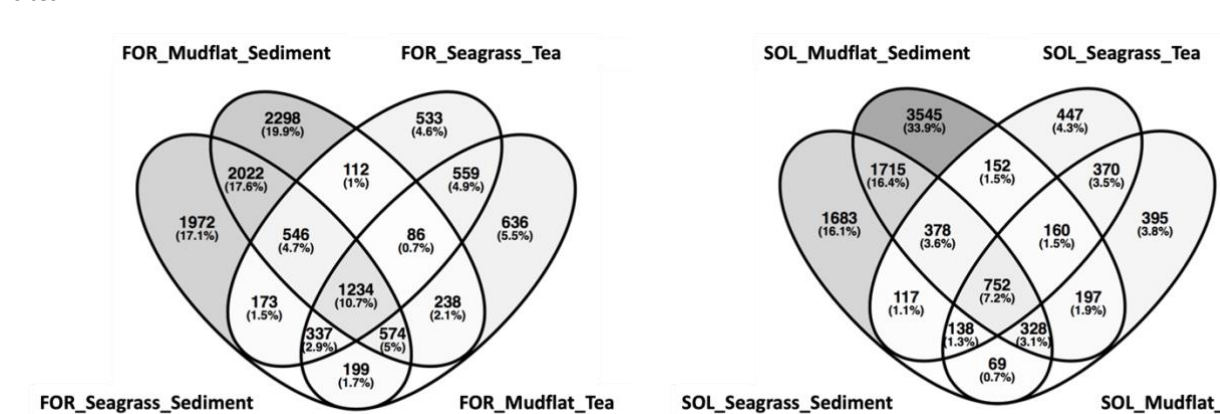
Table 5-3 Total number of Operation Taxonomic Units (OTUs) across all sites, habitat types and substrate types.

| Site | Plot and replicate | Habitat type | Substrate | No. of OTUs present |
|---------------------|--------------------|--------------|-----------|---------------------|
| 16S Neg | N/A | N/A | N/A | 9 |
| 16S Pos | N/A | N/A | N/A | 8 |
| Operational control | A | N/A | Tea | 18 |
| Operational control | B | N/A | Tea | 26 |
| FOR | 1A | Seagrass | Sediment | 3,170 |
| FOR | 1B | Seagrass | Sediment | 3,391 |
| FOR | 2A | Seagrass | Sediment | 3,581 |
| FOR | 2B | Seagrass | Sediment | 2,775 |
| FOR | 1A | mudflat | Sediment | 2,881 |
| FOR | 1B | mudflat | Sediment | 3,155 |
| FOR | 2A | mudflat | Sediment | 3,518 |
| FOR | 2B | mudflat | Sediment | 2,889 |
| FOR | 1A | Seagrass | Tea | 1,163 |
| FOR | 1B | Seagrass | Tea | 2,221 |
| FOR | 2A | Seagrass | Tea | 1,680 |
| FOR | 2B | Seagrass | Tea | 1,388 |
| FOR | 1A | mudflat | Tea | 1,174 |
| FOR | 1B | mudflat | Tea | 1,188 |
| FOR | 2A | mudflat | Tea | 1,516 |
| FOR | 2B | mudflat | Tea | 1,441 |
| SOL | 1A | Seagrass | Sediment | 2,077 |
| SOL | 1B | Seagrass | Sediment | 2,397 |
| SOL | 2A | Seagrass | Sediment | 2,069 |
| SOL | 2B | Seagrass | Sediment | 2,246 |
| SOL | 1A | mudflat | Sediment | 2,379 |
| SOL | 1B | mudflat | Sediment | 2,208 |
| SOL | 2A | mudflat | Sediment | 4,043 |
| SOL | 2B | mudflat | Sediment | 3,120 |
| SOL | 1A | Seagrass | Tea | 746 |
| SOL | 1B | Seagrass | Tea | 996 |
| SOL | 2A | Seagrass | Tea | 999 |
| SOL | 2B | Seagrass | Tea | 1,448 |
| SOL | 1A | mudflat | Tea | 1,330 |
| SOL | 1B | mudflat | Tea | 751 |
| SOL | 2A | mudflat | Tea | 1,372 |
| SOL | 2B | mudflat | Tea | 996 |

Across the two sites, only 12.8% of OTUs were shared across habitat types in sediment samples (Figure 5-5). The largest proportion of OTUs were unique to each site and habitat type, with unique OTUs ranging between 10-16% across the four individual sediment sample conditions. The mudflat areas at SOL had the largest quantity of unique OTUs consistently when compared across sites and to tea samples. When comparing OTUs shared within individual sites across habitat types and substrates, there are fewer common OTUs (10.7%

Venn diagram illustrating the overlap of four sediment types: FOR_Mudflat_Sediment, SOL_Seagrass_Sediment, FOR_Seagrass_Sediment, and SOL_Mudflat_Sediment. The diagram shows the number of samples in each region and their percentage of the total.

| Region | Count | Percentage |
|---|-------|------------|
| FOR_Mudflat_Sediment only | 2014 | 14% |
| SOL_Seagrass_Sediment only | 1433 | 10% |
| FOR_Seagrass_Sediment only | 1815 | 12.6% |
| SOL_Mudflat_Sediment only | 2294 | 16% |
| FOR_Mudflat_Sediment & SOL_Seagrass_Sediment | 159 | 1.1% |
| FOR_Mudflat_Sediment & FOR_Seagrass_Sediment | 1316 | 9.2% |
| FOR_Mudflat_Sediment & SOL_Mudflat_Sediment | 469 | 3.3% |
| SOL_Seagrass_Sediment & FOR_Seagrass_Sediment | 861 | 6% |
| SOL_Seagrass_Sediment & SOL_Mudflat_Sediment | 193 | 1.3% |
| FOR_Seagrass_Sediment & SOL_Mudflat_Sediment | 368 | 2.6% |
| FOR_Mudflat_Sediment & FOR_Seagrass_Sediment & SOL_Mudflat_Sediment | 290 | 2% |
| FOR_Mudflat_Sediment & SOL_Seagrass_Sediment & SOL_Mudflat_Sediment | 1847 | 12.8% |
| FOR_Mudflat_Sediment & FOR_Seagrass_Sediment & SOL_Seagrass_Sediment | 125 | 0.9% |
| FOR_Mudflat_Sediment & SOL_Seagrass_Sediment & FOR_Seagrass_Sediment | 272 | 1.9% |
| SOL_Seagrass_Sediment & FOR_Seagrass_Sediment & SOL_Mudflat_Sediment | 923 | 6.4% |
| FOR_Mudflat_Sediment & SOL_Seagrass_Sediment & FOR_Seagrass_Sediment & SOL_Mudflat_Sediment | 469 | 3.3% |



5.3.2.1 Prokaryote community analysis

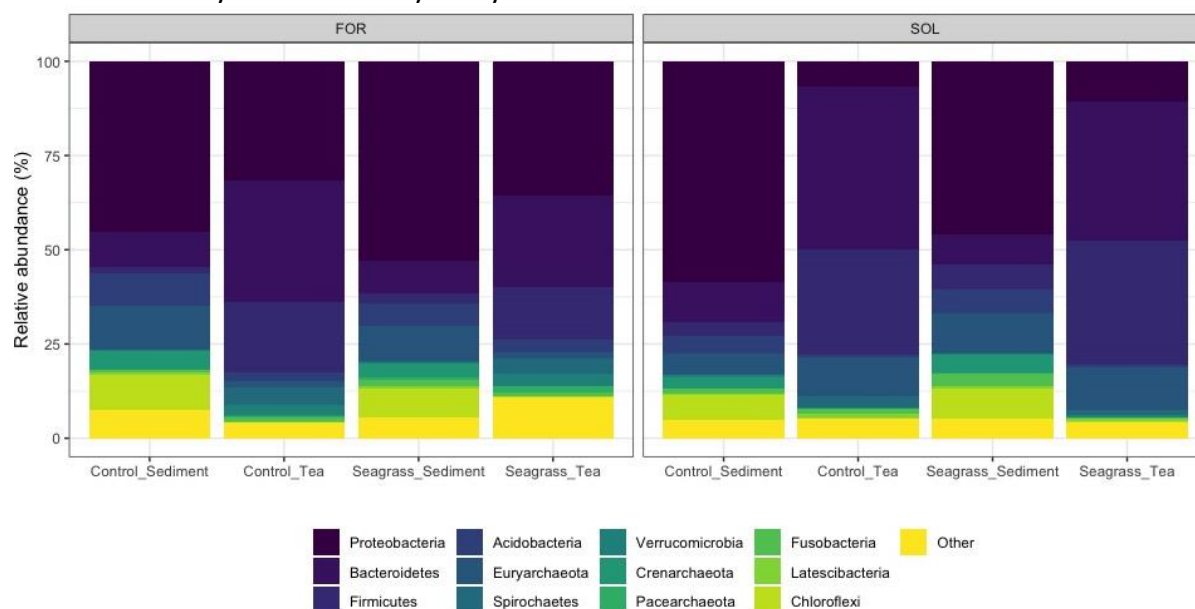


Figure 5-7 Prokaryote community composition for intertidal seagrass and mudflat areas associated with sediment and tea substrates. Taxa are at phylum level.

With 16,569 unique OTUs, the top 500 most relative abundant OTUs were explored. Only three OTUs were identified within both operational tea controls. There were: two types of Proteobacteria, both from the class *Gammaproteobacteria* (order *Enterobacteriales*), and a *Firmicutes*, *Bacilli*. Prokaryote community analysis, unlike Shannon's diversity analysis showed even though the majority of samples shared the same taxa, they were distributed slightly differently across sample conditions (Figure 5-7). The four most abundant phylum associated with sediment included *Proteobacteria* (predominately *Deltaproteobacteria* and *Gammaproteobacteria*), *Bacteroidetes* (*Flavobacteriia* being the largest group), *Acidobacteria* and *Euryarchaeota* (*Methanomicrobia* being the largest class group). The most dominate taxa associated with tea included *Proteobacteria* (predominately *Deltaproteobacteria*), *Bacteroidetes* (*Bacteroidia* being the largest group) and *Firmicutes* (*Clostridia* being the largest group). *Proteobacteria* was consistently the highest taxa across all sample conditions except SOL tea samples. Four taxa, *Crenarchaeota*, *Parcearchaeota*, *Latescibacteria* and *Chloroflexi* were either low in abundance or not present in tea substrates (Table 5-4). Overall, relative abundance of microbial communities were very similar at phylum level across habitat types, less similar across sites and differed greatly between substrates (Figure 5-8).

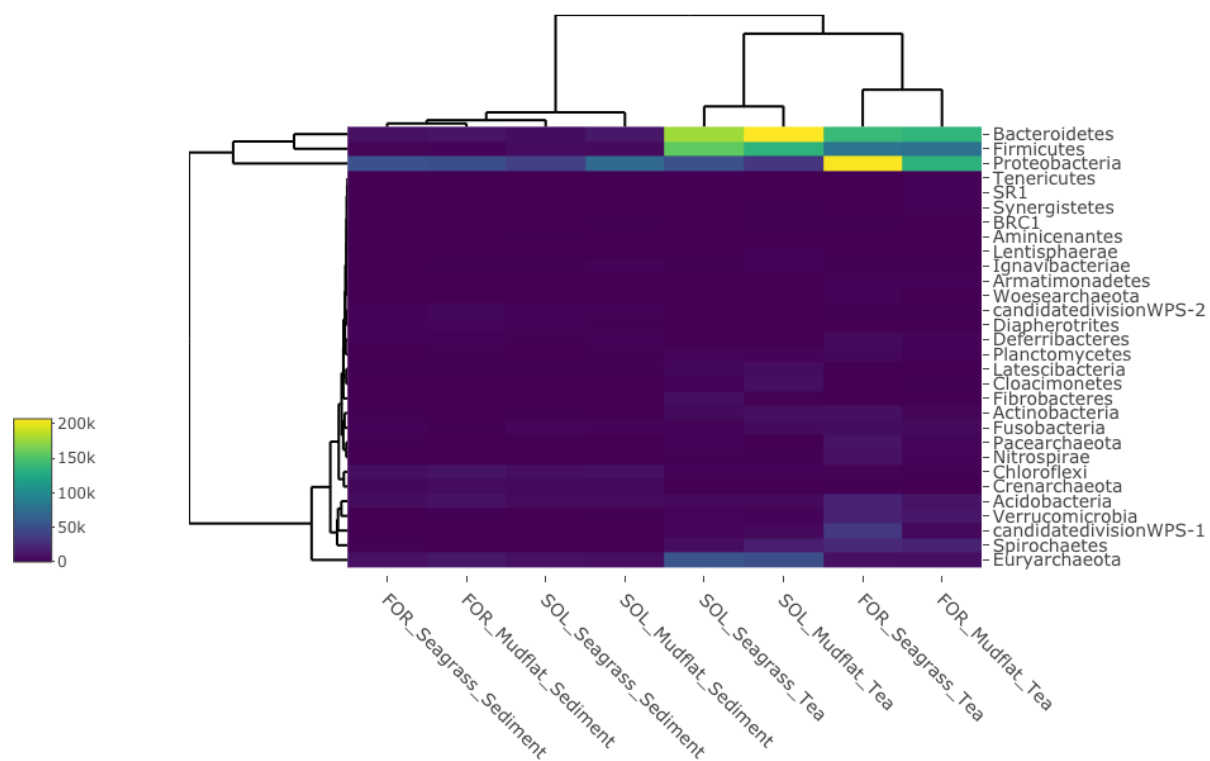


Figure 5-8 Heatmap of taxonomic diversity at Phylum level for most abundant OTUs (500 OTUs) across all sample conditions. Clustering metric Euclidean.

Table 5-4 Prokaryote community composition for intertidal seagrass and mudflat areas associated with sediment and tea substrates. Taxa are at Class level. Percentages represent relative abundance.

| | | Site | FOR | | | | SOL | | | | |
|----------|------------------------|------------------------------------|--------------------------|---------|----------|----------|----------|---------|----------|----------|-----|
| | | Habitat type | Seagrass | Mudflat | Seagrass | Mudflat | Seagrass | Mudflat | Seagrass | Mudflat | |
| Domain | Phylum | Class | Substrate | Tea | | Sediment | | Tea | | Sediment | |
| Bacteria | Acidobacteria | Acidobacteria | | 3% | 2% | 6% | 9% | 1% | 1% | 6% | 4% |
| | Actinobacteria | Actinobacteria | | 1% | 1% | 0% | 0% | 1% | 2% | 0% | 0% |
| | Bacteroidetes | Cytophagia | | 6% | 13% | 1% | 0% | 3% | 5% | 1% | 1% |
| | | Bacteroidia | | 11% | 14% | 2% | 1% | 29% | 32% | 4% | 3% |
| | | Bacteroidetesincertaesedis | | 6% | 4% | 0% | 0% | 1% | 4% | 0% | 0% |
| | | Flavobacteriia | | 2% | 1% | 5% | 8% | 0% | 0% | 2% | 6% |
| | | Sphingobacteriia | | 0% | 0% | 0% | 0% | 4% | 3% | 0% | 0% |
| | | candidatedivisionWPS-1 | WPS-1generaincertaesedis | | 6% | 1% | 0% | 0% | 0% | 1% | 0% |
| | candidatedivisionWPS-2 | WPS-2generaincertaesedis | | 0% | 0% | 2% | 3% | 0% | 0% | 1% | 1% |
| | Chloroflexi | Anaerolineae | | 0% | 0% | 7% | 8% | 0% | 0% | 7% | 6% |
| | Crenarchaeota | Thermoprotei | | 0% | 0% | 4% | 5% | 0% | 0% | 5% | 3% |
| | Deferribacteres | Deferribacteres | | 1% | 0% | 1% | 1% | 0% | 0% | 0% | 1% |
| | Firmicutes | Clostridia | | 12% | 15% | 2% | 1% | 29% | 25% | 6% | 3% |
| | | Bacilli | | 1% | 2% | 0% | 1% | 3% | 1% | 1% | 0% |
| | | Erysipelotrichia | | 1% | 2% | 0% | 0% | 0% | 0% | 0% | 0% |
| | Fusobacteria | Fusobacteriia | | 1% | 1% | 2% | 0% | 0% | 1% | 3% | 1% |
| | Latescibacteria | Latescibacteriageneraincertaesedis | | 0% | 0% | 1% | 1% | 1% | 1% | 1% | 1% |
| | Nitrospirae | Nitrospira | | 1% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| | Proteobacteria | Deltaproteobacteria | | 29% | 25% | 30% | 24% | 9% | 5% | 23% | 28% |
| | | Gammaproteobacteria | | 3% | 4% | 19% | 18% | 1% | 1% | 20% | 27% |
| | | Spirochaetia | | 4% | 5% | 0% | 0% | 1% | 3% | 0% | 0% |
| | | Alphaproteobacteria | | 3% | 3% | 2% | 2% | 1% | 0% | 1% | 2% |
| | | Verrucomicrobia | Opitutae | | 3% | 3% | 0% | 0% | 0% | 0% | 0% |
| Archaea | Euryarchaeota | Methanomicrobia | | 1% | 1% | 5% | 6% | 6% | 9% | 6% | 4% |
| | | Methanobacteria | | 0% | 0% | 3% | 5% | 5% | 1% | 3% | 1% |
| | Pacearchaeota | PacearchaeotalncertaeSedisAR13 | | 2% | 1% | 0% | 0% | 0% | 0% | 0% | 0% |
| Other | | | | 2% | 2% | 7% | 7% | 4% | 4% | 7% | 6% |

5.4 Discussion

5.4.1 Addressing hypotheses

Chapter 4 identified a statistical difference in decomposition rates between habitat types, with tea litter degrading faster in seagrass meadows than mudflat habitats. It was hypothesised that differences in microbial communities could explain the difference in decomposition rates between habitat types because vegetation, spatial variability, tidal variation and oxygenation of sediment substrate, are all factors that typically explain differences in microbial communities in intertidal habitats (Lee et al., 2021). Both habitats were close in proximity so were exposed to the same tidal variations, and despite the majority of study sites recording higher average dry bulk density (DBD (g cm^3)) in mudflat habitats (Table 3-3) both FOR and SOL DBD values were similar regardless of habitat type. Seagrass meadows are also characterised by belowground rhizome and root structures which promote oxygenation, unlike bare mudflats. Therefore, a higher microbial biomass within seagrass meadows would help explain the faster decomposition rates in seagrass habitats from priming effects of the introduced tea litter (although only relative abundance can be inferred from the current results). However, these results do not support the idea that clear differences in microbial communities were driving differences in decomposition. Overall, there was no statistically significant difference in alpha or Shannon's diversity between habitat types. Microbial communities across sediment habitats at FOR shared 38% of OTUs, whereas only 30.3% of OTUs were similar across sediment habitats at SOL. A higher proportion of OTUs were shared across microbial communities associated with tea litter; 42.4% within tea samples at FOR and 40.5% at SOL. However, similar to sediment substrates, microbial communities associated with tea litter were similar across habitat types within sites. SOL tea samples were the most similar (tightly clustered) regardless of habitat types. The similarities between microbial communities across habitat types indicate that at a depth of 15 cm, the composition of microbial communities was not influenced by the presence of seagrass. A possible explanation for the similarity in microbial communities between habitat types could be linked to the depth of tea litter burial, which was below the rhizosphere of *Z. noltii* meadows. Cúcio et al. (2016) found distinct

differences between, and within, rhizosphere microbial communities but did not sample below the rhizosphere. In this study, site and substrate – but not habitat – are the leading factors influencing variation across microbial communities (Figure 5-4).

Microbial communities associated with intertidal mudflats are likely to rely on external nutrient sources for growth which are often limited, whilst seagrass meadows have complex root structures and a continual supply of vegetation to promote regular microbial growth (Hemminga, 1998). The introduction of artificial litter and the unavoidable disturbance during burial at depth was expected to result in more rapid colonisation of tea litter buried in mudflat habitats. However, there was no differences between alpha and beta diversity for microbial communities associated with tea litter buried across habitat types. Irrespective of this, it is interesting to note the differences between microbial colonisation of the tea litter samples from the PCoA. Figure 5-4 shows faster tea colonisation of sediment associated microbial communities at FOR than tea buried at SOL, because the Euclidean distance between the microbial communities when compared to the operational control tea is greater. Microbial communities associated with tea litter buried at SOL are the most distinct and tightly clustered when compared to all other samples subject to environmental conditions. In addition to this, communities within SOL tea samples were also most similar to the operational control tea material. Therefore, the microbial community at FOR is likely to be more mobile and more active, explaining the dissimilarity of the microbial communities in FOR tea samples to the operational controls. Surprisingly, faster rates of decomposition were recorded for tea litter at SOL than FOR. The SOL site also had a lower proportion of different OTUs associated with tea samples than FOR.

These unexpected findings suggest microbial community growth and therefore microbial carbon use efficiency (CUE) could be controlled by additional factors such as sediment temperature, water concentration, oxygenation and salinity (Geyer et al., 2016). Microbial CUE is defined as the underlying microbial population's efficiency in metabolising a variety of compounds to satisfy carbon demand.

Therefore, CUE influences and controls accumulation and loss dynamics within OM stocks. Scottish east coast seagrass meadows have been found to contain higher stocks of OM (Potouroglou, 2016) than west coast meadows (chapter 3) and therefore slower decomposition rates at FOR could be explained by the microbial communities within these meadows requiring lower OM inputs. Another explanation for the faster rates of decomposition found at SOL may be linked to the role of macroinfauna bioturbation continually mixing sediment particles and redistributing these across the sediment column. The consequences of sediment bioturbation for microbial communities include increased oxygenation and pore water mobility which cause physicochemical changes at depth, plus macroinfauna activity enables microbes to capitalise on available exudations and moulted exoskeletons (Lohrer et al., 2004). A Scottish mesocosm study exploring intertidal mudflat ecosystem engineers found that water turbidity and sediment function does little to influence subsurface microbial communities in mudflats. Instead, the study found key drivers of microbial community differences were linked to physical sediment reworking through manual disturbance and from infauna bioturbatory activity as they promoted nutrient release (Wyness et al., 2021). Whilst there are only two disturbance events during this study (burial and retrieval of tea samples), further exploration of macroinfauna communities that promote bioturbation could support the differences in decomposition rates across sites.

5.4.2 Describing microbial communities

As previously stated, *Proteobacteria* was the most dominant phylum across all environmental conditions, except microbial communities associated with tea litter at SOL. Trevathan-Tackett et al. (2020), a study on microbial breakdown of seagrass detritus in Australia, found *Proteobacteria* to be the most dominant taxa associated with buried seagrass litter but explained this as a probable artifact of the methodology they adopted in their study, owing to the 14 day burial duration. Litter samples buried for longer timescales showed that microbial communities associated with seagrass detritus became more diverse. Regardless of this, after 90 days the results show *Deltaproteobacteria* had the highest relative abundance across the majority of environmental conditions; 25 of the 31 *Deltaproteobacteria* were linked to

sulphur reduction with the majority of these belonging to the family *Desulfobacteraceae*, but *Desulfobulbaceae*, *Desulfovibrionaceae* and *Desulfuromondaceae* were also amongst the top 500 OTUs. Members of these families play an important role in sulphur processes and remineralisation of OM in the absence of oxygen within seagrass meadows and have been found in rhizospheres across seagrass meadows in Portugal and France (Cúcio et al., 2016; Probandt et al., 2017). *Gammaproteobacteria*, an essential class for fat decomposition in sediments, was less abundant in tea samples than *Deltaproteobacteria*, although it was the most dominant class found in operational tea controls. *Gammaproteobacteria* was the second most relative abundant class for sediment associated microbial communities across both sites, whilst at SOL and FOR, it only represented 1% and 3% respectively of relative abundance within microbial communities associated with tea. This low level of colonisation of tea litter at SOL could be linked to the dominance of *Clostridia*, belonging to *Firmicutes*, which is also linked to sulphate reduction (Widdel, 2006). *Clostridia* was found in the highest abundance in microbial communities associated with tea litter at SOL than any other environmental condition (29% and 25% in seagrass and mudflat habitats, respectively). However, the taxon has not been successfully classified according to carbon mineralisation rate and does not change in a predictable manner based on carbon availability (Fierer et al., 2007).

Another key taxon important for carbon cycling is *Acidobacteria* due to their ability to degrade complex biopolymers including sugars such as chitin (Ward et al., 2009). All samples contained *Acidobacteria*, though they were lower in relative abundance than *Proteobacteria* and *Bacteroidetes* in sediment associated samples, and had a much lower relative abundance in tea samples (Table 5-4). Sample depth could be a possible explanation for the low abundance, as 15 cm is below the root depth of *Z.noltii* because *Acidobacteria* are known to contribute to biofilm formation and soil structure when abundant in surface sediments (Ward et al., 2009). Further to this, *Acidobacteria* may not have fully established at the time of DNA extraction because bacteria oligotrophic in nature are involved in later stages of decomposition (Fierer

et al., 2007). Similarly, *Verricomicrobia* are typically abundant in intertidal surface sediments because they are resilient to fluctuating hyper saline environments. However, only within tea litter at FOR were they found to make up more than 1% of all abundant species.

Bacteroidetes, including the class *Cytophagia* and *Bacteroidia*, were dominant in tea associated microbial communities. *Cytophagia* and *Flavobacteria* are proficient at degrading biopolymers including cellulose, chitin and pectin (Manz et al., 1996; Reichenbach, 1992). *Bacteroidetes* are highly mobile bacterium characterised as initial metabolisers of labile carbon inputs commonly known as copiotrophic (Fierer et al., 2007). Therefore, it is unsurprising that the taxa were quick to colonise the introduced tea samples. The faster decomposition rates could be explained by the much higher relative abundance of *Bacteroidia* associated with SOL tea litter (29% and 32% in seagrass and mudflat habitats respectively) than at FOR (11% and 14% in seagrass and mudflat habitats respectively).

This study was unable to measure microbial biomass or enzyme activity, however Liu et al. (2017) reported that increasing nutrient enrichment resulted in a boost in sediment microbial biomass as well as enzyme activity in *Thalassia hemprichii* sediments. Similarly, Jones et al. (2003) estimated a higher bacterial abundance within seagrass meadows when compared to bare mudflats and transitional areas using phospholipid fatty acid concentrations in *Thalassia testudinum* meadows. From the evidence provided, it is difficult to determine the key difference between microbial communities that explain the faster decomposition rates of tea litter in Chapter 4 without further analysis. A key disadvantage of using NGS data to study microbial communities associated with the different environmental conditions is that the data analysed is a subsample used for DNA extraction. The data is compositional, only providing information on relative abundance, which unfortunately is independent of total microbial load and could lead to bias (Morton et al., 2019). Therefore, this study is unable to identify if there is a higher bacterial abundance within seagrass areas than mudflats, even though there is a higher

number of OTUs detected in sediments associated with mudflat areas when compared to seagrass areas (Figure 5-5).

5.5 Conclusion

In conclusion, even though there are differences in microbial communities between sites and substrates, microbial community composition is similar across habitat types at a burial depth of 15 cm. Therefore, this study does not help to explain the variation between decomposition of an introduced tea litter within intertidal seagrass meadows and bare adjacent mudflat areas. Rates of microbial colonisation of specific copiotrophic microbes could explain the faster rates of decomposition found across SOL buried tea litter when compared to litter buried at FOR. Within tea litter communities at SOL there was a higher relative abundance of *Bacteroidetes* and *Firmicutes* than present in microbial communities associated with tea litter at FOR. However, there are still large information gaps in addressing the possible reasons for faster rates of decomposition found for tea litter buried in seagrass meadows over mudflat areas across Scottish seagrass meadows explored in Chapter 4.

Chapter 6 The influence of seagrass meadows on sedimentary characteristics and accumulation rates

Abstract

The efficiency with which seagrass meadows trap and retain sediment is believed to be a leading factor explaining the habitat's ability to sequester carbon, however this is understudied in Scottish meadows. Whilst research has studied deposition rates, no studies have explicitly investigated sediment stability and its association with erosion. To help fill this knowledge gap and to quantify the surficial erosivity of fine sediments in Scottish seagrass meadows, sediment erodibility was measured in-situ using a cohesive strength meter (CSM) and shear vane in seagrass and bare mudflat areas. Seagrass sediments showed significantly greater cohesion, as measured with the shear vane, than adjacent mudflats. Surface cohesion, as measured by the CSM, was also generally higher in seagrass meadows, although this difference was not as strong and varied widely between sites, with some areas naturally containing sediments that have a higher erosion threshold than others.

Sediment characteristics such as bulk density and grain size and their relationships with carbon could help explain why efficiency in carbon storage varies across seagrass meadows. Overall, the analysis supported a higher proportion of finer grain sized particles were found in *Zostera marina* meadows than adjacent bare mudflats. However, the influence of *Zostera noltii* on associated sediment grain size was more variable. Regardless of this, when exploring the relationship between sediment characteristics and carbon overall seagrass meadows show a stronger positive relationship than bare mudflat areas, regardless of species.

In addition to this, the study explored sedimentation rates using radionuclide dating techniques. The study relied upon anthropogenic radionuclides, and sediment were analysed for ^{137}Cs , ^{241}Am and ^{210}Pb using gamma assay. However due to a range of possible factors including sample preparation and atmospheric noise the results were inconclusive. Understanding long term sedimentation rates is fundamental for effective management of seagrass meadows which show large variation in its influence on carbon storage.

6.1 Introduction

Coastal habitats, including saltmarshes, seagrass meadows and mudflats, are sites where terrestrial and marine environments meet and transfer materials. Therefore, these marginal habitats lie within zones where steep gradients in oxygen and salinity, along with physical mixing driven by tides, mediate biogeochemical interactions of organic carbon (OC) cycling. Cohesive 'muddy' sediments typical of coastal habitats play a pivotal role in coastal dynamics. Cohesive sediments are a mixture of sediment particles (<2 mm in diameter), organic matter (OM) and water. They contain a high proportion of fine sediments, which have a particles size of <63 µm in diameter. The clay minerals within cohesive sediments cause aggregation of particles which in turn enhances sedimentation (Berlamont et al., 1993).

The supply and deposition of marine and terrestrial sediment and particulate organic matter (POM) in coastal margin environments are controlled largely by atmospheric and hydrodynamic forces (Kirby, 2000). Therefore sedimentation rates vary seasonally, with higher rates of sedimentation typically occurring in spring and summer seasons, whilst in the winter erosion is often heightened due to an increase in the frequency and magnitude of storm events (Bramato et al., 2012). Across Scotland, many intertidal mudflats host *Zostera noltii* and an intertidal variation of *Zostera marina* seagrass meadows. Contrary to the term 'mudflat' implying a planar expanse where freshwater meets marine mud, the geomorphological profiles of intertidal mudflats are often complex. This is in part due to cross-shore transportation caused by either wind generated waves, which tend to leave concave upward profiles, or tidal currents which cause mudflats to accrete over time, making these coastlines optimal habitats for seagrass meadows to establish and thrive (Kirby, 2000). Seagrass meadows utilise dynamic mudflats across Scotland, creating unique microhabitats. In undulating mudflats, *Z.noltii* meadows typically establish on raised areas (indeed they help to create these mounds). In contrast intertidal *Zostera marina* meadows are typically found in shallow depressions which provide the species with full or partial submersion in water even during low tide.

The presence of seagrasses (and other flora and fauna, including microphytobenthos and mussel beds) can generate unevenness in mudflats by creating hotspots for sediment accretion where seagrass is present. The aboveground biomass of seagrasses, regardless of size, modify water flow above and within their canopy. The vegetation canopy reduces current and wave energy, which in turn results in fall out of suspended particulates from the water column (Fonseca and Cahalan, 1992; Koch et al., 2006; Potouroglou et al., 2017; Reidenbach and Thomas, 2018). The fall out of particulate material is important for both OM accumulation and, in the case of subtidal meadows, the high light requirement of seagrass meadows for growth (Boer, 2007; Carr et al., 2016).

6.1.1 Sediment stability/erodibility

Sediments are continually subjected to physical stresses that can cause resuspension of particles. Factors that control intertidal sediment stability are the physical, chemical and biological properties of the surface sediments, the effects of wave energy and tidal amplitude and exposure to climatic conditions (Andersen, 2001; Grabowski et al., 2010; Paterson and Black, 1999; Widdows and Brinsley, 2002). Shear strength is a measure of the sediment's resistance to shear stress which can help describe and predict surface erosion, internal material fractures and deformation as well as complete failure of material stability. Understanding the erosion threshold of the sedimentary material associated with seagrass meadows could help in understanding how to conserve and manage this habitat, for example by identifying threshold sizes below which habitat is no longer stable.

The most common method used to measure the erodibility of fine sediments is laboratory-based flumes. This approach simulates simplified natural flow conditions found in the environment, in a controlled setting using flowing water to apply horizontal shear forces, often at incrementally increasing flow velocity to show the different modes of erosion occurring (Monteith and Pender, 2005). However, the sediment will be subject to disturbance of both its chemical and physical properties when removed from its natural environment. In-situ methods provide instant measurements, reducing the artefactual errors associated with the extraction,

transportation and storage of material required for flume studies. The two main in-situ techniques adopted to determine the shear strength of cohesive sediment are the application of a shear vane, measuring internal shear strength and the cohesive strength meter (CSM), measuring surficial sediment stability (Grabowski, 2014; Hauton and Paterson, 2003; Tolhurst et al., 1999). The shear vane and CSM when combined provide a comprehensive assessment of sediment erodibility in the field.

6.1.2 Grain size influence and bulk density

The main sediment features that influence the erosion threshold of surficial sediments are grain size distribution and bulk density (Avnimelech et al., 2001). The two factors are closely related with sediments containing a higher clay content decreasing in bulk density. Seagrass meadows are often celebrated for their ability to trap sediment particles because their above ground biomass reduces water velocity, causing predominantly fine grain sized particles to fall out of the current and settle within sediment substrate. Sites that have a naturally slow water velocity and a high distribution of leaf biomass provide optimal conditions to increase deposition of finer grain sized particles within a meadow (van Keulen and Borowitzka, 2002). Although the *Zostera* genus is not renowned for having a vast above ground biomass, unlike *Posidonia* and other tropical seagrass species, because of its blade like leaves, when present in dense patches or meadows the genus has still been found to enhance sediment deposition (Potouroglou et al., 2017; Reidenbach and Thomas, 2018).

The effects of physical properties are well documented for a wide variety of seagrass meadows worldwide. In general, research suggests there is a strong negative relationship between grain size in sediments and carbon density, where the lower the median grain size present the higher the carbon density (Dahl et al., 2016; Macreadie et al., 2015; Miyajimar et al., 2017; Röhr et al., 2016). A study in twenty Baltic Sea meadows across Finland and Denmark showed more than 40% of the variation was explained by sediment characteristics, including silt content (Röhr et al., 2016). However, the potential impacts of physical properties on carbon stocks in UK seagrass meadows are far less understood. To date, there has been only one

published study investigating this relationship, which concluded that sediment grain size was not an influencing factor for explaining the variation in carbon stocks of subtidal *Z.marina* meadows on the South coast of England (Green et al., 2018).

6.1.3 Quantifying sedimentation rates

The general understanding of carbon storage within seagrass meadows has increased rapidly in the last decade, with research predominately focused on quantifying carbon stocks (Duarte et al., 2013; McLeod et al., 2011; Nellemann et al., 2009). However, knowledge of carbon stocks alone is insufficient for a proper understanding of carbon dynamics and an applied application of this knowledge through inclusion of seagrass in current carbon mitigation strategies; to achieve this carbon sequestration rates must be studied (Macreadie et al., 2017). Determining sedimentation rates coupled with meadow-specific information including seagrass density and seasonal prevalence and species could provide essential information on both carbon sequestration rates as well as the continued carbon sink capacity of a meadow. Decision makers need to know, with accuracy, if the habitat is a long-term carbon sink or only a temporary site of carbon capture.

There are several approaches used to derive sedimentation rates; the most common and inexpensive methods are in-situ long term measurements of sedimentation rates. These methods vary but typically use spatial grids across seagrass patches to measure sediment surface heights over time or creating traps and measuring sedimentation between tides (Bos et al., 2007; Potouroglou et al., 2017; Reed et al., 1999). Whilst low costs are associated with in-situ field measurements they rely on frequent recordings across a minimum of one year. With this, if experiments have short durations the results can be adversely affected by weather, for these experiments to be robust they require measurements across multiple seasons and years. The most common laboratory-based technique adopted to determine sedimentation rates is the use of naturally occurring radionuclide tracers such as radiocarbon (^{14}C) and lead-210 (^{210}Pb). The measurement of ^{14}C detection by AMS is a method accurate to atom level (Lougheed et al., 2017). ^{210}Pb deposited in aquatic systems is rapidly transported to the underlying sediment where decay occurs post

deposition, giving a depth distribution which, in an accumulating deposit with suitable conditions, allows determination of chronologies using the vertical distribution of the radionuclide tracer. ^{210}Pb , which is a decay product of the Uranium-238 (^{238}U) series (Figure 6-1) is often used to date faster accumulating sediments, most successfully, in sheltered, depositional environments such as lakes/lochs which are not subject to intense physical or biological mixing as the method relies upon accumulation of uniform concentrations of initial and background ^{210}Pb within the sediment profile and/or a constant rate of supply of ^{210}Pb and no post depositional mobility or mixing (Arias-Ortiz et al., 2018). In more dynamic and variable sedimentary environments, such as intertidal mudflats which are home to seagrass meadows, ^{210}Pb is frequently coupled with other anthropogenic radionuclide tracers which act as independent chronological markers to reconstruct temporal accumulation rates where deposition of ^{210}Pb is inconsistent or too low to be accurately measured (Assinder et al., 1993; Harvey et al., 2007; Haslett et al., 2003). Anthropogenic radionuclides, accidentally or deliberately released into the environment can, under suitable circumstances, be used to establish chronologies. Observed peaks from periods of known maximum release can give benchmark dates in profiles which allow accurate dating of accumulating systems. Anthropogenic radionuclides are commonly preserved in sediment sinks, providing records of temporal variations in low-solubility contaminant concentrations within deposited material (Harvey et al., 2007).

| Nuclide | Half-life |
|--|-----------------------------|
| ^{238}U | 4.4683±0.0048 billion years |
| ^{234}Th | 24.1 days |
| ^{234}Pa | 6.69 hours |
| ^{234}U | 245,250±490 years |
| ^{230}Th | 75,690±230 years |
| ^{226}Ra | 1,599±4 years |
| ^{222}Rn | 3.823±0.004 days |
| ^{222}Rn decays to short-lived daughters: | |
| ^{218}Po (3.04 minutes) > ^{214}Pb (26.9 minutes) | |
| ^{214}Bi | 19.7 minutes |
| ^{214}Po | 0.164 mseconds |
| The decay products following ^{214}Po are long-lived radionuclides such as: | |
| ^{210}Pb | 22.6±0.1 years |
| ^{210}Bi | 5.01 days |
| ^{210}Po | 138.4±0.1 days |
| > ^{206}Pb | Stable |

Figure 6-1 Uranium-238 decay chain, including half-lives for series nuclides, with decay modes. '>' represents a jump in the decay chain (adopted from Bourdon et al. (2003)).

6.1.4 Study aim and objectives

This chapter aims to ascertain if there are differences in sediment physical properties between seagrass meadows and adjacent bare mudflats habitats. If differences are found these may help infer which sites are more at risk to erosion and substrate losses which could affect seagrass prevalence as well as the potential impacts this could have on carbon storage. Further to this, the chapter explores sedimentation rates, comparing the differences between seagrass and mudflat areas at one site; rates of sediment accretion are likely to correspond with carbon accumulation in sedimentary systems.

The aim was addressed through the following objectives:

- 1) To conduct a comprehensive assessment of sediment erodibility between seagrass meadows and bare mudflat sites.
- 2) To identify if there are any differences between distribution of finer grain sized particles across locations and habitat types.
- 3) To establish down profile radionuclide records to address differences in sedimentation rates between habitat types and infer, if possible, accumulation rates.

The following hypotheses were formulated and explored to address these objectives:

H_{0a} = Erodibility of sediment substrate forming seagrass patches (susceptibility to erosion) does not differ between seagrass meadows and adjacent bare mudflat habitat patches within a site.

H_{0b} = Erodibility of sediment substrate forming seagrass patches (susceptibility to erosion) does not differ between seagrass meadows across different sites.

H_{0c} = Grain size distribution of sediments does not alter carbon density in seagrass meadows.

H_{0d} = Particle size distribution and dry bulk density of surface sediments do not differ between sediment substrate associated with seagrass beds and mudflat patches.

H_{0e} = The proportion of fine grain size particles within sediments do not explain the differential ability of seagrass to trap carbon at different Scottish sites

H_{0f} = Sediment accumulation rates do not differ between sediments associated with seagrass beds and adjacent mudflat patches.

6.2 Methods

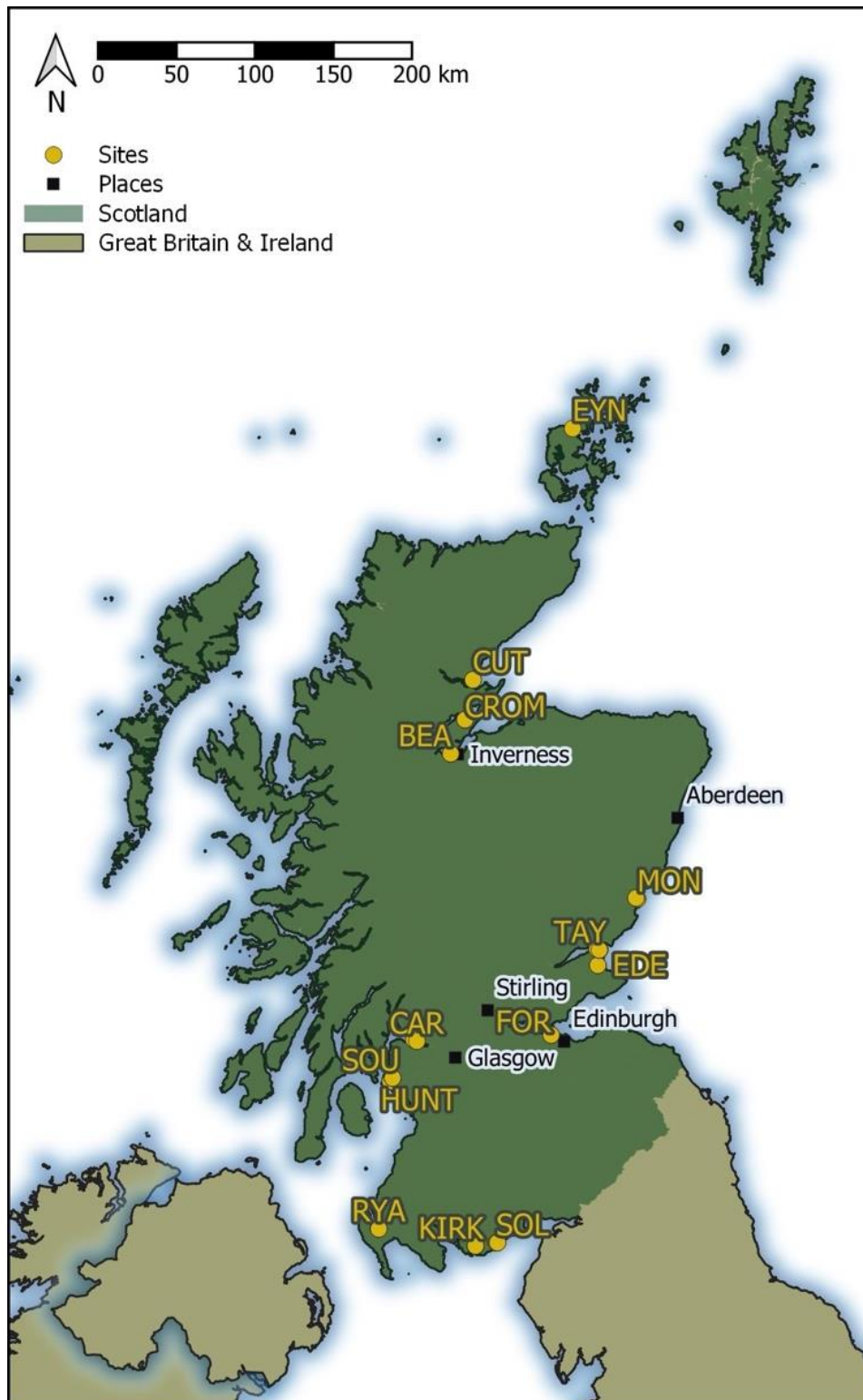
6.2.1 Study area

The in-situ stability study was conducted across six intertidal seagrass meadows (Figure 6-2). Three of the sites are situated on the east coast of Scotland: Firth of Forth (FOR), Eden Estuary (EDE) and Firth of Tay (TAY); three on the west coast of Scotland: Firth of Clyde (CAR), Loch Ryan (RYA) and Solway Firth (SOL). The particle size analysis included the six main sites mentioned above, as well as: the Dornoch Firth (CUT), Cromarty Firth (CROM), Beaulay Firth (BEA), Montrose Basin (MON), Southannan Sands (SOU), Hunterston Sands (HUNT), Kirkcudbright (KIRK) and Eynhallow, Orkney (EYN). Details on how plots were chosen can be found in Chapter 3 (section 3.2.2).

6.2.2 In-situ sediment stability techniques

6.2.2.1 Cohesive strength meter

The CSM is a portable jet test to measure the in-situ relative stability of surficial sediments (Figure 6-3A and B). The device monitors how resistant the sediment is to erosion based on the resuspension of sediment. The CSM system test chamber is inserted into the sediment and filled with water. A downward nozzle jet system pulses water at incrementally increasing jet pressures onto the sediment surface over time (tested in one second intervals). A transmissometer sensor is located 1 cm above the sediment surface within the CSM test chamber and once there is a reduction in the infrared light across the test chamber (>10%) this is an indication that the sediment bed has been eroded. The CSM records internal pressure (P_i) then using the calibration equation for the CSM stagnation pressure (P_{stag}) can be calculated (Figure 6-4; Vardy et al., 2007). The same CSM was calibrated before each experimental period, as the study was completed across two years, 2018 and 2019. The tests were conducted over the same tide point across all sites but on different days. All tests were conducted using CSM testing programme "Fine 1". The CSM was used on both seagrass and mudflat areas at EDE, TAY, FOR and RYA, operational errors with the CSM resulted in SOL and CAR not being included in the study. All seagrass habitats had a paired adjacent mudflat habitats to make up one plot.



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Places: The data were sourced from mapcruzin.com, and have been created from OpenStreetMap data and are licensed under the Open Database 1.0 License. See www.openstreetmap.org for details about the project. This file contains OpenStreetMap data as of 2014-09-03T20:22:02Z courtesy of <http://download.geofabrik.de>

Scotland: Source OS Open Data. Contains OS data © Crown copyright and database right (2021).

Additional Boundaries: These data were extracted from the GADM database (www.gadm.org), version 2.5, July 2015. They can be used for non-commercial purposes only. It is not allowed to redistribute these data, or use them for commercial purposes, without prior consent.

Figure 6-2 Map of sample sites.

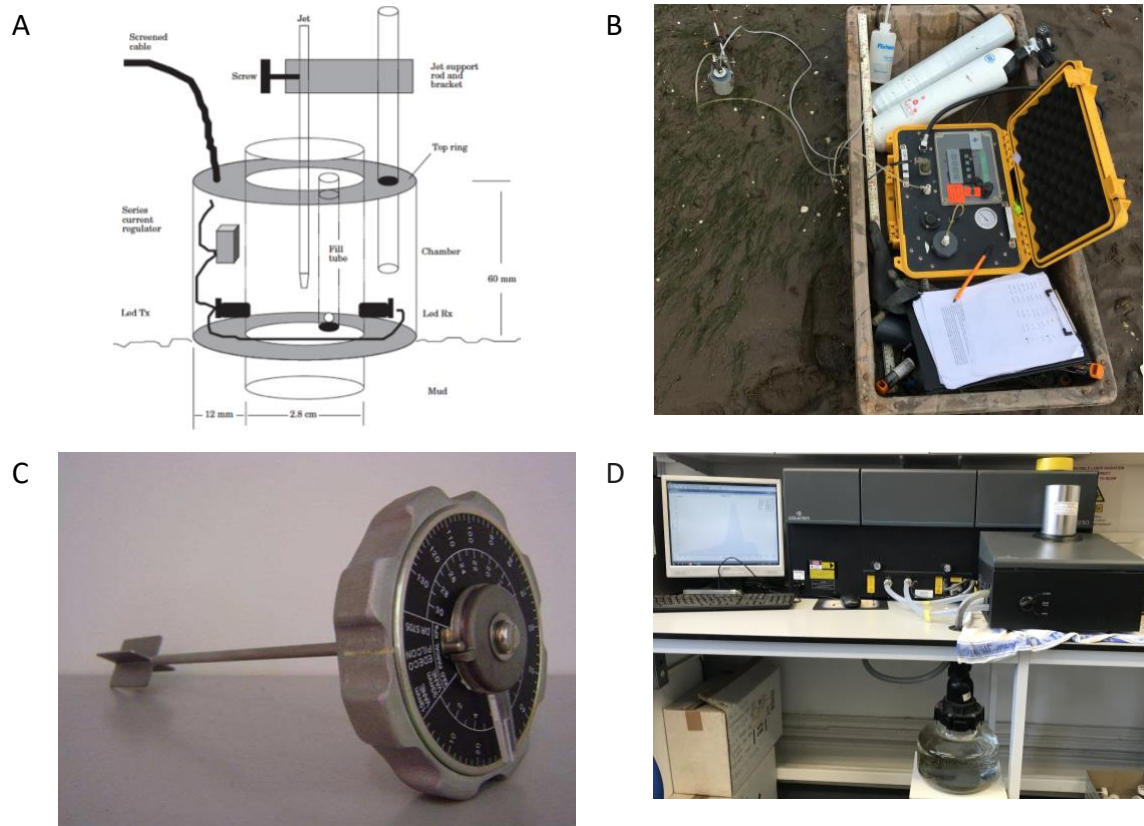


Figure 6-3 A) Diagram of the CSM test chamber (Tolhurst et al., 1999) B) CSM in operation in the field C) Shear vane D) Beckman Coulter LS230, software version 3.39

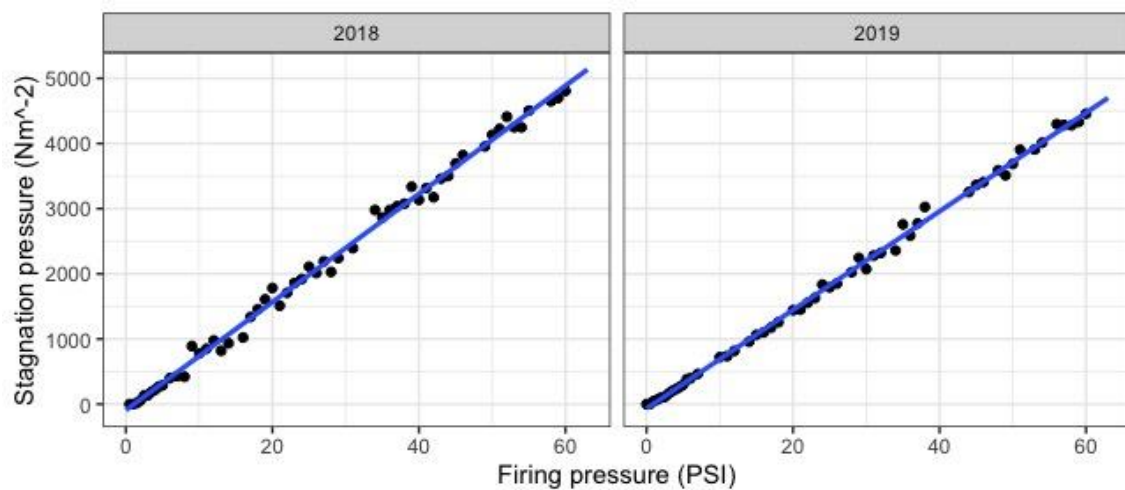


Figure 6-4 CSM calibration plot of stagnation pressure against firing pressure for in-situ experiments conducted in 2018 ($y = 80.612x$, $R^2 = 0.994$) and 2019 ($y = 73.956x$, $R^2 = 0.9989$)

6.2.2.2 Shear vane

The Pilcon hand vane instrument is used for in-situ surveys to estimate the undrained shear strength of fully saturated clays. In this study a 33 mm diameter shear vane blade was used because it is most suitable for cohesive sediment

substrates (Figure 6-3C). The vane blade was pushed into the sediment to a depth of 45 mm and then the vane tester was rotated clockwise at a slow constant rate (1 rotation min^{-1}) until the sediment failed/sheared. The reading on the torque head was then recorded as the shear strength (kilopascal; kPa) of the sediment before being converted to Newton/square meter (Nm^{-2}). The shear vane was used on both seagrass and adjacent bare mudflat plots at all main sites, except for CAR where the method was unsuitable because the substrate was rocky and too shallow in places. Similar, to the CSM tests, seagrass meadows had a paired adjacent mudflat habitat to make up one plot.

6.2.3 Sediment sampling

In total, fourteen sampling locations were selected from both the east and west coast, thirteen sites were intertidal and one subtidal location Eynhallow, Orkney (EYN). Intertidal sediment samples 50 cm in depth were collected at low tide either using a Russian peat corer or a Polyvinyl chloride (PVC) corer (internal diameter 53 mm). At each site one seagrass and one bare adjacent mudflat core were taken. Samples collected via Russian peat corer were transferred on site to a half pipe and wrapped in clingfilm to reduce disturbance whilst samples collected with the PVC corer were sealed and wrapped on site, transported upright and stored in a wet laboratory until sample preparation. The cores were then sliced into 1 cm increments and dried at 60°C before gently being ground with a pestle and mortar prior to analysis.

6.2.3.1 Particle size analysis and dry bulk density

Particle size analysis (PSA) was conducted on sediment <2 mm in size on a Beckman Coulter LS230, software version 3.39 (Figure 6-3). Each sample was first sieved to remove any large or unnecessary particles, such as rocks, shells or seagrass material, and then gently ground with a pestle and mortar to break up any agglomeration. A riffle box was used to ensure sediment was well homogenised and representative of the whole sample. Representative samples were adequately dispersed prior to analysis to ensure consistent wetting and prevent any further agglomeration. The dispersant used was Calgon, which was prepared by adding 35 g of sodium

hexametaphosphate and 7 g of sodium carbonate to 1 l of distilled water and stored in an air-tight labelled container. Calgon (6 ml) and distilled water (25 ml) were added to each sample. Dispersion was also improved by sonicating the samples in an ultrasonic bath for three minutes, prior to using a magnetic stirrer to continually mix the sample as it was pipetted into the Coulter machine. Triplicate readings for each sample were taken and automatically stored in sample files. The dry bulk density (DBD) method is explained in chapter 3. The sediment size scale adopted from Gradistat program version 8 (Blott and Pye, 2001), where sand size particles <2 mm-63 µm, silt sized particles <63-2 µm and clay sized particles are <2 µm. Across all sediments clay sized particles were of the smallest proportion and silt and clay particles are defined as fine grain sized particles throughout. The sites are grouped into coastal locations for statistical analysis.

Table 6-1 Sample summary of total number of individual cores analysed for particle size analysis component of study.

| Coastal location | Site | Seagrass cores | Mudflat cores |
|------------------|------|----------------|---------------|
| EAST | EDE | 1 | 1 |
| | FOR | 2 | 2 |
| | TAY | 2 | 2 |
| | MON | 1 | 1 |
| CLYDE | CAR | 2 | 2 |
| | SOU | 1 | 1 |
| SOLWAY | KIRK | 1 | 1 |
| | SOL | 3 | 3 |
| | RYA | 1 | 1 |
| DORN | BEA | 2 | 2 |
| | CROM | 1 | 1 |
| | CUT | 2 | 1 |
| NORTH | EYN | 2 | 2 |

6.2.3.2 Radionuclide dating

In the intertidal zone, the sediments are often subject to intense physical and biological mixing, giving rise to generally deep mixed layers with major effects on ²¹⁰Pb distribution. Combining ²¹⁰Pb (Goldberg et al., 1978) with other independent anthropogenic radionuclide markers, such as ¹³⁷Cs and ²⁴¹Am discharged from Sellafield nuclear power station into the Irish Sea, may provide information with regard to the mixing patterns for recent intertidal sediments, allowing a useful chronology to be determined. Sediment core samples from seagrass and adjacent

bare mudflat areas at Auchencairn Bay (SOL) were therefore used for this assessment because the meadow's proximity to Sellafield provided optimal conditions to utilise the tracing potential of discharged ^{137}Cs and ^{241}Am to understand the physical and biological processes underpinning sediment deposition and these two contrasting areas. Hence it was anticipated that these radionuclides could be used to infer sedimentation rates in the current work.

Subsamples (10 g) of sieved, lightly ground sediment were weighed loose and sealed in petri dishes to provide a reproducible geometry. The sealed samples were then stored for a minimum of three weeks before counting to ensure radioactive equilibrium between ^{222}Rn and ^{226}Ra . After reaching equilibrium samples were placed in a reproducible position on the end window of the detector. The samples were analysed for ^{210}Pb and/or ^{137}Cs and ^{241}Am by gamma spectroscopy using either a low background Ortec Profile series High-Purity Germanium (HPGe) Coaxial photon detector (GEM-S-XLB-C), an EG&G Ortec LO-AX n-type HPGe gamma photon detector or an Ortec GMX series HPGe Coaxial photon detector (GAMMA-X). The detector was housed in a 10 cm Pb shield with a Cd-Cu lining to reduce x-ray background and was supported on 10 cm of Pb to further reduce background. Background spectra were recorded before and after analysis of each core to correct for any background contribution of ^{210}Pb , ^{214}Bi and ^{214}Pb . No background peaks occur at the detection energies of either ^{137}Cs or ^{241}Am . Analysis times of up to 167 hours were used to obtain counting statistics uncertainties of less than 10%.

Detection efficiencies were established using standards prepared by adding known activities of the radionuclides of interest. Dilutions of certified standard solutions of ^{210}Pb , ^{214}Pb , ^{214}Bi , ^{137}Cs and ^{241}Am (Amersham and Harwell standard solution sources) were added to saltmarsh sediment of an age that no unsupported ^{210}Pb or anthropogenic radionuclides were present. The accuracy and validity of the method was assessed by using a Certified Reference Material.

Due to unresolvable technical problems with the dating approach the discussion for this component of work is in a separate appendix (Radionuclide dating discussion

(Appendix 6).

6.2.4 Statistical analysis

All analyses were conducted in R Studio. All figures were produced using the package ggplot2 (Wickham, 2016). All data was checked for normality and a mixed model ANOVA (lmer, from the lme4 package; Bates et al., 2015) was used to analyse the similarities between coastal locations/sites and habitat types, as well as the interactions between these factors. Where there were also run through a post-hoc comparison of means via the glht function in the multcomp package (Hothorn et al., 2008).

6.3 Results

6.3.1 In-situ techniques

Overall, 26 plots across four sites were tested for CSM-derived erodibility (Table 6-2).

Four tests failed, all at RYA within the mudflat habitat. The tests were not repeated due to time constraints and limited suitable sites, as the site contains large continuous seagrass meadow with few mudflat areas larger than 1m² that were not submerged in water for the total duration of the low tide. Nonetheless these four areas are representative of the site with mudflat areas having highly loose surface sediments. Of the remaining 22 plots, only four had higher critical erosion threshold (CET) values for the mudflat habitat. Seagrass meadows had higher CET values for half of the plots tested with the remaining plots sharing the same CET values across habitats.

Table 6-2 In-situ paired plot of critical erosion thresholds derived from CSM testing across sites.

| Site | Plot | Critical erosion threshold (Nm ⁻²) | | Within plot differences |
|------|------|--|----------|-------------------------|
| | | Mudflat | Seagrass | |
| FOR | 1 | 32.245 | 48.367 | C < S |
| FOR | 2 | 32.245 | 40.306 | C < S |
| FOR | 3 | 24.184 | 32.245 | C < S |
| FOR | 4 | 40.306 | 32.245 | C > S |
| FOR | 5 | 32.245 | 24.184 | C > S |
| EDE | 1 | 8.061 | 8.061 | C = S |
| EDE | 2 | 8.061 | 8.061 | C = S |
| EDE | 3 | 8.061 | 8.061 | C = S |
| EDE | 4 | 8.061 | 40.306 | C < S |
| EDE | 5 | 8.061 | 16.122 | C < S |
| RYA | 1 | 133.121 | 133.121 | C = S |
| RYA | 2 | 199.681 | 44.374 | C > S |
| RYA | 3 | FAILED | 14.791 | - |
| RYA | 4 | FAILED | 7.396 | - |
| RYA | 5 | 7.396 | 14.791 | C < S |
| RYA | 6 | 7.396 | 14.791 | C < S |
| RYA | 7 | FAILED | 7.396 | - |
| RYA | 8 | FAILED | 7.396 | - |
| TAY | 1 | 56.428 | 56.428 | C = S |
| TAY | 2 | 40.306 | 40.306 | C = S |
| TAY | 3 | 40.306 | 72.551 | C < S |
| TAY | 4 | 22.187 | 14.791 | C > S |
| TAY | 5 | 7.396 | 14.791 | C < S |
| TAY | 6 | 7.396 | 22.187 | C < S |
| TAY | 7 | 14.791 | 29.582 | C < S |
| TAY | 8 | 7.396 | 7.396 | C = S |

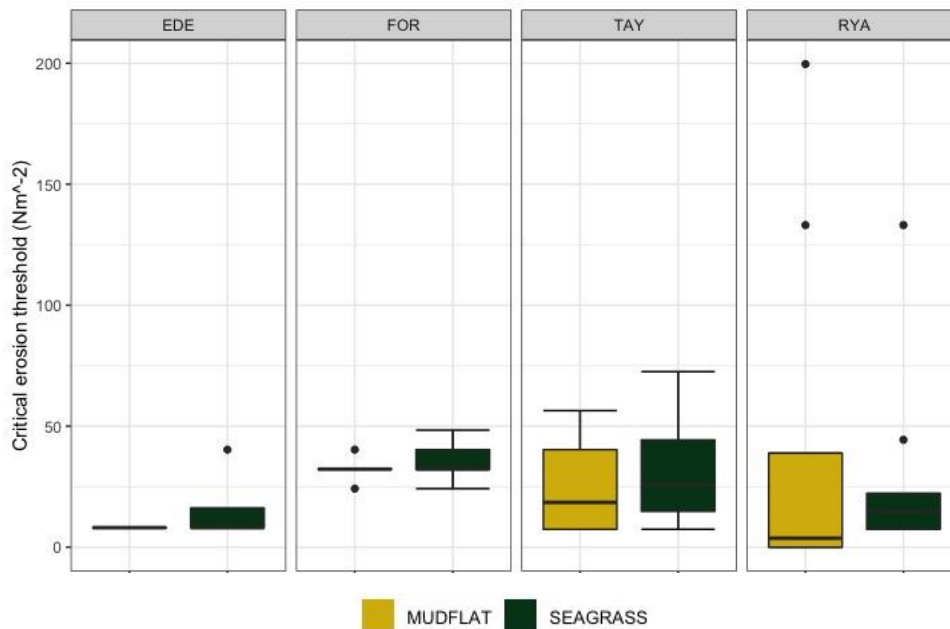


Figure 6-5 Boxplot of critical erosion thresholds for each site and habitat type. The boxplot indicates the median, first and third quartiles, minimum and maximum spread of data for each site and plot type.

There was no significant interaction term between sites and habitat ($df = 3$, $F = 2.6$ $p = 0.066$). Despite the within-plot differences between habitats (Table 6-2), no statistical difference was found between habitat types ($df = 1$, $F = 0.9$ $p = 0.345$). However, there was a significant difference in CET values across the four sites ($df = 3$, $F = 4.8$ $p = 0.006$). Figure 6-5 identifies many of the CET values overlap across sites. EDE and FOR, mudflat habitats were recorded with a smaller range of CET's than seagrass meadows at the same site. Further to this, at EDE, FOR and TAY seagrass maximum CET value range was higher than the adjacent mudflat areas. Whereas RYA was dissimilar from all other sites, with a large range in CET values occurring across mudflat areas, larger than any other site or habitat.

Table 6-3 Statistical model summary for critical erosion threshold of *Z.noltii* meadows and adjacent mudflat areas. Nested ANOVA model used where `csmmodel = lmer(CET~SITE+HABITAT TYPE+SITE*HABITAT TYPE+(1|PLOT), data = CSM_R, REML=TRUE)`.

| | Sum Sq | Mean Sq | NumDF | DenDF | F value | Pr(>F) |
|---------------------|---------|---------|-------|--------|---------|--------|
| Site | 13291.8 | 4430.6 | 3 | 35.319 | 4.82 | 0.006 |
| Habitat type | 837.1 | 837.1 | 1 | 33.571 | 0.91 | 0.347 |
| Site * Habitat type | 7247.4 | 2415.8 | 3 | 33.538 | 2.63 | 0.066 |

Table 6-4 Paired summary of habitat types within the same plot. Values represent Shear vane strength (kPa)

| | EDE | | FOR | | TAY | | SOL | | RYA | |
|---------------------|---------|----------|---------|----------|---------|----------|---------|----------|---------|----------|
| Plot | Mudflat | Seagrass | Mudflat | Seagrass | Mudflat | Seagrass | Mudflat | Seagrass | Mudflat | Seagrass |
| 1 | 5.50 | 3.50 | 5.75 | 6.75 | 6.75 | 9.50 | 11.75 | 9.50 | 5.25 | 9.00 |
| 2 | 4.25 | 4.75 | 5.25 | 8.75 | 5.50 | 6.50 | 10.00 | 11.00 | 4.50 | 8.50 |
| 3 | 5.25 | 7.00 | 9.00 | 12.25 | 6.75 | 8.25 | 9.75 | 12.25 | 3.75 | 9.00 |
| 4 | 3.75 | 4.50 | 10.25 | 9.50 | 7.50 | 8.25 | 9.50 | 9.50 | 4.75 | 8.75 |
| 5 | 3.50 | 6.75 | 7.75 | 9.00 | 7.50 | 8.00 | 8.50 | 9.75 | 4.00 | 8.25 |
| 6 | 4.00 | 6.00 | 7.25 | 5.00 | 7.00 | 7.00 | 9.50 | 11.50 | 6.00 | 6.00 |
| 7 | 11.50 | 7.50 | 5.50 | 7.00 | 6.50 | 7.50 | 10.50 | 11.00 | 5.25 | 7.25 |
| 8 | 5.75 | 10.25 | 6.00 | 6.00 | 6.00 | 7.00 | 8.75 | 12.50 | 5.00 | 7.25 |
| 9 | 6.00 | 7.50 | 4.00 | 6.25 | 9.50 | 7.00 | 10.25 | 12.00 | 4.25 | 6.75 |
| 10 | 6.00 | 7.00 | 6.00 | 8.00 | 6.35 | | 9.25 | 10.50 | 4.00 | 7.25 |
| 11 | 6.25 | 7.25 | 5.00 | 8.00 | 7.00 | 7.00 | 4.50 | 7.25 | 5.50 | 6.75 |
| 12 | 5.00 | 8.00 | 7.00 | 5.25 | 6.50 | 7.50 | 5.75 | 8.50 | 6.50 | 6.50 |
| 13 | 5.00 | 8.00 | 4.00 | 4.75 | 6.00 | 7.00 | 6.00 | 6.50 | 5.00 | 8.50 |
| 14 | 5.50 | 6.50 | 3.75 | 4.75 | 6.25 | 9.50 | 6.00 | 7.25 | 7.00 | 7.00 |
| 15 | 5.00 | 7.00 | 4.50 | 5.75 | 6.50 | 7.00 | 5.00 | 7.00 | 7.50 | 7.00 |
| 16 | 3.50 | 8.00 | 5.00 | 6.00 | - | 7.50 | 6.50 | 7.75 | 5.50 | 7.00 |
| 17 | 4.00 | 7.00 | 4.50 | 8.00 | | | 7.25 | 7.25 | 7.00 | 7.25 |
| 18 | 4.00 | 6.50 | 5.00 | 7.00 | | | 6.50 | 6.00 | 4.00 | 8.00 |
| 19 | 4.00 | 7.00 | 4.50 | 7.00 | | | 6.00 | 8.25 | 5.00 | 7.50 |
| 20 | 5.00 | 7.00 | 5.00 | 7.50 | | | 7.00 | 9.00 | 5.25 | - |
| 21 | | | | | | | 7.00 | 8.75 | | |
| 22 | | | | | | | 6.00 | 7.00 | | |
| 23 | | | | | | | 5.00 | 9.00 | | |
| 24 | | | | | | | - | 10.00 | | |
| Mean | 5.14 | 6.85 | 5.75 | 7.13 | 6.77 | 7.63 | 7.66 | 9.13 | 5.25 | 7.55 |
| Median | 5.00 | 7.00 | 5.13 | 7.00 | 6.50 | 7.50 | 7.00 | 9.00 | 5.13 | 7.25 |
| Max | 11.50 | 10.25 | 10.25 | 12.25 | 9.50 | 9.50 | 11.75 | 12.50 | 7.50 | 9.00 |
| Min | 3.50 | 3.50 | 3.75 | 4.75 | 5.50 | 6.50 | 4.50 | 6.00 | 3.75 | 6.00 |
| st dev | 1.73 | 1.43 | 1.72 | 1.85 | 0.93 | 0.91 | 2.08 | 1.92 | 1.09 | 0.89 |
| Count | 20 | 20 | 20 | 20 | 15 | 15 | 23 | 24 | 20 | 19 |
| Confidence Interval | 0.76 | 0.63 | 0.75 | 0.81 | 0.47 | 0.46 | 0.85 | 0.77 | 0.48 | 0.4 |

Shear vane-derived strength (SVS) of paired habitats within-plots showed, across all sites, that shear strength was higher within seagrass meadows than adjacent mudflat habitat (Table 6-4). Overall, there was a large range in SVS across sites and habitat types (Figure 6-6). The higher SVS was found at SOL for both habitats and the lowest SVS values being found at EDE regardless of habitat type. Overall, there was no significant interaction term for shear vane-derived erodibility between site and habitat type ($df = 4$, $F = 1.5$, $p = 0.115$). There were significant differences between shear vane-derived strength across sites ($df = 4$, $F = 12.5$, $p < 0.001$) and habitat types ($df = 1$, $F = 39.9$, $p < 0.001$). The post-hoc test identified SOL and EDE as the least similar to all the sites, with sediment substrate being the most and least cohesive, respectively. Conversely, habitats at RYA displayed opposing results depending on

the test. The SVS showed that bulk sediment susceptibility to erosion is increased in mudflat areas whilst the CET results indicated that the surface erodibility of mudflat areas varied across the meadow, substantially more than sediment substrate associated with seagrass meadows.

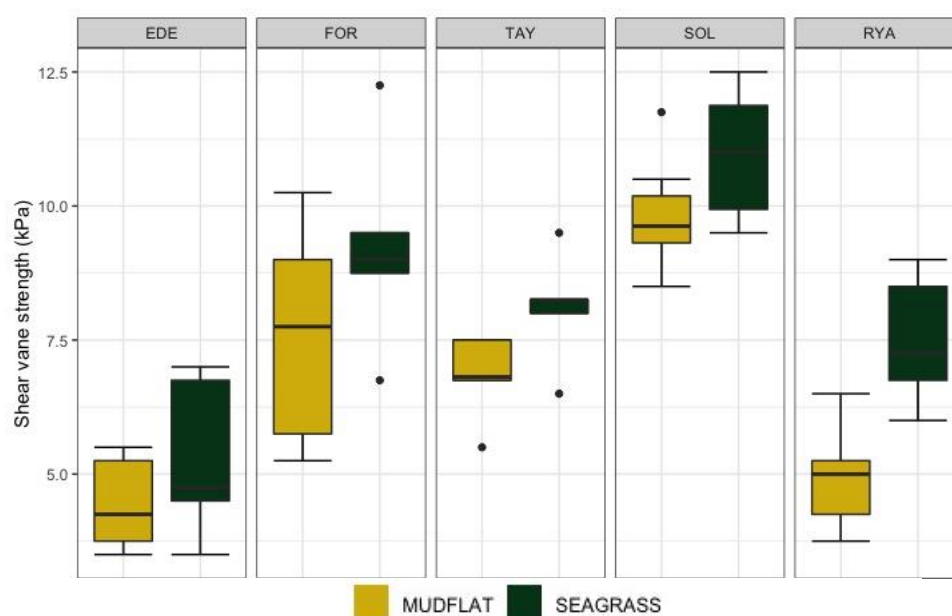


Figure 6-6 Boxplot of shear strength (Nm^{-2}) for each site and plot type. The boxplot indicates the median, first and third quartiles, minimum and maximum spread of data for each site and plot type.

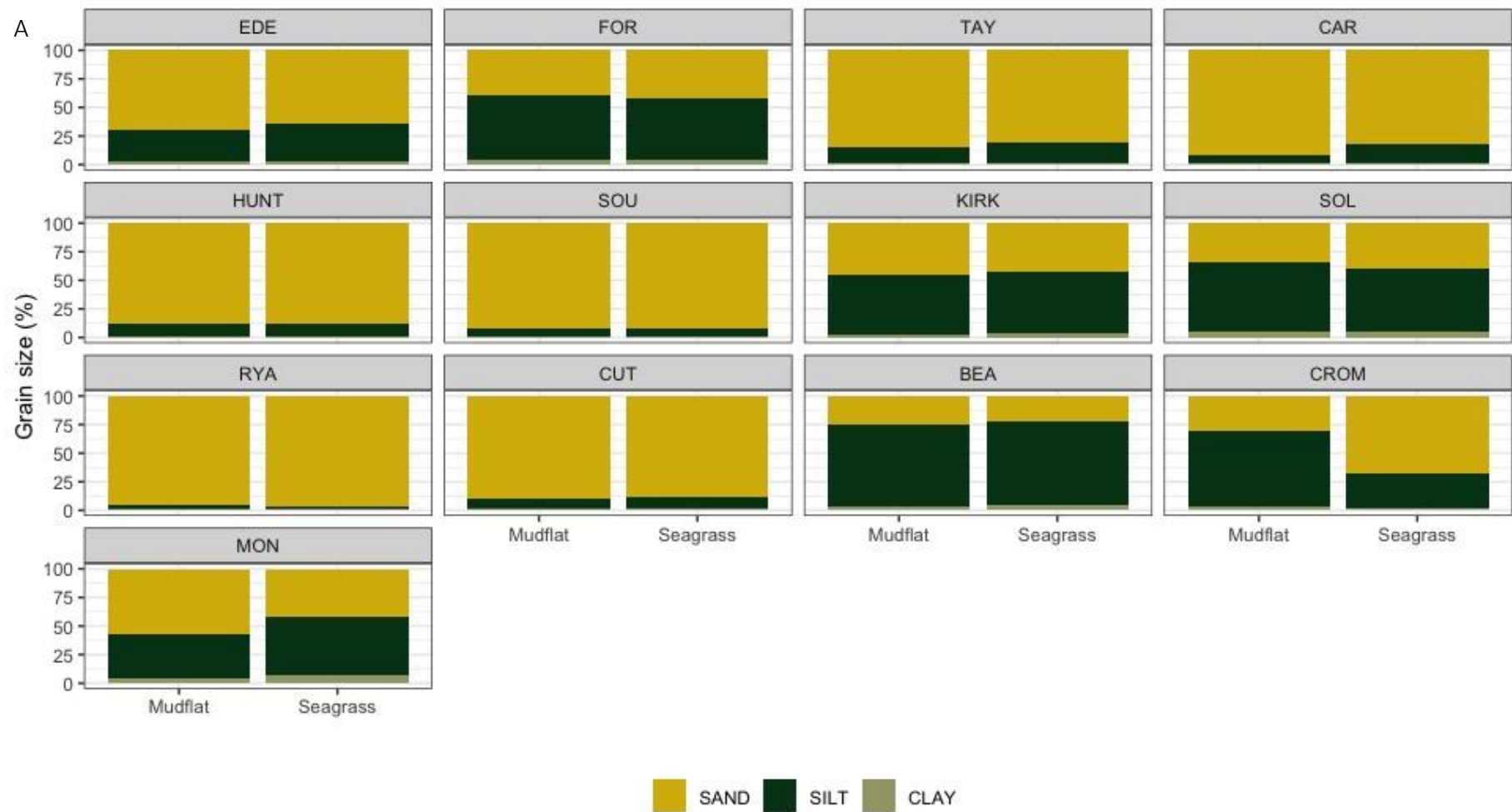
Table 6-5 Statistical model summary for Shear vane strength of *Z.noltii* meadows and adjacent mudflat habitats. Nested Anova model used (`svmodel = lmer(Mm.2 ~ SITE + HABITAT TYPE + SITE * HABITAT TYPE + (1|PLOT), data = SHEARVANE_R, REML = TRUE)`)

| | Sum Sq | Mean Sq | NumDF | DenDF | F value | Pr(>F) |
|---------------------|-----------|-----------|-------|--------|---------|--------|
| Site | 139655427 | 34913857 | 4 | 170 | 12.46 | <0.001 |
| Habitat type | 111757586 | 111757586 | 1 | 164.41 | 39.90 | <0.001 |
| Site * Habitat type | 17015872 | 4253968 | 4 | 164.37 | 1.52 | 0.199 |

6.3.2 Particle size analysis

Across all sites the lowest proportion of particles were clay-sized across all sites. The highest proportion of silt-sized particles were found across FOR, SOL (*Z.noltii* associated areas), BEAU, CROM (mudflat only), MON (seagrass only) and KIRK. EYN associated samples consisted of 94.5-100% of sand-sized particles. Focusing on the finer grain sized particles (silt and clay proportions) across sites and habitats, Figure 6-7 shows large variation regardless of seagrass species associated with the plots. Differences between the proportion of silt sized particles across habitats occurred at a large proportion of sites. The most distinct differences between proportion of fine

grain sized particles occurred at CAR and MON (*Z.noltii*) and SOL and BEA (*Z.marina*) where seagrass plots had the highest concentration and CROM (*Z.noltii*), where mudflat areas had the highest concentration (Figure 6-7).



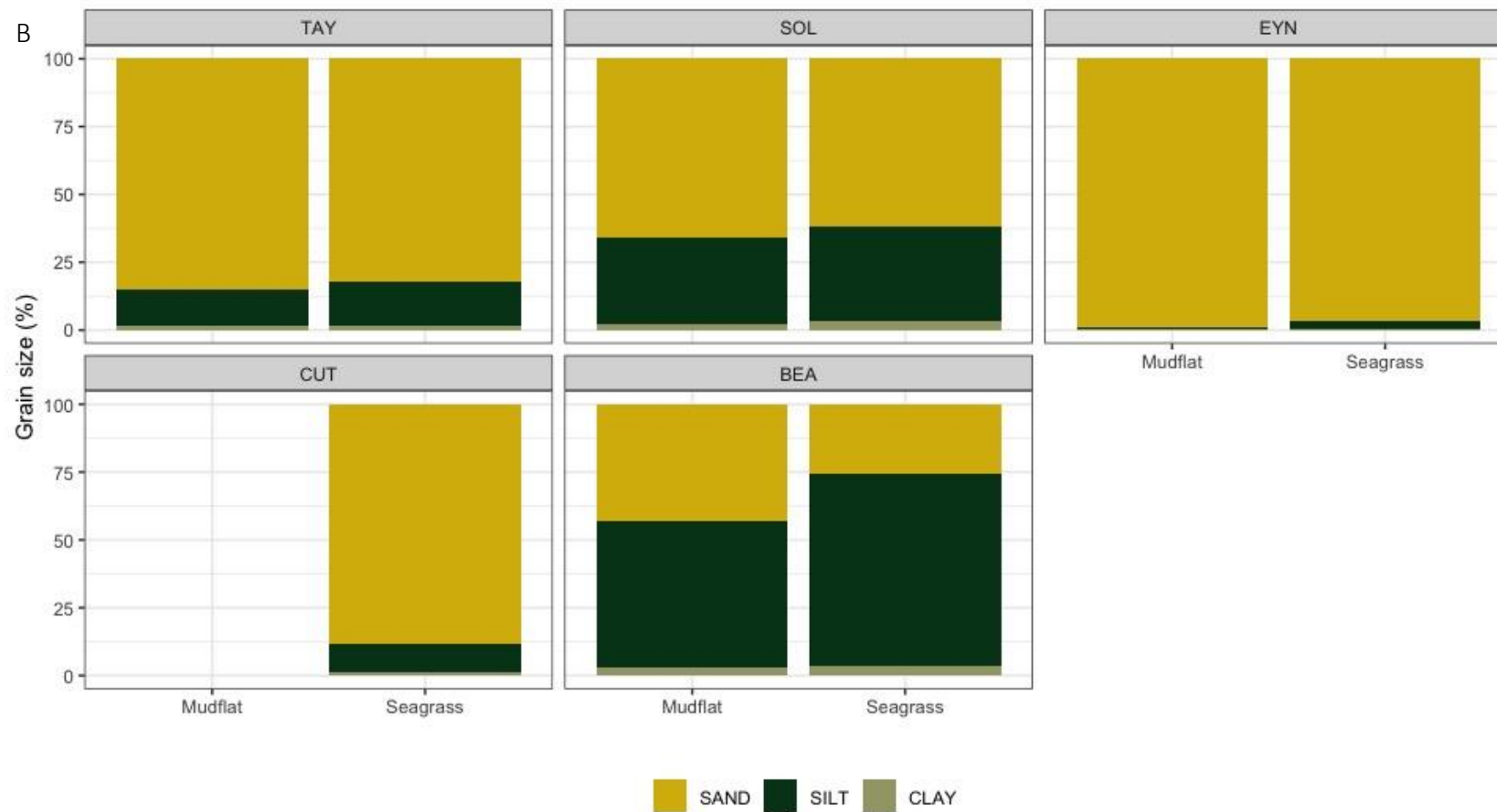


Figure 6-7 Histogram identifying the percentage of mean sand, silt and clay particles found at every site (A) *Z.noltii* meadows and associated mudflat habitats (B) *Z.marina* meadows and associated mudflat habitats.

The dataset was grouped sites by geographical location to increase the statistical power of the analyses. The nested ANOVA determined there was no significant interaction term between coast and habitat type when exploring grain size distribution. (Table 6-6), however there was a significant difference in finer grain sized particles across coastal locations

Table 6-6 Statistical summary for finer grain size distribution within seagrass and mudflat habitats. Nested ANOVA model used (model = lmer(TOTAL_SILTCLAY (%)~COASTAL LOCATION+HABITAT TYPE+COASTAL*HABITAT TYPE+(1|COAST/SITE), data = SILTCLAY_R, REML=TRUE).

| | Sum Sq | Mean Sq | NumDF | DenDF | F value | Pr(>F) |
|-------------------------------|--------|---------|-------|-------|---------|--------|
| Coastal location | 74485 | 18621.3 | 4 | 562.1 | 42.056 | <0.001 |
| Habitat type | 1 | 1.2 | 1 | 2.0 | 0.002 | 0.962 |
| Coastal location*Habitat type | 2061 | 515.3 | 4 | 562.1 | 1.163 | 0.326 |

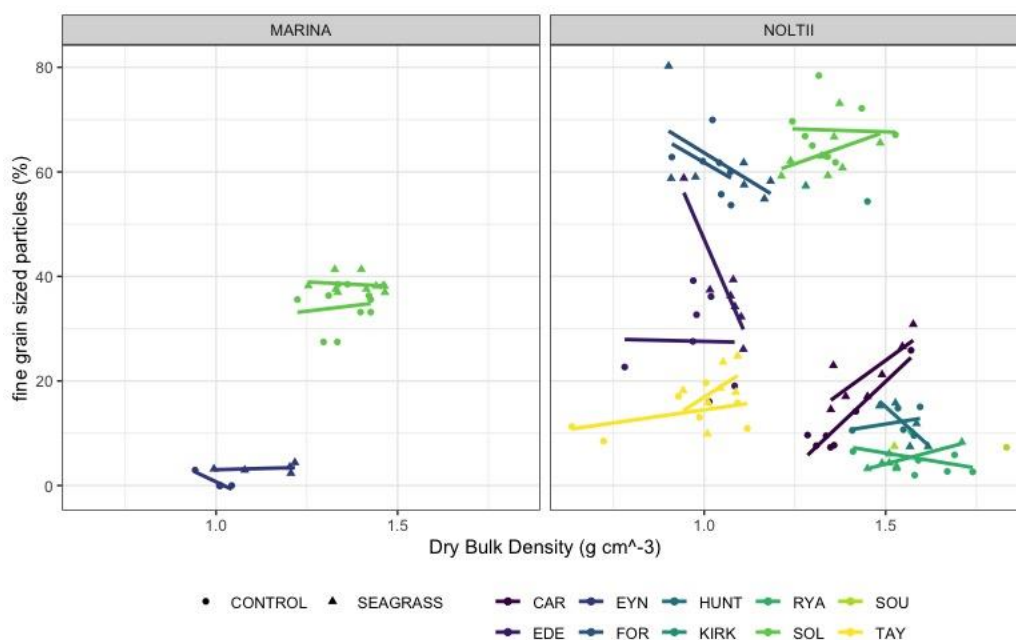
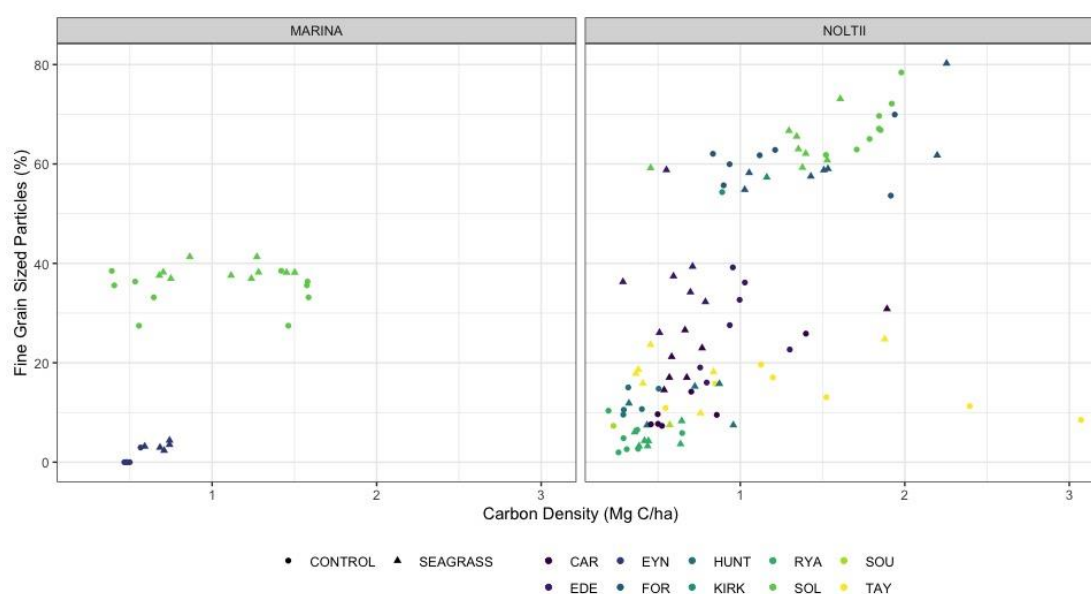


Figure 6-8 Regression relationship between finer grain sized particles and dry bulk density across individual sites.

A weak positive linear relationship could be inferred when looking at the regression relationship (linear) between fine grain sized particles and dry bulk density (seagrass $R^2 = 0.20$ and mudflat $R^2 = 0.06$). However, Figure 6-8 displays all data points are grouped by site and therefore any relationships would be site specific. More sample points are required to make any conclusive statements about this relationship across these sites.

A



B

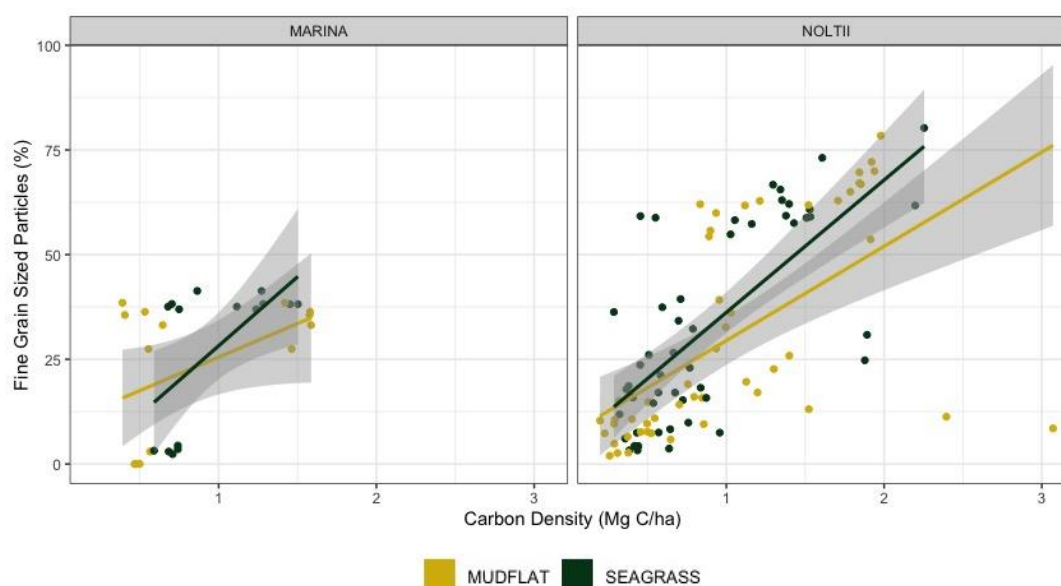


Figure 6-9 (A) Scatterplots where x= carbon density (Mg C/ha) and y = finer grain sized particles for *Z.marina* and *Z.noltii* associated samples, where colour = site and shape = habitat type. (B) Regression relationship between carbon density (Mg C/ha) and fine grain sized particles (<63 μ m). Linear relationships produced included: fine grain sized particles = 0.01 carbon density + 0.51 ($R^2 = 0.23$) for mudflat areas (associated with *Z.marina*); fine grain sized particles = 0.01 carbon density + 0.66 ($R^2 = 0.37$) for seagrass meadows (*Z.marina*); fine grain sized particles = 0.02 carbon density + 0.56 ($R^2 = 0.35$) for mudflat areas (associated with *Z.noltii*); fine grain sized particles = 0.02 carbon density + 0.38 ($R^2 = 0.49$) for seagrass meadows (*Z.noltii*). Samples included in this regression were EDE, FOR, TAY, CAR, HUNT, KIRK, SOL and RYA (*Z.noltii*), SOL and EYN (*Z.marina*).

Exploring the relationship between grain size and carbon density across subsamples, for all datasets, positive relationships were evident, with the total proportion of finer grain sized particles correlating with higher carbon density (Figure 6-9A). However, all R^2 values indicate that the positive relationship between carbon and grain size

only explains, in all instances, less than 50% of the data. *Z.noltii* seagrass areas have the strongest positive relationship with 49% of the data explained by the positive trendline (Figure 6-9B). Exploring this relationship across intertidal meadows only (excluding the EYN dataset) the regression relationship was weaker than with just *Z.noltii* habitats (fine grain sized particles = $0.02 \text{ carbon density} + 0.55$, $R^2 = 0.30$, for mudflat areas and fine grain sized particles = $0.01 \text{ carbon density} + 0.39$, $R^2 = 0.39$, for seagrass areas). Furthermore the relationship presented in Figure 6-9B is a misrepresentation of the actual relationship between the proportion of finer grain sized particles and carbon density. All *Z.marina* associated samples are grouped by site and only two sites were analysed in this dataset (Figure 6-9A). therefore additional sites would need to be studied to draw any conclusions about this relationship in *Z.marina* associated sites.

6.4 Discussion

6.4.1 In-situ erodibility measurements

Overall, in-situ surface erodibility measurements highlighted the strong influence of both habitat type and site on cohesivity and therefore stability of the sediment substrate. Surface CET at both EDE and FOR indicated that the mudflat habitats' erodibility was consistent across multiple plots within the site, whereas there was a larger variation in erodibility within seagrass meadows (Figure 6-5). Exploring within-site differences between habitats showed no significant differences at any site except RYA. Also, it is important to note that all experiments had paired habitats (plot containing seagrass and adjacent mudflat area) and there were differences within plots ($P = 0.055$), even if the differences were not significant. This highlights the importance of seagrass meadows at a small, localised scale, with raw data identifying differences between seagrass and mudflat area CET values within plots across all 4 sites (EDE, FOR, RYA and TAY). For 79% of all sites and plots, seagrass sediments had higher CET values; 14% had the same CET values as mudflat areas and only 7% of comparisons showed higher CET values in mudflats. This indicates greater surface cohesivity in seagrass meadows, even if the susceptibility to erosion across the meadow varied.

Although surface stability tests (CSM) found no general significant influence of habitat type on CET, shear vane strength showed significant differences across habitat types at every site (Figure 6-6) despite there being no significant difference within plots at a microscale. The stabilising effect of roots and rhizomes on the sediment may explain the difference between the CSM and shear vane readings; whilst the first measures only the very shallow, surface sediments, the latter included effects on stability down to 45 mm depth. Consistently, across every site, the highest recorded shear vane strength (kPa) of the sediment substrate was within seagrass areas and the lowest within mudflat areas (Table 6-4). These results clearly demonstrate that *Z.noltii*, similar to other rooted aquatic macrophytes, increase the bulk strength of the sediment and thus has a stabilising effect on the sediment substrate, despite the species relatively shallow root structure when compared to other seagrass species (Duarte et al., 1998).

The in-situ measurements took place during the seagrass growing season (July 2018 and 2019) and consequently they coincided with the optimal conditions for biofilm species colonization in temperate regions including the UK (Cebrián and Valiela, 1999; Valiela, 1995). Biofilm communities are typically found in estuarine mudflat environments and occur through the proliferation of microphytobenthos within surface sediments. Biofilm colonisation, growth and reproduction helps to stabilise sediments (Paterson, 1997). Wilkie et al. (2012) explored sediment particle retention in bare sediment and *Z.noltii* meadows at Tayport, Scotland, over time finding larger sized particles were retained more effectively in seagrass meadows over bare mudflat plots as hypothesised. However the study also found no difference in small particle retention across the two habitat types over the duration of the study (Wilkie et al., 2012). Similarly, this study found no significant differences between habitat CETs. This could be explained by successful establishment of biofilm species across the coastal mudflats, regardless of habitat presence, however this was not measured. Seasonal CET data may have provided a more sound understanding of potential biofilm success, since biofilm reduction is likely to occur during winter because of reduced temperature and shorter day-length which may have effected habitat types differently (Chan et al., 2003; Hillebrand and Sommer, 1997). Despite all the CSM tests being conducted during the seagrass growing season, another possible explanation for the similarities in CET between habitats could be explained by vegetation presence. Bos et al. (2007) noted seasonal differences in both accretion and the removal of sediment within intertidal eelgrasses die back during winter conditions. The study found sediment accretion to be as great as 5-7 mm during the growing season of intertidal *Z.marina*, however after winter most of the previously accreted sediment had been eroded, potentially linked to the lack of vegetation. Further to this bare mudflat areas did not experience the same severity of erosion, if any, over winter (Bos et al., 2007). However, Potouroglou et al. (2017) found the opposite effect at a Scottish seagrass site with seagrass plots retaining sediment even across wintering months and mudflat plots showing strong seasonal pattern variation. Severity of seagrass die-off as well as storm events could cause longer lasting effects on surface sediment stability and therefore seasonal CET data

could help determine if the similarities between habitats was due to seasonal variables or because the entire mudflat shared similar surface cohesivity.

Further analyses including direct investigation of biofilm presence would enhance the scope of this study as well as our understanding of the mechanical stability of sediments (Herman et al., 2001; Irsfeld et al., 2014). The use of a CSM to explore biofilm integrity has only occurred in laboratory conditions with cultured biofilms via the crystal staining method (Irsfeld et al., 2014). As our stability measures were deliberately conducted in-situ to reduce disturbance to sediment substrate the most suitable method to explore biofilm matrix is fluorescence correlation spectroscopy (FCS) to assess the viscosity and porosity of the biofilm (Peulen and Wilkinson, 2011).

6.4.1.1 In-situ measurement considerations

The large range of CET values for mudflat habitats at RYA are most likely to be explained by the sediment dynamics of the loch. The seagrass meadows at EDE, FOR and TAY are very patchy in nature and have a near equal proportion of mudflat to seagrass areas across the site, whereas RYA is a medium-sized dense intertidal bed with a limited number of bare patch areas that were suitable for CSM application. Where bare mudflat areas were present at RYA, they were typically submerged under water due to the undulating nature of the bed. The CSM is unable to perform on the surface of submerged sediment surfaces because of the disturbance caused during preparation to the already saturated and unconsolidated sediment. There was limited suitable sample locations for mudflat habitats as a result of this, and half of the tests failed before water pressure was applied within the CSM chamber, despite areas that were selected not being submerged in water, they were still too saturated for the experiment. These samples were removed from the statistical model.

Further to this, it is important to note that two of the successful mudflat area tests conducted at RYA produced CET values higher than at any other site. There were limited suitable options to conduct tests within the mudflat habitat, which led to two mudflat areas being selected on significantly elevated mounds, resulting in high CET

values. The mounds are likely to be present on the mudflat as historical sand deposits from the loch's active dredging history explaining the anomalies in the results (Figure 6-5). The loch is an important transport hub of both people and goods to and from Ireland. The Stranraer ferry port was active for 149 years which resulted in the loch regularly being dredged until 2011, when the port was moved to a deeper more suitable location in the loch. During low tide, large sediment deposits can be seen across the entire mudflat, *Z.noltii* has established on some of the deposits. Therefore, these sediment deposits do represent the entire mudflat but only a small section of the intertidal seagrass meadow at RYA and should be used with caution.

The differences found between habitats for both shear vane-derived strength and finer grain sized particles could have been explored further to build confidence in the stability of seagrass and thus the continued argument of them as carbon sequestration areas. However, PSA was conducted on the sediment cores used for carbon analysis in Chapter 3. In-situ testing was randomised across the seagrass meadow and not conducted at the same time as the core sampling. Therefore, grain size and shear vane-derived strength cannot be analysed as if they are the same sample. Future hypotheses to explore would be linked to erosion thresholds and the subsequent composure of the sediment substrate, i.e., do higher erosion thresholds occur within the middle of seagrass meadows and are these meadows composed of larger and denser grains that are more resistant to erosion? In addition, exploring relationships between root density and shear vane strength may help explain the contribution of roots (as apart from biofilms or other factors) to sediment stability.

6.4.1.2 Sediment particle size and bulk density

As evident from the in-situ substrate erodibility tests, seagrass does increase the bulk erosion threshold of sediment substrate. This should be closely correlated with grain size and bulk density of surface sediment substrates, because they are both directly and indirectly associated with sediment vulnerability to erosion, resuspension and other mechanical properties (Avnimelech et al., 2001; Jepsen et al., 1997). Jepsen et al. (1997) found that, under flume conditions, erosion rates depended critically on sediment bulk density and decreased rapidly with the

increase in bulk density of the sediment. Chapter 3 found significant differences in DBD across sites, as well as the significant difference between habitat types across full-depth profiles (50 cm). Fine grain size analysis showed differences between coast locations, however no differences between habitat types. The results found for were similar to Wilkie et al. (2012) which found that the deposition and sorting of sediment grains was not influenced by the presence or absence of seagrass (both *Z.noltii* and *Z.marina*) within a Scottish intertidal seagrass bed, but instead was controlled by hydrodynamic conditions within the estuary. The smaller sample size of *Z.marina* meadows may have contributed to the insignificant difference between habitat types. Several studies exploring the influence of *Z.marina* on grain size including Dahl et al. (2016) and Bos et al. (2007), the latter finding significant evidence, from exploring transplanted intertidal *Z.marina* seedlings in a bare intertidal mudflat in the Wadden Sea, that higher silt concentrations were a direct result of seagrass presence rather than grain size being the cause of seagrass establishment. Another

The relationship between surface sediments DBD and particle size explored in this chapter does not indicate a clear relationship between the two factors, despite the expectation that finer grain sized particles will decrease sediment bulk density, regardless of seagrass species associated with the samples (Dahl et al., 2016; Joensuu et al., 2018). The expected decrease in proportion of fine grain sized particles with the increase in sediment bulk density was only seen at EDE and FOR, with seagrass meadows at EDE being significantly different to mudflat habitats, whilst little difference was found between habitats at FOR. Other sites including HUNT, SOL and RYA show clustered values across habitat types with no distinction between habitats. Removing the site, as a factor, the dataset solely exploring habitat types shows strong positive relationships for sediment samples associated with *Z.marina*, but these relationships are misleading because of the site differences and due to the small sample size (EYN and SOL) within the *Z.marina* dataset (Figure 6-8). Further to this, within *Z.noltii* associated sediments no relationship between DBD and the percentage of fine grain sized particles was found. Therefore, there are no clear patterns to suggest seagrass meadows' sedimentary properties follow

expected trends linked to sediment stability within surface sediments. The clustered site data for the surface sediments, whilst unexpected, is similar to that of surficial CET results, where no differences occur between habitat types providing further support that these meadows are more connected and continuous than previously expected, which raises the question: are the mudflats that were selected permanently bare unvegetated areas of the intertidal mudflats or are they simply temporary bare areas, that are part of a dynamic mosaic of seagrass patches. If the latter is true, and the 'bare mudflat' habitat patches have recently supported seagrass growth, then their logical status as a comparator area is undermined. Further analysis to explore this concept would require exploring grain size, bulk density and the surface erosivity across multiple areas within a plot and across seagrass mounds to consider patch size and edge of effects of both habitat types, as well as having data on temporal changes in seagrass distribution.

Sediment properties, particularly bulk density and the proportion of finer grain sized particles within sediments are seen as important indicators of carbon storage (Dahl et al., 2016; Kenworthy and Thayer, 1984). Chapter 3 found weak negative regression relationships between DBD (g cm^3) and OM (%) in *Z.noltii* associated habitats, with higher the DBD values containing a lower proportion of OM (%) within the sediment substrate, the trendline explained 31% of the data in seagrass meadows and 56% of the data in mudflat habitats (Figure 3-7). However, no relationship was found for *Z.marina* associated sediments regardless of habitat type. The linear regression relationships between finer grain sized particles and carbon density were stronger than those between DBD and OM even though in all instances the relationships only explained <50% of the data (Figure 6-9). The trendline showed across that the relationship was strongest in seagrass meadows. Dahl et al. (2016) found a strong association between the higher proportion of fine grain sized particles and OC, and recommended sediment properties such as grain size and bulk density be considered when evaluating management of *Z.marina* generated carbon sinks.

Across the study there is large variation in the influence of seagrass within sites. Therefore, it may be useful to adapt mudflat wide approaches to management rather than species specific or management focusing solely on seagrass meadows. *Z.noltii* meadows vegetation, both biomass and cover are highly variable and change over short periods of time, canopy height of this species is also very low. This study provides a snapshot in time of present seagrass properties and susceptibility to erosion, which misses seasonal variation and the long-term nature of the meadows is not represented. The low canopy level of the continuous patchy seagrass mounds when paired with a shallow water depth could explain the similarities in sediment cohesivity between habitats. Understanding sedimentation rates and therefore the rate of OM accumulation may further help explain the large variation in the influence of seagrass presence on a site. A mudflat scale management approach could be highly effective in the promotion of carbon storage, particularly in areas that are already naturally organically rich due to the sites being underlain by Carboniferous strata (Browne et al., 1999).

6.5 Conclusion

This study demonstrates through in-situ measurement of sediment internal shear strength that seagrass meadows have a strong influence on bulk sediment cohesivity and therefore sediment stability. In-situ surficial erosivity tests showed large within and between site variability, even though the largest proportion of plots indicated CSM-derived strength was greater in seagrass than mudflat habitats, although this was not statistically significant. Proportion of fine grain sized particles within sites varied between habitat types for both seagrass species, although this difference was not statistically significant. Despite this, there was a strong positive influence of the proportion of fine grain sized particles on carbon density and this relationship was strongest for *Z.noltii* meadows.

Seagrass meadows are highly variable environments, yet this research, coupled with evidence from Potouroglou et al. (2017) supports the theory that intertidal seagrass meadows are areas which enhance sedimentation and stability (both surficial and bulk). Therefore, long-term research exploring this relationship across more seagrass meadows and monitoring could help identify the current state of seagrass meadows. Coastal environments are subject to an array of pressures, the loss of stability to a seagrass meadow could have far reaching consequences on both the surrounding environments, coastal biodiversity and ecosystems functions.

Chapter 7 Application of research findings to support the management and protection of seagrass meadows

7.1 Introduction

The research in the preceding chapters has explored carbon in coastal sediments with a particular focus on carbon storage within seagrass meadows. The need to understand blue carbon stocks and fluxes in a wide range of settings, using a relatively easy and low-cost method, was the incentive for the exploration, in chapter two, of the methodological implications of applying loss on ignition (LOI) to determine organic matter (OM) and in turn, organic carbon (OC) stocks within coastal and marine sediments. Understanding carbon within seagrass meadows, and the influence of *Zostera noltii* and *Zostera marina* in enhancing carbon storage over adjacent bare mudflat areas, is critical for management approaches that aim to protect and enhance this habitat for the purpose of carbon storage. As the relationship between seagrass meadows and carbon storage varies greatly, key sedimentary characteristics were investigated to identify if these characteristics explained the differences seen across sites. This chapter aims to summarise the research and identify key issues, as well as highlight the key findings, that are of relevance to policy influencers and policy makers.

7.2 Loss on ignition

The testing of the LOI methodology showed that it is a reliable technique to measure OM from Scottish coastal sediments (Chapter 2). Whilst arguably more advanced and accurate techniques exist, none are as accessible, inexpensive, or rapid. Understanding carbon stocks across a wide range of locations and habitats is fundamental if Scotland, and other countries, intend to realise the potential for blue carbon at a national scale, when individual sites are highly spatially variable. Under such circumstances, of large spatial variability, limited understanding of drivers and policy urgency, the focus should be on 'big picture' sampling, which achieves representativeness and coverage, even if this is at the expense of precision. Chapter 2 demonstrates that LOI conducted at 500°C should be a generally reliable method; combusting most of the OC present without affecting inorganic carbon (IC). However, as demonstrated in the literature review, there is a very wide range of practices in the literature and frequent use of temperatures that may under or over-estimate OC levels. Whilst further testing needs to be conducted to finalise the most appropriate correction factors for samples which contain high proportions of clay and magnesite, that should not delay the immediate application of a standardised method across suitable sediments. This study recommends not to use the method, if either high clay content or high magnesite is present within sediment samples. This will likely represent a small proportion of total sediments across Scotland. In conclusion, this work shows that there needs to be greater clarity in reporting LOI procedures in the literature and that 500°C is an appropriate temperature; by contrast the commonly used temperatures of 450°C and 550°C may under and over-estimate OC respectively.

Conversion of LOI data to percentage OC uses conversion factors derived from a range of sources. Chapter 3 highlights the local conversion factor has a lower strength of the relationship between OM (%) and OC (%) when compared to global equations derived from Fourqurean et al. (2012; Table 3-2). Fourqurean et al. (2014) advises when determining OC (%) via the LOI method to use a locally derived equation where possible. Therefore, to strengthen the relationship for use within Scotland, a collaborative effort is needed to increase the uptake in application across research, as well as strengthen the scientific validation of the approach. This would involve testing the method across the full spectrum

of sediment types (different proportions of clay content). One way in which this might be facilitated is through an open call in the Scottish Blue Carbon Forum (SBCF) for open access of data. The call would also seek to identify which universities/laboratories have easy access to a CHN analyser with the ability to test subsamples, via both the LOI and CHN methods, to improve confidence in the application of this method. If necessary, this equation could be reviewed yearly with new data added to make it more robust.

7.3 Carbon within seagrass meadows

Seagrass meadows have been celebrated for their ability to naturally sequester carbon from both their own vegetation, and from surrounding environments when OM is transported and deposited within them. As a previous study explored the main east coast Scottish intertidal seagrass meadows, this study focused on west coast sites. Across the west coast and the subtidal meadow, OC stocks were determined by species; within *Z.noltii* meadows average OC stock was 44.97 ± 13.85 Mg C/ha and *Z.marina* meadows average stock was 45.57 ± 45.68 Mg C/ha, in the upper 50 cm depth profile. A higher average OC stock was found for east coast sites; 57 Mg C/ha regardless of species (Potouroglou et al., 2021). Large variation in OC stocks were seen across both species within this study, but particularly for *Z.marina* OC stocks. Within mudflat habitats, average OC stocks for *Z.noltii* and *Z.marina* associated plots, were 40.63 ± 22.86 Mg C/ha and 51.84 ± 20.22 Mg C/ha, respectively. Combining data from all ten intertidal sites provides an average OC stock for seagrass sediments of 54.79 ± 35.02 Mg C/ha, and 44.58 ± 26.32 for mudflat area OC sediment stocks (Potouroglou et al., 2021). Sites FOR, TAY (Potouroglou et al., 2021) and HUNT showed large differences between seagrass and mudflat areas, but the difference was not consistent and overall, not significant. All three sites are situated on carboniferous strata and subsequently the presence of coal seams could explain their enhanced carbon stores. Assuming, conservatively, that intertidal seagrasses cover 1600ha of Scottish coasts (based on Burrows et al. (2014)) the mean value for OC stocks is 175,360 Mg in the top 1 m of sediments. There is no regular mapping or monitoring of seagrass meadows across Scotland, so the true extent of seagrass meadows is unclear.

7.3.1 Determination of sediment age using radionuclide dating

Attempts to use radionuclide dating to determine sedimentation rates, and therefore carbon accumulation rates, were unsuccessful. Whilst the Solway Firth has prominent and detectable levels of anthropogenic independent markers within its sediment substrate, there were two main reasons it was not feasible: firstly, the intertidal meadow was positioned in an area where terrestrial inputs of anthropogenic markers remain in the catchment area (waste deposits from the Chernobyl accident) and results suggested there was an additional source of ^{137}Cs as no expected ratios were observed across any of the cores observed. The second reason was linked to both ^{137}Cs and ^{241}Am being found at

detectable levels at the bottom of 50 cm and 76 cm depth cores. The presence of either anthropogenic radionuclide tracer creates a high level of uncertainty over the integrity of the core, as disturbance during extraction may have caused cross contamination. Therefore, it is highly unlikely that intertidal seagrass meadows could be aged using the techniques adopted in this thesis and future efforts to determine sediment age in this way should primarily focus on subtidal seagrass meadows.

7.3.2 Wider context

The recognition of the role of coastal habitats as carbon sinks has led to an enhanced focus on the management, conservation and restoration of these habitats. This study identified how the presence of seagrass positively influences carbon storage, at least at some sites, which is consistent with the global picture and reinforces the notion that seagrass meadows have a part to play in climate mitigation. However, it is important that the carbon sequestration service of Scottish seagrass meadows is not exaggerated in policy discussion. Total OC stocks per hectare within seagrass meadows are comparable to Scottish saltmarshes, English seagrasses and Scottish terrestrial soils, such as brown soils and mineral gleys (Table 7-1). However, forest and peat soils may contain three to five times as much OC within the top 1 m of soil, compared to Scottish seagrass meadows, and these habitats also cover much larger areas across Scotland (Table 7-1). For example, peat soils have a total OC stock of 1232 Mt C whereas seagrass meadows store 0.175 Mt C (Table 7-1). Hence it is clear that intertidal seagrasses will play only a modest role in total OC storage across Scotland, even if the most generous estimates of total coverage and carbon densities are assumed (Table 7-1).

Across six seagrass sites, $\delta^{13}\text{C}$ values provided evidence that the contribution of terrestrial OM to total OM stored ranged from $35.8 \pm 0.16\%$ at RYA to $83.8 \pm 0.05\%$ at EYN, with an average contribution of $55.9 \pm 18.33\%$; although similar, this is slightly higher than the global average of 51% of allochthonous material (Kennedy et al., 2010). Global estimates include much more productive tropical species, which may explain why they contain, on average, a higher proportion of autochthonous material. However, because allochthonous sources are linked to processes in the watershed, these might also be important and changes in land use and management may change the extent of this sink in the future; indeed it could be argued

that the elevated proportional storage found here, based on $\delta^{13}\text{C}$ values, might reflect poor management practices that permit carbon run-off. Hence, one possible policy implication is to focus on better carbon production and storage on land, rather than to emphasise storage of derived material in seagrass. Ultimately, seagrass meadows both in Scotland and globally should be recognised for their potential to sequester and store carbon. However, in Scotland, where only eelgrasses are found, carbon is just one of a plethora of reasons to conserve and protect these habitats and is probably not the most compelling one (Table 7-1), therefore the focus of protection should not be primarily for the purpose of blue carbon mitigation.

7.3.2.1 Carbon budgets

Expressing the value of carbon within Scottish seagrass meadows in monetary terms may help to justify the continued protection of this habitat. Using the current average UK carbon value of ~£33/Mg C (as predicted within Department of Energy and Climate Change (DECC), 2011 for 2021), this study estimates, that in the top 1 m of sediment substrate, the present market value of carbon in Scottish seagrasses is £5.8 million, based on seagrasses occupying an area of 1600 ha. By contrast, peat has a carbon value of ~£40.7 billion (top 1m; Table 7-1). Consequently, peat soils are 7040 times more valuable from a monetary perspective. With the most recent carbon estimates, across the total area and depth of Scottish peatlands, estimated >2500 Mt, the true value is likely to be much higher (Ferretto et al., 2019). These values highlight that seagrass are less important than peat soils for the purpose of climate mitigation, particularly important to acknowledge if the Scottish Government sticks to its commitment, within the Climate change (Scotland) Act of 2019, to reduce carbon emissions by 100% by 2045 (compared to a 1990-1995 baseline). However, seagrass meadows provide numerous important ecosystem services which are not able to be associated with a crude monetary value and therefore should still be managed and protected for these purposes.

Table 7-1 UK-wide average total OC stocks across terrestrial and coastal habitats across Scotland and England.

| Habitat | soil type | Total OC stock Mg C/ha | Area (ha) | Megatonnes C | Reference |
|---------------------------------|---|---------------------------|--------------|--------------|---|
| Scottish Terrestrial soils | Forest soils (Across a range of soil types) | 297 | 130,000 | 407 | Vanguelova et al. (2013) |
| | Peat | 547 | 1,726,320 | 1232 | Rees et al. (2018) |
| | Mineral gleys | 131 | 1,263,040 | 285 | |
| | Peaty gleys | 242 | 1,080,990 | 443 | |
| | Brown soils | 115 | 997,970 | 276 | |
| | mineral podzols | 124 | 928,430 | 250 | |
| | Peaty podzols | 214 | 908,360 | 374 | |
| Scottish Seagrass sediments | Intertidal meadows | 109.6 | 1,600 | 0.175 | This study and Potoroglou et al. (2021) |
| | Subtidal meadow | 73.9 | - | - | This study |
| Scottish bare mudflat sediments | Intertidal | 89.2 | - | - | This study |
| Scottish Saltmarsh Sediments | Total | 1.28×10^{-4} | 6750 | 0.009 | Burrows et al. (2014) |
| | Orkney | 113 | 48.94 | 0.006 | Porter et al. (2020) |
| English seagrass sediments | Intertidal meadows (<i>Z.marina</i> , <i>Z.noltii</i> , <i>Ruppia maritima</i>) | 112.6 | - | - | Lima et al. (2020) |
| | Subtidal meadows | 140.98 | 4887-10,000* | 0.108-0.222* | Green et al. (2018) |
| | Total UK | | 8493 | 1.2 | Green et al. (2020) |

*Figures based on predicted area of UK seagrass meadows

7.4 Factors that influence the composition of the mudflat seagrass matrix

7.4.1 Decomposition and microbial communities

It was anticipated that differences found in OC storage between intertidal seagrass and bare mudflat areas could be explained by factors such as decomposition of OM, microbial community composition and physical sedimentary properties, including clay content and sediment substrate stability. However, the results from the decomposition work were unexpected and challenging to interpret. Despite very little difference between OC stocks between habitat types, and the assumption that seagrass meadows are environments where slow decomposition occurs, when testing a standardised litter (rooibos tea substrate), the litter buried in seagrass sediments decomposed faster than in comparable adjacent mudflats (at least in relatively superficial sediments). The diversity of microbial communities could have been a plausible explanatory factor for this, however this study found the most abundant prokaryotes were similar across habitat types in the earliest stages of decomposition (after 3 months of litter burial). This consistent and significant difference deserves further study. Further study may reveal unknown aspects of seagrass/sediment ecology, for example the influence of the rhizosphere on decomposition (there were suggestions in the data that the pattern of faster decomposition in seagrass plots was reversed at deeper depths, perhaps because these lie below any influence of roots). It may also be an artefact of the types of substrate and intervention used.

7.4.2 Sediment stability

Z.noltii meadows are already known for their ability to positively impact surface elevation in Scotland (Potouroglou et al., 2017); the present study found the species to have a significant, positive influence on sediment substrate cohesivity (from in-situ shear vane-derived strength tests). Surficial erosivity was also explored and whilst there was not a statistically significant influence of *Z.noltii* presence, at plot level, across 50% of plots, seagrass habitats had a higher critical erosion threshold (CET; from cohesive strength meter-derived erodibility), with the next largest subgroup sharing the same CET values across habitats; hence, the lack of significance may reflect type 2 error arising from large, within and between site, variability and the sensitivity of the CSM instrument to micro-scale effects. Despite differences found for other physical properties of seagrass meadows when compared to mudflats, fine grain sized particle content within sediment subsamples did not

vary between habitats for *Z.noltii* areas, similar to another Scottish Study (Wilkie et al., 2012). Similarly, no differences were found for fine grain size particle content between habitats for *Z.marina* areas even though the species, in other studies outside of Scotland, has been found to positively affect the proportion of fine grain sized particles within the sediment substrate which is linked to the larger above ground biomass of the species (Dahl et al., 2016; Röhr et al., 2018).

It is important to carry out long term research and explore these relationships across more seagrass meadows, as seagrass habitats are highly variable. However, this research, coupled with evidence from Potouroglou et al. (2017), supports the theory that Scottish intertidal seagrass meadows enhance sedimentation of particulate OM from allochthonous sources and increase sediment stability, both surficial and bulk. Therefore, damage or loss beyond recovery of seagrass meadows, whether anthropogenic (i.e. coastal pressures, sea level rise) or natural (i.e. wasting disease) could have far reaching negative effects on the stability of coastal mudflats. For example, losses of structural cohesivity could perpetuate the continued degradation of OC due to remobilising particulate OM, which would result in the release of carbon. This increased erosion could result in turbid and unclear water, that in turn, could impact coastal biodiversity and the ecosystem functions that coastal habitats can provide.

7.5 Current protection and legislation

UK seagrasses are protected through several EU directives including the Water Framework Directive (Directive 2000/60/EC, 2010), the Marine (Scotland) Act 2010, they are a habitat of principle importance under the Natural Environment and Rural Communities (NERC) Act 2006 (England and Wales only) and the UK's wildlife and countryside Act 1981.

Across the Scottish Marine Protected Areas (MPAs) network, there are eight historic MPAs (39 in total), 225 sites for nature conservation (encompassing a range of habitats and species), one research MPA, and five area-based measures to protect vulnerable marine ecosystems and species, including the blue ling and sand eels. These all lie within approximately 37% of Scottish seas (Scottish Government, 2021). Seagrass meadows have been recognised as important within numerous conservation acts and policies, including being listed as one of the Features of Conservation Importance (FOCI) under the MPA network. Seagrass meadows are also a threatened and/or declining habitat on the OSPAR convention-declining list and as a sub-feature of intertidal mudflats, sandflats and subtidal sandbanks, for the designation of Special Areas of Conservation under the European Habitats Directive.

Across the UK it is clear seagrass meadows are recognised for the important ecosystem services they provide and there has been a continued effort to conserve and protect this habitat. For example, in 2014 JNCC produced a sensitivity assessment, identifying seagrass beds as 'highly sensitive to a number of human activities' (d'Avack et al., 2014). In 2020 Natural England launched a £2.5 million project to restore, through planting seed bags and seedling bags in the Plymouth Sound Recreation, trailing Advanced Moors Systems (AMS) in the Solent as part of the 5 year LIFE Recreating ReMEDIES Project (Natural England, 2020). Further to this, in Scotland a project to extend the extent of a subtidal *Z. marina* meadow was awarded money by Nature Scot, and most recently a research report has been produced to advice on restoration of seagrass in Scotland (Kent et al., 2021)

Regardless of the recognised importance of seagrass meadows, the spatial extent of seagrasses is poorly mapped, is not monitored, and knowledge of historical distribution is limited. Green et al. (2021) estimated seagrass declines across the UK over the past century

to be as great as 92%, with 39% of seagrasses lost since the 1980's. In the summer of 2018, to provide a more expansive dataset of intertidal seagrass sediment data, 12 historical seagrass meadows were visited during low tide, however only two of those sites had current evidence of seagrass at them: a small very patchy meadow at Ardfarn, within Loch Craignish, and Kirkcudbright (Solway Firth). At the Ardfarn meadow, no intertidal patch of *Z.noltii* was $\geq 1 \text{ m}^2$ in size and therefore sediment cores were not collected, to keep the sampling regime of the study within Chapter 3 consistent. The large uncertainties in the spatial extent of seagrass meadows, yearly variation in meadow size and uncertainty over whether a meadow is healthy, stable or declining, could all result in neglect of the management and protection of these habitats.

7.5.1 Restoration for the purpose of carbon

Peatlands are Scotland's most important natural asset in terms of carbon storage, and will play a key role in the national mitigation of climate change, with the Scottish Government committed to restoring 250,000 ha of degraded peatland by 2030 (Scottish Government, 2020). Examples of peatland restoration projects include two in the Caithness and Sunderland area (called 'The Flows') which used over 18,000 dams to block drainage from 15,600 ha of blanketbog (IUCN UK Peatland Programme, n.d.) and the 'Flows to the Future' project, which aims to install 200,000 dams to block drainage ditches to protect and restore 1813 hectares of blanketbog (Nature Scot, 2017).

These projects are evidence that in instances where peatland has been severely degraded, restoration is a viable option to re-establish peatland function, species and habitats, as well as the storage of carbon still locked within the peatland. Measures to improve water levels and reverse the causes of degradation include grazing reduction, reduction or banning of muirburn to allow for recovery and the blocking of erosion channels. These measures within a terrestrial habitat are practical and plausible. However, adoption of any restoration strategy within an intertidal or subtidal habitat is generally more technically and financially demanding.

The most successful seagrass restoration project to date has recovered 3612 ha of *Z.marina* seagrass in America. Orth et al. (2020) achieved successful habitat restoration of *Z.marina*

within inshore lagoons across a 19 year period, starting in 1999. A total of 70 million *Z.marina* seeds were planted across four lagoons where seagrass had been lost 70 years prior due to wasting disease. Long-term monitoring of carbon stocks showed seagrass substrate, >9 years post establishment, store 1.3 times as much carbon as newly colonised sediments, however it was not clear from the study whether carbon had been measured prior to restoration (Orth et al., 2020). The project is a notable success and demonstrates how seagrass habitat can be actively restored; however, seedling survival has varied substantially year by year with one lagoon being significantly more suitable for seagrass restoration and subsequently 56% of the total area restored is within it.

Saltmarshes, and more recently native oyster habitat, restoration have been successful in Scotland, with examples including the Eden Estuary (Maynard et al., 2011) and the Skinflats RSPB reserve (Jump, 2010), both saltmarshes, and the Dornoch Environmental Enhancement Project (Fariñas-Franco et al., 2018). None of these restoration projects were conducted for the enhancement of carbon. Nonetheless, Taylor (2019) did explore the carbon stocks within restored saltmarshes compared with natural saltmarsh at Eden and found that the unit area carbon storage value increased, although the study concluded that little is to be gained from small scale restoration activities similar to the project at Eden Estuary. In their meta-analysis, van Katwijk et al. (2015) looked at 1786 seagrass restoration trials globally but found a low overall survival rate across trials, estimating ~37% success rate, and also linked this to the scale of the restoration projects, where larger scales studies had the highest rates of success. Similarly, the trial seagrass restoration described by Unsworth et al. (2019) led to an innovative planting procedure using hessian bags but found the proportion of seedling establishment rates were low (3.5%). Orth et al. (2020) found a large, yearly variability in the success of seed establishment. Hence, currently, seagrass restoration is generally very expensive and uncertain.

There have been rising calls for Scotland to commit to restoration of seagrass meadows. In May 2021, Nature Scot's Biodiversity Challenge Fund awarded £154,409 to the charities Seawilding and Project Seagrass, working with the Scottish Association of Marine Science (SAMS), to enhance an existing *Z.marina* meadow by a quarter of a hectare at Loch Craignish. Seawilding has also been involved in another community led project, restoring

native oysters to Loch Craignish. Project Seagrass has experience of restoration projects and is one of the partners of the £350,000 project 'Seagrass Ocean Rescue' (Project seagrass, 2020). 'Seagrass Ocean Rescue' aims to restore *Z.marina* across a two-hectare area in Wales. Based on seed establishment during Unsworth et al. (2019) trials and van Katwijk et al. (2015), it is evident that identifying suitable sites for seagrass restoration requires extensive long-term funding and historical knowledge on the site and the pressures it is subject too. In November 2020, the 'Seagrass Ocean Rescue' project had reached the one million milestone for seeds planted, far exceeding the majority of seagrass restoration trials reviewed by van Katwijk et al. (2015) therefore increasing the probability of establishing a seagrass meadow.

For successful restoration robust frameworks must be in place, clearly identifying the predominant stressors that have caused the decline or complete removal of the seagrass meadow. Mitigation of such stressors would improve the overall chance of success; certainly, in most cases, this is likely to be essential for restoration to succeed. Further to this, despite Scotland's limited mapping of seagrass meadows, monitoring, ideally long term, should be in place before determining site suitability, particularly for intertidal seagrass restoration, as the extent of most meadows vary annually. Understanding the true spatial extent of a meadow, and the amount of natural inter-annual variation, will prevent under-or-over estimations of successful restoration projects. Finally, the prospect of carbon enhancement through the successful restoration of seagrass meadows may be an important and legitimate motivation. However, extensive determination of current carbon stocks is needed to prevent overinflation of the role seagrass meadows have in enhancing carbon, especially in newly established seagrass meadows. Figures associated with carbon storage in seagrass meadows need to be regional where possible when conveying its importance.

7.6 Main thesis conclusions

1. The LOI method remains important in studies of sedimentary carbon, but there are wide variations in applications of this method as reported in the literature, and these variations are likely to have significant implications for the accuracy of the carbon estimates. Authors should take care to clearly state the methods used, and it is recommended that 500°C for six hours is appropriate for most Scottish marine sediment. A full protocol that is widely understood would be of value to marine ecology, and a first suggestion of this is given in Loss on ignition Standard operating procedure
2. Appendix 7.
3. It is now known with confidence that there is high variability in carbon accumulated in intertidal seagrass meadows. Whilst the presence of seagrass does generally enhance carbon, this is not always the case in Scotland and often carbon stocks are similar to adjacent mudflat areas which are unvegetated.
4. The estimated total seagrass OC stock is 175,360 Mg within a 1 m sediment depth profile. The current carbon valuation of this sediment stock is £5.8 million (top 1 m) based on the UK policy appraisal (DECC, 2011).
5. Research here and in Potoroglou (2016) highlights that specific seagrass meadows, particularly those which are underlain by Carboniferous strata, could play a minor role in the mitigation of climate change within Scotland. However, the remaining high levels of uncertainty about seagrass distribution and the drivers of variability in OC stocks between sites, mean policy positions promoting seagrass protection based on carbon storage are risky. This is particularly true given the much greater, and more certain, contribution to carbon storage of other Scottish habitats, particularly peatlands.
6. It was not possible to accurately date intertidal seagrass meadows using radionuclide markers; future application of this technique, in the UK, should focus on subtidal meadows or at least on sites known to experience relatively little water movement or sedimentary disturbance.
7. The processes of carbon decomposition with seagrass sediments and what factors may enhance or slow decomposition, remain poorly understood. The surprising

finding reported here of faster decomposition rates of a standard substrate, may suggest unknown factors that deserve further investigation.

8. There is need for much greater research on subtidal seagrass meadows. As only one subtidal site was assessed in this project it is difficult to determine the role of subtidal meadows in enhancing carbon.
9. Much better monitoring and mapping of both intertidal and subtidal seagrass meadows in Scotland are required to understand if this habitat could play a role in mitigation of climate change, but also to identify current health of seagrass meadows and the provisions they provide at a site-specific level for long-term management.

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Appendices

Appendix 1 Published work references

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My contribution:

All authors contributed to the review design. The team and I identified the five key mechanisms that may affect carbon sequestration and storage. I wrote the entire 'Direct Transfers of Carbon' section for this review paper. I conducted a literature review on Web of Science and read all relevant research in the area which focused on the three vegetated blue carbon habitats (Mangroves, seagrasses and tidal marshes). The section focused on the 'outwelling hypothesis' exploring outwelling specifically from mangrove forests to seagrass meadows, outwelling from tidal marshes and inwelling from seagrass meadows. I also commented on and provided edits to the original manuscript.

Potouroglou, M., Whitlock, D., Milatovic, L., MacKinnon, G., Kennedy, H., Diele, K., Huxham, M., 2021. The sediment carbon stocks of intertidal seagrass meadows in Scotland. *Estuar. Coast. Shelf Sci.* 258, 107442. <https://doi.org/10.1016/j.ecss.2021.107442>

My contribution:

I lead the fieldwork, collected sediment cores and analysed data across Solway Firth, Loch Ryan and Clyde Firth seagrass meadows. I also commented on and provided edits to the original manuscript. The study had been conceived and designed prior to my PhD commencing by Potouroglou and her supervisory team. All organic carbon stock results in Chapter 3 for CAR, HUNT, SOU, KIRK, ROCK, SOL and RYA are published within the paper.

Further to this, my data on Eynhallow seagrass meadows was included in Porter, J., Austin, W., Burrows, M., Clarke, D., Davies, G., Kamenos, N., Riegel, S., Smeaton, C., Page, C., & Want, A. (2020). *Blue carbon audit of Orkney waters*. (Scottish Marine and Freshwater Science; Vol. 11, No. 3). Marine Scotland Science. <https://doi.org/10.7489/12262-1>
And I provided comments on section 3.2.3. of the audit.

Appendix 2: Site grid co-ordinates and TeaComposition coordinates

Site coordinates

General site grid coordinates

| Coast | Site | Latitude | Longitude |
|-------|------|-----------|-----------|
| East | EDE | 56.361185 | -2.866915 |
| East | FOR | 55.984074 | -3.319614 |
| East | TAY | 56.444104 | -2.848729 |
| East | TAY | 56.448517 | -2.874717 |
| East | BEA | 57.481946 | -4.293126 |
| East | CROM | 57.657668 | -4.154386 |
| East | CUT | 57.865593 | -4.079134 |
| East | MON | 56.716105 | -2.482484 |
| West | CAR | 55.950808 | -4.623777 |
| West | HUNT | 55.731465 | -4.880546 |
| West | SOU | 55.750398 | -4.859251 |
| West | RYA | 54.917 | -4.994767 |
| West | KIRK | 54.821567 | -4.053778 |
| West | ROCK | 54.862519 | -3.800824 |
| West | SOL | 54.839096 | -3.851716 |
| West | SOL | 54.840002 | -3.840008 |
| North | EYN | 59.140611 | -3.110861 |

TeaComposition habitat grid coordinates (specific to chapter 4 and 5)

| Study | Sites | Seagrass 1 | | Seagrass 2 | | Mudflat 1 | | Mudflat 2 | |
|------------------|-------|------------|-----------|------------|-----------|-----------|-----------|-----------|-----------|
| long term study | EDE | 56.361183 | -2.866917 | 56.361217 | -2.866783 | 56.361233 | -2.867033 | 56.36125 | -2.86685 |
| | FOR | 55.984267 | -3.319117 | 55.984267 | -3.319 | 55.984233 | -3.319133 | 55.984233 | -3.3191 |
| | TAY | 56.448517 | -2.874717 | 56.4484 | -2.874717 | 56.448567 | -2.874717 | 56.448483 | -2.8745 |
| | CAR | 55.959467 | -4.6541 | 55.9596 | -4.654033 | 55.959517 | -4.654217 | 55.959567 | -4.654267 |
| | SOL | 54.839217 | -3.851517 | 54.839133 | -3.851417 | 54.839233 | -3.851533 | 54.839117 | -3.851367 |
| | RYA | 54.916333 | -4.993633 | 54.916433 | -4.993633 | 54.91635 | -4.9933 | 54.916417 | -4.9927 |
| Short term study | FOR | 55.9843 | -3.319067 | 55.984317 | -3.319017 | 55.984317 | -3.31895 | 55.98435 | -3.318967 |
| | SOL | 54.839217 | -3.851567 | 54.839183 | -3.85145 | 54.839267 | -3.851633 | 54.839233 | -3.851483 |

Summary of methods for site characteristic data

Appendix 3 Salinity, surface temperature, pH and percentage cover

Salinity was determined by collecting samples from pools of surface water in intertidal seagrass beds. The water was then pipetted onto a portable analog refractometer (kern Optics) and the measure was expressed as parts salt per thousand parts seawater (ppt or ‰).

Surface temperature averages were taken from the most recent met office (Met Office, 2021).

pH was measured using an infield soil pH probe (HI-991003 pH electrode sensor)

Quadrats 1m² in size were randomly placed across each of the six main sites twenty times. Photographs (1m above the centre of the quadrat) were taken (so that all fieldworkers across the 6 sites could provide percentage cover measurements) and in-situ percentage cover measurements were taken from two independent fieldworkers (one of the fieldworkers was the same for every single site to reduce in-field differences). The mean and standard deviation of percentage cover of seagrass was noted.

Temperature log

Appendix 4 Site monthly temperatures at a 15 cm burial depth all temperature in celsius (°C)

| | | EDE | | | | FOR | | | | TAY | | | | CAR | | | | SOL | | | | RYA | | | |
|------|-------|-------|-------|-------|--------|--------------|-------|-------|--------|-------------|-------|-------|--------|-------------|-------|-------|--------|-------|-------|-------|--------|-------|-------|-------|--------|
| Year | Month | min | mean | max | st dev | min | mean | max | st dev | min | mean | max | st dev | min | mean | max | st dev | min | mean | max | st dev | min | mean | max | st dev |
| 2017 | July | 14.13 | 15.75 | 19.00 | 1.00 | 13.85 | 15.94 | 19.38 | 1.29 | 13.27 | 15.45 | 19.00 | 1.13 | 13.85 | 16.16 | 22.24 | 1.49 | 15.47 | 18.19 | 24.93 | 1.85 | 15.47 | 17.25 | 21.95 | 1.08 |
| | Aug | 12.30 | 15.57 | 18.71 | 1.01 | 12.59 | 15.53 | 19.09 | 1.22 | 13.17 | 15.49 | 19.47 | 0.95 | 13.37 | 15.85 | 19.57 | 1.17 | 13.65 | 17.29 | 22.72 | 1.56 | 14.61 | 16.53 | 19.38 | 0.76 |
| | Sept | 10.94 | 13.63 | 16.43 | 1.10 | 11.14 | 13.84 | 17.19 | 1.16 | 11.33 | 13.85 | 16.33 | 0.98 | 9.67 | 13.83 | 18.33 | 1.22 | 10.94 | 14.42 | 19.19 | 1.40 | 11.82 | 14.50 | 18.71 | 1.15 |
| | Oct | 5.86 | 11.35 | 13.65 | 1.35 | 6.47 | 11.72 | 14.42 | 1.34 | 8.08 | 11.91 | 13.65 | 0.87 | 6.37 | 11.56 | 13.85 | 1.15 | 6.27 | 12.20 | 15.57 | 1.53 | 9.67 | 12.37 | 14.80 | 0.80 |
| | Nov | 1.55 | 6.20 | 10.65 | 2.20 | 1.76 | 6.85 | 11.04 | 2.07 | 4.10 | 7.77 | 11.14 | 1.60 | 1.87 | 7.25 | 11.82 | 2.06 | 1.11 | 6.87 | 11.92 | 2.48 | 4.31 | 8.36 | 12.01 | 1.71 |
| | Dec | 0.45 | 3.70 | 7.98 | 2.03 | 0.56 | 4.34 | 8.58 | 2.13 | 2.73 | 5.05 | 7.98 | 1.12 | 1.00 | 5.42 | 9.47 | 2.17 | 0.12 | 3.92 | 8.18 | 2.33 | 2.84 | 6.03 | 8.78 | 1.23 |
| 2018 | Jan | 1.11 | 3.61 | 6.57 | 1.06 | 1.00 | 4.13 | 7.28 | 1.15 | 2.20 | 4.32 | 5.96 | 0.66 | 1.00 | 4.81 | 7.88 | 1.32 | 0.56 | 3.93 | 6.98 | 1.41 | 2.30 | 5.48 | 8.28 | 0.96 |
| | Feb | 0.67 | 3.40 | 6.27 | 1.08 | Corrupt file | | | | 1.44 | 4.16 | 6.37 | 0.83 | 0.34 | 4.12 | 7.88 | 1.28 | 0.34 | 3.61 | 6.98 | 1.31 | 1.55 | 4.81 | 6.47 | 0.86 |
| | March | 0.45 | 4.35 | 8.38 | 1.65 | | | | | 1.11 | 4.37 | 8.88 | 1.43 | 0.12 | 5.05 | 11.24 | 2.15 | 0.12 | 4.83 | 11.24 | 2.52 | 1.00 | 5.70 | 11.14 | 1.86 |
| | April | 2.94 | 8.12 | 13.94 | 2.08 | | | | | 3.47 | 7.63 | 12.98 | 1.75 | 2.52 | 8.57 | 15.76 | 2.23 | 3.68 | 9.24 | 15.95 | 2.55 | 4.73 | 8.96 | 13.85 | 1.88 |
| | May | 7.58 | 12.78 | 17.09 | 1.93 | | | | | 7.48 | 12.12 | 17.48 | 1.94 | 8.48 | 13.75 | 21.28 | 2.89 | 8.18 | 14.42 | 21.19 | 2.91 | 9.28 | 13.46 | 19.95 | 2.59 |
| | June | 11.43 | 15.49 | 19.76 | 1.61 | | | | | 11.82 | 14.98 | 19.19 | 1.37 | 11.53 | 17.31 | 25.03 | 3.12 | 12.30 | 18.43 | 26.39 | 2.84 | 13.46 | 17.47 | 22.81 | 2.12 |
| | July | 15.19 | 18.31 | 22.24 | 1.40 | | | | | 15.09 | 17.76 | 29.05 | 1.54 | 14.33 | 18.85 | 25.81 | 2.11 | 16.14 | 20.02 | 27.47 | 2.31 | 15.57 | 19.16 | 25.03 | 1.75 |
| | Aug | 12.59 | 15.78 | 20.52 | 1.55 | | | | | 13.08 | 15.93 | 22.14 | 1.55 | 12.30 | 16.03 | 20.52 | 1.60 | 12.40 | 17.67 | 23.00 | 2.08 | 13.37 | 16.89 | 21.28 | 1.47 |
| | Sept | 8.88 | 12.86 | 17.38 | 1.68 | | | | | 10.16 | 13.35 | 17.76 | 1.42 | 8.78 | 13.18 | 19.57 | 1.87 | 8.98 | 14.16 | 19.38 | 2.07 | 10.36 | 14.04 | 19.85 | 1.81 |
| | Oct | 5.96 | 10.13 | 13.75 | 1.61 | | | | | 7.48 | 10.85 | 13.85 | 1.21 | 4.62 | 10.44 | 14.04 | 1.81 | 4.00 | 10.53 | 14.61 | 2.42 | 5.35 | 11.17 | 14.90 | 1.93 |
| | Nov | 4.93 | 8.26 | 10.65 | 1.16 | | | | | 6.67 | 8.76 | 10.65 | 0.86 | 4.21 | 8.55 | 11.24 | 1.42 | 3.16 | 7.86 | 11.04 | 2.01 | 5.35 | 8.97 | 11.14 | 1.33 |
| | Dec | 2.20 | 5.28 | 7.68 | 1.37 | | | | | 3.79 | 6.20 | 7.88 | 0.89 | 3.16 | 6.61 | 9.28 | 1.34 | 1.98 | 5.67 | 8.28 | 1.51 | 4.10 | 7.39 | 9.57 | 0.88 |
| 2019 | Jan | 0.01 | 4.14 | 7.68 | 1.58 | Corrupt file | | | | 1.55 | 5.10 | 7.38 | 1.17 | 1.33 | 5.51 | 8.98 | 1.66 | 0.12 | 4.15 | 7.98 | 1.92 | 2.62 | 6.17 | 8.98 | 1.26 |
| | Feb | -0.33 | 4.95 | 9.37 | 2.18 | | | | | 1.22 | 5.30 | 9.47 | 1.64 | 0.67 | 6.17 | 10.46 | 2.03 | -0.21 | 5.45 | 10.26 | 2.44 | 2.20 | 6.77 | 10.85 | 1.80 |
| | March | 3.37 | 6.65 | 10.46 | 1.48 | | | | | 4.21 | 6.92 | 11.33 | 1.31 | 3.68 | 7.25 | 13.17 | 1.54 | 3.47 | 7.51 | 14.61 | 1.96 | 5.14 | 8.21 | 13.65 | 1.48 |
| | April | 5.96 | 9.28 | 15.86 | 2.27 | | | | | 6.17 | 9.24 | 15.57 | 2.09 | 4.93 | 9.67 | 17.19 | 2.55 | 4.83 | 9.93 | 17.38 | 2.59 | 6.27 | 10.04 | 15.86 | 1.88 |
| | May | 7.98 | 12.22 | 17.09 | 1.82 | | | | | 8.38 | 11.98 | 17.57 | 1.67 | 7.48 | 12.69 | 20.62 | 2.43 | 7.48 | 13.57 | 20.42 | 2.90 | 8.68 | 13.41 | 19.66 | 2.12 |
| | June | 10.85 | 14.32 | 19.09 | 1.76 | | | | | 11.14 | 14.06 | 18.05 | 1.51 | 11.92 | 15.34 | 25.13 | 2.33 | 11.63 | 16.45 | 23.97 | 2.65 | 11.43 | 15.84 | 24.55 | 2.14 |
| | July | 12.88 | 17.46 | 21.47 | 1.53 | 15.47 | 18.24 | 24.26 | 1.60 | 13.56 | 14.74 | 16.24 | 0.65 | 13.85 | 16.24 | 21.28 | 1.46 | 16.43 | 19.33 | 24.55 | 1.64 | 14.80 | 18.07 | 23.97 | 1.40 |
| | Aug | 13.65 | 16.61 | 20.42 | 1.42 | 14.52 | 17.19 | 21.00 | 1.39 | LOST LOGGER | | | | LOST LOGGER | | | | 15.09 | 18.14 | 22.53 | 1.59 | 13.46 | 17.58 | 24.16 | 1.74 |
| | Sept | 10.65 | 13.80 | 17.00 | 1.10 | 11.53 | 14.35 | 17.95 | 1.17 | | | | | | | | | 12.21 | 14.91 | 17.38 | 1.08 | 12.01 | 15.01 | 19.09 | 1.06 |
| | Oct | 6.06 | 9.94 | 12.98 | 1.59 | 6.47 | 10.44 | 13.85 | 1.49 | | | | | | | | | 5.96 | 10.24 | 13.65 | 1.81 | 6.88 | 11.22 | 15.76 | 1.70 |
| | Nov | 2.41 | 6.43 | 10.55 | 1.73 | 2.73 | 6.62 | 10.36 | 1.66 | | | | | | | | | 1.98 | 6.01 | 10.26 | 1.78 | 4.21 | 7.37 | 11.53 | 1.50 |
| | Dec | 2.73 | 5.41 | 7.78 | 1.03 | 2.94 | 5.78 | 8.38 | 1.08 | | | | | | | | | 1.22 | 5.36 | 7.58 | 1.29 | 4.21 | 6.63 | 8.68 | 0.94 |
| | Jan | 2.41 | 5.43 | 8.18 | 1.00 | 2.41 | 5.88 | 8.48 | 1.12 | LOST LOGGER | | | | LOST LOGGER | | | | 2.09 | 5.61 | 7.38 | 1.06 | 3.79 | 6.68 | 8.98 | 0.89 |
| 2020 | Feb | 2.62 | 4.60 | 7.28 | 0.88 | 2.62 | 5.09 | 7.98 | 0.96 | | | | | | | | | 2.73 | 5.16 | 7.38 | 0.89 | 3.68 | 5.83 | 7.68 | 0.83 |
| | March | 2.62 | 6.02 | 9.67 | 1.25 | 2.84 | 6.50 | 11.43 | 1.42 | | | | | | | | | 2.41 | 6.26 | 9.87 | 1.38 | 4.73 | 7.15 | 12.30 | 1.21 |
| | April | 4.21 | 9.20 | 13.46 | 1.64 | 5.35 | 9.72 | 15.57 | 1.90 | | | | | | | | | 6.78 | 10.04 | 13.94 | 1.54 | 7.78 | 11.04 | 17.28 | 1.69 |
| | May | 7.48 | 12.55 | 19.66 | 2.28 | 8.48 | 13.62 | 22.81 | 2.64 | | | | | | | | | 8.48 | 13.68 | 21.28 | 2.58 | 9.18 | 13.52 | 20.90 | 2.02 |
| | June | 11.24 | 14.88 | 20.14 | 1.57 | 12.01 | 15.51 | 20.62 | 1.64 | | | | | | | | | 12.69 | 17.03 | 23.68 | 2.29 | 11.63 | 16.41 | 23.20 | 2.23 |
| | July | 12.69 | 15.31 | 18.81 | 1.27 | 13.08 | 15.43 | 18.81 | 1.22 | | | | | | | | | 14.33 | 16.79 | 19.76 | 1.15 | 13.56 | 16.16 | 20.81 | 1.29 |
| | Aug | 11.82 | 15.95 | 21.00 | 1.68 | 12.50 | 16.05 | 20.81 | 1.53 | | | | | | | | | 12.69 | 17.65 | 23.58 | 1.94 | 13.37 | 17.32 | 23.39 | 1.97 |
| | Sept | 12.98 | 14.16 | 16.14 | 0.71 | 13.65 | 14.76 | 16.71 | 0.70 | | | | | | | | | 13.85 | 15.24 | 16.71 | 0.62 | 13.56 | 15.05 | 16.62 | 0.63 |

Bioinformatics Script

Appendix 5 USEARCH script used for bioinformatics analysis – license owned by Edinburgh Napier University

```
40011893@sh11b28-170556:~/Desktop/USEARCH$ ./usearch9 -fastq_mergepairs
*_R1_*.fastq -relabel @ -fastqout 16S_ALL_merged.fq
usearch v9.2.64_i86linux32, 4.0Gb RAM (32.8Gb total), 12 cores
(C) Copyright 2013-16 Robert C. Edgar, all rights reserved.
http://drive5.com/usearch License: M.Kaczmarek@napier.ac.uk
```

Merging

```
Fwd 16SNEG_S72_L001_R1_001.fastq
Rev 16SNEG_S72_L001_R2_001.fastq
Relabel reads as 16SNEG.#
```

00:00 156Mb 100.0% 36.3% merged

Merging

```
Fwd 16SPOS_S71_L001_R1_001.fastq
Rev 16SPOS_S71_L001_R2_001.fastq
Relabel reads as 16SPOS.#
```

00:00 156Mb 100.0% 29.6% merged

Merging

```
Fwd BA_S25_L001_R1_001.fastq
Rev BA_S25_L001_R2_001.fastq
Relabel reads as BA.#
```

00:00 156Mb 100.0% 20.2% merged

Merging

```
Fwd BB_S26_L001_R1_001.fastq
Rev BB_S26_L001_R2_001.fastq
Relabel reads as BB.#
```

00:01 156Mb 100.0% 38.7% merged

Merging

```
Fwd DB10_S36_L001_R1_001.fastq
Rev DB10_S36_L001_R2_001.fastq
Relabel reads as DB10.#
```

00:03 156Mb 100.0% 66.5% merged

Merging

```
Fwd DB11_S37_L001_R1_001.fastq
Rev DB11_S37_L001_R2_001.fastq
```


Relabel reads as DB11.#

00:04 157Mb 100.0% 68.7% merged

Merging

Fwd DB12_S38_L001_R1_001.fastq

Rev DB12_S38_L001_R2_001.fastq

Relabel reads as DB12.#

00:05 157Mb 100.0% 68.8% merged

Merging

Fwd DB13_S39_L001_R1_001.fastq

Rev DB13_S39_L001_R2_001.fastq

Relabel reads as DB13.#

00:06 157Mb 100.0% 68.7% merged

Merging

Fwd DB14_S40_L001_R1_001.fastq

Rev DB14_S40_L001_R2_001.fastq

Relabel reads as DB14.#

00:07 157Mb 100.0% 68.9% merged

Merging

Fwd DB15_S41_L001_R1_001.fastq

Rev DB15_S41_L001_R2_001.fastq

Relabel reads as DB15.#

00:08 157Mb 100.0% 68.9% merged

Merging

Fwd DB16_S42_L001_R1_001.fastq

Rev DB16_S42_L001_R2_001.fastq

Relabel reads as DB16.#

00:09 157Mb 100.0% 69.0% merged

Merging

Fwd DB1_S27_L001_R1_001.fastq

Rev DB1_S27_L001_R2_001.fastq

Relabel reads as DB1.#

00:09 157Mb 100.0% 69.1% merged

Merging

Fwd DB2_S28_L001_R1_001.fastq
Rev DB2_S28_L001_R2_001.fastq
Relabel reads as DB2.#

00:09 157Mb 100.0% 69.2% merged

Merging
Fwd DB3_S29_L001_R1_001.fastq
Rev DB3_S29_L001_R2_001.fastq
Relabel reads as DB3.#

00:10 157Mb 100.0% 69.3% merged

Merging
Fwd DB4_S30_L001_R1_001.fastq
Rev DB4_S30_L001_R2_001.fastq
Relabel reads as DB4.#

00:10 167Mb 100.0% 69.3% merged

Merging
Fwd DB5_S31_L001_R1_001.fastq
Rev DB5_S31_L001_R2_001.fastq
Relabel reads as DB5.#

00:11 167Mb 100.0% 69.4% merged

Merging
Fwd DB6_S32_L001_R1_001.fastq
Rev DB6_S32_L001_R2_001.fastq
Relabel reads as DB6.#

00:11 167Mb 100.0% 69.5% merged

Merging
Fwd DB7_S33_L001_R1_001.fastq
Rev DB7_S33_L001_R2_001.fastq
Relabel reads as DB7.#

00:12 167Mb 100.0% 69.2% merged

Merging
Fwd DB8_S34_L001_R1_001.fastq
Rev DB8_S34_L001_R2_001.fastq
Relabel reads as DB8.#
00:12 167Mb 100.0% 68.9% merged

Merging

Fwd DB9_S35_L001_R1_001.fastq
Rev DB9_S35_L001_R2_001.fastq
Relabel reads as DB9.#

00:13 167Mb 100.0% 69.0% merged

Merging

Fwd SB10_S52_L001_R1_001.fastq
Rev SB10_S52_L001_R2_001.fastq
Relabel reads as SB10.#

00:14 167Mb 100.0% 68.9% merged

Merging

Fwd SB11_S53_L001_R1_001.fastq
Rev SB11_S53_L001_R2_001.fastq
Relabel reads as SB11.#

00:16 167Mb 100.0% 68.9% merged

Merging

Fwd SB12_S54_L001_R1_001.fastq
Rev SB12_S54_L001_R2_001.fastq
Relabel reads as SB12.#

00:17 167Mb 100.0% 68.9% merged

Merging

Fwd SB13_S55_L001_R1_001.fastq
Rev SB13_S55_L001_R2_001.fastq
Relabel reads as SB13.#

00:18 167Mb 100.0% 68.7% merged

Merging

Fwd SB14_S56_L001_R1_001.fastq
Rev SB14_S56_L001_R2_001.fastq
Relabel reads as SB14.#

00:18 167Mb 100.0% 68.7% merged

Merging

Fwd SB15_S57_L001_R1_001.fastq
Rev SB15_S57_L001_R2_001.fastq
Relabel reads as SB15.#

00:20 168Mb 100.0% 68.4% merged

Merging

Fwd SB16_S58_L001_R1_001.fastq

Rev SB16_S58_L001_R2_001.fastq

Relabel reads as SB16.#

00:21 168Mb 100.0% 68.4% merged

Merging

Fwd SB1_S43_L001_R1_001.fastq

Rev SB1_S43_L001_R2_001.fastq

Relabel reads as SB1.#

00:21 168Mb 100.0% 68.3% merged

Merging

Fwd SB2_S44_L001_R1_001.fastq

Rev SB2_S44_L001_R2_001.fastq

Relabel reads as SB2.#

00:22 177Mb 100.0% 68.3% merged

Merging

Fwd SB3_S45_L001_R1_001.fastq

Rev SB3_S45_L001_R2_001.fastq

Relabel reads as SB3.#

00:22 177Mb 100.0% 68.3% merged

Merging

Fwd SB4_S46_L001_R1_001.fastq

Rev SB4_S46_L001_R2_001.fastq

Relabel reads as SB4.#

00:22 177Mb 100.0% 68.3% merged

Merging

Fwd SB5_S47_L001_R1_001.fastq

Rev SB5_S47_L001_R2_001.fastq

Relabel reads as SB5.#

00:23 178Mb 100.0% 68.2% merged

Merging

Fwd SB6_S48_L001_R1_001.fastq

Rev SB6_S48_L001_R2_001.fastq

Relabel reads as SB6.#

00:23 178Mb 100.0% 68.2% merged

Merging

Fwd SB7_S49_L001_R1_001.fastq

Rev SB7_S49_L001_R2_001.fastq

Relabel reads as SB7.#

00:24 178Mb 100.0% 68.1% merged

Merging

Fwd SB8_S50_L001_R1_001.fastq

Rev SB8_S50_L001_R2_001.fastq

Relabel reads as SB8.#

00:25 178Mb 100.0% 67.9% merged

Merging

Fwd SB9_S51_L001_R1_001.fastq

Rev SB9_S51_L001_R2_001.fastq

Relabel reads as SB9.#

00:26 178Mb 100.0% 67.9% merged

Totals:

4611264 Pairs (4.6M)

3131563 Merged (3.1M, 67.91%)

1235607 Alignments with zero diffs (26.80%)

1358964 Too many diffs (> 5) (29.47%)

120737 No alignment found (2.62%)

0 Alignment too short (< 16) (0.00%)

4482950 Staggered pairs (97.22%) merged & trimmed

253.06 Mean alignment length

253.08 Mean merged length

0.74 Mean fwd expected errors

3.71 Mean rev expected errors

0.04 Mean merged expected errors

40011893@sh11b28-170556:~/Desktop/USEARCH\$./usearch9 -fastq_filter

16S_ALL_merged.fq -fastq_maxee 1.0 -relabel Filt -fastaout 16S_ALL_filtered.fa

usearch v9.2.64_i86linux32, 4.0Gb RAM (32.8Gb total), 12 cores

00:18 89Mb 100.0% Filtering, 100.0% passed

3131563 Reads (3.1M)

1557 Discarded reads with expected errs > 1.00

3130006 Filtered reads (3.1M, 100.0%)

40011893@sh11b28-170556:~/Desktop/USEARCH\$./usearch9 -fastx_uniques
16S_ALL_filtered.fa -relabel Uniq -sizeout -fastaout 16S_ALL_uniques.fa
usearch v9.2.64_i86linux32, 4.0Gb RAM (32.8Gb total), 12 cores

00:06 950Mb 100.0% Reading 16S_ALL_filtered.fa
00:08 1.6Gb 100.0% DF
00:08 1.7Gb 3130006 seqs, 372796 uniques, 265742 singletons (71.3%)
00:08 1.7Gb Min size 1, median 1, max 72224, avg 8.40
00:10 1.1Gb 100.0% Writing 16S_ALL_uniques.fa
40011893@sh11b28-170556:~/Desktop/USEARCH\$./usearch9 -cluster_otus
16S_ALL_uniques.fa -minsize 2 -otus 16S_ALL_otus.fa -relabel Otu
usearch v9.2.64_i86linux32, 4.0Gb RAM (32.8Gb total), 12 cores

00:47 93Mb 100.0% 16569 OTUs, 3350 chimeras
40011893@sh11b28-170556:~/Desktop/USEARCH\$./usearch9 -usearch_global
16S_ALL_merged.fq -db 16S_ALL_otus.fa -strand plus -id 0.97 -otutabout
16S_ALL_otutab.txt -biomout 16S_ALL_otutab.json
usearch v9.2.64_i86linux32, 4.0Gb RAM (32.8Gb total), 12 cores

00:00 45Mb 100.0% Reading 16S_ALL_otus.fa
00:00 11Mb 100.0% Masking (fastnucleo)
00:01 12Mb 100.0% Word stats
00:01 12Mb 100.0% Alloc rows
00:01 28Mb 100.0% Build index
00:34 172Mb 100.0% Searching, 98.8% matched
3091317 / 3131563 mapped to OTUs (98.7%)
00:34 172Mb Writing 16S_ALL_otutab.txt
00:34 172Mb Writing 16S_ALL_otutab.txt ...done.
00:34 172Mb Writing 16S_ALL_otutab.json
00:34 172Mb Writing 16S_ALL_otutab.json ...done.
40011893@sh11b28-170556:~/Desktop/USEARCH\$./usearch9 -utax 16S_ALL_otus.fa -db
refdb.udb -strand both -fastaout 16S_ALL_otustax.fa
usearch v9.2.64_i86linux32, 4.0Gb RAM (32.8Gb total), 12 cores

00:00 67Mb 100.0% Rows
00:00 67Mb Read taxonomy info...done.
00:00 67Mb Reading pointers...done.
00:00 69Mb Reading db seqs...done.
00:02 216Mb 100.0% 16.6k seqs, 30.4% at phylum, 2.7% genus (P > 0.90)
40011893@sh11b28-170556:~/Desktop/USEARCH\$./usearch9 -alpha_div
16S_ALL_otutab.txt -output 16S_ALL_alpha.txt
usearch v9.2.64_i86linux32, 4.0Gb RAM (32.8Gb total), 12 cores

00:00 10.0Mb 100.0% Reading 16S_ALL_otutab.txt
40011893@sh11b28-170556:~/Desktop/USEARCH\$./usearch9 -beta_div
16S_ALL_otutab.txt
usearch v9.2.64_i86linux32, 4.0Gb RAM (32.8Gb total), 12 cores

00:00 10.0Mb 100.0% Reading 16S_ALL_otutab.txt
00:00 10.0Mb 100.0% bray_curtis
00:00 10.0Mb 100.0% Building tree
00:00 10.0Mb 100.0% bray_curtis_binary
00:00 10.0Mb 100.0% Building tree
00:00 10.0Mb 100.0% euclidean
00:00 10.0Mb 100.0% Building tree
00:00 10.0Mb 100.0% jaccard
00:00 10.0Mb 100.0% Building tree
00:00 10.0Mb 100.0% jaccard_binary
00:00 10.0Mb 100.0% Building tree
00:00 10.0Mb 100.0% manhattan
00:00 10.0Mb 100.0% Building tree
40011893@sh11b28-170556:~/Desktop/USEARCH\$

Radionuclide dating discussion (Appendix 6)

The experimental design behind attempting to use radioisotopes to date intertidal seagrass meadows was robust. Although seagrass meadows are found in more dynamic and variable intertidal sedimentary environments where deposition of ^{210}Pb is inconsistent or too low to be accurately measured (Assinder et al., 1993; Harvey et al., 2007; Haslett et al., 2003), other anthropogenic radionuclide tracers can act as independent chronological markers to reconstruct temporal accumulation rates in coastal and near-shore environments (Brown et al., 1999; Clifton and Hamilton, 1982; MacKenzie et al., 1994, 2004). Potoroglou (2016) showed the difficulty of using ^{210}Pb within an intertidal seagrass meadow at TAY therefore the SOL site was carefully chosen to try and overcome this previous limitation. The proximity of Balcary Bay (within Solway Firth) to Sellafield nuclear fuel reprocessing plant meant anthropogenic radionuclides, released into the environment from planned or accidental origins, could be used to establish sediment chronologies. Authorised discharges of low-level liquid radioactive waste from Sellafield are temporally well-defined, starting in 1952 with maximum release of most radionuclides in the mid-1970s (Gray et al., 1995). By analysing sediment cores for ^{137}Cs and ^{241}Am , characteristic trends in the time-integrated radionuclide concentration profiles may allow chronologies to be developed (MacKenzie et al., 1994; MacKenzie and Scott, 1993).

For this study this method was used to answer the main research question; does seagrass vegetation increase sediment accumulation when compared to mudflat areas, however results were inconclusive. Although subsurface peaks in both ^{137}Cs and ^{241}Am radionuclides were found in sediment cores from SOL, the corresponding $^{137}\text{Cs}/^{241}\text{Am}$ activity ratios were not consistent with the expected ratios for the period of maximum discharge of radionuclides from Sellafield or recently deposited sediment, suggesting an additional source of ^{137}Cs may be masking the 'true' activity concentration profiles at this site. $^{137}\text{Cs}/^{241}\text{Am}$ activity ratios in the north east Irish Sea off-shore sediment are shown to decrease from around 6 in the late 1970s to around 1 by the 1990s (MacKenzie et al., 1998), with recently deposited material having a ratio of 0.25 (Kinch, 2017 pers. comm). The expected ratios were not observed in any of the cores analysed for SOL in this study. As the

cores were intertidal and collected close to shore, it was hypothesised that atmospheric ^{137}Cs deposited and retained in the rich organic soils surrounding the Solway coast after the Chernobyl nuclear power plant accident in 1986 and entering this environment from terrestrial run-off, may have caused the unexpected ratios seen (Clark and Smith, 1988). In addition to this complication of source apportionment a second issue arose which was most probably a sampling issue but could not be overlooked.

When gamma counting a 50 cm depth core at 5 cm intervals, both ^{137}Cs and ^{241}Am were found at detectable levels at the bottom of the core profile. This is not unusual however in order to develop a chronology from a ^{210}Pb dated core coupled with an independent chronology derived from anthropogenic radionuclides such as ^{137}Cs and ^{241}Am , it is preferable to reach a core depth where no above background radionuclides are detected. As this core depth was not reached on the first sampling occasion, two deeper additional cores, one seagrass and one mudflat, were collected to a depth of 76 cm and 75 cm, respectively from the same site. Gamma counting of four lower depth increments (50, 60, 70 and 76 cm) for the deep seagrass core also unfortunately found measurable levels of ^{137}Cs and ^{241}Am present at the bottom of the depth profile (76 cm) after activities of ^{137}Cs and ^{241}Am were found to be below detection level in the previous three samples (50-70 cm). The presence of ^{137}Cs and ^{241}Am at the bottom of the core cast doubt on the integrity of the core sampling and/or core storage procedures. Despite the careful precautions taken to extract and quickly wrap sediment cores on site to avoid cross contamination, mudflats contain pools of surface water throughout and are highly saturated. The disturbance created from extracting a core results in water from within the sediment and on its surface mixing with the core which is unavoidable and may have led to cross contamination, but this is difficult to determine and outside the scope of this study. Therefore, these site-specific challenges prevented the successful dating of SOL cores collected which would have allowed sediment accumulation rates to be determined for vegetated and mudflat areas.

LOSS ON IGNITION – A SUITABLE METHOD FOR DETERMINATION OF ORGANIC MATTER

APPLICATION FOR MARINE AND COASTAL SEDIMENTS

PRINCIPLE OF METHOD

The need to standardise this method to enable widespread application across a range of sediment samples with more certainty in accuracy is essential if country wide habitat carbon inventories are to be produced.

The method estimates sequential weight loss of core sediment subsamples after heating at a range of temperatures which have been selected based on literature and scientific evidence. The subsamples are placed in weighed crucibles and then repeatedly weighed after heating to remove labile, refractory and inorganic matter. Samples must be cool when weighing and must be placed in a desiccator between weighing and combustion to avoid absorption of atmospheric water. The percentage of organic matter determined can then be converted to percentage of organic carbon within the sediment sample using a regression equation. To quantify carbon density the dry bulk density quantified can be used.

EQUIPMENT

- Drying oven
- Balance (weighing in grams to 4 decimal places)
- Drying tins
- Sample spatula
- A known volume spoon or syringe
- Desiccator
- Crucibles
- Muffle furnace (that can reach 950°C)

- Tongs for removing samples from furnace
- heat resistant gloves
- series of sieves (optional)
- sieve shaker (optional)
- pestle and mortar (optional)
- Riffle box (optional)
- Weighing boats (optional)

SAFETY

The greatest hazard during this experiment is being burned during sample removal from the furnace. This method has been tailored to avoid contact with the furnace when it is above 100°C. However precautionary safety gloves which comply with general laboratory safety protocols should be used in the event different practices were conducted.

METHOD

DRY BULK DENSITY – VOLUME APPROACH

This step should not be missed if the aim is to quantify carbon density within a sample.

1. Weigh drying dish
2. Place an aliquot of 5cm³ (or a known amount depending on available tools within your lab) from each subsample (wet) into drying dish
3. weigh sample in drying dish¹
4. Place in pre-heated drying oven at 60°C for 48 hours (or in highly saturated samples, until a constant weight is reached).
5. Place in desiccator to cool and prevent the sample adsorbing atmospheric water
6. Reweigh sample
7. Use the following equation to determine dry bulk density (DBD)

$$\text{DBD (g cm}^{-3}\text{)} = \frac{\text{dried sediment sample weight (g)}}{\text{initial wet sample volume (cm}^3\text{)}}$$

¹ This step, including the initial weight enables the user to determine dry matter and water content on a mass basis if required

8. Place sample in desiccator if intending to use it immediately for LOI application. If not, seal in an air-tight sample bag and leave at room temperature

PRIOR SUBSAMPLE CHECKS FOR CLAY CONTENT AND CARBONATES CONTENT

The main issues with the application of LOI in sediment samples are clay content and inorganic materials being combusted during labile and refractory matter temperatures therefore if it is suspected that the sediments contain a high proportion of clay sized particles or are high in inorganic matter, particularly magnesite. For both, further analysis is required to determine suitable conversion factors.

GRAIN SIZE ANALYSIS

The method applied will depend on available laboratory apparatus and scope of project. This SOP provides details on the most accessible method – mechanical grain size analysis via sieving. This method is carried out with either air dried or oven dried samples. The method separates fine sediment particles from coarser sediment particles. A minimum of three sieve sizes are required to collect sand, silt and clay particles separately.

1. Sieve or remove any large or unnecessary particles, such as rocks, shells or vegetation.
2. If sample has evidence of agglomeration grind gently with rubber pestle and mortar to loosen.
3. If samples are large use a riffle box or other suitable method to provide a non-biased representation of the whole sample, this equipment will reduce the volume of sample without removing an unfair distribution of any of the particles.
4. Weigh subsample and then add to stacked sieves, either use a sieve shaker or shake by hand until sample has passed through the first sieve. Place the remaining soil into a weighing boat and weigh to 4 d.p, repeat this step for each sieve size and record each weight.
5. To calculate the relative percentage of sand, silt and clay in the subsample use the following equations:

$$\text{Sand (\%)} = \frac{\text{Mass of sand (g)}}{\text{Total soil mass (g)}} \times 100$$

$$\text{Silt (\%)} = \frac{\text{Mass of silt (g)}}{\text{Total soil mass (g)}} \times 100$$

$$\text{Clay (\%)} = \frac{\text{Mass of clay (g)}}{\text{Total soil mass (g)}} \times 100$$

6. *In future iterations of this SOP this step will determine the most suitable correction factor for subsamples with certain proportions of clay content which alter LOI of OM significantly*

Please note: It is unlikely that silt and clay fractions of the sediment will be fully separated, particularly if this is done by hand, however it will provide a rough indication of the proportion of clay is high within the subsample. If accurate proportions are sought for full grain size analysis please use a different method.

CARBONATES ANALYSIS

An easily accessible suitable method still needs to be determined.

ORGANIC MATTER DETERMINATION BY LOI

1. Weigh crucible (4 d.p) Sediment samples to determine DBD to be oven dried, for 48hours or until constant weight at 60°C
2. Test samples for clay content. Whilst this study used a laser diffraction particle sizing analyser, dry sieving samples and weighing the proportion of sediment within each sieve is just as effective to provide estimates. XX assumed samples with higher clay content than XX would require a correction factor (and we hope to test this). We would currently assume that all samples with >20% clay sized particles would not be suitable for this method.
3. If samples contain <20% clay content the following combustion methods are advised
 - i. Labile matter: 2g aliquot for 6 hours at 250°C
 - ii. Refractory matter OR total organic matter if labile matter step is missed: for 6 hours at 500°C (same sample)
 - iii. Inorganic matter: 2 hours at 950°C (same sample)
4. OM and IC is calculated using the following equations:

$$\text{labile OM (\%)} = \frac{\text{initial dry sediment weight (g)} - \text{weight of sediment after ignition at 250°C (g)}}{\text{initial dry sediment weight of sample before temperature step (g)}} \times 100$$

$$\text{Refractory OM (\%)} = \frac{\text{initial dry sediment weight at 250°C step (g)} - \text{weight of sediment after ignition at 500°C (g)}}{\text{initial dry sediment weight of sample before temperature step (g)}} \times 100$$

$$5. \text{ Total OM (\%)} = \frac{\text{initial dry weight of sample before temperature step (g)} - \text{weight of remaining sample after burning at relevant temperature (g)}}{\text{Initial dry weight of sample before temperature step (g)}} \times 100$$

$$\text{Inorganic matter} = \frac{\text{initial dry sediment weight at 500°C step (g)} - \text{weight of sediment after ignition at 950°C (g)}}{\text{initial dry sediment weight before temperature step (g)}}$$

6. *In future iterations of this SOP this step will determine the most suitable correction factor to use if the sediment subsample contains high clay content or Magnesite.*

DETERMINATION OF OC (%)

The blue carbon manual (Fourqurean et al., 2014) recommends localised conversion equations to use OM to produce OC values, Potouroglou et al., (2021) has created a Scottish sediment regression equation, for LOI at 500°C;

$$\text{OC (\%)} = (0.41 \times \text{OM}) - 0.13$$

Where OM is total OM, if you have conducted a stepped LOI use TOM based on the 500°C combustion.

DETERMINATION OF CARBON DENSITY

Use OC content (%), previously derived DBD content, and if the samples underwent compaction, due to the sampling protocol used, apply this factor in the following equation:

$$\text{Carbon density} = \left(\text{DBD (g cm}^{-3}\text{)} \frac{\text{OC (\%)}}{100} \right) \times \text{compaction factor}$$

The compaction is derived from the measurements taken before and after extraction, in addition to the measurement taken before the samples were removed from sediment cores before sample preparation.